Variation in Drought Tolerance Attributes Among Tepary Bean (*Phaseolus acutifolius*) Germplasm

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Abstract

Tepary bean (*Phaseolus acutifolius* A. Gray) is an important food legume which originated from South America. In South Africa, it is cultivated by smallholder growers mainly in the drought prone Sekhukhune District of Limpopo Province. Currently, there are no significant breeding efforts aimed at cultivar development of this crop and it remains under-utilized despite the potential of the crop. Therefore, this study evaluated drought tolerance and growth attributes of the tepary bean emphasising on the leaf proline content that are associated with drought tolerance directly or indirectly. The study also determined the drought tolerance and growth relationships as well as identified potentially superior genotypes of tepary bean. The germplasm was evaluated before and after the soil moisture stress treatment which was imposed on the trial by withholding water for 21 days. A 6 x 7 rectangular lattice design replicated three times was used for evaluating 42 genotypes. The results showed that prior to soil moisture stress, there were significant ($P<0.05$) differences among the 42 genotypes for all the six phenotypic parameters that were measured. The highest (1.05 µmol/g dry weight) and lowest (0.32 µmol/g dry weight) leaf proline content (LPC) were observed for genotypes 'Ac-35' and 'Ac-9', respectively. The trial mean for proline was 0.69 µmol/g dry weight. The genotype 'Ac-42' attained the highest (27.85) leaf chlorophyll content (LCC) which was 48.94% higher than the check genotype ('Ac-34'). The genotype 'Ac-33' achieved almost two-fold higher relative water content (RWC) (84.72%) than genotype 'Ac-11' which recorded the lowest (43.12%) RWC. The highest (68.70 mmol $m^{-2}s^{-1}$) stomatal conductance (SC) was three-fold more than for the check genotype (19.90 mmol $m^{-2}\,s^{-1}$). At least four genotypes ('Ac-6', 'Ac-7', 'Ac-22' and 'Ac-28') attained significantly ($P < 0.05$) greater stem height (SH) than the trial mean (28.63 cm). After the soil moisture stress treatment, the results revealed that the LPC ranged from 1.26 to 0.36 µmol/g dry weight that were observed for genotype 'Ac-35' and 'Ac-9', respectively. The LPC showed a positive but not significant ($P > 0.05$) correlation with each of the other remaining attributes both before and after the moisture stress treatment. 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. In both soil moisture conditions, there was no discernible correlation between the SD and the SH. In general, the soil moisture stress lead to a variable increment in the LPC among the genotypes. An independent samples t-test which was used to determine the significance of the change in LPC showed that there was a highly significant ($P < 0.00019$) difference between the measurements of this amino acid before and after soil moisture stress. The results also showed a reduction in LCC during the soil moisture stress period but there was no clear pattern of the influence of the soil moisture stress on both the SC and RWC.

The principal component analysis showed that before the soil moisture stress, the first two principal components accounted for 45.49% of the total variation and three traits (SC, LPC and SH) were highly associated with PC1. In addition, SC contributed the most variation for this component. However, PC2 was highly associated with LPC and RWC. In contrast, PC3 was dominated by SH. The results also showed that after the soil moisture stress, the first two principal components accounted for >50.0% of the total variation. The LPC and SH were highly associated with PC2 but PC3 was dominated by both LCC and SD. In the biplot analysis four genotypes ('Ac-2', 'Ac-19', 'Ac-30' and 'Ac-41') were clustered around the origin prior to the moisture stress treatment while five genotypes ('Ac-3', 'Ac-9', 'Ac-11', 'Ac-28' and 'Ac-35') were distinct and positioned far away from the origin. The genotypes in the right top quadrant (including 'Ac-4', 'Ac-6', 'Ac-7' and 'Ac-28') were associated and characterized by high leaf proline, high degree of stomatal opening and tall shoots. The tallest shoots were associated with the genotypes that were grouped in the left top quadrant while the remainder of the genotypes were characterized by thick stems and grouped in the left bottom quadrant. The tepary bean genotypes were grouped into three main clusters with the majority of the genotypes (64.28%) grouped in cluster III. Cluster I consisted of only seven genotypes including 'Ac-40' (which was associated with high LCC) as well as 'Ac-2', 'Ac-35', and 'Ac-37' (which were characterized by both LPC and RWC). The check (genotype 'Ac-34') was grouped in cluster III in a sub-cluster with genotype 'Ac-20'. This study discusses the implications of the observed variability among the tepary bean genotypes for these phenotypic attributes and growth parameters. 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 that breeders and growers can utilize.

Key words: physiological attributes; correlation; soil moisture stress. germplasm; phenotypic variability; tepary bean

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Dedication

This dissertation is dedicated to both my parents, P. A. and K. A. Nong and late grandparents, Maubane T. S. and Nong T. M.

Declaration

I, Refilwe Aljareau Nong, hereby declare that this dissertation, for the Master Science in Agriculture (Crop Science) in the Department of Plant and Soil Sciences at the University of Venda, hereby submitted by me, has not previously been submitted for a degree at this or any other University. It is my own work, design and execution. All reference material contained therein has been duly acknowledged.

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As supervisors, we agree that the submission of this dissertation.

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List of Abbreviations

ABA = abscisic acid

AC = accession

AMMI = additive main effects and multiplicative interaction

ANOVA = analysis of variance

ARC = Agricultural Research Council

FAO = Food and Agriculture Organization

GGE = genotype main effect and genotype by environment interaction

ITPS = Inter-governmental Technical Panel on Soils

LCC = leaf chlorophyll content

LPC = leaf proline content

LSD = least significant difference

mRNA = messenger ribonucleic acid

N = nitrogen

NSR = number of secondary roots

OA = osmotic adjustment

PCA = principle component analysis

PC1 = first principal component

PC2 = second principal component

PC3 = third principal component

PC4 = fourth principal component

PC5 = fifth principal component

PEG = polyethyle glycol

pH = potential of hydrogen

PRL = primary root length

P5CR = pyrroline-5-carboxylate reductase

RDW = root dry weight

RFW = root fresh weight

RWC = relative water content

SAS = statistical analysis system

SC = stomatal conductance

SD = stem diameter

SDW = shoot dry weight

SFW = shoot fresh weight

SH = shoot height

SNP = single nucleotide polymorphism

SPSS = statistical package for the social sciences

SRL = secondary root length

SWSR = Status of the World's Soil Resources

UV = ultraviolet

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1.0 CHAPTER ONE: GENERAL INTRODUCTION

1.1 Introduction

Tepary bean (*Phaseolus acutifolius*) is a self-pollinating diploid (2n = 2x = 22) legume which originated from the arid and semi-arid region of north western Mexico and south western United States (Nabhan and 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 (Gwata et al., 2016). In South Africa, tepary bean is cultivated in the dryland areas of Limpopo Province particularly in the Sekhukhune region (Fig. 1.1) where growers use traditional varieties. 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 (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 et al., 2016). The seed contains high protein (25.0%) content essential mineral elements such as calcium, iron, copper and zinc among others (Bhardwaj and Hamama, 2004). The seed of tepary bean also contains considerable amounts of oil and fatty acids (Bhardwaj and Hamama, 2005). Tepary bean also fixes nitrogen (N) thus contributing to the improvement of soil fertility (Mohrmann et al., 2017). Due to its high protein content, and resistance to biotic and abiotic stress factors, tepary bean is suitable for cultivation by resource-poor farmers particularly in southern Africa (Porch et al., 2013). The inclusion of legumes in the human diet is important in controlling and preventing various metabolic diseases such as colon cancer, diabetes mellitus and coronary heart disease (Jiri et al., 2017).

Despite its potential significance, tepary bean has generally received limited research priority towards cultivar development compared with other legume crops. Consequently, a limited number of improved cultivars have been released for cultivation (Porch et al., 2013). Tepary been production can be enhanced through development of superior and high yielding genotypes with enhanced resistance to abiotic and biotic stresses. Although tepary bean grows well in hot and dry environments, its productivity may vary among genotypes and environments (Mhlaba et al., 2018).

Climate change has increased the frequency of extreme weather patterns including irregular precipitation, which can cause drought stress resulting in significant reductions in

crop production thus threatening food security (Lesk et al., 2016). Currently, limited water is a major constraint in grain legume production in many African countries. One of the approaches to achieve increased water capture and use efficiency in legumes is through developing better root systems (Ye et al., 2018). Variation in root traits in legumes were reported in previous studies of chickpea (Kashiwagi et al., 2005), common bean (Beebe et al., 2013) and tepary bean (Butare et al., 2011). In addition, significant variability in proline accumulation in crops under soil moisture stress was reported (Mwadzingeni et al., 2016). Therefore, both the root attributes and leaf proline variation are reliable indicators for response to moisture stress in various legumes.

1.2 Problem statement

In South Africa, tepary bean growers currently use traditional varieties. There are no commercial improved varieties of this crop. However, introduced germplasm from Mexico in our research program has not yet been characterized adequately for drought attributes under local agro-ecological conditions. Superior genotypes that can tolerate severe soil moisture stress have not been identified. To date, potential breeding material in the germplasm has not been identified adequately.

1.3 Justification

The identification of superior germplasm of tepary bean which can tolerate severe soil moisture stress will enhance the genetic improvement of the crop in terms of local production. Genotypes that possess the genes for drought tolerance can be utilized in future as parental sources in the breeding program aimed at improving the traits. New improved cultivars of tepary bean will benefit growers and end users in our region.

1.4 Objectives

The main objective of this study was to evaluate the performance of exotic tepary bean germplasm under soil moisture stress conditions.

The specific objectives of the study were to:

(i) determine the variation in drought tolerance attributes and growth parameters among tepary bean genotypes before and after soil moisture stress conditions

(ii) determine the relationships among the drought tolerance attributes and growth parameters in the germplasm

(iii) identify superior genotypes of tepary bean genotypes that tolerate soil moisture stress conditions.

1.5 Hypotheses

The study tested the following null hypotheses:

(i) there was no variation in drought tolerance attributes and growth parameters among tepary bean genotypes that were subjected to soil moisture stress conditions.

(ii) there were no relationships among the drought tolerance attributes and growth parameters in the germplasm

(iii) there were no superior genotypes of tepary bean genotypes that tolerate soil moisture stress conditions in the germplasm.

Fig. 1.1 The location of Sekhukhune district in South Africa where tepary bean is cultivated by smallholder farmers. [*Adapted from:* - https://www.google.com/maps/place/Southern (Accessed 29/07/2021)].

2.0 CHAPTER TWO: LITERATURE REVIEW

2.1 Origin and biology of tepary bean

Tepary bean is a short life cycle legume originating from the arid and semi-arid environments of north-western Mexico and south-western United States (Nabhan and Felger, 1978). It was introduced and cultivated in Africa, Asia, and Europe (Zambre et al., 2006). In Africa, it is cultivated in Botswana, Kenya, Zimbabwe and South Africa (Molosiwa et al., 2014; Jiri and Mafongoya, 2016) by smallholder farmers mainly for subsistence purposes.

Genotypes of tepary bean vary in their flower colour (white and purple); and seed colour (white, black, speckled and tan) (Mohrmann et al., 2017) as well as growth habit (climbing, erect and bushy) (Porch et al., 2017; Ghadimian et al., 2021). Short duration types may mature within 60 - 70 days from the day of sowing (Molosiwa and Kgokong, 2018). Tepary bean plants flower within 27 - 40 d after germination but the growth period may extend to 120 days in cooler areas (Mohrmann et al., 2017). In a recent field study that was conducted in KwaZulu Natal Province (South Africa), tepary bean required 32 d and 52 d to flowering and maturity, respectively (Jiri and Mafongoya, 2016).

2.2 Importance of the crop

Tepary bean seed is often consumed after boiling, steaming, frying or baking. The leaves are eaten in many communities although they are tougher than those of common bean and take relatively longer to cook (Bhardwaj and Hamama, 2004). In Botswana, it is grown by small scale farmers as a source of food for humans while haulms are used as feed for animals (Molosiwa et al., 2014). Tepary bean is grown occasionally for fodder (Bhardwaj, 2013). In Africa, tepary bean is an important food crop that reduces malnutrition and enhance income and livelihoods of resource limited farmers (Parry et al., 2009; Jiri and Mafongoya, 2016). It is tolerant to drought, salinity, heat stress, pests and microorganisms (Heredia-Rodriguez et al., 2019). According to FAO (2015), the dry grain of tepary bean is an affordable alternative source of protein comparable to meat. The crop also fixes nitrogen (N) in the soil which may reduce the application of chemical N fertilizers (Mohrmann et al., 2017). However, the rate of N fixation has not been adequately determined particularly under African agro-ecological conditions.

2.3 Production areas and agronomic practices

Tepary bean recently gained importance in semi-arid parts of Africa such as north-eastern Kenya, Uganda, Zimbabwe, Botswana and South Africa, where most other legumes fail due to drought (Shisanya, 2005; Jiri et al., 2017). In South Africa, it is cultivated mainly in the Sekhukhune District of the Limpopo Province.

It is a short duration crop suited to arid and semi-arid regions as it is tolerant to heat and drought stresses (Rao et al., 2013; Porch et al., 2017). Previous studies showed that the crop could be cultivated in conditions where the maximum daily temperatures reach 32°C and minimum temperatures of 19°C (Rao et al., 2013). After flowering, little to no rain is needed as it can thrive during drought with low annual rainfall requirements ranging from 500 mm - 1700 mm (Mapp et al., 2016). Well-drained soils are preferred while reasonable yield can be obtained on soils with pH 5 - 7 (Shisanya, 2005). It does not tolerate waterlogging and heavy clays (Ahmed et al., 2012). However, the crop is moderately tolerant to saline and alkaline soil conditions (Abiala et al., 2018).

The seed is planted in rows of 60 cm - 90 cm with 10 cm - 45 cm between plants within the row (Heredia-Rodriguez et al., 2019). Earlier work on tepary bean revealed that planting dates significantly affected seed yield, seed weight and harvest index. The time of planting is important in determining the final seed yield and can be a useful agronomic means to effectively control several pests (Ezeaku et al., 2014; Molosiwa and Kgokong, 2018). Therefore, it can be a good cultural control method of pest and diseases among subsistence farmers with limited access to resources (Akande et al., 2012). A study by Molosiwa and Kgokong (2018) indicated that planting early in December resulted in more yields especially under normal rainfall than late planting in February in Southern parts of Botswana.

Effective weed control is one of the prerequisites for high yields. Early control is extremely important because the root system of the plant develops at this stage and some weeds secrete chemical inhibitors which limit plant growth (Sajedi et al., 2015). Weeding using a hand hoe is very essential at early stages of crop growth since weeds compete with crop plants for nutrients, moisture, sunlight, space and act as secondary host for insect-pests and diseases (Rana and Jatav, 2017; Jiri et al., 2017). Mechanical weed control is usually carried out during seedbed preparation removing all weeds as well as ensuring that implements do not damage the crop; this can also be avoided by using row spacing that permits easy access (Liebenberg, 2002). Cultivation between the rows can be advantageous because it loosens the soil and improves aeration and water penetration. Weeds in the row can be hand-pulled while chemical weed control can be implemented before planting (Rana and Jatav, 2017).

Tepary bean is harvested when all the pods have matured, but before they begin to shatter. It can be harvested by hand-pulling (Souter et al., 2017), then threshed by hand using a stick to beat the pods while the wind is used to separate the seeds from the chaff (Liebenberg, 2002).

2.4 Productivity of the crop and constraints to production

Climate change has caused changes in the growing seasons in many parts of the world leading to reduction in crop yield (Ezeaku et al., 2015). Araújo et al., (2015) stated that drought stress is one of the major abiotic constraints limiting crop productivity particularly for small holder farmers and that this affects plant processes resulting in reduced gas exchange and crop growth. Markhart (1985) observeds that some crops tolerate water stress better than others by becoming more efficient at utilizing available water under drought conditions. Parry and Medrano (2005) indicated that growth rates of crops are generally determined by rates of photosynthesis; therefore, higher photosynthesis and improved water use efficiency may result in increased yields under water stress. Cuellar-Ortiz et al., (2008) observed that water shortage occurring in short periods impose a more stressful metabolic state by altering plant photosynthesis, leading to a depletion of sugars and hence poor grain quality and yield. On the other hand, Ashraf (2012) highlighted that excessive rainfall can lead to waterlogging resulting in limited gas exchange between root systems and soil pores. Ashraf (2012) added that when soils are saturated, oxygen requirements rapidly exceed available concentrations and consequently, the roots suffer from absence of oxygen and this reduces nutrient uptake, crop growth and yield. Ahmed et al., (2012) noted that water-logging may cause necrosis, stunting, defoliation, reduced N fixation and plant death.

Abdelrahman et al., (2018) indicateds that increases in soil salinity is one of the major causes of soil degradation in many parts of the world. Tetteh, (2015) pointed out that soil degradation can be caused by inappropriate fertilizer applications and FAO and ITPS (2015) addeds that excessive irrigation with salt-containing water can also lead to soil degradation. Hu et al., (2016) indicated that unsustainable soil management practices are also responsible for soil degradation. Cao et al., (2018) concluded that soil salinity inhibits the normal growth and development of most crop plants thus causing significant yield losses globally. These limitations apply also to leguminous crops such as tepary bean.

2.5 Breeding efforts to improve the crop

Mhlaba et al., (2018) observed that despite the nutritional importance and ability to tolerate biotic and abiotic stresses, tepary bean is neglected and under-utilized because of 7 limited research support. In addition, Gujaria-Verma et al., (2016) observed that this lack of research support is particularly pronounced in the genetic improvement of the crop. Porch et al., (2013) asserted that only a few improved genotypes have been developed and released for cultivation). For instance, Mhlaba et al., (2018) and Molosiwa et al., (2014) stated that farmers in sub-Saharan Africa currently cultivate unimproved varieties which are low yielding. Mwale et al., (2020) argued that increased tepary bean production can be obtained through the development of superior and high-yielding genotypes with enhanced tolerance to abiotic and biotic stresses. Potential target traits include water use efficiency, rooting depth, biomass accumulation and stomatal conductance (Rao et al., 2017; Chater et al., 2017).

Although limited genetic divergence was reported among the domesticated tepary bean genetic pool, Bhardwaj et al., (2002) and Mohamed et al., (2005) noted that genotypic differences exist for agronomic traits. On the other hand, Mohamed et al., (2005) and Türkan et al., (2005) indicated that there are also physiological and biochemical differences in the traits of tepary bean. Thus, Blair et al., (2012) and Porch et al., (2013) claimed that a breeding program involving domesticated and wild tepary bean accessions can be useful for developing breeding populations. Porch et al., (2013) reported that previous breeding efforts improved yield, adaptation, seed size, seed quality, common bacterial blight resistance and rust resistance. Singh and Munoz (1999) added that persistent breeding efforts have successfully transferred genes conferring common bacterial blight resistance but Kusolwa and Myers (2011) noted that these breeding efforts have resulted in bruchid seed pest resistance. Souter et al., (2017) stated that these efforts have led to drought tolerant tepary bean. Thangwana et al., (2021) reported that the use of ethyl methane sulphonate as a mutagenic agent in tepary bean produced early maturing lines that have the potential to escape terminal drought stress.

2.6 Effect of soil moisture stress on the productivity of tepary bean

Soil moisture stress limits agricultural productivity, particularly in African smallholder cropping systems. It does so by affecting different plant processes, resulting in reduced crop growth and productivity. Soil moisture stress influences plant-water relations, affecting water uptake, stomatal functioning and delaying chlorophyll biosynthesis, hence photosynthesis (Hayat et al., 2012). The frequency of soil moisture stress during the cropping seasons is increasing in many parts of the world partly due to the global climate change (Blum, 2011).

The requirement for moisture varies with the stage of the plant development (Pushpavalli et al., 2014). Soil moisture stress during the vegetative growth may result in

minimal yield reduction since the crop can recover and compensate once the stress is withdrawn. However, during the early reproductive stages (early flowering to full bloom) moisture stress causes flower abortion in legumes and consequently a decrease in seed yield (Farooq et al., 2014; Wijewardana et al., 2019). For instance, in soybean, drought stress during the mid-reproductive stages (pod initiation to seed filling) leads to significant loss in grain yield but the crop is less sensitive to drought occurring in the late reproductive growth stages (Pushpavalli et al., 2014).

Moderate to severe drought reduces plant biomass, grain yield and related components of legumes (Baroowa and Gogoi, 2013). According to Beebe et al., (2013) moderate to severe drought stress in common bean reduced canopy biomass and seed yield, harvest index, number of pods as well as seed weight per plant. The magnitude of the reduction depends on the duration and intensity of the stress, genotypic variability and crop developmental stage (Farooq et al., 2016). In addition, the crop response to drought varies depending on the species, duration and intensity of the stress, as well as the timing of the stress with respect to the stage of development of the plant (Vadez et al., 2012). Leguminous crops employ various strategies to cope with soil moisture stress, with variation in response to moisture stress within species (Lynch, 2013). Therefore, the plant genotype is important in its ability to cope with soil moisture stress.

2.7 Methods for inducing soil moisture stress

A range of different methods for inducing moisture stress have been used for different crops including legumes such as chickpea (Pang et al., 2017), cowpea (Nkoana et al., 2019) and tepary bean (Crespo-Muñoz et al., 2018). The different methods were employed at specific growth stages depending on the objectives of the study (Table 2.1). The use of polyethyle glycol (PEG) was used widely in both legumes and cereals such as maize (Bruce et al., 2002) sorghum (Jones and Turner, 1978) and wheat (Morgan et al., 1986). However, the tray method was used mainly for cowpea at the seedling growth stage (De Ronde and Spreeth, 2007; Nkoana et al., 2019).

Table 2.1 Examples of methods that were used for inducing soil moisture stress

Drought inducing method	Crop		Growth stage	References
	Common name	Scientific name		
Box (tray) evaluation in the greenhouse	Cowpea	*Vigna anguiculata*	Seedling	De Ronde and Spreeth, 2007; Nkoana et al., 2019
Field evaluation	Chickpea	*Cicer arietinum*	Reproductive	Pang et al., (2017)
	Tepary bean	*Phaseolus acutifolius*		Urrea and Porch, 2009; Mohamed et al., 2005
	Common bean	*Phaseolus vulgaris*		Muñoz-Perea et al., 2006; Urrea and Porch, 2009
	Rice	*Oryza sativa*	Vegetative	Gaudin et al., 2013
Pot evaluation in the greenhouse	Tepary bean	*Phaseolus acutifolius*	Seedling	Leal-Delgado et al., 2019
	Common bean	*Phaseolus vulgaris*		Türkan et al., 2005
	Cowpea	*Vigna anguiculata*		Nkomo et al., 2020
	Chickpea	*Cicer arietinum*	Flowering	Pouresmael et al., 2013
	Common bean	*Phaseolus vulgaris*		Thinley and Dorji, 2021
	Cowpea	*Vigna anguiculata*		Ajayi, 2022
	Tepary bean	*Phaseolus acutifolius*	Reproductive	Crespo-Muñoz et al., 2018
	Soybean	*Glycine max*		Bellaloui et al., 2011
	Cowpea	*Vigna anguiculata*		Singh et al., 1999
In vitro evaluation using polyethylene glycol (PEG)	Tepary bean	*Phaseolus acutifolius*	Seedling	Jimenez-Galindo et al., 2018; Mohamed and Tawfik, 2007
	Common bean	*Phaseolus vulgaris*		Jimenez-Galindo et al., 2018
	Soybean	*Glycine max*		Bouslama and Schapaugh, 1984
	Pearl millet	*Pennisetum glaucum*		Govindaraj et al., 2010
	Maize	*Zea mays*		Bruce et al., 2002
	Wheat	*Triticum aestivum*		Morgan et al., 1986
	Sorghum	*Sorghum bicolor*		Jones and Turner, 1978

2.8 Drought tolerance mechanisms and their measurements

2.8.1 Genetic factors

The adaptive mechanisms that plants employ during moisture stress conditions which include morphological, physiological, cellular and metabolic adjustments are governed by specific genes that produce proteins that allow moisture stress avoidance or tolerance (Uno et al., 2000; Ndima et al., 2001). Therefore, proteins that are produced protect the macromolecular functioning (Galau et al., 1986), sequestration of ions, binding of water and functioning as molecular chaperones. The synthesis of many proteins during dehydration are regulated by the plant hormone abscisic acid (Bray, 1997) and it has been recorded that most genes require abscisic acid under stressed conditions (Nelson et al., 1994).The drought responsive genes were classified according to their functions such as involvement in regulation of gene expression, metabolism, ion sequestration, and osmolyte synthesis (Bray, 1993).

2.8.2 Root traits

The development and distribution of the root system can be regarded as key factors for more efficient water uptake and thereby for managing the performance of legumes under drought stress (Wasaya et al., 2018). The essential characteristics of drought tolerance such as a deep, wide-spreading, much-branched root systems are essential to access available soil moisture in deep layers of the soil (Fenta et al., 2014). A deep and proliferative root system can avoid drought stress by its ability to acquire more water and nutrients (Ye et al., 2018) in water deficit soils. The benefit of a deep and proliferative root system for drought tolerance was reported in various crops, including wheat (Wasson et al., 2012), chickpea (Chen et al., 2012) and soybean (Sadok and Sinclair, 2011). In chickpea, genotypes with long and dense roots performed well under water deficit environments (Jaganathan et al., 2015). Root length varied significantly among genotypes with total root length ranging from 305 cm to 3824 cm while rooting depth ranged from 38.3 cm to 105 cm (Chen et al., 2017). However, in another study, rooting depth was similar among genotypes that were harvested at 61 days after sowing (Zaman-Allah et al., 2011). Nevertheless, the sensitive genotypes produced shallower root systems than the tolerant ones.

Early development of deep rooting and ability to partition photosynthates to the grain were identified as key mechanisms contributing to improved drought resistance in common bean (Beebe et al., 2013). Greater exploitation of subsoil water due to deeper root systems also improved chickpea yield under drought conditions (Kashiwagi et al., 2015). Root traits

contribute to the survival and productivity of plants under soil water-limited conditions and are strongly associated with drought tolerance in crops (Sadok and Sinclair, 2011). Despite the importance of the root traits in legumes limited efforts have been directed towards developing cultivars with an improved root system in tepary bean.

2.8.3 Relative water content

Relative water content (RWC) is a measure of the amount of water available in plant tissues. RWC is important for preserving water through stomatal features including stomatal closure during moisture stress conditions (Lugojan and Ciulca, 2011). It is also critical for growth and physiological functioning of plants. Plant genotypes maintaining a higher yield under moisture stress conditions can be selected based on variations in RWC (Omae et al., 2005). Tepary bean genotypes that could maintain relatively high values of RWC showed considerable tolerance to drought (Mohamed and Noga, 2002), soybean (Sloane et al., 1990) and common bean (Runkulatile et al., 1993). In cowpea, the RWC of leaves of genotypes from water-stressed treatment was lower than that of the non-stressed genotypes (Hayatu et al., 2014). In other studies, involving tepary bean (Mohamed et al., 2005), faba bean (Siddiqui et al., 2015), common bean (França et al., 2000) and chickpea (Rahbarian et al., 2011; Talebi et al., 2013), RWC was used as a selection criterion for drought tolerance. In addition, the RWC in drought tolerant cowpea genotypes ranged between 50.0% and 67.0% (Ajayi, 2022) while > 40.0% RWC was considered as a reasonable indicator of drought tolerance (Alidu et al., 2019). However, moisture stress at the flowering stage decreased the RWC for both tolerant and susceptible genotypes, but the reduction was lower for the drought tolerant cowpea genotypes which subsequently attained higher seed yield than the susceptible genotypes (Ajayi, 2022). In chickpea, significant genotypic variation in RWC due to soil moisture stress was reported (Pouresmael et al., 2013). Therefore, genotypes that maintain a high RWC in moisture stress environments can be suitable to exploit in tepary bean breeding programs.

2.8.4 Stomatal conductance

The stoma is a critical organ which regulates water and gas exchange between the plant and the external environment. During photosynthesis, optimum absorption of carbon-dioxide is ensured by the stomata through controlling the optimal transpiration (Markhart, 1985). The ability of the stomata to stomatal opening and closure, thus adjusting transpiration in plants under moisture stress conditions is critical for avoiding dehydration (Fang and Xiong, 2015). Higher stomatal conductance or stomatal closure restricts water loss but the degree of

stomatal opening is difficult to measure accurately. Stomatal characteristics in faba bean were determined through microscopy (Khazaei et al., 2013). In general, the leaf porometer can be used to measure stomatal conductance. Markhart, (1985) observed that the stomatal closure in tepary bean during soil moisture resulted in postponing moisture loss in the leaf tissue and concluded that if this trait is incorporated into bean genotypes, it could improve their levels of drought tolerance. In another study, greater stomatal conductance was maintained in tepary bean than in common bean under soil moisture stress induced by PEG treatment (Turkan et al., 2005). However, stomatal conductance in common bean and chickpea reduced with increasing drought stress (Pouresmael et al., 2013; Mathobo, 2017). In addition, higher stomatal density in faba bean, for instance, resulted in diminished drought tolerance and yield in comparison with genotypes that were characterized by low stomatal density as they performed better in stress conditions (Ricciardi, 1989; Khan et al., 2007; Belachew et al., 2019). Khazaei et al., (2019) summarised that faba bean breeders could select for high stomatal conductance during drought to optimize the genetic gain. Therefore, considerable variability for stomatal conductance exists in legumes under soil moisture stress.

2.8.5 Abscisic acid and proline accumulation

Abscisic acid (ABA) is the messenger hormone which mediates drought stress response in plants (Raghavendra et al., 2010). Soil moisture stress causes high levels of ABA to be synthesized (Sauter et al., 2001; Christmann et al., 2007;) in plants which respond by closing their stomata (Rabbani et al., 2003; Mori et al., 2006) and gene expression in response to drought conditions (Yoshida et al., 2019). The hormone also promotes the synthesis and accumulation of proline by affecting the activity of pyrroline-5-carboxylate reductase (P5CR) (Verslues and Bray, 2006; Verbruggen and Hermans, 2008). In addition, ABA is critical for water uptake and transport within the plant through root conductivity enabling the plant to continuously adapt to moisture stress conditions (Ali et al., 2020).

On the other hand, proline (Fig. 2.1) is an amino acid which is synthesized from glutamic acid and used in the biosynthesis of proteins (Kishor et al., 2005). In plants, it is associated with plant adaptation to adverse conditions. As a multifunctional amino acid, proline has diverse roles under stress conditions such as stabilization of proteins, membranes, and subcellular structures, and protecting cellular functions by scavenging reactive oxygen species (Kaur and Asthir, 2015) and maintaining turgidity of cells (Hayat, 2012). The enhanced rate of proline biosynthesis can contribute to the stabilization of redox balance and maintenance of cellular homeostasis by dissipating the excess of reducing potential when the electron transport chain is saturated during adverse conditions (Taiz and Zeiger, 2010) and its

catabolism is connected to oxidative respiration and administers energy to resume growth after stress. Proline accumulates in the leaves with increased drought stress in both sensitive and tolerant cultivars. However, proline accumulates in higher concentrations in the tolerant genotypes than the sensitive genotypes. It can be used to screen genotypes for drought tolerance (Esack et al., 2015).

In tepary bean, drought tolerance was previously linked to proline accumulation (Lazcano-Ferrat and Lovatt, 1999) and antioxidant biosynthesis (Turkan et al., 2005). However, the studies did not focus of the root traits which are critical in crop adaption and productivity under soil moisture limited conditions (Ye et al., 2018). However, in cowpea, genotypes tolerant to soil moisture stress were identified using a combination of root trait and leaf proline response (Nkoana et al., 2020). In a study that was conducted in wheat, the rate of proline accumulation and utilization was considerably higher in the drought-tolerant cultivar than in the drought-sensitive one (Solanki et al., 2014). Genotypic variation in proline accumulation was reported in various legumes including soybean (Masoumi et al., 2011), chickpea (Mafakheri et al., 2010) and cowpea (Hamidou et al., 2007; Nkoana et al., 2020) under varying drought stress levels. Therefore, there is a merit in investigating both the accumulation of proline and root trait in a diversity of tepary bean genotypes under soil moisture stress to identify superior germplasm that can be utilized in breeding activities of the crop. For instance, there is potential to utilize proline as a biochemical marker for drought stress tolerance in crops.

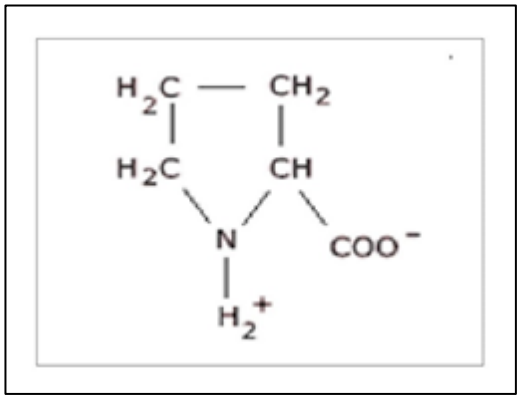

Fig. 2. 1 The structure of proline

2.9 Plant and root trait measurements under drought conditions

Physiological traits such as stomatal conductance, chlorophyll content, relative water content (RWC), osmotic adjustment (OA) and cell membrane stability have been used as indicators of drought tolerance in several crop plants (Bayoumi et al., 2008; Turan and Ekmekçi, 2011). Chlorophyll content was determined using chlorophyll meter (Muñoz et al., 2021). Under drought, chlorophyll content is shown to increase in the leaf, however the genotypes with higher observed chlorophyll content access water deep in the soil profile under moderate or intermediate droughts (Polania et al., 2016). In the field, phenotypes with water spending strategy under drought stress develop deep roots in order to acquire water from deep soil layers resulting in increased carbon assimilation (increased stomatal conductance) and plant growth (Mwale et al., 2020).

The stomatal conductance was used to analyze the stomata (Dipp et al., 2017). Leaf relative water content was determined using the method of Sade et al., (2015) where fully expanded leaves were sampled from the experiment under drought conditions. Fresh weight was recorded and then the leaf samples were placed in a petri dish with distilled water for 4 hours. Turgid weight was recorded after which the samples were placed in an incubator at 70°C for 24hrs to determine dry weight. The relative water content was then calculated (Siddiqui et al., 2015). On the other hand, shoot biomass was determined separately after uprooting the plant prior to oven drying it at 80°C to determine the dry weight (Fenta et al., 2012).

Plant root systems are important for adaptation against biotic and abiotic stresses. Genotyping quantitative traits have been conducted successfully but phenotyping has been a major challenge for plant breeders to improve abiotic stress tolerance in crop plants (Sharma et al., 2016). Root phenotyping methodologies include some degree of automation with imaging and image processing that utilize high resolution scanners for resolving lateral roots (Lopes and Reynolds, 2010). In another study that was conducted under greenhouse conditions, the entire primary root was sampled for visual measurement of primary root lateral branching, taproot length, root system architecture and its fresh weight (Fort et al., 2017). The remaining root biomass was stored separately and oven dried at 60°C for 72 hours and weighed to determine their dry weight.

2.10 Measurement of proline under drought conditions

Recent studies reported that proline analysis was carried out in laboratories; samples of the second top leaves from the flag leaf were harvested from the stressed and non-stressed

plots from the greenhouse. The leaf samples were temporarily stored at low temperatures and freeze dried (Mwadzingeni et al., 2016). The dry leaf tissue was ground and 0.1g samples were homogenized in 10mls of 3% aqueous sulfosalicylic acid. Proline extraction was done following the acid-ninhydrin method followed by UV-visible spectrophotometer readings as described previously (Bates et al., 1973).

2.11 Summary of literature review

The literature review revealed the following:

(i) tepary bean crop is short-season, maturing in about 90 days

(ii) tepary bean is generally regarded as a drought tolerant legume

(iii) soil moisture stress negatively affects water uptake, stomatal functioning and chlorophyll biosynthesis, hence photosynthesis

(iv) during the early reproductive stages, moisture stress causes flower abortion in legumes and consequently a decrease in seed yield

(v) a deep and proliferative root system partly accounts for the ability of tepary bean to avoid the negative effects of drought stress

(vi) root and growth parameters of various legumes including tepary bean have been evaluated successfully to identify drought tolerance under greenhouse conditions

(vii) leaf physiological attributes such as proline content, chlorophyll content, relative water content and stomatal conductance have been used as indicators of drought tolerance in tepary bean and several other field crops

(viii) an increment in both leaf proline and stomatal conductance have been associated with drought tolerance in legumes including tepary bean.

3.0 CHAPTER THREE: MATERIALS AND METHODS

3.1 Genetic materials

There were 42 tepary bean genotypes including two checks that were used in the study. The germplasm originated from Mexico and included a range of seed color types such as black, brown, speckled, white and cream types (Fig. 3.1). In addition, the genotypes included both early and late flowering types as well as distinct testa colors (Table 3.1). The seed size classification varied from small (100 seed weight ≤ 13.0 g) to large (100 seed weight ≥ 17.0 g).

Fig. 3.1 Samples of tepary bean seed genotypes with white (left), grey-speckled (middle) and black (right) testa colors.

Table 3.1 A summary of some descriptors for 42 tepary bean genotypes that were used in the study.

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

3.2 Testing location and trial establishment

The study was conducted at Agricultural Research Council – Vegetable and Ornamental Plants Institute, Roodeplaat South Africa, Pretoria (25.60°S; 28.35°E). In each row for each replication, five seeds per genotype were planted in a 155 cm x 77 cm x 23 cm box (tray) 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) (Fig. 2.1). The soil mixture was irrigated to field capacity after which the excess water could drain before planting (Nkoana et al., 2019). The seeds were planted at a depth of about 3 cm at a spacing of 15 cm between rows and 10 cm within rows. The greenhouse temperatures were maintained at 28°C during the day and 15°C during the night.

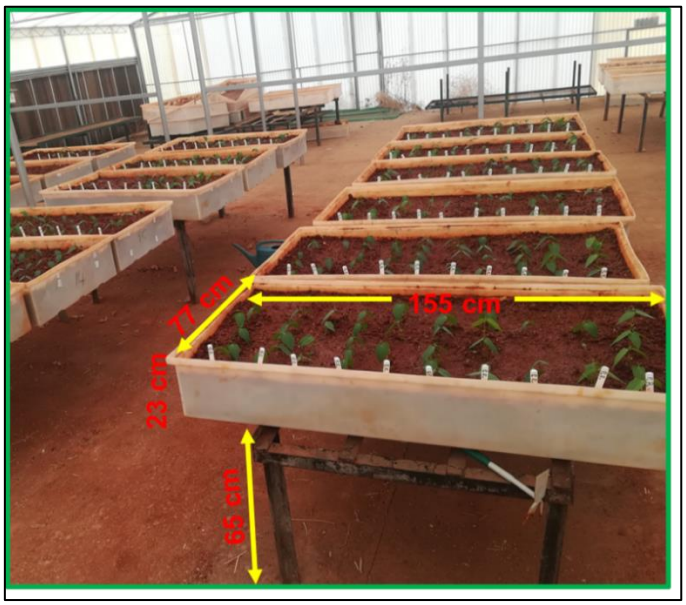

Fig. 3.2 Raised trays filled with a mixture of red top field soil and vermiculite for planting tepary bean that were used in the study.

3.3 Measurement of phenotypic traits

At five weeks after germination, prior to soil moisture stress, six phenotypic traits were measured among the genotypes. The traits were as follows:

(i) stem diameter (SD) (cm)

(ii) stem height (SH) (cm)

(iii) total leaf chlorophyll content (LCC)

(iv) leaf proline content (LPC) (µmol/g dry weight)

(v) relative water content (RWC) (%)

(vi) stomatal conductance (C) (mmol m^{-2} s^{-1}).

The SD was measured with the aid of a venier caliper (Fig. 3.3) and the SH was measured with the aid of a ruler. The LCC was measured using a chlorophyll meter (Minolta Chlorophyll Meter Spad-502, Minolta Co., Ltd., Tokyo, Japan) (Fig. 3.3). The meter quantitatively records numerical leaf color 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 leaf chlorophyll content (Markwell et al., 1995; 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:

RWC = ((fresh weight − dry weight) / (turgid weight − dry weight)) × 100.

To determine the turgid weight, the leaves were submerged in distilled water in dark for 24 h (Singh and Reddy, 2011; Sade et al., 2015). The dry weight was obtained by first oven-drying (at 60°C to constant weight for three days) and weighed. The stomatal conductance (SC) was measured by using a hand leaf porometer (Fig. 3.4) (Rebetzke et al., 2000).

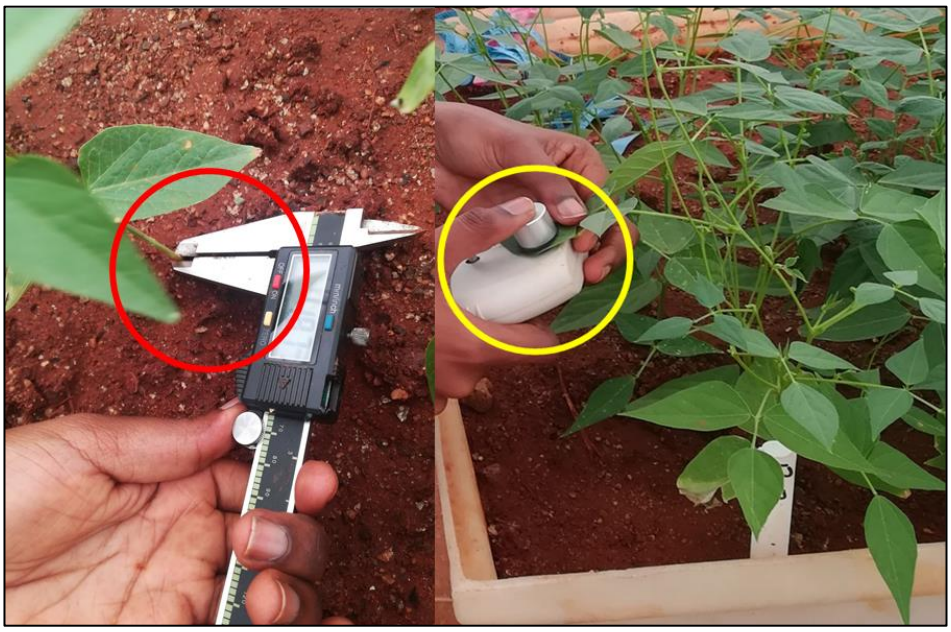

Fig. 3.3 The measuring of the stem diameter with a vernier caliper (red circle) and leaf chlorophyll content with a chlorophyll meter (yellow circle).

Fig. 3.4 The measuring of the stomatal conductance with a leaf porometer.

3.3.1 Leaf sampling and determination of leaf proline content

Fully expanded trifoliate leaves were sampled for proline analysis (Bates et al., 1973; Singh and Reddy, 2011). For the extraction of proline, at least two leaves from each replication were collected at noon and about 0.5 g dry weight of the bulked leaves was homogenized in 10 ml of 3% aqueous sulfosalicylic acid. This was followed by filtration and reaction with 2 ml of acid ninhdrin and 2 ml of glacial acetic acid in a test tube for 1 hour 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 et al., 1973; Nkoana et al., 2019). The chromophore containing toluene was then aspirated from the aqueous phase and warmed to room temperature where the absorbance was read at 520 nm using toluene as a blank. The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:

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

3.4 Experimental design and statistical analysis

The study utilized a 7 x 6 rectangular lattice design replicated three times and the data sets were subjected to analysis of variance (ANOVA) using the statistical software (SAS) program version 9.3 (Bailey and Speed, 1986; 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 and 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 was calculated separately for the treatments followed by the principle component analysis (PCA) based on the correlation matrix using the Statistical Package for the Social Sciences (SPSS) version 23 (Ringnér, 2008; SPSS, 2012). A principal component biplot was constructed to optimally visualize the graphic relationships between the genotypes and the phenotypic traits (Yan and Kang, 2003).

4.0 CHAPTER FOUR: RESULTS

The results of the study revealed interesting findings regarding the performance of tepary bean before and after soil moisture stress. In addition, the results demonstrated a useful procedure that can be applied in studies aimed at screening for drought tolerance in tepary bean. The results are presented in the sections below.

4.1 Trial establishment

The seed germination was high (>90.0%) resulting in good seedling establishment (Fig. 4.1). In addition, the adult plants also showed vigour particularly prior to the soil moisture stress treatment (Fig. 4.1). No leaf diseases were observed on the plants.

Fig. 4.1 Establishment of tepary bean seedlings (left) and vigorous adult plants (right) that were raised in the greenhouse.

4.2 Measurement of phenotypic and growth parameters 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 six phenotypic parameters that were measured (Table 4.1). The highest (1.05 µmol/g dry weight) and lowest (0.32 µmol/g dry weight) leaf proline content (LPC) were observed for genotype 'Ac-35' and 'Ac-9', respectively. The trial mean for proline was 0.69 µmol/g dry weight. However, genotype 'Ac-42' attained the highest (27.85) leaf chlorophyll content (LCC) which was 48.94% higher than the check genotype ('Ac-34'). The genotype 'Ac-33' achieved almost two-fold higher relative water content (RWC) (84.72%) than genotype 'Ac-11' which recorded the lowest (43.12%) RWC (Table 4.1). The trial mean for RWC was 69.66%. The highest (68.70 mmol $m^{-2}s^{-1}$ attained by genotype 'Ac-4') stomatal conductance (SC) was three-fold more than for the check genotype (19.90 mmol m^{-2} s^{-1}). The genotypes also showed significant ($P < 0.05$) variability in stem diameter (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 significantly ($P < 0.05$) greater shoot height (SH) than the trial mean (28.63 cm).

Table 4.1 Variability in leaf physiological and stem parameters among 42 tepary bean accessions before water stress (SD = stem diameter (cm); SH = stem height (cm); LPC = leaf proline content (µmol/g dry weight); LCC = leaf chlorophyll content; RWC = relative water content (%); SC = stomatal conductance (mmol m^{-2} s^{-1})).

Genotype		LPC	LCC	RWC	SC	SD	SH
Designation	Code						
Ac-35	41	1.05	26.11	73.47	21.97	2.27	18.99
Ac-28	34	1.00	17.91	69.38	55.20	2.02	48.60
Ac-6	6	0.99	17.08	70.01	20.77	1.92	57.77
Ac-7	7	0.90	23.68	47.62	47.70	2.11	46.90
Ac-18	20	0.90	24.09	69.56	23.10	2.06	21.21
Ac-14	16	0.88	24.98	78.17	25.70	1.91	34.79
Ac-36	42	0.88	26.73	83.33	25.75	1.83	25.00
Ac-37	43	0.85	22.99	73.16	26.83	1.88	24.03
Ac-41	49	0.84	20.67	65.46	19.00	2.08	29.56
Ac-19	21	0.84	22.81	60.32	34.47	2.20	16.22
Ac-4	4	0.83	25.32	65.64	68.70	1.94	26.69
Ac-15	17	0.83	21.85	78.42	26.87	2.36	19.22
Ac-31	37	0.81	19.37	81.22	28.53	2.46	39.97
Ac-10	10	0.78	25.14	69.31	33.07	2.29	32.93
Ac-32	38	0.78	17.70	80.71	23.47	1.97	19.61
Ac-5	5	0.76	21.91	68.89	22.30	2.10	34.78
Ac-22	24	0.71	21.10	60.35	14.51	1.96	50.48
Ac-39	46	0.70	23.77	76.04	9.90	2.17	19.73
Ac-30	36	0.69	18.29	79.23	40.87	2.16	19.88
Ac-34 (Check)	40	0.68	14.22	76.40	19.90	2.21	31.17
Ac-21	47	0.65	20.98	71.59	28.83	2.02	19.25
Ac-1	23	0.65	21.55	72.87	24.90	2.24	30.67
Ac-3	1	0.64	15.92	53.66	52.40	1.79	15.52
Ac-33	3	0.64	17.41	84.72	27.70	2.28	24.96
Ac-26	39	0.63	17.99	73.59	43.90	1.97	33.11
Ac-20	32	0.63	18.76	76.78	29.00	2.18	28.23
Ac-27	22	0.63	20.23	73.31	47.77	2.47	34.69
Ac-11	33	0.63	19.73	43.12	34.93	1.91	28.10
Ac-2	13	0.62	21.26	63.66	24.40	2.39	41.67
Ac-29	2	0.61	16.12	57.72	35.30	1.99	17.44
Ac-16	35	0.59	21.72	66.94	26.33	2.31	33.97
Ac-42	18	0.57	27.85	73.31	40.30	2.13	26.83
Ac-17	50	0.57	22.6	65.80	22.87	2.17	17.34
Ac-38	19	0.56	18.67	75.53	22.87	1.85	21.26
Ac-40	45	0.55	17.96	46.66	13.40	2.17	32.73
Ac-24	29	0.50	21.00	73.00	16.63	2.07	23.84
Ac-12	14	0.48	19.43	80.30	17.20	2.08	32.97
Ac-25	31	0.45	21.08	63.26	25.90	2.09	29.89
Ac-8	8	0.43	16.50	68.86	17.73	2.07	27.70
Ac-23	25	0.41	22.64	76.62	26.07	2.25	27.51
Ac-13	15	0.40	25.41	67.57	27.47	2.18	17.80
Ac-9	9	0.32	25.96	70.19	17.07	2.12	19.33
Grand mean		0.69	21.11	69.66	31.96	2.11	28.63
Coefficient of variation (%)		25.26	15.67	13.76	42.24	7.92	9.96
Least significant difference (5%)		0.61	6.88	23.35	32.04	0.49	22.52

4.3 Measurement of phenotypic and growth parameters after soil moisture stress

The soil moisture stress imposed for 21 days resulted in a clear variation in the degree of wilting among the genotypes and some of them exhibited partial or permanent wilting (Fig. 4.2). The results also revealed significant ($P<0.05$) differences among the 42 genotypes for all the six phenotypic parameters that were measured after the soil moisture stress treatment (Table 4.2). The LPC ranged from 1.26 to 0.36 µmol/g dry weight that were associated with genotype 'Ac-35' and 'Ac-9', respectively. The trial mean for the LCC was 8.31. The highest RWC (84.61%) and SC (84.0) were observed for the genotype 'Ac-18' and 'Ac-39', respectively (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 due to severe wilting that was induced by the soil moisture stress.

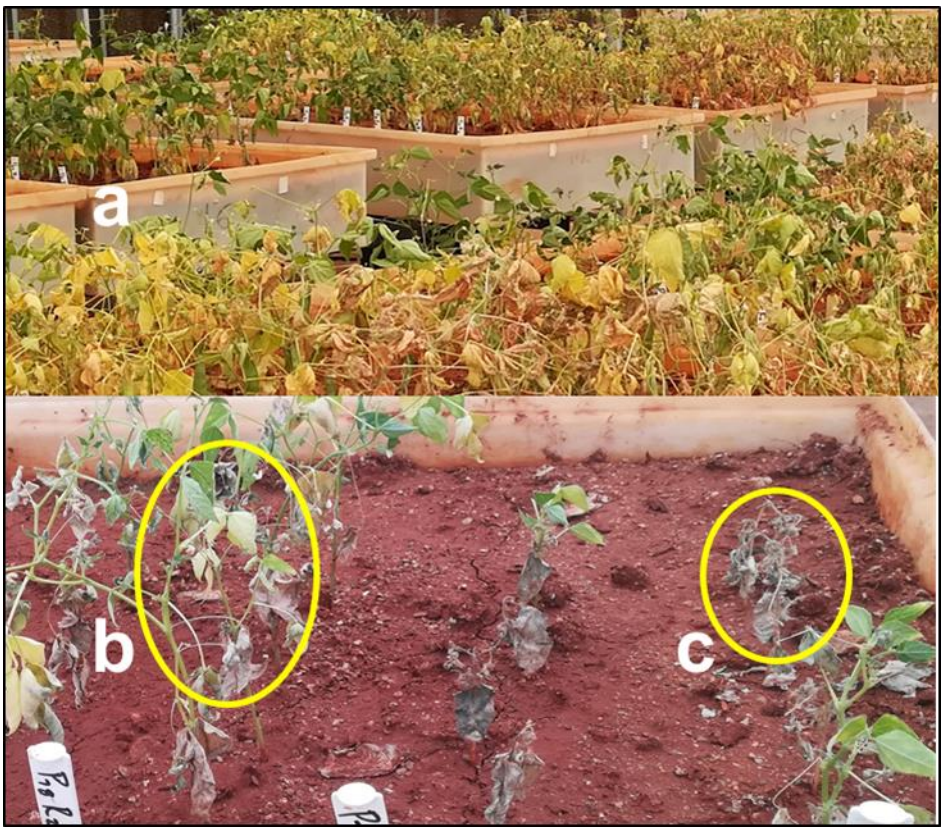

Fig. 4.2 Variation in the degree of wilting (a) partially wilted (b) and permanently wilted (c) tepary bean plants after 21 days of soil moisture stress in the greenhouse.

Table 4.2 Variability in leaf physiological and stem parameters among 42 tepary bean accessions after water stress (LPC = leaf proline content; LCC = leaf chlorophyll content; RWC = relative water content; SC = stomatal conductance SD = stem diameter; SH = shoot height).

Genotype		LPC	LCC	RWC	SC	SD	SH
Designation	Code						
Ac-35	41	1.26	11.42	62.64	24.4	1.76	27.48
Ac-28	34	1.09	11.41	70.47	25.85	1.69	47.98
Ac-6	6	1.08	6.40	52.22	26.10	1.40	52.59
Ac-7	7	1.08	8.08	55.71	26.40	1.75	40.59
Ac-18	20	1.05	13.28	84.61	24.60	1.62	45.32
Ac-14	16	1.00	14.29	76.92	0.00	1.67	35.91
Ac-36	42	0.99	0.00	53.78	12.70	1.52	36.68
Ac-37	43	0.98	13.63	53.78	11.10	1.61	34.00
Ac-41	49	0.97	11.68	55.00	21.60	1.67	44.36
Ac-19	21	0.95	10.85	78.57	14.20	1.85	32.05
Ac-4	4	0.93	10.17	76.19	40.10	1.61	39.72
Ac-15	17	0.92	10.85	51.78	26.00	2.00	30.00
Ac-31	37	0.92	0.00	0.00	0.00	2.13	43.23
Ac-10	10	0.92	11.93	0.00	34.77	1.77	33.23
Ac-32	38	0.92	6.97	57.20	0.00	1.63	39.85
Ac-5	5	0.92	7.23	52.68	21.00	1.77	42.93
Ac-22	24	0.92	6.40	58.33	0.00	1.57	43.41
Ac-39	46	0.90	6.10	50.00	84.00	1.80	33.48
Ac-30	36	0.90	8.61	78.24	33.53	1.80	26.49
Ac-34 (Check)	40	0.90	0.00	0.00	0.00	1.62	27.90
Ac-21	47	0.89	14.67	26.67	14.30	2.00	35.15
Ac-1	23	0.88	9.90	78.57	16.40	1.63	36.51
Ac-3	1	0.86	10.67	68.69	22.77	1.90	33.70
Ac-33	3	0.86	8.56	69.58	27.90	1.25	31.44
Ac-26	39	0.85	4.20	65.00	29.20	1.86	38.91
Ac-20	32	0.85	0.00	0.00	0.00	1.62	50.02
Ac-27	22	0.83	9.23	75.00	26.10	1.66	28.30
Ac-11	33	0.82	17.63	35.71	14.40	2.20	41.77
Ac-2	13	0.80	6.42	66.11	34.70	1.65	33.02
Ac-29	2	0.79	0.00	0.00	0.00	2.04	38.68
Ac-16	35	0.78	6.46	0.00	34.40	1.55	18.54
Ac-42	18	0.77	12.63	0.00	5.30	1.99	42.24
Ac-17	50	0.73	8.01	66.66	32.55	1.73	40.20
Ac-38	19	0.68	7.47	70.45	21.25	1.86	27.78
Ac-40	45	0.68	10.28	66.48	22.30	1.65	26.41
Ac-24	29	0.66	0.00	0.00	0.00	1.75	36.89
Ac-12	14	0.61	0.00	0.00	0.00	1.75	40.13
Ac-25	31	0.49	7.20	50.00	27.70	1.61	43.14
Ac-8	8	0.48	10.63	45.45	7.60	1.73	40.82
Ac-23	25	0.41	0.00	74.64	25.07	2.04	39.78
Ac-13	15	0.38	23.33	58.33	16.50	1.79	28.77
Ac-9	9	0.36	12.28	66.25	12.05	1.75	25.13
Grand mean		0.83	8.31	48.85	19.45	1.74	36.54
Coefficient of variation (%)		23.61	63.70	57.90	81.23	10.72	20.07
Least significant difference (5%)		0.50	6.10	9.63	11.43	0.49	10.37

4.4 Relationships among phenotypic and growth parameters

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 4.3). However, the LCC showed a highly significant (P < 0.01) positive correlation with the RWC but a negative non-significant correlation with SH. In addition, the SC showed a non-significant negative correlation with both the SD and 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 4.4). However, in both soil moisture conditions, there was no discernible correlation between the SD and the SH.

Table 4.3 Pearson's correlation coefficients for six leaf and growth parameters in tepary bean before soil moisture stress. (LPC = leaf proline content; LCC = leaf chlorophyll content; RWC = relative water content; SC = stomatal conductance; SD = stem diameter (cm); SH = shoot height (cm)).

	LPC	LCC	RWC	SC	SD	SH
LPC	1.0000					
LCC	0.0775	1.0000				
RWC	0.1905	0.4006 **	1.0000			
SC	0.1140	0.1622	0.3618 *	1.0000		
SD	0.1939	0.0693	0.2410	-0.1015	1.0000	
SH	0.2650	-0.1852	-0.0877	-0.2083	0.0000	1.0000

** = Highly significant at the 1.0% probability level
* = Significant at the 5.0% probability level

Table 4.4 Pearson's correlation coefficients for six leaf and growth parameters in tepary bean after soil moisture stress. (LPC = leaf proline content; LCC = leaf chlorophyll content; RWC = relative water content; SC = stomatal conductance; SD = stem diameter (cm); SH = shoot height (cm)).

	LPC	LCC	RWC	SC	SD	SH
LPC	1.0000					
LCC	0.0173	1.0000				
RWC	0.1144	0.4005 **	1.0000			
SC	0.1288	0.1622	0.3618 *	1.0000		
SD	-0.1761	0.0700	-0.2414	-0.1039	1.0000	
SH	0.1916	-0.1855	-0.0877	-0.2086	0.0000	1.0000

** = Highly significant at the 1.0% probability level
* = Significant at the 5.0% probability level

4.5 Change in leaf proline, stomatal conductance and relative water content

An independent samples t-test which was used to determine the significance of the change in LPC showed that there was a highly significant ($P < 0.00019$) difference between the measurements of this compound before and after soil moisture stress. In general, the soil moisture stress lead to a variable increment in the LPC among the genotypes (Fig. 4.3). 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' (Fig. 4.3). Only one genotype ('AC-13') showed a negative percent change in LPC after the soil moisture stress treatment.

In general, there was a reduction in LCC during the soil moisture stress period (Fig. 4.4). However, there was no clear pattern in terms of the influence of the soil moisture stress on both the SC and RWC. Some of the genotypes (for example, genotype 'Ac-37') which showed <18.0% change in LPC, maintained significantly higher RWC and SC prior to the stress in comparison with the observations after the stress (Fig. 4.4).

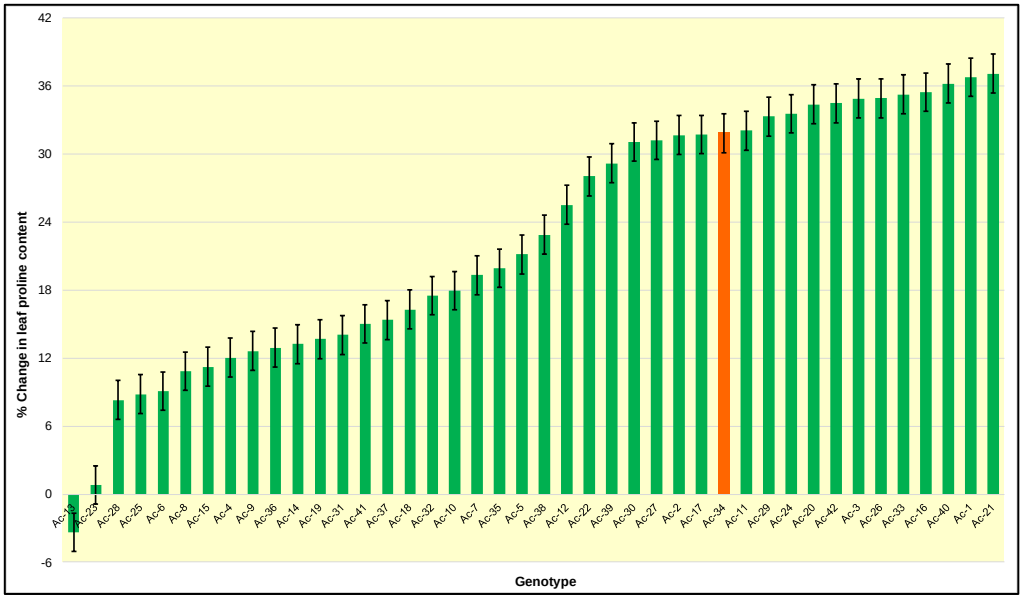

Fig. 4.3 Variation in the percent change in leaf proline content among tepary bean genotypes after soil moisture stress. (Genotype 'AC-34' = check).

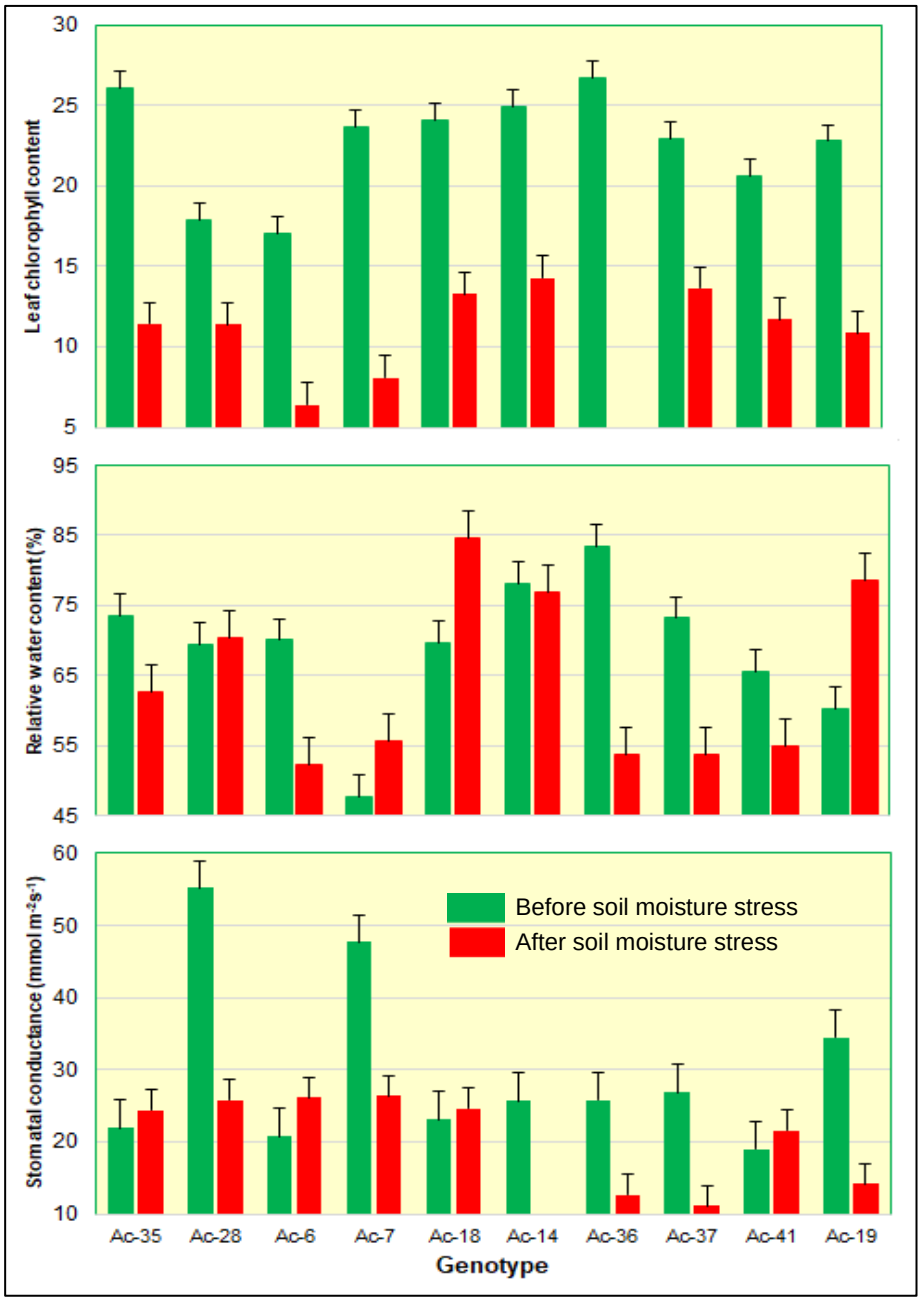

Fig. 4.4 The pattern of leaf chlorophyll content (top), relative water content (middle) and stomatal conductance (bottom) before and after soil moisture stress among the top 10 genotypes (based on leaf proline content) of tepary bean.

4.6 Principal component analysis and principal component biplot

The principal component analysis showed that before the soil moisture stress, the first two principal components accounted for 45.49% of the total variation (Table 4.5). Three traits, namely SC, LPC and SH, were highly associated with PC1. In addition, SC contributed the most variation for this component. However, PC2 was highly associated with LPC and RWC. In contrast, PC3 was dominated by SH. The results also showed that after the soil moisture stress, the first two principal components accounted for >50.0% of the total variation (Table 4.6). Three physiological traits, namely RWC, SC and LCC were highly associated with PC1, but LPC and SH were highly associated with PC2 but PC3 was dominated by both LCC and SD.

In the biplot for the measurements that were carried out before the soil moisture stress, four genotypes (‘Ac-2’, ‘Ac-19’, ‘Ac-30’ and ‘Ac-41’) were clustered around the origin. In contrast, five genotypes (‘Ac-3’, ‘Ac-9’, ‘Ac-11’, ‘Ac-28’ and ‘Ac-35’) were distinct and positioned far away from the origin (Fig. 4.5). The genotypes in the right top quadrant (including ‘Ac-4’, ‘Ac-6’, ‘Ac-7’ and ‘Ac-28’) were associated and characterized by high leaf proline, high degree of stomatal opening and tall shoots. However, the genotypes that were highly associated with the thickest stems as well as leaf chlorophyll and water content (Fig. 4.6) clustered in the top left quadrant. The biplot analysis for the traits that were measured after the soil moisture stress, three genotypes (‘Ac-10’, ‘Ac-25’ and ‘Ac-26’) were grouped close to the origin while four genotypes (‘Ac-6’, ‘Ac-13’, ‘Ac-31’ and ‘Ac-39’) were distinct and positioned far away from the origin (Fig. 4.5). The genotypes in the top right quadrant were highly associated both with LPC and RWC but high leaf chlorophyll was associated with the genotypes that were clustered in the right bottom quadrant. The tallest shoots were associated with the genotypes that were grouped in the left top quadrant while the remainder of the genotypes were characterized by thick stems and grouped in the left bottom quadrant.

Table 4.5 Principal component analysis showing the eigenvector, eigenvalue and cumulative percentage of the first five principal component axes for six phenotypic traits among tepary bean genotypes before soil moisture stress.

Trait	Eigenvector				
	PC1	PC2	PC3	PC4	PC5
Leaf proline content	0.480	0.580	-0.015	-0.292	-0.056
Leaf chlorophyll content	-0.138	0.507	-0.556	0.334	-0.472
Relative water content	-0.410	0.491	0.096	-0.541	0.379
Stomatal conductance	0.508	0.063	-0.329	0.291	0.682
Stem diameter	-0.365	0.349	0.391	0.641	0.286
Shoot height	0.431	0.197	0.649	0.121	-0.288
Eigenvalue	1.522	1.207	1.123	0.895	0.792
Variability (%)	25.368	20.123	18.726	14.922	13.204
Cumulative (%)	25.368	45.491	64.217	79.139	92.344

Table 4.6 Principal component analysis showing the eigenvector, eigenvalue and cumulative percentage of the first five principal component axes for six phenotypic traits among tepary bean genotypes after soil moisture stress.

Trait	Eigenvector				
	PC1	PC2	PC3	PC4	PC5
Leaf proline content	0.159	0.630	0.266	0.410	-0.557
Leaf chlorophyll content	0.462	-0.275	0.551	-0.283	-0.276
Relative water content	0.604	0.082	0.135	-0.291	0.314
Stomatal conductance	0.508	-0.001	-0.199	0.641	0.416
Stem diameter	-0.253	-0.487	0.581	0.485	0.114
Shoot height	-0.272	0.533	0.479	-0.141	0.573
Eigenvalue	1.779	1.321	0.962	0.804	0.693
Variability (%)	29.653	22.023	16.035	13.400	11.544
Cumulative (%)	29.653	51.676	67.711	81.110	92.655

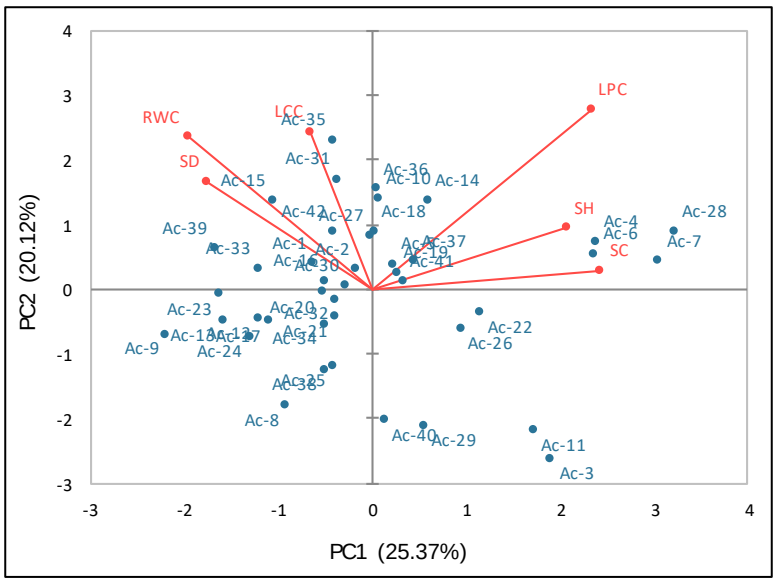

Fig. 4.5 Principal component score plot of PC1 and PC2 describing the variation among 42 tepary bean genotypes estimated using the data set of phenotypic traits before soil moisture stress. (LPC = leaf proline content; LCC = leaf chlorophyll content; RWC = relative water content; SC = stomatal conductance; SD = stem diameter (cm); SH = shoot height (cm)).

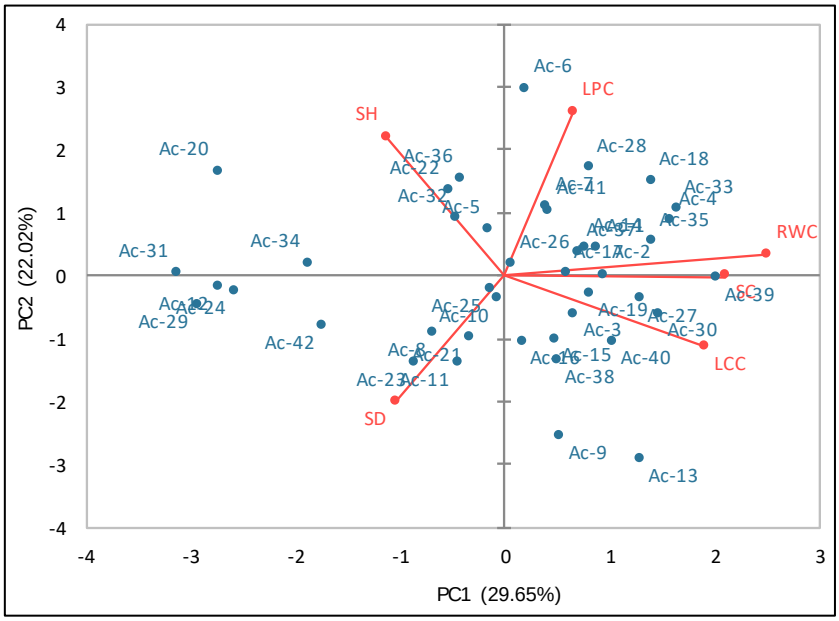

Fig. 4.6 Principal component score plot of PC1 and PC2 describing the variation among 42 tepary bean genotypes estimated using the data set of phenotypic traits after soil moisture stress. (LPC = leaf proline content; LCC = leaf chlorophyll content; RWC = relative water content; SC = stomatal conductance; SD = stem diameter (cm); SH = shoot height (cm)).

4.7 Clustering pattern of the genotypes

The tepary bean genotypes were grouped into three main clusters (Fig. 4.7). 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) as well as 'Ac-2', 'Ac-35', and 'Ac-37' (which were characterized by both LPC and RWC). The check (genotype 'Ac-34') was grouped in cluster III in a sub-cluster with genotype 'Ac-20' (Fig. 4.7). In addition, the bulk (57.0%) of the tallest genotypes were grouped into cluster III.

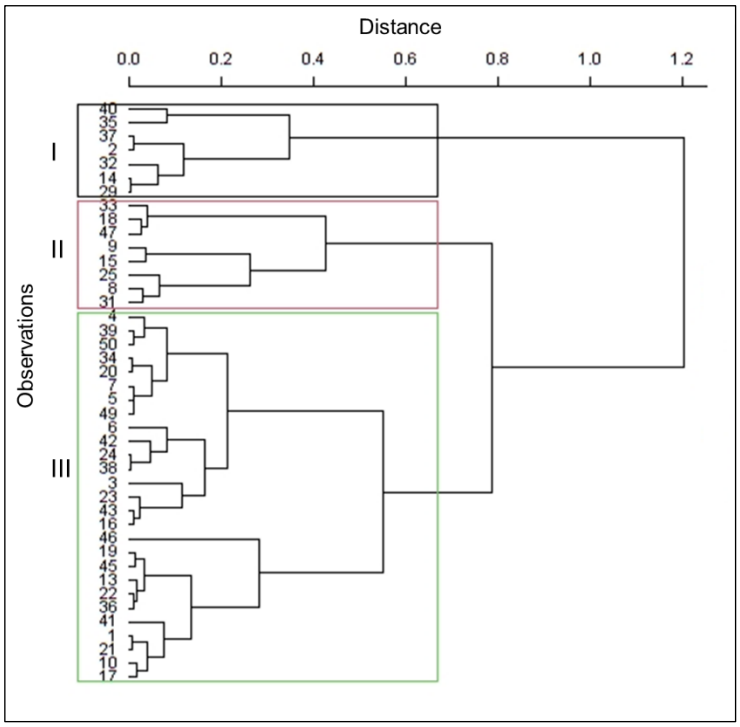

Fig. 4.7 Dendrogram of 42 tepary bean genotypes for six studied variables using hierarchical cluster analysis (ward's method and squared Euclidean distance)

5.0 CHAPTER FIVE: DISCUSSION

The results showed significant phenotypic variation among genotypes tested 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 which was 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 programs of tepary bean. Leaf proline content is an important selection criteria in screening genotypes for drought tolerance (Solanki et al., 2014).

The pattern of relationships between genotypes and the phenotypic 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 physiological and growth parameters that were evaluated and can be used for developing breeding populations. For example, some genotypes were associated with the thickest stems (for instance, 'Ac-8' and 'Ac-11') and high leaf chlorophyll only after the soil moisture stress was imposed indicating that selection for such traits required the stress conditions. However, some of the genotypes exhibited relatively high leaf proline in both moisture stress regimes suggesting that the candidacy of such genotypes in a breeding program aimed at selecting for high proline, can be validated subsequently under soil moisture stress conditions. The observed increase in leaf proline was expected and in agreement with the findings that were reported in previous studies (Masoumi et al., 2011; Solanki and Sarangi, 2014). In a similar study aimed at proline evaluation in cowpea, the genotypes showed highly significant variability in leaf proline content after five weeks of drought stress ranging from 0.39 µmol/g dry weight to 8.81 µmol dry weight (Nkoana et al., 2019). The amino acid is associated with plant adaptation to moisture stress conditions as it protects the plant from adverse environmental stresses (Ashraf et al., 2007; Kaur and 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 (Unyayar et al., 2004; Cechin et al., 2006) and wheat (*Triticum aestivum*) (Vendruscolo et al., 2007; Saeedipour, 2013). In cowpea (*Vigna anguiculata*), which was exposed to water deficit, the up-regulation of the expression of the proline synthesis gene with a concomitant down-regulation of the proline catabolism gene, was reported (Zegaoui et al., 2017). The metabolic role of proline in plants under drought conditions is well documented (Yoshiba et al., 1997; Ashraf and Foolad, 2007; Verbruggen and Hermans, 2008; Szabados and Savouré, 2010). The ability to withstand soil moisture stress was also attributed to profuse branching of the root system (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 signaling 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 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 stabilizing 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. A preliminary study that was conducted to characterize tepary bean root attributes under well-watered conditions found significant variation in primary root length as well as the number of secondary roots (Nong, 2019, *unpublished*) (Appendix 1).

Although the results of this study showed a marked reduction in leaf chlorophyll due to the soil moisture stress among the tepary bean genotypes, other studies reported no change to chlorophyll induced by drought (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. Nonetheless, there were some individual plants that remained green suggesting that the level of chlorophyll in such plants was not affected by the soil moisture stress during the duration of 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 stay-green genes which have been reported widely in other legumes such as bean (Bachmann, et al., 1994) and soybean (Luquez and Guiamét, 2002; Chang et al., 2019) 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 both the relative water content and the stomatal conductance. In a previous study involving polyethylene glycol to induce drought stress in tepary bean, there was a detectable effect on the relative water content (Turkan et al., 2005). Recently, low stomatal conductance was strongly associated with soil moisture stress in tepary bean (Traub et al., 2017). The exposure to soil moisture stress triggers the accumulation of abscisic acid which leads to an efflux of ions from the guard cells followed by stomatal closure (Mori and Murata, 2011) which inadvertently limits photosynthesis by restricting carbon dioxide influx (Chaves, 1991).

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 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 utilized the biplot analysis approach, distinct genotypes were also detected for various crop germplasm including cowpea (Nkoana et al., 2019), white bean (Abel et al., 2019), wheat (Hagos and 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 which 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.

6.0 Chapter Six: Conclusion and Recommendations

Based on the results of this study, tepary bean genotypes showed a wide range of variability for all the traits that were considered before and after soil moisture stress. The PCA revealed three distinct genotypes ('Ac-6', 'Ac-18' and 'Ac-28') 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 prior in the one soil moisture stress regime indicating their diversity in performances 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 soil moisture stress 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. Furthermore, the future selection of tepary bean genotypes that are highly tolerant to drought may also require a concomitant evaluation of their nutritional attributes since it is utilized mainly as a food crop. There could be also some merit in initiatives aimed at developing fodder varieties to optimize food security particularly in the smallholder livestock – cropping farming systems.

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Appendix I

A Short Communication prepared for a local symposium – (July 2021)

A preliminary evaluation of phenotypic traits of tepary bean germplasm

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Abstract

Tepary bean (*Phaseolus acutifolius* A. Gray) is an important grain legume in many cropping systems that are prone to drought. The grain of tepary bean is used mainly for human consumption. In South Africa, unimproved landraces are cultivated mainly in Limpopo Province. To date, there are no significant breeding efforts aimed at cultivar development and the crop remains under-utilized. Therefore, the objectives of this study were to evaluate eight phenotypic traits and their relationships among 42 genotypes of tepary bean in a controlled greenhouse environment. A 6 x 7 rectangular lattice design replicated three times was used in the study. The results indicated that there were highly significant ($P<0.01$) differences in all the phenotypic traits that were measured. The highest number (30) of secondary roots which was observed for genotype 'Ac-39' exceeded the trial mean by 62.87%. In comparison with the check, only three ('Ac-33', 'Ac-39', 'Ac-40') and four ('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. There was a highly significant ($P < 0.01$) positive correlation between the shoot fresh weight and the shoot dry weight among the genotypes 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 secondary root length. These results suggested that the observed phenotypic variability in this germplasm could be exploited for the genetic enhancement of tepary bean. 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 utilization by growers.

Key words: genetic enhancement; germplasm; phenotypic variability; trait

1.0 Introduction

Tepary bean (*Phaseolus acutifolius* A. Gray) (2n = 2x = 22) is a self-pollinating leguminous grain crop originating from the arid and semi-arid region of north western Mexico and south western United States (Nabham and Felger, 1978). Currently, it is cultivated in many African countries including Botswana, Kenya, Malawi, South Africa and Zimbabwe where smallholder farmers use unimproved landraces of the crop (Gwata et al., 2016; Molosiwa et al., 2014). The grain is high (25.0%) in protein and essential mineral elements such as calcium, iron, copper and zinc among others (Bhardwaj and Hamama, 2004). Tepary bean also fixes atmospheric nitrogen thus contributing to the improvement of soil fertility (Mohrmann et al., 2017). Due to 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).

Although tepary bean grows well in hot and dry environments, its productivity may vary among genotypes and environments. Moreover, climate change has increased the frequency of extreme weather patterns including irregular precipitation, which can cause drought stress resulting in significant reductions in crop production thus threatening food security (Lesk et al., 2016). One of the approaches to achieve increased water capture and water use efficiency in legumes is through developing better root systems (Ye et al., 2018). Variability in root traits in legumes was reported in previous studies of chickpea (Kashiwagi et al., 2005), common bean (Beebe et al., 2013) and tepary bean (Butare et al., 2011). However, despite its potential as a major field crop and the abundance of wild relatives, there is no significant breeding effort to date aimed at cultivar development particularly in southern Africa. Consequently, the crop remains under-utilized. Therefore, the objectives of this study were to evaluate eight phenotypic traits and determine their relationships among 42 genotypes of tepary bean in a controlled environment.

2.0 Materials and Methods

Genetic materials

Forty-two genotypes consisting of both large (100-seed weight ≥ 16.0 g) and small seed (100-seed weight ≤ 12.0 g) were used in the study (Table A-1). Most of the seed (>60.0%) was white and only two genotypes (‘Ac-5’ and ‘Ac-8’) possessed black testa (Table A-1).

Table A-1 Descriptors for the tepary bean genotypes that were used in the study. ([§]1Large seed, 100-seed weight ≥ 16.0 g; small seed,100-seed weight ≤ 12.0 g).

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

Testing location, planting and trial management

The study was conducted at the Agricultural Research Council - Vegetable and Ornamental Plants Institute, (25.60°S; 28.35°E), South Africa. The greenhouse temperatures were kept at 30°C during the day and 15°C during the night. Three seeds per genotype were planted manually in the greenhouse in a 155 cm x 77 cm x 23 cm plastic box filled with soil, which was irrigated prior to planting. The seeds were planted at a depth of 4 cm at a spacing of 15 cm between rows and 10 cm within rows. Each genotype consisted of five plants per replication. No chemical or organic fertilisers or pesticides were applied to the plants throughout the season and irrigation was applied as necessary. The weeds were controlled manually.

Phenotypic measurements

At five weeks after germination, two plants per genotype were tagged for identification. In addition, the following phenotypic traits were measured:

(i) number of secondary roots per plant (NSR)

(ii) secondary root length per plant (SRL) (cm)

(iii) root dry weight per plant (RDW) (g)

(iv) root fresh weight per plant (RFW) (g)

(v) primary root length per plant (PRL) (cm)

(vi) shoot height (SH) (cm)

(vii) shoot fresh weight (SFW) (g)

(viii) 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 measured.

Experimental design and data analysis

A 6 x 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 5.0% 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 principle component analysis (PCA) based on the correlation matrix using the Statistical Package for the Social Sciences (SPSS) version 23 (Spss, 2012).

3.0 Results and Discussion

The results showed that there were highly significant (P<0.01) differences in all the phenotypic traits among the tepary bean genotypes (Table 2). The highest NSR (30.0) which was observed for genotype 'Ac-39' exceeded the trial mean by 62.87%. However, only two genotypes ('Ac-4' and 'Ac-29') attained significantly (P < 0.05) higher PRL than the check genotype ('Ac-34') (Table 2). In contrast, when compared with the check, only three ('Ac-33', 'Ac-39', 'Ac-40') and four ('Ac-7', 'Ac-8', 'Ac-40', 'Ac-41') genotypes achieved a significantly (P<0.05) higher SRL and SDW, respectively. In a recent study, the root biomass, showed significant differences between tepary bean types and likely contributed to adaptation to the combined high temperature and acid soil conditions (Adu et al, 2019; Suárez et al., 2022). In addition, increased rooting depth as well as an efficient root system contribute to drought avoidance in legumes (Beebe et al., 2013).

The results also revealed significant (P < 0.05) positive correlations between specific pairs of the phenotypic traits (Table A-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 (Fig. A-1). Similarly, at least 68.0% of the changes in RDW were attributed to the changes in the SRL. 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 grouped into clusters based on their phenotypic trait associations. Genotypes 'Ac-16', 'Ac-24' and 'Ac-38' were clustered close to the origin, suggesting that they possessed a similar genetic relationship for most of the traits. 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 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 utilized as potential parental lines for hybridization in future tepary bean breeding programs 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., 2020; 2021).

Conclusions and recommendations

Firm conclusions based on one season at a single testing location were difficult to draw. Nonetheless, the study affirmed that characterization and evaluation of the tepary bean germplasm for phenotypic traits are useful in discerning genetic variability that can be utilized 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 in order to determine elite germplasm for utilization by tepary bean breeders and growers.

Table A-2 Variability in phenotypic traits among 42 tepary bean accessions (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)).

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
Ac-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	4.65	0.31	0.18	1.33	1.01	13.30
Ac-7	14.00	5.37	6.23	0.65	0.45	2.38	1.87	21.90
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

Table A-3 Pearson's correlation coefficients for eight phenotypic traits among 42 tepary bean genotypes. (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)).

	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

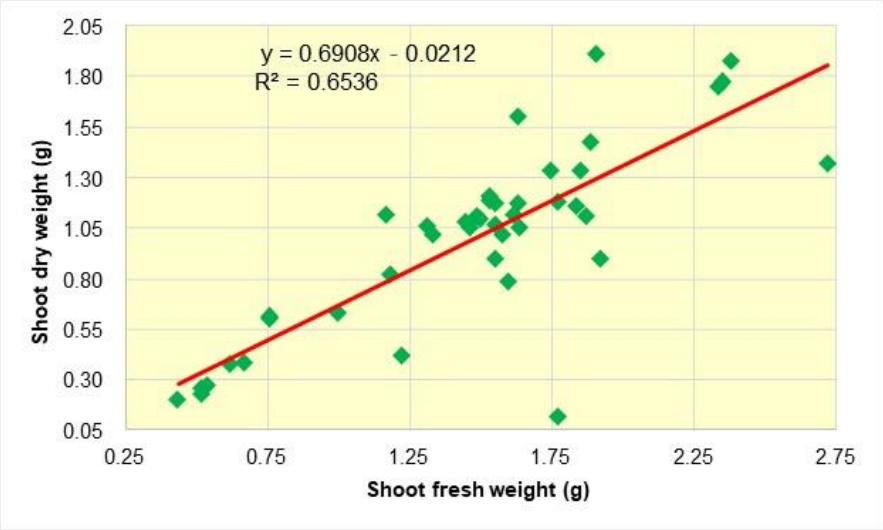

Fig. A-1 The relationship between the shoot dry weight and the shoot fresh weight among 42 tepary bean genotypes.

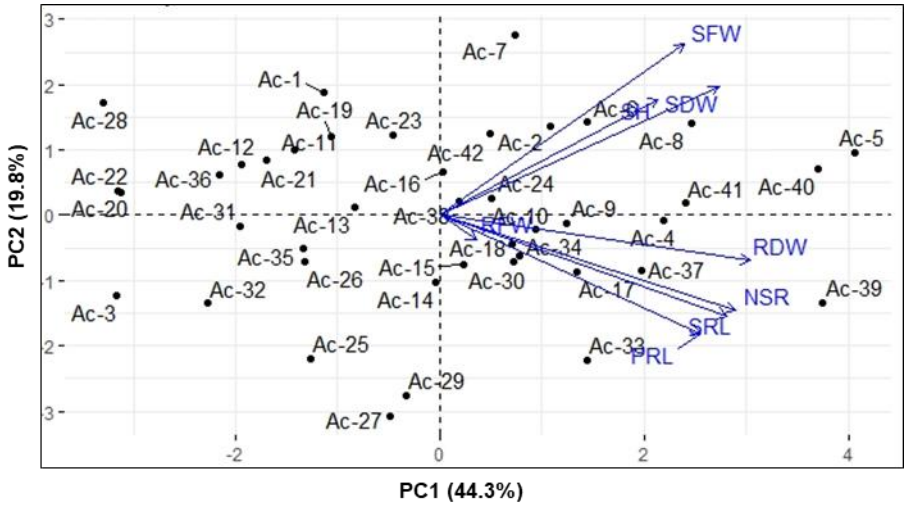

Fig. A-2 Principal component score plot of PC1 and PC2 describing the variation among 42 tepary bean genotypes estimated using the data set of phenotypic traits. (NSR = number of secondary roots; PRL = primary root length; SRL = secondary root length; RFW = root fresh weight; RDW = root dry weight; SFW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

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