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Physiological Responses of Common Bean Genotypes to Drought Stress

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Abstract: Drought compromises edible vegetable production worldwide, including common bean (*Phaseolus vulgaris* L.) an economically important crop that is highly dependent on optimum rainfall or abundant irrigation. In the present study, phenotypic data of 26 Bulgarian common bean mutant lines and cultivars subjected to drought stress has been summarized, and drought stress reaction was evaluated by chlorophyll fluorescence and proteomics approaches. Several basic photosynthetic parameters were examined during treatment to evaluate the drought stress response, and the mutant lines showed different responses. Subsequently, a relationship was found between productivity and photosynthetic performance with the expression of ribulose-1,5-bisphosphate carboxylase through comparative 2D-gel based electrophoresis; accumulation of the well-known stress-related proteins markers dehydrins and small heat shock proteins was established as well. These findings support the further selection of drought tolerant common bean lines for a sustainable agriculture.

Keywords: *Phaseolus vulgaris*; mutant lines; cultivar; drought tolerance; photosynthesis; proteomics; dehydrins; small heat shock proteins

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1. Introduction

Phaseolus vulgaris (L.) commonly known as kidney bean, French bean, dry bean, common bean, or field bean belongs to the Fabaceae family with a genome size of 587 Mbp [1] and chromosome number $2n = 2x = 22$ [2]. The common bean is one of the most nutritious food legume in different countries in Latin America and Eastern and Southern Africa and is ranked first among food legumes [1]. It is a rich and cheap source of proteins (22%), carbohydrates (57%), minerals (0.5 g/100 g of edible portion), vitamin A (221 I.U.), and calcium (50 mg/100 g of edible portion) (FAO: http://faostat.fao.org/ (accessed on 23 January 2023); [1,3]). By virtue of its nutrient density, it complements a protein deficient cereal-based human diet. Moreover, the common bean is a source of income for millions of resource-limited smallholder farmers in tropical regions. Worldwide, it is cultivated in an area of 34.80 million hectares (M ha) with an annual production of 27.54 million tones (MT) (FAOSTAT 2020). South Asia, South-East Asia, and Central America are the major common bean (dry) producers contributing 50% of the global contribution. Among the countries, India ranks first in common bean (dry) production (19.82%, 5.46 MT) followed by Myanmar (11.07%, 3.05 MT) and Brazil (11% 3.03 MT) (FAOSTAT 2020).

Drought stress is emerging as a dreadful threat that substantially reduces the world's food supply [4]. Worldwide common bean producing regions are facing moderate to severe spells of drought during the grain filling stage [5]. It is assumed that drought

stress will further impact the common bean production as the weather gets drier under climate change [5]. Common bean production could be much higher if drought tolerance of exiting cultivars is improved. However, it seems drought will be a major challenge under climate change in the coming years, and crops might face long and intense drought spells. Drought stress seed yield, number of pods and seeds, translocation, and partitioning of assimilates [1,6,7]. Earlier studies have also reported a 60–99% reduction in common bean yield exposed to drought stress during flowering and post-flowering stages [8,9]. Therefore, improving drought tolerance is a critical priority area. The lack of well-adapted local cultivars leads to a deficiency in robust crop production and a reliance on imports. Generally, drought stress reduces osmotic pressure and disrupts water potentials in plant cells, thus causing oxidative stress and cellular damage, and a reduction of photosynthesis, carbon fixation, and primary metabolism. These negative impacts result in decreased survival and yield of crops. Therefore, current breeding programs aim to find physiological evidence corresponding to improved phenotypic traits of plants subjected to drought. As an example, the yield of 24 commercial cultivars of common bean has been determined by statistical analyses at different sites in Chile and Bolivia, and two of them have been characterized in detail [10]. The reported results showed that the tolerant genotype has more plasticity in maintaining stomatal conductance, photosynthetic rate, abscisic acid synthesis, and resistance to photoinhibition. Another study with drought tolerance common bean cultivars highlights that the accumulation of different osmoprotectants and antioxidants parallel with the maintenance of photosynthesis has an impact on common bean yield after drought stress [11]. Considering that molecular chaperons are expressed in response to almost all kinds of stress in plants and involved in diverse cellular functions such as folding, accumulation, translocation and degradation of proteins [12,13], their accumulation in different tolerant bean genotypes represent particular interest for breeding programs as well.

The sustainable approach to mitigate the adverse effects of drought on yield and yield attributing traits is to develop cultivars with improved drought tolerance. Among the crop development approaches employed so far, mutation breeding has been the most successful in developing cultivars with increased drought tolerance [14]. Unlike other modern biotechnological approaches, mutation breeding has more public acceptance and zero ethical and religious criticism by the general public [15,16]. In addition, it offers an opportunity to alter a single trait without changing the entire genetic constitution [17–19]. The Bulgarian common bean collection was extensively studied using molecular markers [20]. The studied phaseolin types—a conservative marker and 22 isoenzyme loci showed a highly narrowed genetic diversity of the National Collection. Bulgaria is a secondary domestication center for beans after the introduction from Andes and Meso-America, which are the primary gene pools of the genus Phaseolus. In the collection of beans very well adapted local genotypes are maintained. Various programs have been undertaken to increase the genetic diversity of beans in the collection [21]. One of these is mutation breeding, which induces numerous beneficial mutations. The mutations were developed into advanced lines and characterized in terms of their productivity, resistance to important bean diseases in the country and Europe.

Keeping in view the success rate and other attributes of mutation breeding, several mutant lines of Bulgarian common bean were subjected to drought and then evaluated for physiological and biochemical alterations using a comparative proteomics approach and measurements of photosynthetic performance. The present study aimed to assess the response of common bean mutant lines with increased productivity to water deficit treatment using physiological and proteomics analyses. This allowed the selection of drought tolerant mutant lines for further cultivar registration. After the classification of common bean mutant lines according to their productivity and morphology by principal component analysis (PCA), we evaluated the photosynthetic performance of the mutant lines that showed maximum separation on PCA and performed a screening by proteomics analysis of enzymes related to primary metabolisms such as ribulose-1,5-bisphosphate carboxylase

and accumulation of well-known molecular chaperones such as dehydrins and small heat shock proteins. The present study concluded with the isolation of mutant lines that could be released as a drought tolerant cultivar after subjecting them to multilocation trails.

2. Materials and Method

2.1. Common Bean Plant Material, Drought Stress, and Multivariate Analysis of Phenotypic Traits

Twenty-three common bean genotypes (23 mutant lines and 3 cultivars) were generated in 2009 by treatment with 0.6 M ethyl methanesulphonate (EMS) of the initial Bulgarian common bean cultivar "Evros" [21] and were subjected to drought stress as described in the earlier works [22]. Plants were exposed to stress during the reproductive period (bud formation—flowering phases). Drought was imposed by water retention for 15 days (without irrigation during the period of treatment).

After four days of applying drought stress in field conditions, we performed measurements of the fast fluorescence of chlorophyll at several drought stages, namely,—D1 (8 days of drought), D2 (12 days of drought), and D3 (14 days of drought). According to previous reports about the productivity of plants in a drought regime, the 12 most productive bean lines were selected, on which a study of photosynthetic parameters was carried out [22]. At the end of drought stress, the relative water content (RWC) of discs with a diameter of 1 cm from corresponding leaves from each line was measured as described previously [23], and the rest of the leaf was sampled in liquid nitrogen and stored at −70 °C for further analyses.

To summarize phenotypic differences between common bean lines subjected to drought stress and select the most appropriate ones for further comparative photosynthetic and proteomics analyses, we used PCA. The impute matrices contain the corresponding sample from each line in triplicates and the columns represent different phenotypic traits namely: length of the plant, fresh weight of the plant, fresh weight of fruits per plant, length of fruit per plant, the width of fruit per plant and weight of common beans as described previously [22]. PCA was performed with MatLab (Mathworks, Natick, MA, USA) software according to standard algorithms.

2.2. Photosynthetic Performance

The functional activity of the photosynthetic apparatus (PSA) in common bean mutant lines was characterized by analyzing the chlorophyll fluorescence parameters. Several parameters such as the maximum quantum yield of photosystem II (Fv/Fm), the ratio of maximum fluorescence to initial fluorescence (Fm/Fo), the ratio between variable fluorescence to initial fluorescence (Fv/Fo), and the time for maximum fluorescence (Tfm) were monitored. A portable fluorometer PEA (Plant Efficiency Analyzer, Hansatech Ltd., Pentney, UK) was used to measure chlorophyll (Chl) fluorescence parameters.

Chlorophyll fluorescence parameters: Fo, Fm—the minimum and maximum dark adapted fluorescence yield, respectively; and Fv—variable fluorescence (Fv = Fm/Fo) were measured. They were used to calculate the ratios Fv/Fo (potential photochemical efficiency) and Fv/Fm (maximum quantum efficiency of PSII) which are considered as indicators for the PSII efficiency in primary photochemical reactions. All the traits were evaluated at the bud formation—the flowering stage between 10:00 and 13:00. Fluorescence parameters were measured on three selected fully expanded uppermost leaves. Leaves were acclimated to dark for 30 min before measurements were taken. The measuring time was 5 s, and the irradiance was 3000 $\mu mol/m^{-2}/s^{-1}$. Plants grown under optimal irrigation conditions were used as controls. Fluorescent characteristics were recorded four times during the period of budding-flowering, at least in three repetitions.

Data measurements were arranged in a matrix and analysed by two-way ANOVA for evaluation of differences in photosynthetic performance among common bean lines in response to drought. The rows correspond to measurements of lines, while the columns represent the measured parameters described above. ANOVA was performed with MatLab software according to standard algorithms.

2.3. Protein Extraction and 2D-PAGE

After PCA, we selected two lines showing the highest separation in PC1, showing the highest differences in their phenotypes after drought stress namely line 22 and line 26. Total leaf proteins were extracted essentially using phenol/sodium dodecyl sulfate (SDS) extraction according to Wang et al. [24] with three washing steps of protein pellets with 0.3 M guanidine hydrochloride in ethanol and one with absolute ethanol. According to manufacturer instructions, the protein concentration was determined spectrophotometrically by a 2D Quant kit (GE Healthcare). Eight hundred micrograms of proteins were precipitated by a 2D Clean up kit (GE Healthcare, Chicago, IL, USA) and applied onto Immobiline DryStrips (IPG strips) 18 cm, pH range 4–7 (GE Healthcare, Chicago, IL, USA) by passive rehydration in Destreak solution (GE Healthcare, Chicago, IL, USA) with 0.5% IPG buffer pH 4–7 (GE Healthcare, Chicago, IL, USA). Samples were focused on a Multiphor II Isoelectric Focusing Unit (GE Healthcare, Chicago, IL, USA) with a total amount of 60,000 Vh. Proteins were then separated onto the second dimension of SDS-PAGE by a Ruby electrophoresis unit (GE Healthcare, Chicago, IL, USA) using 14% gels prepared in multiple gel casters. Three replicates (n = 3) were used for the statistical analyses. Subsequently, the gels were stained with colloidal coomassie blue (CBB) (Biorad, Hercules, CA, USA) and visualized on a scanner (Microtek, Bio-5000 Plus, Taiwan). Gel images were subsequently analysed on Image Master 2D Platinum 7.0 (GE Healthcare, Chicago, IL, USA). The intensity of each spot was first processed by background subtraction and volume percent was used for quantification. To fine-tune spot detection, a threshold of volume% > 0.006 was set. A change in protein expression was summarized by Hierarchical Cluster Analysis (HCA) with MatLab software according to standard algorithms. The impute matrices contained the log2 transformed averaged values of fold change in volume percent of each spot from drought stressed lines compared to the non-stressed controls. Spots corresponding to ribulose-1,5-bisphosphate carboxylase and exhibiting a fold change of increasing or decreasing above 1.5 and p-value < 0.05 were excised by semi-manual spot-picking system SERVA HPE™ ScreenPicker (SERVA Electrophoresis GmbH, Heidelberg, Germany).

2.4. Mass Spectrometry Identification of Selected Spots

The proteins from gel plugs were digested, extracted and spotted onto the MALDI plate by Tecan modular robotic pipetting platform EVO2 (Tecan Trading AG, Männedorf, Switzerland). Gel washing and tryptic digestion were performed by incubations of gel plugs in 50 mM NH_4HCO_3 in 50% MeOH and 100% Acetonitrile (ACN) followed by 40 ng Trypsin (Promega, Madison, WI, USA) in 20mM NH_4HCO_3 (5 ng/µL) respectively. 0.1% TFA in 50% ACN and 7 mg/mL CHCA in 50% ACN/0.1% TFA solutions were used for peptide extraction and spotting respectively. MALDI TOF-TOF analysis was performed by TOF/TOF™ 5800 (AB SCIEX, Redwood City, CA, USA) mass spectrometer in MS and MS/MS mode. For each spot, the 10 most intense peaks of the MS spectrum were selected for MS/MS acquisition. Database interrogation was carried out with ProteinPilot v4.5 (AB Sciex, Redwood City, CA, USA) on an in-house Mascot server version 2.8 (Matrix Science Ltd., London, UK). Spectra were searched against the *Phaseolus vulgaris*-NCBI vulgaris-NCBI_20210810 (73,043 sequences; 28,936,582 residues). Two missed trypsin cleavages were allowed, and trypsin was used as the enzyme (cleavage at the C terminus of Lys/Arg). The mass tolerances were 100 ppm for precursor ions and 0.5 Da for fragment ions. The variable modifications allowed were Tryptophan oxidation and dioxidation, Methionine oxidation and Trp to Kynurenin (W). A fixed modification was set: carbomidomethyl cysteine. Protein scores greater than 56 and individual ions scores > 37 corresponding to $p < 0.05$. Each result has been manually checked for validation.

2.5. Western Blot Analysis of Dehydrins and Small Heat Shock Proteins

For analysis of dehydrins and small HSPs, total protein extracts from lines 22 and 26 (as described above) were resolved on SDS PAGE using 12% gels. Subsequently, proteins were electro-transferred onto a nitrocellulose membrane using a semi-dry transfer

system (Hoefer). Membranes were blocked for one hour with Carbo free blocking solution (Vector Laboratories, Newark, CA, USA) supplemented with 0.05% Tween-20. Afterwards, for immunodetection of dehydrins, the membranes were probed with a primary rabbit polyclonal antibody raised against a synthetic peptide, representing a highly conserved domain (the K segment) of dehydrins (Agrisera) at 1:1000 dilution. For immunodetection of sHSPs, membranes were probed with a primary rabbit polyclonal antibody (Agrisera). Goat anti-rabbit IgG antibody (dilution 1:2000) conjugated to horseradish peroxidase (Abcam) was used as a secondary antibody. After the immunoblot and immunoreactive bands were developed with Peroxidase Activity Assay Kit (Merck KGaA, Darmstadt, Germany). Western blot was performed in triplicates and analysed by Image Quant software (GE Healthcare, Chicago, IL, USA). The signal abundance of the immunodetected bands was normalized to the overall sum of bands from CBB stained gels.

3. Results

3.1. Principal Component Analysis of Phenotypic Traits of Common Bean Mutant Lines Subjected to Drought Stress

To summarize phenotypic differences between common bean lines subjected to drought stress and select the most appropriate ones for further comparative proteomics analyses, we used PCA with previously published data [22]. The results showed a clear grouping of stressed common bean lines with their replicates into four main groups according to the measured phenotypic traits (Figure 1).

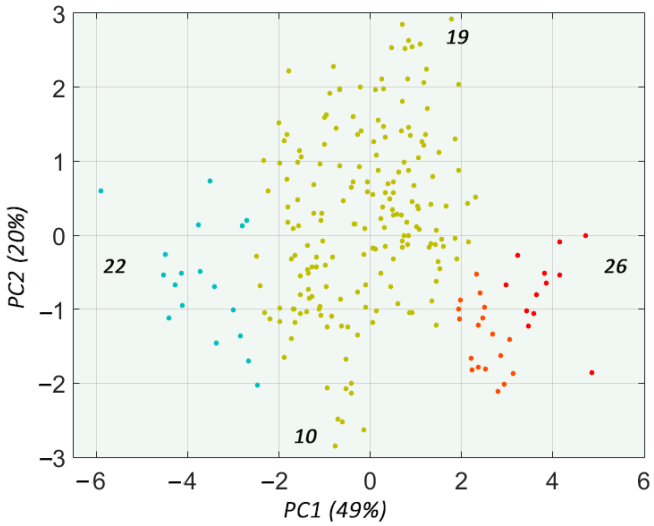

Figure 1. Principal component analysis of phenotypic traits of common bean mutant lines subjected to drought stress. The first two components explain 69% of variance between common bean lines according to length of plant, fresh weight of plant, fresh weight of fruits per plant, length of fruit per plant, width of fruit per plant, and weight of common beans after drought stress. Scores are clustered in four groups in different colours according to their distribution in the PCA score plot. Their corresponding numbers show the bean lines with the highest contribution on the positive and negative PC1 and PC2 scales, respectively.

The main contributing loadings that determine the distribution of scores in PC1 are related to productivity and bean morphology, such as weight of beans, length of fruit per plant, and width of fruit per plant, respectively. The residual orthogonal part of variance was explained by the contributions of plant length and fresh weight of plant in PC2. Lines 14, 22, 24, and 25 were distributed on the negative scale of PC1 due to their low productivity of seeds and short length and width of pods. In contrast, lines 4, 26, and 27 showing higher productivity after drought treatment were grouped in the positive one. Lines 22 and 26 showed the highest distance according to the PC1 scale (Figure 1). Lines 10 and 21 were

separated from lines 3, 18, and 19 in PC2 according to their shortest length and weight of plant; lines 10 and 19 showed the highest distance according to the PC2 scale.

3.2. Photosynthetic Performance of Common Bean Lines during Drought Stress

Given the results from the phenotypic analysis of the common bean lines and collected chlorophyll fluorescence data (Table S1), focused on the photosynthetic performance on four lines showed the highest distance in positive and negative scales of PC axes—10, 19, 22 and 26 (Figure 2).

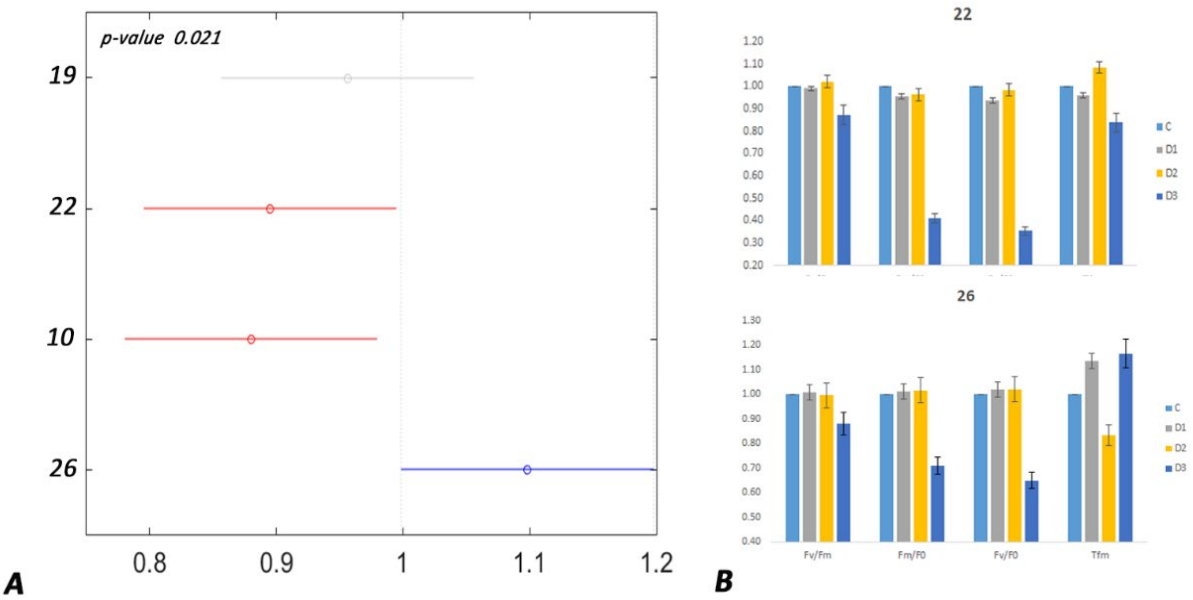

Figure 2. Photosynthetic performance of common bean lines during drought stress. (**A**) Visualization of two-way ANOVA multiple comparisons between the photosynthetic performance of selected common bean line numbers: 19, 22, 10, and 26 shown on the y-axis of the plot. The *p*-value for the model effect (Prob > F) is also shown in the left corner of the plot. The blue bar is the comparison interval for the means of measured photosynthetic parameters e.g., Fv/Fm, Fm/F_0, Fv/F_0, and Tfm of line 26 with the other three lines. The red bars are comparison intervals of lines 22 and 10 that significantly differ from line 26 and grey bar show the comparison interval of line 19, which does not significantly differ from all lines. (**B**) Changes in photosynthetic activity of line 22 (upper bars) and line 26 (lower bars) in response to drought. Bar plots represent the averaged fold changes of each parameter in each state of drought (e.g., D1, D2, D3) compared to the control—well watered state (C). Error bars represent standard errors from triplicates.

Calculated p-value of the probability of the F-distribution of the calculated fluorescent parameters (0.021) from ANOVA shows a significant difference in photosynthetic performance between bean lines (Figure 2A). Visualisation of multiple comparison tests shows that line 26 (blue) is significantly different from lines 22 and 10 (red), and line 19 (grey) does not show significant differences from the others (Figure 2A). Changes in photosynthetic parameters during drought in lines 22 and 26, which show the lowest and highest productivities, respectively, and a significant difference in their photosynthetic response, are represented in Figure 2B. At the first two stages of drought, e.g., D1 and D2, we did not observe significant changes in photosynthetic performance in both lines. However, at the end of drought stress, in parallel with the decreasing of RWC by 30% in both lines (data not shown), different changes in photosynthesis have been found in each line (Figure 2B). As a result of applied drought stress, we found a more pronounced decrease in all measured photosynthetic parameters in line 22 compared to line 26. A slight reduction in the maximum quantum of photosystem II (Fv/Fm) was found in both lines, while a more

pronounced decrease in Fm/Fo and Fv/Fo was observed in line 22 and reached 50 percent. In line 26, Tfm did not change and slightly increased after drought stress.

3.3. Proteomics Analysis

According to the results described above, we selected lines 22 and 26, which show the highest differences in their productivity and photosynthetic responses after drought stress, for further proteomics analyses (Figure 3).

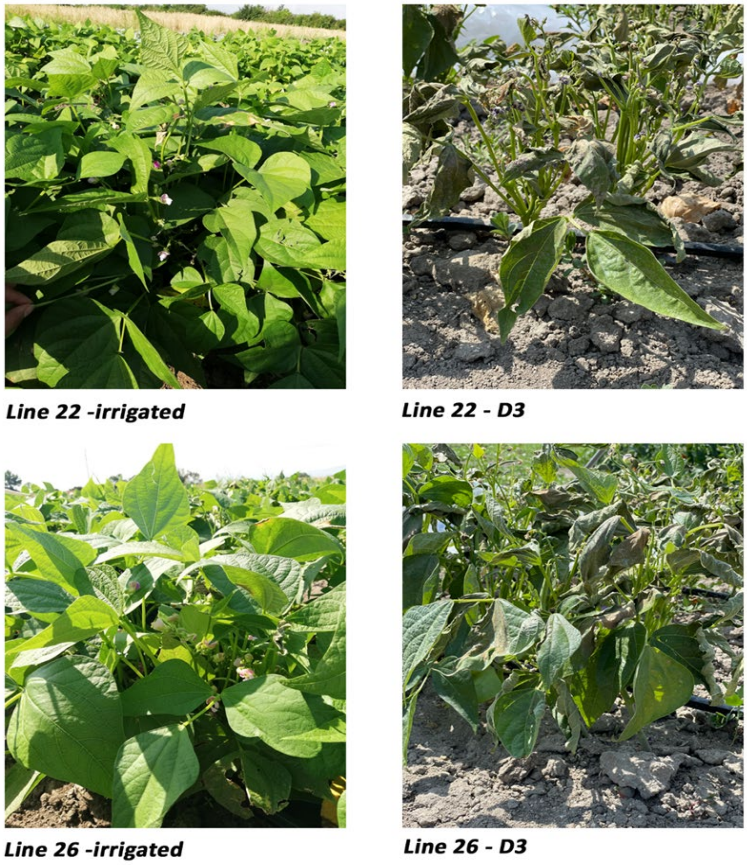

Figure 3. Visualization of common bean mutant lines 22 and 26 in irrigated state and after drought treatment (D3).

Total leaf proteins were extracted from common bean lines 22 and 26 from frozen leaves stored immediately at the end of the applied drought stress, corresponding with the described photosynthetic performance above. The RWC of each leaf sample was around 55%, showing a decrease of 30% compared to control well-watered plants (data not shown). Proteins were separated by two-dimensional polyacrylamide gel electrophoresis, and changes in spot volumes among replicates were analysed (Figure 4A,B).

A total of 360 spots were detected in all gels, and 180 spots were successfully matched among each replicate. HCA/Heat map clustering and visualization of protein expression in each line after drought show several patterns of differences between common bean lines (Figure 4B). Significant changes above 1.5 folds for each line are grouped in several dendrogram clusters. From top to bottom of the clustergram, the red one represents protein spots with increased volume in line 26 in contrast to their decreasing in line 22. The next three coloured clusters on the second main branch consist of spots without significant changes during the drought in both lines, while blue, pink, and orange clusters from the other main branches show spots with increased abundance in line 22 in contrast to line 26. Following changes in photosynthetic performance, we found two spots, corresponding to ribulose-1,5-bisphosphate carboxylase, that showed significant differences among bean lines after drought stress and were excised from 2D gels and targeted for identification by

MALDI-TOF/TOF mass spectrometer (Table S2). As a result, we confirmed that ribulose-1,5-biphosphate carboxylase/oxygenase involved in carbon fixation and photosynthesis decrease in line 22, while in line 26 it increases in response to drought (Figure 4C).

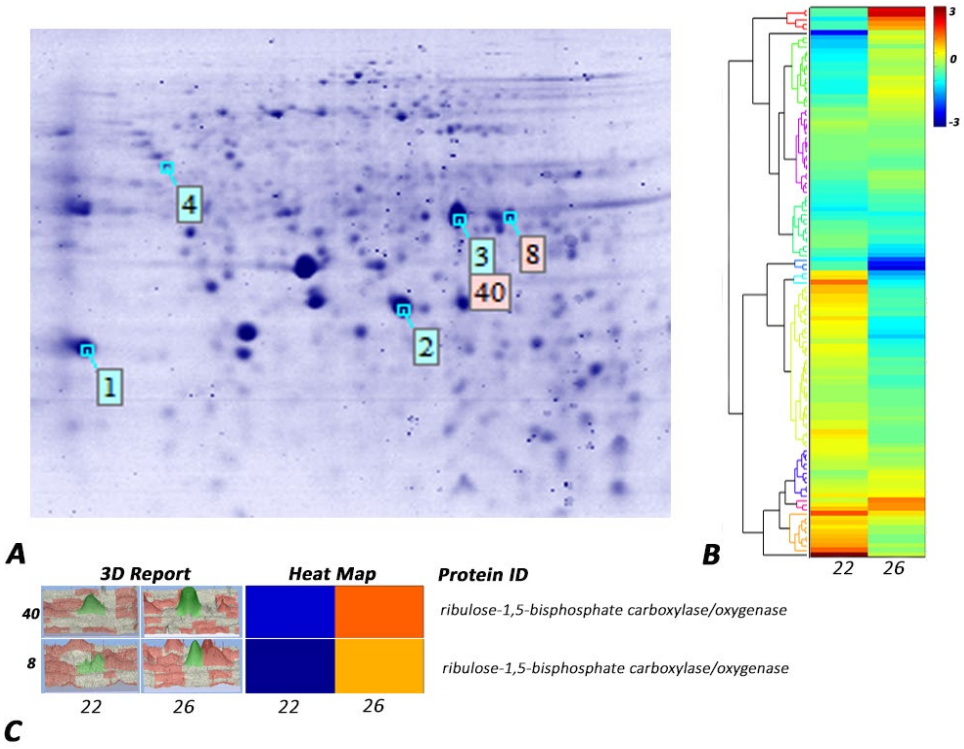

Figure 4. 2D Proteomics analysis. (**A**) Representative 2D PAGE gel of total leaf proteome of common bean line 26 in dried state stained with CBB. Numbers in blue boxes represent the landmarks used for matching between gels, and numbers in pink boxes represent the spots targeted for MS analyses. (**B**) Heat map visualization of the hierarchical clustering of matched spots represented by their log2 transformed averaged meanings of volume% in drought stress normalized to control levels of lines 22 and 26. Different clusters according to their linkage distance are shown with different colors. Color bars represent log2 fold changes of spots after drought stress normalized to their levels in control state (**C**) representation of identified proteins. Each spot number corresponds to the representative gel in (**A**) and is represented by the 3D report for each line in the dried state followed by Heat map visualization and protein ID. Color code for Heat map corresponds to the color bar in (**B**).

Further, to evaluate the expression of two well-known classes of proteins e.g., dehydrins and sHSPs involved in stress response against drought in both mutant lines, we used western blot analysis (Figures 5 and S1).

We found two bands with molecular weights of approximately 33 (d1) and 27 (d2) kDa corresponding to dehydrins immunosignals in line 26, in contrast to line 22, where only the low molecular band (d2) has been detected. In line 26, both bands showed pronounced increases after drought stress, while those detected in line 22 did not show a significant change. We also found expression of sHSPs with a molecular weight of approximately 17 kDa in both lines, which shows accumulation after drought stress.

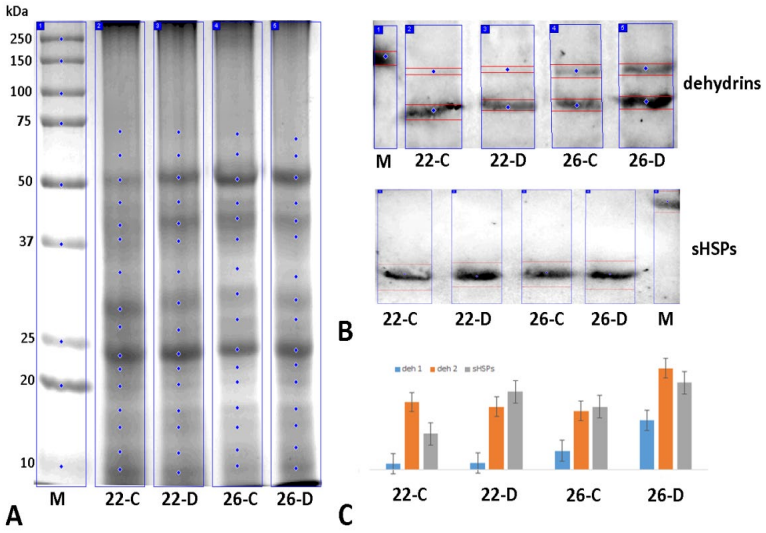

Figure 5. Western blot analysis of expression of dehydrins and small HSPs in common bean mutant lines number 22 and 26 in control (C) and stressed states (D). (**A**) CBB staining of protein extracts from mutant common bean lines in control and stressed states separated by SDS PAGE. Molecular weights of marker (M) are given on left. Each detected band is assigned with blue rhomb. (**B**) Western blot of dehydrins (upper panel) and small HSPs (lower panel). The area of detected immunosignals and marker (M) is defined with red lines and bands are assigned with blue rhomb. (**C**) The mean area of each dehydrin and sHSPs band was normalized to the mean area of the total protein stain. Error bars were calculated from triplicate samples. deh1- upper high molecular band in blue; deh2-lower band in orange; sHSPs small HSP in grey.

4. Discussion

A serious issue in global efforts to ensure food security for the population is climate change that affects agricultural production. In common bean, drought stress is one of the main challenges for farmers that impacts the overall annual production [3,6]. Generating new beneficial mutations combined with highly efficient molecular approaches, such as proteomics, is very much needed to solve this issue. In the present study, the physiological response of 26 Bulgarian common bean mutant lines to water deficit was evaluated by changes in photosynthesis and protein expression.

PCA analysis classified the 26 common bean genotypes subjected to drought according to their productivity and plant morphology. As a result, four main clusters were assessed (Figure 1). After drought stress, lines 26 and 22 were selected based on their highest productivity and lowest productivity, respectively, according to the PC1 results. Furthermore, lines 19 and 10 were separated according to PC2 differences a result of their plant length after water deficit treatment. We found that lines 10 and 22, separated in PC1 and PC2 axes according to their lower plant length and weight of fruit and grain weight, respectively, showed a significant decrease in their photosynthetic performance compared to lines 26 and 19, which showed the highest productivity after drought stress (Figure 2A).

Considering the sensitivity of photosynthetic performance in drought tolerant and drought sensitive species [25,26], we compared the effect of drought stressed mutant common bean lines on PSA in chlorophyll a fluorescence parameters under three drought stages. The Fv/Fm ratio, which characterizes the maximal quantum productivity of the primary photochemical reaction in dark adapted leaves, remained visually unchanged (Figure S1), but the presented mutant lines showed a slight tendency to decrease in all lines when the water was retained at stages D1 and D2 (Figure 2B). The results of no effect on the Fv/Fm ratio in the mutant lines under well-watered and drought stress conditions are in agreement with a similar response observed in common bean [27].

Often the lack of change in the chlorophyll fluorescence ratio may be due to the temperatures experienced by the plants. If the temperatures were within the threshold

range for these species, heat damage to the photosynthetic apparatus might not happen [28]. There have been studies that show that when drought is applied, the Fv/Fm ratio drops right away. This is likely because these species are more sensitive to water stress [29].

This parameter is considered to be a sensitive indicator of plant photosynthetic activity [30], and analysis of chlorophyll fluorescence and measurement of the Fv/Fm ratio can be useful in determining damage to light reaction systems in photosynthetic mechanisms under drought stress conditions. Fv/Fm is a measure of the maximum photochemical efficiency of PSII when all the reaction centres are open. It is established that in healthy leaves, the values of this coefficient are within the limits of 0.83 [31]. By the end of the drought period (D3), a significant decrease was observed in the maximum quantum yield of photosystem II (Fv/Fm) in line 22 (0.702). These results were similar to those observed on some other Bulgarian dry bean cultivars [27,32]. The values of Fv/Fm in these lines maybe indicative of severe disturbances in PS II, and a reduction in photosynthetic efficiency may play a role in yield loss under water stress as it affects carbon assimilation. Drought stress evoked similar effects in parameters Fm/Fo and Fv/Fo (maximum primary yield of the photochemistry of photosystem II), which are more pronounced at line 22. The Fv/Fo ratio, which is used to measure the state and efficiency of the electron transport chain in the process of photosynthesis, is a good way to find out how drought stress affects the state of a plant's photosynthetic machinery. When the values of this fluorescence parameter go down, it means that something is wrong with the way electrons are transferred during photosynthesis. At line 22, the last date of the drought that was used, the decrease in the efficiency of the electron transport processes is more noticeable.

In the studied mutant lines, the results of the performed measurements showed an increase in Fo accompanied by a decrease in the measured parameters Fm and Fv due to drought stress (data not shown). The decreasing trend of Fv/Fm may be attributed to the changes in Fo, which indicates the minimal fluorescence yield. There is a strong increase in the values of this indicator at the end of the drought period, especially at line 22. An increased Fo is a characteristic of PSII inactivation. This is probably due to structural changes in the pigment apparatus of plants resulting from the reduced plastoquinone acceptor (Q_{-A}), unable to be oxidized completely because of the electron flow retardation through PSII [33]. An increase in F0 is accompanied by a drop in Fm at high stress levels, indicating the degradation of the light-harvesting complex of PS II. F_0 and Fm are reliable measures of stress severity. Fluorescence levels increase from F_0 to maximum fluorescence (Fm) when the light intensity is sufficient [34]. The intensity of the water deficit stress, which is relevant to the deactivation of proteins in the *Chl* structure, caused Fm to decline in this study.

After classification of bean mutant lines according to their productivity and comparison of their photosynthetic performance after drought, we used line 22, which shows the lowest productivity and a high decrease in photosynthetic performance, and line 26, with the highest productivity and a slight decrease in photosynthesis after drought stress, for proteomics analyses (Figure 3). Earlier workers have also reported the use of the two-dimensional difference gel electrophoresis (2D-DIGE) technique in identifying the drought responsive proteins in tolerant and susceptible common bean cultivars [35]. Such identified drought-responsive proteins were found to be involved in ATP interconversion, carbon assimilation, translation, proteolysis, and stress and defense-related processes [35]. However, few enzymes are more important to mention, for instance, Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), that can be modulated either by transcriptional and post-translational regulation in plants in response to different environmental fluctuations [36]. Moreover, overexpression of Rubisco in crops for improvement of photosynthesis have been the object of extensive studies for a long time [37]. In this study, we found that the most tolerant bean mutant line with the highest productivity after applied drought stress shows overexpression of two isoforms of Rubisco after drought stress (Figure 4), which also correlates with the smallest change in photosynthetic light reaction compared to other mutant lines. Previous studies have also reported the sensitivity of the expression

levels of Rubisco in common bean plants exposed to drought stress, and depending on the intensity of stress and plant species, some workers reported a vivid reduction in Rubisco activity [36,38] while others observed little or no inhibition [39,40].

Since it was established that dozens of proteins are up-regulated (known as rehydrins) and other dozens are down-regulated (termed hydrins), most of these studies have made gene expression profiles and proteomic studies. Mutant lines with modified expression of these genes can be identified by using comprehensive proteomics profiling and identification by mass spectrometry. Considering the well-established role of dehydrins and small heat shock proteins (sHSPs) in cell protection during drought stress, we evaluated their abundance by western blot analysis in both lines 22 and 26 after drought stress. The results were in line with the findings of Castañeda-Saucedo et al. [41] that reported an increase in dehydrins during drought stress in common bean. Dehydrin accumulation is affected by various environmental factors such as drought, low temperature, and salinity in different plants. They are thermostable, highly hydrophilic proteins with molecular weights in the range of 9–2000 kDa [42]. After the whole genome sequencing of *Phaseolus vulgaris*, a dehydrin with 202 amino acids residues and a molecular weight of approximately 22 kDa has been identified [43]. Our results showed a common immunoreactive band in both lines with a molecular weight of approximately 26 kDa which increased after drought only in line 26 (Figure 5 and Figure S1). Moreover, in line 26, we detected an additional band with a molecular weight of 33 kDa showing increased abundance after the drought as well. However, further studies are needed to evaluate if the detected higher molecular weight dehydrin band in line 26 is due to phosphorylation or differences in the amino acid sequence. All these findings indicate that overexpression of two isoforms of dehydrins in line 26 correlates with drought resistance of this line. Earlier studies have also reported that drought tolerance is based on the synthesis of osmoprotective proteins like dehydrins and chaperones [12,44,45]. To date, many published studies support the role of small heat shock proteins (sHSPs) not only in heat stress but also in almost all stress conditions, including drought. Moreover, the function of sHSPs as molecular chaperones is supported by many in vitro and in vivo assays. [46,47]. In agreement, our results show that sHSP with a molecular weight of 17 kDa is overexpressed in common bean in both mutant lines after drought stress.

5. Conclusions

In the present study, we classified 26 genotypes of common bean according to their plant productivity and morphology after drought stress. We found that the photosynthetic performance of the line with highest productivity remains stable after drought stress, comparing with the line with decreased production caused by drought. Moreover, we found a relation between rates of photosynthesis and accumulation of ribulose-1,5-bisphosphate carboxylase in the tolerant mutant line, thus securing the productivity of the plant in conditions of drought. In parallel, we found that the accumulation of molecular chaperones such as dehydrins and sHSPs is required to ensure plant survival and productivity during drought stress.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13041022/s1. Table S1: Row data of PEA measurements of all common bean mutant lines during drought stress. Table S2: MALDI TOF/TOF report of protein identification. Figure S1: Molecular weight calculation of Dehydrins and sHSPs proteins from western blot analysis.

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