agronomy

Article

Adaptive Responses of Biofortified Common Bean Lines to Acidic Soil and High Temperatures in the Colombian Amazon Region

Juan Carlos Suárez [1,2,*], Amara T. Contreras [2,3], Milan O. Urban [4], Miguel A. Grajales [4], Stephen E. Beebe [4] and Idupulapati M. Rao [4]

[1] Programa de Ingeniería Agroecológica, Facultad de Ingeniería, Universidad de la Amazonia, Florencia 180001, Colombia
[2] Grupo de Investigaciones Agroecosistemas y Conservación en Bosques Amazónicos-GAIA, Centro de Investigaciones Amazónicas CIMAZ Macagual César Augusto Estrada González, Florencia 180001, Colombia
[3] Programa de Maestría en Sistemas Sostenibles de Producción, Facultad de Ingeniería, Universidad de la Amazonia, Florencia 180001, Colombia
[4] International Center for Tropical Agriculture (CIAT), Km 17 Recta Cali-Palmira, Cali 763537, Colombia; m.urban@cgiar.org (M.O.U.); s.beebe@cgiar.org (S.E.B.)
* Correspondence: ju.suarez@udla.edu.co; Tel.: +57-3202804455

Abstract: One of the strategies to combat micronutrient malnutrition is by developing biofortified common bean lines (*Phaseolus vulgaris* L.) capable of tolerating different stress conditions. In this study, the adaptive responses of different biofortified bean lines grown under combined stress of acidic soil and high-temperatures were evaluated in the Colombian Amazon. A total of 247 common bean lines from the Mesoamerican gene pool were used to determine the adaptive response in terms of phenological, physiological, and agronomic behavior under combined stress conditions. The lines tested were obtained from different single crosses, double crosses, and backcrosses between different bean materials, of which 146 were obtained from F_4 families with high iron (Fe) content in seed and 99 common bean lines from F_5 families. Different bean lines had grain yields (GY) higher than 1400 kg ha^{-1} from the F_5 (lines: 859, 805, 865, and 657) and F_4 (lines: 2853 and 2796) families. The superior performance of these lines was related to a higher photosynthate partitioning that has allowed an increase in pod formation (pod partitioning index, PPI) from the canopy biomass (CB) and grain filling (pod harvest index, PHI; harvest index, HI), resulting in higher values of GY. Values of GY were correlated with CB (r = 0.36), PPI (r = 0.6), PHI (r = 0.68), and HI (r = 0.8, $p < 0.001$). This increase in agronomic performance is due to a greater allocation of energy to the photosynthetic machinery (ΦII) and its dissipation in the form of heat (ΦNPQ), with increases in the leaf temperature difference (LTD). Based on the results obtained, six biofortified lines of common bean (lines F5: 859, 805, 865, and 657; lines F4: 2853 and 2796) showed traits of tolerance to combined stress and can serve as progenitors to increase Fe and Zn concentration in the seeds of lines that tolerate the combined stress from acidic soil and high temperature in the Colombian Amazon region.

Keywords: energy use; leaf cooling; phenology; photosynthesis; photosynthate partitioning

check for updates

Citation: Suárez, J.C.; Contreras, A.T.; Urban, M.O.; Grajales, M.A.; Beebe, S.E.; Rao, I.M. Adaptive Responses of Biofortified Common Bean Lines to Acidic Soil and High Temperatures in the Colombian Amazon Region. *Agronomy* **2024**, *14*, 154. https://doi.org/10.3390/agronomy14010154

Received: 6 December 2023
Revised: 25 December 2023
Accepted: 8 January 2024
Published: 10 January 2024

1. Introduction

The Intergovernmental Panel on Climate Change (IPCC) predicts that the climate of the coming decades will be more drastic [1]. An increase in global average temperatures and changes in precipitation patterns are expected [2,3], together with decreased soil water availability and increased CO_2 levels in the atmosphere [4]. These changes and climate variability will impact agricultural land used for food production [5]. Estimates for the year 2050 indicate that 60 to 100% more food will need to be produced [1], which becomes a challenge to address global food security in the coming decades, mainly in crops with high sensitivity to abiotic stress, such as *Phaseolus* beans [6,7]. Maintaining crop yields under stressful conditions is a major challenge to modern agriculture [8]. An urgent need to cope with the effects of climate change on crop production and crop quality is the identification of materials that can provide sources of resistance to stress conditions [9]. Describing and documenting germplasm collections constitutes an added value to the accessions, allowing genetic resources programs to recognize their potential and the genetic variability they conserve, as well as reduce the time invested in the breeding process and the number of genotypes evaluated through pre-breeding [10].

The common bean (*Phaseolus vulgaris* L.) is possibly the most important edible grain legume in the world [11]. It contributes a total of 20–36% protein, as well as 15% of daily calories [12] and has essential amino acids, such as methionine, lysine, and tryptophan, in addition to iron (Fe), calcium (Ca), and zinc (Zn) [10]. Thus, the nutrient-rich grain of the common bean is considered a main source of protein in resource-poor populations [13], especially in developing countries in areas of Latin America, Africa, and parts of Asia [13,14]. However, the high susceptibility of common beans to different stress factors is a major constraint on grain production [15], especially in the tropics [16]. Because of this, breeding priorities in the tropics tend to focus on overcoming productivity constraints to reduce the huge gap between yield potential and yields obtained in farmers' fields [14]. Improved crop varieties adapted to recent and more variable environmental conditions are needed to cope with and mitigate the effects of climate change [17]. Specifically for Colombia, common bean breeding interventions are needed for rural populations living in poverty, including the Amazon region [18], where 7% of school-age children are chronically malnourished with moderate to high risks of Fe deficiency [19].

Advances in plant breeding and phenotyping contributed to elucidating physiological mechanisms related to the description of developmental stages, dry matter accumulation dynamics per organ, source-sink relationships, and plant growth simulation models [20,21], which are of great utility for selecting parents with traits of interest [14] in favor of improving the response of common beans to stress conditions [13,16]. One of the most important breeding traits for genetic enhancement is biofortification, which seeks to biologically increase Fe and Zn concentrations in the grain [22–24]. Results on screening of seeds of bean germplasm indicated an average concentration (mg kg^{-1}) of Fe and Zn of 55 and 28, respectively, with extremes of 102 for Fe and 54 for Zn [25]. One of the possible—however still not identified—causes of these low micronutrient contents may be related to low soil fertility, which could significantly affect the uptake and distribution of these two elements (Fe and Zn) within the plant and their accumulation in the grain [26].

In recent years, bean materials with high Fe and Zn concentration in the grain have been developed using: (i) multiparent advanced generation inter-cross (MAGIC) populations (37.9–87.6 mg kg^{-1} for Fe and 18.5–39 mg kg^{-1} for Zn) [27], and (ii) three Mesoamerican biparental populations from parents with high Fe and Zn accumulation (SMC 40, SMC 44, and SMC 33) crossed with parental lines (SCR 16 and SCR 9) that combine resistance to drought, bean common mosaic virus (BCMV) and bean golden yellow mosaic virus (BGYMV), and these bred lines accumulated between 49.2–108.3 mg kg^{-1} and 16.9–43.4 mg kg^{-1} of Fe and Zn in seed, respectively [20]. Using African multiparental populations that were obtained from AND 620, HRS 454, Gofta, Nakaja, Simama, and MLB 49-89A as parents, which accumulated between 30–115 mg kg^{-1} and 10–60 mg kg^{-1} of Fe and Zn, respectively [28], more than 2800 bean samples were generated for improving Fe and Zn concentration in seed [29]. Likewise, Fe and Zn concentration in seed of materials from different biparental populations have been generated using Cerinza × G 10022 (54–100 mg kg^{-1} for Fe and 23–38 mg kg^{-1} for Zn) [30], G 21242 × G 21078 (28–95 mg kg^{-1} for Fe and 17–49 mg kg^{-1} for Zn) [31], G 14519 × G 4825 (35–97 mg kg^{-1} for Fe and 17–49 mg kg^{-1} for Zn) [32], DOR 364 × G 19833 (40–84 mg kg^{-1} for Fe and 17–42 mg kg^{-1} for Zn) [33]; and 696 × G 19833 (39–79 mg kg^{-1} for Fe and 16–29 mg kg^{-1} for Zn) [34], and also from different bean lines developed in Brazil through breeding with concentrations ranging from 55.7–85.4 mg kg^{-1} for Fe and 29–47.2 mg kg^{-1} for Zn in seed [35]. However, it has been reported that Fe and Zn concentrations in the grain are highly affected by bean genotypes, environments, and genotype-by-environment interactions [36–38].

The above studies contributed to developing and identifying materials that accumulate a high concentration of micronutrients, together with adaptations to drought stress and disease resistance. However, no results are reported on biofortified bean lines that are adapted to the combined stress conditions of acidic soil and high temperatures. An evaluation program of different improved bean lines was initiated in 2018 by the University of Amazonia under the supervision of the CIAT bean breeding program, where initial consideration was given to evaluate the adaptation of advanced lines of ALB: small red kidney and black kidney lines that are adapted to drought and aluminum (Al) toxicity; BFS: small red seeded lines with adaptation to low soil fertility; SAB: cream speckled red seeded lines of the Andean gene pool with drought resistance; and SMR: red seeded lines with drought resistance, with high mineral (Fe) content, and adaptation to acid soil conditions and high temperatures in the western Amazon region of Colombia. This initial evaluation identified three lines (BFS 10, BFS 143, and ALB 210) with adaptations to the combined stress conditions of acidic soil and high temperature based on their physiological attributes and agronomic performance. Likewise, lines such as SEN 46, SEN 48, SEN 70, SMN 99, SMC 140, SMR 139-1G, GGR 147, SMG 21, and SMG 12 have also been identified as promising. These lines have shown greater adaptability with grain yields higher than 1800 kg ha^{-1} and have high mineral contents of Fe and Zn in the grain. Therefore, the objective of this study was to evaluate the adaptive responses of biofortified bean lines to the combined stress conditions of acidic soil and high temperature based on their phenological, physiological, and agronomic attributes for the identification of desirable attributes for further genetic improvement. We tested the hypothesis that biofortified bean lines with increased cooling capacity and energy distribution to the photosynthetic machinery have the ability to increase photosynthate partitioning toward pod formation and grain filling under the combined stress conditions of acidic soil and high temperature.

2. Materials and Methods

2.1. Experimental Site and Meteorological Conditions

The experiment was conducted under field conditions from June to September 2020 and from November 2020 to January 2021 at the Amazon Research Center CIMAZ Macagual of the University of Amazonia. The field site is located in the municipality of Florencia, Caquetá, Colombia, at 1°37′ North latitude and 75°36′ West longitude and an altitude of 250 m above sea level, within a tropical rainforest ecosystem. The average annual rainfall is 3800 mm, with periods of maximum rainfall in April and November, with an average temperature of 25.5 °C and a relative humidity (RH) of 84% and 1700 h of sunshine per year. During the cropping season, there was an average total rainfall of 866 mm, an average humidity of 83.6%, and an average ambient temperature of 36.4 °C, with minimum temperatures ranging from 18.4–26.6 °C and maximum temperatures between 25.0–36.9 °C (Figure 1). The area used for the experiment is characterized by an acidic soil (pH 4.6) with a high Al saturation of over 70% and an exchangeable Al content of 6.3 cmol(+) kg^{-1}. The soil is considered to have low fertility based on the low availability of P (Bray-II) of 2.58 mg kg^{-1} and low organic carbon of 1.35%, together with a low total base saturation of 7.1% (cmol(+) kg^{-1}: Ca, 0.38; Mg, 0.1; K, 0.14; Na, 0.1) and a low cation exchange capacity of 11.3 cmol(+) kg^{-1}. The preparation of the land used for the experiment, as well as the sowing of seed from common bean lines, was carried out manually, as was the control of weeds. No fertilization or irrigation was applied to the experiment, nor were pesticides applied for pest and disease control.

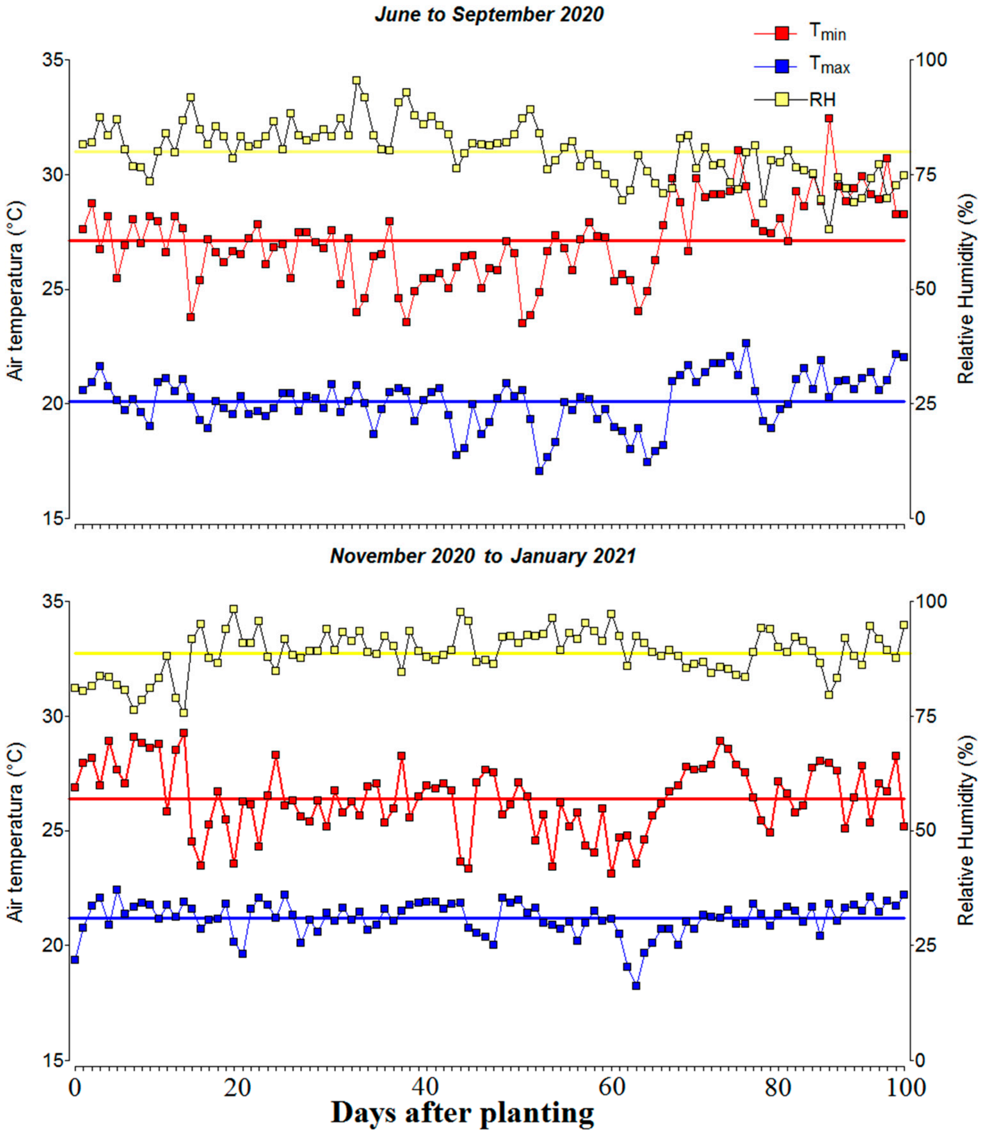

Figure 1. Distribution of maximum/minimum temperatures (T) and relative humidity (RH) during the crop-growing period in two seasons at the Centro de Investigaciones Amazónicas CIMAZ Macagual, Universidad de la Amazonia, Colombia. Red and blue lines indicate the average values of the maximum and minimum air temperatures, respectively. June to September 2020: T_{max}: 27.11 ± 0.27 °C, T_{min}: 20.11 ± 0.11 °C, RH: 79.97 ± 0.60%. November 2020 to January 2021: T_{max}: 26.39 ± 0.15 °C, T_{min}: 21.18 ± 0.07 °C, RH: 88.74 ± 0.48%.

2.2. Plant Material and Experimental Design

A total of 247 common bean lines from the Mesoamerican gene pool were used, as follows: 146 bean lines from F_4 families with above-average seed Fe and Zn concentration (Fe 80.2 ± 0.4 mg kg^{-1}, Zn 28.7 ± 0.3 mg kg^{-1}) and 99 common bean lines from F_5 families with moderate seed Fe and Zn concentration (Fe 63.5 ± 0.7 mg kg^{-1}, Zn 25.6 ± 0.2 mg kg^{-1}) are heterozygous lines with a variety of seed colors ranging from red to black, glossy and opaque, which were obtained from single crosses, double crosses, and backcrosses between different bean lines (the detailed pedigree of lines is presented in Table S1). Two advanced lines of Mesoamerican origin of the common bean (*P. vulgaris*) were used as controls: AMADEUS (TIO CANELA 75 × DICTA 105) (small red) and SER 16 (RAB 651 × TIO CANELA 75) × (RAB 608 × SEA 15) (small red). These bean materials provide sources of resistance to abiotic and biotic stresses as well as increases in micronutrient content and additional agronomic traits that increase biomass partitioning and increase market value.

A completely randomized block design with three replications was used, each block consisting of the 247 genotypes and the two controls (repeated in each row and column per block) to cope with spatial variability within the experimental site. Each experimental unit (plot) consisted of three rows, each three meters apart. In each row, 25 bean plants were planted at a distance of 12 cm between plants, for a total of 75 plants per plot.

2.3. Canopy Biomass, Dry Matter Partitioning Indices, Grain Yield, and Yield Components

At the mid-pod filling growth stage (BBCH 79, 55 days after planting), destructive sampling was carried out in one of the central rows in order to avoid the edge effect. A 0.5 m segment of the row, corresponding to a total of four plants, was taken to determine the canopy biomass (CB). Likewise, at physiological maturity (BBCH 85, 76 days after planting), another 0.5 m row segment corresponding to a total of four plants was taken for destructive sampling to record the dry weight of leaves, stems, pods, and seeds for each plant. The seed number per area (SNA) and the pod number per area (PNA) were also recorded during the sampling at physiological maturity. At the time of harvest, destructive sampling was performed in the central part of each plot. The pods of harvested plants were threshed, and the kernels were cleaned and dried to determine grain yield. The different indices described by Beebe et al. [13] were used based on dry matter distribution information at mid-fill and at harvest. The indices that were calculated were the pod partitioning index (PPI), the pod harvest index (PHI), and the harvest index (HI).

2.4. Phenological Behavior and Pollen Viability

In each plot, days to flowering (DF) and days to physiological maturity (DPM) were determined. The DF corresponds to the number of days it takes for each bean line to reach at least one flower on 50% of the plants planted in the plot, and the DPM relates to the number of days after planting for the pods to lose their green pigmentation on 50% of the plants planted in the plot. Pollen viability (PV) as affected by high temperatures was determined following the methodology of Porch and Jahn [39] and Suzuki et al. [40]. In brief, flower buds (n = 10) were collected before anthesis, and about 100 pollen grains from each flower bud per replicate were tested for percentage of PV by determining the ratio of stained pollen grains to total pollen grains.

2.5. Energy Use Efficiency and Leaf Cooling Capacity under High Temperature Conditions

To measure differences in energy use efficiency among bean lines, the MultispeQ portable device was used (PhotosynQ Inc., East Lansing, MI, USA) [41] that has the capacity to measure different variables of the functioning of the photosynthetic apparatus, fractions of the routes taken by the energy, chlorophyll fluorescence, as well as environmental variables. For this, the default protocol of Photosynthesis RIDES was used, in which the measurement starts automatically once the clamp is opened and closed. During the flowering period (BBCH 65, 44 days after planting), in the central row of each plot, three plants were taken at random, and from each plant, three fully developed leaves located between the seventh and ninth leaves from the base of the plant were taken to perform different measurements. From each selected leaf, three measurements were taken, and these measurements were made between 07:00 and 09:00 h (solar time). Among the environmental variables that were measured simultaneously using MultispeQ were photosynthetically active radiation (PAR), as well as humidity and ambient temperature. Leaf temperature was also measured, and the leaf temperature difference (LTD) was calculated using the difference between leaf temperature and ambient temperature. Different photosynthetic attributes were determined through measurements such as linear electron flow (LEF), which indicates the amount of energy moving through the chloroplast. LEF is mainly related to photosynthetic activity and was calculated by the equation LEF = $(\Phi(\text{PAR}) - \text{Y}\phi\text{II}) \times \text{f}$, where f is the fraction (f = 0.45) of absorbed radiation that is transferred to the PSII centers and ϕII corresponds to the effective quantum yield [41]. ATP synthase activity was calculated by measuring the proton conductance of the thylakoid membrane (gH+) and the amplitude of the electrochromic band shift signal (ESCt) [42] based on the dark-interval relaxation kinetic (DIRK) of electrochromic shift [43]. ESCt estimates proton transport in the transthylakoid membrane in relation to the proton motive force (pmf). Based on the above measurements, the proton flux was estimated as follows: (νH+ = ECSt × gH+) [44].

The different fractions of energy intake were measured, which are related to the following: (i) Energy is received by photosystem II (ΦII), and it indicates the state of the photosynthetic machinery of the plant; (ii) energy is dissipated as heat (ΦNPQ); and (iii) energy is not dissipated, which represents potential damage to the plant (ΦNO), in which all fractions should sum to one (1 = ΦII + ΦNPQ + ΦNO [45]). Likewise, the relative chlorophyll content (R_{Chl}) was measured, and the other physiological variables related to quenching due to non-photochemical dissipation of absorbed light energy (NPQt) were calculated according to Tietz et al. [46]. In addition, different PSI redox states were estimated, according to Kanazawa et al. [42]. PSI redox states are determined by the total active PSI centers (PSIact), the fraction of oxidized (PSIox) and open-state (PSIopen) PSI centers, as well as the over-reduced PSI (PSIor) corresponding to PSI acceptor side limitations resulting from the accumulation of electrons in PSI acceptors during steady-state illumination. Data recorded from the use of MultispeQ (*n* = 2477) [47] were stored on the PhotosynQ platform, and no recorded errors during the sampling time were reported. The database was downloaded, and each of the variables was independently analyzed for outliers using Pearson's standardized residuals. This process provides a measure of how well the model predicts the observation by recording those data that do not present an adequate fit [48].

2.6. Determination of Fe and Zn in Seeds

The seeds of biofortified lines were used to evaluate the adaptive responses in this study. Before sowing the seeds, the seed Fe and Zn concentrations were determined. Atomic absorption spectroscopy (AA-7000, Shimadzu Co., Kyoto, Japan) was used to determine the concentration of seed Fe and Zn using the acid digestion method [49]. In brief, 0.5 g of the seed sample was digested with 3 mL of nitric acid (HNO_3; 65%, P.A., ACS, ISO; PanReac AppliChem, Barcelona, Spain) in a porcelain crucible, heated for 30 min at 150 ± 5 °C until the formation of brown vapor, and then 2 mL of perchloric acid ($HClO_4$; 70%, P.A., ACS, ISO; PanReac AppliChem, Barcelona, Spain) at 200 ± 5 °C until the solution became translucent and white vapor formed, indicating complete digestion of the organic matter. Finally, 3 mL of hydrochloric acid (HCl; 37%, ACS, ISO; PanReac AppliChem, Barcelona, Spain) was added without heating and allowed to cool to room temperature, and the digested sample was transferred to a 50 mL volumetric flask. The transfer was performed using qualitative filter paper (3 hw, 110 mm, 65 g/m^2; Boeco Germany, Hamburg, Germany), and the volume of the solution was brought to 50 mL with deionized water for the determination of seed Fe and Zn concentration.

2.7. Data Analysis

The data of the different phenological and physiological variables and those related to biomass partitioning were graphed using scatter diagrams in order to determine the trend and relationships between the variables, and thus these data are suitable to evaluate differences in the adaptive responses of common bean lines to the combined stress conditions of acidic soil and high temperature. Different variables, such as grain yield (GY), such as ΦII, ΦNPQ, and ΦNO, which are related to energy use, were located on the Y axis; on the X axis, CB, PPI, PHI, ΦII, LEF, and LTD were located. Likewise, a variation of the variable (SNA, PNA, SW, ΦNPQ, LTD, and GY) that is related to the intensity of the color change (from red to violet from highest to lowest value) was included in the dispersion graphs and a magnitude that is related to the size of the circle (PNA, SW, CB, ΦNO, PSIox, PHI, CB, and HI). In each of the scatter plots, a line was drawn on each axis, which corresponds to the mean value of each variable. Likewise, a correlation analysis was carried out using the

Pearson test to determine the relationship between the variables. The scatter plots were made using the ggplot2 package (v3.3.3) [50] in R language software, version R.4.2.0. [51].

3. Results

3.1. Phenotypic Differences in Agronomic Performance

We analyzed the agronomic performance of 247 lines, and we found significant phenotypic differences among the lines. We observed that about 141 lines reached the harvest stage, of which only 116 lines presented a range in grain yield (GY) from 110 to 2109 kg ha^{-1} with a mean value of 593 kg ha^{-1}, and 25 lines presented values below 100 kg ha^{-1}. Lines of the F_5 family (lines: 859, 805, 865, and 657) and F_4 family (lines: 2853 and 2796) achieved grain yields above 1400 kg ha^{-1}. We found that canopy biomass (CB) values ranged from 333 to 3153 kg ha^{-1}, with an average value of 1180 kg ha^{-1}. The lines with the higher values (>2000 kg ha^{-1}) were three from the F_5 family (775, 785, and 827) and seven from the F_4 family (2861, 2863, 3309, 3319, 3597, 3558, 3602, and 3630) (Figure 2a).

In relation to photoassimilate remobilization from vegetative structures to pod formation, pod partitioning index (PPI) values ranged from 5 to 97% (Figure 2b). From the F_5 family, eight lines (885, 887, 755, 859, 795, 667, 849, and 683) and from the F_4 family, four lines (2781, 3730, 2991, and 2796) exhibited PPI values that were higher than 70% ($p < 0.001$) (Figure 2b). For the remobilization of photoassimilates from the pod wall to grain formation, the pod harvest index (PHI) values in nine lines, such as 873, 2853, 665, 861, 3744, 2796, 2781, 789, and 657, were higher than 50% (Figure 2c). The harvest index (HI) value reached up to 49% (Figure 2d). The lines with higher grain yields, such as 2796, 3730, 859, and 2853, also exhibited higher HI values. For the seed number per area (SNA), about 13 lines stood out for producing more than 300 seeds per m^{-2} ($p < 0.001$). The average weight of 100 seeds was between 10 and 54 g, with lines 657, 653, 2796, and 859 presenting values higher than 37 g ($p > 0.001$).

The average seed Fe concentration was 82 ± 2.6 mg kg^{-1} and 65.6 ± 4.7 mg kg^{-1} and Zn of 27.5 ± 2.18 mg kg^{-1} and 26.6 ± 1.4 mg kg^{-1} for the five lines of F_5 (859, 805, 865, 657, and 653) and four lines of F_4 (2853, 2796, 2815, and 3730), respectively. Out of the nine biofortified lines, six lines, including four F_5 lines: 859, 805, 865, and 657, with 86.5 ± 0.5 mg kg^{-1} and 28.5 ± 2.5 mg kg^{-1} of Fe and Zn, respectively, and two F_4 lines: 2853 and 2796, with 61.5 ± 1.9 mg kg^{-1} and 25.5 ± 1.1 mg kg^{-1} of Fe and Zn, respectively, were superior in their seed Fe and Zn concentrations.

3.2. Phenotypic Differences in Phenology and Pollen Viability

During the time of adaptation of the promising lines from the F_4 and F_5 families, days to flowering ranged from 33 to 44 days after planting (DAP), where the F_5 lines reached flowering at 35 DAP and two days later the F_4 lines reached flowering, and this delay in flowering of two days resulted in an average difference in GY of around 115 kg ha^{-1}. Lines such as 795, 841, and 745 (F_5) flowered earlier in response to combined stress conditions. Several lines (715, 3596, 663, 803, 3637, 3741, 849, 3649, 823, 3599, 843, 801, 897, 2824, 3634, 813, 2980, 837, 713, 3315, 3561, 29,17, 679, 891, 755, 2820, and 833) reached the physiological maturity stage earlier. When comparing the average number of days to physiological maturity between the different F4 and F5 lines, we observed that there was no difference between the two groups (71 DAP). However, lines with higher GY values were found to reach maturity slightly later. Pollen viability (PV) ranged from 37% to 95% with an average value of 82.1%, with no difference between the F_4 and F_5 lines. About 144 lines showed PV values above 70%. However, four lines (859, 2853, 3730, and 2796) showed values below 70%.

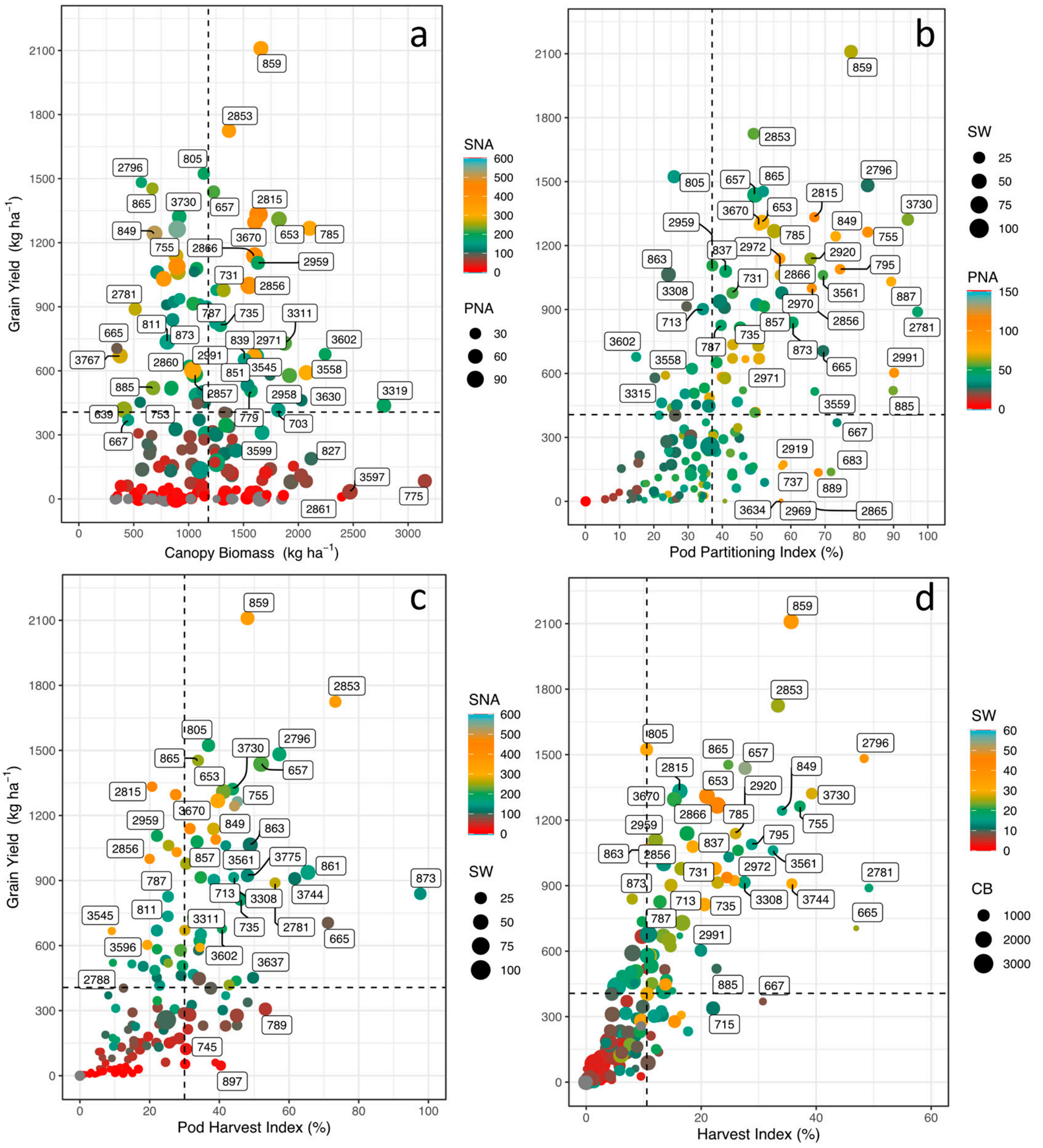

Figure 2. Relationship between grain yield (GY) and dry matter partitioning indices as a function of seed number per area (SNA), pod number per area (PNA), 100-seed weight (SW), and canopy biomass (CB) of biofortified common bean lines grown under the combined stress conditions of acidic soil and high temperature. (**a**) Grain yield and canopy biomass (CB) as a function of the gradient of SNA (change in color gradient from blue to red indicating higher to lower value) and the magnitude of PNA (circle size); (**b**) grain yield and pod partition index (PPI) as a function of the gradient of PNA (change in color gradient from blue to red indicating higher to lower value) and the magnitude of SW (circle size); (**c**) grain yield and pod harvest index (PHI) as a function of the gradient of SNA (change in color gradient from blue to red indicating higher to lower value) and the magnitude of SW (circle size); (**d**) grain yield and harvest index (HI) as a function of the gradient of SW and the magnitude of CB (circle size). The dotted lines on the axes correspond to the means of the dependent and independent variables.

3.3. Phenotypic Differences in Energy Use, Leaf Cooling, and Linear Electron Flow

Analysis of differences in the performance of the photosynthetic apparatus showed that parameters such as LTD, LEF, ECSt, vH+, and R_{Chl} were significantly different among lines ($p < 0.001$). All lines tested were able to adjust their leaf temperature values below ambient temperature (AT) values, which allowed leaf cooling. For example, lines such as 3632 and 835 presented more negative LTD values (−7 of AT; in this study we use "negative" for values, not in the sense of meaning), indicating a marked leaf cooling compared to a high AT value. On the other hand, some lines, such as 3596 and 793, presented lower negative LTD values, and their energy use efficiency related to photosynthetic processes was also low (Figure 3a). However, in the case of linear electron flow (LEF) and vH+ parameters, line 3596 presented the highest values (Figure 3b), as well as line 2962 presented the highest values of ECSt and PSIox. Line 815 showed the highest values of PSIor and PSIox. Chlorophyll fluorescence-derived parameters were significantly influenced by high temperatures and soil acidity ($p < 0.001$). For example, higher values of GY were associated with higher values of PSII quantum yield (ΦII), a situation that occurred with some lines such as 859, 2853, 805, and 2815. In addition, these lines exhibited a lower value of ΦNPQ (Figure 3a). However, the effect of stress was demonstrated in most of the bean lines due to the increase of ΦNPQ and higher allocation to ΦNO, showing a lower distribution of energy to photochemical processes (ΦII), which had a negative effect on GY, as evidenced in most of the lines that exhibited GY values below 1000 kg ha^{-1} ($p < 0.001$, Figure 3a). Results on the relationship between GY and LEF showed that line 865 (GY = 1453 kg ha^{-1}) had a higher electron transport rate combined with a higher proportion of open PSI centers (LEF = 228) (Figure 3b). An opposite relationship was observed with lines 859 and 2853, which showed GY values above 1800 kg ha^{-1} with average values of LEF (132 and 171, respectively) (Figure 3b).

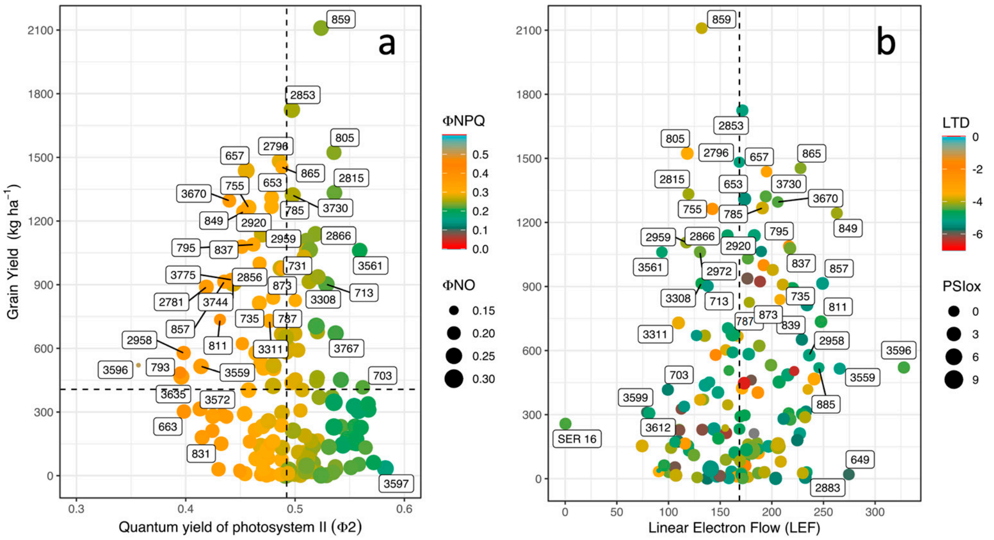

Figure 3. The relationship between grain yield (GY) and two different variables related to the functioning of the photosynthetic apparatus in biofortified common bean lines grown under the combined stress conditions of acidic soil and high temperature. (**a**) Grain yield and quantum yield of photosystem II (ΦII) as a function of the non-photochemical extinction gradient ΦNPQ (change in the color gradient of blue to red indicating higher to lower value) and the magnitude of unregulated processes ΦNO (circle size), parameters affecting the response variable (second); (**b**) grain yield and linear electron flow (LEF) as a function of the leaf temperature difference (LTD) gradient (change in color gradient from blue to red indicating higher to lower value) and magnitude of PSI Oxidized Centers (PSIox) (circle size), parameters affecting the response variable. The dotted lines on the axes correspond to the means of the dependent and independent variables.

Results on energy use showed that the bean lines were used on average at 49% and 22% for photosynthetic processes (ΦII) and dissipation of energy in the form of heat (ΦNPQ), respectively, and the rest for unregulated processes (~27% ΦNO). When we analyzed the effect of high temperatures on the photosynthetic apparatus, we found very specific responses, such as those presented by lines 859, 2815, and 805. These three lines presented

ΦII values higher than 50%, which positively influenced agronomic variables such as PHI and GY. These differences were closely related to LTD, showing values of −3.8, −3.4, and −4.0 °C for 859, 2815, and 805, respectively (Figure 4a). Even though lines 713, 861, 863, 2866, and 3561 eliminated the negative effect of high air temperature on thylakoid membrane function, these lines were not as efficient in mobilizing photoassimilates for grain formation (900 kg ha^{-1}) (Figure 4a). When the behavior of the photosynthetic apparatus of the bean lines that presented higher values of GY was observed in detail, differences were found that were mainly related to the ability to dissipate heat. For example, line 859 presented an LTD value 3 °C lower (less negative) than that presented by genotype 2853. The latter had a significantly higher GY value due to its greater ability to mobilize photoassimilates for grain development. This is probably related to the higher efficiency of PSII (Figure 4a), together with less energy dissipation as heat (Figure 4a). On the contrary, lines 865 and 657 showed lower values of ΦNO with higher (more negative) values of LTD (Figure 4c).

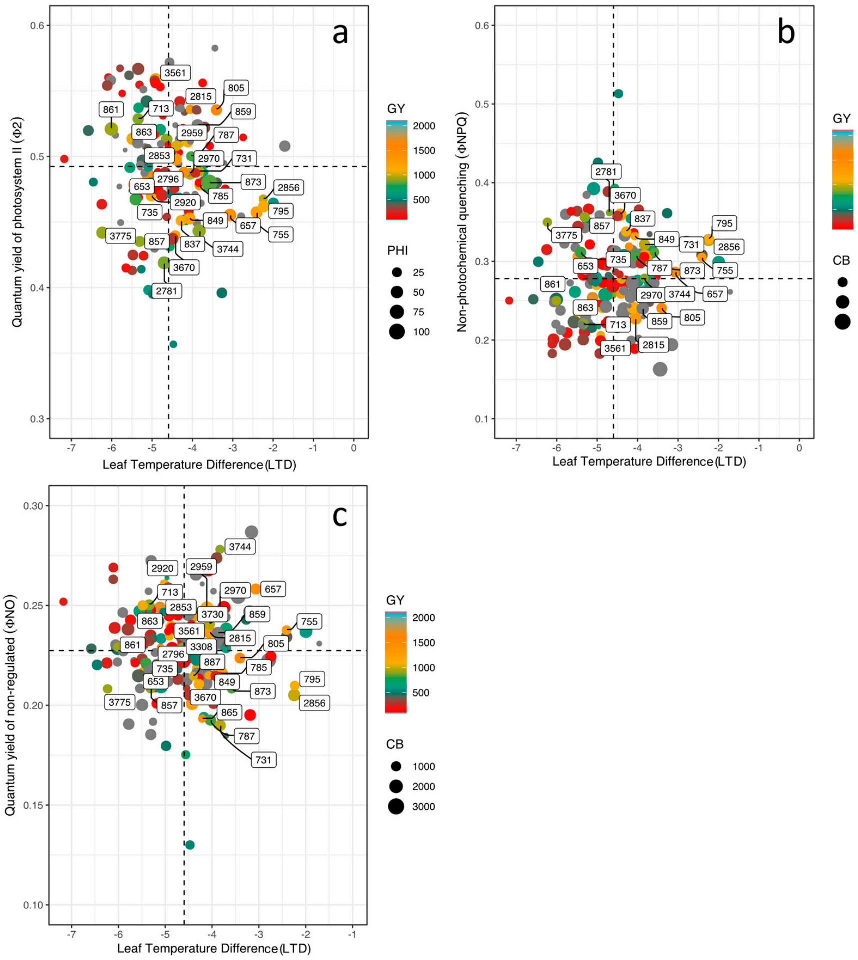

Figure 4. Relationship between leaf temperature difference (LTD) and energy use in photosynthesis as a function of grain yield (GY), pod harvest index (PHI), and canopy biomass (CB) of biofortified common bean lines grown under the combined stress conditions of acidic soil and high temperature. (**a**) Quantum yield of photosystem II (ΦII) and leaf temperature difference (LTD) as a function of GY (change in the color gradient from blue to red indicating higher to lower value) and PPI (circle size); (**b**) quantum yield of regulated non-photochemical energy loss in PSII (ΦNPQ) and leaf temperature difference (LTD) as a function of GY (change in the color gradient from blue to red indicating higher to lower value) and CB (circle size); (**c**) quantum yield of regulated non-photochemical energy loss in PSII (ΦNO) and leaf temperature difference (LTD) as a function of GY (change in the color gradient from blue to red indicating higher to lower value) and CB (circle size). The dotted lines on the axes correspond to the means of the dependent and independent variables.

Observations made on the magnitude of energy moving through the chloroplast when exposed to light in bean lines revealed that the lines that presented higher energy allocation to photosynthetic processes (859, 805, and 2015) (Figure 5a) presented lower energy dissipation in the form of heat (ΦNPQ) (Figure 5b). This photosynthetic response was associated with higher oxidized PSI reaction centers (PSIox) and lower proton flux in the thylakoid membrane (vH+) (Figure 5b). Line 657 showed the highest value of unregulated energy dissipation (ΦNO) (Figure 5c), together with greater values of proton conductance of the thylakoid membrane (gH+), active (PSIact) and open (PSIopen) reaction centers of PSI, chlorophyll content (R_{Chl}), and proton flux (vH+). These superior values of photosynthetic performance contributed to improved pod filling (PPI) and grain filling (PHI) through photosynthate mobilization. Nine lines were found with higher values of GY and lower values of LEF. These lines also exhibited greater ability to mobilize photosynthates, which resulted in superior agronomic performance.

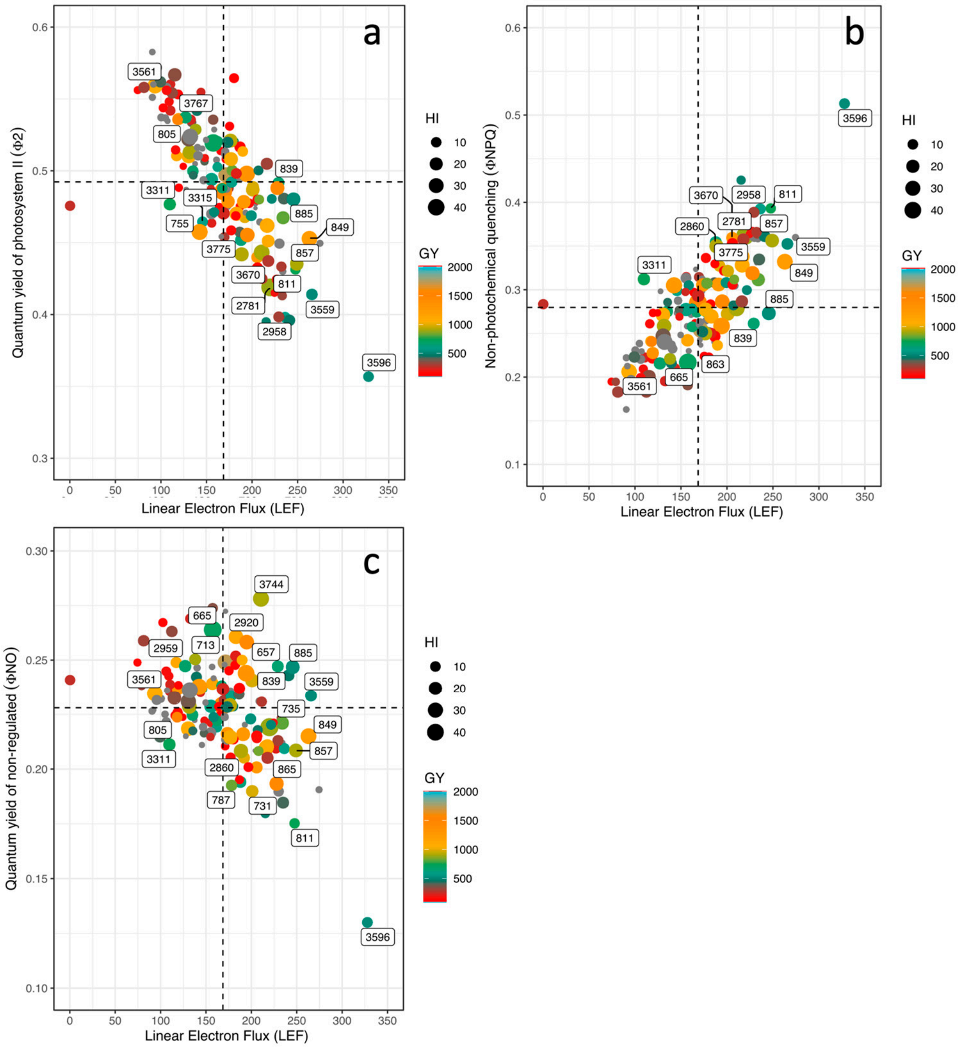

Figure 5. Relationship between different energy pathways and linear electron flux (LEF) as a function of the magnitude of the harvest index (HI) (circle size) and the gradient of grain yield (GY) (change in color gradient from blue to red indicating a higher to lower value) of biofortified common bean lines grown under the combined stress conditions of acidic soil and high temperature. (**a**) Quantum yield of photosystem II (ΦII) and LEF; (**b**) quantum yield of regulated non-photochemical energy loss in PSII (ΦNPQ) and LEF; (**c**) quantum yield of regulated non-photochemical energy loss in PSII (ΦNO) and LEF. The dotted lines on the axes correspond to the means of the dependent and independent variables.

3.4. Correlations between Physiological and Agronomic Traits

Based on the correlation analysis, days to flowering (DF) values were found to be negatively associated with different biomass partitioning indices (PPI, PHI, and HI) (Table 1). GY was found to be correlated with CB (r = 0.36, $p < 0.001$), PPI (r = 0.6, $p < 0.001$), PHI (r = 0.68, $p < 0.001$), and HI (r = 0.8, $p < 0.001$). GY was also related to photosynthetic performance by showing a positive association with LEF (r = 0.11, $p < 0.05$) and PSIact (r = 0.13, $p < 0.05$) and a negative association with ECSt (−0.16, $p < 0.01$) (Table 1). The value of AT was positively related to HI (r = 0.15) and PHI (r = 0.19) and negatively related to DF (r = −0.27), while DPM was positively related to LTD (Table 1). On the other hand, in the relationship between dry matter partitioning indices and the functional aspects of photosynthetic apparatus, we found that leaf cooling (negative values of LTD) positively influenced pod filling (PPI) (r = 0.15) and negatively influenced PSII quantum yield (ΦII) (r = 0.11). With regard to PSII quantum yield (ΦII), we found that it was negatively related to PHI (r = −0.16) and HI (r = −0.16). In addition, a negative effect of ΦII was found on the proton flux of the thylakoid membrane (vH+ = −0.87) (Table 1). At the level of energy use within the chloroplast, LEF was found to favor biomass partitioning (PPI, PHI, and HI) and energy dissipation in the form of heat (NPQt, r = 0.67; ΦNPQ, r = 0.78). However, the redox states of PSI affected PHI positively (PSIact, r = 0.13) and negatively (PSIox, r = −0.03) (Table 1). As for seed Fe content, positive correlations were observed with CB, DF, and VH+, while negative correlations were observed with some indices related to biomass partitioning (GY, PPI, and HI), environmental conditions (AT and PAR), and also those related to the function of the photosynthetic apparatus (gH+ and LEF) and chlorophyll content in the leaf. In the case of seed Zn, the content was positively correlated with PSIact and T, contrary to the negative correlation with gH+.

Table 1. Correlation coefficients ($p < 0.05$) between different phenological, physiological, and agronomic variables of biofortified common bean lines grown under the combined stress conditions of acidic soil and high temperature.

Variables	GY	HI	PHI	DF	DPM	LTD	ΦII	LEF	Seed Fe	Seed Zn
Grain yield (GY)		0.8 ***	0.68 ***					0.11 *	−0.17 *	
Canopy biomass (CB)	0.36 ***	−0.23 ***							0.13 *	
Pod partitioning index (PPI)	0.6 ***	0.73 ***	0.42 ***	−0.15 *		0.15 *		0.18 *		
Pod harvest index (PHI)	0.68 ***	0.75 ***		−0.25 ***			−0.16	0.2 **	−0.17 *	
Harvest index (HI)	0.8 ***		0.75 ***	−0.18 *			−0.16	0.19 **	−0.14 *	
Days to flowering (DF)			−0.25 ***					−0.19 **	0.21 *	
Days to physiological maturity (DPM)						0.2 **	−0.13			
Ambient humidity (AH)						0.68 ***	0.21 ***	−0.31 ***		
Ambient temperature (AT)		0.15 *	0.19 **	−0.27 ***		−0.55 ***	−0.23 ***	0.46 ***	−0.39 *	
Amplitude of the electrochromic band shift signal (ECSt mAU)	−0.16 **	−0.21 **								
Activity of ATP synthase (gH+)				−0.28 ***			0.24 ***		−0.38 *	−0.29 *
Leaf angle (LA)				0.27 ***	0.19 *	0.11 *		−0.15 *		
Leaf temperature difference (LTD)					0.2 **		−0.11 *			
Linear electron flow (LEF)	0.11 *	0.19 **	0.2 **	−0.19 **			−0.78 ***		−0.18 *	
Photosynthetically active radiation (PAR)		0.19 **	0.19 **	−0.15 *			−0.9 ***	0.96 ***	−0.14 *	
Energy dissipated as heat total (NPQt)				−0.13 *			−0.76 ***	0.67 ***		
Fractions of energy received by photosystem II (ΦII)		−0.16 *	−0.16 *			−0.11 *		−0.78 ***		

Table 1. *Cont.*

Variables	GY	HI	PHI	DF	DPM	LTD	ΦII	LEF	Seed Fe	Seed Zn
Fractions of energy not dissipated (ΦNO)			0.01 *	0.16 *			0.35 ***	−0.41 ***		
Fractions of energy dissipated as heat (ΦNPQ)							−0.93 ***	0.78 ***		
Total active PSI centers (PSIact)	0.13 *	0.17 *	0.13 *							0.14 *
Over-reduced PSI (PSIor)					0.14 *					
Fraction of an oxidized PSI center (PSIox)			−0.03 *							
Relative chlorophyll content (R_{Chl})		0.2 **	0.22 ***	−0.22 ***		−0.16 **		0.25 ***	−0.21 *	
Leaf thickness (T)						−0.1	−0.15 *	0.17 **		0.16 *
Proton flux (vH+)		0.15 *	0.16	−0.18 *			−0.87 ***	0.92 ***	0.17 *	

Mean values were used in the correlation analysis, and *, **, and *** represent levels of significance at 0.05, 0.01, and 0.001, respectively.

4. Discussion

4.1. Differences in Crop Development and Photosynthate Remobilization Affect Agronomic Performance

High-throughput phenotyping methods in crops such as common beans can facilitate the exploration of genetic resources by estimating phenotypic traits of importance and identifying accessions of interest for breeding programs [52,53]. This study provides data on phenotypic traits in selected common bean lines developed through breeding using intra- and interspecific crosses. The performance of lines is evaluated based on phenological, physiological, and agronomic responses under the combined stress conditions of acidic soil and high temperatures under field conditions.

Production of CB is the result of the accumulated net photosynthesis of the crop, which is sometimes significantly related to grain yield [15,54]. We found that as many as 11 lines showed a greater capacity to produce CB at the mid-pod filling growth stage compared to the other lines. This trait suggests a higher net CO_2 fixation capacity and greater access to and use of water and nutrients under stress conditions [15,55]. However, the GY values observed in these lines were low, probably due to a greater sensitivity to high temperatures. This condition has a greater impact on productive development since it limits the translocation of assimilates for the formation of pods and grains [56,57]. On the other hand, nine lines consistently presented higher GY values (859, 2853, 805, 2796, 865, 657, 2815, 3730, and 653), with lower CB production, and this suggests a yield advantage of these lines due to their superior capacity for remobilization of photosynthates from vegetative structures to reproductive organs (pods and grains) [58,59].

At the level of photosynthate remobilization, four lines (859, 2796, 2815, and 3730) were characterized by their higher capacity (>70%) to redistribute assimilates stored in vegetative structures for pod formation (PPI) and three lines (2853, 2796, and 657) in their ability to mobilize assimilates from pod walls to grain (>55%). However, we found that the critical point of these lines in general was in the translocation of assimilates from vegetative structures to grains (HI). On the one hand, these assimilate partitioning traits (PPI and PHI) have been previously highlighted as adaptive physiological responses of the common bean [60] and should be considered for heritable effects toward genetic enhancement and varietal development [11]. On the other hand, it should be noted that a reduced efficiency in one of the assimilate translocation steps could result in the sensitivity of other indices to stress conditions, in this case combined heat stress with soil acidity [55]. Furthermore, previous studies have observed that interspecific lines tend to be limited in the last translocation step (PHI), describing this phenomenon as “lazy pod syndrome” as some bean lines fail to complete this critical last step of grain filling [13,15,61–63].

The combined stress conditions of soil acidity and high temperature significantly affected PNA and SNA in general. Three lines (2815, 755, and 2856) presented more than 100 pods per area and around 400 seeds per area; however, these were penalized by abortions of pods and grains in the last stage of grain filling, which directly negatively affected their final values of GY by almost 50%. Interestingly, three lines (859, 2815, and 2853) with higher GY values showed pod penalization, reaching PNA values of 65, 57, and 114 pods, respectively, with a seed number of more than 350 m^{-2}. These lines yielded more not because of their small seed size but because of their superior sink strength to be able to form a greater number of grains under stress conditions, with better mobilization of assimilates towards grain formation and filling (seven to eight grains per pod), which compensates for the loss due to abortion of both pods and grains, and this may be attributed to greater sink strength, an important trait for improved adaptation of new lines [64]. Overall, values of GY contributed to the identification of nine significantly different lines (859, 2853, 805, 2796, 865, 657, 2815, 3730, and 653) in terms of their stress response. This means that the effect of environmental conditions on the evaluated lines was negative; similarly, the stress response of these lines may reflect very different evolutionary strategies that can be reflected in increases or decreases in assimilate partitioning [14,59], as well as a possible segregation between progenies that may have separated the genes again, which may cause transgressive segregation, supporting the behavior of most of the lines of the F_4 and F_5 families evaluated [60].

4.2. Combined Stress Affects Phenology and Pollen Viability

High temperatures have been shown to impact phenology and grain yield in the Western Amazon region of Colombia. In this study, we observed that about 172 lines reached flowering and physiological maturity. Among the lines that obtained the higher GY values, we found that they entered both growth stages without accelerating their phenological cycles. This indicates that GY was not influenced by high temperatures in these two phenological stages. Although earliness has been shown to enhance heat tolerance of grain legumes such as common bean through avoidance mechanisms [65], these lines were not able to adjust their phenological cycle for grain production [59,66]. This was mainly attributed to the impact of high temperatures on biomass partitioning for pod and grain formation, as revealed by HI and PHI values [13,67]. Likewise, pollen viability (PV) values evidenced that high temperature was not a factor that negatively affected the beginning of the reproductive phase to the point of pollen denaturation and floral abortions [39,68]. It has been reported that bean materials with early flowering (fewer days to flowering) may be suitable for more production cycles per year and, in some cases, compensate for some traits related to pod number and yield as part of adaptation to some stresses that are increasingly influenced by climate change [69]. This is considered an important domestication trait for avoiding stress and increasing productivity under conditions of environmental stress [70]. However, we found that sink strength in some lines was limited toward translocation of assimilates from the pod wall to the grain (PHI), which may have been influenced by delayed (not early) reproductive development (in our case, flowering) [11,14,59], as evidenced by the negative correlation we observed between DF and PHI.

4.3. Capacity of Photosynthetic Apparatus for Coping with Soil Acidity and High Temperature

At the physiological level, high temperature is a challenge mainly for the proper functioning of the photosynthetic apparatus, as it affects enzyme kinetics as well as membrane dynamics and organization [71]. Therefore, it is essential for the plant to adapt mechanisms and/or adopt strategies in favor of protecting its photosynthetic apparatus while maximizing light utilization under high temperature conditions [72]. When we look at the ability to regulate leaf temperature, we find that in general, all lines manage to adjust the temperature below the ambient temperature (negative values of LTD). This mechanism is possibly triggered by the higher loss of water at the leaf surface, allowing the leaf surface to be cooler, managing to maintain an optimal metabolism [73], and, as mentioned by Shanker et al. [74], a decrease in the vapor pressure deficit within the leaf. It is possible that heat-stressed plants are trying to minimize the irreversible damage that can be caused by the combined stress. For example, we found that the lines that presented better CB production and GY also showed high LTD values around −7 °C (more negative), while lines such as 859, 805, and 657 with higher yields showed a lower LTD value around −3 °C (less negative). This difference of almost 4 °C is likely related to high water use and low transpiration efficiency [75]. We hypothesize that this is also connected to lower costs in terms of water movement through the plant.

Under stress conditions, we found low relationships between LTD and the quantum yields of PSII ΦNPQ and ΦNO. We assume that the level of stress observed was not severe, which could be the reason for the relatively low relationships observed between LTD and fluorescence parameters. Furthermore, most of the lines that exhibited assimilate translocation limitations showed high ΦNO and low ΦNPQ values, reflecting the inability of these lines to protect themselves against damage from excess illumination, and possibly under more chronic stress, this will lead to photodamage [76,77].

In our study, the bean lines responded to environmental conditions by activating regulatory mechanisms to balance photosystem performance in order to protect the photosynthetic apparatus. For example, a decrease in ΦII was associated with an increase in ΦNO and NPQt under the combined stress, as was an increase in ΦNPQ associated with a lower ΦNO allocation. It appears that the lines tend to favor light-dependent dissipative processes (ΦNPQ) at the expense of fluorescence-related mechanisms to actually mitigate reactive oxygen species (ROS) propagation [78]. This should be attributed to the increased LEF that was reported to be necessary for the activation of NPQ processes [79] and was accompanied by a decrease in ECSt, which is related to the amplitude of the light-driven pmf (proton motive force) [42]. Both high LEF and low ECSt cause, to some extent, an imbalance between proton availability and use for ATP synthesis, resulting in acidification of the thylakoid lumen and generating an effect on the photoprotective processes of ΦNPQ and NPQt [80]. Furthermore, the remarkable increase in NPQt under combined stress indicates the separation of light-harvesting complexes from reaction centers, a mechanism that has been reported as an adaptive response to reduce stress-induced damage [81,82].

On the other hand, in lines with higher GY, a different mechanism was observed; in this case, we found decreases in ECSt and LEF, accompanied by high gH+, which could derive mainly from the decrease in intracellular CO_2 concentration, leading to a decrease in ATP synthase and thus in the plant's ability to utilize the reducing power of NADPH and ATP as the products of LEF [42,79,80,82]. This mechanism could be an adjustment of PSII operational efficiency so that ATP and NADPH production coincide with the decrease in CO_2 assimilation [83], as well as the related ATP-NADPH metabolic consumption by the Calvin cycle and other anabolic pathways within the chloroplast [80]. This adaptation of these lines could also be attributed in part to the reduction of leaf area (in the vegetative phase), which contributes to reaching the balance between water, nutrient, and light energy supply and their consumption, as well as the improvement of PSII quantum yield (ΦII) and the reduction of unregulated processes (ΦNO) [82].

4.4. Differences in Physiological Response between Common Bean Lines Reflect Possible Mechanisms in the Translocation of Assimilates That Favor Better Yields

The specific adaptation level of a bean line depends on its physiological responses and degree of susceptibility to a given stress condition. In this study, we observed that the group of lines is characterized by lower biomass production but high grain yield [84]. When we looked at the correlation analyses on the multiple traits evaluated, we found a positive relationship between GY and the other plant traits such as CB, PPI, PHI, and HI. This clearly indicates the importance of these indices in identifying materials with adaptations to acid soil conditions and heat stress [75]. However, the potential of these lines curiously lies in physiological traits. Specifically in the adaptive response in the adjustment of a lower LEF, in the adjustment capacity of ATP synthase (ECSt), most of PSII active centers, in order to present no impact on PSII quantum efficiency (ΦII), without a concomitant reduction in carbon supply and assimilation rates [77].

The identification of these mechanisms is important because: (1) photosynthesis is the process that contributes most to CB production; (2) an adequate mechanism in energy dissipation is related to a better translocation of assimilates (PHI and HI for pod and grain formation); and (3) variations in electron transport due to heat stress can also be reflected in the yield obtained [74,85–88]. Based on the results of this study, we found that nine biofortified common bean lines (F_5: 859, 805, 865, 657, and 653; F_4: 2853, 2796, 2815, and 3730) have traits with potential for adaptation to the combined stress of acid soils and high temperatures. As mentioned, these biofortified lines presented a mean seed Fe concentration of 82 ± 2.6 mg kg^{-1} and 65.6 ± 4.7 mg kg^{-1} and Zn of 27.5 ± 2.18 mg kg^{-1} and 26.6 ± 1.4 mg kg^{-1} for the five lines of F_5 (859, 805, 865, 657, and 653) and four lines of F_4 (2853, 2796, 2815, and 3730), respectively. Out of the nine biofortified lines, six lines, including four F_5 lines: 859, 805, 865, and 657, with 86.5 ± 0.5 mg kg^{-1} and 28.5 ± 2.5 mg kg^{-1} of Fe and Zn, respectively, and two F_4 lines: 2853 and 2796, with 61.5 ± 1.9 mg kg^{-1} and 25.5 ± 1.1 mg kg^{-1} of Fe and Zn, respectively, were superior in their seed Fe and Zn concentrations. In this sense, it has been reported that Fe and Zn concentrations in shoot tissue are directly related to photosynthetic efficiency [89]. In this study, we found that Fe and Zn concentrations in seed used for planting correlate negatively with some functions of the photosynthetic apparatus, such as those related to ATP synthase function (gH+) and linear electron flow (LEF). This shows the need to further investigate the relationship of photosynthetic traits with seed Fe and Zn concentrations in biofortified bean lines grown in different environments under stress conditions [90]. Further research is also needed to evaluate the stability and segregation of desirable traits, including seed Fe and Zn concentrations, under field conditions. Similarly, it is necessary to evaluate the parental lines from which they are derived under the conditions of this study to determine the percentage of heritability of adaptive responses to these conditions as well as the influence of genetic factors on photosynthesis for exploiting the photosynthetic differences of these materials through bean breeding programs.

5. Conclusions

Superior agronomic performance of different biofortified common bean lines is attributed to a combination of traits related to the following: (i) higher photosynthate partitioning capacity for pod formation and grain filling; (ii) physiologically efficient energy allocation to the photosynthetic machinery (ΦII); and (iii) dissipation of excess energy in the form of heat (ΦNPQ) coupled with increased thermoregulation (LTD). We identified six biofortified bean lines (F_5: 859, 805, 865, and 657; F_4: 2853 and 2796) with yields higher than 1400 kg ha^{-1} under combined stress conditions of acidic soil and high temperature. These bean lines increased canopy biomass (CB) as well as the dry matter allocation ability to increase partitioning into pods, which translated into higher values of grain yield (GY). These are considered desirable plant traits for the genetic enhancement of abiotic stress tolerance in biofortified common bean lines.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14010154/s1. Table S1. List of common bean genotypes used in the study.

Author Contributions: Conceptualization, J.C.S., M.O.U. and I.M.R.; data curation, J.C.S. and A.T.C.; formal analysis, J.C.S., M.O.U. and I.M.R.; investigation, J.C.S., A.T.C. and M.A.G.; methodology, J.C.S., A.T.C., M.O.U., M.A.G., S.E.B. and I.M.R.; funding acquisition, J.C.S.; project administration, J.C.S.; resources, J.C.S., M.O.U. and S.E.B.; supervision, J.C.S., A.T.C., M.O.U., M.A.G., S.E.B. and I.M.R.; writing—original draft, J.C.S., A.T.C., M.O.U. and I.M.R.; writing—review and editing, J.C.S., A.T.C., M.O.U. and I.M.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding, and the article processing charges were funded by Accelerating Varietal Improvement and Seed Systems in Africa (AVISA) project OPP 1198373, funded by the Bill and Melinda Gates Foundation.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Acknowledgments: We are grateful for the financial support from the CGIAR Research Program on Grain Legumes and Dryland Cereals and the Bill and Melinda Gates Foundation for the development of breeding lines of common bean. We would also like to thank all donors who supported this work through their contributions to the CGIAR Fund. MOU is grateful for the support of Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH and the Centre for International Migration and Development (CIM), Germany.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Masson-Delmotte, V.; Zhai, P.; Pörtner, H.-O.; Roberts, D.; Skea, J.; Shukla, P.R.; Pirani, A.; Moufouma-Okia Jefe, W.; Péan, C.; De Operaciones, J.; et al. *Resumen Para Responsables de Políticas. Calentamiento global de 1.5 °C Informe Especial del IPCC Sobre los Impactos del Calentamiento Global de 1.5 °C Con Respecto a los Niveles Preindustriales y las Trayectorias Correspondientes que Deberían Seguir las e*; IPCC: Rome, Italy, 2019; ISBN 9789291693511. Available online: www.ipcc.ch (accessed on 1 March 2023).
2. Rao, I.M.; Beebe, S.E.; Polania, J.; Grajales, M.; Cajiao, C.; Ricaurte, J.; García, R.; Rivera, M. Evidence for genotypic differences among elite lines of common bean in the ability to remobilize photosynthate to increase yield under drought. *J. Agric. Sci.* **2017**, *155*, 857–875. [CrossRef]
3. Lobell, D.B.; Burke, M.B.; Tebaldi, C.; Mastrandrea, M.D.; Falcon, W.P.; Naylor, R.L. Prioritizing climate change adaptation needs for food security in 2030. *Science* **2008**, *319*, 607–610. [CrossRef] [PubMed]
4. Pourkheirandish, M.; Golicz, A.A.; Bhalla, P.L.; Singh, M.B. Global Role of Crop Genomics in the Face of Climate Change. *Front. Plant Sci.* **2020**, *11*, 922. [PubMed]
5. Porch, T.G.; Beaver, J.S.; Brick, M.A. Registration of Tepary Germplasm with Multiple-Stress Tolerance, TARS-Tep 22 and TARS-Tep 32. *J. Plant Regist.* **2013**, *7*, 358–364. [CrossRef]
6. Raggi, L.; Caproni, L.; Carboni, A.; Negri, V. Genome-Wide Association Study Reveals Candidate Genes for Flowering Time Variation in Common Bean (*Phaseolus vulgaris* L.). *Front. Plant Sci.* **2019**, *10*, 962. [CrossRef]
7. Lizana, C.; Wentworth, M.; Martinez, J.P.; Villegas, D.; Meneses, R.; Murchie, E.H.; Pastenes, C.; Lercari, B.; Vernieri, P.; Horton, P.; et al. Differential adaptation of two varieties of common bean to abiotic stress: I. Effects of drought on yield and photosynthesis. *J. Exp. Bot.* **2006**, *57*, 685–697. [CrossRef] [PubMed]
8. Rao, I.M. Digging deep into defining physiological responses to environmental stresses in the tropics: The case of common bean and Brachiaria forage grasses. In *Handbook of Plant and Crop Physiology*; Pessarakli, M., Ed.; CRC Press: Boca Raton, FL, USA, 2021; pp. 1099–1140.
9. Buitrago-Bitar, M.A.; Cortés, A.J.; López-Hernández, F.; Londoño-Caicedo, J.M.; Muñoz-Florez, J.E.; Carmenza Muñoz, L.; Blair, M.W. Allelic diversity at abiotic stress responsive genes in relationship to ecological drought indices for cultivated tepary bean, phaseolus acutifolius a. Gray, and its wild relatives. *Genes* **2021**, *12*, 556. [CrossRef]
10. Ligarreto, G. Componentes de variancia en variables de crecimiento y fotosíntesis en fríjol común (*Phaseolus vulgaris* L.). *Rev. U.D.C.A Actual. Divulg. Científica* **2013**, *16*, 87–96.
11. Assefa, T.; Rao, I.M.; Cannon, S.B.; Wu, J.; Gutema, Z.; Blair, M.; Otyama, P.; Alemayehu, F.; Dagne, B. Improving adaptation to drought stress in white pea bean (*Phaseolus vulgaris* L.): Genotypic effects on grain yield, yield components and pod harvest index. *Plant Breed.* **2017**, *136*, 548–561. [CrossRef]
12. Schmutz, J.; McClean, P.E.; Mamidi, S.; Wu, G.A.; Cannon, S.B.; Grimwood, J.; Jenkins, J.; Shu, S.; Song, Q.; Chavarro, C.; et al. A reference genome for common bean and genome-wide analysis of dual domestications. *Nat. Genet.* **2014**, *46*, 707–713. [CrossRef]
13. Beebe, S.; Rao, I.M.; Blair, M.; Acosta, J. Phenotyping common beans for adaptation to drought. *Front. Physiol.* **2013**, *4*, 35. [CrossRef] [PubMed]
14. Beebe, S. Common bean breeding in the tropics. *Plant Breed. Rev.* **2012**, *36*, 357–426.
15. Polania, J.A.; Poschenrieder, C.; Beebe, S.; Rao, I.M. Effective use of water and increased dry matter partitioned to grain contribute to yield of common bean improved for drought resistance. *Front. Plant Sci.* **2016**, *7*, 660. [CrossRef] [PubMed]
16. Rao, I.M. Advances in Improving Adaptation of Common Bean and Brachiaria Forage Grasses to Abiotic Stresses in the Tropics. In *Handbook of Plant and Crop Physiology*, 3rd ed.; CRC Press: Boca Raton, FL, USA, 2014; pp. 847–889. ISBN 9781466553293.
17. Jarvis, A.; Ramirez, J.; Bonilla-Findji, O.; Zapata, E. Impacts of climate change on crop production in Latin America. In *Crop Adaptation to Climate Change*; Yadav, S.S., Redden, R.J., Hatfield, J.L., Lotze-Campen, H., Hall, A.E., Eds.; Wiley: Chichester, UK, 2011; pp. 44–56.

18. Zapata-Caldas, E.; Hyman, G.; Pachón, H.; Monserrate, F.A.; Varela, L.V. Identifying candidate sites for crop biofortification in Latin America: Case studies in Colombia, Nicaragua and Bolivia. *Int. J. Health Geogr.* **2009**, *8*, 1–18. [CrossRef]
19. ICBF (Instituto Colombiano de Bienestar Familiar). *Encuesta Nacional de la Situación Nutricional en Colombia (ENSIN)*; ICBF: Bogotá, Colombia, 2010.
20. Diaz, S.; Polania, J.; Ariza-Suarez, D.; Cajiao, C.; Grajales, M.; Raatz, B. Genetic correlation between Fe and Zn biofortification and yield components in a common bean (*Phaseolus vulgaris* L.). *Front. Plant Sci.* **2022**, *12*, 739033. [CrossRef]
21. Gonzalez, A.; Lynch, J.; Tohme, J.M.; Beebe, S.E.; Macchiavelli, R.E. Characters related to leaf photosynthesis in wild populations and landraces of common bean. *Crop Sci.* **1995**, *35*, 1468–1476. [CrossRef]
22. Amongi, W.; Nkalubo, S.T.; Ochwo-Ssemakula, M.; Badji, A.; Dramadri, I.O.; Odongo, T.L.; Nuwamanya, E.; Tukamuhabwe, P.; Izquierdo, P.; Cichy, K.; et al. Phenotype based clustering, and diversity of common bean genotypes in seed iron concentration and cooking time. *PLoS ONE* **2023**, *18*, e0284976. [CrossRef]
23. Huertas, R.; Karpinska, B.; Ngala, S.; Mkandawire, B.; Maling'a, J.; Wajenkeche, E.; Kimani, P.M.; Boesch, C.; Stewart, D.; Hancock, R.D.; et al. Biofortification of common bean (*Phaseolus vulgaris* L.) with iron and zinc: Achievements and challenges. *Food Energy Secur.* **2023**, *12*, e406. [CrossRef]
24. Lamptey, M.; Adu-Dapaah, H.; Osei Amoako-Andoh, F.; Butare, L.; Bediako, K.A.; Amoah, R.A.; Tawiah, I.; Yeboah, S.; Asibuo, J.Y. Genetic studies on iron and zinc concentrations in common bean (*Phaseolus vulgaris* L.) in Ghana. *Heliyon* **2023**, *9*, e17303. [CrossRef]
25. Beebe, S. Biofortification of common bean for higher iron concentration. *Front. Sustain. Food Syst.* **2020**, *4*, 573449. [CrossRef]
26. Garg, M.; Sharma, N.; Sharma, S.; Kapoor, P.; Kumar, A.; Chunduri, V.; Arora, P. Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. *Front. Nutr.* **2018**, *5*, 301899. [CrossRef] [PubMed]
27. Diaz, S.; Ariza-Suarez, D.; Izquierdo, P.; Lobaton, J.D.; de la Hoz, J.F.; Acevedo, F.; Duitama, J.; Guerrero, A.F.; Cajiao, C.; Mayor, V.; et al. Genetic mapping for agronomic traits in a MAGIC population of common bean (*Phaseolus vulgaris* L.) under drought conditions. *BMC Genom.* **2020**, *21*, 799. [CrossRef] [PubMed]
28. Kimani, P.M.; Warsame, A.H.M.E.D. Breeding second-generation biofortified bean varieties for Africa. *Food Energy Secur.* **2019**, *8*, e00173. [CrossRef]
29. CIAT. *Breeding Micronutrient Dense Bean Varieties in Eastern Africa. Bean Improvement for the Tropics, Annual Report I-P1 Report*; CIAT: Cali, Colombia, 2008; pp. 15–25.
30. Blair, M.W.; Izquierdo, P. Use of the advanced backcross-QTL method to transfer seed mineral accumulation nutrition traits from wild to Andean cultivated common beans. *Theor. Appl. Genet.* **2012**, *125*, 1015–1031. [CrossRef]
31. Blair, M.W.; Astudillo, C.; Rengifo, J.; Beebe, S.E.; Graham, R. QTL analyses for seed iron and zinc concentrations in an intra-genepool population of Andean common beans (*Phaseolus vulgaris* L.). *Theor. Appl. Genet.* **2011**, *122*, 511–521. [CrossRef]
32. Blair, M.W.; González, L.F.; Kimani, P.M.; Butare, L. Genetic diversity, inter-gene pool introgression and nutritional quality of common beans (*Phaseolus vulgaris* L.) from Central Africa. *Theor. Appl. Genet.* **2010**, *121*, 237–248. [CrossRef]
33. Blair, M.W.; Astudillo, C.; Grusak, M.A.; Graham, R.; Beebe, S.E. Inheritance of seed iron and zinc concentrations in common bean (*Phaseolus vulgaris* L.). *Mol. Breed.* **2009**, *23*, 197–207. [CrossRef]
34. Cichy, K.A.; Caldas, G.V.; Snapp, S.S.; Blair, M.W. QTL analysis of seed Iron, Zinc, and Phosphorus levels in an andean bean population. *Crop Sci.* **2009**, *49*, 1742–1750.
35. Pereira, H.; Del Peloso, M.; Bassinello, P.; Guimarães, C.; Melo, L.; Faria, L. Genetic variability for iron and zinc content in common bean lines and interaction with water availability. *Genet. Mol. Res.* **2014**, *13*, 6773–6785. [CrossRef]
36. Cichy, K.; Chiu, C.; Isaacs, K.; Glahn, R. Dry Bean Biofortification with Iron and Zinc. In *Biofortification of Staple Crops*; Springer: Singapore, 2022; pp. 225–270.
37. Saradadevi, R.; Mukankusi, C.; Li, L.; Amongi, W.; Mbiu, J.P.; Raatz, B.; Ariza, D.; Beebe, S.; Varshney, R.K.; Huttner, E.; et al. Multivariate genomic analysis and optimal contributions selection predicts high genetic gains in cooking time, iron, zinc, and grain yield in common beans in East Africa. *Plant Genome* **2021**, *14*, e20156. [CrossRef]
38. Philipo, M.; Ndakidemi, P.A.; Mbega, E.R. Environmental and genotypes influence on seed iron and zinc levels of landraces and improved varieties of common bean (*Phaseolus vulgaris* L.) in Tanzania. *Ecol. Genet. Genomics* **2020**, *15*, 100056. [CrossRef]
39. Porch, T.G.; Jahn, M. Effects of high-temperature stress on microsporogenesis in heat-sensitive and heat-tolerant genotypes of *Phaseolus vulgaris*. *Plant. Cell Environ.* **2001**, *24*, 723–731. [CrossRef]
40. Suzuki, K.; Tsukaguchi, T.; Takeda, H.; Egawa, Y. Decrease of pollen stainability of green bean at high temperatures and relationship to heat tolerance. *J. Am. Soc. Hortic. Sci.* **2001**, *126*, 571–574. [CrossRef]
41. Kuhlgert, S.; Austic, G.; Zegarac, R.; Osei-Bonsu, I.; Hoh, D.; Chilvers, M.I.; Roth, M.G.; Bi, K.; TerAvest, D.; Weebadde, P.; et al. MultispeQ Beta: A tool for large-scale plant phenotyping connected to the open photosynQ network. *R. Soc. Open Sci.* **2016**, *3*, 160592. [CrossRef]
42. Kanazawa, A.; Ostendorf, E.; Kohzuma, K.; Hoh, D.; Strand, D.D.; Sato-Cruz, M.; Savage, L.; Cruz, J.A.; Fisher, N.; Froehlich, J.E.; et al. Chloroplast ATP synthase modulation of the thylakoid proton motive force: Implications for photosystem I and photosystem II photoprotection. *Front. Plant Sci.* **2017**, *8*, 264832. [CrossRef]
43. Sacksteder, C.A.; Kramer, D.M. Dark-interval relaxation kinetics (DIRK) of absorbance changes as a quantitative probe of steady-state electron transfer. *Photosynth. Res.* **2000**, *66*, 145–158. [CrossRef] [PubMed]

44. Chovancek, E.; Zivcak, M.; Brestic, M.; Hussain, S.; Allakhverdiev, S.I. The different patterns of post-heat stress responses in wheat genotypes: The role of the transthylakoid proton gradient in efficient recovery of leaf photosynthetic capacity. *Photosynth. Res.* **2021**, *150*, 179–193. [CrossRef] [PubMed]
45. Kramer, D.M.; Johnson, G.; Kiirats, O.; Edwards, G.E. New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. *Photosynth. Res.* **2004**, *79*, 209–218. [CrossRef]
46. Tietz, S.; Hall, C.C.; Cruz, J.A.; Kramer, D.M. NPQ(T): A chlorophyll fluorescence parameter for rapid estimation and imaging of non-photochemical quenching of excitons in photosystem-II-associated antenna complexes. *Plant. Cell Environ.* **2017**, *40*, 1243–1255. [CrossRef]
47. Suárez, J.C.; Contreras, A.T. BD Exp. C17d y F5.9_Sco. PROJECT ID: 11013, 4169. [Dataset]. 2023. Available online: https://www.photosynq.org/projects/bd-exp-c17d-y-f5-9_sco (accessed on 5 December 2023).
48. Di Rienzo, J.A.; Macchiavelli, R.; Casanoves, F. *Mixed Models in Infostat. Electronic Version*. 2012. 1994p. Available online: http://www.infostat.com.ar (accessed on 5 December 2023).
49. Bankaji, I.; Kouki, R.; Dridi, N.; Ferreira, R.; Hidouri, S.; Duarte, B.; Sleimi, N.; Caçador, I. Comparison of Digestion Methods Using Atomic Absorption Spectrometry for the Determination of Metal Levels in Plants. *Separations* **2023**, *10*, 40. [CrossRef]
50. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*, 2nd ed.; Springer: Berlin/Heidelberg, Germany, 2016. [CrossRef]
51. R Development Core Team. *"Vigorous Calisthenics" Copyright (C) 2024 The R Foundation for Statistical Computing Platform: x86_64-apple-darwin17.0 (64-bit)*, R version 4.2.0; R Development Core Team: Vienna, Austria, 2023. Available online: https://www.r-project.org/ (accessed on 5 December 2023).
52. Nguyen, N.; Drakou, E.G. Farmers intention to adopt sustainable agriculture hinges on climate awareness: The case of Vietnamese coffee. *J. Clean. Prod.* **2021**, *303*, 126828. [CrossRef]
53. Rodriguez, D.F.C.; Urban, M.O.; Santaella, M.; Gereda, J.M.; Contreras, A.D.; Wenzl, P. Using phenomics to identify and integrate traits of interest for better-performing common beans: A validation study on an interspecific hybrid and its Acutifolii parents. *Front. Plant Sci.* **2022**, *13*, 1008666. [CrossRef] [PubMed]
54. Araus, J.L.; Slafer, G.A.; Reynolds, M.P.; Royo, C. Plant breeding and drought in C3 cereals: What should we breed for? *Ann. Bot.* **2002**, *85*, 925–940. [CrossRef] [PubMed]
55. Polania, J.; Poschenrieder, C.; Rao, I.M.; Beebe, S. Estimation of phenotypic variability in symbiotic nitrogen fixation ability of common bean under drought stress using 15N natural abundance in grain. *Eur. J. Agron.* **2016**, *79*, 66–73. [CrossRef]
56. Farooq, M.; Gogoi, N.; Barthakur, S.; Baroowa, B.; Bharadwaj, N.; Alghamdi, S.S.; Siddique, K.H.M. Drought stress in grain legumes during reproduction and grain filling. *J. Agron. Crop Sci.* **2017**, *203*, 81–102. [CrossRef]
57. Kazai, P.; Noulas, C.; Khah, E.; Vlachostergios, D. Yield and seed quality parameters of common bean cultivars grown under water and heat stress field conditions. *AIMS Agric. Food* **2019**, *4*, 285–302. [CrossRef]
58. Rao, I.M.; Beebe, S.E.; Polanía, J.; Grajales, M.; Cajiao, C.; García, R.; Ricaurte, J.; Rivera, M. Physiological basis of improved drought resistance in common bean: The contribution of photosynthate mobilization to grain. In Proceedings of the Paper presented at Interdrought III: The 3rd International Conference on Integrated Approaches to Improve Crop Production Under Dro, Shanghai, China, 11–16 October 2009.
59. Beebe, S.E.; Rao, I.M.; Cajiao, C.; Grajales, M. Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments. *Crop Sci.* **2008**, *48*, 582–592. [CrossRef]
60. Assefa, T.; Beebe, S.E.; Rao, I.M.; Cuasquer, J.B.; Duque, M.C.; Rivera, M.; Battisti, A.; Lucchin, M. Pod harvest index as a selection criterion to improve drought resistance in white pea bean. *F Crop. Res.* **2013**, *148*, 24–33. [CrossRef]
61. Beebe, S.E.; Rao, I.M.; Blair, M.; Butare, L. Breeding for abiotic stress tolerance in common bean: Present and future challenges. In Proceedings of the 14th Australian Plant Breeding & 11th SABRAO Conference, Brisbane, Australia, 10–14 August 2009.
62. Assefa, T.; Sperling, L.; Dagne, B.; Argaw, W.; Tessema, D.; Beebe, S. Participatory plant breeding with traders and farmers for white pea bean in Ethiopia. *J. Agric. Educ. Ext.* **2014**, *20*, 497–512. [CrossRef]
63. Klaedtke, S.M.; Cajiao, C.; Grajales, M.; Polanía, J.; Borrero, G.; Guerrero, A.; Rivera, M.; Rao, I.; Beebe, S.E.; Léon, J. Photosynthate remobilization capacity from drought-adapted common bean (*Phaseolus vulgaris* L.) lines can improve yield potential of interspecific populations within the secondary gene pool. *J. Plant Breed. Crop Sci.* **2012**, *4*, 49–61.
64. Rao, I.M.; Beebe, S.; Polania, J.; Ricaurte, J.; Cajiao, C.; Garcia, R.; Rivera, M. Can tepary bean be a model for improvement of drought resistance in common bean? *Afr. Crop Sci. J.* **2013**, *21*, 265–281.
65. Hall, A.E. Comparative ecophysiology of cowpea, common bean, and peanut. In *Physiology and Biotechnology Integration for Plant Breeding*; CRC Press: Boca Raton, FL, USA, 2004; pp. 243–287.
66. Rosales-Serna, R.; Kohashi-Shibata, J.; Acosta-Gallegos, J.A.; Trejo-López, C.; Ortiz-Cereceres, J.; Kelly, J.D. Biomass distribution, maturity acceleration and yield in drought-stressed common bean cultivars. *Field Crop. Res.* **2004**, *85*, 203–211. [CrossRef]
67. Polania, J.; Rao, I.M.; Cajiao, C.; Rivera, M.; Raatz, B.; Beebe, S. Physiological traits associated with drought resistance in Andean and Mesoamerican genotypes of common bean (*Phaseolus vulgaris* L.). *Euphytica* **2016**, *210*, 17–29. [CrossRef]
68. Omae, H.; Kumar, A.; Shono, M. Adaptation to high temperature and water deficit in the common bean (*Phaseolus vulgaris* L.) during the reproductive period. *J. Bot.* **2012**, *2012*, 803413.
69. Peláez, D.; Aguilar, P.A.; Mercado, M.; López-Hernández, F.; Guzmán, M.; Burbano-Erazo, E.; Denning-James, K.; Medina, C.I.; Blair, M.W.; De Vega, J.J.; et al. Genotype selection, and seed uniformity and multiplication to ensure common bean (*Phaseolus vulgaris* L.) var. Liborino. *Agronomy* **2022**, *12*, 2285. [CrossRef]

70. Bellucci, E.; Benazzo, A.; Xu, C.; Bitocchi, E.; Rodriguez, M.; Alseekh, S.; Di Vittori, V.; Gioia, T.; Neumann, K.; Cortinovis, G.; et al. Selection and adaptive introgression guided the complex evolutionary history of the European common bean. *Nat. Commun.* **2023**, *14*, 1908. [CrossRef]
71. Yamori, W.; Hikosaka, K.; Way, D.A. Temperature response of photosynthesis in C3, C4, and CAM plants: Temperature acclimation and temperature adaptation. *Photosynth. Res.* **2014**, *119*, 101–117. [CrossRef]
72. Sattari Vayghan, H.; Tavalaei, S.; Grillon, A.; Meyer, L.; Ballabani, G.; Glauser, G.; Longoni, P. Growth temperature influence on lipids and photosynthesis in Lepidium sativum. *Front. Plant Sci.* **2020**, *11*, 515048. [CrossRef] [PubMed]
73. Shanker, A.K.; Bhanu, D.; Sarkar, B.; Yadav, S.K.; Jyothilakshmi, N.; Maheswari, M. Infra red thermography reveals transpirational cooling in pearl millet (*Pennisetum glaucum*) plants under heat stress. *bioRxiv* **2020**. [CrossRef]
74. Shanker, A.K.; Amirineni, S.; Bhanu, D.; Yadav, S.K.; Jyothilakshmi, N.; Vanaja, M.; Singh, J.; Sarkar, B.; Maheswari, M.; Singh, V.K. High-resolution dissection of photosystem II electron transport reveals differential response to water deficit and heat stress in isolation and combination in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Front. Plant Sci.* **2022**, *13*, 892676. [CrossRef] [PubMed]
75. Suárez, J.C.; Urban, M.O.; Contreras, A.T.; Noriega, J.E.; Deva, C.; Beebe, S.E.; Polanía, J.A.; Casanoves, F.; Rao, I.M. Water use, leaf cooling and carbon assimilation efficiency of heat resistant common beans evaluated in Western Amazonia. *Front. Plant Sci.* **2021**, *12*, 644010. [CrossRef]
76. Klughammer, C.; Schreiber, U. Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method. *PAM Appl. Notes* **2008**, *1*, 27–35.
77. Fernández-Calleja, M.; Monteagudo, A.; Casas, A.M.; Boutin, C.; Pin, P.A.; Morales, F.; Igartua, E. Rapid on-site phenotyping via field fluorimeter detects differences in photosynthetic performance in a hybrid—Parent barley germplasm set. *Sensors* **2020**, *20*, 1486. [CrossRef]
78. Tikkanen, M.; Mekala, N.R.; Aro, E.M. Photosystem II photoinhibition-repair cycle protects Photosystem I from irreversible damage. *Biochim. Biophys. Acta-Bioenerg.* **2014**, *1837*, 210–215. [CrossRef]
79. Huang, W.; Yang, S.J.; Zhang, S.B.; Zhang, J.L.; Cao, K.F. Cyclic electron flow plays an important role in photoprotection for the resurrection plant Paraboea rufescens under drought stress. *Planta* **2012**, *235*, 819–828. [CrossRef]
80. Rott, M.; Martins, N.F.; Thiele, W.; Lein, W.; Bock, R.; Kramer, D.M.; Schöttlera, M.A. ATP synthase repression in tobacco restricts photosynthetic electron transport, CO_2 assimilation, and plant growth by overacidification of the thylakoid lumen. *Plant Cell* **2011**, *23*, 304–321. [CrossRef]
81. Basu, S.; Ramegowda, V.; Kumar, A.; Pereira, A. Plant adaptation to drought stress. *F1000Research* **2016**, *5*. [CrossRef]
82. Ben-Jabeur, M.; Gracia-Romero, A.; López-Cristoffanini, C.; Vicente, R.; Kthiri, Z.; Kefauver, S.C.; López-Carbonell, M.; Serret, M.D.; Araus, J.L.; Hamada, W. The promising MultispeQ device for tracing the effect of seed coating with biostimulants on growth promotion, photosynthetic state and water–nutrient stress tolerance in durum wheat. *Euro-Mediterr. J. Environ. Integr.* **2021**, *6*, 1–11. [CrossRef]
83. Baker, N.R. Chlorophyll fluorescence: A probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.* **2008**, *59*, 89–113. [CrossRef] [PubMed]
84. Rao, I.M. Role of physiology in improving crop adaptation to abiotic stresses in the tropics: The case of common bean and tropical forages. In *Handbook of Plant and Crop Physiology*; CRC Press: Boca Raton, FL, USA, 2001; pp. 605–636.
85. Çiçek, N.; Pekcan, V.; Arslan, Ö.; Çulha Erdal, Ş.; Balkan Nalçaiyi, A.S.; Çil, A.N.; Şahin, V.; Kaya, Y.; Ekmekçi, Y. Assessing drought tolerance in field-grown sunflower hybrids by chlorophyll fluorescence kinetics. *Rev. Bras. Bot.* **2019**, *42*, 249–260. [CrossRef]
86. Che, X.; Zhang, T.; Li, H.; Zhang, L.; Liu, J. Effect of hypoxia on photosystem II of tropical seagrass enhalus acoroides in the dark. *Photochem. Photobiol.* **2022**, *98*, 421–428. [CrossRef] [PubMed]
87. Giorio, P.; Sellami, M.H. Polyphasic okjip chlorophyll a fluorescence transient in a landrace and a commercial cultivar of sweet pepper (*Capsicum annuum*, L.) under long-term salt stress. *Plants* **2021**, *10*, 887. [CrossRef] [PubMed]
88. Janeeshma, E.; Kalaji, H.M.; Puthur, J.T. Differential responses in the photosynthetic efficiency of *Oryza sativa* and *Zea mays* on exposure to Cd and Zn toxicity. *Acta Physiol. Plant.* **2021**, *43*, 1–16. [CrossRef]
89. Nsiri, K.; Krouma, A. The Key Physiological and Biochemical Traits Underlying Common Bean (*Phaseolus vulgaris* L.) Response to Iron Deficiency, and Related Interrelationships. *Agronomy* **2023**, *13*, 2148. [CrossRef]
90. Mari, S.; Bailly, C.; Thomine, S. Handing off iron to the next generation: How does it get into seeds and what for? *Biochem. J.* **2020**, *477*, 259–274. [CrossRef]