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Deciphering the morpho-physiological and biochemical responses in *Lablab purpureus* (L.) Sweet seedlings to water stress

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ABSTRACT

Drought is a major crop productivity-limiting factor, which adversely affects growth and yield worldwide. Lablab (*Lablab pupureus* [L.] Sweet), is better adapted to withstand drought stress, however, characterization of lablab in Kenya has not been undertaken and there is limited knowledge on plant response to drought stress. Ten lablab genotypes were evaluated under greenhouse to determine the morpho-physiological and biochemical response at the seedling stage. The study revealed significant differential responses among lablab genotypes to water stress. The interaction between the water stress and the genotypes significantly affected the evaluated traits. Genotypes D1 and D6 showed significantly increased and superior morphological adaptation, respectively, indicating enhanced adaptation for tolerance to water stress. Significant variations of ascorbate peroxidase, guaiacol peroxidase and catalase activities were detected during the experimental period. The plants exhibited varying non-enzymatic antioxidants and osmolytes with an increase in proline, total phenol and water-soluble carbohydrates and a decline in free amino acids and water-soluble proteins under severe water stress. Genotype D10 recorded high antioxidant enzymatic activity and water-soluble carbohydrates, whereas D1 accumulated more proline and proteins, indicating better adaptation in scavenging reactive oxygen species to prevent membrane damage. The higher photosynthetic efficiency of seedlings of genotype D1 and superior morphological adaptation of D6 indicated better agronomic adaptation to severe water stress and could be utilized in plant breeding programmes for tolerance to drought.

1. Introduction

Lablab bean (*Lablab purpureus* (L.) Sweet) is a multipurpose herbaceous annual legume belonging to the *Fabaceae* family with varying chromosome number 2n=20,22,24 (She and Jiang, 2015). It is used for human consumption and forage in livestock production systems with high protein content (20 - 25%), amino acids, vitamins (A, C and Riboflavin), and minerals (such as calcium, iron, and phosphorus) and immature pods and seeds are rich in dietary fibre (Robotham and Chapman, 2017). Lablab bean contains vital phytochemicals which prevents viral infection and fight acute respiratory syndrome like COVID-19 and modulate the immune system activity (Purwanti et al., 2021). Lablab bean plants produces root nodules with nitrogen fixing ability, and an extensive root system which improves the soil physical structure and properties (Naeem et al., 2023).

Global food security is hampered by the changing climatic conditions often associated with high temperatures, floods, and drought leading to low crop yields (Lewandowski et al., 2018). Drought is a major constraint to crop production in developing countries, mainly in Africa and southern Asia, which largely rely on rain-fed agricultural production (Kimani and Beebe, 2011). Severe drought has negative impacts on plant growth, crop physiology and causes biochemical changes leading to a decline in crop yields (Barnabás et al., 2008). Water deficit may occur at any stage of the crop growth and development, and the first effect of drought is on seedling stage, leading to poor stand establishment (Parkash and Singh, 2020). Morpho-physiological changes include reduced growth, diminished chlorophyll content, and damaged photosynthetic apparatus (Chaves et al., 2009). At the cellular level, water limitation will affect vital maintenance functions of turgor pressure, hindering cell expansion and cell wall formation (Yang et al., 2021). Prolonged drought stress consequently leads to reduced fresh and dry weight, leaf number, surface area, root and shoot length, total chlorophyll and relative water content (Dawood et al., 2014). Lablab bean is therefore an important alternative crop when considering food, nutritional and economic security to poor farmers in arid and semi-arid areas and according to Maass et al. (2010), it out-yields other legumes like common beans (*Phaseolus vulgaris*) or cowpea (*Vigna unguiculata*), especially during the dry season (Ewansiha and Singh, 2006).

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Several studies have revealed morphological variability for drought tolerance in lablab beans at different developmental stages (Kokila et al., 2014; Grotelüschen, 2014; Guretzki and Papenbrock, 2014). Some morpho-physiological and biochemical parameters have been established to assess drought tolerance in herbaceous plants, based on classical growth parameters like fresh weight, dry biomass weight and relative water content (RWC) (Yogavathi et al., 2014). Physiological traits have been used for drought tolerance identification like the stomata behavior analyzed by leaf conductivity and infrared thermography (Guretzki and Papenbrock, 2013) and reactive oxygen species and antioxidant enzyme activities (D'souza, and Devaraj, (2011). High throughput phenotyping devices like the MultispeQ Beta have been used to obtain quality data under field or greenhouse conditions to rapidly measure a large number of non-destructive parameters such as leaf pigmentation, photosynthetic traits, light fluorescence and absorbance (Kuhlgert et al., 2016).

Despite these advancements, there is limited knowledge regarding the acclimation of lablab bean seedlings to adaptive mechanisms in plant growth and physio-biochemical processes to water deficit, such as: changes in photosynthetic systems, antioxidant defenses and tissue osmotic potential (Chen et al., 2017). Moreover, majority of the existing well-established lablab breeding programs are conducted outside Africa, and primarily focus on forage enhancement (Sserumaga et al., 2021). Several studies have identified high genetic diversity in lablab in Eastern African accessions (David et al., 2021; Letting et al., 2022), however, there is no documentation regarding adaptation to drought which has delayed its advancement and utilization in plant breeding programmes (Maass et al., 2010). The information on the physiological and biochemical basis for lablab bean adaptation to water stress could help in phenotypic screening and guide introgression into varieties with defined growth strategies. Additionally, assessment of different parameters and their interrelationship under varying water stress regimes would be useful in selecting diverse varieties in breeding for drought tolerance (Sarkar et al., 2016).

Accordingly, this study was conducted to investigate the morphological, physiological and biochemical responses in lablab seedlings subjected to varying water regimes to understand the underlying tolerance mechanisms that would allow effective utilization by breeding programmes to improve lablab production under different water stress levels.

2. Materials and methods

2.1. Materials and study site

Ten extensively grown lablab genotypes obtained from farmers in eastern, coastal, central and rift valley regions of Kenya were used in this study (Table 1). Seeds of the lablab genotypes were obtained from farmers. Pot experiments were conducted under controlled greenhouse conditions: 24 °C/17 °C day/night temperatures, 12/12 h day/night photoperiod and 70 - 80% relative air humidity at the Department of Biochemistry, Faculty of Science and Technology, University of Nairobi.

2.2. ***Experimental design and layout***

The genotypes were arranged in a factorial experiment laid out in a completely randomized design (CRD) with four replications. The two factors were the genotypes (10) and water regimes [well-watered at 100% field capacity (FC), moderately stressed (MS) at 50% field capacity and severely stressed (SS) at field capacity 25%]. The main plots and sub-plots consisted of water regimes and genotypes, respectively.

2.3. Greenhouse evaluation and drought treatments

Plastic pots measuring 10 cm × 10 cm × 8 cm (length, width and height) were filled up with 250 g of homogenized sandy-loam soil. Three seeds per genotype were sown per pot and after germination; the plants were thinned to one seedling per pot. The seedlings were irrigated with 150 ml of water after every two days and grown under natural greenhouse conditions. Ten days after emergence, water stress treatments were imposed on uniformly developed seedlings with eight fully expanded leaves. The 100 % field capacity control group was determined by weighing the pot mass when saturated with water and the total available water by subtracting the mass of pot and soil. The pot water content was maintained by establishing changes in plant biomass according to Li et al. (2008), where an empirical relationship between plant height (P, cm) and fresh weight (F, g) was computed as $F= 0.975 + 0.112\ P$ ($R^2 = 0.968$, $P<0.001$). The water stress treatments were controlled for natural drought by maintaining the weight of the pots in the drought treatment at 50 % and 25% of the well-watered control treatment. In a preliminary experiment, water stress screening was done on the seedlings, revealing that seven days was sufficient/ adequate for the seedlings to exhibit water stress symptoms such as wilting and leaf shedding. Thereafter, the data was taken for physiological, morphological and biochemical parameters on the 100% FC control (well-watered), 50% FC moderately stressed plants and 25% severely stressed treatment.

2.4. Measurement of morphological parameters

Morphological traits were measured on the nineteenth day including the shoot and, root lengths. At least three biological replications were included for each treatment. Shoot length (SL) was obtained by measuring the length of three shoots per replication and averaged. The root length (RL) was obtained by uprooting and measuring the length as described by Guretzki and Papenbrock (2013).

2.5. Measurement of leaf relative water content

Leaf relative water content (RWC) was determined as described by Chen et al. (2015), where the detached leaves were weighed

Table 1
Lablab genotypes used in the study

Genotype	Accession Code	Chromosome number	Origin of collection	Region	Color
D2	11722	22	Mbeere	Eastern	Light brown
D4	21376	22	Mbeere	Eastern	Cream
D6	27002	22	Mbeere	Eastern	Black
D5	13129	22	Machakos	Eastern	Brown
D8	13083	22	Makueni	Eastern	Black
D9	12088	22	Meru	Eastern	Black
D10	11723	22	Limuru	Central	Black
D3	13758	22	Kilifi	Coastal	Black
D7	12187	22	Lamu	Coastal	Brown
D1	10706	22	Nakuru	Rift Valley	Brown

immediately to obtain fresh weight (FW). The leaves were then submerged in 20 ml distilled water in Petri dishes in the dark for 6 h at room temperature to regain turgidity. The leaves were removed and quickly blotted to remove the excess water on the surfaces and weighed to obtain the saturated turgid weight (TW). The leaves were placed in an oven, dried at 70 ° C for 48 h and the dry weight (DW) was recorded.

Leaf relative water content (RWC) was computed using the formula:

$$\text{LRWC } (\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100\ \%$$

2.6. Measurement of photosynthetic parameters

On the eighteenth day between 9 am and 12 noon data were taken on the photosynthetic parameters of the middle fully expanded upper leaf surface of water stressed and non-water stressed plants using MultispeQ Beta device (v1.0 MI, USA) and submitted to the photosynQ platform (http://www,photosynq.org) (Kuhlgert et al., 2016). At least four biological replications were included for each experiment. The measurements include: photosystem II photochemical efficiency (Phi2), photo-protective non-photochemical quenching (PhiNPQ), basal dissipation of non-regulated light energy (PhiNO), mean photochemical efficiency of photosystem II (Fv/Fm), relative chlorophyll content (SPAD), and the total non-photochemical quenching (NPQt). Environmental parameters such as light exposure, light absorbance, Linear Electron Flow (LEF), ambient temperature and relative humidity were captured using MultispeQ and recorded.

2.7. Determination of hydrogen peroxide and malondialdehyde content

Hydrogen peroxide (H_2O_2) in the leaves of plants from each treatment was determined according to Paital (2014). Leaf tissues (0.5 g) were homogenized in an ice bath with 2 ml 0.1 % (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 rpm for 20 minutes and 0.5 ml of the supernatant was added to 0.5 ml 10 mM potassium phosphate buffer (pH 7.0) and 1 ml 1 M potassium Iodide. The absorbance of the supernatant was read at 390 nm and the content of H_2O_2 was calculated using the "extinction coefficient 0.28/mM/cm, and quantified based on the standard curve developed using different concentrations of H_2O_2 (Paital, 2014). The experiment was replicated three times. The level of H_2O_2 was expressed as nmol g^{-1} fresh weight

Malondialdehyde (MDA) content was determined based on Wang et al. (2019). Fresh leaf material (0.1 g) was homogenized in 2 ml 0.1% (w/v) TCA solution. The homogenate was centrifuged at 10,000 rpm for 20 minutes at 4 °C and 0.5 ml of the supernatant added to 1 ml 0.5% (w/v) thiobarbituric acid (TBA) in 20% TCA. The mixture was incubated in a water bath at 95 °C for 30 minutes and the reaction was stopped by quickly placing the eppendorf tubes on ice. The samples were then centrifuged at 10,000 rpm for 5 minutes and the absorbance of the supernatant (0.5 ml) at 532 was read and the value for the non-specific absorption at 600 nm was substracted. Malondialdehyde concentration was calculated with its extinction coefficient 155 mM^{-1} cm^{-1} using the formula:

$$\text{MDA Content} = \frac{\textit{Extraction buffer } (ml) \times \textit{Supernatant } (ml) \times (\textit{Abs } 532 - \textit{Abs } 600)/155)}{\textit{Amount of sample } (g)} \times 1000$$

where 532 nm = maximum absorbance of the TBA-MDA complex; 600 nm = the correction for non-specific turbidity and 155 $mM^{-1}cm^{-1}$ = specific molar extinction coefficient for MDA. The experiment was replicated three times. The MDA content was expressed as expressed as nmol malondialdehyde g^{-1} fresh weight.

2.8. Assays of antioxidant enzyme activities

Antioxidant enzymes extraction was performed according to Fijalkowski and Kwarciak-Kozlowska (2020). Each leaf sample weighing 0.5 g was homogenized in 2 ml of phosphate buffer containing 100 mM potassium phosphate buffer pH (6.8) on ice, 0.2 mM EDTA and 1% (w/v) polyvinylpyrolydone (PVP). The mixture was centrifuged at 10,000 rpm (Mikro 200R German model) for 20 minutes at 4 °C. The supernatants were assayed for guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and catalase (CAT) enzyme activities assays and the assays were replicated three times.

Guaiacol Peroxidase (POD) activity was determined according to Fijalkowski et al. (2020) by adding 50 μl of homogenate to 2 ml of the reaction mixture that contains 25 mM H_2O_2, 25 mM guaiacol and 50 mM sodium acetate buffer (pH 7.0). GPX activity was determined by recording the absorbance at 470 nm which increases due to formation of tetra-guaiacol (coefficient of extinction 26.6 mM^{-1} cm^{-1}). The GPX activity was expressed in units per mg of protein (U/mg protein).

Ascorbate peroxidase (APX) activity was assayed by checking the oxidation of ascorbic acid and recording the change at absorbance of 290 nm. The enzyme extract of 10 μl was mixed with 1 ml of a reaction buffer containing 0.25 mM ascorbic acid, 0.2 mM Tris/HCl buffer (pH 7.8) and 0.5 mM H_2O_2. The ascorbate peroxidase activity was calculated from the extinction coefficient (2.8 mM^{-1} cm^{-1}) ascorbate (Haida et al., 2019). The APX activity was expressed in units per mg of protein (U/mg protein)

Catalase (CAT) activity was determined as described by Haida and Hakiman (2019). The reaction buffer (3 ml) containing 15 mM H_2O_2 and 50 mM phosphate buffer (pH 7.0) was added to 50 μl of the enzyme extract. The activity was calculated from the extinction coefficient (40 mM^{-1} cm^{-1}) for H_2O_2. The activity of catalase was measured as the decrease in absorbance at 240 nm for one minute due to the decomposition of H_2O_2. The CAT activity was expressed in units per mg of protein (U/mg protein).

2.9. Measurement of total phenolics

Total phenolics were determined by Folin-Ciocalteu (F-C) colourimetric method according to Haida and Hakiman (2019). Leaf samples were crushed in 15 ml of 50 % methanol and centrifuged for 2 minutes, then filtered and the volume was made up with methanol (50%) in volumetric flask up to 25 ml. 1 ml of extract mixed with 9 ml of distilled water and 1 ml of Folin-Ciocalteu phenol reagent and vortexed for 30 seconds. The mixture was allowed to settle for 5 minutes then 10 ml of 7.5 % sodium carbonate solution was added to the mixture. The mixture was incubated for 90 minutes at room temperature. The standard was generated by dissolving 10 g of Gallic acid in 100 ml of 50% methanol 100 μg/ml) and then further diluted at different concentrations to 0, 25, 50, 75 and 100 μg/ml to generate a standard curve (Madaan et al., 2011). The absorbance of the standard was determined at 765 nm against blank having phenol reagent without sample. Total phenol content was expressed as μg of GAE/g of extract and the experiment was replicated three times.

$$\textit{Total phenolic content} = \frac{GAE \times V \times D \times 10^{-6}}{W} \times 100$$

Where GAE = Gallic acid equivalents (μg/ml), V = Total volume of sample (ml), D = Dilution factor and W = Sample weight (g)

2.10. Measurement of proline content

The proline level was determined by the method described by Bates et al. (1973). Leaf tissues (0.25 g) were homogenized in 10 ml of 3% aqueous sulphosalicylic acid. The homogenate was centrifuged at 10,000 rpm for 15 minutes and 2 ml of the supernatant was added to 30 ml of glacial acetic, 1.25 g ninhydrin, and 20 ml of 6 M orthophosphoric acid, and incubated for 1 hour at 100 °C. The reaction was terminated in an ice bath and extracted with 4 mL of toluene. The extract was vortexed for 1 minute and the absorbance was measured at 520 nm using toluene as the blank, 1.153 g L-proline as the standard in 10 ml distilled water. A standard curve was generated using different concentrations of proline. The proline content was expressed as μg per gram of fresh weight (μg/g fresh weight). The experiment was replicated three times.

2.11. Measurement of total proteins and free amino acids

Total proteins were estimated according to the method described by Bradford (1976). Briefly, hundred (100) mg leaf samples were homogenized in phosphate buffer. 100 mg Brilliant Blue G-250 was dissolved in 50 ml 95% ethanol followed by 100 ml of 85% phosphoric acid (H_3PO_4), distilled water was added to make total volume of 1 L. The solution was filtered and kept at 4 °C and absorbance was read at 595 nm. For the measurements, 100 μl extract and 5 ml Bradford solution were mixed and incubated for 5 minutes. BSA solution of different concentration was prepared to generate a standard curve and expressed in units per gram of fresh weight (mg/g FW) and the experiment was replicated three times.

Free amino acids were assayed using ninhydrin according to Correia et al. (2005). 0.5g of fresh leaf samples were crushed and 1 ml of supernatant was heated in a boiling water bath of 95 °C for 15 minutes. Two ml of ninhydrin buffer solution containing 0.8 g of ninhydrin was dissolved in 30 ml of 2-methoxyethanol and 10 ml of acetate buffer 4 M, pH 5.5. The buffer solution was added into the supernatant and the mixture was cooled to room temperature, 3 ml of 50% ethanol was added, and the absorbance was read at 570 nm and 440 nm after 10 minutes. The amount of amino acids was determined by reference to a standard curve prepared using different concentrations of aspartic acid units per gram of fresh weight (mg/g FW) and the experiment was replicated three times.

2.12. Determination of carbohydrate content

Carbohydrate content was determined based on Hall (2013). Fresh leaf samples were ground to a fine powder. Approximately 0.2g leaf powder was extracted with ethanol under agitation and centrifuged and the supernatant was collected. The process was repeated three times. The supernatant was combined and diluted with water and the solution was evaporated in a boiling water bath. The samples were dissolved in distilled water and the soluble sugars were measured using by reference to a standard curve prepared by a series of known carbohydrate concentration units per gram of fresh weight (mg/g FW) and the experiment was replicated three times

2.13. Statistical analyses

The analysis of variance (ANOVA) for, morphological, photosynthetic and biochemical traits was carried out to determine the effects of water stress on lablab seedlings using agricolae software package implemented using R version 4.1.2 (R Core Team, 2021). The mathematical representation was given by the model $Y= \mu+ j+k+l +jl + e$where y = yield, μ= mean j = genotypic effects, k = replication, l = water regimes, jl = interaction, e = error. The genotypes were considered random effects whereas the treatments considered fixed effects. The mean differences were separated by least significant differences (LSD) test at $P < 0.05$. Box plots of morphological traits were generated using ggplot2 library on R software and values for all data were expressed as the mean ± SD significant differences based on Tukey's multiple range tests ($P < 0.05$). Principal component analyses (PCA) and biplot graphs were done using factoextra library on R software to determine the contributions of various traits and two tailed Pearson's correlations were calculated to determine the interrelationship of the variables to varying water deficit conditions.

Table 2
Shoot and root traits as influenced by soil moisture levels and the interactions under three replications

		Morphological trait				
Source	**df**	**SL**	**RL**	**SFW**	**SDW**	**RWC**
Block	2	28 ^{ns}	4.820 ^{ns}	0.344 ^{ns}	0.000 ^{ns}	0.7 ^{ns}
Genotype	9	122.2**	21.11**	0.495 *	0.004^{ns}	231.3 *
Treatment	2	1023.4***	57.95**	0.01 ^{ns}	1.947*	332**
G × S	18	47.04***	9.52 ^{ns}	0.295 ^{ns}	0.003 ^{ns}	129.2 ^{ns}
Error	66	9.7	6.17	0.254	0.003	115

SL, shoot length; RL, root length; SFW, fresh weight; SDW, dry weight; RWC, relative water content; G × S, Interaction between the genotype and the stress; ***, significant at 0.001, **, significant at 0.01; *, significant at 0.05; ns, not significant; and df degree of freedom. The values represent mean squares of the source of variation for each of the measured parameters.

3. Results

The results of the ANOVA for the morphological traits are presented in Table 2. The ANOVA revealed significant genotypic differences ($P < 0.01$) for the parameters except shoot dry weight. The parameters were significantly ($P < 0.001$ and $P < 0.01$) affected by water stress except the shoot fresh weight. Genotype by water stress interaction was significant ($P < 0.001$) for the shoot length.

3.1. Morphological changes under water deficit conditions

The mean performance for the morphological traits is presented in Fig. 2. Water stress significantly inhibited the growth of lablab seedlings. The mean shoot length in well-watered control plants was 29.4 cm which significantly declined to 19.9 cm (33%) and 15.4 cm (48%) under moderate and severe water stress, respectively. The mean root length in control plants was 11.15 cm which significantly declined to 8.95 cm (20%) and 7.8 cm (30%) under moderate and severe water stress, respectively. The mean fresh weight in well watered control plants was 1.4 g which significantly declined to 0.9 g (37%) and 0.9 g (42%) under moderate and severe water stress, respectively. The mean dry weight in control plants was 0.3 g which significantly declined to 0.2 g (2%) under moderate water stress and 0.1 g (19%) under severe water stress. The mean plant water content in control plants was 80% which significantly declined to 76% under moderate stress and 76% under severe stress.

3.2. Variance among the photosynthetic traits

The analysis of variance for physiological traits evaluated is presented in Table 3. The results revealed significant genotypic differences ($P \leq 0.01$ and $P \leq 0.05$) for the photosynthetic traits Phi2, PhiNPQ, and LEF. Water deficit had no significant effect on NPQt, Fv/Fm and SPAD. Significant genotype and water stress interactions ($P < 0.05$ and $P < 0.001$) were observed for all the photosynthetic traits, except PhiNO.

The results of mean performance of the photosynthetic parameters and photosynthetic pigments under varying water stress conditions are represented in Table 4. Water stress induced a significant decline in the Phi2 efficiency. The magnitude of decrease was significantly ($P < 0.05$) higher under SS than MS conditions by 15% and 9%,

Table 3
ANOVA of photosynthetic traits of lablab seedlings under varying water stress treatments with four replications

		Photosynthetic trait						
Source	**df**	**Phi2**	**PhiNPQ**	**PhiNO**	**LEF**	**NPQt**	**Fv/Fm**	**SPAD**
Block	3	0.012	0.005	0.004	517	0.328	0.001	26.8
Genotype	9	0.030*	0.024**	0.002^{ns}	2194*	0.821*	0.005*	51.2 ^{ns}
Stress	2	0.087**	0.043**	0.011*	11953***	0.377 ^{ns}	0.003 ^{ns}	7.8 ^{ns}
G × S	18	0.024*	0.021***	0.003^{ns}	3498***	0.817*	0.005***	112.7***
Error	87	0.011	0.007	0.002	772.1	0.378	0.002	32.9

Phi2, photosystem II efficiency; PhiNPQ, photo-protective non-photochemical quenching; PhiNO, basal dissipation of light energy; LEF, Linear Electron Flow; NPQt, total non-photochemical quenching; Fv/Fm, mean photochemical efficiency of photosystem II; SPAD, relative chlorophyll content; G × S interaction between genotype and stress. Df, degree of freedom. ***, significant at $p < 0.001$; **, significant at $p < 0.01$; *, significant at $p < 0.05$; and ns, not significant. The values represent mean squares of the source of variation for each evaluated traits

Table 4
Mean values of photosynthetic parameters of lablab genotypes replicated four times under field capacity, moderate and severe stress conditions

Stress	Genotype	Phi2	PhiNPQ	PhiNO	LEF	NPQt	Fv/Fm	SPAD
Field-Capacity	D1	0.52	0.26	0.22	46.55	1.15	0.69	34.71
	D2	0.59	0.20	0.21	31.44	0.95	0.71	29.51
	D3	0.67	0.14	0.20	13.36	0.71	0.74	37.17
	D4	0.29	0.50	0.20	166.19	2.40	0.59	35.31
	D5	0.57	0.21	0.22	39.24	0.94	0.72	29.94
	D6	0.62	0.18	0.21	29.89	0.85	0.73	31.84
	D7	0.58	0.21	0.21	30.62	1.05	0.71	33.94
	D8	0.36	0.34	0.31	83.27	1.15	0.70	31.21
	D9	0.51	0.26	0.23	48.14	1.09	0.70	31.86
	D10	0.53	0.23	0.24	37.68	0.99	0.71	37.06
Moderate	D1	0.64	0.17	0.19	25.78	0.87	0.72	19.46
	D2	0.62	0.18	0.20	31.34	0.88	0.72	38.41
	D3	0.52	0.22	0.26	46.16	0.84	0.73	35.33
	D4	0.59	0.17	0.25	21.41	0.67	0.75	34.54
	D5	0.58	0.21	0.21	35.79	0.98	0.71	33.92
	D6	0.56	0.20	0.24	58.14	0.85	0.73	37.64
	D7	0.60	0.19	0.21	37.27	0.89	0.72	35.50
	D8	0.52	0.27	0.21	46.95	1.32	0.68	29.99
	D9	0.51	0.25	0.25	74.31	1.00	0.71	28.82
	D10	0.51	0.26	0.23	66.71	1.10	0.70	35.65
Severe	D1	0.68	0.09	0.23	11.79	0.42	0.78	43.74
	D2	0.67	0.14	0.19	12.65	0.75	0.74	36.01
	D3	0.64	0.18	0.18	13.16	1.00	0.71	36.42
	D4	0.63	0.20	0.17	10.03	1.18	0.69	26.46
	D5	0.64	0.18	0.19	21.64	0.96	0.71	26.32
	D6	0.63	0.18	0.19	23.50	0.93	0.72	34.62
	D7	0.57	0.22	0.21	31.82	1.08	0.70	31.06
	D8	0.64	0.17	0.19	23.59	0.93	0.72	29.18
	D9	0.61	0.16	0.23	32.51	0.72	0.74	32.44
	D10	0.46	0.35	0.19	13.65	2.67	0.61	27.59
LSD		**0.086**	**0.066**	**0.037**	**23**	**0.494**	**0.017**	**5.1**
%CV		**18.1**	**37.7**	**20.1**	**76.3**	**57.1**	**5.7**	**22.4**

Phi2, photosystem II efficiency; PhiNPQ, photo-protective non-photochemical quenching; PhiNO, dissipation of light energy; LEF, Linear Electron Flow; NPQt, total non-photochemical quenching; Fv/Fm, photochemical efficiency of photosystem II; and SPAD, relative chlorophyll content. LSD Least Significant Difference, %CV percentage coefficient of variation. Significance at $p < 0.05$.

respectively. The genotype D1 recorded the highest increase while D10 recorded the highest decline of Phi2 under SS conditions ($P < 0.05$). Both photochemical quenching (PhiNPQ) and non- photochemical quenching (NPQt) significantly ($P < 0.05$) declined, especially in genotypes D1, from 0.26 FC to 0.09 under SS conditions compared to D10 which recorded a significant increase of PhiNPQ levels from 0.23 in FC to 0.35 under SS conditions and decreasing SPAD levels (37.06 in FC and 27.59 in SS). A declining quantum yield of basal non-regulated light energy (PhiNO) was observed under severe water stress conditions with mean value of 0.19, whereas the control plants averaged 0.22. Genotypes D1 and D9 showed a higher PhiNO (0.23) under SS conditions. The LEF significantly decreased under SS conditions and exhibited a significant ($p < 0.05$) and negative correlation with Fv/Fm (Table 4), with D9 having a higher LEF (32.51) under SS conditions. Severe water stress led to a significant decline in the Fv/Fm ratio. There was slight reduction in average Fv/Fm ratio (0.711) in moderate water stressed plant as compared to plants grown under severe water stress (0.700). Genotype D1 recorded highest Fv/Fm ratio in the water stressed group ($P < 0.05$). The relative chlorophyll content as indicated by SPAD was significantly ($P < 0.05$) influenced by water stress with slight decline under MS and SS compared to the control plants.

The SPAD content which was 33 under control declined to 32 (1%) under MS and 31 (2%) under SS conditions. Genotype D1 exhibited a high SPAD (43.74), while D4 (26.46) and D5 (26.32) had the lowest under SS conditions. Genotype D1 recorded the highest Phi2 (0.68), PhiNO (0.23), Fv/Fm (0.78), SPAD (43.74) and low PhiNPQ (0.09), NPQt (0.23) and LEF (11.79) compared to the other genotypes under SS conditions. Genotype D1 showed higher values of Phi2 efficiency (0.68) and lower PhiNPQ (0.09) and NPQt (0.42) levels under severe stress ($P<0.05$).

3.2.1. Partitioning of absorbed energy

Genotypic differences were observed in the energy absorbed partitioning of Phi2, PhiNPQ, and PhiNO (Fig. 1). For all genotypes except D10, under severe water stress conditions, the absorbed energy

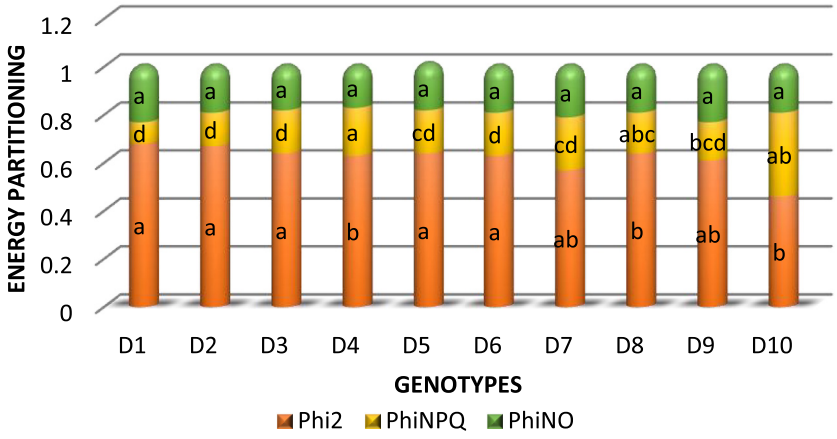

Figure 1. Absorbed Energy partitioning between lablab seedlings under severe water stress replicated four times. Phi2, photosystem II efficiency; PhiNPQ, photo-protective non-photochemical quenching; PhiNO, dissipation of non-regulated light energy. Different letters of the same variable are significantly different at P<0.05

allocated towards photochemical Phi2 pathway was higher than energy devoted to the non-photochemical quenching pathways (PhiNPQ) and non-regulated light energy pathways (NPQt).

3.2.2. Pearson's correlation among the photosynthetic traits

Significant ($p \leq 0.001$) and negative correlations were recorded between photosynthetic traits such as PhiNPQ and Phi2 (r = -0.95), PhiNO and Phi2 (r = -0.5), LEF and Phi2 (r = -0.85), NPQt and Phi2 (r = -0.7) Fv/Fm and PhiNPQ (r = -0.52), Fv/Fm and LEF (r = -0.57) and Fv/Fm and NPQt (r = -0.98) among the severely water stressed plants (Table 5). However, a significant ($p \leq 0.001$) and positive correlation existed between LEF and PhiNPQ (r = 0.81) and NPQt and PhiNPQ (r = 0.86).

3.3. Changes in oxidative, antioxidative and osmotic solutes of lablab seedlings

The results of the analysis of variance for the biochemical traits are presented in Table 6. The results revealed significant ($P < 0.001$, $P < 0.01$, $P < 0.05$) genotypic differences for all the traits except for POD, CAT, phenols and carbohydrates. Genotype by stress interactions were significant ($P < 0.001$ and $P < 0.05$) for all the biochemical traits, except for the POD, APX, CAT and carbohydrates.

3.3.1. Effects of water stress on the reactive oxygen species of lablab seedlings

The mean performance of all the biochemical traits under the different water regimes is presented in Table 7. Water stress induced a significant ($P < 0.5$) increase in the hydrogen peroxide (H_2O_2) activity with different magnitudes of change across the genotypes. The H_2O_2 content increased in all the genotypes. A sharp rise in H_2O_2 levels was recorded in genotype D2 under water stress conditions from 0.83 nmolg^{-1} FW (FC) to 1.553 nmolg^{-1} FW (SS). The contrast of means showed that the H_2O_2 content in MS plants increased by 46 % and by 49 % in SS plants. Water stress caused a significant increase in MDA activity with a reduction under SS conditions. The average MDA content in control plants was 0.2 nmolg^{-1} FW which remained the same under moderate stress conditions. However, there was a significant increase to 0.3 nmolg^{-1} FW (22%) under severe water stress. Genotype D3 which had the most pronounced increase in MDA content under SS with levels significantly ($P < 0.5$) increasing from 0.189 nmolg^{-1} FW (FC) to 0.314 nmolg^{-1} FW (SS).

3.3.2. ***Effects of water stress on enzymatic antioxidants***

The effect of water stress on enzymatic activity is presented in Table 7

The average activity of enzyme POD was significantly higher among the water stressed plants than the control plants ($P < 0.5$). Compared to 1.5 U/mg protein (30%) under control condition, the POD activity increased to 1.8 U/mg protein under moderate stress conditions; and to 2.6 U/mg protein (41%) under severe conditions. Genotype D5 exhibited the highest POD activity under SS (2.617 U/mg protein) compared to the FC (2.588 U/mg protein). The APX activity significantly intensified under SS and MS conditions at ($P < 0.5$). The average APX activity under control conditions was 0.2 U/mg protein which was increased to 0.3 U/mg protein (36%) under moderate stress and 0.5 u/mg protein (49%) under severe stress conditions. Genotype D7 recorded the highest APX activity (0.922) under SS compared to other genotypes, while D4 had the lowest in (0.19) under severe stress. Increased CAT activity was observed under SS ($P < 0.5$). However, it did not show much difference under moderate conditions. The average CAT activity under control conditions was 0.3 U/mg protein, which increased to 0.34 U/mg protein (12%) under severe water stress. Genotype D10 had significantly higher CAT activities under water stress, increasing from 0.297 U/mg protein under control conditions to 0.326 U/mg protein under SS.

3.3.3. Effects of water stress on non-enzymatic antioxidants and osmotic solutes

Water stress significantly increased the proline content ($P < 0.5$), among lablab genotypes under moderate and severe stress relative to the control plants. The average proline content in control plants was 0.3 μgg^{-1} FW of leaf tissue which increased to 0.35 μgg^{-1} FW (18%) under moderate stress and 0.42 μgg^{-1} FW (39%) under SS conditions. Genotype D7 recorded a two-fold increase under SS conditions. Genotype D9 had the lowest PROL content (0.239 μgg^{-1} FW) under SS. The phenols content significantly increased under water stress ($P < 0.5$). However, there was no significant difference under moderate conditions. The average PHE content under control conditions was 0.2 μg GAE g^{-1} FW of leaf sample and increased to 0.3 μg GAE g^{-1} FW (21%) under severe stress in the genotypes D1, D8 and D9 had a reduction in PHE content, with mean values of 0.237 μg GAE g^{-1} FW, 0.153 μg GAE g^{-1} FW and 0.208 μg GAE g^{-1} FW under SS, respectively. Accessions D4, D5 and D8 showed higher phenols content under SS relative to their controls, with mean values of 0.530 μg GAE g^{-1} FW, 0.43 μg GAE g^{-1} FW and 0.313 μg GAE g^{-1} FW, respectively. The average protein content significantly increased with increasing water stress ($P < 0.5$). In control plants, the protein content was 0.15 mgg^{-1} FW of leaf tissue, when grown under moderate stress, declined by 2% and 40% under severe stress, respectively. The decrease was more pronounced in D10, having the lowest PROT content (0.048 mgg^{-1} FW) under SS conditions, while D1 and D2

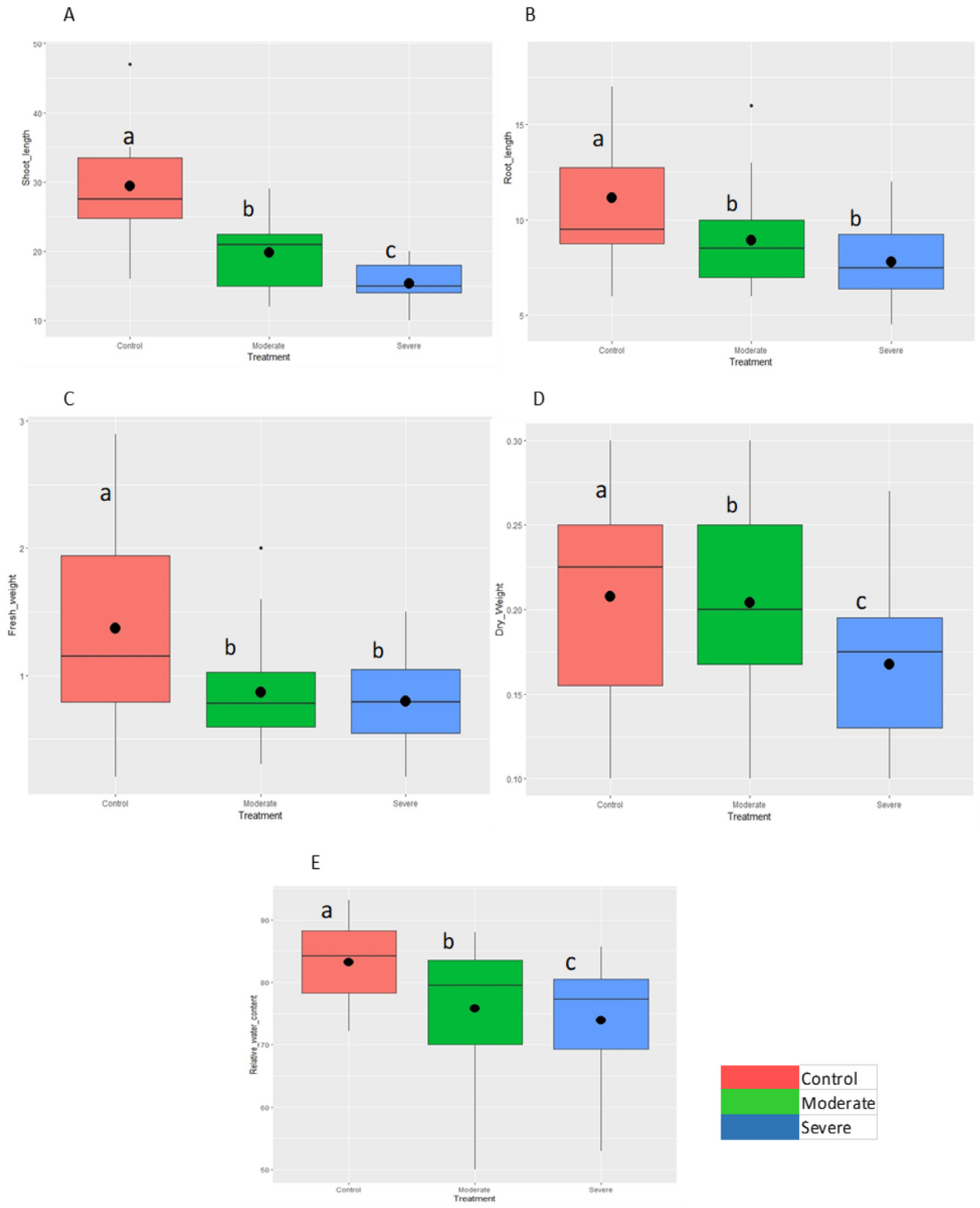

Figure 2. Effect of water stress on the morphological traits and relative water content of lablab seedlings. A=shoot length (cm), B= root length (cm), C= fresh weight (g), D= dry weight (g), E= relative water content (%). Values for all data are expressed as the mean ± and were repeated at least three times. Different lowercase letters indicate significant differences based on Tukey's multiple range tests ($P < 0.05$).

maintained higher protein content (0.449 mgg^{-1} FW and 0.105 mgg^{-1} FW). The average total free amino acids (FAA) content significantly increased ($P < 0.5$) with increasing water stress. In control plants, the FAA content was 0.06 mgg^{-1} FW of leaf tissue. The FAA content in MS and SS conditions was 0.15 mgg^{-1} FW and 0.5 mgg^{-1} FW respectively which increased by 6.2% and 70%, respectively. Genotype D10 recorded the highest decline in FAA of 94 % (0.024 mgg^{-1} FW) and D6 the lowest (0.199 mgg^{-1} FW, 39 %). The average water-soluble carbohydrates content increased. However, there was no significant difference between the MS and SS plants. In the control plants, the carbohydrate content was 2.8 mgg^{-1} FW of leaf tissue which increased to 3 mgg^{-1} FW under MS and SS conditions. Genotypes D1 and D10 performed better in terms of carbohydrate content under water stress (3.24 mgg^{-1} FW and 3.34 mgg^{-1} FW, respectively) compared to the FC plants. Notably, D2 which had highest carbohydrate content under control conditions recorded the lowest (2.66 mgg^{-1} FW) under severe stress conditions. No change in carbohydrate content was detected in D5 between FC and SS conditions (2.88 mgg^{-1} FW).

Table 5
Correlation coefficient of photosynthetic traits of lablab bean seedlings under severe stress

	Phi2	PhiNPQ	PhiNO	LEF	NPQt	Fv/Fm	SPAD
Phi2	1						
PhiNPQ	**-0.95****	1					
PhiNO	-0.5**	0.2 ^{ns}	1				
LEF	**-0.85****	**0.81****	0.41 ^{ns}	1			
NPQt	**-0.7****	**0.86****	-0.19 ^{ns}	0.47 ^{ns}	1		
Fv/Fm	**0.75****	**-0.92****	0.17 ^{ns}	-0.57**	**-0.98****	1	
SPAD	0.06^{ns}	-0.16 ^{ns}	0.24 ^{ns}	0.07 ^{ns}	-0.26 ^{ns}	0.29 ^{ns}	1

Phi2, photosystem II efficiency; PhiNPQ, photo-protective non-photochemical quenching; PhiNO, basal dissipation of light energy; LEF, Linear Electron Flow; NPQt, total non-photochemical quenching; Fv/Fm, mean photochemical efficiency of photosystem II; SPAD, relative chlorophyll content; **, significance at P<0.5 and ns, not significant

3.3.3. Principal component and biplot analyses

The principal component analysis (PCA) of the all the variables measured in the study are shown in Table 8. The biplot in Fig. 3 shows the distribution of genotypes and variables measured in the first and second principal components.

The PCA analysis transformed the data into 10 dimensions representing the proportions of the data variability (Table 8). The first principal component (PC1) contributed the most variability and principal component (PC10) contributed the least variability. A cumulative variability of 91.06%, 91.53% and 92.25% comprised the first six components of the well-watered, moderate stress and severe stress, respectively.

The PCA biplot analysis among the well-watered plants (Fig. 3a) showed that dimension (Dim1) accounted for 33.6% while dimension 2 (Dim2) constituted 20.4% of the cumulative variability. The discriminating traits among the well-watered plants were primarily based on the physiological traits except plant water content (PWC). The analysis also depicted that all the discriminating traits were positively contributing except Phi2, Fv_Fm and PWC were negatively contributing under well-watered conditions.

PCA biplot for moderately stressed plants (Fig. 3b) showed that Dim1 accounted for 29.1 while Dim2 accounted for 25.1% of the cumulative variability. The highest discriminating traits were Phi2, Fv_Fm, FW, LEF, PhiNPQ, NPQt and carbohydrates. All the highest discriminating traits among were contributing positively except Phi2 and Fv_Fm reflecting negative contributions. Biplot analysis for severe stressed plants showed that Dim1 and Dim2 accounted for 27.2% and 23.7% of the cumulative variability, respectively. The traits PhiNO, Fv_Fm, PhiNPQ, NPQt FW and DW were present furthest away from the biplot origin and they depicted positive contribution except for PhiNO, Fv_Fm, FW and DW showing negative contribution.

Genotypes D4, D8 and D3 were present farthest away from the biplot origin implying better performance compared to other genotypes under. D9, D5 and D1 were closer to the biplot origin reflecting that these genotypes had the least variability for studied traits under well-watered conditions (Fig. 3a). Genotypes D1, D10 and D4 were most distinct from the biplot origin indicating most variability with reference to other genotypes. D7 and D5 were located nearest to the biplot origin implying the least variability under moderate stress conditions (Fig. 3b). Genotypes D10, D9, D8, D1 and D5 were located farthest away from the origin of the biplot showing better performance relative to other genotypes under severe water stress conditions (Fig. 3c).

4. Discussion

Drought stress is the most destructive hazard affecting crop production and understanding the genotypic variation in lablab beans for drought resistance is a prerequisite for breeding for high crop yields. In the current study, we investigated the influence of water stress in lablab seedlings based on morphological, physiological and biochemical response. The lablab genotypes showed variability in their morphological, physiological and biochemical mechanisms to water stress, therefore, this diversity may be exploited in breeding for resistance to drought.

Growth inhibition is the most common and important characteristic of the effects of water stress on plants. Water stress significantly reduced the shoot and root length, and the dry and fresh weights of the seedlings compared to the control plants suggesting the sensitivity of the lablab seedlings to water stress. The limited water availability negatively impacted solute transport reducing plant growth and decreased expansion and elongation of stems and leaves (Farooq et al., 2020). Genotypes D6, D7, D8 and D9 had long root lengths under water stress compared to other genotypes under severe stress suggesting an adaptation to water stress as reported previously (Fenta et al., 2014). The root formation increased in length into the soil in search for moisture to increase the uptake of water to sustain growth and survival (Fenta *et al*., 2014). The plasticity of roots and their volume and distribution influenced the plants' capacity to absorb and utilize soil moisture and nutrients to promote growth and development of the above ground biomass (Chen et al., 2015). Under drought situations, the RWC can accurately reflect the water content of plants, indirectly reflecting their tolerance to drought (Meher et al., 2018). In the present study, drought stress significantly reduced the RWC in lablab seedlings. However, genotypes D9 and D10 had significantly higher RWC, implying different abilities of lablab to absorb soil moisture. This is due to the vitality of plant root epidermis inversely related to the ambient moisture level and the ability of the plants to accumulate and osmotically adjust to maintain tissue turgor. This corroborates with the findings of other studies in which RWC slightly increased under water stress in onion plants (Csiszár et al., 2007; Caldwell et al., 2003). Genotype D6 exhibited higher mean values of SL, RL, FW and DW in comparison to the other genotypes, indicating an adaptive advantage to water stress which was linked to deeper root systems which allowed improved soil water exploration under drought.

Photosynthesis is a key factor for maintaining plant growth and the efficiency of Phi2 photochemistry was affected by water stress. Water stress often led to reduction of the Phi2 efficiency activities to

Table 6
ANOVA table showing the effects of water stress on biochemical traits of lablab bean seedlings in three replicates

Source	Df	H_2O_2	MDA	POD	APX	CAT	PHE	PROL	PROT	FAA	CARB
R	2	0.001	0.181	0.872	0.000	0.0021	0.006	0.001	0.013	0.000	5.525
G	9	0.059***	0.012*	0.213^{ns}	0.142*	0.004^{ns}	0.008^{ns}	0.024***	0.108**	0.014**	0.065^{ns}
S	2	0.998***	0.022***	5.96***	0.367*	0.009^{ns}	0.022*	0.140***	0.067**	0.069***	0.047^{ns}
G*S	18	0.021***	0.005***	0.151^{ns}	0.087^{ns}	0.009^{ns}	0.132*	0.006***	0.130***	0.011***	0.118^{ns}
Error	66	0.002	0.000	0.199	0.065	0.007	0.005	0.001	0.008	0.002	0.143

H_2O_2, hydrogen peroxide; MDA, malondialdehyde; GPX, guaiacol peroxidase; APX; ascorbate peroxidase; CAT, catalase; PHE, phenols; PROL, proline; PROT, protein; FAA, total free amino acid; CARB, carbohydrates; R ,replication; G, Genotype; S, stress; G × S, interaction between the genotype and the stress; and df, degree of freedom. ***, significant at 0.001; **, significant at 0.01; *, significant at 0.05; and ns, not significant. The values represent mean squares of the source of variation for each of the measured parameters.

Table 7
Mean values of various biochemical traits of lablab seedlings replicated three times under three water regimes

Water stress		H_2O_2 (nmol g^{-1} FW)	MDA (nmol g^{-1} FW)	POD (Umg^{-1} protein)	APX (Umg^{-1} protein)	CAT (Umg^{-1} protein)	PHE (µg GAEg^{-1} FW)	PROL (µgg^{-1} FW)	PROT (mgg^{-1} FW)	AA (mgg^{-1} FW)	CARB (mgg^{-1} FW)
Control		0.8^{a}	0.21^{a}	1.53^{a}	0.27^{a}	0.3^{a}	0.26^{a}	0.23^{a}	0.15^{a}	0.06^{a}	2.87^{b}
Moderate		1.2^{b}	0.22^{a}	1.81^{a}	0.34^{a}	0.31^{a}	0.35^{b}	0.23^{a}	0.25^{b}	0.15^{b}	2.87^{b}
Severe		1.18^{b}	0.27^{b}	2.59^{b}	0.53^{b}	0.34^{b}	0.42^{c}	0.29^{b}	0.25^{b}	0.5^{b}	3^{a}
Control	**D1**	9.20	2.40	2.64	0.13	0.43	330.00	3.90	0.16	0.08	2.87
	D2	8.30	2.00	2.77	0.09	0.36	390.00	2.30	0.09	0.11	3.06
	D3	8.60	1.90	2.45	0.11	0.32	290.00	2.40	0.16	0.13	2.94
	D4	8.40	3.50	2.87	0.1	0.32	200.00	1.90	0.18	0.1	3.37
	D5	8.30	2.60	2.59	0.16	0.31	130.00	1.70	0.12	0.06	2.88
	D6	7.60	2.00	2.27	0.16	0.33	240.00	2.40	0.63	0.33	2.77
	D7	7.40	3.80	2.46	0.95	0.27	260.00	2.70	0.46	0.1	2.68
	D8	7.50	3.00	2.7	0.24	0.31	300.00	2.10	0.91	0.19	2.99
	D9	7.70	2.50	2.29	0.29	0.47	200.00	2.10	0.23	0.05	2.61
	D10	7.60	3.80	2.83	0.48	0.3	230.00	2.20	0.14	0.4	2.6
Moderate	**D1**	12.60	1.70	1.64	0.27	0.41	320.00	2.50	0.15	0.08	3.09
	D2	15.60	1.70	1.04	0.34	0.25	490.00	2.10	0.09	0.04	2.85
	D3	10.20	2.20	1.22	0.37	0.24	350.00	3.00	0.12	0.03	3.22
	D4	10.60	3.20	1.33	0.55	0.27	310.00	2.50	0.18	0.03	2.86
	D5	11.70	2.30	1.64	0.45	0.32	390.00	1.80	0.4	0.05	3.07
	D6	11.00	2.60	1.6	0.31	0.24	380.00	2.40	0.16	0.06	2.72
	D7	11.30	2.10	1.79	0.26	0.36	410.00	2.10	0.09	0.02	2.8
	D8	12.50	1.90	1.62	0.29	0.42	320.00	2.30	0.09	0.01	2.72
	D9	10.80	1.70	1.74	0.25	0.27	190.00	2.30	0.1	0.13	2.64
	D10	11.70	2.10	1.71	0.33	0.34	310.00	1.80	0.12	0.04	2.68
Severe	**D1**	11.70	1.30	1.74	0.27	0.19	500.00	2.40	0.45	0.07	3.24
	D2	15.50	1.90	1.78	0.35	0.28	440.00	2.80	0.11	0.09	2.66
	D3	12.10	3.10	1.28	0.36	0.29	470.00	2.30	0.12	0.06	2.67
	D4	12.20	2.20	1.94	0.19	0.35	430.00	5.30	0.13	0.04	2.77
	D5	12.20	1.60	2.62	0.23	0.3	450.00	4.30	0.1	0.04	2.89
	D6	11.40	2.10	1.74	0.77	0.33	470.00	2.90	0.1	0.2	3.07
	D7	13.20	2.90	1.88	0.92	0.28	510.00	2.40	0.17	0.02	2.8
	D8	11.10	2.20	1.34	0.71	0.27	400.00	3.10	0.14	0.02	3.16
	D9	10.10	1.60	1.66	0.65	0.39	240.00	1.50	0.09	0.02	2.95
	D10	10.70	3.00	2.17	0.86	0.33	340.00	2.10	0.05	0.02	3.34
	LSD	**0.06**	**0.04**	**0.56**	**0.28**	**0.09**	**0.05**	**0.08**	**0.2**	**0.05**	**0.43**
	%CV	**4.2**	**20.5**	**26.7**	**50.3**	**21.8**	**9.8**	**22.8**	**50.5**	**44.3**	**14.7**

H_2O_2, hydrogen peroxide; MDA, lipid peroxidation; POD, guaiacol peroxidase; APX, ascorbate peroxidase; CAT, catalase; PROL, proline; PHE, phenols; PROT, protein; AA free amino acid; CARB, water soluble carbohydrates; LSD, Least Significant Difference; %CV, and percentage coefficient of variation. Significance at $p < 0.05$

Table 8
The principal components of the measured traits and their eigenvalues, variability and cumulative variability of lablab genotypes subjected to three different water treatments

	Stress	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Eigen value	FC	7.06	4.28	2.85	2.14	1.72	1.07	0.88	0.67	0.32	0.00
	MS	6.11	5.28	2.60	2.49	1.43	1.31	0.78	0.75	0.25	0.00
	SS	5.72	4.99	3.81	1.86	1.64	1.36	1.01	0.38	0.24	0.00
Proportion of variance	FC	33.64	20.40	13.59	10.17	8.17	5.09	4.21	3.21	1.52	0.00
	MS	29.09	25.15	12.40	11.85	6.80	6.25	3.73	3.57	1.17	0.00
	SS	27.24	23.74	18.16	8.83	7.79	6.47	4.80	1.81	1.15	0.00
Proportion of cumulative variance	FC	33.64	54.04	67.63	77.80	85.97	91.06	95.27	98.48	100.00	100.00
	MS	29.09	54.23	66.63	78.48	85.27	91.53	95.26	98.83	100.00	100.00
	SS	27.24	50.99	69.15	77.98	85.77	92.25	97.04	98.85	100	100.00

PC, principal component; FC, field capacity; MS, moderate stress; SS, severe stress. Significance at $p < 0.05$.

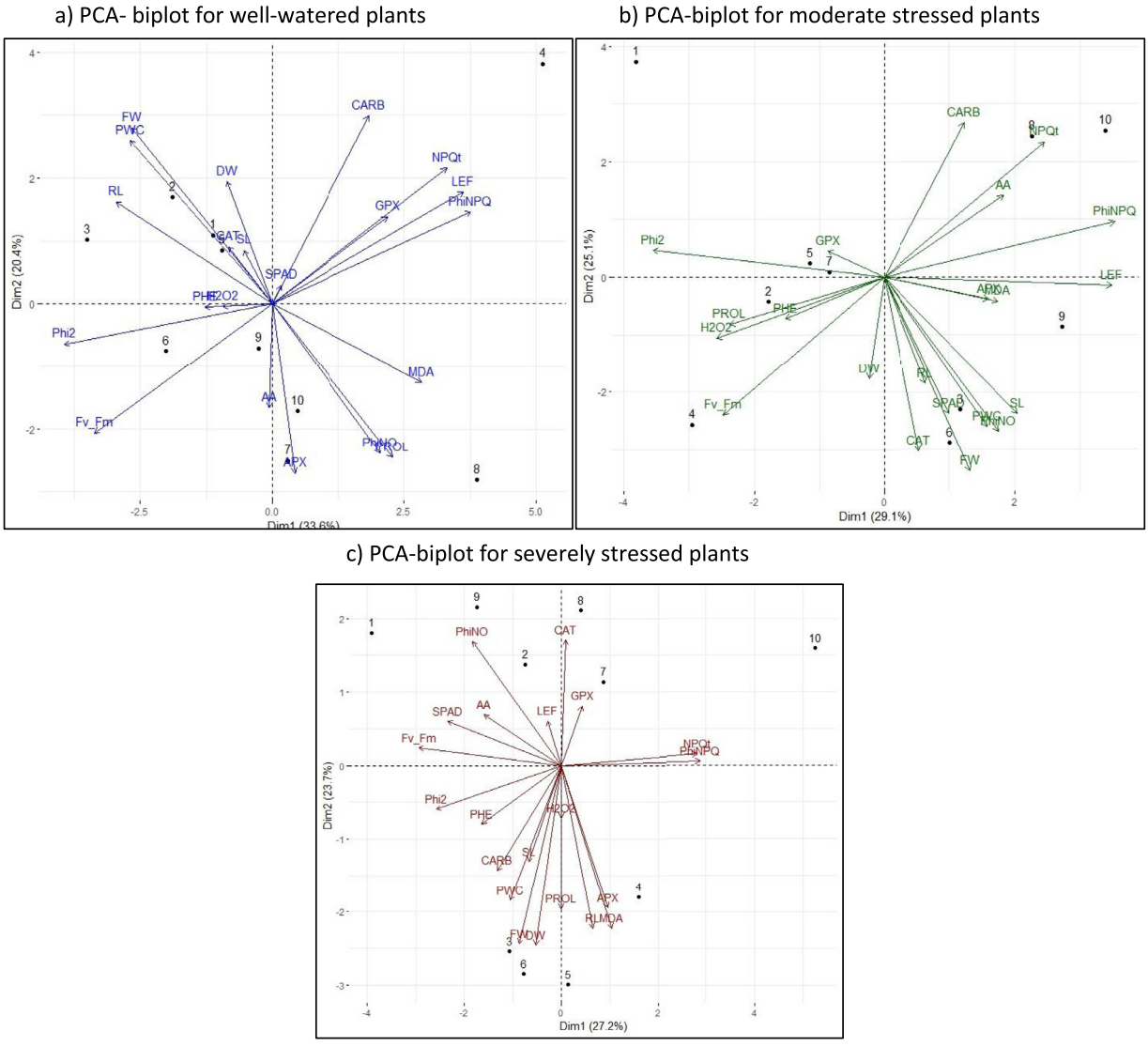

Figure 3. Biplot graphs of PCA for various traits of lablab genotypes under varying water stress conditions. Numbers in black inside the graphs represent lablab genotypes D1-D10. Phi2, photosystem II efficiency; PhiNPQ, photo-protective non-photochemical quenching; PhiNO, basal dissipation of light energy; LEF, Linear Electron Flow; NPQt, total non-photochemical quenching; Fv/Fm, mean photochemical efficiency of photosystem II; SPAD, relative chlorophyll content; SL, shoot length; RL, root length; FW, fresh weight; DW, dry weight; PWC, plant water content; H_2O_2, hydrogen peroxide; MDA, lipid peroxidation; POD, guaiacol peroxidase; APX, ascorbate peroxidase; CAT, catalase; PHE, phenols; PROL, proline; AA, free amino acid; CARB, water soluble carbohydrates. Significance at $p < 0.05$.

enable production of ATP and NADPH to match the decreasing CO_2 intake due to closure of the stomata (Zhao et al., 2020). Compared to other genotypes, D1 and D10 were the least affected by decline in Phi2 activity under severe stress suggesting higher photo-protective efficiency under drought stress. Such genotypes could be ideal for selection in breeding for drought tolerance due to their high efficiency in utilizing less energy for photosynthesis. Similarly, high efficiency of Phi2 under water stress was observed in Arabidopsis (Chen et al., 2015), and barley (Fernández-Calleja et al., 2020) winter wheat (Zhao et al., 2020). Contrastingly, genotypes D4 and D9 were greatly

affected by water stress indicating a higher sensitivity to water stress demonstrating the genotypic role of photosynthetic traits in resistance to water stress.

Our results showed the interaction among the genotypes and water stress had a significant effect in the maximal quantum yield of PSII photochemistry (Fv/Fm) ratio. The reduction in severely stressed plants was higher than in the moderately stressed and control genotypes indicating photoinhibition of Phi2 under severe stress. Severe water stress led to the closure of the Phi2 reaction center which resulted to a restriction of electron transfer leading to a decrease in the amount of light energy available for the photochemical reactions within the Phi2 reaction center (Stefanov and Terashima, 2008). Genotype D1 had the least decline in Fv/Fm and Phi2 value under severe stress implying high capacity to regulate electron transport as a drought tolerance mechanism. Similar to findings by Barboricova et al. (2022) and Shin et al. (2021).

A significant decline was noted in both the photo-chemical (PhiNPQ) and non-photochemical quenching parameters (NPQt), respectively, with increase in water stress severity. Photochemical quenching represents the fraction of open PSII reaction centers and a small decline suggests increased thermo stability of Phi2 (Jumrani et al., 2019). In our study, genotype D10 had high values for PhiNPQ and NPQt under severe stress compared to other genotypes, thus, a high PhiNPQ and NPQt may be a mechanism by genotype D10 to decrease photosynthetic electron transport. Negative correlations were observed between the Phi2 efficiency and the PhiNPQ and NPQt which accurately reflects the changes in the photosynthetic systems of the plants and the degree of stress suffered under water stress. To increase Phi2 efficiency, the plants favored the photosynthetic processes of Phi2 photochemical reactions at the expense of the photoprotective quenching mechanisms related to heat dissipative processes thus an increased stability of Phi2 reaction centers in water-stressed plants (Kuhlgert et al., 2016; Dramadri et al., 2021).

High chlorophyll content can be used as a physiological trait for selecting genotypes tolerant water stress (Jumrani et al., 2019). In this study, the relative chlorophyll content significantly declined under severe water stress compared to well-watered plants. This is similar to the findings of other studies in which chlorophyll content decreased as the time of water stress increased (Alidu et al., 2019; Dramadri et al., 2021). Severe water stress led to closure of stomata aperture limiting water loss through evaporation and entry of atmospheric carbon dioxide compared to their respective control plants. In the present study, genotype D1 had high chlorophyll content under severe water stress compared to other genotypes, suggesting D1 maintained higher rate of photosynthesis and hence better yields under water stress conditions. The differential response of lablab genotypes to water stress suggests varying mechanisms for drought tolerance. This is probably due to the genetic differences that exist in the photosynthetic response of each genotype under water stress.

Water stress tends to increase the generation of reactive oxygen species (ROS) and can result to lipid peroxidation, oxidative damage and ultimately cell death (Mhamdi and Van Breusegem, 2018). In this study, we found a significant rise in the levels of hydrogen peroxide and MDA when lablab seedlings were exposed to water stress. This indicates that the antioxidant enzyme defense system was weakened, and there was an enhancement in lipid peroxidation. Genotype D3 had the highest increase in malondialdehyde (MDA) content under severe water stress which showed the lack of adaptive capacity to scavenge reactive oxygen species which could lead to formation of free radicals and damage to the cell membrane. Contrastingly, genotype D1 had the lowest accumulation of MDA under severe water stress suggesting better adaptive capacity to scavenge ROS. Malondialdehyde (MDA) content is frequently used as a measure for assessing lipid peroxidation and its low levels has been linked with increased tolerance in plants (Zhang and Kirkham, 1994).

The balance of ROS in plants is maintained through the coordination of the antioxidant enzyme and non-enzymatic antioxidant systems. When plants are subjected to drought stress, the balance of ROS is disrupted (Zhang et al., 2018). The excessive accumulation of ROS can damage cells and cause oxidative mamage of cell membranes, which may lead to plant death (Zhang et al., 2018). There is an active ROS scavenging system in plants, in which SOD, POD, CAT and APX are crucial antioxidant enzymes for scavenging ROS (Zhang et al., 2018). Studies have shown that an increase in the activity of antioxidant enzymes is correlated with improved tolerance to drought stress in plants (Zhang et al., 2018). In the present study, the increased POD, APX and CAT lead to scavenging of the ROS reducing photo-oxidative damage and lipid peroxidation (Farooq et al., 2020). Among the antioxidant enzymes, the highest increase was observed in POD activity, suggesting that this enzyme played a crucial role in mitigating the harmful effects of ROS as water stress increased. This is similar to findings by Jumrani and Bhatia (2019) on soybeans subjected to water stress. With an increase in water stress, APX and CAT content were lower than POD. Under severe water stress, the ascorbate content was found to decrease. Ascorbate is a water-soluble antioxidant that interacts with ROS and also serves as a substrate for APX. The reduction in ascorbate levels under water stress suggests that it may be utilized for the detoxification of ROS, indicating its active role in combating oxidative stress. Similarly, the decline in CAT content with increasing water stress indicated its role in scavenging for free radicals and repair the damage caused by ROS under abiotic stresses (Khan et al., 2017). Genotype D1 exhibited a three-fold increase in CAT under severe water stress implying that it had a greater ability to scavenge hydrogen peroxide generated in peroxisomes during photorespiratory oxidation and β-oxidation of fatty acids (Zhang et al., 2019). Total phenolic compounds are commonly induced in plants as a response to abiotic stress. These compounds play a vital role in mitigating oxidative damage by effectively scavenging ROS (Sarkar et al., 2016). In this study, the increase in water stress led to an increase in phenolic content. However, genotype D7 had significantly high phenolic content under severe water stress compared to the control suggesting better antioxidant capacity to scavenge ROS.

During water stress conditions, the accumulation of free amino acids aids in the osmotic adjustment of plants allowing them to better cope with water scarcity and maintain cellular hydration (Blum, 2017). In this study, there was an increase in free amino acids among severe water stressed plants suggesting that the accumulation of amino acids is an active process, induced by the onset of water stress. Free proline is the main osmoprotectant in leaves and roots. In this study the proline content significantly increased under severe water stress, with D7 recording twice the amount of proline in plants under water stress relative to the control plants, which implied better inherent ability for tolerance against water stress. The genotype D9 was more prone to water stress as evidenced by the high reduction in phenolic compounds and proline content probably due to a damaging effect on membrane integrity, which could lead to increased lipid peroxidation that is often associated with water stress in plants (Mohammadi et al., 2018).

To enhance resistance against abiotic stress, plants accumulate significant amounts of soluble proteins and soluble carbohydrates. This accumulation serves to improve the concentration of cell sap, which helps maintain cell turbidity and prevents excessive plasma dehydration. By increasing the solute concentration, plants are better equipped to withstand challenging environmental conditions and maintain proper cellular functioning (Cohen et al., 2010). In this study, we found that with severe water stress, there was an overall increase in soluble proteins and soluble carbohydrates. Similarly, studies have reported increased in soluble protein with the onset of water stress (Guo et al., 2018). Genotype D1 showed a 3-fold increase in protein suggesting better tolerance to abiotic stress. With

increasing drought stress level, increase in soluble sugar can be beneficial in maintaining cell turgor, by improving water-holding and water-absorbing capacity during drought stress. Genotype D1 maintained higher protein content, in water stress which indicated improved tolerance as it helped in osmotic balance alleviating the stress impact. On the other hand, there was a two-fold increase in the water-soluble carbohydrate content in D10 implying better osmotic and oxidative stress adaptation to water stress.

5. Conclusions

In this study, the effect of water stress on the morpho-physiological and biochemical traits of lablab seedlings was investigated. Severe water stress had deleterious effects on the photosynthetic apparatus of lablab seedlings. Water stress reduced the shoot and root length, fresh and dry weight and the relative water content of lablab seedlings. Severe water stress increased the ROS resulting to activation of enzymatic and non-enzymatic antioxidant systems. However, genotype D6 depicted greater morphological capacity to water stress while genotype D1 had superior physiological adaptation with enhanced osmotic capacity and genotype D10 had greater resilience in scavenging ROS to prevent membrane damage. These results could provide essential genetic variability for lablab improvement and crucial in choosing appropriate lablab varieties and desirable parents for plant breeding programmes focusing on drought resistance research.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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