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Research Article

CROCUS SATIVUS: FROM LIGHT TO SHADE: EFFECTS ON PHOTOSYNTHESIS AND PHOTOSYNTHETIC APPARATUS

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ABSTRACT. Saffron is a plant with great economic and medicinal interests, and it requires optimal conditions for the success of its cultivation. Usually, it grows in full sun. However, no sources are documenting the degree of shade tolerance of this species. As a result, an experiment was carried out in 2016 and 2017, in the Experimental station of the Faculty of Sciences of Oujda in eastern Morocco, to study the effect of different levels of shading on chlorophyll pigments and the photosynthetic capacity of saffron (*Crocus sativus*). Saffron was grown for 24 months under different types of shading: 0% (control), 30%, 50%, and 70%. The results obtained from the study showed that shade significantly affected the response of the saffron plant. Plants grown under 30%, 50%, and 70% shade indicated that length, leaf area, leaf area ratio (LAR), chlorophyll *a*, *b*, and *a+b* content, and chlorophyll fluorescence (Fv / Fm, Fv / F0) were significantly elevated compared to plants growing in full sun. Leaf water status showed that leaf water potential-ψf decreased proportionally with the level of shading applied. Regarding the yield of stigmas, it was found that exposure of plants to 30% shade significantly improved the production of this spice.

Keywords: *Stigmas, fluorescence, chlorophyll, shade, qujda, saffron*

INTRODUCTION

Saffron, dried stigmas of the flower of *Crocus sativus* L., considered among the main products of Moroccan terroirs. It is the most expensive spice in the world. Indeed, its price is 10 times higher than that of vanilla and 50 times higher than that of cardamom [1]. Saffron, as a cultivated plant, is a rustic plant that grows from sea level to almost 2000 m [2]. Solar radiations are the part of main abiotic factors for agricultural production [3, 4]**.** They are considered as being one of the environmental factors that is the most important for survival, growth, reproduction and distribution of plants. Luminous intensity affects the central processes of plants such as morphology, physiology and biochemistry [5,4]; [6] to herbaceous kinds [7,8] and woody [9]. Until now, the effects of shadow on the growth of plants can be classified in two big categories: one is positive, as in the case of *Cucumis sativus* [10], of *Trichloris crinita* [11] and of *Capsicum chinense* [12]**,** the other one is negative, as in cases of *Eustoma grandiflorum* [13] and of *Liatris spicata* [14].

In the shade, plants often exhibit morphological and physiological adaptations that allow them to adapt to conditions of low light [15], such as a thinner leaf, a higher chlorophyll (Chl) content per unit leaf mass with an increase in Chlorophyll *b* [16]. In

recent years, chlorophyll fluorescence measurements have been recognized as an informative and useful indicator characterizing different light responses of photosynthesis. Shade significantly affects the performance and structure of the photosynthetic apparatus [17]. It blocks energy transport from PSII to PSI, reduces the leaf thickness of spongy tissues, resulting in low chlorophyll fluorescence [18,17]. Obviously, an optimal level of shading must be determined for each plant. This is also the case for saffron, especially since no study has determined the optimal light intensity for its growth.

The objective of this study was to determine the optimal light intensity for saffron growth by testing the effect of different shading levels on yield, chlorophyll content, and photosynthetic activity.

Overall, the main objective of this document is to provide information for agronomists to develop appropriate planting schemes in which saffron plants receive optimal light intensity for growth.

MATERIALS AND METHODS

Site Characteristics

The experiment was conducted in open fields at the Experimental Research Station of the Faculty of Sciences of Oujda, located at 661 m above sea level and 34° 39' 06 -71' north and 01° 53'58 -80' west (GPS Back Track Bushnell). The semi-arid climate is characterized by temperate winters [19].

Plant material and growing conditions

The saffron corms, 2.5 cm in diameter, used in this trial were provided from a saffron field at the experimental research station of the Faculty of Sciences of Oujda planted on 21/09/2015. During the two years of the experiment, the plants were irrigated with a quantity of water equivalent to 100% ET0 (reference evapotranspiration) from the city of Oujda, taking into account the rainfall of the city. The water used for watering has an electrical conductivity (EC) of 0.7 mS/cm.

The measured parameters

Morphological parameters

The stages of the plant development have been recorded. In addition, the number (LN) and cm length (LL) of leaves of five plants per treatment and per repeat were measured monthly. The leaves were digitized using a flat scanner and the leaf area (LA, cm^2) was measured using the 2010 AutoCAD software. The measurement of leaf area is a determining factor in such growing mechanisms as radiation interception, water and energy exchange [20].

Concentration of photosynthetic pigments

Fully developed leaves from each treatment were sampled. Chlorophyll (Chl a, Chl b, and Chl a + b) was extracted by grinding leaves in 80% acetone in the dark at room temperature. The supernatant was then separated and analyzed by a spectrophotometer (Ray Leigh, Vis-7220G) at wavelengths of 663, 645 and 470 nm as described by [21].

Chlorophyll Fluorescence

Chlorophyll fluorescence was measured with a modulated fluorometer (Hansatech, England). Leaves were then adapted to darkness for 30 min and maximum fluorescence (Fm), variable fluorescence (Fv), and initial fluorescence (F0) were measured. The maximum quantum yield of PS II Fv / Fm was calculated using the formula; Fv / Fm = (Fm - F0) / Fm [22]. Fv / F0, reflects the efficiency of electron donation to PSII controllers and the photosynthetic quantum conversion rate in PSII controllers. Fv / F0, was calculated using the formula; Fv / F0 = (Fm - F0) / F0 [23].

Leaf water potential (ψF)

This measure represents the strength by which water is retained in the plant. The leaf water potential is measured according to the Scholander method [19].

Determination of growth indices

Calculations of growth components such as relative growth rate (RGR), the central parameter of Plant Growth Analysis, and net assimilation rate (NAR) and leaf area ratio (LAR) were calculated with reference to the work [24], these three indices are defined and linked as follows:

- *Relative growth rate or RGR ($mg.g^{-1}.j^{-1}$).*

It is the rate of growth relative to the average weight of the plant during the Δt interval. This criterion allows comparison of the growth rate of plants of various sizes since the average plant weights considered during the observation interval are taken as the unit.

$$\boldsymbol{RGR = (W2\text{-}W1)/\ W1(T2\text{-}T1)}$$
Eqn. 1

- *The net assimilation rate or NAR ($mg.\ cm^{-2}.\ j^{-1}$).*

This is the growth rate relative to the mean leaf area during the Δt interval.

$$\boldsymbol{NAR = (W2–W1)(lnA2–lnA1)/(A2–A1)(T2–T1)}$$
Eqn. 2

This expression is analogous to that of carbon dioxide assimilation through photosynthesis, since it relates the amount of dry matter produced per unit time at the leaf surface.

- *Leaf area ratio or LAR ($cm^2.g^{-1}$).*

This is the ratio of leaf area to total plant weight. This criterion measures an aspect of plant morphology. In the same time interval Δt

$$\boldsymbol{LAR = (LA_1+LA_{2)}\ /\ (W2+W1)}$$
Eqn. 3

Experimental design

The plantation was carried out in August 2016 in the open field under green plastic shade with three shade levels (30%, 50% and 70%). The shading cloth was attached to metal arches forming mini-measurement tunnels (width 1 m x height 0.4 m x 3 m length). The level of shading was measured using a luxmeter (Lutron; LM-8000). Growth and production of saffron under these shading levels were compared with those planted in full sun (without shading nets). The experimental device adopted is a complete random block (BAC), it consists of 3 blocks with a total of 120 saffron Tufts, (10 Tufts / treatment * 4 treatments * 3 repetitions), the blocks indicate the repetitions and the sub-blocks represent the treatments.

Statistical Analyses

The results are subjected to a descriptive statistical analysis and an analysis of variance (ANOVA), using the software "GraphPad Prism for Windows version 7" and the comparison of the means is made by the test of Duncan at the 5% probability threshold. When necessary, data were transformed to conform assumptions of normality and homogeneity of variance using the logarithmic transformation natural.

RESULTS AND DISCUSSION

Plant growth, morphology and water status

Leaf morphology was significantly affected by shade treatment. In the first year there was an increase in leaf number as a function of the percentage of shade applied, while in the second year the number of leaves decreased with the percentage of shade applied, but in general, the number of leaves / plant in shade of 30% was significantly greater than the other treatments ($p < 0.05$) throughout the trial period (Fig1A). Plants grown under 50% and 70% shade had the largest leaf area, while leaves from plants grown in full sun were the smallest (Fig. 1C). Similarly shading had a significant effect on leaf length ($p < 0.05$), plants grown at high shading levels (50% and 70% shade) developed longer leaves (Fig. 1B).

Crop growth parameters: relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) were significantly affected by variations in light intensity. In both seasons, plants grown under more than 50% shade had higher LAR than the 30% shade treatments (Fig. 1D), which in turn had the highest RGR and NAR values (Fig. 1E &1F). On average over both seasons, planting saffron under 70% shade resulted in a significant reduction of RGR by 31.8 and 48% and NAR by 48.8 and 65.6%, respectively, for the first and second seasons.

The results also revealed that leaf water potential-ψf -, was significantly affected by shade level. Saffron plants grown under 70% and 30% shade showed a significant increase in dehydration tolerance in terms of decreased leaf water potential, where the lowest values were recorded in 70% shade (-3.9 MPa) (Fig.2K).

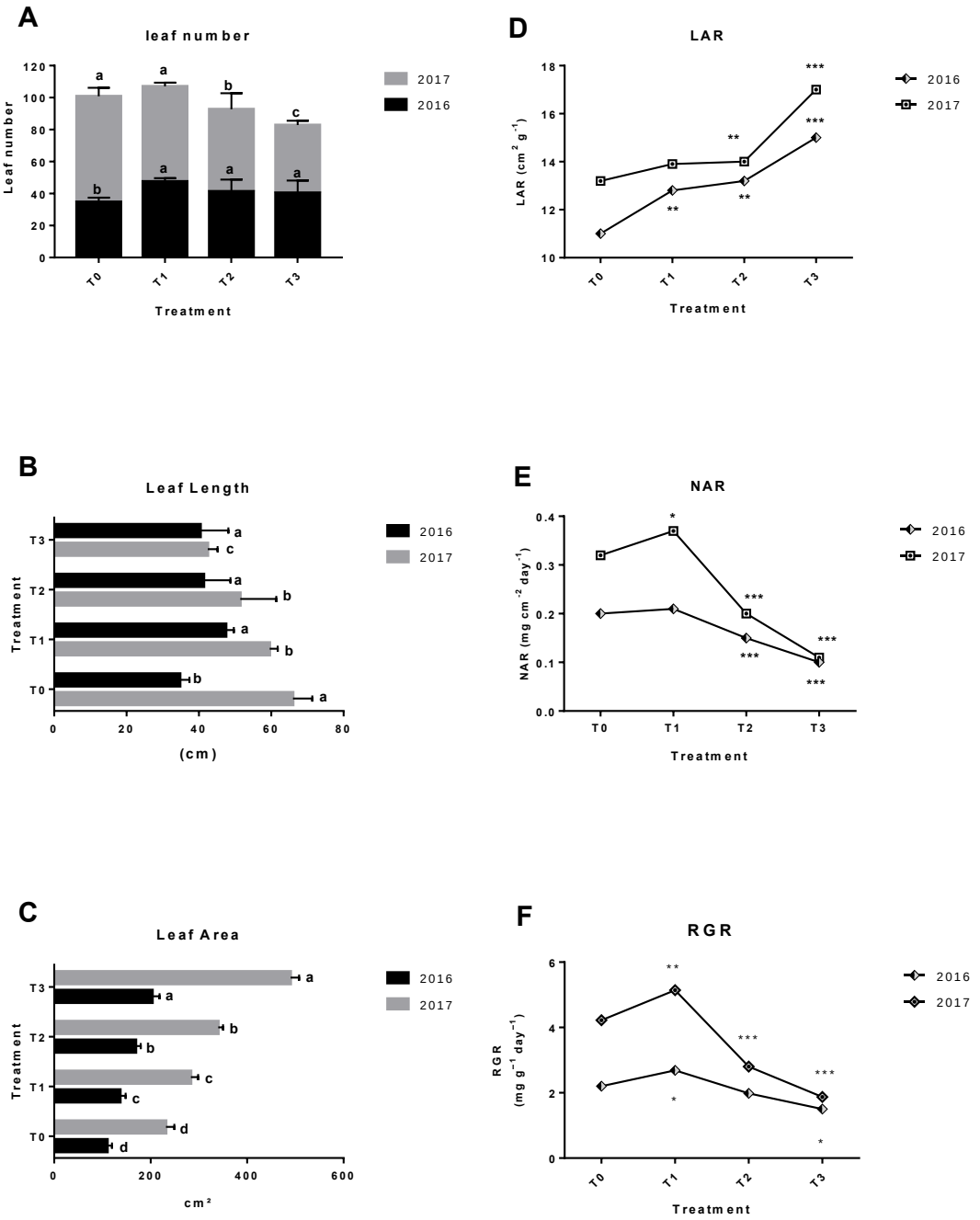


Fig. 1. *Shading effects on saffron morphological and growth parameters. LN; LL (A, B); LA(C); LAR(D); NAR(E); RGR(F). The data are the average of the nine measurements taken during the experiment. *'** and *** indicate significant differences at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively. Different letters indicated significant differences between treatments ($p < 0.05$).*

Chlorophyll content and fluorescence Characteristics

Different shade treatments significantly (p <0.05) affected the chlorophyll content (Chl *a*, Chl *b*, and Chl *a* + *b*) of saffron leaves. Shaded plants had the highest Chl *a*, Chl *b*, and Chl *a* + *b*. The Chl *a* content was much higher than Chl *b* content for all treatments (Fig. 2G, 2H, 2I). A similar trend was observed for the chlorophyll fluorescence (Fv / Fm, Fv / F0), which showed a strong positive correlation with light intensity (Fig. 2J). Very highly significant differences (P = 0.001) were observed between Fv / Fm in plants grown in 70% shade compared with plants grown in full sun.

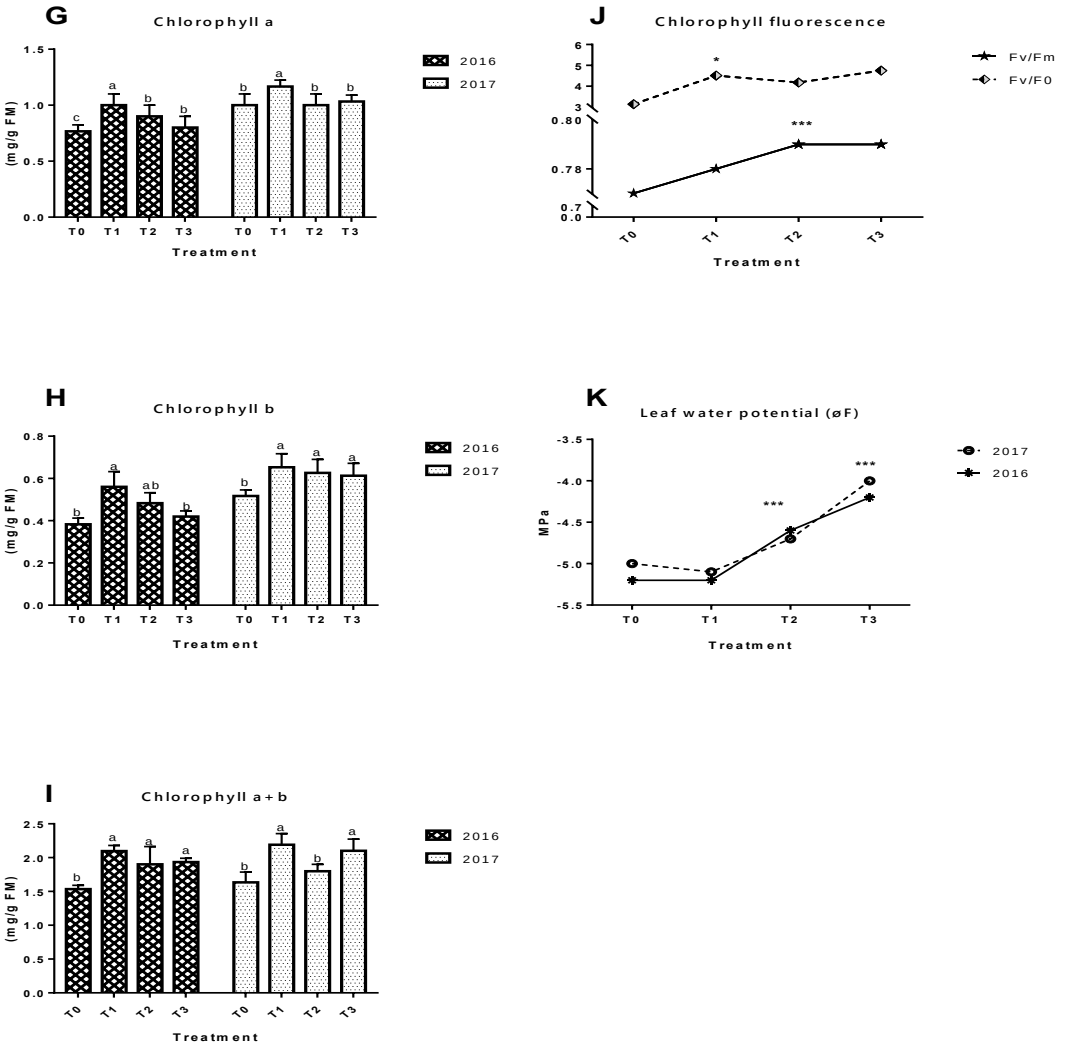


Fig. 2. *Shading effects on chlorophyll and photosynthetic parameters of saffron. (G). chl a; (H). chl b; (I). chl a+b; (J). Fv/Fm and Fv/F0; (K). ψf. The data are the average of the nine measurements taken during the experiment. *'** and *** indicate significant differences at p ≤ 0.05, p ≤ 0.01 and p ≤ 0.001, respectively, at one-way ANOVA. Different letters indicated significant differences between treatments (p < 0.05).*

Evaluation of organ weight and relative organ weight of study animals

The mean values of hepatic tissue weight and relative organ weight of study animals in normal control Group I, standard control Group II, test control Group III, and treatment Groups IV and V were given in Figure 2. Comparatively, the mean organ weight and relative organ weight of test control Group III showed significant reduction relative to control Groups I and II while the treatment Groups IV and V showed non- significant reduction.

Changing the microclimate by subjecting *C. sativus* plants to different levels of shading resulted in observable morphological and physiological changes. The morphology of the plant shows some docility and adaptability to changing environmental conditions [25]. Previous studies have shown that shading conditions favor stem growth and the development of larger leaves to maximize light capture [26,3]. Overall, this study showed that growth characteristics as well as morphological parameters such as leaf number, leaf length, and leaf area ratio (LAR) increased with increasing shade level. Plants grown at high shade levels resemble etiolated plants, which have an unusually high appearance [27]. According to Mendes et al [28]**,** and Krishnakanth et al [29], increase in the apical activity is an adaptive response of the plant to overcome the stress of low light. Similarly, the relatively high leaf area and leaf straightening modification of plants growing under low light levels - 50% and 70% - may be a response of the plant to increase its ability to receive sufficient light [30,31]. The highest number of leaves/plants was observed in the 30% shade treatment, suggesting that at 30% shade the saffron plants can maintain their optimal growth. This result is also supported by De Carvalho Gonçalves et al [32] who reported that intermediate light conditions (about 50% of sunlight) resulted in higher levels of biomass production in some species. Results on leaf water potential were more negative in strong light relative to shade, which is in perfect agreement with the work of Henriksson [33], which suggests that shade mitigates drought effects. This is particularly beneficial in a Mediterranean climate characterized by high air temperatures and recurrent dry periods, which are expected to occur more frequently in the future as a result of global warming [34]**.** Growth parameters such as RGR, NAR, and LAR provide a better understanding of the plant's physiological response to stress [35]. These parameters reflect the growth of a plant canopy, the efficiency of solar radiation interception, and hence photosynthesis. Each herbaceous species has a different response to plant growth rate [36]. In this study, the highest RGR, NAR values were obtained for plants receiving more than 50% light. Consistent with this result, Casierra-Posada et al [37] reported a 320.6% increase in LAR in *Fragaria sp* plants exposed to a green polypropylene coating compared with plants exposed to a clear coating. A higher LAR in shade is considered as adaptive because such an adjustment improves light capture in shade [38,39]. The decrease in NAR is offset by the increase in LAR (increase in leaf area) as reported by [40]. In addition, Yasari E. [41] stated that reducing NAR could reduce RGR at a later stage. In our study, significant changes were also observed in the levels of Chl *a*, Chl *b*, and Chl *a* + *b*. Chlorophyll levels increased with increasing light intensity, and our results are consistent with those reported by Wittmann et al [42]; Fan et al [21], Raza et al [43]**.** Although shade-grown plants are not directly exposed to the sun, they produce additional chlorophyll *a* and *b* to capture diffuse radiation to produce the carbohydrates needed for plant growth, as indicated by Beneragama and Goto [44]**.** These increases in chlorophyll concentration are obvious responses of plants grown under conditions of low light intensity. This response has been interpreted as a mechanism used by plants to optimize quantum assimilation when irradiance is reduced [45].

Photosynthetic function can also be evaluated using various Chl fluorescence ratios [46,47,48]. The two ratios, Fv / Fm [49] and its related more sensitive form Fv / Fo [46], provide a measure of the potential efficiency of photosystem II of dark-adapted leaves. Fv / Fm is a reliable parameter for estimating the photochemical activity of PSII. Under stress-free conditions, it should be close to 0.83 [50]. Studies have shown that Fv / Fm is similar and sometimes higher [51] in shaded plants. These results are consistent with those of the current study indicating that shading increases chlorophyll and fluorescence (Fv / Fm, Fv / F0), which can serve as an indicator of plant photosynthetic efficiency.

CONCLUSION

C. sativus is a plant that grows in the sun but can withstand shade quite well. It can grow in the shade of trees, giving it the opportunity to be integrated into the intercropping system. Similarly, given the short life cycle of saffron, the use of intercropping systems will allow efficient use of inputs, reduce production costs, and improve soil fertility. This study showed that with reduced light, *C. sativus* can grow in shade by changing the composition and proportion of its photosynthetic pigment. This change allows efficiency to improve photosynthetic electron transfer and a subsequent reduction in energy loss. We also showed that leaf morphology adapted to the entire shade by significantly increasing leaf area, chlorophyll content, and chlorophyll fluorescence (Fv / Fm, Fv / F0).

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