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Effect of Drought Stress on Agronomic Traits and Total Leaf Proteins in Different Bottle Gourd [*Lagenaria siceraria* (Molina) Standl.] Genotypes

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Abstract

Breeding drought-tolerant genotypes using genetic and biochemical tools is an important mitigation strategy to improve stress response and yields in bottle gourd [*Lagenaria siceraria* (Molina) Standl.]. This current study evaluated the variations among bottle gourd genotypes for potential breeding purposes by establishing the relationship between agronomic traits and the protein profile required for the plants' resilience against drought stress. The study assessed 12 bottle gourd accessions grown under non-stressed (NS) control conditions and different levels of drought stress (DS) induced by withholding irrigation for 7, 14, and 21 days, using a 12 × 2 × 3 factorial experiment in a randomized complete block design with 3 replicates. Agronomic traits such as the total number of male and female flowers per plant, sex ratio, fruit number and fruit yield per plant (FYPP), and total protein analysis were determined in

bottle-gourd plants immediately after the period of stress. Results showed significant differences ($p < 0.001$) among the genotypes × environmental effect for most agronomic traits except the number of days to first flower (DTFF). Among the genotypes, BG-70 and BG-78 recorded the highest FYPP under drought stress conditions, with BG-70 showing similar results even under NS conditions. A positive correlation was found among all the agronomic traits and the total protein contents of the genotypes, especially after 14 days of drought stress. Overall, the results implied that the significant improvements in agronomic traits and unique protein expressions observed in BG-70 and BG-78 potentially confer tolerance to drought stress. Moreover, the high and unique proteins found in all genotypes (BG-48, BG-58, BG-52, BG-70, BG-78, and BG-81) warrant further research on their interaction with the stress, especially when coupled with improved agronomic traits, which could assist in identifying drought stress tolerant genotypes.

Keywords
Agronomic traits; drought stress; fruit yield; protein concentration; protein expression

1. Introduction

Bottle gourd [*Lagenaria siceraria* (Molina) Standl.] is an essential crop in arid and semi-arid areas of sub-Saharan Africa. The crop can grow in harsh environments characterized by poor soils, limited water supply, and high temperatures, suggesting the potential ability to tolerate drought [1, 2]. This crop exhibits resistance to several abiotic factors, including heat and salt stress, compared to other cucurbit crops [3-5]. Three physiological strategies generally used by plants in response to drought stress include escape, avoidance, and tolerance, comprising mechanisms like antioxidant defense, solute accumulation, osmotic adjustment, stomatal conductance, and increased ratio of root-to-shoot growth [6-11]. However, the plant may use multiple mechanisms to cope with drought stress [12, 13]. In South Africa, the drought escape mechanism was reported for landraces such as BG-79, BG-31, BG-67, BG-52, and BG-78 in a study where yield-based selection for drought tolerance in selected bottle gourds was conducted [2].

The landraces used early flowering and maturation to avoid severe impact from drought stress. Fruit number and yield levels were moderately reduced. However, these landraces proved valid for future use in developing short-cycle varieties that can avoid drought [7, 14-18]. Other physiological processes that are generally affected by exposure to drought stress include crop yield, which is commonly determined by reproductive components such as the number of fruits per plant, plant height, and number of leaves per plant [19, 20]. In previous studies conducted in areas such as India and Bangladesh, drought-tolerant bottle gourd varieties were identified through phenotypic evaluation, in which fruit yield was targeted as an essential trait for selection [1, 8, 9]. The conclusions drawn from these studies highlight the importance of developing varieties showing high performance in both water stress and non-stressed conditions by exploiting their internal physiological and genomic capabilities [21-29]. In addition, varieties with high yields under drought or heat stress conditions were selected as drought tolerant and used for cultivation during dry seasons [21-23, 30].

Biochemical processes such as plant cell water content, cellular expansion, photosynthesis, chlorophyll synthesis, nutrient carbohydrate metabolisms, and cellular respiration are susceptible to drought stress [31-36], and it is imperative to understand how these processes are altered due to drought stress to mitigate proper control management strategies [6, 10]. In bottle gourd, biochemical analysis such as enzyme expression has been proposed as a valuable complementary strategy for selecting drought-tolerant genotypes [11, 36, 37]. Other approaches have investigated biochemical processes, such as the expression of osmosensors and phospholipid cleavage enzymes that are important for sensing water shortage [12-14, 38-40]. The synthesis of aquaporins and/or secondary metabolites required by plants to survive under drought stress has been documented, suggesting their importance in the selection and understanding of biochemical changes linked to drought stress [10, 41]. Alterations in protein synthesis and overall changes in protein profile are some of the basic metabolically stimulated processes that influence drought tolerance in bottle gourd and other cucurbit crops [15, 42, 43]. The direct relationship between the accumulations of drought-induced proteins and physiological adaptation to water limitation has been demonstrated in crops closely related to bottle gourd, such as barley, wheat, maize, muskmelon, and wild melon [14, 16-19, 44]. The expression of such proteins can be used as the selection markers for drought tolerance [45-48]. The increased synthesis of proteins in drought-exposed crops is important in controlling and increasing solute concentration in the cytoplasm [20].

In addition, dehydrin synthesized in response to drought possesses a cytoprotective role in macromolecule stabilization that proved vital for preventing further damage and denaturation of cellular proteins caused by drought stress [19, 39, 49-51]. Heat shock proteins, proteinase inhibitors, thiol proteases, and osmotin proteins also accumulate under drought stress to maintain proteins in their functional conformation and prevent degradation during the changing environment [18, 19, 21-23]. As alluded to above, it is evident that the expression of such proteins serves as potential biochemical markers for the development of superior genotypes. Therefore, this study aimed to determine the level of drought tolerance among bottle gourd genotypes using phenotypic and proteomic analyses and establish correlations between protein content and agronomic traits under normal and water-stress conditions. Such evaluations could contribute new insights to years of informal selection by small-holder farmers as there is no sufficient documentation of formal breeding of this crop. These indicate the need for the identification of phenotypic and biochemical parameters to guide breeding programs of bottle gourd and related crops for inducing tolerance to drought stress.

2. Materials and Methods

2.1 Plant Materials and Crop Establishment

A total of 12 accessions of bottle gourd [*Lagenaria siceraria* (Molina) Standl.], namely: BG-27, BG-31, BG-48, BG-52, BG-58, BG-67, BG-70, BG-78, BG-79, BG-80, BG-81, and GC were used in this study. The experiment was conducted in a greenhouse (growth tunnel conditions) at the University of Limpopo (-25°36'54"S, 28°0'59.76"E, 1312 m above sea level, South Africa) using a 12 × 2 × 3 factorial experiment in a randomized complete block design with three replicates for both the control and stress intensity established. A total of three seeds were sown per genotype in 2 L polyethylene plastic pots containing approximately 2 kg of loamy soil collected from Syferskuil Experimental farm (-23°53'9.60"S, 29°44'16.80"E, 1312 m above sea level) of the University of

Limpopo. Seeded pots were watered daily, maintaining soil moisture content at approximately 40% v/v field capacity with a bi-weekly alteration of a modified Hoagland nutrient solution as described by Ali et al. [41] and tap water until the development of the sixth fully expanded leaf that occurred at approximately 25 days after planting.

2.2 Drought Treatments, Agronomic Traits and Leaf Protein Profile in Bottle Gourds

After 25 days of planting, the plants were subjected to induced drought stress by withholding irrigation for 7, 14 and 21 days, while non-stressed (NS) plants were also watered daily depending on soil moisture content for a similar period. Soil moisture content was monitored daily using an electronic soil moisture meter (HydroSense soil-water sensor, Campbell Scientific Africa). Following these water stress treatments, all sets of plants from each block were randomly selected and sampled for evaluation of growth, agronomic traits, and protein expression profiles. The following agronomic data were recorded: days to first flower (DTFF), the total number of male flowers (NMF), and total number of female flowers (NFF) per plant. Sex ratio (SR) was calculated as the total number of male flowers per plant to the total number of female flowers per plant. The number of fruits per plant (NFPP), single fruit weight (kg) (FW), and fruit yield per plant (kg) (FYPP) were also recorded.

2.2.1 Protein Extraction and Quantification

For protein analysis, fully expanded leaves from each plant under DS and NS conditions were collected into 50 ml centrifuge tubes containing liquid nitrogen to maintain protein integrity during sample collection and transporting to the laboratory. Upon arrival, the samples were kept at -80°C until analysed for total protein content. Leaf proteins were extracted from 0.1 g ground powder using 1 ml of extraction buffer containing 50 mM Tris-HCl (pH 7.5), 100 mM potassium chloride (KCl) and 10% glycerol. The extraction mixture was homogenized for 10 minutes using a mini-bead-beater (Biospec Bartlesville, USA) and centrifuged at 14 000 ×g for 5 min at 4°C using a Neofuge 15R centrifuge (Vacutec California, USA). The leaf protein supernatant was collected and then quantified using the Bicinchoninic Acid (BCA) Protein Assay Kit (Pierce, Rockford, IL, USA). The working reagent of the BCA™ Protein Assay Kit contained bicinchoninic acid that was used to monitor cuprous ions produced in the reaction of protein with alkaline Cu^{2+}. A stable purple colour was produced, which was directly proportional to the protein concentration in each sample and the total proteins were assessed as reported by Smith et al. [42]. To determine protein concentration in test samples, a standard curve was constructed using bovine serum albumin (BSA) of known concentration (0.00-2.5 mg/ml), as shown in Figure 1. Each sample was mixed with the working reagent (sample to working reagent ratio of 1:20), placed in a Nunc® 96 well plate (Nunc Roskilde, Denmark), and covered and incubated at 37°C for 30 minutes. The color change was measured at 562 nm using DU® 730 Life Science UV/Vis spectrophotometer (Beckman Coulter).

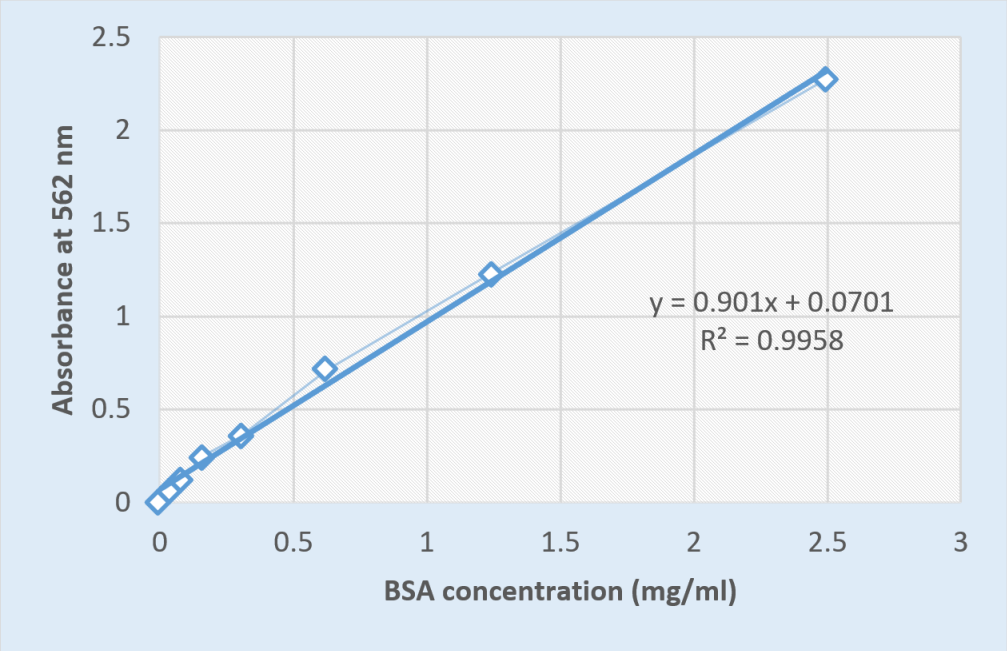

Figure 1 Standard curve showing the absorbance reading of the BSA standard protein of known concentration range of 0-2.5 mg/ml used to quantify the unknown protein concentration in leaf samples.

Analysis of protein band patterns was conducted using 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli [43]. Equal volumes of each leaf extract were mixed with reducing sample buffer [(125 mM Tris-HCl; 4% (w/v) SDS; 20% (v/v) glycerol; 10% (v/v) 2-mercaptoethanol, pH 6.8)] and the mixture was heated at 100°C for 5 minutes. About 25 µg of protein was loaded in each agarose gel well according to the genotype and the treatment. Generally, a pre-stained molecular weight marker (MWM) of mixed proteins with sizes ranging from 3 to 260 kDa was loaded in the first lane. In the second lane, the DS sample collected after 7 days of drought exposure, labeled As, was loaded, followed by NS sample collected after 7 days, labeled Ac. The fourth lane contained DS samples collected after 14 days of drought stress (Bs), followed by NS samples collected after 14 days labeled as Bc. In the sixth lane, DS samples collected after 21 days of drought stress (Cs) were loaded, followed by the 7th lane of NS samples, also collected after 21 days labeled as Cc. After loading, the proteins were separated at a constant current (18 mA per gel) until the loading dye reached the bottom of the gel. The gel was then stained with Coomassie Blue [(0.125% (w/v) Coomassie Blue R-250; 50% (v/v) methanol; 10% (v/v) acetic acid)] overnight and de-stained using de-stain I solution [(50% (v/v) methanol; 10% (v/v) acetic acid)] followed by the de-stain Π solution [(7% (v/v) acetic acid; 5% (v/v) methanol)]. The gels were then analyzed for unique proteins using a Gene Tool from the Syngene system that calculated the molecular weights of each protein band on the gels using a molecular weight marker as a reference.

2.3 Statistical Data Analysis

The study used a factorial experiment in a randomized complete block design with three replications. Data from different irrigation regimes, including NS, 7, 14, and 21 days of drought stress,

were evaluated using analysis of variance (ANOVA) performed using GenStat version 18 [44]. The Least Significant Difference (LSD) test compared treatment means at the 5% significance level.

2.3.1 Trait Correlations Analysis

The magnitude of the variation relationships among agronomic traits and protein concentrations were determined using GenStat version 18 [44]. The Least Significant Difference (LSD) test was used to compare treatment means at the 5% level of significance, and BLUPs estimates were used for Pearson correlation coefficients to determine the associations between assessed agronomic traits using SPSS version 25 (SPSS Inc., Chicago, IL, USA, 2018). Principal Component Analysis (PCA) based on the correlation matrix was performed using SPSS version 25 (SPSS Inc., Chicago, IL, USA, 2018). The best Linear Unbiased Predictors (BLUPs) were calculated using META-R (Multi Environment Trail Analysis with R for Windows) Version 6.0 [45], and the following BLUPs were estimated using the linear model:

$$Yijkl = \mu + Loci + Repj\ (Loci) + Blockk\ (LociRepj) + Genl + Loci \times Genl + \varepsilon ijkl$$

Where;

- Yijkl = the trait of interest,
- μ = overall mean effect,
- Loci = effects of the ith environment,
- Repj = effects of the jth replicate,
- Block (Repi) = effects of the kth incomplete block within the jth replicate,
- Loci × Genl = environment × genotype interaction,
- Genj = effects of the lth genotype,
- εijkl = error associated with the ith replication, jth incomplete block, and the kth genotype, assumed to be normally and independently distributed, with mean zero and homoscedastic variance σ2. Genotypes, environment, and interactions were treated as random factors that affected the calculation of BLUPs.

2.3.2 Stress Tolerance Index and Geometric Mean Productivity

To select for high-yielding genotypes under DS and NS conditions, stress tolerance index (STI) and geometric mean productivity GMP was calculated using the formula below, where Ys refers to fruit yield of a test genotype under drought-stress (DS) conditions; Yp refers to fruit yield of a test genotype under non-stressed (NS) conditions, and Xp referring to mean yield of test genotypes under non-stressed conditions as described by Fernandez [46]:

1) $STI = [(Yp \times Ys)/Xp^2]$
2) $GMP = (Yp \times Ys)$

3. Results

3.1 Effect of Drought on Agronomic Traits in Bottle Gourd

This study evaluated agronomic and total protein variations of 12-bottle gourd accessions grown under induced water stress for 7, 14, and 21 days, compared with the control NS plants. The study

results based on the decreased soil moisture content due to withheld irrigation, as illustrated in Table 1 and Table 2 showed that imposed drought stress negatively influenced the development and expression of different agronomic traits and total proteins recorded in bottle gourds, respectively. The analysis of variance based on the magnitude of stress on the relationship among agronomic traits revealed that significant effects ($p < 0.001$) were observed for all parameters except for the number of days to first flower (DTFF) production and the number of female flowers (NFF) per plant. A non-significant effect was also observed for the sex ratio (SR) determined from the total number of male and female flowers per plant. Nonetheless, the genotype × environmental effect factor appeared also significant for all agronomic traits except for DTFF and NFF (Table 2). BLUPs estimates for the assessed agronomic traits under DS and NS conditions of different drought intensities were used to determine the means, least significant differences (LSD) at 5% significant levels, and coefficient of variance (CV) obtained for all genotypes. The results indicated that DTFF was relatively the same across the different genotypes (Table 1). Bottle gourd genotypes BG-70 and BG-78 recorded the highest NFF (an average total of 17 NFF per plant). Meanwhile, BG-48 and GC recorded the least NNF of 8 per plant, and BG-70 and BG-78 recorded the highest FYPP of 3 kg per plant. The findings further revealed that DTFF was also relatively the same across all the genotypes under NS conditions except for BG-37, which recorded at least 27 days of first flower production per plant. Furthermore, NS controls in BG-81 and BG-58 genotypes recorded the highest NFF of 37 and 33, respectively, in addition to genotypes BG-52 and GC, which recorded the lowest NFF of 14. BG-27 and BG-48 also recorded the highest FYPP of 23 and 16 kg, respectively, compared to DS bottle gourd plants.

Table 1 BLUPs estimates of the 12 bottle gourd genotypes for agronomic traits evaluated under drought stress and non-stress conditions.

Drought Stress										
Genotype	**DTFF**	**NMF**	**NFF**	**SR**	**NFPP**	**FW**	**FYPP**	**Prot-7**	**Prot-14**	**Prot-21**
BG-48	45.00	19.29	7.66[a]	5.29[a]	2.76[a]	0.13[a]	0.73[a]	2.32[a]	2.45[a]	1.01[a]
BG-58	41.67	101.03	12.27[b]	7.67	2.76[a]	0.13[a]	0.73[a]	2.41[b]	2.14[b]	0.89
BG-27	42.67[a]	14.60	8.84	3.12	5.31	0.13[a]	0.87	2.43[b]	2.45[a]	1.62
BG-31	42.67[a]	88.79	12.60[b]	6.53[b]	7.86	0.23	1.55	2.30[a]	2.18[b]	1.08[b]
BG-52	43.33[b]	19.62	13.42	1.91	4.67[b]	0.15[a]	0.92[b]	2.30[a]	2.26[c]	1.15
BG-67	44.67c	72.18	12.43[b]	5.63[a]	4.99[b]	0.16[a]	0.97[b]	2.09	2.14[b]	1.04[ba]
BG-70	44.33[b]	90.73	17.05[c]	9.80	10.41	0.31	2.62	2.32[a]	2.38[d]	1.11
BG-78	47.00	85.79[a]	16.88	4.17[c]	6.26	0.52	2.59	2.50	2.60	1.57[c]
BG-79	44.33[b]	44.05	9.63	5.82[a]	2.76[a]	0.17[ba]	0.78	2.45[b]	2.36[d]	1.57[c]
BG-80	44.67[c]	19.90	12.10[b]	2.14	3.71[c]	0.11[a]	0.73	2.30[a]	2.25[c]	1.42
BG-81	42.00	83.16	7.99[a]	20.57	1.48	0.12[a]	0.65	2.41[b]	2.46[a]	1.78[d]
GC	42.67[a]	20.21	8.15	4.87	3.71[c]	0.14[a]	0.81	2.18	2.29[c]	1.20
Grand Mean	43.75	54.94	11.56	6.46[b]	4.72[b]	0.19[cb]	1.16	2.33[a]	2.33[d]	1.29
LSD	0.42	8.74	9.78	5.37	1.59	0.21	2.14	0.52	0.53	0.27
CV	7.10	8.81	70.46	51.78	20.16	75.97	153.64	15.75	11.68	9.17
P value	>0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	>0.001	>0.001	<0.001
Non-Stress										
BG-48	37.00[d]	108.75	26.67	4.31[c]	14.56	1.15	16.30	1.17	1.42	1.62
BG-58	34.67	67.26	32.67	2.47[d]	8.06[d]	1.24	10.26	1.56[c]	1.65	1.76[d]
BG-27	37.00[d]	167.43[b]	25.67[d]	6.69[b]	14.89	1.51	22.45	1.94	2.07[e]	2.39
BG-31	27.00	164.17	25.00[d]	6.58[b]	6.76	1.29	8.51[c]	1.56[c]	1.60	1.94
BG-52	37.00[d]	167.69[b]	14.00	11.65	8.71	0.82	6.93	1.44	1.51	1.68
BG-67	37.67[e]	54.29	25.67[d]	2.54[d]	3.51[c]	1.42	5.34	1.01	1.14	1.55[c]
BG-70	37.67[e]	101.51	17.33[c]	5.88[ae]	12.29	0.48	6.09	1.72	1.94	2.15

BG-78	36.67^{e}	85.26^{a}	22.33^{e}	4.05^{c}	7.08	1.57	11.20	1.49	2.06^{e}	2.58
BG-79	36.33	62.80	23.33^{e}	3.05	16.51^{e}	0.78	12.68	1.61	2.21	2.36
BG-80	37.67^{e}	100.55	16.67	6.02	8.06^{d}	1.30	10.44	1.59^{c}	1.85	1.94
BG-81	37.00^{d}	189.12	36.67	7.80	16.51^{e}	0.51	8.11^{c}	2.29	2.09	2.42
GC	36.67^{e}	169.50	14.00	11.69	8.06^{d}	0.58	4.89	1.71	1.47	1.63
Grand Mean	36.03	119.86	25.83	6.06	10.42	1.05	10.27	1.59	1.75	2.00
LSD	0.45	24.36	0.35	3.41	2.04	0.35	4.39	0.15	0.25	0.32
CV	14.10	11.00	96.56	34.29	11.61	13.52	18.14	4.11	6.28	6.88
P value	>0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: DTFF = the days to first flower; NMF = the number of male flowers; NFF = the number of female flowers; SR = sex ratio; NFPP = the number of fruits per plant; FW = fruit weight; FYPP = fruit yield per plant (kg); CV = coefficient of variation; LSD = least significant difference; Prot-7 = protein concentration at 7-days of drought stress; Prot-14 = protein concentration at 14-days of drought stress; Prot-21 = protein concentration at 21-days of drought stress. Values accompanied by similar superscripts are not significant at a 5% LSD confidence level.

Table 2 Analysis of variance showing mean squares and significant tests for agronomic traits and total protein content evaluated under non-stressed and drought-stressed conditions in all bottle gourd genotypes.

Source of Variation	Df	DTFF	NMF	NFF	SR	NFPP	FW	FYPP	Prot-7	Prot-14	Prot-21
Replicate	2	8.01^{ns}	54.00^{ns}	453.00^{ns}	14.39^{ns}	1.10^{ns}	0.04^{ns}	2.94^{ns}	0.05^{ns}	1.04^{ns}	0.16^{ns}
Gen	11	21.01^{ns}	4059.10**	255.5^{ns}	64.73**	29.53**	0.35**	43.53**	0.17^{ns}	0.38*	0.45^{ns}
Env	17	1073.39**	75855.10**	3669.4**	2.88^{ns}	583.68**	13.43**	1491.22**	6.62**	4.04**	0.16^{ns}
Gen × Env	11	12.18^{ns}	7159.70**	467.4^{ns}	68.42**	50.35**	0.26**	52.63**	0.11^{ns}	0.08^{ns}	0.02^{ns}
Residual	48	37.43	3518.00	344.80	28.15	1.15	0.03	4.93	0.08	0.11	0.28

Note: df, degrees of freedom; * and ** denote significant differences at 5 and 1% probability levels, respectively; ns, not significant; DTFF = days to first flower; NMF = the number of male flowers; NFF = the number of female flowers; SR = sex ratio; NFPP = the number of fruits per plant; FW = fruit weight (kg); FYPP = fruit yield per plant (kg); Prot-7 = protein concentration at 7-days of drought stress; Prot-14 = protein concentration at 14-days of drought stress; Prot-21 = protein concentration at 21-days of drought stress. Gen = genotype and Env = environment.

3.2 Effect of Drought on Total Protein Expressions in Bottle Gourd

The protein patterns across the 12 bottle gourd genotypes at different drought stress intensities were also evaluated. A slight difference in protein patterns was observed across the genotypes subjected to both DS and NS conditions. Findings made in this study suggest differences in the protein concentrations expressed with some commonality at approximately 25, 35, and 45 kDa. Comparison of the DS genotypes with their control samples at different soil moisture levels also indicated that some proteins appeared unique in the genotype exposed to drought stress. According to the results, genotypes BG-48, BG58, BG-52, BG-70, BG-78, and BG-81 expressed unique proteins presumably associated with drought stress tolerance, whereas BG-27, BG-31, BG-67, BG-79, BG-80, and GC under the same DS treatments did not express any unique proteins when compared to the NS control treatments (unpublished data). Protein bands such as 130 kDa and 60 kDa were absent in water-stressed BG-81 plants compared to plants of the same genotype grown under NS conditions. The molecular weights of those uniquely expressed proteins are shown in Table 3 and Figure S1, Figure S2, and Figure S3.

Table 3 Uniquely expressed protein bands found in selected bottle gourd genotypes exposed to 7, 14, and 21 days of drought stress.

Genotypes	Prot-7	Prot-14	Prot-21
BG-70	46 kDa	---	---
BG-58	38 kDa	38 kDa	---
BG-52	38 kDa	38 kDa	---
BG-78	130 kDa	130 kDa	---
BG-81	43 kDa	43 kDa	43 kDa
BG-48	46 kDa	14 kDa	14 kDa
	17 kDa	---	---
	14 kDa	---	---

Note: Prot-7 = protein concentration at 7-days of drought stress; Prot-14 = protein concentration at 14-days of drought stress; and Prot-21 = protein concentration at 21-days of drought stress.

The protein expression induced by DS conditions across the three soil moisture levels was observed in only six of the bottle gourds used (BG-48, BG58, BG-52, BG-70, BG-78, and BG-81) out of the twelve genotypes investigated for drought tolerance. Proteins expressed were of varying sizes that may be linked with the imposed stress. However, all six genotypes expressed the supposed drought stress-linked proteins when water was withheld for 7 days, while genotypes BG-48 and BG-81, as well as BG-48, expressed these proteins when plants were subjected to drought for 14 and 21 days (Table 3), respectively. When protein concentrations (Table 2) were also evaluated in these genotypes, significant stress effects were observed after 14 days of water withdrawal. Although this was expected since drought was involved for all levels, total protein concentrations (Table 1) were relatively higher (>2 mg/ml) after 7 and 14 days of water stress. The results also showed that protein concentration was reduced (≤1 mg/ml) for genotypes BG-48, BG-58, BG-31, BG-52, BG-67, BG-70, BG-80, and GC when water was withdrawn for 21 days. All bottle gourd genotypes recorded a

gradual increase in protein concentration under NS conditions. The genotype BG-78 recorded the highest protein concentration of 3 mg/ml under normal watering conditions.

3.3 Trait Correlations and Statistic Analysis of STI, GMP and PCA

To ascertain the characteristic effect of drought stress on bottle gourd yield and total protein content, recorded data was subjected to linear correlation analysis and the determination of stress tolerance index, geometric mean productivity, and principal component analysis. According to the results, a strong correlation was established between assessed agronomic traits and protein concentrations of plants grown under DS and NS conditions, as proved by the Pearson's correlation coefficients shown in Table 4. Results also showed a weak to moderate correlation between NMF, collectively with NFF (r = 0.3), FW (r = 0.4), FYPP (r = 0.4) and SR (r = 0.5). A moderately high correlation was recorded between NFPP, FW (r = 0.5), and FYPP (r = 0.6). FYPP followed this with NMF (r = 0.4), NFPP (r = 0.6), and FW (r = 0.9) for all water-stressed genotypes. A weak correlation was also observed in DS plant traits between FYPP with DTFF (r = 0.3), NFF (r = 0.1), SR (r = 0.1), and protein concentration analyzed from 7-day (r = 0.2) and 14-day (r = 0.1) water-stressed plants. When agronomic traits were assessed against protein expression, a positive correlation was observed between NMF with Prot-7, NFPP with Prot-14, and FYPP with Prot-21. Similar observations were made in bottle-gourd plants grown under normal water conditions without stress. Agronomic trait and protein content positive correlation was observed for NMF/SR (r = 0.8), FYPP (r = 0.4), NFPP (r = 0.5) and FW (r = 0.6). Weak correlations were also observed between FYPP and DTFF (r = 0.04), NMF (r = 0.03), and then between FYPP from plants treated as control with DS plants subjected to 7-day (r = 0.2) and 14-day (r = 0.1) without irrigation (Table 4). Furthermore, the results in terms of stress tolerance index (STI) and geometric mean productivity (GMP) values presented in Table 5 below showed that the genotypes BG-78, BG-31 and BG-70 had the significant tolerance indicator values. Their STIs were 0.28, 0.19, 0.13 and 0.15 (for the abovementioned bottle gourd genotypes). Meanwhile, GMP recorded for BG-78, BG-27, BG-31, and BG-70 were 5.39, 4.42, 3.63 and 3.99 in the order of the genotypes.

Table 4 Pearson's correlation coefficients (r) describing the association of phenotypic traits and protein concentration of 12 bottle gourd genotypes evaluated under drought-stressed (upper diagonal) and non-stress (lower diagonal) water conditions.

	DTFF	NMF	NFF	SR	NFPP	FW	FYPP	Prot-7	Prot-14	Prot-21
DTFF		-0.03^{ns}	0.21^{ns}	-0.16^{ns}	0.14^{ns}	0.30^{ns}	0.26^{ns}	0.20^{ns}	0.13^{ns}	-0.19^{ns}
NMF	-0.09^{ns}		0.336*	0.495**	0.32^{ns}	0.374*	0.353*	-0.07^{ns}	0.05^{ns}	-0.09^{ns}
NFF	0.01^{ns}	0.11^{ns}		-0.349*	0.439**	0.17^{ns}	0.12^{ns}	0.08^{ns}	0.11^{ns}	0.14^{ns}
SR	0.05^{ns}	0.760**	-0.397*		-0.13^{ns}	0.01^{ns}	0.07^{ns}	0.07^{ns}	0.11^{ns}	0.13^{ns}
NFPP	0.15^{ns}	0.23^{ns}	0.24^{ns}	0.01^{ns}		0.503**	0.636**	0.11^{ns}	-0.07^{ns}	-0.01^{ns}
FW	-0.10^{ns}	-0.30^{ns}	-0.17^{ns}	-0.341*	-0.378*		0.937**	0.26^{ns}	0.12^{ns}	0.02^{ns}
FYPP	0.04^{ns}	0.03^{ns}	-0.06^{ns}	-0.16^{ns}	0.511**	0.552**		0.22^{ns}	0.09^{ns}	-0.06^{ns}
Prot-7	-0.09^{ns}	0.423*	-0.03^{ns}	0.30^{ns}	0.24^{ns}	-0.18^{ns}	0.04^{ns}		0.592**	0.526**
Prot-14	-0.01^{ns}	-0.01^{ns}	-0.15^{ns}	-0.03^{ns}	0.22^{ns}	0.10^{ns}	0.21^{ns}	0.679**		0.31^{ns}
Prot-21	-0.03^{ns}	0.07^{ns}	-0.13^{ns}	0.06^{ns}	0.17^{ns}	0.05^{ns}	0.13^{ns}	0.655**	0.858**	

Note: * and ** denote significant at 5 and 1% probability level of t-values based on a two-tailed test, respectively; ns, non-significant; DTFF = days to first flower; NMF = the number of male flowers; NFF = the number of female flowers; SR = sex ratio; NFPP = the number of fruits per plant; FW = fruit weight (kg); FYPP = fruit yield per plant (kg); Prot-7 = protein concentration at 7-days of drought stress; Prot-14 = protein concentration at 14-days of drought stress; and Prot-21 = protein concentration at 21-days of drought stress.

Table 5 Stress tolerance index (STI) and geometric mean productivity (GMP) used to evaluate drought tolerance in 12 selected bottle gourd genotypes.

Genotype	TSI	GMP
BG-48	0.11	3.45
BG-58	0.07	2.74
BG-27	0.19	4.42
BG-31	0.13	3.63
BG-52	0.06	2.52
BG-67	0.05	2.28
BG-70	0.15	3.99
BG-78	0.28	5.39
BG-79	0.09	3.14
BG-80	0.07	2.76
BG-81	0.05	2.30
GC	0.04	1.99

The rooted component matrix in Table 6 also shows the proportion of total variance that was observed in this study. This variance was explained by different principal components and their correlations with variable agronomic traits. Results revealed four principal components that were found necessary from the DS treatment conditions, contributing 76.61% of the total variation observed in this study. The first two principal components contributed to the highest variation, with a cumulative contribution of 49.04%. FW and FYPP recorded the strongest positive correlation of 0.91 and 0,93 loading into the first principal component. In contrast, protein contents obtained after 7, 14, and 21 days without irrigation gave 0.78, 0.87, and 0.76 loading into the second principal component, respectively. In the third principal component analysis, NMF and SR recorded a high correlation of 0.77 and 0.81. Meanwhile, NFF was also highly correlated at 0.92 in the fourth principal component. Furthermore, the results showed that under NS conditions, five principal components were important, contributing to 88.54% of the total variation observed in this study. The first two principal components were the most influential, with a cumulative contribution to the total variation of 49.23%. The 7, 14, and 21-day drought-stressed plants recorded positive correlations of 0.88, 0.79, and 0.80 loading into the first principal component, respectively (Table 6).

Table 6 Rotated component matrix of different phenotypic traits and protein concentration of 12 bottle gourd genotypes evaluated across drought-stressed and non-stressed conditions.

Agronomic Traits	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4	PC5
DTFF	0.53	0.07	-0.49	-0.03	-0.03	-0.03	0.27	0.07	0.88
NMF	0.34	-0.08	0.77	0.32	0.51	-0.64	0.09	0.35	-0.29
NFF	0.08	0.11	-0.07	0.92	-0.14	-0.05	0.70	-0.41	-0.36
SR	0.05	0.15	0.81	-0.42	0.43	-0.71	-0.31	0.41	0.09
NFPP	0.58	-0.06	0.09	0.59	0.45	0.01	0.79	0.18	0.08
FW	0.91	0.11	0.11	0.12	-0.20	0.73	-0.30	0.41	-0.18
FYPP	0.93	0.05	0.14	0.10	0.21	0.57	0.37	0.69	-0.09
7-day drought	0.11	0.78	-0.01	-0.06	0.88	-0.02	-0.07	-0.18	-0.11
14-day drought	0.24	0.87	-0.10	-0.02	0.79	0.47	-0.13	-0.23	0.09
21-day drought	-0.22	0.76	0.15	0.19	0.80	0.37	-0.17	-0.26	0.07
Explained Variance (Eigenvalue)	2.96	1.94	1.58	1.17	2.78	2.14	1.57	1.29	1.07
Proportion of Total Variance (%)	29.65	19.39	15.83	11.34	27.83	21.40	15.70	12.92	10.69
Cumulative Variance (%)	29.65	49.04	64.88	76.61	27.83	49.23	64.93	77.85	88.54

Note: PC1-PC4 = Four principal components of drought-stressed bottle gourd genotypes. PC1-PC5 = Five principal components in bottle gourd genotypes grown under non-stressed water conditions.

4. Discussion

Drought stress reduces the ability of plants to live, grow, and produce satisfactory yields. This phenomenon negatively affects a series of physiological and biochemical processes that, when triggered, result in abnormal plant functioning, impeding growth, reproduction, and productivity. The changes caused by drought in plants may also result in plant death. However, the supreme goal in crop improvement is to confer tolerance to stress, adaptability, and resistance to this and other kinds of abiotic and biotic stress constraints [24]. Achieving drought tolerance in crops, including bottle gourds, requires characterization and understanding of the biochemical responses of the existing germplasms to drought stress. This can be achieved through phenotypic and proteomic change evaluations since these biological processes provide insights into the plant's interaction with stress. In line with the above sentiments, the differences observed in agronomic traits and protein content of bottle gourd plants subjected to DS and NS water conditions in this study revealed that the genotypes were highly variable and could serve as a rich source of genetic materials to study the diversity required for drought tolerance in bottle gourd breeding (Table 1 and Table 2). Further, the results obtained in this study on the responses of the 12 genotypes tested against imposed water conditions could be used to identify the germplasm's potential for a high tolerance to drought stress. Moreover, crop yield is an important agro-economic trait easily influenced by several yield components, genes responsible for growth, stress response, yield, and the environment [25, 26]. According to the results of this study, BG-70 and BG-78 genotypes showed more tolerance to drought as identified based on their yield potential under both DS and NS conditions. Bottle gourd genotype BG-70 had a relatively high fruit yield per plant despite being exposed to the stress, and this was also observed under control conditions, considered a drought-tolerant genotype (Table 2). Its tolerance to both conditions suggests that this genotype could be best suited for cultivation under low and high rainy conditions. The performance of the genotype BG-70 was also per the only early report by Blum [27], wherein the plants produced higher yields with or without environmental constraints. On the other hand, genotype BG-78 also presented similar results, which is not ideal for production only in dry conditions, as it produced a high yield under NS conditions (Table 2). It should be noted that, in the current study, a drought escape mechanism was used by the genotypes BG-58, BG-27, BG-31, and BG-81. These genotypes used early flowering to avoid severe impact from exposure to drought stress (Table 2). BG-31 showed one of the highest fruit yields per plant, indicating that the drought escape mechanism effectively reduced the impact of drought. The stress tolerance index (STI) and geometric mean productivity (GMP), defined as an advanced index to identify suitable genotypes with higher yields under contrasting environments [28, 29], were also studied. Observations provided the basis through which trait correlations × genotype × environmental factors determined bottle gourds such as BG-70 and BG-78 as important selections of germplasm resources required for breeding purposes. Accordingly, BG-78, BG-27, BG-31, and BG-70 had the highest values for STI and GMP, as shown in Table 5, indicating their high drought tolerance levels and yield potential compared to the other studied genotypes. Similarly, Eid and Sabry reported that these drought tolerance indices guided the selection of wheat genotypes for improved yields under drought-stress environments [29]. Moderate to highly significant and positive correlations were recorded between FYPP, NMF, NFPP, and FW. In addition, positive correlations were recorded between FYPP, DTFF, NFF, and SR (Table 4). These correlations suggest the direct contribution of these yield components to overall yield and should be considered

important target traits during selection. Correlation analyses aiding in the simultaneous selection of multiple traits for improving yield have been observed in other previous studies of bottle gourd [30, 31] and cucumber [32]. The observed positive correlation between the different agronomic traits, especially fruit yield per plant and the protein concentration, provides evidence of a direct relationship and dependence between the two agronomic traits. Furthermore, apart from the agronomic traits, protein accumulation can also be used as a selection marker for drought tolerance. The correlation between fruit yield per plant and protein accumulation, especially after 7 and 14 days of drought stress (Table 4), indicates a robust protein synthesis related stress response, activated in the early stages of drought and growth compared to the NS plants. This observation was in line with previous reports by Munne-Bosch and Alegre [33], Li et al. [34], and Luo et al. [35], which indicated that not all growth stages are equally sensitive to drought as the seedling establishment phase proved to be the most critically affected stage. However, at the late development stage, the plant becomes less sensitive to drought stress [36]. Many proteins induced in the early stage of drought stress are involved in root morphogenesis and carbon/nitrogen metabolism that contribute to drought avoidance via the enhancement of root growth [16]. At the later stages of growth, lignin synthesis-related proteins, and molecular chaperones serve as important enhancers of physical desiccation and keepers of protein integrity expressed to improve drought tolerance [16]. Fruit weight and yield per plant recorded the highest positive loading into the first principal component under drought stress conditions. Meanwhile, 7-day, 14-day, and 21-day droughts had high positive loading into the first and second principal components under drought and non-stress conditions, respectively (Table 6). Generally, characters with high values in PC1 hold the highest variation, and the importance of selecting genotypes based on such characters is further emphasized. Therefore, the high values observed in PC1 for yield and fruit weight suggest their importance for selection in drought-stressed environments. Also, selecting fruit weight as an important yield component would benefit simultaneous selection for complementary genes adding up to yield. Under non-stressed conditions, the protein concentration proved to be important for selection as this had any variations required for crop improvement and may result in the increased survival rate of the bottle gourd plants. Lastly, protein levels could be genotype-specific in the current study, especially when subjected to drought compared to non-stressed plants. This indicates that these proteins might be expressed and synthesized to protect and prevent cell damage from drought stress. The fact that some of the genotypes did not express high levels of proteins compared to the non-stressed plants indicates that drought tolerance may be genotype-specific in bottle gourd. The results showed that the identified drought-tolerant genotypes were BG-48, BG-58, BG-52, BG-70, BG-78, and BG-81 (Table 5). The genotype-specific response to drought was also reported in muskmelon and some unique proteins that were synthesized by the tolerant genotypes were identified as those involved in polypeptide synthesis, photosynthesis, nucleotide biosynthesis, stress response, transcription regulation, metabolism, energy, and DNA binding [10, 18, 24]. In the current study, based on the molecular weight of the protein band at 43 kDa (Table 3), rubisco activase could be known to have a molecular weight of approximately 42 to 45 kDa. This enzyme is found in the chloroplast and is responsible for regulating the activity of ribulose 1,5-bisphosphate (RuBP) during photosynthesis [36]. The expression of such proteins is important to maintain photosynthesis during a drought-stress environment. The other drought-induced proteins identified in the present study are believed to be dehydrins, known to have molecular sizes ranging from 9-2000 kDa. These proteins stabilize membranes, enzymes, and nucleotides in cells under drought-

stress environments [19]. In addition, the current study identified proteins running at 14 and 17 kDa. These could be heat-shock proteins known to have a molecular weight range of 15 to 30 KDa. The heat-shock proteins are important in refolding misfolded proteins, thereby contributing to maintaining cellular homeostasis, especially during drought-stress environments [37-39]. Contrary to the expressed proteins, in the current study, the synthesis of some proteins was inhibited in drought-stressed plants such as BG-81 (Figure S3), perhaps to prevent any further catabolic reactions in the cells, which is important to improve adaptability under drought stress environments [18, 40]. Overall, these study findings also revealed some limitations, and we make the following recommendations. The proteomic analysis conducted in the present study importantly identified the different levels of proteins by comparing them to the molecular sizes from the previous studies that evaluated the effect of drought stress in plant proteomics. However, it was noted that the extent to which this approach was used became limited and provided scarce information on the uniquely expressed proteins due to drought stress.

5. Conclusions

The present study evaluated the level of drought stress tolerance among bottle gourd genotypes using phenotypic and proteomic analyses. In addition, correlations between protein content and agronomic traits under drought-stressed conditions were established. Drought reduced yield levels. However, BG-70 performed better in drought-stressed and non-stressed conditions. In addition, BG-70 and BG-78 showed the best performance for yield per plant under a drought environment. These genotypes are ideal to cultivate in water-stressed environments. Fruit yield per plant positively correlated with protein concentration as an indication of increased accumulation of proteins in response to drought. These results thus imply that protein concentration can ultimately be considered a tool for effectively selecting germplasm for breeding purposes. However, protein-based selection may be limited, mainly focusing on protein concentration. Further studies are required to quantify the threshold concentration linked to drought or non-stressed conditions. Based on the expression of unique proteins linked to drought stress tolerance, BG-48, BG-58, BG-52, BG-70, BG-78, and BG-81 were identified as promising genotypes for drought tolerance in bottle gourd.

Author Contributions

P. Mangena was responsible for writing and editing, organizing data and completing the manuscript. P. Mkhize was responsible for providing data, funding for the project; conceptualization, analyzing the data, and contributed to editing. All authors have read and agreed to the published version of the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Figure S1: Leaf proteomic profiles for BG-48, BG-58, BG-27 and BG-31 bottle gourd genotypes subjected to different intensities of drought stress. Each gel represents different genotype as per the labelling. In each gel lane 1 = MWM. Lane 2 = As (DS sample at 7-day drought stress), Lane 3 = Ac (NS sample), Lane 4 = Bs (DS sample at 14-day drought stress), Lane 5 = Bc (NS sample), Lane 6 = Cs (DS sample at 21-day drought stress), Lane 7 = Cc (NS sample).
2. Figure S2: Leaf proteomic profiles for BG-52, BG-67, BG-70 and BG-78 bottle gourd genotypes subjected to different intensities of drought stress. Each gel represents different genotype as per the labelling. In each gel lane 1 = MWM. Lane 2 = As (DS sample at 7-day drought stress), Lane 3 = Ac (NS sample), Lane 4 = Bs (DS sample at 14-day drought stress), Lane 5 = Bc (NS sample), Lane 6 = Cs (DS sample at 21-day drought stress), Lane 7 = Cc (NS sample).
3. Figure S3: Leaf proteomic profiles for BG-79, BG-80, BG-81 and GC bottle gourd genotypes subjected to different intensities of drought stress. Each gel represents different genotype as per the labelling. In each gel lane 1 = MWM. Lane 2 = As (DS sample at 7-day drought stress), Lane 3 = Ac (NS sample), Lane 4 = Bs (DS sample at 14-day drought stress), Lane 5 = Bc (NS sample), Lane 6 = Cs (DS sample at 21-day drought stress), Lane 7 = Cc (NS sample).

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