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# Soil calcium deficiency in Sorana bean (*Phaseolus vulgaris* L.) ecotype reveals adaptive strategies through differential physiological and yield responses

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A R T I C L E I N F O

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A B S T R A C T

The common bean (*Phaseolus vulgaris* L.), a key crop within the Fabaceae family, is one of the most widely grown and consumed legumes in the world. However, many genotypes and landraces remain understudied, including the Sorana ecotype, traditionally cultivated in Italy along the Pescia river. It is well-adapted to alluvial, sandy soils with low calcium content. To investigate its adaptive mechanisms to calcium deficiency, we grew Sorana bean plants under control (2 mM $Ca^{2+}$), moderate (0.4 mM), and severe (0.2 mM) calcium deficiency conditions, from sowing to pod harvest. Both calcium-deficient conditions negatively affected plant biomass, photosynthetic pigment levels, polyphenol content, and stomatal conductance. Interestingly, moderate calcium deficiency enhanced yield, harvest index, and pod harvest index, indicating great sink strength and a shift in resource allocation. Bean skin thickness, a defining trait of this ecotype, was also influenced by calcium availability. At the molecular level, abscisic acid-related genes showed differential expression depending on calcium concentration, suggesting a threshold-dependent activation of stress-response pathways. Our results indicate that Sorana adapts to calcium-poor environments by prioritizing seed production over vegetative growth. This strategy, along with its distinctive agronomic traits, positions Sorana as a valuable genetic resource for breeding programs aimed at improving crop resilience and yield under suboptimal soil conditions.

## 1. Introduction

Fabaceae family is an important group of species, which represents the third largest family of higher plants, with ~20,000 species, most of which are of ecological, agronomic, and economic importance (Smýkal et al., 2015). The family is distributed worldwide and given their high protein content (ranging from 20 % to 40 % in seed grain legumes), they provide one-third of all dietary protein (Gepts et al., 2005). Within the Fabaceae family, the common bean (*Phaseolus vulgaris* L.) was first domesticated in Perú and Mexico around 8000 years ago and currently is one of the most widely grown and consumed legumes in the world (Zhang et al., 2022). Common bean genotypes can be cultivated in a wide range of climates and in all continents except for Antarctica (Nicoletto et al., 2019). However, nearly half of the world's dry bean production is provided by the American continent and, in Europe, cultivation is largely focused on countries surrounding the Mediterranean basin. Worldwide, common bean is estimated to occupy 37.75 million of hectares with a mean dry yield production of 755.1 Kg per hectare in 2023 (FAO, 2023). Despite its wide range of growing environments, when compared to other bean species like cowpeas (*Vigna unguiculata* L.), the common bean is less adapted to extreme environments characterised by high temperatures, low fertility and acidic soils,

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or low rainfall (Beebe, 2012). Furthermore, environmental differences can impact yield quality (Nicoletto et al., 2019), which is in turn associated with different genomic loci (Wu et al., 2020).

In addition to common bean varieties, local landraces are of great ecological and economical value, since they are well-adapted to specific environments due to a great genetic diversity (De Ron et al., 2004; Kwak and Gepts, 2009; Catarcione et al., 2023) and have specific properties that are valued by local communities (Loko et al., 2018). In Italy, there are myriads of landraces differently adapted to restricted soil types and climatic conditions that confer them interesting traits with potential use in agriculture (Piergiovanni and Lioi, 2010). Nevertheless, many of these ecotypes are currently poorly studied and characterized, due to their limited distribution areas (Dinelli et al., 2006). One particular example is the Sorana bean landrace, which grows in alluvial sandy soils characterized by reduced insolation period with high humidity. This landrace is grown on the banks of the Pescia river, whose waters are poor in mineral salts, nitrates and especially calcium (Verreschi, 1994). This bean is mainly characterized by its pearly white colour and its extremely thin skin (Verreschi, 1994). Its great digestibility, due to the thin skin, and its organoleptic properties increase its market value, fluctuating between 20 and 30 € per kilogram (Verreschi, 1994). To recognise its value and prevent any fraudulent actions, in 2002, the Sorana bean received the Protected Geographical Indication (PGI) from the European Union, becoming one of the few ecotypes receiving this mention and leading to strict rules for its production (Piergiovanni and Lioi, 2010; Belletti et al., 2017). Despite this, the adaptive traits of this landrace to the soil conditions where it is grown, with special emphasis on low calcium levels, have been poorly studied so far.

Calcium is an essential macronutrient that plays a vital role in stabilizing cell membranes, maintaining cell wall structure and regulating various cell functions, such as signalling and nutrient sensing. Although calcium deficiency is relatively rare compared to other macronutrients, it can occur specially in acidic and sandy soils or highly rainfall areas (White and Broadley, 2003; Olle and Bender, 2009; De Bang et al., 2021). Among the different symptoms caused by calcium deficiency in plants, reports have been made of stunted root and plant growth, as well as increased disease incidence or influence on antioxidative responses (Ramalho et al., 1995; Schmitz-Eiberger et al., 2002; Palta, 2010; Zhang and Zwiazek, 2016). Calcium deficiency is especially noteworthy in fruits, as it leads to unique symptoms, such as blossom-end rot in tomatoes, peppers, and zucchini (Ikeda and Kanayama, 2014). Furthermore, the availability of calcium in the soil is particularly important for crop production, as it can influence the uptake and balance of other essential nutrients (Saito and Uozumi, 2020; Weng et al., 2022). For instance, potassium, iron, magnesium or nitrogen transporter activities in *Arabidopsis* are regulated by $Ca^{2+}$-dependent calcineurin B like (CBL) – CBL protein kinase pathways (Riveras et al., 2015; Liu et al., 2017; Kudla et al., 2018; Wang et al., 2018). Considering the importance of $Ca^{2+}$ signalling in response to abiotic stresses, calcium nutrition also plays a role in improving plant stress responses to abiotic stresses, such as drought and salinity (Kumari et al., 2022). Related to perception and signalling of calcium deficiency, abscisic acid (ABA) exogenous treatment has shown to increase calcium concentrations in tomato fruit, therefore suggesting its involvement in calcium uptake and reallocation (de Freitas et al., 2011). At the leaf level, calcium homeostasis, signalling, and transport are also of crucial importance for the correct functioning of guard cells, and therefore, plant photosynthesis (Jezek and Blatt, 2017). Voltage activated ion channels, such as vacuolar $Ca^{2+}$-ATPases (ACAs), $Ca^{2+}/H^{+}$ antiporters (CAXs), TPC1, $H^{+}$-pyrophosphatases (AVPs) or GORKs play important roles in calcium control of stomatal dynamics (Jezek and Blatt, 2017 and references therein).

In this work, we characterized the responses of *P. vulgaris* Sorana ecotype to calcium deficiency, which is traditionally grown in calcium poor soils in the region of Tuscany, Italy. We aimed to identify putative mechanisms of adaptation of this particular ecotype to nutritional deficiency. For this purpose, we supplied common bean plants (Sorana ecotype) with different concentrations of calcium in pot conditions and evaluated yield and several biometric, biochemical, physiological and molecular parameters. We hypothesized that the Sorana ecotype is better adapted to deficiency-level concentrations of Ca in the soil than to concentrations generally considered optimal for plant growth. Therefore, Sorana plants grown under Ca-deficiency conditions would have improved growth, yield and overall better plant status as compared to plants grown under standard calcium conditions. Our findings improve the understanding of adaptative traits to calcium deficiency in *P. vulgaris* and highlight the necessity to better characterize landraces that may serve as valuable genetic resources for breeding programs aiming to improve crop resilience and nutritional efficiency under suboptimal soil conditions.

## 2. Materials and methods

### 2.1. Experimental design

Calcium deprivation experiments were conducted from September to November 2020 under semi-controlled conditions in the greenhouse of DAGRI Department at the University of Florence (lat. 43°48′58.6″ N, long. 11°11′58.1″ E), where environmental conditions were monitored. Plants were grown using non-supplemented lighting (average 500 μmol $m^{-2}$ $s^{-1}$ PAR), mean air temperature of 28 °C (maximum 34.5 °C, minimum 24.9 °C), mean air humidity of 46 % (maximum 60.5 %, minimum 34.4 %), and an average photoperiod of 15:9 (L:D). *P. vulgaris* Sorana ecotype seeds were pre-germinated in petri dishes containing wet filter paper until radicle emergence (5–6 days) and then transplanted to pots and grown using half strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) in a hydroponic system where plants were watered using a circulating bench sub-irrigation system with outflow occurring after 15 min. The electrical conductivity (EC) and pH value of the solution reservoir were monitored daily with a portable conductivity meter and adjusted with $HNO_3$ to maintain the pH at values between 6 and 6.5. Concomitantly to the transplant, three different experimental conditions were tested: half-strength Hoagland's solution (2 mM $Ca^{2+}$, CTRL), calcium deprivation at 1:5 (C1, 0.4 mM $Ca^{2+}$), and calcium deprivation at 1:10 (C2, 0.2 mM $Ca^{2+}$). In total, 20 plants (two per pot) per treatment and ecotype were grown (Fig. S1). For biometric and yield parameters, sampling was performed at harvest (70 days post treatment, dpt), while for the rest of parameters studied, sampling occurred at four different time points: 7 dpt, 14 dpt, 21 dpt and 28 dpt.

### 2.2. Biometric and yield analysis

At harvest, the fresh and dry weight of the total plant and the pod yield in plants from all conditions was determined. For fresh weight, the entire plant and pods were weighed immediately after harvesting. Then, the plants and pods were dried at 50 °C for 72 h in an oven, and the dry weight was measured. Dried beans were separated from the pods to annotate their total weight in each condition. The harvest index (HI) was calculated as the ratio of the dry weight of the dried shelled beans to the total dry weight of the plant, expressed as a percentage (Pinto et al., 2018). The pod harvest index (PHI) was calculated as the ratio of the dry weight of the shelled beans to the total dry weight of the pods, also expressed as a percentage (Assefa et al., 2013). These indices provide an estimate of the efficiency of biomass allocation of *P. vulgaris* Sorana ecotype in different conditions.

### 2.3. Microscopy observations

Sorana bean skin was peeled off, cut with a scalpel in small pieces (about 0.5 cm) and fixed in 2.5 % (v/v) glutaraldehyde in 10 mM Na-phosphate buffer (pH 7.2) overnight at 4 °C. After rinsing with the same buffer, samples were post-fixed in 1 % osmium tetroxide (OsO4) for one hour, washed with ddH2O, and then dehydrated in an ethanol

series (30, 50, 70 and 90 % for 15 min each step, and 2 times 100 % for 30 min each step) at room temperature. Dehydrated skin segments were infiltrated in 3:1 (v/v) absolute ethanol/London Resin White resin (E/LRW) for one hour, 1:1 E/LRW for one hour,1:3 E/LRW for one hour and 100 % London Resin white overnight at 4 °C according to Balestrini et al. (1996). The samples were then embedded in gelatin capsules and polymerized for 24 h at 60 °C. Semi-thin sections (1.5 μm) were cut using an ultramicrotome, stained with 1 % toluidine blue in 1 % Na borate and analysed under a microscope equipped with a camera (Leica).

### 2.4. Leaf gas exchange determinations

Net photosynthetic rates and stomatal conductances were measured on young, fully expanded leaves for each treatment at the described time points. An open gas exchange system Li-6400 (LiCor Inc., Lincoln, NE, USA) was used as described in Palm et al. (2017). Leaf gas exchange measurements were taken on four plants from each treatment and time point under the following stabilized conditions: $CO_2$ concentration: 400 μmol $CO_2$ $mol^{-1}$; Flow rate: 300 mmol $s^{-1}$; PAR: 1000 μmol $m^{-2}$ $s^{-1}$; Relative air humidity (RH): 40–50 %; Temperature: 20 °C. Chlorophyll fluorescence was measured on the same leaves used for gas exchange measurements using the integrated fluorescence chamber head (Li-6400–40; Li-Cor Inc.) of the open gas exchange system. Maximum quantum yield (Fv/Fm) was determined after overnight dark acclimation of leaves using a dark leaf clip. Effective quantum yield (ΦPSII) was measured on light adapted- leaves and was calculated as ΦPSII = (Fm'-Fs)/Fm', expressing the proportion of the light absorbed by chlorophyll associated with PSII used in photochemistry (Maxwell & Johnson, 2000). Photosystem II efficiency (Fv'/Fm') of light adapted leaves was measured immediately following the collection of gas exchange values and after a saturating pulse of 7000 μmol $m^{-2}$ $s^{-1}$ was applied. Fluorescence data were used also to calculate the Non Photochemical Quenching (NPQ) as NPQ = (Fm- Fm')/Fm' (Redondo-Gómez et al., 2006); and the Electron Transport Rate (ETR) as ETR = ΦPSII * PFDa *0.5, where PFDa is the absorbed light in mmol photon $m^{-2}$ $s^{-1}$ and 0.5 is an index that accounts for the energy partitioning between photosystems (Baker, 2008).

### 2.5. RNA extraction and cDNA synthesis

Total RNA extraction was achieved using the Plant/Fungi Total RNA Purification Kit (Norgen Biotek Corp) according to the manufacturer's protocol from 100 mg of ground leaf tissue. RNA integrity and quality were checked on an agarose gel and by using a TECAN spectrophotometer Infinite 200 (TECAN, Austria) with a specialized NanoQuant Plate™. cDNA was synthesised from 2 μg of total RNA using the Bio-Rad iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions.

### 2.6. Gene selection and primer design

To assess the molecular response to calcium deprivation, eleven *P. vulgaris* genes were selected based on their involvement in calcium-related pathways in guard cells, stomatal dynamics, and homeostasis processes (Ng et al., 2001; Jezek and Blatt, 2017). Specifically, the genes *PvACA.L* and *PvACA4* are members of the $Ca^{2+}$-ATPase (ACA) gene family. They code for P-type $Ca^{2+}$-ATPases that play a role in restoring basal cytosolic calcium levels after signalling events. In Arabidopsis, *AtACA4*, localized to the tonoplast, allows to sequester calcium into vacuoles (Geisler et al., 2000). The gene *PvCAX11* putatively code for a $Ca^{2+}/H^+$ antiporter responsible for sequestering calcium into the vacuole, thereby contributing to calcium homeostasis and playing a role in regulating cytosolic calcium levels during signalling events that affect growth and development (Pittman and Hirschi, 2024). The gene *PvABI5* is a bZIP-type transcription factor that operates downstream of the ABA signalling pathway, and it is regulated by ABA and stress-related kinases (Finkelstein and Lynch, 2000). The gene *PvSLAC1* (Slow Anion Channel-Associated 1) is involved in stomatal regulation and encodes a S-type anion channel in guard cells that is activated by ABA and other stress signals to mediate anion efflux, leading to stomatal closure (Vahisalu et al., 2008). The gene *PvTPC1*, homologue to the *AtTPC1*' two-pore channel 1' gene of *Arabidopsis thaliana* encodes a depolarization-activated Ca-permeable channel localized into the vacuolar membrane. It facilitates the release of $Ca^{2+}$ from vacuoles into the cytosol, contributing to Ca signalling in response to environmental stress (Peiter et al., 2005). In plants, the SnRK family, particularly the sub-family SnRK2s characterized by the OST1 (Open Stomata 1) domain like the gene *PvSnRK2.OST1*, are involved in ABA-dependent stress signalling. In *P. vulgaris*, a comprehensive genome-wide identification revealed 42 SnRK genes (Cervera-Torres et al., 2022). The gene *PvGORK* (Gated Outwardly-Rectifying $K^+$ channel) belongs to the SKOR/GORK gene family, expressed in guard cells, coding for channels that mediates K+ efflux involved in stomatal closure (Hosy et al., 2003). The genes *PvAVP1* and *PvAVP2* putatively encode vacuolar $H^+$-pyrophosphatases (V-PPases) that hydrolyse pyrophosphate (PPi) to drive protons into the vacuole, generating a proton gradient that supports secondary transport of ions and metabolites (Schilling et al., 2017). In *Arabidopsis*, *AVP2* has been reported to be K+ insensitive and Ca2 + hypersensitive (Drozdowicz et al., 2000). The gene *PvANN1* (Annexin 1) putatively codes for a calcium-binding protein that associates with membranes in a calcium-dependent manner, and annexins may also act as calcium-permeable channels (Mortimer et al., 2008). The RT-qPCR primer pairs for each selected gene (Table S1) were designed using the Primer 3 software (http://primer3.ut.ee/) and then double-checked using Netprimer software (http://www.premierbiosoft.com/netprimer/).

### 2.7. RT-qPCR and gene expression analysis

The cDNA was diluted 1:5 and used for quantitative gene expression analysis on a Rotor-Gene Q system (Qiagen). Each RT-qPCR was set up in a 15 μL reaction volume, containing 7.5 μL of Rotor-Gene SYBR® Green Master Mix, 5.5 μL of primer mix (prepared by combining 16 μL of each 10 μM primer with 168 μL of nuclease-free water), and 2 μL of diluted cDNA. The thermal cycling protocol included an initial reverse transcription step at 55 °C for 10 min, followed by a 10-minute denaturation at 95 °C, and then 40 amplification cycles consisting of 15 s at 95 °C and 1 min at 60°C. Reactions were carried out with two biological replicates and two technical replicates per sample, except for the T1 sample, which had three biological replicates. Gene expression levels were normalized against two *P. vulgaris* reference genes, i.e. *PvACT11* (Borges et al., 2012) and *PvCUL4* (O'Rourke et al., 2014) (Table S1), using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Expression values were reported as relative expression ratios.

### 2.8. Biochemical determinations

At four different time points (7 dpt, 14 dpt, 21 dpt and 28 dpt), leaves were taken from the midpoint of the central leaf axis from six plants per condition, frozen in liquid nitrogen, and finely grounded to a powder with the help of a pestle and mortar. Subsequently, 1 mL of cold methanol was added to 10 mg of grounded leaves, followed by shaking for 30 min and centrifugation at 10,000 x g for 10 min. The resulting supernatant was utilized for absorbance readings at 665, 652, and 470 nm to determine the levels of Chlorophyll *a* (Chl*a*), *b* (Chl*b*), and carotenoids, respectively. Absorbance readings were performed using a TECAN spectrophotometer with a 96-well black multi-plate reader. Pigment quantification was conducted based on the equations provided by Wellburn (1994).

Total polyphenol content was determined according to Ainsworth and Gillespie (2007). Briefly, 20 mg of leaf tissue powder were

homogenized with 2 mL of ice-cold 95 % (vol/vol) methanol. Samples were then centrifuged at 13,000 g at room temperature and the supernatant was collected. Then, 200 μL of 10 % (vol/vol) Folin-Ciocalteu reagent was mixed with 100 μL of supernatant and 800 μL of 700 mM $Na_2CO_3$. 200 μL of this mixture was used for absorbance reading at 765 nm, using a TECAN spectrophotometer with a 96-well black multi-plate reader (TECAN, Austria).

### 2.9. Statistical analyses

Biometric and pulse coat parameters were analysed using Kruskal–Wallis one-way analysis of variance and least significant difference (LSD) post hoc test. Biochemical and leaf gas-exchange parameters were analysed using two-way ANOVA (Treatment × Time), followed by Tukey-adjusted pairwise comparisons using estimated marginal means (emmeans). Gene expression data were statistically analysed by using "rtpcr" package (Mirzaghaderi, 2025), through 2-way repeated measures ANOVA with Satterthwaite's method, assessing the effects of the main factors "treatment" and "time", as well as their interaction, at $p \leq 0.05$. A Bonferroni corrected *t*-test was also used to assess differences between control and treated samples within each of the four time points ($p \leq 0.05$).

## 3. Results

### 3.1. Biometric and yield parameters

First, we evaluated the effect of calcium deficiency on biometric and yield parameters in *P. vulgaris* Sorana landrace. All calcium deprivation treatments significantly reduced total plant biomass, both fresh and dry mass (Figs. 1A, 1B). On the other hand, fresh pod bean yield per plant was not significantly affected in C1 (0.4 mM $Ca^{2+}$) conditions but was strongly reduced (55.78 % lower) in the C2 (0.2 mM $Ca^{2+}$) treatment (Fig. 1C). However, dry bean yield per plant significantly increased for C1 (28.1 % higher), while it decreased for C2 treatment (38.43 % lower), both compared to the control conditions (Fig. 1D). Considering individual pods, both C1 and C2 treatments significantly increased their dry mass, 52.14 % and 67.36 %, respectively (Fig. 1E). After pod shelling, bean dry mass was also significantly higher in C1 and C2 conditions, in both cases with ~100 % increase on average (Fig. 1F). To evaluate the efficiency of Sorana ecotype to convert its biomass into harvestable seeds, we calculated the harvest and pod harvest indices (HI, and PHI, respectively) per plant. Only C1 conditions significantly increased the HI from 13.06 % to 29.41 % and the PHI from 50.75 % to 62.95 %, when compared to control conditions (Figs. 1G, 1H).

The reduced thickness of coat layers is a unique characteristic of the

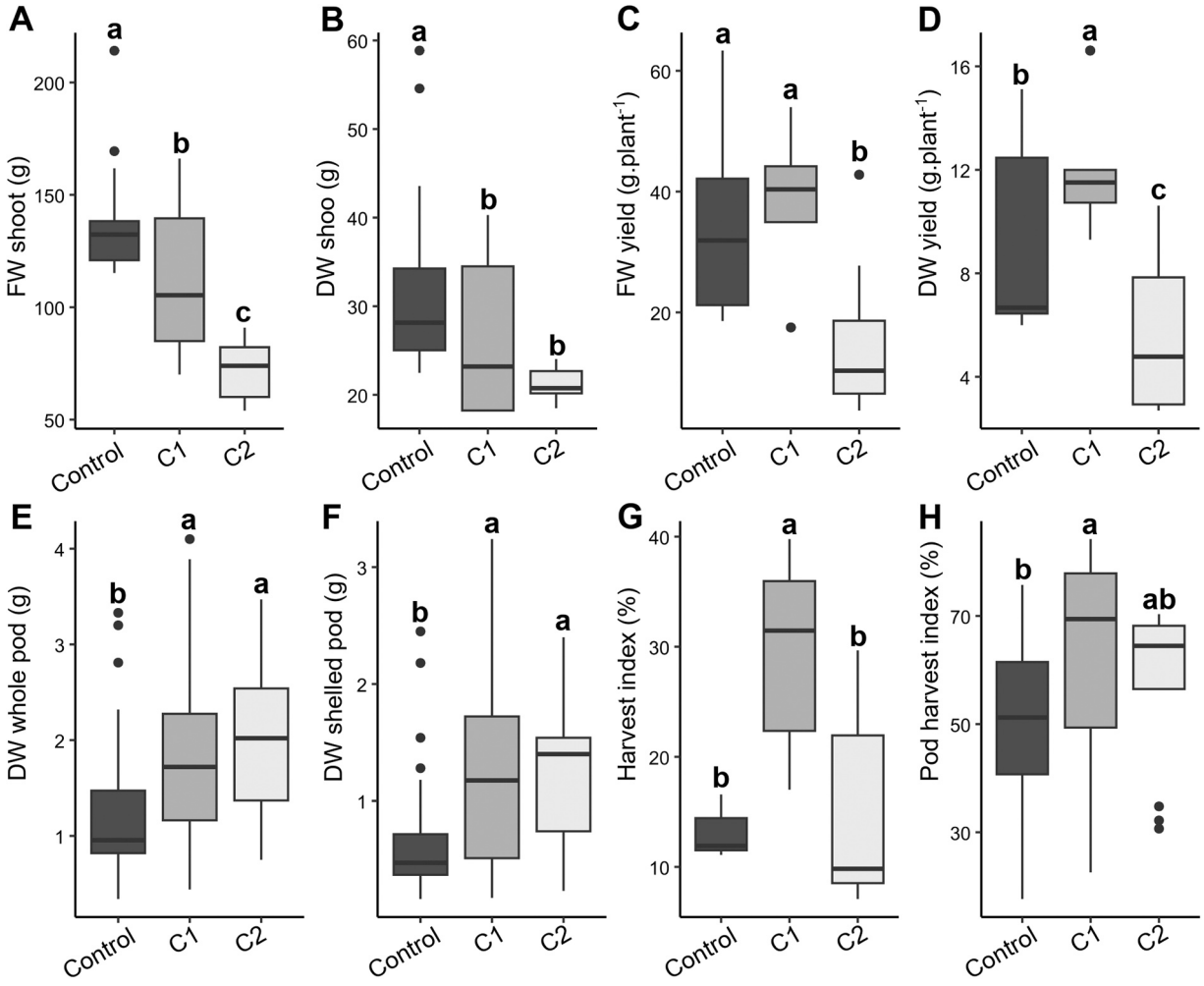


**Fig. 1.** Biomass, yield and (pod) harvest indices of Sorana bean (*P. vulgaris*) plants under different calcium deficiency conditions. Boxplots indicating shoot fresh (A) and dry (B) weight; fresh (C) and dry (D) mass of the total yield; Dry weight of the whole (E) and shelled pods (F); Harvest Index (G) and Pod Harvest Index (H). Whiskers represent the limits of the 1.5 interquartile range. Different letters represent different levels of statistical significance according with a significance of $p < 0.01$, based on Kruskal–Wallis one-way analysis of variance and least significant difference (LSD) post hoc test. Control = 2 mM $Ca^{2+}$; C1 = 0.4 mM $Ca^{2+}$; C2 = 0.$^2$ mM $Ca^{2+}$. n = 8 plants for fresh and dry weight; n = 20 plants for yield-related measurements.

"Fagiolo di Sorana", affecting its digestibility and, in turn, its high economic value. To evaluate how calcium deficiency affects this trait, we also evaluated the morphology of the pulses. The seed coat layer is composed of an outer macrosclerid layer and an inner osteosclerid cell layer (Fig. 2A - C). We found that, although the outer macrosclereid layer was not significantly different between calcium conditions (Fig. 2D), the inner osteosclerid layer was significantly thinner (~20 %) in both calcium deficiency conditions (Fig. 2E). This contributed to a reduction in the total seed coat thickness, making it significantly thinner in C1 and C2 conditions than in control conditions (Fig. 2F). Overall, biometric and yield results indicate a shift in resource allocation from vegetative growth to reproduction under calcium moderate stress.

### 3.2. Leaf gas exchange parameters

The reduced plant biomass and higher yield observed in mild $Ca^{2+}$ deficiency conditions were accompanied by a few significant differences in leaf gas exchange parameters during the experiment (Table S2). Maximum quantum yield was the only parameter completely stable during the whole experiment, with no significant differences according to the time, calcium treatment and its interaction (Fig. 3D; Table S2). Other parameters, such as Non-Photochemical Quenching (NPQ) or photosystem II efficiency of light-adapted leaves (Fv'/Fm') were only significantly affected by treatment duration (Figs. 3E, 3G; Table S2). The net assimilation rate (An), the average effective quantum yield ($\Phi_{PSII}$) and the electron transport rate (ETR) showed similar behaviour during the experiment. They showed a significant, clear pattern throughout time, reaching maximum values at 7 dpt and then dropping until 28 dpt (Figs. 3A, 3F, 3H; Table S2). Regarding the different treatments, when considering the interaction between time and treatment, no significant differences were found at any time point, despite finding consistently lower values for C2 plants at most of time points (Figs. 3A, 3F, 3H). Conversely, stomatal response was statistically affected by calcium treatments at 28 dpt, both for C1 (85.7 % decrease) and C2 (77.1 % decrease) treatments (Fig. 3B). Consequently, we also observed that at 28dpt, intrinsic water use efficiency significantly increased, but only for C1 treated plants (Fig. 3C).

### 3.3. Gene expression analysis

In order to investigate the stomatal conductance differences between calcium stressed and control plants, we evaluated the gene expression of genes involved in calcium-related pathways in guard cells, stomatal dynamics, and homeostasis processes (Ng et al., 2001; Jezek and Blatt, 2017). Among these genes, we found that *PvAVP1*, *PvGORK, PvSLAC1* and *PvSnRK2.OST1* were the most expressed genes in leaves, while the other genes presented overall lower expression values (Fig. 4). The 2-way ANOVA showed no significant effect of treatment, time, or their interaction (Table S3) for genes *PvACA.L*, *PvACA4*, *PvANN1*, *PvGORK*, *PvTPC1*. However, at 7 dpt, C1 samples showed significant down-regulation compared to controls for *PvACA4*, *PvACA.L, PvANN1*, while at 28 dpt, upregulation of C2 samples compared to controls was observed for the expression data of *PvANN1* (Fig. 4). For *PvAVP1* and *PvAVP2* expression, 2-way ANOVA showed the significant effect of time ($p < 0.05$), although it was more pronounced for *PvAVP1* than for *PvAVP2* (Table S3). At 28 dpt, a positive trend of upregulation was observed for both genes in C1 and C2 samples, and for *PvAVP2* even significantly different compared to controls (Fig. 4). For *PvCAX11*, time and the interaction between treatment and time showed significant effects ($p < 0.05$), with C1 and C2 samples showing down-regulation of this gene compared to controls at 14 and 28 dpt (Fig. 4). Despite its overall low level of expression, the gene *PvABI5* showed a clear and strong up-regulation in C2 samples only at 28 dpt (time, treatment and their interaction were statistically significant, Table S3). The gene

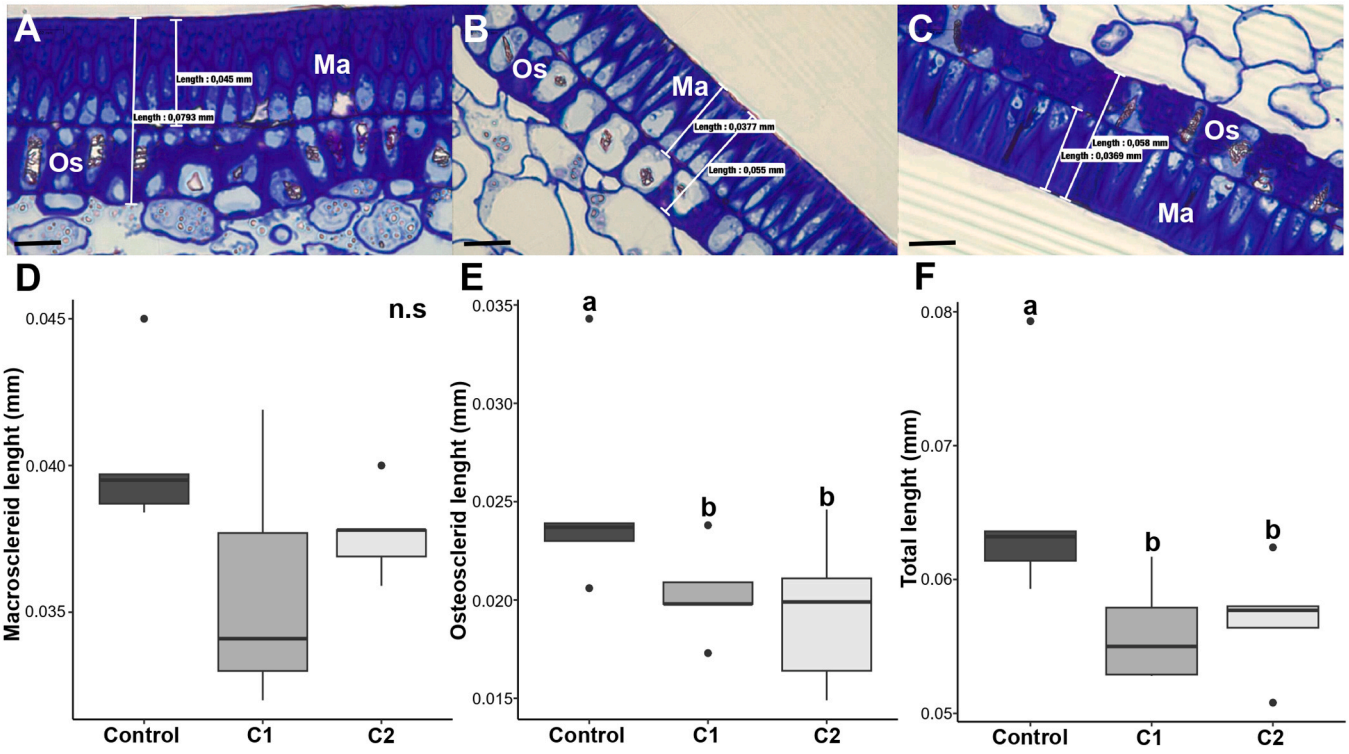


**Fig. 2.** Pulse coat thickness of Sorana bean (*P. vulgaris*) under different calcium deficiency conditions. Phase-contrast microscopy images of pulse bean coat from control (A), C1 (B) and C2 (C) treatments.Ma = macrosclerid layer; Os = Osteoslclerid layer. Scale bar = 20 μm. Boxplots indicating macrosclereid length (D), osteosclereid length (E) and total coat length (F). Whiskers represent the limits of the 1.5 interquartile range. Different letters represent different levels of statistical significance according with a significance of $p < 0.01$, based on Kruskal–Wallis one-way analysis of variance and least significant difference (LSD) post hoc test. Control = 2 mM $Ca^{2+}$; C1 = 0.4 mM $Ca^{2+}$; C2 = 0.$^2$ mM $Ca^{2+}$. n = 5 pulses.

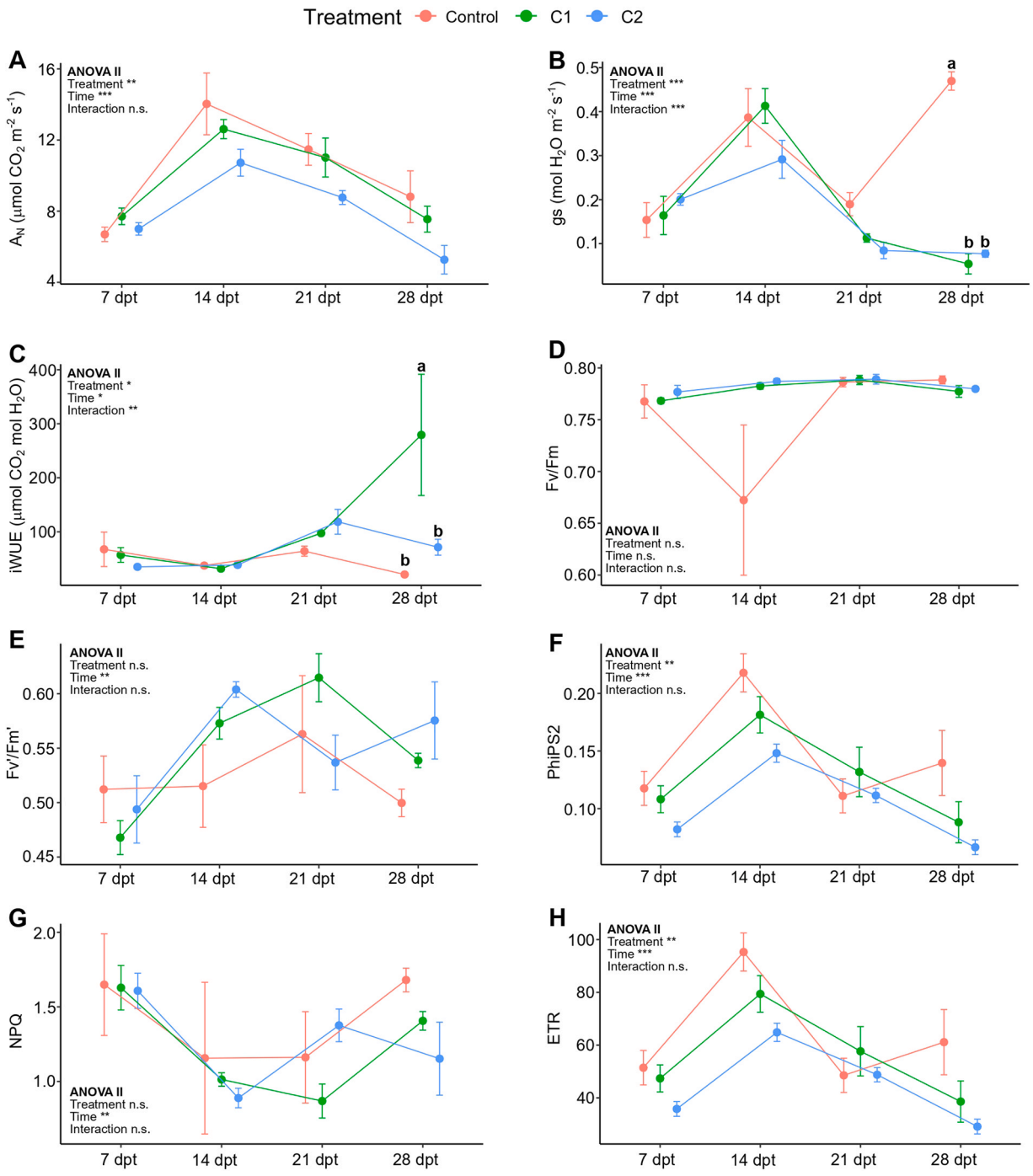


**Fig. 3.** Effects of different calcium treatment on gas-exchange parameters of Sorana bean (*P. vulgaris*) plants. Line plots depict the average values ± standard errors of neat carbon assimilation (A), stomatal conductance (B), intrinsic water use efficiency (C), maximum quantum yield efficiency (D), photosystem II efficiency of light-adapted leaves (E), average effective quantum yield (F), non-photochemical quenching (G) and electron transport rate (H) at 7-, 14-, 21- and 28-days post treatment (dpt). Different letters represent different levels of statistical significance according to a two-way ANOVA (Treatment × Time), followed by Tukey-adjusted pairwise comparisons using estimated marginal means (emmeans). Control = 2 mM $Ca^{2+}$; C1 = 0.4 mM $Ca^{2+}$; C2 = 0.2 mM $Ca^{2+}$. Red line = Control; Green line = C1; Blue line = C2. n = 4 plants.

*PvSnRK2.OST1* displayed the overall highest level of relative expression, particularly from 14 dpt until the end of the experiment (Fig. 4). The 2-way ANOVA showed significant effect of time and time x treatment interaction (Table S3). At 7 dpt and 28 dpt, this gene showed significant up-regulation in C2 samples compared to control ones (Fig. 4). *PvSLAC1* showed a significant pattern of regulation over time ($p < 0.001$) and showed a significant interaction between treatment and time ($p < 0.05$) (Table S3). The magnitude of *PvSLAC1* expression was 10 times higher than that of the other differentially expressed genes at 7 dpt and 28 dpt in C2 samples compared to controls (Fig. 4). In summary, C2 treatment was the condition that caused more significant changes at molecular level with respect to control conditions, with 6 upregulated and 1 downregulated genes, concentrated mainly at the beginning (7 dpt) and end (28 dpt) of the experimental time, and in the highly expressed genes *PvSLAC1* and *PvSnRK2.OST1* (Fig. 4). On the other hand, C1 treatment provoked milder changes, with the downregulation of 5 genes mainly at

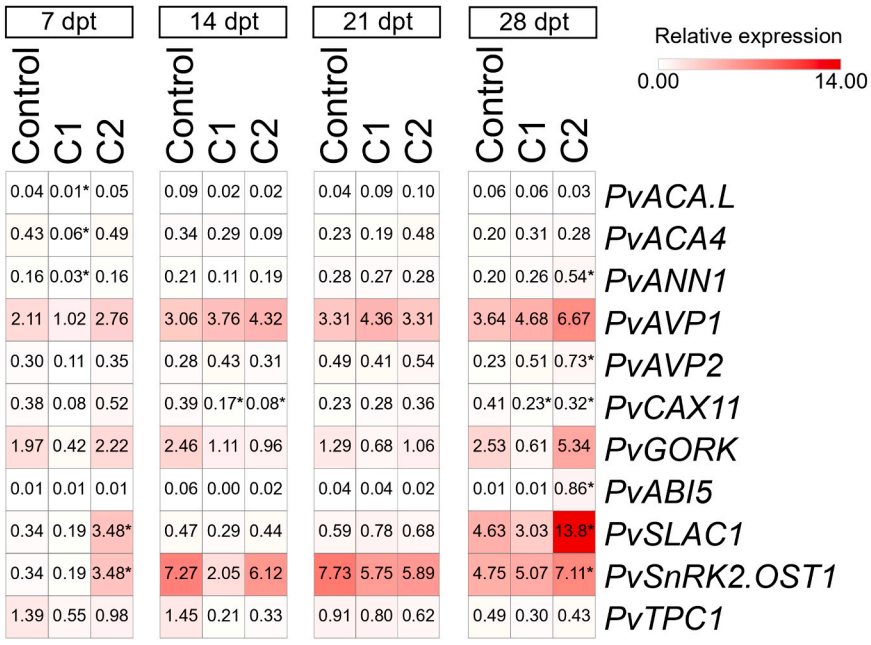


**Fig. 4.** Relative expression of *P. vulgaris* genes related to voltage dependent ion channels and abscisic acid pathway, in response to calcium deficiency conditions. Heatmap depicts the relative expression of selected *P. vulgaris* genes (see Materials and Methods section) in leaves at 7-, 14-, 21- and 28-days post treatment (dpt). The intensity of the red colour corresponds to gene expression levels. Asterisks represent significant differences ($p < 0.05$) with respect to the control at the same time point, as determined by a two-way ANOVA (Treatment × Time), followed by Tukey-adjusted pairwise comparisons using estimated marginal means (emmeans). Control = 2 mM $Ca^{2+}$; C1 = 0.4 mM $Ca^{2+}$; C2 = 0.$^{2}$ mM $Ca^{2+}$. n = 3 biological replicates.

the beginning of the experiment (7 dpt) and concentrated in the lowly expressed *PvACA.L*, *PvACA4*, *PvANN1* and *PvCAX11* genes (Fig. 4).

### 3.4. Biochemical parameters

Motivated by the reduction in total plant biomass observed in Fig. 1, we also decided to evaluate the effect of calcium deficiency on well-established biochemical markers of plant status. The 2-way ANOVA analysis with interaction terms revealed that both the calcium treatment and the time, as well as their interaction, significantly influenced chlorophyll *a*, chlorophyll *b*, and polyphenol levels similarly in Sorana bean plants (Fig. 5; Fig. S2; Table S4). Overall, treatments C1 and C2 significantly reduced chlorophyll a, b and polyphenol content compared to the control (Fig. S2). These main effects were modified by time, since at 7, 21 and 28 dpt (days post treatment) a general trend towards a decrease in chlorophyll *a* and *b* levels was found in calcium deficiency conditions, which in some cases presented significant differences. At 14 dpt, maximum values were reached (Fig. 5; Fig. S2), and no significant differences in chlorophyll *a* and *b* for both calcium deprivation treatments were observed (Fig. 5). In the case of polyphenols, they consistently reached lower levels in C1 and C2 conditions, although these were more pronounced at 14 dpt than at other moments of the experiment (Fig. S2). Carotenoid content was significantly influenced only by the calcium treatment, since it remained remarkably constant over the time of the experiment (Fig. 5; Fig. S2). Both C1 and C2 conditions caused an overall significant decrease in carotenoid content, which was especially pronounced at 21 dpt (Fig. 5; Fig. S2).

## 4. Discussion

### 4.1. Calcium deficiency negatively affects P. vulgaris Sorana ecotype's growth and physiology

Calcium deficiency is a significant soil problem that affects plant growth and yield, especially in acidic soils and high rainfall areas (Jing et al., 2024). The existing literature on integrating biometric, physiological, molecular, and biochemical responses of plants to calcium deficiency is scarce. Here, we report the effects of calcium deficiency in *P. vulgaris* Sorana ecotype, a local landrace well adapted to calcium deficiency in soils. To the best of our knowledge, no physiological results of calcium deficiency in *P. vulgaris* have been reported yet, with the exception of hypocotyl collapse of *P. vulgaris cv.* Pinto dark-grown seedlings (Helms, 1971). This fact underscores the novelty of our work but also prevent us to further discuss about the evolutionary adaptations of the specific Sorana ecotype with respect to modern commercial *P. vulgaris* cultivars. Future research should aim to compare the results here reported on Sorana ecotype with other cultivars, to elucidate to which extent the calcium deficiency adaptations below discussed are specific to Sorana ecotype or shared among different genotypes. Our results of biometric, biochemical, and leaf gas-exchange parameters suggested a negative response of the overall plant health and status to calcium deficiency, as shown by decreased total plant biomass, reduced phenolic content, and decreased photosynthetic pigments, as well as certain leaf gas-exchange parameters. Specific alterations to root architecture or biomass were not recorded, preventing us to elucidate how the sensing organ (roots) responds to calcium deficiency. Nonetheless, these results are in line with those previously reported in the literature. In tomato (*Solanum lycopersicum*), calcium deficiency is of particular interest, since it results in growth impairment and blossom-end rot symptoms (Ikeda and Kanayama, 2014), together with increased lipid peroxidation and altered antioxidant defense system, such as superoxide dismutase and peroxidase enzymes (Schmitz-Eiberger et al., 2002). In *Tulipa gesneriana*, calcium deficiency resulted in stem toppling, flower abortion and reduction of root length and leaf area (Inkham et al., 2023), while in poplar both excess and deficiency of calcium resulted in impaired plant height and leaf gas exchange parameters (Weng et al., 2022). The observed negative effects in plant physiology seemed to be more severe in the lowest calcium treatment (C2) and are probably related with the observed patterns of gene expression of voltage dependent transporters and ABA-signalling genes. Despite its overall low levels of expression, *PvCAX11* was downregulated at two different times in both calcium deficiency

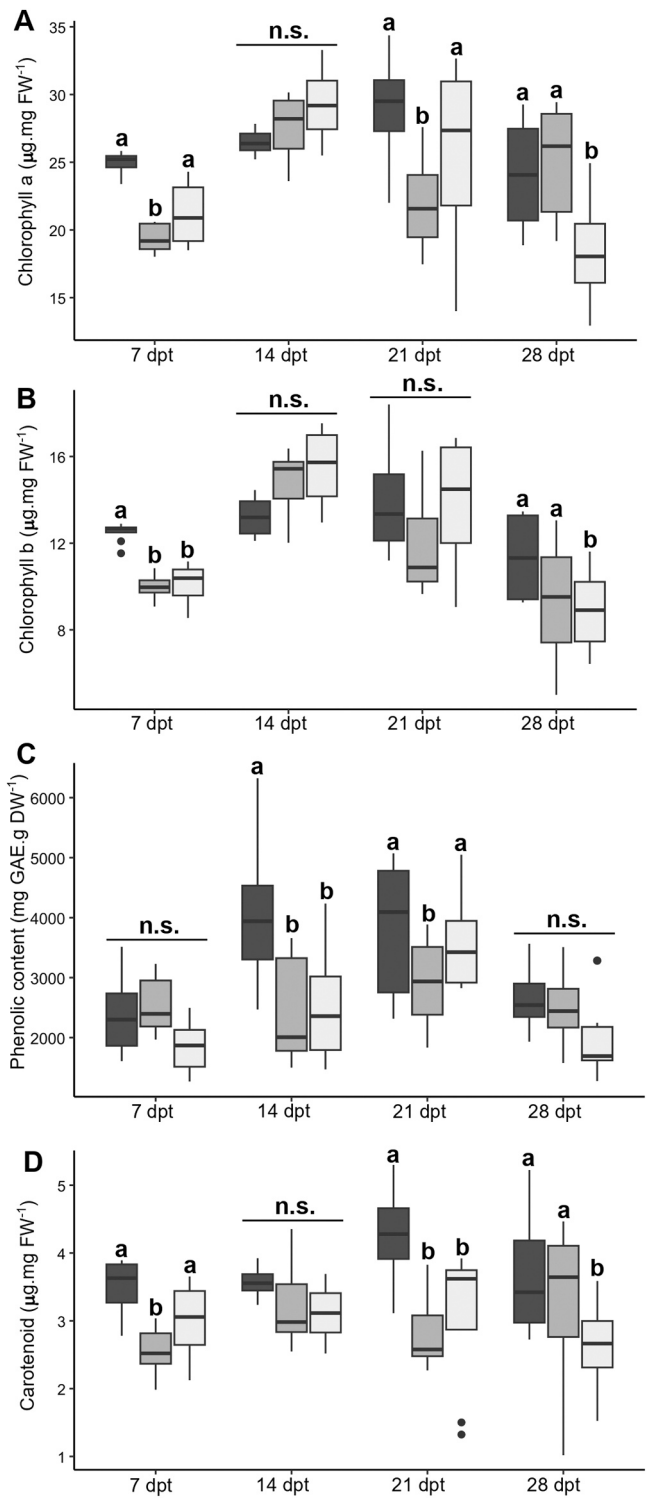


**Fig. 5.** Effects of different calcium treatment on biochemical markers of Sorana bean (*P. vulgaris*) plants. Boxplots depict the interaction between the effects of treatment and time over chlorophyll a (A), chlorophyll *b* (B), polyphenols (C), and carotenoids (D). Whiskers represent the limits of the 1.5 interquartile range. Grey scale represents different factor levels, where dark grey represents control conditions, medium grey C1 conditions and light grey C2 conditions. Different letters represent different levels of statistical significance according to a two-way ANOVA (Treatment × Time), followed by Tukey-adjusted pairwise comparisons using estimated marginal means (emmeans). Significant differences among groups are reported always with respect to control conditions of its specific time point. Control = 2 mM $Ca^{2+}$; C1 = 0.4 mM $Ca^{2+}$; C2 = 0.2 mM $Ca^{2+}$. n = 8 plants.

treatments. CAX transporters use the proton-motive force to drive $Ca^{2+}$ export from the cytosol, primarily into the vacuole (Zhai et al. 2013). Reduced CAX activity may strengthen stress resistance, as *cax1* mutants display increased tolerance to freezing (Catalá et al., 2003). According to Wdowiak et al. (2024), high activity of CAX transporters can lower cytosolic $Ca^{2+}$ levels and weaken $Ca^{2+}$-dependent signalling. In this line, it could be argued that the observed downregulation of *PvCAX11* may be a plant response to counter the low concentrations of calcium in the cytosol. Considering the functional overlap among CAX transporters in *Arabidopsis* (CAX1 and CAX3 often compensate for one another; Cheng et al., 2005), a more in-depth analysis of this family should be performed to reveal possible compensatory mechanisms and better understand this phenomenon.

### 4.2. *Gene expression analysis suggests a threshold-dependent activation of ABA-mediated stress response to calcium deficiency*

The combined results related to ABA-signalling expression pattern and stomatal conductance were of special interest. For the most severe calcium deficiency (C2), stomatal closure at 28 dpt could be related to the upregulation of *PvSnRK2.OST1*, *PvABI5* and *PvSLAC1*, which share a common pattern of induction in C2 treatment at later stages of the experiment. *PvSnRK2.OST1* is known to phosphorylate SLAC1 (Lee et al., 2009); *PvSLAC1* is an anion channel controlling turgor pressure in the aperture-defining guard cells of plant stomata (Chen et al., 2010); finally, *PvABI5* is an ABA-regulated transcription factor (Finkelstein and Lynch, 2000). Altogether, it suggests that, in the most severe calcium deficiency conditions, an ABA-mediated stress occurred. Stomatal closure under drought stress is known to be triggered by two partially overlapping pathways: a $Ca^{2+}$-dependent pathway, which involve primarily calcium signalling, and a $Ca^{2+}$-independent (or ABA-dependent) pathway (Kim et al., 2010). The $Ca^{2+}$-independent pathway operates through ABA-activated SnRK2 kinases, such as OST1, which phosphorylate and induced the activity of the S-type anion channel SLAC1 (Lee et al., 2009). Therefore, in this scenario, the upregulation of *PvSnRK2. OST1*, *PvABI5* and *PvSLAC1* suggest that severe calcium deficiency in bean activates the independent $Ca^{2+}$ signalling pathway, mediated by ABA, focused on the activity of the anion channel *PvSLAC1*. The induction of *PvAVP2*, which plays a role as a $Ca^{2+}$-sensitive pump (Schilling et al., 2017), together with the up-regulation of *PvSLAC1* and *PvSnRK2. OST1*, also suggests the boosting of vacuolar proton pumping after ABA signal in these conditions. On the other hand, we found an absence of regulation of *PvSnRK2.OST1*, *PvABI5* and *PvSLAC1* in the moderate C1 treatment, despite observing stomatal closure at late stages of the stress in this condition. This apparent contradiction suggests that in the C1 treatment, stomatal closure may have been mediated through a different mechanism, probably because calcium deficiency was not severe enough to trigger the ABA-dependent pathway. It is plausible, that in the C1 treatment, where the calcium levels are reduced but the double than in C2, there is still enough calcium available to act as a signalling molecule and this stomatal closure was mediated, thus, by the $Ca^{2+}$-dependent pathway. In this route, increases in cytosolic calcium can directly activate CDPKs, which also target SLAC1, promoting stomatal closure independently of the ABA-OST1 signalling module (Geiger et al., 2010). The distinction between these diverse signalling responses observed in C1 and C2, despite finding similar stomatal closure phenotype, highlights a threshold-dependent activation of ABA-mediated stress responses, where more severe deficiency (C2) leads to full activation of ABA-responsive gene expression and downstream signalling elements, while moderate deficiency (C1) triggers partial or alternative adaptive mechanisms that still need to be investigated.

### 4.3. *Adaptive trade-offs in Sorana bean: sink strength and seed coat thickness under calcium deficiency*

Despite the impairment in growth, photosynthetic pigments and

certain gas-exchange parameters, we observed increases in yield and harvest indices at moderate levels of calcium deficiency (C1). This suggests that the yield increase observed under moderate calcium stress is not directly correlated with photosynthetic performance but likely to changes in resource allocation. A similar phenomenon has been also reported for other environmental stresses, such as drought. Hageman et al. (2020) found that drought affected resource partitioning in 20 different bean genotypes. Interestingly, those genotypes with greater sink strength (i.e. greater leaflet growth or yield) were the most drought resistant. In rice, moderate water deficit can enhance the remobilization of stored assimilates and accelerate grain-filling, resulting in increased yield (Yang et al., 2001). In common bean, PHI is the metric showing the strongest correlation to yield under drought, which quantifies remobilization efficiency of total pod resources into seeds (Hageman and Van Volkenburgh, 2021). Our results show that Sorana bean plants respond to the lack of calcium in the soil probably by reallocating assimilated resources to seed production due to a strong sink strength. This results in increased yield and harvest and pod harvest indices (HI and PHI) under moderate calcium stress that are in line with standard values found in commercial common bean cultivars, which range between 12 % and 65 % (Scully and Wallace, 1990). The fact that Sorana ecotype improves this value under stressful conditions reflects the adaptation of this ecotype to their natural areas, although this benefit was primarily found only at moderate levels of deficiency, suggesting a dose-dependent response of this trait. As stated above, studies on other ecotypes are needed to understand how genotypic diversity in *P. vulgaris* responds to calcium deficiency. Additionally, the molecular and physiological mechanisms involved in this process remain unexplored and future research may be focused to better understand them. Hageman and Van Volkenburgh (2021) proposed that sink strength maintenance under drought could be mediated by ABA signalling, which, in light of our molecular results, might be an hypothesis worth to test also under calcium stress conditions.

When plants were subjected to calcium deficiency treatments we also observed thinner pulse coat thickness, which is one of the defining and most appreciated traits of Sorana pulses (Verreschi et al., 1994). A thinner seed coat might be considered a disadvantage in wild environments, since it shortens seed dormancy, making seeds less resilient against unfavourable germination conditions (Finch-Savage and Leubner-Metzger, 2006). However, domesticated beans normally present shorter dormancy periods than their wild counterparts, due to lower physical seed coat impermeability, which favors faster water uptake and germination (Soltani et al., 2021; Vidak et al., 2022). In Sorana beans, thus, resource allocation favoring seed growth rather than coat thickness can also be considered an adaptation to the highly fluvial, low fertility soils, improving water imbibition and giving an advantage for fast germination. The genetic mechanisms behind this adaptation remain to be resolved, but Soltani et al., (2021) pinpointed the role of a pectin acetylesterase as a major responsible of decreased physical seed dormancy in beans, making it a candidate gene to further study.

### 4.4. Conclusions

In conclusion, although our initial hypothesis maintained that the adaptation of Sorana ecotype to low calcium in soil would be supported by improved performance of these plants in these conditions, we found negative effects of calcium deficiency on biometric, biochemical and gas-exchange parameters accompanied by positive effects on yield and bean production efficiency. Therefore, we propose that this ecotype adapts to the natural calcium deficiency conditions found in its cultivated areas by reallocating resources and favouring seed production over plant growth and coat thickness, which results in a extremely thin skin, a hallmark of this ecotype. It is noteworthy that, while the extremely thin skin is known to be a specific trait of this genotype, it remains unknown whether the growth-yield trade-off here observed is a specific trait of Sorana ecotype or a common adaptative strategy of different *P. vulgaris* genotypes. Finally, at the molecular level, severe calcium deficiency induced the expression of key ABA-responsive genes, suggesting that ABA-mediated, $Ca^{2+}$-independent stomatal closure was activated under high-stress conditions. In contrast, moderate deficiency also led to stomatal closure but without induction of these genes, implying the involvement of a $Ca^{2+}$-dependent signalling pathway. This divergence underscores a threshold-dependent activation of stress pathways based on calcium availability. Overall, this study highlights the trade-off strategy of Sorana bean from vegetative growth to reproduction and positions this ecotype as a valuable model for understanding calcium stress adaptations, emphasizing the the need for further research on genotype-specific mechanisms of stress tolerance in legumes.

## CRediT authorship contribution statement

**Leonardo Sabbatini:** Methodology, Investigation. **Marzia Vergine:** Writing – review & editing, Writing – original draft, Investigation. **Emily Rose Palm:** Writing – review & editing, Methodology, Investigation. **Werther Guidi Nissim:** Writing – review & editing, Methodology, Investigation. **Fabiano Sillo:** Writing – review & editing, Methodology, Investigation, Formal analysis. **de Rose Silvia:** Methodology, Investigation, Formal analysis. **Elisa Azzarello:** Methodology, Investigation. **Stefano Mancuso:** Supervision, Funding acquisition. **Raffaella Balestrini:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Federico Vita:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **José Eduardo Marqués-Gálvez:** Writing – review & editing, Writing – original draft, Visualization, Validation, Formal analysis, Data curation.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.plantsci.2025.112840.

## Data availability

Data will be made available on request.

## References

Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat. Protoc. 2 (4), 875–877. https://doi.org/10.1038/nprot.2007.102.

Assefa, T., Beebe, S.E., Rao, I.M., Cuasquer, J.B., Duque, M.C., Rivera, M., et al., 2013. Pod harvest index as a selection criterion to improve drought resistance in White pea bean. Field Crops Res. 148, 24–33. https://doi.org/10.1016/j.fcr.2013.04.008.

Baker, N.R., 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu. Rev. Plant Biol. 59 (1), 89–113. https://doi.org/10.1146/annurev.arplant.59.032607.092759.

Balestrini, R., Hahn, M.G., Faccio, A., Mendgen, K., Bonfante, P., 1996. Differential localization of carbohydrate epitopes in plant cell walls in the presence and absence of arbuscular mycorrhizal fungi. Plant Physiology 111 (1), 203–213. https://doi.org/10.1104/pp.111.1.203.

Beebe, S., 2012. Common bean breeding in the tropics. Plant Breed. Rev. 36 (1), 357–426. https://doi.org/10.1002/9781118358566.ch5.

Belletti, G., Marescotti, A. and Brazzini, A. (2017) Old world case study: the role of protected geographical indications to foster rural development dynamics: the case of Sorana Bean PGI. In: The importance of place: Geographical indications as a tool for local and regional development, pp.253–276. https://doi.org/10.1007/978-3-319-53073-4_10.

Borges, A., Melotto, M., Tsai, S.M., Caldas, D.G.G., 2012. Changes in spatial and temporal gene expression during incompatible interaction between common bean and anthracnose pathogen. J. Plant Physiol. 169 (12), 1216–1220. https://doi.org/10.1016/j.jplph.2012.04.003.

Catalá, R., Santos, E., Alonso, J.M., Ecker, J.R., Martínez-Zapater, J.M., Salinas, J., 2003. Mutations in the $Ca^{2+}/H^{+}$ transporter CAX1 increase CBF/DREB1 expression and the cold-acclimation response in arabidopsis. Plant Cell 15 (12), 2940–2951. https://doi.org/10.1105/tpc.015248.

Catarcione, G., Paolacci, A.R., Alicandri, E., Gramiccia, E., Taviani, P., Rea, R., et al., 2023. Genetic diversity and population structure of common bean (Phaseolus vulgaris L.) landraces in the lazio region of Italy. Plants 12 (4), 744. https://doi.org/10.3390/plants12040744.

Cervera-Torres, C., Arthikala, M.K., Lara, M., Blanco, L., Nanjareddy, K., 2022. Comprehensive analysis of phaseolus vulgaris SnRK gene family and their expression during rhizobial and mycorrhizal symbiosis. Genes 13 (11), 2107. https://doi.org/10.3390/genes13112107.

Chen, Y.H., Hu, L., Punta, M., Bruni, R., Hillerich, B., Kloss, B., et al., 2010. Homologue structure of the SLAC1 anion channel for closing stomata in leaves. Nature 467 (7319), 1074–1080. https://doi.org/10.1038/nature09487.

Cheng, N.H., Pittman, J.K., Shigaki, T., Lachmansingh, J., LeClere, S., Lahner, B., et al., 2005. Functional association of arabidopsis CAX1 and CAX3 is required for normal growth and ion homeostasis. Plant Physiol. 138 (4), 2048–2060. https://doi.org/10.1104/pp.105.061218.

De Bang, T.C., Husted, S., Laursen, K.H., Persson, D.P., Schjoerring, J.K., 2021. The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants. N. Phytol. 229 (5), 2446–2469. https://doi.org/10.1111/nph.17074.

de Freitas, S.T., Shackel, K.A., Mitcham, E.J., 2011. Abscisic acid triggers whole-plant and fruit-specific mechanisms to increase fruit calcium uptake and prevent blossom end rot development in tomato fruit. J. Exp. Bot. 62 (8), 2645–2656. https://doi.org/10.1093/jxb/erq430.

De Ron, A.M., Casquero, P.A., González, A.M., Santalla, M., 2004. Environmental and genotypic effects on pod characteristics related to common bean quality. J. Agron. Crop Sci. 190 (4), 248–255. https://doi.org/10.1111/j.1439-037X.2004.00098.x.

Dinelli, G., Bonetti, A., Minelli, M., Marotti, I., Catizone, P., Mazzanti, A., 2006. Content of flavonols in Italian bean (Phaseolus vulgaris L.) ecotypes. Food Chem. 99 (1), 105–114. https://doi.org/10.1016/j.foodchem.2005.07.028.

Drozdowicz, Y.M., Kissinger, J.C., Rea, P.A., 2000. AVP2, a sequence-divergent, $K^{+}$-insensitive $H^{+}$-translocating inorganic pyrophosphatase from arabidopsis. Plant Physiol. 123 (1), 353–362. https://doi.org/10.1104/pp.123.1.353.

FAO. 2023. Agricultural production statistics 2010–2023. FAOSTAT Analytical Briefs, No. 96. Rome. https://openknowledge.fao.org/handle/20.500.14283/cd3755en.

Finch-Savage, W.E., Leubner-Metzger, G., 2006. Seed dormancy and the control of germination. –23 N. Phytol. 171, 501. https://doi.org/10.1111/j.1469-8137.2006.01787.x.

Finkelstein, R.R., Lynch, T.J., 2000. The arabidopsis abscisic acid response gene ABI5 encodes a basic leucine zipper transcription factor. Plant Cell 12 (4), 599–609. https://doi.org/10.1105/tpc.12.4.599.

Geiger, D., Scherzer, S., Mumm, P., Marten, I., Ache, P., Matschi, S., et al., 2010. Guard cell anion channel SLAC1 is regulated by CDPK protein kinases with distinct $Ca^{2+}$ affinities. Proc. Natl. Acad. Sci. 107 (17), 8023–8028. https://doi.org/10.1073/pnas.0912030107.

Geisler, M., Frangne, N., Gomes, E., Martinoia, E., Palmgren, M.G., 2000. The ACA4 gene of arabidopsis encodes a vacuolar membrane calcium pump that improves salt tolerance in yeast. Plant Physiol. 124 (4), 1814–1827. https://doi.org/10.1104/pp.124.4.1814.

Gepts, P., Beavis, W.D., Brummer, E.C., Shoemaker, R.C., Stalker, H.T., Weeden, N.F., et al. 2005. Legumes as a model plant family. In: Genomics for Food and Feed report of the cross-legume Advances Through Genomics Conference. https://doi.org/10.1104/pp.105.060871.

Hageman, A.N., Urban, M.O., Van Volkenburgh, E., 2020. Sensitivity of leaflet growth rate to drought predicts yield in common bean (*phaseolus vulgaris*). Funct. Plant Biol. 47 (9), 792–802. https://doi.org/10.1071/FP19332.

Hageman, A., Van Volkenburgh, E., 2021. Sink strength maintenance underlies drought tolerance in common bean. Plants 10 (3), 489. https://doi.org/10.3390/plants10030489.

Helms, K., 1971. Calcium deficiency of dark-grown seedlings of phaseolus vulgaris L. Plant Physiol. 47 (6), 799–804. https://doi.org/10.1104/pp.47.6.799.

Hoagland, D.R., Arnon, D.I., 1950. The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular No. 347. University of California, Berkeley, CA, USA.

Hosy, E., Vavasseur, A., Mouline, K., Dreyer, I., Gaymard, F., Porée, F., et al., 2003. The arabidopsis outward $K^{+}$ channel GORK is involved in regulation of stomatal movements and plant transpiration. Proc. Natl. Acad. Sci. 100 (9), 5549–5554. https://doi.org/10.1073/pnas.0733970100.

Ikeda, H., Kanayama, Y., 2014. Blossom-end rot in fruit vegetables. In: Abiotic Stress Biol. Hortic. Plants. Springer Japan, Tokyo, pp. 117–126. https://doi.org/10.1007/978-4-431-55251-2_9.

Inkham, C., Wichapeng, W., Panjama, K., Ruamrungsri, S., 2023. Exploring the role of calcium in the physiology of tulipa: a comparative study across different cultivars. Horticulturae 10 (1), 13. https://doi.org/10.3390/horticulturae10010013.

Jezek, M., Blatt, M.R., 2017. The membrane transport system of the guard cell and its integration for stomatal dynamics. Plant Physiol. 174 (2), 487–519. https://doi.org/10.1104/pp.16.01949.

Jing, T., Li, J., He, Y., Shankar, A., Saxena, A., Tiwari, A., et al., 2024. Role of calcium nutrition in plant physiology: advances in research and insights into acidic soil conditions—A comprehensive review. Plant Physiol. Biochem. 210, 108602. https://doi.org/10.1016/j.plaphy.2024.108602.

Kim, T.H., Böhmer, M., Hu, H., Nishimura, N., Schroeder, J.I., 2010. Guard cell signal transduction network: advances in understanding abscisic acid, $CO_2$ and $Ca^{2+}$ signaling. Annu. Rev. Plant Biol. 61 (1), 561–591. https://doi.org/10.1146/annurev-arplant-042809-112226.

Kudla, J., Becker, D., Grill, E., Hedrich, R., Hippler, M., Kummer, U., et al., 2018. Advances and current challenges in calcium signaling. N. Phytol. 218, 414–431. https://doi.org/10.1111/nph.14966.

Kumari, V.V., Banerjee, P., Verma, V.C., Sukumaran, S., Chandran, M.A.S., Gopinath, K. A., et al., 2022. Plant nutrition: an effective way to alleviate abiotic stress in agricultural crops. Int. J. Mol. Sci. 23 (15), 8519. https://doi.org/10.3390/ijms23158519.

Kwak, M., Gepts, P., 2009. Structure of genetic diversity in the two major gene pools of common bean (Phaseolus vulgaris L., Fabaceae). Theor. Appl. Genet. 118, 979–992. https://doi.org/10.1007/s00122-008-0955-4.

Lee, S.C., Lan, W., Buchanan, B.B., Luan, S., 2009. A protein kinase-phosphatase pair interacts with an ion channel to regulate ABA signaling in plant guard cells. Proc. Natl. Acad. Sci. 106 (50), 21419–21424. https://doi.org/10.1073/pnas.0910601106.

Liu, K., Niu, Y., Konishi, M., Wu, Y., Du, H., Chung, H.S., et al., 2017. Discovery of nitrate–CPK–NLP signalling in central nutrient–growth networks. Nature 545, 311–316. https://doi.org/10.1038/nature22077.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method. Methods 25 (4), 402–408. https://doi.org/10.1006/meth.2001.1262.

Loko, L.E.Y., Orobiyi, A., Adjatin, A., Akpo, J., Toffa, J., Djedatin, G., et al., 2018. Morphological characterization of common bean (Phaseolus vulgaris L.) landraces of central region of Benin republic. J. Plant Breed. Crop Sci. 10 (11), 304–318. https://doi.org/10.5897/JPBCS2018.0766.

Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence—a practical guide. J. Exp. Bot. 51 (345), 659–668. https://doi.org/10.1093/jexbot/51.345.659.

Mirzaghaderi, G., 2025. rtpcr: a package for statistical analysis and graphical presentation of qPCR data in R. PeerJ 13, e20185. https://doi.org/10.7717/peerj.20185.

Mortimer, J.C., Laohavisit, A., Macpherson, N., Webb, A., Brownlee, C., Battey, N.H., et al., 2008. Annexins: multifunctional components of growth and adaptation. J. Exp. Bot. 59 (3), 533–544. https://doi.org/10.1093/jxb/erm344.

Ng, C.K.Y., McAinsh, M.R., Gray, J.E., Hunt, L., Leckie, C.P., Mills, L., et al., 2001. Calcium-based signalling systems in guard cells. N. Phytol. 151 (1), 109–120. https://doi.org/10.1046/j.1469-8137.2001.00152.x.

Nicoletto, C., Zanin, G., Sambo, P., Dalla Costa, L., 2019. Quality assessment of typical common bean genotypes cultivated in temperate climate conditions and different growth locations. Sci. Hortic. 256, 108599. https://doi.org/10.1016/j.scienta.2019.108599.

O'Rourke, J.A., Iniguez, L.P., Fu, F., Bucciarelli, B., Miller, S.S., Jackson, S.A., et al., 2014. An RNA-Seq based gene expression Atlas of the common bean. BMC Genom. 15, 1–17. https://doi.org/10.1186/1471-2164-15-866.

Olle, M., Bender, I., 2009. Causes and control of calcium deficiency disorders in vegetables: a review. J. Hortic. Sci. Biotechnol. 84 (6), 577–584. https://doi.org/10.1080/14620316.2009.11512568.

Palm, E., Nissim, W.G., Giordano, C., Mancuso, S., Azzarello, E., 2017. Root potassium and hydrogen flux rates as potential indicators of plant response to zinc, copper and nickel stress. Environ. Exp. Bot. 143, 38–50. https://doi.org/10.1016/j.envexpbot.2017.08.009.

Palta, J.P., 2010. Improving potato tuber quality and production by targeted calcium nutrition: the discovery of tuber roots leading to a new concept in potato nutrition. Potato Res. 53, 267–275. https://doi.org/10.1007/s11540-010-9163-0.

Peiter, E., Maathuis, F.J., Mills, L.N., Knight, H., Pelloux, J., Hetherington, A.M., et al., 2005. The vacuolar $Ca^{2+}$-activated channel TPC1 regulates germination and stomatal movement. Nature 434 (7031), 404–408. https://doi.org/10.1038/nature03381.

Piergiovanni, A.R., Lioi, L., 2010. Italian common bean landraces: history, genetic diversity and seed quality. Diversity 2 (6), 837–862. https://doi.org/10.3390/d2060837.

Pinto, R.A., Patto, M.A., Martins, E.S., Abreu, A.D.F.B., 2018. Inheritance of harvest index in common bean. Crop Breed. Appl. Biotechnol. 18 (3), 252–258. https://doi.org/10.1590/1984-70332018v18n3a38.

Pittman, J.K., Hirschi, K.D., 2024. CAX control: multiple roles of vacuolar cation/$H^{+}$ exchangers in metal tolerance, mineral nutrition and environmental signalling. Plant Biol. 26 (6), 911–919. https://doi.org/10.1111/plb.13698.

Ramalho, J.C., Rebelo, M.C., Santos, M.E., Antunes, M.L., Nunes, M.A., 1995. Effects of calcium deficiency on coffea arabica. Nutrient changes and correlation of calcium levels with some photosynthetic parameters. Plant Soil 172, 87–96. https://doi.org/10.1007/BF00020862.

Redondo-Gómez, Wharmby, S., Castillo, C., Mateos-Naranjo, J.M., Luque, E., De Cires, C. J., et al., 2006. Growth and photosynthetic responses to salinity in an extreme halophyte, sarcocornia fruticosa. Physiol. Plant 128 (1), 116–124. https://doi.org/10.1111/j.1399-3054.2006.00719.x.

Riveras, E., Alvarez, J.M., Vidal, E.A., Oses, C., Vega, A., Gutiérrez, R.A., 2015. The calcium ion is a second messenger in the nitrate signaling pathway of arabidopsis. Plant Physiol. 169 (2), 1397–1404. https://doi.org/10.1104/pp.15.00961.

Saito, S., Uozumi, N., 2020. Calcium-regulated phosphorylation systems controlling uptake and balance of plant nutrients. Front. Plant Sci. 11, 44. https://doi.org/10.3389/fpls.2020.00044.

Schilling, R.K., Tester, M., Marschner, P., Plett, D.C., Roy, S.J., 2017. AVP1: one protein, many roles. Trends Plant Sci. 22 (2), 154–162. https://doi.org/10.1016/j.tplants.2016.11.012.

Schmitz-Eiberger, M., Haefs, R., Noga, G., 2002. Calcium deficiency-influence on the antioxidative defense system in tomato plants. J. Plant Physiol. 159 (7), 733–742. https://doi.org/10.1078/0176-1617-0621.

Scully, B.T., Wallace, D.H., 1990. Variation in and relationship of biomass, growth rate, harvest index, and phenology to yield of common bean. J. Am. Soc. Hortic. Sci. 115 (2), 218–225.

Smýkal, P., Coyne, C.J., Ambrose, M.J., Maxted, N., Schaefer, H., Blair, M.W., et al., 2015. Legume crops phylogeny and genetic diversity for science and breeding. Crit. Rev. Plant Sci. 34 (1–3), 43–104. https://doi.org/10.1080/07352689.2014.897904.

Soltani, A., Walter, K.A., Wiersma, A.T., et al., 2021. The genetics and physiology of seed dormancy, a crucial trait in common bean domestication. BMC Plant Biol. 21, 58. https://doi.org/10.1186/s12870-021-02837-6.

Vahisalu, T., Kollist, H., Wang, Y.F., Nishimura, N., Chan, W.Y., Valerio, G., Kangasjärvi, J., 2008. SLAC1 is required for plant guard cell S-type anion channel function in stomatal signalling. Nature 452 (7186), 487–491. https://doi.org/10.1038/nature06608.

Verreschi, V., 1994. I fagioli di sorana. SP 44.

Vidak, M., Lazarević, B., Javornik, T., Šatović, Z., Carović-Stanko, K., 2022. Seed water absorption, germination, emergence and seedling phenotypic characterization of the common bean landraces differing in seed size and color. Seeds 1 (4), 324–339. https://doi.org/10.3390/seeds1040027.

Wang, X., Hao, L., Zhu, B., Jiang, Z., 2018. Plant calcium signaling in response to potassium deficiency. Int. J. Mol. Sci. 19, 3456. https://doi.org/10.3390/ijms19113456.

Wdowiak, A., Podgórska, A., Szal, B., 2024. Calcium in plants: an important element of cell physiology and structure, signaling, and stress responses. Acta Physiol. Plant 46 (12), 108. https://doi.org/10.1007/s11738-024-03733-w.

Wellburn, A.R., 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144 (3), 307–313. https://doi.org/10.1016/S0176-1617(11)81192-2.

Weng, X., Li, H., Ren, C., Zhou, Y., Zhu, W., Zhang, S., et al., 2022. Calcium regulates growth and nutrient absorption in poplar seedlings. Front. Plant Sci. 13, 887098. https://doi.org/10.3389/fpls.2022.887098.

White, P.J., Broadley, M.R., 2003. Calcium in plants. Ann. Bot. 92 (4), 487–511. https://doi.org/10.1093/aob/mcg164.

Wu, J., Wang, L., Fu, J., Chen, J., Wei, S., Zhang, S., et al., 2020. Resequencing of 683 common bean genotypes identifies yield component trait associations across a north–south cline. Nat. Genet 52 (1), 118–126. https://doi.org/10.1038/s41588-019-0546-0.

Yang, J., Zhang, J., Wang, Z., Zhu, Q., Wang, W., 2001. Remobilization of carbon reserves in response to water deficit during grain filling of rice. Field Crops Res 71 (1), 47–55. https://doi.org/10.1016/S0378-4290(01)00147-2.

Zhai, J., Xu, H., Cong, X., Deng, Y., Xia, Z., Huang, X., et al., 2013. $Ca^{2+}/H^{+}$ exchange in the plasma membrane of arabidopsis thaliana leaves. Acta Physiol. Plant 35 (1), 161–173. https://doi.org/10.1007/s11738-012-1059-y.

Zhang, H., Mascher, M., Abbo, S., Jayakodi, M., 2022. Advancing grain legumes domestication and evolution studies with genomics. Plant Cell Physiol. 63 (11), 1540–1553. https://doi.org/10.1093/pcp/pcac062.

Zhang, W., Zwiazek, J.J., 2016. Responses of reclamation plants to high root zone ph: effects of phosphorus and calcium availability. J. Environ. Qual. 45 (5), 1652–1662. https://doi.org/10.2134/jeq2016.01.0026.