Genetic Study of Pod Shattering Resistance in Soybean (*Glycine max* (L.) Merrill) Plant Populations Derived from Exotic x Local Germplasm

By

Nevhudzholi Khuliso Marline

Student Number: 11629675

A dissertation submitted in fulfilment of the requirements for the degree of Master of Science in Agriculture (Plant Production) (MSc Agric)

Department of Plant Production

School of Agriculture

University of Venda

South Africa

Supervisor: Prof. E.T. Gwata

Co-supervisor: Prof. G.R.A. Mchau

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Abstract

Soybean (*Glycine max* (L.) Merrill) is an important legume crop which is cultivated in many countries in the world. It is used for both human consumption and animal feed. In addition, it can be processed into several industrial products such as lubricants and biodiesel. The soybean plant contributes to soil fertility through biological nitrogen fixation. However, the production of soybean is limited by several abiotic stresses including pod shattering which can cause up to 90.0% yield loss. The trait also limits mechanical harvesting as the impact of machines on the canopy results in seed loss. Tropical soybean types which are compatible with indigenous soil rhizobia ubiquitous in African soils, are generally susceptible to pod shattering thus limiting their adoption by growers. The tropical types are desirable in soybean production systems which are limited by inorganic nitrogenous (chemical) fertilizers. Therefore the objectives of this study were to (i) widen the genetic pool for resistance to pod shattering using the cross between resistant x susceptible soybean germplasm (ii) determine the mode of inheritance for resistance to pod shattering in F_2 soybean plant populations segregating for pod shattering and (iii) determine the broad sense heritability for resistance to pod shattering in the soybean germplasm. The new information regarding the genetics of the trait would be useful in designing efficient genetic improvement programs aimed at introgressing resistance to pod shattering into tropical germplsm. Two crosses between distinct parental lines of susceptible tropical (TGx-14) and non-tropical resistant types (LS-82 and PAN-637) were performed in order to generate F_2 progenies that were subsequently evaluated for pod shattering using the field screening and oven methods. Six quantitative traits that are associated with pod shattering including the number of branches (NB), pod clearance (PCL) and pod length (PL) were measured in the F_2 segregating populations. At maturity, the proportion of shattered pods and duration to first pod shattering (DFPS) per genotype were measured. The traits associated with pod shattering showed wide phenotypic variability. In the cross LS-82 x TGx-14, the mean PCL was <20% of the mean plant height. The plant populations from both crosses developed short pods (<5.0 cm) but the cross involving TGx-14 x PAN-637 showed a marked superiority over the cross LS-82 x TGx-14 in a number of the traits associated with pod shattering. There were marked differences between the NB and

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PL suggesting that the choice of the parental genotypes for hybridization was important in a genetic improvement program aimed at broadening the genetic base for these traits. Pod cracking at the pod-filling stage (before maturity) was observed in the F_2 progenies derived from the cross LS-82 x TGx-14. The segregation pattern of F_2 populations indicated a segregation ratio of 13 susceptible : 3 resistant in the cross involving LS-82 x TGx-14 suggesting that two major genes along with inhibitory epistasis were involved in controlling pod shattering in soybean. Both evaluation methods indicated that at least two gene pairs were involved in controlling pod shattering in the crosses that were used. Both methods were practical for screening for pod shattering in soybean. The broad sense heritability was high (0.82) in the progenies derived from LS-82 x TGx-14. Therefore, the study determined that few genes governed pod shattering in soybean.

Key words: hybridization; inheritance; pod cracking; pod shattering; soybean.

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Dedication

This dissertation is dedicated to my daughter Phodzo Wanga Nevhudzholi as an inspiration for her to dedicate her life to research and my mother Mashudu Jeniffer Kwinda whose presence in my life encourages me to be the best person I can be.

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Declaration

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

Student: Nevhudzholi Khuliso Marline

Signature: Nevhudzholi.KM Date: 2016/09/09

As supervisor, I agree to the submission of this dissertation:

Supervisor: Prof. E.T. Gwata

Signature: ..... Date: 04/04/2016

Co-Supervisor: Prof. G.R.A. Mchau

Signature: ..... Date: 04 April 2016

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List of Abbreviations

100-SW =	100 seed weight
DFPS =	duration to first pod shattering
F2 =	second filial generation
Hbs =	broad sense heritability
NPP =	total number of pods per plant
P1 =	first parental line
P2 =	second parental line
PCL =	pod clearance
PHT =	plant height
PW =	total pod weight per plant
TGx =	tropical *Glycine max*
Vg =	genetic variance
Vp =	phenotypic variance
Ve =	environmental variance

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1.0 CHAPTER ONE: GENERAL INTRODUCTION

1.1 Introduction

Soybean (*Glycine max* (L.) Merrill) is an important leguminous crop which is native to Asia (Kato *et al.*, 1998). The crop was first domesticated in the eleventh century in China (Hymowitz and Shurtleff, 2005). Soybean is a highly self-pollinating crop with an average of less than 1.0% cross-pollination (Cianzio, 2007) which may be a result of insects migrating from one plant to another. It was introduced to Africa probably by Asian traders who frequently visited the east coast of Africa (Giller and Dashiell, 2006).

The grain of soybean is high in protein (40.0%) and oil (20.0%) (Gwata and Nziramasanga, 2001). Consequently, it is used for both human consumption and animal feed. In addition, the grain can be processed into several industrial products such as lubricants and biodiesel (Table 1.1). Soybean is also useful in improving soil fertility through biological nitrogen fixation (Giller *et al.*, 2011; Sanginga, 2003).

Table 1.1 Some major uses of soybean.

Component of soybean	General use	Example	References
Grain	Food	(i) Human food	Greenberg and Hartung , 1998
		(ii) Animal feeds	Amaefule and Obiola, 2001
Oil	Industrial products	(i) Bioodiesel	Maluleke, 2008
		(ii) Ink	Erhan, 1999; Erhan *et al.*, 1992
		(iii) Lubricants	Ranles and Wright, 1992
		(iv) Food canning	Greenberg and Hartung , 1998
		(v) Food preparation	Greenberg and Hartung , 1998
Whole plant	Soil management	(i) Biological nitrogen fixation	Sanginga 2003
		(ii) Cover crop	Rufino et al., 2006

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The production of soybean is limited by a number of biotic and abiotic stresses. For instance leaf rust (*Phakopsora pachyrhizi*) was reported to cause up to 90% yield loss in east Africa (Tukamuhabwa *et al.*, 2000). The crop may also be attacked by insect pests at both the vegetative and reproductive stages (Randall, 1997). For example, cabbage loopers (*Trichoplusia spp.*) and stink-bugs (*Halyomorpha halys*) attack the crop at the vegetative and reproductive growth stages, respectively. Soybean is also sensitive to moisture stress particularly at the flowering stage. A study conducted in Iran, reported 44.0% grain yield loss as a result of moisture stress (Kobraei *et al.*, 2011). In dry and hot weather matured soybean pods may also shatter, resulting in considerable yield loss (Bhor *et al.*, 2014; Tukamuhabwa *et al.*, 2002; IITA, 1986).

Pod shattering may be defined as the opening of mature pods along ventral and dorsal sutures which results in scattering of seeds onto the ground (Tsuchiya, 1987). The trait has been observed in other crop species including cowpea (*Vigna unguiculata*) (Andargie *et al.*, 2011; Mohammed *et al.*, 2010), rape seed (*Brassica napus*) (Child *et al.*, 2003; Squires *et al.*, 2003; Morgan *et al.*, 2000), pigeonpea (*Cajanus cajan*) (Nahdy *et al.*, 1999; Venkataratnam and Sheldrake, 1985) and pea (*Pisum sativum*) (Weeden *et al.*, 2002).

In soybean, the shattering of pods limits mechanical harvesting as the impact of machines on the canopy results in seed loss. Delayed harvesting of this crop also results in shattering of pods (Zhang and Boahen, 2010) which subsequently leads to yield loss. The loss in grain yield due to pod shattering can be as high as 100%, particularly in tropical soybean types (IITA, 1986). The shattering (or splitting) of mature pods in soybean is influenced by several environmental factors (Bailey *et al.*, 1997) ranging from high temperatures to humidity (Agrawal *et al.*, 2002). High temperatures in particular, promote dehydration of the pod wall and splitting of the dehiscence zone (Bhor *et al.*, 2014) thus leading to the scattering of seeds onto the ground (Fig. 1.1).

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However pod shattering was selected against by plant breeders in many crops including soybean (Tukamuhabwa *et al.*, 2002) and oilseed rape (Kadkol *et al.*, 1984). Therefore the trait is generally absent from improved modern soybean cultivars (non-tropical types). However, in comparison, the non-tropical soybean types require inoculation with specific commercial inoculants at planting in order to achieve effective nodulation but the tropical types are compatible with indigenous soil rhizobia ubiquitous in African soils (Abaidoo *et al.*, 2000). In addition, the commercial inoculants are often scare particularly in smallholder soybean production systems in southern Africa (Mpepereki *et al.*, 2000).

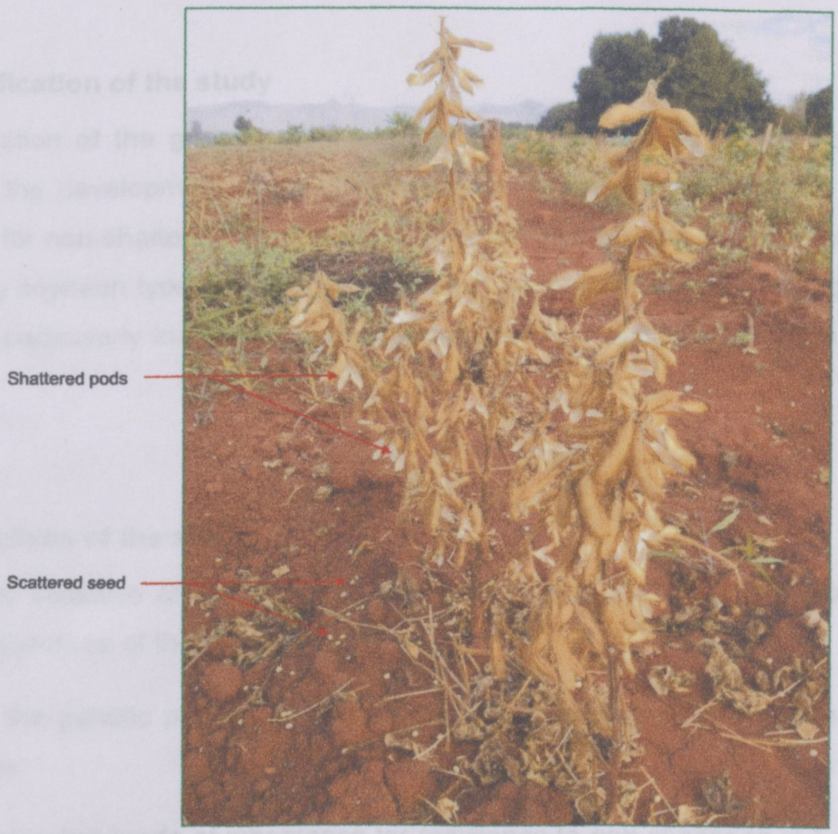

Fig.1.1 Pod shattering in susceptible soybean genotypes scatters the seed onto the ground in the field.

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1.2 Problem statement

Exotic (introduced) soybean genotypes in our breeding program (in the School of Agriculture at the University of Venda) are highly susceptible to pod shattering. These soybean types are desirable in soybean production systems which are limited by either commercial inoculants or inorganic nitrogenous (chemical) fertilizers. The genetic improvement of the exotic (tropical types of) soybean particularly in terms of resistance to pod shattering could benefit smallholder growers who utilize these soybean types. However, the manipulation of the pod shattering trait in soybean requires adequate information regarding its genetic control.

1.3 Justification of the study

Determination of the genetic parameters of pod shattering in our germplasm will enhance the development of an efficient soybean improvement program aimed at breeding for non-shattering tropical soybean types. The genetic improvement of non-shattering soybean types will reduce the loss in grain yields thus benefiting soybean growers, particularly in the resource-poor smallholder cropping systems in southern Africa.

1.4 Objectives of the study

The broad objective of this study was to evaluate pod shattering in soybean. The specific objectives of the study were to:

(i) widen the genetic pool for pod shattering using resistant x susceptible soybean germplasm

(ii) determine the mode of inheritance for resistance to pod shattering in F_2 soybean plant populations segregating for pod shattering

(iii) determine the broad sense heritability for resistance to pod shattering in the soybean germplasm.

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1.5 Hypotheses

The study tested the null (H_o:) hypotheses that:

(i) there was no variability in the progenies derived from resistant to pod shattering x susceptible soybean germplasm

(ii) the mode of inheritance for resistance to pod shattering in F_2 soybean plant populations segregating for the trait was not controlled by a few genes

(iii) the broad sense heritability for resistance to pod shattering was low (<0.5) in the soybean germplasm.

1.6 Dissertation outline

This dissertation is arranged into six chapters. The first chapter introduces the problem of pod shattering in soybean and outlines the objectives of the study as well as the hypotheses that were tested in the study. Chapter two concentrates on the literature review of relevant aspects of pod shattering in soybean. This chapter is followed by Chapter three covering the materials and methods that were used in the study. Chapter four and chapter five focus on the presentation and discussion of the results respectively. In chapter six, the dissertation draws some conclusions about the findings of the study. The chapter also makes some recommendations based on the findings of the study. The last part of the dissertation is devoted to the references that were cited throughout the document. Some appendices have been included in order to provide more detailed information where necessary without interfering with the flow of the dissertation. Because of the close relationship between the main body of the study and the other parts of the dissertation, there were inevitable overlaps in some of the concepts or references cited particularly in the short manuscript in the appendix.

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2.0 CHAPTER TWO: LITERATURE REVIEW

2.1 Soybean crop

Soybean [*Glycine max* (L.) Merrill] is a legume crop which originated from Manchuria in China (Hymowitz and Shurtleff 2005; Kato *et al.,* 1998). It is a diploid species with 2n = 40 chromosomes. Soybean is a highly self-pollinating crop with an average of less than 1.0% cross-pollination (Cianzio, 2007) which may be a result of insects migrating from one plant to another.

2.2 Major uses of soybean

The grain of soybean is an important source of protein for human consumption and poultry feed. The crop is also used for forage purposes in many countries (Saberi *et al.*, 2014; Blount *et al.*, 2009; Tefera *et al.*, 2009; Bilgili *et al.*, 2005; Rao *et al.*, 2005). The oil from soybean is an important raw material for various industrial products (Hymowitz and Newell, 1981) such as paints, ink and crayons. The oil is also used as a raw material in manufacturing antibiotics, varnishes, adhesives and lubricants (Erhan, 1999; Erhan *et al.*, 1992; Randles and Wright, 1992).

The crop contributes to soil fertility through biological nitrogen fixation (Mpepereki *et al.*, 2003; Sanginga, 2003; Giller *et al.,* 1997; Giller and Cadisch, 1995; Daramola *et al.*, 1993) and is useful in crop rotations in many cropping systems worldwide (Ferreira *et al.*, 2000; Edwards *et al.*, 1988). The crop can contribute up to 200.0 kg N/ha (Woomer, 2010). In a study that examined the impact of residual N from soybean on the yield of a subsequent corn crop, Kasasa *et al.*, (1999) reported that the yield of corn increased by >10.0% in some areas. This indicated that soybean contributed significant quantities of relatively cheap organic nitrogenous fertilizer, thus reducing the cost of soybean production.

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2.3 Nutritional value of soybean

Soybean is a highly nutritious crop which contains proteins, vitamins and minerals, unsaturated lipids as well as carbohydrates. In addition, the grain of soybean contains high protein content (40.0%) and high-quality oil (20.0%) (Hymowitz, 2004; Gwata and Nziramasanga, 2001). However, there is variation in the amount of oil reported by some researchers (Shivananda *et al.,* 2000) most probably due to differences in variety, location and season (O'Dell, 1979). Soybean protein contains lysine and hence is a good supplement to cereals as a substitute of animal protein (Lokuruka, 2010; Rockland, 1981). The high protein content and biological value of soybean increase its economic importance over other oil crops. The soybean grain provides a cheap source of protein particularly for vulnerable resource poor communities in many parts of Africa.

The oil from soybean is free of cholesterol with unsaturated fatty acids constituting over 85.0% of total fatty acids (Lokuruka, 2010). Therefore, it is highly desirable in human diets (Malik *et al.,* 2006). Soybean oil production is ranked highest in the international trade among major oil seed crops and constitutes 48.0% of world oil production (Singh and Hymowitz, 1999). Soybean is rich in isoflavones which are known to reduce cancer and other diseases in humans (Messina *et al.*, 1994).

2.4 Nodulation types of soybean

Soybean is classified into two types according to the nodulation behaviour. The non-tropical (or non-promiscuous) soybean type requires a specific strain of rhizobium to form active nodules while the tropical (or promiscuous) type is compatible with a wide range of indigenous African rhizobial strains (Gwata *et al.*, 2003; Pulver *et al.*, 1985) that are ubiquitous in African soils (Abaidoo *et al.*, 1999). The promiscuous types form effective nodules with cowpea-type rhizobia (Pulver *et al.*, 1985). However, the non-promiscuous types form no nodules or ineffective nodules with cowpea type rhizobia. Effective nodules are able to fix atmospheric nitrogen and hence increase soil fertility. The tropical (promiscuous) soybean types are desirable in small-holder cropping systems particularly where production inputs such as

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chemical nitrogenous fertilizers may be unaffordable. However, some tropical soybean types are highly susceptible to pod shattering (Tefera *et al.*, 2009) which results in yield loss (Tukamuhabwa *et al.*, 2002). Therefore, the genetic improvement of the resistance to pod shattering in these tropical soybean types could increase their productivity.

2.5 Soybean production

Soybean is produced world-wide. The cultivation was initiated probably in northern China where the crop was domesticated around 2500 BC (Ngata, 1959). Currently, soybean is produced world-wide.

2.5.1 Global soybean production

The total production of soybean world-wide was about 258.4 million metric tons (FAOSTAT 2012). The bulk of soybean was produced in the United States of America (USA) and Brazil (Figure 2.1). In Africa, Nigeria and South Africa were the leading producers (Table 2.1). On average the grain yields in Africa were lower than those obtained in the Americas (Figure 2.2).

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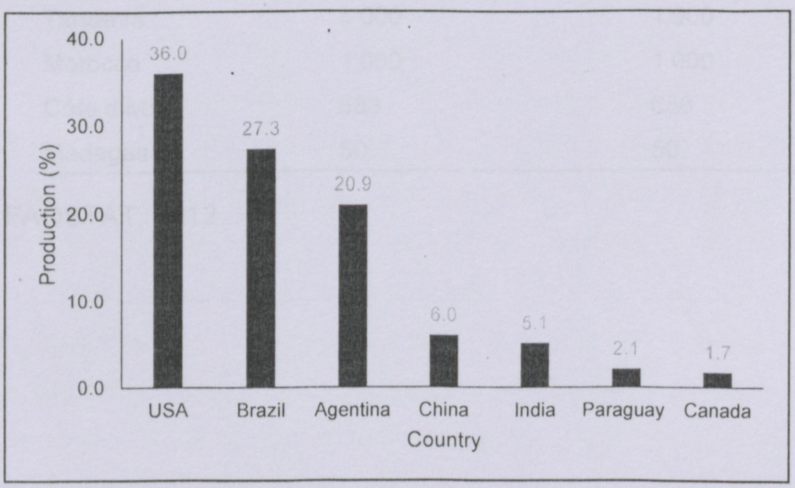

Fig. 2.1 Global production of soybean in 2010 (FAOSTAT, 2012).

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Table 2.1 Three-year (2006-2008) average area and production of soybean in African countries.

Country	Cultivated Area (ha)	Grain Production (Mt)
Nigeria	625 667	592 000
South Africa	199 323	317 332
Uganda	146 667	176 333
Zimbabwe	60 679	96 008
Malawi	71 333	50 000
Rwanda	42 788	27 046
Egypt	7 981	25 932
DR Congo	33 492	16 177
Zambia	10 000	1 200
Benin	18 820	10 771
Cameroon	12 000	7 000
Ethiopia	6 826	6 685
Burkina Faso	4 177	5 853
Mali	3 274	4 131
Liberia	7 867	3 183
Burundi	3 700	3 000
Gabon	2 100	2 200
Kenya	2 504	2 092
Tanzania	5 000	1 900
Morocco	1 000	1 000
Côte d'Ivoire	683	686
Madagascar	50	50

Source: FAOSTAT, 2012.

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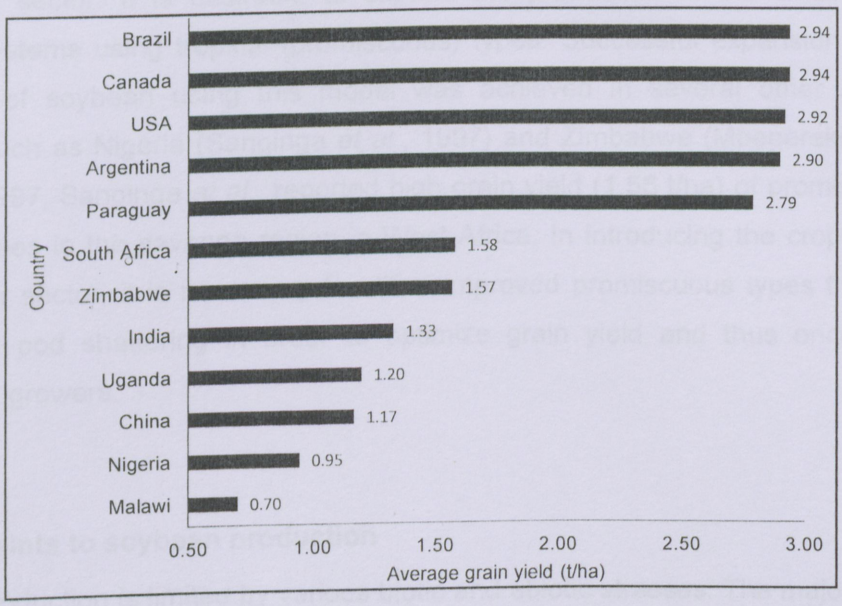

Fig. 2.2 Average grain yield (t/ha) in some soybean producing countries. (FAOSTAT, 2012).

2.5.2 Soybean cultivation in South Africa

For optimum production in South Africa, soybean is planted at the beginning of the summer season in early to mid-November. The seed is planted at about 3.0 cm deep depending on the soil type (Department of Agriculture, Forestry and Fisheries, 2010). The seed is planted in rows spaced at about 60.0 cm (Nieuwenhuis and Nieuwelink, 2005). Within the row, the seed is planted at 5.0 – 10.0 cm. The crop is cultivated generally under rain-fed cropping systems but is sensitive to moisture stress during the reproductive stage particularly at flowering. Temperatures above 30.0 °C affect yield adversely while temperatures below 13.0 °C during flowering inhibit flowering and seed formation. The optimal temperature for all growth stages is about 25.0 °C. Soil temperatures of about 15.0 °C stimulate germination. The crop requires 500.0 to 900.0 mm of rainfall during the growing season for optimal yield (Nieuwenhuis and Nieuwelink, 2005).

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The production of soybean in South Africa is concentrated in the large scale commercial sector. It is desirable to expand the production to the small-holder cropping systems using tropical (promiscuous) types. Successful expansion of the production of soybean using this model was achieved in several other African countries such as Nigeria (Sanginga *et al.*, 1997) and Zimbabwe (Mpepereki *et al.*, 2000). In 1997, Sanginga *et al.*, reported high grain yield (1.56 t/ha) of promiscuous soybean types in the savanna region in West Africa. In introducing the crop to the small-holder sector, it is necessary to utilize improved promiscuous types that are resistant to pod shattering in order to optimize grain yield and thus encourage adoption by growers.

2.6 Constraints to soybean production

Soybean production is limited by various biotic and abiotic stresses. The major biotic constraints include diseases such as leaf rust (*Phakopsora pachyrhizi*) (Bromfield, 1984) and insect pests (Vennila *et al.*, 2007). In Uganda the yield loss in soybean due to leaf rust was 26.9 - 36.3% in commercial varieties (Kabuki *et al.*, 2002). However some soybean cultivars which are tolerant to the diseases were reported (Wolfe, 1985).

Soybean production is also limited by semi-loopers (*Animis flava fab*) (Vennila *et al.*, 2007). Young larvae feed on leaf lamina leaving holes on the leaf while adult larvae leave only the midrib and veins. Spider mites also reduce soybean yield by as much as 60.0% and can accelerate soybean senescence as well as pod shattering (Cullen and Schramm, 2009).

Drought stress, particularly at the flowering stage, leads to flower abortion or shrivelled seeds. In Iran for instance, drought stress caused a yield loss of approximately 44.0% in soybean (Kobraei *et al.*, 2011). Drought also induces shattering of pods in soybean. Moisture stress during seed development may cause pods to weaken and hence open easily. Pod shattering may be caused by different

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environmental conditions such as low humidity, high temperatures, rapid temperature change as well as drying and wetting (Tsuchiya, 1987). In addition, the green stem syndrome (in which stems and leaves remain green after pod maturity) delays harvesting (Egli and Brueni, 2006) particularly mechanical harvesting. The cause of this syndrome is not understood fully. Apparently, the abortion of flowers or seed during drought stress results in the redistribution of sugars and nutrients back in the stems and leaves leading to delayed drying out of stems and leaves. According to Egli and Brueni (2006), removal of 25.0 to 50.0% of pods delayed drying of stems and leaves by at least 4 - 30 d which may result in pod shattering while farmers wait for stems to dry before using a combined harvester to harvest the crop.

2.7 Pod shattering in soybean

Pod shattering in soybean reduces grain yield and quality. According to Tukamuhabwa *et al.*, (2002), delayed harvesting caused approximately 0.2 t/ha yield loss in a soybean field in Uganda. Pod shattering in combination with late harvesting in susceptible varieties may cause up to 100% loss in grain yield (IITA, 1986). In 1987, Tsuchiya postulated that the pod shattering trait was inherited from wild soybean (*Glycine soja)* which scatters its seed as soon as the pods mature. The trait is influenced by several environmental factors (Bailey *et al.*, 1997; Tukamuhabwa *et al.*, 2000) and is aggravated by rapid fluctuations in temperature and humidity (Agrawal *et al.*, 2002). High temperatures in particular, promote dehydration of the pod wall and splitting of the dehiscence zone (Bhor *et al.*, 2014).

2.7.1 Mechanism of pod shattering

The pod shatters at maturity when it opens either dorsally or ventrally, or on both dorsal and ventral sutures, scattering seeds especially under dry conditions (Tsuchiya, 1987). Pod shattering results from a series of highly coordinated sequence of biochemical events in the pod tissue (Agrawal *et al.,* 2002). Phenylalanine and heat shock proteins play an important role in determining the degree of susceptibility or resistance of soybean cultivars to pod shattering.

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According to Manglik *et al.,* (1998), the application of trans-cinnamic acid and cobalt chloride ($CoCl_2$) reduced phenylalanine activities which resulted in reduced pod shattering susceptibility. Probably, the selection of genotypes that are low in phenylalanine can produce cultivars with improved resistance to pod shattering. Plant growth regulators also reduced the degree of pod shattering in soybean (Gulluogu *et al.,* 2006). Cell wall degrading enzymes such as hydrolytic [cellulase (β-1, 4-glucanase) and poly-galactouranase)] and oxidoreductase (peroxidase and polyphenol oxidase) influenced pod shattering resistance (Agrawal *et al.,* 2002). These enzymes move from non-shattering zones to shattering zones in susceptible cultivars and *vice-versa* in resistant cultivars when pods have reached harvest maturity. Therefore, the genes that synthesize these proteins (enzymes) are turned on at specific growth stages, hence they can possibly be modified through biotechnological techniques to remain switched off in order to improve resistance to pod shattering in soybean.

2.7.2 Evaluation methods

The dehiscence of pods in soybean was studied using a variety of methods in both the open field and laboratory. The field screening method for assessing pod shattering in soybean was used widely in previous studies (Romkaew and Umezaki, 2006; Helms, 1994; Tiwari and Bhatnagar, 1993; Caviness, 1965). In this method, harvesting of mature soybean plants from the field was delayed in order to allow pod shattering under natural conditions in the field. One of the main assumptions in the method was that delayed harvesting would enable susceptible genotypes to shatter.

In other similar studies that were aimed at evaluating resistance to pod shattering in soybean, the oven method was applied (Bhor *et al.*, 2014; Romkaew and Umezaki, 2006; Tukamuhabwa *et al.*, 2002; Tiwari and Bhatnagar, 1993; Tsuchiya and Sunada, 1977). In this approach, mature soybean pods were harvested and induced to shatter by oven-drying at various temperatures including 44.0 °C (Bhor *et al.*, 2014), 60.0 °C (Romkaew and Umezaki, 2006) and 80.0 °C (Tukamuhabwa *et al.*, 2002). Other studies involved keeping mature pods in a desiccator for about 35 d

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(Romkaew and Umezaki, 2006; Caviness, 1965; Metcalfe *et al.*, 1957). In other crops, particularly oilseed rape, the mechanical cracking method was used widely to assess the degree of pod shattering (Squires *et al.*, 2003; Morgan *et al.*, 2000; Davis and Bruce, 1997; Kwon *et al.*, 1991). All these approaches appeared to be adequate for distinguishing between genotypes that were resistant or susceptible to pod shattering. However, each method presented some drawbacks such that more than one approach was used in a single study in order to affirm the findings.

2.7.3 Genetic control of pod shattering

Conflicting results regarding the genetic control of resistance to pod shattering in soybean were reported in only a limited number of studies in literature. In a study that focused on introgressing the trait using selective crosses followed by generation advancement to F_3; it was concluded that the trait was controlled by classical dominant epistasis (Tukamuhabwa *et al.*, 2000). Tsuchiya and Sunada (1980) observed that susceptibility to pod shattering was partially dominant but Tiwari and Bhatnagar (1992) observed contradicting results in F_1 soybean populations with some crosses showing dominance but others showing partial dominance for non-shattering. Bhor *et al.*, (2014) observed that pod shattering was controlled by inhibitory epistasis as indicated by a 13 (susceptible) : 3 (resistant) Mendelian ratio which was reported in the study.

In comparison with other legumes, a similar 13:3 (susceptible : resistant) segregation ratio was observed in cowpea (Mohammed *et al.*, 2010). Similarly, in rapeseed, the F_2 population derived from interspecific hybridization between *Sinapsis alba* x *Brassica napus* also showed a 13:3 segregation ratio for pod shattering (Wang *et al.*, 2007). In general, most commercial cultivars (non-promiscuous) that are cultivated in many parts of the world are resistant to pod shattering since soybean breeders deliberately selected for improved resistance to pod shattering.

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2.7.4 Heritability of resistance to pod shattering and maternal effect

By definition, heritability is the proportion of variation in a phenotypic trait in a population that can be attributed to the genetic variation among the individuals which constitute the population. There are two forms of heritability namely the broad and narrow sense heritability. Most studies in soybean determined the broad sense heritability partly because it is less demanding in terms of developing the various prerequisite filial generations for determining the narrow sense heritability. For instance, a moderately high (0.59) broad sense heritability was reported for resistance to pod shattering in soybean (Agdew *et al.*, 2012). However, other studies reported high (≥0.9) broad sense heritability (Bailey *et al.*, 1997; Tiwari and Bhatnagar, 1991; Tsuchiya, 1987) suggesting that the trait is highly heritable. These studies demonstrated that the heritability of resistance to pod shattering was variable depending on the set of soybean germplasm studied.

Maternal effect is defined as a phenomenon where progeny phenotypes are altered as a function of the environment created by or experienced by the maternal parent (Gilsinger, 2010). In a study focusing on pod shattering, Tukamuhabwa *et al.* (2002) observed no maternal effect in soybean. In the study, the authors reported that the F_1 plants varied from two to twelve but produced enough F_2 seed for replicated experiments. The authors attributed the low number of F_1 plants to the unpredictability of cleistogamy behaviour in the parental lines. In general, the species is highly sensitive to emasculation during artificial crossing and aborts the emasculated flowers easily thus leading to a low number of successful crosses.

2.8 Summary

The literature review showed that:

(i) soybean is a useful grain crop which is used for human consumption and manufacturing of industrial products

(ii) in general, some tropical (promiscuous) soybean types are susceptible to pod shattering, thus reducing the grain yield

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(iii) pod shattering in soybean is influenced by several environmental factors

(iv) pod shattering in soybean is controlled by a few genes and is highly heritable

(v) a variety of methods was used for evaluating pod shattering in soybean and other field crops

(vi) the mechanism of pod shattering in soybean also involves a series of biochemical events in which the distribution of cell wall degrading enzymes as well as hydrolytic enzymes contributes to the shattering of pods.

Therefore, there is merit in investigating the genetic control and heritability of pod shattering in soybean using tropical (promiscuous) germplasm. The knowledge obtained from such a study will be useful in designing an efficient soybean improvement program particularly aimed at introgressing shattering resistance from non-promiscuous germplasm into promiscuous types.

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3.0 CHAPTER THREE: MATERIALS AND METHODS

3.1 Testing location

The study was conducted at the University of Venda research farm at Thohoyandou (22.9° S; 30.48° E; 600.0 m.a.s.l.) in the screen-house, field and laboratory. Daily maximum temperatures at the location during summer often exceed 33.0 °C and the annual rainfall (about 500.0 mm) is highly seasonal with 95.0% occurring between October and March. The soils at the location are deep (>150.0 cm), red and well drained clays with an apedal structure and about 60.0%.

3.2 Genetic materials

The study used three soybean genotypes that have contrasting behaviour with regards to pod shattering resistance. Genotypes LS-82 and PAN-637 were previously classified as resistant in the field while genotype TGx-14 showed high susceptibility to pod shattering (Nevhudzholi *et al.*, 2013). Genotype LS-82 originated from the United States of America (U.S.A.) but TGx-14 originated from Nigeria (Tefera *et al.*, 2009). The TGx- (Tropical *Glycine max*) series of soybean genotypes is generally susceptible to pod shattering. The third genotype (PAN-637), was a local old commercial cultivar had no history of pod shattering at the test location.

3.3 Hybridization and genetic advancement

Five seeds of each cultivar were planted in pots (Fig. 3.1) in a screen-house during November 2013. Each pot was filled with field top-soil. The planting was staggered over a seven-week period in order to ensure synchronisation of flowering between the parental combinations. At flowering, two crosses were performed manually between LS-82 x TGx-14 and the reciprocal as well as between TGx-14 x PAN-637. The successful crosses were confirmed using both physiological and morphological markers (see section 4.1).

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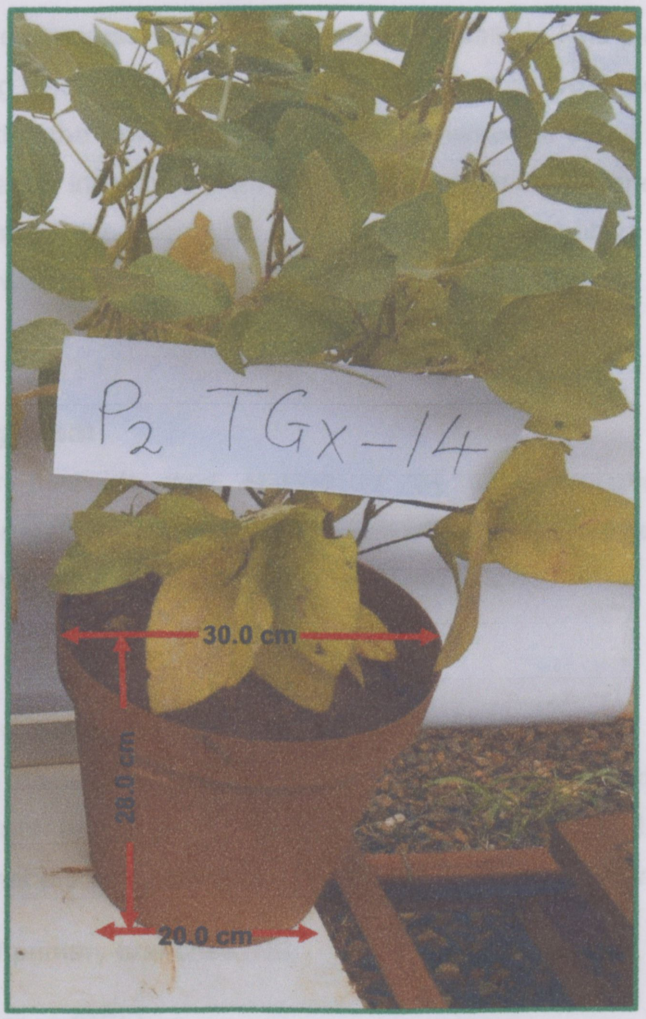

Fig. 3.1 Pots in the screen-house were used for raising soybean plants.

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The seed from the successful F_1 crosses was harvested manually at maturity and planted in the screen-house in pots (as described above) during the 2014 off-season (July 2014) in order to raise F_2 seed. There was adequate seed for raising F_2 plants for both crosses. The F_2 seed from each cross was partitioned into two portions of approximately 70 seeds each in order to conduct a replicated field experiment for evaluating pod shattering in the field (Tukamuhabwa *et al.*, 2002) as well as obtain samples for the oven method.

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3.4 Planting

The F_2 seed of each cross was space planted manually in field plots under rain fed conditions during December 2014. Each plot consisted of two 8.0 m rows spaced at 1.0 m apart. Within the row, the seed was space planted at about 12.0 – 15.0 cm in order to allow each individual plant to express its optimum genetic potential. For each cross, a randomised complete block design (Tukamuhabwa *et al.*, 2002) with two replications was used.

3.5 Crop management

No fertiliser or pesticide was applied to the crop throughout the season. Supplementary irrigation was applied as necessary. Weeds were controlled manually and standard soybean management practices were followed.

3.6 Agronomic measurements

At the pod filling stage, each genotype was tagged for identification. At physiological maturity, the plant height (PHT) of each plant was measured. In addition the following traits that are associated with pod shattering were measured:

(i) the number of primary branches (NB)

(ii) the pod clearance (PCL)

(iii) the duration to 75% maturity (75%DM)

(iv) the pod length (PL) and

(v) the pod weight (PW)

The pod clearance was measured from the base of the plant to the height of the lowest pod on the stem. In addition to these traits, the duration to first pod shattering per plant was recorded. The plants were left in the field for 65 d after maturity in order to allow adequate time for pod shattering. Prior to harvesting, the total number of pods per plant and the number of shattered pods per plant were recorded in order to determine the percent of shattered pods per plant.

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3.7 The oven method

At physiological maturity, batches of at least five pods per genotype were harvested and stored in brown paper bags (5.0 cm x 8.0 cm x 18.0 cm) that were kept at room temperature for 10 d in order to equilibrate the pod moisture as described previously (Romkaew and Umezaki, 2006). The pods (in batches of five) were oven dried at 80.0 °C for 12 h (Tukamuhabwa *et al.*, 2002) after which they were scored for pod shattering using a scale of 0 – 5 (where 0 = no shattering and 5 = all pods shattered). In the scale, the 0 – 2 range was classified as resistant to pod shattering while the 3 – 5 range was classified as susceptible.

3.8 Data analysis

The mode of inheritance for pod shattering was determined by Chi-square (X^2) tests (Jackson *et al.*, 2005; Sisson, 1976) for the goodness-of-fit of observed F_2 segregation data to the expected ratios. The monogenic (3:1) and digenic (9:7; 13:3 and 15:1) models were tested using the equation:

$$X^2 = \sum \frac{(\text{observed - expected})^2}{\text{expected}}$$

The broad sense heritability (H_{bs}) was determined using the following equations (Falconer and Mackay, 1996; Mayo, 1987):

$$H_{bs} = V_g/V_p \quad \text{(i)}$$

$$V_p = V_g + V_e \quad \text{(ii)}$$

where:

V_g = genetic variance

V_p = phenotypic variance

V_e = environmental variance

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In this approach, since the two parental lines were true breeding, they were assumed to be homozygous and their corresponding variances were considered to be the result of environmental influences.

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4.0 CHAPTER FOUR: RESULTS

This chapter presents the findings of the study covering all the three specific objectives. The first section of the chapter presents the evidence of variation in the newly developed germplasm using the non-shattering x shattering soybean genotypes. In the second part of the chapter, the results of the mode of inheritance are presented while the last part of the chapter covers the findings on the heritability of the resistance to pod shattering trait.

4.1 Hybridization of parental genotypes

The F_1 seed was harvested and planted in pots (filled with top soil from the field) in the screen-house in order to confirm the successful crosses as well as raise the F_2 seed. The cross between LS-82 x TGx-14 (designated cross #1) was performed successfully and confirmed by morphological and physiological markers (Table 4.1). The F_1 plants from this cross consisted of purple flowers which indicated that the cross was successful since purple flower colour is dominant over white (Nevhudzholi *et al.*, 2015; Gwata *et.,* 2005; Hartwig and Hinson, 1962). In the reciprocal cross (TGx-14 x LS-82), the medium (or intermediate) duration to maturity (Nevhudzholi *et al.*, 2015) (Fig 4.1), as well as the black hylum (Specht and Williams, 1978) of the F_1 plants were used for confirming the cross. Similarly, the successful cross between TGx-14 x PAN-637 (designated cross #2) was confirmed by the medium duration to maturity (Table 4.1). However, the reciprocal cross succumbed to unfavourable conditions (particularly high temperatures during the time of performing the crosses) resulting in pod abortion. Similar difficulties in obtaining enough F_1 hybrid seed in soybean was also reported (Tukamuhabwa *et al.*, 2000).

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Table 4.1 Physiological and morphological markers used for confirming the successful crosses.

Parental line	Morphological or physiological marker	Reference
LS-82	(i) black hylum	Specht and Williams, 1978
	(ii) early maturity	Nevhudzholi *et al* ., 2015
	(iii) white flowers	Gwata *et al* ., 2005; Hartwig and Hinson, 1962
	(iv) low branching	Nelson, 1996
TGx-14	(i) brown hylum	Specht and Williams, 1978
	(ii) late maturity	Nevhudzholi *et al* ., 2015
	(iii) purple flowers	Gwata *et al* ., 2005; Hartwig and Hinson, 1962
	(iv) high branching	Nelson, 1996
PAN-637	(i) early maturity	Nevhudzholi *et al* ., 2015
	(ii) white flowers	Gwata *et al* ., 2005; Hartwig and Hinson, 1962
Cross		
LS-82 x TGx-14	(i) purple flowers	Gwata *et al* ., 2005; Hartwig and Hinson, 1962
	(ii) intermediate branching	Nelson, 1996
	(iii) medium maturity	Nevhudzholi *et al* ., 2015
TGx-14 x LS-82	(i) medium maturity	Nevhudzholi *et al* ., 2015
	(ii) black hylum	Specht and Williams, 1978
TGx-14 x PAN-637	(i) medium maturity	Nevhudzholi *et al* ., 2015

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Fig. 4.1 Medium duration to maturity in a successful F_1 plant (middle) derived from a cross between LS-82 (***left***: early maturity) x TGx-14 (***right***: late maturity).

4.2 Field evaluation of pod shattering traits in F_2 populations

The germination of the F_2 seed in the field was generally poor. Seven quantitative traits that are associated with pod shattering in the F_2 segregating plant populations derived from LS-82 x TGx-14 and the reciprocal cross showed normal distribution curves that are expected of quantitative traits (Poelhman and Sleper, 1995; Brown and Caligari, 2008). For instance the frequency distribution curves for pod weight (Fig. 4.2) and pod length per plant (Fig. 4.3) were normal with no obvious discontinuities.

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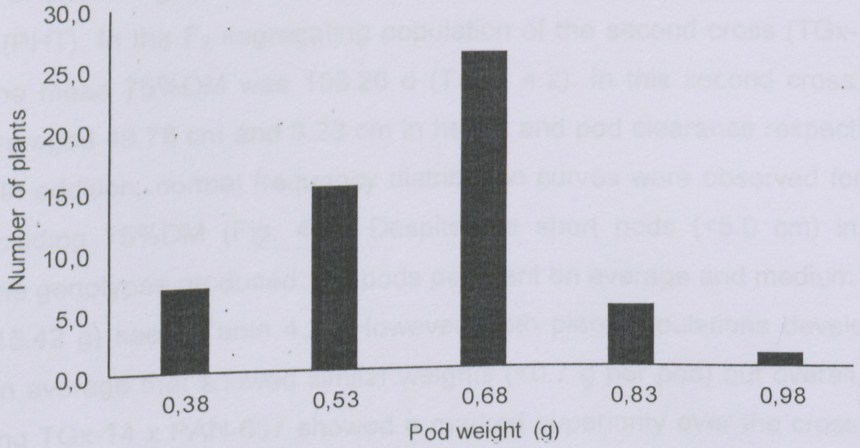

Fig. 4.2 Frequency distribution of pod weight in the F_2 soybean populations derived from LS-82 x TGX-14.

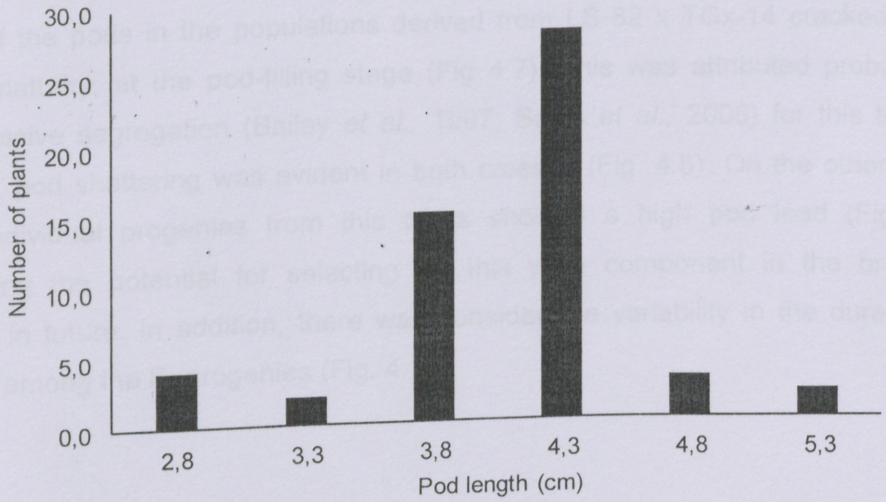

Fig. 4.3 Frequency distribution of pod length in the F_2 soybean populations derived from LS-82 x TGX-14.

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In the cross LS-82 x TGx-14, the 75%DM was about 100 d (Table 4.2). On average, the pods in the segregating populations of this cross required at least 4 d 75%DM to the initiation of shattering. The mean pod clearance (PCL) was <20% of the mean plant height (PHT). In the F_2 segregating population of the second cross (TGx-14 x PAN-637), the mean 75%DM was 105.26 d (Table 4.2). In this second cross, the progenies averaged 49.78 cm and 8.28 cm in height and pod clearance respectively (Table 4.2). In addition, normal frequency distribution curves were observed for the attributes including 75%DM (Fig. 4.5). Despite the short pods (<5.0 cm) in the population, the genotypes produced 105 pods per plant on average and medium size (100-SW = 15.42 g) seed (Table 4.2). However, both plant populations developed short pods on average that showed similar weights (<0.7 g per pod) but overall, the cross involving TGx-14 x PAN-637 showed a marked superiority over the cross LS-82 x TGx-14 in a number of the traits associated with pod shattering (Fig. 4.6). Marked differences between the two soybean crosses were observed for PHT and NB as well as PL (Fig. 4.6) suggesting that the choice of the parental genotypes for hybridization was important in a genetic improvement program aimed at broadening the genetic base for these traits in the species.

Some of the pods in the populations derived from LS-82 x TGx-14 cracked (split) before maturity, at the pod-filling stage (Fig 4.7). This was attributed probably to transgressive segregation (Bailey *et al.*, 1997; Salas *et al.*, 2006) for this trait. At maturity, pod shattering was evident in both crosses (Fig. 4.8). On the other hand, some individual progenies from this cross showed a high pod load (Fig. 4.9) suggesting the potential for selecting for this yield component in the breeding material in future. In addition, there was considerable variability in the duration to maturity among the F_2 progenies (Fig. 4.10).

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Table 4.2 Four statistical parameters for six characters that are associated with pod shattering in F_2 soybean plant populations derived from two crosses.

Parameter	PHT	NB	PCL	75%DM	PL	PW
Cross: LS-82 x TGx-14						
Mean	42.21	5.74	7.90	100.55	4.26	0.67
Variance	246.57	6.17	15.57	60.15	0.23	0.02
S.D.	15.70	2.48	3.95	7.76	0.48	0.14
C.V.	37.20	43.24	49.98	7.71	11.27	21.64
Cross: TGx-14 x PAN-637						
Mean	49.93	6.63	8.20	105.26	4.13	0.62
Variance	262.80	4.69	9.34	32.35	0.27	0.02
S.D.	16.21	2.17	3.06	5.69	0.52	0.14
C.V.	32.47	32.67	37.24	5.40	12.57	22.48

PHT = plant height (cm); NB = number of primary branches per plant; PCL = pod clearance (cm); 75%DM = duration to 75% maturity (d); PL = pod length (cm); PW = pod weight (g).

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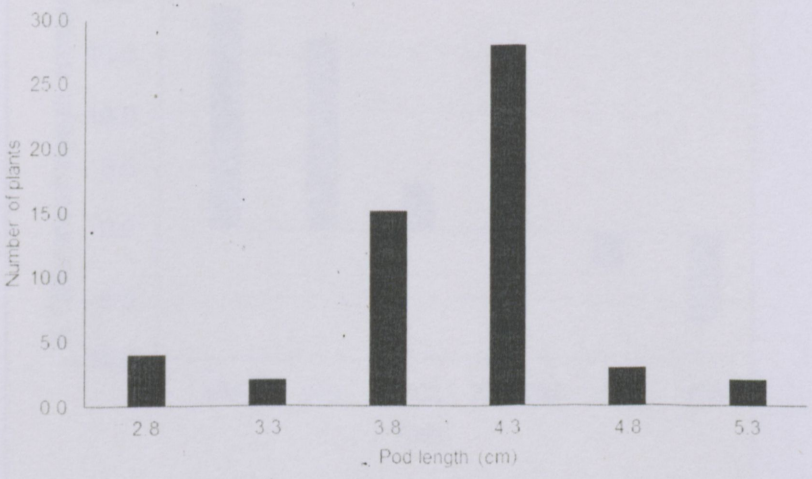

Fig. 4.4 Frequency distribution of pod length in the F_2 soybean populations derived from TGX-14 x PAN-637.

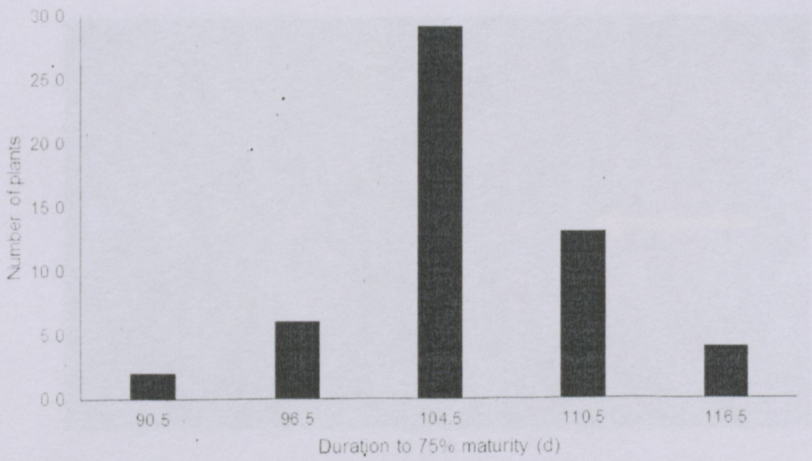

Fig. 4.5 Frequency distribution of duration to maturity in the F_2 soybean populations derived from TGX-14 x PAN-637.

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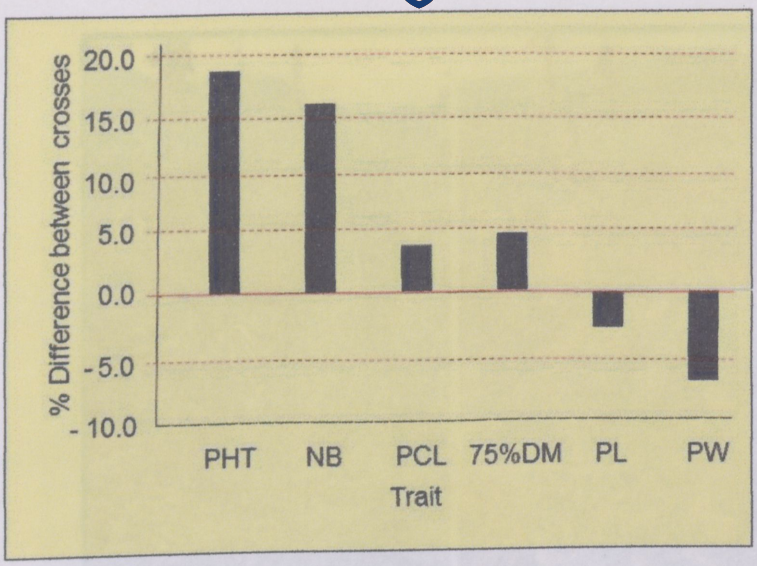

Fig. 4.6 Magnitude of differences between two distinct crosses in six traits that are associated with pod shattering in soybean. [PHT = plant height (cm); NB = number of primary branches per plant; PCL = pod clearance (cm); 75%DM = duration to 75% maturity (d); PL = pod length (cm); PW = pod weight (g)].



Fig. 4.7 Pod splitting at the pod-filling in some F_2 soybean genotypes derived from LS-82 x TGX-14.

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Fig. 4.8 Pod shattering in a mature genotype derived from the cross (a) TGx-14 x LS-82 and (b) TGx-14 x PAN-637.

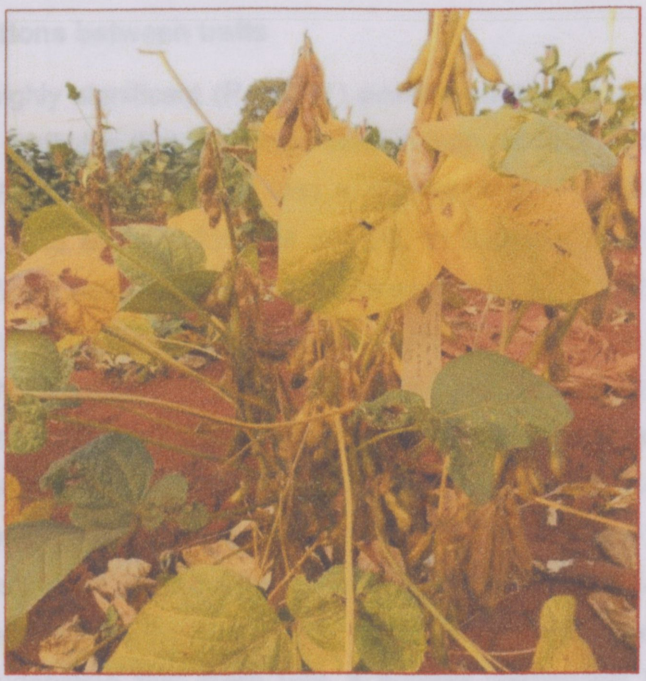

Fig. 4.9 High pod load per plant in an individual genotype from an F_2 plant population derived from LS-82 x TGx-14.

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Fig. 4.10 Variability in the duration to maturity within an F_2 plant population derived from LS-82 x TGx-14.

4.2.1 Correlations between traits

There were highly significant ($P < 0.01$) positive correlations between PHT and all the other five attributes that were measured in both crosses (Table 4.3). In the cross involving LS-82 x TGx-14, there was a significant ($P < 0.05$) positive correlation between PCL and each of 75%DM and PL. In addition, the number of branches showed significant positive correlations with each of PCL, 75%DM, PL and PW in the cross involving LS-82 x TGx-14. In the second cross (TGx-14 x PAN-637), PL showed a highly significant ($P < 0.01$) correlation with each of the other five attributes (Table 4.3). It was interesting to note that there was a highly significant ($P <0.01$) correlation between PL and PW in each set of the F_2 plant populations from the two different parental combinations (Table 4.3). The coefficient of determination (R^2) which was estimated in the linear relationship between PL and PW for the F_2 population derived from the cross LS-82 x TGx-14 was considerably high (>70.0 %) (Fig. 4.11) suggesting that there was a notable association between the genes controlling pod length and pod weight.

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Table 4.3 Coefficient of correlation (*r*) among attributes that are associated with poding in F_2 soybean plant populations derived from two crosses.

	PHT	NB	PCL	75%DM	PL	PW
Cross: LS-82 x TGx-14						
PHT	1.0000					
NB	0.6403**	1.0000				
PCL	0.6481**	0.3000*	1.0000			
75%DM	0.5568**	0.2828*	0.3742**	1.0000		
PL	0.469**	0.3873**	0.4000**	0.2646	1.0000	
PW	0.4472**	0.3464**	0.2236	0.2646	0.8544**	1.0000
Cross: TGx-14 x PAN-637						
PHT	1.0000					
NB	0.781**	1.0000				
PCL	0.469**	0.2449	1.0000			
75% DM	0.4582**	0.3000	0.4000*	1.0000		
PL	0.500**	0.3605**	0.3873*	0.4582**	1.0000	
PW	0.4582**	0.3000	0.3742*	0.4472**	0.8484**	1.0000

** = Highly significant at the 1.0% probability level

* = Significant at the 5.0% probability level

PHT = plant height; NB = number of primary branches per plant; PCL = pod clearance (cm); 75%DM = duration to 75% maturity (d); PL = pod length (cm); PW = pod weight (g).

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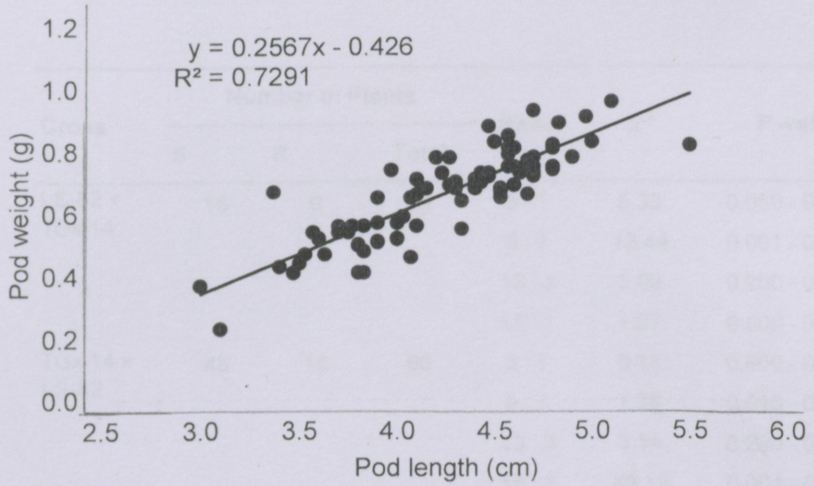

Fig. 4.11 Relationship between pod length and pod weight in a segregating soybean F_2 population derived from LS-82 x TGx-14.

4.2.2 Mode of inheritance of pod shattering

Based on the field evaluation (or natural shattering of the pods at maturity), the segregation pattern of F_2 populations indicated a segregation ratio of 13 susceptible : 3 resistant in the cross involving LS-82 x TGx-14 (Table 4.5). Nonetheless, there was only a limited number of plants available. However, in the reciprocal cross, the segregation pattern fitted both the 3:1 and 13:3 ratios but when the data were pooled to constitute at least 82 observations, the segregation pattern fitted best the 13:3 model (Table 4.5) which was in agreement with the observations reported recently in a similar study (Bhor *et al.*, 2014). In addition, the frequency distribution for both the proportion of shattered plants and duration to first pod shattering (Fig. 4.12) clearly indicated a skewed distribution pattern which strongly suggested epistatis (Anbessa *et al.*, 2006).

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Table 4.4 Segregation pattern for pod shattering in soybean F_2 populations based on shattering in the field.

Cross	Number of Plants			Ratio	X^2	*P*-value
	S	R	Total			
LS-82 x TGx-14	16	0	16	3 : 1	5.33	0.050 - 0.0100
				9 : 7	12.44	0.001 - 0.0100
				13 : 3	3.69	0.200 - 0.0500
				15 : 1	1.07	0.500 - 0.2000
TGx-14 x LS-82	48	18	66	3 : 1	0.18	0.900 - 0.5000
				9 : 7	7.28	0.010 - 0.0010
				13 : 3	3.14	0.200 - 0.0500
				15 : 1	49.13	0.001 - 0.0001
Pooled	64	18	82	3 : 1	5.51	0.050 - 0.0100
				9 : 7	15.83	0.001 - 0.0001
				13 : 3	0.55	0.500 - 0.2000
				15 : 1	34.50	0.001 - 0.0001
TGx-14 x PAN-637	47	7	54	3 : 1	4.17	0.050 - 0.0100
				9 : 7	20.80	0.001 - 0.0001
				13 : 3	1.19	0.500 - 0.2000
				15 : 1	4.15	0.050 - 0.0100

S = Susceptible to pod shattering; R = resistant to pod shattering

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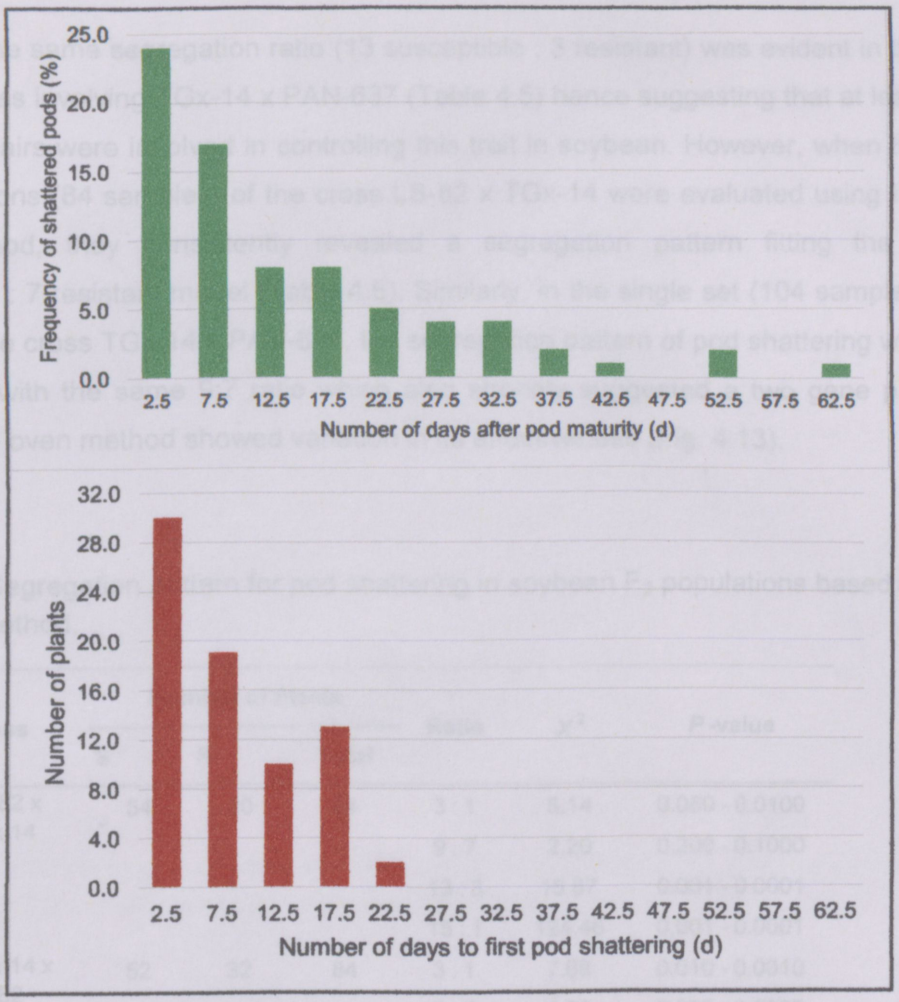

Fig. 4.12 Skewed frequency distribution curves for the proportion of shattered pods (top) and the number of shattered plants (bottom) in F_2 progenies derived from LS-82 x TGx-14 that were evaluated in the field at Thohoyandou during the 2013/2014 season.

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Similarly, the same segregation ratio (13 susceptible : 3 resistant) was evident in the second cross involving TGx-14 x PAN-637 (Table 4.5) hence suggesting that at least two gene pairs were involved in controlling this trait in soybean. However, when the F_2 populations (84 samples) of the cross LS-82 x TGx-14 were evaluated using the oven method, they consistently revealed a segregation pattern fitting the 9 susceptible : 7 resistant model (Table 4.6). Similarly, in the single set (104 samples) involving the cross TGx-14 x PAN-637, the segregation pattern of pod shattering was consistent with the same 9:7 ratio which also strongly suggested a two gene pair model. The oven method showed variation in its effectiveness (Fig. 4.13).

Table 4.5 Segregation pattern for pod shattering in soybean F_2 populations based on the oven method.

Cross	Number of Plants			Ratio	X^2	*P*-value
	S	R	Total			
LS-82 x TGx-14	54	30	84	3 : 1	5.14	0.050 - 0.0100
				9 : 7	2.20	0.200 - 0.1000
				13 : 3	15.87	0.001 - 0.0001
				15 : 1	124.46	0.001 - 0.0001
TGx-14 x LS-82	52	32	84	3 : 1	7.68	0.010 - 0.0010
				9 : 7	1.09	0.300 - 0.2000
				13 : 3	20.63	0.001 - 0.0001
				15 : 1	145.38	0.001 - 0.0001
Pooled	106	62	168	3 : 1	12.70	0.001 - 0.0001
				9 : 7	3.20	0.500 - 0.3000
				13 : 3	36.35	0.001 - 0.0001
				15 : 1	269.43	0.001 - 0.0001
TGx-14 x PAN-637	63	41	104	3 : 1	11.54	0.001 - 0.0001
				9 : 7	0.79	0.500 - 0.3000
				13 : 3	29.18	0.001 - 0.0001
				15 : 1	195.32	0.001 - 0.0001

S = Susceptible to pod shattering; R = resistant to pod shattering

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Fig. 4.13 Variation in pod shattering in the oven method indicating a set of samples with (a) both shattered and intact pods and (b) only intact pods.

4.2.3 Heritability of pod shattering

The broad sense heritability (H_{bs}) for pod shattering based on the number of days to first pod shattering in the field for the cross derived from LS-82 x TGx-14 (84.0%) was considerably higher than that for the cross derived from TGx-14 x PAN-637 (60.0%) (Table 4.6). The H_{bs} for pod shattering in both crosses based on the proportion of shattered pods in the oven method was > 75.0% (Table 4.6) which was in agreement with findings that were reported in similar work previously (Tukamuhabwa *et al.*, 2002).

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Table 4.6 Estimation of broad sense heritability for pod shattering in F_2 soybean plant populations.

Cross	Population	Variance	
		Number of days to first pod shattering in the field	Percent of pod shattering in the oven
LS-82 x TGx-14	P_1	8.00	120.00
	P_2	7.80	320.00
	F_2	48.99	1 230.07
	H_{bs}	0.84	0.82
TGx-14 x PAN-637	P_1	7.80	320.00
	P_2	15.50	280.00
	F_2	29.20	1 181.44
	H_{bs}	0.60	0.75

P_1 = first parental line; P_2 = second parental line; F_2 = second filial generation; H_{bs} = broad sense heritability.

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5.0 CHAPTER FIVE: DISCUSSION

In this chapter, the major findings that were reported in chapter three and their implications are discussed. In many instances, findings from similar studies are compared critically with those from the present study. In addition, possible explanations for the observed results are proposed where possible.

5.1 Phenotypic variability

The results that were reported in this study revealed a number of new insights with regards to pod shattering in soybean. Firstly, the variability in the traits associated with pod shattering that was observed in the F_2 segregating population indicated that there was potential for selecting for such traits in subsequent filial generations. For instance, the mean number of primary branches (NB) increased by >15.0% in the population derived from the cross TGx-14 x PAN-637 compared to the population derived from the cross LS-82 x TGx-14. This suggested that the parental choice is important in breeding for this trait which is directly related to pod load since the pods develop on the branches. The average NB (approximately 6.0) reported in this study was also consistent with the findings reported elsewhere (Malik *et al.*, 2006; Rasaily *et al.*, 1986). In a study conducted to assess the genetic variability in yield characters among soybean genotypes, the number of branches per plant was significantly correlated to the number of pods per plant (Malik *et al.*, 2006). The number of branches per plant was also influenced by late planting (Settimi and Board, 1988) which suggested that in soybean, this attribute is an important component of grain yield.

The results also showed that there were genotypic differences in terms of pod weight. In a soybean improvement program aimed at increasing pod weight per plant, the cross LS-82 x TGx-14 would likely produce heavier pods than the cross between TGx-14 x PAN-637. Furthermore, the findings from this study demonstrated a highly significant positive relationship between pod weight and pod length in the F_2 populations from both crosses. This close association between the two pod traits

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suggested that there is probably a linkage between the genes controlling these traits. In addition, this positive correlation between the two traits indicated that selection programs for either trait can lead to the inadvertent selection of the other trait

5.2 Pod cracking

The results of this study revealed pod cracking which is a new phenomenon that has not been documented before in our soybean breeding program (at the School of Agriculture, University of Venda). Pod cracking exposes the seed to pathogens and premature weathering thus leading to poor seed quality. Potentially, seed sprouting in the split pod is also likely (Lanclos, 2003). It appears that the parental line LS-82 (originating from the USA) was the source of the pod cracking gene(s) since the phenomenon was not observed in any of the progenies arising from the second cross (TGx-14 x PAN-637) which did not involve LS-82. Nonetheless a proper genetic study of the pod cracking trait in soybean is merited in order to design an effective genetic improvement program since it reduces seed quality. Although the pod splitting was a new phenomenon in the soybean breeding program (in our School), it was observed previously in the field by farmers in the U.S.A. (Lanclos, 2003). The pod splitting showed varietal differences and was attributed mainly to variable weather patterns particularly dry conditions during pod initiation and pod set followed by heavy rain during the seed filling stage. In this case, it appears that progenies derived from this cross (LS-82 x TGx-14) were sensitive to the dry conditions prevailing at the testing location which necessitated supplementary irrigation in order to minimize seed abortion.

However, while it is tempting to single out LS-82 as a possible source of the pod splitting trait, the genotype obtained the largest seed (> 18.0 g per 100 seed) in a preliminary study aimed at estimating yield loss in selected soybean germplasm (Appendix I). This indicated that the cultivar possesses some potentially useful agronomic attributes. However, small, narrow pods of soybean were associated with reduced shattering (Bara *et al.*, 2013). In this study, no pod cracking was observed in the parental genotype itself. Therefore, there will be merit in studying the genetics of pod cracking in soybean. In particular, it will be interesting to determine if pod

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cracking is linked to the seed size and degree of shattering that was associated with those genotypes that cracked. The genotypic variation in cracking could also be of interest.

Another possible explanation for the pod cracking that was observed in this study was transgressive segregation since it showed in neither parent. By definition, transgressive segregation is when segregating hybrids often exhibit phenotypes that are extreme or novel relative to the parental lines (Rieseberg *et al.*, 2003). In a survey of 171 studies that reported on phenotypic variation in segregating hybrid populations, transgressive segregation occurred most frequently in the populations of domesticated plant populations (Rieseberg *et al.*, 1999). High frequencies of transgressive segregation in yield traits among F_2 and F_3 soybean populations were also reported previously (Aditya *et al.*, 2013) and they were attributed to the involvement of additive and additive x additive epistatic effects as well as dominance effects in the genetic control of yield (Massey *et al.*, 2008). In rice, transgressive segregation of the tiller angle was attributed to complementary gene action (Xu *et al.*, 1998). The pod cracking trait could be under similar epistatic effects but this remains speculative until corroborative emperical evidence is obtained.

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5.3 Mode of inheritance

The 13 (susceptible) : 3 (resistant) ratio which was found for the pod shattering trait using the field screening method in this study was in agreement with similar previous studies (Bhor *et al.*, 2014; Mohammed, 2010) .The interpretation of this ratio was that two major genes along with inhibitory epistasis (Bohr *et al.*, 2014) were involved in the mode of inheritance of pod shattering in soybean. In other words, the 13 : 3 ratio suggested complete dominance at both gene pairs, but one gene, when dominant, epistatic to the second and the second gene, when homozygous recessive, epistatic to the first implying that the genes required for pod shattering resistance would be one dominant (S_) and one recessive (Sstt).

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The results of the oven method which was applied in this study differed considerably from those obtained from the field evaluation method. This was probably due to variation in the moisture content of the pods. The method assumed that moisture reduction in the mature pods that were harvested and stored at room temperatures for 15 d in order to equilibrate the moisture (Bhor *et al.*, 2014; Tiwari and Bhatnagar, 1997) proceeded at the same rate in all the pods regardless of their degree of maturity. At harvesting, mature pods for use in the oven method were determined visually and the period for moisture equilibration was imposed arbitrarily such that the stability of the moisture content of the pods could have influenced the observed segregation ratio of 9 (susceptible) : 7 (resistant). Using both the oven method and the desiccator method, Romkaew and Umezaki (2006) reported that the moisture content of soybean pods which were intact was always higher than that of the dehisced pods. The authors demonstrated varietal differences in the reduction rate in the pod moisture content at different relative humidity levels below or above 50.0%. In this study, the relative humidity over the equilibration period was not measured. Equally important, the authors concluded that both the oven and desiccator methods were useful for assessing pod dehiscence but produced nearly the same results. Therefore there is no consensus as to whether the field screening method and the oven method can produce congruent results particularly considering the complexity of pod shattering (Agrawal *et al.*, 2003; 2002). The precise mechanism that caused the observed dehiscence of pods was not established. However, in other studies, the pod shattering was attributed to the breakdown of the middle lamella by the enzyme endopolygalacturonase (Christiansen, *et al.*, 2002). In oilseed rape, resistance to pod shattering was attributed to a failure of cell degradation in the dehiscence zone and to the presence of extra vascular tissue within this zone (Morgan *et al.*, 1998).

Estimation of the number of genes or loci (Bjarco and Line, 1987; Das and Griffey,1994) requires that the (i) crosses were derived by mating randomly selected parental lines from appropriate populations (ii) segregating genes are not linked and both dominance and epistasis are absent, all loci have equal effects and genes combine randomly. In this study, the parental lines were selected deliberately based on their pod shattering behaviour. Therefore, the number of genes or loci in this study could not be determined accurately without violating these assumptions and

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thus leading to an underestimation or overestimation of both parameters (Lande, 1981).

5.4 Heritability

The broad sense heritability (H_{bs}) for resistance to pod dehiscence as measured by the duration to the initiation of pod shattering was high (0.82) in progenies that were derived from LS-82 x TGx-14 and moderately high (0.60) in the second cross. Similarly, the H_{bs} for resistance to pod shattering was high (≥ 0.75) in both crosses when estimated using the oven method. These high values for H_{bs} were in agreement with the high values (≥ 0.90) that were previously reported in similar studies (Bailey *et al.*, 1997; Tiwari and Bhatnagar, 1991; Tsuchiya, 1987; Caviness, 1969). Therefore, it is reasonable to conclude that the H_{bs} for resistance to pod shattering in soybean is high and the trait is highly heritable. The heritability together with the phenotypic variance and selection intensity can be used for predicting the response to selection (Johnson *et al.*, 1955). This could be useful in breeding for resistance to pod shattering in soybean. Likely, on the basis of the results of this study, a higher genetic advance (or response to selection) for pod shattering resistance could be expected in breeding populations derived from the cross LS-82 x TGx-14 (cross #1) than in those progenies derived from TGx-14 x PAN-637 (cross #2) since the heritability of the first cross was >30.0% higher than that of the second cross.

The results also demonstrated that the heritability for pod shattering resistance varied with the parental combinations as well as with the method of evaluation. From a plant breeding point of view, the field evaluation approach appeared to be more practical than the oven method particularly in large-scale screening activities for pod shattering. Soybean genotypes selected using this field screening approach could be evaluated further over multiple locations in subsequent evaluation cycles in order to confirm their resistance to the trait. In addition, there is insufficient information regarding the impact on seed viability of the high temperature (80.0 °C) that was used in the oven method. This information would be important for instance in a program where the oven-dried seed may be required for further breeding

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activities. Exposure of soybean seed to high temperatures negatively influenced its viability (Egli *et al.*, 2005).

Although this study contributed some new information about pod dehiscence in soybean, there were a few drawbacks in the study. For instance, the measurement of pod shattering in a segregating population in the field, did not differentiate accurately between the ages of the pods yet the process of pod development, pod filling and pod maturity differs among soybean genotypes. Pod maturity was determined visually. In addition, the field evaluation method assumed that there were no extreme fluctuations in the environmental conditions such as temperature, humidity and wind over the entire period of the evaluation. In the oven method, as indicated above it was not feasible to quantitate the moisture in the pods after the equilibration period. Nonetheless, both methods were practical for the purposes of screening for pod shattering in a soybean improvement program.

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6.0 CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

The results of this study revealed a wide range of phenotypic variability for several traits associated with pod shattering in soybean. The variability suggested that the parental choice is important in breeding for the traits. The results also showed genotypic differences in terms of pod weight with the cross LS-82 x TGx-14 producing heavier pods than the cross TGx-14 x PAN-637. The findings also revealed a highly significant positive relationship between pod weight and pod length in the F_2 populations from both crosses suggesting a close association between the genes controlling these traits such that selection of one trait can lead to the inadvertent selection of the other trait.

The new F_2 progenies derived from the cross LS-82 x TGx-14 also showed pod splitting at the pod-filling stage. Since it was absent from the F_1 progenies, it was attributed to transgressive segregation (Riesenberg *et al.,* 2003). In contrast, this phenomenon was absent from the second cross (TGx-14 x PAN-637) thus further suggesting that LS-82 was the possible source of the gene(s) controlling this trait.

Based on the field screening method, there was strong evidence supporting the 13 (susceptible) : 3 (resistant) segregation model for pod shattering ratio which was in agreement with similar previous studies (Bhor *et al.*, 2014; Mohammed, 2010). This model implied that two major genes along with inhibitory epistasis (Bohr *et al.*, 2014) were involved in controlling pod shattering in soybean. However, the results based on the oven method differed from those obtained from the field evaluation method and fitted 9 (susceptible) : 7 (resistant) mode of inheritance for pod shattering. The disparity was attributed a number of limitations including inconsistent pod moisture content after the equilibration period. Nonetheless, both methods indicated that at least two gene pairs were involved in controlling pod shattering in the crosses that were used. The broad sense heritability was in the moderate to high category as reported in other similar studies (Rieseberg *et al.*, 1999).

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It is recommended that future studies interested in the Mendelian genetics of this trait should take into consideration the age of the pods as well as the pod moisture content (Romkaew and Umezaki, 2006) after equilibration. Although both methods were practical for screening for pod shattering in a soybean, the field method appeared to be relatively more reliable. In addition, genotypes that showed resistance to pod shattering should be selected in future advanced filial generations of the two populations. Apart from evaluating their productivity of such genotypes, it will be useful to evaluate their level of promiscuous nodulation using rhizobia from target production areas. The combination of genes for resistance to shattering, promiscuous (tropical type) nodulation and high productivity in the newly developed germplasm can benefit smallholder soybean growers significantly.

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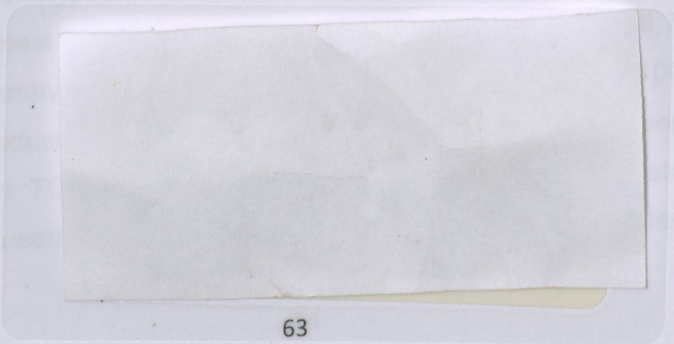

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Appendix I

A Short Communication prepared for a local journal

A Preliminary Agronomic Evaluation of Selected Soybean [*Glycine max* (L) Merrill] Germplasm

[a]Nevhudzholi KM, [a]Gwata ET and [b]Mchau GRA.

[a]Department of Plant Production, University of Venda, School of Agriculture, P. Bag X5050, Thohoyandou 0950, South Africa

[b]Department of Horticulture, School of Agriculture, University of Venda, P. Bag X5050, Thohoyandou 0950, South Africa

Abstract

Soybean (*Glycine max*) is an important grain legume world-wide. Tropical types of soybean that nodulate promiscuously with indigenous soil rhizobia are desirable smallholder cropping systems in southern Africa. However, tropical types of soybean are generally susceptible to pod shattering resulting in considerable yield losses. However, pod shattering is influence by several environmental factors. Therefore, the specific objectives of the study were to determine the (i) agronomic performance and (ii) the proportion of grain yield loss due to pod shattering in a selected set of soybean cultivars using a representative typical ecotope in southern Africa. During harvesting, a range of agronomic attributes were measured including the total number of pods per plant, pod length, percent yield loss (%YL) and grain yield. A five-fold difference in PC was observed between the tallest and shortest genotypes. On average, the genotypes produced about 41.0 pods per plant but 'Magoye' which developed the highest number of pods attained the highest %YL (68.76%) through pod shattering. Genotype 'LS-82' achieved the largest (18.41 g per 100 seed) seed while 'TGx-14' produced the smallest seed. There was a marked contrast in pod shattering between 'TGx-14' and the check ('PAN-637') which suggested that these cultivars could be used as parental lines in genetic studies that are interested in pod

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shattering. In soybean production systems that are prone to considerable fluctuations in environmental factors during harvesting periods, early harvesting of the shattering genotypes will be necessary.

Key words: attributes; ecotope; pod shattering; tropical soybean type.

Introduction

Soybean (*Glycine max*) is an important grain legume which is used mainly for human consumption and animal feeds. It is also used in crop rotations in order to replenish soil fertility through biological nitrogen fixation particularly in smallholder cropping systems in where inorganic nitrogenous fertilizer may be limited (Giller *et al.*, 2011). In such cropping systems, tropical types of soybean that nodulate promiscuously (Gwata *et al.*, 2005; Sanginga *et al.*, 2002) with indigenous soil rhizobia are desirable. However, tropical types of soybean are generally susceptible to pod shattering (Tukamuhabwa *et al.*, 2002) which results in considerable losses in yield.

The shattering of pods in soybean is influenced by several environmental factors (Bailey *et al.*, 1997; Tukamuhabwa *et al.*, 2000). Pod shattering in soybean is aggravated by rapid fluctuations in temperature and humidity (Agrawal *et al.*, 2002). High temperatures in particular, promote dehydration of the pod wall and splitting of the dehiscence zone (Bhor *et al.*, 2014).

In the semi-arid region of the Limpopo Province (South Africa), there is a paucity of information regarding the potential yield loss due to pod shattering in soybean. Such information is critical for the adoption of tropical types of soybean particularly by smallholder growers in the region. Therefore, this study examined the agronomic performance of soybean. The specific objectives of the study were to determine the (i) agronomic performance and (ii) the proportion of grain yield loss due to pod shattering in a selected set of soybean cultivars. The study tested the hypotheses

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that the (i) agronomic performance was similar among the cultivars and (ii) proportion of yield loss was equal among the cultivars.

Materials and Methods

Testing location

The study utilized a typical representative ecotope (Mzezewa *et al.*, 2010) located at Thohoyandou (22^0 58' S, 30^0 26' E; 596 m a.s.l.) in the Limpopo Province (South Africa). During summer, daily maximum temperatures at the location often exceed 33.0 ^oC and the annual rainfall (about 500.0 mm) is highly seasonal with 95.0% occurring between October and March. The soils at the location are predominantly deep (>150 cm), red and well drained clays with an apedal structure and about 60.0%. The soil reaction is acidic with a pH 5.0) (Table A-1).

Table A-1 Soil characteristics at the testing location at Thohoyandou (Limpopo Province, South Africa).

Characteristic	Type/Content
Soil texture	Clay
Soil pH	6.50
Ca(Aoc) mg/kg	432.34
Mg(Aoc) mg/kg	113.54
Na(Aoc) mg/kg	15.77
K(Aoc) mg/kg	76.93
P(Bray 1) mg/kg	2.20
Clay (%)	48.00
Silt (%)	18.00
Sand (%)	34.00

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Genetic material

Six distinct soybean genotypes (including one standard check) consisting of both tropical types (predominantly susceptible to shattering) and non-tropical types (mostly non-shattering) were used in the study (Table A-2). The genotypes also differed in duration to maturity.

Table A-2 Soybean genotypes used in the study.

Genotype	Origin	Type	Notes
1 LS-82	USA	non-tropical	- generally non-shattering - early maturing - round pods - large seed
2 Magoye	Australia	tropical	- moderate shattering - flat pods - late maturing
3 PAN-637	South Africa	non-tropical	- non-shattering - early maturity
4 STG	Nigeria	tropical	- shows the stay green trait - small seed
5 TGx-14	Taiwan	tropical	- highly shattering - late maturity
6 4-LF	Unknown	non-tropical	- produces four-lobed leaves

Planting and crop management

Healthy seed of each genotype was planted manually in single row plots measuring 1.5 m long and spaced at 0.6 m apart. Within the row, seed was planted at 0.62 m apart. No commercial rhizobial inoculant or fertilizer was applied during or after planting. Standard soybean crop management practices were followed throughout the season.

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Agronomic measurements

During harvesting, the following agronomic attributes were measured:

(i) plant height (PHT)

(ii) pod clearance (PCL)

(iii) number of primary branches (NBR) per plant

(iv) total number of pods (TNP) per plant

(v) pod length (PL)

(vi) pod width (PW)

(vii) single pod weight (SPW)

(viii) 100 seed weight (100-SW)

(ix) percent yield loss (%YL)

(x) grain yield (GY).

The proportion of grain loss due to pod shattering was measured. Pod shattering was measured by harvesting separately each of six randomly selected mature plants (two per replicate) before the initiation of pod shattering. Each plant was placed carefully in a polythene bag without shaking the pods and suspended on a firm string in a screen-house (Fig. A-1) in order to trap the shattered seed which was then collected after thirty days for weighing. The remainder of the seed from intact or partially spilt pods from each genotype was also collected for weighing separately.

Experimental design and data analysis

The experiment was laid out as a randomised complete block design replicated three times. Data sets of each quantitative variable were subjected to the analysis of variance in SAS followed by mean separation (Duncan, 1955).

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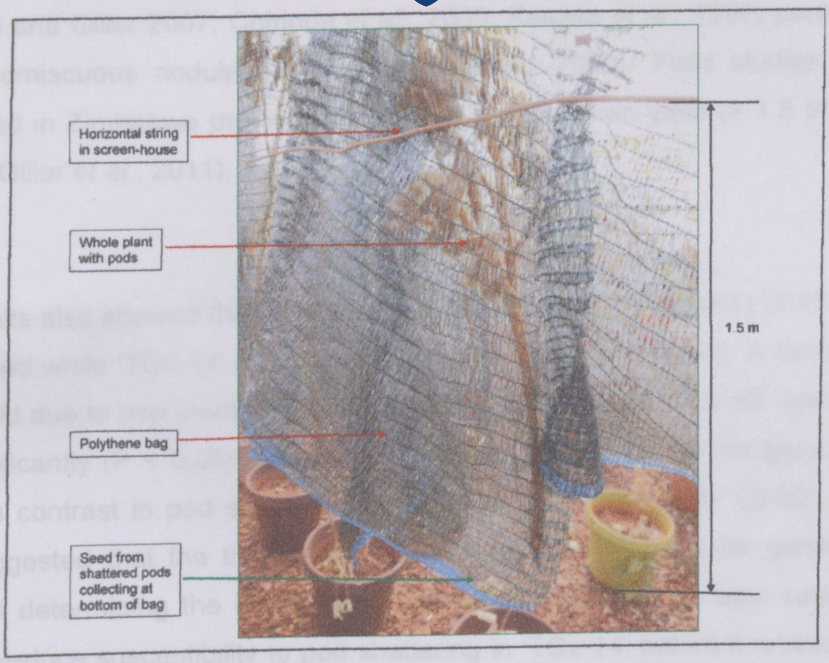

Fig. A-1 A mature soybean plant suspended in a polythene bag in a screen-house.

Results and discussion

There were highly significant ($P < 0.001$) differences in the majority of the agronomic traits that were measured among the six soybean genotypes (Table A-3) indicating the diversity of the germplasm. The shortest plant height (20.5 cm) was observed for genotype 'LS-82'. A five-fold difference in pod clearance was observed between the tallest and shortest genotypes (Table A-3). On average, the genotypes produced about 41.0 pods per plant but 'Magoye' which developed the highest number of pods (Table A-3) attained the highest (68.76%) yield loss (Table A-4) through pod shattering. This was consistent with observations that were reported in similar studies (Tukamuhabwa *et al.*, 2002). From a soybean production stand point, delayed harvesting of this genotype should be avoided if cultivated in a similar agro-ecological environment. However, this observation about the yield loss in this genotype should be interpreted with caution for two major reasons. Firstly, only a single location was used for the study. Secondly, the genotype is widely used as a commercial cultivar in other parts of southern Africa including Malawi and Zimbabwe

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(Zengeni and Giller 2007; Chirinda *et al.*, 2003; Kasasa *et al.*, 1999) partly because of its promiscuous nodulation (Mpepereki *et al.*, 2000). Field studies that were conducted in Zimbabwe previously indicated a good grain yield (> 1.5 t/ha) for this cultivar (Giller *et al.*, 2011).

The results also showed that genotype 'LS-82' achieved the largest (18.41 g pair 100 seed) seed while 'TGx-14' produced the smallest seed (TableA-3). A minimal loss in grain yield due to pod shattering (<1.0%) was similar between 'LS-82' and 'PAN-637' but significantly ($P < 0.05$) different from the loss observed for the genotype 'TGx-14'. This contrast in pod shattering between 'TGx-14' and both 'LS-82' and 'PAN-637' suggested that the three genotypes as parental material for genetic studies aimed at determining the inheritance of the trait. This findings also suggested the need to reduce susceptibility to pod shattering in 'TGx-14' before it is recommended for wide spread cultivation by growers in agro-ecologies that are prone to extreme fluctuations in temperatures and humidity (Agrawal *et al.*, 2002).

Table A-3 The means of eight agronomic traits of six soybean genotypes.

Cultivar	PHT (cm)	PCL (cm)	NB	TNP	PL (cm)	PW (cm)	SPW (g)	100-SW (g)
TGx-14	73.76 a	23.23 a	3.46 ab	39.85 abc	3.86 b	0.81 c	0.47 c	10.75 e
4-LF	71.27 a	18.47 b	4.53 a	45.40 ab	3.74 b	0.86 b	0.50 c	11.57 d
STG	65.50 b	25.10 a	3.27 b	46.00 ab	3.55 c	0.79 c	0.46 c	11.18 de
Magoye	41.97 c	9.70 c	2.87 b	50.60 a	3.74 b	0.87 b	0.57 b	13.14 c
PAN-637 (Check)	26.20 d	6.30 cd	3.67 ab	36.07 bc	4.41 a	1.05 a	0.74 a	16.89 b
LS-82	20.50 e	4.67 d	3.67 ab	29.58 c	4.41 a	1.03 a	0.75 a	18.41 a
Mean	50.34	14.72	3.58	41.69	3.94	0.90	0.58	13.56
C.V. (%)	11.57	32.32	37.09	40.68	4.88	7.81	14.62	7.01
R^2 (%)	93.67	75.79	28.98	27.2	78.06	70.24	68.44	90.95
Significance	**	**	*	**	**	**	**	**

Means followed by the same letters in each column are not significantly different ($P < 0.05$)

** = Highly significant at the 1.0% probability level

* = Significant at the 5.0% probability level

PHT = plant height; PCL = pod clearance (cm); NB = number of primary branches per plant; TNP = total number of pods per plant; PL = pod length (cm); PW = pod weight (g); SPW = single pod weight (g); 100-SW = 100 seed weight (g).

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Table A-4 Grain yield and percent yield loss due to pod shattering in selected soybean genotypes.

Cultivar	Grain Yield (t/ha)	Percent Yield Loss (%)
TGx-14	0.91 a	17.69 b
4-LF	0.78 a	24.16 b
STG	0.52 ab	0.02 c
Magoye	0.48 ab	34.65 ab
PAN-637 (Check)	0.46 ab	68.76 a
LS-82	0.32 b	0.13 c
Mean	0.61	24.26
C.V.	32.01	81.51
R^2 (%)	72.98	73.1
Significance	*	**

Means followed by the same letters in each column are not significantly different ($P < 0.05$)

** = Highly significant at the 1.0% probability level

* = Significant at the 5.0% probability level

Conclusions and recommendations

Unequivocal conclusions based on the results of this study were difficult to draw because of the limited number of locations and seasons that were used in the study. However, the large contrast in pod shattering among the soybean genotypes suggested that suitable parental crossing combinations could be used in genetic studies that are interested in pod shattering. In soybean production systems that are prone to considerable fluctuations in environmental factors during harvesting periods, early harvesting of the shattering genotypes would be recommended strongly.

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