Stem stomata of the wild almond, *Prunus arabica,* regulate stem photosynthesis in response to different environmental stimuli

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Abstract

World population is estimated to reach 9.5 billion by the year 2050, and accordingly, the demand for food production. Due to climate change, plant-based food production faces changes in environmental conditions, including elevation of the atmospheric [CO_2] and increase in global temperatures. Identifying and integrating traits contributing to plant resilience, growth, and productivity under harsh environmental conditions is in great demand. Almonds are one of the most important crops in the world. The need for almonds is growing, and consequently, the almond industry is expanding across the globe. *Prunus arabica* is a wild almond species native to the Fertile Crescent Mountains (including Israel). Unlike the commercial almond species *Prunus dulcis*, its stomatous stems remain green for several years, assimilating a considerable amount of CO_2. This distinctive trait, termed stem photosynthetic capability (SPC), was previously characterized and genetically mapped in the almond genome. Stomatal conductance regulation has been intensely studied in leaves, yet very little is known about stomatal responses in stems to different stimuli. We tested *P. arabica* stems' under various environmental conditions, known as stomatal ques in leaves. Our results confirm that *P. arabica* stems possess fully functional stomata that respond to CO_2 shifts, light, and temperature changes. The unique SPC trait of *P. arabica* may be beneficial and further used in almond breeding programs. Additional research is being conducted to test SPC's contribution to plant resilience, growth, and productivity in the face of climate change.

Keywords: CO_2, light, temperature, climate, conductance, transpiration

INTRODUCTION

World population is estimated to reach 9.5 billion by the year 2050 (USCB, 2015), and accordingly the demand for food (Long et al., 2015). Yet, with climate change, plants face harsh environmental conditions, including scarcity of water (Seckler et al., 1998; Turner, 2001), increase in global mean temperatures (IPCC, 2021), temperature extremes (Perkins-Kirkpatrick and Lewis, 2020), and elevation of the atmospheric [CO_2]. These climate changes affect plant physiology and influence agricultural food production, which calls for better fit and improved cultivars for future farming.

Almonds are one of the most important agricultural crops in the world and are known for their high nutritional (Chen et al., 2018; King et al., 2008) and health value (Estruch et al., 2018; Hyson et al., 2002; Martínez-González et al., 2008). The demand for almonds is growing, and consequently the expansion of the almond industry worldwide (Shlomi, 2020; Tuchman and Kahal, 2019). During the development of agriculture and in the process of breeding for crop improvement, many traits that were preserved in wild varieties disappeared and do not exist in commercial varieties. With climate change, identifying various traits contributing to

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plant resilience, growth, and productivity under harsh environmental conditions is in great demand. In recent years a rare wild almond species, *Prunus arabica* (Olivier) Meikle (*P. arabica*), has been added to the Newe Ya'ar collection, which contains over 150 different almond types. *P. arabica* is a distinctive almond species and is native to the Fertile Crescent Mountains, including a few specimens that were found in Israel (CatalogueOfLife, 2022). One of its noticeable morphological traits attracted our attention. Unlike other almond species, its stems remain green (un-barked) all year-round (Brukental et al., 2021; Sorkheh et al., 2009). Physiological and genetical based study revealed that *P. arabica* assimilates a considerable amount of CO_2 throughout the year via stem photosynthesis capability (SPC). The trait was genetically mapped to LG 1 and LG 7 (Brukental et al., 2021).

There are two types of photosynthesis via the stems: 1) stem recycling photosynthesis (SRP), which involves the reassimilation of CO_2 from respiration (Ávila et al., 2014; Nilsen, 1995) in barked stems with no stomata (Ávila et al., 2014; Ávila-Lovera et al., 2019; Saveyn et al., 2010), and 2) stem photosynthetic capability (SPC) (Brukental et al., 2021) (also termed stem net photosynthesis (SNP) (Ávila et al., 2014)), which involves the uptake of atmospheric CO_2 via stomatal pores within stem epidermis.

SRP contribution to plant carbon gain (Sprugel and Benecke, 1991) is minor (recycle 7 to 123% of the respired CO_2), while SPC can reach 60% of leaves' photosynthetic rates (Ávila et al., 2014), and even more (Brukental et al., 2021).

Stomatal pores regulate gas exchange between the atmosphere and the interior of the leaf. Each stomatal pore is regulated by two guard cells that change their volume by turgor regulation. This mechanism enables the control of CO_2 uptake for photosynthesis and the limitation of water loss via transpiration for optimal water use efficiency. Various physiological and environmental stimuli regulate stomatal conductance, including CO_2 concentration [CO_2] (Hetherington and Woodward, 2003; Mott, 1988; Zhang et al., 2018), light (Assmann and Jegla, 2016), and temperature (Aparecido et al., 2020; Marchin et al., 2016; Mott and Peak, 2010). Stomatal conductance regulation has been intensively studied in leaves, yet very little is known on stem stomatal responses to different stimuli.

In this study, stomatal conductance responses of the SPC-type stems of the wild *P. arabica* almond to different environmental stimuli were analyzed. Our results showed that *P. arabica* stem stomata open in response to low concentrations of CO_2, and close in response to transition from light to dark and temperature increases. These results further support the physiological role of stomata in SPC-type stems.

MATERIALS AND METHODS

Plant material

We used trees of the wild almond species *Prunus arabica* (Olivier) grafted on GF.677 rootstock and planted in the Helmslli almond orchard at Newe Ya'ar Research Center, Israel (Figure 1). The trees were grown under an intensive agricultural regime, irrigated, and fertilized under the standard growth conditions of cultivated almond.

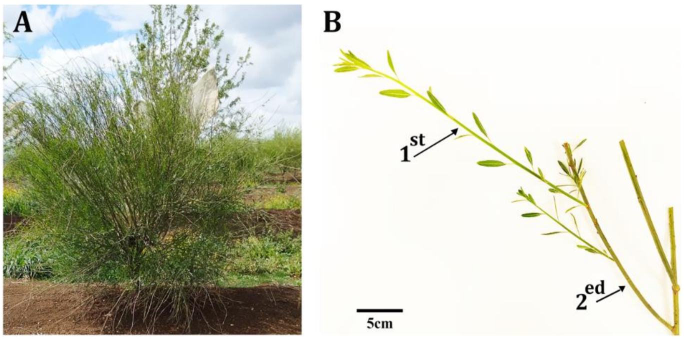

Figure 1. The wild almond *P. arabica* tree shows unique architecture. Representative pictures of *P. arabica* whole tree (A) and its 1st and 2nd-year stems (B).

Gas exchange measurements

Gas exchange measurements were conducted on *P. arabica* stems in the orchard. Leaves were removed from the stems two days prior to the measurements to eliminate any wounding stress effect. Boundary layer conductance to water vapor was estimated using representative stem segments wrapped in wet filter paper (Kollist et al., 2007).

1. Stem stomatal conductance responses to low levels of CO_2.

Stems were acclimated under 900 ppm CO_2 until stability of stomatal conductance (g_s) was reached (usually within 30 min). Then CO_2 was decreased to 100 ppm for an additional 35 min. Air temperature was 20°C and VPD_{stem} was 1.5 kPa. Data was logged every 30 s.

2. Stem stomatal conductance responses to transition from light to dark.

Stems were acclimated under a light intensity of 650 $\mu mol\ m^{-2}\ s^{-1}$. After stability of g_s was reached, the light was turned off, and data was logged every 60 s. VPD_{stem} was kept at 1.3 kPa and air temperature at 22°C.

3. Stem stomatal conductance responses to temperature increases.

Gas exchange parameters were recorded under 28, 35, and 40°C (n=6). The environmental controls of the CIRAS-3 maintained chamber temperature, and VPD_{stem} was kept at 3 kPa. Data was logged after reaching a steady state of photosynthetic rate (A) and g_s for each temperature (after approximately 5 min).

Statistics

Regression analysis was used to describe gas exchange responses to temperature increases using JMP® pro 15.0 software (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

The wild *P. arabica* almond tree possesses a unique architecture and green stems all year round

In nature, *P. arabica* shows a bushy growth pattern (Brukental et al., 2021). *P. arabica* scions were grafted on a GF.677 rootstock in our orchard and trained on one trunk. Representative pictures show the distinctive bushy tree architecture of the *P. arabica* (Figure 1A). *P. arabica* possesses few leaves during spring and summer, which are restricted to the current year stem growth. Its leaves are rigid, greyish, and relatively small when compared to commercial cultivars. It is important to note that *P. arabica* tends to drop its leaves during the dry season in nature. In our fertigated orchard, the leaves remained vital and intact until November. One of the most distinctive features of the wild *P. arabica* is its green stems that stay green all year round (Figure 1B).

Stomata of *P. arabica* stems respond to different environmental stimuli

For optimal performance and maximal efficiency, plants regulate their stomatal conductance in response to their environment. CO_2, light, and temperature are a few of the fundamental environmental stimuli that signal the plant and induce stomatal movements (Driesen et al., 2020). Stomata in plant leaves open in response to low CO_2 (Zhang et al., 2018) and close in response to the transition from light to dark (Assmann and Jegla, 2016). Stomatal conductance responses to temperature were found to vary between different studies. Few studies showed that temperature increases induce stomatal closure (Hamerlynck and Knapp, 1996; Lahr et al., 2015; Mott and Peak, 2010; Slot et al., 2016) while others showed stomatal opening (Aparecido et al., 2020; Drake et al., 2018; Feller, 2006; Marchin et al., 2016; Urban et al., 2017). These differences in stomatal conductance responses to temperature result from the differences in regulatory mechanisms between different species and the variability in the environmental conditions during the experiment (Marchin et al., 2016; Schulze et al., 1973). To test the functionality of the stomata of *P. arabica* 2nd-year stems, we measured stem g_s in response to changes in CO_2 levels, transition from light to dark, and temperature increases.

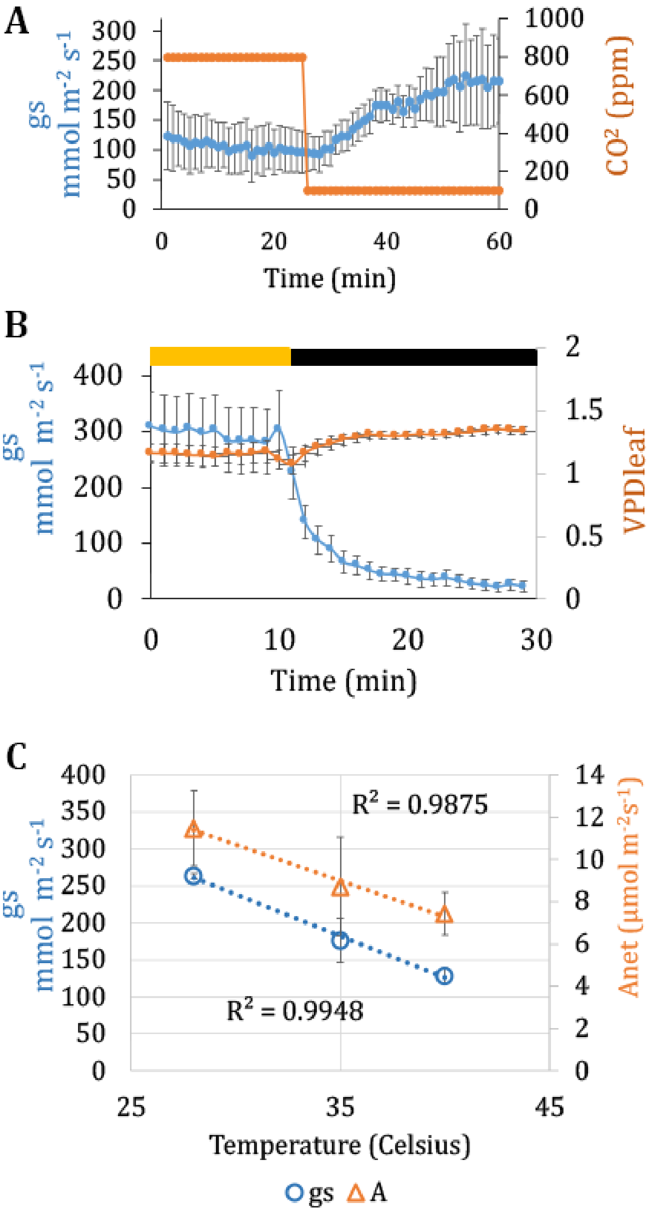

Figure 2. Stomata of *P. arabica* stems respond to environmental stimuli, including changes in $[CO_2]$, light to dark transition, and elevated temperatures. Gas exchange stomatal conductance responses (g_s) were conducted on intact *P. arabica* stems to (A) transition in CO_2 levels, from 900 to 100 ppm (B) light (yellow horizontal bar) to dark (black horizontal bar) transition or (C) increase in air temperature (from 28 to 35 and 40°C). (A-C) Data presented as mean ± se of 6 stems (n=6). In (C) *P-value* for the slope of g_s and A_{net} regression line was 0.045 and 0.071, respectively.

Figure 2A describes the stomatal response of *P. arabica* 2nd-year stems to a step-change in $[CO_2]$ from 900 to 100 ppm. Under 900 ppm CO_2, g_s stabilized at approximately 100 mmol m^{-2} s^{-1}. Transition to 100 ppm $[CO_2]$ increased g_s gradually to about 220 mmol m^{-2} s^{-1} within 30 min. These results show that, like stomata on plant leaves that open in response to low $[CO_2]$, stomata on *P. arabica* stems respond significantly and in a similar manner. Figure 2B describes the stomatal response of *P. arabica* 2nd-year stems to a rapid transition from light to dark. Under light intensity of 650 µmol m^{-2} s^{-1}, stem g_s stabilized around 300 mmol m^{-2} s^{-1}. Turning off the light in the cuvette resulted in a rapid decline of g_s in the first 3 min. The decline in g_s continued slower and reached values close to zero, representing complete stomatal closure. Throughout the measurement, VPD_{stem} remained practically stable at around 1.3 kPa. This ensures that the decline in g_s is not an artifact due to diminished driving force after the light was switched off. Next, stomatal response of *P. arabica* 2nd-year stems to temperature increments was measured (Figure 2C). To test the direct effect of temperature on stomatal conductance response and eliminate the effect of water vapor-pressure-deficit (VPD), which is dependent on temperature and has been found to affect stomatal conductance (Grossiord et al., 2020), all measurements were conducted under constant VPD (VPD_{stem}=3 kPa). The response of g_s and A_{net} to temperature was measured under 28, 25, and 40°C. Results show a significant decline in g_s from 250 mmol m^{-2} s^{-1} under 28°C to 125 mmol m^{-2} s^{-1} under 40°C, with a *P*-value of 0.045 for the slope of g_s regression line. Net CO_2 assimilation rate (A_{net}) also showed some reduction. A_{net} declined from 11 µmol m^{-2} s^{-1} under 28°C to 7 µmol m^{-2} s^{-1} under 40°C. Yet this reduction was not significant, with *P*-value of 0.071 for the slope of A_{net} regression line. The observed stomatal closure in response to elevated temperature further support for conserved mechanisms, which preserve water under high temperatures conditions, thus maintaining high CO_2 assimilation rates.

CONCLUSIONS

Taken together, these results support the functionality and regulation of stomata of the wild *P. arabica* SPC-type stem to environmental stimuli, as [CO_2], light/dark, and temperature. Mechanism that comprises acclimation and adaptation to confront the harsh environment.

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