UNIVERSITY OF ZULULAND

Morpho-agronomic and genetic variation, and segregation patterns of *Phaseolus vulgaris* landraces from selected provinces of South Africa

by

Valencia Vuyisile Ndlangamandla

201509692

Submitted to the Faculty of Science, Agriculture and Engineering in fulfilment of the requirements for the degree of Masters in the Department of Botany at the University of Zululand

Supervisor: Dr. NR Ntuli

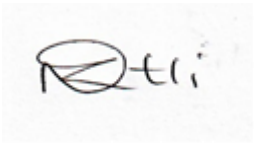
Date submitted: 2021

Declaration

The research described in this dissertation was carried out in the Department of Botany at the University of Zululand, KwaDlangezwa, under the supervision of Dr. N.R. Ntuli. I, **Valencia Vuyisile Ndlangamandla**, declare that the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work. These studies have not otherwise been submitted in any form for any degree or diploma at any University. Where use has been made of work of others, it is duly acknowledged in the text.

Valencia Vuyisile Ndlangamandla

I certify that the above statement is correct.



Dr. N.R Ntuli

Abstract

Phaseolus vulgaris L. (dry beans) of Central American origin is a self-pollinating crop with a low frequency of crossing. It is planted for its edible leaves, immature pods, and dried seeds throughout the world. In South Africa, local communities grow a variety of *P. vulgaris* landraces. Landraces are significant for breeding purposes because they contain important germplasm. However, studies on variation in morphology and genetics among *P. vulgaris* landraces are limited in South Africa. Thus, this study aimed to determine the morpho-agronomic and genetic variations among *P. vulgaris* landraces. *P. vulgaris* landraces collected from the various rural communities of four selected provinces in South Africa were grown in a randomized complete block design with three replications over two seasons.

Significant variations were recorded in germination percentages, vegetative and reproductive traits. The vegetative and reproductive traits correlated positively with each other, and with both traits. The first five informative principal components explained 88.749% and 91.678% of the total variation in the morpho-agronomic and segregation patterns, respectively. The landraces were clustered in a biplot and dendrogram based on their seed coats, shape, similar morpho-agronomic traits, and their area of origin. The 12 parents of *P. vulgaris* produced offspring that are different from their parents in seed colour, shape, and size.

The genetic diversity analysis with simple sequence repeat (SSR) markers revealed the range of genetic diversity, observed heterozygosity, and polymorphic information content as 0.00–0.65, 0.00–0.05, and 0.00–0.58, respectively. The population structure divided the 40 landraces into two subpopulations namely Mesoamerican and Andean gene pools. Although there was considerable overlap among the landraces, numerous Mesoamerican landraces carried certain seed features or genes from the Andean gene pool, indicating a significant amount of mixing. Although, the populations showed an overlap among the landraces as several from the Mesoamerican group carried some seed traits or genes from the Andean gene pool, as they showed a high level of admixture. The grouping of landraces in a principal coordinate analysis (PCoA) and dendrogram had a similar clustering to the population structure. The landraces demonstrating admixture were also grouped in the same cluster (dendrogram) and

similar quadrants (PCoA). The findings of the variance in morpho-agronomic and genetics of *P. vulgaris* landraces can be used to improve, conserve them, and increase their productivity.

Publications

Ndlangamandla, V.V, and Ntuli, N.R, 2019. Variation on growth and yield traits among selected *Phaseolus vulgaris* landraces in KwaZulu-Natal, South Africa. *Biodiversitas*, 20(6):1597–1605.

Ndlangamandla, V.V., Ntuli N.R., 2021. Morpho-agronomic and genetic variation among *Phaseolus vulgaris* landraces from selected provinces of South Africa. *Journal of Crop Science and Biotechnology*. https://doi.org/10.1007/s12892-021-00116-2

Dedication

I dedicate this dissertation to my lovely parents, Dinah Ndlangamandla, and Victor Khumalo for support and encouragement. To my brother Bonginkosi Ndlangamandla for giving me the motive to carry on even when the going gets tough. I am so grateful and blessed to have a family like you in my life. To my supervisor Dr. N.R Ntuli for her guidance, supervision, and encouragement throughout my research.

Acknowledgments

- I am thankful to the National Research Foundation (NRF) and the Botany Department for their financial assistance.
- I wish to express my deep sense of gratitude and heartfelt thanks to my supervisor, Dr. N.R Ntuli for her guidance, supervision, and encouragement throughout my research. I have learned a lot from her, I feel proud and blessed for having her as my supervisor.
- I am grateful to my mentor, Ms. Z. Mbhele for her timely help and assistance during my field trials and data collection.
- My gratitude goes to communities that provided me with the seeds.
- I am thankful to my academic family, Noluthando Nkosi, Nontobeko Zwane, Lungelo Buthelezi, Samkelisiwe Ngubane, Prince Sokhela, and Khulekani Mbatha for their support during my field trials, data collection, and laboratory experiments.
- I am grateful to my friends, Msizi Shandu, Noxolo Nkunzi, Nonkululeko Madonsela, and Nokukhanya Gumede for their support during my field trials and data collection and also their encouragement towards my studies.
- To my family, Bonginkosi Ndlangamandla, Lee-Anne Mntungwa, Pearl Mntungwa, Thabani Ncube, Ntombifuthi Mntungwa, Thandekile Salelwayo, Wonderboy Manana, Njabulo Manana, and my parents (Dinah Ndlangamandla and Victor Khumalo). I can feel their support and love far away. I am grateful for the support you have towards finishing my degree.

Abbreviations

%:	Percentage
^{0}C:	Degrees Celsius
x g:	Gravity force
50% F:	50 percent flowering
µg/mg:	Micrograms per miligrams
µl:	Microliter
µM:	Micromolar
A:	Absent
A:	Adenine
AN:	Allele number
AS:	Allele size
B:	Brown
B:	Benoni
B-50B50M-Cl:	Benoni-50% brown 50% maroon-Cylindrical
Br:	Bushbuckridge
Br- 100LB-Cl:	Bushbuckridge-100% light brown- Cylindrical
Bk:	Black
By:	Brownish yellow
bp:	Base pairs
C:	Cream
C:	Cytosine
CC:	Chlorophyll content
Cl:	Cylindical
cm:	Centimeter
cm^2:	Square centimeters
Cu:	Cuboidal
CV:	Coefficient of variance
D:	Durban
D-50C50Gy-K:	Durban-50% cream 50% grey- Kidney
D-100By-Cl:	Durban-100% brownish yellow- Cylindrical
D-90C10DB-Cu:	Durban-90% cream 10% dark brown- Cuboidal

D-90C10LR-Cl:	Durban- 90% cream 10% light-red- Cylindrical
D-90C10p-Cl:	Durban- 90% cream 10% pink- Cylindrical
D-100C-Cl:	Durban-100% cream- Cylindrical
D-100C-Cu:	Durban- 100% cream- Cuboidal
D-50LB50B-Cu:	Durban- 50% light brown 50% brown- Cuboidal
D-90LB10B-Cu:	Durban- 90% light brown 10% brown- Cuboidal
D-90LB10M-Cu:	Durban- 90% light brown 10% maroon- Cuboidal
D-100LP-Cu:	Durban- 100% light purple- Cuboidal
D-50M50LB-Cl:	Durban-50% maroon 50% light brown- Cylindrical
D-90M10LB-Cl:	Durban-90% maroon 10% light brown- Cylindrical
D-50P50LB-Cl:	Durban- 50% purple 50% light brown- Cylindrical
D-100p-Cu:	Durban- 100% pink- Cuboidal
D-50RB50LB-Cl:	Durban- 50% reddish brown 50% light brown- Cylindrical
DAP:	Days after planting
DB:	Dark brown
DFF:	Days to first flowering
DNA:	Deoxyribonucleic acid
dNTPs:	Deoxyribonucleotide triphosphate
DP:	Dark purple
E:	Eshowe
E-50Bk50C-Cl:	Eshowe-50% black 50% cream-Cylindrical
E-90Bk10C-Cl:	Eshowe-90% black 10% cream-Cylindrical
E-100Bk-Cl:	Eshowe-100% black-Cylindrical
D-50GB50LB-Cl:	Eshowe- 50% greenish 50% light brown- Cylindrical
D-100GB-Cl:	Eshowe- 50% greenish 50% light brown- Cylindrical
E-50Gy50C-Cl:	Eshowe- 50% grey 50% cream- Cylindrical
E-50LR50C-K:	Eshowe-50% light red 50% cream- Kidney
E-90LB10M-Cu:	Eshowe- 90% light brown 10% maroon- Cuboidal
E-50M50C-K:	Eshowe- 50% maroon 50% cream- Kidney
E-90M10C-Cl:	Eshowe- 90% maroon 10% cream- Cylindrical
D-90P10C-Cl:	Eshowe- 90% pink 10% cream- Cylindrical
E-50YG-Cl:	Eshowe-50% yellowish green- Cylindrical
E-100YG-Cl:	Eshowe- 100% yellowish green- Cylindrical
Em:	Empangeni

Em-50B50LB-Cl:	Empangeni- 50% brown 50% light brown- Cylindrical
Em-50Bk50C-Cu:	Empangeni- 50% black 50% cream- Cuboidal
Em-50GB50LB-Cu:	Empangeni- 50% greenish brown 50% light brown- Cuboidal
Em-100LB-Cl:	Empangeni- 100% light brown- Cylindrical
Em-50M50LB-Cl:	Empangeni- 50% maroon 50% light brown- Cylindrical
Em-50P50C-Cl:	Empangeni- 50% purple 50% cream- Cylindrical
Em-100C-Cu:	Empangeni- 100% cream- Cuboidal
Em-100YG-Cl:	Empangeni- 100% yellowish green- Cylindrical
FC:	Flower colour
G:	Greenish
G:	Guanine
GD:	Genetic diversity
Gy:	Grey
g:	Grams
He:	Expected heterozygosity
Ho:	Observed heterozygosity
HSM:	Hundred seed mass
ISM:	Individual seed mass
K:	Kidney
K value:	Kinship matrix
kg:	Kilograms
KN:	KwaNdebele
KN-50B50M-Cl:	KwaNdebele- 50% brown 50% maroon- Cylindrical
KN-100W-Cl:	KwaNdebele- 100% white- Cylindrical
LA:	Leaf area
LB:	Light brown
LC:	Leaf colour
LP:	Light purple
LR:	Light red
LSD:	Least significance differences
M:	Mtubatuba
M:	Maroon
M:	Mottled

M-90LB10M-Cl:	Mtubatuba- 90% light brown 10% maroon- Cylindrical
M-90C10M-Cl:	Mtubatuba- 90% cream 10% maroon- Cylindrical
M-90M10C-Cl:	Mtubatuba- 90% maroon 10% cream- Cylindrical
MAF:	Major allele frequency
m:	Meter
ml:	Mililiter
mg:	Miligram
$MgCl_2$:	Magnesium chloride
mg cm^{-2}:	Milligram per square centimeters
min:	Minute
mm:	Milimeter
mm^2:	Square milimeters
mM:	Milimolar
MPC:	Mature pod colour
N:	Nelspruit
N-100C-K:	Nelspruit- 100% cream- Kidney
N-100DP-K:	Nelspruit- 100% dark purple- Kidney
N-100M-K:	Nelspruit- 100% maroon- Kidney
N-100LP-K:	Nelspruit- 100% light purple- Kidney
NB:	Number of branches
ng:	Nanograms
ng/µl:	Nanogram per microliter
NP:	Number of pods
NSP:	Number of seeds per pod
NSPl:	Number of seeds per plant
O:	Oval
P:	Purple
P:	Polokwane
P-50M50C-O:	Polokwane- 50% maroon 50% cream- Oval
P-90M10LB-O:	Polokwane- 90% maroon 10% light brown- Oval
PCR:	Polymorphic chain reaction
PCoA:	Principal coordinate analysis
P. coccineus:	*Phaseolus coccineus*
PGH:	Plant growth habit

PH:	Plant height
PIC:	Polymorphic information content
PL:	Pod length
P. lunatus:	*Phaseolus lunatus*
pmol:	Picomoles
PS:	Pod Shape
PS:	Port Shepstone
PS-100Byp-Cl:	Port Shepstone- 100% brownish yellow pink- Cylindrical
PS-50DB50LB-Cl:	Port Shepstone- 50% dark brown 50% light brown- Cylindrical
PS-90DB10LB-Cl:	Port Shepstone- 90% dark brown 10% light brown- Cylindrical
PS-90LB10B-Cl:	Port Shepstone- 90% light brown 10% brown- Cylindrical
PS-90LB10M-Cl:	Port Shepstone- 90% light brown 10% maroon- Cylindrical
PS-50M50LB-Cl:	Port Shepstone- 50% maroon 50% lightbrown- Cylindrical
PS-90M10LB-Cl:	Port Shepstone- 90% maroon 10% light brown- Cylindrical
PS-100YG-Cl:	Port Shepstone- 100% yellowish green- Cylindical
P value:	Probability value
P. vulgaris:	*Phaseolus vulgaris*
PW:	Pod width
RB:	Reddish brown
S:	Sample size
S:	Stripped
SC:	Stem colour
SCC:	Seed coat colour
SCP:	Seed coat pattern
SD:	Seed diameter
SG:	Seed germination
SL:	Seed length
SP:	Seed pattern
Sp:	Spotted

SS:	Seed shape
SSRs:	Simple sequence repeats
ST:	Seed thickness
SW:	Seed width
T:	Thymine
rpm:	Revolutions per minute
W:	White
YG:	Yellowish green

Table of contents

Content Page

List of tables

List of figures

Chapter 1

1. Introduction

Phaseolus vulgaris L. of Central American origin (Gioia *et al*., 2019), is an important food legume in the *Fabaceae* family (Mayo-Prieto *et al*., 2019). It is commonly known as the common bean, string bean, field bean, French bean, and kidney bean (Musango *et al.*, 2016). It is also a self-pollinating crop with a low frequency of crossing (Burle *et al*., 2010). *P. vulgaris* is regarded as an important field crop in South Africa (Muedi *et al*., 2015). The major South African provinces for small-scale farming of *P. vulgaris* production are the Eastern Cape, KwaZulu-Natal, and Mpumalanga (Fourie, 2002). It provides a cheap source of protein to people in developing countries (Jannat *et al*., 2019). *P. vulgaris* is a very nutritional crop because of its high protein content and a high quantity of fiber provides vital nutrients and complex carbohydrates (Guidoti *et al*., 2018). Landraces are varieties of plants domesticated from the wild through natural and artificial selection (Abdollahi *et al*., 2016). Landraces help small-large scale farmers or agricultural programs to adapt to new challenges such as climate change (Padilla-Chacón *et al*., 2019).

The germination of *P. vulgaris* is both hypogeal and epigeal (Musango *et al.*, 2016). *P. vulgaris* shows either climbing or bushy growth types, where plants with a climbing growth habit are usually taller and produce more numerous branches than the bushy plants (Stoilova *et al*., 2005; Checa *et al*., 2006). During the vegetative phase, the plant height of common bean landraces range from 20 to 123 cm (Stoilova *et al*., 2005). The leaf colour is either light green, green, or dark green, and the shape is either oval or triangular (Loko *et al*., 2018). The flower colour is either white, cream, scarlet, red, yellow, white-pink, purple, or light purple (Romero-Arenas *et al.*, 2013; Musango *et al.*, 2016).

The pod colour is either black, purple, green, or red (Loko *et al*., 2018). The number of seeds per pod ranges from four to five (Musango *et al.*, 2016). The pod shape is either straight, curved, or slightly curled. Curled pods are shorter (6 cm) whereas straight pods are longer (13 cm) (Loko *et al*., 2018). The seed colour is either white,

red, yellow, brown, black, purple, or grey (Jannat *et al*., 2019). The seed shapes can be as follows: round, oval, elliptical, rhomboid, kidney-shaped, or cylindrical shape (Romero-Arenas *et al.*, 2013). An increase in days to 50% flowering, plant height, number of seeds per plant, pod length, and seed weight is associated with an increase in seed yield (Negahi *et al.*, 2014).

Genetic variability in landraces is crucial to determine the existing diversity, identify genotypes adapted to the climatic conditions of specific environments, and support improvement strategies (Cabral *et al*., 2018). Estimates of genetic diversity can be obtained by using DNA markers such as simple sequence repeats (SSRs) also known as microsatellites (Gioia *et al*., 2019). Simple sequence repeats are defined as small stretches of repeated DNA usually of two to six nucleotides (Mishra *et al*., 2014). Simple Sequence repeats are frequently used to determine genetic diversity in *P. vulgaris* because of their abundance and even distribution in the genome, their co-dominant inheritance, as well as their high levels of polymorphism and reproducibility (Gioia *et al*., 2019).

1.1 Problem statement

Phaseolus vulgaris is a small- and large-scale crop grown in South Africa. It occupies an important place in human nutrition, especially in the diets of low-income earners of developing countries. *P. vulgaris* is considered the poor man's meat. There are many *P. vulgaris* landraces grown by local communities in South Africa. Landraces contain important germplasm for breeding purposes. However, there are no records of morpho-agronomic and genetic variation among these landraces in the country. Therefore, this study focuses on identifying different *P. vulgaris* landraces grown by local communities and determining their morpho-agronomic and genetic variation.

1.2 Aim(s) of the study

This study aimed to investigate the morpho-agronomic and genetic variability among *Phaseolus vulgaris* landraces from selected provinces of South Africa.

1.3 Research objectives

The objectives of this study were to determine:

- Variation in growth and yield traits among *Phaseolus vulgaris* landraces of selected provinces of South Africa.
- Variation in seed coat colour, pattern, and size among the parents and progenies of *Phaseolus vulgaris* landraces.
- Genetic relationships among *Phaseolus vulgaris* landraces revealed by simple sequence repeat (SSR) markers.

1.4 Research questions

The research questions of the study were as follows:

- How will the growth and yield characteristics vary among *Phaseolus vulgaris* landraces from selected provinces of South Africa?
- How will the seed coat colour, pattern, and size vary among the parents and progenies of *P. vulgaris* from selected provinces of South Africa?
- What is the genetic relationship among *Phaseolus vulgaris* landraces from selected provinces of South Africa?

1.5 Hypotheses

HA_1: There is a high variation in growth and yield traits among *Phaseolus vulgaris* landraces from selected provinces of South Africa.

$H0_1$: There is a low variation in growth and yield traits among *Phaseolus vulgaris* landraces from selected provinces of South Africa.

HA_3: Parents and progenies from selected provinces of South Africa have high variation in their seed coat colour, pattern, and size.

$H0_3$: Parents and progenies from selected provinces of South Africa have low variation in their seed coat colour, pattern, and size.

HA_2: *Phaseolus vulgaris* landraces from selected provinces of South Africa vary widely in their genetic makeup.

$H0_2$: *Phaseolus vulgaris* landraces from selected provinces of South Africa do not vary in their genetic constituents.

Chapter 2

2. Literature review

This literature briefly reviews the taxonomy and uses of *Phaseolus vulgaris* L. It also defines landraces and states the types of landraces that exist. It further reviews the variation among growth and yield traits and also the genetic analysis of *P. vulgaris* landraces using simple sequence repeat (SSR) markers.

2.1 Taxonomy, domestication, and uses

Phaseolus vulgaris L. is commonly known as common bean, string bean, field bean, French bean, and kidney bean (Musango *et al*., 2016). It belongs to subclass Rosidae, order Fabales, family Fabaceae and subfamily Papilionoidea (Romero-Arenas *et al.*, 2013; Ntuli, 2018). *P. vulgaris* is a warm-season legume and is a self-pollinating species with a low average cross-pollinating rate (3%) (Ntuli, 2018). Domestication is an extensive process that transforms a wild plant into a crop (Bitocchi *et al*., 2017).

P. vulgaris originated in Mexico approximately four to six million years ago (Bitocchi *et al*., 2017) and was domesticated in Mesoamerica and Andes 8000 years ago (Cichy *et al*., 2015). *P. vulgaris* is an important crop in many developing countries (Darkwa *et al*., 2016) and is regarded as an important field crop in South Africa (Muedi *et al*., 2015). *P. vulgaris* is produced commercially in Mpumalanga (56%), Free State (28%), North West (7%), KwaZulu-Natal (5%), and Gauteng (4%) provinces. The major areas for small-scale farmer *P. vulgaris* production are Mpumalanga, Eastern Cape, and KwaZulu-Natal (Fourie, 2002).

Summer and spring are the seasons suitable for the cultivation of *P. vulgaris* with optimum growth temperature ranging from 16^0 to 30^0 C (Ntuli, 2018). The leaves are commonly cooked as a vegetable (Nahar *et al*., 2009). The pods are cooked like green beans and they provide minerals and nutrients (Saleem *et al*., 2016). The seeds are a

powerful source of elements needed for healthy nutrition in human beings (Iriti *et al*., 2019). *P. vulgaris* has a high content of protein, carbohydrates, vitamins, minerals, and fibre fibre (Darkwa *et al*., 2016). In many developing countries it serves as a substitute for meat due to its high iron content (Ntuli, 2018).

2.2 Landraces of *Phaseolus vulgaris*

Landraces, also known as local genotypes, are populations generated through natural or artificial selection (Ekbic and Hasancaoglu, 2018). Landraces are members of a crop species with wide genetic diversity, which usually have identifiable characteristics, are given local names, and have not undergone proper crop improvement (Stoilova *et al*., 2013; Abdollahi *et al*., 2016). Landraces are classified into primary and secondary landraces. Primary landraces are crops that have never been subjected to formal plant breeding and have developed their unique characteristics in their original environment (Zeven, 1998).

Primary landraces are further classified into autochthonous and allochthonous landraces (Zeven, 1998). An autochthonous landrace is a variety that is grown in the original location, where it developed its unique characteristics, and allochthonous is an introduced variety that is locally adapted but that has developed its unique characteristics through grower selection in another region (Casañas *et al*., 2017). Secondary landraces are the ones that developed in the formal breeding sector and are likely to be genetically distinct from the original bred material (Zeven, 1998).

Landraces play an important role in ensuring quality and well-managed crops in agricultural production (Casañas *et al*., 2017). They act as a source of genetic diversity and specific traits are often used by plant breeders to create new variation (Ntuli, 2018). *P. vulgaris* landraces are associated with intermediate to high yield and also contribute to food security (Jannat *et al*., 2019). Landraces act as a source of traits for more efficient nutrient uptake and utilization, as well as for useful genes for adaptation to stressful environments such as water stress and high temperatures (Kouam *et al*., 2017).

The landraces are distinguished into two gene pools namely Mesoamerican and Andean (Gioia *et al*., 2019). In the domestication process, the two gene pools show differences in agronomic traits such as seed size, phaseolin, shape, and growth habit (Lei *et al*., 2020). The Andean gene pool is composed of large-seeded (> 40 g 100 seed mass) light and dark red kidney, white kidney, yellow, green, and bushy cranberry beans. While the Mesoamerican gene pool includes either small (< 25 g 100 seed mass) or medium (25–40 g 100 seed mass) with seed coat colour of black, white, navy, reds, and pinks (Elsadr *et al*., 2011; Gioia *et al*., 2019). The Mesoamerican gene pool has greater genetic diversity compared with the Andean (Gioia *et al*., 2019). Several landrace genes have been related to the origins of domestication. Several landrace genes have been related to the origins of domestication (Bitocchi *et al*., 2017).

2.3 Morphological traits

2.3.1 Germination percentage

Germination is the most important stage in plant life, which determines successful crop production (Aguilar-Benítez *et al*., 2014). Seed germination percentage depends on environmental factors such as optimum temperature, light, salinity, and water availability (Ntuli, 2018). In the Ugandan *P. vulgaris* landraces, the seed germination showed consistency where all the landraces emerged five days after planting (Okii *et al*., 2014). Germination percentages range among *P. vulgaris* landraces from Nepal from 84.0–93.8% (Kalauni *et al*., 2019) and the landraces from Brazil range from 89–94% (Lima *et al*., 2005).

2.3.2 Stem traits

P. vulgaris can either have a climbing, semi-climbing, erect, or bushy growth habit (Abdollahi *et al*., 2016; Loko *et al*., 2018). Their growth habit can either be determinate or indeterminate. The *P. vulgaris* growth habits are classified into four major classes, namely: plants with terminal reproductive buds, determinate erect branches and without climbing ability (Group 1); erect growth but terminal vegetative growing buds are indeterminate (Group 2); indeterminate but more prostrate with terminal growth

buds (Group 3); and tall indeterminate plants with long vines, terminal vegetative buds and strong climbing ability (Group 4) (Checa *et al*., 2006).

The stem colour is either green, green with pink pigmentation, or green with purple pigmentation (Loko *et al*., 2018). The stem diameter of *P. vulgaris* from Valencia, Spain shows variation with a range of 2.75–8.40 mm (Arteaga *et al*., 2019). While the stems of Nigerian landraces range from 2.3 to 2.8 cm (Nwadike and Terkimbi, 2015). In Portugal and Bulgaria, *P. vulgaris* landraces are either shorter (19.5 cm) or taller (121.3 cm), where the climbing landraces are taller than the bushy ones (Stoilova *et al*., 2013). However, the plant height of Turkish landraces ranges from 25.25 to 361.50 cm (Yeken *et al*., 2019).

The number of branches in the Turkish landraces is either fewer (3.20) or numerous (10.78) (Yeken *et al*., 2019), or ranges from 4 to 14 in Uganda (Okii *et al*., 2014). Almost all landraces with climbing growth habits have numerous branches compared with those with bushy growth habits (Stoilova *et al*., 2005).

2.3.3 Leaf traits

The number of leaves per plant among Nigerian *P. vulgaris* landraces ranges from 18.5–37.8 (Nwadike and Terkimbi, 2015). The leaf area can be either broad 3030 mm^2 or narrower 1333 mm^2 among landraces in Ethiopia (Yohannes *et al*., 2020). The leaf chlorophyll content has variation from 47.1 to 51.3 $nmol/cm^2$ among *P. vulgaris* landraces from Ethiopia (Egu and Tesfaye, 2018). However, the chlorophyll content of landraces from Romania ranges from 13.71 to 28.49 (Modiga and Jitareanu, 2017).

2.3.4 Flower traits

The colour of flowers varies among Mexican and Ugandan *P. vulgaris* landraces and can be either white, scarlet, pink, purple, light purple, or white with lilac edges or with red stripes (Okii *et al*., 2014; Ekbic and Hasancaoglu, 2018). Days to first flowering among the landraces also varies from 41 to 55 days in Turkey (Ekbic and Hasancaoglu, 2018) and 33 to 41 days in Zimbabwe for days to 50% flowering

(Musango *et al*., 2016). However, the days to first flowering range from 43 to 56 days among the Ethiopian landraces (Yohannes *et al.*, 2020).

2.3.5 Pod traits

The colour of immature pods is either pure green, green with purple, carmine, red or purple, whereas the mature pods are either yellow, yellow with purple, carmine, red or pink stripes (Ntuli, 2018). The pod shape varies from straight, slightly-curled to curled (Loko *et al*., 2018). The number of pods per plant had a wide variation among the *P. vulgaris* landraces in Portugal and Bulgaria with a range of 6.4–20.8 (Stoilova *et al*., 2005). The pods are either shorter (6 cm) or longer (13 cm) and are either narrower (0.8 cm) or wider (1.0 cm) among the *P. vulgaris* landraces from India (Singh *et al*., 2017). Furthermore, the pod length and width of landraces from Benin ranges from 55–266.7 mm and 11.3–19.0 mm, respectively (Loko *et al*., 2018).

2.3.6 Seed traits

The seed coat's main colours of *P. vulgaris* landraces are as follows: white, cream, brown, yellow, green, yellowish-green, red, black, purple, or bicolour (Gioia *et al*., 2019). The seeds are either round, oval, kidney, or cuboidal in shape (Loko *et al*., 2018). The seed coats are either shiny, intermediate, or opaque (Ntuli, 2018). The seed width is either narrower (5.26 mm) or wider (10.04 mm) among the landraces in India (Dutta *et al*., 2016). The seeds are also either shorter (10.0 mm) or longer (16.7 mm), thinner (4.2 mm), or thicker (8.2 mm) in Iran (Marzooghian *et al*., 2013). The seed size of Ethiopian *P. vulgaris* landraces has comparable ranges of 9.04–15.97 mm in seed length, 5.81–10.21 mm in width, and 4.18–8.46 mm in seed thickness (Bareke, 2019). The number of seeds per pod among the landraces ranges from 3–6 seeds in Zimbabwe (Musango *et al*., 2016). The number of seeds ranges from 10–62 seeds per plant in Iran (Marzooghian *et al*., 2013). The hundred seed mass of *P. vulgaris* is either lighter (29.82 g) or heavier (55.35 g) among the landraces in Turkey (Yeken *et al*., 2018).

2.4 Segregation patterns of seed coat colours of *Phaseolus vulgaris*

Seed coat colour is one of the most significant traits of *P. vulgaris* (Zhu *et al*., 2017). The seed coat colours of *P. vulgaris* are classified as white, black, yellowish, green, brown, purple, red, and grey (Loko *et al*., 2018). The pigments responsible for the wide variations in colour of seed coats are tannins, flavonoids, and anthocyanins (Caldas and Blair, 2009). The variation in seed coat colours within *P. vulgaris* landraces are caused by mutation followed by segregation (Musango *et al*., 2016). The seeds are domesticated from two gene pools Mesoamerican and Andean in Central and South America respectively (Cichy *et al*., 2015; Musango *et al*., 2016). The large-seeded light and dark red kidney, white kidney, bush cranberry, most green, and yellow beans are classified in the Andean gene pool. Whereas, the Mesoamerican gene pool includes the small-seeded black, white, and navy beans (Giola *et al*., 2019).

2.4.1 Factors influencing seed coat colours of *Phaseolus vulgaris*

The factors for *P. vulgaris* for seed coat colour are classified as the following: the Ground-factor gene, complementary colour gene, and modifying genes (intensifying factors) (Zhu *et al*., 2017). The Ground-factor gene is a dominant gene, usually indicated as *P,* is necessary for the plant to be able to produce seed coat colour (McClean *et al*., 2018). Complementary genes also known as chromogen factors are dominant genes that complement basic genes to produce a pale seed coat colour (McClean *et al*., 2002). Modifying genes are genes that interact with colour genes to form different seed coat colours. Modifying genes have intensifying effects (darkening effects) upon pale colours formed by the action of the colour genes but do not impact colour in them (Musango *et al*., 2016).

There are different gene symbols used by various researchers for the same gene (Bassett, 2003). The dominant *P* allele is believed to be the most significant basal colour gene or ground-factor for seed colouration and has multiple alleles that modify seed colour in various ways (Zhu *et al*., 2017). The recessive *p* allele is pleiotropic to other genes and *pp* homozygotes produce white seeds together with white flowers (McClean *et al*., 2018). The *C*, *T*, *D*, and *J* genes for seed coat colour are only

expressed in the presence of gene *P,* which is hyperstatic to all seed coat colour and pattern genes for both seed coat and flower colours (Caldas and Blair, 2009).

The *C* gene is known as the primary locus for seed coat pattern, has multiple alleles, and exists in a complex locus with the *R* gene for dominant red seed coat, the genes are usually presented as [*R-C*] (Bassett, 2003; Zhu *et al*., 2017). The linkage between the two genes ([*R-C*]) is unbreakable, hence they are presented in brackets (Bassett, 2003). The pattern gene *T* is required to express seed coats with partly coloured patterns (coloured vs white) (Bassett and Miklas, 2007). Gene *D* influences the hilum colour, the hilum colour is controlled by various genes that control seed coat colour (Caldas and Blair, 2009). The dominant *J* gene represents the seed coat colour for fully developed immature seeds, and the recessive *j* produces pale coat colour for immature seeds (Grahic *et al*., 2013).

Genes *G*, *B*, *V*, and *Rk* are modifier genes (Caldas and Blair, 2009). The modifier gene *G* from Gelbe stands for yellow in German, and gene *B* for brown expressing mineral brown (Bassett, 2003) and also expresses yellow to yellowish-brown (Grahic *et al*., 2013). The *G* also stands for colour modifying gene for greenish to greenish-yellow (Grahic *et al*., 2013). The seed coats of *V* genotypes contain anthocyanin pigments whereas the seed coats of *vv* genotypes contain flavonol pigments (McClean *et al*., 2002). The modifier gene *V* stands for the violet factor known to control the amounts of anthocyanins made in the seed coats of dark coloured (bluish, violet to black) *P. vulgaris* (Zhu *et al*., 2017). The red kidney locus *Rk* controls the recessive red seed coat colour (light red colour) and garnet brown (dark red kidney) (Bassett, 2003).

The seed pattern of *P. vulgaris* is controlled by the action of two independent genes, *C* and *T*. Dominant *C* is a complex locus consisting of many linked genes responsible for the different seed patterns such as striping, specking, and mottling. The dominant *T* with recessive *tt* genotype along with epistatic interactions produce partially coloured seed coat patterns (McClean *et al*., 2002). The inheritance of seed coat colour is controlled by two dominant genes where black seed colour was hyperstatic to white seed colour (Hacisalihoglu and Settles, 2013). In allelic terms of black seed is controlled by combination *AABB* and white seed coat colour is controlled by *aabb.*

White seed coat colour needs recessive homogenous genes in a locus while black seed colour is influenced by *P* or *A* (dominant factor) (Zhu *et al*., 2017).

2.5 Genetic analysis

Molecular markers are DNA sequences that are readily detected and whose inheritance can be monitored (Mishra *et al*., 2014). Molecular markers are useful because they can reveal differences between individuals of the same or different species (Gioia *et al*., 2019). They have been used to tag genes of economic importance in many crop species (Vidak *et al*., 2017). Deoxyribo Nucleic Acid markers are divided into the following classes: hybridization-based, polymerase chain reaction (PCR) based, and DNA sequence-based (Mishra *et al*., 2014). Molecular markers are an important tool to describe and determine genetic diversity among *P. vulgaris* (Vidak *et al*., 2017).

2.5.1 Simple sequence repeat analysis

Simple sequence repeats (SSRs) also known as microsatellites are defined as small stretches of repeated DNA usually of two to six nucleotides (Mishra *et al*., 2014). They are tandemly repeated and located in a given pattern between segments of non-repeated DNA (Gioia *et al*., 2019). Simple sequence repeats are ideal for distinguishing between closely related germplasm, and are highly informative markers that detect length polymorphisms at loci with simple sequence repeats (Vidak *et al*., 2017). Their advantages for diversity studies include uniform genome coverage, high levels of polymorphism, codominance, and an easy-to-implement specific PCR-based assay (Gioia *et al*., 2019).

Simple sequence repeats are commonly composed of mononucleotide (A), dinucleotide (AT), trinucleotide (ATC), and tetranucleotide (AGGT) repeats (Córdoba *et al*., 2010; Vidak *et al*., 2017). Based on their location in the genome, SSRs can be classified as nuclear (nuSSR), mitochondrial (mtSSR), or chloroplastic (cpSSR) (Vidak *et al*., 2017). Simple sequence repeats can be easily characterized in almost any molecular laboratory with inexpensive equipment and primer pairs that can be

electronically distributed and readily ordered from many sources (Córdoba *et al*., 2010).

The SSR motifs $(ATA)_8$, $(CA)_{12}$, $(CAC)_8$, and $(GA)_1$ are enriched at the 15 microsatellite loci and a *P. vulgaris* genomic library. The first set of 68 SSRs has been obtained using GA enriched genomic libraries. Thus SSRs are concluded to be a valuable genetic marker for assessing genetic diversity in common bean and useful for mapping and also molecular characterization (Vidak *et al*., 2017). The characterization of the common bean genome is necessary for understanding the genes and molecular processes involved in agronomic adaptation, nutritional traits, disease or insect resistance, drought tolerance, and also yield (Córdoba *et al*., 2010).

Simple sequence repeats are mainly composed of dinucleotide repeats with the AT/TA and AG/TC. Trinucleotide motifs are the next most common with many being AT-rich such as the ATA/TAT and AGA/TCT. These microsatellite loci along with AT/TA and AG/TC motifs tend to be the longest SSRs (Córdoba *et al*., 2010).

2.5.2 Genetic traits

2.5.2.1 Number of alleles of Phaseolus vulgaris

An allele is a variant of a particular gene that brings variations to a specific trait (Pashley, 1994). Due to their multi-allelic nature, SSRs are useful resources for genetic diversity and relationship evaluation, marker-assisted selection, breeding, genetic linkage, and population genetics (Kapoor *et al*., 2020). A total of 72 alleles is detected in *P. vulgaris* landraces from Turkey, and the number of alleles per locus ranges from one to six with an average number of 2.4 alleles per locus (Bilir *et al*., 2019). More than 97% of the amplification products show polymorphism, indicating high variation at the DNA level among the landraces (Bilir *et al*., 2019).

In *P. vulgaris* from Turkey, there is a high polymorphism with a mean of 14.8 alleles per locus and ranges from 6 to 29 alleles in the germplasm (Bilir *et al*., 2019). The total number of alleles in the Andean and Mesoamerican groups among the Italian *P.*

vulgaris is 343 with an average of 5.6 alleles per locus with all SSR markers analyzed being polymorphic ranging from 2 to 22 alleles (Gioia *et al*., 2019). The frequency of the major allele ranges from 0.17 to 0.81 with a mean of 0.46 among the landraces from Southern Italy (Scarano *et al*., 2014).

2.5.2.2 Polymorphic information content (PIC), expected and observed heterozygosity

The genotypic variances within species of the marker, which could be a relative amount ranging from zero to one, are known as polymorphic information content (PIC).Avval, 2017). The SSRs with a higher number of repeats tend to be more polymorphic (Kapoor *et al*., 2020). The PIC value ranging from 0.00 to 0.50 means that there is no allelic variation and when the PIC is almost 1.00, agreater variety of alleles is indicated (Avval, 2017). The PIC of the Brazilian landraces ranges from 0.00 to 0.58 with a mean of 0.42 (Burle *et al*., 2010). The PIC values ranged from 0.055 to 0.721 over 13 loci and seven loci had a PIC greater than 0.5 with a mean value of 0.492 among the *P. vulgaris* landraces in China (Wang *et al*., 2012).

Heterozygosity describes the probability that a random individual chosen from the population is different at a locus (Nazareno *et al*., 2017). The expected heterozygosity and observed heterozygosity ranged from 0.057 to 0.814 and from 0.026 to 0.531 with mean values of 0.453 and 0.165, respectively in the Chinese landraces (Wang *et al*., 2012). The expected heterozygosity (H_e) values range from 0.480 to 0.910. The observed heterozygosity values range from 0.108 to 0.971 among *P. vulgaris* in Turkey (Bilir *et al*., 2019). The overall mean observed heterozygosity is 0.452 with the highest heterozygosity value of 0.971 and the lowest value of 0.108 among Turkish landraces (Bilir *et al*., 2019). The observed heterozygosity (H_o) is low ranging from 0.000 to 0.099 with a mean value of 0.006 across all markers in the Italian *P. vulgaris* landraces (Gioia *et al*., 2019).

The division of a basic population into smaller groups as a result of the gene flow between individuals to as population structure Nkhata *et al*., 2020). Population structure is essential in the context of genetic diversity and breeding to examine the

genetic composition and relatedness of the individuals within each group (Lei *et al*., 2020).

Chapter 3

Variation in growth and yield traits among *Phaseolus vulgaris* landraces from selected provinces of South Africa.

Part of this chapter was published as follows:

- Ndlangamandla, V.V., Ntuli, N.R., 2019. Variation on growth and yield traits among selected *Phaseolus vulgaris* landraces in KwaZulu-Natal, South Africa. *Biodiversitas*, 20(6):1597–1605.
- Ndlangamandla, V.V., Ntuli, N.R., 2021. Morpho-agronomic and genetic variation among *Phaseolus vulgaris* landraces from selected provinces of South Africa. *Journal of Crop Science and Biotechnology*. https://doi.org/10.1007/s12892-021-00116-2

3.1 Introduction

Phaseolus vulgaris is a crop plant of the Fabaceae family that is domesticated from Central America (Maras *et al*., 2013). It is the most significant legume with vigorous growth and a high total yield (Ntuli, 2018). It is grown worldwide for its edible green immature pods and dry seeds (Gioia *et al*., 2019). *P. vulgaris* is a primary source of protein, fiber content, complex carbohydrates, iron, zinc, magnesium, and potassium in both developing and developed countries (Guidoti *et al*., 2018). A landrace is defined as populations generated through natural or artificial selection (Ekbic and Hasancaoglu, 2018). *P. vulgaris* landraces are characterized by seed size, colour, and pattern (Gioia *et al*., 2019).

P. vulgaris landraces vary in their vegetative and reproductive traits. Germination percentage among the landraces ranges either from 84.0 to 93.8 (Kalauni *et al*., 2019). Their growth habit is either climbing, semi-climbing, erect, or bushy (Abdollahi *et al*., 2016; Loko *et al*., 2018). The colour of the stems is either green, green with pink pigmentation, or green with purple pigmentation (Loko *et al*., 2018). The stem diameter of the Egyptian *P. vulgaris* landraces differs significantly as some have thinner (4.2 mm) or thicker stems (10.5 mm) (Nassar *et al*., 2010). In Portugal and Bulgaria, some *P. vulgaris* landraces plants have shorter stems (19.5 cm) whereas others have taller stems (123.4 cm) (Stoilova *et al*., 2005). *P. vulgaris* landraces in Uganda have numerous branches or fewer branches, 14 and 4 respectively (Okii *et al*., 2014).

The plants of *P. vulgaris* landraces in Egypt have either fewer leaves (20) or numerous leaves (28). They also have either narrower (2038.4 mm^2) or broader leaves (2956.6 mm^2) (Nassar *et al*., 2010). The colour of flowers of *P. vulgaris* landraces are the following: white, scarlet, pink, purple to light purple (Ekbic and Hasancaoglu, 2018) or white with lilac edges or with red stripes (Okii *et al*., 2014). *P. vulgaris* landraces in Turkey require from 41 to 55 days to flower (Ekbic and Hasancaoglu, 2018).

The pod shape is either straight, slightly curled, or curved (Ekbic and Hasancaoglu, 2018). The seeds of *P. vulgaris* landraces are either white, cream, brown, yellow,

green, yellowish-green, red, black, and purple or bicolour (Loko *et al*., 2018). The seeds of *P. vulgaris* are either narrower (5.26 mm) or wider (10.04 mm) in India (Dutta *et al*., 2016). The seeds are also either longer (16.7 mm) or shorter (10.0 mm), thinner (4.2 mm), or thicker (8.2 mm) in Iran (Marzooghian *et al*., 2013). The seeds among the *P. vulgaris* landraces in Turkey are either lighter (29.82 g) or heavier (55.35 g) (Yeken *et al*., 2018).

There are many *P. vulgaris* landraces grown by rural communities in South Africa. Few studies have reported the diversity of morpho-agronomic traits of *P. vulgaris* landraces, but no literature has recorded such variation among these landraces in the country. Therefore, the objective of this study focused on evaluation of the variation in morpho-agronomic traits among *P. vulgaris* landraces from selected provinces of South Africa, which will contribute significantly towards hunger alleviation, nutritional benefits, and income generation, particularly for rural communities.

3.2 Materials and Methods

3.2.1 Study site and field trial design

Seeds of *P. vulgaris* landraces were collected from rural communities of KwaZulu-Natal: Durban (29.8587^0 S, 31.0218^0 E), Empangeni (28.7532^0 S, 31.8935^0 E), Eshowe (28.8947^0 S, 31.4628^0 E), Mtubatuba (28.4059^0 S, 32.2143^0 E) and Port Shepstone (30.7277^0 S, 30.4473^0 E), Limpopo: Polokwane (23.8962^0 S, 29.4486^0 E), Mpumalanga: Bushbuckridge (24.8398^0 S, 31.0464^0 E), KwaNdebele (25.2542^0 S, 28.4230^0 E) and Nelspruit (25.4753^0 S, 30.9694^0 E) and Gauteng: Benoni (26.1511^0 S, 28.3696^0 E) provinces (Figure 3.1). Table 3.1 and Figure 3.2 describes the 38 landraces used in this study, whose names are created from the: area of the collection - percentage of seed coat colour - seed shape. The study was conducted at the University of Zululand, KwaDlangezwa campus, Orchard Unit farm (28.8524^0 S, 31.8491^0 E) (Figure 3.1). The landraces were sown from August to November. *P. vulgaris* landraces were planted in a randomized complete block design with three replications (Table 3.2).

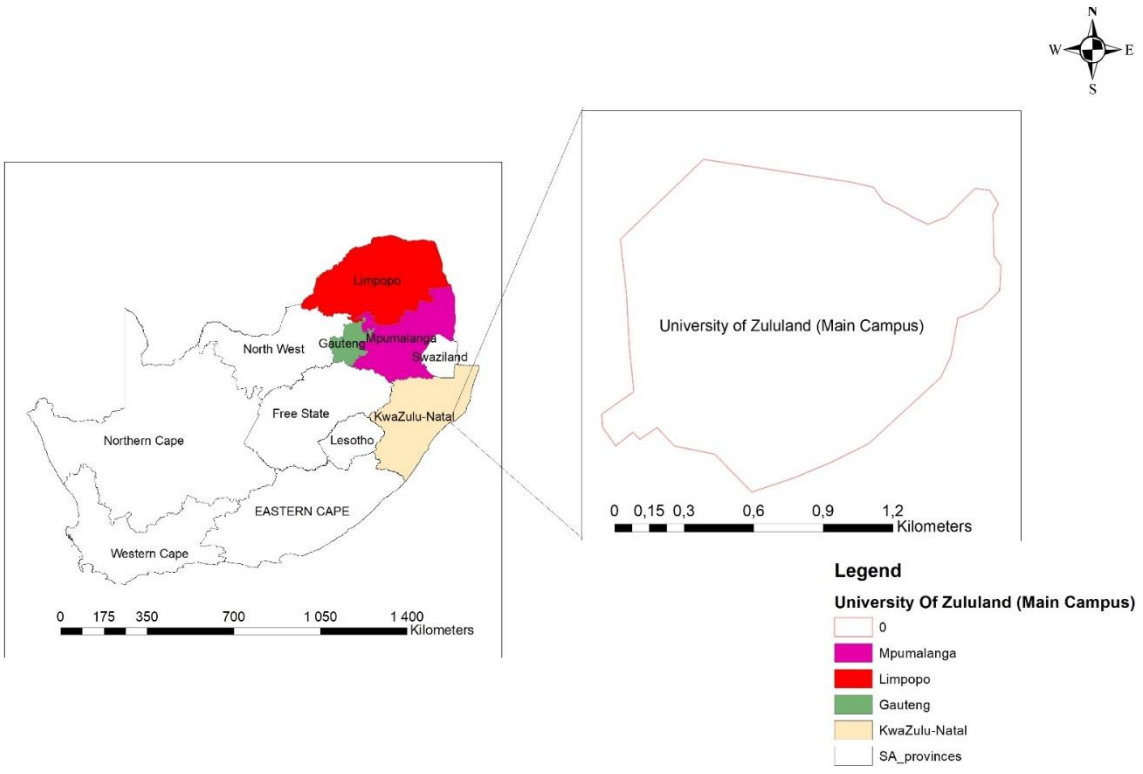


Figure 3.1: Study site and areas of collection.

Table 3.1: *Phaseolus vulgaris* landraces, area of origin, and seed morphology.

No	Landraces	Area of collection	Seed coat colour percentage	Seed pattern	Seed size	Seed shape
1	*B-50B50M-Cl*	Benoni	50% B 50% M	stripped	Medium	Cylindrical
2	*Br- 100LB-Cl*	Bushbuckridge	100% LB	absent	Large	Cylindrical
3	*D-100By-Cl*	Durban	100% By	absent	Medium	Cylindrical
4	*D-50C50Gy-K*	Durban	50% C 50% Gy	spotted	Small	Kidney
5	*D-90C10LR-Cl*	Durban	90% C 10% LR	stripped	Medium	Cylindrical
6	*D-100C-Cl*	Durban	100% C	absent	Medium	Cylindrical
7	*D-90LB10B-Cu*	Durban	90% LB 10% B	stripped	Small	Cuboidal
8	*D-50M50LB-Cl*	Durban	50% M 50% LB	stripped	Large	Cylindrical
9	*D-90M10LB-Cl*	Durban	90% M 10% LB	spotted	Large	Cylindrical
10	*D-50P50LB-Cl*	Durban	50% P 50% LB	mottled	Large	Cylindrical
11	*D-50RB50LB-Cl*	Durban	50% RB 50% LB	mottled	Medium	Cylindrical
12	*D-100YG-Cl*	Durban	100% YG	absent	Medium	Cylindrical
13	*E-100Bk-Cl*	Eshowe	100% Bk	absent	small	Cylindrical
14	*E-50LR50C-K*	Eshowe	50% LR 50% C	spotted	Medium	Kidney
15	*E-90LB10M-Cu*	Eshowe	90% LB 10% M	spotted	Medium	Cuboidal
16	*E-50M50C-K*	Eshowe	50% M 50% C	spotted	Medium	Kidney
17	*E-90M10C-Cl*	Eshowe	90% M 10% C	spotted	Medium	Cylindrical
18	*E-50YG-Cl*	Eshowe	50% YG	absent	Medium	Cylindrical
19	*E-100YG-Cl*	Eshowe	100% YG	absent	Medium	Cylindrical
20	*Em-50Bk50C-Cu*	Empangeni	50% Bk 50 C	spotted	Small	Cuboidal
21	*Em-50M50LB-Cl*	Empangeni	50% LB 50% M	stripped	Small	Cylindrical
22	*Em-100LB-Cl*	Empangeni	100% LB	absent	Small	Cylindrical

Table 3.1 continued

No	Landraces	Area of collection	Seed coat colour percentage	Seed texture	Seed size	Seed shape
23	*Em-100YG-Cl*	Empangeni	100% YG	absent	Small	Cylindrical
24	*KN-50B50M-Cl*	KwaNdebele	50% B 50% M	stripped	Small	Cylindrical
25	*KN-100W-Cl*	KwaNdebele	100% W	absent	Small	Cylindrical
26	*M-90LB10M-Cl*	Mtubatuba	90% LB 10% M	stripped	Medium	Cylindrical
27	*N-100DP- K*	Nelspruit	100% DP	absent	Large	Kidney
28	*N-100LP-K*	Nelspruit	100% LP	absent	Large	Kidney
29	*P-50M50C-O*	Polokwane	50% M 50% C	stripped	Medium	Oval
30	*PS-50DB50LB-Cl*	Port Shepstone	50% DB 50% LB	spotted	Medium	Cylindrical
31	*PS-90DB10LB-Cl*	Port Shepstone	90% DB 10% LB	spotted	Medium	Cylindrical
32	*PS-90LB10B-Cl*	Port Shepstone	90% LB 10% B	stripped	Medium	Cylindrical
33	*PS-90LB10M-Cl*	Port Shepstone	90% LB 10% M	stripped	Medium	Cylindrical
34	*PS-50M50LB-Cl*	Port Shepstone	50% M 50% LB	spotted	Medium	Cylindrical
35	*PS-90M10LB-Cl*	Port Shepstone	90% M 10% LB	spotted	Medium	Cylindrical
36	*PS-100YG-Cl*	Port Shepstone	100% YG	absent	Small	Cylindrical
37	*Phaseolus coccineus*	Benoni	100% W	absent	Large	Kidney
38	*Phaseolus lunatus*	Benoni	50% M 50% C	absent	Large	Kidney

Landrace names are currently unique to authors and are coined from: area of the collection – the percentage of seed coat colour(s) - seed shape. Percentage of seed coat colour: B, brown. Bk, black. By, brownish-yellow. C, cream. DB, dark brown. DP, dark purple. Gy, grey. LB, Light brown. LP, light purple. LR, light red. M, maroon. P, purple. RB, reddish-brown. W, white. YG, yellowish-green.

Table 3.2: Field layout of 38 *Phaseolus vulgaris* landraces.

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
38	**37**	**36**	**35**	**34**	**33**	**32**	**31**	**30**	**29**	**28**	**27**	**26**	**25**	**24**	**23**	**22**	**21**	**20**	**19**	**18**	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**10**	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**
24	**13**	**21**	**27**	**18**	**9**	**3**	**36**	**37**	**38**	**19**	**7**	**6**	**29**	**5**	**4**	**8**	**10**	**2**	**20**	**14**	**17**	**1**	**16**	**26**	**20**	**23**	**25**	**30**	**28**	**32**	**31**	**11**	**33**	**12**	**15**	**29**	**25**

Landraces are explained in Table 3.1. 1, *N-100DP- K*. 2, *N-100LP-K*. 3, *Br-100LB-Cl*. 4, *P-50M50C-Cl*. 5, *Em-100LB-Cl*. 6, *Em-50M50LB-Cl*. 7, *Em-100YG-Cl*. 8, *Em-50Bk50C-Cu*. 9, *KN-100W-Cl*. 10, *KN-50B50M-Cl*. 11, *B-50B50M-Cl*. 12, *P. coccineus*. 13, *P. lunatus*. 14, *PS-50DB50LB-Cl*. 15, *PS-50M50LB-Cl*. 16, *PS-90M10LB-Cl*. 17, *PS-90DB10LB-Cl*. 18, *M-90LB10M-Cl*. 19, *PS-90LB10B-Cl*. 20, *PS-90LB10M-Cl*. 21, *E-50YG-Cl*. 22, *E- 100YG-Cl*. 23, *E-90LB10M-Cu*. 24, *E-50M50C-K*. 25, *E-50LR50C-K*. 26, *E-90M10C-Cl*. 27, *E-100Bk- Cl*. 28, *D-100YG-Cl*. 29, *D-90M10LB-Cl*. 30, *D-50M50LB-Cl*. 31, *D-50P50LB-Cl*. 32, *D-50RB50LB-Cl*. 33, *D-100By-Cl*. 34, *D-50C50Gy-K*. 35, *D-90LB10B-Cu*. 36, *D-90C10LR-Cl*. 37, *D-100C-Cl*. 38, *PS-100YG-Cl*.

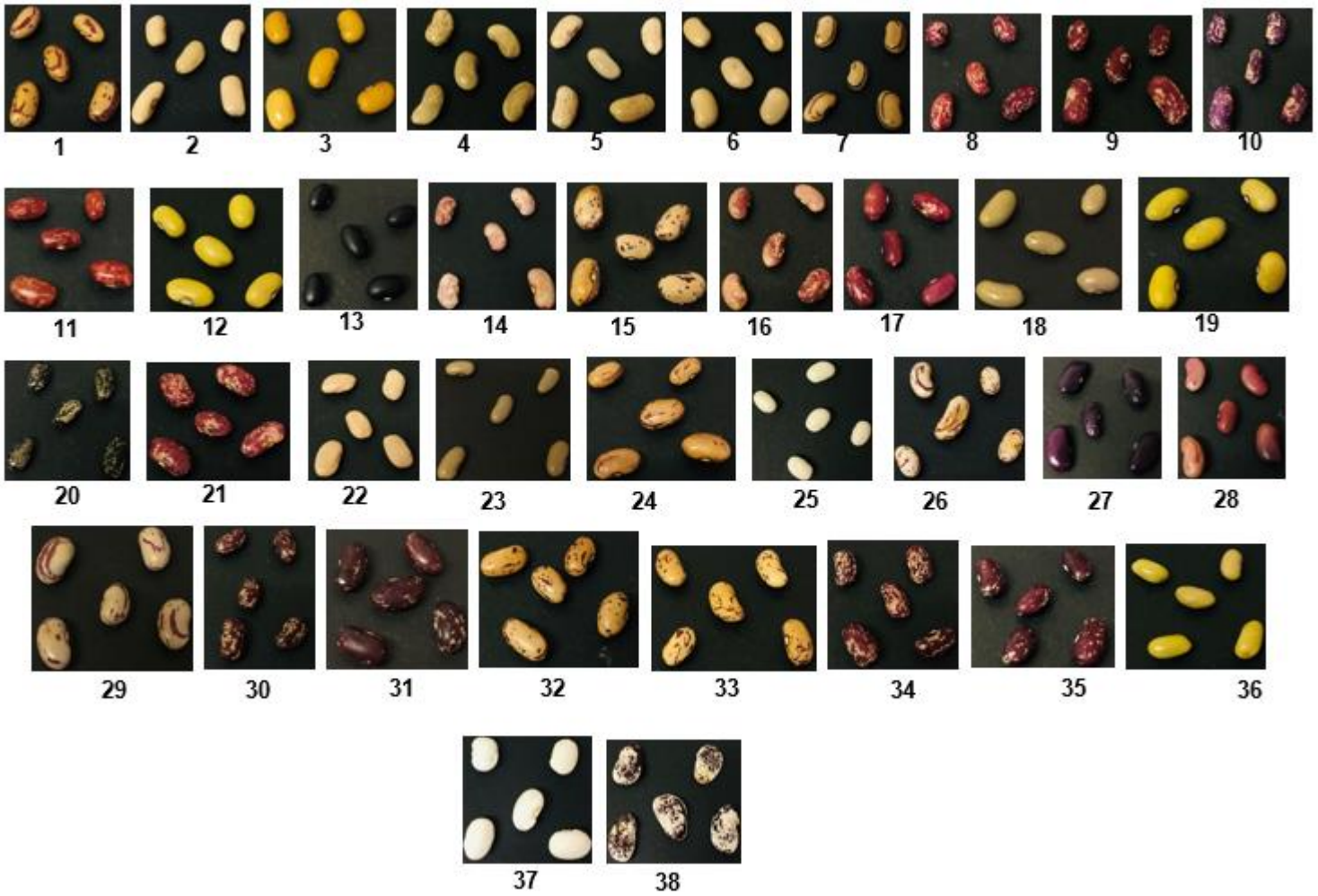


Figure 3.2: Seed morphology among *Phaseolus vulgaris* landraces from selected areas of South Africa.

The experimental field was 50 m in length and 5 m in width. The experimental plots were 140 cm in length, 140 cm in width, and 50 cm apart. Each landrace was sown in four rows of 120 cm long, with an inter-plant spacing of 10 cm and inter-row spacing of 10 cm (Table 3.2). In each row, 10 seeds were planted.

3.2.2 Data collection

Data for the qualitative and quantitative characteristics were recorded on an individual plant using five randomly selected plants per plot. The inner plants were used for measurements to eliminate border effects. The five randomly selected plants were tagged and recorded consistently at three days' intervals for the morpho-agronomic traits. The vegetative traits were measured before flowering (33 days after planting) to eliminate interference with the flowering period. However, the plant height and number of branches were determined at harvest (101 days after planting). The qualitative traits were scored according to Key access and utilization descriptors for *P. vulgaris* resources(Balkaya and Ergun, 2008).

3.2.2.1 Germination percentage and stem traits

The germination percentage was counted at 14 days after planting using the following formula: GP (%) = (number of germinated seeds /total number of seeds sown) × 100 (Abdel-Haleem and El-Shaleny, 2015). Growth habits and stem colour were determined for each landrace. Plant growth habit (PGH): 1–determinate bush, 2–semi -climbing, 3–climbing, 4–indeterminate climbing. Stem colour (SC): 1–green, 2–green with pink or purple pigmentation. The plant height (PH) (cm) from the scar of cotyledonous leaves to the stem apex was measured using a ruler. The stem diameter (mm) was measured between the scar of the cotyledonous leaves and the first set of true leaves, using Vernier calipers. The number of branches was counted manually.

3.2.2.2 Leaf traits

The colour of leaves and leaf veins was determined for each landrace. Leaf colour (LC): 1–green, 2–green with pink or purple pigmentation. The number of leaves per plant was determined by direct counting. The chlorophyll content (mg cm^{-2}) was

measured using a CCM- 200 plus chlorophyll content meter with a measurement area of 0.71 cm^2 on two points of each lobe of the second leaf from the apex. An average for all points was recorded as the final value for each plant (Pereyra *et al.*, 2014). The leaf area (LA) [Area (mm^2) = length (mm) × width (mm)] of the middle leaf lobe was measured using a ruler (Bhatt and Chanda, 2003).

3.2.2.3 Flower traits

The colour of the flowers was determined among the *P. vulgaris* landraces. Colour of flowers (FC): 1–white, 2–cream, 3–purple, 4–white with purple edges, 5–pink, 6–cream with purple edges. The days of first flowering and 50% flowering were recorded (from the date of sowing to the date on which approximately 50% tillers produced flowers) for landraces.

3.2.2.4 Pod traits

The colour and shape of the mature pods for the landraces were determined. Mature pod colour (MPC): 1–dark purple, 2– purple stripe on yellow, 3–normal green, 4–pale yellow to white, 5–pink stripe on yellow, 6–pink. Pod shape (PS): 1–straight, 2–semi-curled, 3–curled. The number of pods per plant was determined by direct counting. Vernier calipers were used to measure the pod length (mm) from the tip to the highest point on the pod, as well as the pod width (mm).

3.2.2.5 Seed traits

The colour and shape of the seeds were determined among landraces. Seed coat pattern (SCP): 0–absent, 1–mottled, 2–striped, 3–spotted. Seed coat colour (SCC): 1 – white, 2–cream, 3–yellowish-green, 4–50% yellowish-green, 5–black, 6– 50% cream 50% black, 7–dark-purple, 8–purple, 9–90% dark-purple 10% cream, 10–90% purple 10% cream, 11–light brown, 12–brownish-yellow, 13–maroon, 14–90% dark brown 10% light brown, 15–90% light brown 10% maroon, 16–90% cream 10% light red, 17–90% maroon 10% light brown, 18–90% light brown 10% brown, 19–50% cream 50% light red, 20–50% dark brown 50% light brown, 21–50% brown 50% maroon, 22–50% cream 50% maroon, 23–50% cream 50% grey, 24–50% reddish-brown 50% light

brown, 25–light purple, 26–reddish purple, 27–90% cream 10% pink, 28–pink, 29–90% cream 10% dark brown, 30–50% light brown 50% brown, 31–90% black 10% cream, 32– 50% greenish-brown 50% light brown, 33–50% light brown 50% maroon, 34 – 50% purple 50% cream. Seed shape: 1–cylindrical, 2–round, 3–cuboid, 4–kidney, 5–oval. The number of seeds per pod and plant was determined by direct counting. The seed length (mm), width (mm), and thickness (mm) were measured using Vernier calipers. The hundred and total seed mass (g) were measured using a Mettler PC 2000 weighing scale.

3.2.3 Data analysis

Data were analyzed using GenStat Release version 12.1 for quantitative characteristics. The means of the different traits were compared using Tukey's 95% confidence intervals test ($P \geq 0.05$). Variability of quantitative traits between landraces was evaluated by calculating the coefficient of variation. Correlation and principal component analysis (at a significant level of ≥ 0.6), biplots (scatter plots), and agglomerative hierarchical clustering (dendrogram) among traits were determined using XLSTAT (2019.1).

3.4 Results

Phaseolus vulgaris landraces showed significant variation for vegetative traits (P <001) (Table 3.3). Landraces with similar seed coat colour from different environments showed significant differences in germination percentage, stem diameter, the number of leaves, and the number of branches. Landraces with different seed coat colour, intensity, and shape from the same environments differed significantly in almost all growth and yield traits. The reproductive traits of *P. vulgaris* also differed significantly (Table 3.4). Landraces with a similar seed coat colour but from different environments differed in some traits.

3.4.1 Germination percentage and stem characteristics

P. vulgaris landraces *E-100Bk-Cl* and *D-100By-Cl* showed the highest (100%) and lowest (40%) germination percentages, respectively (Table 3.3). The outgroup *Phaseolus coccineus* had the lowest germination percentage (27%) compared with the *P. vulgaris* landraces. Although the germination percentage of Eshowe landrace *E-100Bk-Cl* was the highest, it was similar to the majority of other landraces, but was only higher than those of landraces *D-100ByCl, D-50M50LB-Cl, D-90M10LB-Cl, D-50P50LB-Cl, D-50RB50LB-Cl, D-100YG-Cl* from Durban *E-50LR50C-K* from Eshowe, *KN-50B50M-Cl* from KwaNdebele, *N-100LP-K* from Nelspruit, *P-50C50M-O* from Polokwane, and the outgroup *P. coccineus.*

Landraces from Durban and Eshowe with different seed coats differed significantly among each other in the germination percentage, but those from Empangeni, Nelspruit, and Port Shepstone did not. Among landraces from Durban, the highest (91%) and lowest (40%) germination percentages were recorded for landraces *D-100C-Cl* and *D-100By-Cl,* respectively. In those from Eshowe, *E-100Bk-Cl* had the highest germination percentage (100%) and *E-50LR50C-K* had the lowest (73%). The lowest to highest germination percentage ranges among landraces from Empangeni, KwaNdebele, Nelspruit, and Port Shepstone were 87.67%–92%, 77.00%–95.00%, 69.33%–81.33%, and 79.33%–91.33%, respectively.

Table 3.3: Variation in germination percentage [14 days after planting (DAP)] and vegetative traits (33 DAP) among *Phaseolus vulgaris* landraces.

Landraces	GP	PH	PGH	SD	SC	LC	LA	CC	NL	NB
B-50B50M-Cl	79.67$^{a–f}$	78.80$^{b–i}$	1	3.00^{f}	1	1	6590$^{a–d}$	13.08^{efg}	15.11$^{e–l}$	2.93$^{d–h}$
Br- 100LB-Cl	92.00^{abc}	92.33$^{b–f}$	3	3.53$^{a–f}$	2	1	5756$^{a–e}$	17.28$^{a–g}$	26.89^{cde}	2.93$^{d–h}$
D-100By-Cl	40.00^{jk}	76.60$^{b–i}$	2	3.73$^{a–f}$	2	1	2961^{f}	15.50$^{b–g}$	24.00$^{c–h}$	3.07$^{d–h}$
D-50C50Gy-K	90.00$^{a–d}$	75.20$^{c–i}$	1	3.40$^{a–f}$	2	1	5338$^{c–f}$	16.41$^{b–g}$	26.89^{cde}	3.00$^{d–h}$
D-90C10LR-Cl	86.67$^{a–f}$	86.90$^{b–g}$	1	3.40$^{a–f}$	2	1	6228$^{a–d}$	18.82$^{a–g}$	17.22$^{d–l}$	3.13$^{d–h}$
D-100C-Cl	91.00^{abc}	80.67$^{b–i}$	1	3.47$^{a–f}$	2	1	4520^{def}	21.51^{abc}	19.11$^{d–l}$	2.93$^{d–h}$
D-90LB10B-Cu	83.00$^{a–f}$	81.20$^{b–h}$	2	3.00^{f}	2	1	6046$^{a–e}$	17.98$^{a–g}$	28.11^{cd}	2.87$^{d–h}$
D-50M50LB-Cl	49.00$^{h–k}$	82.90$^{b–h}$	1	3.67$^{a–f}$	1	1	7914^{ab}	15.48$^{b–g}$	19.11$^{d–l}$	3.80$^{b–g}$
D-90M10LB-Cl	65.33$^{f–i}$	80.27$^{b–i}$	1	3.40$^{a–f}$	1	1	8144^{a}	16.27$^{b–g}$	16.33$^{d–l}$	2.87$^{d–h}$
D-50P50LB-Cl	44.33^{ijk}	72.27$^{e–i}$	1	3.80$^{a–f}$	1	1	6339$^{a–d}$	17.54$^{a–g}$	12.11$^{g–l}$	3.93$^{b–f}$
D-50RB50LB-Cl	67.67$^{d–h}$	73.13$^{d–i}$	2	4.00$^{a–d}$	1	1	4868^{def}	19.63$^{a–f}$	16.67$^{d–l}$	4.00$^{b–f}$
D-100YG-Cl	67.00$^{e–i}$	80.33$^{b–i}$	1	4.07^{abc}	1	1	5895$^{a–e}$	16.26$^{b–g}$	14.11$^{f–l}$	4.07$^{b–e}$
E-100Bk-Cl	100.00^{a}	59.00^{hi}	1	3.27$^{c–f}$	2	1	4179^{def}	14.77$^{c–g}$	17.00$^{d–l}$	2.07^{h}
E-50LR50C-K	73.00$^{b–g}$	75.47$^{b–i}$	1	3.77$^{a–f}$	1	1	4150^{def}	16.45$^{b–g}$	14.78$^{e–l}$	2.53^{fgh}
E-90LB10M-Cu	91.67^{abc}	100.00^{b}	1	3.93$^{a–e}$	1	1	4749^{def}	17.01$^{a–g}$	6.78^{l}	2.80$^{d–h}$
E-50M50C-K	89.33$^{a–e}$	79.13$^{b–i}$	1	4.27^{a}	1	1	5292$^{c–f}$	12.93^{efg}	9.67^{kl}	2.13^{h}
E-90M10C-Cl	89.33$^{a–e}$	83.20$^{b–h}$	1	3.80$^{a–f}$	1	1	7700^{abc}	16.67$^{a–g}$	13.22$^{f–l}$	2.67$^{d–h}$
E-50YG-Cl	91.00^{abc}	78.40$^{b–i}$	1	3.33$^{b–f}$	1	1	5906$^{a–e}$	14.57$^{c–g}$	11.78$^{h–l}$	2.33^{gh}
E-100YG-Cl	89.33$^{a–e}$	95.00$^{b–e}$	1	3.80$^{a–f}$	1	1	6084$^{a–e}$	19.78$^{a–e}$	12.33$^{g–l}$	2.73$^{d–h}$
Em-50Bk50C-Cu	87.67$^{a–f}$	63.07^{ghi}	3	3.47$^{a–f}$	2	1	5399$^{c–f}$	14.23$^{d–g}$	22.56$^{c–j}$	3.47$^{b–h}$
Em-50M50LB-Cl	88.00$^{a–f}$	67.80$^{f–i}$	1	4.00$^{a–d}$	1	1	5866$^{a–e}$	15.46$^{b–g}$	14.22$^{f–l}$	3.20$^{d–h}$
Em-100LB-Cl	89.33$^{a–e}$	72.20$^{e–i}$	3	4.00$^{a–d}$	2	2	5250$^{c–f}$	14.49$^{c–g}$	31.56^{bc}	3.27$^{c–h}$

Table 3.3 continued:

Landraces	GP	PH	PGH	SD	SC	LC	LA	CC	NL	NB
Em-100YG-Cl	92.00^{abc}	94.07$^{b–e}$	1	3.13^{def}	1	1	4469^{def}	18.61$^{a–g}$	13.78$^{f–l}$	2.87$^{d–h}$
KN-50B50M-Cl	77.00$^{b–f}$	75.80$^{b–i}$	2	3.07^{ef}	1	1	4521^{def}	13.32^{efg}	21.89$^{c–k}$	4.13^{bcd}
KN-100W-Cl	95.00^{ab}	56.20^{i}	1	3.67$^{a–f}$	1	1	3761^{ef}	13.37^{efg}	21.11$^{c–k}$	4.87^{b}
M-90LB10M-Cl	79.33$^{a–f}$	90.60$^{b–f}$	1	3.40$^{a–f}$	1	1	6014$^{a–e}$	12.83^{efg}	19.44$^{c–k}$	2.87$^{d–h}$
N-100DP- K	81.33$^{a–f}$	99.87^{b}	3	3.87$^{a–f}$	2	1	6446$^{a–d}$	21.41$^{a–d}$	25.33$^{c–f}$	3.00$^{d–h}$
N-100LP-K	69.33$^{c–h}$	99.13^{bc}	3	4.20^{ab}	2	1	5824$^{a–e}$	18.90$^{a–g}$	24.44$^{c–g}$	3.47$^{b–h}$
P-50M50C-O	50.33$^{g–j}$	90.67$^{b–f}$	3	4.00$^{a–d}$	1	1	5288$^{c–f}$	18.34$^{a–g}$	21.22$^{c–k}$	4.73^{bc}
PS-50DB50LB-Cl	90.00$^{a–d}$	83.27$^{b–h}$	2	3.60$^{a–f}$	1	1	6486$^{a–d}$	15.16$^{c–g}$	11.44$^{i–l}$	3.27$^{c–h}$
PS-90DB10LB-Cl	91.00^{abc}	88.27$^{b–f}$	1	3.87$^{a–f}$	1	1	5133^{def}	11.99^{g}	13.33$^{f–l}$	2.67$^{d–h}$
PS-90LB10B-Cl	83.67$^{a–f}$	84.53$^{b–g}$	1	3.47$^{a–f}$	1	1	4805^{def}	18.18$^{a–g}$	14.00$^{f–l}$	2.60$^{e–h}$
PS-90LB10M-Cl	89.33$^{a–e}$	88.27$^{b–f}$	1	3.93$^{a–e}$	1	1	5859$^{a–e}$	12.56^{fg}	15.44$^{e–l}$	3.00$^{d–h}$
PS-50M50LB-Cl	91.33^{abc}	79.93$^{b–i}$	1	3.90$^{a–e}$	1	1	5992$^{a–e}$	15.39$^{b–g}$	10.78^{jkl}	3.07$^{d–h}$
PS-90M10LB-Cl	83.00$^{a–f}$	81.87$^{b–h}$	1	3.93$^{a–e}$	1	1	5215^{def}	14.19^{efg}	13.00$^{f–l}$	2.80$^{d–h}$
PS-100YG-Cl	79.33$^{a–f}$	76.53$^{b–i}$	1	3.40$^{a–f}$	1	1	4363^{def}	19.78$^{a–e}$	23.22$^{c–i}$	3.80$^{b–g}$
P. coccineus	27.00^{k}	97.73^{bcd}	4	3.20$^{c–f}$	1	1	4332^{def}	22.37^{ab}	49.89^{a}	10.13^{a}
P. lunatus	78.00$^{a–f}$	148.44^{a}	3	3.23 $^{c–f}$	1	1	3754^{ef}	23.77^{a}	40.89^{ab}	9.67^{a}
Mean	79.35	83.93		3.63			5442	16.73	19.18	3.52
P- value	<.001	<.001		<.001			<.001	<.001	<.001	<.001
CV%	8.8	20.5		16.9			31.6	30.1	34.8	29.9

Landraces are explained in Table 3.1. Traits: GP, germination percentage (%); PH, plant height (cm); PGH, plant growth habit: 1, determinate bush; 2, semi-climbing; 3, climbing; 4, indeterminate climbing; SD, stem diameter (mm); SC, stem colour: 1, green; 2, green with purple pigmentation; LC, leaf colour: 1, green; 2, green with purple pigmentation; LA, leaf area (mm^2); CC, chlorophyll content (mg cm^2); NL, number of leaves; NB, number of branches. Means followed by different letter(s) within a column differ significantly ($P < 0.05$) according to Turkey's LSD.

Landraces with similar seed coat colour but different origins differed in germination percentage. Among the landraces sown from 50% maroon and 50% cream, seeds of Eshowe landraces (*E-50M50C-K*) also germinated better than those of Polokwane landraces (*P50M50C-O*).

The majority (68%) of the *P. vulgaris* landraces were bushy, while the minority (32%) had a climbing growth habit (Table 3.3). The outgroup *Phaseolus coccineus* showed an indeterminate climbing growth habit. Most of the stems (65.79%) were pure green, whereas 34.21% were green with purple pigmentation. Almost all landraces had soft stems. However, stems of landraces from Polokwane (*P-50M50C-O*) were hard (woody).

Phaseolus lunatus was the tallest (148.44 cm) of them all. Among *P. vulgaris* landraces, *E-90LB10M-Cu* was the tallest (100.00 cm), but *KN-100W-Cl* was the shortest (56.20 cm). Landraces *E-90LB10M-Cu* from Eshowe and *N-100DP-K* from Nelspruit had the tallest stems, it was comparable to the majority of other landraces, however, was only taller than landraces *D-50C50Gy-K, D-50P50LB-Cl*, and *D-50RB50LB-Cl* from Durban*, E-100Bk-Cl* from Eshowe, *Em-50Bk50C-Cu, Em-50LB50M-Cl,* and *Em-100LB-Cl* from Empangeni and KwaNdebele landrace *KN-100W-Cl* with shorter stems.

Significant differences in plant height were recorded among landraces from Empangeni and Eshowe only (Table 3.3). In the landrace from Eshowe, *E-90LB10M-Cu* had the tallest stems (100.00 cm), while *E-100Bk-Cl* had the shortest (59 cm). Again, in the landrace from Empangeni, *Em-100YG-Cl* had the tallest plants (94.07 cm), whereas *Em-50Bk50C-Cu* had the shortest plants (63.07 cm). The shortest to tallest stem ranges among landraces from Durban, KwaNdebele, Nelspruit, and Port Shepstone were 72.27–86.90 cm, 56.20–78.80 cm, 99.13–99.87 cm, and 76.53–88.27 cm, respectively.

Landraces *B-50B50M-Cl* and *D-90LB10B-Cu* had the thinnest stems (3.00 mm) while *E-50M50C-K* had the thickest stem (4.27 mm) among the landraces (Table 3.3). However, stems of landrace *E-50M50C-K* were only thicker than those of *B-50B50M-*

Cl, D-90LB10B-Cu, E-100Bk-Cl, and *E-50YG-Cl, Em-100YG-Cl, KN-50B50M-Cl,* and also the outgroups *P. coccineus* and *P. lunatus.*

The stem diameter differed significantly among the Durban, Eshowe, and Empangeni landraces. In the landrace from Durban, *D-100YG-Cl* had the thickest stems (4.07 mm) whereas *D-90LB10B-Cu* had the thinnest stems (3.00 mm). For the Eshowe landraces, *E-50M50C-K* had the thickest stems (4.27 mm) while *E-100Bk-Cl* had the thinnest stems (3.27 mm). Once more, landrace from Empangeni, *Em-100LB-Cl* and *Em-50M50LB-Cl* had the thickest (4.00 mm) stems while *Em-100YG-Cl* had the thinnest (3.13 mm) stems. However there is no significant difference between the stem diameter in the Empangeni landraces.The differences for stem diameter between the landraces from KwaNdebele, Nelspruit, and Port Shepstone were insignificant, with the thinnest to thickest stems ranged from 3.07–3.67mm, 3.87–4.20 mm, and 3.40–3.93 mm, respectively.

P. coccineus and *P. lunatus* had the most numerous branches 10.13 and 9.67, respectively compared with the *P. vulgaris* landraces. However, landrace *KN-100W-Cl* produced numerous branches (4.13) whereas *E-100Bk-Cl* produced fewer branches (2.07) among the *P. vulgaris* (Table 3.3). Among the *P. vulgaris* landraces *D-50M50LB-Cl, D-50P50LB-Cl, D-50RB50LB-Cl,* and *D-100YG-Cl* from Durban, *KN-50B50M-Cl,* and *KN-100W-Cl* from KwaNdebele, *P-50M50C-O*, and *PS-100YG-Cl* from Port Shepstone had the most numerous branches. While the rest of the landraces produced fewer branches.

The landraces from Durban, Eshowe, Empangeni, KwaNdebele, Nelspruit, and Port Shepstone have branches ranging from 2.87–4.07, 2.07–2.80, 2.87–3.47, 4.13–4.87, 3.00–3.47, and 2.60–3.80, respectively. Significant differences among landraces with similar seed coat colour but different locations were recorded in stem diameter, where Durban landrace *D-100YG-Cl* had thicker stems than landrace from Empangeni *Em-100YG-Cl.*

3.4.2 Leaf traits

The leaves were green for almost all the landraces except for *Em-100LB-Cl* which had green leaves with purple venation (Table 3.3). The leaves were trifoliate for almost all landraces but the Polokwane landrace (*P-50M50C-O*) had pentafoliate leaves. Landrace *D-90M10LB-Cl* had broad leaves (8144 mm^2) while *D-100By-Cl* had narrow leaves (3761 mm^2). *P. lunatus* also had the narrower leaves (3754 mm^2) similar to landraces *D-100By-Cl* and *KN-100W-Cl* (Table 3.3). Landrace *D-90M10LB-Cl* had the broadest leaves which was significantly greater than that of the landraces *D-100By-Cl, D-50C50Gy-K, D-100C-Cl,* and *D-50RB50LB-Cl* from Durban, *E-100Bk-Cl, E-50LR50C-K, E-90LB10M-Cu,* and *E-50M50C-K* from Eshowe, *Em-50Bk50C-Cu, Em-100LB-Cl,* and *Em-100YG-Cl* from Empangeni, *KN-50B50M-Cl,* and *KN-100W-Cl, P-50M50C-O* from Polokwane, *PS-90DB10LB-Cl, PS-90LB10B-Cl, PS-90M10LB-Cl,* and *PS-100YG-Cl* from Port Shepstone and also the outgroups (*P.coccineus* and *P. lunatus*). Whereas, the remaining landraces were similar to *D-90M10LB-Cl* with broader leaves.

Landrace from Durban, *D-90M10LB-Cl* had broader (8144 mm^2) leaves whereas *D-100By-Cl* had narrower (2961 mm^2) leaves. Similarly, Eshowe landrace *E-90M10C-Cl* had broader leaves (7700 mm^2) while *E-50LR50C-K* had narrower leaves (4150 mm^2). The leaf area range among the *P. vulgaris* landraces from Empangeni, KwaNdebele, Nelspruit, and Port Shepstone were 4469–5866 mm^2, 3761–4521 mm^2, 5824–6446 mm^2, and 4363–6486 mm^2, respectively.

P. lunatus leaves had the highest chlorophyll content (23.77 mg cm^2) compared with the *P. vulgaris* landraces (Table 3.3). Among the *P. vulgaris* landraces, *D-100C-Cl* had the highest chlorophyll content (21.51 mg cm^2) whereas *PS-90DB10LB-Cl* had the lowest chlorophyll content (11.99 mg cm^2). The outgroups *P. coccineus* and *P. lunatus* along with landrace *D-100C-Cl* had significantly higher chlorophyll contents than landraces *B-50B50M-Cl* from Benoni, *E-50M50C-K* from Eshowe, *Em-50Bk50C-Cu* from Empangeni, *KN-50B50M-Cl* and *KN-100W-Cl* from KwaNdebele, *M-90LB10M-Cl* from Mtubatuba, *PS-90DB10LB-Cl, PS-90LB10M-Cl,* and *PS-90M10LB-Cl* from Port Shepstone. However, the rest of the landraces had a similarly high chlorophyll content as landrace *D-100C-Cl*.

The chlorophyll content of landraces from Port Shepstone differed significantly. While the landraces from Durban, Eshowe, Empangeni, and Nelspruit landraces did not. Within the Port Shepstone landraces, *PS-100YG-Cl* had the highest chlorophyll (19.78 mg cm^2) whereas *PS-90DB10LB-Cl* had the lowest (11.99 mg cm^2). The lowest to highest chlorophyll content ranges among the Durban, Eshowe, Empangeni, KwaNdebele, and Nelspruit landraces were 15.48–21.51 mg cm^2, 12.93–19.78 mg cm^2, 14.23–18.61 mg cm^2, 13.32–13.37 mg cm^2, and 18.90–21.41 mg cm^2, respectively.

The outgrouped landraces *P. coccineus* and *P. lunatus* produced numerous leaves 49.89 and 40.89, respectively. Landrace *Em-100LB-Cl* produced numerous leaves (31.56) while *E-90LB10M-Cu* produced fewer leaves (6.78) among the *P. vulgaris* landraces (Table 3.3). The outgrouped landraces *P. coccineus* and *P. lunatus* and also landrace *Em-100LB-Cl* yielded numerous leaves outperforming landraces *B-50B50M-Cl, D-90C10LR-Cl, D-100C-Cl, D-50M50LB-Cl, D-90M10LB-Cl, D-50P50LB-Cl, D-50RB50LB-Cl,* and *D-100YG-Cl* from Durban, *E-100Bk-Cl, E-50LR50C-K, E-90LB10M-Cl, E-50M50C-K, E-90M10C-Cl, E-50YG-Cl,* and *E-100YG-Cl* from Eshowe, *Em-50M50LB-Cl* and *Em-100YG-Cl* from Empangeni, *PS-50DB50LB-Cl, PS-90DB10LB-Cl, PS-90LB10M-Cl, PS-50M50LB-Cl,* and *PS-90M10LB-Cl* from Port Shepstone, and were comparable to the remaining landraces.

The number of leaves among the Durban, Empangeni, and Port Shepstone landraces differed significantly. The leaves of landrace *D-90LB10C-Cl* from Durbanwere numerous (28.11) whereas *D-50P50LB-Cl* had fewer leaves (12.11). The landrace from Empangeni, *Em-100LB-Cl,* had numerous leaves (31.56) while *Em-100YG-Cl* had fewer leaves (13.78). Similarly, the landrace from Port Shepstone, *PS-100YG-Cl* had numerous leaves (23.22) whereas landrace *PS-50M50LB-Cl* had fewer leaves (10.78). The number of leaves among the landraces from Eshowe, KwaNdebele and Nelspruit were 6.78–17.00, 21.11–21.89, and 24.44–25.33, respectively.

The landraces with 90% light brown and 10% maroon and with 90% light brown and 10% brown differed in the number of leaves produced by plants. Among the landraces with 90% light and brown 10% maroon seeds, those originating from Mtubatuba (*M-*

90LB10M-Cl) had numerous leaves compared with plants from Eshowe (*E-90LB10M-Cu*) and Port Shepstone (*PS-90LB10M-Cl*). This was similar to landraces with 90% light brown and 10% brown seeds where the landrace from the Durban area (*D-90LB10B-Cu*) had numerous leaves compared with Port Shepstone plants (*PS-90LB10B-Cl*).

3.4.3 Flower traits

Flower colour varied between cream, cream with pink edges, pink, purple, white, and white with pink edges (Table 3.4). The earliest first flower [36.00 days after planting (DAP)] was recorded in *E-100Bk-Cl* and *N-100DP-K* and days to 50% flowering (38.00 DAP) was recorded in *E-100Bk-Cl* whereas *P-50M50C-O* recorded the most delayed first flower (64.33 DAP) and *P. lunatus* and *B-50B50M-Cl* were the latest (70.00 and 67.00, respectively) to 50% flowering (Table 3.4). *P. vulgaris* landraces *E-100Bk-Cl* from Eshowe and *N-100DP-K* from Nelspruit had the earliest maturity, similar to the majority of other landraces but was earlier than *B-50B50M-Cl* from Benoni, *KN-50B50M-Cl,* and *KN-100W-Cl* from KwaNdebele, *P-50M50C-O* from Polokwane, and *P. lunatus.*

The landraces from Durban origin differed significantly in both the number of days to first and 50% flowering. Within the Durban group, *D-100YG-Cl* and *D-90M10LB-Cl* had the earliest first flowering (37.67 DAP) whereas *D-90LB10B-Cu* was delayed (48.33 DAP). *D-90M10LB-Cl* had the earliest 50% flowering (39.67 DAP) while *D-50C50Gy-K* and *D-90LB10B-Cu* had the latest (48.00 DAP). Again, landraces from the Eshowe area differed significantly in first and 50% flowering. *E-100Bk-Cl* had the earliest first and 50% flowering, i.e. 36.00 and 38.00 DAP, respectively. However, landrace *E-50LR50C-K* had the latest first and 50% flowering, 43.33 and 44.67 DAP, respectively.

Landraces from Empangeni differed significantly in 50% flowering but, the days to first flowering did not. *Em-100YG-Cl* had the earliest 50% flowering (40.67 DAP) whereas *Em-100LB-Cl* was delayed (47.33 DAP). The earliest to latest first flowering ranges among the landraces from Empangeni, Port Shepstone, and Nelspruit were 38.00–41.67, 38.00–43.33, and 36.00–37.33 DAP, respectively.

Table 3.4: Variation in reproductive traits among *Phaseolus vulgaris* landraces.

Landraces	DFF	50%F	FC	MPC	PS	PL	PW	NP	NSP	NSPl	SL	ST	SW	TSM	HSM
B-50B50M-Cl	62.67^{ab}	67.00^{a}	2	5	1	120.0^{a}	12.20^{cde}	3.13^{i}	4.73$^{b–g}$	9.80^{g}	11.10$^{g–l}$	3.60$^{c–h}$	5.50$^{e–l}$	3.64^{de}	33.10^{e}
Br- 100LB-Cl	37.33^{ghi}	40.33^{hij}	5	4	3	115.3$^{a–e}$	11.20$^{d–j}$	4.93$^{d–i}$	4.20$^{c–i}$	14.60$^{c–g}$	12.60$^{d–g}$	3.00$^{g–j}$	6.00$^{c–i}$	6.96^{cde}	33.15^{e}
D-100By-Cl	41.00$^{d–h}$	46.00$^{c–f}$	1	4	2	103.1$^{d–k}$	10.07$^{h–l}$	3.00^{i}	4.93$^{a–e}$	13.93$^{c–g}$	10.20$^{j–m}$	3.70$^{c–g}$	5.30$^{e–m}$	6.25^{cde}	57.87^{cde}
D-50C50Gy-K	41.00$^{d–h}$	48.00^{c}	2	2	2	116.0$^{a–d}$	10.07$^{h–l}$	4.87$^{d–i}$	6.07^{a}	18.13$^{c–g}$	10.40$^{j–m}$	2.90$^{g–j}$	4.50$^{j–m}$	3.99^{de}	29.85^{e}
D-90C10LR-Cl	40.00$^{e–i}$	43.33$^{e–i}$	4	5	1	106.1$^{a–k}$	11.87$^{c–f}$	3.07^{i}	4.20$^{c–i}$	11.00^{fg}	12.10$^{e–i}$	3.50$^{d–h}$	5.70$^{d–j}$	3.71^{de}	35.63^{e}
D-100C-Cl	38.00^{ghi}	47.00^{cde}	5	2	2	111.9$^{a–h}$	10.60$^{e–l}$	5.07$^{d–i}$	4.80$^{b–g}$	16.13$^{c–g}$	9.70^{lm}	2.70^{hij}	4.80$^{i–m}$	6.82^{cde}	32.93^{e}
D-90LB10B-Cu	47.00^{cde}	48.33^{c}	4	2	1	110.7$^{a–i}$	10.07$^{h–l}$	5.07$^{d–i}$	5.00$^{a–d}$	23.87$^{a–d}$	9.30^{mno}	2.40^{j}	4.60$^{j–m}$	7.73^{cde}	35.08^{e}
D-50M50LB-Cl	38.00^{ghi}	40.00^{hij}	6	5	1	114.9$^{a–e}$	11.93$^{c–f}$	15.48$^{b–g}$	4.80$^{b–g}$	16.20$^{c–g}$	11.40$^{f–k}$	4.10$^{b–e}$	6.10$^{c–h}$	8.55^{cd}	53.70^{de}
D-90M10LB-Cl	37.67^{ghi}	39.67^{ij}	4	5	1	120.0^{a}	9.87$^{i–l}$	2.93^{i}	4.07$^{c–i}$	10.13^{g}	13.00^{def}	4.10$^{b–e}$	6.30$^{c–f}$	4.43^{de}	46.27^{de}
D-50P50LB-Cl	44.00^{cde}	43.67$^{d–h}$	3	2	1	109.1$^{a–j}$	11.07$^{d–j}$	2.93^{i}	3.93$^{c–i}$	10.93^{fg}	13.00^{def}	3.70$^{c–g}$	5.60$^{d–k}$	5.73^{de}	54.32^{de}
D-50RB50LB-Cl	39.33$^{f–i}$	47.67^{c}	4	2	2	102.1$^{e–k}$	9.93$^{i–l}$	5.00$^{d–i}$	4.93$^{a–e}$	17.87$^{c–g}$	10.70$^{h–m}$	3.00$^{g–j}$	5.40$^{e–l}$	6.06^{de}	38.10^{e}
D-100YG-Cl	37.67^{ghi}	40.00^{hij}	5	4	2	113.9$^{a–f}$	11.87$^{c–f}$	9.13^{abc}	4.80$^{b–g}$	25.00^{abc}	10.10$^{j–m}$	3.70$^{c–g}$	5.10$^{f–m}$	11.86^{c}	26.88^{e}
E-100Bk-Cl	36.00^{i}	38.00^{j}	3	2	3	93.0^{k}	8.93^{l}	5.13$^{d–i}$	3.87$^{d–i}$	16.93$^{c–g}$	7.80^{op}	3.10$^{f–j}$	4.10^{m}	4.44^{de}	31.42^{e}
E-50LR50C-K	43.33^{cd}	44.67$^{e–i}$	1	4	1	103.9$^{c–k}$	11.80$^{c–g}$	4.13$^{f–i}$	4.00$^{c–i}$	13.67$^{d–g}$	13.30^{de}	4.00$^{b–f}$	5.50$^{e–l}$	5.10^{de}	36.27^{e}
E-90LB10M-Cu	38.33^{ghi}	39.67^{ij}	4	5	1	105.7$^{b–k}$	9.93$^{i–l}$	2.87^{i}	3.87$^{d–i}$	8.87^{g}	9.60^{lmn}	4.40^{bcd}	5.20$^{e–m}$	3.80^{de}	45.80^{de}
E-50M50C-K	38.00^{ghi}	39.67^{ij}	2	5	1	106.7$^{a–k}$	10.33$^{f–l}$	2.93^{i}	5.00$^{a–d}$	10.93^{fg}	10.70$^{h–m}$	3.80$^{c–g}$	4.90$^{h–m}$	2.89^{e}	26.58^{e}
E-90M10C-Cl	37.67^{ghi}	40.00^{hij}	6	5	2	118.6^{ab}	10.13$^{g–l}$	4.80$^{d–i}$	5.13^{abc}	17.93$^{c–g}$	10.70$^{h–m}$	3.00$^{g–j}$	4.30^{lm}	5.48^{de}	26.58^{e}
E-50YG-Cl	37.67^{ghi}	39.67^{ij}	5	4	1	111.6$^{a–h}$	11.00$^{d–k}$	3.93$^{f–i}$	4.93$^{a–e}$	14.20$^{c–g}$	10.40$^{j–m}$	3.80$^{c–g}$	5.00$^{g–m}$	3.96^{de}	31.83^{e}
E-100YG-Cl	37.67^{ghi}	40.00^{hij}	5	4	1	116.6$^{a–d}$	11.07$^{d–j}$	4.40$^{e–i}$	5.13^{abc}	16.93$^{c–g}$	9.80^{klm}	3.00$^{g–j}$	4.40^{klm}	4.99^{de}	31.92^{e}
Em-50Bk50C-Cu	39.33$^{f–i}$	43.00$^{f–i}$	3	2	3	96.5^{jk}	9.53^{jkl}	7.47$^{b–g}$	5.00$^{a–d}$	34.07^{a}	10.10$^{j–m}$	2.50^{ij}	5.40$^{e–l}$	6.86^{cde}	56.77^{cde}
Em-50M50LB-Cl	39.33$^{f–i}$	41.00$^{g–j}$	4	5	1	113.8$^{a–g}$	10.73$^{d–k}$	3.93$^{f–i}$	4.67$^{b–h}$	14.53$^{c–g}$	11.40$^{f–k}$	4.10$^{b–e}$	5.70$^{d–j}$	5.84^{de}	29.20^{e}
Em-100LB-Cl	41.67$^{d–g}$	47.33^{cd}	1	1	2	100.0$^{f–k}$	9.33^{kl}	7.53$^{b–f}$	5.60^{ab}	31.53^{ab}	8.00^{nop}	2.70^{hij}	4.30^{lm}	6.64^{cde}	38.70^{de}

Table 3.4 continued:

Landraces	DFF	50%F	FC	MPC	PS	PL	PW	NP	NSP	NSPl	SL	ST	SW	TSM	HSM
Em-100YG-Cl	38.00^{ghi}	40.67^{hij}	1	4	2	99.7$^{h–k}$	10.40$^{f–l}$	4.20$^{f–i}$	3.73$^{e–i}$	12.40^{efg}	10.40$^{j–m}$	3.00$^{g–j}$	5.00$^{g–m}$	4.52^{de}	43.53^{de}
KN-50B50M-Cl	59.00^{b}	61.33^{b}	1	5	1	108.9$^{a–j}$	12.93^{c}	3.87^{ghi}	3.07^{ij}	8.93^{g}	13.90^{cd}	4.90^{ab}	6.80^{cd}	6.18^{de}	43.31^{de}
KN-100W-Cl	59.67^{b}	60.67^{b}	1	4	2	96.9^{ijk}	8.93^{l}	7.87$^{b–e}$	4.87$^{a–f}$	22.93$^{a–e}$	7.50^{p}	3.00$^{g–j}$	4.10^{m}	8.64^{cd}	38.30^{e}
M-90LB10M-Cl	41.67$^{d–g}$	45.67$^{c–f}$	4	5	1	115.4$^{a–e}$	10.60$^{e–l}$	4.60$^{e–i}$	3.87$^{d–i}$	13.40$^{d–g}$	11.60$^{f–j}$	3.30$^{e–j}$	5.40$^{e–l}$	6.19^{de}	47.33^{de}
N-100DP- K	36.00^{i}	40.33^{hij}	3	4	1	115.3$^{a–e}$	11.93$^{c–f}$	5.00$^{d–i}$	3.47^{hi}	13.53 $^{d–g}$	16.70^{b}	4.50^{bc}	7.10^{c}	6.92^{cde}	41.50^{de}
N-100LP-K	37.33^{ghi}	40.33^{hij}	3	4	1	115.0$^{a–e}$	12.33^{cd}	6.00$^{c–i}$	3.60^{ghi}	18.20$^{c–g}$	13.90^{cd}	3.40$^{e–i}$	6.20$^{c–g}$	8.58^{cd}	92.00^{c}
P-50M50C-O	64.33^{a}	67.00^{a}	2	5	2	113.1$^{a–h}$	13.13^{c}	6.07$^{b–i}$	3.67$^{f–i}$	15.13$^{c–g}$	12.30$^{d–h}$	4.50^{bc}	6.40^{cde}	17.72^{b}	43.63^{de}
PS-50DB50LB-Cl	40.00$^{e–i}$	41.00$^{g–j}$	4	5	1	115.1$^{a–e}$	11.53$^{c–i}$	4.67$^{d–i}$	4.67$^{b–h}$	15.27$^{c–g}$	11.00$^{g–l}$	3.20$^{e–j}$	5.00$^{g–m}$	5.77^{de}	43.31^{de}
PS-90DB10LB-Cl	38.33^{ghi}	41.00$^{g–j}$	4	5	1	111.5$^{a–h}$	11.73$^{c–h}$	11.99^{a}	4.67$^{b–h}$	14.60$^{c–g}$	11.10$^{g–l}$	3.10$^{f–j}$	5.10$^{f–m}$	5.76^{de}	39.39^{de}
PS-90LB10B-Cl	39.00$^{f–i}$	39.67^{ij}	4	5	1	110.7$^{a–i}$	10.33$^{f–l}$	4.47$^{e–i}$	4.33$^{c–h}$	12.87 $^{d–g}$	11.60$^{f–j}$	3.70$^{c–g}$	5.30$^{e–m}$	5.07^{de}	33.10^{e}
PS-90LB10M-Cl	39.00$^{f–i}$	39.67^{ij}	4	5	1	115.1$^{a–e}$	10.73$^{d–k}$	4.13$^{f–i}$	4.67$^{b–h}$	18.00$^{c–g}$	10.80$^{h–m}$	4.10$^{b–e}$	4.60$^{j–m}$	7.18^{cde}	41.42^{de}
PS-50M50LB-Cl	38.00^{ghi}	40.00^{hij}	2	5	2	114.2$^{a–e}$	11.53$^{c–h}$	3.73^{hi}	4.47$^{b–h}$	12.20^{efg}	10.60$^{i–m}$	3.80$^{c–g}$	4.70$^{j–m}$	4.22^{de}	33.10^{e}
PS-90M10LB-Cl	38.00^{ghi}	40.00^{hij}	1	5	1	117.7^{abc}	10.80$^{d–k}$	4.73$^{d–i}$	5.00$^{a–d}$	16.73$^{c–g}$	11.10$^{g–l}$	3.70$^{c–g}$	4.90$^{h–m}$	6.65^{cde}	37.21^{e}
PS-100YG-Cl	43.33^{def}	48.33^{c}	1	4-6	2	96.1^{jk}	10.13$^{g–l}$	8.27$^{a–d}$	4.47$^{b–h}$	23.80$^{a–d}$	10.10$^{j–m}$	3.20$^{e–j}$	4.50$^{j–m}$	7.53^{cde}	29.42^{e}
P. coccineus	40.00$^{e–i}$	44.67$^{c–g}$	1	4	3	104.0$^{c–k}$	18.80^{b}	11.87^{a}	3.07^{ij}	22.07$^{b–f}$	15.50^{bc}	5.60^{a}	10.00^{b}	30.21^{a}	175.05^{b}
P. lunatus	60.00^{ab}	70.00^{a}	1	3	3	113.8$^{a–g}$	28.27^{a}	9.67^{ab}	2.13^{j}	9.80^{g}	30.00^{a}	5.70^{a}	18.30^{a}	23.29^{b}	219.30^{a}
Mean	42.06	45.03				109.51	11.52	5.21	4.41	16.24	11.67	3.62	5.71	7.37	49.18
P- value	<.001	<.001				<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
CV%	3.4	2.6				9.0	31.6	48.7	19.2	48.8	8.1	14.3	13.0	54.1	22.2

Landraces are explained in Table 3.1. Traits: DFF, days to first flowering; 50% F, days to 50% flowering; FC, flower colour; PC, pod colour; PS, pod shape; PL, pod length (mm); PW, pod width (mm); NP, number of pods per plant; NSP, number of seeds per pod, NSPl, number of seeds per plant; TSM, total seed mass (g); HSM, hundred seed mass (g); SL, seed length (mm); SW, seed width (mm); ST, seed thickness (mm). Flower colour (FC): 1, white; 2, cream; 3, purple; 4, white with pink edges; 5, Pink; 6, cream with pink edges. Mature pod colour (MPC): 1, dark purple; 2, purple stripe on yellow; 3, Normal green; 4, pale yellow to white; 5, pink stripe on yellow; 6, pink. Pod shape (PS): 1, straight; 2, semi-curled; 3, curled. Means followed by a different letter(s) within a column differ significantly ($P < 0.05$) according to Turkey's LSD.

Nelspruit and Port Shepstone landraces showed insignificant differences in first and 50% flowering. The earliest to latest 50% flowering ranges among the landraces from Nelspruit and Port Shepstone were 40.33, and 39.67–48.33 DAP, respectively. Plants sown from 100% yellowish-green seeds differed significantly in first and 50% flowering. The earliest first (37.67 DAP) and 50% flowering (40.00 DAP) was recorded in landrace *D-100YG-Cl* and *E-100YG-Cl* whereas *PS-100YG-Cl* was delayed in both first (43.33 DAP) and 50% flowering (48.33).

Plants sown from 50% brown and 50% maroon and 100% light brown seeds differed significantly in 50% flowering, but the first flowering did not. *KN-50B50M-Cl* had the earliest 50% flowering (61.33 DAP) whereas *B-50B50M-Cl* was delayed (67.00 DAP). *Br-100LB-Cl* had the earliest (40.33 DAP) 50% flowering whereas *Em-100LB-Cl* was delayed (47.33 DAP). The landraces with 50% maroon and 50% cream, and 90% light brown and 10% brown seeds showed significant differences in first and 50% flowering. *E-50M50C-K* had the earliest first flower (38.00 DAP) and 50% flowering (39.67 DAP) whereas *P-50M50C-O* had delayed in both first (64.33 DAP) and 50% (67.00 DAP) flowering. *PS-90LB10B-Cl* had the earliest first flower (39.00 DAP) and 50% (39.67 DAP) flowering while *D-90LB10B-Cu* was delayed in first (47.00 DAP) and 50% (48.33 DAP) flowering.

3.4.4 Pod traits

Pod colour varied from dark purple, normal green, purple stripe on yellow, pale yellow to white, pink to pink stripe on yellow. Pod shape also varied from curled, semi-curled to straight (Table 3.4). Landrace *PS-90DB10LB-Cl* and *P. coccineus* had numerous (12 pods per plant) pods whereas landraces *D-90M10LB-Cl*, *D-50P50LB-Cl*, *E-50M50C-K*, *D-100By-Cl*, *E-90LB10M-Cu* and *B-50B50M-Cl* had the fewest (3 pods per plant) pods (Table 3.4). *P. lunatus, D-100YG-Cl* from Durban, and *PS-100YG-Cl* from Port Shepstone also produced many more pods than the majority of the *P. vulgaris* landraces.

In the landraces from Durban and Port Shepstone, the number of pods differed significantly. *D-50M50LB-Cl* had numerous (15.48) pods whereas landraces *D-90M10LB-Cl* and *D-50P50LB-Cl* had fewer pods. Again, *PS-90DB10LB-Cl* had

numerous (11.99) while *PS-90DB10LB-Cl* had (3.73) fewer pods. The number of pods among the landraces from Eshowe, Empangeni, and Nelspruit landraces ranged from2.87–5.13, 3.93–7.53, and 5.00–6.00, respectively.

Plants with 100% yellowish-green and 50% maroon 50% light brown seeds, differed significantly in the number of pods produced. *D-100YG-Cl* had numerous (9.13) pods whereas *Em-100YG-Cl* had fewer (4.20) pods. Again, *D-50M50LB-Cl* had numerous (15.48) while *PS-50M50LB-Cl* had fewer (3.73) pods.

Landrace *B-50B50M-Cl* and *D-90M10LB-Cl* had the longest (120 mm) pods but *E-100Bk-Cl* had the shortest (93 mm) (Table 3.4). The pods of the Benoni landrace *B-50B50M-Cl* was the longest, it was similar to the majority of landraces but, it had longer pods than *D-100By-Cl,* and *D-50RB50LB-Cl* from Durban, *E-100Bk-Cl, E-50LR50C-K, E-90LB10M-Cu,* and *E-50M50C-K* from Eshowe, *Em-50Bk50C-Cu, Em-100LB-Cl,* and *Em-100YG-Cl* from Empangeni, *KN-100W-Cl* from KwaNdebele, *PS-100YG-Cl* from Port Shepstone, and the *P. coccineus* outgroup.

Among the Durban, Eshowe, Empangeni, and Port Shepstone landraces, the pod length differed significantly, but the landraces from Nelspruit did not. *D-90M10LB-Cl* had the longest (120.0 mm) whereas *D-50RB50LB-Cl* had the shortest (102.1 mm) pods. Again, *E-90M10C-Cl* had the longest (118.6 mm) pods whereas *E-100Bk-Cl* had the shortest (93.0 mm). *Em-50LB50M-Cl* had the longest (113.8 mm) pods whereas *Em-50Bk50C-Cu* had the shortest (96.5 mm). Again, *PS-90M10LB-Cl* had the longest (117.7 mm) pods whereas *PS-100YG-Cl* had the shortest (96.1 mm). The shortest to longest pod ranges among the Nelspruit landraces were 115.0–115.3 mm.

Plants sown from 100% yellowish-green seeds, differed significantly in pod length. However, landraces sown from 50% maroon and 50% light brown, 50% brown and 50% maroon, 90% light brown and 10% maroon and 90% light brown and 10% brown, and 100% light brown seeds did not. *E-100YG-Cl* had the longest (116.6 mm) pods while *PS-100YG-Cl* had the shortest (96.1 mm).

P. lunatus had the widest pods outperforming all the landraces (28.27 mm). Among the *P. vulgaris* landraces, *P-50M50C-O* was the widest (13.13 mm) but *KN-100W-Cl*

and *E-100Bk-Cl* were the narrowest (8.93 mm) (Table 3.4). The pod width of the Polokwane landrace *P-50M50C-O* was the widest, it was comparable to other *P. vulgaris* landraces but was wider than the majority of the landraces *Br-100LB-Cl* from Bushbuckridge, *D-100By-Cl, D-50C50Gy-K, D-100C-Cl, D-90LB10B-Cu, D-90M10LB-Cl, D-50P50LB-Cl,* and *D-50RB50LB-Cl* from Durban, *E-100Bk-Cl, E-90LB10M-Cu, E-50M50C-K, E-90M10C-Cl, E-50YG-Cl,* and *E-100YG-Cl* from Eshowe, *Em-50Bk50C-Cu, Em-50M50LB-Cl, Em-100LB-Cl,* and *Em-100YG-Cl* from Empangeni, *KN-100W-Cl* from KwaNdebele, *M-90LB10M-Cl* from Mtubatuba, and *PS-90LB10B-Cl, PS-90LB10M-Cl, PS-90M10LB-Cl,* and *PS-100YG-Cl* from Port Shepstone.

Among the Durban and Eshowe landraces, the pod width differed significantly. However, the Empangeni, Nelspruit, and Port Shepstone landraces did not. *D-50M50LB-Cl* had the widest (11.93 mm) pods while *D-90M10LB-Cl* had the narrowest (9.87 mm). *E-50LR50C-K* had the widest (11.80 mm) while *E-100Bk-Cl* had the narrowest (8.93 mm) pods. The narrowest to widest pod ranges among the landraces from Empangeni, Nelspruit, and Port Shepstone were 9.33–10.73, 11.93–12.33, and 10.13–11.73, respectively.

Landraces sown from 100% yellowish-green, 100% light brown, and 50% maroon and 50% cream seeds differed significantly in pod width. *D-100YG-Cl* had the widest (11.87 mm) pods whereas *PS-100YG-Cl* had the narrowest (10.13 mm). *Br-100LB-Cl* had the widest (11.20 mm) pods while *Em-100LB-Cl* had the narrowest (9.33 mm) pods. *P-50M50C-O* had the widest (13.13 mm) pods whereas *E-50M50C-K* had the narrowest (10.33 mm).

3.4.5 Seed traits

Landrace *D-50C50Gy-K* had numerous seeds (6.07 per pod) while the outgroup *P. lunatus* had fewer seeds (2.13 per pod) and *KN-50B50M-Cl* and *N-100DP-K* had fewer seeds (3.07 and 3.47 per pod, respectively) among the *P. vulgaris* landraces (Table 3.4). Durban landrace *D-50C50Gy-K* produced more seeds per pod than landraces *Br-100LB-Cl* from Bushbuckridge, *D-90C10LR-Cl*, *D-90M10LB-Cl*, and *D-50P50LB-Cl* from Durban, *E-100Bk-Cl, E-50LR50C-K*, and *E-90LB10M-Cu* from Eshowe, *Em-*

100YG-Cl from Empangeni, *KN-50B50M-Cl* from KwaNdebele, *M-90LB10M-Cl* from Mtubatuba, *N-100DP-K*, and *N-100LP-K* from Nelspruit, and *P-50M50C-O* from Polokwane. In many seeds, the remaining landraces were comparable to *D-50C50Gy-K*.

Landrace *Em-50Bk50C-Cu* had numerous seeds (34.07 seeds per plant) whereas *E-90LB10M-Cu* and *KN-50B50M-Cl* (8.87 and 8.93 seeds per plant) had fewer seeds (Table 3.4). *Em-50Bk50C-Cu* produced significantly more seeds per plant than the majority of the landraces (*B-50B50M-Cl* from Benoni*, Br-100LB-Cl* from Bushbuckridge*, D-100By-Cl, D-50C50Gy-K, D-90C10LR-Cl, D-100C-Cl, D-50M50LB-Cl, D-90M10LB-Cl, D-50P50LB-Cl,* and *D-50RB50LB-Cl* from Durban, *E-100Bk-Cl, E-50LR50C-K, E-90LB10M-Cu, E-50M50C-K, E-90M10C-Cl, E-50YG-Cl,* and *E-100YG-Cl* from Eshowe, *Em-50M50LB-Cl,* and *Em-100YG-Cl* from Empangeni, *KN-50B50M-Cl* from KwaNdebele, *M-90LB10M-Cl* from Mtubatuba, *N-100DP-K* and *N-100LP-K* from Nelspruit, *P-50M50C-O* from Polokwane, *PS-50DB50LB-Cl, PS-90DB10LB-Cl, PS-90LB10B-Cl, PS-90LB10M-Cl, PS-50M50LB-Cl,* and *PS-90M10LB-Cl* from Port Shepstone and the outgroups *P. lunatus* and *P. coccineus* .

The landraces from the Durban area differed significantly in the number of seeds per pod and per plant. *D-50C50Gy-K* had numerous (6.07) seeds while *D-50P50LB-Cl* had fewer (3.93) seeds. *D-100YG-Cl* had numerous (25.00) seeds per plant whereas *D-90M10LB-Cl* had fewer (10.13) seeds. The landraces from Eshowe also differed significantly in the number of seeds per pod, but the number of seeds per plant did not. *E-90M10C-Cl* and *E-100YG-Cl* had numerous (5.13) seeds per pod whereas landraces *E-100Bk-Cl* and *E-90LB10M-Cu* had fewer (3.87) seeds. The numeb of seeds per plant among the landraces from Eshowe ranged from 8.87–17.93.

The Empangeni landraces differed significantly in the number of seeds per pod and per plant. *Em-100LB-Cl* had numerous (5.60) seeds per pod whereas *Em-100YG-Cl* had fewer (3.73) seeds. *Em-50Bk50C-Cu* had numerous (34.07) seeds per plant while *Em-100YG-Cl* had fewer (12.40) seeds. The least to most seeds per pod and per plant ranges among the landraces from Nelspruit were 3.47–3.60, and 13.53–18.20, respectively. The landraces from Port Shepstone differed significantly in the number of seeds per plant, but the number of seeds per pod did not. *PS-100YG-Cl* had

numerous (23.80) seeds per plant whereas *PS-50M50LB-Cl* had fewer (12.20) seeds. The least to most seeds per pod ranges among the Port Shepstone landraces were 4.33–5.00.

The plants sowed from 50% brown and 50% maroon, and 50% maroon and 50% cream seeds, differed significantly in the number of seeds per pod, but the number of seeds per plant did not. *B-50B50M-Cl* had numerous (4.73) seeds per pod whereas *KN-50B50M-Cl* had fewer (3.07) seeds. *E-50M50C-K* had numerous (5.00) seeds per pod while *P-50M50C-O* had fewer (3.67) seeds. The plants with 100% light brown seeds, differed significantly in the number of seeds per pod and per plant. *Em-100LB-Cl* had numerous (5.60) seeds per pod whereas *Br-100LB-Cl* had fewer (4.20) seeds. Again, *Em-100LB-Cl* had numerous (31.53) seeds per plant while *Br-100LB-Cl* had fewer (14.60) seeds.

P. lunatus had the longest seeds (30 mm) of them all. Among the *P. vulgaris* landraces, *N-100DP-K* had the longest (16.70 mm) seeds whereas *KN-100W-Cl* had the shortest (7.50 mm) (Table 3.4). Landrace *N-100DP-K* from Nelspruit had the longest seeds, it was similar to the outgroup (*P. coccineus*) but longer than all of the *P. vulgaris* landraces.

The Durban, Eshowe, Empangeni, and Nelspruit landraces, differed significantly in seed length. However, the Port Shepstone landraces did not. *D-90M10LB-Cl* and *D-50P50LB-Cl* from Durban had the longest (13.00 mm) seeds, but *D-90LB10B-Cu* had the shortest (9.30 mm). *E-50LR50C-K* from Eshowe origin had the longest (13.30 mm) seeds whereas *E-100Bk-Cl* had the shortest (7.80 mm). *Em-50LB50M-Cl* from the Empangeni area had the longest (11.40 mm) seeds while *Em-100LB-Cl* had the shortest (8.00 mm). *N-100DP-K* had the longest (16.70 mm) seeds whereas *N-100LP-K* had the shortest (13.90 mm). The shortest to longest seed ranges among the landraces from Port Shepstone was 10.10–11.60 mm.

The landraces with 50% brown and 50% maroon, 100% light brown, and 90% maroon, and 10% light brown seeds differed significantly in seed length. *KN-50B50M-Cl* had the longest (13.90 mm) seeds while *B-50B50M-Cl* had the shortest. *Br-100LB-Cl* had the longest (12.60 mm) whereas *Em-100LB-Cl* had the shortest (8.00 mm). *D-*

90M10LB-Cl had the longest (13.00 mm) seeds while *PS-90M10LB-Cl* had the shortest (11.10 mm).

P. lunatus showed insignificant differences with *P. coccineus* and *KN-50B50M-Cl* but, had the thickest seeds (5.70 mm) outperforming the remaining landraces. Among the *P. vulgaris* landraces, *KN-50B50M-Cl* had the thickest (4.90 mm) seeds while *D-90LB10B-Cu* had the thinnest (2.40 mm) (Table 3.4). The KwaNdebele landrace *KN-50B50M-Cl* had thicker seeds than landraces *B-50B50M-Cl* from Benoni, *Br-100LB-Cl* from Bushbuckridge, *D-100By-Cl*, *D-50C50Gy-K, D-100C-Cl, D-90C10LR-Cl, D-90LB10B-Cu, D-50P50LB-Cl* and *D-50RB50LB-Cl* from Durban, *E-100Bk-Cl, E-50M50C-K, E-90M10C-Cl, E-50YG-Cl* and *E-100YG-Cl* from Eshowe, *Em-100LB-Cl* and *Em-100YG-Cl* from Empangeni, *KN-100W-Cl* from KwaNdebele, *M-90LB10M-Cl* from Mtubatuba, and *PS-50DB50LB-Cl, PS-90DB10LB-Cl, PS-90LB10B-Cl, PS-50M50LB-Cl, PS-90M10LB-Cl* and *PS-100YG-Cl* from Port Shepstone but it was similar to the outgroups and landraces *D-50M50LB-Cl, D-90M10LB-Cl, E-50LR50C-K, E-90LB10M-Cl, Em-50M50LB-Cl, N-100DP-K, P-50M50C-O,* and *PS-90LB10M-Cl.*

The Durban, Eshowe, Empangeni, Nelspruit, and Port Shepstone landraces, differed significantly in seed thickness. *D-50M50LB-Cl* and *D-90M10LB-Cl* from Durban had the thickest (4.10 mm) seeds, but *D-90LB10B-Cu* had the thinnest (2.40 mm). *E-90LB10M-Cu* had the thickest (4.40 mm) seeds while *E-90M10C-Cl, E-100YG-Cl* and *E-100Bk-Cl* had the thinnest (3.00 mm) seeds. *Em-50LB50M-Cl* from the Empangeni area had the thickest (4.10 mm) seeds while *Em-50Bk50C-Cu* had the thinnest (2.50 mm) seeds. *N-100DP-K* produced the thickest (4.50 mm) seeds whereas *N-100LP-K* had the thinnest (3.40 mm). *PS-90LB10M-Cl* had the thickest (4.10 mm) seeds whereas *PS-90DB10LB-Cl* produced the thinnest (3.10 mm).

P. lunatus produced the widest seeds (18.30 mm) of them all. In the *P. vulgaris* landraces, seeds of *N-100DP-K* were the widest (7.10 mm), while *E-100Bk-Cl* and *KN-100W-Cl* were the narrowest (4.10 mm) (Table 3.4). Landrace *N-100DP-K* from Nelspruit had the widest seeds than the majority of the landraces (*B-50B50M-Cl* from Benoni*, D-100By-Cl, D-50C50Gy-K, D-90C10LR-Cl, D-100C-Cl, D-90LB10B-Cu, D-50P50LB-Cl, D-50RB50LB-Cl,* and *D-100YG-Cl* from Durban, *E-100Bk-Cl, E-50LR50C-K, E-90LB10M-Cu, E-50M50C-K, E-90M10C-Cl, E-50YG-Cl,* and *E-*

100YG-Cl from Eshowe, *Em-50Bk50C-Cu*, *Em-50M50LB-Cl*, *Em-100LB-Cl,* and *Em-100YG-Cl* from Empangeni, *KN-100W-Cl* from KwaNdebele, *M-90LB10M-Cl* from Mtubatuba and *PS-50DB50LB-Cl, PS-90DB10LB-Cl, PS-90LB10B-Cl, PS-90LB10M-Cl, PS-50M50LB-Cl, PS-90M10LB-Cl,* and *PS-100YG-Cl* from Port Shepstone). But, it was comparable to landraces *Br-100LB-Cl* from Bushbuckridge, *D-50M50LB-Cl,* and *D-90M10LB-Cl* from Durban, *KN-50B50M-Cl* from KwaNdebele, *N-100LP-K* from Nelspruit, and *P-50M50C-O* from Polokwane.

The Durban, Eshowe, and Empangeni landraces, differed significantly in seed width. However, the Nelspruit and Port Shepstone landraces did not. *D-90M10LB-Cl* and *D-50M50LB-Cl* from Durban had the widest (6.30 mm and 6.10 mm, respectively) seeds, but *D-50C50Gy-K* had the narrowest (4.50 mm) seeds. *E-50LR50C-K* had the widest (5.50 mm) seeds than other landraces in the Eshowe group. *Em-50LB50M-Cl* from the Empangeni area had the widest (5.70 mm) seeds while *Em-100LB-Cl* narrowest (4.30 mm) seeds. The narrowest to widest seed ranges among the Nelspruit and Port Shepstone landraces were 6.20 – 7.10 mm, and 4.50–5.30 mm, respectively.

The landraces with 50% brown and 50% maroon, 90% maroon and 10% light brown, and 50% maroon and 50% cream seeds differed significantly in seed width. *KN-50B50M-Cl* had the widest (6.80 mm) seeds while *B-50B50M-Cl* had the narrowest (5.50 mm) seeds. *D-90M10LB-Cl* had the widest (6.30 mm) seeds whereas *PS-90M10LB-Cl* had the narrowest (4.90 mm). *P-50M50C-O* had the widest (6.40 mm) whereas *E-50M50C-K* had the narrowest (4.90 mm) seeds.

P. coccineus displayed the largest value for the total seed mass (TSM), i.e. 30.21 g which outperformed both *P. lunatus* and all the *P. vulgaris* landraces. Among the *P. vulgaris* landraces *P-50M50C-O* yielded the biggest total seed mass (17.72 g) but *E-50M50C-K* had the lowest value for the TSM (2.89 g) (Table 3.4). *P-50M50C-O* from Polokwane produced the biggest total seed mass, it was similar to the outgroup *P. lunatus* but was bigger than the remaining *P. vulgaris* landraces.

The landraces from Durban differed significantly in the TSM. *D-100YG-Cl* had the biggest value (11.86 g) TSM whereas *D-90C10LR-Cl* had the lowest value (3.71 g). The biggest to lowest TSM ranges among the landraces from Eshowe, Empangeni,

Nelspruit, and Port Shepstone were 2.89–5.48, 4.52–6.86, 6.92–8.58, and 4.22–7.53 g, respectively. The landraces with 100% yellowish-green and 50% maroon, and 50% cream seeds showed significant differences in TSM. *D-100YG-Cl* had the biggest (11.86 g) TSM while *Em-100YG-Cl* had the lowest (4.52 g) TSM. Again, *P-50M50C-O* had the biggest (17.72 g) whereas *E-50M50C-K* had the lowest (2.89 g).

The *P. lunatus* had the highest 100-seed mass (219.30 g) compared with all the landraces. Among the *P. vulgaris* landraces, *N-100LP-K* had the highest 100-seed mass (92.00 g) whereas *E-50M50C-K* and *E-90M10C-Cl* both had the least (26.58 g) (Table 3.4). The Nelspruit landrace *N-100LP-K* had the highest 100-seed mass outperforming all the *P. vulgaris* landraces.

The plants from Nelspruit differed significantly in 100-seed mass. *N-100LP-K* had the highest (92.00 g) 100-seed mass whereas *N-100DP-K* had the lowest (41.50 g). The lowest to highest 100-seed mass ranges among the landraces from Durban, Eshowe, Empangeni, and Port Shepstone were 26.88–57.87, 26.58–45.80, 29.20–56.77, and 29.42–43.31 g, respectively.

3.4.6 Correlation among morpho-agronomic traits of *Phaseolus vulgaris*

Positive correlations were significant among the measured morpho-agronomic traits (Table 3.5). Stem diameter and leaf area correlated positively with each other. Plant height had a positive correlation with pod width and they both correlated with seed length, seed width, and 100-seed mass. Leaf area and pod length correlated positively with each other. The number of branches showed a positive correlation with the number of pods and they both further correlated with total seed mass. The number of branches also correlated positively with pod width, seed length, width and thickness, and 100-seed mass. A positive correlation was also recorded between the days to first flowering and 50% flowering. The number of pods had a positive correlation with the number of seeds per plant and total seed mass.

Table 3.5: Correlation of morpho-agronomic traits among *Phaseolus vulgaris* landraces

Traits	GP	SD	PH	LA	CC	NB	DFF	50%F	NP	PL	PW	NSP	NSPl	SL	SW	ST	TSM
SD	-0.077																
PH	-0.126	0.259															
LA	0.008	**0.620**	-0.032														
CC	-0.285	0.132	0.583	-0.154													
NB	-0.508	-0.063	0.519	-0.302	0.518												
DFF	-0.163	-0.291	0.121	-0.249	-0.021	0.406											
50%F	-0.169	-0.318	0.217	-0.315	0.114	0.498	**0.955**										
NP	-0.238	-0.255	0.224	-0.315	0.385	**0.734**	0.204	0.277									
PL	-0.041	0.523	0.374	**0.612**	-0.031	-0.070	-0.038	-0.028	-0.307								
PW	-0.305	0.090	**0.796**	-0.204	0.509	**0.835**	0.393	0.487	0.485	0.202							
NSP	0.122	-0.071	-0.379	0.187	-0.321	-0.378	-0.207	-0.285	-0.136	-0.143	-0.437						
NSPl	0.026	-0.391	-0.362	-0.086	-0.037	0.099	-0.120	-0.086	**0.654**	-0.447	-0.221	0.283					
SL	-0.233	0.103	**0.749**	-0.162	0.482	**0.714**	0.427	0.495	0.337	0.243	**0.888**	-0.480	-0.351				
SW	-0.307	0.092	**0.782**	-0.203	0.526	**0.810**	0.369	0.476	0.438	0.151	**0.963**	-0.457	-0.235	**0.920**			
ST	-0.485	0.249	0.528	-0.107	0.264	**0.627**	0.296	0.292	0.184	0.261	**0.701**	-0.389	-0.461	**0.677**	**0.702**		
TSM	-0.560	0.033	0.511	-0.265	0.515	**0.930**	0.338	0.408	**0.795**	-0.027	**0.766**	-0.301	0.196	0.597	**0.716**	0.595	
HSM	-0.470	0.104	**0.712**	-0.286	0.518	**0.915**	0.391	0.477	0.550	0.072	**0.922**	-0.448	-0.139	**0.822**	**0.918**	**0.706**	**0.878**

Traits: GP, germination percentage; SD, stem diameter; PH, plant height; LA, leaf area; CC, chlorophyll content; NB, number of branches; DFF, days to first flowering; 50% F, 50% flowering; NP, number of pods; PL, pod length; PW, pod width, NSP, number of seeds per pod; NSPl, number of seed per plant; SL, seed length; SW, seed width; ST, seed thickness; TSM, total seed mass; HSM, 100-seed mass. Significant values ≥ 0.6 are in bold.

Pod width showed a positive correlation with seed thickness and total seed mass. Seed length and seed width correlated positively with each other and they both correlated positively with seed thickness and 100-seed mass. Seed width had a positive correlation with total seed mass. Seed thickness and 100-seed mass correlated positively with each other. A positive correlation was also recorded between total seed mass and 100-seed mass.

3.4.7 Principal component analysis

The first five informative principal components (PC1–PC5) explained 81.785% of the total variation (Table 3.6). The first component (PC1) showed a positive correlation with plant height, the number of branches, pod width, seed length, width and thickness, total seed mass, and 100-seed mass that described 44.397% of the total variation. The second component (PC2) described 17.401% of the total variation and correlated positively with stem diameter, leaf area, pod length and showed a negative correlation with the number of pods and the number of seeds per plant.

The third component (PC3) correlated positively with days to first and 50% flowering that described 10.565% of the total variation. Germination percentage was positively correlated with the fourth component (PC4) and described 5.550% of the total variation. The number of seeds per pod correlated positively with the fifth component (PC5) and described 3.872% of the total variation.

Table 3.6: Principal component coefficients of traits for different *Phaseolus vulgaris* landraces

Traits	PC1	PC2	PC3	PC4	PC5
GP	-0.457	0.023	0.232	**0.601**	0.089
SD	0.050	**0.776**	-0.335	0.016	-0.077
PH	**0.740**	0.386	-0.092	0.224	0.141
LA	-0.281	**0.636**	-0.260	0.322	-0.127
CC	0.582	0.028	-0.382	-0.006	-0.182
NB	**0.915**	-0.239	-0.169	-0.031	-0.003
DFF	0.479	-0.268	**0.698**	0.145	-0.062
50%F	0.569	-0.292	**0.644**	0.189	-0.107
NP	0.565	**-0.602**	-0.426	0.248	-0.072
PL	0.115	**0.788**	0.039	0.278	-0.085
PW	**0.950**	0.105	0.003	0.132	0.136
NSP	-0.512	-0.159	-0.183	0.107	**0.709**
NSPl	-0.170	**-0.734**	-0.485	0.318	-0.110
SL	**0.884**	0.201	0.148	0.131	0.091
SW	**0.940**	0.116	0.015	0.095	0.130
ST	**0.747**	0.306	0.074	-0.359	0.149
TSM	**0.864**	-0.239	-0.312	-0.032	-0.014
HSM	**0.965**	-0.005	-0.082	-0.045	0.071
Eigenvalue	7.992	3.132	1.902	0.999	0.697
Variability(%)	44.397	17.401	10.565	5.550	3.872
Cumulative(%)	44.397	61.798	72.362	84.877	88.749

Traits: GP, germination percentage; SD, stem diameter; PH, plant height; LA, leaf area; CC, chlorophyll content; NB, number of branches; DFF, days to first flowering; 50% F, 50% flowering; NP, number of pods; PL, pod length; PW, pod width, NSP, number of seeds per pod; NSPl, number of seed per plant; SL, seed length; SW, seed width; ST, seed thickness; TSM, total seed mass; HSM, 100-seed mass. Significant values ≥ 0.6 are in bold.

3.4.8 Cluster analysis

In a biplot, almost all traits correlated positively with PC1, except for leaf area, germination percentage, number of seeds per pod, and number of seeds per plant (Figure 3.3). The biplot further clustered the landraces with similar morphological traits into three different groups. Group I was composed of the outgrouped landraces *P. coccineus* and *P. lunatus*. Group II included landraces *D-50M50LB-Cl* and *D-50P50LB-Cl* from the Durban area, *N-100DP-K,* and *N-100LP-K* from Nelspruit, *KN-*

50B50M-Cl from KwaNdebele, and *P-50M50C-Cl* from Polokwane. Landraces *D-50M50LB-Cl*, *KN-50B50M-Cl* and *P-50M50C-O* had similar seed coats, which differed in colour intensity and area of origin. *N-100DP-K* and *N-100LP-K* also had similar seed coat colours, but differed only in colour intensity (Table 3.1). All the remaining landraces formed Group III.

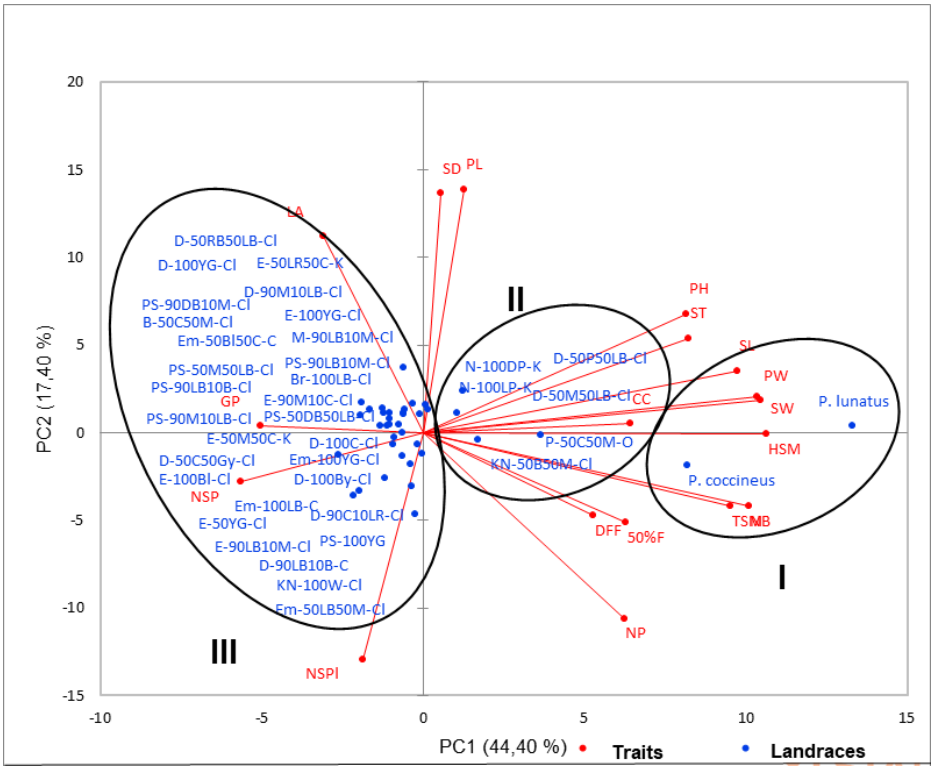


Figure 3.4: Biplot based on the first two principal components (PC) for morpho-agronomic traits of *Phaseolus vulgaris* landraces.

Landraces are explained in Table 3.1. Traits: GP, germination percentage; SD, stem diameter; PH, plant height; LA, leaf area; CC, chlorophyll content; NB, number of branches; DFF, days to first flowering; 50% F, 50% flowering; NP, number of pods; PL, pod length; PW, pod width, NSP, number of seeds per pod; NSPl, number of seeds per plant; SL, seed length; SW, seed width; ST, seed thickness; TSM, total seed mass; HSM, 100-seed mass.

The relationship between landraces was further illustrated by a dendrogram based on Euclidean distance, which grouped them into four clusters (Figure 3.4). Cluster I was divided into two sub-clusters (IA and IB). Sub-cluster IA was composed of *D-100By-Cl, D-50C50Gy-Cl*, *D-90C10LR-Cl*, *E-100Bk-Cl*, *E-50LR50C-Cl*, *E-90LB10M-Cu, Em-100YG-Cl,* and *PS-90LB10B-Cl.* These *P. vulgaris* landraces were associated with greater germination percentage, earlier flower formation, and shorter seeds (Table 3.3 and 3.4).

Sub-cluster IB consisted of *Br-100LB-Cl*, *D-50M50LB-Cl, D-90M10LB-Cl, D-50P50LB-Cl, E-50M50C-K, E-90M10C-Cl, E-50YG-Cl, E-100YG-Cl, Em-50LB50M-Cl, M-90LB10M-Cl, N-100DP-K, N-100LP-K, PS-90DB10LB-Cl PS-50M50LB-Cl, PS-90LB10M-Cl,* and *PS-90M10LB-Cl*. Cluster II was composed of *P-50M50C-O*, *KN-50B50M-Cl,* and *B-50B50M-Cl.* These landraces were associated with greater plant height, stem diameter, leaf area, pod length, and longer, wider and thicker as well as heavier seeds (Table 3.3 and 3.4) as well as similar seed coat colour, but *KN-50B50M-Cl*, and *B-50B50M-Cl* differed in colour intensity from *P-50M50C-O* (Table 3.1).

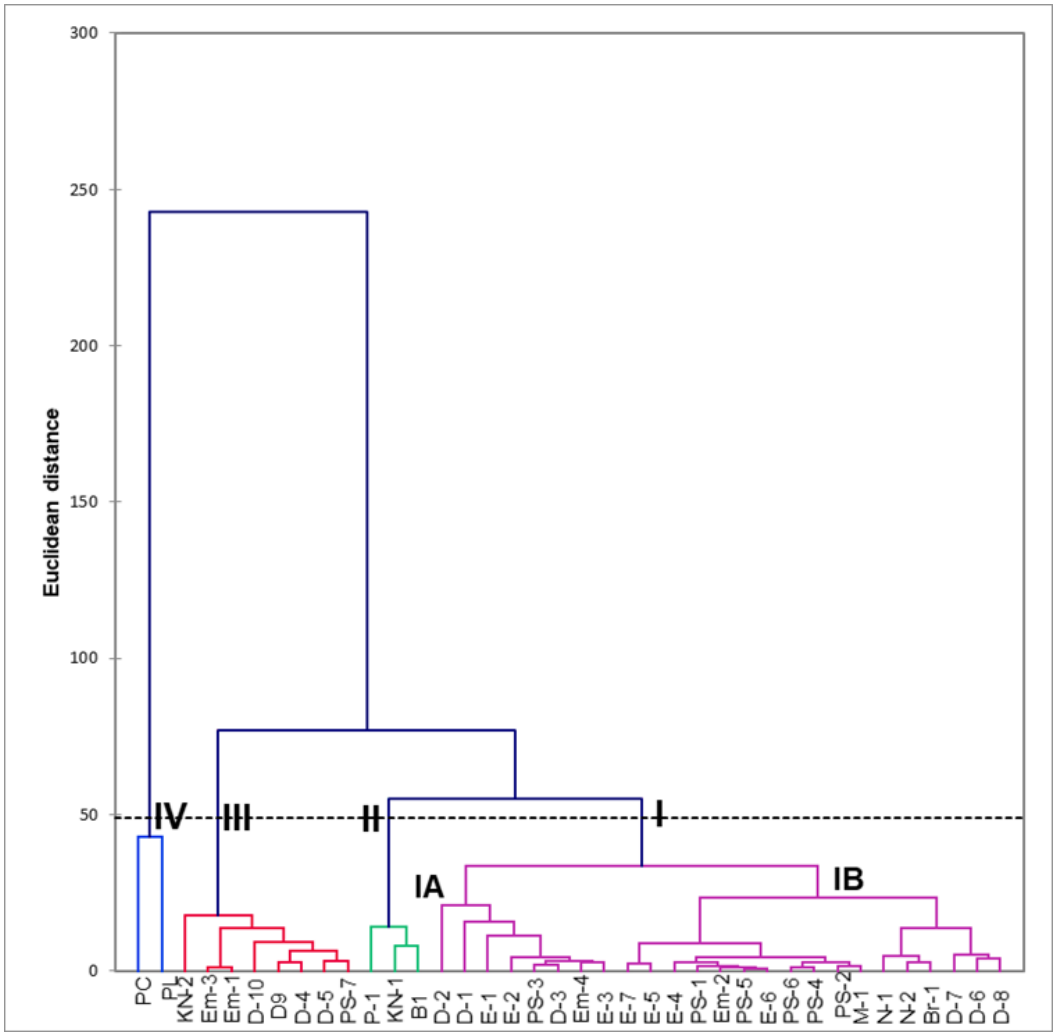


Figure 3.3: Dendrogram grouping of *Phaseolus vulgaris* landraces based on Euclidean distances

The description for landraces is in Table 3.1. Landraces from Benoni: **B-1**, *B- 50B50M-Cl*; **PL**, *Phaseolus lunatus;* **PC**, *Phaseolus coccineus.* Landrace from Bushbuckridge: **Br-1**, *Br-100LB-Cl*. Landraces from Durban: **D-1**, *D-100By-Cl*; **D-2**, D-50C50Gy-K; **D-3**, *D-90C10LR-Cl*; **D-4**, *D-100C-Cl*; **D-5**, *D-90LB10B-Cu*; **D-6**, *D-50M50LB-Cl*; **D-7**, *D-90M10LB-Cl*; **D-8**, *D-50P50LB-Cl*; **D-9**, *D-50RB50LB-Cl*; **D-10**, *D-100YG-Cl*. Landraces from Eshowe: **E-1**, *E-100Bk-Cl*; **E-2**, *E-50LR50C-K*; **E-3**, *E-90LB10M-Cu*; **E-4**, *E-50M50C-K*; **E-5**, *E-90M10C-Cl*; **E-6**, *E-50YG-Cl*; **E-7**, *E-100YG-Cl*. Landraces from Empangeni: **Em- 1**, *Em-50Bk50C-Cu*; **Em-2**, *Em-50M50LB-Cl*; **Em-3**, *Em-100LB-Cl*; **Em-4**, *Em-100YG-Cl*. Landraces from KwaNdebele. **KN-1**, *KN-50B50M-Cl*; **KN-2**; *KN-100W-Cl*. Landrace from Mtubatuba. **M-1**, *M-90LB10M-Cl*. Landraces from Nelspruit. **N-1**, *N-100DP-K*; **N-2**, *N-100LP-K*. Landrace from Polokwane. **P-1**, *P-50M50C-O*. Landraces from Port Shepstone. **PS- 1**, *PS-50DB50LB-Cl*; **PS-2**, *PS-90DB10LB-Cl*; **PS-3**, *PS-90LB10B-Cl*; **PS-4**, *PS-90LB10M-Cl*; **PS-5**, *PS-50M50LB-Cl*; **PS-6**, *PS-90M10LB-Cl*; **PS-7**, *PS-100YG-Cl*.

Cluster III consisted of *D-100C-Cl*, *D-90LB10B-Cu*, *D-50RB50LB-Cl*, *D-100YG-Cl*, *Em-50Bk50C-Cu*, *Em-100LB-Cl, KN-100W-Cl,* and *PS-100YG-Cl.* These landraces had narrower leaves, numerous seeds per pod and plant as well as lighter 100-seed mass (Table 3.3 and 3.4). Cluster IV was comprised of the outgroups *P. coccineus* and *P. lunatus*. The outgroups were associated with greater stem diameter, leaf area, chlorophyll content, pod length and seed length, width, and thickness as well as numerous leaves, branches, heavy 100-seed mass, and fewer seeds per pod (Table 3.3 and 3.4).

3.5 Discussion

3.5.1 Germination percentage and stem traits

The germination percentage of *Phaseolus vulgaris* landraces ranged from 40–100 % with an average of 79.35 % (Table 3.3). The study of *P. vulgaris* landraces from Brazil and Nepal (Lima *et al*., 2005; Kalauni *et al*., 2019) had higher ranges of 89–94% and 84–93.8% with an average of 92.00 and 89.3%, respectively. The higher germination percentage may be due to the diversity in the seed characteristics of the landraces or the climatic conditions of these countries (Lima *et al*., 2005).

Landraces of the same origin (Durban and Eshowe) with different seed coat colour, intensity, and shape differed in seed germination percentage (Table 3.3). Variability in seed traits was probably the main difference for these seeds regardless of their same origin (Zilio *et al*., 2013), and thus they differed in their germination percentage. Such differences in landraces with different seed traits was also evident in plant height and stem diameter of landraces from Durban, Eshowe, and Empangeni (Table 3.3). Among South African *P. vulgaris* landraces, 65.79% produced pure green stems while 34.21% were green with purple pigmentation. Similar findings were reported in the mid-hills of Nepal (Kalauni *et al*., 2019).

A range in the plant height from 56.20–100.00 cm (Table 3.3) was within the range of 19.5–123.4 cm obtained among Portuguese and Bulgarian landraces (Stoilova *et al*., 2005). The significant variation in plant height might indicate heterogeneity among the

landraces, which is essential for breeding purposes (Razvi *et al*., 2017). The stem diameter range from 3.00–4.27 mm (Table 3.3) was narrower than the 2.75–8.40 mm found among landraces from Valencia in Spain (Arteaga *et al*., 2019). Early measurements in the current study are probably the cause for the lower variation in stem diameter (21 days after planting and before flowering), whereas the Spanish landraces were measured at maturity (Arteaga *et al*., 2019). The variation in stem size was also probably caused by the different landraces investigated (Nwadike and Terkimbi, 2015).

A range in the number of branches from 2.07–4.13 (Table 3.3) was lower than the number of branches from 3.20–10.78 reported among a comparable study from Turkey (Yeken *et al*., 2019). The outgroups, *P. coccineus* and *P. lunatus*, as well as landraces *E-90LB10M-Cu* and *N-100DP-K,* had the tallest plants, numerous branches and also climbing growth habits. Similar results were recorded among *P. vulgaris* landraces from Bulgaria and Portugal, where plants with climbing growth habits had taller plants with numerous branches (Stoilova *et al*., 2013).

Differences among landraces with similar seed coat colour but different environments were recorded in germination percentage (*D-100YG-Cl*, *E-100YG-Cl*, *Em-100YG-Cl*, and *PS-100YG-Cl*) and stem diameter (*Br-100LB-Cl* and *Em-100LB-Cl*) (Table 3.3). The variability could probably result from diverse conditions in their locations (Bareke, 2019). The germination percentage and plant height had insignificant differences in landraces with 50% brown and 50% maroon (*B-50B50M-Cl* and *KN-50B50M-Cl*) and with 100% light brown seeds (*Br-100LB-Cl* and *Em-100LB-Cl*), which are similar to those recorded in Bursa and Bulikesir in North-western Turkey (Yeken *et al*., 2018).

This insignificance possibly suggests that the landraces with different seed coat colours do not respond differently to environmental conditions in terms of germination percentage and plant height (Zilio *et al*., 2013). In landraces with 50% maroon 50% cream, *P-50M50C-O* from Polokwane had thicker hard (woody) stems than plants from Eshowe (*E-50M50C-K*) with the same colour. This variation in stems might be due to the diversity in origins. Polokwane in the Limpopo province is a semi-arid area with low rainfall (Mpandeli and Maponya, 2013). The stems were probably hard and thicker

to adapt to the hot and dry conditions in the parental environment (Mansoor *et al*., 2019).

3.5.2 Leaf traits

The leaves showed green with sliver veins and green with purple veins among *P. vulgaris* landraces from South Africa. The leaf colour of the landraces from the Mid-hills of Nepal only showed green and purple veins (Kalauni *et al*., 2019). The variation in leaf colour can be attributed to differences in the genotypic and phenotype of landraces investigated between these countries (Zilio *et al*., 2013). A range in the number of leaves from 18.5–37.8 of the Nigerian landraces (Nwadike and Terkimbi, 2015) was higher than the number of leaves from 13.78–23.22 (Table 3.3). This higher range recorded might be due to different planting seasons, the South African landraces were planted in the warm and dry season (August–November) while the Nigerian trial was planted duringthe wet cropping season (Nwadike and Terkimbi, 2015).

A range in leaf area from 3761–8144 mm^2 (Table 3.3) was broader than the 1333–3030 mm^2 obtained among landraces from southern Ethiopia (Yohannes *et al*., 2020). The explanation for the broader leaves in this study could be related to the significant quantity of plant resources required for leaf area increment in the vegetative stage, whereas Ethiopian landraces' leaf area was narrower as it was measured after blooming began. The decrease in leaf area after the initiation of flowering could be due to low plant resources (water or phosphate) in the reproductive stage (Trindade *et al*., 2010). A range in the chlorophyll content from 11.99–21.51 mg cm^2 (Table 3.3) was comparable to that of Northeast Romania with a range of 13.71–28.49 (Modiga and Jitareanu, 2017).

Variability in leaf area of similar landraces from different origins (Durban and Empangeni) (Table 3.3) possibly resulted from diverse environmental conditions in the respective areas (Stoilova *et al*., 2013). The Durban landrace *D-90M10LB-Cl* outperformed the Port Shepstone *PS-90M10LB-Cl* with similar seed coat colour in leaf area. The Port Shepstone landraces were probably growing under environmental stress, which caused a decrease in leaf growth (Trindade *et al*., 2010). This variability

supports breeding efforts to improve plant adaptation to diverse environments (Trindade *et al*., 2010; Razvi *et al*., 2017). The genetic diversity, exhibited by differences in seeds coat colour, was displayed as differences in leaf number and the different environments as documented in Nigeria by Nwadike and Terkimbi, 2015.

3.5.3 Flower traits

The flower colour was responsible for the huge diversity in *P. vulgaris* landraces, this is also true for the colour of pods (Musango *et al*., 2016). The green with purple pigmented stems produced white with purple edges, pink, white, purple, and cream flowers. The green stems produced various flower colours (Table 3.4). The shoot and petal colours are controlled by either one gene or a group of linked genes (Okii *et al*., 2014). The days to the first flowering of the South African landraces range from 36.00–43.33 days after planting (DAP) (Table 3.4) which was within the range of 30.3–50.3 found among Portuguese and Bulgarian landraces (Stoilova *et al*., 2005).

A range in the days to 50% flowering from 38.00–44.67 DAP (Table 3.4) was similar to the range of 31.00–41.00 recorded among Zimbabwean landraces (Musango *et al*., 2016). Eshowe (*E-100Bk-Cl*) and Nelspruit (*N-100DP-K*) landraces, due to their short flowering cycle, might be growing under environmental stress (Lorts *et al*., 2019). Plants that are stressed by the environment modify their structure and employ plant resources in the reproductive stage to develop quickly (Darkwa *et al*., 2016). The results are in agreement with the findings of Nwadike and Terkimbi (2015). This is also true of landraces with comparable seed coats but distinct geographical settings. A landrace from Eshowe (*E-50M50C-K)* with 50% maroon and 50% cream seeds matured earlier than a landrace from Polokwane (*P-50M50C-O*) with comparable seeds.

3.5.4 Pod traits

The white flowers produced pale yellow to white, dark purple, pink, and normal green pods. Cream flowers generated pink and purple stripes on yellow pods. Purple flowers gave pale yellow to white and purple stripes on yellow pods. White with purple edged flowers generated pink stripes on yellow and pale yellow to white pods (Table 3.4).

This variation probably resulted from mutation and segregation (Musango *et al*., 2016). A range in the number of pods from 3–12 (Table 3.4) was fewer than the 6.4–13.9 pods found among landraces from Bulgaria and Portugal (Stoilova *et al*., 2005). The fewer pods could be due to the weather conditions in KwaZulu-Natal which was characterized by high temperatures (25 to 30^0C) when these landraces were in flowering and pod formation. During the flowering and pod filling phases temperatures often rise quickly and landraces develop under drought stress which limits the productivity of the plants (Stoilova *et al*., 2013).

Variability in the number of pods among landraces with the same seed coat colour but from different locations were recorded (*D-100YG-Cl* and *Em-100YG-Cl*; *D-50M50LB-Cl* and *PS-50M50LB-Cl*) which were similar to those reported among brown seeded landraces from Valencia, Spain (Arteaga *et al*., 2019). Significant differences in the number of pods from landraces with different seed coats but the same environments (Durban and Port Shepstone) might be due to the genotypic variation of *P. vulgaris* landraces along with their area of origin (Alemu *et al*., 2018).

In the landraces from the current study the pod length and width ranged from 93–120 mm and 9–13 mm, respectively. They were within the range among *P. vulgaris* landraces from Benin (55–266.7 mm and 11.3–19.0 mm, respectively) (Loko *et al*., 2018). The variation in pod sizes might be due to the differences in the types of landraces studied between these countries (Bagheri *et al*., 2017). Plants sown from 100% light brown seeds differed in pod length, a landrace from Bushbuckridge (*Br-100LB-Cl*) had longer pods than a landrace from Empangeni (*Em-100LB-Cl*) with a similar seed coat colour. The variability in pod length could be due to the seed size, *Br-100LB-Cl* had longer seeds whereas *Em-100LB-Cl* had smaller seeds (Table 3.4). Similarly, a study conducted in Ethiopia indicated that large seeds generally produce longer pods (Bareke, 2019).

3.5.5 Seed traits

The number of seeds per pod ranged from 3–6 (Table 3.4). Similar ranges were obtained from the landraces of Zimbabwe and India, i.e. 3.56–5.56 and 5.36–5.73, respectively (Musango *et al*., 2016; Razvi *et al*., 2017). The number of seeds, *viz.* 10–

34 (Table 3.4) was within the range of 19.9–36.3 documented among Portuguese and Bulgarian landraces (Stoilova *et al*., 2005). Landraces from the same environment (Durban) with different seed coats differed in the number of seeds per pod (Table 3.4). *D-50C50Gy-K* was the smallest in seed size but had numerous seeds whereas *D-50P50LB-Cl* was the largest in seeds however, had fewer seeds (Table 3.4). Thus, this study revealed that longer and wider seeds have few numbers of seeds per pod. A similar observation was reported in Valencia, Spain (Arteaga *et al*., 2019). This was also true among landraces sown from 50% brown and 50% maroon; 50% maroon and 50% cream seeds which originated from different locations (Table 3.4).

In Benin, the seed length and width ranged from 6–16 mm and 3.1–7.3 mm, respectively (Loko *et al*., 2018) and was within the range of 7.30–16.70 and 4.10–7.10, respectively attained among the South African landraces (Table 3.4). A range in the seed thickness from 2.40–4.90 mm (Table 3.4) was thinner than the 5.04–10.42 mm of Turkish seeds (Ekbic and Hasancaoglu, 2018). The 100-seed mass of South African landraces ranged from 26.58–92.00 g (Table 3.4) and was higher than the 4.00–51.00 g obtained among *P. vulgaris* landraces from Benin (Lima *et al*., 2005). The significant variation in the 100-seed mass was probably caused by genetic diversity and inter-plant competition (Lima *et al*., 2005; Musango *et al*., 2016).

Landraces with white seeds but from different origins of North-western Turkey,i.e. Yalova (YLV-28), Canakkale (CNK-4), and Bursa (BLCK-7) showed insignificant differences in 100-seed mass (Yeken *et al*., 2018). The results correspond with the current study where landraces with 100% light brown seeds from Bushbuckridge (*Br-100LB-Cl*) and Empangeni (*Em-100LB-Cl*) showed insignificancy in 100-seed mass (Table 3.4). These results may indicate that among the landraces with similar seed coats, the environmental conditions do not influence the seed mass (Zilio *et al*., 2013). The explanation also true for landraces with various seed coat colors from the same region (Table 3.4).

3.5.6 Correlation among morpho-agronomic traits

The stem diameter correlated positively with leaf area (r = 0.620), this correlation suggested that selection of thicker stems in South Africa will improve the leaf area

(Table 3.5). In similar findings among *P. vulgaris* landraces from Southern Ethiopia the stem diameter correlated positively with various traits (Yohannes *et al*., 2020).

Plant height and pod width correlated positively with each other and further correlated with seed length, width, and 100- seed mass (Table 3.5). These results were evident where the taller plants with wider pods had longer (< 16.00 mm) and wider seeds (< 7.00 mm) and also the highest 100- seed mass (< 90.00 g) (Table 3.3 and 3.4). This could indicate that landraces with taller plants and wider pods, such as KwaNdebele's *KN-50B50M-Cl*, Nelspruit's *N-100DP-K* and *N-1000LP-K*, and *P-50M50C-O* from Polokwane, are suitable landraces for future plant breeding since they can enhance plant growth.The results of the current study correspond to those of *P. vulgaris* from Nilgiris, where plant height correlated with 100-seed mass and where short plants had 100-seed mass < 60.0 g whereas tall plants had 100-seed mass > 60.0 g (Jose *et al*., 2009).

The number of pods had a positive correlation with the number of seeds per plant (r = 0.654) and total seed mass (r = 0.795) (Table 3.5). The number of pods in a similar study of *P. vulgaris* in Lesotho correlated with the number of seeds per pod (Morojele *et al*., 2016). This suggests that landraces with numerous pods will lead to landraces with many and heavy seeds. The number of branches showed a positive correlation with the number of pods (r = 0.734) and they both further correlated with total seed mass (r = 0.930 and r = 0.795, respectively) (Table 3.5).

The number of branches also correlated positively with pod width (r = 0.835), seed length (r = 0.713), seed width (r = 0.810) and thickness (r = 0.627) and also 100-seed mass (r = 0.915) (Table 3.5). These findings were similar to that of Langat *et al*. (2019) , when landraces in Kenya were under unstressed conditions, the number of branches correlated positively with numerous traits. The *P. vulgaris* from South Africa demonstrated the highest correlation (r = 0.955) in terms of days to first flowering and 50% flowering (Table 3.5). This could indicate that breeding of these traits could lead to an increase or improvement of another trait, which is significant for breeding purposes (Razvi *et al*., 2017).

The number of pods had a positive correlation with the number of seeds per plant (r = 0.654) and total seed mass (r = 0.795) (Table 3.5), which is similar to results reported in India (Razvi *et al*., 2017). This could indicate that landraces *PS-90DB10LB-Cl*, *D-100YG-Cl*, *Em-50Bk50C-Cu*, and *Em-100LB-Cl* have the ability to produce a lot of pods and seeds (Table 3.4). The pod width showed a positive correlation with seed thickness (r = 0.701) and total seed mass (r = 0.766) (Table 3.5). In *P. vulgaris* from Ethiopia, the pod width positively correlated with seed length (r = 0.569) (Loko *et al*., 2018).

Seed length and width had a strong positive correlation with each other (r = 0.920) and they both correlated positively with seed thickness (r = 0.677 and r = 0.702, respectively) and 100-seed mass (r = 0.822 and r = 0.918, respectively) (Table 3.5). In a similar study of *P. vulgaris* landraces from Ethiopia, the seed length had a strong correlation with seed width (r = 0.766) and thickness (r = 0.706) (Loko *et al*., 2018). The seed thickness and 100-seed mass also correlated with each other (r = 0.706) (Bareke, 2019).

3.5.7 Principal component analysis

In the current study, the principal component analysis (PCA) summarised the variation of growth and yield traits in five principal components. To select the appropriate number of factors, a factor loading with an Eigen total value of ≥ 0.60 was used (Akhshi *et al*., 2015). The first five principal components (PC1-PC5) explained 88.749% of the total variation. The traits of these components could be considered in differentiating *P. vulgaris* landraces (Al-Ballat and Al-Araby, 2019).

Principal component 1 consisted of morpho-agronomic traits including plant height, number of branches, pod width, seed length, width, thickness, total seed mass, and 100-seed mass. Principal component 1 incorporated almost all growth factors and only one yield factor. This indicates the significance of these traits in producing vigorously growing plants. In a comparable study of Iran *P. vulgaris* landraces, PC1 composed of numerous growth traits and few yield factors (Akhshi *et al*., 2015).

The number of pods and number of seeds per plant correlated negatively with PC2 in the current study, but in the *P. vulgaris* landraces from India they correlated positively with PC1 (Panchbhaiya *et al*., 2017). Therefore, the negative correlation of number of pods and number of seeds per plant with PC2 suggest that such traits are not suitable for use in breeding programs of improving *P. vulgaris* yield. The days to first flowering and 50% flowering correlated positively with PC3. According to the traits of this PC, it includes components for the duration of the growth cycle (early or late maturity). This PC indicates the essence of these characteristics in adaptation to stressful environments (high temperatures and low humidity) (Stoilova *et al*., 2013).

3.5.8 Cluster analysis

In the cluster analysis biplot and dendrogram, landraces were clustered based on seed coat colour, shape, similar morpho-agronomic traits, and also area of origin. Group I (in the biplot) and Cluster IV (in the dendrogram) was formed by the outgroups, *viz*. *Phaseolus coccineus* and *Phaseolus lunatus*. The grouping of these landraces was possibly due to their indeterminate climbing growth habit associated with taller plants, numerous branches, and longer, wider, thicker, and heavier seeds, and their delay in the days to flowering as well as the fact that are different species. Group II included *D-50M50LB-Cl* and *D-50P50LB-Cl* from Durban and *N-100DP-K* and *N-100LP-K* from Nelspruit which were clustered in sub-cluster IB. Group II was also composed by *P-50M50C-O* from Polokwane and *KN-50B50M-Cl* from KwaNdebele, although they were classified in separate clusters in the dendrogram (Figure 3.2 and 3.3).

The grouping of *D-50M50LB-Cl*, *P-50M50C-O*, and *KN-50B50M-Cl* in the biplot might be due to their shared similarity in seed coat colours (but differed in colour intensity) as well as longer, wider, and thicker seeds and leaves with high chlorophyll content. This is also true for *N-100DP-K*, *N-100LP-K*, and *D-50P50LB-Cl* (Figure 3.2). The results were similar to that of *P. vulgaris* landraces in Bulgaria and Portugal, where the landraces with similar seed coat colour but different shape, colour intensity, and area of origin were clustered together (Stoilova *et al*., 2013).

Group III included the majority of the South African *P. vulgaris* landraces based on a similar grouping of landraces by their area of origin and morpho-agronomic traits. *D-*

100C-Cl associated with *D-90LB10B-Cu*, *D-50RB50LB-Cl*, *Em-50Bk50C-Cu*, *Em-100LB-Cl*, *KN-100W-Cl,* and *PS-100YG-Cl* in a dendrogram (Cluster III) (Figure 3.3). The clustering might be possibly due to their similarity in numerous and longer pods which yielded smaller, lighter, and a high number of seeds (Table 3.4). Similar to the current findings, landraces from Ethiopia with small seeds, a higher number of pods, and seeds clustered together (Bareke, 2019).

D-50M50LB-Cl, *Em-50M50LB-Cl*, and *PS-50M50LB-Cl* associated with *Br-100LB-Cl*, *D-90M10LB-Cl*, *D-50P50LB-Cl*, *E-50M50C-K*, *E-90M10C-Cl*, *E-50YG-Cl*, *E-100YG-Cl*, *M-90LB10M-Cl*, *N-100DP-K*, *N-100LP-K*, *PS-90DB10B-Cl*, *PS-90M10LB-Cl*, and *PS-90LB10M-Cl* were clustered in sub-cluster IB (Figure 3.2). These landraces might be clustered due to tall plants, numerous and broader leaves, longer, wider, and thicker seeds, and also heavier seeds compared with other landraces. Plant height and seed traits are considered highly heritable traits (Musango *et al*., 2016), thus these landraces might be essential in plant breeding programs.

In the current study, landraces with 50% brown 50% maroon seeds namely *B-50B50M-Cl* and *KN-50B50M-Cl* were clustered in cluster II and Group II in the biplot. Landraces *D-100YG-Cl,* and *PS-100YG-Cl* with 100% yellowish-green seeds were grouped in a biplot (Group II) and cluster III. The landraces with 50% maroon and 50% light brown (*D-50M50LB-Cl*, *Em-50M50LB-Cl*, and *PS-50M50LB-Cl*) were clustered in sub-cluster IB (Figure 3.2 and 3.3). A comparable study of *P. vulgaris* landraces from Poland and also Bulgaria and Portugal yielded clustering of *P. vulgaris* with the same seed coat colour but from different environments (Stoilova *et al*., 2013; Boros *et al*., 2014).

3.6 Conclusion

Significant differences were recorded in vegetative and reproductive traits among *P. vulgaris* landraces with various seed traits and originating from different provinces of South Africa. Landraces *N-100DP-K*, *N-100LP-K*, *E-90LB10M-Cu, D-90M10LB-Cl*, *D-90LB10C-Cl*, *D-100YG-Cl*, and *E-100Bk-Cl* were the most productive in growth and yield traits. *E-100Bk-Cl* had the highest germination percentage and earliest days to

flowering. In growth traits *B-50B50M-Cl*, *D-90M10LB-Cl* and *N-100DP-Cl* had the most vigorously growing shoots, leaves as well as longer pods and seeds. In the yield traits landraces *D-90LB10C-Cl*, *PS-90DB10LB-Cl*, *D-100YG-Cl*, and *N-100LP-K* had many branches and leaves and also numerous pods and seeds. These landraces could be selected due to their excellent performance, and they can adapt to new environments and mature faster than other *P. vulgaris* from South Africa.

The appropriate traits for studying diversity among *P. vulgaris* landraces were positively correlated with components of high heritability component (PC1) in the principal component analysis (PCA). The essential traits were plant height, number of branches, pod width, seed length, width and thickness, and also total and 100-seed mass. Thus, breeding towards South African *P. vulgaris* with tall landraces could potentially result in plants with numerous branches, wider pods, and longer, wider, and thicker seeds. The use of *N-100DP-K*, *N-100LP-K Br-100LB-Cl*, and *E-90LB10M-Cu* in future plant breeding and large-scale farming will probably lead to high growth and yielding landraces. Landraces of *P. vulgaris* could be further tested in various locations to screen for morpho-agronomic traits and their adaptation to biotic constraints such as terminal drought, excess soil moisture, uncertainty in rainfall, and soil acidity.

Chapter 4

Variation in seed traits of F_1 progeny that segregated from the selected parents of *Phaseolus vulgaris* landraces

4.1 Introduction

Phaseolus vulgaris L. is a crop that forms an important part of people's diets worldwide (Hacisalihoglu and Settles, 2013). Its seeds have major macronutrients (proteins and starch) and are also rich in micronutrients (vitamin B5, vitamin B9, iron, and zinc) (Hacisalihoglu and Settles, 2013). It is domesticated from two gene pools, *viz.* Mesoamerican and Andean in Central and South America, respectively (Musango *et al*., 2016). The Andean gene pool includes large-seeded light and dark red kidney, white kidney, bush cranberry, green and yellow beans, and the Mesoamerican includes small-seeded black, white, navy, red, and pink beans (Giola *et al*., 2019). *P. vulgaris* landraces are grouped according to seed coat background colours, *viz.* , pinto bean (brown stripes on the background), carioca bean (Khaki stripes), turtle bean (black), and cranberry bean (dark red speckles) (Elsadr *et al*., 2011).

P. vulgaris varieties are classified by seed size, seed coat colour, and shape (Hacisalihoglu and Settles, 2013). The seed coat colours are as follows: white, red, brown, yellow, purple, black, grey, while others can be bicolour (Zhu *et al*., 2017). The seed sizes are classified as small (< 25 g 100 seed mass), medium (25–40 g 100 mass), or large (> 40 g 100 seed mass) (Gioia *et al*., 2019). The seeds are either round, oval, kidney, or cuboidal in shape (Loko *et al*., 2018). Variation in seed coat colours of *P. vulgaris* landraces are caused by mutation and segregation of alleles (Musango *et al*., 2016). The seed coat colours are also controlled by a group of genes/ alleles that appear to regulate flavonol and anthocyanin biosynthetic pathways (McClean *et al*., 2002).

The seed coat colours of *P. vulgaris* are controlled by genes or factors named ground-factor gene, complementary colour genes, and modifying genes (McClean *et al*., 2002). The *P* locus, which is an important basic colour gene, has multiple alleles for seed coat and flower colours and is also known as the ground factor for all seed coat

colour genes (Caldas and Blair, 2009). Whereas, the *C* and *T* loci also have a large multiple allelic series of dominant seed coat patterns (Bassett, 2003). A dominant gene indicated as *P* is the dominant acting gene, which has a darkening influence upon the colour produced by the combined action of basic gene and colour genes (McClean *et al*., 2018).

C, *D*, and *J* are genes for seed colour and are only expressed in the presence of gene *P*, which is hyperstatic to all seed coat colour genes. While, genes *G*, *B*, *V*, and *Rk* are modifier genes that influence the variety (Caldas and Blair, 2009). The patterns controlled by *C* have a dark pattern colour contrasted with cream as the light colour, except when modified by alleles for recessive red colours controlled by red kidney locus *Rk* (Bassett, 2003*).* Gene *D* influences the hilum colour, which is controlled by various genes that control seed coat colour (Caldas and Blair, 2009). The dominant *J* gene represents the seed coat colour of immature seeds, and the recessive *j* produces pale coat colour for immature seeds (Grahic *et al*., 2013).

The red kidney locus *Rk* controls the recessive red seed coat colours (light red kidney and garnet brown (dark red kidney) and has a stable expression with *c* (Bassett, 2003*).* A dominant gene *R* produces a red seed coat colour and dominant (*G* and *B*) gives yellow to brown or greenish and *V* for bluish or violet to black (anthocyanin colours) (McClean *et al*., 2018). The *G* from Gelbe stands for yellow in German (Bassett, 2003). The recessive gene is mostly pleiotropic to other genes and the homogenous *pp* produces a white seed coat colour (McClean *et al*., 2018). The seeds of *P. vulgaris* sometimes segregate into three colours, i.e. black, grey, and white in a 12:3:1 ratio, which is indicative of a duplicated dominant epistasis model. The epistatic effects play an important role in the inheritance of the seed coat colour (Zhu *et al*., 2017).

In South Africa, *P. vulgaris* landraces are broadly grown from seeds saved from the previous harvest. Studies on seed coat colour segregation among these landraces are still not documented in the country. Therefore, the objective of this study was to characterize *P. vulgaris* landraces using variation in seed coat colour, which will allow plant breeders to identify valuable seeds for future plant breeding.

4.2 Materials and Methods

The study site and experimental design are explained in Chapter 3. Table 4.1 and Figure 4.1 describes the 12 landraces (that were segregated to form some progenies) used in this study, whose names were created from the: area of the collection - the percentage of seed coat colour–seed shape. The parents (P_1) were self-pollinated to produce progenies (F_1).

4.2.1 Flower and pod colour

The colour of the flowers and mature pods were determined among the *P. vulgaris* landraces. Flower colour (FC): white, cream, purple, pink, and cream with purple edges. Mature pod colour (MPC): dark purple, purple stripe on yellow, pale yellow to white, pink stripe on yellow, and pink.

4.2.2 Seed traits

The colour, pattern, and shape of the seeds were determined among landraces. Quantitative seed traits included the measurement of the following five characteristics: the seed length (SL) (mm), width (SW) (mm) and thickness (ST) (mm) as well as individual seed mass (ISM) (g) and 100-seed mass (HSM) (g). For each landrace, the seed length, width, and thickness were measured on 10-seeds based on a similar seed coat colour, using Vernier calipers. The SL was measured from the highest tip to the bottom tip of the seed, ST as the distance between the lowest and the highest points of the seed when it lays down on a horizontal surface, and SW distance from the hilum to the opposite side (Arteaga *et al*., 2019). The ISM and HSM were measured using a Mettler PC 2000 weighing scale.

Table 4.1: *Phaseolus vulgaris* parental landraces, area of origin, and seed morphology.

Landraces	Area of collection	Seed coat colour percentage	Seed pattern	Seed size	Seed shape
D-50C50Gy-K	Durban	50% C 50% Gy	spotted	Small	Kidney
D-100C-Cl	Durban	100% C	absent	Medium	Cylindrical
D-90LB10B-Cu	Durban	90% LB 10% B	spotted	Small	Cuboidal
D-100YG-Cl	Durban	100% YG	absent	Medium	Cylindrical
E-100Bk-Cl	Eshowe	100% Bk	absent	small	Cylindrical
Em-50Bk50C-Cu	Empangeni	50% Bk 50 C	spotted	Small	Cuboidal
Em-100LB-Cl	Empangeni	100% LB	absent	Small	Cylindrical
M-90LB10M-Cl	Mtubatuba	90% LB 10% M	stripped	Medium	Cylindrical
N-100LP-K	Nelspruit	100% LP	absent	Large	Kidney
P-50M50C-O	Polokwane	50% M 50% C	stripped	Medium	Oval
PS-90M10LB-Cl	Port Shepstone	90% M 10% LB	stripped	Medium	Cylindrical
PS-100YG-Cl	Port Shepstone	100% YG	absent	Small	Cylindrical

Landrace names are currently unique to authors and are coined from: area of the collection – the percentage of seed coat colour(s) - seed shape. Percentage of seed coat colour: B, brown. Bk, black.C, cream. DB, dark brown. DP, dark purple. Gy, grey. LB, Light brown. LP, light purple. M, maroon. YG, yellowish-green.

Figure 4.1: Seed colour, shape, and size of 12 parents of the *Phaseolus vulgaris* landraces.

4.3 Results

4.3.1 Segregation of *Phaseolus vulgaris* landraces based on seed coat colour and shape

All parents of the *P. vulgaris* landraces had the same flower colour as their respective progenies (Table 4.2). The same trend was recorded in the colour of mature pods, except for the parental and progeny *PS-100YG-Cl* with pale to white pods but progeny *PS-100Byp-Cl* had pink pods.

The parental landrace *E-100Bk-Cl* (P_1) from Eshowe yielded the most numerous (seven) progenies, whereas the parent *Em-50Bk50C-Cu* (P_1) from Empangeni produced five progenies. Parental landraces *D-50C50Gy-K* (P_1), *D-100C-Cl* (P_1), and *D-90LB10B-Cu* (P_1) from Durban, as well as *N-100LP-K* (P_1) from Nelspruit, yielded four progenies. The following parental landraces: *D-100YG-Cl* (P_1) from Durban, *Em-100LB-Cl* (P_1) from Empangeni, *M-90LB10M-Cl* (P_1) from Mtubatuba, *P-50M50C-O* (P_1) from Polokwane, as well as *PS-90M10LB-Cl* (P_1) and *PS-100YG-Cl* (P_1) from Port Shepstone, each brought forth two progenies (Table 4.2 and Figure 4.3).

Almost all parental landraces produced a variety of progenies, but among them, there was a progeny that was the same as the parent (Table 4.2 and Figure 4.3). The exception was recorded on landrace *D-100C-Cl* from Durban, which produced an offspring *D-100C-Cu* that was closely related to it but had a cuboidal instead of a parental cylindrical shape. Also, progenies *D-100p-Cu* and *D-100RP-Cl* from the same parent did not produce any pattern similar to the parent but had different seed coat colours of 100% pink and 100% reddish-purple, respectively. Some of the seeds of progenies *D-100C-Cl* and *D-100p-Cu* were produced from the same pod (Figure 4.2).

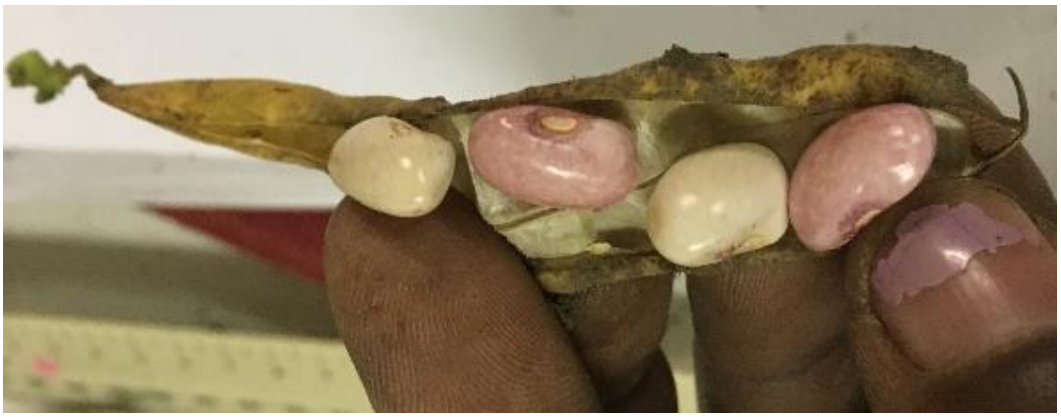


Figure 4.2: Segregation of seeds in the same pod.

The parental landrace *E-100Bk-Cl* (P_1) from Eshowe yielded an offspring *E-100GB-Cl* that did not have any pattern as they had only one primary colour but differed in seed coat colour (100% greenish-brown). Again, the Empangeni parent *Em-50Bk50C-Cu* (P_1) produced an offspring *Em-50GB50LB-Cl* that had a spotted seed pattern closely related to the parent but had a different seed coat (50% greenish-brown and 50% light brown) and cylindrical shape instead of cuboidal shape. The Durban parental landrace *D-50C50Gy-K* (P_1) produced offspring *D-90LB10M-Cl* which shared a similar spotted seed pattern but varied in seed colour (90% light brown and 10% maroon) and cylindrical shape.

Table 4.2: Description of parent and F_1 generation of *Phaseolus vulgaris* landraces according to reproduction traits.

Parents	F_1 progeny	FC	MPC	SP	SL	SW	ST	ISM	HSM
D-50C50Gy-K	*D-50C50Gy-K* **(P_1)**	C	PSY	Sp	8.90^{b}	4.10^{a}	2.40^{c}	0.24^{b}	24.00^{b}
	1.D-50C50Gy-K	C	PSY	Sp	10.40^{a}	2.90^{b}	4.50^{a}	0.30^{a}	29.85^{a}
	2.D-100C-Cu	C	PSY	A	9.30^{b}	4.50^{a}	2.70^{bc}	0.30^{a}	29.70^{a}
	3.D-90LB10M-Cl	C	PSY	Sp	9.30^{b}	4.10^{a}	2.90^{bc}	0.31^{a}	31.10^{a}
	4.D-100LP-Cu	C	PSY	A	8.00^{c}	4.80^{a}	3.20^{b}	0.32^{a}	32.30^{a}
	Mean				**9.18**	**4.08**	**3.14**	**0.29**	**29.39**
	CV%				**7.6**	**14.1**	**17.3**	**14.2**	**14.2**
	P-value				**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
D-100C-Cl	*D-100C-Cl* **(P_1)**	p	PSY	A	9.90^{b}	4.00^{c}	2.80^{b}	0.26^{c}	26.00^{c}
	1.D-100C-Cu	p	PSY	A	9.70^{b}	4.80^{ab}	2.70^{b}	0.33^{b}	32.93^{b}
	2.D-90C10p-Cl	p	PSY	Sp	11.90^{a}	5.30^{a}	4.10^{a}	0.45^{a}	44.60^{a}
	3.D-100p-Cu	p	PSY	A	9.70^{b}	4.50^{bc}	2.30^{b}	0.32^{b}	32.10^{b}
	4. D-100RP-Cl	p	PSY	A	9.40^{b}	4.20^{c}	2.80^{b}	0.30^{bc}	29.50^{bc}
	Mean				**10.12**	**4.56**	**2.94**	**0.33**	**33.03**
	CV%				**8.4**	**9.7**	**20.8**	**8.9**	**8.9**
	P-value				**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
D-90LB10B-Cu	*D-90LB10B-Cu* **(P_1)**	CPE	PSY	Sp	8.90^{ab}	4.40^{bc}	2.40^{a}	0.22^{b}	21.90^{b}
	1.D-90LB10B-Cu	CPE	PSY	Sp	9.30^{ab}	4.60^{ab}	2.40^{a}	0.35^{a}	35.08^{a}
	2.D-90C10DB-Cu	CPE	PSY	S	9.70^{a}	5.00^{a}	2.90^{a}	0.34^{a}	34.00^{a}
	3.D-100C-Cu	CPE	PSY	A	9.60^{a}	4.50^{abc}	3.00^{a}	0.34^{a}	34.30^{a}
	4.D-50LB50B-Cu	CPE	PSY	M	8.50^{b}	4.00^{c}	2.40^{a}	0.24^{b}	25.30^{b}
	Mean				**9.20**	**4.50**	**2.62**	**0.30**	**30.12**
	CV%				**7.4**	**8.8**	**21.2**	**11.9**	**12.8**
	P-value				**0.002**	**<.001**	**0.032**	**<.001**	**<.001**

Table 4.2: continued

Parent	Landraces	FC	MPC	SP	SL	SW	ST	ISM	HSM
D-100YG-Cl	*D-100YG-Cl* **(P_1)**	p	YW	A	9.90^{a}	5.20^{a}	4.10^{a}	0.44^{a}	43.60^{a}
	1.D-100YG-Cl	p	YW	A	10.10^{a}	5.10^{a}	3.70^{a}	0.27^{b}	26.88^{b}
	2.D-100C-Cl	p	YW	A	8.10^{b}	4.20^{b}	2.30^{b}	0.23^{b}	23.20^{b}
	Mean				**9.37**	**4.83**	**3.37**	**0.31**	**31.23**
	CV%				**6.0**	**7.9**	**16.4**	**11.3**	**11.3**
	P-value				**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
E-100Bk-Cl	*E-100Bk-Cl* **(P_1)**	P	PSY	A	8.60^{bc}	4.70^{a}	3.70^{a}	0.30^{b}	30.40^{b}
	1.E-100Bk-Cl	P	PSY	A	7.80^{c}	4.10^{a}	3.10^{ab}	0.32^{ab}	31.42^{b}
	2.E-90Bk10C-Cl	P	PSY	Sp	9.60^{ab}	4.70^{a}	3.40^{ab}	0.37^{ab}	37.40^{ab}
	3.E-50Bk50C-Cl	P	PSY	M	10.10^{a}	4.60^{a}	3.30^{ab}	0.36^{ab}	36.40^{ab}
	4.E-50GB50LB-Cl	P	PSY	Sp	8.50^{bc}	4.30^{a}	3.20^{ab}	0.29^{b}	29.10^{b}
	5.E-50Gy50C-Cl	P	PSY	Sp	9.30^{ab}	4.80^{a}	3.90^{a}	0.41^{a}	40.70^{a}
	6.E-100GB-Cl	P	PSY	A	9.30^{ab}	4.70^{a}	3.40^{ab}	0.35^{ab}	34.50^{ab}
	7.E-90P10C-Cl	P	PSY	M	9.00^{abc}	4.50^{a}	2.80^{b}	0.32^{ab}	32.10^{ab}
	Mean				**9.03**	**4.55**	**3.35**	**0.34**	**34.00**
	CV%				**11.1**	**12.8**	**18.3**	**19.0**	**19.0**
	P-value				**<.001**	**0.132**	**0.007**	**0.002**	**0.002**
Em-100LB-Cl	*Em-100LB-Cl* **(P_1)**	W	DP	A	7.90^{b}	4.10^{a}	2.10^{b}	0.20^{c}	19.50^{c}
	1.Em-100LB-Cl	W	DP	A	8.00^{b}	4.30^{a}	2.70^{a}	0.39^{a}	38.70^{a}
	2.Em-50LB50M-Cl	W	DP	Sp	10.00^{a}	4.10^{a}	2.80^{a}	0.25^{b}	24.90^{b}
	Mean				**8.63**	**4.17**	**2.53**	**0.28**	**27.70**
	CV%				**5.4**	**8.8**	**14.6**	**9.5**	**9.5**
	P-value				**<.001**	**0.387**	**<.001**	**<.001**	**<.001**

Table 4.2: continued

Parent	Landraces	FC	MPC	SP	SL	SW	ST	ISM	HSM
Em-50Bk50C-Cu	*Em-50Bk50C-Cu* **(P_1)**	P	PSY	Sp	8.50^{cd}	3.90^{d}	2.30^{c}	0.22^{e}	22.00^{e}
	1.Em-50Bk50C-Cu	P	PSY	Sp	10.10^{ab}	10.10^{a}	2.50^{bc}	0.57^{a}	56.77^{a}
	2.Em-50B50LB-Cl	P	PSY	S	10.90^{a}	4.30^{cd}	2.60^{bc}	0.29^{cd}	29.00^{cd}
	3.Em-50GB50LB-Cl	P	PSY	Sp	9.50^{bc}	3.80^{d}	2.20^{c}	0.23^{de}	23.30^{de}
	4.Em-50P50C-Cu	P	PSY	S	7.80^{d}	5.20^{b}	3.20^{ab}	0.40^{b}	40.30^{b}
	5.Em-100C-Cu	P	PSY	A	8.60^{cd}	4.80^{bc}	3.60^{a}	0.33^{c}	32.50^{c}
	Mean				**9.23**	**5.35**	**2.73**	**0.34**	**33.98**
	CV%				**9.2**	**12.6**	**21.3**	**14.3**	**14.3**
	P-value				**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
M-90LB10M-Cl	*M-90LB10M-Cl* **(P_1)**	W	pSY	S	13.20^{a}	5.50^{b}	4.10^{a}	0.55^{a}	55.30^{a}
	1.M-90C10M-Cl	W	pSY	S	11.60^{b}	5.40^{b}	3.30^{b}	0.48^{a}	47.33^{b}
	2.M-50M50C-Cl	W	pSY	S	12.20^{b}	6.00^{a}	3.10^{b}	0.48^{a}	48.20^{ab}
	Mean				**12.33**	**5.63**	**3.50**	**0.51**	**50.3**
	CV%				**6.7**	**7.6**	**13.0**	**13.7**	**13.6**
	P-value				**0.001**	**0.012**	**<.001**	**0.046**	**0.035**
N-100LP-K	*N-100LP-K* **(P_1)**	p	YW	A	15.60^{b}	6.30^{b}	3.70^{ab}	0.48^{d}	47.50^{d}
	1.N-100LP-K	p	YW	A	13.90^{c}	6.20^{b}	3.40^{ab}	0.92^{a}	92.00^{a}
	2.N-100C-K	p	YW	A	15.70^{ab}	6.40^{b}	3.10^{bc}	0.60^{c}	60.40^{c}
	3.N-100DP-K	p	YW	A	13.40^{c}	5.90^{b}	2.60^{c}	0.47^{d}	47.10^{d}
	4.N-100M-K	p	YW	A	17.00^{a}	7.40^{a}	4.00^{a}	0.80^{b}	79.60^{b}
	Mean				**15.12**	**6.44**	**3.36**	**0.65**	**65.3**
	CV%				**7.1**	**8.9**	**17.9**	**11.5**	**11.5**
	P-value				**<.001**	**<.001**	**<.001**	**<.001**	**<.001**

Table 4.2 continued:

Parent	Landraces	FC	MPC	SP	SL	SW	ST	ISM	HSM
P-50M50C-O	*P-50M50C-O* **(P_1)**	C	pSY	S	11.60[a]	6.10[a]	4.60[a]	0.57[a]	56.70[a]
	1.P-50M50C-O	C	pSY	S	12.30[a]	6.40[a]	4.50[a]	0.44[b]	48.44[b]
	2.P-90M10LB-O	C	pSY	S	11.80[a]	6.90[a]	4.10[a]	0.56[a]	55.80[a]
	Mean				**11.90**	**6.47**	**4.40**	**0.52**	**53.6**
	CV%				**13.0**	**13.3**	**19.0**	**10.9**	**11.9**
	P-value				**0.592**	**0.140**	**0.387**	**<.001**	**0.018**
PS-90M10LB-Cl	*PS-90M10LB-Cl* **(P_1)**	W	pSY	S	10.80[b]	4.90[a]	3.20[b]	0.40[a]	39.60[a]
	1.PS-90M10LB-Cl	W	pSY	S	11.10[ab]	4.90[a]	3.70[a]	0.37[a]	37.21[a]
	2.PS-90LB10M-Cl	W	pSY	S	11.50[a]	4.80[a]	3.40[ab]	0.40[a]	40.20[a]
	Mean				**11.13**	**4.87**	**3.43**	**0.39**	**39.00**
	CV%				**5.0**	**7.8**	**12.7**	**10.2**	**10.1**
	P-value				**0.036**	**0.796**	**0.058**	**0.186**	**0.229**
PS-100YG-Cl	*PS-100YG-Cl* **(P_1)**	W	YW	A	8.00[b]	4.60[a]	2.80[a]	0.26[b]	26.30[b]
	1.PS-100YG-Cl	W	YW	A	10.10[a]	4.50[a]	3.20[a]	0.30[ab]	29.42[ab]
	2.PS-100Byp-Cl	W	p	M	8.50[b]	4.90[a]	2.90[a]	0.32[a]	31.60[a]
	Mean				**8.87**	**4.67**	**2.97**	**0.29**	**29.11**
	CV%				**7.5**	**11.4**	**12.5**	**11.3**	**11.3**
	P-value				**<.001**	**0.246**	**0.067**	**0.007**	**0.007**

Landraces' names for both parents and progeny are currently unique to authors and are coined from: area of the collection – the percentage of seed coat colour(s) - seed shape. **P_1** parental seed. Area, D, Durban; E, Eshowe; Em, Empangeni; M, Mtubatuba; N, Nelspruit; P, Polokwane; PS, Port Shepstone. Seed coat colour(s), B, brown; Bk, black; Byp, brownish-yellow with pink; C, cream; DB, dark brown; DP, dark purple; GB, greenish-brown; Gy, grey; LB, light brown; LP, light purple; M, maroon; p, pink; P, purple; RP, reddish-purple; YG, yellowish-green. Seed shape, Cl, cylindrical; Cu, Cuboidal; K, kidney; O, oval. Traits: Flower colour (FC); C, cream; CPE, cream with purple edges; p, pink; P, purple; W, white. Mature pod colour (MPC); DP, dark purple; pSY, pink stripe on yellow PSY, purple stripe on yellow; YW, pale yellow to white. Seed pattern (SP), A, absent; M, mottled; S, stripped; Sp, spotted. SL, seed length (mm); SW, seed width (mm); ST, seed thickness (mm); ISM, individual seed mass (g); HSM, 100-seed mass (g). Means followed by a different letter(s) within a column differ significantly ($P < 0.05$).

Figure 4.3: Seed morphology of parents and progenies of *Phaseolus vulgaris*

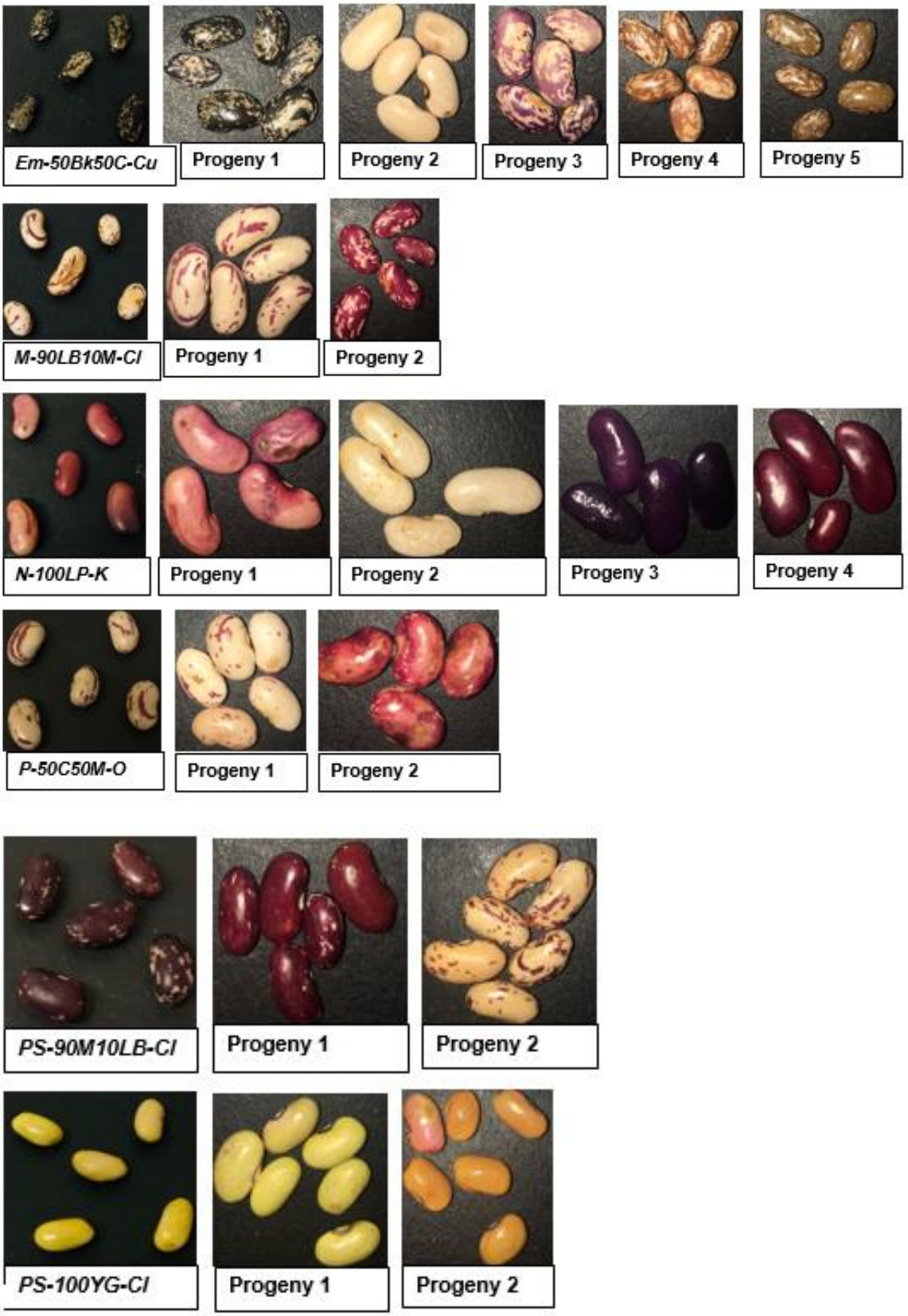


Figure 4.3 Continued:

4.3.2 Seed traits of the segregated *Phaseolus vulgaris* landraces

4.3.2.1 Seed length

A significant variation was recorded in seed length for landraces, between each parent and its progeny and/or among offspring of the same parent, apart from landraces from Polokwane that showed insignificant differences (Table 4.2). The longest to shortest seed ranges of Polokwane landraces were 11.60–12.30 mm. The Durban progeny *D-50C50Gy-K* from the parental landrace *D-50C50Gy-K* (P_1) had the longest (10.40 mm) seeds, whereas progeny *D-100LP-Cu* had the shortest (8.00 mm). *D-50C50Gy-K* had longer seeds than the parental landrace *D-50C50Gy-K* (P_1) and progenies *D-100C-Cu, D-100LP-Cu,* and *D-90LB10M-Cl.* However, the parent *D-50C50Gy-K* (P_1) along with progenies *D-100C-Cu*, and *D-90LB10M-Cl* had longer seeds than the progeny *D-100LP-Cu*.

For the Durban landraces sowed from 100% cream (*D-100C-Cl*) seeds, progeny *D-90C10p-Cl* had the longest seeds at 11.90 mm, and *D-100RP-Cl* had the shortest seeds at 9.40 mm (Table 4.2). The progeny *D-90C10p-Cl* had longer seeds than the parent *D-100C-Cl* (P_1) and progenies *D-100C-Cl*, *D-100RP-Cl*, and *D-100p-Cl*. Furthermore, the parent *D-100C-Cl* (P_1) had a similar seed length with progenies *D-100C-Cu*, *D-100RP-Cl*, and *D-100p-Cu.*

In the landraces from Durban sown from 90% light brown 10% brown (*D-90LB10B-Cu)* seeds, progeny *D-90C10DB-Cu* had significantly longer (9.70 mm) seeds than progeny *D-50LB50B-Cu* (8.50 mm) (Table 4.2). The progeny *D-90C10DB-Cu* had a similar seed length to the parental landrace *D-90LB10B-Cu* (P_1) and progenies *D-90LB10B-Cu*, and *D-100C-Cu.* However, the progeny *D-90C10DB-Cu* had longer seeds than progeny *D-50LB50B-Cu.* Also, the parent seed *D-90LB10B-Cu* (P_1) along with progeny *D-90LB10B-Cu* had almost the same seed length as *D-50LB50B-Cu.*

Progeny *D-100YG-Cl* sown from 100% yellowish-green seeds (*D-100YG-Cl*) had longer seeds than progeny *D-100C-Cl* with a range of 8.10–10.10 mm (Table 4.2). The

parental landrace *D-100YG-Cl* (P_1) shared a similar length with progeny *D-100YG-Cl.* They both had longer seeds than progeny *D-100C-Cl*.

Wide variations were observed among Eshowe landraces sown from plants with 100% black (*E-100Bk-Cl*) seeds as progeny *E-50Bk50C-Cl* had the longest (10.10 mm) seeds while progeny *E-100Bk-Cl* had the shortest (7.80 mm) (Table 4.2). Progeny *E-50Bk50C-Cl* had similarly long seeds as progenies *E-90Bk10C-Cl*, *E-50Gy50C-Cl*, *E-100GB-Cl*, and *E-90P10C-Cl.* Seeds from these landraces were significantly longer than the parental seed *E-100Bk-Cl* (P_1) and progenies *E-100Bk-Cl* and *E-50GB50LB-Cl*. However, the shorter landraces *E-100Bk-Cl* (P_1), *E-100Bk-Cl*, and *E-50GB50LB-Cl* had similar seed lengths with progeny *E-90P10C-Cl*. The landraces from Empangeni sown from parent *Em-100LB-Cl* (P_1) also varied in seed length, the progeny *Em-50LB50M-Cl* had long seeds at 10.00 mm whereas the parental landrace *Em-100LB-Cl* (P_1) had the shorter at 7.90 mm (Table 4.2). The progeny *Em-50LB50M-Cl* had a longer seed length than the parental landrace *Em-100LB-Cl* (P_1) and progeny *Em-100LB-Cl* which had similarly short seeds.

Significant differences were recorded among the Empangeni landraces sown from 50% black 50% cream (*Em-50Bk50C-Cu*) seeds, where the progenies *Em-50B50LB-Cu* and *Em-50Bk50C-Cu* had the longest seed length whereas progeny *Em-50P50C-Cl* had the shortest with a range of 7.80–10.90 mm (Table 4.2). The progenies *Em-50Bk50C-Cu* and *Em-50B50LB-Cl* had longer seeds than the parental landrace *Em-50Bk50C-Cu* (P_1) and progenies *Em-100C-Cu*, *Em-50P50C-Cu,* and *Em-50GB50LB-Cl.* However, the progeny *Em-50B50LB-Cl* had the same seed length as progeny *Em-50Bk50C-Cu*, and *Em-50Bk50C-Cu* further shared a similar seed length with *Em-50GB50LB-Cl.* Furthermore, the parent *Em-50Bk50C-Cu* and progenies *Em-100C-Cu*, and *Em-50GB50LB-Cl* also shared similar seed lengths.

Among the landraces from Mtubatuba, parental landrace *M-90LB10M-Cl* (P_1) had the longest seed length at 13.20 mm while progenies *M-90C10M-Cl* and *M-50M50C-Cl* had the shortest 11.60 and 12.20 mm (Table 4.2). Progenies *N-100M-K* and *N-100C-K* had longer seeds than progenies *N-100LP-K*, and *N-100DP-K*. But, *N-100C-K* had a similar seed length with the parental landrace *N-100LP-K* (P_1). Again, progenies *N-100LP-K* and *N-100DP-K* had the same shorter seeds(Table 4.2) .

The progenies *PS-90LB10M-Cl* and *PS-90M10LB-Cl* had seeds of a same length however, *PS-90LB10M-Cl* had longer seeds than the seeds of the parental landrace *PS-90M10LB-Cl* (P_1) and progeny *PS-90M10LB-Cl* had a similar length to the parent (Table 4.2). In the landraces also from Port Shepstone, progeny *PS-100YG-Cl* had longer seeds than the parent *PS-100YG-Cl* (P_1) and the progeny *PS-100Byp-Cl* both have seeds that are similar in length.

4.3.2.2 Seed width

Significant variations were recorded in seed width for almost all landraces, between each parent and its progeny, and/or among offspring of the same parent. However, the landraces sown from plants with 100% black (*E-100Bk-Cl*), 100% light brown (*Em-100LB-Cl*), 50% maroon and 50% cream (*P-50M50C-O*), 90% maroon and 10% light brown (*PS-90M10LB-Cl*) as well as 100% yellowish-green (*PS-100YG-Cl*) seeds showed insignificant variations (Table 4.2). The widest to narrowest seed ranges among the landraces sown from 100% light brown seeds (*Em-100LB-Cl*), and 50% maroon and 50% cream (*P-50M50C-O*) was 4.10–4.30 mm, and 6.10–6.90 mm, respectively. Similarly, the Port Shepstone parent and progenies sowed from 90% maroon and 10% light brown (*PS-90M10LB-Cl*), and 100% yellowish-green (*PS-100YG-Cl*) the widest to narrowest seeds ranges were 4.80–4.90 and 4.50–4.90 mm, respectively.

The widest (4.80 mm) and the narrowest (2.90 mm) seed width were recorded for progenies *D-100LP-Cu* and *D-50C50Gy-K*, respectively among landraces sown from 50% cream and 50% grey (*D-50C50Gy-K*) seeds (Table 4.2). The progeny *D-100LP-Cu* had similarly long seeds as its parent *D-50C50Gy-K* (P_1) and progenies *D-100C-Cu*, and *D-90LB10M-Cl*. They all outperformed progeny *D-50C50Gy-K*. Seeds of progeny *D-90C10p-Cl* sown from plants with 100% cream (*D-100C-Cl*) seeds were wider at 5.30 mm while the parental landrace *D-100C-Cl* (P_1) was narrower at 4.00 mm (Table 4.2). Again, *D-90C10p-Cl* had a similar seed length with *D-100C-Cu. D-100C-Cu* further shared a similar length with *D-100p-Cu.* However, the progeny *D-100p-Cu* had similarly short seeds as parental seed *D-100C-Cl* and progeny *D-100RP-Cu*.

Again, in landraces sown from 90% light brown 10% brown (*D-90LB10B-Cu*) seeds, the progeny *D-90C10DB-Cu* had the widest seeds at 5.00 mm whereas *D-50LB50B-Cu* had the narrowest at 4.00 mm (Table 4.2). The wider seeds of *D-90C10DB-Cu* had similar width with progenies *D-90LB10B-Cu* and *D-100C-Cu*. *D-90LB10B-Cu* and *D-100C-Cu* also had similar seed width with the parental landrace *D-90LB10B-Cu* (P_1). However, the parent *D-90LB10B-Cu* (P_1) shared a similar seed width with the narrowest seeds produced by progeny *D-50LB50B-Cu*. Among plants sown from 100% yellowish-green (*D-100YG-Cl*) seeds, the parent *D-100YG-Cl* (P_1) had wider seeds at 5.20 mm than *D-100C-Cl* which were narrower at 4.20 mm (Table 4.2). The parental landrace *D-100YG-Cl* (P_1) and progeny *D-100YG-Cl* had seeds of a similar width which were greater than *D-100C-Cl.*

In landraces from Empangeni sown from 50% black and 50% cream seeds(*Em-50Bk50C-Cu*), seeds of progeny *Em-50Bk50C-Cu* grew wider at 10.10 mm while *Em-50P50C-Cu* were narrower at 3.80 mm (Table 4.2). Progeny *Em-50Bk50C-Cu* outperformed the parental landrace *Em-50Bk50C-Cu* (P_1) and other progenies *Em-100C-Cu*, *Em-50P50C-Cu*, *Em-50B50LB-Cl*, and *Em-50GB50LB-Cl*. The parent *Em-50Bk50C-Cu* (P_1) had the same width as progenies *Em-50B50LB-Cl and Em-50GB50LB-Cl*. In the landraces from Mtubatuba Progenies *M-90C10M-Cl* and *M-50M50C-Cl* had the widest (6.00 mm) and narrowest (3.80 mm) seeds, respectively. The progeny *M-90C10M-Cl*, on the other hand, exhibited similar width to the parental seed *M-90LB10M-Cl* (P1). The Nelspruit progeny *N-100M-K* had the widest seeds at 7.40 mm (Table 4.2). *N-100M-K* outperformed the parental landraces and the rest of the progenies in terms of seed width.

4.3.2.3 Seed thickness

The seed thickness differed significantly in almost all landraces, between each parent and its progeny and/or among offspring of the same parent. However,, the parents and progenies sowed from *D-90LB10B-Cu, P-50M50C-O,* and *PS-100YG-Cl* did not (Table 4.2). The thickest to thinnest seed ranges among plants with 50% maroon and 50% cream (*P-50M50C-O*), 100% yellowish-green (*PS-100YG-Cl*), and 90% light

brown and 10% brown seeds [*D-90LB10B-Cu* (P_1)] were 4.10–4.60 mm, 2.80–3.20 mm, and 2.40–3.00 mm, respectively.

The progeny *D-50C50Gy-K* produced the thickest (4.50 mm) seeds while parent *D-50C50Gy-K* (P_1) had the thinnest (2.40 mm) (Table 4.2). The offspring *D-50C50Gy-K* outperformed the parental landrace and progenies (*D-100C-Cu*, *D-90LB10M-Cl*, and *D-100LP-Cu*). The parental landrace *D-50C50Gy-K* (P_1) shared similar seed thickness with progenies *D-100C-Cu* and *D-90LB10M-Cl*. The progeny *D-100LP-Cu* had thicker seeds than the parental seed, whereas *D-100C-Cu* and *D-90LB10M-Cl* had similar seed thickness.

Progeny *D-90C10p-Cl*, recording 4.10 mm, had the thickest seeds (Table 4.2). Progeny *D-90C10p-Cl* had thicker seeds than the parent *D-100C-Cl* and other landraces (*D-100C-Cu*, *D-100RP-Cl*, and *D-100p-Cu*) from the same parent. The parental landrace [*D-100YG-Cl* (P_1)] at 4.10 mm produced the thickest seeds but was showed insignificant differences with progeny *D-100YG-Cl*. While progeny *D-100C-Cl* had the thinnest at 2.30 mm (Table 4.2). Furthermore, parent *D-100YG-Cl* (P_1) and progeny *D-100YG-Cl* with the same yellowish-green seed coat and cylindrical shape, both had thicker seeds than *D-100C-Cl.*

Progeny *E-50Gy50C-Cl* and parent *E-100Bk-Cl* (P_1) had the thickest seeds than progeny *E-90P10C-Cl* and they both further shared similar seed thickness with other progenies (*E-100Bk-Cl*, *E-90Bk10C-Cl*, *E-50Bk50C-Cl*, *E-50GB50LB-Cl*, and *E-100GB-Cl*) (Table 4.2). *Em-100LB-Cl* and *Em-50LB50M-Cl* progenies have thicker seeds than the parental landrace *Em-100LB-Cl* (P1).

The progeny *Em-100C-Cu* shared similar thickness with progeny *Em-50P50C-Cl* and they outperformed the parental landrace *Em-50Bk50C-Cu* (P_1) and progeny *Em-50GB50LB-Cl*. However, progeny *Em-50P50C-Cl* further had similar seed thickness with *Em-50Bk50C-Cu* and *Em-50B50LB-Cl*.

The parental seed *M-90LB10M-Cl* (P_1) with 4.10 mm had the thickest seeds and progeny *M-50M50C-Cl* had the thinnest at 3.10 mm (Table 4.2). The parental landrace' seeds grew thicker than their offspring (*M-50M50C-Cl,* and *M-90C10M-Cl*). Progeny

N-100M-K at 4.00 mm recorded the thickest seeds whereas *N-100DP-K* had the thinnest at 2.60 mm (Table 4.2). The progeny *N-100M-K* had seeds of a similar thickness as parental landrace *N-100LP-K* (P_1) and *N-100LP-K* and they had thicker seeds than progenies *N-100DP-K* and *N-100C-K*. The seed thickness of the progeny *N-100C-K* was similar to that of the *parent N-100LP-K* (P1).The landrace *PS-90M10LB-Cl* and parent *PS-90M10LB-Cl* (P_1) differed as the parent had the thinnest seeds. However, *PS-90M10LB-Cl* had similar thicker seeds as progeny *PS-90LB10M-Cl*.

4.3.2.4 Individual and 100-seed mass

The individual and 100-seed mass differed significantly among almost all landraces, between each parent and its progeny, and/or among offspring of the same parent. The landraces with 90% light brown and 10% maroon seeds (*M-90LB10M-Cl*) showed insignificant differences in individual seed mass and landraces *PS-90M10LB-Cl* in 100-seed mass (Table 4.2). The highest to lowest individual seed mass value ranges among landraces sown from 90% light brown and 10% brown (*M-90LB10M-Cl*) were 0.55–0.48 g. Progeny *D-100LP-Cu* had similarly heavy seeds as *D-50C50Gy-K*, *D-100C-Cu*, *D-90LB10M-Cl*, and *D-100LP-Cu* and they all differed from the parental landrace *D-50C50Gy-K* (P_1) with lighter seeds.

D-90C10p-Cl had a greater individual and 100-seed mass than the parental landrace *D-100C-Cl* (P_1) and other progenies (*D-100C-Cu*, *D-100RP-Cl*, and *D-100p-Cu*). Again, *D-100C-Cu* and *D-100p-Cu* outperformed the parent *D-100C-Cl* (P_1) as they had bigger individual seed mass and heavier 100-seed mass than the parent. But, the parent *D-100C-Cl* (P_1) had a similar individual and 100-seed mass as *D-100RP-Cl*.

Progenies (*D-90LB10B-Cu*, *D-100C-Cu*, and *D-90C10DB-Cu*) had greater seed mass (individual and 100-seed mass) than the parental landrace *D-90LB10B-Cu* (P_1) and progeny *D-50LB50B-Cu* (Table 4.2). *D-90LB10B-Cu* (P_1) and progeny *D-50LB50B-Cu* shared similar individual and 100-seed mass.

The parental seed *D-100YG-Cl* (P_1) produced the biggest value for individual seed mass at 0.44 g and heavier 100-seed mass at 43.60 g whereas progeny *D-100C-Cl*

and *D-100YG-Cl* had the lowest value (0.23 g, and 0.27 g) and the least (23.20 g, and 26.88 g) 100-seed mass (Table 4.2). *E-50Gy50C-Cl* had produced heavier seeds compared to the parental seed *E-100Bk-Cl* (P_1) and progeny *E-50GB50LB-Cl.* But, parent *E-100Bk-Cl* (P_1) shared a similar seed mass with progenies (*E-100Bk-Cl*, *E-90Bk10C-Cl*, *E-50Bk50C-Cl*, *E-100GB-Cl*, and *E-90P10C-Cl*) (Table 4.2).

The progeny *Em-100LB-Cl* measuring at 0.39 g and 38.70 g had the biggest individual and heaviest 100-seed mass, and parent *Em-100LB-Cl* (P_1) had the lowest individual seed mass and lightest 100-seed mass at 0.20 g and 19.50 g, respectively (Table 4.2). The progeny *Em-100LB-Cl* outperformed the parent *Em-100LB-Cl* (P_1) with the same seed coat and shape, and also progeny *Em-50LB50M-Cl*.

Progeny *Em-50Bk50C-Cu* had the biggest value for individual seed mass and heaviest 100 seed mass, recording 0.57 g and 56.77 g, respectively and the parental landrace *Em-50Bk50C-Cu* (P_1) had the lowest value at 0.22 g and lightest 100 seed mass at 22.00 g (Table 4.2). Landrace *Em-50Bk50C-Cu* had a significantly bigger seed mass compared to parent *E-50Bk50C-Cu* (P_1) (with the same 50% black and 50% cream and cuboidal seed shape) and other progenies (*Em-100C-Cu*, *Em-50P50C-Cu*, *Em-50B50LB-Cl*, and *Em-GB50LB-Cl*). Again, the progenies *Em-100C-Cu*, *Em-50P50C-Cu*, *Em-50B50LB-Cl*, and *Em-50GB50LB-Cl* outperformed the parental landrace *E-50Bk50C-Cu* (P_1).

The parental seed *M-90LB10M-Cl* (P_1) recorded the heaviest 100 seed mass at 55.30 g and g and M-90LB10M-Cl (P1) had a similar 100-seed mass as progeny M-50M50C-Cl while progeny *M-90C10M-Cl* had the lightest at 47.33 (Table 4.2).The progeny *N-100LP-K,* recording 0.92 g, and 92.00 g, had the biggest individual seed mass and heaviest 100-seed mass, respectively while progeny *N-100DP-K* had the lowest value at 0.47 g and lightest 100-seed mass at 47.10 g. However, the parental seed *N-100LP-K* (P_1) shared a similar mass with the least performing progeny *N-100DP-K*.

Parent *P-50M50C-O* (P_1), at 0.57 g had the biggest value and heaviest 100-seed mass at 56.70 g and had a similar seed mass as progeny *P-90M10LB-O*, and progeny *P-50M50C-O* (with the same 50% maroon and 50% cream and oval shape) had the lowest individual seed mass at 0.44 g and least 100-seed mass at 48.44 g (Table 4.2).

Progeny *PS-100Byp-Cl* had the biggest value (0.32 g) and heaviest 100 seed mass (31.60 g) while the parental landrace *PS-100YG-Cl* (P_1) had the lowest individual seed mass at 0.26 g and least 100-seed mass at 26.30 g. But, *PS-100YG-Cl* also shared a similar seed mass as progeny *PS-100Byp-Cl* with greater seed mass.

4.3.3 Correlation among the quantitative seed traits

Positive correlations were significant among the measured seed traits except for seed thickness (Table 4.3). Seed width and seed length correlated positively with each other and they both further correlated positively with the individual seed mass and 100-seed mass. A strong positive correlation was recorded between individual seed mass and 100-seed mass.

Table 4.3: Correlation among seed traits of 12 segregated *Phaseolus vulgaris* landraces

Traits	SW	SL	ST	ISM
SL	**0.835**			
ST	0.599	0.541		
ISM	**0.874**	**0.789**	0.578	
HSM	**0.872**	**0.787**	0.575	**1.000**

Traits: SW, seed width (mm); SL, seed length (mm); ST, seed thickness (mm); ISM, individual seed mass (g); HSM; 100-seed mass (g). Significant values ≥ 0.6 are in bold.

4.3.4 Principal component analysis

The first two informative principal components explained 91.678% of the total variation (Table 4.4). The first principal component (PC1) correlated positively with all measured seed traits. The second principal component (PC2) was only positively correlated with seed thickness.

Table 4.4: Principal component coefficients of seed traits for segregated *Phaseolus vulgaris* landraces

Traits	PC1	PC2
SW	**0.941**	-0.090
SL	**0.888**	-0.126
ST	**0.712**	**0.701**
ISM	**0.959**	-0.157
HSM	**0.957**	-0.160
Eigenvalue	4.018	0.566
Variability (%)	80.357	11.321
Cumulative (%)	80.357	91.678

Traits: SW, seed width (mm); SL, seed length (mm); ST, seed thickness (mm); ISM, individual seed mass (g); HSM, 100-seed mass (g). Significant values ≥ 0.6 are in bold.

4.3.5 Cluster analysis

In a biplot, all seed traits correlated positively with PC1. The biplot further clustered *P. vulgaris* parents and progenies into four groups (Figure 4.4). Group I was formed by *D-100YG-Cl* (P_1) and *D-90C10p-Cl* from Durban, *M-90LB10M-Cl* (P_1) from Mtubatuba, *P-50M50C-O* (P_1) and *P-90M10LB-O* from Polokwane, and *N-100LP-K* (P_1) from Nelspruit. Landraces with 50% cream and 50% maroon (*P-50M50C-O*) and 90% maroon and 10% light brown (*P-90M10LB-O*) seeds had similar seed coats and shapes but differed in colour intensity. Group II contained *N-100LP-K*, *N-100M-K*, and *N-100C-K* from Nelspruit and *P-50M50C-O* from Polokwane. Group III was composed of a singlet, *N-100DP-K* originating from Nelspruit.

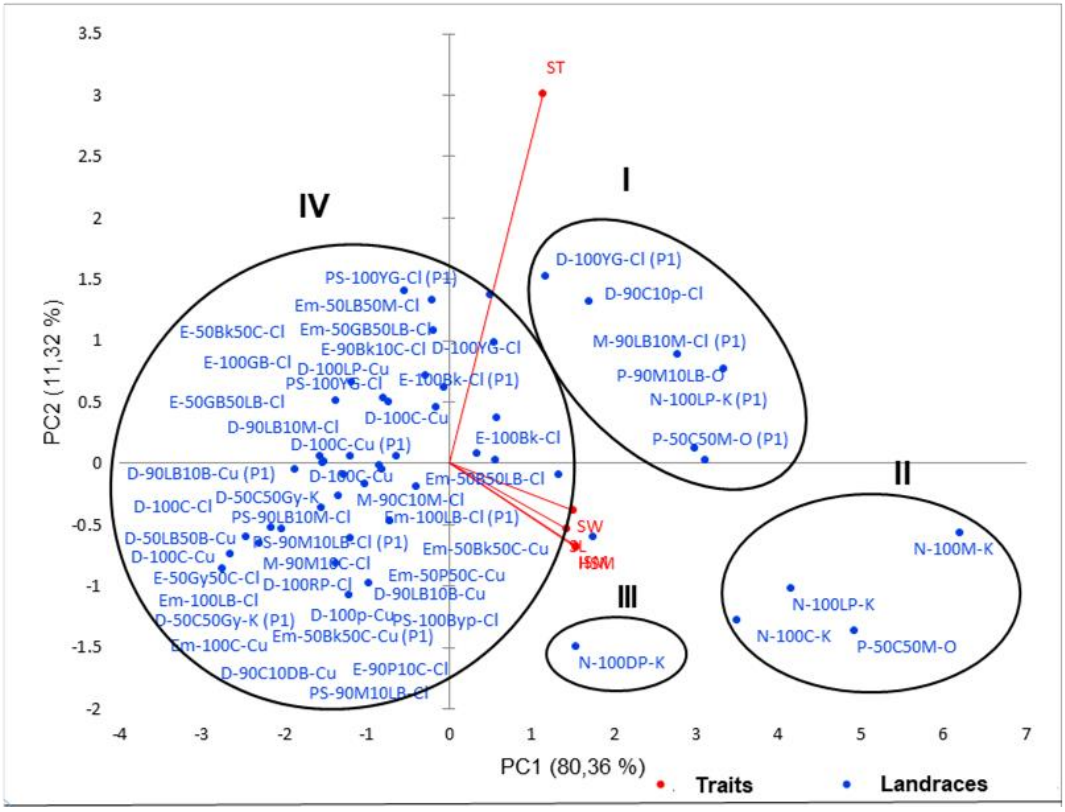


Figure 4.4: Biplot based on the first two principal components (PC) for seed traits and 12 segregated *Phaseolus vulgaris* landraces and progenies.

Landraces are explained in Table 4.1. Traits: SL, seed length; ST, seed thickness; SW, seed width; ISM, individual seed mass; HSM, hundred seed mass.

All the remaining landraces, both parents and progenies, associated themselves in Group IV. Most of these landraces correlated negatively with PC1, except for *D-100C-Cl* (P_1), *D-100C-Cu*, *D-90LB10M-Cl*, *D-100LP-Cu*, and *D-100YG-Cl* from Durban, *E-100Bk-Cl* (P_1), *E-100Bk-Cl*, *E-90Bk10C-Cl*, *E-50Bk50C-Cl*, *E-50GB50LB-Cl*, and *E-100GB-Cl* from Eshowe, *Em-50GB50LB-Cl*, and *Em-50LB50M-Cl* from Empangeni and also *PS-100YG-Cl* (P_1) and *PS-100YG-Cl* from Port Shepstone.

The relationship between parents and progenies was further illustrated by a dendrogram using Euclidean distance (Figure 4.5). Landraces were grouped into three main clusters. Cluster I contained *N-100M-K*, *N-100LP-K*, and *N-100DP-K* from the Nelspruit area and *P-50M50C-O* from Polokwane, *N-100LP-K* (P_1) with its progeny *N-100C-K* from Nelspruit, *M-90LB10M-Cl* (P_1) with its progenies *M-90LB10M-Cl* and *M-90M10C-Cl* from Mtubatuba, and *P-50M50C-O* (P_1) with progeny *P-90M10LB-O* from Polokwane .

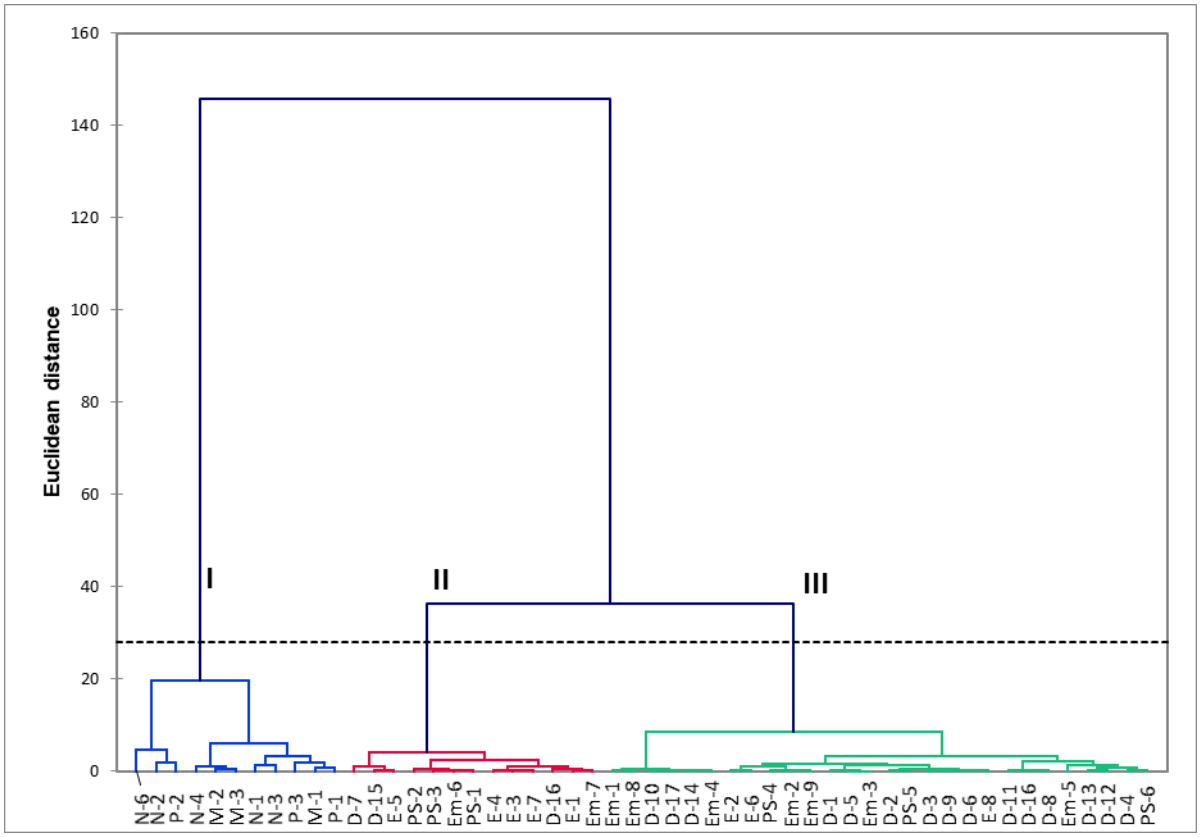


Figure 4.5: Dendrogram grouping of segregated *Phaseolus vulgaris* landraces and progenies based on Euclidean distances

Description for landraces is in Table 4.1. Landraces from Durban: **D-1**, *D-50C50Gy-K* (P_1); **D-2**, *D-50C50Gy-K*; **D-3**, *D-90LB10M-Cl*; **D-4**, *D-100LP-Cu*; **D-5**, *D-100C-Cu*; **D-6**, *D-100C-Cl* (P_1); **D-7**, *D-90C10p-Cl*; **D-8**, *D-100p-Cu*; **D-9**, *D-100RP-Cl*; **D-10**, *D-90LB10B-Cu* (P_1); **D-11**, *D-90LB10B-Cu*; **D-12**, *D-90C10DB-Cu*; **D-13**, *D-100C-Cu*; **D-14**, *D-50LB50B-Cu*; **D-15**, *D-100YG-Cl* (P_1); **D-16**, *D-100YG-Cl*; **D-17**, *D-100C-Cl.* Landraces from Eshowe: **E-1**, *E-100Bk-Cl* (P_1); **E-2**, *E-100Bk-Cl*; **E-3**, *E-90Bk10C-Cl*; **E-4**, *E-50Bk50C-Cl*; **E-5**, *E-50Gy50C-Cl*; **E-6**, *E-50GB50LB-Cl*; **E-7**, *E-100GB-Cl*; **E-8**, *E-90P10C-Cl*. Landraces from Empangeni. **Em-1**, *Em-100LB-Cl* (P_1); **Em-2**, *Em-100LB-Cl*; **Em-3**, *Em-50LB50M-*Cl; **Em-4**, *Em-50Bk50C-Cu* (P_1); **Em-5**, *Em-50Bk50C-Cu*; **Em-6**, *Em-50B50LB-Cl*; **Em-7**, *Em-50GB50LB-Cl*; **Em-8**, *Em-50P50C-Cu*; **Em-9**, *Em-100C-Cu*. Landraces from Mtubatuba: **M-1**, *M-90LB10M-Cl* (P_1); **M-2**, *M-90C10M-Cl*; **M-3**, *M-90M10C-Cl*. Landraces from Nelspruit: **N-1**, *N-100LP-K* (P_1); **N-2**, *N-100LP-K*; **N-3**, *N-100C-K*; **N-4**, *N-100DP-K*; **N-5**, *N-100M-K*. Landraces from Polokwane: **P-1**, *P-50M50C-O* (P_1); **P-2**, *P-50M50C-O*; **P-3**, *P-90M10LB-O*. Landraces from Port Shepstone: **PS-1**, *PS-90M10LB-Cl* (P_1); **PS-2**, *PS-90M10LB-Cl*; **PS-3**, *PS-90LB10M-Cl*; **PS-4**, *PS-100YG-Cl* (P_1); **PS-5**, *PS-100YG-Cl*; **PS-6**, *PS-100Byp-Cl*.

Cluster II was formed by *D-90C10p-Cl* and *D-100C-Cu* both originating from Durban and also *E-50Gy50C-Cl* from Eshowe, *D-100YG-Cl* from Durban, *E-100Bk-Cl* (P_1) with progenies *E-50Bk50C-Cl*, *E-90Bk10C-Cl*, and *E-100GB-Cl* from Eshowe, *Em-50B50LB-Cl* and *Em-50GB50LB-Cl* from Empangeni and also *PS-90M10LB-Cl* (P_1) with its progenies *PS-90LB10M-Cl* and *PS-90M10LB-Cl*.

Cluster III was composed of *D-90LB10B-Cu* (P_1) with progeny *D-100C-Cl* from Durban, *E-100Bk-Cl* from Eshowe, and *Em-50Bk50C-Cu* (P_1), *Em-100LB-Cl* (P_1), and *Em-50P50C-Cu* from Empangeni, *D-50C50Gy-K* (P_1) with its progenies *D-100C-Cu*, *D-90LB10M-Cl* and *D-100LP-Cu*, *D-100C-Cl* (P_1) with progenies *D-100C-Cu*, *D-100p-Cu* and *D-100RP-Cl,* and also *D-90LB10B-Cu*, *D-90C10DB-Cu* and *D-100YG-Cl* from Durban, *E-50GB50LB-Cl* and *E-90P10C-Cl* from Eshowe, *Em-100LB-Cl*, *Em-100C-Cu*, *Em-50LB50M-Cl* and *Em-50Bk50C-Cu* from Empangeni, and also *PS-100YG-Cl* (P_1) with its progenies *PS-100YG-Cl* and *PS-100Byp-Cl* from Port Shepstone.

4.4 Discussion

4.4.1 Segregation of *Phaseolus vulgaris* landraces based on seed coat colour and shape

The variation in phenotypic traits was observed among 12 *Phaseolus vulgaris* landraces from different environments, *viz*. Durban, Eshowe, Empangeni, Mtubatuba, Nelspruit, Polokwane, and Port Shepstone (Table 4.2 and Figure 4.3). The high diversity in the seed coats was probably caused by specific genes or epistatic gene interaction (Musango *et al*., 2016). In *P. vulgaris* landraces from South Africa, eight genes (*P*, *C*, *T*, *G*, *B*, *V*, *Rk*, and [*C-R*]) were probably involved in the determination of seed coat colour. A comparable study with landraces from Florida had multiple genes involved in seed coat colour determination (Bassett, 2005).

The parents of *P. vulgaris* yielded numerous progenies that varied in the seed coat colour, pattern, and shape (Table 4.2 and Figure 4.3). These variations may have resulted from mutation followed by segregation (Borker and More, 2010). This might show heterogeneity among parents and offspring of *P. vulgaris* (Razvi *et al*., 2017), which is important for breeding purposes and large-scale farming. Farmers in various environments choose seeds with a wide range of seed coat colours (Musango *et al*., 2016).

The parents produced offspring that are different, the genes responsible for seed coat colour were probably largely heterozygous for several loci (Musango *et al*., 2016). The variation may also have resulted from environmental conditions (phenotypic plasticity), the progenies possibly have changed their phenotype in response to the current environment and might be independent of the expected genetic contribution of the parental plant (Lorts *et al*., 2019).

The parental landrace *E-100Bk-Cl* (P_1) and progenies *E-100Bk-Cl* and *E-100GB-Cl* were possibly controlled by gene pattern *T* (Bassett, 2003), thus they did not show any pattern and had only one primary colour. Gene *T* expresses a coloured seed coat and unpatterned (self-coloured) seeds (Bassett, 2005). However, the progenies *E-*

90Bk10C-Cl, *E-50GB50LB-Cl,* and *E-50Gy50C-Cl* from the same parent had spotted seed patterns. Again, progenies *E-50Bk50C-Cl* and *E-90P10C-Cl* also from the same parent had a mottled pattern (Table 4.2). The diversity in spotted seed patterns among the progenies was possibly due to the gene *C/C* which expressed the light background colour (cream) overlaid with a darker pattern colour while the spotted pattern in the progeny *E-50Gy50C-Cl* was maybe due to *c/c* for lighter pattern colour (McClean *et al*., 2002). Furthermore, the mottled may have resulted from *C/c* gene which expresses the subtle seed coat mottling effects (Bassett, 2003).

The ground-factor gene *P* with all other genes generated the phenotypic expression in the *P. vulgaris* seed (Zhu *et al*., 2017). The colour modifying gene *V* (with *G B V*) probably generated the black (*E-100Bk-Cl*, *E-90Bk10C-Cl,* and *E-90Bk10C-Cl*) seed coat colour and might also have produced purple/violet (*E-90P10C-Cl*) seeds (with *G b V*) (Bassett, 2003). The greenish-brown (*E-100GB-Cl*, and *E-50GB50LB-Cl*) seed coat colour was possibly expressed by modifying gene *B,* in association with *G B v* (Bassett, 2003). The grey-white (*E-50Gy50C-Cl*) seeds might have transpired when the genes are homozygous at p^{gri} and another allele at *P* (Zhu *et al*., 2017). In the study of *P. vulgaris* landraces from Florida in the USA, the black seed coat colour carries numerous genes *viz. P*, [*Cr*], *J*, *G*, *B*, *V*, and *Rk* (Bassett, 2005). Hence, this might be the reason for the number of most segregated progenies and their diversity in seed coat colour among the South African *P. vulgaris* landrace.

The parent *Em-50Bk50C-Cu* (P_1) had the same spotted seed pattern as progenies *Em-50Bk50C-Cu* and *Em-50GB50LB-Cl*, although progeny *Em-50GB50LB-Cl* varied in seed coat and shape. But, progenies *Em-50P50C-Cu* and *Em-50B50LB-Cl* from the same parent had a striped pattern (Table 4.2). This variation in the pattern was maybe caused by the seed pattern gene *C/C* (McClean *et al*., 2002). The progeny *Em-100C-Cu* did not produce any seed pattern, thus the recessive gene pattern *tt* was possibly responsible for the partly coloured unpatterned seed coat (Bassett, 2005). The genes responsible for diversity in seed coats were probably dominant genes *P*, *C*, *T*, *G*, *B*, and *V* (McClean *et al*., 2018) similar to those found in landrace sown from *E-100Bk-Cl* (P_1) plants.

Landraces sowed from *D-50Gy50C-K* (P_1) probably had both dominant (*P*), p^{gri}, *V*, *B*, and *Rk* genes (McClean *et al*., 2002; Zhu *et al*., 2017). Modifying gene *V* with *G b V* may have interacted with recessive *tt* (for unpatterned seed coat) to produced purple/violet seeds with a slightly paler colour (*D-100LP-Cu*) (Bassett, 2003). The 90% light brown and 10% maroon (*D-90LB10M-Cl*) seed coats may have resulted from the red kidney locus *Rk* which controls the dark red seed colour coat (maroon) (Bassett, 2005) and further interacted with the modifying gene *B* (with *G B v*) to produce brown seeds (Bassett, 2003; Zhu *et al*., 2017). The spotted pattern in 50% grey and 50% cream seeds (*D-50Gy50C-K*) might have resulted from the recessive homozygous *c/c* genotypes responsible for slightly paler colour in the seed coat pattern (McClean *et al*., 2002). While the spotted pattern in *D-90LB10M-Cl* may have resulted from gene *C/C* which expressed the overlaid darker pattern colour (Bassett, 2003).

The seed coat colors in parent *D-100C-Cl* (P1) and progenies *D-100C-Cu*, *D-100p-Cu*, and *D-100RP-Cl*, as well as *D-90C10p-Cl*, were most likely caused by the ground-factor dominant *P* and recessive *p*, *Rk*, and gene *R* (McClean *et al*., 2002). The recessive allele *pp* may have expressed the no colour (cream) seed coat in *D-100C-Cl* (P_1) and *D-100C-Cu* (McClean *et al*., 2018). The 100% pink (*D-100p-Cu*) and 90% cream and 10% pink (*D-90C10p-Cl*) seed colour was probably due to the red kidney (*Rk*) locus that controls recessive red seed coat (light-red) (Bassett, 2003). Though, progeny *D-90C10p-Cl* from the same parent had a stripped seed coat pattern. The gene *R* probably expressed the dominant red (*D-100RP-Cl*) colour and a little more blue (Bassett, 2003). This might also mean that *R* was linked within the *C* locus ([*C-R*]), responsible for the red seed coat colours (McClean *et al*., 2002).

The genes *P*, *C*, *B*, and recessive *p* were probably responsible for the seed coat colours in *D-90LB10B-Cu* landraces (McClean *et al*., 2002). The progenies (*D-90LB10B-Cu, D-50LB50B-Cu,* and *D-90C10DB-Cu*) may have been controlled by gene *B* with *G B v* for the brown seed coat colour, *D-90LB10B-Cu, D-90C10DB-Cu*, and *D-50LB50B-C* (Bassett, 2003). But, the spotted and striped pattern possibly resulted from the gene pattern *C* responsible for the spotted and striped distinguished by light background overlaid with a darker seed pattern colour (McClean *et al*., 2002). The mottled pattern in *D-50LB50B-Cu* was probably due to the *C/c* gene which expresses subtle seed coat mottling effects (Bassett, 2003). Furthermore, progeny *D-*

100C-Cu might have lacked a dominant gene *P*, where recessiveness resulted in a cream seed coat colour (Bassett, 2005; McClean *et al*., 2018).

The progenies *M-90C10M-Cl* and *M-90M10C-Cl* had the same seed coats but differed in seed colour intensity and primary colour (cream) which differed from the parental plant. This may have resulted from the environmental conditions, the progenies *M-90C10M-Cl* and *M-90M10C-Cl* sown in Empangeni, might have changed their phenotype in response to the current environment and are independent of the expected parental genes (Lorts *et al*., 2019). The parental seed (*M-90LB10M-Cl)* from Mtubatuba was probably controlled by gene *B* with *G B v* while the progenies might be controlled by gene *R* linked with *C* ([*C-R*]) to produce the dark red colour with a striped seed pattern and cream background (Bassett, 2003).

The parental landrace *N-100LP-K* (P_1) with progenies *N-100LP-K*, *N-100C-K*, *N-100DP-K*, and *N-100M-K* did not produce any seed pattern and they only had one primary seed colour (Table 5.2). The dominant gene *T* may have expressed the unpatterned (self-coloured) in the parent *N-100LP-K* (P_1) and progenies *N-100LP-K*, *N-100DP-K*, and *N-100M-K* (Bassett, 2003). Modifying gene *V* with *G b V* possibly expressed purple/violet (*N-100LP-K* and *N-100DP-K*) seeds (Bassett, 2003). *N-100C-K* may have lacked the ground-factor *P* thus produced no colour which resulted in a cream seed coat (McClean *et al*., 2002). The red kidney locus *Rk* might have produced dark red kidney seeds (*N-100M-K*) (Bassett, 2003).

The parent *D-100YG-Cl* (P_1) along with its progenies (*D-100YG-Cl* and *D-100C-Cl*) did not produce any seed pattern as they had only one primary seed colour (Table 5.2). The absent pattern in the parent *D-100YG-Cl* (P_1) along with its progenies (*D-100YG-Cl* and *D-100C-Cl*) may have resulted from the recessive gene *tt* which expressed a partly coloured seed coat (yellowish-green), however, *D-100C-Cl* may have lacked gene *P* that produced seed colour in *P. vulgaris* (Bassett, 2005). The gene *Gy* probably produced yellowish-green seeds (*D-100YG-Cl*). Furthermore, the cream seeds (*D-100C-Cl*) were probably controlled by the recessive gene *pp* (Bassett, 2003).

Parent *Em-100LB-Cl* (P_1) and progeny *Em-100LB-Cl* seed patterns probably resulted from gene pattern *tt* (unpatterned lighter seed coats) (Bassett, 2003). Gene *C* might

have expressed the spotted seed pattern with overlaid darker red (maroon) seed pattern colour (McClean *et al*., 2002). Gene *B* with *G B v* was responsible for the brown seed coat colour (*Em-100LB-Cl*) linked with gene *c/c* for paler colour in brown (light brown) (Bassett, 2003). However, the bicolour in the progeny *Em-50LB50M-Cl* may have resulted from the gene *C* which probably expressed the spotted seed pattern with overlaid darker red (maroon) seed pattern colour (Bassett, 2005).

The striped seed pattern in plants sown from *P-50M50C-O* and *PS-90M10LB-Cl* probably was due to the gene pattern *C* (McClean *et al*., 2002). The seed coat colour of landraces from Polokwane and Port Shepstone were probably controlled by similar genes namely, *P*, *C*, *R*, and *B* (McClean *et al*., 2002; Bassett, 2003). The dominant gene *R* may have expressed the dominant dark red (maroon), modifying gene *B* with *G B v* produced brown seeds (Bassett, 2003) and the recessive homozygous *pp* probably produced no colour (cream) (McClean *et al*., 2018).

The gene *tt* was possibly responsible for the unpatterned seed coat in *PS-100YG-Cl* (P_1) and *PS-100YG-Cl* (Bassett, 2003). While, the gene *C/c* could be responsible for the mottled pattern in progeny *PS-100Byp-Cl* (McClean *et al*., 2002). The genes probably responsible for the diversity in seed coats were *P*, *Gy*, and [*C-R*] (McClean *et al*., 2002). Gene *Gy* may be responsible for the greenish-yellow or yellowish seed coat colour (Bassett, 2003). Brownish-yellow with pink was possibly due to modifying gene *B* with *g B v* which produced buffy citrine (brownish-yellow) (Bassett, 2003), the pink colour was maybe due to the linked gene [*C-R*] which was responsible for the red seed coat colour or even light red (McClean *et al*., 2002). The variation in seed coat colour between the parent and offspring (*PS-100Byp-Cl*) may be due to the differences between the current environment (Empangeni) and the original parental environment (Port Shepstone), the offspring may have the ability changed its phenotype in response to the current environment (Lorts *et al*., 2019).

4.4.2 Seed traits of the segregated *Phaseolus vulgaris* landraces

The South African *P. vulgaris* landraces indicated two eco-geographical gene pools *viz.*; Mesoamerican and Andean with wide variability (Gioia *et al*., 2019). The majority (75%) of the landraces belonged to the Mesoamerican gene pool, whereas the

minority (25%) belonged to the Andean gene pool (Table 4.2). The Mesoamerican group included small-seeded (27.70 to 34.0 g 100-seed mass) formed by following groups; *D-50C50Gy-K* (P_1), *D-100C-Cl* (P_1), *D-90LB10B-Cu* (P_1), *D-100YG-Cl* (P_1), *E-100Bk-Cl* (P_1), *Em-100LB-Cl* (P_1), *Em-50Bk50C-Cu* (P_1), *PS-90M10LB-Cl* (P_1), and *PS-100YG-Cl* (P_1). However, the Andean included large-seeded or medium (50.3 to 65.3 g 100-seed mass) formed by parents *M-90LB10B-Cl* (P_1), *N-100LP-K* (P_1), and *P-50M50C-O* (P_1).

4.4.2.1 Seed size and mass

The progeny *D-50C50Gy-K* had the longest and thickest seeds compared with parent *D-50C50Gy-K* (P_1), and other progenies (Table 4.2). Again, progeny *D-100LP-Cu* from the same parent had the biggest value and highest 100-seed mass than parent *D-50C50Gy-K* (P_1) (Table 4.2). These findings were similar to those of *P. vulgaris* landraces in Spain, where descendents (F_1) of the Montcau bean had the longest seeds (18.8 mm) and highest 100-seed mass (76.2 g) compared with the parent Montcau (P_1) (15.5 mm and 45.2 g, respectively) (Rivera *et al*., 2015).

Progeny *D-100LP-Cu* sown from plants with 100% cream [*D-100C-Cl* (P_1)]) had the longest, widest, and thickest seeds, as well as the biggest value and highest 100-seed mass, compared with other progenies and the parental seed [*D-100C-Cl* (P_1)] (Table 4.2). The difference in seed size could be related to increased genetic distances between parents and progenies (Musango *et al*., 2016). The parent of plants of 100% yellowish-green seeds [*D-100YG-Cl* (P_1)] along with progeny *D-100YG-Cl* had longer, wider and thicker seeds as well as the highest 100-seed mass whereas progeny *D-100C-Cl* had shorter, narrower, and lighter seeds (Table 4.2). This diversity was probably caused by the genetic factors associated with pigmentation (gene *P*) (Singh *et al*., 2017). The seed size of *P. vulgaris* landraces can be associated with seed coat pigmentation and pattern, and the larger seed size may be linked to the ground-factor gene *P* (McClean *et al*., 2018). Progeny *D-100C-Cl* probably lacked the gene *p*, hence the cream seed coat had a smaller seed size (Singh *et al*., 2017).

In landraces sown from plants with 100% black [*E-100Bk-Cl* (P_1)] seeds, progeny *E-50Bk50C-Cl* (mottled seed pattern) had the longest seeds while progeny *E-100Bk-Cl*

(self-coloured) had the shortest seeds (Table 4.2). In a comparable study on *P. vulgaris* from Orono, Maine in the United States the pigmented progenies which differed in seed size showed an association of seed size differences with seed pattern (Sax, 1923). Progeny *E-50Gy50C-Cl* had heavier seeds while progeny *E-50GB50C-Cl* had lighter seeds (Table 4.2). These variabilities between parents and progenies for mass probably resulted from genetic factors and environmental conditions (Lorts *et al*., 2019). The environment has a strong effect to alter the seeds resulting in different seed sizes and mass (Singh *et al*., 2017).

The parent and progenies sowed from 100% light brown seeds, progeny *Em-50LB50M-Cl* with a spotted seed pattern, had longer and thicker seeds, whereas the parent *Em-100LB-Cl* (P_1) had shorter seeds. Again, progeny *Em-100LB-Cl* had heavier seeds, while parent *Em-100LB-Cl* (P_1) had lighter seeds (Table 4.2). Diversity in seed patterns may have played a significant role in the differences in seed size between the parental landrace and progenies sown from 100% light brown (*Em-100LB-Cl)* seeds (McClean *et al*., 2018). In a similar study, the patterned *P. vulgaris* seeds have larger and heavier parents and progenies than unpatterned seeds (Sax, 1923).

Landraces with 50% black and 50% cream seeds (*E-50Bk50C-Cu*) showed high diversity in seed colour and size, where progeny *E-50B50LB-Cu* had longer seeds, while the parent *E-50Bk50C-Cu* (P_1) had shorter seeds (Table 4.2). In a similar study, domesticated progenies differed from the wild parent in their longer seeds and increased variability of seed colour (Morales-Santos *et al*., 2017). Progeny *E-50Bk50C-Cu* had the heaviest seeds while the parent *E-50Bk50C-Cu* (P_1) with the same seed colour and shape had lighter seeds (Table 4.2). Diversity in the seed mass may have resulted due to the differences in the current progeny and the parental environmental conditions (Lorts *et al*., 2019).

Parental landrace *M-90LB10B-Cu* (P_1) from Mtubatuba had the longest, thickest as well as heaviest seeds, and thus outperformed progenies *M-90LB10M-Cl* and *M-50M50LB-Cl* (Table 4.2). This could have also resulted from the genetic effect and environmental conditions (Lei *et al*., 2020). It could be possible that the current progenies were under environmental stress (Lorts *et al*., 2019). This was evident as

progenies *M-90LB10M-Cl* and *M-50M50LB-Cl* (differed in seed coat from the parent) resulted in shorter, thinner, and lighter seeds.

In Nelspruit landraces with 100% light purple (*N-100LP-K*) seeds, the progeny *N-100M-K* had the longest, widest and thickest seeds and outperformed parent *N-100LP-K* (P1) and other progenies. However, the progeny *N-100LP-K* (similar to the parent) had the heaviest seeds and outperformed the parental landrace and other landraces (Table 4.2). In a comparable study, the pigmented *P. vulgaris* varied in seed size and mass, F_1 had larger and heavier seeds compared with the parent (P_1) (Sax, 1923). The increased variability in seed size in the self-fertilizing *P. vulgaris* might be due to the genetic effect (Lei *et al*., 2020). This was also true for Port Shepstone landraces sown from 100% yellowish-green seeds.

4.4.3 Correlation among the seed traits of 12 *Phaseolus vulgaris* landraces

A positive correlation between seed width and seed length was found and they both further positively correlated with individual seed mass as well as 100-seed mass in the twelve segregated *P. vulgaris* from different environments. Similarly, the 100-seed mass, seed length, and seed width of *P. vulgaris* landraces in China significantly positively correlated with each other (Lei *et al*., 2020). The correlated findings suggested that the breeding procedure towards longer and wider seeds in the current study will result in heavy seeds. This shows good potential for future large-scale farming. Farmers prefer *P. vulgaris* seeds with wide variation that are large and heavy (Musango *et al*., 2016).

4.4.4 Principal component analysis

No study has recorded clustering of seed traits and segregated landraces in the principal component analysis, biplot, and dendrogram. Principal component 1 incorporated all growth factors for seeds (seed length, width, thickness, as well as individual and 100-seed mass). This might indicate the significance of seed traits in the breeding programs for longer, wider, thicker, and heavier seeds in *P. vulgaris* landraces.

4.4.5 Cluster analysis

Group I was formed by parent *D-100YG-Cl* (P_1) and progeny *D-90C10p-Cl* from Durban, *M-90LB10B-Cl* (P_1) from Mtubatuba, parent *P-50M50C-O* (P_1) and its progeny *P-90M10LB-O* from Polokwane, and parent *N-100LP-K* (P_1) from Nelspruit. This group consisted of large seeds that outperformed other landraces in the same group based on the seed size and 100-seed mass.

Group II contained progenies *N-100LP-K*, *N-100M-K*, and *N-100C-K* from Nelspruit and progeny *P-50M50C-O* from Polokwane. This group also consisted of larger seeds based on the seed size and 100-seed mass. Progeny *N-100DP-K* formed Group III separated from other *P. vulgaris* landraces. This separation could be due to the shorter, narrower, and lower 100-seed mass compared with larger seeds from the same group.

All the remaining landraces, both parents and progenies, associated themselves in Group IV. Most of these landraces correlated negatively with PC1, except for *D-100C-Cl* (P_1), *D-100C-Cu*, *D-90LB10M-Cl*, *D-100LP-Cu*, and *D-100YG-Cl* from Durban, *E-100Bk-Cl* (P_1), *E-100Bk-Cl*, *E-90Bk10C-Cl*, *E-50Bk50C-Cl*, *E-50GB50LB-Cl*, and *E-100GB-Cl* from Eshowe, *Em-50GB50LB-Cl*, and *Em-50LB50M-Cl* from Empangeni and also *PS-100YG-Cl* (P_1) and *PS-100YG-Cl* from Port Shepstone. Based on the seed size and 100-seed mass, this relationship consisted of small and lighter seeds. This was evident as most of the landraces in Group IV correlated negatively with PC1. Principal component 1 had a strong positive correlation with seed length, width, thickness as well as individual and 100-seed mass (Table 4.4).

The dendrogram revealed the clustering of parents and progenies based on their seed size and mass. Cluster I was composed of parents along with their progenies from different areas (*N-100LP-K* (P_1), *N-100C-K, N-100DP-K, N-100LP-K,* and *N-100M-K* from Nelspruit, *M-90LB10B-Cl* (P_1), *M-90LB10M-Cl,* and *M-50M50LB-Cl* from Mtubatuba, and also *P-50M50C-O* (P_1), *P-50M50C-O,* and *P-90M10LB-O* from Polokwane). These landraces were probably clustered together because of their longer, wider, thicker, and heavier seeds (Table 4.2). *E-100Bk-Cl* (P_1) with progenies

E-50Bk50C-Cl, *E-90Bk10C-Cl*, *E-50Gy50C-Cl,* and *E-100GB-Cl* from Eshowe, *Em-50B50LB-Cl* and *Em-50GB50LB-Cl* from Empangeni and also *PS-90M10LB-Cl* (P_1) with its progenies *PS-90LB10M-Cl* and *PS-90M10LB-Cl*) in Cluster II were probably based on the seed size and mass (medium seeds).

Cluster II was composed of parents and progenies that outperformed other landraces in the same group however, they were smaller than landraces in Cluster I. According to Lei *et al*. (2020) seeds between 20-40 g 100-seed mass are medium whereas seeds with 100-seed mass greater than 40 g are large. The remaining landraces were clustered in Cluster III. This close clustering could be due to the smaller and lighter seeds (Table 4.2). In China, *P. vulgaris* landraces with less than 25 g 100-seed mass are considered small (Lei *et al*., 2020).

4.5 Conclusion

The twelve segregated *P. vulgaris* landraces showed variation in seed size and mass as well as seed coat colour and pattern. The parental environment and the current progeny's environment played a significant role in this variation. Some of the *P. vulgaris* seeds can change their phenotype in response to the current environment or its parental origin. Landrace *E-100Bk-Cl* with 100% black seed coat colour may be important for future large-scale farming as they produce huge diversity in seed coat colours. Farmers in different environments prefer different bean types/colours. The dominant ground-factor *P* (pigment) is essential for seed coat in *P. vulgaris* and also played an important role in seed size. This was evident as almost all *P. vulgaris* landraces that resulted in larger and heavier seeds were pigmented. The biplot and dendrogram clustered the parents and progenies mainly according to their similarity in seed size and mass. The selection of large-seeded landraces *M-90LB10M-Cl*, *N-100LP-K*, and *P-50C50M-O* in the current study can lead to suitable seed growth and mass future large-scale agriculture and breeding. It is recommended to further do the genetic testing stocks for seed coat colour and patterns to test for the actual seed coat colour and pattern genes.

Chapter 5

Genetic variation and population genetic structure on selected *Phaseolus vulgaris* landraces revealed by simple sequence repeat markers

Part of this chapter was published as follows:

- Ndlangamandla, V.V., Ntuli, N.R., 2021. Morpho-agronomic and genetic variation among *Phaseolus vulgaris* landraces from selected provinces of South Africa. *Journal of Crop Science and Biotechnology*. https://doi.org/10.1007/s12892-021-00116-2

5.1 Introduction

Phaseolus vulgaris L. of the Fabaceae family originated from Central America (Gioia *et al*., 2019). *P. vulgaris* is commonly known as dry beans, kidney beans, and common beans (Musango *et al*., 2016). It is a diploid (2n = 2x = 22) and a self-pollinating crop (Burle *et al*., 2010). *P. vulgaris* is a species that originated in the American continent (Gioia *et al*., 2019). It has two distinct gene pools namely the Mesoamerican and Andean gene pools (Musango *et al*., 2016). The gene pools show variation in agronomic traits such as seed size, phaseolin, and shape as well as growth habits (Lei *et al.,* 2020). *P. vulgaris* is one of the important grain legumes of eastern and southern Africa (Asfaw *et al*., 2009).

Molecular markers are used to reveal variations among the *P. vulgaris* landraces at the Deoxyribose Nucleic Acid (DNA) level, providing a more reliable tool for germplasm diversity assessment (Bilir *et al*., 2019). Simple Sequence Sequence Repeats (SSRs) also known as microsatellites are small stretches of repeated DNA, usually of one to six nucleotides (Mishra *et al*., 2014). They are commonly composed of: mononucleotide (A), dinucleotide (AT), trinucleotide (ATC) and tetranucleotide (AGGT) repeats (Córdoba *et al*., 2010). They are used frequently in *P. vulgaris* because of their high levels of polymorphism and reproducibility (Gioia *et al*., 2019).

Molecular characterization using SSRs is important to increase the efficient use of germplasm for crop breeding (Bilir *et al*., 2019). They are used to study the intra-specific diversity and the genetic structure in the Mesoamerican and Andean gene pools in *P. vulgaris* (Almeida *et al*., 2020). To analyze the genetic makeup and relatedness of individuals within a population, population structure is required in the context of genetic diversity and breeding. (Lei *et al*., 2020)

In *P. vulgaris* landraces from Turkey, there is a high polymorphism, where the number of alleles ranges from 6 to 29 with a mean of 14.8 alleles per locus (Bilir *et al*., 2019). The observed heterozygosity (H_o) ranges from 0.000 to 0.099 with the mean value of 0.006 across all markers for *P. vulgaris* landraces in Italy (Gioia *et al*., 2019). The

polymorphic information content (PIC) values range from 0.055 to 0.721 over 13 loci and seven loci have a PIC greater than 0.5 with the mean value of 0.492 (Wang *et al*., 2012).

Diversity studies have mainly been limited to morpho-agronomic traits and no comprehensive marker evaluation of *P. vulgaris* has been documented in South Africa. Thus, the objective of this study was to determine the genetic diversity among *P. vulgaris* landraces using SSR marker analyses combined with morpho-agronomic studies. Hence, genetic diversity study among various *P. vulgaris* landraces will help to identify landraces that are suitable for future breeding programs.

5.2 Materials and methods

The genetic diversity among *Phaseolus vulgaris* landraces was analyzed using seven simple sequence repeat (SSR) markers (Table 5.1).

5.2.1 Plant material

The seeds of *P. vulgaris* were collected from rural communities of four selected provinces (Gauteng, KwaZulu-Natal, Limpopo, and Mpumalanga). The seeds were planted at the University of Zululand, KwaDlangezwa campus, Orchard unit farm (Figure 5.1). The young leaves were picked and kept in a -80^{0}C freezer and were then freeze-dried for 24 hours.

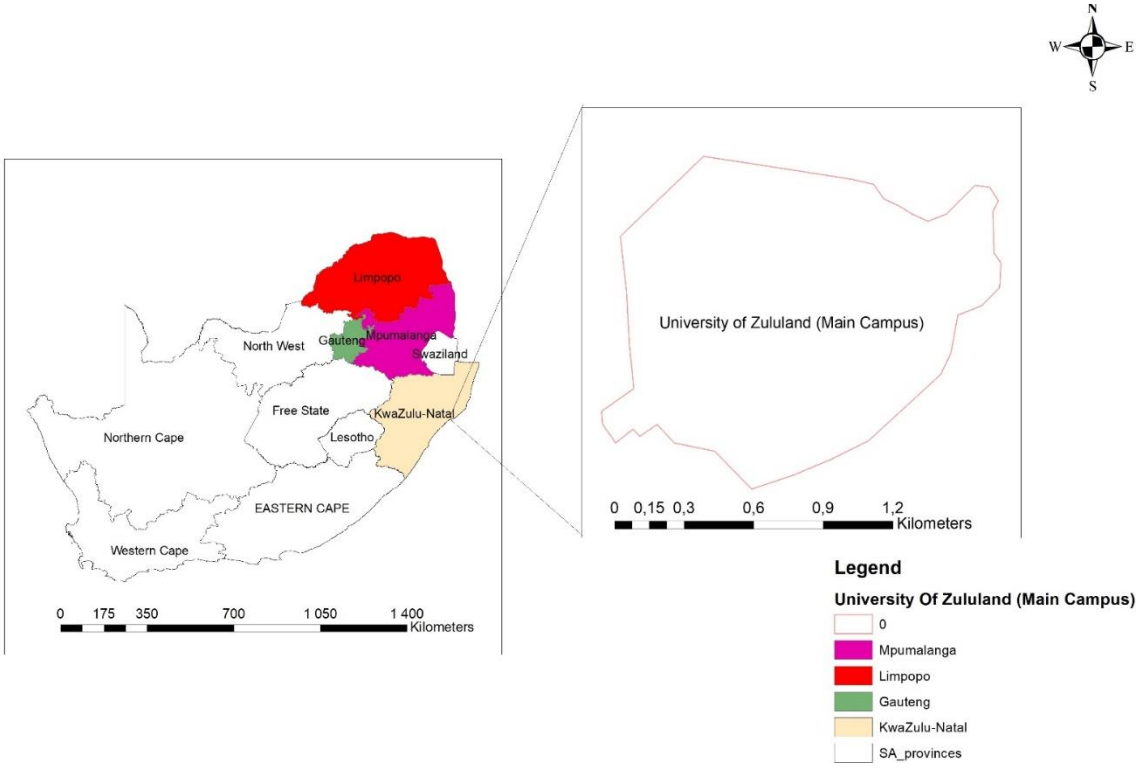


Figure 5.1: Map showing the area of collection and study site

5.2.2 DNA extraction protocol

Deoxyribose nucleic acid (DNA) was extracted from young leaves of *Phaseolus vulgaris* using the Zymo kit according to the instruction provided by the manufacturer. The DNA was extracted by Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa.

The dried leaf samples were finely cut and 150 mg of the sample was added to a ZR BashingBead™ Lysis Tube (2.0 mm). A 750 µl of BashingBead™ buffer was added to the tube and capped tightly. The tube containing bashing Bead™ buffer and the samole was secured in a bead beater fitted with a 2 ml tube holder assembled and processed at a maximum speed for 5 minutes. The ZR BashingBead™ Lysis tube was centrifuged in a microcentrifuge at 10 000 × g for 1 minute. A 400 µl aliquot of the supernatant was discarded after being transferred to a Zymo-spinTM III-F filter. An amount of 1200 µl of Genomic lysis buffer was added to the filtrate in the collection tube and mixed well.

An 800 µl aliquot of the mixture was transferred to a Zymo-spin™ IICR column in a collection tube and centrifuged at 10 000 × g for 1 minute. The flow-through was discarded from the collection tube. Again, 800 µl of the mixture was transferred to the Zymo-spin™ IICR column in a collection tube and centrifuged at 10 000 × g for 1 minute. A 200 µl of DNA pre-wash buffer was added to the Zymo-spin™ IICR column in a new collection tube and centrifuged at 10 000 × g for 1 minute. A 500 µl of gDNA wash buffer was added to Zymo-spin™ IICR column and centrifuged at 10 000 × g for 1 minute.

The Zymo-spin™ IICR column was transferred to a clean 1.5 ml microcentrifuge tubeat 10 000 × g for 30 seconds to elute the DNA. A Zymo-spin III-HRC filter was placed in a clean collection tube and a 600 µl prep solution was added. The mixture was again centrifuged at 8 000 × g for 3 minutes. The eluted DNA was transferred to a prepared Zymo-spin™ III-HRC spin filter in a clean 1.5 ml microcentrifuge tube and centrifuged at exactly 16 000 × g for 3 minutes.

5.2.3 Simple Sequence Repeat (SSR) amplification

The Polymerase Chain Reaction (PCR) amplifications were performed in an Eppendorf Mastercycler® in 50 ng/µl of DNA template in two separate 10 µl volume reactions. The reactions contained 4 µl of DNA template, 0.8 µl of deoxyribonucleotide triphosphate (dNTPs) (2.5 mM), 1 µl of 10 x buffer and 0.06 µl of Taq polymerase (Inqaba Biotec). In the first reaction, 1 µl of $MgCl_2$ (50 mM), 1 µl of forward and reverse primers (5 µM) and 1.14 µl of ultra-pure water were included. In the second reaction, a 1.2 µl of $MgCl_2$ (50 mM) and 1.5 µl of both forward and reverse primers were added to make up the master mix. Forward primers were labelled with M13 FAM (blue), T7 565 (red), pGEX5 550 (yellow) fluorescent dyes. The PCR conditions consisted of denaturing at 94^0 C for 2 minutes, nine cycles at 93^0 C for 15 seconds, annealing at 65^0 C for 20 seconds, and the extension at 72^0 C for 30 seconds.

The annealing temperature of each cycle decreases by 1^0 C with the final 30 cycles at 55^0 C and the final elongation step at 72^0 C for 5 minutes. The PCR products were separated by capillary electrophoresis analysis performed on an ABI3500 genetic analyzer. Allele size was determined for each SSR locus by using GeneMarker HID version 2.9.5. Of all primers tested, seven produced constituent amplification of well-defined alleles sizes and were selected for further analysis (Table 5.1).

Table 5. 1: Seven forward and reverse SSR markers of *Phaseolus vulgaris*

Marker	Dye used	Primer sequences (5'-3')	SSR sequence	AS
F:PV-atcc001	M13 FAM	ATGCATGTTCCAACCTTCTC	(ATCC)3(AG)2(TAC)3	171
R:PV-atcc001	M13 FAM	GGAGTGGAACCCTTGCTCTCACTGC		
F:PV-ctt001	pGEX5 550	GAGGGTGTTTCACTATTGTCACTGC	(CTT)3(T)3(CTT)6	152
R:PV-ctt001	pGEX5 550	TTCATGGATGGTGGAGGAACAG		
F:PV-ag001	T7 565	CAATCCTCTCTCCTCTCATTTCCAATC	(GA)11	157
R:PV-ag001	T7 565	GACCTTGAAGTCGGTGTCGTTT		
F:PV-atgc002	M13 FAM	AGCTTTCACACTATGACACCACTGG	(ATGC)4	144
R:PV-atgc002	M13 FAM	TGCGACATGAGAGAGAAAGACAGGG		
F:PV-ggc001	M13 FAM	GGGAGGGTAGGGAAGCAGTG	$(TA)_{22}$	239
R:PV-ggc001	M13 FAM	GCGAACCACGTTGATGAATGA		
F:PV-ccct001	pGEX5 550	CACCAATGTCTCCGGCGCA	$(CCCT)_3$	150
R:PV-ccct001	pGEX5 550	CGGTTGCCGTCGAATGTGAT		
F:PV-at003	T7 565	ACCTAGAGCCTAATCCTTCTGCGT	$(AT)_6$	139
R:PV-at003	T7 565	GAATGTGAATATCAGAAAGCAAATGG		

F, the forward primer sequences; R, the reverse primer sequences; AS, Allele size (bp); bp, base pairs.

5.2.3 Data analysis

Genetic analysis *viz.* allele number and frequency, gene diversity, heterozygosity, and the polymorphism information content (PIC) were calculated in PowerMarker software version 3.25. To clarify the gene differentiation between landraces, Nei's genetic distance was evaluated. The population genetic structure was detected using a defined number of pre-set populations K, each of which is characterized by a set of allele frequencies at each locus, using the Bayesian model-based clustering approach. The STRUCTURE version 2.3.4 program was used to detect population genetic structure using a defined number of pre-set populations K, each of which is characterized by a set of allele frequencies at each locus.The Evanno test is recommended to help with the identification of the best-fitting number of populations within a sample.

The structure program was set as follows: the analysis was run with 10 simulations per K value from K = 1 to 10, using a burn-in period length of 5 000 and after burn-in 50 000 replicates. The most expected value of K for each test was detected by ΔK (Evanno *et al*., 2005) using the Structure Harvester (Earl and Vonholdt, 2012), online (http://tayloro.biology.ucla.edu/struct_harvest/). Bar plots were generated with mean results of runs for the K value using STRUCTURE v 2.3.4. The principal coordinate analysis (PCoA) was performed using GenAlEx v 6.4 software. The dendrogram was obtained using the unweighted pair group method of arithmetic mean (UPGMA) in PowerMarker and then generated with the Mega software for displaying genetic relations among the *P. vulgaris* landraces.

5.3 Results

The seven simple sequence repeat markers produced reliable results when applied to the 40 *Phaseolus vulgaris* landraces and the outgroups, *Phaseolus coccineus* and *Phaseolus lunatus*. The reliability was based on clear, constituent amplification of well-defined expected alleles.

5.3.1 Allele number and major allele frequency of SSRs

The seven SSR loci analysed produced a total of 51 alleles with a mean of 3.64 alleles per marker (Table 5.2). The number of alleles ranged from one to six, where the reverse marker PV-atcc001 and the forward and reverse marker of PV-ccct001 had the fewest alleles (one allele). While the forward marker PV-ag001 and also the forward marker of PV-ggc001 produced numerous alleles (six alleles).

Table 5. 2: Genetic variability within *Phaseolus vulgaris* landraces for seven SSR markers

Marker	S	AN	MAF	GD	Ho	PIC
F:PV-atcc001	40	2	0.80	0.32	0.00	0.27
R:PV-atcc001	40	1	1.00	0.00	0.00	0.00
F:PV-ctt001	40	5	0.53	0.6	0.00	0.52
R:PV-ctt001	40	5	0.48	0.65	0.00	0.58
F:PV-ag001	40	6	0.60	0.58	0.00	0.53
R:PV-ag001	40	5	0.75	0.41	0.05	0.39
F:PV-atgc002	40	3	0.83	0.30	0.00	0.28
R:PV-atgc002	40	3	0.80	0.34	0.00	0.30
F:PV-ggc001	40	6	0.75	0.42	0.00	0.40
R:PV-ggc001	40	5	0.71	0.46	0.03	0.42
F:PV-ccct001	40	1	1.00	0.00	0.00	0.00
R:PV-ccct001	40	1	1.00	0.00	0.00	0.00
F:PV-at003	40	4	0.55	0.54	0.00	0.44
R:PV-at003	40	4	0.75	0.40	0.00	0.35
Mean	**40**	**3.64**	**0.75**	**0.36**	**0.01**	**0.32**

F, the forward marker; R, the reverse marker; S, sample size; AN, allele number; MAF, major allele frequency; GD, genetic diversity; Ho, observed heterozygosity; PIC, polymorphic information content.

The major allele frequency ranged from 0.48 to 1.00 with a mean of 0.75 (Table 5.2). Reverse marker PV-ctt001 had the minimum allele frequency (MAF = 0.048) whereas the reverse marker of PV-atcc001 as well as the forward and reverse marker of PV-ccct001 had the maximum allele frequency (MAF = 1.00).

5.3.2 Genetic diversity, observed heterozygosity and polymorphic information content

The genetic diversity ranged from 0.00 to 0.65 with a mean of 0.36 (Table 5.2). The reverse marker PV-atcc001 had the highest genetic diversity (0.65) whereas the reverse marker PV-atcc001, and the forward and reverse markers PV-ccct001 were the lowest (GD = 0.00). Almost all markers showed observed heterozygosity of zero, except for the reverse markers PV-ggc001 (Ho = 0.03) and PV-ag001 (Ho = 0.05) (Table 5.2). The highest polymorphism (PIC = 0.58) was recorded in the reverse marker PV-ctt001, while the lowest (PIC = 0.00) was found in reverse marker PV-atcc001, and the forward and reverse marker of PV-ccct001 (Table 5.2).

5.3.3 Genetic distance between *P. vulgaris* landraces, based on SSR markers

The genetic distance varied from 0.00 to 0.79. The Durban landrace *D-100By-Cl* had the closest genetic distance (GD = 0.00) with landraces *D-100YG-Cl*, *E-50YG-Cl, N-100LP-K,* and *PS-100YG-Cl* from Durban, Eshowe, Nelspruit, and Port Shepstone, respectively. The genetic distance between *E-50M50C-K* and *E-50M50LB-Cl*, *E-90M10C-Cl, Em-50M50LB-Cl*, and *KN-50B50M-Cl* from Eshowe, Empangeni, and KwaNdebele, respectively, was 0.00.

Table 5. 3: Nei's genetic distance of *Phaseolus vulgaris* landraces using seven SSR markers

L	B-1	Br-1	D-1	D-4	D-10	D-2	D-6	D-8	D-9	D-3	D-5	D-7	E-1	E-7	E-2	E-4	E-6	E-3	E-5	Em-3	Em-4	Em-1
Br-1	0.43																					
D-1	0.5	0.5																				
D-4	0.57	0.29	0.64																			
D-10	0.5	0.5	0	0.64																		
D-2	0.43	0.5	0.57	0.5	0.57																	
D-6	0.36	0.64	0.14	0.64	0.14	0.43																
D-8	0.36	0.64	0.16	0.66	0.16	0.43	0.02															
D-9	0.57	0.57	0.07	0.71	0.07	0.57	0.21	0.24														
D-3	0.5	0.64	0.14	0.71	0.14	0.5	0.14	0.16	0.07													
D-5	0.64	0.36	0.42	0.36	0.43	0.5	0.57	0.59	0.5	0.57												
D-7	0.57	0.64	0.36	0.57	0.36	0.5	0.36	0.31	0.36	0.36	0.71											
E-1	0.43	0.64	0.29	0.43	0.29	0.36	0.21	0.24	0.36	0.36	0.43	0.43										
E-7	0.43	0.57	0.07	0.64	0.07	0.5	0.07	0.09	0.14	0.07	0.5	0.36	0.29									
E-2	0.5	0.64	0.21	0.79	0.21	0.5	0.21	0.16	0.21	0.14	0.64	0.21	0.43	0.14								
E-4	0.36	0.64	0.14	0.64	0.14	0.43	0	0.02	0.21	0.14	0.57	0.36	0.21	0.07	0.21							
E-6	0.5	0.5	0	0.64	0	0.57	0.14	0.16	0.07	0.14	0.43	0.36	0.29	0.07	0.21	0.14						
E-3	0.43	0.57	0.07	0.64	0.07	0.5	0.07	0.09	0.14	0.07	0.5	0.36	0.29	0	0.14	0.07	0.07					
E-5	0.36	0.64	0.14	0.64	0.14	0.43	0	0.02	0.21	0.14	0.57	0.36	0.21	0.07	0.21	0	0.14	0.07				
Em-3	0.57	0.29	0.64	0	0.64	0.5	0.64	0.66	0.71	0.71	0.36	0.57	0.43	0.64	0.79	0.64	0.64	0.64	0.64			
Em-4	0.43	0.57	0.07	0.64	0.07	0.5	0.07	0.09	0.14	0.07	0.5	0.36	0.29	0	0.14	0.07	0.07	0	0.07	0.64		
Em-1	0.5	0.5	0.29	0.36	0.29	0.36	0.36	0.38	0.36	0.36	0.36	0.36	0.14	0.29	0.43	0.36	0.29	0.28	0.36	0.36	0.29	
Em-2	0.36	0.64	0.14	0.64	0.14	0.43	0	0.02	0.21	0.14	0.57	0.36	0.21	0.07	0.21	0	0.14	0.07	0	0.64	0.07	0.36

Table 5.3: Continued

L	B-1	Br-1	D-1	D-4	D-10	D-2	D-6	D-8	D-9	D-3	D-5	D-7	E-1	E-7	E-2	E-4	E-6	E-3	E-5	Em-3	Em-4	Em-1	Em-2
KN-2	0.57	0.29	0.5	0.29	0.5	0.57	0.64	0.66	0.57	0.64	0.36	0.71	0.5	0.57	0.71	0.64	0.5	0.57	0.64	0.29	0.57	0.36	0.64
KN-1	0.36	0.64	0.14	0.64	0.14	0.43	0	0.02	0.21	0.14	0.57	0.36	0.21	0.07	0.21	0	0.14	0.07	0	0.64	0.07	0.36	0
M-1	0.21	0.57	0.29	0.57	0.29	0.36	0.14	0.16	0.36	0.29	0.5	0.43	0.21	0.21	0.36	0.14	0.29	0.21	0.14	0.57	0.21	0.29	0.14
N-1	0.43	0.57	0.21	0.71	0.21	0.64	0.36	0.38	0.14	0.21	0.5	0.43	0.5	0.29	0.36	0.36	0.21	0.29	0.36	0.71	0.29	0.43	0.36
N-2	0.5	0.5	0	0.64	0	0.57	0.14	0.16	0.07	0.14	0.43	0.36	0.29	0.07	0.21	0.14	0	0.07	0.14	0.64	0.07	0.29	0.14
N-3	0.57	0.57	0.14	0.79	0.14	0.57	0.29	0.24	0.14	0.21	0.57	0.21	0.43	0.21	0.07	0.29	0.14	0.21	0.29	0.79	0.21	0.43	0.29
N-4	0.36	0.5	0.14	0.57	0.14	0.43	0.14	0.16	0.21	0.14	0.43	0.43	0.21	0.07	0.21	0.14	0.14	0.07	0.14	0.57	0.07	0.21	0.14
PC	0.71	0.66	0.66	0.74	0.66	0.74	0.66	0.66	0.66	0.66	0.79	0.66	0.74	0.66	0.66	0.66	0.66	0.66	0.66	0.74	0.66	0.74	0.66
PL	0.57	0.64	0.57	0.71	0.57	0.79	0.57	0.52	0.64	0.64	0.71	0.57	0.64	0.57	0.57	0.57	0.57	0.57	0.57	0.71	0.57	0.64	0.57
P-1	0.21	0.36	0.43	0.36	0.43	0.43	0.29	0.31	0.5	0.43	0.57	0.5	0.36	0.36	0.5	0.29	0.43	0.36	0.29	0.36	0.36	0.36	0.28
PS-7	0.5	0.5	0	0.64	0	0.57	0.14	0.16	0.07	0.14	0.43	0.36	0.29	0.07	0.21	0.14	0	0.07	0.14	0.64	0.07	0.29	0.14
PS-1	0.36	0.5	0.14	0.57	0.14	0.43	0.14	0.16	0.21	0.14	0.43	0.43	0.21	0.07	0.21	0.14	0.14	0.07	0.14	0.57	0.07	0.21	0.14
PS-5	0.57	0.57	0.57	0.42	0.57	0.21	0.5	0.45	0.57	0.57	0.5	0.29	0.29	0.57	0.43	0.5	0.57	0.57	0.5	0.43	0.57	0.29	0.5
PS-2	0.43	0.71	0.29	0.79	0.29	0.43	0.14	0.14	0.29	0.21	0.71	0.36	0.36	0.21	0.21	0.14	0.29	0.21	0.14	0.79	0.21	0.5	0.14
PS-3	0.36	0.36	0.14	0.5	0.14	0.57	0.29	0.31	0.21	0.29	0.43	0.5	0.29	0.21	0.36	0.29	0.14	0.21	0.29	0.5	0.21	0.29	0.29
PS-4	0.5	0.5	0.07	0.71	0.07	0.57	0.21	0.16	0.14	0.29	0.5	0.29	0.36	0.14	0.14	0.21	0.07	0.14	0.21	0.71	0.14	0.36	0.21
PS-6	0.09	0.5	0.38	0.5	0.38	0.45	0.24	0.26	0.45	0.38	0.57	0.52	0.31	0.31	0.45	0.24	0.38	0.31	0.24	0.5	0.31	0.38	0.24

Table 5.3: Continued

L	KN-2	KN-1	M-1	N-1	N-2	N-3	N-4	PC	PL	P-1	PS-7	PS-1	PS-5	PS-2	PS-3	PS-4
KN-1	0.64															
M-1	0.57	0.14														
N-1	0.57	0.36	0.36													
N-2	0.5	0.14	0.29	0.21												
N-3	0.64	0.29	0.43	0.29	0.14											
N-4	0.5	0.14	0.14	0.36	0.14	0.29										
PC	0.59	0.66	0.66	0.71	0.66	0.66	0.66									
PL	0.64	0.57	0.57	0.64	0.57	0.57	0.57	0.64								
P-1	0.36	0.29	0.21	0.5	0.43	0.57	0.29	0.66	0.57							
PS-7	0.5	0.14	0.29	0.21	0	0.14	0.14	0.66	0.57	0.42						
PS-1	0.5	0.14	0.14	0.36	0.14	0.29	0	0.66	0.57	0.29	0.14					
PS-5	0.57	0.5	0.43	0.64	0.57	0.43	0.5	0.74	0.71	0.5	0.57	0.5				
PS-2	0.79	0.14	0.29	0.43	0.29	0.29	0.29	0.66	0.64	0.43	0.29	0.29	0.5			
PS-3	0.36	0.29	0.29	0.36	0.14	0.29	0.14	0.66	0.57	0.29	0.14	0.14	0.57	0.42		
PS-4	0.57	0.21	0.36	0.29	0.07	0.07	0.21	0.66	0.5	0.5	0.07	0.21	0.5	0.28	0.21	
PS-6	0.5	0.24	0.09	0.38	0.38	0.52	0.24	0.68	0.57	0.14	0.38	0.24	0.52	0.38	0.24	0.45

L, landraces; the description for landraces is in Table 3.1. Landraces from Benoni: **B-1**, *B- 50B50M-Cl*; **PL**, *Phaseolus lunatus;* **PC**, *Phaseolus coccineus.* Landrace from Bushbuckridge: **Br-1**, *Br-100LB-Cl*. Landraces from Durban: **D-1**, *D-100By-Cl*; **D-2**, D-50C50Gy-K; **D-3**, *D-90C10LR-Cl*; **D-4**, *D-100C-Cl*; **D-5**, *D-90LB10B-Cu*; **D-6**, *D-50M50LB-Cl*; **D-7**, *D-90M10LB-Cl*; **D-8**, *D-50P50LB-Cl*; **D-9**, *D-50RB50LB-Cl*; **D-10**, *D-100YG-Cl*. Landraces from Eshowe: **E-1**, *E-100Bk-Cl*; **E-2**, *E-50LR50C-K*; **E-3**, *E-90LB10M-Cu*; **E-4**, *E-50M50C-K*; **E-5**, *E-90M10C-Cl*; **E-6**, *E-50YG-Cl*; **E-7**, *E-100YG-Cl*. Landraces from Empangeni: **Em- 1**, *Em-50Bk50C-Cu*; **Em-2**, *Em-50M50LB-Cl*; **Em-3**, *Em-100LB-Cl*; **Em-4**, *Em-100YG-Cl*. Landraces from KwaNdebele. **KN-1**, *KN-50B50M-Cl*; **KN-2**; *KN-100W-Cl.* Landrace from Mtubatuba. **M-1**, *M-90LB10M-Cl*. Landraces from Nelspruit. **N-1**, *N-100DP-K*; **N-2**, *N-100LP-K*. Landrace from Polokwane. **P-1**, *P-50M50C-O*. Landraces from Port Shepstone. **PS- 1***, PS-50DB50LB-Cl*; **PS-2**, *PS-90DB10LB-Cl*; **PS-3**, *PS-90LB10B-Cl*; **PS-4**, *PS-90LB10M-Cl*; **PS-5**, *PS-50M50LB-Cl*; **PS-6**, *PS-90M10LB-Cl*; **PS-7**, *PS-100YG-Cl*.

The Eshowe landraces *E-90LB10M-Cu* and *E-100Bk-Cl*, as well as Empangeni's *Em-100YG-Cl*, were the genetically closest at zero. The closest genetic difference between Durban landrace *D-100C-Cl* and Empangeni landrace *Em-100LB-Cl* was zero. Furthermore, the Nelspruit *N-90DP10C-K* landrace was the most closely related to the Port Shepstone landrace *PS-50DB50LB-Cl* (GD = 0.00).

The Durban landrace *D-100C-Cl*, as well as the *E-50LR50C-K*, and *PS-90DB10LB-Cl* from Eshowe and Port Shepstone, had the farthest genetic distance (GD = 0.79). The genetic distance between *Em-100LB-Cl* from Empangeni and *E-50LR50C-K*, *N-100LP-K*, and *PS-90DB10LB-Cl* from Eshowe, Nelspruit, and Port Shepstone, respectively, was 0.79. Also, the outgroup *P. lunatus* and the Durban landrace *D-50C50Gy-K* had the farthest genetic distance (GD = 0.79). The genetic distance between the outgroup *P. coccineus* and the Durban landrace *D-90LB10B-Cl* was also the farthest (0.79). *KN-100W-Cl* from KwaNdebele, along with Port Shepstone landrace *PS-90DB10LB-Cl*, had the farthest genetic distance (GD = 0.79).

5.3.4 Population structure among *Phaseolus vulgaris*

The Evanno test found a sharp strong maximum for Delta K at K = 2 in the plots of L (K) versus Delta (Figure 5.2). Thus, clustering the *P. vulgaris* landraces into two sub-populations.

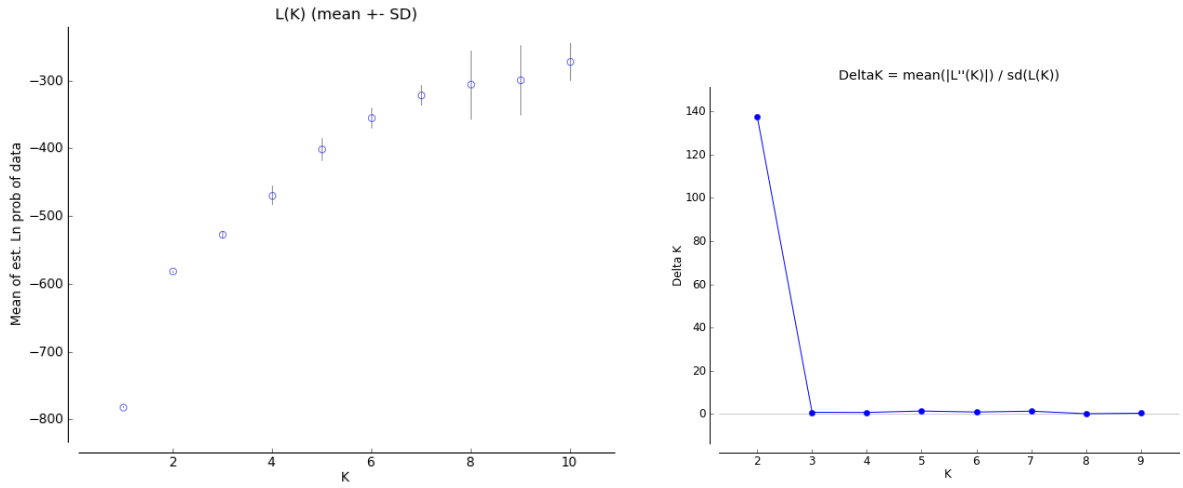


Figure 5.2: The Evanno test showing plot parameters of L (K) and Delta against the likely subpopulations of the 40 landraces.

The population structure grouped the genetic relationships of the South African landraces into subpopulations and admixtures as shown in K = 2 and K = 3 (Figure 5.3). The structure analysis clustered the 40 landraces into two sub-populations (K2.1 (red), and K2.2 (green) based on their morpho-agronomic traits at K = 2. K2.1 (red) contained landraces *D-100C-Cl* from Durban, *Em-100LB-Cl* (Empangeni), *KN-100W-Cl* from KwaNdebele, and *Br-100LB-Cl* from Bushbuckridge as well as the outgroups *Phaseolus coccineus* and *Phaseolus lunatus*. K2.2 (green) included the Durban landraces *D-100By-Cl, D-90C10LR-Cl, D-50M50LB-Cl, D-50P50LB-Cl, D-50RB50LB-Cl,* and *D-100YG-Cl,* the Eshowe landraces *E-50LR50C-K, E-90LB10M-Cu, E-50M50C-K, E-90M10C-Cl, E-50YG-Cl,* and *E-100YG-Cl,* Empangeni landraces *Em-50M50LB-Cl,* and *Em-100YG-Cl, KN-50B50M-Cl* from KwaNdebele, Nelspruit landraces *N-90DP10C-K, N-100LP-K,* and *N-100P-K* as well as the Port Shepstone landraces *PS-50DB50LB-Cl, PS-90LB10M-Cl,* and *PS-100YG-Cl.*

The following *P. vulgaris* landraces were found in the admixtures: *D-90LB10B-Cu,* and *D-50C50Gy-K* from Durban, Port Shepstone landrace *PS-50M50LB-Cl,* and Benoni landrace *B-50B50M-Cl*, they were shared in between K2.1 and K2.2 (98% red and 2% green). *P-50M50C-O* from Polokwane was shared in between K2.1 and K2.2 (95% red and 5% green) and the Port Shepstone *PS-90M10LB-Cl* (82% red and 18% green). Again, the Empangeni landrace *Em-50Bk50C-Cu* was shared in between K2.1 and K2.2 (80% red and 20% green) and the landrace *D-90M10LB-Cl* from Durban (60% red and 40% green). However, the Port Shepstone landraces *PS-90DB10LB-Cl,* and *PS-90LB10B-Cl, M-90LB10M-Cl* from Mtubatuba, and the Nelspruit landrace *N-100DP-K* were shared in between K2.1 and K2.2 (78% green and 22% red), and the Eshowe landrace *E-100Bk-Cl* (50% green and 50% red).

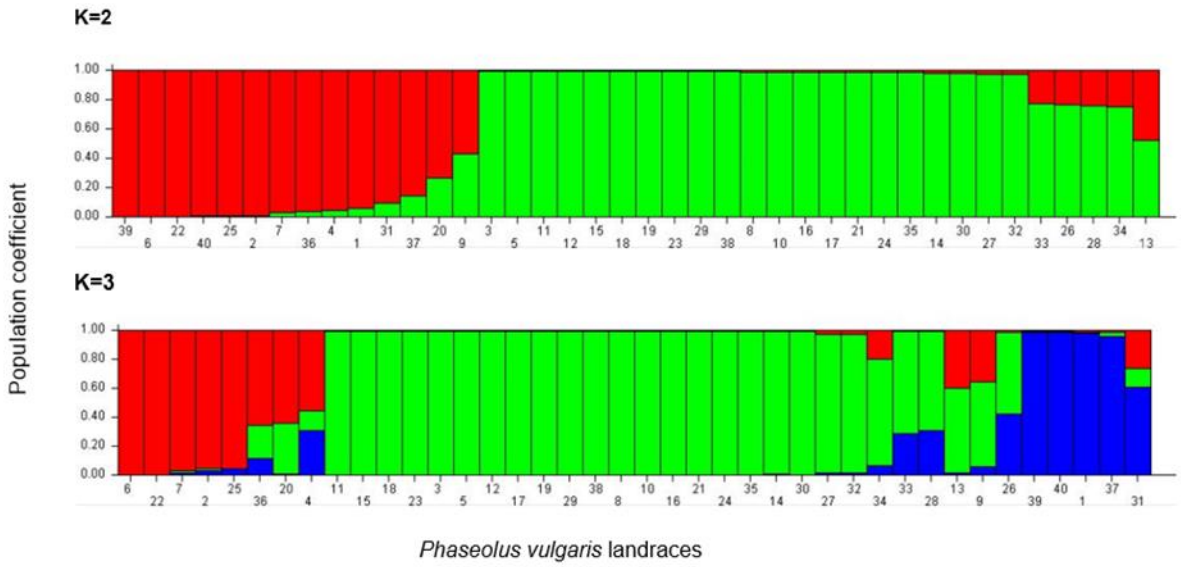


Figure 5.3: Population structure for 38 *P. vulgaris* landraces from selected provinces of South Africa revealed by SSR analysis.

K = 2 above; K2.1 (red), K2.2 (green), K = 3 below; K3.1 (red), K3.2 (green), K3.3 (blue). Landraces: **1**, *B-50B50M-Cl;* **2**, *Br-100LB-Cl;* **3**, *D-100By-Cl;* **4**, *D-50C50Gy-K;* **5**, *D-90C10LR-Cl;* **6**, *D-100C-Cl;* **7**, *D-90LB10B-Cu;* **8**, *D-50M50LB-Cl;* **9**, *D-90M10LB-Cl;* **10**, *D-50P50LB-Cl*; **11**, *D-50RB50LB-Cl*; **12**, *D-100YG-Cl*; **13**, *E-100Bk-Cl*; **14**, *E-50LR50C-K*; **15**, *E-90LB10M-Cu*; **16**, *E-50M50C-K*; **17**, *E-90M10C-Cl*; **18**, *E-50YG-Cl*; **19**, *E-100YG-Cl*; **20**, *Em-50Bk50C-Cu*; **21**, *Em-50M50LB-Cl*; **22**, *Em-100LB-Cl*; **23**, *Em-100YG-Cl*; **24**, *KN-50B50M-Cl*; **25**; *KN-100W-Cl*; **26**, *M-90LB10M-Cl*; **27**, *N-90DP10C-K;* **28,** *N-100DP-K*; **29**, *N-100LP-K;* **30**, *N-100P-K*; **31**, *P-50M50C-O*; **32**, *PS-50DB50LB-Cl*; **33**, *PS-90DB10LB-Cl*; **34**, *PS-90LB10B-Cl*; **35**, *PS-90LB10M-Cl*; **36**, *PS-50M50LB-Cl*; **37**, *PS-90M10LB-Cl*; **38**, *PS-100YG-Cl*; **39**, *Phaseolus coccineus;* **40**, *Phaseolus lunatus.*

The further clustering of the population at K = 3 resulting in the separation of South African landraces into three sub-populations. The first group [K3.1 (red)]) included *D-100C-Cl* and *Em-100LB-Cl* from Durban and Empangeni. The second group K = 2 (green) composed of Durban landraces *D-50RB50LB-Cl, D-100By-Cl, D-50M50LB-Cl, D-90C10LR-Cl, D-50P50LB-Cl* and *D-100YG-Cl,* Eshowe landraces *E-50LR50C-K, E-90LB10M-Cu, E-50M50C-K, E-90M10C-Cl, E-50YG-Cl,* and *E-100YG-Cl,* Empangeni landraces *Em-50M50LB-Cl,* and *Em-100YG-Cl, KN-50B50M-Cl* from KwaNdebele, Nelspruit landraces *N-100LP-K* and *N-100P-K,* as well as *PS-90LB10M-Cl* and *PS-100YG-Cl.* The Benoni landrace *B-50B50M-Cl* as well as the outgroups *P. coccineus* and *P. lunatus* formed group K3.3 (blue). The majority of the landraces were admixtures, the landraces *Br-100LB-Cl* from Bushbuckridge, *D-90LB10B-Cl* from Durban, and *KN-100W-Cl* from KwaNdebele were shared between K3.1 and K3.3 (98% red and 2% blue). However, landraces *PS-90M10LB-Cl* (98% blue and 2%

green) and *Em-50Bk50C-Cu* (62% red and 38% green) were found in between K3.1 and K3.2. The Mtubatuba landrace *M-90LB10M-Cl* was found in between K3.2 and K3.3 (60% green and 40% blue).

The following landraces were shared between K3.1, K3.2, and K3.3: *N-90DP10C-K* from Nelspruit and *PS-50DB50LB-Cl* from Port Shepstone had 98% green, 1% red, and 1% blue, and *PS-90LB10B-Cl* had 73% green, 22% red, and 5% blue. While the Durban landrace *D-90M10LB-Cl* shared 57% green, 38% red, and 5% blue, and the Port Shepstone landrace shared 62% red, 28% green, and 10% blue. Again, the Durban *D-50C50Gy-K* had 58% red, 30% blue, and 12% green and Polokwane landrace *P-50M50C-O* shared 58% blue, 30% red, and 12% green.

5.3.5 Principal coordinate analysis of *P. vulgaris* landraces revealed by SSR markers

In the principal coordinate analysis (PCoA) the *P. vulgaris* landraces were grouped based on the genotypic distance, where different landraces were colour-coded according to their area of origin and the two outgroups (Figure 5.4). The first two components of the principal coordinates accounted for 44.45% of the total variation. In the upper portion of the first quadrant, landraces *D-50RB50LB-Cl* from Durban, *N-100P-K,* and *N-100DP-K* from Nelspruit, *PS-90LB10M-Cl* from Port Shepstone as well as the admixtures formed by *D-100By-Cl,* and *D-100YG-Cl* from Durban, *E-50YG-Cl* from Eshowe, *N-100LP-K* from Nelspruit, and Port Shepstone landrace *PS-100YG-Cl* were clustered closer together. In the lower portion of the quadrant, the following landraces were clustered closer together; *D-90C10LR-Cl* from Durban, *E-50LR50C-K* from Eshowe, and also the admixtures formed by landraces *E-90LB10M-Cu* and *E-100YG-Cl* from Eshowe, *Em-100YG-Cl* from Empangeni.

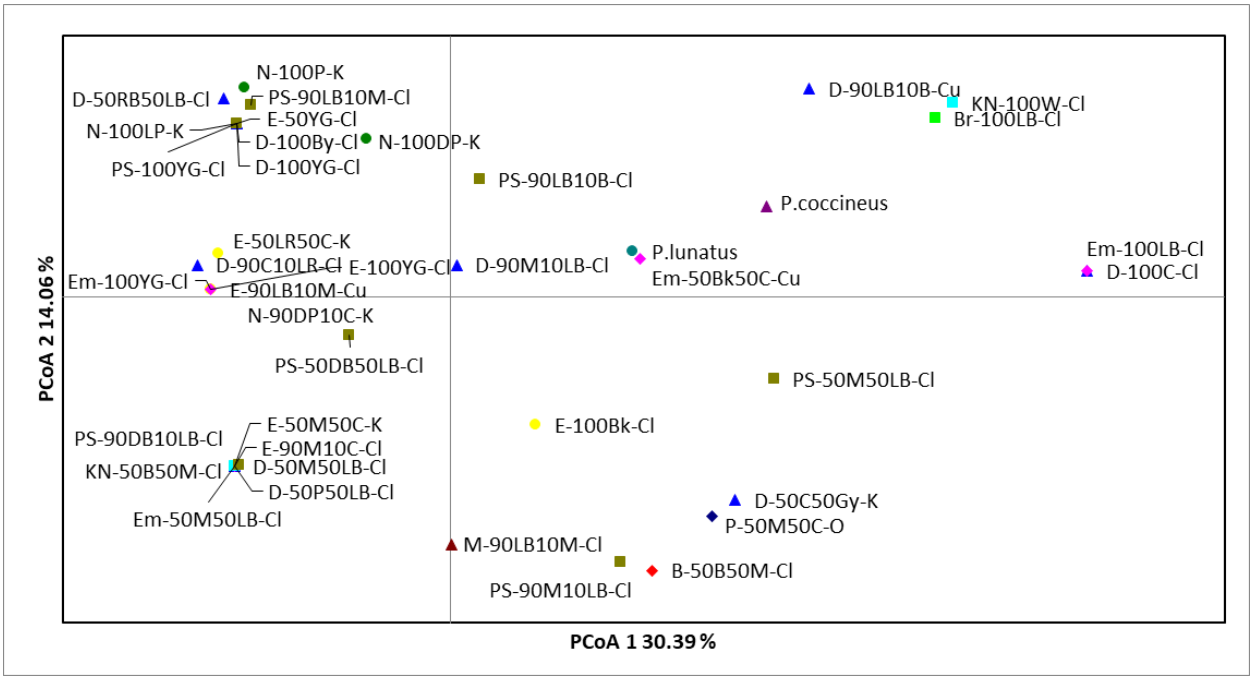


Figure 5.4: Principal coordinate analysis (PCoA) of *P. vulgaris* landraces from SSR markers based on the genotypic distance.

The landraces were divided into twelve populations based on their area of origin: diamond red- landraces from Benoni; square green- landraces from Bushbuckridge; triangle navy blue- landraces from Durban; circle yellow- landraces from Eshowe; diamond purple- landraces from Empangeni; square light blue- landraces from KwaNdebele; triangle maroon- landraces from Mtubatuba; circle dark green- landraces from Nelspruit; diamond navy blue- landraces from Polokwane; square yellowish-green- landraces from Port Shepstone; triangle dark purple and circle bluish-green represent the outgroups *P. coccineus* and *P. lunatus* respectively.

In the second quadrant, landraces were scattered apart. Landraces *D-90LB10B-Cu* from Durban, *KN-100W-Cl* from KwaNdebele, and *Br-100LB-Cl* from Bushbuckridge were grouped closer together. The Empangeni landrace *Em-100LB-Cl* and *D-100C-Cl* from Durban were grouped closer together. The outgroup *P. lunatus* and *Em-50Bk50C-Cu* from Empangeni were clustered closely. Whereas, *PS-90LB10B-Cl* from Port Shepstone, *D-90M10LB-Cl* from Durban as well as the outgroup *P. coccineus* were further apart from all the landraces in the quadrant.

In the third quadrant, *N-90DP10C-K* from Nelspruit and *PS-50DB50LB-Cl* from Port Shepstone formed an admixture in the upper portion. *KN-50B50M-Cl* from KwaNdebele was clustered together with the admixture formed by landraces *D-50M50LB-Cl* and *D-50P50LB-Cl* from Durban, *E-50M50C-K* and *E-90M10C-Cl* from Eshowe, *Em-50M50LB-Cl* from Empangeni as well as *PS-90DB10LB-Cl* from Port Shepstone. The landraces were scattered in the fourth quadrant, *D-50C50Gy-K* from

Durban, and *P-50M50C-O* from Polokwane were closely associated. Again, *B-50B50M-Cl* from Benoni and *PS-90M10LB-Cl* from Port Shepstone were associated. However, the Port Shepstone *PS-50M50LB-Cl, E-100Bk-Cl* from Eshowe, and *M-90LB10M-Cl* from Mtubatuba were further apart.

5.3.6 The phylogenetic relationship between *P. vulgaris* landraces

The phylogenetic relationship was further illustrated by the dendrogram using the unweighted pair group method of arithmetic mean (UPGMA) diagram based on Nei's genetic distance (Figure 5.5). The dendrogram clustered the landraces into seven clusters.

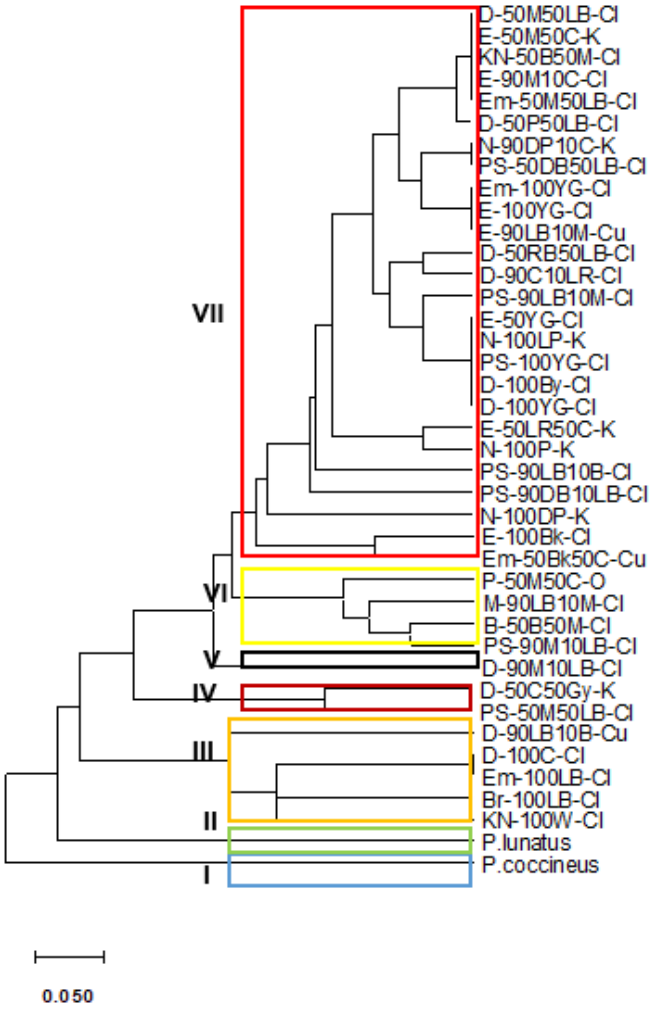


Figure 5.5: Unweighted Pair Group Method of Arithmetic mean (UPGMA) dendrogram based on Nei's genetic distance of *P. vulgaris* landraces using SSR markers.

Cluster I was made up of *P. coccineus* from the outgroup. Cluster II was generated only by the outgroup *P. lunatus*. Landraces *KN-100W-Cl*, *Br-100LB-Cl*, *D-100C-Cl*, and *D-90LB10B-Cu* were found in Cluster III. *D-50C50Gy-K* and *PS-50M50LB-Cl* were in Cluster IV, while *D-90M10LB-Cl* was in its own cluster (Cluster V). *PS-90M10LB-Cl*, *B-50B50M-Cl*, *M-90LB10M-Cl*, and *P-50M50C-O* made up Cluster VI. The rest of South African landraces were found in Cluster VII, including *Em-50Bk50C-Cu*, *E-100Bk-Cl*, *N-100DP-K*, and others *PS-90DB10LB-Cl, PS-90LB10B-Cl, N-100P-K, E-50LR50C-K, D-100YG-Cl, D-100By-Cl, PS-100YG-Cl, N-100LP-K, E-50YG-Cl, PS-90LB10M-Cl, D-90C10LR-Cl, D-50RB50LB-Cl, E-90LB10M-Cu, E-100YG-Cl, Em-100YG-Cl, PS-90DB50LB-Cl, N-90DP10C-K, D-50P50LB-Cl, Em-50M50LB-Cl, E-90M10C-Cl, KN-50B50M-Cl, E-50M50C-K* as well as *D-50M50LB-Cl.*

5.4 Discussion

5.4.1 Allele number and major allele frequency of simple sequence repeats

A total of 51 alleles with an average of 3.64 alleles per locus ranged from 1–6 as detected by seven Simple Sequence Repeat (SSR) markers were found among the South African *Phaseolus vulgaris* landraces (Table 5.2). The 13 SSR markers among *P. vulgaris* landraces in Turkey had a higher average number of alleles of 14.8 and a higher range (6–29) than the alleles in the current study (Bilir *et al*., 2019). The genetic differences in allelic numbers between the two countries could be due to the diversity in the structure, motif, length, and genomic content of SSR loci as well as the number of markers analysed (Blair *et al*., 2006). Forward and reverse markers of PV-ag001 and PV-ggc001 followed by forward and reverse makers of PV-ctt001 produced numerous alleles (six alleles). This could probably mean that these SSR markers detected a high degree of polymorphism (Burle *et al*., 2010).

The frequency of major alleles in the current study (Table 5.2) ranged from 0.48 to 1.00 with an average of 0.75, which was greater than the range (0.17–0.81) and average (0.46) of major alleles in *P. vulgaris* landraces in Southern Italy (Scarano *et al*., 2014). These findings suggest that alleles in South African landraces are common and there are wide genetic distinctions.

5.4.2 Genetic diversity, observed heterozygosity and polymorphic information content

Genetic diversity that ranged from 0.00–0.65 among the *P. vulgaris* landraces in South Africa (Table 5.2) was within a range from 0.00–0.96 found among *P. vulgaris* landraces from Brazil (Burle *et al*., 2010). The observed heterozygosity that ranged from 0.00–0.05 over seven SSR loci in the current study (Table 5.2) was within a range from 0.00–0.099 identified in 58 SSR loci among landraces in Italy (Gioia *et al*., 2019). The low levels of heterozygozity in *P. vulgaris* alleles in South Africa could indicate that the landraces have low genetic variation. These differences were probably caused

by unequal numbers (7 and 58) of detected SSR loci. The lower heterozygosity values in the current study could have been due to the fact that *P. vulgaris* is a naturally self-pollinating plant and most loci were probably homozygous (Nkhata *et al*., 2020).

The polymorphic information content (PIC) values show how beneficial specific markers are in genetic diversity research (Nkhata *et al*., 2020). The PIC that ranged from 0.00–0.58 among the *P. vulgaris* landraces in the current study (Table 5.2) was within a range from 0.00–0.96 recorded among the Brazilian landraces (Burle *et al*., 2010). This variation in PIC between South Africa and Brazil could have resulted from high mutation rates which lead to variability at SSRs loci (Blair *et al*., 2006). The low PIC values indicate that the genetic diversity of these selected *P. vulgaris* landraces in South Africa was relatively low.

The reverse marker PV-ctt001 had the highest genetic diversity (0.65) and PIC (0.58) followed by forward markers PV-ctt001 (GD = 0.65 and PIC = 0.58), PV-ag001 (GD = 0.58 and PIC = 0.53), and PV-at003 (GD = 0.54 and PIC = 0.44). This could probably mean that these SSR markers have high polymorphism among *P. vulgaris* landraces in South Africa and could be ideal for genetic mapping and characterizing genetic diversity for future seed breeding and conservation (Burle *et al*., 2010).

5.4.3 Genetic distance between *P. vulgaris* landraces, based on SSR

The existence of variability among 38 *P. vulgaris* landraces and the two outgroups (*Phaseolus coccineus* and *Phaseolus lunatus*) was revealed by correlation based on genetic distance that ranged from 0.00–0.79 (Table 5.3). Landraces with similar seed coat colour but different colour intensity and areas of origin (*E-50M50C-K, E-50M50LB-Cl, E-90M10C-Cl, Em-50M50LB-Cl,* and *KN-50B50M-Cl*) were the closest in the genetic distance, therefore had the highest degree of similarity. The high similarity could be due to the almost similar mature seed coat colours *E-50M50C-K* (50% maroon 50% cream*, E-50M50LB-Cl* and *Em-50M50LB-Cl* (50% maroon 50% light brown)*, E-90M10C-Cl* (90% maroon 10% cream)*,* and *KN-50B50M-Cl* (50% brown 50% maroon), which are probably controlled by the same gene for seed colour (Bassett, 2003).

Landraces with different seeds coat colours and origins (*E-90LB10M-Cu*, *E-100Bk-Cl*, and *Em-100YG-Cl*) also had the highest degree of similarity. The similarity could have resulted from a similar area of origin as Eshowe and Empangeni are geographically close to each other, where both are located on the north coast of the KwaZulu-Natal province. The results were similar to the Turkish genotypes, i.e. Mus and Bitlis demonstrated close genetic distance and the genotypes were geographically close to one another (Bilir *et al*., 2019).

The farthest genetic distance and lowest degree of similarity between landraces *D-100C-Cl*, *E-50LR50C-K*, and *PS-90DB10LB-Cl* from Durban, Eshowe, and Port Shepstone, respectively, was probably due to the low rates of gene flow detected by the SSR markers among these KwaZulu-Natal landraces (Musango *et al*., 2016). The decrease in similarity could be explained in terms of increasing genetic distances between *KN-100W-Cl* and *PS-90DB10LB-Cl* that could have resulted from major differences in the area of origin, where Port Shepstone is in moist, coastal areas of KwaZulu-Natal and KwaNdebele is in dry, inland regions of Mpumalanga.

5.4.4 Population structure among *Phaseolus vulgaris*

The population structure of *Phaseolus vulgaris* landraces in the current study was represented graphically for K = 2 (Figure 5.3). The highest delta value occurred at K = 2, which indicated that the 38 *P. vulgaris* and the two outgroups (*Phaseolus coccineus* and *Phaseolus lunatus*) of the current study could be divided into two subpopulations with admixed landraces between the subpopulations (Figure 5.2 and 5.3). The results were similar to the population structure of *P. vulgaris* germplasm in Malawi, where delta K was the highest at K = 2 (Nkhata *et al*., 2020). At the K = 3 levels, the population was modelled to evaluate more genetic variations of the subpopulations and the admixtures.

The 40 landraces evaluated in the current study were grouped into two subpopulations based on the Bayesian genotype clustering approach. This might have resulted from the domestication of *P. vulgaris* from two gene pools described as Mesoamerican and Andean (Musango *et al*., 2016). The population structure of the current study showed

an overlap among landraces, as several landraces from the Mesoamerican gene pool were identified as carrying some seed traits or genes from the Andean gene pool (Figure 5.3). This may have occurred as a result of the use of Andean landraces as dominant donor parents in certain breeding programs, resulting in certain genes being shared between the two gene pools. (Almeida *et al*., 2020).

At K2.1 (red), the subpopulation was composed of landraces *D-100C-Cl, Em-100LB-Cl, KN-100W-Cl,* and *Br-100LB-Cl* as well as the outgroups *P. coccineus* and *P. lunatus* (Figure 5.3). This population of medium-sized seeds (32.93–38.70 g 100-seed mass) could probably belong to the 100% Mesoamerican gene pool based on the seed size (100 seed mass), and seed colour (Table 3.4). Studies of *P. vulgaris* in Italy and Washington indicated that the Mesoamerican gene pool is composed of either small (< 25 g) or medium (25–40 g) sized seeds based on 100 seed mass, with black, white, navy, reds, and pinks, while, the Andean gene pool has large-seeded (> 40 g) varieties, with light and dark red kidney, white kidney, bushy cranberry or yellow, white and green seeds (Elsadr *et al*., 2011; Gioia *et al*., 2019). However, *P. lunatus* probably belonged to the 100% Andean gene pool based on the large seeds (219.30 g 100 seed mass) and the cranberry seed colour (50% cream and 50% maroon) (Table 3.4; Elsadr *et al*., 2011).

In the current study, *P. coccineus* had the seed size and shape of Andean gene pool, i.e. large, white, kidney shaped seeds with 175.05 g 100-seed mass (Table 3.4). But, in the Slovenian germplasm, the *P. coccineus* was restricted to the Mesoamerican gene pool (Sinkovic *et al*., 2019). The landraces in subpopulation K2.1 were probably grouped because of the medium leaves, and lighter seeds. The outgroups *P. coccineus* and *P. lunatus* , on the other hand, had heavier seeds. (Table 3.3 and 3.4). Landraces *D-100C-Cl, Em-100LB-Cl, KN-100W-Cl,* and *Br-100LB-Cl,* as well as the *P. coccineus,* shared similar seed coat colour (100% cream, 100% light brown, and 100% white) but different colour intensity. Nevertheless, the genetic diversity between them was low (GD = 0.36) except for *P. lunatus* (Table 5.3).

The K2.2 (green), included *D-100By-Cl, D-90C10LR-Cl, D-50RB50LB-Cl, D-100YG-Cl, E-50LR50C-K, E-90LB10M-Cu, E-50M50C-K, E-90M10C-Cl, E-50YG-Cl, E-100YG-Cl, Em-50M50LB-Cl, Em-100YG-Cl, KN-50B50M-Cl, N-90DP10C-K, N-*

100LP-K, N-100P-K, PS-50DB50LB-Cl, PS-90LB10M-Cl, and *PS-100YG-Cl.* This subpopulation consisted of middle-seeded (> 25 g 100 seed mass) and large-seeded (> 40 g 100-seed mass) seeds, which could belong in both the 100% Mesoamerican and 100% Andean gene pools (Gioia *et al*., 2019). In a comparable study, the landraces (MS13, MS20, and MS30) in *P. vulgaris* germplasm from Zimbabwe consisted of both Mesoamerican and Andean gene pools based on seed size and colour which showed an overlap of gene pools (Musango *et al*., 2016).

The landraces *D-100By-Cl, E-90LB10M-Cu, Em-100YG-Cl, KN-50B50M-Cl, N-90DP10C-K, N-100LP-K, N-100P-K,* and *PS-50DB50LB-Cl* might belong to the 100% Andean gene pool based on their large seeds (43.31–92.00 g 100-seed mass). While the remaining landraces of this subpopulation might belong to the 100% Mesoamerican group based on their middle-sized seeds (26.58–38.10 g 100-seed mass) (Table 3.4). These landraces were probably grouped because of their similar morpho-agronomic traits such as taller plants, the majority had a bushy growth type and three had climbing growth habits, thicker stems, higher chlorophyll content, fewer branches, fewer number of pods and seeds per pod/plant (Table 3.3 and 3.4). Although landraces *N-90DP10C-K* and *N-100P-K* were not included for morpho-agronomic traits, they showed similar characteristics with the above-mentioned landraces.

The admixtures were composed of landraces *D-90LB10B-Cu, PS-50M50LB-Cl, D-50C50Gy-K, B-50B50M-Cl, P-50M50C-O, PS-90M10LB-Cl, Em-50Bk50C-Cu, D-90M10LB-Cl, PS-90DB10LB-Cl, M-90LB10M-Cl, N-100DP-K, PS-90LB10B-Cl,* and *E-100Bk-Cl.* The admixed landraces showed a mixture of Mesoamerican and Andean gene pools (Figure 5.3). *E-100Bk-Cl* (100% black) showed 50% of the Andean gene and 50% of the Mesoamerican, which was different from other landraces based on the Bayesian analysis. This was similar to the population structure reported among *P. vulgaris* germplasm from Brazil (Almeida *et al*., 2020). Because the seed coat colors are controlled by the interaction of several genes operating individually or in the presence of epistatic genes to create segregated offspring from white to black, these landraces most likely possessed a combination of domesticated gene pools (Zhu *et al*., 2017). Among *P. vulgaris* from South Africa and Zambia, the red mottled, yellow,

and black seeds had admixed pools and may serve as a bridge to transfer important traits from Mesoamerican to Andean genotypes (Cichy *et al*., 2015).

The admixtures *B-50B50M-Cl, D-50C50Gy-K, D-90LB10B-Cu, E-100Bk-Cl, PS-90DB10LB-Cl, PS-90LB10B-Cl,* and *PS-50M50LB-Cl* had medium-sized seeds based on 100 seed mass and might have belonged in the Mesoamerican gene pool. While, the *D-90M10LB-Cl, Em-50Bk50C-Cu, M-90LB10M-Cl, N-100DP-K,* and *P-50M50C-O* admixtures had large seeds and might have belonged to the Andean gene pool. These admixed seeds might be due to the hybridization between the Mesoamerican and Andean gene pools (Delfini *et al*., 2021) or the importation of *P. vulgaris* seeds from South America and Europe to Africa (Cichy *et al*., 2015). The further clustering of the population at K = 3 resulted in the separation of the K2.1 (red) into two subpopulations K3.1 (red) and K3.3 (blue), while the other landraces in K2.2 (green) remained the same in K = 3 (K3.2) (Figure 5.3).

5.4.5 PCoA and phylogenetic relationship between *P. vulgaris* landraces

In the PCoA and phylogenetic diagram for *P. vulgaris*, the results revealed a similar clustering pattern to the Bayesian population clustering analysis (Figures 5.4 and 5.5). Landraces *D-50RB50LB-Cl, N-100P-K, N-100DP-K, PS-90LB10M-Cl* as well as the admixtures *D-100By-Cl, D-100YG-Cl, E-50YG-Cl, N-100LP-K,* and *PS-100YG-Cl* in the upper portion of the first quadrant (PCoA) had the closest distance and were clustered in the Cluster VII of the dendrogram.This high degree of similarity was probably due to their similar vegetative and reproductive traits such as taller plants, thicker stems, numerous leaves as well as their earlier days to flowering, longer and wider pods, and numerous seeds per plant (Table 3.3 and 3.4). The results were similar to the comparative study of *P. vulgaris* landraces from Zimbabwe, where the landraces from different gene pools were clustered together due to the morphological and agronomic traits (Musango *et al*., 2016).

The lower portion of the first quadrant (PCoA) and Cluster VII (dendrogram) was composed of *E-50LR50C-K, D-90C10LR-Cl,* and admixtures *Em-100YG-Cl, E-90LB10M-Cu,* and *E-100YG-Cl.* This clustering possibly resulted from high rates of gene flow among the populations, which might have resulted from similar geographical

areas as Durban, Eshowe and Empangeni are all coastal areas of the KwaZulu-Natal province. According to the clustering analysis among *P. vulgaris* from Turkey, the populations that demonstrated high similarity and high gene flow were geographically close (Bilir *et al*., 2019). These results show large variations in seed coats but had a high degree of similarity that probably emerged from gene introgressions due to random bee pollination in the field in the same geographical areas (Musango *et al*., 2016) or through natural cross-pollination (Nkhata *et al*., 2021).

KN-100W-Cl, Br-100LB-Cl, and *D-90LB10B-Cu* had the farthest distances with *Em-100LB-Cl,* and *D-100C-Cl* and also landraces *PS-90LB10B-Cl, D-90M10LB-Cl, P. coccineus* as well as *P. lunatus* and *Em-50Bk50C-Cu* (Figure 5.4). This degree of dissimilarity probably resulted from the variation in seed coat colour, seed shape (cuboidal to cylindrical), and possibly the different gene pools. These variations can be attributed to large genetic differences between the two groups as a result of parental race differences; Andean origin and Mesoamerican origin based on seed weight (Gioia *et al*., 2019). This was also true for the scattering of *P. vulgaris* landraces in the fourth quadrant of PCoA (Figure 5.4).

Although, *Br-100LB-Cl, KN-100W-Cl,* and *D-90LB10B-Cu* from different origins (Bushuckridge, KwaNdebele, and Durban, respectively) were genetically close based on their close distance in the PCoA (Figure 5.4) and by associating together in cluster III on the dendrogram (Figure 5.5). This might have resulted from the similar gene pool (Mesoamerican) based on their middle-sized seeds (100-seed mass) and was probably influenced by the similar morpho-agronomic traits (Table 3.4). These results were similar to the study of *P. vulgaris* landraces from Zimbabwe (Musango *et al*., 2016). The grouping of *Em-100LB-Cl* and *D-100C-Cl* in the PCoA (Figure 5.4) was probably due to the similar seed coat colour (but different intensity), seed shape (cylindrical), and also the similar geographical location (coastal of KwaZulu-Natal). It was supported by the observation that they cluster together in one group on the dendrogram (Figure 5.5). *Em-100LB-Cl* and *D-100C-Cl* probably shared similar seed coat colour genes (gene *c/c*) responsible for the lighter or paler brown colour in the seed coats (McClean *et al*., 2002).

The third quadrant (PCoA) and Cluster VII (dendrogram) included *N-90DP10C-K,* and *PS-50DB50LB-Cl* (admixtures), *PS-90DB10B-Cl,* and *KN-50B50M-Cl,* and also admixtures (*E-50M50C-K, E-90M10C-Cl, E-50M50LB-Cl, D-50P50LB-Cl,* and *Em-50M50LB-Cl*) (Figure 5.4 and 5.5). These landraces were genetically close but originated from different geographical areas (Nelspruit, and KwaNdebele in Mpumalanga province, Port Shepstone, Eshowe, Durban, and Empangeni in the KwaZulu-Natal province), this probably resulted from the exchange or introduction of planting material (seeds) between farmers in different provinces (Nkhata *et al*., 2020). The sharing of the ancestry between these landraces was probably due to the intergene crossing in breeding or natural hybridization (Scarano *et al*., 2014). These results might indicate that landraces such as *E-50M50LB-Cl, Em-50M50LB-Cl, KN-50B50M-Cl, E-5M50C-K,* and *E-90M10C-Cl* were sown from the same parental seed or parents with similar seed coat colour.

The outgroups *P. coccineus* and *P. lunatus* were characterized as the most dissimilar landraces followed by the *E-100Bk-Cl* and *Em-50Bk50C-Cu* in the PCoA (Figure 5.4). The results were also supported by the phylogenetic diagram (dendrogram) as the outgroups formed their clusters, Cluster I for *P. coccineus* and Cluster II for *P. lunatus.* While *E-100Bk-Cl* and *Em-50Bk50C-Cu* were grouped in Cluster VII (Figure 5.5). Taller climbing plants, thicker stems, numerous leaves and branches, as well as longer, thicker, and wider seeds with heavier mass and the fact that they are separate species, may distinguish the outgroups from the rest of the landraces (Table 5.4 and 5.5).

E-100Bk-Cl and *Em-50Bk50C-Cu* were grouped together on the dendrogram but had the farthest distance in the PCoA. The grouping was possibly due to their similar morpho-agronomic traits (Table 3.3 and 3.4) and they may also share the same seed coat gene (*[Cr] Z J G B V Rk*) that expresses the black seed coat (Bassett, 2003). The farthest distance which shows the high rate of dissimilarity between the two landraces probably resulted from the variation in gene pools, *E-100Bk-Cl* might belong to the Mesoamerican based on the middle-sized seeds (100 seed mass) and *Em-50Bk50C-Cu* due to the large seed belonged to the Andean gene pool (Table 3.4; Gioia *et al*., 2019).

5.5 Conclusion

The results obtained in this study indicated that the majority of the SSR markers had lower genetic diversity, observed heterozygosity, and polymorphic information content than those reported in other studies, which suggests a limited number of rare variants among the *P. vulgaris* landraces with different origins. This was probably due to the fact that *P. vulgaris* are self-pollinating with a low frequency of crossing. However, they further revealed that the reverse and forward marker PV-ctt001, and forward markers PV-ag001 and PV-at003 have high polymorphism and genetic diversity in the *P. vulgaris*. These markers could be ideal for determining genetically homogenous/ heterogeneous landraces for future breeding and conservation and also in marker-assisted selection studies. The population structure of the current study showed an overlap among landraces, as several landraces from the Mesoamerican gene pool were identified as carrying some seed traits or genes from the Andean gene pool (many landraces were represented as admixtures). This was also supported by the principal coordinate analysis and the dendrogram, as the admixture landraces were grouped in different quadrants of the PCoA and clustered together in the dendrogram (cluster VII). In the South African landraces, it can be concluded that the morpho-agronomic traits are not showing what is truly represented by the genes. The *P. vulgaris* landraces could further be evaluated in the mitochondrial DNA analysis to screen for ancestry origin.

Chapter 6

Conclusions and Recommendations

This study recorded significant variations in vegetative and reproductive traits among the *P. vulgaris* landraces from different provinces of South Africa. Landraces *E-100Bk-Cl* and *N-100DP-K* had the highest germination percentage and earliest days to first and 50% flowering. *E-100Bk-Cl* might be appropriate for growth under stressful environments. The plants growing under stress change their structure and use plant resources towards fast maturity and inhibit growth traits leading to shorter plants, narrower leaves, and shorter and narrower pods and seeds. However, in *P. vulgaris* production, the early flowering trait is an essential component of seed yield and results in early harvesting. Early flowering has also been recognized as a beneficial agronomic trait of crops for early maturity, yield uniformity, and overall crop production. Thus, *E-100Bk-Cl* and *N-100DP-K* could be recommended for the selection of early harvesting landraces for plant breeding, and small or large-scale farming.

Landrace *E-90LB10M-Cu* recorded the tallest plants similar to the outgroups *Phaseolus coccineus* and *Phaseolus lunatus*. *E-50M50C-K* had the thickest stems and landrace *D-90M10LB-Cl* produced broader leaves. Again, *D-100C-Cl* had the highest chlorophyll content and *Em-100LB-Cl* yielded numerous leaves. *B-50B50M-Cl* produced the longest pods outperforming all the landraces, *P-50M50C-O* had the widest pods and *PS-90DB10LB-Cl* produced numerous pods per plant. *D-50C50Gy-K* had numerous seeds per pod while *Em-50Bk50C-Cu* had numerous seeds per plant. *N-100DP-K* had the longest and widest seeds; *KN-50B50M-Cl* produced the thickest seeds, and *N-100LP-K* yielded the heaviest seeds. These landraces might be selected for cultivation and breeding towards tall landraces with broader and numerous leaves, numerous pods and seeds as well as longer and bigger pods and seeds in South Africa. These are the characteristics that make leaves, pods, and seeds suitable for human consumption as vegetables, green beans, and cooked dry beans.

This study revealed a positive correlation between the plant height and pod width, which further correlated with seed length, width, and 100-seed mass. These traits

probably suggest that breeding towards tall plants with wider pods in South Africa will result in longer, wider, and heavier seeds. Therefore, *KN-50B50M-Cl, N-100DP-K, N-100LP-K,* and *P-50M50C-O* are potential landraces and can be recommended for future plant breeding, large-scale farming, and potential large seed companies. The principal component analysis supported this, since PC1 incorporated practically all growth variables and fewer yield traits, and may be used to analyze variability among *P. vulgaris* landraces.

The South African *P. vulgaris* landraces were clustered using a biplot and dendrogram based on their seed coat colour, shape, similar morpho-agronomic traits, and their area of origins. This probably means that the morpho-agronomic traits especially seed traits are good indicators for studying variability among *P. vulgaris* landraces. The clustering also shows that the landraces in the current study can adapt to different environments. Thus, breeding towards landraces that grow vigorously and form high yields in different environments and can be recommended for future plant breeding programs to improve plant adaptation to diverse environments.

The study on the segregation patterns of seed coat colour and the seed morphology of the parents and the progenies recorded the diversity of *P. vulgaris* landraces from South Africa. The twelve segregated landraces probably had multiple genes (*P, C, T, G, B, B, Rk,* and [*C-R*]) that are involved in the seed coat determination. The parents of *P. vulgaris* produced offspring with different seed colour, shapes, and sizes. This variation could result from parents that have different genetic makeups. Therefore, the following landraces *D-50C50Gy-K, D-100C-Cl, D-90LB10B-Cu, D-100YG-Cl, E-100Bk-Cl, Em-100LB-Cl, Em-50Bk50C-Cu, M-90LB10M-Cl, N-100LP-K, P-50C50M-O, PS-90M10LB-Cl,* and *PS-100YG-Cl* could be exploited, enriched, or used directly in seed breeding programs and conservation.

The analysis of *P. vulgaris* landraces with different seed coat colours and from different environments of South Africa using simple sequence repeat markers revealed that *P. vulgaris* landraces have a limited number of rare variants as they showed low genetic diversity, observed heterozygosity, and polymorphic information content. This might likely be due to that *P. vulgaris* are self-pollinating species with a low frequency of cross-pollinating. The principal coordinate analysis (PCoA) showed that some

Mesoamerican and Andean groups carry seed traits or genes from each other, as most landraces were represented as admixtures. This was also supported by the population structure and dendrogram. *D-100By-Cl, D-100YG-Cl, E-50YG-Cl, N-100LP-K, PS-100YG-Cl, E-90LB10M-Cu, E-100YG-Cl, Em-100YG-Cl, D-50M50LB-Cl, D-50P50LB-Cl, E-50M50C-K, E-90M10C-Cl, Em-50M50LB-Cl, PS-90DB10LB-Cl, N-90DP10C-K,* and *PS-50DB50LB-Cl* were all clustered as admixtures in similar quadrants of the PCoA. In the population structure, these landraces were grouped in K2.1 (green) and Cluster VII in the dendrogram. The landraces consisted of middle-seeded (> 25 g 100 seed mass) and large-seeded (> 40 g 100 seed mass) seeds, which could belong in both Mesoamerican and Andean gene pools. The study could be further recommended for the screening of the ancestral gene or origin, using mitochondrial DNA analysis.

References

Abdel-Haleem, A., El-Shaieny, H., 2015. Seed germination percentage and early seedling establishment of five (*Vigna Unguiculata* L. (Walp) genotypes under salt stress. *European Journal of Experimental Biology,* 5(2): 22–32.

Abdollahi, A., Tahmasebpour, B., Dehghanian, H., 2016. Factor analysis of phonological and morphological traits in common bean (*Phaseolus vulgaris* L.). *Biological Forum– An International Journal*, 8(1): 132–134.

Aguilar-Benítez, G., Peña-Valdivia, C.B., Vega, J.R., Castro-Rivera, R., Ramírez-Tobías, H.M., 2014. Seed germination and early root growth in common bean and maize landraces and improved cultivars at different water stress levels. *International Journal of Applied Science and Technology*, 4(4): 118–127.

Akhshi, N., Nazarian Firouzabadi, F., Cheghamirza, K., Dorri, H.R., 2015. Coefficient analysis and association between morpho-agronomical characters in common bean (*Phaseolus vulgaris* L.). *Cercetari Agronomice*, 48(4): 164.

Al-Ballat, I.A., Al-Araby, A.A., 2019. Correlation and path coefficient analysis for seed yield and some of its traits in common bean (*Phaseolus vulgaris* L.). *Egypt Journal of Horticulture*, 46(1):41–51.

Alemu, A., Nebiyu, A., Getachew, M., 2018. Growth and yield of common bean (*Phaseolus vulgaris* L.) cultivars as influenced by rates of phosphorus at Jimma, Southwest Ethiopia. *Journal of Agricultural Biotechnology and Sustainable Development*, 10(6):104–115.

Almeida, C.P.D., Paulino, J.F.D.C., Morais Carbonell, S.A., Chiorato, A.F., Song, Q., Vittori, V.D., Rodriguez, M., Papa, R., Benchimol-Reis, L.L., 2020. Genetic diversity, population structure, and Andean introgression in Brazilian common Bean cultivars after half century of genetic breeding. *Genes*, 11: https://doi:10.3390/genes11111298

Arteaga, S., Yabor, L., Torres, J., Solbes, E., Muñoz, E., Díez, M.J., Vicente, O., Boscaiu, M., 2019. Morphological and agronomic characterization of Spanish landraces of *Phaseolus vulgaris* L. *Agriculture*, 9: 149. https://doi:10.3390/agriculture9070149

Asfaw, A., Blair, M.W., Almekinders, C., 2009. Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) landraces from the East

African highlands. *Theoretical and Applied Genetics*, 120: 1–12. https://DOI10.1007/s00122-009-1154-7

Avval, S.E., 2017. Assessing polymorphism information content (PIC) using SSR molecular markers on local species of *Citrullus colocynthis*. Case study: Iran, Sistan-Balouchestan province. *Journal of Molecular Biology Research*, 7(1): 42–49.

Balkaya, A., Ergün, A., (2008) Diversity and use of pinto bean (*Phaseolus vulgaris*) populations from Samsun, Turkey, New Zealand. *Journal of Crop and Horticultural Science*, 36: (3)189–197, DOI: 10.1080/01140670809510235

Bareke, T., 2019. Diversity and genetic potential of various morphological traits among common bean (*Phaseolus vulgaris*, *Fabaceae*) landraces. *Biodiversitas*, 20(11): 3237–3245. https://DOI:10.13057/biodiv/d201116

Bassett, M.J., 2003. Allelism between the P and Stp genes for seedcoat color and pattern in common bean. *Journal of the American Society for Horticultural Science*, 128(4): 548–551.

Bassett, M.J., 2005. Inheritance of the virgata pattern of partly colored seed coats in 'Early Giant'common bean. *Euphytica*, 141(1-2):139–145.

Bassett, M.J., Miklas, P.N., 2007. A new gene, bic, with pleiotropic effects (with TPV) for bicolor flowers and dark olive brown seed coat in common bean. *Journal of the American Society for Horticultural Science*, 132(3): 352–356.

Bhatt, M. and Chanda, S.V., 2003. Prediction of leaf area in *Phaseolus vulgaris* by non-destructive method. *Bulgarian journal Plant Physiology*, 29(2): 6–100.

Bilir, Ö., Özmen, C.Y., Özcan, S., Kibar, U., 2019. Genetic analysis of Turkey common bean (*Phaseolus vulgaris* L.) genotypes by simple sequence repeats markers. *Russian Journal of Genetics*, 55(1): 61–70. https://DOI:10.1134/S1022795419010034

Bitocchi, E., Rau, D., Bellucci, E., Rodriguez, M., Murgia, M.L., Gioia, T., Santo, D., Nanni, L., Attene, G., Papa, R., 2017. Beans (*Phaseolus* ssp.) as a model for understanding crop evolution. *Frontiers in Plant Science*, 8: 722. https://doi:10.3389/fpls.2017.00722

Blair, M.W., Giraldo, M.C., Buendia, H.F., Tovar, E., Duque, M.C., Beebe, S.E., 2006. Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics*, 113: 100–109. https://DOI10.1007/s00122-006-0276-4

Boros, L., Wawer, A., Borucka, K., 2014. Morphological, phenological and agronomical characterisation of variability among common bean (*Phaseolus vulgaris* L.) local populations from the national centre for plant genetic resources: Polish genebank. *Journal of Horticultural Research*, 22(2): 123–130. https://DOI:10.2478/johr/2014-0029

Burle, M.L., Fonseca, J.R., Kami, J.A., Gepts, P., 2010. Microsatellite diversity and genetic structure among common bean (*Phaseolus vulgaris* L.) landraces in Brazil, a secondary center of diversity. *Theoretical and Applied Genetics*, 121: 801–813. https://DOI10.1007/s00122-010-1350-5

Cabral, P.D.S, de Souza, L.C, da Costa, G.F, Silva, F.H.C, Soares, T.C.B, 2018. Investigation of genetic diversity of common bean (*Phaseolus vulgaris*) cultivars using molecular markers. *Genetics and Molecular Research*, 14(4): DOI http://dx.doi.org/10.4238/gmr18106

Caldas, G.V., Blair, M.W., 2009. Inheritance of seed condensed tannins and their relationship with seed-coat color and pattern genes in common bean (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics*, 119: 131–142.

Casañas, F., Simo, J., Casals, J., Prohens, J., 2017. Toward an evolved concept of landrace. *Frontiers in Pplant sScience,* 8: 145. doi: 10.3389/fpls.2017.00145.

Checa, O., Ceballos, H., Blair, M.W., 2006. Generation means analysis of climbing ability in common bean (*Phaseolus vulgaris* L.). *Journal of Heredity,* 97(5):456–465.

Cichy, K.A., Porch, T.G., Beaver, J.S., Cregan, P., Fourie, D., Glahn, R.P., Grusak, M.A., Kamfwa, K., Katuuramu, D.N., McClean, P., Mndolwa, E., 2015. A *Phaseolus vulgaris* diversity panel for Andean bean improvement. *Crop Science*, 55: 2149–2160.

Córdoba, J.M., Chavarro, C., Rojas, F., Muñoz, C., Blair, M.W., 2010. Identification and mapping of simple sequence repeat markers from common bean (*Phaseolus vulgaris* L.) bacterial artificial chromosome end sequences for genome characterization and genetic–physical map integration. *The Plant Genome*, 3:154–165. https://10.3835/plantgenome2010.06.0013

Darkwa, K., Ambachewa, D., Mohammed, H., Asfawa, A., Blairc, M.W., 2016. Evaluation of common bean (*Phaseolus vulgaris* L.) genotypes for drought stress adaptation in Ethiopia. *The Ccrop Jjournal*, 4: 367–376.

Delfini, J., Moda-Cirino, V., dos Santos Neto, J., Ruas, P.M., Sant'Ana, G.C., Gepts, P., Gonçalves, L.S.A., 2021. Population structure, genetic diversity and genomic selection signatures among a Brazilian common bean germplasm. *Scientific rReports*, 11:2964. https://doi.org/10.1038/s41598-021-82437-4

Dutta, S.K., Singh, S.B., Chatterjee, D., Boopathi, T., Singh, A.R., Saha, S., 2016. Morphological and genetic diversity of pole type common bean (*Phaseolus vulgaris* L.) landraces of Mizoram (India). *Indian Journal of Biotechnology*, 15: 550–559.

Earl, D., Vonholdt, B., 2011. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4: 359–361.

Egu, A.M, Tesfaye, K., 2018. Morpho-physiological characterization related to drought tolerance of common bean (*Phaseolus vulgaris* L.) genotypes. *International Journal of Advanced Biological and Biomedical Research*, 7(1): 39 – 46.

Ekbic, E., Hasangcaoglu, E., 2018. Morphological and molecular characterization of local common bean (*Phaseolus vulgaris* L.) genotypes. *Applied Ecology and Environmental Research*, 17(1): 841–853. http://dx.doi.org/10.15666/aeer/1701.841853

Elsadr, H.T., Wright, L.C., Pauls, K.P., Bett, K.E., 2011. Characterization of seed coat post-harvest darkening in common bean (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics*, 123:1467–1472.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using software STRUCTURE: A simulation study. *Molecular Ecology*, 14: 2611–2620.

Feeding of Rural communities in Puebla-Mexico *International Journal of Biological Sciences*, 2(28): 59–65.

Fourie, D., 2002. Distribution and severity of bacterial diseases on dry beans (*Phaseolus vulgaris* L.) in South Africa. *Journal of Phytopathology*, 150: 220–226.

Gioia, T., Logozzo, G., Marzario, S., Zeuli, P.S., Gepts, P., 2019. Evolution of SSR diversity from wild types to US advanced cultivars in the Andean and Mesoamerican domestications of common bean (*Phaseolus vulgaris*). *PloS One*, 14(1): https://doi.org/10.1371/journal.pone.0211342

Grahic, J., Gasi, F., Kurtovic, M., Karic, L., Djikic, M., Gadzo, D., Podrug, A., 2013. Level of cross-pollination among common bean (*Phaseolus. vulgaris)* landraces from Bosnia and Herzegovina examined through seed coat colour. In *Proceedings of the 24th International Scientific-Expert-Conference of Agriculture and Food Industry, Sarajevo, Bosnia and Herzegovina,* 386–390.

Guidoti, D.T., Gonela, A., Vidigal, M.C.G., Conrado, T.V., Romani, I., 2018. Interrelationship between morphological, agronomic and molecular characteristics in the analysis of common bean genetic diversity. *Acta Scientiarum. Agronomy*, 40: https://Doi:10.4025/actaasciagron.v40il.33032

Hacisalihoglu, G., Settles, A.M., 2013. Natural variation in seed composition of 91 common bean genotypes and their possible association with seed coat color. *Journal of pPlant Nnutrition*, 36(5): 772–780.

Iriti, M., Scarafoni, A., Pierce, S., Castorina, G., Vitalini, S., 2019. Soil application of effective microorganisms (EM) maintains leaf photosynthetic efficiency, increases seed yield and quality traits of bean (*Phaseolus vulgaris* L.) plants grown on different substrates. *International Journal of Molecular Ssciences*, 20: 2327. doi:10.3390/ijms20092327.

Jannat, S., Shah, A.H., Shah, K.N., Kabir, S., Ghafoor, A., 2019. Genetic and nutritional profiling of common bean (*Phaseolus vulgaris* L.) germplasm from Azad Jammu and Kashmir and exotic accessions. *JAPS, Journal of Animal and Plant Sciences*, 29(1): 205–214.

Jose, F.C., Mohammed, M.S., Thomas, G., Varghese, G., Selvaraj, N., Dorai, M., 2009. Genetic diversity and conservation of common bean (*Phaseolus vulgaris* L., Fabaceae) landraces in Nilgiris. *Current Science*, 97 (2): 227–235.

Kalauni, S., Pant, S., Luitel, B.P., Bhandari, B., 2019. Evaluation of pole-type French bean (*Phaseolus vulgaris* L.) genotypes for agro-morphological variability and yield in the mid-hills of Nepal. *Acta Scientific Aagriculture*, 3(12): 113–121.

Kapoor, M., Mawal, P., Sharma, V., Gupta, R.C., 2020. Analysis of genetic diversity and population structure in Asparagus species using SSR markers. *Journal of Genetic Engineering and Biotechnology*, 18: 50. https://doi.org/10.1186/s43141-020-00065-3

Kouam, E.B., Ndomou, M., Gouado, I., Pasquet, R.S., 2017. Assessment of the genetic diversity of cultivated common beans (*Phaseolus vulgaris* L.) from

Cameroon and Kenya using allozymes markers. *Journal of Experimental Biology and Agricultural Sciences*, 5(1): 87–97.

Langat, C., Ombori, O., Leley, P., Karanja, D., Cheruiyot, R., Gathaara, M., Masila, B., 2019. Genetic variability of agronomic traits as potential indicators of drought tolerance in common beans (*Phaseolus vulgaris* L.). *International Journal of Agronomy*, https://doi.org/10.1155/2019/2360848

Lei, L., Wang, L., Wang, S., Wu, J., 2020. Marker-trait association analysis of seed traits in accessions of common bean (*Phaseolus vulgaris* L.) in China. *Frontiers in Ggenetics*, 11: 698. https://doi:10.3389/fgene.2020.00698

Lima, E.R., Santiago, A.S., Araújo, A.P., Teixeira, M.G., 2005. Effects of the size of sown seed on growth and yield of common bean cultivars of different seed sizes. *Brazilian Journal of Plant Physiology*, 17(3): 273–281.

Loko, L.E.Y., Orobiyi, A., Adjatin, A., Akpo, J., Toffa, J., Djedatin, G., Dansi, A., 2018. Morphological characterization of common bean (*Phaseolus vulgaris* L.) landraces of Central region of Benin Republic. *Journal of Plant breeding and Crop Science*, 10(11): 304–318. https://DOI:10.5897/JPBCS2018.0766

Lorts, C., Lynch, J.P., Brown, K.M., 2019. Parental effects and provisioning under drought and low phosphorus stress in common bean. *Food and Energy Security*, 9: e00192. https://doi.org/10.1002/fes3.192

Mansoor, U., Fatima, S., Hameed, M., Naseer, M., Ahmad, M.S.A., Ashraf, M., Ahmad, F. and Waseem, M., 2019. Structural modifications for drought tolerance in stem and leaves of *Cenchrus ciliaris* L. ecotypes from the Cholistan Desert. *Flora*, 261: 151485. https://doi.org/10.1016/j.flora.2019.151485

Maras, M., Šuštar-Vozlič, J., Kainz, W., Meglič, V., 2013. Genetic diversity and dissemination pathways of common bean in central Europe. *Journal of the American Society for Horticultural Science*, 138(4): 297–305.

Marzooghian, A., Moghaddam, M., Valizadeh, M., Kooshki, M.H., 2013. Genetic diversity of common bean genotypes as revealed by seed storage proteins and some agronomic traits. *Plant Breeding and Seed Science*, 67: 125–137. https://DOI:10.2478/v10129-011-0075-1

Mayo-Prieto, S., Marra, R., Vinale, F., Rodríguez-González, A., Woo, S.L., Lorito, M., Gutiérrez, S., Casquero, P.A., 2019. Effect of *Trichoderma velutinum* and *Rhizoctonia solani* on the metabolome of bean plants (*Phaseolus vulgaris*

L.). *International Journal of Molecular Sciences*, 20(3): 549. https://doi:10.3390/ijms20030549

McClean, P.E., Bett, K.E., Stonehouse, R., Lee, R., Pflieger, S., Moghaddam, S.M., Geffroy, V., Miklas, P., Mamidi, S., 2018. White seed color in common bean (*Phaseolus vulgaris*) results from convergent evolution in the P (pigment) gene. *New Phytologist*, 219: 1112–1123.

McClean, P.E., Lee, R.K., Otto, C., Gepts, P., Bassett, M.J., 2002. Molecular and phenotypic mapping of genes controlling seed coat pattern and color in common bean (*Phaseolus vulgaris* L.). *Journal of Heredity*, 93(2): 148–152

Mishra, K.K., Fougat R.S., Ballani A., Thakar V., Jha Y., Madhumati, B., 2014. Potential and application of molecular markers techniques for plant genome analysis. *International Journal of Pure and Applied Bioscience*, 2(1):169–188.

Modiga, B.A, Jitareanu, C.D, 2017. Stomatic conductance and chlorophyll content index and leaf area of some beans local cultivars from North-east of Roomania, under salt stress. *Research Journal of Agricultural Science*, 49(4): 35–39.

Morales-Santos, M.E., Peña-Valdivia, C.B., García-Esteva, A., Aguilar-Benítez, G., Kohashi-Shibata, J., 2017. Seed and seedlings physical characteristics and seeds germination of wild and domesticated common bean (*Phaseolus vulgaris* L.) and their progeny. *Agrociencia*, 51: 43–62.

Morojele, M.E., Rhupu, R., Sefume, L., 2016. Variability in yield and yield components among common bean (*Phaseolus vulgaris* L.) genotypes. *Global Journal of Agricultural Research*, 4(5): 1–9.

Mpandeli, N.S., Maponya, P.I, 2013. Coping with climate variability in Limpopo province, South Africa. *Peak Journal of Agricultural Sciences*, 1(4): 54–64.

Muedi, H.T., Fourie, D., McLaren, N.W., 2015. Distribution and severity of bacterial brown spot on dry beans in South Africa: An update. *South African Journal of Science*, 111(3-4):1–6. https://dxdoi.org/10.17159/sajs.2015/20140015

Musango, R., Kudzai, K., Mhungu, S., Tibugar, H., 2016. Phenotypic characterization of common bean (*Phaseolus vulgaris* L.) accessions conserved at the genetic resources and biotechnology institute. *Journal of Biodiversity and Environmenta Sciences*, 8(6): 26–36.

Nahar, K., Ali, M.H., Ruhul Amin, A.K.M., Hasanuzzaman, M., 2009. Moisture content and germination of bean (*Phaseolus vulgaris* L.) under different storage conditions. *Academic Journal of Plant Sciences*, 2(4): 237–241.

Nassar, R.M.A., Ahmed, Y.M., Boghdady, M.S., 2010. Botanical studies on *Phaseolus vulgaris* L. I-Morphology vegetative and reproductive growth. *International Journal of Botany,* 6(3): 323–333.

Nazareno, A.G., Bemmels, J.B., Dick, C. W., Lohmann, L.G., 2017. Minimum sample sizes for population genomics: an empirical study from an Amazonian plant species. *Molecular Ecology Resources*, 17(6): 1136–1147. https://doi:10.1111/1755-0998.12654

Negahi, A., Bihamta, M.B., Negahi, Z., Alidoust, M., 2014. Evaluation of genetic variation of some agronomical and morphological traits in Iranian and exotic common bean (*Phaselous vulgaris* L.). *Agricultural Communications*, 2(3): 22–26.

Nkhata, W., Shimelis, H., Melis, R., Chirwa, R., Mzengeza, T., Mathew, I., and Shayanowako, A., 2020. Population structure and genetic diversity analyses of common bean germplasm collections of East and Southern Africa using morphological traits and high-density SNP markers. *PLoS One,* 15(12): e0243238. https://doi.org/10.1371/journal.pone.0243238

Ntuli, N.R., 2018. Morpho-Agronomic variation among *Phaseolus vulgaris* landraces: A Review. *Rediscovery of landraces as a resource for the future*, Grillo, O. Eds. IntechOpen: London – United Kingdom.

Nwadike, C., Terkimbi, T., 2015. Effects of planting date on performance of common bean (*Phaseolus vulgaris* L.) landraces of the Jos plateau: A preliminary studies. *International Journal of Current Research and Academic Review*, 3(8): 309–324.

Okii, D., Tukamuhabwa, P., Odong, T., Namayanja, A., Mukabaranga, J., Paparu, P., Gepts, P., 2014. Morphological diversity of tropical common bean germplasm. *African Crop Science Journal*, 22(1): 59–67.

Padilla-Chacón, D., Valdivia, C.P., García-Esteva, A., Cayetano-Marcial, M.I., Shibata, J.K., 2019. Phenotypic variation and biomass partitioning during post-flowering in two common bean cultivars (*Phaseolus vulgaris* L.) under water restriction. *South African Journal of Botany*, 121: 98–104. https://doi.org/10.1016/j.sajb.2018.10.031

Panchbhaiya, A., Singh, D.K., Jatav, V., Sanganamoni, M., Verma, P. (2017). Studies on variability, heritability and genetic advance for yield and yield contributing characters in french bean (*Phaseolus vulgaris* L.) germplasm under Tarai region of Uttarakhand. *Journal of Applied and Natural Science*. 9(4): 1926–1930.

Pashley, M., 1994. A level students: their problems with gene and allele. *Journal of Biological Education,* 28(2): 120–126.

Pereyra, M.S., Davidenco, V., Núñez, S.B., Argüello, J.A., 2014. Chlorophyll content estimation in oregano leaves using a portable chlorophyll meter: relationship with mesophyll thickness and leaf age. *Agronomia and Aambiente,* 34(1-2): 77–84. https://doi.org/10.1016/j.sajb.2018.10.031

Razvi, S.M., Khan, M.N., Bhat, M.A., Ahmad, M., Ganaie, S.A., Sheikh, F.A., Najeeb, S., Parry, F.A., 2018. Morphological variability and phylogenetic analysis in common bean (*Phaseolus vulgaris* L.). *Legume Research-An International Journal*, https://DOI:10.18805/LR-3421

Rivera, A., Roselló, S., Casañas, F., 2015. Seed curvature as a useful marker to transfer morphologic, agronomic, chemical and sensory traits from Ganxet common bean (*Phaseolus vulgaris* L.). *Scientia Horticulturae*, 197:476–482.

Romero-Arenas, O., Damian, H.M.A., Rivera, T.J.A., Baeza, S.A., Huerta, L.M., Cabrera, H.E., 2013. The Nutritional value of Beans (*Phaseolus vulgaris* L.) and its importance for

Saleem, Z.A., Ahmed, S., Hasan, M.M., 2016. *Phaselous vulgaris* LINN.: Botany, medical uses, phytochemistry and pharmacology. *World Journal of Pharmaceutical Research*, 5(11): 1611–1616.

Sax, K., 1923. The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. *Genetics*, 8: 552–560.

Scarano, D., Rubio, F., Ruiz, J.J., Rao, R., Corrado, G., 2014. Morphological and genetic diversity among and within common bean (*Phaseolus vulgaris* L.) landraces from the Campania region (Southern Italy). *Scientia Horticulturae*, 180: 72–78. http://dx.doi.org/10.1016/J.scientia.2014.10.013

Singh, V., Singh, A.K., Moharana, D.P., Singh, B., Jaiswal, D.K., Singh, D.K., 2017. Assessment of quantitative differences among diverse genotypes of French bean (*Phaseolus vulgaris* L.) for yield and yield attributing traits*. Journal of Pharmacognosy and Phytochemistry,* 8: 42. https://doi:10.3389/fpls.2017.00042

Stoilova, T., Pereira, G., Tavares de Sousa, M.M., Carnide, V., 2005. Diversity in common bean landraces (*Phaseolus vulgaris* L.) from Bulgaria and Portugal. *Journal of Central European Agriculture,* 6(4): 443–448.

Stoilova, T., Pereira, G., de Sousa, M., 2013. Morphological characterization of a small common bean (*Phaseolus vulgaris* L.) collection under different

environments. *Journal of Central European Agriculture*, 14(3): 854–864. https://DOI:10.5513/JCEA01/14.3.1277

Trindade, R.S., Araújo, A.P., Teixeira, M.G., 2010. Leaf area of common bean genotypes during early pod filling as related to plant adaptation to limited phosphorus supply. *Revista Brasileira de Ciência do Solo*, 34: 115–124.

Vidak, M., Carović-Stanko, K., Barešić, A., Grdiša, M., Šatović, Z., Liber, Z., 2017. Microsatellite markers in common bean (*Phaseolus vulgaris* L.). *Journal of Central European Agriculture*, 18(4): 902-917. https://DOI:10.5513/JCEA01/18.4.1983

Wang, H., Ding, Y., Hu, Z., Lin, C., Wang, S., Wang, B., Zhang, H., Zhou, G., 2012. Isolation and characterization of 13 new polymorphic microsatellite markers in the *Phaseolus vulgaris* L. (Common bean) Genome. *International Jjournal of Mmolecular Ssciences*, 13: 11189–11193. https://doi:10.3390/ijms130911188

Yeken, M.Z., Kantar, F., Çancı, H., Özer, G., Çiftçi, V., 2018. Breeding of dry bean cultivars using *Phaseolus vulgaris* landraces in Turkey. *International Journal of Agriculture and Wildlife Science*, 4(1): 45–54. https://doi:10.24180/ijaws.408794

Yeken, M.Z., Nadeem, M.A., Karakoy, T., Baloch, F.S., Çiftci, V., 2019. Determination of Turkish Ccommon Bbean Ggermplasm for Mmorpho-agronomic and mMineral vVariations for bBreeding pPerspectives in Turkey. *KSU Journal of Agriculture and Nature*, 22*:* 38–50. https://DOI:10.18016/ksutarimdoga.vi.549996

Yohannes, S., Loha., Gessese, M.K., 2020. Performance evaluation of common Bean (*Phaseolus vulgaris* L.) genotypes for yield and related traits at Areka, Southern Ethiopia. *Advances in Agriculture*, https://doi.org/10.1155/2020/1497530

Yu, K., Park, S.J., Poysa, V., Gepts, P., 2000. Integration of simple sequence repeat (SSR) markers into a molecular linkage map of common bean (*Phaseolus vulgaris* L.). *Journal of Heredity*, 91(6): 429–434.

Zeven, A.C., 1998. A review of definitions and classifications. *Euphytica,* 104:127–139.

Zhu, J., Wu, J., Wang, L., Blair, M.W., Wang, S., 2017. Novel alleles for black and gray sSeed color genes in common bBean. *Crop Science*, *57*(3): 1603–1610. https://doi:10.2135/cropsci2016.05.0356

Zilio, M., Souza, C.A., Coelho, C.M.M., Miquelluti, D.J., Michels, A.F., 2013. Cycle, canopy architecture and yield of common bean genotypes (*Phaseolus vulgaris*)

in Santa Catarina State. *Acta Scientiarum. Agronomy*, 35(1): 21–30. https://Doi.10.40.25/actasciagron.v35i1.15516