

ABA accumulation and PsAO gene expression in field pea under water deficit

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Abstract

The plant hormone abscisic acid (ABA) plays a crucial role in plant responses to drought and other abiotic stresses, facilitating adaptation mechanisms under water-deficit conditions. This study aimed to investigate the response of field pea (*Pisum sativum*) varieties to drought stress by evaluating ABA concentrations, stomatal conductance, and the expression of *PsAO* genes during the third leaf pair stage. Drought stress was simulated by withholding irrigation to impose moderate and severe levels of water deficit. A statistically significant increase in ABA concentration was observed in all tested pea varieties under stress conditions. Under moderate drought, stomatal responses varied among genotypes; however, severe drought triggered accelerated stomatal closure across all varieties. The cultivar Dukat exhibited the highest stomatal sensitivity, which corresponded with a tenfold increase in ABA concentration, suggesting a strong reliance on chemical (ABA-mediated) drought signaling. In contrast, Javor showed only a modest (2.5-fold) increase in ABA, despite reduced stomatal conductance, indicating a likely reliance on hydraulic signals for drought response. Gene expression analysis revealed that *PsAO2* and *PsAO3* genes were upregulated under drought, while *PsAO1* expression remained relatively unchanged compared to control plants. Notably, *PsAO3* expression was consistently elevated under both moderate and severe drought, suggesting that this gene may play a central role in conferring drought tolerance in field pea. These findings highlight the importance of ABA biosynthesis and signaling, particularly via *PsAO3*, in the adaptation of pea plants to water-deficit conditions.

Keywords: Drought, Field pea, ABA, Gene expression.

1. Introduction

Pea (*Pisum sativum* L.) is the second most important food legume worldwide which, together with other legumes, provides one-third of the entire amount of protein for human and

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animal consumption. Field pea serves as a significant source of fodder and forage for livestock, as well as a key component in the production of edible and industrial oils (Petrović et al., 2016). Like other pulses, field pea exhibits moderate sensitivity to various abiotic stressors, particularly drought (Petrović et al., 2021). Drought is defined as the prolonged absence of sufficient moisture, which is essential for normal plant growth and the completion of its life cycle (Latif, 2014). It is a major abiotic factor that adversely affects crop plant growth and development, primarily reducing vegetative growth, shoot and root length, and leaf area (Petrović et al., 2021). Water deficit reduces plant growth through the inhibition of various physiological and biochemical processes, such as reduction of the rate of photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism, and hormones (Latif, 2014). One of the initial responses of plants to water deficit is stomatal closure, a process regulated by the plant hormone abscisic acid (ABA). ABA plays a crucial role in the plant's response to drought stress by mediating cellular signaling that regulates water movement from the root to the leaf (Alves and Setter, 2004). Under drought conditions, the accumulation of ABA helps plants adapt to drought and other abiotic stress factors (Tuteja, 2007; Sah et al., 2016). The constant presence of ABA in low concentrations is necessary for the normal growth and differentiation of plant cells. Increased ABA concentration is necessary for stomatal closure, seed dormancy, and aging (Sah et al., 2016; Wu et al., 2022). ABA is very intensively studied worldwide because it has been established that it represents a universal "stress hormone" whose accumulation, especially in roots, helps plants adapt to abiotic stress factors, such as drought, high and low temperatures, salt or ion stress (Nakashima and Yamaguchi-Shinozaki, 2013; Liu et al., 2017). It is one of the most important signaling molecules in plants, which plays multiple roles in regulating many developmental processes and adaptive stress processes (Santner et al., 2009; Cutler et al., 2010; Wu et al., 2022).

In different plant tissues and organs, the intensity of biosynthesis and ABA concentrations can vary dramatically during different stages of development, depending on the changing environmental conditions (Xiong and Zhu, 2003). ABA biosynthesis primarily occurs in chloroplasts and other plastids. The concentration of free ABA in the cytosol is regulated through processes such as degradation, conjugation, and transport. For example, cytosolic ABA increases during drought as a result of synthesis in the leaf, redistribution within the mesophilic cells, transport from the shoots, and recirculation from other leaves. The concentration of ABA decreases after water rehydration due to degradation and reduction in the rate of synthesis, but also due to translocation from leaves to other organs (Zdunek and Lips, 2001).

The primary objective of studying plant responses to abiotic stresses, such as drought, salinity, and cold, is to develop crops with enhanced tolerance to these stress factors. Plants usually respond to water deficit in the soil or in the growth substrate by closing the stoma and reducing leaf growth in order to reduce its transpiration surface (Wilkinson and Davies, 2008; Goodger et al., 2005). These stress responses can be initiated by the so-called chemical (Davies and Zhang, 1991) or hydraulic signals (Gowing et al., 1990). Absciscic acid (ABA) has the main role in sending chemical signals related to drought stress.

A lot of research has been done to clarify molecular mechanisms behind plant adaptation to abiotic stresses. Now the most of the genes involved in ABA biosynthesis have been identified. Water deficit stimulates changes in the expression profiles of different gene classes, most of which are associated with ABA signaling pathways (Zdunek-Zastocka et al., 2004; Zdunek-Zastocka, 2008). Metabolic pathways leading to free active ABA have been described (Wu et al., 2022). Aldehyde oxidase (AO; EC 1.2.3.1) plays a crucial role in the final step of absciscic acid (ABA) biosynthesis, and it is also involved in the biosynthesis of other important phytohormones, including indole-3-acetic acid (IAA). The enzyme is localized in the cytosol and catalyzes the oxidation of indole-3-acetaldehyde (IAAld) to IAA and absciscic aldehyde (ABAld) to ABA (Seo et al., 2000; Wu et al., 2022). AO is a molybdoenzyme, meaning it contains a molybdenum cofactor (Moco) at its catalytic site, which is essential for its enzymatic function. Three AO isoforms were isolated from the leaves and roots of peas: PsAO1, PsAO2, and PsAO3 (Zdunek-Zastocka et al., 2004; Zdunek-Zastocka, 2008). The activity of PAO1 was dominant in the leaves of seedlings and young leaves of mature plants, while PAO3 showed the highest band intensity in old leaves and roots. PsAO2 mRNA was not affected by salinity or ammonium treatment. PsAO3 could oxidize absciscic aldehyde, a precursor of ABA, indicating the possible involvement of this isoform in ABA synthesis in pea plants (Zdunek-Zastocka, 2008).

Due to the limited information available on the physiological responses of Serbian field pea (*Pisum sativum* L.) varieties to drought, this study aimed to evaluate the effects of water deficit on selected physiological and biochemical parameters. Specifically, it investigated differences in absciscic acid (ABA) concentration, stomatal conductance, and the expression of ascorbate oxidase (AO) isoforms among selected cultivars at both the early seedling stage and the stage of the third pair of leaves. In addition, the study sought to identify the most active AO isoform under drought conditions in field pea plants.

2. Materials and Methods

2.1 Plant material

The study was conducted on seven genus of *Pisum* genotypes: Junior, Dukat, Partner, Trezor, Javor, Pionir and Mraz, selected based on morphological characteristics and whether they are spring or winter genotypes, taken from the collection of the Institute of Field and Vegetable Crops, Novi Sad, Serbia (five spring and two winter cultivars). Seeds were sterilized with 0.1% Sodium hypochlorite (w/v) for 1 min and then thoroughly rinsed with distilled water (Asmat et al., 2019).

2.2 Drought stress experiment

The drought stress experiment was conducted under controlled conditions (phytotron chamber), with a photoperiod of 14h, brightness (PAR) 300 $\mu\text{mol}/\text{m}^2$, temperature 25/18 °C and 70% relative humidity of air. The plants were grown in 1 L pots filled with commercial substrate (Potground H, Klasmann-Deilmann, Germany) and irrigated to maintain an optimal soil water content of 36% until the development of the third pair of leaves. Drought stress was then induced by withholding irrigation, reducing the substrate water content to 18% for moderate drought (treatment T1) and to 9% for severe drought (treatment T2). Control plants were maintained under optimal soil water conditions, with irrigation adjusted to achieve a substrate water content of 36%. The amount of water in the substrate was measured by the Theta Probe (ThetaProbe, type ML2X, Delta-T Devices Ltd., England).

2.3 Measurement of the stomatal conductance

Stomatal conductance (gs) was measured on the abaxial surface of the third leaf pair using a SC-1 Leaf Porometer (Decagon Devices Inc., USA). Five plants per treatment were used for measurements. Measurements were made for each variety in the control, as well as in two different drought treatments (T1 and T2). Values are expressed in mmol/m^2 .

2.4 ABA concentration measurement

Before extraction, frozen plant leaves were ground to a fine powder in pre-chilled steel cylinders, with liquid nitrogen, by a mixer mill (A-11 basic, IKA). ABA extraction was done from 0.5g of plant tissue mixed with 1.5ml of water and 2% PVP without further purification (Asch, 2000). Cross-reaction of the antibody with other compounds in the extract was avoided by briefly boiling plant tissue in water before extraction and then in a Thermomixer comfort (Eppendorf) at 4 °C, in a dark room, during the night. Before the analysis, the cross-reaction

test confirmed that the isolated ABA extract does not contain other substances that react with the antibody without being antigenic, i.e. abscisic acid. ABA content in the leaves of the investigated plants was measured by the ELISA method using MAC 252 monoclonal antibody for ABA according to Asch (2000). Plate contents (Nunc: F96 Maxisorp immuno plate) were read at 405 nm by an ELISA reader (Sunrise, Tecan).

2.5 Gene expression analysis

RNA was extracted from 100 mg of frozen plant tissue, using RNeasy Plant Mini Kit (Qiagen, Germany), according to the manufacturer's manual. The quality and quantity of the extracted RNA were checked with an UV/VIS spectrophotometer (Evolution 100, Thermo Scientific, USA), by measuring the absorbance at 260 nm (A260) and 280 nm (A280). The A260/A280 ratio is a widely used method to assess the purity of RNA samples, specifically to check for protein contamination. It is based on UV absorbance measurements and provides useful information about the quality of the RNA. The ratio for the isolated RNA ranged between 1.8 and 2.0, indicating high purity. The use of Ambion 171 DNA-free DNase Treatment and Removal Reagents before cDNA synthesis is an essential step to ensure the removal of genomic DNA from the RNA sample. cDNA synthesis was carried out with 100 ng of total RNA in a final reaction volume of 20 μ l, using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) following the manufacturer's protocol. The synthesized cDNA, using it as a template in a PCR reaction is a key step for amplifying specific genes of interest.

The amplification was carried out on Mastercycler gradient (Eppendorf, Germany), using a premix of 2 $\times$ PCR Master Mix, (Fermentas, Lithuania) containing 4 mM MgCl₂, 0.4 mM dNTP, 0.05 units μ l⁻¹ Taq DNA Polymerase (recombinant in a final volume of 25 μ l. The temperature programme was as follows: denaturation at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 sec, 53 – 57 °C for 30 sec, and 72 °C for 1 min, and final extension at 72 °C for 10 min.

After performing PCR, amplification products were analyzed by electrophoresis on a 1% agarose gel with ethidium bromide (0.5 μ g/ml). The expected fragment sizes were estimated by comparison with the FastRuler™ Low Range DNA Ladder (Fermentas, Lithuania). Gels were visualized under UV light using a transilluminator, and images were captured with the BioDoc Analyze documentation system (Biometra, Germany).

2.6 Statistical analysis

Percentage data were arcsine-transformed before statistical analysis. Analysis of variance (ANOVA) was performed for all investigated parameters using SigmaPlot 10.0 (Systat

Software Inc., San Jose, CA, USA). We followed the statistical methodology described in Marchin et al., 2020. Significant differences among the mean values were compared by Student's t-test ($P < 0.05$). Figures were drawn using Sigmaplot. Genotypes and treatments were compared by means of the two-factor analysis of variance (ANOVA) with significance levels of $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

3. Results

3.1 Stomatal conductance (g_s)

Stomatal conductance was measured for each variety on the third pair of leaves under control conditions and two drought treatments (T1 and T2) (Table 2). Notable differences were observed between the T1 and T2 treatments, with all varieties exhibiting significantly reduced stomatal conductance under severe drought conditions (T2).

The conductivity of the stoma was the lowest in cultivar Pionir at the moderate drought (T1) (50.04 mmols^{-2}), while cultivar Trezor had the highest conductivity (80.64 mmols^{-2}). In the case of severe drought (T2), cultivar Dukat showed the highest conductivity values of stomatal conductance (37.96 mmols^{-2}), while Junior had the lowest value ($16.08 \text{ mmol s}^{-2}$).

Regression analysis was conducted in order to interpret treatment effects correctly. A sigmoidal fourth-order regression pattern was made to draw the curve of the dependence of the stomatal conductance in relation to the volume of water content in the substrate (Figure 1). The regression model explains a significant portion of the variance in stomatal conductance under moderate drought (T1), with statistical significance from $P < 0.001$. Dukat showed the highest tolerance in this treatment with faster closing of stomata and decreased conductance by 66.98% compared to conductance under optimal conditions. Similar results were found in the cultivar Pionir (reduction in conductivity by 63.30%). Under the same - T1 treatment, the stomas of cultivar Junior had a significantly higher conductivity of stoma than other varieties (52.98% compared to control) so they were more open. However, with a further decline of water in the substrate and stronger stress (T2 treatment), the stoma of this cultivar closed faster and the lowest conductance was measured (11.20% compared to the control). Comparing the reactions of stomatal cells in cultivar Partner with other cultivars, the slowest decrease in conductance was observed in this particular cultivar.

3.2 ABA Accumulation in leaves of field peas

Measurements of ABA accumulation (Table 3) were made in plant leaves under control and in plants exposed to drought stress (moderate and severe).

In all cultivars, a statistically significant increase in ABA content, compared to the control, was observed at both drought treatments (Table 3). Compared to control, under the conditions of moderate and severe stress, the lowest increase in ABA concentration was found in cultivar Javor (moderate 95.62%, severe 147.26%), and the highest was found in cultivar Dukat (moderate 637.27%, severe 917.04%). Comparing the drought treatments, the lowest increase in ABA at T2 compared to T1 was observed in cultivar Mraz (13.08%) and the highest in cultivar Pionir (55.33%).

Among the tested cultivars, Dukat exhibited the highest accumulation of the abscisic acid (ABA) stress hormone under both drought treatments, with the differences being statistically significant compared to other cultivars. Conversely, Javor consistently showed the lowest ABA content across both treatments. Furthermore, the most pronounced increase in ABA concentration was observed under drought conditions compared to the optimal water regime, indicating a strong drought-responsive hormonal adjustment.

The dependence of ABA content in leaves and water content in the soil is shown in Figure 2. A statistically significant increase in the ABA concentration in leaves in reducing soil water content is evident at both drought treatments in all varieties ($P < 0.001$). Variety Dukat showed the highest accumulation of ABA while accumulation of ABA was the lowest in variety Javor, compared to other varieties (Table 3).

The results of stoma cell conductivity and changes in the conduction of stoma cells are also shown in correlation with ABA content in leaves (Figure 3).

The results showed different sensitivity of the stoma cells of the tested cultivars to the changes in ABA concentration. In this case, the accumulation of ABA in leaves greatly affected Dukat's stoma and less the cultivar Javor's stoma.

Dukat showed the highest accumulation of ABA in leaves, while the lowest accumulation was measured in Javor. Dukat also showed the highest sensitivity of stoma cells in drought treatments because the stomatal conductivity decreased, compared to conductivity under optimal conditions. Based on all listed above Dukat was the most resistant cultivar.

3.3 The influence of drought on the expression of the genes involved in the synthesis of ABA

The expression levels of three genes: *PsAO1*, *PsAO2*, and *PsAO3* were analyzed in two field pea (*Pisum sativum*) genotypes: Dukat, which showed the highest ABA accumulation, and Javor, which showed the lowest, relative to control conditions. Gene expression was assessed in leaf tissue collected from plants grown under control conditions and those subjected to severe

drought stress. The *Arabidopsis thaliana* 18S rRNA gene was used as the internal reference for normalization.

Changes in the expression levels of the transcript of three genes for AO in the leaves of selected pea cultivars are presented in Figure 4.

Compared to the control, the ABA content increased by 917% under drought conditions in Dukat and by 147% in the Javor (Table 3). For both cultivars, the increased expression of the *PsAO2* and *PsAO3* genes was observed under the influence of drought, but it was more expressed in Dukat than in Javor. The expression of the *PsAO1* gene in both cultivars was similar to the expression in the control group. It can be assumed that *PsAO1* and *PsAO3* play a greater role than *PsAO2* in the synthesis of ABA in leaves of selected pea cultivars under drought stress.

4. Discussion

Different environmental and internal signals including drought, salt stress, light, temperature, CO₂ concentration, relative humidity, phytohormones, and microbes affect and regulate the function of the stomata on the plant leaves (Bawa et al., 2023; Driesen et al., 2020; Wachendorf and Küppers, 2017). The stomatal response is a crucial aspect of plant defense against abiotic stress. As one of the first reactions in response to stress, it emphasizes the essential role of stomatal regulation in maintaining water balance and minimizing water loss, particularly in drought and high-temperature conditions. Research continues to deepen our understanding of how ABA and other regulatory pathways control stomatal function and help plants survive in challenging environmental conditions. (Matkowski and Daszkowska-Golec, 2019; Ferguson 2019; Kolist et al., 2019). Stomatal conductance (g_s) is a key physiological parameter that estimates the rate of gas exchange and transpiration through the leaf stomata, particularly under abiotic stress conditions such as drought or high temperatures. Early stomatal closure in response to mild drought can be positively correlated with drought tolerance in pea genotypes. However, the timing and extent of stomatal response must be finely tuned—too early or too severe closure can hurt productivity (Agurla et al., 2018).

The rate of gas exchange and transpiration through the leaf stomata is estimated by stomatal conductance (g_s). Generally, stomatal conductance is higher when stomata are open wider and lower when stomata are closed or narrower. Our study of the stomatal cell conductance in seven field pea cultivars under drought stress showed differences between cultivars, and also between treatments. All cultivars had significantly lower stomatal conductance ($P < 0.0001$) in conditions of severe drought. In order to explain the obtained results

regression analysis was conducted. The curve of the dependence of the stomatal conductance in relation to the volume of water content in the substrate showed that the greatest differences in the reactions of the stoma of the investigated cultivars were expressed under moderate drought (T1). However, with a further decline of water in the substrate, the stoma of all cultivars closed faster. R-squared values ranged from 0.9652 to 0.9935. This means that 85% of the variance in stomatal conductance can be explained by the independent variables in the model, suggesting a strong relationship between the predictors and stomatal conductance. The fastest partial closure of the stomata in the cultivar Dukat, in conditions of drought, may represent a significant adaptive reaction that allows this cultivar to maintain the highest degree of stoma openness in comparison to other cultivars, in conditions of severe drought. This enables the assimilation process to be continued, which results in the highest drought resistance index of both the aerial part and the roots (Petrović, 2021). The stomatal response observed in Dukat is an intriguing aspect of its drought tolerance strategy. However, to assess whether this trait is generalizable across other species, it's important to consider the genetic and physiological diversity within legumes. Similar results of changes in stoma conductivity were determined by Maksimović et al. (2010), by measuring stomatal conductance on the leaves of pea plants under the influence of salinity, as well as Sassi et al. (2010), who investigated the effect of osmotic stress on bean plants. Many legumes, such as common beans (Polania et al., 2022) and chickpeas (Pang et al., 2016), also exhibit adaptive stomatal responses to drought, suggesting that this trait may be a common mechanism for drought resilience in the family. However, the effectiveness of these responses can vary based on environmental conditions and specific genetic backgrounds. Therefore, while similar patterns may be observable in other legumes, it is crucial to conduct comparative studies to understand the nuances of stomatal behavior and its adaptive significance in different cultivars and species.

Many signals induce stomatal closure. Among these ABA is a best-known signal that regulates water status and stomatal movement. The amount of ABA in the plant tissue is regulated in several metabolic steps, both in biosynthesis and inactivation steps (Brookbank et al., 2021). Under drought conditions, plants produce and accumulate increased amounts of ABA in the guard cells, and this induces stomatal closure to conserve water (Bharath et al., 2021). Measurement of ABA concentration in the leaves of tested field pea cultivars exposed to drought in this work also confirmed these claims. In all cultivars, a statistically significant increase in ABA content, compared to the control, was observed, at both drought treatments. Dukat showed the highest accumulation of ABA stress hormone in both treatments compared

to other cultivars, and this difference is statistically significant. In both treatments, the lowest content of ABA, compared to other cultivars, was found in Javor. In addition, the maximum increase in the concentration of ABA was determined under drought compared to the optimal water regime conditions. There is a clear relationship between ABA response and drought tolerance. In pea genotypes, more efficient ABA perception and signalling are often key traits in drought-tolerant lines. Breeding programs increasingly use these traits as selection criteria for improving drought resilience.

The results of stomatal conductance measurements, together with changes in ABA content in the leaves, revealed differential sensitivity of stomatal cells among the tested cultivars in response to ABA accumulation. In particular, ABA accumulation had a pronounced effect on stomatal closure in Dukat, while the stomata of Javor exhibited lower responsiveness to changes in ABA levels. Dukat displayed the highest stomatal sensitivity under drought stress, as indicated by a significant reduction in stomatal conductance compared to well-watered conditions. Taken together, these findings suggest that Dukat possesses enhanced drought resistance, likely mediated through more efficient ABA-regulated stomatal control. This response is clearly influenced by chemical signaling, particularly through the accumulation of abscisic acid (ABA). In contrast, Javor exhibited only a minimal increase in ABA concentration under drought stress, while still showing a reduction in stomatal conductance. This suggests that, in Javor, stomatal regulation may be primarily governed by hydraulic signals rather than chemical cues. Hydraulic signaling involves changes in leaf water status, often resulting from reduced root water uptake and decreased turgor pressure in leaf cells (Goodger et al., 2005). These changes can inhibit leaf growth and promote stomatal closure. According to the concept of chemical drought signaling (Gowing et al., 1990), the root system acts as a critical "sensor" of soil conditions—detecting not only reductions in water availability but also changes in the soil's mechanical composition. Roots perceive environmental stress and initiate a complex chemical communication network with the shoot. This involves ABA, ethylene, cytokinins, sugars, and ions, each playing roles in: modulating stomatal behavior, reprogramming growth and metabolism and activating systemic stress responses. The results of Davies and Zhang (1991) showed that ABA begins to accumulate at the root in the first stages of water deficiency. As a result of the changed pH values of xylem juice and under water deficit, the synthesized compounds, as chemical stress signals, are transported by the xylem to shoots, where they cause the closure of the stoma and reduce the growth of leaf cells (Bahrn et al., 2002; Davies et al., 2005; Schachtman and Goodger, 2008; Wilkinson and Davies, 2008). ABA-driven closure is a

more direct, specific response, where the plant actively synthesizes a hormone (ABA) to signal closure under stress conditions. Hydraulic signals are more of a passive, mechanical response to physical changes in water status, where the plant detects internal water loss and reacts accordingly by closing the stomata. In summary, while both mechanisms lead to stomatal closure in response to water stress, ABA-driven closure involves a hormonal signaling pathway that triggers biochemical changes in the guard cells, while hydraulic signals result from physical changes in the plant's water status, which lead to stomatal closure as a protective mechanism against excessive water loss (Wilkinson and Davies, 2008).

It should also be noted that ABA biosynthesis is related to carotenoid biosynthesis. Reduced ABA concentration in the leaves may therefore be the result of the metabolic pathway in drought conditions being oriented in the direction of the synthesis of carotenoids as antioxidant agents necessary for metabolism. Other authors suggest a potential trade-off between ABA production and antioxidant capacity. Specifically, during stress conditions (e.g., drought or high salinity), is noted an upregulation of key genes involved in ABA biosynthesis—*NCED3*, for example—which diverts carotenoid precursors like 9-cis-violaxanthin and 9'-cis-neoxanthin toward ABA synthesis. Interestingly, this increase in ABA biosynthesis coincided with a reduction in xanthophyll cycle activity and lower expression of *VDE* (violaxanthin de-epoxidase) and *ZEP* (zeaxanthin epoxidase), which are critical for non-photochemical quenching (NPQ)—a major photoprotective, antioxidant mechanism. These changes were also accompanied by measurable decreases in total carotenoid content and antioxidant enzyme activities (like SOD and APX), suggesting that as more carotenoids are diverted into ABA synthesis, there's less available for direct ROS quenching or photoprotection. This supports the hypothesis that under stress, plants may prioritize hormonal signaling (ABA) over maintaining maximum antioxidant capacity, possibly as a short-term adaptation to optimize water-use efficiency and stomatal regulation (Cazzonelli, 2011; Ruiz-Sola et al., 2014; Van Norman et al., 2014; McAdam et al., 2016; Mercado-Reyes et al., 2024).

The cellular and molecular mechanisms underlying ABA-induced stomatal closure have been extensively studied and are now relatively well understood. Most of the genes involved in ABA biosynthesis have now been identified. Water deficit stimulates changes in the expression profiles of different gene classes, most of which are associated with ABA signaling pathways (Zdunek-Zastocka et al., 2004; Zdunek-Zastocka, 2008). According to Yao et al. (2023) understanding the function of the *AAO* gene family is of great significance for insight into plants' response to abiotic stresses. Analysis of the expression of *PsAO1*, *PsAO2*, and *PsAO3*

was performed in selected field pea genotypes, in which the highest and lowest ABA accumulation occurred in the leaves of pea plants exposed to severe drought treatment, compared to the control. It can be assumed that the genes *PsAO2* and *PsAO3* play a more important role in the synthesis of ABA in the leaves of plants that were in an older vegetative phase of development than *PsAO1*, while in the seedlings the *PsAO2* gene has less importance in the synthesis of ABA, compared to other genes. This is especially expressed in Dukat where, under the influence of severe drought, an 90 - fold increased concentration of ABA was measured in the leaves of the plants compared to the control. Accumulation of ABA in this cultivar also contributes to faster closing of stomata, which reduces transpiration, as well as activation of antioxidant mechanisms (Petrović et al., 2023). An increase in ABA concentration was also measured in Javor, but slightly less than in Dukat, so the stomata of this cultivar closed slower, and the activation of antioxidant mechanisms was reduced. The results of this study are consistent with the results of Zdunek-Zastocka (2010) and Zdunek-Zastocka and Sobczaka (2013), who showed that the most important gene, whose expression increases in pea plants exposed to drought, is *PsAO3*. When compared with other ABA-related genes, such as *NCED3* (a key gene in ABA biosynthesis), which was upregulated, and *ABI5* (an ABA signaling component), which showed also an increase, *PsAO1* gene expression level aligns closely with major components of the ABA pathway. This suggests a potential functional association or regulatory coordination between *PsAO2* and *PsAO3* and core ABA signaling elements (Barrero, 2006).

CONSLUSIONS

The findings of this study underscore the link between drought tolerance and specific physiological and molecular adaptations in selected field pea (*Pisum sativum*) genotypes. The genotypes exhibited distinct responses to drought stress, with notable differences in both physiological and molecular mechanisms depending on the severity of drought. Under moderate drought, stomatal behavior varied among genotypes, whereas all genotypes responded to severe drought with accelerated stomatal closure, a classical water-conserving defense mechanism. A clear correlation was observed between leaf ABA concentration and stomatal conductance. In Dukat, stomatal conductance was strongly affected by a tenfold increase in ABA content, suggesting a dominant role of ABA-mediated “chemical signaling” in this cultivar’s drought response. In contrast, Javor showed minimal stomatal sensitivity despite a 2.5 - fold increase in ABA, indicating a response more reliant on “hydraulic signals,” likely driven by changes in leaf water status and turgor pressure. At the molecular level, all

three analyzed genes (*PsAO1*, *PsAO2*, and *PsAO3*), which are involved in the biosynthesis of the key ABA-producing enzyme aldehyde oxidase, were expressed under drought conditions. Notably, *PsAO3* expression consistently increased under drought, suggesting it plays a central role in ABA biosynthesis during stress and may be a critical component of the drought response pathway in pea. These findings highlight the complexity of drought adaptation mechanisms in pea and point to *PsAO3* as a potential target for future research and breeding programs. However, further studies are needed to elucidate the localization and regulation of ABA transporters, as well as the detailed dynamics of ABA biosynthesis and catabolism under drought conditions.

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Table 1. Sequences of oligonucleotide primers.

Gene	Sequence (5'–3')	Annealing temperature (°C)	Reference
18S rRNK	F: CCAGGTCCAGACATAGTAAG R: GTACAAAGGGCAGGGACGTA	55	
PsAO1	F: GACAGTTCTGCAAGAAAAACCAAGTGG R: CAGCACCAGCTATAGATTTCATGCTC	57	Zdunek-Zastocka, 2008
PsAO2	F: GTCAAGGCCCTGAGTTTGCACA R: GGAGTTGGTTTTAGATGCATCTCTTG	57	
PsAO3	F: CAGCTGGATGGAGTAAGAGATG R: GTGCTGTAACATATGTTATGTGG	55	

Table 2. Mean values of the stomatal conductivity by varieties in the control and drought treatment group.

Variety	Treatment	g_s (mmol/s ²)
Mraz	Control	124.13 ± 2.04
	T1	60.86*** ± 1.10
	T2	25.08*** ± 0.57
Junior	Control	143.61 ± 2.57
	T1	76.08*** ± 0.94
	T2	16.08*** ± 0.83
Javor	Control	166.57 ± 6.74
	T1	64.70*** ± 1.22
	T2	30.66*** ± 0.74
Dukat	Control	194.00 ± 14.96
	T1	64.06*** ± 3.95
	T2	37.96*** ± 1.31
Pionir	Control	147.17 ± 5.46
	T1	50.04** ± 0.74
	T2	22.62*** ± 0.86
Partner	Control	131.80 ± 4.98
	T1	66.48** ± 1.26
	T2	36.64** ± 1.61
Trezor	Control	189.33 ± 10.05
	T1	80.64** ± 1.44
	T2	28.54** ± 1.34

*** - P<0.001, ** - P<0.01.

Table 3. ABA concentrations in plant leaves (ng/g fresh weight) under the influence of drought treatment.

Variety	Treatment	ABA (ng/g FW)
Mraz	Control	187.00 ± 11.23
	T1	486.20*** ± 9.81
	T2	549.80*** ± 15.23
Junior	Control	171.00 ± 13.72
	T1	389.00*** ± 26.63
	T2	458.40*** ± 15.24
Javor	Control	146.00 ± 8.84
	T1	285.60*** ± 11.71
	T2	361.00*** ± 10.16
Dukat	Control	88.00 ± 7.44
	T1	648.80*** ± 19.80
	T2	895.00*** ± 27.21
Pionir	Control	164.00 ± 7.66
	T1	454.00*** ± 15.29
	T2	705.20*** ± 46.19
Partner	Control	161.00 ± 9.44
	T1	456.00*** ± 20.04
	T2	657.80*** ± 26.89
Trezor	Control	146.00 ± 10.78
	T1	356.40*** ± 8.12
	T2	506.00*** ± 24.00

*** - P < 0.001.

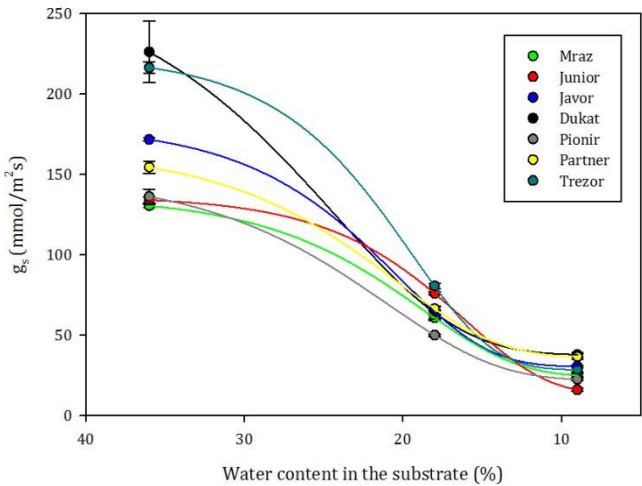


Fig. 1. Changes in stomatal conductivity (g_s) depending on the content of water in the substrate.

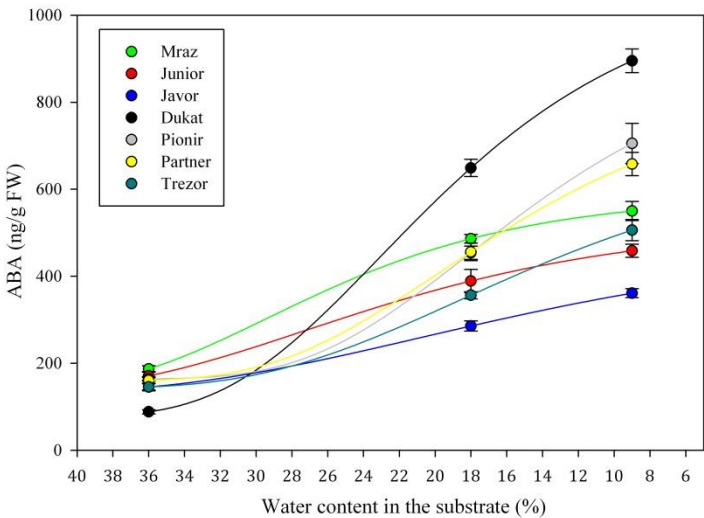


Fig. 2. Changes in the concentration of ABA in leaves depending on the water content of the soil.

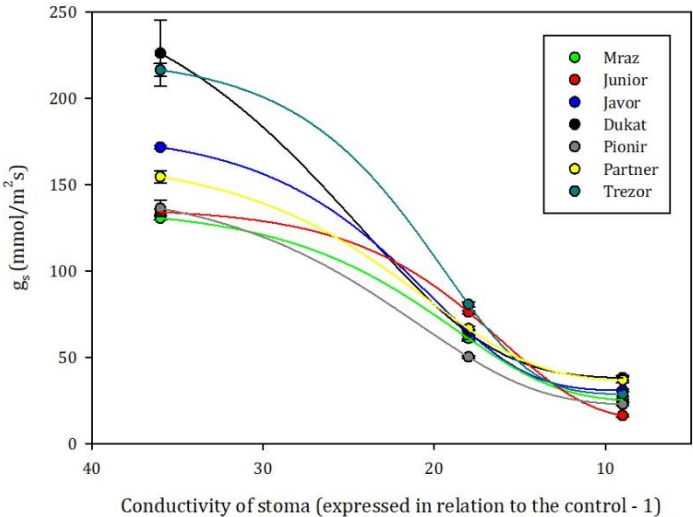


Fig. 3. The ratio of ABA concentration in leaves and conductivity of stoma (expressed in relation to the control = 1).

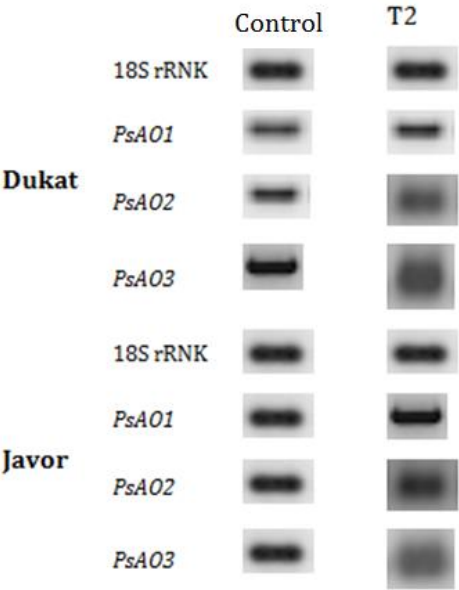


Fig. 4. Effect of drought on the expression of the *PsAO1*, *PsAO2* and *PsAO3* genes in plant leaves of selected field pea varieties.