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Drought tolerance screening of plum rootstocks based on physiological and biochemical traits

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Abstract: Drought-tolerant rootstocks with better performance regarding water deficit is important for sustaining orchard productivity, especially in regions where water availability is unpredictable. By selecting appropriate rootstocks, fruit growers can mitigate the adverse effects of insufficient water on yields. However, the response of specific rootstocks to drought remains unknown. Our study examined the drought tolerance of five plum rootstocks (Wavit, Torinell, Adesoto, Penta, and St. Julien) focusing on their physiological and biochemical responses. To assess their tolerance under drought conditions, we evaluated leaf relative water content (RWC), chlorophyll fluorescence, lipid peroxidation, hydrogen peroxide (H_2O_2), proline, and phenolic content. The results showed that Torinel exhibited the highest performance index (PI_{ABS}), maximum PSII photochemical efficiency (F_v/F_m), RWC, lowest lipid peroxidation and H_2O_2 during the drought-stress condition. Based on our results, we identified Torinel as a rootstock with a great ability to withstand drought, suggesting that it could be applied in the breeding program to increase plum resistance to drought. The study provides insights into the drought tolerance of different plum rootstocks, identifying which ones are better suited for cultivation in water-limited environments.

Keywords: climate change; water scarcity; oxidative stress; photosynthesis; *Prunus domestica* L.

Abiotic stressors present great challenges to agricultural production, as they impact numerous aspects of plants morphology, physiology, and biochemistry, leading to adverse effects on growth, development, and yield (dos Santos et al. 2022). Among various abiotic stress factors, drought is one of the most significant, since it plays a crucial role in sustaining crop growth and productivity. To conserve water, plants often close their stomata in response to drought, thereby limiting gas exchange and reducing the possibility of absorbing CO_2. Consequently, photosynthesis declines (Viljevac et al. 2013), which negatively affects plant growth (Wang et al. 2018). During drought conditions, plants produce reactive oxygen species (ROS) within their cells where overproduction of ROS can lead to dysfunction in cellular structures and molecules which cause oxidative stress (Kar 2011). Excessive formation of ROS, particularly H_2O_2, indicates oxidative stress that leads to cellular damage and lipid peroxidation, prompting plants to accumulate various defensive compounds such as proline and phenolic compounds, which act as osmoprotectants and antioxidants to enhance drought tolerance and reduce oxidative damage (Sarker and Oba 2018). Indicator of plant hydration during water scarcity, lower relative water content (RWC), reflects cell dehydration. As a consequence of cellular dehydration, a decrease in turgor pressure occurs, leading to the shrinkage and wilting of plant tissues (Seleiman et al. 2021).

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If plants lack access to water, they are unable to obtain nutrients, leading to disruptions in nutrient uptake and transport within the plants, resulting in nutrient deficiencies (Bista et al. 2018). Plums are an important stone fruit grown in temperate climates around the world. They are valued for their nutritional content because they possess a high content of antioxidants, vitamins, and minerals, which contribute significantly to human health (Walkowiak-Tomczak 2008). Due to their versatility, plums can be used in processed foods like jams, jellies, juices, making them highly valuable ingredients in the food industry (Birval et al. 2017). Unlike other fruit species, the introduction of new vegetative rootstocks in plum cultivation has been lacking, with *Prunus cerasifera* L. cv. Myrobalana being the most commonly used rootstock in Europe (Biško et al. 2019, Zezulová et al. 2022). Rootstock selection is a very important part of fruit production since it affects tree health (Bowman and Albrecht 2020), yield (Caruso et al. 2020), fruit quality (Martins et al. 2021), and adaptability to specific growth conditions (Solonkin et al. 2022). Therefore, fruit growers must carefully consider their choice of rootstock to ensure long-term and successful fruit production. It was observed that modern, new rootstocks demonstrate increased vigour, a better ability to withstand biotic and abiotic stressors, enhanced plant yield, and improved fruit quality (Russo et al. 2007, Pavlousek 2011, Denardi et al. 2016). Although in previous studies researchers explored the qualitative and nutritional characteristics of plum cultivars cultivated on various rootstocks (Butac et al. 2015, Zezulova et al. 2022), there is still missing data regarding the influence of abiotic stressors on rootstocks, as this aspect has not been thoroughly examined. Considering the challenges that fruit production, including plum growing, faces with drought, which is emerging as a significant concern, our study examined five different plum rootstocks for drought tolerance. The objective was to evaluate their tolerance, and to distinguish the most tolerant and sensitive rootstocks.

MATERIAL AND METHODS

Plant material and experimental conditions. This study included five different rootstocks: Wavit, Torinell, Adesoto, Penta, and St. Julien, chosen for their compatibility with a wide range of European plum cultivars (Moreno et al. 1995, Czinege et al. 2012). Wavit, derived as a clone of *Prunus domestica* L. cv. Wangenheim from a seedling, produces a semi-vigorous tree with high yields and robust fruit size, exhibiting resilience to winter conditions (Stefanova et al. 2009). Torinell, a hybrid of Reine Claude P 99 and Reine Claude de Bavay is used for intensive production systems and robust cultivars, featuring good compatibility with most cultivars and an earlier fruit onset. Adesoto is a vigorous plum rootstock from *Prunus insititia* L. Bullace, known for its robust root system and drought resistance, and is propagated through woody cuttings. Penta, a cross between Tetra (*Prunus domestica* L.) and Adara (*Prunus cerasifera* L.), induces semi-dwarf to dwarf trees. St. Julien, a *Prunus insititia* L. rootstock, is widely used in plum cultivation due to its semi-vigorous growth and suitability for various plum cultivars, having become the standard for growing plums (Nečas et al. 2023). The trial took place in the greenhouse at the Agricultural Institute Osijek, Croatia (45°32'32.18"N, 18°33'22.36"E) in August 2023. Bare-rooted rootstocks were obtained from a commercial nursery and put into 4-L pots with a soil mix of 65% white peat, 35% black peat, 150 L clay/m^3, and 1 500 g nitrogen-phosphorous-potassium fertiliser/m^3. Rootstocks were adequately watered using drip irrigation for 16 weeks to establish roots, formation of new leaves, and acclimate to environmental conditions. At the beginning of the experiment (April 2023), each rootstock was watered daily with 0.5 L in the morning, and as the rootstocks grew, watering was increased to 1 L daily during the summer months to ensure sufficient hydration for all rootstocks. During the study, the temperatures in the greenhouse varied between 22 °C and 38 °C daily, and humidity ranged from approximately 70% in the morning to 35% during the day. Following the acclimation period, we conducted an experiment with a total of ten plants per rootstock, separated into two sets: five plants experienced water shortage by not being watered (referred to as drought stress treatment plants, DS), while the other five plants were regularly irrigated (referred to as control plants, CTRL). Following 6 days of drought treatment, we measured chlorophyll fluorescence on three developed leaves of each plant. The same leaves were harvested, frozen, and stored at –80 °C for further examination. Additionally, fresh leaf samples were gathered for assessing RWC. Each treatment group was subjected to either fifteen or five independent biological replicates per rootstock and treatment, depending on the specific analysis.

Determination of *Chl* fluorescence parameters. We measured the photochemical performance of photosystem II (PSII) using fast chlorophyll fluorescence kinetics. Measurements were carried out with a Handy-PEA fluorimeter (Plant Efficiency Analyser, Hansatech Instruments Ltd., Norfolk, UK) on both control and drought-stressed plants exposed to water deficit between 8:00 and 9:30 a.m. During measurements, the temperature was recorded at 26 ± 2 °C, humidity at 70 ± 10%, and light intensity at 250 ± 50 µmol/m^2/s. Prior to measurements, leaves were exposed to darkness using leaf clips for 30 min. Afterwards, they were subjected to a saturating light pulse lasting 1 s to obtain the transient rise of OJIP chlorophyll fluorescence. The dynamics of the fluorescence increase were recorded, and parameters were computed and analysed using the JIP test (Strasser et al. 2000). For analysis, 9 JIP parameters were chosen: maximum quantum yield of PSII ($F_v/F_m = TR_0/ABS = [1^{-}(F_0/F_m)$; performance index of PSII based to absorption (PI_{ABS}) = $(RC/ABS) \times (TR_0/DI_0) \times [ET_0/(TR_0 - ET_0)]$); Q_A reducing RCs per PSII antenna *Chl* ($RC/ABS = (F_v/F_m) \times (V_J/M_0)$); flux ratio trapping per dissipation ($TR_0/DI_0 = F_v/F_0$); electron transport further than primary acceptor Q_A ($ET_0/(TR_0 - ET_0)$); absorption per active RC ($ABS/RC = M_0 \times (1/V_J) \times [1/(F_v/F_m)]$); trapping per active RC ($TR_0/RC = M_0 \times (1/V_J)$); electron flux reducing end electron acceptors at the PSI acceptor side per RC ($RE_0/RC = 1/M_0(1/V_J)(1V_I)$); dissipation per active RC ($DI_0/RC = (ABS/RC) - (TR_0/RC)$.

Transient fluorescence curves. OJIP transient's data were normalised between the F_0 and F_m phases, and the chlorophyll fluorescence transients with O-J-I-P phases were plotted on a logarithmic scale. Fluorescence between OP steps was expressed as V_{OP} ($W_{OP} = (F_t - F_0)/(F_m - F_0)$ for all rootstocks in drought treatment. To better evaluate the changes in fluorescence intensity between the OJ and OK steps, we doubled normalised fluorescence values between F_0 and F_J (2 ms) and between F_0 and F_K (0.3 ms). To visualise the K (~ 300 µs) and L band (~ 150 µs), ChlF transient data were expressed as $V_{OJ} = [F_t - F_0)/(F_J - F_0]$ and $V_{OK} = [F_t - F_0)/(F_K - F_0]$. Data were plotted as difference kinetics between drought treatments and the reference (control treatment): $[\Delta V_{OK} = V_{OK(drought\ treatment)} - V_{OK(control)}]$ and $[\Delta V_{OJ} = V_{OJ(drought\ treatment)} - V_{OJ(control)}]$ (Yusuf et al. 2010, Martins et al. 2017). Results are expressed in relative units (rel. u.).

Determination of the relative water content. Following the assessment of chlorophyll fluorescence, leaves were collected to determine the RWC. Leaf discs measuring 1 × 1 cm were excised to measure their fresh weight. After measuring the fresh weight, the leaf discs were placed in distilled water at 8 °C for a period of 24 h. Turgid weights of the leaf discs were recorded after the 24-h soaking period. Subsequently, the leaf discs were exposed to a 24-h drying process at 80 °C to determine the dry weight. The RWC was calculated using the formula: RWC (%) = (FW – DW)/(TW – DW) × 100, where FW, DW, and TW represented the fresh weight, dry weight, and turgid weight of the leaf discs, respectively.

Determination of lipid peroxidation and H_2O_2 content. For extraction, 0.1 g of tissue powder (grounded in liquid nitrogen) was mixed with 1 mL of 0.1% trichloroacetic acid (TCA), and was left for 15 min on ice. After centrifugation at 14 000 × *g* for 15 min, obtained supernatants were utilised to determine the lipid peroxidation and H_2O_2 content.

The lipid peroxidation level in leaf samples was quantified by measuring malondialdehyde (MDA) content using the following method (Verma and Dubey 2003). 0.5 mL of supernatant was collected and mixed with 1 mL 20% TCA containing 0.5% (*w*/*v*) thiobarbituric acid (TBA). The mixture was heated at 95 °C for 30 min, then cooled in an ice bath and centrifugated at 14 000 × *g* for 15 min. Subsequently, the absorbance of the supernatant was measured at 532 nm and 600 nm using a Specord 200 spectrophotometer (Analytic Jena, Jena, Germany). The MDA level was expressed as nmol/g of fresh weight.

The method described by Velikova et al. (2000) was employed to assess hydrogen peroxide levels in leaf tissue. 0.25 mL of the obtained supernatant was combined with 0.25 mL of 10 mmol potassium phosphate buffer (pH 7.0) and 0.5 mmol of 1 mol potassium iodide. After a 20-min incubation period in darkness, the absorbance was measured at 390 nm using a microplate spectrophotometer (Epoch, BioTek, Winooski, USA). Verification of H_2O_2 content was carried out using a H_2O_2 standard curve and expressed as µmol/g FW.

Total phenolics and proline content. For extraction, 0.1 g of tissue powder (grounded in liquid nitrogen) was mixed with 1 mL of 80% ethanol and was left for 1 h in an ultrasound bath at 25 °C. After centrifugation at 14 000 × *g* for 15 min, supernatants were obtained to determine the total phenolic and proline content.

The total phenolic content was determined through a modified Folin-Ciocalteu method (Singelton and Rossi 1965). In this procedure, 5 µL of the supernatant was mixed with 795 µL of distilled water and 50 µL of Folin-Ciocalteu reagent, which was diluted at a 1:1 ratio with water. Following a 5-min incubation period, 150 µL of a 20% sodium carbonate solution was added to the mixture. The resulting mixture was homogenised and kept in darkness at room temperature (20–25 °C) for 60 min. The absorbance was measured at 765 nm using an Epoch microplate spectrophotometer. The total phenolic content was calculated from the gallic acid standard curve and expressed as mg/g FW.

The proline content in micromoles per gram of fresh weight (µmol/g FW) was determined by the method of Woodrow et al. (2017). Samples (50 µL) and proline standards were placed in reaction tubes, with 100 µL of a reaction mixture (ninhydrin 1% (*v*/*v*) in acetic acid 60% (*v*/*v*) and ethanol 20% (*v*/*v*)). The mixtures were heated at 95 °C for 20 min, centrifuged, and transferred to a polypropylene microplate and the absorbance at 520 nm was measured using an Epoch microplate spectrophotometer. Quantification of proline content was carried out using a proline standard curve.

Statistical analyses. The data were shown as the mean ± standard error (SE) of fifteen (for *Chl* fluorescence parameters) and five biological replicates for RWC, MDA, H_2O_2, proline, and phenolics. Two-way analysis of variance (ANOVA) was used to statistically analyse the data, and the differences between means in the interaction of treatments and rootstocks were examined using post hoc analysis, the least significant difference (*LSD*) test, with a probability of $P < 0.05$.

RESULTS AND DISCUSSION

This study examined various biochemical and physiological changes in the leaves of five different plum rootstocks under water deficit conditions. We also analysed parameters commonly used for screening and evaluating tolerance to various abiotic stresses. RWC is an indicator crucial for understanding the hydration status of the plant, representing the balance between water availability and transpiration in leaf tissue (Jin et al. 2017). A decrease in RWC serves as an early sign of water deficiency in plants and is often used to identify the water deficit tolerance of different cultivars (Soltys-Kalina et al. 2016, Masheva et al. 2022). In our study, we observed that 6 days without water led to a significant decrease in RWC in Adesoto, Penta, St. Julien, and Wavit leaves, indicating a substantial reduction in leaf water content in these rootstocks (Figure 1A). The RWC values of these four rootstocks declined to 53% (Adesoto), 59% (Penta), 55% (St. Julien), and 54% (Wavit) of the control levels. However, RWC in the leaves of Torinel remained unchanged, indicating that the water content in the leaves of this rootstock was stable despite the water deficit (Figure 1A). Previous studies have shown that leaf RWC is directly related to the tolerance of plants to stress, since plants with higher RWC levels generally exhibit better tolerance (Siddiqui et al. 2016, Meetam et al. 2022). Therefore, the highest RWC in drought-stressed Torinel rootstocks indicates that this rootstock is more capable of dealing with drought stress conditions.

Lipid peroxidation, one of the most commonly used parameters for detecting oxidative stress in plants, is often measured by MDA content, whose concentration rises in plant tissues subjected to stress and reflects the extent of lipid peroxidation within the cells (Khaleghi et al. 2019). Plants with higher levels of MDA content indicate greater membrane permeability and are more sensitive to drought than those with lower MDA content (Pandey et al. 2010). The levels of MDA content significantly increased in drought-stressed plants compared to control plants in all investigated rootstocks (Figure 1B). During drought treatment, Torinel showed the lowest MDA content among the rootstocks, with the smallest increment, when compared to control plants. This suggest that it has better capabilities for drought stress tolerance. Rootstocks Adesoto and St. Julien exhibited the highest MDA content under drought conditions, implying their cellular membranes are more vulnerable and impaired. Therefore, they are considered more sensitive to drought. Drought-induced damage was also confirmed by a significant increase in H_2O_2 accumulation in all investigated rootstocks, with the highest accumulation observed in Wavit (175%) and the lowest in Torinel (35%) (Figure 1D). Increase in reactive oxygen species, such as H_2O_2, resulting in oxidative stress (Khojerdi et al. 2016) and elevated levels of H_2O_2 in plants experiencing drought stress were also observed in many previous studies (Sanchez-Rodrıguez et al. 2010, Cao et al. 2017). Proline helps plants cope with water deficits by scavenging ROS. It accumulates in plants to maintain cellular turgor pressure by acting as an osmolyte, thus helping to counteract osmotic stress and prevent dehydration by balancing water levels within the cells (Delauney and Verma 1993). As shown in Figure 1C, all rootstocks showed a significant increase in the amount of free proline in their leaves during drought treatment, with the highest accumulation observed in Wavit and the

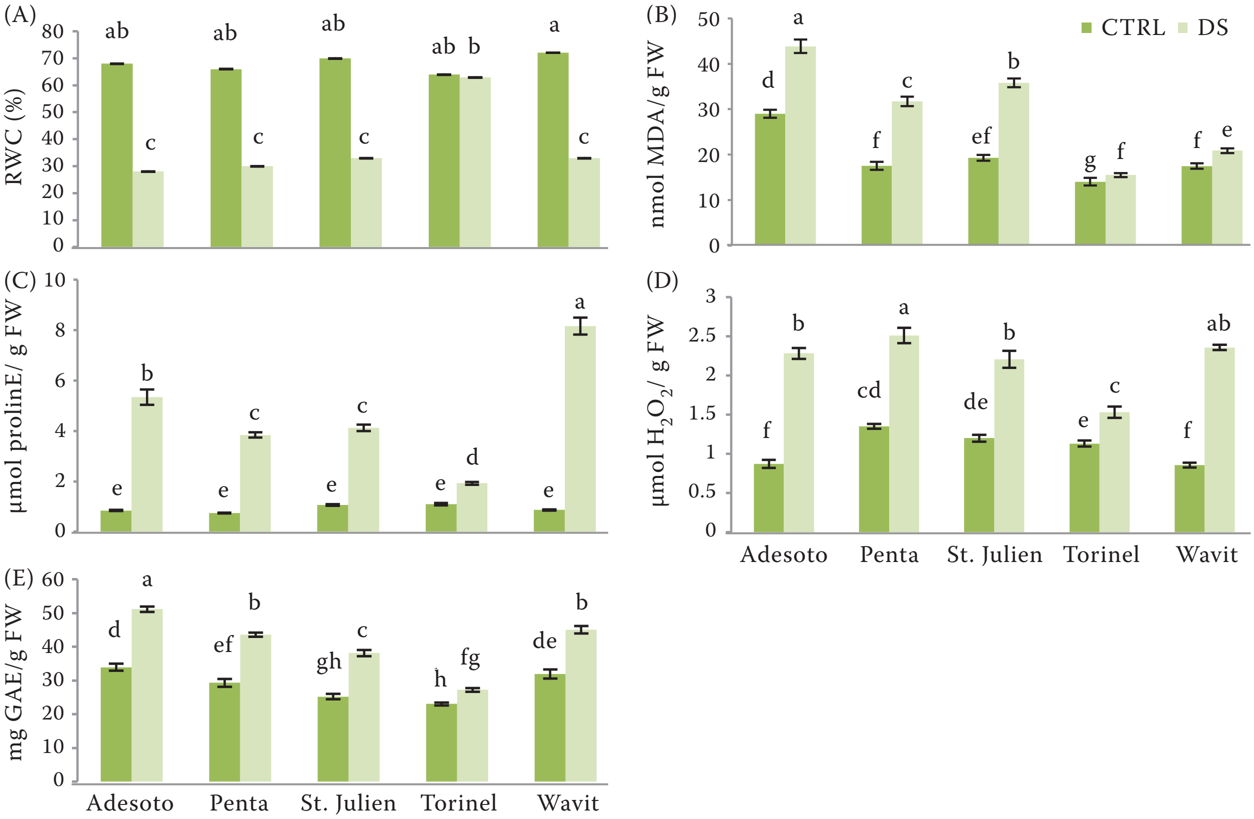

Figure 1. The impact of drought on (A) relative water content (RWC); (B) malondialdehyde (MDA); (C) proline; (D) hydrogen peroxide (H_2O_2), and (E) phenolics (GAE) contents in the leaves of five plum rootstocks. The data is presented as means ± standard error, with $n = 5$. Distinct letters in the figures indicate significant differences among the treatments and rootstocks as determined by the *LSD* (least significant difference) test ($P < 0.05$). CTRL – control plants; DS – drought stress treatment; FW – fresh weight

lowest in Torinel. Previous research has shown that drought-tolerant varieties typically have increased proline levels compared to drought-sensitive cultivars (Türkan et al. 2005, Anjum et al. 2012). Despite these findings, the beneficial effects of proline accumulation are not always pronounced or relevant to abiotic stresses. We noticed a higher accumulation of proline content in drought-sensitive rootstocks, unlike the drought-tolerant Torinel, where proline accumulation was the smallest. Similarly, Cicevan et al. (2016), Mihaljević et al. (2021) also did not find a connection between proline levels and tolerance to drought. Rampino et al. (2006) found that drought-sensitive genotypes accumulated more proline levels, which increased immediately, while the increase in resistant genotypes was delayed.

All rootstocks exhibited an increase in phenolic content, with Adesoto and St. Julien showing the highest increments (by 51%) and Torinel displaying the smallest increase (18%) (Figure 1E). Phenolics, as secondary metabolites, have a vital function in alleviating drought stress in plants. Through their antioxidative properties, which enable them to eliminate ROS, they protect plant cells from oxidative damage while maintaining cellular integrity and functionality (Nakabayashi et al. 2014). They are synthesised in response to drought, and drought-tolerant cultivars often produce higher levels of phenolic compounds than sensitive cultivars (Kumar et al. 2023). The lowest production of total phenolics in Torinel compared with other rootstocks implies that it possesses additional mechanisms or adaptations that help its survival in drought conditions without phenolic compounds for protection against oxidative stress. Similar to our results, Varela et al. (2016) also reported reduced polyphenol levels in *L. chilense* and the absence of correlation with antioxidant capacity in leaves, suggesting that secondary metabolite production is not a significant characteristic of drought avoidance in this species.

PSII is considered the most sensitive component of the photosynthetic apparatus. Therefore, understanding its responses to stress is important for understanding the plant's adaptation and tolerance to stressful environmental conditions (Guidi et al. 2019). Lack of water reduced the photochemical efficiency of PSII in most investigated rootstocks. A significant decline in F_v/F_m parameter was observed after 6 days of drought in all rootstocks except Torinel. St. Julien showed the greatest decrease among all the rootstocks, with a decline of 43% (Figure 2A). During the water deficit, a significant decrease in the PI_{ABS} parameter was noticed in Adesoto, Penta, St. Julien, and Wavit, with the greatest decline (93%). Minor changes were observed for Torinel, but they were not significant (Figure 2B). The chlorophyll fluorescence parameters F_v/F_m and PI_{ABS}, which are commonly used in screening for drought tolerance, showed that Torinel maintained stable photosynthetic activity under drought conditions, whereas other investigated rootstocks experienced significant declines in these parameters, indicating reduced photosynthetic activity. Among these rootstocks, a values of F_v/F_m were below 0.75, which, according to previous researches, implies that their photosynthetic apparatus was not functional and that damage to photosystem II has occurred (Zhuang et al. 2020, Jin et al. 2023). Our findings align with previous studies, where drought stress decreased the photochemical activity of PSII, leading to a reduction in leaf F_v/F_m as well as lower PI_{ABS} values, indicating higher levels of drought sensitivity (Rapacz et al. 2019, Plich et al. 2020).

During drought stress, several parameters related to photosynthesis, including absorption, trapping, electron transport, and dissipation, exhibit specific behaviours that reflect their adaptive responses to reduce the negative impact of water deficit on photosynthesis. In our study, we found that the decrease in PI_{ABS} of Adesoto, Penta, and Wavit under drought conditions was primarily attributed to significant increases in ABS/RC (Figure 1A), TR_0/RC (Figure 3B), DI_0/RC (Figure 3D), coupled with a decrease in ET_0/RC (Figure 3C). In contrast, the values of these parameters remained unchanged in Torinel. The increase in

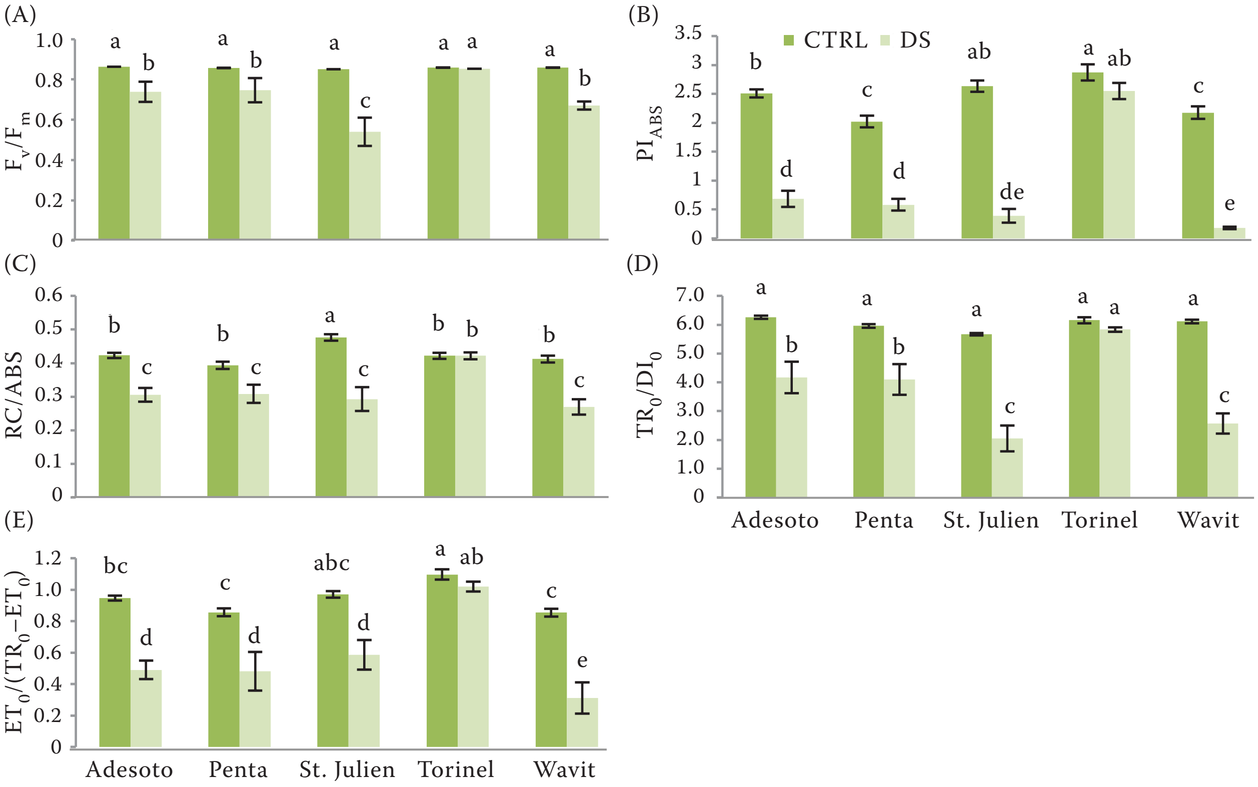

Figure 2. (A) Maximum PSII photochemical efficiency (F_v/F_m); (B) performance index (PI_{ABS}); (C) RC/ABS; (D) TR_0/DI_0, and (E) $ET_0/(TR_0–ET_0)$ of drought stressed leaves in five plum rootstocks. The data is presented as means ± standard error, with n = 15. Distinct letters in the figures indicate significant differences among the treatments and rootstocks as determined by the *LSD* (least significant difference) test ($P < 0.05$) CTRL – control plants; DS – drought stress treatment

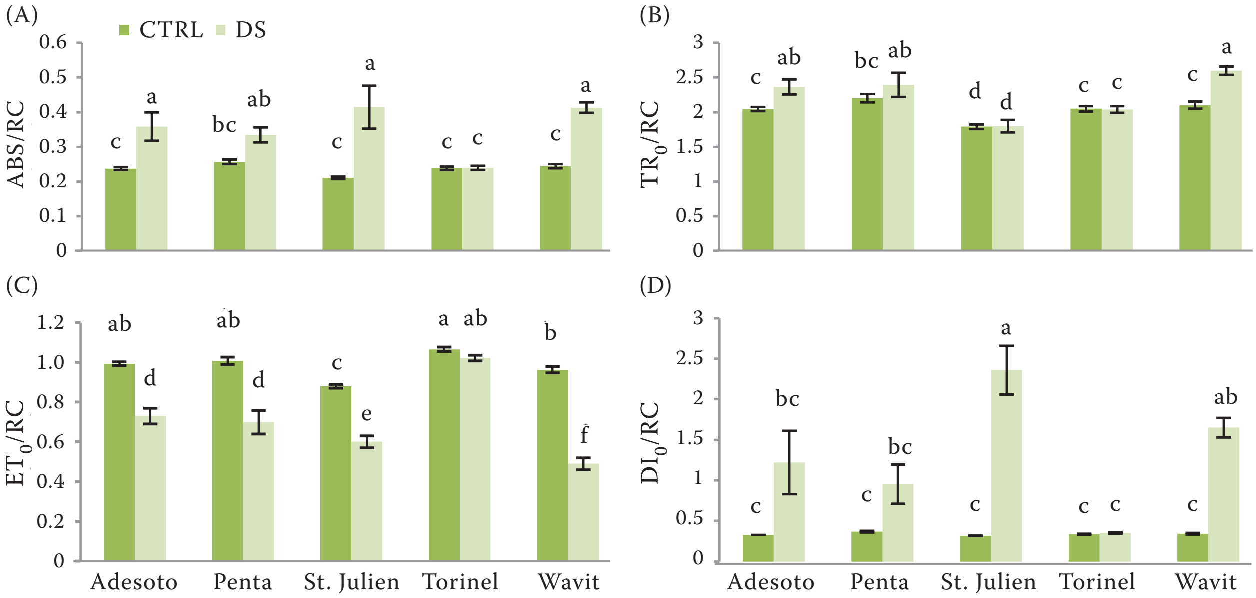

Figure 3. (A) ABS/RC; (B) TR_0/RC; (C) ET_0/RC; and (D) DI_0/RC of drought-stressed leaves in five plum rootstocks. The data is presented as means ± standard error, with $n = 15$. Distinct letters in the figures indicate significant differences among the treatments and rootstocks as determined by the *LSD* (least significant difference) test ($P < 0.05$). CTRL – control plants; DS – drought stress treatment

specific fluxes per RC ABS/RC, TR_0/RC, and DI_0/RC is a common response of plants to drought stress, typically resulting from damaged OEC and impaired electron transport beyond Q_A. An increase in ABS/RC values suggests that active reaction centres become less effective due to increased inhibition of electron transport from Q_A^- to Q_B and transformation of RCs into silent RCs, which is evident by the decreased values of ET_0/RC and increased energy dissipation (DI_0/RC) (Zheng et al. 2019). Greater reductions in the parameter ET_0/RC (Figure 3C) observed in drought-stressed plants of Adesoto, Penta, St. Julien, and Wavit suggest a suppression of electron transport per RC. In contrast, unchanged values of ET_0/RC in Torinel indicate a better photosynthetic electron transport system under drought conditions than the other rootstocks. Previous research observed similar increases in parameters ABS/RC, DI_0/RC, and TR_0/RC, along with decreases in ET_0/RC and PI_{ABS} in drought-sensitive wheat plants (Ghaffar et al. 2023) and sunflower plants (Markulj Kulundžić et al. 2023). PI_{ABS} parameter consists of three components: TR_0/DI_0, RC/ABS and $(ET_0/(TR_0 - ET_0)$. These components significantly decreased when subjected to drought treatment in Adesoto, Penta, St. Julien, and Wavit rootstocks (Figures 1C–E). These declines led to a reduction in the overall PI_{ABS} values of investigated rootstocks. In contrast, in Torinel, these parameters remained unchanged, with the highest PI_{ABS} and F_v/F_m values, indicating that its photosynthetic apparatus can maintain efficiency despite stressful conditions. In the study of Badr and Brüggemann (2020) and Sousaraei et al. (2021), it was also observed that drought-stressed plants with the highest PI_{ABS} and F_v/F_m values exhibited better drought tolerance.

After six days without water, the OJIP curves changed their shape from the typical OJIP curve in all rootstocks except for Torinel. Significant increases in J and I steps were observed, particularly pronounced in the Wavit rootstock, which exhibits the highest J and I steps while Torinel maintains a consistent shape (Figure 4). Similarly, previous studies on drought-stressed maize leaves have also observed an increase in the J and I steps (Zhou et al. 2019). The increase in J and I steps is a consequence of the electron transfer inhibition from the primary electron acceptor Q_A to the secondary electron acceptor Q_B, as well as from Q_B to plastoquinone (PQ) within the PSII reaction complex (Strasser et al. 2004, Gao et al. 2018).

The differential curves for the L and K bands are presented separately to examine and show modifications in OJIP fluorescence rise kinetics in more detail. In drought treatment, the positive L and K

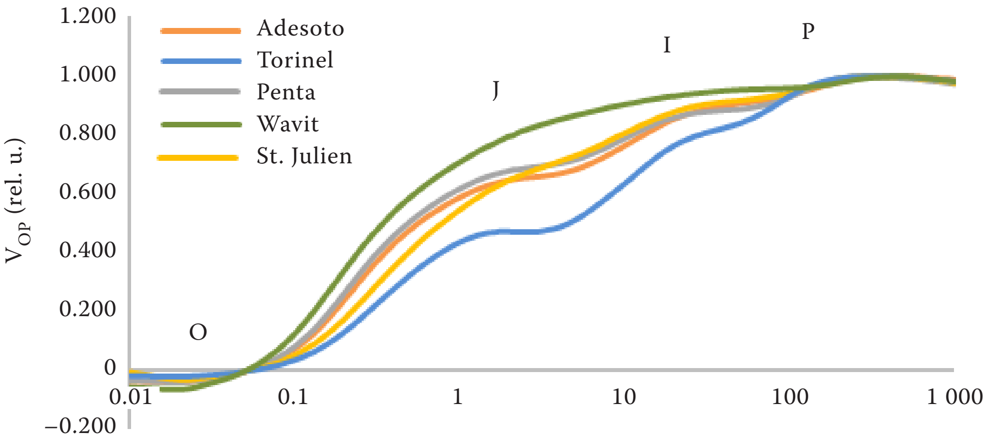

Figure 4. Double normalised OJIP chlorophyll *a* fluorescence rise measured on drought-stressed leaves of five plum rootstocks. $V_{OP} = [(F_t - F_0)/(F_P - F_0)]$. O step – minimal fluorescence intensity when all PSII RC are open; J step – fluorescence intensity at 2 ms; I step – fluorescence intensity at 30 ms; P step – maximal fluorescence intensity when all PSII RC are closed

bands appeared in Adesoto, Penta, St. Julien, and Wavit rootstocks, with the highest peak of the L and K bands observed in the Wavit rootstock. However, drought stress measurements did not reveal any significant L and K band changes in the Torinel rootstock (Figure 5A, B). Double normalisation of chlorophyll *a* fluorescence kinetics using the L and K bands has been shown in previous studies to be a valuable tool for assessing the potential drought tolerance of plants (Oukarroum et al. 2007, Gomes et al. 2012). The amplitude of the K

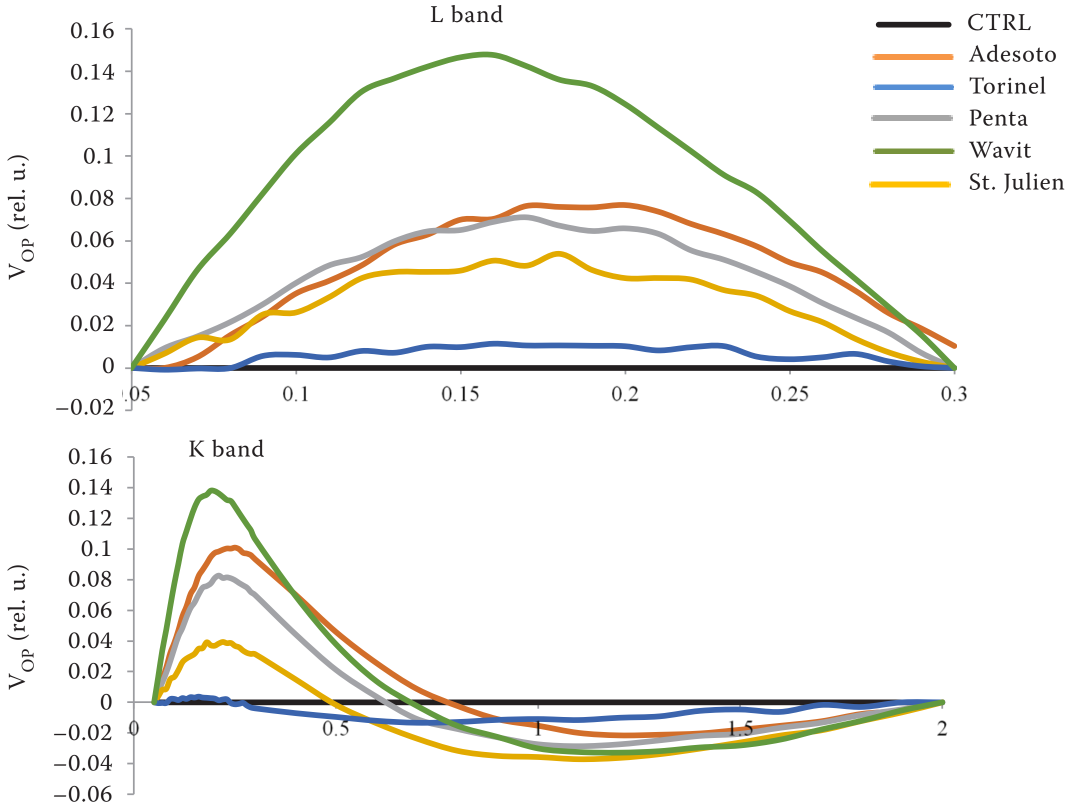

Figure 5. Effects of drought stress on (A) O–K (L band) and (B) O–J (K band) phase kinetic curves in the leaves of five plum rootstocks, submitted to water deficit for 6 days. [$\Delta V_{OK} = V_{OK}$(drought) – V_{OK}(control)]; [$\Delta V_OJ = V_{OJ}$(treatment) – V_{OJ}(control)]. CTRL – control plants; DS – drought stress treatment

peak, or the change in its intensity, correlates with the degree of injury of OEC (De Ronde et al. 2004). The positive K band values in drought-sensitive rootstocks within our research also signified a decline in OEC performance, attributed to disruptions the electron flow from the OEC to the PSII reaction center (Gomes et al. 2012). Among all investigated rootstocks, Wavit exhibited the highest amplitude, implying the most substantial reduction in the activity of OEC on the electron donor side. Torinel did not show a significant increment in the K step, meaning there was no disruption of the OEC (Figure 5A). The L band is used in drought stress research to indicate energy connectivity or grouping between PSII units in plants (Gomes et al. 2012). A positive L band indicates that the PSII units are less grouped, meaning that under drought stress, the ability of PSII units to efficiently transfer and utilise energy decreases (Straser and Stirbet 1998). This decrease in energetic connectivity is associated with lower stability within the system and was noted in Adesoto, Penta, St. Julien and Wavit, who showed the greatest amplitude of the L band. Torinel showed higher connectivity, improved utilisation of excitation energy and greater stability within the system, as indicated by the slightly positive L band (Figure 5B). Falqueto et al. (2017) noted similar results when evaluating the PSII activity of two rubber tree clones during drought conditions.

The results show that Torinel rootstock is the most tolerant of the investigated drought conditions among the tested rootstocks. The potential of Torinel under water deficit conditions requires further confirmation through field experiments to verify its practical usefulness in natural environments. These findings are useful for breeding projects focused on creating plum cultivars that can withstand drought, as they provide guidelines for the selection of superior rootstock for further field evaluation and commercialisation.

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