

Research Article

# Effect of genotypes and artificial shading during flowering stage on the productivity patterns of Faba bean (*Vicia faba* L.) under Mediterranean conditions

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#### Abstract

In agroforestry systems, higher-positioned crops frequently tend to impose shade stress on lower-positioned ones, with consequent negative repercussions on the agronomic characteristics of the latter. The present study was conducted under field conditions over two cropping seasons 2020-2021 and 2021-2022, to evaluate the mechanisms of physiological (e.g., stomatal conductance and water relative content) and biochemical (e.g., proline and chlorophyll content) variations, as well as grain yield and its components of different Faba bean varieties subjected to shade stress. Six varieties of *Vicia faba* L. (Aguadulce, Hiba, Zina, Alfia17, Defes, and Extra hative) were subjected to three levels of artificial shade stress S1 (0%), S2 (50%), and S3 (90%) during the flowering stage. The results showed that variety, shade treatment, and year significantly affected above-ground biomass and yield attributes. Substantial reductions in grain yield of 31.17% and 53.34% were observed under S2 and S3, respectively, compared with S1, demonstrating the adverse impact of shading on this parameter. Significant differences between the studied varieties were noticed. Among the varieties tested, Hiba and Zina showed the highest grain yield under shade stress conditions. Shading decreased stomatal conductance for all varieties tested, influencing proline synthesis in response to this abiotic stress. The results suggest that incorporating high-performance varieties Hiba and Zina into agroforestry systems can provide significant added value to local farmers since they are the most tolerant to shade stress, which can improve the performance of the tree-plant association in terms of yield and productivity.

Keywords: Artificial shade, Proline, Stomatal conductance, Vicia faba L., Yield

#### INTRODUCTION

Agroforestry is emerging as a promising land management strategy, especially in the context of climate change adaptation (Amassaghrou *et al*, 2023). It offers effective methods for carbon sequestration (Mayer *et* 

*al*, 2022), soil enhancement (Rousseau *et al*, 2012), and the improvement of air and water quality (Dollinger & Jose, 2018; Pent & Fike, 2021). These systems are crucial for enhancing yields and optimizing the use of resources on land plots (Shafiq *et al*, 2020). In Morocco, annual crops are often integrated into agroforestry

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systems centered around olive trees. Legume crops, in particular, are advantageous compared to cereals and non-legume forages. Due to their indeterminate growth habit, legumes can continue to grow and increase yield potential under favorable conditions. They can fix atmospheric nitrogen and have low sensitivity to the nitrogen competition aspects with tree platforms in agroforestry areas. Furthermore, as noted by Amassaghrou *et al* (2023), unlike cereals, olive yields are not significantly affected by the presence of legumes, and the land equivalent ratio surpasses 1.

Faba bean (Vicia faba L.) is a legume crop of significant importance in the daily diets of humans and animals due to the prominent protein aspect (20-30%) (Qahtan, 2021) and its bioactive compounds (Krause et al, 2023). However, many Faba bean cultivars are sensitive to abiotic stress, which negatively impacts crops (Essa et al, 2023). In agroforestry systems, variations in plant population density and the height of intercropped plants can induce shading stress and alter the microclimate of the small culture (Wang et al, 2021a). Faba bean is particularly vulnerable to insufficient light when intercropped with taller plants, affecting its normal growth. Light intensity is crucial for plant growth, development, and physiological processes. However, excessive shading can reduce plant quality, vield, and productivity (Gao et al, 2020). Shading conditions significantly impact key plant traits, including flowering duration, length, yield hypocotyl and overall (Muhammad et al, 2021). Reduced light quality and intensity during growth phases can decrease photosynthetic efficiency by damaging the chloroplast ultrastructure, thus reducing chlorophyll synthesis (Shafig et al, 2020). Insufficient light has been shown to decrease grain number, grain weight, and grain filling rate in maize (Zea mays L.) (Hu et al, 2023). Studies have also shown that light interception through artificial shade structures can reduce soybean yields by impacting the number of pods and affecting crop yield during flowering and pod formation (Rivest et al, 2009). Shading stress affects soybeans at morphological, physiohormonal, biochemical, metabolic, and molecular levels, as demonstrated by various research studies (Barro et al, 1989; Du et al, 2018; Hussain et al, 2019).

While trees in agroforestry systems share soil nutrients with crops, they also mitigate drought stress for crops by casting shade and creating a more suitable microclimate (Mugunga *et al*, 2017) and moderating temperature (Peng *et al*, 2015), though this effect is reduced in drier conditions (Coussement *et al*, 2018). This leads to enhanced yield and crop quality (Fida *et al*, 2021) and improves physiological performance (Mensah *et al*, 2023). Shading under drought conditions generally enhances plant growth and survival, reduces transpiration requirements, and prevents excessive temperatures by lowering air temperatures (Naseer *et al*, 2023).

Faba bean is a valuable crop for agroforestry systems where light deficit is a limiting factor. However, there is a lack of data on its response to shade under Mediterranean field conditions. Therefore, this research aimed to evaluate the effects of shade stress on yield and its components in different Faba bean varieties and to study the biochemical and physiological responses of Faba bean (*V. faba* L.) under this constraint to identify the most tolerant varieties.

#### MATERIALS AND METHODS

#### **Trial description**

Trials were conducted at the National Institute of Agricultural Research's experimental station in Douyet, INRA-Morocco, over two consecutive growing seasons: 2020-2021 (Y1) and 2021-2022 (Y2). The experimental site, located at 34°04'N, 5°07'W, is in the favorable zone of the Saïs plain in the Province of Moulay Yaacoub, Wilaya of Fez-Meknes, at an altitude of 416 meters. The soil is silty clay, composed of 48.50% silt, 39.90% clay, and 11.60% fine sand, primarily dark Vertisols. The soil's chemical characteristics include a pH of 7.80, 3.63 mg/kg of organic matter, 11.89 mg/kg of available  $P_2O_5$ , and 478.05 mg/kg of available K<sub>2</sub>O. The climate is Mediterranean, with a dry season extending from May to October.

#### **Plant material**

Four major Faba bean varieties: Hiba (Hb), Aguadulce (Ag), Extra hative (Eh), and Defes (Df), and two minor Faba bean varieties: Zina (Zn) and Alfia17 (Af17) were tested. Manual seeding was carried out on December 21, 2020, and December 14, 2021, during the 1st and 2nd years, respectively, where the area had been previously prepared by deep 3-disc plowing. Before this experiment, the land had been rotated with cereals. Then, Fertilizers were applied and spread to improve soil quality, such as the base fertilizer NPK "10-30-10" (200kg/ha). Plant-related biotic agents (diseases/ pests/weeds) were intensively treated to prevent yield loss.

#### Experimental study design

The experiment was designed as a split plot with two replications. Shade treatments (S1, S2, and S3) were assigned to the main plots, while Faba bean varieties were assigned to subplots. Each elementary plot, measuring 21.6 m by 4 m, consisted of six rows per variety (36 rows per plot) with 0.60 m inter-row spacing. There were 3-meter-wide alleys between replicates and treatments. Each plot covered an area of 86.4 m<sup>2</sup>, and the total experimental area was 811.8 m<sup>2</sup>. Each year, the experiment was conducted on different plots within the experimental site.



Fig. 1. Photographs of the experimental trials. (A) full sunlight treatment S1 (0%); (B) Shade treatment S2 (50%); (C) Shade treatment S3 (90%)

Subplots designated for shading treatment were covered with Aluminet shading, a material made from highdensity polyethylene with a metalized layer for enhanced durability. These shade nets were stretched over wooden supports using metal wire, positioned 1 meter above the ground, and constructed to shade the plants from the top and sides while leaving the south side open for air movement. The shading bands were arranged to reduce the intensity of photosynthetically active radiation (PAR) by 50% and 90%, measured by a ZDS-10 Luxmeter, China, resulting in three treatments: no shading (S1), 50% shading (S2), and 90% shading (S3) (Fig. 1). Shading was applied from the flowering stage to maturity, beginning 81 days after seeding in Y1 and 127 days after seeding in Y2. The delay in Y2 was due to more severe weather conditions compared to Y1, which slowed Faba bean development and delayed the flowering phase.

### Sampling and measurement

#### Physiological and biochemical measurements

The measurements were carried out during two agricultural seasons Y1 and Y2. Stomatal conductance and photosynthetic performance were measured in situ on five randomly selected plants in the field, while leaf and plant samples were taken for growth, physiological, and biochemical assessments. The plant and leaf samples were harvested manually in the four central rows/six rows per variety over a length of 0.5m from the border of each plot.

Leaf sampling was carried out by selecting mature leaves without any visible damage. To obtain consistent results for the assessment of various biochemical and physiological parameters, leaf sampling was carried out between 9:00 and 10:00 am. Plastic bags were used to pack the leaves and immediately taken to the laboratory for estimation of photosynthetic pigments, proline, and relative water content. It should be noted that the leaf samples were all of the same physiological age, thus assuring homogeneity in all physiological and biochemical measurements. Physiological and biochemical measurements were only performed during Y2 due to the unavailability of equipment during the first year. Stomatal conductance, photosynthetic performance measurement using SPAD, relative water content, chlorophyll content, and proline content analyses were carried out between 23/04/2022 and 27/04/2022, corresponding to a period of 21 days after the shade nets were installed during Y2. All measurements were carried out for each treatment, each variety, and each repetition.

## Evaluation of physiological and biochemical parameters

#### Stomatal conductance (SC)

Stomatal conductance (mm/s) is a response to ambient  $CO_2$  concentration, air vapor pressure difference in the leaves, leaf temperature, and water status (Oukaltouma *et al*, 2020). It was measured on three plants (leaves) per variety and treatment. Measurements were taken at midday under 21°C±13°C and 82% relative humidity on the leaves using a Porometer (Model SC1, Decagon Devices, version 2012).

#### SPAD value

The SPAD value was measured with a SPAD-502 device (Konica Minolta, Japan) (Xie *et al*, 2021). Measurements were taken from the central portion of each leaf, avoiding the midribs. Three readings were recorded per leaf, and the average chlorophyll value for each leaf was then calculated.

#### **Relative water content (RWC)**

The fresh weight (FW) of leaf discs from three leaves per variety and per treatment was determined. They were then immersed for 24 hours in distilled water to reach full turgor. After wiping the surface water from the leaf discs, their turgor weight (TW) was measured. Samples were then dried for 72 hours at 70°C and their dry weight (WS) was determined. RWC was determined using the following formula of Galmés *et al* (2007).

RWC(%) = [(FW - DW) / (TW - DW)] X 100 Eq.1

#### **Proline content**

0.5 grams of leaves were mixed with 10 ml of 3% water -based sulfosalicylic acid and filtered through filter paper. In a test tube, 2 ml of the filtered sample were combined with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin, then heated in boiling water at 100°C for 1 hour. The mixture was subsequently cooled on ice to halt the reaction. Next, toluene (4 ml) was added to the mixture, and the reaction was stirred for 20 s. Toluene served as a control, and its absorbance for the chromophore was measured after aspiration and returning to room temperature. The reaction's absorbance was measured at 528 nm. Proline content was expressed in milligrams per gram of fresh weight (mg/g FW), using a standard curve for comparison (Bates *et al.*, 1973).

#### Chlorophyll a, b, and t contents

Chlorophyll pigments were measured using faba bean leaves. The samples were grounded in 5 ml of 80% acetone, and centrifuged at 3000 r/min for 5 min to extract the chlorophyll contents. The liquid above was gathered and assessed using a spectrophotometer at 663 and 645 nm (Muhammad *et al.*, 2021). Chlorophyll a (Chl a) and b (Chl b) and total chlorophyll (Chl t) were quantified in mg/g FW using formulas established by Arnon (1949):

| Chlorophyll a = $-2.69(A645) + 12.7(A663)$       | Eq.2 |
|--|------|
| Chlorophyll b = $-4.68(A663) + 22.9(A645)$       | Eq.3 |
| Chlorophyllt (total) = $8.02(A663) + 20.2(A645)$ | Eq.4 |

#### Yield parameters

At the maturity stage, plant sampling for yield analysis was carried out by randomly selecting five plants per variety. These plants intended for analysis of yield and its components were harvested on 08/06/2021 and 15/06/2022, representing 87 days and 70 days, respectively, after the installation of shade treatments during Y1 and Y2 cropping seasons. Yield components included plant height (cm), NTS (number of total stems), NP (number of pods), NS (number of seeds), and WSS (100-seed weight) (g). The harvest index (HI) was computed as the grain yield (YS) (Kg/ha) and total above-ground biomass (AB) ratio (Kg/ha).

#### Statistical processing

Datasets were statistically processed using the IBM SPSS software (version 21.0). Differences between crop growth variables and yield components were analyzed using three-way ANOVA implementing: 1) the

different varieties tested (Ag, Eh, Hb, AL17, Zn, and Df), 2) shade treatments (S1, S2, and S3), and 3) years (2021 vs. 2022) as fixed factors. A Tukey multiple comparison test was performed to highlight homogenous groups between the treatments. A Principal component analysis (PCA) was conducted to explore the relationships between shading proprieties and Faba bean physiological aspects using sklearn.decomposition.PCA module in Python (version 3.12.4) integrated into Py-Charm IDE (version 2024.1).

#### RESULTS

## Effect of shading and variety on Faba bean productivity

Results of a three-way ANOVA showed that the effects of variety (V), shade treatment (S), and year (Y) on grain yield (YS) and above-ground biomass (AB) were significant, whereas the effect on harvest index (HI) was not significant. All S\*Y, S\*V, Y\*V, and S\*Y\*V interactions were not-significant for YS and HI. However, the interaction between S\*V was significant for AB. Shading, represented by conditions S2 and S3, had a negative effect on yields YS and above-ground biomass AB (Table 1). Main seed yield recorded a value of 1036.46 Kg/ha under S2 and 701.38 Kg/ha under S3 compared with varieties grown in full sun (S1) with a yield of 1504.63 Kg/ha. Considerable reductions of 31.17% and 53.34% under S2 and S3, comparatively to S1, respectively, were observed. Regarding AB, it reached a yield of 2847.8 Kg/ha under S2 and 2013.89 Kg/ha under S3, compared to S1 with an above-ground biomass of 4117.48 Kg/ha, resulting in a reduction of 30.78% under S2 and 51.12% under S3, comparatively to S1 respectively. These observations underline the significant negative impact of shading on grain and above-ground biomass yields compared with full sun conditions. The obtained results showed significant differences between the studied varieties for YS and AB, except for HI, in which no significant difference was observed between the Faba bean varieties (Table 1). HB and ZN had the highest grain yields and aboveground biomass of all the studied varieties, with an average of 1368.05 Kg/ha for HB and 3815.97 Kg/ha for ZN, respectively. However, it should be noted that EH had the lowest grain yield, at 810.19 kg/ha, whereas DF had the lowest above-ground biomass, at 1899.31 kg/ ha, compared with the other varieties. A significant decrease in YS and AB was observed between the two crop years Y1 and Y2. In fact, grain yield (YS) and above-ground biomass were reduced by 29.77% and 13.74%, respectively, between Y1 and Y2 (Table 1).

Furthermore, It should be noted that in Y1, the DF variety showed the most stable grain yield under the S3 treatment, reaching 590.28 kg/ha, whereas the EH variety showed similar results under the S2 treatment, with



**Fig 2.** Effect of artificial shading and variety on Faba bean grain yield in 2020-2021 (A) and 2021-2022 (B) cropping seasons. Yield values marked with different asterisks reflect statistically valid differences at P < 0.05, as determined by the S-K-N test; no shading (S1), 50% shading (S2), and 90% shading (S3:. Ag: aguadulce, EH: extra hative, HB: hiba, AF17: alfia17, DF: defes and ZN: zina. YS: seed yields

a yield of 666.67 kg/ha (Fig. 2). These results were comparable to those of their controls in full sun, which were 1402.78 kg/ha for the DF variety and 1798.61 kg/ ha for the EH variety, respectively (Fig. 2A). In Y2, under shade conditions S2 and S3, variety EH recorded the lowest grain yields, reaching 444.44 kg/ha and 250 kg/ha, respectively, compared with their control, which yielded 645.83 kg/ha (Fig. 2B). On the other hand, the ZN variety produced an exceptional amount of grain yield under S2 and S3 conditions, with yields of 1493.06 kg/ha and 1034.72 kg/ha, respectively (Fig. 2B).

## Effect of shade and variety on Faba bean yield components

Three-way ANOVA revealed that variety (V), shade treatment (S), and year (Y) significantly affected Faba

bean height, NP, and NS. However, year and variety had a non-significant effect on WSS and NTS, respectively. The interactions Y\*V and S\*Y showed a nonsignificant effect on NTS. However, the S\*V interaction had a non-significant effect on NTS. Finally, the S\*Y\*V interaction significantly affected only the height and NS. In general, shading (S2 and S3) had an unfavorable impact on Faba bean crops. It reduced all yield components associated with the Faba bean varieties tested. NP, NS, and WSS showed a prominent difference between all applied treatments, with the S2 shade treatment generating reductions of 37.21%, 36.26%, and 21.70% for NP, NS, and WSS, respectively (Table 2). On the other hand, the S3 shade treatment produced even more significant reductions in yield components: 54.04%, 64.21%, and 41.51% for NP, NS, and WSS respectively. Although plants cultivated in full sun (S1) (68.6 cm) had almost the same height as those grown in 50% shade (68.7 cm). Notably, shading increased the height of Faba beans grown under S2. Thus, the number of NTS stems remained unchanged between S2 and S3 (2.21) compared with S1 (3.17) (Table 2). The obtained results showed significant differences between the studied varieties for different yield parameters, except for NTS, in which no significant differences were detected between Faba bean varieties. In terms of plant height, AG and DF recorded the lowest value (75.45 cm), while the highest height value was recorded for the variety EH (78.3 cm) (Table 2). Both varieties (AG and EH) produced the lowest NP value with 4.33 pods per plant and the lowest NS value with 14.45 seeds per plant. However, the highest values of NP and NS were recorded in variety AF17 with values of 11.66 pods per plant and 25.19 seeds per plant, respectively (Table 2). A lower reduction in WSS (2.47%) was observed in crop years Y1 and Y2. Furthermore, height, NTS, NP, and NS were decreased from Y1 to Y2.

#### Effect of shading on the physiological and biochemical parameters of Faba bean Chlorophyll a, b, and t content

The data shown in Fig. 3 indicate that the various varieties reacted in a significantly non-different way to shade -induced stress (Fig. 3A). The ChI content in S2 and S3 (1.09 mg/g FW) was similar to that in Control S1 (1.06 mg/g FW). Under treatment S2, chlorophyll content varied ranging from 1.001 mg/g FW in the DF variety to 1.15 mg/g FW in the ZN variety. However, under treatment S3, chlorophyll content varied from 0.89 mg/g FW in the AG variety to 1.27 mg/g FW in the DF variety.

Shading had a positive impact on the chlorophyll b content (Fig. 3B). This content varied from 0.37 mg/g FW under full sun (S1) to 0.44 mg/g FW under S2 and then to 0.49 mg/g FW under S3. More specifically, variety HB showed the highest chlorophyll b content under S3 (0.83 mg/g FW) compared with its control under S1

| Treatment      | Seed yield<br>(YS) (Kg/ha) | Above-ground biomass<br>(AB) (Kg/ha) | Harvest index<br>(HI) |
|----------------|----------------------------|--------------------------------------|-----------------------|
| Shade          |                            |                                      |                       |
| S1 (0%)        | 1504.63 c                  | 4117.48 c                            | 0.38 a                |
| S2 (50%)       | 1036.46 b                  | 2847.80 b                            | 0.31 a                |
| S3 (90%)       | 701.38 a                   | 2013.89 a                            | 0.34 a                |
| Variety        |                            |                                      |                       |
| AG             | 993.06 ab                  | 2650.46 b                            | 0.36 a                |
| EH             | 810.19 a                   | 3221.06 bc                           | 0.24 a                |
| HB             | 1322.92 b                  | 3737.27 с                            | 0.36 a                |
| DF             | 946.76 ab                  | 1899.31 a                            | 0.33 a                |
| AF17           | 998.84 ab                  | 2555.56 b                            | 0.40 a                |
| ZN             | 1413.19 b                  | 3894.68 c                            | 0.37 a                |
| Cropping Year  |                            |                                      |                       |
| 2020-2021 (Y1) | 1270.06                    | 3213.73                              | 0.37                  |
| 2021-2022 (Y2) | 891.59                     | 2772.38                              | 0.32                  |

**Table 1.** Mean comparison of productivity traits for six Faba bean varieties under three shade levels during the two cropping years 2020-2021 and 2021-2022

Letters indicate homogeneous groups with potentially significant differences at  $P \le 0.05$ ; no shading (S1), 50% shading (S2), and 90% shading (S3); Ag: aguadulce, EH: extra hative, HB: hiba, AF17: alfia17, DF: defes and ZN: zina. YS: seed yields.

 Table 2. Mean comparison of yield components for six Faba bean varieties under three shade levels during two cropping seasons

| Traitement     | Plant Height<br>(cm) | Number of total stems (NTS) | Number of<br>pods (NP) | Number of<br>seeds (NS) | 100-seed<br>weight<br>(WSS) (g) |
|----------------|----------------------|-----------------------------|------------------------|-------------------------|---------------------------------|
| Shade          |                      |                             |                        |                         |                                 |
| S1 (0%)        | 68.65 b              | 3.17 b                      | 12.07 c                | 32.20 c                 | 1,06 c                          |
| S2 (50%)       | 68.77 b              | 2.20 a                      | 7.59 b                 | 20.49 b                 | 0,83 b                          |
| S3 (90%)       | 56.26 a              | 2.21 a                      | 5.54 a                 | 11.52 a                 | 0,62 a                          |
| Variety        |                      |                             |                        |                         |                                 |
| AG             | 57.7 a               | 2.50 a                      | 3.96 a                 | 15.48 a                 | 1.02 c                          |
| EH             | 78.3 d               | 2.39 a                      | 4.71 a                 | 13.43 a                 | 0.78 b                          |
| HB             | 64.1 bc              | 2.37 a                      | 9.00 b                 | 23.35 c                 | 0.83 b                          |
| DF             | 57.2 a               | 2.44 a                      | 8.66 b                 | 19.04 b                 | 0.81 b                          |
| AF17           | 60.1 ab              | 2.45 a                      | 11.66 c                | 25.19 c                 | 0.61 a                          |
| ZN             | 66.4 c               | 2.46 a                      | 8.65 b                 | 21.79 bc                | 0.78 b                          |
| Cropping Year  |                      |                             |                        |                         |                                 |
| Y1 (2020-2021) | 71.46                | 2.71                        | 10.32                  | 25.88                   | 0.81                            |
| Y2 (2021-2022) | 55.84                | 2.14                        | 5.24                   | 13.34                   | 0.79                            |

Letters indicate homogeneous groups with potentially significant differences at  $P \le 0.05$ . no shading (S1), 50% shading (S2) and 90% shading (S3). Ag: aguadulce, EH: extra hative, HB: hiba, AF17: alfia17, DF: defes and ZN: zina. YS: seed yields

(0.39 mg/g FW). In contrast, varieties EH and AF17 recorded the highest values under S2 (0.57 mg/g FW) compared with their respective chlorophyll b contents under S1 full sun (0.44 mg/g FW and 0.36 mg/g FW).

No significant difference was spotted for variety EH between the shading treatments, for variety AG between treatments S1 and S2, or for variety ZN between treatments S2 and S3 (Fig. 3C). However, a significant difference was observed in HB. This variety had a higher total chlorophyll (Chl t) content under the S3 treatment, reaching 1.95 mg/g FW, compared with its control (1.66 mg/g FW) and treatment S2 (1.4 mg/g FW). Furthermore, the total chlorophyll content of variety AF17 under S2 was higher, at 1.71 mg/g FW, than that observed in full sun S1 (1.4 mg/g FW) and S3 (1.64 mg/g FW).

#### Relative water content (RWC)

The RWC ranged of control plants from 65% for variety A17 to 50% for variety Hb (Fig. 4). no significant differ-

ence in shading levels was observed for the variety ZN. Shade level S2 improved the RWC of variety DF, resulting in a 13.06% increase over its full-sun control. Similarly, shade level S3 showed a beneficial effect, increasing the RWC of variety HB by 19.80% when compared with its full-sun control. RWC decreased the least in variety AF17 (26.98%) under S3 and in variety AG (45.74%) under S2 compared with their respective controls.

#### **Proline content**

The highest proline content has been observed in the AG variety (especially in its leaves) (0.47 mg/g FW) under 90% shade treatment, compared with its full-sun control (0.23 mg/g FW) (Fig. 5). The variety DF showed a significant increase in proline content, rising from 0.08 mg/g FW under treatment S1 to 0.199 mg/g FW under treatment S3. However, the ZN variety significantly reduced proline content, dropping by 67.34% under S3 treatment compared with its control under S1. The effect of treatment S2 resulted in a substantial reduction in the proline content of HB variety, with a decrease of 46%. Varieties DF and AF17 also showed a reduction in proline content, reaching percentages of 30.62% and 30.20% respectively.

#### Stomatal conductance (SC)

The samples under abiotic stress conditions such as shading, recorded a significant reduction in stomatal conductance compared with plants grown in full sun (Fig. 6). Specifically, stomatal conductance was reduced by 64.04% under 50% shade and by 70.44% under 90% shade. No significant difference was observed between the stomatal conductance of samples cultivated in different shading levels, S2 and S3. Indeed, the average SC for all varieties tested was similar under S2 (0.38 mm/s) compared with S3 (0.31 mm/ s). Furthermore, the same effect was observed between the shading and full sun treatments for the DF variety. On the other hand, significant differences were spotted between the varieties within each shade treatment. Variety Df showed the highest stomatal conductance under S2 (0.541 mm/s) and S3 (0.44 mm/s), while variety Eh recorded the lowest value, with 0.226 mm/s and 0.152 mm/s under S2 and S3, respectively.

#### Photosynthetic performance

The results revealed a significant difference between shading treatments for each variety studied. The application of 50% shade had a variable impact on the chlorophyll content of the different varieties (Fig. 7). Specifically, there was a significant increase in chlorophyll content for varieties AG, EH, and AF17, with increases of 20.72%, 4.50%, and 12.68% respectively. In contrast, a decrease in chlorophyll content was observed in varieties HB, DF, and ZN, with decreases of 17.74%,



**Fig 3.** Effect of shading levels on chlorophyll content; Chlorophyll a (A), Chlorophyll b (B), and total Chlorophyll (C) contents of different varieties under shade treatment. Values marked with different asterisks reflect statistically valid differences at  $P \le 0.05$ , as determined by the S-K-N test

14.42%, and 8.28%, respectively. At a shading level of 90%, the chlorophyll content was virtually uniform among all the varieties examined. AG had the highest content, reaching 40.4 mg/g FW, whereas ZN had the lowest, at 34.77 mg/g FW (Fig. 7).

## Relationships between shading proprieties and Faba bean physiological aspects

APC was assessed to explore the similarities between samples under study. The examined attributes were



**Fig 4.** RWC of different varieties under shade treatment. Values marked with different asterisks reflect statistically valid differences at  $P \le 0.05$ , as determined by the S-K-N test



**Fig. 6.** Stomatal conductance of different varieties under shade treatments. Values marked with different asterisks reflect statistically valid differences at  $P \le 0.05$ , as determined by the S-K-N test

concentrated in two groups: the first comprised Chl a, Chl b, and Ch T, while the second consisted of proline, RWC, Ch SPAD, and SC. The first two components accounted for 54.87% (PC1) and 48.23% (PC2) (Fig. 8). The PC1 retained the parameters of the first group (Chl a and b and Ch T), which correlated positively with Chl a, Chl b, and Ch T. Therefore, a negative correlation was observed between the first principal component and proline, RWC, Ch SPAD, and SC. Concerning the second principal component, the positive part keeps all assessed parameters except Chl b, which is found in the negative part of the second principal component. Concerning similarities of the studied samples cultivated under different conditions, the first PC allowed the discrimination of two groups; the first PC had similar properties in terms of Chl a, Chl b, and Ch T. The first group was composed of S2Ag, S2Hb, S2Df, S3Ag, S1Ag, S1Af17, and S1Zn. This group was found in the positive part of PC1. Other samples were located in the negative part of PC1.



**Fig 5.** Proline content of different varieties under shade treatments. Values marked with different asterisks reflect statistically valid differences at  $P \le 0.05$ , as determined by the S-K-N test



**Fig 7.** Chlorophyll content by SPAD of different varieties under shade treatments. Values marked with different asterisks reflect statistically valid differences at  $P \le 0.05$ , as determined by the S-K-N test.

#### DISCUSSION

#### Effect of shading on Faba bean productivity

This study revealed that shading adversely affected Faba bean grain yield (Fig. 1 and 2). The extent of this impact varied based on the level of shading and the variety of Faba beans examined. The findings indicated a negative correlation between shading intensity and grain yield of rice (Oryza sativa L.), likely due to reduced leaf photosynthetic rates, photosynthesis, and nutrition metabolism enzymes (Song et al, 2022). The primary reason for the negative impact of shade stress on crop yield is the downregulation of leaf photosynthesis (Song et al, 2022). In response to a shaded environment, plants often elongate their stems to reach more light. However, sustaining this growth requires most of the plant's energy, which is then diverted from reproductive organ (grain) development. The present findings align with those of Pan et al (2016), Xie et al (2021), and Wang et al (2021b), who also observed a



**Fig 8.** *Principal component analysis (PCA) of the analyzed samples using the assessed parameters as an input: ChI a, Ch b, Ch T, Proline, SC, and RWC* 

decrease in grain yield of Rice (Oryza sativa L.) and peanut (Arachis hypogaea L.) respectively with increased shading levels. The negative effect of shadowing on above-ground dry biomass may be explained by changing resource distribution, when plants are shaded, they can reallocate their resources to those parts of the plant that are best adapted to capturing light. This effect can potentially suppress seed production in Favor of stem and leaf growth. Shade stress considerably reduces grain yield and, consequently, the harvest index, which may be due to reduced 100-grain weight and number of grains per pod. The harvest index showed variation across the different shading treatments and varieties that were tested (Table 1). The results obtained are consistent with those documented by El Naim et al. (2015) for Faba Bean (V. faba L.). The findings also showed that Faba bean grown in mixed crops had a harvest index of 24.07%, which was significantly higher than the harvest index of 18.08% for Faba bean grown as a single crop. This suggests that intercropped Faba bean can allocate a larger portion of its nutrients to the grain rather than to overall plant growth (Nurgi et al, 2023).

#### Effect of shading on plant yield and its components Alterations in light conditions can impact the development of plants, their photosynthetic processes, and the storage and accumulation of nutrients, leading to re-

duced crop quality and yield (Wang et al., 2014). Prior research has demonstrated that shade stress can lower the harvest of different crops, such as wheat and soybean (Wang et al., 2021c) and spring corn (Wang et al., 2021b). In addition, the decreased amount concerning crop-yielding aspects is strongly linked to the type of crop, amount of shade, severity of shade, and length of shade exposure. Plant yield is the most important parameter defining agricultural production. However, many environmental conditions produce a series of plant changes that negatively affect this desirable trait (Oukaltouma et al., 2020). In the present study, shade stress significantly reduced the plant yield (YP) of all varieties tested and the reductions were more evident under 90% shading (S3), with a significant difference between varieties compared with S2 (50% shading) and S1 (0% shading) (Table 1). Indeed, during the 2021 crop year, the EH variety showed less tolerance than the other varieties (Table 1). Shade treatments S2 and S3 significantly reduced the yield of the samples under study. The lowest tolerance of both treatments was observed for the variety AF17 during the growing year 2022 (Table 1). Consequently, the effect of shading on Faba beans varies according to the varieties used, the level of shading applied, and the environmental conditions of each crop year. Rivest et al. (2009) demonstrated that artificial shade structures could decrease soybean (Glycine max L.) yield during the reproductive period by impacting pod numbers. These findings indicate that shading has a greater impact on yield during flowering and pod development than seed filling. As per the results of (Wu *et al.*, 2017), shade treatment led to a 60% decrease in rice yield. Similarly, tea (*Camellia sinensis* L.) showed similar trends of declining indicators in shaded conditions within the same context (Wang *et al.*, 2012).

Naim et al. (2020) found that winged bean (Psophocarpus tetragonolobus L.) plants under 30% shading generally yielded more than those under 0% or 60% shading. These results are consistent with Khalid et al. (2019), who noted that plants in light shade could achieve higher yields due to improved stomatal function, which enhances the availability of essential bioactive compounds for seed formation and development. The present study further demonstrated that shade stress significantly reduced pod number, seed number, and individual seed weight across all six Faba bean varieties examined (Table 2). The reduction was more pronounced under 90% shading (S3) compared to 50% shading (S2) (Table 1). These findings are similar to those reported by Wang et al. (2014) for purple-fleshed sweet potato (Ipomoea batatas L.). Low shading and light intensity during flowering periods decrease photoassimilate availability. leading to flower abortion and fewer pods per plant in intercropping systems of maizefaba bean (Nurgi et al., 2023). The results are also in agreement with studies on soybean and sage (Salvia officinalis L.) (Zervoudakis et al., 2012; Iqbal et al., 2019). However, Naim et al. (2020) discovered an increase in pod number, seed number, and other yield components of winged bean (P. tetragonolobus) in moderately shaded plants compared with unshaded plants. In Japonica rice (O. sativa L.), plants experiencing shade stress (60%) exhibit noticeable morphological alterations, including elongation of stems and petioles (Wu et al., 2017). These alterations in structure allow plants to take in increased light energy and avoid being shaded by other plants.

In this study, the two shading treatments S2 and S3, significantly increased the height of most varieties in 2021 (Table 2). The growth rate was higher in AF17 compared to other cultivars under shade stress (Table 2). This result could be attributed to shade stress management by photosynthesis allocation under shade stress. This shade-sensitive variety redirected a larger share of its photosynthetic products towards elongating the main stem to capture more light. Similar findings have been reported in several studies (Wang et al., 2014; Hussain et al., 2019). However, Naim et al. (2020) found that bean plants performed better in unshaded conditions. Unshaded plants exhibited greater height and more branches than those under moderate or heavy shading, indicating that winged beans grow most effectively without shade. This outcome aligns

with research on other legume crops like chickpeas (Lake & Sadras, 2014; Iqbal *et al.*, 2019).

## Effect of shading on physiological and biochemical parameters of Faba bean

Light significantly impacts plant development morphology, anatomy, and other aspects of plant physiology. Appropriate light intensity is a fundamental prerequisite for development and normal plant growth. Excessive shade, often as much as 80%, negatively impacts plant growth, development, yield, and productivity (Gao et al., 2020). S2 shade treatment positively affected the chlorophyll content of AG, EH, and AF17 Faba bean varieties (Fig. 3). The results are consistent with those reported by Angadi et al. (2022). In this study, SPAD values were maintained at higher levels throughout the growing season due to continued leaf development, which was interpreted as a manifestation of the tolerance of these two varieties to reduced light levels (Fig. adaptation This attempts to boost PAR 7). (photosynthetically active radiation) absorption and improves the overall efficiency of the photosynthetic assimilation process (Chai et al., 2018).

In contrast, the S3 shading treatment was found to have a detrimental effect on all the varieties examined (Fig. 7). Similar results were obtained in a study conducted by (Hu *et al.*, 2023), who discovered that shading up to 60% caused a prominent decrease in SPAD entities in maize, leading to a considerable reduction in photosynthetic rate. This negative influence of shading on the rate of photosynthesis is mainly due to the direct reduction in incident radiation. In addition, biotic stressors considerably diminish the SPAD value of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and oilseed rape (*Brassica napus* L.) (Ploschuk *et al.*, 2021).

It has been shown that under 90% shade (S3), the chlorophyll a, b, and t levels of *V. faba* varieties were higher than those of control plants and those grown in 50% shade (S2) (Fig. 3). Varieties AF17 and ZN showed the highest levels of chlorophyll contents under shading treatments S2 and S3 compared with those grown in full sun (S1) (Fig. 3). Increased chlorophyll content in the leaves of plants, including both a and b types of chlorophyll, allows more light energy absorption and capture under low light conditions, therefore increasing light-use efficiency (Wang *et al.*, 2021b).

Gao *et al.* (2020) found that *Astragalus elata* plants under 50% shading had significantly higher levels of chlorophyll contents (a, b, and t) than the control treatment. However, excessive shading can negatively affect photosynthesis, thereby impacting plant morphogenesis, growth, and development of peanut (*Arachis hypogaea* L.). (Wang *et al.*, 2021c). Wang *et al.* (2021b) also reported that 50% shading notably increased chlorophyll contents (a, b, and total) in peanut leaves. Nevertheless, chlorophyll levels under shaded conditions were still lower than those in unshaded conditions. Chlorophyll content serves as an indicator of oxidative stress in plants (Muhammad *et al.*, 2021).

The analysis of proline levels in Faba bean varieties suggested that proline accumulation increased because of abiotic constraints, particularly shade stress (Fig. 5). Higher shading levels were associated with increased proline content, indicating that reduced light exposure may compromise seedling plasma membrane stability. These results concur with those evoked by (Gao et al., 2020), who demonstrated that the free proline content of A. elata plants subjected to 80% shading was significantly superior to that of other shading levels. In addition, proline accumulation increased under low-light conditions, disrupting the balance between the production and elimination of reactive oxygen species (ROS) within the cell. This, in turn, leads to changes in other physiological characteristics of the plant. Soybean (G. max L.) grown under shaded conditions exhibited higher proline levels than those grown in full sunlight, indicating that shaded plants were better at safeguarding their cells from damage (Muhammad et al., 2021).

Measurements of relative water content (RWC) in soybean leaf tissue showed that plant water status was less favorable in plants exposed to direct light compared with those under shade (Muhammad *et al.*, 2021). These results agree with the theory that shadegrown plants require less water than those exposed to full sunlight (Li *et al.*, 2011) since they can preserve and require less water for transpiration (Shafiq *et al.*, 2020). The present study's findings demonstrated that shading treatments S2 and S3 decreased the RWC of all Faba bean varieties compared with those grown in full sun S1 (Fig. 4).

Stomata are vital for plants to take in carbon dioxide (CO<sub>2</sub>) necessary for energy production and photosynthesis. Additionally, they help reduce water loss by closing under hot or dry conditions. Shading can significantly impact the leaf temperature of Corvina (Vitis vinifera L.), reducing leaf temperature and lowering stomatal conductance (Ferrara et al., 2023). Da Silva et al. (2019) found that shading treatments can influence temperature, radiation, and humidity within the plant canopy, reducing transpiration and stomatal conductance of Conilon coffee (Coffea canephora). Shading up to 45% has been shown to increase stomatal conductance in Forage peanuts (Arachis pintoi red chili), likely due to improved environmental conditions, particularly soil moisture (Rodrigues da Cruz et al., 2020). Ahmed et al. (2023) observed a positive correlation between shading levels and stomatal conductance across various bell pepper (Capsicum annuum L.) varieties. This effect is likely due to high temperatures in exposed areas and lower light levels in shaded areas, which affect

the dynamics of stomatal opening and closing (Kostaki *et al.*, 2020). Plants in shaded conditions receive less incident photosynthetically active radiation (PAR), causing stomata to remain open longer due to insufficient saturation (Gómez-Bellot *et al.*, 2023).

#### Conclusion

The present study evaluated the effect of shading on six Faba bean (V. faba L.) varieties to select the most tolerant ones to this abiotic stress. In general, morphological, physiological, and biochemical parameters were appropriate for assessing variations between bean varieties in their tolerance to shading. In summary, the two shading treatments S2 (50%) and S3 (90%), considerably affected Faba bean grain yield. However, compared with control (S1), shading treatment S2 was less damaging than S3 on the grain yield for most tested varieties. Significant variations were observed between Faba bean genotypes, effectively supporting identifying interesting varieties for farmers adopting agroforestry. Also, those genotypes may be useful for breeding programs of shade-tolerant varieties. Under both shading conditions, Zina and Hiba were the best-performing grain yield varieties, demonstrating greater shade stress tolerance. By contrast, shade stress reduced the grain yield of Extra hative and Defes varieties, respectively, indicating a lower tolerance to the light deficit. Yield components NP, NS, and WSS were the most affected by shading and were very useful for assessing morphological variation between shading treatments and different bean varieties. Shading decreased stomatal conductance and proline content in all the varieties tested, which explains the response of Faba bean plants to attenuate the adverse effects of shade stress. However, relative water content, Ch a, Ch b, and Ch t were the least affected by shade. This may explain the adaptability of the different varieties to reducing light levels. The differences highlighted between V. faba varieties can be used to search for genetic differences and genes involved in the tolerance of varieties to light deficit, which can also be used in breeding programs.

#### **Conflict of interest**

The authors declare that they have no conflict of interest.

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