ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

ASTU Research Grant Final Report

Assessment Diversity of Common Bean (*Phaseolus vulgaris* L.) for D-pinitol Accumulation Using Ethiopian Germplasm

BY

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Abstract

Common bean (*Phaseolus vulgaris* L., 2n =2x= 22) is the world's most important food crop that is grown in the tropics and subtropics, has an insulin-like function and help in regulating body's metabolism. The study was undertaken to determine the pattern of D-pinitol accumulation among common bean accessions, identifying cultivars among accessions for D-pinitol accumulation and for further research investigation of anti-diabetic and anticancer drugs design. In this study, the field work survey was conducted by collecting of the germplasm from different common bean growing regions of Ethiopia. The highest amount of D-pinitol accumulations was observed for seed samples collected from West Wollega, Nejo Chokorsa district (861.3 μg/ml). The next highest accumulation was recorded by seed samples obtained from Boji and Gimbi, respectively. Comparing the seed samples collected from the Eastern part of Ethiopia (East Harerghe, West Harerghe and Somali) with that of the Western part (West Wollega and Benshangul Gumuz), samples obtained from western part of Ethiopia, particularly West Wollega accumulated the highest amount of D-pinitol. Therefore, the study confirmed that seed of common bean was one of the sources for D-pinitol, and there is a need of further investigation, sequencing and modifications the genes responsible for D-pinitol accumulation.

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1. Introduction

1.1. Background and Justifications

Common bean (*Phaseolus vulgaris* L., 2n =2x= 22) is the world's most important food crop that grown in the tropics and subtropics. The area covered by the crop has been increasing due to its role in low input agricultural systems and contain high protein content (Mwale *et al*., 2008). In nutritional terms, beans are often called the 'meat of the poor' due to their role as an economical alternative in the diets of people who cannot afford animal products. It has high proteins and it is rich in minerals (especially iron and zinc) and vitamins (Piergiovanni and Lioi, 2010). The varieties of common bean grain can be classified into two major gene pools based on seed size differences, with Andean beans being large seeded and Mesoamerican beans being small seeded (Blair *et al*., 2010).

In Ethiopia, it is one of the major food and cash crops and it has considerable national economic significance and also traditionally ensures food security. It ranks third as an export commodity in Ethiopia, contributing about 9.5% of total export value from agriculture. It is often grown as a cash crop by small scale farmers. The majority of common bean producers in Ethiopia are small scale farmers, and it is used as a major food legume in many parts of the country where it is consumed in different types of traditional dishes. The common bean is among pulses that take the largest share of all pulses in terms of area coverage. It is solely an important legume in the Ethiopian lowlands as a rotation crop, particularly in the Rift valley where farmers grow white bean for export (Abebe Hinkossa *et al*., 2013). Besides, it has a short maturity period, only about three months, it fills gaps in household food needs during the hunger period when there is no other product to be sold (Amare, 1989; Ayele, 1990).

Medical research of D-pinitol has indicated that it has an insulin-like function and help in regulating body's metabolism (Poongothai and Sripathi, 2012). In addition to these, D-pinitol is also effective to treat cancers. It positively modulates the antioxidant activity and reduces the lipid peroxidation by quenching and detoxifying the free radicals (Mininath *et al.*, 2015).

1.2. Statement of the Problem

Leguminous plants such as soybean is a rich sources of D-pinitol, however, there is no any investigations (informations) on the patterns of accumulations of D-pinitol in common bean. Therefore, this study was focused on the genetic diversity studies for the assessment of the patterns of D-pinitol accumulation in common beans (*Phaseolus vulgaris* L.) using Ethiopian germplasm.

1.3. Objectives

1.3.1. General Objective

The main objective of the present study is to determine the patterns of D-pinitol accumulation in common beans (*Phaseolus vulgaris* L.) of Ethiopia

1.3.2. Specific Objectives

1. to determine the pattern of D-pinitol accumulation among common bean verities.
2. To indicate the best cultivar (s) among accessions for D-pinitol accumulation

2. Literature Review

2.1. Origin in Common Bean

The genus *Phaseolus* is of American origin and comprises over 30 species (Singh, 2001). Only five of these species, namely, *P. Acutifolius* A. Gray (tepary bean), *P. coccineus* L. (scarletrunner bean), *P. lunatus* L. (Lima bean), *P. polyanthus* Green man (year-long bean), and *P. vulgaris* L. (common bean) were domesticated. Among these species, common bean is the most widely grown, occupying more than 85% of production area sown to all *Phaseolus* species in the world (Gepts *et al*., 1986).

Diversity among *Phaseolus* species in relation to common bean is organized into primary, secondary, tertiary, and quaternary gene pools. The primary gene pool of common bean comprises both cultivars and wild populations. The latter is the immediate ancestors of common bean cultivars (Singh, 2001). Wild populations of common bean are distributed from northern Mexico to north western Argentina) (Gepts *et al*., 1986). Moreover, common bean is a non-centric crop, with multiple domestication sites throughout the distribution range in Middle and Andean South America. Hybrids between the wild and cultivated beans are fully fertile and have no major barriers (Singh, 2001).

Different market classes of snap bean cultivars are largely determined on the basis of pod shape (flat, cylindrical, or oval), color (dark green, light green, yellow, or purple), and length (or sieve size). Among snap bean cultivars, there can be a large variation in growth habit and adaptation traits (Gepts, 1990).Similarly, large variation in growth habit, phenological traits, seed size, shape, color, and canning and cooking qualities are found among dry bean cultivars (Tar'an *et al*, 2002).

Genotypic differences in drought resistance have been reported for common bean (Abebe *et al*., 1998). The most effective selection criterion, among various morphological, physiological, phenological, yield, and yield related traits for identifying drought resistant genotypes were mean seed yield (the arithmetic and geometric) of drought stress and nonstress environments (Abebe *et al*., 1998; Tilahun Amede *et al*., 2004). Among *Phaseolus* species, the tepary bean, *P. acutifolius* A. Gray, has the highest level of drought resistance (Muñoz-Perea *et al.*, 2007).In dry bean, drought resistance was reported in the races Durango, Mesoamerica, and Jalisco (Terán and Singh, 2002). The highest level of drought resistance among these races occurs in the race Durango, which originated in the semiarid central and northern highlands of Mexico (Muñoz-Perea *et al*., 2007). Race Durango cultivars also predominate in North America including the USA (Singh, 2007)**.** Similarly, a diverse group of cultivars has been evaluated for drought resistance in Brazil, Colombia (Singh and Terán, 1995), Mexico, the Rift Valley of East Africa (Abebe *et al*., 1998), and the USA (Acosta-Gallegos and Adams, 1991).

2.2. Common Bean Production in Ethiopia

The common bean is the world's most important food legume. Beans offer a low cost alternative to beef and milk because bean seed is rich in protein, iron, fibers, and complex carbohydrates (Mwale, 2008). An adaptation of bean to the range of environmental conditions around the world has resulted in the evolution of extensive genetic variation for nutrient efficiency in this crop. Consequently, its rich and apparent genetic diversity for stress tolerance makes it an excellent crop model plant (Gómez, 2004).

Even though the exact time of its introduction is controversial, it is generally believed that common bean was probably brought to Ethiopia in the 16th century (Gepts, 1990) by the Portuguese. In Ethiopia, the common bean is among pulses that take the largest share of all pulses in terms of area coverage, with an increasing trend for the last ten years. It is solely an important legume in the Ethiopian lowlands as a rotation crop, particularly in the Rift valley where farmers grow white bean for export (Ayele, 1990). A common bean is a principal food crop, particularly in southern and eastern part of Ethiopia, where it serves as a main source of dietary protein and supplements other staple foods in production areas. Besides, it has a short maturity period of only about three months and, hence, it fills gaps in household food needs during the hunger period and income gap when there is no other produce to be sold (Amare, 1989; Ayele, 1990).

The common bean is also the main component of the crop system because of its role in double cropping, ability of shade tolerance, early maturity (Shimelis W/Hawariat *et al*., 1990; TenawWorkayehu and Yeshi Chiche, 1990) and the role in soil fertility due to its symbiotic association with rhizobia (Broughton *et al*., 2003). In drought prone area, it is a risk aversion crop (Alelign Kafyalew, 1990). Common bean plays a great role as a source of proteins and carbohydrates; however, the productivity has been very low due to biotic and abiotic stresses prevailing in farmers' fields. The drought is considered as one of the most important abiotic stresses that contribute to significant yield losses in sub-Saharan Africa in general (Wortmann and Allen, 1994) and Ethiopia in particular (Abebe *et al*., 1998).

2.2.1. Breeding Techniques for Common Bean Improvement

The Ethiopian national common bean improvement program has been trying to release promising bean varieties through crop improvement techniques. Crop improvement is achieved through the introduction, using different selection methods and hybridizing plants with desired traits (Gómez, 2004). The introduction is the early plant breeding method and it is taking of the superior plant varieties with desired traits from one area to the newer area for immediate use after evaluating in certain areas within shorter period of time (Tilahun Amede *et al*., 2004). This method is not currently applied in Ethiopia (Tar'an and Singh, 2002). Selection is the preferential survival and reproduction or preferential elimination of individuals with certain genetic compositions by means of natural or artificial controlling factors. It is the most commonly used breeding technique in Ethiopia probably due to the use of already existing natural variation to select the best performing genotype, no need of cost for creating artificial variation (Wortmann and Allen, 1994).

The majority of common bean varieties released in Ethiopia are developed by using selective breeding methods like mass selection; pure line selection, bulk selection and backcross method. In addition to selection, sometimes hybridization is used to cross two genotypes with dissimilar but with desirable traits to produce hybrids. Recently, gene pyramiding common bean against diseases like ALS, CBB and anthracnose is undertaken at SARI (Setegn Gebeyehu, 2006). In addition, assessing the diversity of common bean diseases like CBB and ALS is also underway. Gene pyramiding is arranging multiple genes leading to simultaneous expression more than one gene in a cultivar to produce long lasting resistance expression of genes in which success depends on the number of genes to be transferred and the distance between the target gene and the flanking markers and the number of genotypes selected in each breeding generation the nature of germplasm, etc. (Singh, 2002).

Since 1970's, more than 50 common bean varieties have been released for different ecologies of specific common bean traits. In these five decades, though the improved status of common bean is not constant; the number of varieties released in every ten years has been increasing linearly (Wortmann and Allen, 1994). For the future, the common bean improvement is expected to be enhanced with the support of molecular breeding method by which specific trait to be inserted is done relatively easily provided that skilled manpower and all necessary materials are available. Due to its economic and agro-ecological importance, the improved status of common bean should be boosted through sharing the responsibility of breeding system according to the agro-ecology and preference criteria of the farmers. In addition to this, it is one of the major legume crops which bring foreign currency to the country and thus the common bean crop improvement system has to be encouraged by the higher officials more than what is being done now (Gepts, 1990).

2.3. Properties and Medicinal Values of D-pinitol

The name pinitol derives from "pine", as it was first isolated from a pine tree. It also occurs prevalently in other plants, largely from leguminous plants. D-pinitol (D-3-*O*-methyl-chiro-inositol) is a natural, sugar-like molecule found in a variety of plant foods. It is considered as one part of healthy diet. Foods high in D-pinitol are legumes, leaf vegetables, and citrus fruits, but the molecule does not appear to be naturally found in animal products, and it is not produced in the human body (Poongothai and Sripathi, 2012).

D-Pinitol is effective in lowering blood glucose levels with no side effects and toxicities, and it is also one of the insulin mimickers in the food supplement industry (Poongothai and Sripathi, 2012). Furthermore, D-pinitol also has antioxidant, antilipidemic and acts as feeding stimulant activities which improve diabetic control and reduce associated risk factors (Mininath*et al.*, 2015). Moreover, it is known for antidiabetic, anti-inflammatory and, which can facilitate glycogen or circulating sugar into metabolically active tissues (Dewangan *et al.*, 2014).

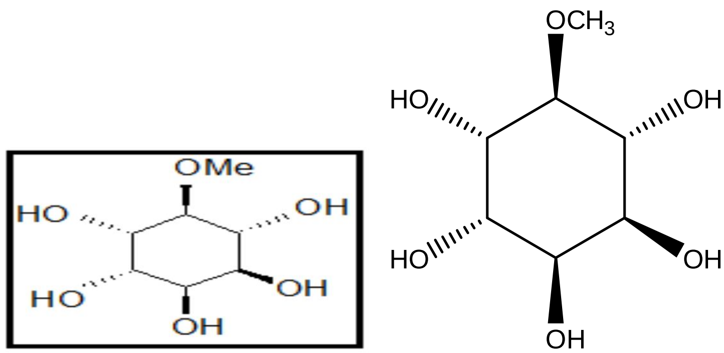


Figure 1. Structure of D-pinitol **(**Source: Poongothai and Sripathi (2012)

D-Pinitol is synthesized in two reactions: O-methylation and epimerization, beginning with *myo*-inositol as shown below (Streeter *et al.*, 2001). *Myo*-inositol is first converted to ononitol through an inositol methyl transferase (IMT) (Chiera, *et al.*, 2006). This IMT takes the methyl group from S-Adenosyl- L-methionine and transfers it to *myo*-inositol forming ononitol. Ononitol is then converted to pinitol through an unknown epimerase (McDonald *et al.*, 2012).

IMT epimerase

***Myo*-inositol ------------ Ononitol ---------------- D-pinitol**

Research on genetic diversity of D-pinitol accumulation in soybean (*Glycine max* (L.) shown, two to three-fold differences in pinitol accumulation in leaf blades of accessions. Furthermore, the previous studies shown that D-pinitol accumulation was uniquely associated with adaptation to drought stress or water deficits. As the studies revealed that plants from regions of low rainfall were accumulating larger increments of D-pinitol than plants from regions of relatively high rainfall (Streeter *et al.*, 2001).

The gradient shifted during plant development, with consistently higher concentrations of pinitol in the uppermost leaves. This result and other lines of evidence indicated that shifting patterns of pinitol accumulation were due to translocation of the skeletons from lower to upper nodes. Pinitol, proline, and sugars are accumulated in leaf blades on soybean plants subjected to drought, but the molar concentration of pinitol in stressed plants was greater than the concentrations of proline or sugars (Streeter *et al*, 2001).

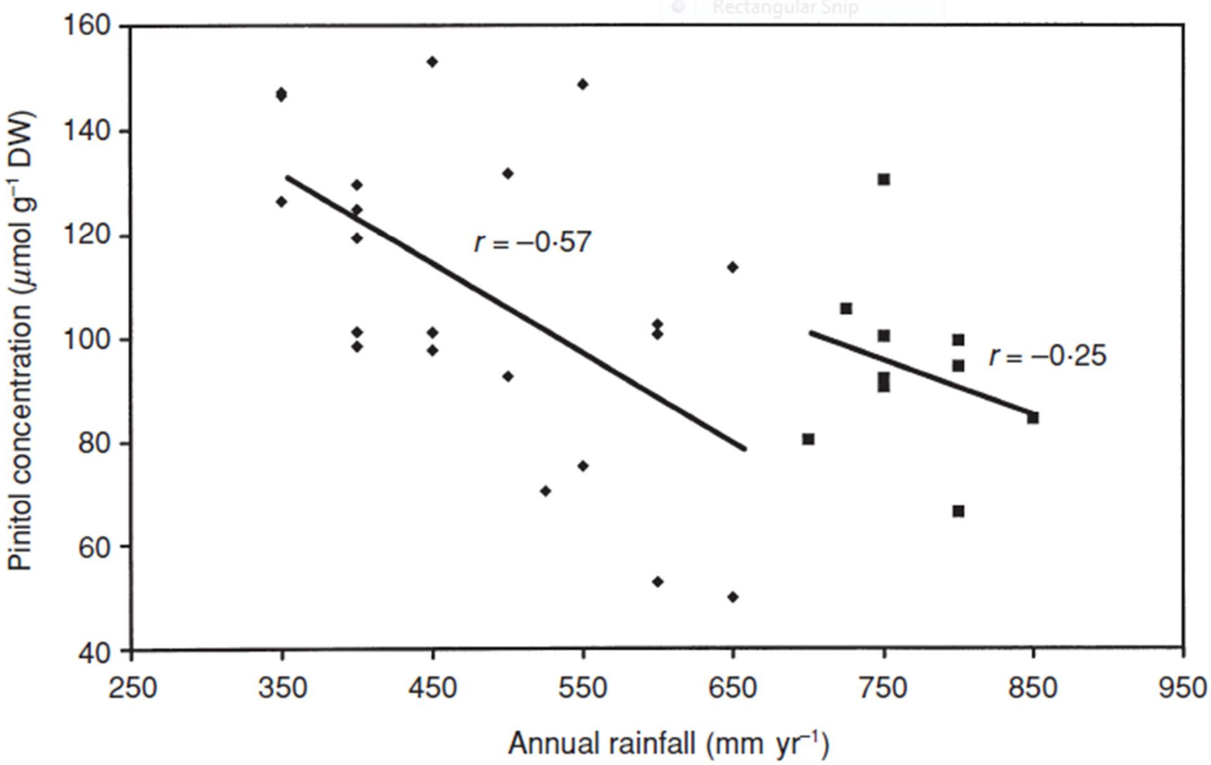


Figure 2. Relationship between D-pinitol accumulation and annual rainfall in leaf blades of Soyabean

Medical research into D-pinitol has indicated that it has an insulin-like function and help in regulating body's metabolism (Poongothai and Sripathi, 2012). In addition to these, D-pinitol is also effective to treat cancers. It positively modulates the antioxidant activity and reduces the lipid peroxidation by quenching and detoxifying the free radicals (Mininath *et* *al.*, 2015). Even though the previous research efforts resulted in the D-pinitol accumulation in Soybean, patterns of accumulation of D-pinitol in common bean still remains the subject of investigation. Therefore, this study will be focused on the assessment of the patterns of D-pinitol accumulation in common beans (*Phaseolus vulgaris* L.) using Ethiopian germplasm.

3. Materials and Methods

3.1. Plant Materials

The seed samples were collected from different common bean growing regions of Ethiopia including Oromia (East and west Harerghe, West Wollega, Central Rift valley), SNNP (Omo, Hadiya), Somali (Shinile), Benishangul Gumuz (Assosa) and Amhara (Debra Tabore). Seed samples of each germplasm were extracted to obtain the D-pinitol (Table 1).

Table 1. Common bean collection regional states, zones, woredas, coordinates and altitudes

Regional states	Zones	Woredas	Co-ordinates		Altitude
			Latitude	Longitude	
Oromiya	West Harerghe (Chiro)	Ancher	09°19’ N	42°27’ E	1889m
		Boke	09°19’ N	42°27’ E	1889m
		Chiro zuria	09°19’ N	42°27’ E	1889m
		Darolebo	09°19’ N	42°27’ E	1889m
		Hirna	09°19’ N	42°27’ E	1889m
Oromiya	East Harerghe	Bedeno	09°29’ N	42°34’ E	1870m
		Haromaya	09°29’ N	42°34’ E	1870m
		Meta	09°04’ N	42°45’ E	1872m
Somali regional state	Somali (Shinile)	Afdem	09°41′ N	41°51′ E	1080m
		Alejer	09°41′ N	41°51′ E	1080m
		Bike	09°41′ N	41°51′ E	1080m
Oromiya	Boji Chokorsa (West Wollega)	Boji	9°30′ N	35°35′ E	1535m
		Bila	9°30′ N	35°35′ E	1535m
		Muklemi	9°30′ N	35°35′ E	1535m
		Lalo	9°30′ N	35°35′ E	1535m
		Gute	9°30′ N	35°35′ E	1535m
		Laga Gumbi	9°30′ N	35°35′ E	1535m
Oromiya	Nejo (West Wollega)	Gori	9°30′ N	35°30′ E	1830m
		Warajiru	9°30′ N	35°30′ E	1830m
		Najjo Abaya	9°30′ N	35°30′ E	1830m
		Nagoya hip-hop	9°30′ N	35°30′ E	1830m
		Alaltu	9°30′ N	35°30′ E	1830m
Oromiya	Gimbi (West Wollega)	Gimbi	9°10′ N	35°50′ E	1920m
		Bikiltu	9°10′ N	35°50′ E	1920m
		Chuta	9°10′ N	35°50′ E	1920m
Oromiya	Mendi (West Wollega)	Manasibu	9°80’ N	35°10’ E	1821m
		Kondala	9°80’ N	35°10’ E	1821m
		Sasiga	9°80’ N	35°10’ E	1821m
		Babo Gmbel	9°80’ N	35°10’ E	1821m
Benshangul-Gumuz	Assosa	Assosa zuria	10° 57' N	36° 30' E	2332m
		Sherkole	10° 57' N	36° 30' E	2332m
		Guba	10° 57' N	36° 30' E	2332m

3.2. D-pinitol Standard Optimization and Sample Dilution

The Caro pinitol® was used as standard for this study. The standard was dissolved in double distilled water for UV-vis measurement and the maximum wavelength of the absorbance was 203nm. The standard was prepared with the concentration of 35 μg/mL, 40 μg/mL, 45μg/mL and 50 μg/mL to check the absorbance linearity at 203 nm. The absorbance of the serially diluted standard of 35 μg/mL, 40 μg/mL, 45μg/mL and 50 μg/mL at 203 nm were 0.293, 0.325, 0.357, 0.386, respectively increases linearity (Table 2).

The 25 mg of ethanol extract was dissolved in 25 ml detergent water for UV-vis measurement as a stock solution of each sample. Then 100 μl of the stock solution was mixed with 5 ml of laboratory reagent water.

3.2.1. D-pinitol Quantification

Thirty two (32) seed samples collected from Oromia, South nation nationality peoples state of Ethiopia, Somali, Benishangul Gumuz and Amhara were quantified and compared with the standard as references.

3.2.2. D-Pinitol Extraction

The experiment was conducted at applied chemistry laboratory, Adama Science and Technology University, from March, 2017 to June, 2017. The collected seed samples were air dried and pulverized. The dried and pulverized seed samples were extracted by using ethanol in a Soxhlet extractor. The ethanol extract was filtered and dried under vacuum on a rotary evaporator. The crude ethanol extract was further extracted successively with n-hexane, chloroform and ethyl acetate to remove non-polar and medium polar natural products from the ethanol extract. The defatted ethanol extract was subjected to quantitative UV-vis analysis. Each sample was analyzed in triplicate. For determination of concentrations, initially a standard curve was constructed by preparing a series of standard solutions of an authentic D-pinitol sample and measuring its absorbance at 203nm.

4. Results and Discussion

4.1. D-pinitol standard optimization

The seed samples of the plant material were subjected to quantitative UV-vis analysis to determination its concentrations. The D-pinitol standard curve was constructed using 35 µg/mL, 40 µg/mL, 45 µg/mL and 50 µg/mL by serial dilution from a 1mg/ml pinitol standard (Table 2).

Table 2. Shows a series of standard serial dilutions used to determine the absorbance D-pinitol in common bean

Concentration of serial dilution (µg/mL)	Absorbance (A)
35	0.293
40	0.325
45	0.357
50	0.386

The best-fit line (R_2), was found to be 0.9994, indicating the D-pinitol's concentration was significant. As it demonstrated in figure 3 that using the calibration curve the concentration of D-pinitol in samples collected from various localities were calculated by measuring their respective absorbance at 203 nm by using the linear equation determined from the calibration curve.

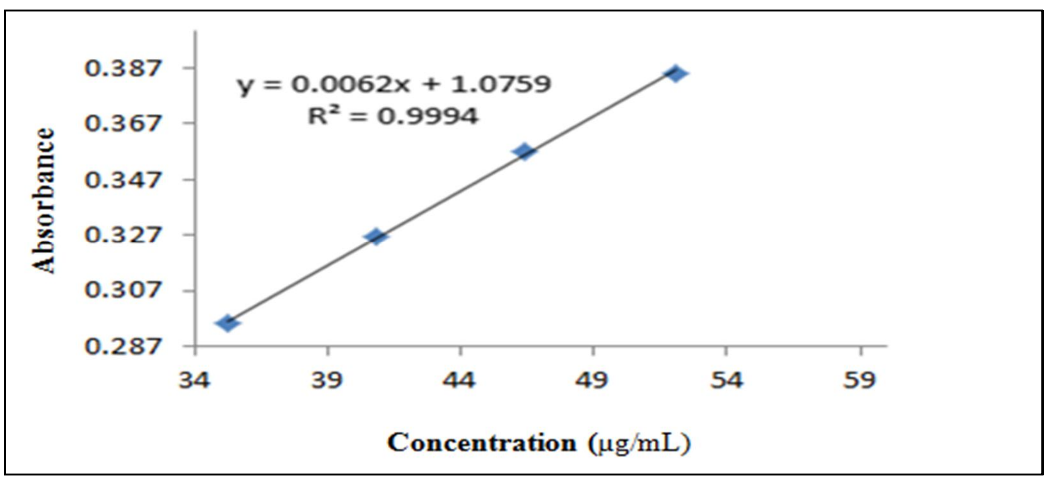
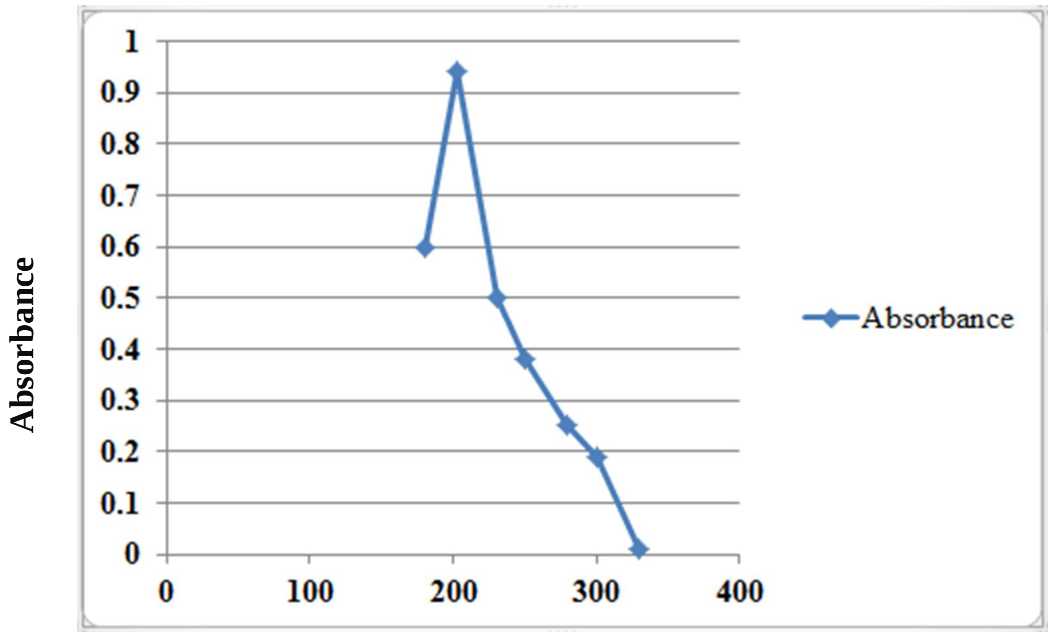


Figure 3**. D-pinitol standard absorbance at 203 nm (standard/ calibration curve)**

4.2. D-pinitol Standard Absorption and Wavelength (nm)

The maximum absorption of the D-pinitol standard at 203nm is 0.94 (Figure 4). The absorption of a selected representative sample (West Wollega-Nejo) is 0.38 at 203 nm.



Wavelength, λ (nm)

Figure 4. Absorbance and wavelength of representative samples of common bean

4.3. D-pinitol Quantification and the Absorbance in Common Bean

The absorbance of each samples were determined, and the concentration of D-pinitol in each sample was calculated using the formula from the trend line equation of y = 0.0062 + 0.0759. The amount of D-pinitol accumulations showed high significant variation for seed samples collected from different woredas of West Harerghe zone. Accordingly, based on the absorbance measurements and concentration calculations evident that from seed samples obtained of West Harerghe zone, samples collected from Darolebo district was accumulated the highest amount of D-pinitol (501.3 µg/mL), followed by samples collected from Boke district (271.7 µg/ml) (Figure 5).

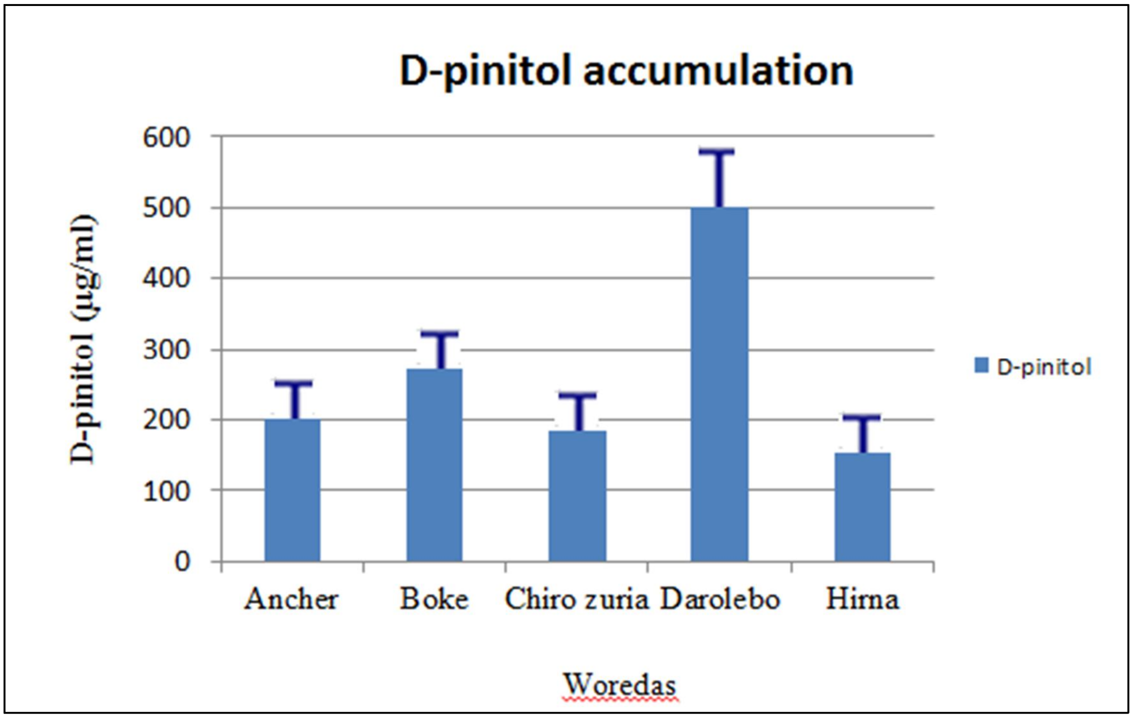


Figure 5. D-pinitol accumulation in common bean collected from West Harerghe

Similarly, three different common bean seed samples were obtained from East Harerghe and from the absorbance measurements and concentration calculations, evident that the seed samples collected from Meta district accumulated the highest amount of D-pinitol (457.9 µg/ml) followed by samples collected from Bedeno district (257.1 µg/ml) (Figure 5).

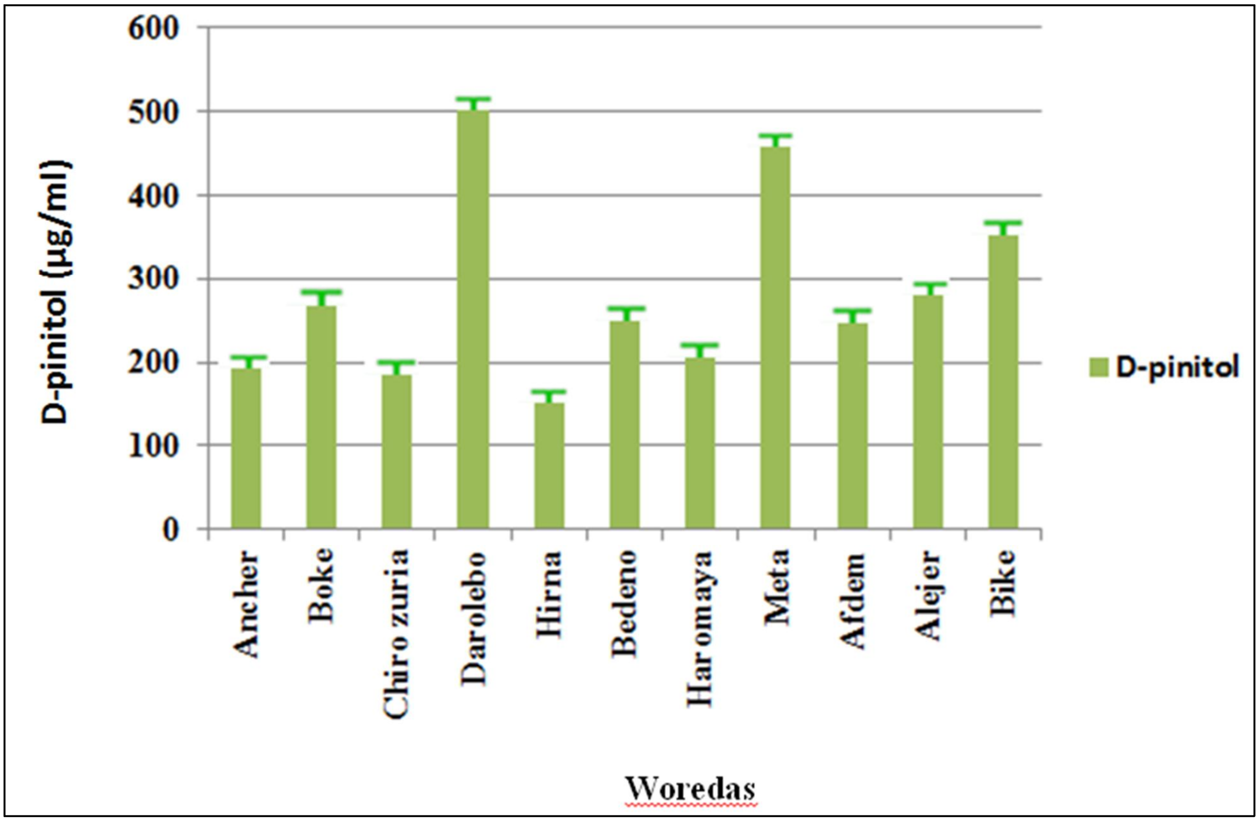


Figure 6. The D-pinitol accumulation of seed samples of common bean collected from Eastern Ethiopia (West Harerghe, East Harerghe and Somali zone)

Furthermore, three different samples were obtained from Somali regional state around Shinile (Figure 6) and the absorbance measurements and concentration calculations showed that seed samples obtained from Bike site was found to accumulated the highest amount of D-pinitol (355.0 µg/ml) and the samples found from Alejer was the second in the amount of D-pinitol accumulations (281.5 µg/ml) (Table 3).

Table 3. Comparison of the D-pinitol accumulation for samples collected from different regions of Ethiopia

Sample collection site	**Woreda**	**Amount of powder used for soaking (gm)**	**D-Pinitol (µg) accumulation**
West Harerghe (Chiro)	Ancher	100	200.2±0.05
	Boke	100	271.7±0.09
	Chiro zuria	100	184.5±0.26
	Darolebo	100	501.3±0.08
	Hirna	100	153.5±1.70
East Harerghe	Bedeno	100	257.1±1.32
	Haromaya	100	211.8±0.97
	Meta	100	457.9±2.38
Somali (Shinile)	Afdem	100	246.0±1.35
	Alejer	100	281.5±0.33
	Bike	100	355.0±0.78
Boji Chokorsa	Boji	100	432.7±0.02
	Bila	100	422.9±0.96
	Muklemi	100	375.3±0.02
	Lalo	100	613.2±0.05
	Gute	100	350.5±0.63
	Laga Gumbi	100	79.4±0.70
Nejo (West Wollega)	Gori	100	30.0±0.01
	Warajiru	100	725.8±43.04
	Najjo Abaya	100	322.1±3.76
	Nagoya hip-hop	100	111.0±0.35
	Alaltu	100	861.3±32.5
Gimbi (West Wollega)	Gimbi	100	468.7±3.08
	Bikiltu	100	461.4±5.71
	Chuta	100	550.5±12.4
Mendi (West Wollega)	Manasibu	100	457.7±2.24
	Kondala	100	323.9±0.48
	Sasiga	100	164.9±0.15
	Babo Gmbel	100	162.0±0.12
Benshngul-Gumuz	Assosa	100	79.4±0.07
	Sherkole	100	83.0±0.03
	Guba	100	314.8±0.65

When comparing the common bean samples collected from Eastern and Western Harerghe, it was observed that samples collected from Darolebo, West Harerghe showed the highest amount of D-pinitol (501.3 μg/ml). The next highest accumulation was by samples from Meta, East Harerghe (457.9 μg/ml). The seed samples collected from Hirna were found to accumulate the lowest amount (153.5 μg/ ml). Streeter *et al*. (2001) suggested that soybean seed genotypes collected different geographical regions accumulated various amount of D-pinitol. He further suggested that pinitol concentration in stressed Plant was 30–40% greater than in well-watered plants. According to Phang *et al* (2008), soybean plants accumulate metabolites such as D-ononitol (an intermediate of D–pinitol) that act as compatible solutes in response to osmotic stress caused by salt or drought to lower the cellular osmotic potential without affecting normal metabolic reactions.

In additions, twenty one different common bean seed samples were obtained from West Wollega zones including Boji Chokorsa, Nejo, Gimbi and Mand areas, and the result of absorbance measurements and concentration calculations showed that samples from Nejo, Alaltu area accumulated the highest amount of D-pinitol (861.3 μg/ml), while samples obtained from Warajiru (Nejo) and Lalo (Boji) deserved the second (725.8 μg/ml) and third (613.2 μg/ml), respectively among the West wollega collections. Tadele and Girmy (2018) explained that soybean plants had significantly higher pinitol contents in seeds, leaves and stems. Phillips *et al*. (1982) reported that D-pinitol content was obtained from seed samples of soybean. The Highest D-pinitol concentration is occurs in seed coats and lower in the axis and cotyledons of soybean seeds (Horbowicz and Obendorf, 1994).

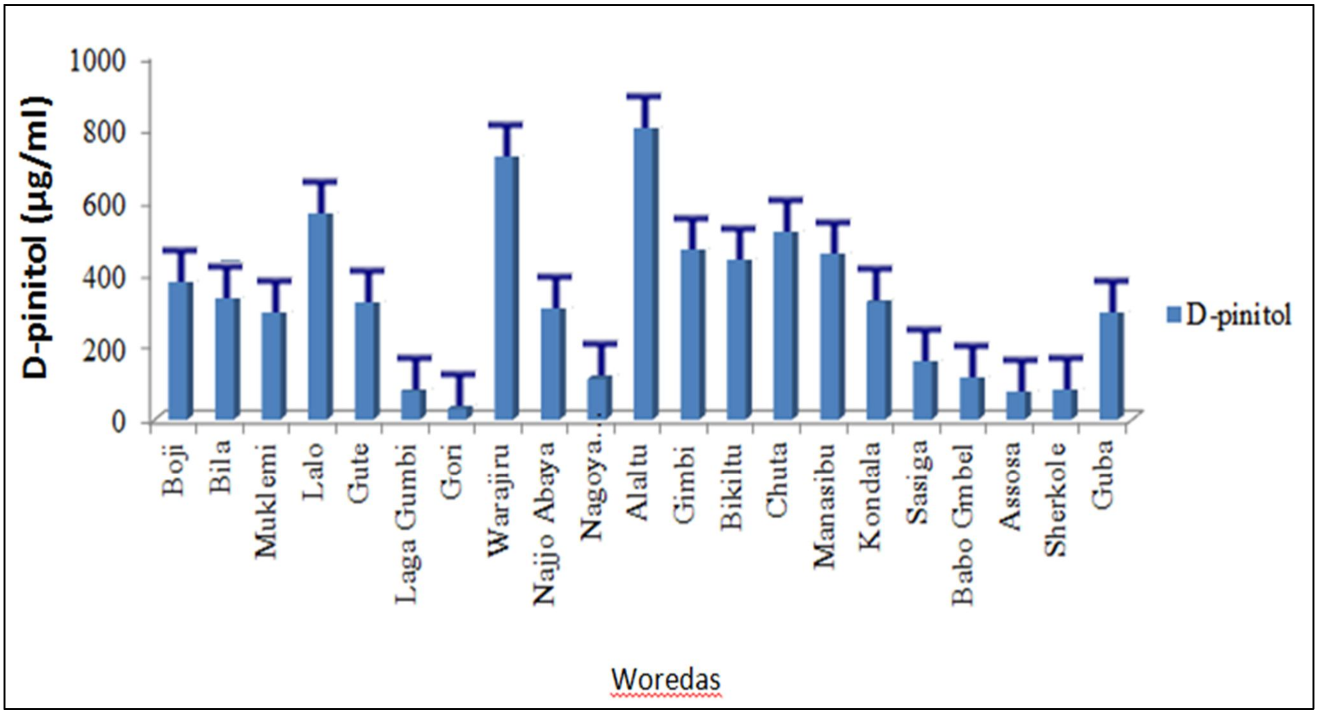


Figure 7. Shows the differences in D-pinitol accumulation of common bean collected from different regions of Oromiya

According to Table 3, three different seed samples of common beans were collected from Benshangul Gumuz, Assosa areas and showed that the D-pinitol accumulation was the highest amount for samples obtained from Guba district (314.8 µg/ml), whereas the samples obtained from Assosa district was accumulated the lowest amount of D-pinitol in its seeds (79.4 µg/ml).

5. Conclusions and Recommendation

5.1. Conclusions

D-pinitol is an important bioactive molecules that is an accumulate metabolites related to plant tolerance to drought stress. The result of this study revealed high and significant variation that the highest amount of D-pinitol accumulations was observed in samples collected from West Wollega, Nejo (861.3 μg/mL). The next highest accumulation was by Boji and Gimbi samples, respectively.

Comparing the seed samples of common bean collected from eastern part of Ethiopia (East Hererghe, West Hererghe and Somali) with that of the Western part (West wollega), it is evident that the samples collected from western parts of Ethiopia, particularly West Wollega (Nejo) accumulated the highest amount of D-pinitol.

5.2. Recommendations

The present study used only few common bean seed samples collected from limited locations. Further detail work and investigations may be needed to cover of the common bean growing areas.

6. References

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Approval of Investigators

We hereby declare that the research report entitled "**Assessment Diversity of Common Bean (*Phaseolus vulgaris* L.) for D-pinitol Accumulation Using Ethiopian Germplasm**" is our original work; all sources are duly acknowledged and the report is compiled by incorporating the necessary comments and suggestions given by the reviewers.

	Name	Signature	Date
Principal Investigator:	Dr. Hailemichael Tesso	___________	___________________
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Approval of Reviewers

I hereby confirm that (PI)Dr. **Hailemichael Tesso** has accomplished his/her work as per the approved proposal and incorporated all the comments given by the reviewers in his/her terminal report of the project entitled **Assessment Diversity of Common Bean (*Phaseolus vulgaris* L.) for D-pinitol Accumulation Using Ethiopian Germplasm** and hence the report qualifies for submission as standard research output.

	Name	Signature	Date
Reviewer 1.	__________________________________	___________	___________________
Reviewer 2.	__________________________________	___________	___________________

Approval: **School Ethical Review Board (School Scientific Committee)**

	Name	Signature	Date
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3.	________________________________	__________________	_____________
4.	________________________________	__________________	_____________