




Impact of soil moisture stress on growth and physiological traits of tepary bean genotypes



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Background: In South Africa, tepary bean is cultivated by smallholder growers, mainly in the Sekhukhune District (Limpopo province), which is prone to drought. Currently, there are no significant breeding efforts aimed at cultivar selection and crop development, and the crop remains underutilised despite its potential value.

Aim: To evaluate diverse tepary bean collections using physiological and growth attributes.

Setting: Agricultural Research Council – Vegetable, Industrial and Medicinal Plants, South Africa in drought screening glasshouse.

Methods: A 6 × 7 rectangular lattice experimental design was replicated three times.

Results: Results showed that there were significant ($p < 0.05$) differences among the tepary bean collections tested for all the selected traits that were measured. The highest (1.05 $\mu\text{mol/g}$ dry weight) and lowest (0.32 $\mu\text{mol/g}$ dry weight) leaf proline contents were observed for genotypes 'Ac-35' and 'Ac-9', respectively. The genotype 'Ac-33' achieved almost twofold higher relative water content (84.72%) than the rest of the accessions tested.

Conclusion: The current study was conducted in a greenhouse as a rapid method to determine the differences in response to drought among several tepary bean genotypes. The genotypes showed a wide range of variability for all the trait attributes that were considered before and after soil moisture stress. The principal component analysis revealed three distinct genotypes ('Ac-6', 'Ac-13' and 'Ac-31') under the moisture stress regime that can be considered for further investigation, particularly under field conditions to determine their grain yield potential. There would be merit in conducting further studies to determine the yield potential of the genotypes selected in this study in multiple field-testing locations.

Contribution: There would be merit in conducting further studies to determine the yield potential of the genotypes selected in this study in multiple field-testing locations.

Keywords: physiological attributes; correlation; soil moisture stress; germplasm; phenotypic variability; trait.

Introduction

Tepary bean (*Phaseolus acutifolius*) is a self-pollinating diploid ($2n = 2x = 22$) legume, which originated from the arid and semi-arid region of northwestern Mexico and southwestern United States (Nabhan & Felger 1978). It then spread to many African countries, including Botswana, Kenya, Malawi, South Africa and Zimbabwe, where smallholder farmers use unimproved landraces of the crop (Molosiwa et al. 2014; Thangwana, Gwata & Zhou 2021). It is a summer annual crop and possesses unique genetic attributes such as tolerance to drought and heat, making it suitable for cultivation in arid and semi-arid environments (Baath et al. 2020). Tepary bean is traditionally grown for dry seed production. It is sometimes consumed as sprouts or green beans and the leaves are also consumed while haulms are used for animal feed (Bhardwaj 2013; Porch et al. 2017; Small 2014).

The grain of tepary bean provides affordable sources of protein for human consumption and is valuable for income generation, particularly in the smallholder cropping systems in southern Africa (Gwata, Shimelis & Matova 2016). It was reported that the seed contains varying concentration of protein, calcium, iron, copper, zinc as well as considerable amounts of oil and fatty acids (Bhardwaj & Hamama 2004, 2005; Heredia-Rodriguez et al. 2019; Mapp et al. 2016). Tepary bean also fixes atmospheric nitrogen, thus contributing to the improvement of soil fertility (Mapp et al. 2016; Mohrmann et al. 2017; Shisanya 2004) and soil structure. Because of its drought tolerance gene and low input requirements, and resistance to biotic

and abiotic stresses, tepary bean is suitable for cultivation by resource-poor farmers, particularly in southern Africa (Gwata et al. 2016; Jiri, Mafongoya & Chivenge 2017) at marginal areas. However, despite its valuable potential, tepary bean has generally received limited research attention towards the influence of soil moisture stress on vegetative growth for early identification of potential drought-tolerant genes and cultivar development (Abid et al. 2016). Consequently, a limited number of improved cultivars have been released for cultivation (Porch et al. 2013). Although tepary bean grows well in harsh arid conditions, its productivity can vary among genotypes, environments and management practices (Ghadimian et al. 2021). This variation suggests that superior and high-yielding genotypes can be selected for breeding purposes (Mohamed, Mohamed & Schmitz-Eiberger 2005; Muñoz 2021). Moreover, the grain yield potential is negatively affected under soil moisture stress growing conditions (Buitrago-Bitar et al. 2021; Suárez et al. 2022). Hence, characterisation and selection of tepary bean genetic resources for drought tolerance at early juvenile stages using growth and physiological traits are important for the adaptation of the crop. The current climate change has increased the frequency of extreme drought conditions, resulting in significant reductions in crop production as well as productivity, thus threatening food and nutritional security (Lesk, Rowhani & Ramankutty 2016). Currently, limited water is a major constraint in grain legume production in many African countries. However, leguminous plants such as tepary bean utilise a variety of mechanisms to cope with soil moisture stress (Araújo, Beebe & Crespi 2015; Hayat et al. 2012; Turner, Wright & Siddique 2001; Ye et al. 2018). In previous studies, an improvement in the ability to achieve increased water capture and use efficiency in tepary bean was attributed to the development of better root systems (Butare et al. 2011) as well as the ability to mobilise photosynthates from stems and leaves for grain development (Rao et al. 2013). Under receding soil moisture conditions, some field legumes can maintain turgor as well as metabolism by synthesising osmolytes or compatible solutes, including ureides (Sinclair & Serraj 1995) and leaf proline (Kishor et al. 2005; Solanki & Sarangi 2014). In addition, their adaptive mechanisms can include the adjustment of membrane composition, protein–protein and protein–lipid interactions (Chaves, Maroco & Pereira 2003). Variation in leaf proline accumulation during soil moisture stress conditions was reported in several legumes, including chickpea (Mafakheri et al. 2010), cowpea (Nkoana, Gerrano & Gwata 2019a), peanut (Solanki & Sarangi 2014), soybean (Akitha & Giridhar 2015) and tepary bean (Türkan et al. 2005). Therefore, this study was designed to examine variability in growth and leaf physiological parameters among tepary bean genotypes that were subjected to soil moisture stress conditions at early growth stage. The identification of superior germplasm of tepary bean, which can tolerate soil moisture stress, will enhance the genetic improvement of the crop in future breeding programmes aimed at developing new cultivars.

Materials and methods

Genetic materials

There were 42 tepary bean genotypes, including two checks, used in the study (Table 1), which were obtained from

TABLE 1: A summary of some descriptors of 42 tepary bean genotypes that were used in the study.

Genotype		Seed	Testa color	Useful notes
Designation	Code			
Ac-1	1	Medium	Cream	Normal flowering, white flowers, climbing, medium pods
Ac-2	2	Large	Cream	White flowers, climbing, long pods
Ac-3	3	Medium	White	White flowers, climbing, long pods
Ac-4	4	Medium	White	Late flowering, white flowers, climbing, long pods
Ac-5	5	Small	Black	Purple flowers, climbing, long pods
Ac-6	6	Medium	White	Early flowering, white flowers, semi-erect, small pods
Ac-7	7	Small	White	Early flowering, white flowers, semi-erect, medium pods
Ac-8	8	Medium	Black	Early flowering, purple flowers, semi-erect, long pods
Ac-9	9	Small	Brown	White flowers, climbing, medium pods
Ac-10	10	Small	Cream	White flowers, climbing, medium pods
Ac-11	13	Medium	White	White flowers, climbing, medium pods
Ac-12	14	Small	White	Early flowering, white flowers, climbing, medium pods
Ac-13	15	Small	White	White flowers, climbing, long pods
Ac-14	16	Small	White	Late flowering, white flowers, semi-erect, medium pods
Ac-15	17	Medium	Speckled	Early flowering, purple flowers, erect, small pods
Ac-16	18	Medium	White	White flowers, climbing, medium pods
Ac-17	19	Medium	White	White flowers, climbing, long pods
Ac-18	20	Small	White	White flowers, climbing, medium pods
Ac-19	21	Large	White	White flowers, semi-erect, long pods
Ac-20	22	Medium	White	Late flowering, white flowers, climbing, long pods
Ac-21	23	Medium	White	White flowers, semi-erect, long pods
Ac-22	24	Small	White	White flowers, semi-erect, long pods
Ac-23	25	Small	White	White flowers, climbing, medium pods
Ac-24	29	Small	White	White flowers, semi-erect, small pods
Ac-25	31	Small	Cream	White flowers, climbing, long pods
Ac-26	32	Small	White	White flowers, climbing, long pods
Ac-27	33	Small	White	White flowers, climbing, medium pods
Ac-28	34	Medium	Cream	Late flowering, white flowers, semi-erect, medium pods
Ac-29	35	Small	Cream	Late flowering, purple flowers, semi-erect, medium pods
Ac-30	36	Medium	Brown	White flowers, semi-erect, medium pods
Ac-31	37	Medium	Speckled	Purple flowers, climbing, long pods
Ac-32	38	Large	White	White flowers, climbing, medium pods
Ac-33	39	Small	White	Late flowering, white flowers, climbing, medium pods
Ac-34 (check)	40	Small	White	Late flowering, white flowers, climbing, long pods
Ac-35	41	Small	White	White flowers, climbing, long pods
Ac-36	42	Medium	White	Early flowering, white flowers, climbing, medium pods
Ac-37	43	Medium	White	White flowers, climbing, medium pods
Ac-38	45	Small	White	Late flowering, purple flowers, semi-erect, medium pods
Ac-39	46	Small	Cream	Early flowering, white flowers, semi-erect, long pods
Ac-40	47	Medium	White	White flowers, semi-erect, long pods
Ac-41	49	Large	White	White flowers, semi-erect, long pods
Ac-42	50	Small	White	Early flowering, white flowers, climbing, long pods

Mexico. The genotypes have diversified range of seed colour types such as black, brown, speckled, white and cream types. In addition, the genotypes included both early- and late-maturity groups. The seed size varied from small (100-seed weight \leq 13.0 g) to large (100-seed weight \geq 17.0 g).

Testing location, trial establishment and measurement of parameters

The study was conducted at the Agricultural Research Council – Vegetable, Industrial and Medicinal Plants research station (25.60°S; 28.35°E), Pretoria, South Africa, in the 2019/2020 crop season. In each row for each replication, five seeds per genotype were planted in a 155.0 cm \times 77.0 cm \times 23.0 cm plastic box that was placed on a metal table (65.0 cm high) in a greenhouse and filled with a mixture of red top field soil and vermiculite (1:1). The soil mixture was irrigated to field capacity after which the excess water could drain before planting (Nkoana et al. 2019a). The seeds were planted at a depth of about 3.0 cm at a spacing of 15.0 cm between rows and 10.0 cm within rows. The greenhouse temperatures were maintained at 28°C during the day and 15°C during the night with the average relative humidity ranging from 40% to 50%.

At 5 weeks after germination, prior to soil moisture stress, the following growth and physiological traits that are associated with response to soil moisture deficit (Baroowa & Gogoi 2013; Lazcano-Ferrat Lovatt 1999; Singh & Reddy 2011) were measured:

- Stem diameter (SD) (cm)
- Shoot height (SH) (cm)
- Total leaf chlorophyll content (LCC)
- Leaf proline content (LPC) ($\mu\text{mol/g}$ dry weight)
- Relative water content (RWC) (%)

The SD was measured at the base of the plant and the SH was measured from the base to the shoot apex. Leaf chlorophyll content was measured using a chlorophyll meter (Minolta Chlorophyll Meter Spad-502, Minolta Co., Ltd., Tokyo, Japan). The meter quantitatively records numerical leaf colour units ranging from high (green) to low (yellow) and determines the transmittance of light through the sample leaf at two wavelengths (650 nm and 920 nm), after which the instrument automatically calculates a numerical value, which is linearly related to the LCC (Gwata et al. 2004). For determining the RWC, two fully expanded and mature leaf samples per genotype were detached to measure the leaf fresh, turgid and dry weights, after which the RWC was determined as follows:

$$\text{RWC} = (\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight}).$$

To determine the turgid weight, the leaves were submerged in distilled water in the dark for 24 h (Sade, Galkin & Moshelion 2015; Singh & Reddy 2011). The dry weights of the samples were obtained following oven drying (at 60°C to constant weight for 3 days).

Leaf sampling and determination of leaf proline content

Fully expanded trifoliolate leaves were sampled for proline analysis (Singh & Reddy 2011). For the extraction of proline, at least two leaves from each replication were collected in the afternoon and freeze-dried. The 0.5 g dry weight of the bulked powder leaves was homogenised in 10.0 mL of 3% aqueous sulfosalicylic acid. This was followed by filtration and reaction with 2.0 mL of acid ninhydrin and 2.0 mL of glacial acetic acid in a test tube for 1 h at 100°C. The reaction was subsequently terminated in an ice bath followed by the extraction of the reaction mixture with 4 mL toluene and mixing vigorously with a test tube stirrer for 20 seconds (Bates, Waldren & Teare 1975; Nkoana et al. 2019a). The chromophore containing toluene was then aspirated from the aqueous phase and warmed to room temperature where the absorbance was read at 520.0 nm using toluene as a blank. The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:

$$\mu\text{moles proline/g of fresh leaves} = (\mu\text{g proline/mL} \times \text{mL toluene} / 115.55 \mu\text{g}/\mu\text{mole}) / (\text{g sample}) / 5.$$

Experimental design and statistical analysis

The study utilised a 7 \times 6 rectangular lattice design replicated three times, and the data sets were subjected to analysis of variance using the Statistical Analysis Software (SAS) program, version 9.3 (SAS 2000), followed by mean separation using Fisher's least significant difference (LSD) test. The correlations between the phenotypic traits were determined using Pearson's correlation method (Boslaugh & Watters 2008). The Student *t*-test (for independent samples) was applied to determine the statistical significance of the difference between the LPC measurements before and after the soil water stress treatment. The principal component analysis (PCA) (based on the correlation matrix) was performed using the Statistical Package for the Social Sciences (SPSS) version 23 (Ringnér 2008; SPSS 2012). A principal component biplot was constructed to optimally visualise the graphic relationships between the genotypes and the five traits (Yan & Kang 2003).

Ethical considerations

Ethical clearance to conduct this study was obtained from the University of Venda, Faculty of Science, Engineering and Agriculture Research Ethics Committee (No. FSEA/21/PSSC/02/1707).

Results

Genotypic performance before soil moisture stress

The results showed that prior to soil moisture stress, there were significant ($p < 0.05$) differences among the 42 genotypes for all the growth and physiological parameters that were measured (Table 2). The highest (1.05 $\mu\text{mol/g}$ dry weight) and lowest (0.32 $\mu\text{mol/g}$ dry weight) LPC were

observed for genotypes 'Ac-35' and 'Ac-9', respectively. The trial mean for proline was 0.69 $\mu\text{mol/g}$ dry weight. However, genotype 'Ac-42' attained the highest (27.85) LCC, which was 48.94% higher than the check genotype ('Ac-34'). The genotype 'Ac-33' achieved almost twofold higher RWC (84.72%) than genotype 'Ac-11', which recorded the lowest (43.12%) RWC (Table 2). The trial mean for RWC was 69.66%. The genotypes also showed significant ($p < 0.05$) variability in SD, which ranged from 2.47 cm (for genotype 'Ac-27') to 1.79 cm (for genotype 'Ac-3'). At least four genotypes ('Ac-6', 'Ac-7', 'Ac-22' and 'Ac-28') attained

TABLE 2: Variability in growth and physiological parameters among 42 tepary bean accessions before water stress.

Designation	Genotype Code	LPC ($\mu\text{mol/g}$ dry weight)	LCC	RWC (%)	SD (cm)	SH (cm)
Ac-35	41	1.05	26.11	73.47	2.27	18.99
Ac-28	34	1.00	17.91	69.38	2.02	48.60
Ac-6	6	0.99	17.08	70.01	1.92	57.77
Ac-7	7	0.90	23.68	47.62	2.11	46.90
Ac-18	20	0.90	24.09	69.56	2.06	21.21
Ac-14	16	0.88	24.98	78.17	1.91	34.79
Ac-36	42	0.88	26.73	83.33	1.83	25.00
Ac-37	43	0.85	22.99	73.16	1.88	24.03
Ac-41	49	0.84	20.67	65.46	2.08	29.56
Ac-19	21	0.84	22.81	60.32	2.20	16.22
Ac-4	4	0.83	25.32	65.64	1.94	26.69
Ac-15	17	0.83	21.85	78.42	2.36	19.22
Ac-31	37	0.81	19.37	81.22	2.46	39.97
Ac-10	10	0.78	25.14	69.31	2.29	32.93
Ac-32	38	0.78	17.70	80.71	1.97	19.61
Ac-5	5	0.76	21.91	68.89	2.10	34.78
Ac-22	24	0.71	21.10	60.35	1.96	50.48
Ac-39	46	0.70	23.77	76.04	2.17	19.73
Ac-30	36	0.69	18.29	79.23	2.16	19.88
Ac-34 (check)	40	0.68	14.22	76.40	2.21	31.17
Ac-21	47	0.65	20.98	71.59	2.02	19.25
Ac-1	23	0.65	21.55	72.87	2.24	30.67
Ac-3	1	0.64	15.92	53.66	1.79	15.52
Ac-33	3	0.64	17.41	84.72	2.28	24.96
Ac-26	39	0.63	17.99	73.59	1.97	33.11
Ac-20	32	0.63	18.76	76.78	2.18	28.23
Ac-27	22	0.63	20.23	73.31	2.47	34.69
Ac-11	33	0.63	19.73	43.12	1.91	2.10
Ac-2	13	0.62	21.26	63.66	2.39	41.67
Ac-29	2	0.61	16.12	57.72	1.99	17.44
Ac-16	35	0.59	21.72	66.94	2.31	33.97
Ac-42	18	0.57	27.85	73.31	2.13	26.83
Ac-17	50	0.57	22.60	65.80	2.17	17.34
Ac-38	19	0.56	18.67	75.53	1.85	21.26
Ac-40	45	0.55	17.96	46.66	2.17	32.73
Ac-24	29	0.50	21.00	73.00	2.07	23.84
Ac-12	14	0.48	19.43	80.30	2.08	32.97
Ac-25	31	0.45	21.08	63.26	2.09	29.89
Ac-8	8	0.43	16.50	68.86	2.07	27.70
Ac-23	25	0.41	22.64	76.62	2.25	27.51
Ac-13	15	0.40	25.41	67.57	2.18	17.80
Ac-9	9	0.32	25.96	70.19	2.12	19.33
Grand mean	-	0.69	21.11	69.66	2.11	28.63
Coefficient of variation (%)		36.36	20.20	20.55	14.46	18.84
Least significant difference (5%)		0.61	6.88	23.35	0.49	22.52

LPC, leaf proline content; LCC, leaf chlorophyll content; RWC, relative water content; SD, stem diameter; SH, shoot height.

significantly ($p < 0.05$) greater shoot height (SH) than the trial mean (28.63 cm).

Genotypic performance after soil moisture stress

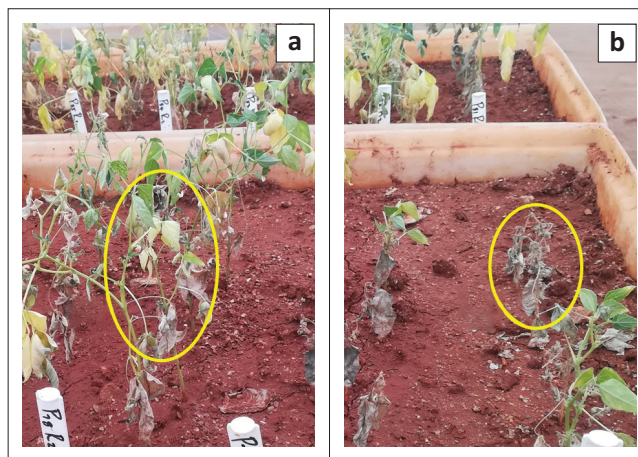
The soil moisture stress that was imposed for 21 days resulted in a clear variability in the degree of wilting among the genotypes with some exhibiting partial or permanent wilting (Figure 1). The results also revealed significant ($p < 0.05$) differences among the 42 genotypes for all the five growth and physiological parameters that were measured after the soil moisture stress treatment (Table 3). The LPC ranged from 1.26 to 0.36 $\mu\text{mol/g}$ that were associated with genotypes 'Ac-35' and 'Ac-9', respectively. The trial mean for the LCC was 8.31. The highest RWC (84.61%) was observed for the genotype 'Ac-18' (Table 3). The widest stems (2.20 cm) were observed for genotype 'Ac-11'. Only three genotypes ('Ac-6', 'Ac-20' and 'Ac-28') attained significantly ($p < 0.05$) greater SH than the trial mean (28.63 cm). However, genotype 'Ac-20' recorded an insignificant LCC because of severe wilting that was induced by the soil moisture stress.

Relationships among the growth and physiological traits

Prior to the soil moisture stress, the LPC showed a positive but not significant ($p < 0.05$) correlation with each of the other remaining attributes (Table 4). However, the LCC showed a highly significant ($p < 0.01$) positive correlation with the RWC but a negative correlation with SH. Similarly, after the soil moisture stress, the LCC maintained a highly significant ($p < 0.01$) positive correlation with the RWC but a negative correlation with the SH (Table 5). However, in both soil moisture conditions, there was no discernible correlation between the SD and the SH.

Change in leaf proline content

An independent samples *t*-test, which was used to determine the significance of the change in LPC, showed that there



Source: Photograph taken by the author R.A. Nong, on 18 November 2019 in Pretoria, South Africa. Used with permission. No unauthorised duplication allowed.

FIGURE 1: Partially wilted (a) and permanently wilted (b) plants of tepary bean after 21 days of soil moisture stress (left) in the greenhouse.

TABLE 3: Variability in growth and physiological parameters among 42 tepary bean accessions after water stress.

Genotype		LPC ($\mu\text{mol/g}$ dry weight)	LCC	RWC (%)	SD (cm)	SH (cm)
Designation	Code					
Ac-35	41	1.26	11.42	62.64	1.76	27.48
Ac-28	34	1.09	11.41	70.47	1.69	47.98
Ac-6	6	1.08	6.40	52.22	1.40	52.59
Ac-7	7	1.08	8.08	55.71	1.75	40.59
Ac-18	20	1.05	13.28	84.61	1.62	45.32
Ac-14	16	1.00	14.29	76.92	1.67	35.91
Ac-36	42	0.99	0.00	53.78	1.52	36.68
Ac-37	43	0.98	13.63	53.78	1.61	34.00
Ac-41	49	0.97	11.68	55.00	1.67	44.36
Ac-19	21	0.95	10.85	78.57	1.85	32.05
Ac-4	4	0.93	10.17	76.19	1.61	39.72
Ac-15	17	0.92	10.85	51.78	2.00	30.00
Ac-31	37	0.92	0.00	0.00	2.13	43.23
Ac-10	10	0.92	11.93	0.00	1.77	33.23
Ac-32	38	0.92	6.97	57.20	1.63	39.85
Ac-5	5	0.92	7.23	52.68	1.77	42.93
Ac-22	24	0.92	6.40	58.33	1.57	43.41
Ac-39	46	0.90	6.10	50.00	1.80	33.48
Ac-30	36	0.90	8.61	78.24	1.80	26.49
Ac-34 (check)	40	0.90	0.00	0.00	1.62	27.90
Ac-21	47	0.89	14.67	26.67	2.00	35.15
Ac-1	23	0.88	9.90	78.57	1.63	36.51
Ac-3	1	0.86	10.67	68.69	1.90	33.70
Ac-33	3	0.86	8.56	69.58	1.25	31.44
Ac-26	39	0.85	4.20	65.00	1.86	38.91
Ac-20	32	0.85	0.00	0.00	1.62	50.02
Ac-27	22	0.83	9.23	75.00	1.66	28.30
Ac-11	33	0.82	17.63	35.71	2.20	41.77
Ac-2	13	0.80	6.42	66.11	1.65	33.02
Ac-29	2	0.79	0.00	0.00	2.04	38.68
Ac-16	35	0.78	6.46	0.00	1.55	18.54
Ac-42	18	0.77	12.63	0.00	1.99	42.24
Ac-17	50	0.73	8.01	66.66	1.73	40.20
Ac-38	19	0.68	7.47	70.45	1.86	27.78
Ac-40	45	0.68	10.28	66.48	1.65	26.41
Ac-24	29	0.66	0.00	0.00	1.75	36.89
Ac-12	14	0.61	0.00	0.00	1.75	40.13
Ac-25	31	0.49	7.20	50.00	1.61	43.14
Ac-8	8	0.48	10.63	45.45	1.73	40.82
Ac-23	25	0.41	0.00	74.64	2.04	39.78
Ac-13	15	0.38	23.33	58.33	1.79	28.77
Ac-9	9	0.36	12.28	66.25	1.75	25.13
Grand mean	-	8.31	8.31	48.85	1.74	36.54
Coefficient of variation (%)		29.70	29.70	23.24	10.72	20.07
Least significant difference (5%)		6.10	9.63	9.63	0.49	10.37

LPC, leaf proline content; LCC, leaf chlorophyll content; RWC, relative water content; SD, stem diameter; SH, shoot height.

was a highly significant ($p < 0.00$) difference between the measurements of this compound before and after soil moisture stress. In general, the soil moisture stress led to a

TABLE 4: Pearson's correlation coefficients for five growth and physiological parameters among 42 tepary bean accessions before water stress.

Parameter	LPC ($\mu\text{mol/g}$ dry weight)	LCC	RWC (%)	SD (cm)	SH (cm)
LPC	1.0000	-	-	-	-
LCC	0.0775	1.0000	-	-	-
RWC	0.1905	0.4006*	1.0000	-	-
SD	0.1939	0.0693	0.2410	1.0000	-
SH	0.2650	-0.1852	-0.0877	0.0000	1.0000

LPC, leaf proline content; LCC, leaf chlorophyll content; RWC, relative water content; SD, stem diameter; SH, shoot height.

*Highly significant at the 1.0% probability level.

TABLE 5: Pearson's correlation coefficients for five growth and physiological parameters among 42 tepary bean accessions after water stress.

Parameter	LPC ($\mu\text{mol/g}$ dry weight)	LCC	RWC (%)	SD (cm)	SH (cm)
LPC	1.0000	-	-	-	-
LCC	0.0173	1.0000	-	-	-
RWC	0.1144	0.4005*	1.0000	-	-
SD	-0.1761	0.0700	-0.2414	1.0000	-
SH	0.1916	-0.1855	-0.0877	0.0000	1.0000

LPC, leaf proline content; LCC, leaf chlorophyll content; RWC, relative water content; SD, stem diameter; SH, shoot height.

*Highly significant at the 1.0% probability level.

variable increment in the LPC among the genotypes (Figure 2). The highest percent change in the LPC, which was observed for genotype 'Ac-21', was significantly ($p < 0.05$) higher than the change that occurred in the check genotype 'Ac-34'. Only one genotype ('Ac-13') showed a negative percent change in LPC after the soil moisture stress treatment.

Principal component analysis

The principal component analysis showed that before the soil moisture stress, the first two principal components accounted for 51.29% of the total variation (Table 6). Two traits, namely, LPC and SH, were highly associated with PC1. However, PC2 was highly associated with LPC and RWC. In contrast, PC3 was dominated by SD. The results also showed that after the soil moisture stress, the first two principal components accounted for 56.70% of the total variation (Table 7). Similarly, two physiological traits, namely LPC and SH, were highly associated with PC1, but LPC and SH were highly associated with PC2.

Principal component biplot

In the biplot analysis, four genotypes ('Ac-16', 'Ac-19', 'Ac-20' and 'Ac-32') were clustered around the origin prior to the moisture stress treatment, while four genotypes ('Ac-3', 'Ac-6', 'Ac-9' and 'Ac-35') were distinct and positioned far away from the origin indicating their peculiar alleles (Figure 3). The genotypes in the right top quadrant (including 'Ac-6', 'Ac-7', 'Ac-14' and 'Ac-28') were associated and characterised by high leaf proline, and long stems, while the genotypes that were found at the top left quadrant were associated together with attributes of RWC, LCC and the thickness (Figure 3). There was no association of recorded traits with the tested genotypes that were positioned to the third and fourth quadrant of the biplot (bottom left and bottom right quadrant), indicating that the

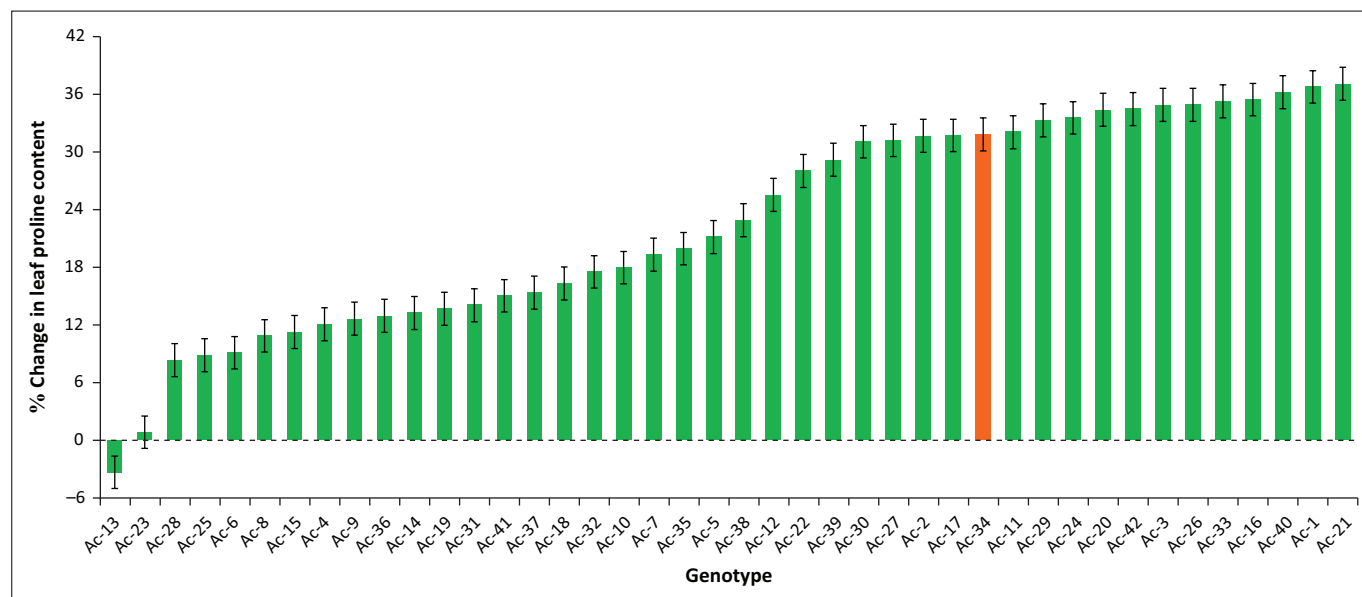


FIGURE 2: Variation in the percent change in leaf proline content among tepary bean genotypes after soil moisture stress (genotype 'Ac-34' = check).

TABLE 6: Principal component analysis showing the eigenvector, eigenvalue and cumulative percentage of the first five principal component axes for five phenotypic traits among tepary bean genotypes before soil moisture stress.

Trait	Eigenvector				
	PC1	PC2	PC3	PC4	PC5
Leaf proline content	0.453	0.614	-0.284	0.188	-0.549
Leaf chlorophyll content	-0.309	0.437	-0.559	-0.534	0.340
Relative water content	-0.427	0.469	0.150	0.683	0.329
Stem diameter	-0.373	0.347	0.639	-0.421	-0.394
Shoot height	0.615	0.303	0.419	-0.188	0.565
Eigenvalue	1.359	1.205	1.062	0.878	0.495
Variability (%)	27.176	24.108	21.251	17.553	9.911
Cumulative (%)	27.176	51.285	72.536	90.088	100.000

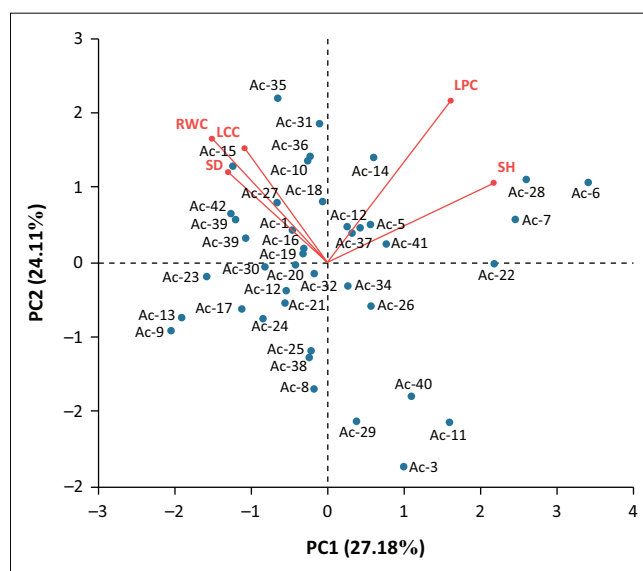
PC, principal component.

TABLE 7: Principal component analysis showing the eigenvector, eigenvalue and cumulative percentage of the first five principal component axes for five phenotypic traits among tepary bean genotypes after soil moisture stress.

Trait	Eigenvector				
	PC1	PC2	PC3	PC4	PC5
Leaf proline content	0.166	0.629	0.295	0.691	0.110
Leaf chlorophyll content	0.591	-0.277	0.439	0.021	-0.617
Relative water content	0.681	0.080	0.042	-0.356	0.634
Stem diameter	-0.299	-0.486	0.686	0.154	0.424
Shoot height	-0.264	0.533	0.498	-0.610	-0.159
Eigenvalue	1.514	1.321	0.950	0.726	0.489
Variability (%)	30.272	26.427	19.009	14.513	9.778
Cumulative (%)	30.272	56.700	75.709	90.222	100.000

PC, principal component.

influence of those traits was minimal (Figure 3). The biplot analysis for the traits that were measured after the soil moisture stress showed that four genotypes ('Ac-2', 'Ac-17', 'Ac-26' and 'Ac-39') were grouped close to the origin, while five genotypes ('Ac-6', 'Ac-13', 'Ac-20', 'Ac-29' and 'Ac-31') were distinct and positioned far away from the origin (Figure 4). The genotypes in the top right quadrant were highly associated both LPC and RWC, but high LCC was associated with the genotypes that were clustered in the right bottom quadrant (Figure 4). The longest shoots were associated with the genotypes that were grouped in



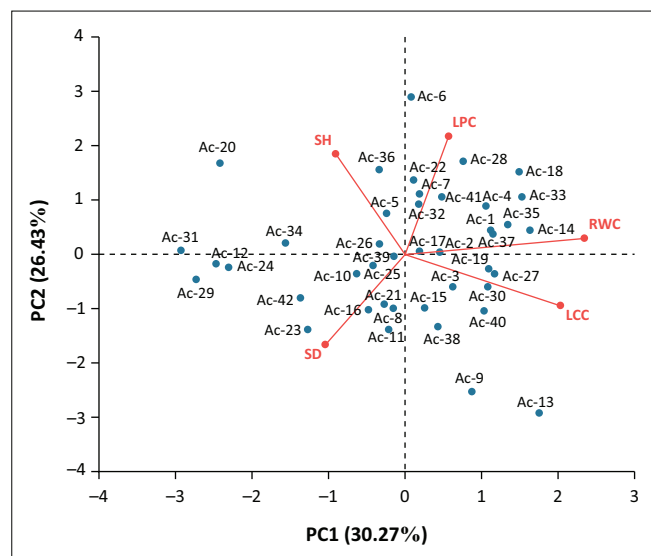
LPC, leaf proline content ($\mu\text{mol/g}$ dry weight); LCC, leaf chlorophyll content; RWC, relative water content (%); SD, stem diameter (cm); SH, shoot height (cm).

FIGURE 3: Principal component score plot of PC1 and PC2 describing the variation among 42 tepary bean genotypes estimated using the data set of growth and physiological parameters before soil moisture stress.

the left top quadrant, while the remainder of the genotypes were characterised by thick stems and grouped in the left bottom quadrant of the biplot (Figure 4).

Clustering pattern of the genotypes

The tepary bean genotypes were grouped into three main clusters (Figure 5). Most of the genotypes (64.28%) were grouped in cluster III, while cluster I consisted of only seven genotypes, including 'Ac-40' (which was associated with high LCC after the soil moisture stress treatment) as well as 'Ac-35' and 'Ac-37' (which were characterised by both LPC and RWC after the soil moisture stress treatment). The check (genotype 'Ac-34') was grouped in cluster III in a subcluster with genotype 'Ac-20' (Figure 5).



LPC, leaf proline content ($\mu\text{mol/g}$ dry weight); LCC, leaf chlorophyll content; RWC, relative water content (%); SD, stem diameter (cm); SH, shoot height (cm).

FIGURE 4: Principal component score plot of PC1 and PC2 describing the variation among 42 tepary bean genotypes estimated using the data set of growth and physiological parameters after soil moisture stress.

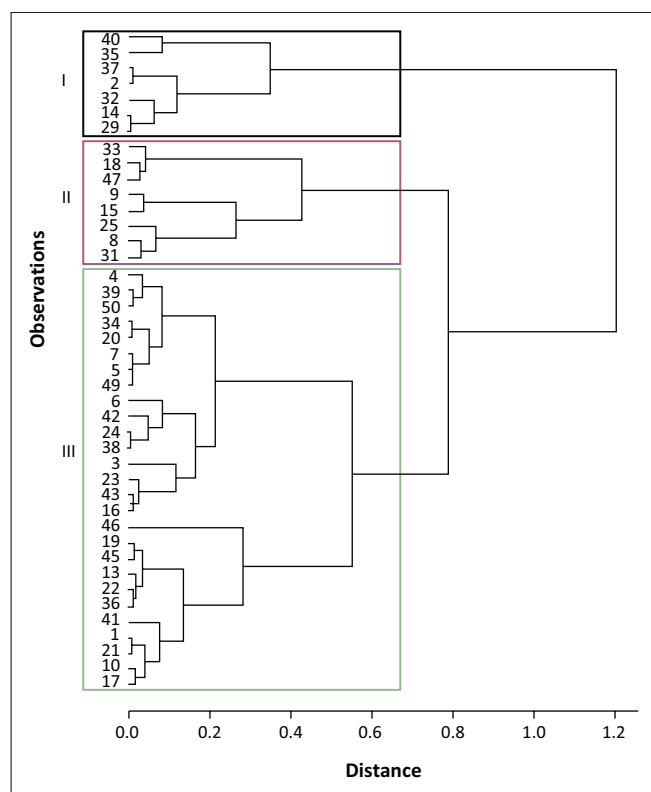


FIGURE 5: Clustering pattern of the 42 tepary bean genotypes.

Discussion

The results showed significant genetic variation in response to soil moisture stress, which suggested the potential for selection of parental lines for improving tepary bean for specific traits. The significant genotypic variability observed in the study indicated that the germplasm contained genotypes with considerable levels of soil moisture stress tolerance that can be exploited in drought tolerance breeding programmes of tepary bean. Leaf proline content is an

important selection criterion in screening genotypes for drought tolerance (Solanki & Sarangi 2014).

The pattern of relationships between genotypes and the growth and physiological attributes was influenced by the prevailing soil moisture status. The genotypes were scattered in the biplot before and after the soil moisture stress was imposed, indicating that the genotypes were genetically diverse for the growth and physiological parameters that were evaluated and could be used for developing new breeding populations. For example, there were some genotypes that were associated with the thickest stems (for instance, 'Ac-8' and 'Ac-11') and high LCC after the soil moisture stress was imposed, indicating that selection for such traits required the stress conditions. This suggested that those genotypes can maintain water in the plant system for the next growth and development stage in drought-prone areas of the country. However, some of the genotypes exhibited relatively high leaf proline in both moisture stress regimes. The observed increase in leaf proline was expected and it agreed with the findings that were reported in previous studies (Masoumi et al. 2011; Solanki & Sarangi 2014). In a similar study aimed at proline evaluation in cowpea, the genotypes showed highly significant variability in LPC after 5 weeks of drought stress ranging from 0.39 $\mu\text{mol/g}$ dry weight to 8.81 μmol dry weight (Nkoana et al. 2019a). The amino acid is associated with plant adaptation to moisture stress conditions, as it protects the plant from adverse environmental stresses (Ashraf and Foolad 2007; Kaur & Asthir 2015). Drought-tolerant genotypes that were associated with elevated leaf proline levels under soil moisture stress were identified in a wide range of field crops, including sunflower (Cechin et al. 2006; Unyayar, Kelep & Unal 2004) and wheat (*Triticum aestivum*) (Vendruscolo et al. 2007). In cowpea (*Vigna unguiculata*), which was exposed to water deficit, the upregulation of the expression of the proline synthesis gene with a concomitant downregulation of the proline catabolism gene was reported (Zegaoui et al. 2017). The metabolic role of proline in plants under drought conditions is well-documented (Ashraf & Foolad 2007; Szabados & Saviouré 2010; Verbruggen & Hermans 2008; Yoshida et al. 1997). Previously, the ability to withstand soil moisture stress was also attributed to profuse branching of the root system in the genus *Phaseolus* (Butare et al. 2011). In this study, the metabolic role of proline likely contributed to the ability of some of the genotypes to withstand soil moisture stress since the space for profuse root branching was limited by the trays that were used. The metabolism of proline apparently enhances cellular signalling processes by increasing the formation of reactive oxygen species in the mitochondria via the electron transport chain, hence promoting cellular survival or apoptosis (Liang et al. 2013). Probably, the metabolic function of total proline together with other inactive metabolites (such as trehalose, or sorbitol or mannitol among others), which were not measured in this study, also contributed to stress tolerance by maintaining membrane integrity or stabilising proteins as well as balancing cellular redox during the soil moisture stress period (Hasanuzzaman et al. 2019). Nonetheless, the variation in root morphological traits could be a useful additional criterion to evaluate.

Although the results of this study showed a marked reduction in leaf chlorophyll because of the soil moisture stress among the tepary bean genotypes, other studies reported no change to chlorophyll under soil moisture stress conditions (Cechin et al. 2006). Probably, this discrepancy could be attributed to the differences in the duration and methodology that was used to induce the soil moisture stress. In this study, the leaves of most genotypes turned yellow (chlorotic), indicating diminished chlorophyll content. Nonetheless, there were some individual plants that remained green, suggesting that the amount of chlorophyll in such plants was not affected by the soil moisture stress during the stress treatment. In addition, it was tempting to conclude that such genotypes were tolerant to drought. However, further validation of the genotypes will be merited before concluding unequivocally that such genotypes tolerated soil moisture stress. Nonetheless, the individual plants that remained green throughout the stress period could be of interest as potential sources of the stay-green genes, which have been reported widely in other legumes such as bean (Bachmann et al. 1994) and soybean (Chang et al. 2019; Luquez & Guiamét 2002) as well as in cereals (Spano et al. 2003; Yoo et al. 2007). Furthermore, there were other attributes, apart from the leaf chlorophyll, that were used to evaluate the response of the genotypes to soil moisture stress, although the results revealed that there was no clear pattern in terms of the impact of the moisture stress on the RWC. In a previous study involving polyethylene glycol to induce drought stress in tepary bean, there was a detectable effect on the RWC (Türkan et al. 2005).

The genotypes that were distinct and found far away from the origin in the principal component biplot analysis indicated that they probably possessed some peculiar genes that can be used in the genetic enhancement of tepary bean. In addition, when evaluated in multiple locations, such genotypes that were located far away from the origin were more responsive to environmental fluctuations and therefore classified as specifically adapted genotypes (Teressa et al. 2021). In similar previous studies that utilised the biplot analysis approach, distinct genotypes were also detected for various crop germplasm, including cowpea (Nkoana et al. 2019a; Nkoana, Gerrano & Gwata 2019b), white bean (Abel, Berhanu & Dagmawit 2019), wheat (Hagos & Abay 2013) and sorghum (Teressa et al. 2021). In addition, the cluster analysis further differentiated the genotypes into three main groups with subgroups, thus providing a better understanding of the differences and similarities that existed between them. The genetic distances between some of the genotypes suggested the presence of genetic diversity in the germplasm, which is valuable in the selection of parental lines for improving tolerance to soil moisture stress in tepary bean.

Conclusion

Based on the results of this study, tepary bean genotypes showed a wide range of variability for all the growth and physiological attributes that were considered before and after soil moisture stress. The PCA revealed three distinct

genotypes ('Ac-6', 'Ac-13' and 'Ac-31') under the moisture stress regime that can be considered for further investigation, particularly under field conditions to determine their grain yield potential. The classification of tepary bean genotypes was not consistent when they were evaluated in the two soil moisture stress regimes, indicating their diversity in performance depending on the soil moisture status. The current study was conducted in a greenhouse as a rapid method to determine the differences in response to drought among several tepary bean genotypes. There will be merit in conducting further studies to determine the yield potential of the genotypes selected in this study in multiple field testing locations.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article. The author, A.S.G., serves as an editorial board member of this journal. The peer review process for this submission was handled independently, and the author had no involvement in the editorial decision-making process for this manuscript. The authors have no other competing interests to declare.

Authors' contributions

R.A.N. contributed towards the conceptualisation, data curation, investigation, methodology, validation, writing of the original draft, review and editing. A.S.G. contributed to the investigation, methodology, data curation, supervision, validation, writing, review and editing. E.T.G. contributed to the conceptualisation, investigation, methodology, data curation, formal analysis, review and editing.

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Data availability

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