CORRELATION ANALYSIS OF GROWTH, ECO-PHYSIOLOGICAL TRAITS, CARBON ASSIMILATION AND NITROGEN FIXATION PARAMETERS IN B16 BAMBARA GROUNDNUT LANDRACE UNDER DROUGHT STRESS

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Abstract. Bambara groundnut, a resilient legume that thrives in harsh environments, serves as a suitable model for examining drought responses. The purpose of this study is to investigate the complex relationships between growth parameters, eco-physiological traits, carbon assimilation, and nitrogen fixation in Bambara groundnut landraces under drought conditions. The growth parameters measured in the study include biomass accumulation; root-to-shoot ratio, as well as the eco-physiological traits (photosynthesis, stomatal conductance, transpiration rate, intercellular CO_2 concentration and water use efficiency intrinsic); carbon assimilation (net photosynthetic rate) and nitrogen fixation (nodule number and activity). Using correlation coefficient analysis, we aim to explore the effects of drought stress on these variables and their interdependencies. Pearson correlation coefficients were determined and showed the relationship between the paired parameters. The study reveals that correlations between these parameters under drought conditions, is an effective means of gaining a comprehensive understanding of the Bambara groundnut response to drought.

Keywords: *Bambara groundnut, correlation analysis, drought stress, growth parameters, eco-physiological traits, plant growth-promoting rhizobacteria*

Introduction

Leguminous plants are highly valued for their nutritional and economic importance globally, and they offer valuable insights into how plants adapt to changing climates (Udeh et al., 2020). Although they are often referred to as “poor man’s meat,” legumes play a vital role in the diets and well-being of many people, particularly in sub-Saharan African rural communities. This is due to their high protein and carbohydrate content, as well as the various phenolic compounds that are released when they are consumed, either cooked or uncooked (Udeh et al., 2020; Aderinola et al., 2024). However, the growth of these plants and the production of crops are often limited by various abiotic stresses, such

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as high temperatures, salinity, flooding, and drought, as reported by Singh et al. (2024). Drought, in particular, causes a significant disruption of normal growth and development in crops due to the unavailability of water, as stated by Katerova et al. (2024). It also results in dehydration stress, which significantly contributes to the decline in crop growth and yield. However, crops develop a variety of mechanisms, including dehydration escape, dehydration avoidance, and dehydration tolerance, which are genetically induced responses to mitigate the effects of dehydration resulting from drought. Dehydration avoidance entails the closure of stomata, the modification of roots to extract water from the soil, and a reduction in leaf surface area to decrease water loss (Zahedi et al., 2025).

Bambara groundnut which is a highly nutritious legume and has nutraceutical properties (Udeh et al., 2022), exhibits the capacity to employ dehydration-escape mechanisms in response to drought stress conditions. Additionally, it reduces the duration of its vegetative growth phase and initiates flowering at an earlier stage. Moreover, it allocates a greater proportion of resources to the development of its roots, enabling more effective utilization of the available soil volume (Berchie et al., 2012). This adaptive strategy is essential for survival in water-stressed conditions and can result in increased photosynthesis and carbon assimilation (Yang et al., 2021). Studies have revealed that drought impacts some of BGN's physiological and growth traits, despite the plant's well-known resistance to drought. Stomatal conductance, internal carbon dioxide (CO_2) concentration, transpiration rate, symbiotic N_2 fixation, plant biomass, metabolite synthesis, and carbon assimilation characteristics, among other parameters (Haider et al., 2024). The ability of BGN to thrive in drought conditions may be linked to the functions of certain N-fixing bacteria and plant growth-promoting rhizobacteria (PGPR).

Plant growth-promoting rhizobacteria contribute to plant development by means of nitrogen fixation and the mobilization of essential nutrients, while also mitigating the harmful consequences of environmental stresses such as drought (Khan et al., 2020; Vocciante et al., 2022; Vishnupradeep et al., 2022). *Bradyrhizobium japonicum* and *Bacillus subtilis* have been documented as plant growth-promoting rhizobacteria (PGPR) that contribute significantly to plant development by improving nitrogen fixation and releasing antimicrobial substances that inhibit the spread of harmful bacteria in plants (Kinsella et al., 2009). However, there is still a paucity of studies on their roles in the amelioration of drought effect on BGN's growth and the relationship between growth, physiological parameters, and symbiotic N_2 fixation as well as carbon assimilation properties that could play a crucial in the crop's adaptation. Carbon assimilation (shoot C content, %C and C/N, $\delta^{13}C$), growth parameters (plant height, number of leaves, and root length), eco-physiological parameters (photosynthesis, stomata conductance, transpiration rate, and intercellular CO_2 concentration), and nitrogen fixation indices delta 15N ($\delta^{15}N$), N concentration (%N), percentage of N derived from atmospheric N fixation (%Ndfa), shoot N content, amount of nitrogen fixed (N-fixed), soil N uptake, and correlation analysis (CA) were all examined in this study.

A common method for examining the connections and relationships between different phenomena is correlation analysis. A common technique for determining the degree of a linear relationship between two variables is Pearson correlation analysis. The relationship is shown as a table or graph using a correlation coefficient. The relationship between the various physiological processes of Bambara groundnuts under drought conditions is not well understood. In order to explain the different dynamics of the crop when exposed to drought conditions, Hence, the study is set out to ascertain the relationship between the growth, eco-physiological, carbon assimilation, and nitrogen fixation of Bambara groundnut using correlation analysis. Because it is comparatively underutilized in comparison to major cash crops and is recognized for its climate-smart characteristics, nitrogen-fixing capacity, and capacity to thrive in challenging environmental conditions, the Bambara groundnut was selected as the subject of this study.

Materials and methods

Experimental site

The experiment for this research was carried out in a fully automated greenhouse located at the Florida Science Campus of the University of South Africa in Gauteng Province, South Africa. The growth conditions in the greenhouse were between 24-34°C Day and 18-24°C night, also the relative humidity ranges from 86-96%.

Experimental design and treatments

With four replicates and a fully randomized design, the experiment employed a 4 × 3 × 2 binomial arrangement. *Bacillus subtilis* BD234 strain (BA) on its own, *Bradyrhizobium japonicum* (BR) on its own, co-inoculation of *B. subtilis* BD234 and *B. japonicum* strain, ammonium nitrate (NH_4NO_3) as a positive control, and no inoculation (negative control) were the inoculation treatments. As explained by Silva et al. (2019), the three simulated drought stress levels were 100% of field capacity (PC) (well-watered/control), 50% of field capacity (PC) (moderate stress), and 25% of field capacity (PC) (severe stress). B16 Bambara groundnut landrace was used in this study. The field capacity of each pot was estimated by the gravimetric method (Green et al., 2004). At first, a certain amount of soil was poured into each pot based on weighting. Then 4 pots were randomly selected and saturated. Pot weights were recalculated after 48 h of drainage and the soil was dried for 24 h at 105°C. According to *Equation 1*, the soil moisture content at 100% of PC was calculated as the difference between the soil weight after drainage (PCW) and soil weight after drying (DW) (Abbaspour and Babaee, 2017).

$$\text{Pot capacity (PC)} = \frac{\text{soil weight in pot capacity (PCW)} - \text{weight of dry soil (DW)}}{\text{weight of dry soil (DW)}} \times 100 \qquad \text{(Eq.1)}$$

For each experiment, 12 pots per treatment and a total of 60 pots per Bambara groundnut landraces were used. The size of the pots used for the experiment were 25 cm height (21 cm diameter top and 15.5 cm base) were filled with 7 kg (70% of topsoil and 30% vermiculite) which was sterilized and used as the growth media for the crop. For the experiment, a total of 120 seeds were chosen, sterilized for 30 min with 3.5% sodium hypochlorite, rinsed four times with sterile distilled water, and then imbibition with Mili-Q water for the night. Prior to planting, 70% ethanol was sprayed on the pots to sterilize them.

Inoculant preparation, irrigation, and drought treatments

Following the determination of three pot capacities, seeds were sown in 25 cm pots filled with growth media. Two milliliters of each of the two microbial inoculants (*B. japonicum* and *B. subtilis*) were applied to Bambara groundnut seeds prior to planting. The plants were irrigated twice a week with deionized water and a standard nutrient solution of 10 mL per pot containing N-free nutrient (Broughton and Dilworth, 1970). The positive control was nitrogenated with 0.5 mM ammonium nitrate (NO_3 treatment).

At the onset of flowering, drought was imposed through withholding water supply for 14 days. Gas exchange and other physiological parameters were collected on drought stressed plants (day 14 of no moisture supply) which was sufficient for the 3 days full recovery of Bambara groundnut plants subjected to severe drought stress. Outside the drought period, soil water content was maintained at pot capacity (800 mL) with daily irrigation through to the flowering growth stage and monitored every 2 days interval at 9:00 a.m. and 3:00 p.m. using tensiometer installed at 10 cm depth.

Data collection

Growth response parameter measurements were carried out as described by Silva et al. (2019). Data was collected at physiological maturity. At the physiological maturity stage, the number of leaves and plant height, along with the fresh and dry biomass of the plant's roots and shoots, were measured (after being uprooted, the plants were thoroughly rinsed under running tap water. Subsequently, the roots were detached from the stems, and the fresh weight of the roots was recorded before allowing them to dry at room temperature). During flowering stage, a portable infrared gas analyzer (LI 6400 XT, version 6.2) was used to collect eco-physiological parameters such as photosynthetic rates (A), stomatal conductance (gs), intercellular CO2 concentration (Ci), transpiration rate (E), and intrinsic water use efficiency (WuEi). A temperature of 25°C, a photosynthetic photon flux density of 1000 µmol $m^{-2}.s^{-1}$, a CO2 concentration of 400 $\mu mol.mol^{-1}$, a gas flow of 500 $\mu mol.s^{-1}$, and other conditions that were present in the chamber were also included. Between eight and ten in the morning, measurements were made. Each pot's replicate was used for the measurement. The ratio of A to gs was calculated as an instantaneous indicator of water-use efficiency. Nodule number per plant and nodule dry weight were recorded after drought stress. Nutrient uptake, amount of nitrogen fixed, carbon assimilation data were also determined.

Determination of mineral elements in Bambara groundnut shoot

Approximately 1 g of ground sample was ashed in a porcelain crucible at 500°C for the entire night in order to identify the mineral elements (P, K, Ca, Mg, Na, Cu, Mn, Fe, Al, and B) in Bambara groundnut shoots. The ash was then dissolved in 5 mL of analytical-grade 6 M HCl and baked for 30 min at 50°C. After that, 35 mL of de-ionized water was added. Whatman No. 1 filter paper was used to filter the mixture. Inductively coupled plasma mass spectrometry (ICP-MS) was used to measure the concentration of mineral elements in plant extracts from four replicate samples (Ataro et al., 2008). Standard solutions with certificates of analysis were used to verify the quality of the data that was gathered. After every ten samples, a known standard was used to monitor each element instead of analyte isotopes. A wet digestion method using 65% high-purity grade nitric acid was used to measure sulfur. In a 250 mL glass beaker, 1 g of ground plant material was digested for the entire night with 20 mL of 65% nitric acid. After that, the extract-filled beaker was set on a sand bath and slowly boiled until about 1 mL of extract remained. Ten milliliters of high-purity grade 4 M nitric acid were then added, and the mixture was boiled for 10 min. After cooling and removing the beaker from the sand bath, the extract was thoroughly cleaned in a 100 mL volumetric flask before being filtered through Whatman No. 2 filter paper. Using a calibrated ICP-MS, direct aspiration was used to determine the S content of the sample (FSSA, 1974; Teixeira et al., 2017).

Correlation between measured experimental parameters

Correlation analysis was used to examine the relationship between growth, eco-physiological, carbon assimilation, and nitrogen fixation parameters. *Table 1* displays the correlation between the following parameters: carbon assimilation, nitrogen fixation (%N, $\delta^{15}N$, Ndfa, Shoot/N, N-fixed, Soil N uptake, $\delta^{13}C$, %C, C/N, Shoot C content), plant growth parameters (plant height, number of leaves, and root length), and eco-physiological indices (photosynthesis, stomata conductance, transpiration, and Ci). Plant growth indices (plant height, number of leaves, and root length) were examined by the authors. The correlation analysis examined carbon assimilation (%N, shoot/N, %C, and C/N), eco-physiological parameters (photosynthesis, stomata conductance, Transpiration rate and intercellular CO_2 concentration), and nitrogen fixation indices ($\delta^{15}N$, $\delta^{13}C$, Ndfa, N-fixed, soil N uptake, and shoot C content).

Correlation pattern

The authors investigated the relationship between the nitrogen fixation, carbon assimilation, eco-physiological factors, and plant growth indices by creating a relationship pattern and determining the degrees of significance of the correlation coefficient using STATISTICA version 12 (StatSoft Inc., Tulsa, OK, USA). All pairwise correlation values are shown in *Table 1*. Any number between +1 and - 1 can be the correlation coefficient (r) value. Whether the variable is positively or negatively associated is shown by the sign of the correlation coefficient, which can be either positive or negative (Ogugua et al., 2023). The significance of the link is indicated by the coefficient's true value. A one-way statistical analysis was used in this study to assess the nutritional availability of all parameters that were studied. The EzCorrGraph application (https://ezcorrgraph.firebaseapp.com) was utilized for the purpose of creating a correlation diagram and graphical visualization of strong positive and negative correlations (de Campos and Licht, 2021). This app was developed using HTML5 and JavaScript, allowing it to generate the correlation diagram from either a correlation matrix or a list of pairs with a user-friendly and straightforward approach (Ogugua et al., 2023).

Results and discussion

The synopsis of the analysis (*Table 1*) showed the association between the eco-physiological parameters (photosynthesis, stomatal conductance, transpiration, and internal CO_2), plant growth parameters (plant height, fresh shoot and root, shoot and root dry matter, and number of leaves and root length), nitrogen fixation indices ($\delta^{15}N$, $\delta^{13}C$, Ndfa, N-fixed, soil N uptake, and shoot C content), and carbon assimilation (%N, shoot/N, %C, and C/N) of the uniqwe Bambara groundnut landrace exposed to drought.

From the result of the study, a significant differences between eco-physiological parameters (photosynthesis, stomatal conductance, Trmmol, and Ci), plant growth parameters (plant height, fresh shoot and root weight, shoot and root dry matter, and number of leaves and root length), nitrogen fixation indices ($\delta^{15}N$, $\delta^{13}C$, Ndfa, N-fixed, soil N uptake, and shoot C content), and carbon assimilation (%N, shoot/N, %C, and C/N) were palpable (*Table 1*).

Table 1. *Correlation analysis growth indices, eco-physiological parameters, carbon assimilation and nitrogen fixation indices*

	Photo	Cond	Trmmol	Ci	WuEi	Fresh shoot	Shoot DM	Fresh RW	Root DM	P/Height	N/Leaves	R/Length	%N	δ^{15}N (‰)	Ndfa (%)	Shoot/N (mg)	N-Fixed	Soil N uptake	δ^{13}C (‰)	%C	C/N	Shoot (mg) C content
Photo	1.00***	0.50*	**-0.94****	-0.58*	0.48***	**-0.99*****	-0.38***	**-0.85*****	0.03***	**0.99*****	-0.45***	**-0.88*****	**-0.84*****	**0.91*****	0.33***	-0.42***	**-0.73*****	-0.37***	-0.15***	0.24***	-0.12***	-0.31**
Cond		1.00***	**-0.76*****	0.42**	-0.52***	-0.64***	**-0.99*****	0.03***	**0.88*****	0.63***	**-0.99*****	**-0.85*****	**-0.89*****	0.10***	**0.98*****	**-0.99*****	**-0.96*****	**-0.99*****	**0.79*****	**0.96*****	**0.80*****	**-0.98*****
Trmmol			1.00***	0.28***	-0.16**	**0.99*****	0.67***	0.63***	-0.36***	**-0.98*****	**0.73*****	**0.99*****	**0.97*****	**-0.73*****	-0.63***	**0.70*****	**0.91*****	0.66***	-0.19***	-0.55***	-0.22***	0.61***
Ci				1.00***	**-0.99*****	0.43***	-0.53***	**0.92*****	**0.80*****	-0.44***	-0.46***	0.13***	0.05***	**-0.86*****	0.58***	-0.49**	-0.14**	-0.54***	**0.89*****	0.65***	**0.88*****	-0.59***
WuEi					1.00***	-0.32***	0.63***	**-0.87*****	**-0.86*****	0.33***	0.56***	-0.01***	0.07***	**0.79*****	-0.67***	0.59***	0.25***	0.63***	**-0.94*****	**-0.74***	**-0.93****	0.68*
Fresh shoot						1.00***	0.53***	**0.75*****	-0.20**	**-0.99*****	0.60**	**0.95*****	**0.92*****	**-0.83****	-0.49**	0.57**	**0.83****	0.53**	-0.02**	-0.40**	-0.05**	0.47**
Shoot DM							1.00***	-0.16***	**-0.94*****	-0.52***	**0.99*****	**0.77*****	**0.82*****	0.03***	**-0.99*****	**0.99*****	**0.91*****	**0.99*****	**-0.86*****	**-0.99*****	**-0.87*****	**0.99*****
Fresh RW								1.00***	0.50***	**-0.76*****	-0.08***	0.50***	0.43***	**-0.99*****	0.22***	-0.12***	0.26***	-0.17***	0.65***	0.30***	0.62***	-0.23***
Root DM									1.00***	0.19***	**-0.90****	-0.50**	-0.57**	-0.38**	**0.95****	**-0.92*****	**-0.71****	**-0.94*****	**0.98****	**0.98*****	**0.99*****	**-0.96*****
P/Height										1.00***	-0.59*	**-0.94*****	**-0.92*****	**0.84****	0.48***	-0.56***	**-0.83****	-0.52**	0.01***	0.39**	0.04*	-0.46*
N/Leaves											1.00**	**0.82****	**0.86****	-0.05***	**-0.99*****	**0.99*****	**0.94*****	**0.99*****	**-0.81****	**-0.97*****	**-0.83****	**0.99*****
R/Length												1.00***	**0.99*****	-0.61***	**-0.74*****	**0.80*****	**0.97*****	**0.77*****	-0.34*	-0.67*	-0.37***	**0.73**
%N													1.00***	-0.55*	**-0.79*****	**0.85*****	**0.98*****	**0.82****	-0.41***	**-0.73****	-0.44**	**0.78*****
δ^{15}N (%)														1.00***	-0.08**	-0.02*	-0.39*	0.03**	-0.53***	-0.17***	-0.51***	0.10***
Ndfa (%)															1.00***	**-0.99*****	**-0.89*****	**-0.99*****	**0.89*****	**0.99****	**0.90*****	**-0.99****
Shoot/N (mg)																1.00***	**0.93*****	**0.99*****	**-0.84*****	**-0.98*****	**-0.85*****	**0.99***
N-Fixed																	1.00***	**0.91*****	-0.57***	**-0.84*****	-0.60*	**0.88***
Soil N uptake																		1.00***	**-0.86*****	**-0.99*****	**-0.88*****	**0.99*****
δ^{13}C (%)																			1.00***	**0.92*****	**0.99*****	**-0.89*****
%C																				1.00***	**0.94*****	**-0.99*****
C/N																					1.00***	**-0.91****
Shoot (mg) C content																						1.00***

Asterisks indicate significance. *P < 0.05, **P < 0.01, ***P < 0.001

%N (N concentration), δ^{15}N (%) (delta ^{15}N), Ndfa (%) (percentage N derived from fixation), shoot/N (shoot N content), N-fixed (Nitrogen fixed), soil N uptake (soil nitrogen uptake), δ^{13}C (delta ^{13}C), Ci (internal CO_2), WuEi (water use efficiency), Root DM (dry matter), N/leaves (number of leaves), R/length (root length), %C (C concentration); +.70 or higher (very strong positive); + 0.40 to + 0.69 (strong positive); + 0.30 to + 0.39 (moderate positive). + 0.01 to + 0.19 (no correlation); 0 (zero correlation); −0.10 to −0.19 (no relationship); −0.20 to −0.29 (weak negative); −0.30 to −0.39 (moderate negative); −0.40 to −0.69 (strong negative); −0.70 or higher (very strong negative); + 0.20 to + 0.29 (weak positive); + 0.01 to + 0.19 (no correlation); 0 (zero correlation). Note: Fresh root weight (Fresh RW); Root dry matter (Root DM)

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Photosynthesis showed a strong correlation with Trmmol (-0.94) vs fresh weight (-0.99), root length (-0.88), fresh root (-0.86), plant height (+0.99), δ15N (0.91), %N (-0.84), and N-fixed (-0.73) (*Fig. 1*). The strong relationship recorded in this study on photosynthesis vs biomass (fresh weight, root length, fresh root, and plant height) and transpiration confirms the earlier submissions, which stated that an important strategy to increase crop yield is by improving the photosynthesis efficiency of crops such as Bambara groundnut. From the result of our study, the more the photosynthesis of Bambara groundnut leaves reflects a hypothetical increase in the yield and biomass production of the plant, the findings of this study represent the core of a possible second green revolution for solving the food disaster (Kendabie et al., 2020; Gao et al., 2023; Jaiswal and Dakora, 2025).

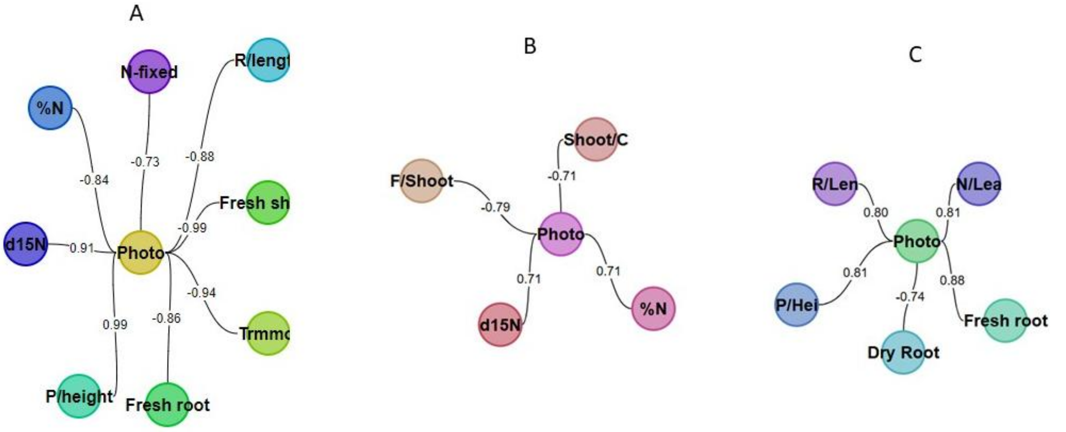

Figure 1. *Relationship between the photosynthesis vs plant height, fresh shoot and root weight, root length,* $\delta^{15}N$*, %N, Trmmol and N-fixed. The correlation coefficient is the value that exists between two strong parameters that are significance at* $P < 0.05$*,* $P < 0.01$*,* $P < 0.001$*. (Note: A = Severe; B = Moderate; C; Well-water)*

It has been apparent from several studies that photosynthesis is important for plant growth. Studies from different fields of plant science have investigated and many more are still investigating the biophysical and biochemical constraints on photosynthesis, with the aim of discovering ways to increase the efficiency of both light and dark reactions (Ahmed et al., 2020; Li et al., 2023; Cabon et al., 2024; Tang et al., 2024). They also strive to reduce respiration or make it more efficient to maximize photosynthate availability (Burgess, 2023). Studies in this area have also aimed to optimize the uptake and utilization of nutrients and water transport (Lynch, 2011) and to understand the molecular mechanisms governing cell division (Holz et al., 2024; Yang et al., 2024).

The goal of these investigations is to modify or influence these processes and gain a deeper understanding of the systems biology of the unique Bambara groundnut landrace at the supracellular level, with the aim of boosting the growth and productivity of this neglected crop. Plant biomass and yield can act as indicators of a plant's ability to effectively integrate various processes and organs (Zahedi et al., 2025). The main objective of these studies is to gain a deeper understanding of the intricate web of physiological and morphological characteristics and how drought (environmental variations) affects them, which will ultimately improve our knowledge of plant growth and development (Hagn et al., 2024; Sun et al., 2024; Zahedi et al., 2025).

Evidence suggests that the rate of photosynthesis (A) and stomatal conductance (g) in leaves are related in various environments. This relationship led to the suggestion of a messenger from the mesophyll that controls stomatal performance. As A is influenced by the intercellular concentration of CO_2 (ci), which in turn is impacted by g, the correlation between A and g may be partially influenced by ci if g has some measure of independence. Strong positive correlation ($P < 0.05$, $P < 0.01$, $P < 0.001$) was evident on the stomatal conductance vs number of leaves per plant (-0.99), shoot (-0.78) and root dry matter (+0.88), root length (-0.86), Ndfa and N-fixed (-0.98), C/N (+0.80), soil N uptake (-0.99), shoot C content (-0.98), δ13C (+0.78), %C (+0.98), shoot N content (-0.99), %N (-0.89), and Trmmol (-0.78) (*Fig. 2*). From the result of this study, the stomatal conductance plays a significant role in improving the plant biomass (number of leaves, shoot and root dry matter and root length). The study findings agree with Engineer et al. (2016) earlier reports of Liu et al. (2022); Li et al. (2023) and Cordak et al. (2025) that the CO_2 concentrations also affect leaf development and leaf area in some plants. The response is said to have a direct bearing on water conservation, which enhances plant biomass (Engineer et al., 2016; Cordak et al., 2025). Also, stomatal conductance has a direct influence on transpiration rate and photosynthesis, while N_2 fixation is indirectly affected. This process therefore plays a significant role in water and nutrient transport, thereby affecting plant growth and productivity (Torralbo et al., 2019; Liu et al., 2022; Li et al., 2023; Cordak et al., 2025).

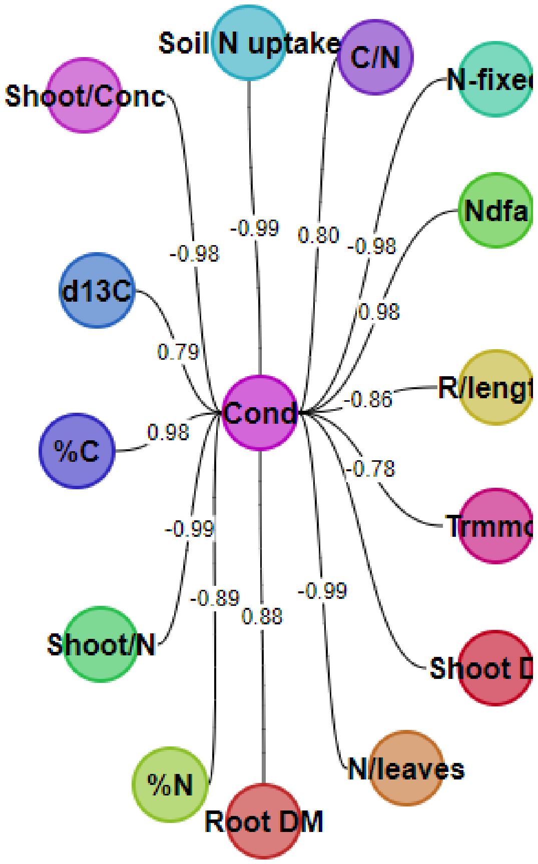

Figure 2. *A nodal network showing the relationship between stomatal conductance vs shoot and root dry matter, number of leaves, root length, Ndfa, N-fixed, C/N, soil N uptake, shoot C content, $\delta^{13}C$, %C, shoot N content, %N, Trmmol. The correlation coefficient is the value that exists between two strong parameters that are significance at $P < 0.05$, $P < 0.01$, $P < 0.001$*

In line with the findings of our study, some studies has strongly suggested that transpiration aids uptake and translocation of different nutrients and water (Plett et al., 2020; Chen et al., 2023) this was evident in our study as transpiration (Trmmol) vs plant height (-0.98), fresh shoot (+0.99), root length (+0.99), %N (+0.97), shoot N content (+0.70), number of leaves per plant (+0.73), N-fixed (+0.91) and $\delta^{15}N$ (-0.73) (*Fig. 3*) showed a robust relationship on Bambara landrace under drought conditions its importance to plant nutrition and physiological function. The connection between Trmmol and root length in relation to %N was previously documented by Novák and Vidovič (2003) and Zhang et al. (2022). According to their findings, plants can compensate for low nitrogen supply caused by reduced transpiration during drought stress by modifying their root morphology and physiological behaviors. Transpiration affects the rate of nutrient availability for direct assimilation by the root. The strong correlation recorded in this study with transpiration vs %N has earlier been reported by Novák and Vidovič (2003).

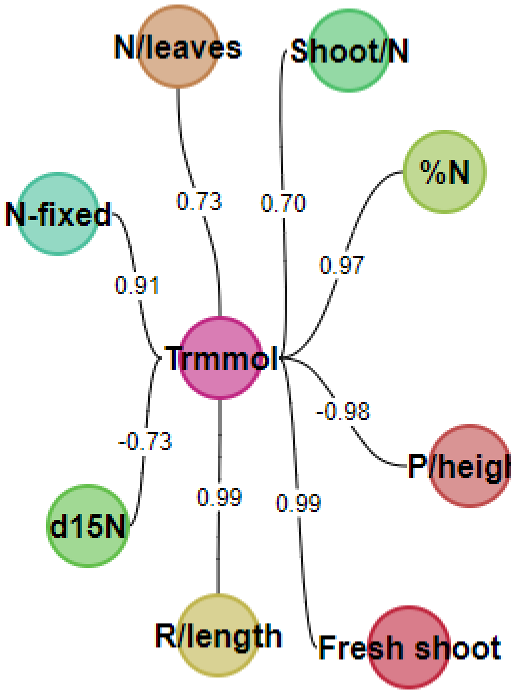

Figure 3. *Trmmol vs plant height, fresh weight, root length, %N, shoot/N, number of leaves, N-fixed, δ15N. The correlation coefficient is the value that exists between two strong parameters that are significance at P < 0.05, P < 0.01, P < 0.001*

Intercellular CO_2 is known to be one of the key parameters in photosynthesis and its increase in the atmosphere is reported to control the plant response especially during drought (Wei et al., 2022). Its relationship with fresh root weight, root dry matter, WuEi, $\delta^{13}C$, C/N and $\delta^{15}N$ is evident in this study. The intercellular CO_2 showed strong correlation with the fresh root weight (+0.92), root dry matter (+0.80), WuEi (-0.99), $\delta^{13}C$ (+0.89), C/N (+0.88) and $\delta^{15}N$ (-0.88) (*Fig. 4*). Ci is said to enhance the root system of plant which permits more uptake of nutrients and water from the soil to improve yield. It is widely acknowledged that the process of carbon isotope influence occurs during the fixation of CO_2 during stomatal conductance and photosynthesis of C_3 plants such as Bambara groundnut (Basu et al., 2021).

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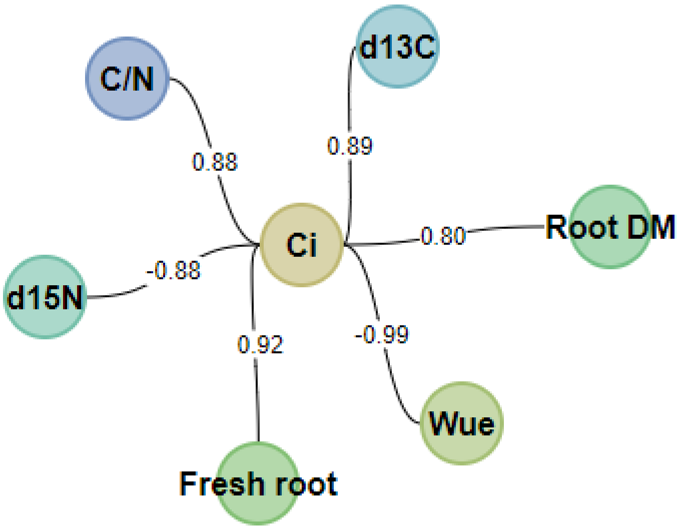

Figure 4. *Ci vs fresh root weight, root dry matter, WuEi, δ^{13}C, C/N, δ^{15}N. The correlation coefficient is the value that exists between two strong parameters that are significance at $P < 0.05$, $P < 0.01$, $P < 0.001$*

Water use efficiency (WUEi) depicts the correlation between plant productivity and water utilization, which is influenced by environmental factors and the crop itself and is governed by both. As evident in our study, the WUEi portrayed strong association with the fresh root weight (-0.87), root dry matter (-0.88) (*Fig. 5*). Since the roots act as the main uptake point for water and nutrient circulation to the plant, they are central for enhancing yield and WUEi of crops (Chen et al., 2020; Fang et al., 2021). Therefore, the root is considered important part for maintaining crop productivity while limiting water consumption (Yan et al., 2022). In line with our study, Prince et al. (2022) and Wang et al. (2024) reported root parameters to have regulated WUEi in alfalfa and sorghum during drought stress. Formal investigations into stable isotope studies have yielded valuable insights into the critical processes of plant metabolism, providing a clear comprehension of water transportation through the soil-plant-atmosphere continuum. According to Werner et al. (2012) and Schmidt and Gleixner (2020), these studies have been instrumental in elucidating the essential mechanisms governing the exchange of water vapor between the soil and the atmosphere. This was evident in our study as WUEi showed strong association on the δ^{13}C (-0.94), C/N (-0.93), δ^{15}N (+0.79), %C (-0.74) as presented in (*Table 1*; *Fig. 5*).

The Bambara groundnut landrace's fresh shoots showed a high degree of correlation with plant height, shoot dry matter, and root length. These correlations were strong, with coefficients of + 0.99, + 0.95, and moderate + 0.53 respectively. These findings support Song's earlier submission in 2017, which suggested that a well-developed root system is crucial for promoting the appropriate growth and development of the shoot, this is in affirmation with the result of our study. Also, fresh shoot of the Bambara groundnut showed strong correlation with %N (+0.92), δ^{15}N (+0.83) and N-fixed (+0.83) (*Fig. 6*). In line with our study, Qiao et al. (2021) observed a positive correlation between the shoot biomass and total N-fixed of *Medicago truncatula*. The strong correlation between increases in shoot biomass and N-fixed of soybean agrees with earlier studies (Li et al., 2017); similar trend on the %N (+0.92), δ^{15}N was reported in the studies (Yan et al., 2020).

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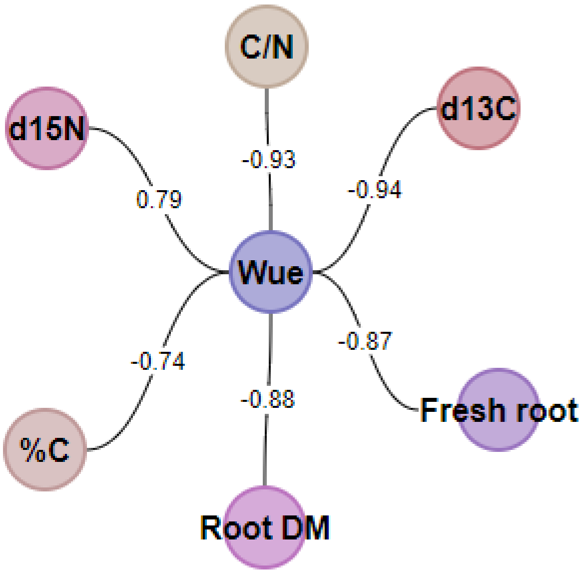

Figure 5. *WuEi vs fresh root weight, root dry matter, δ^{13}C, C/N, δ^{15}N, %C. The correlation coefficient is the value that exists between two strong parameters that are significance at $P < 0.05$, $P < 0.01$, $P < 0.001$*

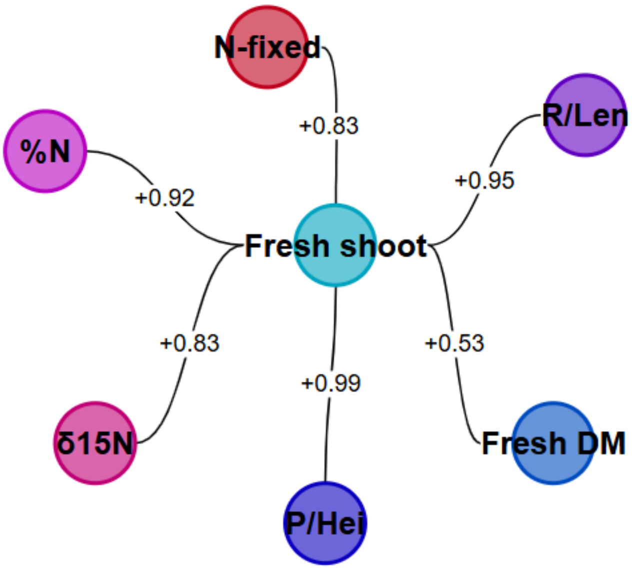

Figure 6. *Fresh shoot vs plant height (plant height), shoot dry matter (fresh DM), root length (root length), %N, δ^{15}N, N-fixed. The correlation coefficient is the value that exists between two strong parameters that are significance at $P < 0.05$, $P < 0.01$, $P < 0.001$*

Findings of the study demonstrated a significant association between the Bambara groundnut shoot dry matter and factors such as the number of leaves per plant (+0.99), root dry matter (-0.94), and root length (+0.77), which aligns with Song's findings from 2017. A well-developed root system is crucial for promoting healthy shoot growth and development. Our results also revealed a strong correlation between shoot dry matter and other factors, including shoot N content (+0.99), N-fixed (+0.91), soil N uptake (+0.99), C/N (-0.87), %C (-0.99), shoot C content (-0.99), δ^{13}C (-0.86), Ndfa (-0.99), and %N

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(+0.82) (*Fig. 7*). Anglade et al. (2015) reported a close relationship between shoot dry matter and the amount of N_2 fixed in the shoot in legumes, which has been investigated by previous studies (Schipanski and Drinkwater, 2012; Unkovich, 2012). Anglade et al. (2015) and Rani et al. (2023) emphasized that determining strong relationships between shoot N yields (or shoot DM) and the amount of fixed N provides a useful empirical rule for farmers, managers, and researchers to estimate net N inputs.

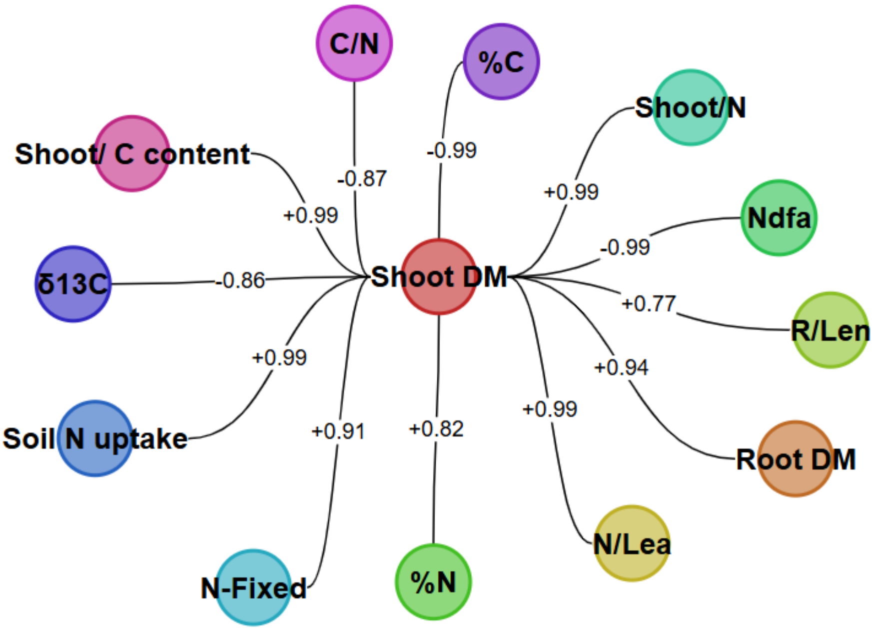

Figure 7. *Shoot DM vs number of leaves, root dry matter, root length, shoot/N, N-fixed, soil N uptake, C/N, %C, shoot C content, $\delta^{13}C$, Ndfa, %N. The correlation coefficient is the value that exists between two strong parameters that are significance at $P < 0.05$, $P < 0.01$, $P < 0.001$*

Conclusion

The study examined the relationship between the Bambara groundnut’s growth, eco-physiological, carbon assimilation, and nitrogen fixation parameters under drought conditions using correlation analysis. According to the correlation analysis, the relationship between growth, physiological, and biochemical strategies is essential for improving the drought resilience of Bambara groundnut. Sustaining growth during drought requires maintaining effective nitrogen fixation and carbon assimilation. By identifying important traits for enhancing drought tolerance and directing future agricultural practices, an understanding of these relationships helps to promote food security. The findings show the intricate interrelationships between different physiological functions in drought-stressed Bambara groundnut. This study also emphasizes how important it is to comprehend how these factors relate to one another in order to improve the drought resistance of Bambara groundnut. However, further studies are recommended to reveal the metabolite profile changes that occur in Bambara groundnut under drought conditions.

Author contributions. ELU, KCM, SAK conceptualized the manuscript; UVO Analyzed and interpreted the data; UVO and ELU wrote the manuscript; MN reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of interests. The authors declare that they have no competing interests.

Data availability. The article contains all of the data used in the study; any additional data not included in the article can be obtained from the corresponding author upon reasonable request.

REFERENCES

[1] Abbaspour, N., Babaee, L. (2017): Effect of salicylic acid application on oxidative damage and antioxidant activity of grape (Vitis vinifera L.) under drought stress condition. – International Journal of Horticultural Science and Technology 4(1): 29-50.

[2] Aderinola, T., Ajayeoba, T., Akanni, G., Uzomah, A., Onyeaka, H., Adeboye, A. (2024): Legume-based functional foods in West Africa for managing non-communicable diseases: a comprehensive review of dietary strategies. – Nutrire 49(2): 47.

[3] Ahmed, K. B. M., Khan, M. M. A., Siddiqui, H., Jahan, A. (2020): Chitosan and its oligosaccharides, a promising option for sustainable crop production-a review. – Carbohydrate Polymers 227: 115331.

[4] Anglade, J., Billen, G., Garnier, J. (2015): Relationships for estimating N_2 fixation in legumes: incidence for N balance of legume-based cropping systems in Europe. – Ecosphere 6(3): 1-24.

[5] Ataro, A., McCrindle, R. I., Botha, B. M., McCrindle, C. M. E., Ndibewu, P. P. (2008): Quantification of trace elements in raw cow's milk by inductively coupled plasma mass spectrometry (ICP-MS). – Food Chemistry 111(1): 243-248.

[6] Basu, S., Ghosh, S., Chattopadhyay, D. (2021): Disentangling the abiotic versus biotic controls on C3 plant leaf carbon isotopes: inferences from a global review. – Earth-Science Reviews 222: 103839.

[7] Berchie, J. N., Opoku, M., Adu-Dapaah, H., Agyemang, A., Sarkodie-Addo, J. (2012): Evaluation of five bambara groundnut (Vigna subterranea (L.) Verdc.) landraces to heat and drought stress at Tono-Navrongo, Upper East Region of Ghana. – African Journal of Agricultural Research 7(2): 250-256.

[8] Broughton, W. J., Dilworth, M. J. (1970): Control of leghaemoglobin synthesis in Snake beans. – Journal of Biochemistry 125: 1075-1080.

[9] Burgess, A. J. (2023): Breath of green life: reduction in plant day and night respiration under elevated CO2. – Plant Physiol 192(1): 31-33.

[10] Cabon, A., Ameztegui, A., Anderegg, W. R., Martínez-Vilalta, J., De Cáceres, M. (2024): Probing the interplay of biophysical constraints and photosynthesis to model tree growth. – Agricultural and Forest Meteorology 345: 109852.

[11] Chen, Y., Palta, J., Prasad, P. V., Siddique, K. H. (2020): Phenotypic variability in bread wheat root systems at the early vegetative stage. – BMC Plant Biology 20: 1-16.

[12] Chen, Z., Li, H., Zhang, W. H., Wang, B. (2023): The roles of stomatal morphologies in transpiration and nutrient transportation between grasses and forbs in a temperate steppe. – Annals of Botany 132(2): 229-239.

[13] Cordak, A. S., Kooperman, G. J., Zarakas, C. M., Swann, A. L., Koven, C. D. (2025): The role of leaf area changes within plant CO2 physiological impacts on the global hydrological cycle. – Geophysical Research Letters 52(2): e2024GL110904.

[14] de Campos, F. F. and Licht, O. A. B. (2021): Correlation diagrams: Graphical visualization of geochemical associations using the EzCorrGraph app. – Journal of Geochemical Exploration 220: 106657.

[15] Engineer, C. B., Hashimoto-Sugimoto, M., Negi, J. Israelsson-Nordström, M., Azoulay-Shemer, T., Rappel, W. J., Iba, K., Schroeder, J. I. (2016): CO2 sensing and CO2 regulation of stomatal conductance: advances and open questions. – Trends Plant Sci. 21(1): 16-30.

[16] Fang, Y., Liang, L., Liu, S., Xu, B., Siddique, K. H., Palta, J. A., Chen, Y. (2021): Wheat cultivars with small root length density in the topsoil increased post-anthesis water use and grain yield in the semi-arid region on the Loess Plateau. – European Journal of Agronomy 124: 126243.

[17] FSSA (1974): Manual of Soil Analysis Methods. – FSSA publication no. 37, Pretoria.

[18] Gao, X., Chai, H. H., Ho, W. K., Mayes, S., Massawe, F. (2023): Deciphering the molecular basis for photosynthetic parameters in Bambara groundnut (Vigna subterranea L. Verdc) under drought stress. – BMC Plant Biology 23(1): 287.

[19] Green, C. H., Foster, C., Cardon, G. E., Butters, G. L., Brick, M., Ogg, B. (2004): Water Release from Crosslinked Polyacrylamide. – Colorado State University, Ft. Collins, CO 7, pp. 252-260.

[20] Hagn, L., Schuster, J., Mittermayer, M., Hülsbergen, K. J. (2024): A new method for satellite-based remote sensing analysis of plant-specific biomass yield patterns for precision farming applications. – Precision Agriculture 25(6): 2801-2830.

[21] Haider, S., Bibi, K., Munyaneza, V., Zhang, H., Zhang, W., Ali, A., Ahmad, I. A., Muhammad, M., Xu, F., Yang, C. (2024): Drought-induced adaptive and ameliorative strategies in plants. – Chemosphere 364: 143134.

[22] Holz, M., Zarebanadkouki, M., Benard, P., Hoffmann, M., Dubbert, M. (2024): Root and rhizosphere traits for enhanced water and nutrients uptake efficiency in dynamic environments. – Frontiers in Plant Science 15: 1383373.

[23] Jaiswal, S. K., Dakora, F. D. (2025): Maximizing photosynthesis and plant growth in African legumes through rhizobial partnerships: the road behind and ahead. – Microorganisms 13(3): 581.

[24] Katerova, Z., Todorova, D., Vaseva, I. I., Shopova, E., Petrakova, M., Iliev, M., Sergiev, I. (2024): Effects of melatonin pre-and post-drought treatment on oxidative stress markers and expression of proline-related transcripts in young wheat plants. – International Journal of Molecular Sciences 25(22): 12127.

[25] Kendabie, P., Jørgensen, S. T., Massawe, F., Fernandez, J., Azam-Ali, S., Mayes, S. (2020): Photoperiod control of yield and sink capacity in Bambara groundnut (Vigna subterranea) genotypes. – Food and Energy Security 9(4): e240.

[26] Khan, N., Ali, S., Tariq, H., Latif, S., Yasmin, H., Mehmood, A., Shahid, M. A. (2020): Water conservation and plant survival strategies of rhizobacteria under drought stress. – Agronomy 10(11): 1683.

[27] Kinsella, K., Schulthess, C. P., Morris, T. F., Stuart, J. D. (2009): Rapid quantification of Bacillus subtilis antibiotics in the rhizosphere. – Soil Biology and Biochemistry 41(2): 374-379.

[28] Li, Y., Yu, Z., Liu, X., Mathesius, U., Wang, G., Tang, C., Wu, J., Liu, J., Zhang, S., Jin, J. (2017): Elevated CO2 increases nitrogen fixation at the reproductive phase contributing to various yield responses of soybean cultivars. – Frontiers in Plant Science 8.

[29] Li, F., He, C., Chang, Z., Ma, C., Yu, J., Liu, L., Zhang, Y., Hao, L. (2023): Effects of elevated carbon dioxide on plant growth and leaf photosynthesis of annual ryegrass along a phosphorus deficiency gradient. – Frontiers in Plant Science 14: 1271262.

[30] Liu, L., Hao, L., Zhang, Y., Zhou, H., Ma, B., Cheng, Y., Tian, Y., Chang, Z., Zheng, Y. (2022): The CO2 fertilization effect on leaf photosynthesis of maize (Zea mays L.) depends on growth temperatures with changes in leaf anatomy and soluble sugars. – Frontiers in Plant Science 13: 890928.

[31] Lynch, J. P. (2011): Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. – Plant Physiology 156: 1041-1049.

[32] Novák, V., Vidovič, J. (2003): Transpiration and nutrient uptake dynamics in maize (Zea mays L.). – Ecological Modelling 166(1-2): 99-107.

[33] Ogugua, U. V., Kanu, S. A., Ntushelo, K. (2023): Relationship between different physiological processes of Tomato seedlings exposed to acid mine water uncovered using correlation analysis. – Heliyon. https://doi.org/10.1016/j.heliyon.2023.e18975.

[34] Plett, D. C., Ranathunge, K., Melino, V. J., Kuya, N., Uga, Y., Kronzucker, H. J. (2020): The intersection of nitrogen nutrition and water use in plants: new paths toward improved crop productivity. – Journal of Experimental Botany 71(15): 4452-4468.

[35] Prince, S., Anower, M. R., Motes, C. M., Hernandez, T. D., Liao, F., Putman, L., Mattson, R., Seethepalli, A., Shah, K., Komp, M., Mehta, P. (2022): Intraspecific variation for leaf physiological and root morphological adaptation to drought stress in alfalfa (Medicago sativa L.). – Frontiers in Plant Science 13: 795011.

[36] Qiao, Y., Miao, S., Jin, J., Mathesius, U., Tang, C. (2021): Differential responses of the sunn4 and rdn1-1 super-nodulation mutants of *Medicago truncatula* to elevated atmospheric CO2. – Annals of Botany 128(4): 441-452.

[37] Rani, M., Kaushik, P., Bhayana, S. and Kapoor, S. (2023): Impact of organic farming on soil health and nutritional quality of crops. – Journal of the Saudi Society of Agricultural Sciences 22(8): 560-569.

[38] Schipanski, M. E. and Drinkwater, L. E. (2012): Nitrogen fixation in annual and perennial legume-grass mixtures across a fertility gradient. – Plant and Soil 357: 147-159.

[39] Schmidt, H. L., Gleixner, G. (2020): Carbon Isotope Effects on Key Reactions in Plant Metabolism and 13C-Patterns in Natural Compounds. – In: Griffiths, H. (ed.) Stable Isotopes. Garland Science, London, pp. 13-25.

[40] Singh, N., Raghuvanshi, R. S., Patidar, T., Chauhan, S. S. (2024): Resilience in legumes: understanding plant responses to abiotic stress and biotechnological solutions. – African Journal of Biological Sciences 6(16): 2663-2187.

[41] Song, J. (2017): The relationship of root system with the growth and development of bulbs and shoots in lilies. – HortScience 52(2): 245-250.

[42] Sun, Y., Robert, C. A., Thakur, M. P. (2024): Drought intensity and duration effects on morphological root traits vary across trait type and plant functional groups: a meta-analysis. – BMC Ecology and Evolution 24(1): 92.

[43] Tang, X., Zhao, J., Zhou, J., Zhu, Q., Sheng, X., Yue, C. (2024): Elevated co2 shifts photosynthetic constraint from stomatal to biochemical limitations during induction in Populus tomentosa and Eucalyptus robusta. – Plants 14(1): 47.

[44] Teixeira, P. C., Donagemma, G. K., Fontana, A., Teixeira, W. G. (2017): Manual of Soil Analysis Methods. – Embrapa, Brasília, DF.

[45] Torralbo, F., González-Moro, M. B., Baroja-Fernández, E., Aranjuelo, I. and González-Murua, C. (2019): Differential regulation of stomatal conductance as a strategy to cope with ammonium fertilizer under ambient versus elevated CO2. – Frontiers in Plant Science 10: 450214.

[46] Udeh, E. L., Nyila, M. A., Kanu, S. A. (2020): Nutraceutical and antimicrobial potentials of Bambara groundnut (Vigna subterranean): a review. – Heliyon 6(10).

[47] Unkovich, M. (2012): Nitrogen fixation in Australian dairy systems: review and prospect. – Crop and Pasture Science 63(9): 787-804.

[48] Vishnupradeep, R., Bruno, L. B., Taj, Z., Karthik, C., Challabathula, D., Kumar, A., Freitas, H., Rajkumar, M. (2022): Plant growth promoting bacteria improve growth and phytostabilization potential of Zea mays under chromium and drought stress by altering photosynthetic and antioxidant responses. – Environmental Technology and Innovation 25: 102154.

[49] Vocciante, M., Grifoni, M., Fusini, D., Petruzzelli, G., Franchi, E. (2022): The role of plant growth-promoting rhizobacteria (PGPR) in mitigating plant’s environmental stresses. – Applied Sciences 12(3): 1231.

[50] Wang, K., Nan, L. L., Xia, J., Wu, S. W., Yang, L. L. (2024): Metabolomics reveal root differential metabolites of different root-type alfalfa under drought stress. – Frontiers in Plant Science 15: 1341826.

[51] Wei, Z., Abdelhakim, L. O. A., Fang, L., Peng, X., Liu, J., Liu, F. (2022): Elevated CO2 effect on the response of stomatal control and water use efficiency in amaranth and maize plants to progressive drought stress. – Agricultural Water Management 266: 107609.

[52] Yan, G., Han, S., Zhou, M., Sun, W., Huang, B., Wang, H., Xing, Y., Wang, Q. (2020): Variations in the natural 13 C and 15 N abundance of plants and soils under long-term N addition and precipitation reduction: interpretation of C and N dynamics. – Forest Ecosystems 7: 1-13.

[53] Yan, M., Zhang, L., Ren, Y., Zhang, T., Zhang, S., Li, H., Chen, Y., Zhang, S. (2022): The higher water absorption capacity of small root system improved the yield and water use efficiency of maize. – Plants 11(17): 2300.

[54] Yang, S. Y., Lin, W. Y., Hsiao, Y. M., Chiou, T. J. (2024): Milestones in understanding transport, sensing, and signaling of the plant nutrient phosphorus. – The Plant Cell 36(5): 1504-1523.

[55] Yang, Y. J., Bi, M. H., Nie, Z. F., Jiang, H., Liu, X. D., Fang, X. W., Brodribb, T. J. (2021): Evolution of stomatal closure to optimize water-use efficiency in response to dehydration in ferns and seed plants. – New Phytologist 230(5): 2001-2010.

[56] Zahedi, S. M., Karimi, M., Venditti, A., Zahra, N., Siddique, K. H., Farooq, M. (2025): Plant adaptation to drought stress: the role of anatomical and morphological characteristics in maintaining the water status. – Journal of Soil Science and Plant Nutrition 25(1): 409-427.

[57] Zhang, J., Hu, Z., Zhang, C., Tao, Y., Chen, X., Griffiths, B. S., Liu, M. (2022): Roots with larger specific root length and C:N ratio sustain more complex rhizosphere nematode community. – Plant and Soil 477(1): 693-706.