Identifying Bioactive Compounds in Common Bean (*Phaseolus vulgaris* L.) Plants under Water Deficit Conditions

Maria José Gómez-Bellot†, Lilisbet Guerrero ‡, José Enrique Yuste §, Fernando Vallejo ‡ and María Jesús Sánchez-Blanco †,*

1 Department of Irrigation, CEBAS-CSIC, University Campus of Espinardo–Edif. 25, 30100 Espinardo, Murcia, Spain; mjgb@cebas.csic.es (M.J.G.-B.); lilig396@gmail.com (L.G.)
2 Metabolomics Platform, CEBAS-CSIC, University Campus of Espinardo–Edif. 25, 30100 Espinardo, Murcia, Spain; jyuste@cebas.csic.es (J.E.Y.); fvallejo@cebas.csic.es (F.V.)
* Correspondence: quechu@cebas.csic.es; Tel.: +34-968-396-200 (ext. 445318)

Abstract: Deficit irrigation (DI) strategies are becoming increasingly common in areas where water resources are limited. The application of moderate levels of DI can result in water savings with a small reduction in yield but with a higher quality of the product. The aim of this work was to evaluate the effect of applying a certain level of water deficit (40% water holding capacity) on the yield and quality of the common bean (*Phaseolus vulgaris* L.), specifically the cultivar ‘Triunfo-70’. Bioactive compounds were investigated by applying an LC-MS-based untargeted metabolomics approach as an analytical tool for identifying novel markers associated with a water deficit in beans. The results showed that beans harvested 30 days after DI application experienced water stress, as indicated by the decrease in the leaf water potential and gas exchange values (stomatal conductance and photosynthesis). In addition, the number of pods per plant was significantly reduced by the DI treatment. The water deficit induced significant alterations in various bioactive compounds (including organic acids, polyphenols, hydroxybenzoic acids, lipids, and phospholipids) when compared to the control treatment. Additionally, twelve new biomarkers were identified in this study for the first time in the common bean under DI. These findings suggested that DI acted as an elicitor, increasing phenylpropanoid metabolism, while concurrently reducing the production of compounds associated with fatty acid metabolism. Additionally, new metabolites were tentatively identified in common beans. This study represents the successful application of the untargeted metabolomics approach to finding bioactive secondary metabolites in beans under different irrigation conditions.

Keywords: irrigation; beans; untargeted metabolomics; LC-MS

1. Introduction

When water becomes scarce or expensive, producers may choose to implement a stress management approach to reduce their water consumption [1]. Deficit irrigation (DI) strategies are increasingly common in areas where water resources are limited [2]. In addition, the application of DI leads to greater water savings with a small reduction in yield but with a higher product quality [3]. DI consists of applying a quantity of irrigation water lower than the total water requirement of the crop at some growth stages or sometimes during the whole growing season [4]. Therefore, under DI, plants are subjected to a certain degree of water stress, which tends to have lower evapotranspiration. In some cases, this leads to the development of certain water stress symptoms such as leaf wilting, reduced leaf area, and stunted growth and changes in physiological processes, such as stomatal conductance, leaf water potential, photosynthesis, and leaf temperature [5]. Hence, it is useful to measure indicators related to the water status of the plant and other processes that are highly sensitive to a water deficit [6]. In general, the application of a DI strategy in crops resulted in a yield reduction due to smaller fruit size and weight, but quality indices such
as sugars, ascorbic acid, and anthocyanin content increased due to water reductions [7,8]. Crops such as melons, cucumbers, and tomatoes have demonstrated higher water use efficiency without much loss of yield but with higher product quality. In this sense, Alipour and Amini [9] reported that applying 80% of the water required for the common bean can lead to higher water productivity compared to the full water requirement. When irrigation was restored, there was an increase in the yield, number of pods per plant, number of seeds in each pod, and plant height, but grain protein decreased. In fact, ID increased the protein concentration in red beans and was parallel to an increase in the grain’s starch–protein ratio [10].

The Fabaceae family contains about 70 plant species, including Phaseolus (frijolus, wild bean), that are all native to the Americas, mainly Mesoamerica. Phaseolus is one of the most economically important legume genera. Among the domesticated species is the common bean, *P. vulgaris*, which is now cultivated worldwide in tropical, semi-tropical, and temperate climates [11,12]. The common bean (*Phaseolus vulgaris* L.) cultivar “Triunfo-70” has generated great interest as a functional food because its consumption has been associated with positive effects on human health. Different studies have shown that the reduction in cardiovascular disease, diabetes, and obesity through the consumption of bean seeds is associated with the phenolic compounds present in these seeds [13–15], and they have been classified in the top 10 common vegetables in relation to antioxidant content and activity [16,17].

Metabolomics is a rapidly emerging field aiming to identify and quantify cellular low-molecular-weight molecules (metabolites). Together with genomics, transcriptomics and proteomics, metabolomics provides valuable insights into the composition of organisms. Mass spectrometry-based metabolomics approaches can enable the detection and quantification of many thousands of metabolite features simultaneously because of wide analyte coverage, high sensitivity, high selectivity, and high throughput [18].

Untargeted metabolomic methods are designed to generate a global picture of the metabolome, providing for the simultaneous measurement of numerous metabolites from biological samples without bias. The objective is the identification of metabolites to discriminate between treatments and predict class memberships [19]. In order to enable the large-scale determination of unknown compounds, the use of high-throughput analytical techniques, such as high-resolution mass spectrometry (UHPLC-QTOF), is essential [19].

Common beans are rich in proteins, fiber, phenolic compounds, and other bioactive molecules that could be separated and processed to obtain value-added ingredients with biological potential. Due to this relevance, the effect of different irrigation conditions of bean cultures on bioactive metabolite accumulation through an LC-MS-based “untargeted metabolomics” approach was assessed. To the best of our knowledge, no information is available since this study is the first to describe an untargeted metabolomics approach to identify bioactive secondary metabolites from common beans to discriminate among different irrigation conditions.

Consequently, the objective was to evaluate the physiological and biochemical responses of deficit irrigation in *Phaseolus vulgaris* L. to assess whether a certain degree of water deficit could enhance the quality yield and the total bioactive metabolites.

2. Materials and Methods

2.1. Plant Material and Experiment Conditions

The experiment was performed on seeds of *Phaseolus vulgaris* L., cultivar “Triunfo-70”, collected from a nursery. In order to obtain the plants, two seeds per socket were planted 5 mm deep in a polystyrene tray to ensure germination and were placed in a controlled growth chamber. The temperature inside the growth chamber was 24 °C during light conditions and 18 °C during dark conditions, with a relative humidity of 60%, a photosynthetic photon flux density of 350 µmol m$^{-2}$ s$^{-1}$, and photoperiod of 16/8 h (light/dark). Two weeks later, 60 seedlings were transplanted into plastic pots (1.5 L) filled with a commercial soilless substrate composed of peat, coconut fiber, and perlite (67/30/3,
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After transplantation, 2 g L\(^{-1}\) of Osmocote Plus (14:13:13 N, P, K plus microelements) was applied to the plants. All the plants were irrigated manually three times per week using tap water with an electrical conductivity of 1.0 dS m\(^{-1}\). The field capacity of the substrate was calculated according to Álvarez and Sánchez-Blanco [20]. Each pot was weighed before each irrigation event, and the volume of irrigation water required to refill the pot to its threshold level was calculated and added to each plant. Twelve days after transplantation, two irrigation treatments were applied: control irrigation at 100% water-holding capacity and deficit irrigation at 40% water-holding capacity. The duration of the experiment was 30 days from the beginning of the treatments. The experimental plot consisted of two treatments with three replicates per treatment. Therefore, there were thirty plants per treatment and ten plants per replicate.

2.2. Water Status Indicators

The leaf water potential (\(\Psi_{\text{leaf}}\)) was measured at 4 and 30 DAT (days after the application of treatments) in six plants per treatment (two plants per replication). The leaf water status was measured by collecting mature leaves and placing them in a pressure chamber (Model 3000; Soil Moisture Equipment Co., Santa Barbara, CA, USA) according to Scholander et al. [21] within 20 s of collection and pressurizing them at a rate of 0.02 MPa s\(^{-1}\) [22].

The leaf photosynthetic rate (\(P_n\)) and stomatal conductance (\(g_s\)) were measured at 4 and 30 DAT in six plants per treatment (two plants per replication) using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA). The reference of CO\(_2\) was set at 400 ppm, the photosynthetically active radiation (PAR) was at 1500 \(\mu\)mol m\(^{-2}\) s\(^{-1}\), and the speed of the circulating airflow inside the system was set at 500 \(\mu\)mol s\(^{-1}\).

2.3. Plant Yield and Nutritional Content

At the end of the experiment, 30 DAT, the pod yield (expressed as dry weight of pods per plant, number of pods per plant, and number of seeds per pod) was determined for six plants per treatment (two plants per replication). The inorganic mineral content of the dry beans was determined for nine plants per treatment (three samples per replication) by means of emission spectrophotometry at the end of the experiment. The samples were placed in an oven at 80 °C for drying. Then, the samples were ground and sieved through a 2 mm nylon mesh. The nutrient concentrations of the samples were determined in an extract digested with HNO\(_3\):HClO\(_4\) (2:1, \(v/v\)) using an inductively coupled plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL, Thermo Fisher Scientific, Markham, ON, Canada).

2.4. Untargeted Metabolomics Analysis by UPLC-QTOF

Lyophilized samples were weighed (200 mg) and extracted using 500 \(\mu\)L of 80/20 aqueous:methanol (\(v/v\)) solvent. Then, the samples were sonicated for 30 min in an ultrasonic bath, centrifuged at 11,500 \(\times\) g for 15 min, and filtered through a 0.45 \(\mu\)m filter (Agilent Technologies, Santa Clara, CA, USA). The samples were injected into a reverse-phase Poroshell 120 EC-C18 column (Agilent Technologies, Santa Clara, CA, USA), according to the operating conditions that were previously described [23] Detection was performed in both positive and negative ionization modes, and the data were acquired using Mass Hunter Workstation software (version B.08.00, Service Pack 1, Agilent). Feature extraction statistical analysis and tentative identification were carried out according to our previous publications [23–26].

2.5. Statistical Analyses

In the experiment, plants (n = 60) were randomly allocated to each treatment. There were 30 plants per treatment, with 3 replications per treatment. Each replication consisted of 10 plants placed in one tray. The data from Figures 1–3 and Table 1 were analyzed using
the Student’s $t$-test to verify whether there were significant differences using IBM SPSS Statistics 25. A value of $p < 0.05$ was considered to be statistically significant.

![Figure 1](image1.png)

**Figure 1.** Leaf water potential ($\Psi_{\text{leaf}}$) in bean plants submitted through control and deficit irrigation treatments. Different lowercase letters indicate significant differences between treatments according to the Student’s $t$-test.

![Figure 2](image2.png)

**Figure 2.** Net photosynthetic rate ($P_n$) (A) and stomatal conductance ($g_s$) (B) of bean plants irrigated through control and deficit irrigation treatments. Different lowercase letters indicate significant differences between treatments according to the Student’s $t$-test.
Figure 2. Net photosynthetic rate (Pn) (A) and stomatal conductance (gs) (B) of bean plants irrigated through control and deficit irrigation treatments. Different lowercase letters indicate significant differences between treatments according to the Student’s t-test.

Figure 3. Dry weight of pods per plant (A), number of pods per plant (B), and number of seeds per pod (C) in bean plants irrigated through control and deficit irrigation treatments. Different lowercase letters indicate significant differences between treatments according to the Student’s t-test.

Table 1. Mineral concentration of pods in bean plants irrigated through control and deficit irrigation treatments. Values are means ± SEM (n = 9 plants).

<table>
<thead>
<tr>
<th>Mineral Content</th>
<th>Control</th>
<th>DI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (g/100 g)</td>
<td>2873.8 ± 452.6</td>
<td>2113.7 ± 333.1</td>
<td>ns</td>
</tr>
<tr>
<td>Cu (mg/Kg)</td>
<td>2.59 ± 0.45</td>
<td>3.21 ± 0.27</td>
<td>ns</td>
</tr>
<tr>
<td>Fe (mg/Kg)</td>
<td>65.83 ± 5.21</td>
<td>75.93 ± 4.12</td>
<td>ns</td>
</tr>
<tr>
<td>K (g/100 g)</td>
<td>17,905.3 ± 1615.6</td>
<td>19,820.7 ± 480.1</td>
<td>ns</td>
</tr>
<tr>
<td>Mg (g/100 g)</td>
<td>1847.4 ± 113.5</td>
<td>1994 ± 94.23</td>
<td>ns</td>
</tr>
<tr>
<td>Na (g/100 g)</td>
<td>22.17 ± 3.19</td>
<td>25.54 ± 3.88</td>
<td>ns</td>
</tr>
<tr>
<td>P (g/100 g)</td>
<td>3885.7 ± 231.0</td>
<td>4456.8 ± 59.9</td>
<td>a *</td>
</tr>
<tr>
<td>Zn (mg/Kg)</td>
<td>36.53 ± 2.61</td>
<td>42.75 ± 2.05</td>
<td>a *</td>
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Table 1. Cont.

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<td>Control</td>
</tr>
<tr>
<td>N (mg/kg)</td>
<td>27,276.8 ± 793.7</td>
</tr>
<tr>
<td>C (mg/Kg)</td>
<td>431,193.1 ± 2032.2</td>
</tr>
<tr>
<td>C/N</td>
<td>15.88 ± 0.42</td>
</tr>
</tbody>
</table>

Different lowercase letters indicate significant differences between treatments according to the Student’s t-test. P, probability level: * p ≤ 0.05; p > 0.05 non-significant differences are indicated by “ns”.

As previously reported by Tomás-Navarro et al. [24], the data matrix, feature extraction, deconvolution, and alignment were carried out on an Agilent Profinder B.06.00 (Agilent Technologies, Waldbronn, Germany). Finally, the MassHunter MSC (Molecular Structure Correlator, Agilent Technologies, Waldbronn, Germany) program was used to correlate MS/MS fragment ions with the proposed molecular structures for that compound.

According to previous publications [23–26], pre-processed CEF files were exported into the Mass Profiler Professional (MPP) software package (revision B.14.09.01, Agilent Technologies, Santa Clara, CA, USA) for statistical analysis. The final list of features was used for metabolite identification with purchased METLIN databases and according to the exact mass [26].

In order to find distinctive differences in the irrigation variable, a data matrix with two groups was created. The full data matrix was based on 1260 entities. A PLS-DA model was created to evaluate the classification of the samples into groups (Figure 4). The calculated PLS-DA model, based on 8 samples and 3 components, described 99.6% of the variance ($R^2 = 0.991$) according to the cross-validation prediction of $Q^2 = 0.601$. The first principal component, PC1, explained 89.02% of the total variability, demonstrating that the differences in the sample metabolomes might be affected by the irrigation. Additionally, the VIP (variable importance in projection) score was used to measure the importance of the entities, resulting in a filtered list of 179 entities according to a VIP > 1.

Figure 4. PLS-DA model of full dataset. (i) Red dot: control samples; (ii) yellow dot: samples under irrigation condition (DI).
**3. Results**

### 3.1. Leaf Water Potential and Gas Exchange

At 4 days after the application of the treatments (DAT), the plants subjected to DI treatment showed a non-significant decrease of 8% in the leaf water potential compared to those plants subjected to the control treatment, while at 30 DAT, a significant decrease of 57% was observed in the same plants (Figure 1).

At 4 DAT, a non-significant reduction of 20% in the net photosynthetic rate and stomatal conductance was observed in the plants subjected to DI compared to the control treatment (Figure 2). At 30 DAT, the net photosynthetic rate values of the plants irrigated with DI decreased to half of those from the control treatment (Figure 2A), while the stomatal conductance decreased by 57%, showing statistical differences between the treatments (Figure 2B).

### 3.2. Plant Yield and Nutritional Content

At 30 DAT, the dry weight of the pods was reduced only numerically by the DI treatment without significant differences (Figure 3A). The number of pods per plant was significantly reduced by the DI treatment (Figure 3B), while the number of seeds per plot showed similar values between both treatments (Figure 3C). At 30 DAT, the concentrations of Ca, Cu, Fe, Ca, Mg, Na, C, N, and C/N in the pods were not modified by the DI treatment, while the P and Zn concentrations were higher in the pods from DI treatment (Table 1).

### 3.3. Metabolite Identification

The univariate statistic layer was applied after the multivariate analysis. A total number of 179 metabolites were statistically significant (T-test unpaired; corrected p-value cut-off: 0.05; p-value computation: Asymptotic; Multiple Testing Correction: Benjamini-Hochberg) (Figure 5). The databases showed a list of 14 metabolites that were tentatively identified [27] unregulated between both treatments (Table 2). Among them, 12 new biomarkers (3–14, Table 1) were identified for the first time in beans under DI treatments. The metabolites tentatively identified belong mainly to the classes of dihydrofurans (1), hydroxybenzoic acids (2), polyphenols (3–6), lipids, and phospholipids (7–14).

#### Table 2. Compounds tentatively identified in DI versus control.

<table>
<thead>
<tr>
<th>ID</th>
<th>Compound Name</th>
<th>Formula</th>
<th>RT (min)</th>
<th>Ionization</th>
<th>Mass (ppm)</th>
<th>Error (ppm)</th>
<th>Fragments</th>
<th>Regulation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ascorbic acid</td>
<td>C6H8O6</td>
<td>1.3</td>
<td>[M–H]−</td>
<td>175.0240</td>
<td>1.2</td>
<td>UP</td>
<td>HMDB0000044</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2,4-Dihydroxybenzoic acid</td>
<td>C7H6O4</td>
<td>2.3</td>
<td>[M–H]−</td>
<td>153.0188</td>
<td>1.7</td>
<td>109.0295</td>
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<tr>
<td>3</td>
<td>Dihydromyricetin</td>
<td>C15H20O8</td>
<td>4.8</td>
<td>[M–H]−</td>
<td>319.0452</td>
<td>−0.4</td>
<td>301.0354</td>
<td>UP</td>
<td>HMDB0308822</td>
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<tr>
<td>4</td>
<td>Cyanidin 3-(4-acetylglucoside)</td>
<td>C22H23O12</td>
<td>4.9</td>
<td>[M–H]−</td>
<td>490.1115</td>
<td>−0.7</td>
<td>221.0061</td>
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<tr>
<td>5</td>
<td>7,3′,4′-Trihydroxyflavone</td>
<td>C16H16O5</td>
<td>6.5</td>
<td>[M–H]−</td>
<td>269.0449</td>
<td>−1.3</td>
<td>213.0188</td>
<td>UP</td>
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<tr>
<td>6</td>
<td>Kaempferol 4′-rhamnoside</td>
<td>C21H20O10</td>
<td>6.5</td>
<td>[M–H]−</td>
<td>431.0978</td>
<td>−1.6</td>
<td>285.0399</td>
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<td>7</td>
<td>9,12,13,TriHODE</td>
<td>C18H20O5</td>
<td>8.1</td>
<td>[M–H]−</td>
<td>327.2165</td>
<td>1.1</td>
<td>211.1329</td>
<td>DOWN</td>
<td>LMFA02000220</td>
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<tr>
<td>8</td>
<td>PC(5:0/5:0)</td>
<td>C18H35NO5P</td>
<td>8.3</td>
<td>[M–H]+</td>
<td>426.2254</td>
<td>−0.9</td>
<td>339.1214</td>
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<td>9</td>
<td>Sebacic acid</td>
<td>C10H16O4</td>
<td>8.4</td>
<td>[M–H]−</td>
<td>201.1130</td>
<td>1.2</td>
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<td>10</td>
<td>9,10-Dihydroxy-8-oxo-12- octadecenoic acid</td>
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<td>[M–H]−</td>
<td>327.2171</td>
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<td>11</td>
<td>Kudzusaponin SA1</td>
<td>C24H32O15</td>
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<td>[M–H]−</td>
<td>811.4478</td>
<td>1.3</td>
<td>473.3636</td>
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<td>12</td>
<td>Dodecanedioic acid</td>
<td>C12H22O4</td>
<td>10.4</td>
<td>[M–H]−</td>
<td>229.1440</td>
<td>0.5</td>
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<tr>
<td>13</td>
<td>Jasmonic acid</td>
<td>C_{12}H_{18}O_3</td>
<td>10.7</td>
<td>[M–H]^-</td>
<td>209.1178</td>
<td>2.1</td>
<td>165.1279</td>
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<tr>
<td>14</td>
<td>5,8,12-Trihydroxy-9-octadecenoic acid</td>
<td>C_{18}H_{34}O_5</td>
<td>10.9</td>
<td>[M–H]^-</td>
<td>329.2336</td>
<td>0.2</td>
<td>311.2211</td>
<td>DOWN</td>
<td>LMFA01050543</td>
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Metabolites were confirmed by comparison with pure standards, exact mass, isotopic pattern, fragments (20 ev of collision energy), libraries (Metlin, MoNA and Lipid Maps), and bibliography.

Figure 5. Volcano Plot of the metabolites upregulated and downregulated in the control and DI irrigation groups.

4. Discussion

4.1. Physiological Response

Deficit irrigation (DI) has been used as an agricultural technique with the aim of saving water. Its application consists of facilitating crop development with less water than is necessary to cover the evapotranspiration process. However, it is essential to know the degree of water deficit to which plants can be subjected without inducing a significant reduction in their development and yield [6]. In bean crops, it has been proved that saving 20% of irrigation water, through the application of DI, could be achieved without a
significant reduction in the yield, since the small decrease in the moisture content within the root zone was not enough to cause water stress while saving 40% of irrigation caused water stress, provoking the lowest yields [28]. In our experiment, a 60% saving in irrigation considerably affected the physiological response of the plants, as observed in the leaf water potential values. This measurement has been widely used to assess the water status of plants in different crops [6,29,30]. In our study conditions, the leaf water potential of the plants was significantly reduced after four weeks of deficit irrigation, with slightly more negative values than those found in the common bean by Wakrim et al. [31] who reduced the irrigation to 50% of the transpiration rate for 25 days, indicating the sensitivity of this parameter to the lack of water. Stomatal conductance is a measure of the degree of stomatal opening and can be used as an indicator of plant water status. Huang et al. [32] indicated that stomatal adjustment is a common strategy in plants to mediate drought, causing a lower Pn [33]. Bean plants subjected to DI at 4 DAT showed a similar photosynthesis rate ($P_n$) and stomatal conductance ($g_s$) to the control plant; however, differences were found in the $g_s$ and Pn at 30 DAT. DeLaat [34] stated that a lower leaf water potential ($\Psi_{leaf}$) is associated with low stomatal conductance and transpiration values, which limits the photosynthesis rate.

Regarding the mineral content of plants, beans are an important source of mineral elements such as copper, phosphorus, aluminum, iron, zinc, and other minerals [35]. In this study, DI did not change the leaf mineral concentration except for Zn and P, which accumulated more under DI than under the control. Zn plays an essential structural and/or enzymatic role in transcription and other cellular functions. Smith et al. [36] reported that the leaf Zn concentration increased in bean plants under water stress, thus indicating that nutrient concentration could be identified as a tolerance trait to abiotic stress. In fact, drought-resistant cultivars may be more efficient in photo-assimilate production and translocation to the different bean tissues, especially for micronutrients such as Zn and Fe and macronutrients such as P and Ca [37]. Under situations of mineral deficit or excess, plants accumulate a series of metabolic compounds such as organic acids, sugars, amino acids, and enzymes that improve resistance to certain environmental stresses [38].

4.2. Yield and Quality

In general, grain yield is influenced by a complex of different morphological, physiological, and phenological characteristics, which in turn are affected by the soil moisture [39]. The number and weight of seeds per pod and, more specifically, the number of pods per plant are the characteristics that most affect bean yield [40–42]. Asemanrafat and Honar [10] reported that under water stress conditions, the number of pods per plant and the number of beans in each pod are factors that are significantly decreased. In our case, the number of pods per plant was the only parameter affected by deficit irrigation. These results are in concordance with several studies that affirmed that moderate drought stress may have a negative effect on the pod number per plant but no effect on the seed size or seed number per pod in bean genotypes that are tolerant to drought [43–45].

4.3. Metabolism

To the best of our knowledge, there is no published information based on “untargeted metabolomics” in beans related to irrigation treatments. Specifically, just ascorbic acid and 2,4-dihydroxybenzoic acid were previously described as metabolites present in kidney and black beans, respectively, without any water deficit treatment by UHPLC-QTOF [46,47]. In our study, 12 new biomarkers were identified in beans from DI treatment. According to Wang et al. [48] malonylation is closely related to lipids, fatty acids, alkaloids, and jasmonate metabolism as a consequence of water. In this way, our results showed significant changes in phospholipids and fatty acids exposed to a water deficit [48]. Thus, our topical finding would be that when water stress is applied to the bean plants, some particular phospholipids decrease, with the deactivation of fatty acid metabolism. In particular, PC(5:0/5:0) and 5,8,12-trihydroxy-9-octadecenoic acid decreased in plants under DI. These
findings agreed with those previously described by Navari-Izzo et al. [49]. Metabolomic analysis of an Arabidopsis drought-tolerant variety showed that the levels of PC decreased under the late stages of water stress, while flavonoids increased [50].

Trihydroxy unsaturated fatty acids with 18 carbons have been reported as plant self-defense substances. Their production in nature is found mainly in plant systems. It was reported that Pseudomonas aeruginosa PR3 converted linoleic acid into two compounds: 9,10,13-trihydroxy-11(E)-octadecenoic acid (9,10,13-THOD) and 9,12,13-trihydroxy-10(E)-octadecenoic acid (9,12,13-THOD); 7, Table 2) [51]. Another compound that was decreased was 9,10-dihydroxy-8-oxo-12-octadecenoic acid, an oxidized polyunsaturated omega-6 long-chain fatty acid derived from linoleic acid [52–54].

Conversely, severe drought increased antioxidant secondary phenolic compounds, [55] which might contribute to scavenging ROS production during a water deficit [55]. Thus, six metabolites including ascorbic acid, 2,4-dihydroxybenzoic acid, dihydromyricetin, cyanidin 3-(4-acetylglucoside), 7,3’,4’-trihydroxyflavone, and kaempferol 4’-rhamnoside were identified as upregulated metabolites (Table 2). The increase in phenolics in stressed plants is in agreement with recent reports on other plant species [56,57]. Indeed, these secondary metabolites can protect plant cells from oxidative stress caused by a water deficiency [55,58,59]. Metabolomic analysis of Arabidopsis and rice showed that the levels of antioxidants such as ascorbic acid and flavonoids were increased under early water stress [50,60]. In medicinal and spice plants, drought-mediated induction of aromatic products might be utilized as a novel strategy to enhance the yield of these valuable compounds [61]. Therefore, our findings suggest that DI treatment led to notable differences in the metabolome compared to the control. On the one hand, an enhancement in functional phenolic compounds was revealed, which are known for their positive effects on fruit quality and human health. On the other hand, DI treatment caused a reduction in the production of compounds associated with fatty acid metabolism. These results are a starting point to further investigate the alteration and identification of new biomarkers related to deficit irrigation strategies in order to improve the nutritional quality of bean crops without reducing plant production and yield.

5. Conclusions

Untargeted metabolomics aims to discover and identify bioactive plant compounds. The total metabolome of the beans was clearly altered and different metabolic pathways were affected by deficit irrigation. A group of newly discovered metabolites from different families was found to be related to these alterations. Irrigation water deficit could be a promising way to achieve a dual effect of increased bioactive compounds and reduced fatty acids in the common bean. Further studies will be necessary to corroborate this result.

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