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Light Intensity and Phenotypic Response in Two *Vicia faba* L. Varieties

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ABSTRACT

The impacts of different light intensities on the phenotypic plasticity of several plant features in two *Vicia faba* varieties (Sakha1 and Giza Blanca) were explored for the genotype-environment interaction of specific traits. Plants from both genotypes were grown for 28 days in four distinct light conditions: 550 mol $m^{-2}s^{-1}$, 850 mol $m^{-2}s^{-1}$, 1200 mol $m^{-2}s^{-1}$, and 1800 mol $m^{-2}s^{-1}$, then after, various measurements were conducted. For variables measured including leaf width, shoot fresh and dry weight, and root fresh and dry weight, both genotypes showed comparable plasticity responses, showing no genotype-environment interaction for these traits. However, Sakha1 outperformed Giza Blanca in terms of leaf count, stomatal density of the abaxial surface, and root/shoot ratio as light intensity increased. The plasticity of a particular leaf area implied morphogenetic regulation for both genotypes and showed that leaves tended to expand their leaf area in low light conditions in order to capture more light. In contrast, these plants developed significantly thicker leaves when exposed to high light intensities.

Keywords: Genotype-environmental interaction; Light intensity; Phenotypic plasticity; *Vicia faba* L.

INTRODUCTION

Taking into consideration the wide changes in the climate which affect both function and distribution of plants, one-way plants will respond to these changes is through environmentally induced shifts in (phenotypic plasticity) (Nicotra *et al*., 2010). Phenotypic plasticity is a quantitative trait which refers to the ability of a genotype to exhibit changes in a specific trait across different environments. It is a particularly important characteristic to enable sessile plants to acclimate to rapid changes in their environment (Wang, 2016). Plasticity can be quantified for sets of conspecific plants whether they are genetically identical or not. If they are not identical, then the component of the environmental to trait differences represents the average plasticity of the genotypes present and is commonly referred to as genotype - environment (G×E) interaction (Sambandan *et al.*, 2008). Moreover, the more varied the genotypes that are under investigation and the environments in which they are measured, the more phenotypic variation that is expected to be observed. The contrary of plasticity is the term canalization which refers to the tendency of a genotype to produce the same phenotype regardless of this micro environmental variation (Hallgrimsson *et al.*, 2002).

Quantifying plasticity of a trait for a single genotype can be divided into two approaches: The first is the nominal; accordingly, measuring of phenotypic plasticity is based on the concept of the reaction norm that describes the pattern of trait values of a genotype across multiple environments (Sommer *et al*., 2017). In contrast to nominal measures of plasticity, approaches for relative quantification of plasticity, data for a single trait from multiple genotypes over the same set of environments are employed. However, the degree of plasticity could be described according to the amount of the change in phenotype; the phenotype is plastic when the change is large, the phenotype is stable when this change is almost zero (Lalejini *et al.*, 2021).

For many cases, the phenotypic responses to environmental stress may be the consequence of growth reduction due to resource limitations (Gratani *et al*., 1997; Dorn *et al*., 2000). Physiological, morphological, and anatomical plasticity may have a different role in plant adaption to environmental changes. Plasticity for physiological traits may allow plants to grow and reproduce in spatially or temporally variable environments (Kuiper *et al*., 1988; Gratani *et al*., 2006). However, Plants grown in conditions of high resource are larger than those grown in low resource conditions if both are measured at the same age. In addition, high resource conditions may change plant characteristics over time more rapidly than is the case in low resource conditions (Benner and Bazzaz, 1985; Lacey, 1986; Funk, 2013).

One example of plant phenotypic plasticity could be the modification of leaf traits to the light intensity (Dengler, 1980). Light is one of the most important environmental factors for plant growth (Naoya *et al*., 2008). In addition, plant growth, morphogenesis and other physiological responses depends mainly on the intensity and quality of light (Rajapakse *et al*., 1992; Fukuda *et al*., 2008; Li and Kubota, 2009). Thus, the appearance of the foliage can change greatly in response to light stress as plants may alter the pigments, structure, and orientation of leaves to cope with high light stress (Smith *et al*., 1997). Also, physiological changes at the levels of the leaf have evolved to adjust the various light environments (Zhang *et al*., 2003). As is known, the increases in net photosynthesis rate (Pn) correlates with increases in light intensity. The increase in light intensity causes increasing in both of stomatal index and density (Volenikova and Ticha, 2001). The photosynthetic efficiency is attributed to presence of functional stomata on both sides of the leaf (Abdelhakam 2021). However, (Pan and Guo, 2016) revealed a decreases of net photosynthesis rate in response to excessive high light intensity. Whereas, to mitigate light damage caused by excessive light energy and to ensure the proceeding of photosynthesis, acclimating to high light intensity is related to many morpho-physiological characteristics, such as the reduction in specific leaf area in order to protect the plant from high irradiance; increase in leaf thickness, due to the quantity of layers or growth of palisade tissue; deep development of spongy layer (Givnish *et al*., 2004; Matos *et al*., 2009; Morais *et al*., 2004; Sims and Pearcy, 1994; Wentworth *et al*., 2006). Plants which grow under low

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irradiances, can improve the rate of photosynthesis by increasing both of plant length and specific leaf area (SLA) and decreasing branching to capture light more efficiently (Steinger *et al*., 2003). Understanding phenotypic plasticity will be important for predicting the future changes in species distribution, community composition, and agricultural productivity under the conditions of global change (Kleunen *et al*., 2007; Lande, 2009). Therefore, the objectives of this study were to analyse the differences between two genotypes of *Vicia faba* in terms of morphology, shoot and root dry and fresh weights, and stomatal densities in order to determine whether or not these features are plastic and to clarify the extent to which these genotypes interact with the environment, particularly under various light intensities.

MATERIALS AND METHODS

Plant materials and growth conditions

Seeds of two varieties of *Vicia faba* L.(Giza Blanca and Sakha 1) obtained from the Agricultural Research Center (Giza, Egypt) were germinated in 10 cm×10 cm pot containing 250 g of clay soil in the greenhouse of Botany and Microbiology Department, Faculty of Science, Damietta University. The growth conditions were 18-20/12-15^{o}C day/ night temperature, 65–80 % relative humidity (RH) and 14/10 day/ night photoperiod. The pots were divided into eight groups, as each variety was grown under four different light intensities (550, 850, 1200 and 1800 µmol m^{-2} s^{-1}). Light intensity was adjusted using mesh which limits light penetration and light meter was used for measuring light intensity. Plants were irrigated twice a week. When the plants were 28 d, all measurements were carried out.

Morphological measurements

For each group, the total number of leaves per plant was counted. Leaf length and width for the expanding leaf were measured using a ruler. Heights of whole shoots were also measured. Measurements were made for five plant replicates for each treatment.

Shoot and root fresh and dry weights and their ratio

Whole shoots of five plants from each group were harvested and used for fresh and dry weight measurements. For root samples, the plants were removed carefully from soil, washed briefly to remove soil remains. All samples were collected in pre-weighed plastic bags; the bags were sealed immediately and weighed to obtain the fresh weight (FW). The samples were dried at 60 ^{o}C for 2 d and weighed (dry weight, DW). Root/shoot ratio was calculated for the dry weights.

Leaf area and specific leaf area

Excised expanding and expanded leaves were collected in pre-weighed plastic bags and weighed for fresh weights. These leaves were photographed to determine the leaf area using Photoshop V 6.0. Five replicates were used for each group. The specific leaf area was calculated as follow:

$$\textbf{Specific leaf area} = \frac{leaf\ area}{FW\ cm^2}\ gFW^{-1}$$

Where, FW is the fresh weight of the leaf.

Stomata density and stomatal index

Determination of stomatal density and their index were carried out to determine the effect of light intensity in which, twenty expanding foliage leaves of each group were used for stomatal measurements. The epidermises of both the adaxial and abaxial surfaces were separated using razor then mounted on slides. The epidermis samples from each side were selected for mapping of all stomata. Stomatal index and stomatal density for both adaxial and abaxial surfaces were calculated as follows:

$$Stomatal\ density = \frac{number\ of\ stomata}{leaf\ field\ area}$$

$$Stomatal\ index = \frac{number\ of\ stomata}{Number\ of\ epidermal\ cells}$$

Statistical analyses

To evaluate the impact of light intensity statistical analyses were conducted using two-way ANOVA analyses. Fisher's least significant difference (LSD) post hoc test was performed using Sigma Plot V11.0 (2008) at significant level of $p \leq 0.05$. The normality of results was confirmed by using XLSTAT (2011). All data were represented are in mean, of five replica, ± standard error (Mean ± SEM).

RESULTS

Plant growth and morphology

The effects of different light intensities on the two varieties of *V. faba* were monitored by measuring changes in leaf number, leaf length, leaf width, stem length, the two genotypes exhibit increasing in leaf number with the increase of light intensity (Fig. 1A). Under all different light inten-sities, the number of leaves in Giza Blanca was significantly more than Sakha 1. Both genotypes showed decreasing in leaf width with the increase of light intensity (Fig. 1B). Giza Blanca showed no difference in leaf width at both 550 µmole/m²s and 850 µmole/m²s light intensities as well as Sakha 1. Both genotypes were the same at light intensities 1200 µmole/m²s and 1800 µmole/m²s. There were no significant differences in both genotypes in the leaf width under different light intensities. Simi-larly, Leaf length of both genotypes decreases with the increase of light intensity. At light intensity 550 µmole/m²s, both genotypes have the same leaf length (Fig. 1C). A different pattern was shown in (Fig. 1D) as both genotypes exhibit the same stem length at all light intensities except for light inten-sity 1200 µmole/m²s where Sakha 1 give the maximum value of stem length.

Fresh weights and dry weights of shoot and roots

Shoot FW of both genotypes increased with the increase in light intensity (Fig. 2A). Shoot FW in Giza Blanca was more than in Sakha 1 at all light intensities,

but both exhibited the same pattern. The same trend was observed for shoot DW (Fig. 2C). Shoot DW in Giza Blanca was more than in Sakha at all light intensities. Giza Blanca showed the same root FW at both light intensities 550 µmole/m²s and 850 µmole/m²s and the same root FW at 1200 µmole/m²s and 1800 µmole/m²s (Fig. 2B). Meanwhile, Root FW in Sakha 1 increased significantly with the increase of light intensity. Root FW in Giza Blanca was higher than in Sakha at all light intensities. Under all different light intensities, root DW in Giza Blanca was significantly more than Sakha 1 (Fig. 2D). With the increase of light intensity, increases in the ratio of the root/shoot dry weights in both genotypes (Fig. 3). However, there was no significant difference between the two varieties in this ratio at both light intensities 550 µmole/m²s and 850 µmole/m²s. Root/Shoot Ratio in Sakha 1 was higher than Giza Blanca under both light intensities 1200 µmole/m²s and 1800 µmole-per m²s.

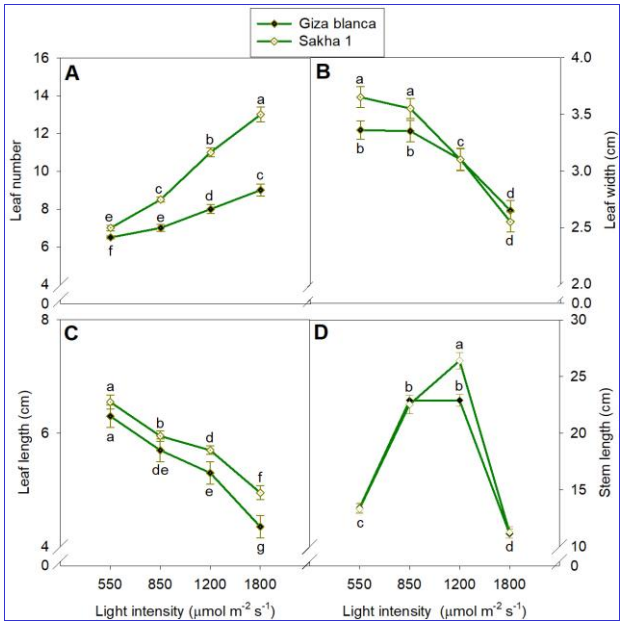

Figure (1): Response of some morphological parameters of two varieties of *Vicia faba* (Giza Blanca and Sakha 1) to different light intensities. A, leaf number; B, leaf width; C, leaf length and D, stem length. Data are means of five replicates ± SE. Data labelled with different letters are significantly different at $p \leq 0.05$.

Leaf area and specific leaf area

A decrease in the leaf area with the increasing of light intensity in both genotypes was observed. Leaf area in Sakha 1 was significantly higher than it in Giza Blanca under all light intensities (Fig. 4, IA). The same trend was recorded for the two genotypes when measuring the specific leaf area with different light intensity (Fig. 4IB). Sakha 1 recorded slightly higher specific leaf area than Giza Blanca at all light intensities

Stomatal traits

Stomatal traits were measured for both varieties in the adaxial and abaxial surfaces. At the adaxial surface, both genotypes exhibited increase in the stomatal density with the increasing of light intensity. Stomatal density in Giza Blanca was slightly higher than Sakha 1 at all light intensities (Fig. 4, IIA). Contrarily, at the abaxial surface, Sakha 1 was significantly higher in stomatal density than Giza Blanca (Fig. 4, IIB).

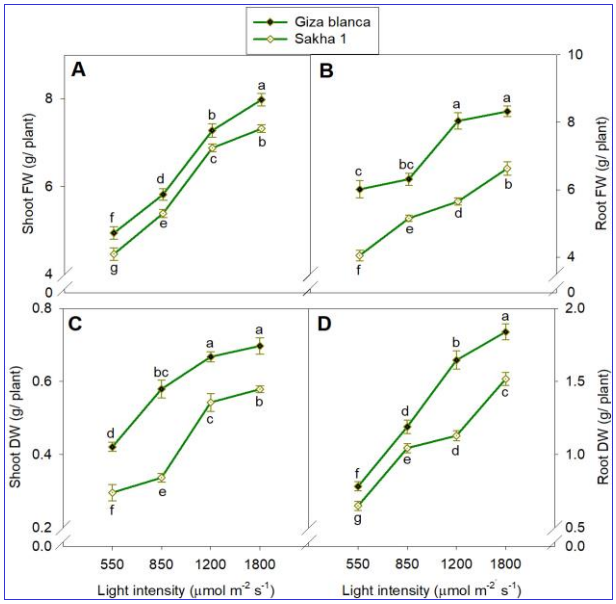

Figure (2): Response of biomass measured parameters to varying light intensities in two varieties of *Vicia faba* (Giza Blanca and Sakha 1).A, shoot FW; B, root FW; C, shoot DW and D, root DW. Data are means of five replicates ± SE. Data labelled with different letters are significantly different at $p \leq 0.05$.

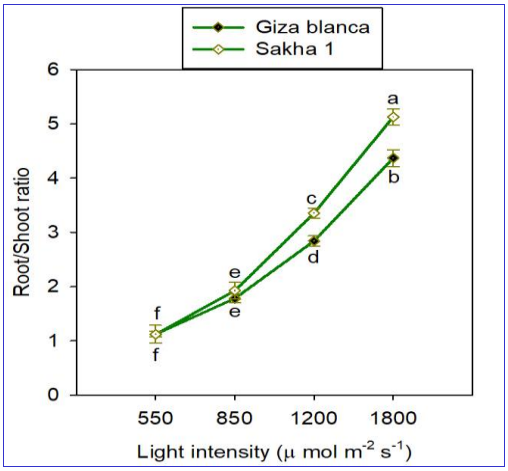

Figure (3): Response of Root/shoot DW ratio in two varieties of *Vicia faba* (Giza Blanca and Sakha 1) to different light intensities. Data are means of 5 replicates ± SE. Data labelled with different letters are significantly different at $p \leq 0.05$.

The increase in the light intensity led to the increase in stomatal index in both genotypes at both surfaces (Fig.4, III). At the adaxial surface, although Sakha 1 exhibit the same stomatal index at both light intensities 850 µmole/m²s and 1200 µmole/m²s, Sakha 1 has a significant increase in stomatal index more than Giza Blanca under all light intensities (Fig. 4, IIIA). The same trend was shown for the abaxial surface (Fig. 4, IIIB). Sakha 1 also exhibited a significant increase in the stomatal index than Giza Blanca at all light intensities.

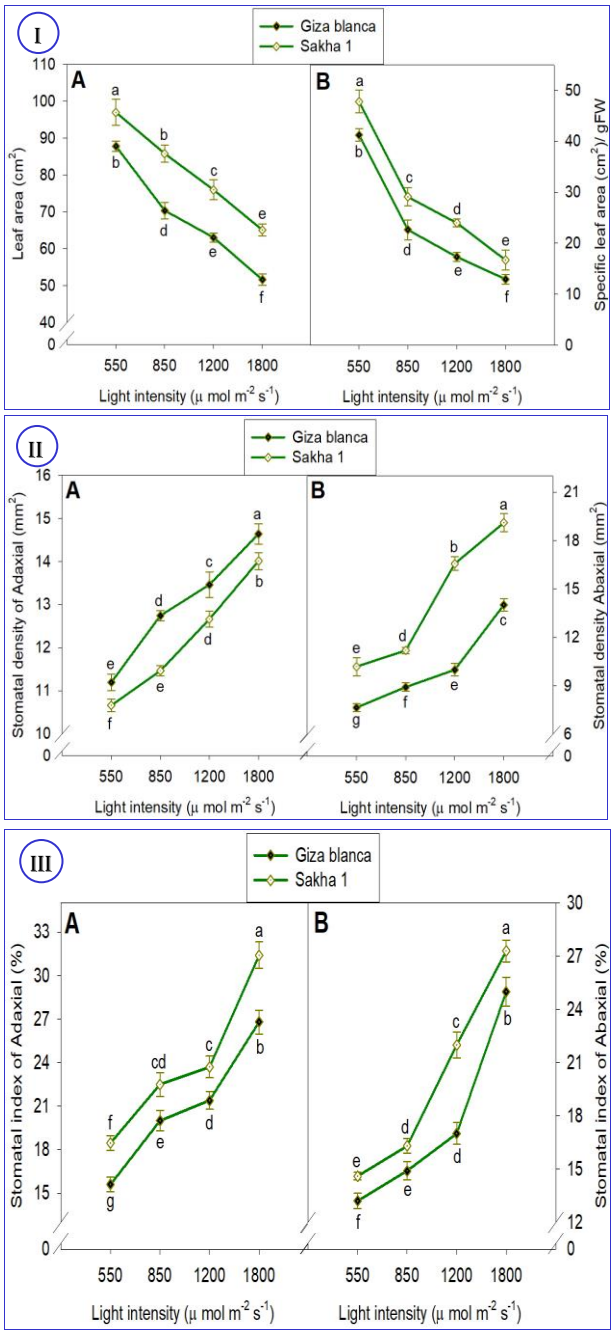

Figure (4): Impact of different light intensities on the measured parameters of two varieties of *Vicia faba* (Giza Blanca and Sakha 1). I, leaf area (A) and specific leaf area (B); II, the percentage of stomatal density for adaxial (A) and abaxial (B) surfaces; III, the percentage of stomatal index for adaxial (A) and abaxial (B) surfaces. Data are means of five replicates ± SE. Data labelled with different letters are significantly different at $p \leq 0.05$

DISCUSSION

To determine whether there were any variations between the genotypes tested in terms of how well they could alter their phenotype in response to environmental inputs, we assessed the response parameters of distinct genotypes. Considering that the reaction standard for a given genotype can be seen as a line on a plot of environmental value versus phenotypic value as well as a non-linear response with environment (Laitinen and Nikoloski, 2019). Another plasticity response that we observed was the active and passive responses. The passive response can reflect resource limitation, whereas the active response changes allocation to offset loss in fitness in environment (Nicotra *et al.*, 2010). Meanwhile, the response of morphological parameters to different light intensities revealed that the light regulates the structure of plants by signals from the environment (Hoenecke *et al*., 1992; Franklin *et al*., 2005; Kim *et al*., 2007).

Leaves are considered as a critical interface between the plants and environment (Tsukaya, 2005; Nicotra *et al.*, 2011). In the present work, leaf number parameter of both varieties showed a plasticity response with the increase of the light intensity as they gave best number at 1800 µmol/m²s and its plasticity was higher in Sakha 1 than Giza Blanca (Fig. 1A). Although both genotypes showed response, the amount of product produced in response to the environment differed; Sakha 1 has more sensitive photoreceptor than Giza Blanca.

Leaves under high irradiance had a significantly smaller leaf length and width (Fig 1B, C), compared to the leaves grown under low irradiance. This response could be termed a passive response (Nicotra *et al*., 2010) which can reflect resource limitation. However, the plasticity response with the increase of light intensity in both varieties indicates no genotype-environment interaction for this trait. Differences in light intensity as well as quality can induce plasticity of stem elongation (Barišić *et al*., 2006). The results of the stem length indicated a trait that changes non-linearly with environment (Fig. 1D). However, linear plasticity responses of plant height to different light intensities in other herbaceous species were recorded (Mahall *et al*., 1981; Barišić *et al*., 2006).

Response of fresh and dry weight of shoot and root and root/shoot ratio showed a significant plasticity response at high light intensity in both varieties as well as Shoot DW (Fig. 2C). The same results was recorded by (Fan et al., 2013) who assumed that the high irradiation promoted the higher fresh weight, dry weight and health index of the young plants. In contrast to others who observed lack of plasticity of leaf weight allocation in herbaceous species (Evans and Hughes, 1961; Hughes and Cockshull, 1971).

In Root FW results (Fig. 2B), Giza Blanca showed the same plasticity response at 1200 µmole/m²s and 1800 µmole/m²s and also at 550 µmole/m²s and 850 µmole/m²s, this means that there is no significantly plastic response between all light intensities. However, Sakha 1 had a gradual plasticity response with the increase of irradiance. Similarly, (Barišić *et al*., 2006) indicated that differences in light intensity as well as quality can induce plasticity of root weight allocation. Another Agreement of our results by (Patterson *et al*., 1978) who observed the same patterns of plasticity root weight allocation in *Abutilon theophrasti* and with other herbaceous species (Mahall *et al*., 1981; Barišić *et al*., 2006). Root DW parameter in both varieties showed a

gradual plastic response with the increase of light intensity In all the pervious parameters in figure (2), Giza Blanca showed slightly higher values than Sakha 1, so it was suggested that the two varieties showed a nearly plasticity response at all light intensities which suggested the absence of the genotype-environmental interaction (G x E) of these traits. However, the response of the weights of shoot and roots could be considered as an adaptive plasticity response which are generally, but not necessarily, active and require a specific signal perception-transduction system allowing plants to change their development (Nicotra *et al*., 2010). Despite the fact that our findings indicated larger leaf number with high light and high shoot and root weights concurrently, a further finding was that the greater leaf number may have somewhat compensated the low weight under low light (Nicotra *et al*., 2010). The increase of the root/shoot ratio in both genotypes with the increase of light gave an indication that the increase in root dry weight was higher than that in shoot dry weight (Fig. 3). This could be elucidated by the fact that an increase in root weight, because an increase in water uptake which might be required in the high photosynthetic rate resulted at the high light intensity. Both varieties were similar in their response at 550 µmol/m²s and 850 µmol/m²s but it showed a significantly higher plasticity response in Sakha 1 than in Giza Blanca with the increase of light intensity at 1200 µmol/m²s and 1800 µmol/m²s.

Leaf area and specific leaf area are influenced by different light intensities in which positive significant responses were recorded. Results obtained from figure (4, IA) indicated a significantly decrease in leaf area with the increase of light intensity in Sakha 1 more than Giza Blanca. This reduction in leaf area could be to reduce water loss under conditions of high light intensity. However, both genotypes exhibited a plasticity response to this trait. Similarly, Differences in light intensity was recorded to induce plasticity of leaf area (Morgan and Smith, 1981). Specific leaf area exhibits a vast variation to cope with the change in the environment in which the plants are grown. Light intensity is one of the most important factors that affects the specific leaf area (Friend, 1966; Casal *et al*., 1987; Andrade *et al*., 1993; Rebetzke *et al*., 2004).

Results obtained demonstrated the response of specific leaf area parameter to the different light intensities. In both varieties, along with the increase of the light intensity, SLA always gradually decreased, and the decrease in SLA may reduce the light energy absorption. The same results were recorded by (Sims and Pearcy, 1994; Wentworth *et al*., 2006). Smaller SLA would protect the photosynthetic structures by allowing avoidance or decrease of light inhibition, as well as self-adaptation to changes in light. Both genotypes showed plasticity response to different light intensity in terms of SLA. However, Sakha 1 showed higher values of SLA than Giza Blanca under all light intensities. Light induced differences in SLA may reflect not only differences in mesophyll thickness (Yun and Taylor, 1986) but also differences in leaf vascularization (Charles-Edwards *et al*., 1974), which will affect leaf water status.

Stomatal index and their density also varied based exposed light intensities. This can explained as the development of stomata appears to be related with light intensity (Lee *et al*., 2007). The variation in stomatal density acts as an adaptation to cope with the suddenly environmental conditions (Xu and Zhou, 2008). The response of stomatal density to different light intensities at the adaxial and abaxial surfaces (Fig. 4, II) showed that the stomatal density increased with the increase of the light intensity for the two genotypes. Both genotypes recorded plasticity for this trait. The difference was that Giza Blanca in this parameter recorded higher stomatal density at all light intensities than Sakha 1 at the adaxial surface mean-while Sakha 1 recorded the higher value and the higher plasticity response for the abaxial surface. Our results agreed with the studies in which a quantitative analysis demonstrated that the stomatal frequency increased as light intensity increased (Gorton *et al*., 1993; Thomas *et al*., 2003). This increase in stomatal frequency of leaf also might have resulted in an increase in stomatal conductance with the increase in light intensity (Lee *et al*., 2007). The same trend was observed in the results of the stomatal index (Fig. 4, III). Both varieties showed an obvious increase in stomatal index with the increase in light intensity. However, Sakha 1 had higher stomatal index than Giza Blanca at both abaxial and adaxial surfaces at all light intensities

CONCLUSION

The two genotypes of *Vicia faba*, Sakha 1 and Giza Blanca, had a significant plasticity in several morpho-logical phenotypes in response to different light irradiance. This plasticity enables the plants to adapt to the difference in the environmental conditions especially light intensity. As it is important to identify which traits are likely to show important plasticity responses to changing environmental conditions which will help to develop predictors to enable us to generalize about the sorts of species likely to exhibit these plasticity responses. The plastic responses of the traits studied in this work indicate the kind of plasticity and whether the genotype-environment interaction is present or absent for each trait.

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دراسة تأثير اختلاف شدة الاضاءة على المرونة المظهرية فى سلالتين من نبات الفول

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اهتمت الدراسة باستكشاف تأثيرات شدة الضوء علي مرونة صفات النباتات. تمت الدراسة علي سلالتين من نبات الفول (سخا1 وجيزة بلانكا).تم زراعة سلالتين الفول المختاره تحت شدة اضاءة مختلفة وهى : 1800 µmol/m²s,1200µmol/m²s,850µmol/m²s,550µmol/m²s وتم إجراء قياسات مختلفة. بالنسبة للمتغيرات التي تم قياسها بما في ذلك عرض الورقة، والوزن الطازج والجاف للنبات، ووزن الجذر الطازج والجاف ، أظهرت النتائج ان كلا من السلالتين أظهرا نفس الاستجابة المظهرية لعرض الورقة والوزن الطازج والجاف لكلا من الساق والجذر مما يدل عدم ظهور أي تفاعل بين بيئة النمط الوراثي لهذه الصفات.و لكن مع زيادة درجة الحرارة, أظهر سخا1 مرونة مظهرية أعلي من جيزة بلانكا من حيث عدد الأورق وكثافة الثغور ونسبة الثغور وإن المرونه في منطقة ورقة معينة تتضمن تنظيمًا مورفوجينيًا لكلا النوعين الجينيان محل الدراسة وأظهرت أن الأوراق تميل إلى توسيع مساحة أوراقها في ظروف الإضاءة المنخفضة من أجل امتصاص المزيد من الضوء استجابة لذلك. وهذا عكس النباتات التي تنمو تحت شدة إضاءة عالية والتي تميل لتكوين أوراق سميكة. ولكننا فى حاجة لمزيد من التجارب لتوضيح دور العوامل الجينية في التحكم في مرونة صفات النباتات.