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Abstract

© 2020 American Society for Photobiology Combined enhanced UV-B radiation and drought may induce different morphological and physiological alterations in plants than either abiotic stress alone. We evaluated morphology, biomass, and primary and secondary metabolism changes in seedlings of two common bean cultivars, IAC Imperador (drought-resistant) and IAC Milênio. To test the hypothesis that cultivars responded differently to combined stresses in a controlled environment, seedlings of the examined bean cultivars were exposed to UV-B and/or drought treatments for three weeks. The cultivars behaved differently, especially to the drought treatment, suggesting that they use different mechanisms to cope with unfavorable environmental conditions. IAC Imperador showed a stronger protective response, modifying wax composition and primary metabolism, and improving its resistance to UV-B radiation. For IAC Imperador, the accumulation of cuticular wax and alkane was higher under combined stress but production of primary alcohols was reduced, suggesting a possible fatty acyl switch. Root/shoot length and biomass ratios increased in both cultivars, particularly for the combined stress, indicating a common plant response. We show that these two bean cultivars responded more strongly to UV-B and combined stress than drought alone as evident in changes to their chemistry and biology. This shows the importance of investigating plant morphological and physiological responses to combined stress.

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**UV-B and Drought Stress Influenced Growth and
Cellular Compounds of Two Cultivars of
Phaseolus vulgaris L. (Fabaceae)**

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ABSTRACT

Combined enhanced UV-B radiation and drought may induce different morphological and physiological alterations in plants than either abiotic stress alone. We evaluated morphology, biomass, primary and secondary metabolism changes in seedlings of two common bean cultivars, IAC Imperador (drought-resistant) and IAC Milênio. To test the hypothesis that cultivars responded differently to combined stresses in a controlled environment, seedlings were exposed to UV-B and/or drought treatments for three weeks. The cultivars behaved differently, especially to the drought treatment, suggesting that they use different mechanisms to cope with unfavorable environmental conditions. IAC Imperador showed a stronger protective response, modifying wax composition and primary metabolism, and improving its tolerance to UV-B radiation. For IAC Imperador, the accumulation of cuticular wax and alkane was higher under combined stress but production of primary alcohols was reduced, suggesting a possible fatty acyl switch. Root/shoot length and biomass ratios increased in both cultivars, particularly for the combined stress, indicating a common plant response. We show that these two bean cultivars responded more strongly to UV-B and combined stress than drought alone as evident in changes to their chemistry and biology. This

41 shows the importance of investigating plant morphological and
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INTRODUCTION

Anthropogenic activities, especially those related to the emission of atmospheric pollutants, are causing climate change, such as global warming through increasing atmospheric CO₂ levels. Climate change can affect, among other conditions, precipitation cycles, increasing the frequency of drought episodes (1) and resulting in negative impacts on agricultural productivity (2). The release of atmospheric pollutants have increased the quantity of damaging ultraviolet-B radiation (UV-B; 280-315 nm) reaching Earth's surface due to stratospheric ozone depletion. This ozone depletion, caused by emission of chlorofluorocarbons (CFCs) and other gases, has increased UV-B-induced DNA damage (3), lowered production (4) and has enhanced secondary plant compound levels (4–6).

Plants live in environments subjected to constant transformations and are exposed to biotic and abiotic factors that can interrupt growth and development. While research has focused on understanding single environmental factors on plant growth, plants naturally face exposure to multiple stresses simultaneously. These may be antagonistic or synergistic for different plant processes. Understanding the consequences of multi-stress exposure is important, but not often investigated (5, 6). This is particularly relevant as climatic change intensifies environmental stresses (7). Therefore, the development of new cultivars resistant to environmental stress is vital to maintain agricultural production and sustain human populations (8). Resistant cultivars are physiologically capable of coping with, at least, one prevailing stress (9) as a result of metabolic and structural adaptation (10). We investigated whether changes to one environmental stress confers resistance to other stresses.

Combining a water deficit with increased UV-B radiation may cause different effects on plants than either stress alone. Responses will depend on the intrinsic tolerance of a species to these

two abiotic factors and the period of exposure to the stresses, potentially leading to either irreversible damage or induced resistance. As both stresses (drought and increased UV-B radiation) can cause growth inhibition in plants, plant responses may be intensified when they are experienced simultaneously, although a response to one stress could also relieve the effect of the other. For example, increased UV-B radiation could attenuate the effect of drought on the photosynthetic rate (10, 11). Alternatively, water stress together with increased UV-B radiation could result in greater synthesis of UV-B absorbing compounds, such as flavonols than would otherwise form with enhanced UV-B alone (12).

This study aimed to evaluate the effect of combined drought and enhanced UV-B radiation on the physiology and morphology of seedlings of the common bean, *Phaseolus vulgaris*. We also aimed to identify differences in response between two cultivars. We assessed plant growth, phenolic compounds and primary metabolite profiles, and wax deposition of two bean cultivars called Instituto Agronômico de Campinas (IAC) Milênio and IAC Imperador. Replicate cultivars were treated with enhanced UV-B radiation, drought or combined UV-B/drought under controlled laboratory conditions. As IAC Imperador grows more quickly and uses 30% less water than IAC Milênio according to IAC (13, 14), we predicted that the former cultivar will show a more plastic response to drought and UV-B conditions than the latter.

MATERIALS AND METHODS

To explore physiological and morphological changes in two cultivars of the common bean (*Phaseolus vulgaris* L., Fabaceae) in response to enhanced UV-B radiation and drought, we used plants under laboratory conditions.

Plant material: Seeds of two bean cultivars were obtained from the Instituto Agronômico de Campinas (IAC). Seeds of IAC Imperador and IAC Milênio contain approximately 21% protein and are resistant to two phytopathogenic diseases, anthracnose (*Colletotrichum lindemuthianum*) and fusariosis (*Fusarium oxysporum*). IAC Imperador has a quick maturity life cycle of 70–75 days and production of 2,266 kg ha⁻¹ (13), reached with lower rates of irrigation (approximately 30% less water) than other bean cultivars. IAC Milênio has a life cycle of 90–95 days, and production of 2,831 kg ha⁻¹ (14). As plants were only grown for three weeks (21 days), differences in the time to maturity of these two cultivars is likely to have a relatively small effect on our results, although it cannot be ruled out completely.

Experimental design: Seeds were sown in 40 pots (four seeds/pot) with a mixture of vermiculite:soil (3:1). Germination was carried out in a chamber (FANEM 347-CDG, Brazil) with a 12/12 h light/dark photoperiod at constant 25°C. The same chamber was used for the experiment with the two UV-B treatments performed first followed by the non UV-B treatments. All other conditions were consistent. For the control (C) and drought (D) groups, the chamber was equipped with four white fluorescent lamps (Osram, Germany; 20 W; range of 100–300 µmol m⁻² s⁻¹ measured with a Li-Cor Biosciences Model Li-250A sensor) and each pot (10 per treatment) received 10 mL of distilled water every two days. The drought condition (D)

113 was initiated by suspending the water supply as soon as epigeal
114 germination was observed. For the other treatments, seeds
115 received UV-B supplementation (UVB) for 2 h during the first half
116 of the light period, corresponding to a daily dose of 10.2 kJ m^{-2}
117 (measured by MACAM Ultraviolet Radiometer (Scotland) connected to
118 an UV-B specific sensor), by replacing one of the fluorescent
119 lamps by a UV-B lamp (TL 20W/01 RS SLV/25, Philips, Netherlands).
120 For the UVB treatment each pot continued to received 10 mL of
121 distilled water every two days. For plants exposed to drought
122 and UV-B (UVB+D), watering was suspended as soon as epigeal
123 germination was observed. Pots were randomly rotated daily for
124 all treatments.

125 **Plant sampling:** After three weeks, the first trifoliate leaf was
126 harvested from all plants (ten plants per treatment) and analyzed
127 for biomass, waxes, primary and phenolic metabolites. This
128 ensured that all leaves were of the same developmental age. One
129 leaflet was separated and dried at 45°C ; the other two leaflets
130 were frozen in nitrogen and kept at -80°C until analysis.
131 Leaflets from two randomly chosen individuals of the same
132 treatment were combined to achieve an appropriate mass for
133 chemical analyses ($n=5$).

134 **Biomass analysis:** After sampling of the first trifoliate leaf, plants
135 were harvested and roots washed to remove soil particles then
136 excess water removed using blotting paper. Root and shoot lengths

were measured, separated, dried at 45°C for five days, and the dry weight determined. Root/shoot biomass and length ratios were calculated.

Cuticular wax analysis: Dried leaflets were first measured to obtain the surface area using the software Image J 1.49. After that, they were washed twice in dichloromethane (CH₂Cl₂) for 20 s at room temperature (~ 25°C) to extract cuticular waxes (modified from 15). The extracts were filtered and dried in a rotary evaporator under vacuum at 40°C. The wax was further dried to a constant mass in a desiccator. The total wax content was calculated as $\mu\text{g cm}^{-2}$.

Cuticular waxes were derivatized using pyridine (50 μL , CAS 110-86-1, Synth) and *N,O*-bis-(trimethylsilyl)-trifluoroacetamide (50 μL , BSTFA, CAS 25561-30-2, Sigma-Aldrich) for 1 h at 70°C (modified from 16), and analyzed by gas chromatography (6850 Network GC System - Agilent) coupled to mass spectrometry (Agilent 5975C VL MSD) (GC-MS) equipped with a HP5-MS capillary column (Agilent, length 30 m, ID 250 μm , 0.25 μm film thickness). The initial column temperature was adjusted to 100°C for 5 min, and ramped at 5°C min⁻¹ to a final temperature of 320°C, with a total run time of 49 min. The injection volume was 1 μL with helium as a carrier gas at 1 mL min⁻¹. The injector, ion source, and quadrupole temperatures were 300°C, 230°C, and 150°C, respectively. MS detection was

performed with electron ionization (EI) at 70 eV, working in the full-scan acquisition mode ranging between 50-600 m/z at 2.66 scan s^{-1} .

Wax compounds were identified by comparison of mass fragmentation using NIST digital library spectra (v2.0, 2008) and fragmentation pattern. Peaks with relative percentages lower than 1% in the chromatogram were not considered for the analysis.

Primary metabolites analysis: Analysis of primary metabolites was made according to Lisec et al. (17), with modifications. Frozen leaflets (50 mg) were extracted with 700 μL of pre-cooled methanol ($-20^{\circ}C$) and briefly stirred. Afterwards, 60 μL of ribitol (0.2 mg mL^{-1} , used as an internal standard) was added and incubated for 10 min at $70^{\circ}C$ and 950 rpm (142 g). The mixture was centrifuged (10 min at 11,000 g), before the supernatant was transferred to glass tubes, and chloroform (375 μL at $-20^{\circ}C$) and distilled water (750 μL at $4^{\circ}C$) added. After stirring, the mixture was centrifuged for 15 min at 2,200 g and the polar phase was collected.

The polar phase was derivatized using methoxyamine hydrochloride (28 μL , CAS 593-56-6, Sigma-Aldrich) for 2 h at $37^{\circ}C$ and *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (48 μL , MSTFA, CAS 24589-78-4, Sigma-Aldrich) for 30 min at $37^{\circ}C$. Primary metabolites were analyzed by GC-MS (as above) equipped

with the VF-5ms column (Agilent, length 30 m, ID 250 μm , 0.25 μm film thickness) and a pre-column (0.25 mm x 10 m). The initial column temperature was adjusted to 70°C for 5 min, and ramped at 5°C min⁻¹ to a final temperature of 295°C, with a total run time of 50 min. The injection volume was 1 μL with helium as a carrier gas at 1 mL min⁻¹. The injector, ion source, and quadrupole temperatures were 230°C, 200°C, and 150°C, respectively. MS detection was performed with electron ionization (EI) at 70 eV, working in the full-scan acquisition mode ranging between 50–600 m/z at 2.66 scan s⁻¹.

Compound identification was made by comparison of mass fragmentation using NIST digital library spectra (v2.0, 2008) and through Linear Retention Index (LRI). An alkane mixture ranging from C₈ to C₂₈ was injected every ten samples. The alkane mixture consisted of a standard solution of alkanes C₈–C₂₀ (Sigma-Aldrich) with addition of alkane standards C₂₁–C₂₈ (PolyScience), except for the C₂₇ homologue. The Linear Retention Index (LRI) was calculated for each compound using the alkane standard mixture according to Viegas and Bassoli (18) and compared with the Golm Metabolome Database (GMD) (<http://gmd.mpimp-golm.mpg.de/>), European Mass Bank database (<https://massbank.eu/MassBank/Index>), and literature (19).

Phenolic compounds analysis: Frozen leaflets were extracted with acetone:water (8:2) in an ultrasonic bath for 30 min. The

209 samples were centrifuged at 14,000 rpm (20,817 g) for 3 min and
210 the supernatant transferred to a fresh tube. The extraction
211 procedure was repeated until exhaustion. Extracts were pooled
212 and concentrated under vacuum (SpeedVac concentrator,
213 Vacufuge® plus) to reduce the proportion of organic solvent
214 and water was removed through freeze-drying (Liofilizador K202,
215 Liotop). Samples were dissolved in water to solubilize polar
216 compounds. The aqueous fraction was collected and subsequently
217 freeze-dried (20).
218 Dried aqueous fractions were then weighed, diluted in water to
219 achieve a concentration of 5 mg mL⁻¹, and analyzed by High-
220 Performance Liquid Chromatography coupled to a diode array
221 detector (HPLC-DAD 1260 - Agilent) through a C18 column (Zorbax
222 Eclipse Plus, 4.6 x 150 mm, 3.5 µm) at 45°C. The mobile phase
223 had a constant flow of 1 mL min⁻¹ and a gradient elution of 0.1%
224 acetic acid (A) and acetonitrile (B), starting with 100% (A),
225 decreasing to 85% (A) for the next 6 min, then to 70% (A) for
226 20 mins, decreasing to 0% (A) for the next 1 min and finishing
227 with an isocratic phase for the last 4 min. Absorbance
228 detection was performed at λ = 280 and 352 nm. A post-run of 5
229 min was applied to return to initial conditions.
230 Compounds detected at 280 and 352 nm were classified as non-
231 flavonoid phenolics and flavonoids respectively, according to
232 their UV absorption spectra. Non-flavonoid compounds were

expressed as mg of gallic acid equivalents (GAE) per g of fresh weight (FW), while flavonoids were expressed as mg of rutin equivalents (RE) g⁻¹ fresh weight (FW). Total phenolics were obtained from the sum of flavonoid and non-flavonoid fractions and are expressed mg g⁻¹ FW. Only peaks with an abundance above 20 mAU were considered.

Statistical analysis: Two factor permutational multivariate analyses of variance (PERMANOVAs) were used to identify changes in biomass variables and composition of cuticular waxes, primary metabolites and phenolic compounds amongst the two cultivars and the stress treatments (PRIMER v7, PRIMER-E Ltd). For waxes and primary metabolites, Bray Curtis dissimilarity indices were used to measure differences amongst samples. For the biomass variables and phenolic compounds, data were first normalized as each compound was measured on a different scale, then Euclidean distances were calculated. Pairwise comparisons were performed within the PERMANOVA module to identify where differences lay. Data were visualized in non-metric multidimensional scaling ordinations (nMDS) for bootstrapped group averages with 95% confidence intervals (no. bootstraps per group = 38). Individual compounds were then compared using univariate statistics by Kruskal-Wallis rank test followed by a *post-hoc* Dunn test, using R software (version 3.5.3). Heatmaps were made using the Morpheus platform (<https://software.broadinstitute.org/morpheus/>). To elucidate differences between control and treatments, heatmap analysis were performed by transforming obtained data, using the formula below. As a result, data in heatmaps are presented as values normalized to their respective control.

$$TD = C - \log_2(T * 1000)$$

TD: Transformed data, C: Control data, T: Treatment data

RESULTS

Growth and biomass production

Differences in biomass allocation patterns were evident amongst *P. vulgaris* L. cultivars (Pseudo $F_{1,67} = 4.971$, $p = 0.004$) and within treatments (Pseudo $F_{3,67} = 15.467$, $p = 0.001$). The pattern of change in biomass allocation within treatment was the same for each cultivar (interaction term NS: Pseudo $F_{3,67} = 0.920$, $p = 0.502$). Pairwise comparisons identified differences amongst all pairs of treatments, which are well depicted in the ordination (Fig. 1).

<Figure 1>

In general, when compared to control, treatments affected the growth and biomass production of both cultivars (see Supplementary Materials). Seedlings were significantly shorter when exposed to UV-B radiation in comparison to the control (Fig. 2a). For IAC Imperador, seedling shoot length was 29.7% and 28.1% shorter for combined stress (UVB+D) and UVB, respectively. Shoots of IAC Milenio seedlings were 38.9% (UVB+D) and 34.5% (UVB) of the length of the control. Although neither drought nor UV-B appeared to affect root length separately, roots were significantly longer under the combined stress than the UVB treatment alone for the IAC Imperador cultivar (Fig. 2b). Consequently, it is unsurprising that shoot length strongly influences the root/shoot length ratio resulting in a significant increase in both

UVB treatments for IAC Milênio and only UVB+D for IAC Imperador compared to the control (Fig. 2c).

<Figure 2>

In contrast with shoot length, no significant difference in shoot dry biomass was observed between the treatments for either cultivar (Fig. 2d). However, except for drought treatment of IAC Imperador, root dry biomass of both cultivars increased when submitted to stressful conditions (Fig. 2e). This increase subsequently increased root/shoot dry mass, especially for UVB treatments, resulting in the UVB+D treatment being significantly higher than the control for both cultivars and UVB alone also being higher in IAC Milênio (Fig. 2f).

Cuticular waxes

The effect of treatment on the composition of waxes varied between bean cultivars (Pseudo $F_{3,30} = 9.886$, $p = 0.001$) (Fig. 3a). The two cultivars did not differ in cuticular wax content for controls ($p = 0.111$), but differed for all other treatments ($p < 0.025$) (see Supplementary Materials). Total wax content was similar for both *P. vulgaris* cultivars under control conditions with $40.74 \pm 34.87 \mu\text{g cm}^{-2}$ for IAC Imperador and $31.92 \pm 7.52 \mu\text{g cm}^{-2}$ for IAC Milênio (Fig. 4a and see Supplementary Materials). IAC Milênio tended to have more primary alcohols (PA C_{26} and PA C_{28}), while IAC Imperador tended to have more alkane C_{31} (Table 1, Fig. 4b).

<Figure 3>

<Figure 4>

Wax compound classes found in *P. vulgaris* were alkanes, primary alcohols, fatty acids and steroids (Table 1, Fig. 4b). Primary alcohols and alkanes were the most abundant classes, comprising between 89-95% of compounds. Fatty acids and steroids were detected only under enhanced UV-B (UVB and UVB+D) for both cultivars (Table 1). For IAC Imperador, while alkanes increased across stress treatments (D, UVB, UVB+D), primary alcohols decreased in abundance (Table 1). For IAC Milênio, no difference was found for any treatment.

<Table 1>

A total of 18 wax compounds were identified: 10 alkanes (C₂₄-C₃₃), five primary alcohols (C₂₄-C₃₂), one fatty acid (hexadecanoic acid), and two steroids (stigmasterol and β -sitosterol) (Table 1). Among the alkanes, nonacosane (C₂₉) and hentriacontane (C₃₁) were the dominant compounds in both cultivars. For IAC Imperador, shorter chain alkanes (C₂₄ to C₂₆) appeared only under UVB treatment. For both cultivars, the primary alcohols were distributed in an even homologous series with hexacosanol (C₂₆) and octacosanol (C₂₈) as major homologues.

Primary metabolites

Twenty-three primary metabolites were detected, comprising soluble sugars, amino acids, and carboxylic acids (Table 2). Overall, there were differences in primary metabolite composition between all treatments (Pseudo $F_{3,30} = 3.786$, $p = 0.001$) and between the two cultivars (Pseudo $F_{1,30} = 3.716$, $p = 0.002$) (see Supplementary Materials). Pairwise tests identified all treatments differed from all others in composition, except the control and drought treatments (Fig. 3b).

<Table 2>

For IAC Milênio, there was no significant difference in total soluble sugars, amino acids or total carboxylic acid contents (Fig. 5) (see Supplementary Materials). IAC Imperador also showed no significant difference in total soluble sugars (Fig. 5a). However, for IAC Imperador the total amino acid and carboxylic acid contents were significantly higher for UVB+D treatment when compared to UVB alone (Fig. 5b, 5c). In addition, amino acid content was also lower in the UVB treatment when compared to the drought treated plants (Fig. 5b).

<Figure 5>

Concentrations of fructose and glucose were similar in all treatments for both cultivars (Tables 3 and 4). For IAC Milênio significantly higher levels of sucrose (3-fold) were found in the UVB+D treatment than in the control (Table 4). On the other hand, for IAC Imperador, ribonic acid was found only in treatments submitted to drought stress (D and UVB+D) (Table 3).

<Table 3>

<Table 4>

For amino acids, aspartic acid was found in all UV-B irradiated plants of both cultivars (Tables 3 and 4), with the exception of the UVB treated IAC Imperador (Table 3). Moreover, drought and UVB+D treated IAC Imperador showed higher glutamic acid content than UVB alone; and drought treatment in this cultivar also presented a higher content of 5-oxoproline when compared with UVB (Table 3). For IAC Imperador and IAC Milênio, GABA was found in both treatments with UV-B irradiation (UVB and UVB+D), but not in control or drought only treatments (Tables 3 and 4).

In the IAC Imperador cultivar, tartaric acid was present in all treatments, [except the](#) UVB treatment. In addition, UVB+D showed a higher content of malic acid than UVB and D

applied singly (Table 3). Succinic acid was detected in both cultivars; however, in IAC Imperador, it was not detected in the UVB treatment (Tables 3 and 4).

Phenolic compounds

Two distinct groups of phenolic compounds were identified: 1) non-flavonoid phenolics represented by maximum absorption between 220 and 326 nm, and 2) flavonoids detected by the presence of two peaks typical for flavonoids, one at 240-266 nm and another at 328-360 nm. However, no difference (see Supplementary Materials) in overall phenolic composition was found between cultivars or treatments (Fig. 3C).

DISCUSSION

We evaluated the morphological and biochemical responses of two cultivars of the common bean, *Phaseolus vulgaris*, to elevated UV-B radiation and drought conditions. The combined UV-B and drought treatment on both cultivars was greater than the individual stressors, especially for biomass allocation and wax composition. We showed that the two cultivars, IAC Imperador and Milênio, differed in their response to drought and UV-B, showing changes to plant growth, biomass, composition of cuticular waxes and primary metabolites. As predicted, IAC Imperador was able to withstand the drought treatment with a lower impact on plant shoot and root growth compared to IAC Milênio but showed a shift to alkanes in cuticular waxes. In

contrast, cuticular wax composition did not change in IAC Milênio in response to either drought or UVB treatments.

Growth and biomass production

Both cultivars showed similar patterns of response to stresses with more pronounced changes in growth in response to combined stress (UVB+D), than either stress treatment separately (D or UVB). However, these responses were not additive, resulting in a more subdued increase.

These results differ from published studies and show that responses to combined stresses can be species specific. Additive effects of UV-B radiation and drought have been observed for willows (21), whereas for pea, wheat, maize, and soybean, growth was lower, but not additive, in response to combined stress (22, 23). This suggests the presence of some mechanism that mitigates the adverse effects caused by the combined stress conditions in these crop plants.

Water is a limiting factor at the initial phase of plant growth, affecting elongation and expansion (24), and roots commonly become more pronounced and deeper due to an increase of assimilate supply for this organ, to foster more efficient use of soil water (25–27). Sartori et al. (28) described reductions in photosynthetic capacity and carbon assimilation in plants of IAC Imperador with only two days of water restriction. In our study, shoot biomass did not change in either cultivar under any treatment and changes under stress were largely associated with increasing allocation to root biomass. For both cultivars, root biomass increased with the combined stresses, also evident in increases in length and biomass shoot/root ratios. At these young stages, roots represented about 16% of the total biomass allocation in controls, increasing to about 20% with combined stress. Omae et al. (29) observed shoot reduction in different bean cultivars submitted to water stress and correlated this feature with the water retention capacity in drought situations. We did not see this in our seedlings.

In both cultivars, the effect of UVB on biomass seemed more pronounced than the effect of drought, with shorter shoots observed under UVB than drought treatment, although this was not significant. This allocation of more biomass to roots in plants growing under enhanced UV-B radiation has been reported elsewhere (30).

While the same general response pattern to treatment was verified for both cultivars in growth and biomass parameters, IAC Milênio responded more to drought stress alone than IAC Imperador in respect of changes to root growth under drought (Fig. 2e-f), suggesting that IAC Milênio invests more in morphological changes for acclimation to drought.

Cuticular waxes

High levels of UV-B radiation (31–33) and water deficit (34–39) can increase the amounts of total waxes. However, differences among control and treatments were not detected for either cultivar of *P. vulgaris* in our study, suggesting that high levels of cuticular protection may already be present in these plants and are not induced by higher exposure to either stress. Similar patterns of wax content across treatments in response to UV-B irradiation were also observed in *Brassica napus* L. leaves (39) and fruits (40), and in needles of different species of pine (*Picea* spp.) (41). The absence of increased wax amounts under UV-B irradiation does not indicate a lack of UV-B protection. Despite the reported correlation of increased wax content in the presence of UV-B light, the varied optical properties of cuticular wax crystals are also an important factor in controlling reflection of excess incoming radiation (35, 42, 43), probably being more so than cuticle thickness alone.

Large changes were found in cuticular wax composition across treatments (Fig. 3a). IAC Milênio had a minor response to stresses, while IAC Imperador responded more strongly (Fig. 4b). IAC Imperador produced more alkanes, suggesting an investment to protect the plant

413 against environment changes; IAC Imperador may increase its drought-resistance through
414 accumulating compounds, such as alkanes, with higher hydrophobicity to protect the cells
415 against stresses.

416 There are several indications suggesting that wax composition is a very important attribute
417 related to water retention in the cuticle. Alkanes are the most hydrophobic wax constituents,
418 being more efficient for plant water retention than alcohols and ketones (44). IAC Imperador
419 did not show increases in wax content, but exhibited an enhanced proportion of alkanes,
420 mainly C₂₉ and C₃₁, together with a reduction of primary alcohols, across treatments,
421 particularly under combined stress (UVB+D). This might improve water retention particularly
422 when drought was associated with enhanced UV-B radiation. In contrast, IAC Milênio showed
423 no change in alkane proportions. Apparently, in IAC Milênio, changes in cuticular waxes as a
424 protective mechanism against drought and UVB stress are not triggered.

425 The presence of hexadecanoic acid and steroids (β -sitosterol and stigmasterol) in the waxes of
426 both cultivars seem to be UV-B dependent. This is the first time that a possible correlation
427 between the presence of fatty acids and steroids associated with enhanced UV-B irradiation
428 has been reported. Further studies are necessary to confirm this idea and verify if these
429 compounds improve protection for plants.

430 **Primary metabolites**

431 Primary metabolites such as lipids, proteins, sugars and amino acids are essential for many
432 plant physiological processes, such as photosynthesis, protein synthesis and nutrient
433 absorption (45). Changes in environmental conditions can impact primary metabolite
434 production (46). It is known that drought treatment can alter primary metabolites, increasing
435 carbohydrates levels, such as sucrose (46). The two bean cultivars had different primary

metabolite content (Fig. 3b) associated with a higher production of sucrose in IAC Imperador in all treatments, except UVB+D which showed similar concentrations for both cultivars.

Resistance in IAC Imperador may be mediated through a significant change in cellular metabolites.

Increases in non-structural carbohydrate concentrations in plants growing under high doses of UV-B light is suggested to be due to mitochondrial damage, resulting in a decrease of respiratory utilization of substrate, favoring the accumulation of sugars (47). Pea plants (*Pisum sativum*), submitted to high doses of UV-B ($12.9 \text{ kJ m}^{-2} \text{ day}^{-1}$) were characterized by an 89% increase of total soluble sugars in comparison to those under ambient UV-B (48). Such a response was not apparent in the cultivars used in this study, which did not show changes in total soluble sugars content when submitted to a higher dose of UV-B radiation alone. These cultivars may have similar protective mechanisms against UV-B radiation and water stress. Often under water stress, the content of total soluble sugar can increase or decrease depending on the level of stress to which a particular plant is exposed, decreasing carbon assimilation and consequently growth. Soluble sugars have a similar function as amino acids, protecting membranes against dehydration by reducing the osmotic potential and regulating water transport. Water stress affects the total soluble sugar content differently in a range of legumes according to genotype. For *Cajanus cajan* (L.) Millsp., for instance, the accumulation of total soluble sugars was not influenced by water stress levels, whereas changes were apparent for other species (49). Although some of these sugars were detected in both cultivars, there was also no significant difference overall in these compounds in any treatments relative to the control (Fig. 5a).

Both drought and UV-B irradiation may have contributed to the higher content of amino acids in IAC Imperador under the combined stress. Increased total amino acids can occur under drought stress and is responsible for osmotic adjustment, avoiding tissue dehydration or even protecting subcellular structures against the deleterious effects of drought (49-51). The total amino acids increased in IAC Imperador submitted to combined stress when compared to UVB radiation alone, [indicating the importance of drought stress to amino acid accumulation](#). GABA (gamma-aminobutyric acid) is a well-known intermediate for amino acid metabolism and, in plants, it is accumulated in response to a wide range of environmental stresses. Thus, GABA can act as a signal to maintain cellular metabolism or, under water stress, can act as a potential osmolyte assisting in the absorption of water. In other cultivars of common beans under combined stresses, GABA also acts as a significant antioxidant, helping ameliorate the deleterious effects of these stresses (52). In both *P. vulgaris* cultivars in our study, GABA was detected only in plants subjected to UV-B irradiation, suggesting that it might also act as an antioxidant compound here.

Phenolic compounds

UV-B radiation incidence and drought were expected to cause changes in phenolic compound production (11) as drought triggers accumulation of H₂O₂ and lipid peroxidation, which leads to an overexpression of genes that regulate flavonoid production (53). However no difference was found in total phenolics or flavonoid concentrations in response to drought treatment in either cultivar. [In contrast, we found a low flavonoid content which did not vary between control and UVB treatments. Flavonoid biosynthesis is correlated with the incidence of UV-B, UV-A and visible light \(54\).](#) Experiments carried out in growth chambers, as in this study, lack UV-A radiation and have low visible light compared to sunlight, which may weaken the ability

of a plant to respond appropriately to UV radiation (55). However, our other results show that the plants were able to enhance other UV photoprotection, so [low flavonoid content may be](#) the normal response. Field trials under natural, filtered and/or supplemental UV conditions would resolve this.

Heatmap and biosynthetic pathways shifts

Differences between treatments are highlighted in the heatmap with identified connections to the biosynthetic pathways (Fig. 6). The greater production of sucrose in both cultivars submitted to stressful conditions may be detrimental for IAC Imperador as it was coupled with declines of sucrose monomers, fructose and glucose. These precursor compounds appear to be depleted in IAC Imperador under drought conditions when ribonic and tartaric acid synthesis is enhanced, while the synthesis of *myo*-inositol seems to decrease. On the other hand, IAC Milênio, despite also showing a greater amount of sucrose and ribonic acid in UVB treatments, seems to accumulate both fructose and glucose. We can verify that IAC Milênio produces *myo*-inositol when submitted to stressful conditions, while IAC Imperador does not increase production of this compound.

<Figure 6>

It is likely that part of both fructose and glucose production was directed to the glycolysis pathway to form glyceraldehyde 3-P, pyruvic acid and acetyl-CoA. Acetyl-CoA is either shifted to the citric acid cycle, malonate or to mevalonate pathways. It seems that acetyl-CoA was used as a by-product of mevalonate to synthesize steroids to be deposited as wax constituents; this pattern was seen in both cultivars when submitted to UV-B radiation. In IAC Milênio, other products of the glycolysis pathway, such as phosphoenolpyruvate perhaps, together with erythrose 4-P from the pentose phosphate pathway, seem to shift to the shikimate

pathway to produce phenolic compounds when submitted to the stressful conditions. This kind of pattern was not seen in IAC Imperador which either slightly reduced or maintained the same quantity of phenolics.

In the malonate pathway some acetyl-CoA might be used to produce hexadecanoic acid in plants treated with UV-B radiation, e.g. in the case of IAC Imperador, or submitted to UV-B and UVB+D treatments as in IAC Milênio. While fatty acyl groups are the initial substrates in wax synthesis for either primary alcohols or alkanes (56), evidently, under the stressful UVB+D condition, the IAC Imperador cultivar switched the use of these fatty acyls from the acyl reduction pathway to the decarboxylation pathway. This shift was not observed in IAC Milênio which maintained the same proportion of both waxy compounds.

IAC Milênio may shift acetyl-CoA to the citric acid cycle instead of the malonate pathway, producing more carboxylic acids and amino acids, such as citric acid, succinic acid, aspartic acid, glutamic acid and GABA, when submitted to UV-B radiation, instead of investing in waxy alkanes and primary alcohols. Probably, in IAC Imperador, some acetyl-CoA was used as a product of the citric acid cycle to produce GABA in the UV-B light treatments, reducing the synthesis of other compounds of this cycle, such as malic, citric, succinic, and glutamic acids.

Apparently, IAC Milênio invests in phenolic compounds as well as carboxylic and amino acid synthesis as its main chemical defense against those stresses. Conversely, IAC Imperador, the cultivar with water-deficiency resistance, invests in synthesis of waxy constituents mainly under combined stresses, which reinforces the resilience of this cultivar.

CONCLUSION

In general, both cultivars showed more pronounced alterations under combined UV-B and drought (UVB+D) stress, than under UV-B and drought separately. Biomass and growth responses to drought alone were greater for IAC Milênio, which seemed to reallocate resources from shoots to roots possibly in order to optimize water absorption.

Under UVB+D the IAC Imperador cultivar invested resources in alkane synthesis at the expense of primary alcohols. This cultivar also invested in the synthesis of ribonic acid, a soluble sugar that can function to reduce osmotic potential under drought treatments. Both cultivars also invested in the production of GABA, a compound that is known for its antioxidant activity, in both UVB and UVB+D treatments. Neither cultivar showed an increase of phenolic compounds in seedlings irradiated with UV-B light.

Both cultivars showed a range of other characteristics to combat multiple stresses, increasing the synthesis of waxes and primary compounds and investing more in the root system. These changes were unidirectional for both cultivars, albeit with IAC Imperador having a greater degree of change in the composition of protective compounds against drought, such as alkanes and sucrose. This suggests that improved resistance of IAC Imperador to drought conditions is mediated through significant changes in its cellular metabolites. In contrast, IAC Milênio had greater degrees of change in its growth and biomass parameters, showing that both cultivars use different mechanisms to cope with unfavorable conditions.

In summary, both cultivars seem to acclimate to applied stresses. IAC Milênio has a more plastic body development response to the treatments than IAC Imperador, which appeared to have more constitutive resistance, with more pronounced changes in metabolite synthesis. Further studies are necessary to infer which physiological mechanisms and genetic expression are involved and if these can help to improve resilience in other crops.

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SUPPLEMENTARY MATERIALS

Table S1 and Table S2 can be found at DOI: 10.1562/2006-xxxxxx.s1.

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FIGURE CAPTIONS

Figure 1. Non-metric multidimensional scaling ordination plots of means of growth and biomass parameters and their 95% confidence interval ranges for *Phaseolus vulgaris* L. cultivars IAC Imperador (**Imp**) and IAC Milênio (**Mil**) submitted to four treatments (**C**: control, **D**: drought, **UVB**: enhanced UV-B radiation, and **UVB+D**: enhanced UV-B radiation plus drought).

Figure 2. Growth and biomass data from *Phaseolus vulgaris* L. cultivars IAC Imperador and IAC Milênio submitted to four treatments (**C**: control, **D**: drought, **UVB**: enhanced UV-B radiation, and **UVB+D**: UV-B plus drought). Data are presented as means $\pm$ standard deviation. For all treatments $n=10$, except UVB of Imperador ($n=9$) and UVB+D of Milênio ($n=6$). Data were compared among treatments for the same cultivar. Different letters represent significantly different means ($P < 0.05$). *indicates significant differences among groups that were not resolved in the *post-hoc* Dunn test.

Figure 3. Non-metric multidimensional scaling ordination plots of means and their 95% confidence interval ranges of a) Waxes, b) Primary Metabolites, and c) Phenolics in *Phaseolus vulgaris* L. cultivars IAC Imperador (**Imp**) and IAC Milênio (**Mil**) submitted to four treatments (**C**: control, **D**: drought, **UVB**: enhanced UV-B radiation, and **UVB+D**: UV-B plus drought). Means are depicted as squares (**Imp**) or circles (**Mil**).

Figure 4. Wax content and composition for leaves of *Phaseolus vulgaris* L. cultivars IAC Imperador and IAC Milênio submitted to four treatments (**C**: control, **D**: drought, **UVB**:

enhanced UV-B radiation, and **UVB+D**: UV-B plus drought). a) Wax content. Data are presented as the mean $\pm$ standard deviation. For all treatments $n=5$, except UVB-D of Milênio ($n=3$) Data were compared among treatments for the same cultivar. Different letters represent significantly different means ($P < 0.05$). b) Mean relative percentage of four major wax classes. NI = not identified.

Figure 5. Content of primary metabolites (μg of compound per g of fresh weight, $\mu\text{g g}^{-1}$ FW) of leaves from *Phaseolus vulgaris* L. cultivars IAC Imperador and IAC Milênio submitted to four treatments (**C**: control, **D**: drought, **UVB**: enhanced UV-B radiation, and **UVB+D**: UV-B plus drought). Data are presented as means $\pm$ standard deviation. For all treatments $n=5$, except UVB-D of Milênio ($n=4$). Data were compared among treatments for the same cultivar. Different letters represent significantly different means ($P < 0.05$). For each class, the following compounds of Table 2 were considered: total soluble sugars (compounds 11, 15, 16, 17, 18, 21, and 23), total amino acids (compounds 3, 5, 6, 7 and 8) and total carboxylic acid (compounds 1, 4, 9 and 14).

Figure 6. Biosynthesis scheme associated with the heatmap of changes in 13 metabolites and 6 classes of compound levels in leaves from *Phaseolus vulgaris* L. cultivars IAC Imperador and IAC Milênio in response to four treatments (**C**: control, **D**: drought, **UVB**: enhanced UV-B radiation, and **UVB+D**: UV-B plus drought). The $\log_2$ fold changes are shown in the heatmap results: Red indicates an increase in comparison to control, while blue indicates a decrease.

Table 1. Relative percentage of leaf wax compounds detected by GC-MS of two cultivars (IAC Imperador and IAC Milênio) of *Phaseolus vulgaris* L. submitted to four treatments. Imperador: control (C, n=5), drought (D, n=5), UV-B (UVB, n=5), and UV-B plus drought (UVB+D, n=5). Milênio: control (C, n=5), drought (D, n=5), UV-B (UVB, n=5), and UV-B plus drought (UVB+D, n=3).

Cultivar	Imperador				Milênio			
Treatment	C	D	UVB	UVB+D	C	D	UVB	UVB+D
<i>Total alkanes</i>	40.70 ± 10.61^b	52.25 ± 11.96^{ab}	61.26 ± 6.76^{ab}	87.50 ± 16.82^a	33.50 ± 10.95 ^a	33.36 ± 14.01 ^a	40.56 ± 15.53 ^a	20.12 ± 2.79 ^a
C ₂₄	0.00	0.00	2.49 ± 1.40	0.00	0.00	0.00	0.00	0.00
C ₂₅	0.00	0.00	4.73 ± 0.66	0.00	0.00	0.00	0.00	0.00
C ₂₆	0.00	0.00	5.99 ± 0.97	0.00	0.00	0.00	0.00	0.00
C ₂₇	1.58 ± 0.93	3.58 ± 2.25	8.87 ± 1.60	0.00	0.24 ± 0.53	1.72 ± 0.66	0.00	0.00
C ₂₈	1.17 ± 1.07	3.63 ± 2.41	9.48 ± 1.58	0.00	0.00	1.67 ± 0.60	0.00	0.00
C ₂₉	13.14 ± 1.82^b	20.22 ± 7.24^{ab}	13.25 ± 2.23^b	30.20 ± 6.32^a	17.79 ± 7.37 ^a	12.65 ± 4.64 ^a	17.81 ± 7.62 ^a	8.81 ± 1.49 ^a
C ₃₀	1.11 ± 1.02	3.38 ± 2.19	6.58 ± 1.11	0.00	0.00	1.60 ± 0.72	0.00	0.00
C ₃₁	18.78 ± 6.97^{ab}	17.26 ± 11.15^{ab}	9.27 ± 1.65^b	57.30 ± 12.93^a	14.62 ± 4.66 ^a	12.80 ± 5.72 ^a	22.76 ± 8.07 ^a	11.31 ± 1.34 ^a
C ₃₂	0.71 ± 0.97	0.00	0.57 ± 1.28	0.00	0.00	0.41 ± 0.91	0.00	0.00
C ₃₃	4.22 ± 2.47	4.17 ± 1.34	0.00	0.00	0.86 ± 1.38	2.51 ± 1.06	0.00	0.00
<i>Total primary alcohols</i>	57.63 ± 7.57^a	28.55 ± 22.99^{ab}	13.15 ± 8.81^{ab}	9.53 ± 15.71^b	66.50 ± 10.95 ^a	66.29 ± 13.97 ^a	40.06 ± 21.55 ^a	65.50 ± 6.52 ^a
C ₂₄	0.00	0.00	0.50 ± 1.13	0.00	0.00	0.00	0.57 ± 0.82	0.60 ± 1.04
C ₂₆	38.31 ± 5.44^a	18.62 ± 14.46^{ab}	7.03 ± 4.20^b	5.53 ± 9.11^b	44.58 ± 7.58^a	41.76 ± 11.73^{ab}	22.40 ± 10.15^b	40.84 ± 5.94^{ab}
C ₂₈	19.32 ± 2.81^a	9.52 ± 8.24^{ab}	5.04 ± 3.18^{ab}	4.00 ± 6.60^b	20.31 ± 5.06 ^a	22.60 ± 3.77 ^a	14.14 ± 8.12 ^a	22.45 ± 1.74 ^a
C ₃₀	0.00	0.40 ± 0.90	0.58 ± 1.30	0.00	0.75 ± 1.04	1.26 ± 0.79	1.64 ± 2.27	1.62 ± 1.42
C ₃₂	0.00	0.00	0.00	0.00	0.85 ± 1.27	0.68 ± 1.00	1.32 ± 1.88	0.00
<i>Hexadecanoic acid</i>	0.00	0.00	0.56 ± 1.25	0.00	0.00	0.00	0.47 ± 1.05	2.10 ± 1.82
<i>Total steroids</i>	0.00	0.00	2.96 ± 4.16	2.23 ± 4.99	0.00	0.00	6.41 ± 4.24	5.85 ± 0.67
Stigmasterol	0.00	0.00	1.32 ± 1.83	2.23 ± 4.99	0.00	0.00	2.92 ± 1.85	1.98 ± 1.74
β-sitosterol	0.00	0.00	1.64 ± 2.33	0.00	0.00	0.00	3.49 ± 3.33	3.86 ± 1.36
<i>Not identified</i>	1.67 ± 3.74	19.20 ± 23.40	22.08 ± 7.88	0.74 ± 1.01	0.00	0.35 ± 0.78	12.49 ± 15.87	9.53 ± 5.09

Data are presented as mean ± standard deviation. For all treatments n = 5, except UVB+D of Milênio (n=4). Data were compared among treatments for the same cultivar. Different letters in a row indicate statistically significant differences (*P* < 0.05), with bold font indicating classes where significant differences were found. All *p* values are in Table S1 of supplementary material.

Table 2. Primary metabolites analyzed by GC-MS in leaves from two cultivars (IAC Imperador and IAC Milênio) of *Phaseolus vulgaris* L.

Peak	RT (min)	Probable compounds	Match (NIST02)	LRI	LRI (Nagai et al. 2017)	LRI (Mass bank)	LRI (GMD)
1	17.723	Succinic acid (2TMS)	937	1313.4	1314.1	1303.7	1310.7
2	20.569	NI	-	1421.1	-	-	-
3	20.610	Aspartic acid (2TMS)	896	1422.7	1423.9	-	1422.4
4	22.198	Malic acid (3TMS)	935	1485.6	1484.9	1478.0	1479.3
5	23.023	5-oxoproline (2TMS)	909	1519.3	1519.7	1519.7	1521.7
6	23.157	GABA (3TMS)	866	1524.9	1524.5	1526.6	1527.5
7	23.315	Glutamic acid (2TMS)	784	1531.5	1531.4	-	1528.2
8	25.319	Glutamic acid (3TMS)	-	1616.03	-	1607.8	1614.6
9	25.684	Tartaric acid (4TMS)	929	1632.1	-	1620.9	1628.9
10	27.544	Ribitol (5TMS) - internal standard	917	1715.0	1715.8	1710.9	1712.7
11	28.406	Ribonic acid (5TMS)	741	1755.0	-	-	1751.5
12	28.536	NI	-	1761.1	-	-	-
13	28.846	NI	-	1775.5	-	-	-
14	29.616	Citric acid (4TMS)	906	1811.9	1811.3	1803.9	1803.9
15	30.619	Fructose <i>O</i> -methyloxime (5TMS) ¹	903	1860.9	1861.3	1855.5	1853.9
16	30.820	Fructose <i>O</i> -methyloxime (5TMS) ¹	875	1870.7	1870.5	1864.3	1863.1
17	31.123	Glucose <i>O</i> -methyloxime (5TMS) ²	948	1885.5	1885.4	1881.6	1880.5
18	31.497	Glucose <i>O</i> -methyloxime (5TMS) ²	907	1904.0	1903.6	1898.5	1902.4
19	31.969	NI	-	1928.1	-	-	-
20	32.665	NI	-	1963.8	-	-	-
21	34.826	<i>Myo</i> -inositol (6TMS)	922	2077.7	2076.0	2083.6	2080.2
22	38.753	NI	-	2199.8	-	-	-
23	43.762	Sucrose (8TMS)	880	2614.7	-	2614.8	2623.0

RT: retention time. Match: NIST02 library. LRI: linear retention index. GMD: Golm Metabolome Database.

-: linear retention index not found in the database.

NI: not identified. TMS: trimethylsilyl. GABA: gamma-aminobutyric acid.

¹ Fructose stereoisomers produced by methoximation reaction.

² Glucose stereoisomers produced by methoximation reaction.

Table 3. Concentration of primary metabolites (µg of compound/g of fresh plant, µg g⁻¹ FW) detected by GC-MS in leaves of *Phaseolus vulgaris* L. cultivar IAC Imperador submitted to four treatments: control (C, n=5), drought (D, n=5), UV-B (UVB, n=5), and UV-B plus drought (UVB+D, n=5).

Peak	Probable compounds	Treatments			
		C	D	UVB	UVB+D
1	Succinic acid (2TMS)	11.81 ± 26.41	9.20 ± 20.58	0.00	34.00 ± 34.17
2	NI	91.60 ± 43.50 ^a	64.78 ± 7.21 ^a	114.17 ± 91.60 ^a	112.94 ± 21.90 ^a
3	Aspartic acid (2TMS)	0.00	0.00	0.00	47.60 ± 5.36
4	Malic acid (3TMS)	559.66 ± 215.99^{ab}	413.56 ± 27.51^b	249.18 ± 262.49^b	867.98 ± 243.80^a
5	5-oxoproline (2TMS)	90.95 ± 22.83^{ab}	120.44 ± 46.31^a	26.91 ± 37.94^b	84.88 ± 24.69^{ab}
6	GABA (3TMS)	0.00	0.00	18.85 ± 26.44	27.83 ± 45.44
7-8	Glutamic acid ¹	92.14 ± 20.35^{ab}	133.39 ± 47.52^a	23.87 ± 36.07^b	147.72 ± 42.31^a
9	Tartaric acid (4TMS)	7.63 ± 17.06	24.26 ± 22.94	0.00	85.30 ± 90.85
11	Ribonic acid (5TMS)	0.00	5.11 ± 11.43	0.00	32.29 ± 4.38
12	NI	38.72 ± 54.24	66.51 ± 61.84	0.00	62.33 ± 60.45
13	NI	158.20 ± 97.57^b	217.89 ± 38.74^{ab}	259.59 ± 57.87^{ab}	309.19 ± 82.39^a
14	Citric acid (4TMS)	69.53 ± 47.24	116.26 ± 57.96	0.00	221.57 ± 111.07
15-16	Fructose <i>O</i> -methyloxime (5TMS) ¹	267.76 ± 163.11 ^a	197.31 ± 162.97 ^a	152.75 ± 69.00 ^a	168.65 ± 61.06 ^a
17-18	Glucose <i>O</i> -methyloxime (5TMS) ²	517.10 ± 506.79 ^a	236.16 ± 230.44 ^a	226.91 ± 90.82 ^a	335.36 ± 217.22 ^a
19	NI	0.00	0.00	19.87 ± 44.43	94.65 ± 20.45
20	NI	306.72 ± 73.67 ^a	307.50 ± 107.59 ^a	263.61 ± 45.87 ^a	361.98 ± 79.47 ^a
21	<i>Myo</i> -inositol (6TMS)	939.06 ± 259.57 ^a	843.76 ± 283.97 ^a	718.99 ± 71.18 ^a	1098.09 ± 210.07 ^a
22	NI	0.00	13.47 ± 30.11	126.19 ± 97.34	184.27 ± 228.34
23	Sucrose (8TMS)	672.26 ± 534.17 ^a	905.05 ± 353.53 ^a	838.21 ± 147.71 ^a	1377.96 ± 333.25 ^a

NI: not identified. TMS: trimethylsilyl. GABA: gamma-aminobutyric acid.
Data are presented as mean ± standard deviation.
Only compounds with letters were statistically analyzed. Different letters within a row indicate statistically significant differences (*P* < 0.05).
¹ Glutamic acid content corresponds to the sum of glutamic acid 2TMS and glutamic acid 3TMS.
² Fructose content corresponds to the sum of the two stereoisomers.
³ Glucose content corresponds to the sum of the two stereoisomers.
All *p* values are in Table S1 of supplementary material.

Table 4. Concentration of primary metabolites (μg of compound/g of fresh plant, $\mu\text{g g}^{-1}$ FW) detected by GC-MS in leaves of *Phaseolus vulgaris* L. cultivar IAC Milênio submitted to four treatments: control (C, n=5), drought (D, n=5), UVB (UVB, n=5), and UVB plus drought (UVB+D, n=4).

Peak	Probable compounds	Treatments			
		C	D	UVB	UVB+D
1	Succinic acid (2TMS)	7.59 $\pm$ 16.98 ^a	16.80 $\pm$ 23.11 ^a	29.64 $\pm$ 44.38 ^a	41.90 $\pm$ 1.88 ^a
2	NI	97.29 $\pm$ 47.44 ^a	76.56 $\pm$ 42.22 ^a	60.94 $\pm$ 23.31 ^a	77.16 $\pm$ 33.93 ^a
3	Aspartic acid (2TMS)	0.00	0.00	11.79 $\pm$ 26.37	32.23 $\pm$ 40.78
4	Malic acid (3TMS)	664.14 $\pm$ 266.81 ^a	764.66 $\pm$ 496.43 ^a	580.09 $\pm$ 165.99 ^a	771.78 $\pm$ 287.82 ^a
5	5-oxoproline (2TMS)	78.75 $\pm$ 65.57 ^a	74.88 $\pm$ 19.26 ^a	57.10 $\pm$ 38.07 ^a	66.66 $\pm$ 13.93 ^a
6	GABA (3TMS)	0.00	0.00	17.27 $\pm$ 23.78	37.51 $\pm$ 30.00
7-8	Glutamic acid ¹	23.12 $\pm$ 32.17 ^a	40.81 $\pm$ 38.64 ^a	80.52 $\pm$ 63.02 ^a	36.96 $\pm$ 44.59 ^a
9	Tartaric acid (4TMS)	74.14 $\pm$ 39.75 ^a	39.83 $\pm$ 62.80 ^a	43.00 $\pm$ 30.11 ^a	58.01 $\pm$ 16.69 ^a
11	Ribonic acid (5TMS)	5.73 $\pm$ 12.82 ^a	6.05 $\pm$ 13.53 ^a	19.76 $\pm$ 18.06 ^a	16.23 $\pm$ 18.79 ^a
12	NI	0.00	0.00	0.00	16.15 $\pm$ 36.11
13	NI	112.50 $\pm$ 106.33 ^a	172.60 $\pm$ 73.04 ^a	233.94 $\pm$ 79.13 ^a	215.92 $\pm$ 36.28 ^a
14	Citric acid (4TMS)	39.14 $\pm$ 37.57 ^a	34.76 $\pm$ 47.76 ^a	72.48 $\pm$ 50.38 ^a	115.41 $\pm$ 85.60 ^a
15-16	Fructose <i>O</i> -methyloxime (5TMS) ²	175.45 $\pm$ 138.98 ^a	247.80 $\pm$ 101.96 ^a	131.42 $\pm$ 107.01 ^a	225.38 $\pm$ 187.54 ^a
17-18	Glucose <i>O</i> -methyloxime (5TMS) ³	172.23 $\pm$ 156.01 ^a	365.65 $\pm$ 152.09 ^a	154.46 $\pm$ 139.35 ^a	424.43 $\pm$ 475.73 ^a
19	NI	0.00	0.00	39.00 $\pm$ 54.12	43.19 $\pm$ 51.18
20	NI	213.17 $\pm$ 72.34 ^a	232.30 $\pm$ 15.34 ^a	193.47 $\pm$ 33.36 ^a	254.75 $\pm$ 117.48 ^a
21	<i>Myo</i> -inositol (6TMS)	421.55 $\pm$ 269.31 ^a	981.20 $\pm$ 415.90 ^a	959.36 $\pm$ 336.28 ^a	980.58 $\pm$ 348.82 ^a
22	NI	0.00	0.00	83.28 $\pm$ 119.70	140.53 $\pm$ 163.72
23	Sucrose (8TMS)	419.46 $\pm$ 155.55 ^b	646.77 $\pm$ 269.63 ^{ab}	619.34 $\pm$ 150.05 ^{ab}	1320.27 $\pm$ 759.66 ^a

NI: not identified. TMS: trimethylsilyl. GABA: gamma-aminobutyric acid.

Data are presented as mean $\pm$ standard deviation.

Only compounds with letters were statistically analyzed. Different letters within a row indicate statistically significant differences ($P < 0.05$).

¹ Glutamic acid content corresponds to the sum of glutamic acid 2TMS and glutamic acid 3TMS.

² Fructose content corresponds to the sum of the two stereoisomers.

³ Glucose content corresponds to the sum of the two stereoisomers.

All p values are in Table S1 of supplementary material.

Figure 1. Non-metric multidimensional scaling ordination plots of means of growth and biomass parameters and their 95% confidence interval ranges for *Phaseolus vulgaris* L. cultivars IAC Imperador (Imp) and IAC Milênio (Mil) submitted to four treatments (C: control, D: drought, UVB: enhanced UV-B radiation, and UVB+D: enhanced UV-B radiation plus drought).

79x39mm (300 x 300 DPI)

Figure 2. Growth and biomass data from *Phaseolus vulgaris* L. cultivars IAC Imperador and IAC Milênio submitted to four treatments (C: control, D: drought, UVB: enhanced UV-B radiation, and UVB+D: UV-B plus drought). Data are presented as means $\pm$ standard deviation. For all treatments $n=10$, except UVB of Imperador ($n=9$) and UVB+D of Milênio ($n=6$). Data were compared among treatments for the same cultivar. Different letters represent significantly different means ($P < 0.05$). *indicates significant differences among groups that were not resolved in the post-hoc Dunn test.

189x199mm (300 x 300 DPI)

Figure 3. Non-metric multidimensional scaling ordination plots of means and their 95% confidence interval ranges of a) Waxes, b) Primary Metabolites, and c) Phenolics in *Phaseolus vulgaris* L. cultivars IAC Imperador (Imp) and IAC Milênio (Mil) submitted to four treatments (C: control, D: drought, UVB: enhanced UV-B radiation, and UVB+D: UV-B plus drought). Means are depicted as squares (Imp) or circles (Mil).

80x109mm (300 x 300 DPI)

Figure 4. Wax content and composition for leaves of *Phaseolus vulgaris* L. cultivars IAC Imperador and IAC Milênio submitted to four treatments (C: control, D: drought, UVB: enhanced UV-B radiation, and UVB+D: UV-B plus drought). a) Wax content. Data are presented as the mean $\pm$ standard deviation. For all treatments $n=5$, except UVB-D of Milênio ($n=3$). Data were compared among treatments for the same cultivar. Different letters represent significantly different means ($P < 0.05$). b) Mean relative percentage of four major wax classes. NI = not identified.

87x109mm (300 x 300 DPI)

Figure 5. Content of primary metabolites (μg of compound per g of fresh weight, $\mu\text{g g}^{-1}$ FW) of leaves from Phaseolus vulgaris L. cultivars IAC Imperador and IAC Milênio submitted to four treatments (C: control, D: drought, UVB: enhanced UV-B radiation, and UVB+D: UV-B plus drought). Data are presented as means $\pm$ standard deviation. For all treatments $n=5$, except UVB-D of Milênio ($n=4$). Data were compared among treatments for the same cultivar. Different letters represent significantly different means ($P < 0.05$). For each class, the following compounds of Table 2 were considered: total soluble sugars (compounds 11, 15, 16, 17, 18, 21, and 23), total amino acids (compounds 3, 5, 6, 7 and 8) and total carboxylic acid (compounds 1, 4, 9 and 14).

Figure 6. Biosynthesis scheme associated with the heatmap of changes in 13 metabolites and 6 classes of compound levels in leaves from *Phaseolus vulgaris* L. cultivars IAC Imperador and IAC Milênio in response to four treatments (C: control, D: drought, UVB: enhanced UV-B radiation, and UVB+D: UV-B plus drought). The log₂ fold changes are shown in the heatmap results: Red indicates an increase in comparison to control, while blue indicates a decrease.

35x59mm (300 x 300 DPI)