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INTERACTION OF ABIOTIC AND BIOTIC STRESS ON PHOTOSYNTHETIC EFFICIENCY AND YIELD IN FABA BEAN (*VICIA FABA* L.)

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Abstract

Faba bean (Vicia faba L.) is an important grain legume whose productivity is increasingly constrained by the combined effects of abiotic and biotic stresses under field conditions. This study evaluated the interaction between these stress factors in relation to photosynthetic efficiency and yield performance in thirteen experimental faba bean breeding lines grown in Romania. The field experiment was conducted in 2025 using a randomized complete block design with three replications. Abiotic stress response was assessed through chlorophyll fluorescence parameters, particularly the maximum quantum efficiency of photosystem II (Fv/Fm), and the chlorophyll content index (SPAD), while biotic stress was evaluated based on natural infestation by the black bean aphid (Aphis fabae). Considerable variability was observed among genotypes, with Fv/Fm values ranging from 0.57 to 0.79 and SPAD values from 10.22 to 23.77. Seed yield varied between 308.2 g m^{-2} and 1057.5 g m^{-2}. Lines L7, L17, L21, and L4 maintained higher photosynthetic efficiency, lower aphid infestation, and superior yield stability under stress conditions. These results highlight the value of integrating physiological indicators and field observations in selecting faba bean breeding material adapted to multiple stress environments.

Key words*: Aphis fabae, breeding, chlorophyll fluorescence, physiological response, SPAD index.*

INTRODUCTION

Faba bean (*Vicia faba* L.) is an important grain legume cultivated worldwide for both human consumption and animal feed due to its high protein content and nutritional value. In addition to its role as a food and feed crop, the faba bean contributes significantly to sustainable agricultural systems through biological nitrogen fixation, improving soil fertility and reducing the need for synthetic nitrogen fertilizers (Jensen et al., 2010; Köpke & Nemecek, 2010). Because of these agronomic and ecological benefits, the crop has gained increasing importance in diversified and climate-resilient cropping systems.

Despite its importance, faba bean productivity is frequently limited by environmental stresses that negatively affect plant growth, physiology, and yield formation. Among these constraints, abiotic stresses such as drought, high temperatures, and nutrient imbalances represent major limiting factors in many production regions (Lawlor & Cornic, 2002; Farooq et al., 2017). These stresses can impair physiological processes, particularly photosynthesis, by affecting stomatal conductance, chlorophyll stability, and the efficiency of the photosynthetic apparatus. Reduced photosynthetic performance ultimately limits carbon assimilation and biomass accumulation, leading to significant yield reductions (Flexas et al., 2004; Ashraf & Harris, 2013). Recent studies have also highlighted the importance of understanding physiological responses to environmental stress in faba bean for improving breeding strategies targeting stress-tolerant genotypes (Shapiguzov et al., 2025).

In addition to abiotic factors, faba bean crops are also affected by biotic stresses, including fungal diseases and insect pests. Among the most important insect pests of faba bean is the black bean aphid (*Aphis fabae* Scopoli), which feeds on plant sap and can cause considerable yield losses by reducing plant vigor and transmitting plant viruses (Nuessly et al., 2004). Aphid feeding may influence plant physiological processes by altering assimilate distribution, reducing photosynthetic activity, and inducing stress responses in host plants (Rubiales et al.,

2015). Experimental evidence indicates that aphid infestation may also trigger biochemical and oxidative stress responses in faba bean plants, highlighting the importance of physiological indicators for assessing plant tolerance to pest attack (Tošić et al., 2025).

Under field conditions, plants are rarely exposed to a single stress factor. Instead, crops frequently experience multiple stresses simultaneously, and the interaction between abiotic and biotic stresses can result in complex plant responses that differ from those observed under individual stress conditions (Mittler, 2006; Atkinson & Urwin, 2012). Environmental constraints such as drought or heat stress may alter plant defence mechanisms and influence plant-insect interactions, potentially increasing plant susceptibility to herbivorous pests (Gill & Tuteja, 2010). Increasing climatic variability is expected to further intensify these interactions, affecting both pest dynamics and crop physiological performance (Gebretsadik et al., 2025)

Photosynthesis is particularly sensitive to environmental constraints and therefore represents an important indicator of plant stress responses. Measurements of chlorophyll fluorescence are widely used as non-destructive methods to assess the efficiency of the photosynthetic apparatus and detect early stress effects in plants. Indicators such as the maximum quantum efficiency of photosystem II (Fv/Fm) and chlorophyll content index (SPAD) are increasingly applied in plant physiology and breeding programs as rapid tools for identifying genotypes with improved tolerance to environmental stresses (Maxwell & Johnson, 2000; Murchie & Lawson, 2013).

Although numerous studies have investigated abiotic stress and pest damage separately, relatively limited information is available regarding their combined effects on photosynthetic efficiency and yield performance in faba bean under field conditions. For example, several studies have analysed the physiological responses of faba bean to drought or heat stress (Siddiqui et al., 2015; Farooq et al., 2017) while others have focused on pest pressure and resistance mechanisms in legumes (Sillero et al., 2010; Rubiales et al., 2015). However, the interaction between environmental stress and insect infestation remains insufficiently explored in faba bean cropping systems. A better understanding of these interactions is essential for identifying breeding material capable of maintaining stable productivity under multiple stress environments. Therefore, the objective of this study was to evaluate the interaction between abiotic stress conditions and natural aphid infestation in relation to photosynthetic efficiency and yield performance in experimental faba bean breeding lines grown under field conditions in Romania. By integrating physiological indicators such as chlorophyll fluorescence and chlorophyll content with field observations of aphid infestation and yield performance, the study aims to identify breeding material with improved tolerance to combined stress factors.

MATERIALS AND METHODS

Plant material and experimental design

The study was conducted in 2025 at the Vegetable Research and Development Station (VRDS) Buzău, Romania, under field conditions. Thirteen experimental faba bean (*Vicia faba* L.) breeding lines (L1-L10, L17, L21, L22), originating from the local breeding program, were evaluated.

The experiment was arranged in a randomized complete block design with three replications. Sowing was performed on 10 March 2025, on modelled soil, with a spacing of 10 cm between plants and 15 cm between rows. Each experimental plot consisted of uniformly managed plants, and all agronomic practices were applied according to standard local recommendations. Harvest was carried out in mid-July, at full physiological maturity.

Assessment of abiotic stress

Abiotic stress response was evaluated through measurements of photosynthetic performance and chlorophyll content. Chlorophyll fluorescence was measured on fully expanded, healthy leaves using a portable fluorometer (Plant Stress Kit Chlorophyll Fluorometers, ADC Bioscience Ltd) after dark adaptation for 20 minutes. The minimum fluorescence (Fo), maximum fluorescence (Fm), and variable fluorescence (Fv = Fm − Fo) were recorded, and the maximum quantum efficiency of photosystem II was expressed as the Fv/Fm ratio, following widely used protocols for

chlorophyll fluorescence analysis (Maxwell & Johnson, 2000; Murchie & Lawson, 2013).
Chlorophyll content was estimated non-destructively using a portable chlorophyll meter (CCM-200+ Chlorophyll Content Meter, ADC Bioscience Ltd) and expressed as SPAD values. Multiple readings were taken from the middle section of the leaf blade for each genotype. Measurements were performed during similar phenological stages and under comparable environmental conditions to ensure consistency among genotypes.

Assessment of biotic stress
Biotic stress was evaluated based on natural aphid (*Aphis fabae*) infestation occurring during the growing season. The intensity of infestation was visually assessed on representative plants using a five-point scale, according to the method described by Barcanu et al. (2022). This approach allowed the classification of breeding lines according to their susceptibility to aphid infestation under natural field conditions.

Statistical analysis
Experimental data were analysed using descriptive statistical methods. Mean values and coefficients of variation were calculated for the analysed traits. Comparative analysis among breeding lines was performed in order to identify genotypes showing superior physiological performance, tolerance to aphid infestation, and yield stability under field conditions.

RESULTS AND DISCUSSIONS

Climatic conditions and stress context
The 2025 growing season at the Vegetable Research and Development Station (VRDS) Buzău was characterized by climatic variability that created a complex stress environment for faba bean development (Figure 1). Early spring conditions were relatively favourable, with moderate temperatures and sufficient soil moisture supporting crop establishment. During April and May, increased precipitation promoted vegetative growth but also favoured the occurrence of natural aphid infestations (*Aphis fabae*). From June onward, higher temperatures and reduced rainfall coincided with critical phenological stages, including flowering and pod formation, intensifying heat and water stress. These conditions provided a suitable field context for evaluating genotype responses to combined abiotic and biotic stress.

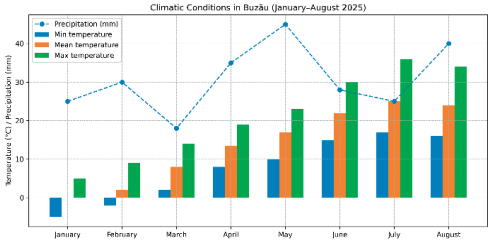

Figure 1. Climatic conditions recorded in Buzău during the 2025 growing season (January-August)

Photosynthetic efficiency under abiotic stress
The evaluation of the faba bean breeding lines presented in Table 1 revealed considerable variability among genotypes in their physiological responses to abiotic stress conditions occurring during the 2025 growing season. Photosynthetic performance was assessed using the maximum quantum efficiency of photosystem II (Fv/Fm) and the SPAD chlorophyll content index, two widely used indicators for evaluating plant responses to environmental stress.

Table 1 Photosynthetic performance (Fv/Fm) and chlorophyll content (SPAD) of faba bean breeding lines evaluated under field stress conditions in 2025

Line	Fv/Fm (mean)	Fv/Fm ≥ 0.80 (%)	SPAD (mean)	SPAD (CV%)	Abiotic stress tolerance
L1	0.61	23.53	20.62	66.91	Moderate
L2	0.66	0.00	20.59	63.02	Low
L3	0.66	5.88	14.57	55.96	Low
L4	0.79	44.44	18.50	34.19	Moderate
L5	0.68	22.22	19.07	61.90	Moderate
L6	0.61	11.11	10.22	79.29	Low
L7	0.72	33.33	23.62	55.97	Moderate
L8	0.60	5.56	12.26	62.64	Low
L9	0.57	38.89	11.59	72.28	Low
L10	0.76	16.67	13.85	48.21	Intermediate
L17	0.75	27.78	23.77	46.04	Moderate
L21	0.76	17.65	22.05	43.93	Moderate
L22	0.73	16.67	16.99	52.46	Intermediate

*Note: Fv/Fm – maximum quantum efficiency of photosystem II; SPAD – chlorophyll content index measured with a chlorophyll meter; CV – coefficient of variation. Abiotic stress tolerance classification was based on the combined interpretation of Fv/Fm values and the SPAD index.

Under optimal conditions, Fv/Fm values in most C3 plants typically range between 0.78 and 0.83, while lower values generally indicate stress-induced damage or photoinhibition of the

photosynthetic apparatus (Maxwell & Johnson, 2000; Baker, 2008). In the present study, most breeding lines exhibited Fv/Fm values below the optimal range, suggesting that plants were exposed to significant environmental constraints, particularly during the reproductive stages when high temperatures and reduced precipitation occurred.

Among the tested genotypes, L17 exhibited one of the most balanced physiological responses, combining a relatively high Fv/Fm value (0.75) with the highest SPAD value (23.77). These results indicate a strong ability to maintain both photosystem II efficiency and chlorophyll stability under stress conditions. Similar responses were observed in lines L7 (Fv/Fm = 0.72; SPAD = 23.62) and L21 (Fv/Fm = 0.76; SPAD = 22.05), which also maintained relatively high chlorophyll content and stable photosynthetic activity. Line L4, despite presenting the highest Fv/Fm value (0.79), showed a somewhat lower SPAD value (18.50), suggesting that although the photosynthetic apparatus remained functionally efficient, some reduction in chlorophyll content occurred.

In contrast, several lines showed clear signs of physiological stress. Line L6 (Fv/Fm = 0.61; SPAD = 10.22) and line L8 (Fv/Fm = 0.60; SPAD = 12.26) displayed both reduced photosynthetic efficiency and low chlorophyll content, indicating a higher sensitivity to stress conditions. The lowest Fv/Fm value was recorded in line L9 (0.57), suggesting possible photoinhibition or damage to photosystem II under heat and water deficit conditions. Similar reductions in Fv/Fm under drought or heat stress have been widely reported in legumes and are often associated with oxidative stress and damage to the photosynthetic machinery (Flexas et al., 2004; Gill & Tuteja, 2010).

Interestingly, some genotypes such as L10 and L22 maintained relatively high Fv/Fm values (0.76 and 0.73, respectively), but showed lower SPAD readings. This pattern suggests that although the functionality of the photosynthetic apparatus was partially preserved, chlorophyll degradation may have occurred under stress conditions, potentially limiting long-term photosynthetic capacity and yield formation.

Overall, the results highlight significant physiological variability among the tested breeding lines. Genotypes L17, L7, L4, and L21 showed relatively stable physiological performance compared with the other breeding lines, maintaining both relatively high Fv/Fm values and chlorophyll content under field stress conditions. Such traits are strongly associated with improved tolerance to drought and heat stress and represent valuable selection criteria in faba bean breeding programs targeting adaptation to increasingly variable climatic conditions (Khan et al., 2010; Farooq et al., 2017).

Biotic stress impact and interaction with physiological traits

During the growing season, natural infestations of the black bean aphid (*Aphis fabae*) were observed in the experimental faba bean plots (Figure 2). Aphids are among the most important insect pests affecting faba bean crops, as they feed on phloem sap, reducing plant vigor and interfering with photosynthetic activity and assimilate distribution (Nuessly et al., 2004; Rubiales et al., 2015).



Figure 2. Aphid infestation (*Aphis fabae*) on faba bean plants under field conditions

Most breeding lines exhibited good tolerance to aphid infestation (score 2), including L1, L3, L4, L6, L7, L8, L17, L21, and L22**,** where less than 25% of foliage was affected. These genotypes generally maintained stable productivity under field conditions. For example, L7 recorded the highest seed yield among the evaluated breeding lines (1057.5 g m^{-2})**,** followed by L1 (627.5 g m^{-2}), while L6, L21, and L22 also showed relatively stable yields between 500 and 550 g m^{-2}. The ability of these lines to maintain productivity under moderate pest pressure suggests better physiological resilience and sustained photosynthetic activity.

In contrast, L2 and L9 showed moderate susceptibility (score 3), with 25-50% of leaves affected. Their productive performance was more variable, indicating partial tolerance but

reduced physiological stability under biotic stress.

The highest susceptibility was recorded in L5 and L10 (score 4), where more than half of the foliage was affected by aphid infestation. These genotypes also recorded the lowest yields, with L5 producing 308.2 g m^{-2} and L10 reaching 405.7 g m^{-2}. Aphid feeding can significantly reduce plant productivity by removing photo assimilates and inducing physiological stress, which ultimately limits reproductive development and seed filling (Blackman & Eastop, 2000; Dedryver et al., 2010).

Interestingly, natural predators such as lady beetles (Coccinellidae) were frequently observed in the experimental plots (Figure 3). These insects are efficient biological control agents of aphids and likely contributed to regulating pest populations, reducing the need for chemical intervention.



Figure 3. *Coccinella septempunctata* (lady beetle) on faba bean plant, a natural predator of aphids

Overall, the results indicate substantial variability among faba bean genotypes in their response to aphid infestation. Lines L7, L1, L17, L21, and L22 combined good tolerance to aphid attack with stable yield performance, making them promising candidates for breeding programs targeting improved resilience to combined biotic and abiotic stresses.

Interaction between abiotic and biotic stress

The results obtained in this study indicate a clear interaction between abiotic stress conditions and the susceptibility of faba bean genotypes to aphid infestation. Genotypes that maintained higher photosynthetic efficiency and chlorophyll content under environmental stress, such as L7, L17, and L21, generally showed lower levels of aphid damage and more stable yield performance.

In contrast, lines characterized by reduced physiological stability, reflected by lower Fv/Fm and SPAD values, tended to exhibit higher susceptibility to aphid infestation and reduced productivity. Similar interactions between abiotic stress and pest susceptibility have been reported in legume crops, where environmental stress weakens plant physiological defenses and increases vulnerability to insect herbivores (Mittler, 2006; Atkinson & Urwin, 2012).

These findings highlight the importance of integrating physiological indicators such as photosynthetic efficiency with field-based pest assessments when selecting breeding material adapted to increasingly variable environmental conditions.

CONCLUSIONS

This study revealed considerable variability among the evaluated faba bean breeding lines in their physiological and agronomic responses to combined abiotic and biotic stress conditions under field environments. Photosynthetic efficiency (Fv/Fm) ranged from 0.57 to 0.79, while seed yield varied between 308.2 and 1057.5 g m^{-2}, indicating differences in the physiological stability and productivity of the tested genotypes.

Breeding lines maintaining relatively higher photosynthetic efficiency and chlorophyll content, particularly L7, L17, L4 and L21, showed improved yield performance and lower susceptibility to aphid infestation (*Aphis fabae*). In contrast, genotypes characterized by reduced physiological performance tended to exhibit lower productivity under stress conditions.

The results highlight the importance of integrating physiological indicators, such as chlorophyll fluorescence and the chlorophyll content index, with field observations of pest pressure in the evaluation of faba bean breeding material. These indicators can support the identification of genotypes with improved tolerance to multiple stress factors and more stable productivity under variable environmental conditions.

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