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A preliminary evaluation of phenotypic traits of tepary bean (*Phaseolus acutifolius* A. Gray)



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Scan this QR code with your smart phone or mobile device to read online. **Background:** Tepary bean (*Phaseolus acutifolius* A. Gray) is an underutilised grain legume crop and important source of food, nutrition and income. To date, there are no significant breeding efforts aimed at cultivar development and the crop remains under-utilised and under-researched.

Aim: Therefore, the aim of this study was to evaluate eight phenotypic traits and their relationships among 42 genotypes of tepary bean in a controlled drought screening greenhouse environment.

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

Method: A 6 × 7 rectangular lattice design replicated three times was used in the study.

Results: There were highly significant (p < 0.01) differences in all the phenotypic traits that were measured. The highest number (30) of secondary roots was recorded for genotype 'Ac-39', which exceeded the trial, mean value by 62.87%. In comparison with the check, only Ac-33', 'Ac-39', 'Ac-40' and 'Ac-7', 'Ac-8', 'Ac-40', 'Ac-41' genotypes achieved a significantly (p < 0.05) higher secondary root length (SRL) and shoot dry weight (SDW), respectively. A highly significant (p < 0.01) positive association was observed between the shoot fresh weight and the SDW suggesting that there was a strong linear relationship between the two parameters. Similarly, at least 68.0% of the changes in root dry weight were attributed to the changes in the SRL.

Conclusion: These results suggested that the observed phenotypic variability in this germplasm which could be exploited for the enhancement of tepary bean.

Contribution: There will be merit in validating these results on a field basis together with grain yield evaluation and genotyping over multiple locations and seasons to determine elite germplasm for production and utilisation by growers.

Keywords: genetic enhancement; germplasm; phenotypic variability; trait; root.

Introduction

Tepary bean (*Phaseolus acutifolius* A. Gray) (2n = 2x = 22) is a valuable crop for subsistence farmers in Southern Africa. It is a self-pollinating leguminous grain crop that originated from the arid and semi-arid region of north-western Mexico and south western United States (Moghaddam et al. 2021; Nabhan & Felger 1978). The crop is mostly cultivated in Southern Africa, where smallholder farmers use landraces with low yield potential (Gwata, Shimelis & Matova 2016; Thangwana, Gwata & Zhou 2021). Moreover, the farmers cultivate unimproved varieties, which are low yielding and poorly adapted to climate changes especially drought stress (Molosiwa et al. 2014). In addition, there is no documented or registered tepary bean breeding programme in South Africa and the surrounding region. The grain is high (25.0%) in plant-based protein and essential mineral elements such as calcium, iron, copper and zinc, among others (Bhardwaj & Hamama 2004). Tepary bean is a nutrition dense legume crop (Porch et al. 2017), especially for resource poor communities in tropical and sub-tropical regions of the globe. Furthermore, tepary bean fixes atmospheric nitrogen, thus contributing to the improvement of soil fertility (Mohrmann et al. 2017) and soil microbial diversity. As a result of its high protein content and resistance to biotic and abiotic stresses, tepary bean is suitable for cultivation by resource-poor farmers particularly in southern Africa (Porch et al. 2013).

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Although the tepary bean grows well in hot and arid regions, its production and productivity are influenced by the genetic potential and environmental factors. The increased temperatures and damaging solar radiation during flowering and fruiting result in diminished yield, thus posing food security risks (Gross & Kigel 1994; Nabhan 2020; Porch & Jahn 2001). Moreover, climate change has increased the frequency of extreme weather patterns including irregular precipitation that can cause drought stress resulting in significant yield reductions of the crop, thus threatening food, nutrition and income security (Lesk, Rowhani & Ramankutty 2016; Li, Braga-Junqueira & Reyes-Garcia 2021). One of the approaches to achieve increased water capture and water use efficiency in legumes is through developing good root systems (Ye et al. 2018). Phenotypic variability in root traits in legumes was reported in previous studies in chickpea (Kashiwagi et al. 2005), common bean (Beebe et al. 2013; Polania et al. 2021) and tepary bean (Butare et al. 2011). The various root features of interest have been described previously (Burridge et al. 2016; Kashiwagi et al. 2005). Despite its potential as a major field crop and the abundance of wild relatives, there is no significant breeding effort that has been carried out, to date, aimed at cultivar development particularly in southern Africa. Consequently, the crop remains under-utilised. Therefore, the aim of this study was to evaluate 42 tepary bean landraces using eight phenotypic traits and determine their trait association in a controlled environment

Research methods and design

Plant materials

A total of 42 genotypes of tepary bean consisting of both large (100-seed weight \geq 16.0 g) and small seed (100-seed weight \leq 12.0 g) sizes were used in the study (Table 1). The seeds of most of the genotypes were white (> 60.0%) and only two genotypes ('Ac-5' and 'Ac-8') possessed black testa (Table 1).

Testing location, planting and trial management

The study was conducted at the Agricultural Research Council - Vegetable, Industrial and Medicinal Plants, (25.60°S; 28.35°E), South Africa in drought screening glasshouse. The glasshouse temperatures were kept at 30°C during the day and 15°C during the night. The average relative humidity in the greenhouse ranged from 45% to 55% during the study period. Ten seeds per genotype were planted manually (at two seeds per planting station or hole) in the glasshouse in a 155 cm \times 77 cm \times 23 cm plastic box filled with a mixture of red top soil and vermiculite mix (1:1) ratio, which was irrigated to field capacity and the excess water was allowed to drain prior to planting. The seedlings were thinned subsequently to one per station resulting in five seedlings per genotype in each replication. The plastic box evaluation method was used in previous similar studies aimed at screening the cowpea germplasm (De Ronde & Spreeth 2007; Nkoana, Gerrano & Gwata 2019). The seeds were planted at a depth of 4 cm at a spacing of 15 cm between adjacent rows and 10 cm within rows.

Genotype code	Seed			
	Size	Colour		
AC-1	Medium	Cream		
AC-2	Large	Cream		
AC-3	Medium	White		
AC-4	Medium	White		
AC-5	Small	Black		
AC-6	Medium	White		
AC-7	Small	White		
AC-8	Medium	Black		
AC-9	Small	Brown		
AC-10	Small	Cream		
AC-11	Medium	White		
AC-12	Small	White		
AC-13	Small	White		
AC-14	Small	White		
AC-15	Medium	Speckled		
AC-16	Medium	White		
AC-17	Medium	White		
AC-18	Small	White		
AC-19	Large	White		
AC-20	Medium	White		
AC-21	Medium	White		
AC-22	Small	White		
AC-23	Small	White		
AC-24	Small	White		
AC-25	Small	Cream		
AC-26	Small	White		
AC-27	Small	White		
AC-28	Medium	Cream		
AC-29	Small	Cream		
AC-30	Medium	Brown		
AC-31	Medium	Speckled		
AC-32	Large	White		
AC-33	Small	White		
AC-34 (Check)	Small	White		
AC-35	Small	White		
AC-36	Medium	White		
AC-37	Medium	White		
AC-38	Small	White		
AC-39	Small	Cream		
AC-40	Medium	White		
AC-41	Large	White		
AC-42	Small	White		

Large seed, 100-seed weight \geq 16.0 g; small seed, 100-seed weight \leq 12.0 g.

No chemical or organic fertilisers or pesticides were applied to the plants throughout the season. The weeds were controlled manually. Irrigation, using tap water, was applied daily before the stress was imposed. The drought stress treatment was imposed at the vegetative (seedling) stage, which often coincides with early-season drought in the region. On an average, tepary bean flowers in 35–42 days after sowing depending on the genotype (Suárez et al. 2022).

Measurement of phenotypic traits

The plants were allowed to grow until the appearance of the first three trifoliate leaves (5 weeks after planting). At 5 weeks after germination, three plants per genotype were tagged (for data collection) in the middle of each row and the following phenotypic traits were measured during the experiment:

TABLE 2: Variability in phonotypic traits among 42 to

- Number of secondary roots per plant (NSR).
- Secondary root length per plant (SRL) (cm).
- Root dry weight per plant (RDW) (g).
- Root fresh weight per plant (RFW) (g).
- Primary root length per plant (PRL) (cm).
- Shoot height (SH) (cm).
- Shoot fresh weight (SFW) (g).
- Shoot dry weight (SDW) (g).

Following separation of the shoots and the roots and subsequent oven-drying at 75°C for 72 h, both the SDW and RDW were weighed and the values were recorded.

Experimental design and data analysis

A 6 × 7 rectangular lattice design replicated three times was used in the study. The data sets for all the traits were subjected to analysis of variance followed by mean separation using the least significant difference at the 5% probability level. To determine the magnitude of the relationships and identify influential traits, the Pearson's correlation coefficients (r) were calculated separately for the treatments followed by the principal component analysis (PCA) based on the correlation matrix using the Statistical Package for the Social Sciences (SPSS) version 23 (SPSS 2012).

Ethical considerations

This study followed all ethical standards for research without direct contact with human or animal subjects.

Results and discussion

The analysis of variance results showed that there were highly significant (p < 0.01) differences in all the phenotypic traits among the tested tepary bean genotypes assessed during the early seedling growth stage (Table 2), suggesting the presence of phenotypic variability. This was consistent with reports that drought stress can occur at different plant growth and development stages such as seedling establishment, post-emergence growth, flowering stage, reproduction, and grain filling stages (Shavrukov et al. 2017). The highest NSR (30.0) was observed in genotype 'Ac-39' followed by the genotype Ac-27, while the lowest was observed in Ac-28. The two genotypes ('Ac-4' and 'Ac-29') attained significantly (p < 0.05) higher PRL compared with the check genotype ('Ac-34') (Table 2). In contrast, when compared with the check, only three genotypes ('Ac-33', 'Ac-39', 'Ac-40') and four genotypes ('Ac-7', 'Ac-8', 'Ac-40', 'Ac-41') achieved a significantly (p < 0.05) higher SRL and SDW, respectively. In a recent preliminary study, the RDW showed significant differences among the tested tepary bean genotypes suggesting that tepary bean expressed unique genes, which can be combined with other traits of interest to improve drought tolerance trait for adaptation and likely, this contributed to adaptation to the combined effect of high temperature and

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Genotype code	NSR	PRL	SRL	RFW	RDW	SFW	SDW	SH
Ac-39	30.00	7.50	11.30	1.21	0.32	1.55	1.17	24.30
Ac-27	25.33	7.23	9.17	0.64	0.27	0.52	0.22	14.83
Ac-29	24.67	9.57	6.30	0.54	0.26	0.62	0.37	15.97
Ac-10	24.33	6.37	8.47	0.56	0.27	1.46	1.05	22.13
Ac-2	23.33	5.14	7.95	0.62	0.07	1.63	1.60	25.80
Ac-40	23.00	6.60	11.10	1.57	1.13	2.35	1.77	23.40
Ac-5	22.33	8.10	10.07	1.63	1.18	2.72	1.37	27.53
Ac-6	22.33	5.77	8.03	0.53	0.23	1.89	1.47	26.57
Ac-17	22.00	7.17	7.80	1.31	1.03	1.53	1.20	15.80
Ae-33	22.00	6.30	11.40	1.42	1.21	1.17	1.11	11.40
Ac-41	22.00	6.63	9.77	1.29	0.88	1.91	1.71	17.33
Ac-15	21.67	5.23	7.67	1.06	0.40	1.64	1.05	14.17
Ac-24	21.33	5.43	8.83	0.67	0.27	1.87	1.10	18.17
Ac-16	20.67	4.73	6.90	0.68	0.43	1.77	1.18	17.70
Ac-38	20.67	5.27	7.90	0.39	0.19	1.55	0.89	20.97
Ac-30	20.33	6.83	9.47	0.48	0.18	1.60	0.78	18.90
Ac-34	20.33	6.47	8.13	0.81	0.31	1.31	1.06	18.53
Ac-14	20.00	5.07	9.03	0.72	0.22	1.18	0.82	15.40
Ac-37	19.67	7.80	10.07	1.00	0.39	1.55	1.07	20.63
Ac-4	19.33	8.53	8.13	1.02	0.38	1.85	1.33	21.23
Ac-8	19.00	6.80	7.50	1.12	0.82	2.34	1.74	22.00
Ac-9	19.00	6.67	8.27	0.93	0.72	1.63	1.17	19.50
Ac-26	17.67	5.00	7.13	0.38	0.18	1.00	0.63	15.67
Ac-25	17.00	6.00	9.57	0.39	0.17	0.52	0.25	15.50
Ac-42	16.67	4.57	8.23	0.56	0.22	1.75	1.33	22.10
Ac-18	16.33	6.87	7.90	1.33	1.03	1.53	1.19	15.43
Ac-23	16.33	5.33	6.30	0.55	0.22	1.84	1.16	19.40
Ac-19	16.00	5.13	5.20	0.41	0.15	1.58	1.01	20.27
Ac-31	15.33	5.13	6.30	0.31	0.10	1.22	0.42	16.86
Ac-1	15 .00	4.23	4.97	0.41	0.21	1.92	0.89	21.50
Ac-21	15.00	3.93	5.90	0.46	0.20	1.49	1.10	14.27
Ac-35	15.00	3.27	10.23	0.52	0.29	0.76	0.61	19.17
Ac-11	14.33	3.27	7.13	0.65	0.28	1.62	1.11	14.87
Ac-13	14 33	6.24	6.46	0.67	0.42	1 50	1.09	14 27
Ac-3	14.00	5.40	4 90	0.28	0.16	0.44	0.20	13 73
Ac-36	14.00	4.60	4.50	0.20	0.10	1 2 2	1.01	12 20
AC-30	14.00	4.00 E 27	6.22	0.51	0.10	1.55	1.01	21.00
AC-7	12.00	2.27	0.25	0.03	0.45	2.30	1.07	12.40
AC-12	13.00	2.80	6.84	0.62	0.37	1.45	1.08	12.40
Ac-22	12.00	3.90	4.30	0.24	0.10	0.76	0.60	16.50
Ac-32	12.00	6.20	7.30	0.45	0.24	0.54	0.27	16.00
Ac-20	11.67	3.27	5.43	0.24	0.09	0.67	0.38	19.43
Ac-28	10.67	2.80	3.60	0.22	0.07	1.77	0.11	18.87
Mean	18.42	5.69	7.66	0.71	0.39	1.47	0.99	18.42
Coefficient of variation (%)	43.86	13.52	18.24	1.69	0.92	3.50	2.37	43.86
Least significant difference (5.0%)	5.58	1.71	2.74	0.21	0.36	0.88	0.62	6.98

NSR, number of secondary roots; SRL, secondary root length (cm); RFW, root fresh weight (g); RDW, root dry weight (g); PRL, primary root length (cm); SH, shoot height (cm); SFW, shoot fresh weight (g); SDW, shoot dry weight (g).

acid soil conditions as reported previously (Adu et al. 2019; Suárez et al. 2022). In addition, increased rooting depth as well as an efficient root system contributed to drought avoidance in legumes (Beebe et al. 2013). The existence of significant differences among the tested tepary bean genotypes for the traits studied indicated that some genotypes tolerated moisture stress better than others did.

The results also revealed significant (p < 0.05) positive correlations between specific pairs of the phenotypic traits (Table 3). For instance, there was a highly significant

(p < 0.01) positive correlation between the SDW and the SFW among the genotypes indicating that there was a strong linear relationship between the two parameters (Table 3; Figure 1). Similarly, at least 68.0% of the changes in RDW were attributed to the changes in the SRL. These positive relationships among traits would help the breeder to improve these traits simultaneously when selecting the tepary bean genotypes for drought tolerance in a breeding programme. In another study involving phenotyping of chickpea (*Cicer aritinum*), the root traits of plants that were raised in cylinders almost matched the relationships that were determined under field conditions (Vadez et al. 2008).

The genotypes that were used in the study varied in seed size (from small to large) (Table 1). However, the study focussed on screening the genotypes for drought tolerance at the vegetative stage irrespective of genotypic seed characteristics. Screening germplasm for a specific trait is a standard procedure from the plant breeding perspective because the genes of interest may not be linked (or associated) with seed size at all. At least, this was one of the underlying assumptions in the study. Moreover, legumes employ various morpho-physiological, physiobiochemical and molecular mechanisms to cope with drought stress (Khatun et al. 2021).

TABLE 3: Pearson's correlation coefficients for eight phenotypic traits among 42 tepary bean genotypes.

Traits	NSR	PRL	SRL	RFW	RDW	SFW	SDW	SH
NSR	1.0000	-	-	-	-	-	-	-
PRL	0.6683**	1.0000	-	-	-	-	-	-
SRL	0.6922**	0.4940**	1.0000	-	-	-	-	-
RFW	0.1581	0.0100	0.0608	1.0000	-	-	-	-
RDW	0.4888**	0.6079**	0.6816**	0.0412	1.0000	-	-	-
SFW	0.2054	0.1170	0.1559	0.1020	0.4209**	1.0000	-	-
SDW	0.3367*	0.2044	0.3336*	0.0818	0.5209**	0.8084**	1.0000	-
SH	0.3526*	0.1918	0.2419	0.1565	0.1931	0.5804**	0.4510**	1.0000

NSR, number of secondary roots; PRL, primary root length (cm); SRL, secondary root length (cm); RFW, root fresh weight (g); RDW, root dry weight (g); SFW, shoot fresh weight (g); SDW, shoot dry weight (g); SH, shoot height (cm).

*, significant at 5% probability level; **, highly significant at 1% probability level.

The PCA biplot grouped the genotypes into different clusters in the quadrant based on their phenotypic trait associations (Figure 2). Genotypes 'Ac-16', 'Ac-24', 'Ac-10', 'Ac-18' and 'Ac-38' were clustered close to the origin, suggesting that they possessed a similar genetic relationship for most of the traits. The genotypes positioned in the first quadrant were highly associated with the phenotypic traits such as SFW, SDW, and SH. These traits were highly positively associated with each other as the angle between them was less than 90° (Figure 2). In contrast, the genotypes 'Ac-3', 'Ac-5', 'Ac-20', 'Ac-22', 'Ac-28', 'Ac-39' and 'Ac-40' were positioned far from the origin indicating that they possessed unique genes or alleles in comparison with the rest of the germplasm that was evaluated. In this regard, these genotypes appeared to be the most genetically distinct based on the eight phenotypic traits that were measured and could be utilised as potential parental lines for hybridisation in future tepary bean breeding programmes aimed at improving the traits of interest. A similar approach for determining the phenotypic root traits in cowpea successfully identified superior cowpea genotypes that were tolerant to soil moisture stress (Nkoana et al. 2019). However, other studies focused on the postflowering drought soil moisture stress to select superior genotypes of common been (Mideksa 2016). In addition, the integration of agronomic and biotechnological strategies



FIGURE 1: The relationship between the shoot dry weight and the shoot fresh weight among 42 tepary bean genotypes.



NSR, number of secondary roots; PRL, primary root length; SRL, secondary root length; RFW, root fresh weight; RDW, root dry weight; SFW, shoot fresh weight; SDW, shoot dry weight; SH, shoot height. FIGURE 2: Principal component score plot of PC1 and PC2 describing the variation among 42 tepary bean genotypes estimated using the data set of phenotypic traits.

was proposed as a realistic avenue for developing legume cultivars that tolerate moisture stress drought-tolerant legume cultivars (Nadeem et al. 2019). Therefore, the preliminary findings that were reported in this study can contribute to the understanding of tepary bean and its requirements for genetic enhancement. Nonetheless, because of the shallow boxes that were used in the study which, most probably, restricted full expression of the root growth, it is important to approach the results of the root traits with caution (Chen et al. 2022; Rich et al. 2020; Schwinning & Ehleringer 2001; Xu et al. 2015).

The variability among the genotypes in response to soil moisture stress indicated the potential of the tepary bean germplasm to be utilised as a possible donor of alleles for tolerance (Singh 2001). In previous studies, common bean (*Phaseolus vulgaris* L.) was backcrossed successfully to tepary bean to develop drought and disease tolerant interspecific hybrids (Muñoz et al. 2003; Souter et al. 2017). Likely, such improved germplasm may be adopted widely by local farmers in drought-prone areas. In addition, the significant positive correlations between some of the root traits that were observed in this study agreed with the results that were reported for other similar legumes that were evaluated under soil moisture stress (Dayoub et al. 2021; Kumar et al. 2012; Priya et al. 2021).

Conclusions and recommendations

Firm conclusions based on one season at a single testing location were difficult to draw. Nonetheless, the study affirmed that characterisation and evaluation of the tepary bean germplasm for phenotypic traits are useful in discerning genetic variability that can be utilised in future breeding of the crop aimed at improving the tepary bean value chain. In addition, there will be merit in validating these results on a field basis together with grain yield evaluation and genotyping over multiple locations and seasons to expedite the selection of elite germplasm for utilisation by tepary bean end users.

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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.

Authors' contributions

R.A.N. was contributed towards the investigation, methodology, validation; writing of the original draft. A.S.G.

contributed towards the data curation; supervision, writing, reviewing and editing. E.T.G. was responsible for the conceptualisation, formal analysis of data as well as reviewing, editing and supervision.

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Data availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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