# GENETIC VARIATION IN BLACK GRAM AND GUAR FOR PHENOLOGY, PHYSIOLOGY, GROWTH, AND YIELD UNDER IRRIGATED AND RAINFED CONDITIONS OF NORTHERN GREAT PLAINS

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By

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#### Title

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#### ABSTRACT

Field trials were conducted in 2022 and 2023 to evaluate the feasibility of growing black gram (*Vigna mungo*) and guar (*Cyamopsis tetragonoloba*) and to identify superior accessions for semiarid conditions of Northern Great Plains, U.S. Significant genetic variability was observed among 21 black gram and 18 guar accessions under rainfed and irrigated conditions. Black gram accession PI425187 yielded the highest grain per plant (8.6 g) under rainfed conditions, while PI377397 and PI377406 performed best under irrigated (6.5 g). Number of pods per plant and seeds per pod were highly correlated with yield per plant. The highest guar yield per plant obtained were (8.1 g) in rainfed and (6.6 g) in irrigated conditions. These guar yields were, however, significantly below the yield reported in the southern great plains, U.S. The study demonstrates the feasibility of cultivating black gram in NGP conditions. More multi-location trials are recommended to identify superior accessions.

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## DEDICATION

This thesis is dedicated to my parents, brother, sister, and brother-in-law for their love,

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# LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BNF	Biological Nitrogen Fixation
CAGR	Compound Annual Growth Rate
IMARC	International Market Analysis Research and consulting group
N	Nitrogen
ND	North Dakota
NDAWN	North Dakota Agricultural Weather Network
NGP	Northern Great Plains
R <sup>2</sup>	Coefficient of Determination
U.S	United States of America
USDA	United States Department of Agriculture
WREC	Williston Research Extension Center

#### **1. INTRODUCTION**

Black gram (*Vigna mungo var. mungo (L.) Hepper*) and guar (*Cyamopsis tetragonoloba (L.) Taub*) are popular warm season semi-arid pulse crops belonging to the Fabaceae (Leguminosae) family. These two pulse crops have long been integral to Southeast Asian diets since ancient times. Guar has become mainstream in North America due to the use of guar gum in the fracking industry (Abidi et al., 2015), while black gram has the potential for use in plant-based protein and diversified industrial food products (Singh et al., 2018). Both legume crops can be used for different purposes in cropping systems including a cover crop, sole crop, intercropping and double crop.

They also possess other attractive features such as high nutritional value, easy digestibility, tolerance to drought and salinity, high market demand and more (Mudgil et al., 2014; Ravelombola et al., 2021; Nair et al., 2024). This would benefit the agricultural industry by allowing farmers to diversify their crop rotations, increase their profitability, and minimize the risk of crop loss. Furthermore, both crops have been relatively understudied in terms of genetic research compared to other crops. Commercial cultivation of guar and black gram faces several challenges due to limited information on specific agronomic practices, genetic variation within genotypes, as well as the lack of improved varieties (Pathak, 2015; Ghafoor et al., 2001). The assessment of genetic diversity among genotypes is crucial for optimizing the utilization of guar.

The overarching objectives of this research are to evaluate the feasibility of growing black gram and guar accessions in Northern Great Plains of the U.S. under rainfed and irrigated

conditions and to identify new specialty/leguminous cash crops and varieties that promote agricultural sustainability through increased crop diversification and farm income.

#### **1.1. Literature Review**

#### 1.1.1. Northern Great Plains

The Northern Great Plains (NGP) of the U.S. is known for its young and fertile soils, which are a hub for growing various crops such as grains, oilseeds, and pulses. This region includes most of Montana, North Dakota, South Dakota, and parts of Wyoming and Nebraska in the U.S., and it is spread into Canada, including Manitoba, Saskatchewan, Alberta, and British Columbia. The weather in this area has cold and dry winters, warm to hot summers, and varying precipitation. It covers over 52 million hectares of cropland and delivers more than \$92 billion in annual agricultural output (Shafer et al., 2014). NGP represents over 24.6% of the cropland area planted in the U.S. (Wienhold et al., 2018). It produces various crops such as canola (*Brassica napus L.*), sunflower (*Helianthus annuus L.*), barley (*Hordeum vulgare L.*), dry edible bean (*Phaseolus vulgaris L.*), wheat (*Triticum aestivum L.*), sugar beet (*Beta vulgaris L.*), corn (*Zea mays L.*), and soybean (*Glycine max (L.) Merr.*). Additionally, the region contributes to the cultivation of other crops, albeit in smaller quantities, including oats (*Avena sativa L.*), flax (*Linum usitatissimum L.*), safflower (*Carthamus tinctorius L.*), potato (*Solanum tuberosum L.*), and sorghum (*Sorghum bicolor L. Moench*) (Wienhold et al., 2018).

Recent agricultural trends in the eastern regions of the NGP indicate a noticeable transition toward increased corn and soybean cultivation, displacing traditional wheat production and grasslands (Wright & Wimberly, 2013). Croplands in the eastern parts of the NGP are rainfed, and conservation efforts target strategies to reduce runoff. In the central part of the Northern Plains, irrigation is widespread, driven by the Ogallala aquifer. Conversely, in the arid western regions of the NGP, crops are cultivated under dryland conditions, such as wheat-fallow systems. Notably, these areas often adopt no-till practices to reduce soil erosion (Hansen et al., 2012). In addition, irrigation in its western parts is facilitated through reservoirs fed by melting snow from nearby mountains, emphasizing the diverse agricultural strategies employed across the NGP to adapt to varying environmental conditions.

In the NGP, the traditional cereal-fallow system has been replaced by legume and oilseed-based systems (Hansen et al., 2012; Lupwayi & Kennedy, 2007). The cultivation of soybeans primarily concentrates in the southeastern region of the NGP, particularly in the subhumid areas of South and North Dakota. Dry peas, lentils, and chickpeas are predominantly produced in the semiarid regions of the NGP (Cutforth et al., 2007). Since 2011, there has been a notable expansion in the harvested area for these pulses due to increased demand for gluten-free food in both domestic and international markets. Montana has experienced a significant surge of 128 percent in the combined area for these pulses, whereas ND has witnessed an impressive increase of 274 percent (Bond, 2017).

#### **1.1.2.** Issue in Legume Production in the Northern Great Plains

North Dakota is the nation's leading producer of specialty crops such as pea (*Pisum sativum L.*), and dry bean (*Phaseolus vulgaris L.*) as well as the second-largest producer of lentil (*Lens culinaris Medik*) (USDA-NASS, 2023). These crops have played a vital role in promoting soil health, and farm income of North Dakotans. The historical data reveals considerable fluctuations in peas and lentil acreage in ND (Figure 1.1). One of the main reasons for such fluctuation in the acreage of these crops has been disease spread, mainly white mold (*Sclerotinia sclerotiorum (Lib.) de Bary*) and root rot caused by *Aphanomyces euteiches and Fusarium* 

*solani* which frequently became epidemic in NGP (Gossen et al., 2016; Biller & Draper, 2001; Kalil et al., 2020).

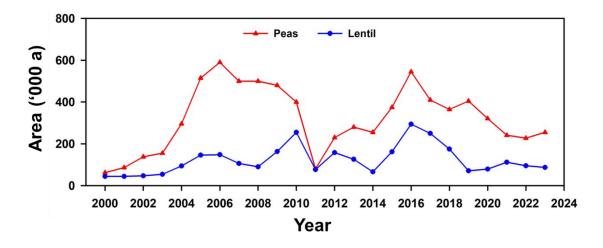


Figure 1.1: Pea and lentil acreage in ND (2000-2023).

White mold (*Sclerotinia sclerotiorum (Lib.) de Bary*) may result in yield losses of up to 60 % for pea and up to 35% for lentils (Antwi-Boasiako et al., 2022; Kahlon et al., 2018) leading to substantial economic losses, with annual estimates reaching \$12 million attributed to *S. sclerotiorum* in pulse crops (Ashtari Mahini et al., 2020). Data on the comparative efficacy of fungicides for the control of white mold on field peas and lentils is not currently available. The present recommendation for dealing with white mold is to have 4 to 5 years of rotations with resistant crops to prevent the buildup of sclerotia in the soil.

Root rot caused by a complex of Fusarium spp., can infect plants at any stage of their growth and cause yield losses in peas as high as 80% (Gaulin et al., 2007). To date, neither resistant cultivars of peas and lentils nor effective fungicides are available to combat root rot. In North Dakota, root rot in peas and lentils may be caused by *Rhizoctonia solani*, Fusarium species, *Aphanomyces euteiches*, and Pythium species (Kalil et al., 2020). Lentil and peas are recommended to be rotated every 3 to 4 years. However, if persistent *A. euteiches*-produced

oospores are present, it may be advisable to extend the rotation cycle to a duration of 6 to 8 years (Zitnick-Anderson et al., 2020).

Growers are seeking means to manage this disease or alternative non-sensitive crops. Thus, it is imperative to identify new legume crops and develop varieties that are not sensitive to these pathogens. Consideration should be given to the fact that for identified crops to be embraced by farmers, they must seamlessly integrate into existing crop rotations and be cultivable without requiring modifications or the purchase of new farm equipment.

The second important reason for the fluctuation in legume acres is global demand and supply. For example, following a shortfall in Indian lentil production called "Dal Crisis," lentil and pea production in 2015/16 in the USA increased by more than 50 % over the previous year (Bond, 2017). However, as speculated, Indian lentil and pea production returned to its normal status and crop acreage dropped significantly in 2019-2020 due to lower export demand. Lentil export volume to India declined from 154.6 million pounds in 2018/19, the second largest lentil export destination, to 16.1 million pounds in 2019/20 (Lucier & Davis, 2020). Several factors can contribute to legume price spikes and volatility, including reduced production due to unfavorable weather and a decrease in cultivated area as farmers shift to growing competing crops such as wheat, soybeans, and corn (Davis et al., 2022). However, a lack of crop diversity can lead to low profitability, disease susceptibility, soil degradation, and long-term sustainability. On the other hand, diversifying with specialty crops can help farmers decrease disease pressures, exploit new niche markets, reduce economic risks, and strengthen the domestic economy by reducing imports.

Drought is the most significant abiotic problem in the NGP, increasing from east to west t across North Dakota. Most crops in the western parts of North Dakota are cultivated under

dryland conditions. Although the soil in this region can be highly productive crop choice is limited because of low rainfall and a short growing season of 110-130 frost-free days (Daigh et al., 2021). With cold constraints in the early and late stages and potential threats of drought or heat in the middle of the growing season, one should be careful in selection and exploitation of new crop species.

#### 1.2. Black Gram

#### 1.2.1. Black Gram as Alternative Crop

Black gram, a short-duration pulse crop primarily cultivated in southern Asia during various seasons of rainfed and irrigated conditions, presents an advantageous option for farmers in the Northern Great Plains. Agronomic studies have shown that black gram is a suitable legume choice for various cropping systems, including intercropping, multiple cropping, sole cropping, and within crop rotations (Harisudan et al., 2009; Varathrajan et al., 2022; Paudel, 2016). Black gram, a warm season legume, is easily cultivable and can be mechanically planted and harvested. It shares common features with soybean, such as nitrogen fixation, which reduces the need for additional nitrogen application and can be machine-harvested using the same combine harvester (Singh et al., 2020). It can be produced in semi-arid to subhumid lowland tropics and subtropics (Mane et al., 2018). Black gram can thrive in lowlands and, under favorable conditions, can even grow from sea level up to 1800 m above sea level (Arora & Mauria, 1989).

Black gram, a short-duration and hardy legume, is stress-tolerant and well-suited for the summer cropping system, withstanding temperatures of up to 42°C (Banerjee et al., 2021). It is more salt tolerant than Vigna radiata (Raptan et al., 2001). Unlike soybean, black gram has relatively greater tolerance to excess water during germination and early seedling stages (Subbarao et al., 2001). With its dense vegetative growth and ground coverage, black gram

serves as an excellent cover forage, preventing soil erosion (Bochalya et al., 2022). Moreover, black grams contribute to soil health benefits, such as nitrogen acquisition from the atmosphere, adding nitrogen to subsequent crops. Farmers in semi-arid regions can successfully cultivate black gram under low moisture and fertility conditions. Its short growth cycle (70-90 days) (Nair et al., 2024) and low water requirements (Khorsand et al., 2021) make it an ideal crop for integration into farming systems in the NGP, U.S.

According to the findings of the IMARC Group, a leading market research company that offers management strategy and market research worldwide, the global black gram market reached 3 million metric tons in 2022 and is projected to exhibit a compound annual growth rate (CAGR) of 6.5% from 2023 to 2028. This growth trajectory is anticipated to lead to a market volume of 4.5 million metric tons by 2028 (IMARC Group, 2022). The report highlights a substantial opportunity for marketing black gram and indicates that several factors have contributed to the significant growth of the black gram market in recent decades. These include growing popularity of South Asian cuisine among Western consumers, the ever-growing world population and their changing dietary habits because of increasing health awareness, and the widespread utilization of black gram as both a green manuring crop and as nutritive fodder for dairy cattle. The demand for black gram in the food and beverage industry is also a key driver of its market expansion. Black gram has found extensive use in various food products such as dips, sauces, bread, curries, stews, bakery items, snacks, and soups. Food companies such as BAM and Swad incorporate black gram as an ingredient in their products such as pasta, papad, and snacks, further boosting its market presence and consumption.

#### **1.2.2. Black Gram in Sustainable Cropping System**

Climate change has a significant impact on agriculture, particularly through the increased frequency and severity of droughts and heat waves (Lesk et al., 2016). Climate change impacts can be heat stress, water stress, pests, and diseases, which can reduce crop yields and affect crop quality (Lobell et al., 2011; Daryanto et al., 2019). Cycles of drought are common in the NGP including North Dakota, with semi-arid conditions dominating the western half of the state. Many crops in North Dakota are under dryland conditions and therefore rely heavily on rainfall for soil moisture. Drought has resulted in considerable losses for the state's agriculture economy. The state incurred a \$223 million loss in 2002 because of drought-related crop losses (Jossi, 2002). The same drought in 2006 engendered agricultural damages of over \$425 million, with crop insurance indemnity payments totaling around \$309 million. The state pays an estimated \$228 million in losses each year due to drought conditions (NCSL, 2008). In North Dakota, there are reportedly nearly 302,000 acres of yearly active irrigated land. This is only 1.1% of the state's total cultivated land limited the utility of irrigation to mitigate climate impacts.

Black gram is a heat and drought-tolerant warm season legume crop, which will be helpful in agricultural adaptation to climate change (Jothimani & Arulbalachandran, 2020). Its capacity to withstand drought is evidenced by its ability to maintain higher mid-day leaf water potential, leaf area, photosynthesis rate, leaf chlorophyll, and stomatal conductance, all of which collectively contribute to enhanced seed yield (Baroowa et al., 2015). Moreover, it can thrive in arid and semiarid areas and is adaptable to various soil types, making it suitable for cultivation in diverse environments (Dwivedi & Singh, 2020). Black gram is a good fit in diverse cropping systems, particularly with water-intensive crops such as rice and wheat fallows, due to its attractive features such as short duration, nitrogen fixation, and relative drought & heat tolerance.

Commonly, farmers intercrop black gram with corn (*Zea mays L.*), sorghum (*Sorghum bicolor L. Moench*) cotton (*Gossypium hirsutum L.*), pigeon pea (*Cajanus cajan (L.) Huth*), and rice (*Oryza sativa L.*) to improve soil fertility by lowering the leaching of nutrients, minimizing pest and disease incidence, weed control, soil erosion, and enhancing yield of main crops (Nair et al., 2023; Harisudan et al., 2009).

Black gram can be used to improve soil fertility as an effective green manure option even in scenarios where the crop fails due to various factors, be it biotic or abiotic, or when growers opt against harvesting due to unfavorable market prices. It has desirable characteristics such as rapid growth, short duration, early initiation of biological nitrogen fixation (BNF), high nitrogen accumulation rate, a wide range of ecological adaptability, timely release of nutrients, photoperiod insensitivity, and most importantly, ease of incorporation. Vigna species at an age of 45 days have been found to fix approximately 75 kg of nitrogen per hectare (Meena et al., 2018). The crop referred to as a "mini-fertilizer factory" possesses a remarkable capacity to maintain and enhance soil fertility through the process of fixing atmospheric nitrogen in symbiosis with rhizobium bacteria residing in root nodules. Nonetheless, the efficiency of nitrogen fixation in black gram exhibits considerable variation, which is contingent on factors such as host genotype, rhizobia efficiency, soil, and climatic conditions. Previous studies have shown a range of nitrogen fixation rates, such as 36 kg ha<sup>-1</sup> (Lengwati et al., 2020), 125 to 143 kg ha<sup>-1</sup> (Gopalakrishnan et al., 2015), and 273 mg N plant<sup>-1</sup> (Senaratne & Ratnasinghe, 1993).

#### **1.2.3. Black Gram's Health Benefits and Uses**

Many sectors of U.S. agriculture are keenly interested in the rise of new plant-based protein sources, including analogs of meat, egg, and dairy (Tonsor et al., 2022). Pulses, which include beans, lentils, and chickpeas, are particularly noteworthy for their high protein and low-

fat contents. Additionally, they are rich in fiber, folate, iron, and potassium, all of which are nutrients that are often under-consumed in the U.S. (Winham et al., 2022; Havemeier et al., 2017). Among pulses, black gram stands out as a popular and nutritious option, containing 25g of protein, 58g of carbohydrates, 983 mg of potassium, 216 mg of folate, and 7.5 mg of iron per 100g (USDA-ARS, 2021).

Black gram is consumed as a whole grain, dehusked cotyledons, or milled flour. Dehusked cotyledon is a crucial ingredient in fermented dishes such as idli and dosa. It is also used in non-fermented meals such as cooked dal, hopper, papad, and waries (spicy hollow balls) (Batra & Millner, 1974). Milled flour can be used as a raw material in the food industry, especially for snacks and cookies. Black gram soup possesses medicinal properties, healing health issues such as dyspepsia and gastric conditions (Suvan et al., 2020). It can replace eggs in cake mixtures and baked goods due to its effective foaming functionality (Joseph et al., 1993). In addition, black gram is widely used in treatments related to beauty and wellness. It also substitutes for soap, resulting in soft and smooth skin, and is utilized in cosmetic formulations, particularly in the preparation of facial masks (Zia-Ul-Haq et al., 2014). Whole black gram flour pastes are often employed for skin and hair treatments, either alone or in combination with sandalwood or fenugreek paste.

Black gram is a valuable dietary source due to its high fiber content and low glycemic index. Research has shown that black gram consumption can help modulate lipid homeostasis and blood glucose control, particularly in individuals with diabetes mellitus and those who consume a high-saturated-fat diet. Black gram inhibits  $\alpha$ -amylase, an enzyme responsible for carbohydrate digestion, leading to delayed carbohydrate absorption and lower peak postprandial plasma glucose concentrations (Kaur et al., 2015).

#### **1.2.4. Botany**

Black gram (*Vigna mungo var. mungo (L.) Hepper*), a member of the Fabaceae (Leguminosae) family, goes by several names such as black lentil, white lentil, urad bean, and black matpe bean. It has chromosome number 2n = 2x = 22 and it is a self-pollinated pulse crop. It is highly valued in the Indian subcontinent and is extensively cultivated in South and Southeast Asian countries such as Afghanistan, Bangladesh, India, Pakistan, Nepal, Myanmar, the Philippines, Sri Lanka, and Thailand (Kaewwongwal et al., 2015). The domestication of black gram is believed to have occurred in India, where it was derived from the wild black gram, *Vigna mungo (L.) Hepper var. silvestris Lukoki, Marechal & Otoul* (Chandel et al., 1984).

This densely hairy annual legume grows upright, sub-erect, or trailing. Modern cultivars of black gram have been developed with erect and semi-erect plant types. It possesses a well-developed taproot and widely branching stems. Two distinct pod orientations, namely main stem bearing and sympodial bearing, are observed in black gram. The plant attains a height of 35 to 50 cm (Beniwal & Tomer, 2019). The inflorescence emerges on the peduncle and bears small, yellow, papilionaceous flowers. It has large trifoliate hairy leaves that are either ovate or lanceolate in shape. The pod is cylindrical, erect, 4-7 cm long, 0.5 cm broad, with a short, hooked beak and hairy. The color of black gram pod is black. It contains 4-10 ellipsoid black or mottled seeds (Heuzé et al., 2016). However, three different pod colors have been reported: black, brown, and straw color, with black being the most prominent and dominant over the straw and brown pod colors (Nair et al., 2024). Black gram contrasts with mung bean (*Vigna radiata L.*) by producing shorter, stout, immensely hairy pods and larger oblong seeds that range from blackish to olive green in color.

#### **1.3.** Genetic Diversity Studies in Black Gram

Evaluation of the feasibility of cultivating a crop in a specific environment and improving grain yield under various biotic and abiotic stresses necessitates the examination of genetic diversity within germplasm collections. This involves analyzing inheritance patterns of key morphological and agronomic traits. The primary limitation of black-gram genetic improvement is the narrow genetic diversity among genotypes. This is mostly due to the consistent use of a few closely related parents in breeding programs (Jayamani & Sathya, 2013). Understanding the nature and extent of genetic variability present in quantitative traits is a key factor in yield improvement success (Priya et al., 2021). Additionally, evaluating the relationship between yield and other yield attributes will aid in selecting the best genotypes. Evaluation of the extent of genetic diversity between genotypes would facilitate efficient use of genetic variation in agronomic and genetic improvement programs for black gram (Ghafoor et al., 2001).

#### 1.3.1. Genetic Variation in Agronomic Traits among Black Gram Genotypes

There have been studies on genetic diversity in black gram for agronomic and morphological traits. However, these experiments were predominantly carried out in India, Pakistan, and other South Asian countries, given that black gram is primarily cultivated in that region of the world. Reddy et al. (2022) evaluated 8 black gram varieties for agronomic traits under semi-arid conditions and reported significant differences among the black gram varieties for all the growth and yield attributes. Out of the eight black gram varieties, T-9 showed the highest growth attributes, followed by LBG-904 and LBG-752. On the other hand, LBG-904 had the highest yield attributes, followed by TBG-129, PU-31, VBN-8, and T-9. Another study was conducted by Singh et al. (2021) to evaluate 12 black gram genotypes for agronomic traits. The results showed no significant differences among genotypes for any of the traits except for 100 seed weight. Baral et al. (2022) reported a large variation in various agronomic traits among 10 genotypes in Nepal. Variations in plant height at maturity, number of days to reach 50% flowering, number of primary branches, length of branches, number of pods bearing peduncles, length of the longest peduncle, length of pods, number of seeds per pod, 1000 seed weight, and grain yield per plant were identified. Ghafoor and Ahmad (2005) evaluated 111 genotypes of black gram for agronomic traits and found that days to flowering, days to maturity, number of branches per plant, number of pods per plant, biomass per plant, grain yield per plant, and harvest index displayed high genetic variance. In contrast, pod length, number of seeds per pod, and 100-seed weight exhibited low genetic variance. Panda et al. (2017) found significant differences in yield traits in 50 black gram genotypes, including released and selected varieties, elite cultures, and promising local varieties during the 2015-2016 rabi season (winter) in Odisha.

#### **1.3.2.** Physiological Responses of Black Gram Genotypes

Chlorophyll content plays a significant role in the photosynthetic capability of plants. As the intensity of drought stress increases, plants experience a rapid loss of leaf chlorophyll content (Kiani et al. 2008). The decrease in chlorophyll content in leaves under drought stress may be caused by various factors such as excessive swelling of chloroplast membranes, distortion of lamellae vesiculation, and the appearance of lipid droplets (Zaeifizade & Goliov, 2009). Furthermore, Baroowa and Gogoi (2013) observed that the total chlorophyll content decreases with the duration of drought stress in black gram. Another study conducted by Gurumurthy et al. (2019) found that no significant differences in chlorophyll content among 10 black gram genotypes under well-watered (control: 66% of field capacity) and water stress conditions (stress: 40% of field capacity). Kumar Joshi et al. (2021) reported a statistically significant

difference in chlorophyll content and canopy temperature among 11 black gram genotypes under water stress and irrigated conditions. They found that one genotype, IC426766, had lower SPAD readings and higher canopy temperatures when irrigated, but higher SPAD readings and lower canopy temperatures when drought conditions experienced.

#### **1.4. Guar**

#### **1.4.1. Guar as Alternative Crop**

Guar is a deep-rooted legume that integrates well into crop rotation systems with other crops such as wheat (Shrestha et al., 2023). Guar does not shatter, stands well in the field, and has a high seed yield combined with favorable plant architecture that makes it easy to mechanically harvest (Reis et al., 2021), as well as cultivate and plant using existing farm equipment (Tripp et al., 1977). Farmers in arid and semi-arid regions can successfully grow and cultivate guar even in degraded soils with poor fertility and limited rainfall, as guar does not demand high inputs and intensive management needs (Abidi et al., 2015).

The Northern Great Plains exhibit diverse climatic conditions; however, recent trends indicate a shift towards warmer and drier weather, characterized by high temperatures, and reduced precipitation, being deep rooted legume, hardy, fast growing, short duration, soil improving and drought tolerant, guar can easily integrate into a summer cropping system (Alexander et al., 1988, Tripp et al., 1977).

Guar is tolerant to soil salinity, and alkalinity (MacMillan et al., 2021). Disease risks are relatively low for the cluster bean grown in arid and semi-arid regions. There are only two major diseases that affect guar, Alternaria leaf blight (Target spot), and Bacterial leaf blight. Alternaria leaf blight is caused by fungus, *Alternaria tenuissima Samuel Paul Wiltshire*, that occurs with repeated days of cool and wet weather. It can be controlled with timely fungicide applications.

Bacterial leaf blight is a seed disease that can cause the plant to begin premature defoliation. It is caused mainly by contaminated seeds which can be greatly reduced if steps are taken to purchase seeds free of contaminants. However, guar variety 'Brooks' is high yielding and resistant to Alternaria leaf spot and Bacterial leaf blight (Tripp et al., 1977).

Guar is economically beneficial as a multipurpose crop. Depending on the cultivation region, all plant components are used. Guar can be grown for vegetables, gum, feed, forage, cover crop and green manure to build organic matter in soil (Abidi et al., 2015). Industrial use is popular in the U.S., whereas vegetable use and cattle feeding are prevalent in Asian countries. Guar is becoming increasingly popular as an industrial with strong demand due to increased demand for guar gum globally and domestically. Total worldwide guar production is estimated to be around 3.4 million metric tons per year (Ravelombola et al., 2021). India contributes approximately 80% of the world's guar production, followed by Pakistan (15%), while the remaining 5% is produced in the U.S. (specifically northern Texas and southwestern Oklahoma), Australia, and South Africa (Gresta et al., 2014).

Furthermore, the global guar gum market is expected to grow at a compound annual growth rate of 6.9% between 2021 and 2028, reaching a market value of USD 1111.8 million by 2028, up from USD 787.6 million in 2019 (Research Nester, 2022). There are reports suggesting that guar gum can be produced at costs lower than U.S. import prices. It was found that producing guar gum at a cost as low as \$0.45 per kg is below the five-year average U.S. import price of \$0.99 per kg and the minimum selling price of \$1.44 per kg for guar gum (Antonanzas et al., 2023; Summers et al., 2021). This presents a significant opportunity for farmers to explore new niche markets, reduce economic risks, and strengthen the domestic economy by minimizing imports.

#### 1.4.2. Guar in Sustainable Cropping System

Guar is one of few deserts crop capable of withstanding the impact of hotter and drier weather, as well as limited rainfall during the growing season. It integrates well into multiple cropping systems and is highly demanded as an industrial cash crop. With low input and management requirements, guar has significant economic benefits to farming systems overall (Abidi et al., 2015). The severe drought in Texas had a significant impact on the agriculture sector, resulting in a staggering \$7.62 billion revenue loss in 2011 (Ray et al., 2018). Despite challenges, guar managed to achieve profitable production during that period.

Guar is a low CO<sub>2</sub> emission crop, emitting less than 3000 kg of CO<sub>2</sub> eq. ha<sup>-1</sup>, in comparison to other crops such as potato (5428 kg CO<sub>2</sub> eq. ha<sup>-1</sup>), corn (5796 kg CO<sub>2</sub> eq. ha<sup>-1</sup>), pea (3209 kg CO<sub>2</sub> eq. ha<sup>-1</sup>), soybean (3999 kg CO<sub>2</sub> eq. ha<sup>-1</sup>), and faba bean (3217 kg CO<sub>2</sub> eq. ha<sup>-1</sup>) (Greta et al., 2014; Köpke & Nemecek, 2010). Its environmental sustainability and economic viability make it a good option for diversification in the cropping system. Guar is a deep-rooted legume considered a soil-improving crop with biological nitrogen-fixing properties (34 to 54 kg N ha<sup>-1</sup>) (Shrestha et al., 2023). As a legume, guar can be used for green manure, even if the main crop fails due to biotic or abiotic factors, or if the producer decides against harvesting due to low prices. Additionally, it serves as a cover crop during fallow periods. In fact, guar was introduced in the U.S. in 1903, specifically for purposes such as green manure and cattle feeding (Abidi et al., 2015).

Guar is known to form nodules with rhizobia, and according to Stafford and Lewis (1980), the application of rhizobium inoculant to guar seeds in field conditions in Northern Texas resulted in a 36% increase in nodules per plant compared to seeds left uninoculated. This increase was accompanied by a rise in plant productivity. The researchers noted that the number

of nodules varied depending on the timing of observation, with more nodules observed at 8 weeks after planting compared to observations at 4 or 12 weeks. During the 8-week observation period, the number of nodules per plant ranged from 0.5 to 8.9. Interestingly, water stress did not impact nodule number; however, there was a significant reduction in nodule fresh weight (Venkateswarlu et al., 1983). Despite water limitations, guar demonstrated the ability to nodulate and effectively fix nitrogen.

Guar is commonly used in crop rotation with cotton, grain sorghum, small grains, vegetables, and flax due to its ability to improve soil health and enhance soil fertility by fixing atmospheric nitrogen. Crops following guar in rotation are likely to experience 15% yield increase (Tripp et al., 1977). Acharya (2020) found that guar-cotton rotations generate an average net return of \$319.70, with a low likelihood of losing money at 10.70%. These findings suggest that incorporating guar as a primary crop in a rotation system could significantly increase farm profitability, resource use efficiency, and long-term sustainability.

#### **1.4.3. Botany**

Guar (*Cyamopsis tetragonoloba* (*L.*) *Taub*), commonly known as calcutta-lucerne, cluster-bean, and siam-bean, is a summer annual legume crop belonging to the pea family (Fabaceae) (USDA-NRCS, 2020). The cluster bean is autogamous and derives its name from the ability to bear pods in clusters. Guar is a self-pollinated crop with 2n=14 chromosomes (Boghara et al., 2016). The genus *Cyamopsis* comprises four species: *C. tetragonoloba* (*L.*) *Taub., C. serrata Schinz, C. senegalensis Guill & Perr, and C. dentate Tarre*, an interspecies hybrid of *C. serrata* and *C. senegalensis* (Gaikwad et al., 2023). Cultivated (*Cyamopsis tetragonoloba*) is primarily cultivated in India and Pakistan for human consumption and animal fodder. However, *C. tetragonoloba* was domesticated from the wild African species *C. senegalensis* and later introduced to the South Asian subcontinent by traders between the 9th and 13th centuries A.D (Mudgil et al., 2014).

The plant exhibits both indeterminate and determinate growth habits, with erect, suberect basal branching. Some erect genotypes have zero to two branches (Reis et al., 2021). A peculiar characteristic of guar is its asynchronous flowering and pod-setting, occurring from four to six weeks after seedling emergence until the plant's death. Guar flowers are purple, pink, or mixed, about 8 mm long, and form in axillary racemes (Mudgil et al., 2014). It shows variation in plant height, ranging from 0.4 to 3.0 m, and maturity range from 70 days (for determinate genotypes) to 190 days (for indeterminate genotypes) (Gresta et al., 2016). The color of guar species seeds is in light-grey, pink, white, or black colors.

#### 1.4.4. Significance of Guar Gum

The guar seed consists mainly of three layers, the seed coat (16–18%), the germ (43–46%), and the endosperm (34–40%) (Kumar et al., 2017, Mudgil et al., 2014). The germ portion is mainly composed of protein, while galactomannan, a polymer made up of mannose and galactose present in the endosperm, is a key reason for guar cultivation (McCleary et al., 1985). Guar gum typically contains 75% soluble fiber, 7.6% insoluble fiber, 2.16% crude protein, 0.78% total lipids, 0.54% ash, and 9.55% moisture (Srinivasan, 2020). Although guar gum may not provide essential nutrients, its low-calorie content and high fiber content contribute to a feeling of satiation (Tahmouzi et al., 2023). Its insoluble fiber content improves digestion, as it absorbs water in the intestines, producing a mild laxative effect, avoiding constipation, reducing diverticulitis and colon cancer (Saeed et al., 2012). Additionally, guar gum can help regulate blood glucose levels and reduce the glycemic index after a high-carbohydrate meal by binding to starch in the intestinal lumen, thereby delaying glucose release and slowing starch breakdown

(Zeece, 2020). Furthermore, it contributes to the easing of irritable bowel syndrome symptoms, prebiotic effects, and enhances the production of short-chain fatty acids (Souza et al., 2023).

Guar gum possesses multifunctional properties, including thickening, emulsifying, binding, and gelling capabilities, solubility in cold water, wide pH stability, film-forming ability, and biodegradability (Thombare et al., 2016). That is why it has a significant demand in diversified industries, such as food, paper, cosmetics, pharmaceuticals, oil well drilling, explosives, and the textile industry (Tahmouzi et al., 2023; Garcia et al., 2023). Guar gum, due to its viscous colloidal dispersions, can be used as colon specific drug carrier in the form of coating and matrix tablets (Prabaharna, 2011). Guar gum is a common additive to stabilize, emulsify, and thicken various products, such as body lotions, yogurt, canned coconut or almond milk, and soups, enhancing their texture and overall quality. Guar meal is a byproduct obtained after the extraction of galactomannan, which is rich in protein, may be excellent supplement additive for livestock, broilers, and fish. However, it contains saponins. These compounds have been studied for their antibacterial and anti-protozoal activities, and feed ingredient in poultry feed (Saeed et al., 2017; Gresta et al., 2016; Nidhina & Muthukumar, 2015).

The market expansion of guar gum and its derivatives is mainly driven by the oil and gas industries, where they are widely used in various applications, including drilling fluid formulations, dispersants, and green corrosion inhibitors (Research Nester, 2022). Guar gum is commonly used as drilling fluid in hydraulic fracturing operations. Fluid is highly viscous and has excellent proppant-carrying properties, which means it can easily transport the sand or other materials used to prop open the fractures into rock (Zhang et al., 2022). The presence of sand in the cracks prevents collapse, creating porosity that enables the smooth flow of oil and natural gas through the fractures, ready for extraction to the surface (Mudgil et al., 2014). The U.S. produced

over 5 million barrels of crude oil per day in 2008, with roughly 10% of that amount coming from hydraulic fracturing. By 2015, however, output had increased to slightly over 9 million barrels per day, with 51% of that production coming from hydraulic fracturing (Thapa et al., 2018). That has led to U.S. as biggest consumer and importer of guar gum in the world.

#### **1.5. Genetic Diversity Studies in Guar**

Germplasm evaluation is an essential process aimed at identifying and selecting genotypes with high yield potential, wide adaptability and genotypes showing superior resistance to heat, drought, and salinity. It also involves pinpointing desirable sources of resistance to diverse diseases and pests. Guar has not been studied as extensively in terms of genetics and agronomics as other crops. The commercial cultivation of guar is facing difficulties due to a lack of efficient plant type, longer growth period, limited information on specific agronomic practices, and a shortage of improved varieties (Pathak, 2015). Its cultivation is limited to certain areas and regions. The drought-resilient characteristics of guar, coupled with current US market needs, make it a strong candidate for a new alternative agricultural crop; however, there is a lack of research on different climatic environmental condition and the expansion of guar cultivation to different regions in the U.S.

To improve guar breeding programs, traits such as higher seed yield, disease resistance, and early maturity are necessary (Kumar et al., 2017). By exploring genetic diversity and selecting for these traits, we can make significant strides in improving guar.

#### **1.5.1.** Genetic Variation of Agronomic Traits among Guar Genotypes

Research on germplasm evaluation, focused on yield and yield-attributing traits, has been conducted on guar. Many studies report a high level of genetic diversity for growth and yield traits, such as plant height, flowering days, pod clusters, branching, pod length, seed number per

pod, number of pods per plant, and yield per plant, along with morphological traits (Jukanti et al., 2015; Singh et al., 2021; Boghara et al., 2016; Kgasudi et al., 2020; Santhosha et al., 2017; Vir & Singh, 2015). Morris (2010) characterized 73 accessions collected from India, Pakistan, and the USA, reporting significant genetic variability in pod length (32 to 110 mm), 100 seed weight (2.3 to 4.8 g), seed number per accessions (80 to 9358) and number of days to 50% maturity (96 to185 days). Plant height and branches per plant are crucial traits due to their role in bearing more clusters and branches. Ramanjanevulu et al. (2018) evaluated 6 guar genotypes for agronomic traits under rainfed conditions in Telangana, India. He observed significant variations in yield and its yield component except for 1000 seed weight and concluded that plants with short height, more no. of branches, clusters and pods per plant are the important traits to be given priority to develop high yielding cultivars. Another study by Manivannan et al. (2015) observed a large variation for days to maturity, plant height, pods per plant and cluster per plant among 42 guar accessions in India. Comparatively, low variation was noticed for seeds per pod, 100-seed weight, secondary branches per plant, pod length, pods per cluster and days to 50 percent flowering. Further, he concluded that variability found among guar accessions can be attributed to the genetic differences and the environment in which these accessions were grown. Similarly, Kumar et al. (2013) evaluated 23 released and elite genotypes of guar from different parts of India and revealed that the presence of ample amount of variation in cotyledon size, root length, hypocotyl length, epicotyl length, pubescence, plant height, leaf margin, branching habit, growth habit, days to 50 % flowering, days to maturity, flower color, pod size, number of pods per cluster and number of clusters per plant for all the 23 genotypes. Khalid et al. (2017) evaluated 100 accessions of the guar collected from the guar growing areas such as Punjab, KPK, Sindh, and Baluchistan of Pakistan. He found a large variation in germination percentage, days to 50%

flowering, plant height, branches plant per plant, clusters plant per plant, days to maturity and grain yield per plot. To enhance seed yield in guar, effective parameters for selection may include pods per plant, branch numbers, plant height, and 100-seed weight. Pathak (2011) found seed yield was significantly and positively correlated with plant height, seeds per pod, pods per plant, and primary branches per plant. Similarly, the number of seeds per pod, number of pods per plant, number of pods per cluster, number of clusters per plant, days to 50% flowering, and days to maturity showed positive and significant correlations with seed yield per plant (Vir & Singh, 2015). However, all this germplasm research has been concentrated on warm dryland climatic conditions. Thus, there is still much to explore to enhance our understanding of guar genetic variability in the NGP climatic environment.

#### 1.6. Summaries

- The Northern Great Plains of the U.S. is characterized by cold dry winters and mild to hot dry summers with varied rainfall.
- Pulse acreage in North Dakota fluctuated significantly from year to year due to factors such as disease pressure in peas and lentils, necessitating crop rotation every 4-5 years, as well as global demand and supply dynamics.
- It is imperative to identify new pulse crops to ensure the sustainability of agriculture in North Dakota.
- Black gram and guar are two pulse crops with considerable economic, nutritional, and soil health benefits, and they could be integrated into the existing cropping pattern.
- Two studies were undertaken, given below, to assess the feasibility of growing black gram and guar under rainfed and dryland conditions in the Northern Great Plains.

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# 2. GENETIC VARIATION IN BLACK GRAM FOR PHENOLOGY, PHYSIOLOGY, GROWTH, AND YIELD UNDER IRRIGATED AND RAINFED CONDITIONS OF NORTHERN GREAT PLAINS

#### 2.1. Abstract

Black gram (*Vigna mungo*) is a drought tolerant and short duration pulse crop. Field trials were conducted to evaluate the feasibility of growing black gram and identify superior accessions for semiarid conditions of Northern Great Plain U.S. Twenty-one black gram accessions were seeded at the NDSU Williston Research Extension Center's rainfed and irrigated sites. The experimental design was an alpha lattice design with four replications. The interaction effect of year × accessions was observed on plant stand, live seed emergence, days to  $1^{st}$  flowering in both conditions. Black gram accession PI425187 yielded the highest grain per plant (8.56 g) under rainfed conditions, while PI377397 and PI377406 performed best under irrigated (6.5 g). Pods per plant and number of seeds per pod were highly correlated yield per plant. The study demonstrates the feasibility of growing black gram in NGP conditions. More multi-location trials are recommended to identify superior accessions.

## **2.2. Introduction**

Black gram (*Vigna mungo var. mungo (L.) Hepper*), also known as black lentil, white lentil, and black matpe bean, belongs to the fabaceae family. Black gram, a short-duration pulse crop primarily cultivated in southern Asia during various seasons of rainfed agro-ecosystems, presents an advantageous option for farmers in the Northern Great Plains. Because of early maturity, farmers can harvest black grams earlier than corn and soybean (Singh, 2018). It thrives within an ideal temperature range of 25°C to 32°C; however, it can withstand temperatures up to 42°C, further facilitating its optimal growth and development (Banerjee et al., 2021). It can be used as a part of double-cropping, as a standalone full-season crop in case of early crop failure, as a high-value cash crop, or as a special cover crop (Singh, 2018). It fixes nitrogen from the air into the soil, can be used as a green manure crop, and possesses a deep root system and higher foliage cover compared to peas and lentils. These attributes effectively control soil erosion and compete with weeds. Black gram is a good source of high protein (25 g) and low fat (1.3 g). It also contains phosphorous (345 mg), folate (216 mg), and iron (8.7 mg) per 100g, all of which are low in consumption in the U.S. (Nair et al., 2024; Winham et al., 2022; Havemeier et al., 2017). It is consumed either as whole grain or as dehulled splits or as flour. Flour is commonly used in making various south Indian fermented food products such as dosa, idli, and other products like making dal, curries, sweets, and snacks (Sunil et al., 2018). Its seed may be used in the food industry as a functional food and nutraceutical, as well as in the cosmetic and pharmaceutical industries (Nair et al., 2024).

Black gram is drought tolerant legume crop. However, it is most sensitive to water stress during critical stages such as the late flowering and early pod-filling stages, affecting yield and yield-attributing characteristics (Nilanthi et al., 2015; Nair et al., 2024). Drought has different effects on black gram, depending on the growth stage. Baroowa and Gogoi (2016) found that drought had the greatest negative impact on black gram yield during the early reproductive stage, whereas plant height, leaf area, and leaf number were most affected during the vegetative stage. The writer argued that when plants experience water scarcity, their ability to exchange gases in their leaves is disrupted. This disruption has several negative consequences, including limiting the size of the plant's source and sink tissues, impairing phloem loading, assimilate translocation, and dry matter partitioning. These effects lead to a decline in crop productivity. Similarly, Huang et al. (1975) reported that water stress may be observed as either a direct physiological result of

dehydration or an indirect effect on a plant's activity through a lower assimilate supply for biological N<sub>2</sub> fixation.

Another study conducted by Nilanthi et al. (2015) revealed that vegetative-stage stress significantly limited plant height, wet and dry weight of shoots and roots, and root length. Reproductive-stage stress, on the other hand, notably decreased the number of pods, wet and dry weight of pods, and wet and dry weight of seeds. Pandiyan et al. (2017) found that under water stress, three black gram genotypes experienced reduced plant height and lack of branches in most plants. The few plants that did have branches produced shorter and unproductive branches. Furthermore, there was a significant decrease in the number of pods per plant, pod length, and number of seeds per pod. Additionally, the hundred seed weight, single plant yield, and dry matter production were all lower under irrigated conditions. Anitha et al. (2015) found that imposing water deficit stress during the flowering stage of black gram genotypes resulted in a decrease in yield attributes such as pod number per plant, pod weight, seed number, and seed weight. However, there was significant variation among 17 black gram genotypes. Furthermore, the study revealed that higher yields and yield parameters were recorded under well-irrigated conditions than under moisture-stressed conditions.

Black gram yield under irrigated areas may range from 1000 to 1300 kg ha<sup>-1</sup> as compared to 700 to 900 kg ha<sup>-1</sup> under dryland conditions depending on climate, soil, genotypes, and specific management practices. The water requirement of black gram during its crop growth ranges from 400 to 500 mm, depending on varieties and agro-climatic conditions (Ray et al., 2023). However, excessive water can lead to the development of more branches and leaves in black gram, prolonging the vegetative period even after flowering, which results in a reduction of seed yield (Kumar et al., 2018). Therefore, it is necessary to provide optimum water according to

the requirements of black gram. In the case of black gram, limited research on germplasm evaluation has been conducted under irrigated conditions. In India and Pakistan, black gram is primarily grown during the kharif season (June to October), coinciding with high rainfall. However, Aman and Singh (2022) found high genetic variation among genotypes in terms of the number of clusters per plant, number of primary branches per plant, number of pods per plant, and grain yield per plant under irrigated conditions. They observed positive and significant correlations between grain yield per plant and several factors, including the number of primary branches per plant, number of clusters per plant, number of pods per cluster, number of pods per plant, number of seeds per pod, pod length, 1000 grain weight, biological yield, and harvest index.

However, this evaluation of genotypes or germplasms research mainly focuses on the climatic conditions and adaptability of India, Pakistan, and south Asian countries. Hence, there is still much to be explored to enhance our understanding of black gram genetic variability in the U.S. climatic environment.

# 2.3. Objectives

The overarching objectives were to evaluate the feasibility of growing black gram under rainfed and irrigated conditions of the Northern Great Plains of the U.S. and to identify the superior accessions. The secondary objective was to identify black gram traits (phenological, growth, physiological and yield component) responsible for adaptation and higher yield.

#### **2.4.** Plant Materials

A total of 21 black gram accessions were evaluated in this study (Table 2.1). The selection and evaluation of black gram accessions were initiated by Dr. Gautam Pradhan in 2016. The processes involved screening 287 exotic black gram germplasms from 16 countries

worldwide under high tunnel conditions. The accessions were obtained from the USDA-ARS Plant Genetic Resources Conservation Unit: Griffin, GA, USA. From the initial screening, 89 accessions that flowered and produced seeds were identified. Further evaluations, selection, and seed multiplication were conducted by Dr. Pradhan under greenhouse conditions in 2017, under irrigated field conditions in 2018, and rainfed field conditions in 2020.

Accessions	Country of origin
PI 164441	India
PI 164727	India
PI 183462	India
PI 288602	India
PI 298910	Australia
PI 308573	Venezuela
PI 360949	India
PI 374134	India
PI 374135	India
PI 376871	Thailand
PI 377387	India
PI 377390	India
PI 377391	India
PI 377394	India
PI 377396	India
PI 377397	India
PI 377406	India
PI 383310	India
PI 425187	Australia
PI 425189	Australia
PI 425190	Australia

Table 2.1: Accession number and country of origin of 21 black gram evaluated in the study.

Source: USDA-ARS Plant Genetic Resources Conservation Unit, Griffin, Georgia, USA.

# 2.5. Black Gram under Rainfed Conditions

# 2.5.1. Methodologies

#### 2.5.1.1. Experimental Site

A two-year field research trial was conducted in 2022 and 2023 at Williston Research Extension Center's (WREC) dryland site, Williston, North Dakota. It is located near the Missouri River in a valley, 5 miles from Williston city with an elevation of 640 m. The research site is characterized by Williams-bowbells loam soil. In 2022, black gram was seeded in WREC field no. 10 (103.7376992°W 48.1261452°N), where the previous crop was oats. In 2023, WREC field no. 3 (103.7382385°W 48.1358058°N) was used where the previous crop was wheat. Air temperature and precipitation data was recorded from the NDAWN station located at WREC (Table 2.2).

	Max	x Air Te	mp	Min	in Air Temp			Precipitation		on
Month	Norm.	2022	2023	Norm.	2022	2023		Norm.	2022	2023
		°C			°C				mm	
June	25	24	27	11	11	14		72	46	32
July	29	29	28	14	15	14		64	49	46
August	29	30	28	13	15	14		44	15	69
September	24	25	24	8	8	11		35	10	46
Total								215	120	193

Table 2.2: Mean monthly air temperatures and precipitation during black gram growing season in 2022 and 2023 in Williston, ND.

Note: Norm. = Normal, represents a 30-year average from 1990-2021. Data obtained from North Dakota Agricultural Weather Network.

# 2.5.1.2. Experimental Design

The field trials were carried out in an alpha lattice design replicated 4 times. Each

replication included 21 accessions randomly assigned to 3 blocks, with 7 experimental unit plots

within each block. The plot size was 5.5m in length and 1.2m in width. The row spacing was 19 cm, and there were 7 rows in the plots.

### 2.5.1.3. Seeding and Crop Management

The black gram seeds were inoculated with Exceed® Superior Legume Inoculant (70.9 g for 22.68 kg of seeds), which contains a brady rhizobium strain of rhizobia. Accessions were sown using a GPS-based autosteered seven-row no-till plot seeder that maintained a seed depth of 1 inch. The targeted plant population at harvest was 333,333 plants ha<sup>-1</sup>. Planting was performed on June 2, 2022, and June 4, 2023. Only starter fertilizer (N-P-K-S-Zn: 12-14-0-10-1) was applied during planting. In the 2022 trial, a pre-emergence herbicide, Valor EZ @ 88 ml /a in October 2021, and Spartan Charge @ 103 ml/a on May 6, 2022, were used to keep the experimental plots weed-free. Similarly, for 2023 trial, pre-emergence herbicides Valor EZ @ 88 ml /a in October 2022 and AIM eC @ 47 ml /a + Agsaver Glyphosate 41% PLUS @ 945 ml /a + MSO @ 2pt/a + Ammonium Sulphate @ 0.9 kg/a on June 1, 2023, were applied. During the growing seasons of both 2022 and 2023, weeds were manually removed without causing adverse effects on plant growth and development. The crops were harvested on September 22, 2022, and September 25, 2023, following physiological maturity in both years.

In 2023, hailstorm damage occurred in the black gram trial on August 1, resulting in significant damage to the crop yield. At the time of the event, black gram accessions were at the flowering to early pod initiation stage. The type of injuries observed included defoliation, loss of flowers, stand loss, stem damage, and torn-off branches in Figure 2.2. Black gram accessions were able to recover and produce yield due to presence of green parts in the plants and intact roots in the soil (Figure 2.2 and Figure 2.3). However, the hailstorm significantly affected various growth, yield and yield attributing characteristics, such as plant height, number of pods

per plant, number of pod clusters per plant, ultimately resulting in a reduction in yield per plant and grain yield.



Figure 2.1: Black gram trial before August 1, 2023, under rainfed conditions, WREC.



Figure 2.2: Black gram trial after August 1, 2023, under rainfed conditions, WREC.



Figure 2.3: Black gram at harvest under rainfed conditions, WREC.

## 2.5.1.4. Data Collection and Phenotypic Measurement

Plant stand were manually counted from all 7 rows of experimental plot 20 days after sowing to calculate live seed emergence percent. The live seed emergence percentage was calculated as plant stand divided by number of pure live seed (PLS) sown per plot multiplied by 100 (Carson & Clay, 2016). The pure live seed per experimental unit (Plot) was determined to be 228 for all accessions to achieve the desired in field plant population of 333333 plant ha<sup>-1</sup>.

The flowering date was recorded when the first flower appeared in the plot, and the number of days to flower was subsequently computed. Five plants were randomly selected and tagged after 1 month of sowing in each experimental plot, ensuring there were neighboring plants surrounding it. Leaf chlorophyll content was measured using atLEAF handheld digital chlorophyll meters from tagged plants. One trifoliate leaf was tagged for repeated measurements of leaf chlorophyll content at LEAF value. The measurements were conducted on accessions at the flowering stage, with data collected weekly for three consecutive weeks in August. Chlorophyll content was not recorded because of hailstorm-induced leaf destruction in 2023. At maturity, several parameters were measured from five tagged plant, measurements include above-ground biomass weight, plant height, pod clusters per plant, primary branches, number of pods per plant, pod length, number of seeds per pod, yield per plant, thousand seed weight and harvest index. Theoretical grain yield in kg ha<sup>-1</sup> was computed based on grain yield per plant by considering the targeted plant population at harvest. This calculation was performed to enable comparison with yields reported by other researchers, who typically use kg ha<sup>-1</sup> as the unit of yield. Five pods from the sample were chosen for pod length. Plant height was measured from the ground base to the tip of the stem using a measuring scale. However, in 2023, height was measured from the ground base to the top of the plant because of hailstorm damage.

Black gram grain samples (10 g) were uniformly milled using a laboratory mill- IKA A Basic and sent to a commercial laboratory to quantify the total nitrogen using Pregl-Dumas's method (Jones & Case, 1990). Seed protein concentration was calculated by multiplying the total grain N concentration to a N-to-protein conversion factor of 6.25.

#### 2.5.1.5. Data Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, North Carolina, USA) software package. Data were checked for normality and outliers using histograms, scatter plots, Q-Q Plots, and plots of residuals. Analysis of variance (ANOVA) was performed in SAS using the PROC GLIMMIX procedure. Accessions and year were treated as fixed effects. Replication, replication within year, and block within replication × year were considered as random effects. When significant, the means were separated using the least square mean test at a significance level of P<0.05. Correlation and multiple regression analysis were performed using PROC CORR and PROC REG procedures in SAS, respectively.

## 2.6. Results and Discussion

## 2.6.1. Analysis of Variance

Analysis of variance showed statistically significant effect of year, accessions, and interaction effect of year  $\times$  accessions on several measured traits (Table 2.3). The interaction effect of year  $\times$  accessions was observed on plant stand, seed emergence, days to 1<sup>st</sup> flowering, thousand seed weight, yield per plant, and harvest index. Other measured parameters did not exhibit a significant interaction effect of year  $\times$  accessions. Pod length and protein content were significantly different among accessions. The significant effect of the year was evident on the number of pods per plant and seeds per pod. Moreover, there was no significant effect of year, accessions, and the interaction effect of year  $\times$  accession for primary branches.

Table 2.3: Sources of variation, degrees of freedom, and levels of significance for the ANOVA of measured parameters of black gram under rainfed conditions in 2022 and 2023.

SOV	DF	PS	LSE	FD	NP	PCP	PB	SP	PL	TW	YPP	HI	PC
Year	1	***	***	***	**	*	ns	*	ns	ns	**	ns	ns
Accessions	20	***	***	***	***	ns	ns	ns	***	***	**	***	***
Year ×													
Accessions	20	***	***	***	***	ns	ns	ns	ns	*	***	***	ns

Note: ns, \*, \*\*, \*\*\* = not significant, significant at (P $\leq$ 0.05), (P $\leq$ 0.01), and (P $\leq$ 0.001), respectively. SOV = Source of variation, DF = Degrees of freedom, PS = Plant stands, LSE = Live seed emergence, FD = Days to 1<sup>st</sup> flowering, NP = Number of pods per plant, PCP = Pod clusters per plant, PB = Primary branches, SP = Seeds per pod, PL= Pod length, TW = Thousand seed weigh, YPP= Yield per plant, HI=Harvest index, PC= Protein content.

#### 2.6.1.1. Germination, Plant stand and Live Seed Emergence Percent

There was a significant effect of year, accessions, and interaction effect of year × accession on plant stand and percent seed emergence (Table 2.3). In all accessions, a lower in plant stands and seed emergence percentages was observed in 2022 compared to those in 2023 (Table 2.4). When averaged across accessions, the seed emergence was (54.4 %) in 2023, whereas it was only (18.8 %) in 2022. Similarly, the plant stand was (49.8) in 2022 while it was (233) in 2023. The utilization of older seeds in 2022 might be the reason for the lower seed emergence percentage. In 2022, we used 2-3 years old seeds, whereas in 2023, fresh seeds from 2022 were utilized. The age of seeds can diminish their lifespan, germination, and vigor, resulting in delayed or failed germination as well as poor seedling development (de Vitis et al., 2020).

Furthermore, seed emergence of a field crop is known to be influenced by soil temperature and air temperature (Beegum et al., 2023). The optimum temperature for black gram germination is typically between 28 °C to 30 °C (Lawn & Ahn, 1985). The average maximum air temperature was 24 °C in 2022 whereas in 2023, it was 27 °C in June as shown in (Table 2.2). Seed emergence varied from 5.5% for PI425189 to 34.8% for PI183462 in 2022. It ranged from (43.5 %) for PI425187 to (63-64 %) for PI377387, PI377397, and PI3883310 in 2023.

Furthermore, plant stand varied from 83.6 for PI183462 to (19.6-24.6) for PI425189 and

PI425190 in 2022, whereas plant stand ranged from (364.6) for PI360949 to (163.5) for

PI308573. The highest seed emergence percentage (64 %) recorded in 2023 was still a very low

number and work should be done to increase this number significantly.

Table 2.4: Germination, plant stand and live seed emergence of black gram accessions in 2022 and 2023 under rainfed conditions.

	Germi	ination	Plant s	tand	Live seed er	nergence
	(%	%)	(no.	.)	(%)	)
Accessions	2022	2023	2022	2023	2022	2023
PI164441	87	80	42.9 nop	189.5 gh	15.4 fg	52.0 abc
PI164727	91	57	47.6 no	267.3 c	19.1 efg	54.7 abc
PI183462	91	68	83.6 j	236.0 de	34.8 cde	56.6 ab
PI288602	91	59	60.3 kl	235.6 de	24.6 def	48.5 abc
PI298910	80	76	62.4 k	201.3 fg	20.5 efg	52.8 abc
PI308573	89	89	43.8 nop	163.5 i	16.7 fg	51.8 abc
PI360949	80	44	63.7 k	364.6 a	20.6 efg	55.1 abc
PI374134	93	78	35.4 p	199.3 fg	14.4 fg	54.4 abc
PI374135	93	77	47.1 no	204.9 fg	17.8 efg	55.0 abc
PI376871	87	61	57.4 kl	251.8 cd	20.0 efg	53.9 abc
PI377387	78	55	60.4 kl	326.2 b	20.7 efg	64.9 a
PI377390	93	96	65.3 k	171.4 hi	25.6 def	56.9 ab
PI377391	82	73	41.9 nop	219.4 ef	14.9 fg	56.4 ab
PI377394	93	77	51.4 ln	201.8 fg	21.5 efg	53.7 abc
PI377396	89	56	49.9 no	268.9 c	20.8 efg	54.4 abc
PI377397	93	71	50.4 lno	250.6 cd	20.2 efg	64.0 a
PI377406	87	67	56.0 kl	219.5 ef	21.4 efg	51.5 abc
PI383310	84	68	41.2 op	259.4 cd	15.3 fg	63.2 a
PI425187	89	75	41.8 nop	167.3 i	14.9 fg	43.5 bcd
PI425189	93	94	19.6 q	177.8 hi	5.5 g	47.6 abc
PI425190	98	46	24.6 q	315.9 b	10.4 fg	52.0 abc
Year mean			49.8 B	233.0 A	18.8 B	54.4 A

Note: Same small letter in a column or a row indicates statistically non-significant at p=0.05. Same capital letter in a row indicates statistically non-significant at p=0.05.

### 2.6.1.2. Plant Height

Plant height was analyzed in separate years due to the use of different methods for measuring height. Plant height was measured from the ground surface to the tip of the stem in 2022. However, canopy height was taken at harvest in 2023 due to a hailstorm that broke stems.

Analysis of variance showed that accessions were statistically significant for plant height (Table 2.5). Accession PI164727 had the highest plant height (27.8 cm), while PI377406 had the lowest (13.3 cm) with mean (21.5 cm) in 2022. Similarly, accession PI298910 had the highest plant height (27.2 cm) while accession PI377406 had the lowest plant height (15.1 cm) with mean height (21.7 cm) in (Table 2.5).

Our similar results regarding significant genetic variability among black gram accessions for plant height were also reported by (Panda et al., 2017; Baral et al., 2022; Senthamizhselvi et al. 2019; Shanthi et al. 2019; Bag et al., 2014; Chippy et al., 2021). Panda et al. (2017) reported that the height of black gram genotypes in India varied from (20 cm to 39 cm). Similarly, Chippy et al. (2021) observed a mean height ranging from (22 cm to 59 cm) among 102 genotypes evaluated in India.

## 2.6.1.3. Days to 1<sup>st</sup> Flowering

Analysis of variance revealed significant effect of year, accessions, and interaction effect of year  $\times$  accession on days to 1<sup>st</sup> flowering (Table 2.3). All the accessions flowered 6 to 16 days earlier in 2023 compared to 2022 (Table 2.5). When averaged across accessions, days to 1<sup>st</sup> flowering was (59 days) in 2022 while it was decreased to (47.7 days) in 2023. The early flowering of accessions in 2023 could be due to the increase of air temperature that led to early germination and growth of black gram. Tang et al. (2023) observed soybean flowers early by 2-7 days at 30 °C compared with 25 °C, suggesting that legumes accelerate flowering at high temperature. In our research trial site, the average maximum air temperature was 24°C and 29°C

in June and July 2022 respectively, while in June and July 2023, it was 27°C and 28°C (Table

2.2).

	Plant h	eight	Days to 1 <sup>st</sup> fle	owering
-	(cn	n)	(days)	)
Accessions	2022	2023	2022	2023
PI164441	23.5 cdef	21.6 efg	64.4 a	51.4 cdefgh
PI164727	27.8 a	23.2 cde	62.8 a	48.2 fghij
PI183462	21.7 efg	22.1 def	55.5 abcdefg	45.5 hij
PI288602	22.1 efg	21.2 efgh	65.0 a	48.6 fghij
PI298910	26.5 ab	27.2 a	63.9 a	49.4 defghij
PI308573	25.4 abc	25.9 ab	62.2 ab	52.0 cdefgh
PI360949	19.8 gh	19.5 ghi	49.6 defghij	48.5 fghij
PI374134	23.7 cdef	21.1 efgh	58.4 abcd	47.0 ghij
PI374135	23.9 bcde	21.4 efgh	59.5 abc	47.8 fghij
PI376871	22.6 def	24.1 bcd	59.6 abc	50.7 cdefghij
PI377387	22.9 cdef	22.7 def	62.3 ab	46.8 hij
PI377390	17.3 hi	20.6 fgh	57.6 abcde	46.0 hij
PI377391	17.7 hi	21.4 efgh	56.2 abcdef	43.4 ij
PI377394	15.2 ij	19.1 hi	52.7 bcdefgh	46.1 hij
PI377396	22.7 def	21.6 efg	62.5 ab	46.1 hij
PI377397	15.2 ij	17.3 ij	51.2 cdefghi	44.8 hij
PI377406	13.3 ј	15.1 ј	48.8 efghij	43.0 j
PI383310	21.2 fg	22.0 def	62.2 ab	49.3 defghij
PI425187	23.2 cdef	22.1 def	62.3 ab	48.6 fghij
PI425189	21.6 efg	25.4 abc	62.5 ab	48.0 fghij
PI425190	24.8 bcd	22.1 def	65.1 a	51.8 cdefgh
Year mean	21.5	21.7	59.0 A	47.7 B

Table 2.5: Plant height and days to 1<sup>st</sup> flowering of black gram accessions in 2022 and 2023 under rainfed conditions.

Note: Same small letter in a column or a row indicates statistically non-significant at p=0.05. Same capital letter in a row indicates statistically non-significant at p=0.05.

Days to 1<sup>st</sup> flowering ranged from the longest for accessions PI425190, PI288602,

PI164441, PI298910 and PI164727 (62.8 to 65.1 days) to the lowest for PI377406 (48.8 days).

Similarly, accessions PI308573 and PI425190 flowered late (52 and 51.8 days) while PI377406 flowered early (43 days).

Ghafoor and Ahmad (2005) found genetic variability among 111 genotypes for days to flowering with mean (44 days) in Pakistan. Similarly, Chippy et al. observed (30-44 days) to flowering among 102 genotypes evaluated in India. Baral et al. (2022) noted mean days to flowering was ranged from (26 days to 29 days) among 10 genotypes in Nepal.

# 2.6.1.4. Number of Pods per Plant

There was a significant effect of year, accessions, and interaction effect of year × accession on number of pods per plant (Table 2.3). In all accessions, a few numbers of pods per plant were observed in 2023 compared to 2022 (Table 2.6). Hailstorm was the main reason for such an observation. Black gram accessions were at the stage of peak flowering and pod initiation when the hailstorm occurred on August 1, 2023. It caused defoliation and the loss of flowers, with pod-bearing branches torn off. When averaged across accessions, number of pods per plant was (22.1) in 2022 while it was (10.3) in 2023. Accession PI425187 recorded the highest number of pods per plant (40.6), while accession PI298910 recorded the lowest (13.2) in 2022. Accession PI164441 produced a higher number of pods per plant (15.4), while the fewest were observed in PI425190 (5.84) in 2023. Analysis of variance demonstrated presence of genetic variability among accessions for pod bearing capacity. This finding wwas also reported by (Panda et al., 2017; Senthamizhselvi et al., 2019; Shanthi et al., 2019; Bag et al., 2014). Panda et al. (2017) reported numbers of pods per plant ranging from (16) to (43) among 50 black gram in India.

# 2.6.1.5. Thousand Seed Weight

Analysis of variance showed significant effect of accessions, and interaction effect of year  $\times$  accession on thousand seed weight and effect of year was non-significant (Table 2.3). In all accessions, thousand seed weight was higher in 2023 compared to 2022 (Table 2.6).

Table 2.6: Number of pods per plant and thousand seed weight of black gram accessions in 2022 and 2023 under rainfed conditions.

	Number of pod	s per plant	Thousand see	ed weight
	(no.)		(g)	
Accessions	2022	2023	2022	2023
PI164441	14.9 cdefghijk	15.4 cdefghijk	37.7 fghi	46.2 abcdefghi
PI164727	30.8 ab	10.1 hijk	42.9 bcdefghi	49.3 abcde
PI183462	15.3 cdefghijk	9.1 ijk	45.0 abcdefghi	53.7 ab
PI288602	18.1 bcdefghij	13.8 defghijk	36.4 hi	43.0 bcdefghi
PI298910	13.2 efghijk	9.5 hijk	46.8 abcdefg	55.0 a
PI308573	21.0 bcdefgh	12.1 efghijk	36.3 i	43.2 abcdefghi
PI360949	27.9 abc	7.2 k	43.6 abcdefghi	44.2 abcdefghi
PI374134	24.9 abcdef	9.1 ijk	40.9 cdefghi	44.6 abcdefghi
PI374135	22.4 bcdefg	10.0 hijk	41.2 cdefghi	44.1 abcdefghi
PI376871	20.4 bcdefghi	10.2 ghijk	43.3 abcdefghi	47.1 abcdef
PI377387	14.0 defghijk	8.6 jk	45.3 abcdefghi	44.4 abcdefghi
PI377390	25.4 abcde	12.8 efghijk	40.9 cdefghi	48.1 abcdef
PI377391	18.2 bcdefghij	12.0 fghijk	44.6 abcdefghi	48.0 abcdef
PI377394	27.9 abc	10.8 ghijk	39.0 efghi	46.6 abcdefgh
PI377396	26.7 abcd	9.7 hijk	42.4 bcdefghi	46.2 abcdefghi
PI377397	32.3 ab	9.7 hijk	40.5 cdefghi	42.9 bcdefghi
PI377406	31.6 ab	11.0 ghijk	36.8 ghi	39.6 defghi
PI383310	28.1 abc	12.4 efghijk	43.6 abcdefghi	44.2 abcdefghi
PI425187	40.6 a	12.4 efghijk	41.0 cdefghi	44.1 abcdefghi
PI425189	15.2 cdefghijk	9.4 hijk	49.5 abcd	55.0 a
PI425190	18.1 bcdefghij	5.8 k	50.8 abc	52.3 abc
Year mean	22.1 A	10.3 B	42.3 A	46.8 A

Note: Same small letter in a column or a row indicates statistically non-significant at p=0.05. Same capital letter in a row indicates statistically non-significant at p=0.05. It was observed that the hailstorm in 2023 caused a reduction in both the yield per plant and the number of pods per plant. The observed increase in bold seed could be a result of the plant's response to prioritize seed development as a sink for available resources during the stressful conditions caused by the hailstorm. When averaged across accessions, thousand seed weight was (42.3 g) in 2022, whereas it was (46.8 g) in 2023. Accessions PI425190 recorded the highest thousand seed weight (50.8 g), while the lowest were PI308573 (36.3 g) in 2022. Similarly, accessions PI298910 and PI425189 recorded the highest thousand seed weights (55 g) and the lowest was PI377406 (39.6 g).

Analysis of variance revealed that the existence of genetic variability among tested accessions for thousand grain weight. In agreement with this finding, variations on thousand seed weight due to genotypes were also reported by (Panda et al., 2017; Baral et al., 2022; Senthamizhselvi et al., 2019; Bag et al., 2014). Panda et al. (2017) found thousand seed weight ranging from (27 g to 53 g) among 50 genotypes evaluated in India. Baral et al. (2022) observed thousand seed weight ranging from (40 g to 45 g) among 10 genotypes evaluated in Nepal. Moreover, Chippy et al. (2021) demonstrated thousand seed weight varying from (30 g to 66 g) among 102 genotypes evaluated in India.

## 2.6.1.6. Yield per Plant

Analysis of variance showed that year, accessions, and interaction effect of year × accessions were statistically significant for yield per plant and grain yield per hectare (Table 2.3). When averaged across accessions, the yield per plant was (5.3 g) in 2022 and (2.0 g) in 2023 (Table 2.7). In all accessions, a decline in yield per plant was evident in 2023 compared to those in 2022. The substantial decreases in yield per plant in 2023 were due to a hailstorm that occurred on August 1 of that year. The hailstorm struck during the peak reproductive stage of the

black gram accessions, resulting in losses of flowers, defoliation, stem breakage and torn-off branches. Despite the damage, the black gram accessions were able to recover, thanks to the presence of green parts in the plants and intact roots in the soil. Nevertheless, the hailstorm had a substantial impact on growth and yield attributing characteristics such as the plant height, pod number and cluster number, ultimately leading to a reduction in yield per plant.

Table 2.7: Yield per plant and	grain yield of black gram	accessions in 2022 and 2023 under
rainfed conditions.		

	Yield per	plant	Grain yield		
	(g)		(kg ha <sup>-1</sup>	)	
Accessions	2022	2023	2022	2023	
PI164441	2.8 bcdefghij	4.0 abcdefgh	920.4 bcdefghij	1319.9 abcdefgh	
PI164727	7.0 abc	1.8 fghij	2343.2 abc	596.2 fghij	
PI183462	3.6 abcdefghij	1.9 fghij	1205.9 abcdefghij	621.7 fghij	
PI288602	4.2 abcdefgh	2.7 bcdefghij	1415.9 abcdefgh	909.9 bcdefghij	
PI298910	3.0 bcdefghij	2.1 defghij	1018.2 bcdefghij	716.1 defghij	
PI308573	4.5 abcdefg	2.7 bcdefghij	1506.4 abcdefg	890.9 bcdefghij	
PI360949	7.5 ab	1.2 ј	2499.4 ab	386.5 j	
PI374134	5.5 abcde	1.5 ghij	1843.0 abcde	497.5 ghij	
PI374135	5.0 abcdef	1.7 fghij	1680.5 abcdef	571.6 fghij	
PI376871	4.5 abcdef	2.0 fghij	1518.4 abcdef	675.0 fghij	
PI377387	2.9 bcdefghij	1.2 ј	980.0 bcdefghij	406.7 j	
PI377390	5.5 abcdef	2.4 cdefghij	1836.3 abcdef	811.1 cdefghij	
PI377391	4.3 abcdefg	2.2 defghij	1451.3 abcdefg	731.4 defghij	
PI377394	6.0 abcde	2.1 efghij	2005.6 abcde	713.1 efghij	
PI377396	6.9 abc	1.9 fghij	2320.1 abc	633.6 fghij	
PI377397	7.1 ab	1.4 hij	2374.5 ab	479.7 hij	
PI377406	4.9 abcdef	1.3 ij	1629.8 abcdef	435.2 ij	
PI383310	6.5 abcd	2.3 defghij	2158.2 abcd	784.3 defghij	
PI425187	8.6 a	2.4 defghij	2857.0 a	791.3 defghij	
PI425189	3.9 abcdefghi	2.1 efghij	1306.7 abcdefghi	701.9 efghij	
PI425190	4.8 abcdef	1.2 ј	1615.4 abcdef	387.7 ј	
Year mean	5.2 A	2.0 B	1737.4 A	669.6 B	

Note: Same small letter in a column or a row indicates statistically non-significant at p=0.05. Same capital letter in a row indicates statistically non-significant at p=0.05. Accession PI425187 had the highest yield per plant (8.56 g), while PI164441 had the lowest (2.8 g) in 2022. Accession PI164441 had the largest grain yield (4.0 g), while the lowest was (1.2 g) for PI360949, PI377387 and PI425190 in 2023. The study revealed genetic variability among black gram accessions for yield per plant. Panda et al. (2017) found that yield per plant varied from (2 g to 9 g) among 50 genotypes in India supported our finding. The theoretical yield, calculated in kg ha<sup>-1</sup>, indicated that in 2022, all accessions produced >900 kg of grains per hectare. However, in 2023, despite hail damage, 13 out of 21 accessions still managed to produce >600 kg of grains per hectare (Table 2.7). These yields surpassed the average yield of 598 kg ha<sup>-1</sup> reported in India (Nair et al., 2024), where black gram is native.

## 2.6.1.7. Primary Branches

There was non-significant effect of year, accessions, and interaction effect of year  $\times$  accession on number of primary branches (Table 2.3). The overall mean number of primary branches per plant was (4.6), when averaged across year and accessions. This observation revealed absence of genetic variability among black gram accessions for primary branches in both years.

#### 2.6.1.8. Harvest Index

Analysis of variance showed that accessions, and interaction effect of year  $\times$  accessions were statistically significant for harvest index and effect of year was non- significant (Table 2.3 and Table 2.8). Accession PI377390 recorded the highest harvest index (0.38) while the lowest was for accession PI164441 (0.13) in 2022. Accession PI374134 recorded a higher harvest index (0.31), while the lowest was observed for in accession PI377387 (0.17) in 2023.

	Harvest inc	lex
Accessions	2022	2023
PI164441	0.13 g	0.30 abcdef
PI164727	0.34 abcde	0.21 defg
PI183462	0.34 abcde	0.24 abcdefg
PI288602	0.21 defg	0.25 abcdefg
PI298910	0.22 bcdefg	0.19 efg
PI308573	0.21 defg	0.22 bcdefg
PI360949	0.38 ab	0.22 cdefg
PI374134	0.33 abcdef	0.31 abcdef
PI374135	0.32 abcdef	0.22 bcdefg
PI376871	0.24 abcdefg	0.22 bcdefg
PI377387	0.24 abcdefg	0.17 fg
PI377390	0.38 a	0.25 abcdefg
PI377391	0.35 abcd	0.25 abcdefg
PI377394	0.32 abcdef	0.23 abcdefg
PI377396	0.31 abcdef	0.23 abcdefg
PI377397	0.38 abc	0.25 abcdefg
PI377406	0.33 abcdef	0.20 defg
PI383310	0.31 abcdef	0.29 abcdefg
PI425187	0.28 abcdefg	0.21 defg
PI425189	0.23 abcdefg	0.21 defg
PI425190	0.20 defg	0.21 defg
Year mean	0.29 A	0.23 A

Table 2.8: Harvest index of black gram accessions in 2022 and 2023 under rainfed conditions.

Note: Same small letter in a column or a row indicates statistically non-significant at p=0.05. Same capital letter in a row indicates statistically non-significant at p=0.05.

## 2.6.1.9. Protein Content

Analysis of variance revealed that accessions were statistically significant for grain protein content. The analysis demonstrated that year as well as interaction effect of year  $\times$  accessions was non- significant for protein content (Table 2.9).

	Protein content	Pod length
Accessions	(%)	(cm)
PI164441	21.1 bcdef	4.3 efg
PI164727	22.9 a	4.4 defg
PI183462	22.6 ab	4.9 a
PI288602	21.2 bcdef	4.1 g
PI298910	21.4 bcdef	4.7 abc
PI308573	22.0 abcde	4.6 abcde
PI360949	20.6 f	4.7 abcd
PI374134	21.9 abcdef	4.6 abcde
PI374135	21.4 bcdef	4.6 abcde
PI376871	21.4 bcdef	4.4 cdefg
PI377387	22.2 abc	4.4 cdefg
PI377390	22.1 abcd	4.4 cdefg
PI377391	22.2 abcd	4.6 abcde
PI377394	21.7 abcdef	4.6 abcde
PI377396	21.5 bcdef	4.7 abcd
PI377397	21.2 bcdef	4.5 bcde
PI377406	22.1 abcde	4.1 fg
PI383310	20.8 def	4.4 cdef
PI425187	20.9 cdef	4.4 cdefg
PI425189	20.7 ef	4.9 ab
PI425190	21.7 abcdef	4.5 bcde
Mean	21.6	4.5

Table 2.9: Protein content and pod length of black gram accessions in 2022 and 2023 under rainfed conditions.

Note: Same small letter indicates statistically non-significant at p=0.05.

Accession PI164727 had a higher amount of protein (22.9 %), whereas a lower amount was recorded for accession PI36049 (20.6 %), with a mean value of (21.6 %). The presence of genetic variability for protein content among accessions which was reported by (Suvan et al., 2019; Chippy et al., 2021). Chippy et al. (2021) reported protein content varied from (17 % to 28 %) among 150 black gram genotypes evaluated in India.

#### 2.6.1.10. Pod Length

Analysis of variance revealed that accessions were statistically significant for pod length while year as well as interaction effect of year × accessions was non- significant (Table 2.3). Thus, the longest pod length of each accession, averaging across years, was measured for PI183462 (4.9 cm), and the shortest pod length in PI288602 (4.1cm), with a mean of (4.5 cm) (Table 2.9). Baral et al. (2022) noted black gram pod length ranging from (4.2 cm to 5.1 cm) with mean (4.5 cm) among 10 genotypes evaluated in Nepal. Panda et al. (2017) observed pod length ranging from (4 cm to 5.8 cm) among 50 genotypes evaluated in India. These results supported our finding and confirmed genetic variability exists among accessions for pod length.

#### 2.6.1.11. Pod Clusters per Plant

There was a significant effect of year on pod clusters per plant. Accessions and interaction effect of year × accession on pod clusters per plant was non-significant (Table 2.3). When averaged across accessions, pod clusters per plant was (12.1) in 2022, whereas it was only (5.7) in 2023 (Table 2.10). The difference on pods clusters per plant between two years was due to hailstorm damages occurred under rainfed condition in 2023. The hailstorm caused huge loss of flowers which led to less pod initiation in black gram.

#### 2.6.1.12. Number of Seeds per Pod

Analysis of variance demonstrated significant effect of year on number of seeds per pod and interaction effect of year  $\times$  accession on number of seeds per pod was non-significant (Table 2.3). When averaged across accessions, number of seeds per pod was (5.3) in 2022 while it was only 3.9 in 2023 (Table 2.10). The reduction of number of seeds per pod could be related to hailstorm damages occurred in 2023. Unfilled pods were observed in 2023 (not quantified).

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	Pod clusters	Seeds per pod
	(no.)	(no.)
2022	12.1 A	5.3 A
2023	5.7 B	3.9 B

Table 2.10: Number of pod clusters per plant and number of seeds per pod of black gram accessions in 2022 and 2023 under rainfed conditions.

Note: Same capital letter in a column indicates statistically non-significant at p=0.05.

## 2.6.1.13. Leaf Chlorophyll Content

The leaf chlorophyll content atLEAF value indicates the photosynthetic capacity of leaves and the health status of plants. Analysis of variance revealed statistically significant differences among accessions for leaf chlorophyll content atLEAF values in the significance level at (P<0.001) (Figure 2.4). Analysis of variance revealed time was statistically significant for leaf chlorophyll content atLEAF value in 2022 (Figure 2.5).

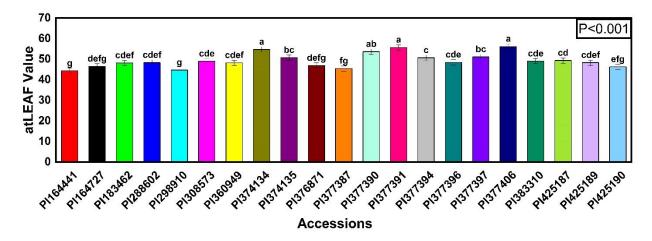


Figure 2.4: Leaf chlorophyll content atLEAF value of black gram accessions under rainfed conditions in 2022.

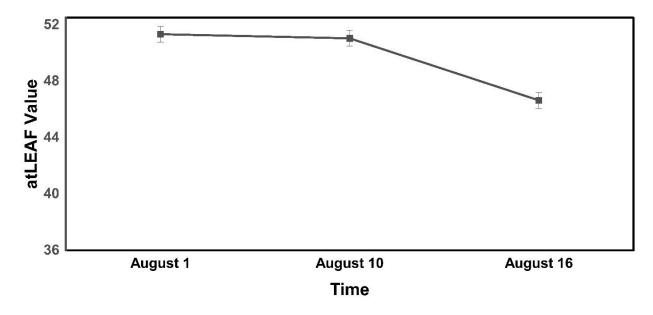


Figure 2.5: Trend of leaf chlorophyll content atLEAF value of black gram accessions under rainfed conditions in 2022.

Accessions PI425187 and PI374134 exhibited the highest chlorophyll atLEAF values (53.8) respectively, while accession had the lowest atLEAF value at (39.3). The atLEAF value greater than (35) is considered as good health and good grade (Novichonok et al., 2016). Averaged across accessions, the trend of chlorophyll content showed atLEAF value was 51.3 on August 1. By August 10, it remained the same at (51), but then declined to (46.64) by August 16, 2022, (Fig 2.5). These results indicated a decrease in chlorophyll due to less precipitation (14.47 mm) in August 2022 (Table 2.2). Baroowa and Gogoi (2013) documented a decrease in leaf chlorophyll content of black gram and green gram over time due to drought.

#### 2.6.2. Correlation

Correlation coefficient is a statistical measure of strength association and relationship between two variables. It is an indirect selection of superior genotypes or varieties with desirable characters. Studying correlations between different characters can assist plant breeders in understanding how improving one character may lead to simultaneous changes in others. Consequently, identifying important yield components, their nature, and the magnitude of their associations with economic yield proves invaluable in selecting high-yielding genotype with

desired characters. The phenotypic correlation between 10 variables of 21 black gram accessions

were listed year wise in (Table 2.11 and 2.12).

Table 2.11: Pearson correlation coefficients of black gram variables under rainfed conditions in 2022.

	PS	PH	FD	PB	PCP	NP	PL	SP	TW	YPP
PS										
PH	-0.19									
FD	-0.32 **	0.60 ***								
PB	-0.24 *	0.47 ***	0.40 ***							
PC	0.02	0.07	-0.18	0.25 *						
NP	-0.05	-0.07	-0.29 **	0.12	0.93 ***					
PL	-0.03	0.17	0.01	-0.16	-0.05	-0.04				
SP	-0.01	0.17	0.10	0.25 *	0.14	0.02	0.11			
TW	-0.49 ***	0.27 **	0.21	-0.11	-0.24 *	-0.18	0.44 ***	-0.11		
YPP	-0.16	0.05	-0.21	0.15	0.88 ***	0.92 ***	0.13	0.28 *	0.03	

<sup>\*\*</sup>- significant at 0.05, <sup>\*\*\*</sup>- significant at 0.01, <sup>\*\*\*\*</sup>- significant at 0.001 level of probability Note: PS: Plant Stand, PH: Plant height(cm), FD: Days to 1<sup>st</sup> flowering, PCP: Pod clusters per plant, PB: Primary branches, NP: Number of pods per plant, PL: Pod length(cm), SP: Number of seeds per pod, TW: Thousand seed weight(g), YPP: Yield per plant(g).

Yield per plant exhibited a significant and strong positive correlation with pod clusters and number of pods per plant in 2022 (Table 2.11) which indicated that increase in number of pod clusters and number of pods per plant would have positive impact on yield per plant. The result of the presented study is in line with the findings of other researchers. Shanthi et al. (2019) observed strong positive phenotypic correlation of pod clusters and number of pods per plant with yield. Yield per plant exhibited a significant yet weak positive correlation with seeds per pod, while displaying non-significant weak positive relationship with plant height, primary branches, pod length, and thousand seed weight. Conversely, there was a negative correlation with days to 1<sup>st</sup> flowering, and plant stand. Plant stand showed negative and significant correlation with days to 1<sup>st</sup> flowering, primary branches and thousand seed weight. It means increase in plant stand reduce primary branches and thousand seed weight. The strongest

correlation was found between the number of pods per plant and pod clusters per plant.

Table 2.12: Pearson correlation coefficients of black gram variables under rainfed conditions in 2023.

	PS	PH	FD	PB	PCP	NP	PL	SP	TW	YPP
PS										
PH	-0.14									
FD	0.03	0.40 ***								
PB	-0.06	-0.05	0.10							
PCP	-0.24 *	0.22	-0.12	-0.56 ***						
NP	-0.36 ***	0.09	-0.03	0.60 ***	0.09					
PL	-0.20	0.33 ***	-0.03	-0.06	0.06	-0.05				
SP	-0.39 ***	0.35 ***	0.40 ***	0.11	0.21	0.36 ***	0.04			
TW	-0.10	0.24 *	0.15	0.32 ***	-0.52 ***	0.03	0.03 ***	-0.07		
YPP	-0.40 ***	0.28 **	0.18	0.59 ***	0.01	0.91 ***	0.91	0.60 ***	0.27 **	

'\*'- significant at 0.05, '\*\*'- significant at 0.01, '\*\*\*'- significant at 0.001 level of probability Note: PS: Plant Stand, PH: Plant height(cm), FD: Days to 1<sup>st</sup> flowering, PCP: Pod clusters per plant, PB: Primary branches, NP: Number of pods per plant, PL: Pod length(cm), SP: Number of seeds per pod, TW: Thousand seed weight(g), YPP: Yield per plant(g).

Yield per plant showed a statistically significant and strong positive association with primary branches, number of pods per plant, seeds per pod in 2023 (Table 2.12). There was statistically significant yet weak positive correlation with plant height and thousand seed weight. Yield per plant displayed non-significant correlation with flowering days, pod clusters per plant, and pod length. Strong and significant correlations were observed between yield per plant and the number of pods per plant in both years. It indicated that a greater number of pods per plant leads to higher yields. Similar results were reported by Shanthi et al. (2019) and Chippy et al. (2021). There was a positive correlation between plant height and yield per plant in both years. This observation is consistent with the findings of (Chippy et al., 2021; Senthamizhselvi et al., 2019). Plant stand showed negative and significant correlation with pod clusters per plant, number of pods per plant, number of seeds per pod and yield per plant. It revealed a positive correlation with days to 1<sup>st</sup> flowering. As mentioned earlier, the different correlations observed in 2023 compared to 2022 were due to hailstorm episode in 2023.

## 2.6.3. Multiple Regression

The multiple linear regressions were estimated to measure the relationship and the change in magnitude of the grain yield due to the other yield attributing characteristics (Table 2.13 and Table 2.14). Yield per plant is fixed as a dependent variable and other yield attributing variables as independent variable.

For 2022 year, the multiple linear regression equation could be written as

Yield per plant (g) = -9.33 + 0.01 plant height(cm) - 0.02 primary branches + 0.02 pod clusters + 0.21 number of pods per plant + 0.28 pod length (cm) + 0.74 number of seeds per pod + 0.10 thousand seed weight(g).

Variables	DF	Coefficients	Std. error	t-stat
Intercept	1	-9.33 ***	0.69	-13.51
PH	1	0.01	0.01	0.46
PB	1	-0.02	0.04	-0.58
PCP	1	0.02	0.03	0.48
NP	1	0.21 ***	0.01	16.29
PL	1	0.28	0.16	1.79
SP	1	0.74 ***	0.05	14.23
TW	1	0.10 ***	0.01	9.63
$\mathbb{R}^2$			0.98	
Observation			83	

Table 2.13: Multiple regression studies between yield and other variables of black gram in 2022.

'\*'- significant at 0.05, '\*\*'- significant at 0.01, '\*\*\*'- significant at 0.001 level of probability Note: PH: Plant height(cm), PB: Primary branches, PCP: Pod clusters per plant, NP: Number of pods per plant, PL: Pod length(cm), SP: Number of seeds per pod, TW: Thousand seed weight(g). For 2023, the multiple linear regression equation could be written as

Yield per plant (g) = -3.70 - 0.01 plant height(cm) - 0.01 primary branches - 0.01 pod clusters + 0.20 number of pods per plant -0.02 pod length (cm) + 0.39 number of seeds per pod + 0.04 thousand seed weight(g).

Variables	DF	Coefficients	Std. error	t-stat
Intercept	1	-3.70 ***	0.27	-13.84
PH	1	0.01	0.01	1.85
PB	1	0.01	0.01	0.91
PCP	1	0.01	0.01	0.69
NP	1	0.20 ***	0.01	27.6
PL	1	-0.02	0.06	-0.35
SP	1	0.39 ***	0.02	17.74
TW	1	0.04 ***	0.00	11.6
$\mathbb{R}^2$			0.98	
Observation			84	

Table 2.14: Multiple regression studies between yield and other variables of black gram in 2023.

'\*'- significant at 0.05, '\*\*'- significant at 0.01, '\*\*\*'- significant at 0.001 level of probability Note: PH: Plant height(cm), PB: Primary branches, PCP: Pod clusters per plant, NP: Number of pods per plant, PL: Pod length(cm), SP: Number of seeds per pod, TW: Thousand seed weight(g).

The R<sup>2</sup> value of (0.98) in each year depicts a good model fit, implying that the independent variables are responsible for 98 percent of the grain yield. Independent variables like number of pods per plant, number of seeds per pod, and thousand seed weight were found positive statistically significant corelated with yield per plant (Table 2.13 and 2.14). The slope coefficient of the number of seeds per pod had the highest impact in yield per plant, one per cent increase in seeds per pod, there would be a significant increase in the yield per plant by 0.74 and 0.39 per cent, other variables being held constant. In 2022, when there was a one per cent increase in the variables viz., number of pods per plant, pod length and thousand seed weight there would be an increase in the yield per plant by 0.21, 0.28 and 0.10 per cent, respectively.

Likewise, with one per cent increase in the independent variables such as number of pods per plant and thousand seed weight there would be an increase in the yield per plant by 0.20 and 0.04 per cent in 2023. Plant height, primary branches and pod clusters per plant showed negative impact in yield per plant in 2023.

## 2.7. Summaries

The evaluation of 21 black gram accessions over two years reveals a wide range of desirable genetic variability and significant differences were observed among accessions in traits such as plant stand, plant height, flowering days, pod clusters per plant, number of pods per plant, pod length, thousand seed weight, yield per plant, harvest index, and protein content, while traits like primary branches and number of seeds per pod showed non-significant. Accession PI425187 had the highest yield per plant (8.56 g), while PI164441 had the lowest (2.8 g) in 2022. Accession PI164441 had the largest grain yield (4.0 g), while the lowest was (1.2 g) for PI360949, PI377387 and PI425190 in 2023. Accession PI164727 had a higher amount of protein (22.9 %), whereas a lower amount was recorded for accession PI36049 (20.6 %).

The correlation study revealed that yield per plant was correlated with all several yield attributing character but highly and significant correlated with number of pods per plant in both years. Regression study predicted that yield per plant was influenced by all independent variables measured but number of pods per plant, number of seeds per pod, thousand seed weight were found positively statistically significant correlated and had large impact in yield per plant in both years. Selection of accession with higher pods number and grain yield would be economical. In 2023, a hailstorm damaged and reduced the yield potential of black gram accessions. However, the black gram accessions produced a higher grain yield compared to the average yield in India. Thus, this study demonstrated that it is feasible to grow black gram under no-till, rainfed

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conditions in the NGP of the U.S. However, more research should be conducted to enhance percent seed germination of black gram accessions and more multilocation trials should be conducted to identify superior germplasm that perform best in wider environmental conditions of Northern Great plains, U.S.

## 2.8. Black Gram under Irrigated Conditions

## 2.8.1. Methodologies

### 2.8.1.1. Experimental Site

A two-year field research trial was conducted at the Williston Research Extension Center's Nesson Valley Irrigation Research Site, located in Nesson Valley, Ray, North Dakota. The farm is situated approximately 31 miles east of Williston on Highway 1804 and has an elevation of 580 meters. The research site is characterized by the lihen loamy fine sand soil type. In 2022, black gram was seeded in field span no. 3 (103.0984443°W 48.1670423°N), and in 2023, the trial was conducted in field span no. 4 (103.1082228°W 48.1669003°N). Spring wheat was the preceding year's crop in both locations. Air temperature and precipitation data were recorded from the NDAWN stations located at Hofflund.

	Max	Air Te	mp	Min	Air Te	mp		Pre	cipitatio	on	
	°C				°C			mm			
Month	Norm.	2022	2023	Norm.	2022	2023		Norm.	2022	2023	
June	24	23	27	11	10	13		81	59	51	
July	28	27	27	14	14	13		71	68	42	
August	28	28	28	14	14	13		38	4	66	
September	72	23	24	7	8	9		33	10	38	
Total								223	141	197	

Table 2.15: Mean monthly air temperatures and precipitation during black gram growing season in 2022 and 2023 in Nesson, ND.

Note: Norm. = Normal, represents a 30-year average from 1990-2021. Data obtained from North Dakota Agricultural Weather Network.

## 2.8.1.2. Seeding and Crop Management

Black gram accessions seeds were inoculated with Exceed® Superior Legume Inoculant (70.9 g for 22.68 kg of seeds), which contains a bradyrhizobium strain of rhizobia. Seeds were seeded using a GPS-based autosteered seven-row plot seeder maintaining a seed depth of 1 inch. The targeted plant population at harvest was 333,333 pure live seeds per hectare. Planting took place on June 3, 2022, and June 2, 2023. Starter fertilizer (N-P-K-S-Zn: 12-14-0-10-1) was applied during planting. In the 2022 and 2023 trial, Gramoxone @ 591 ml /a, Roundup @ 591 ml/and class act 2% on May 23, 2022, were used to keep the experimental plots weed-free. The crops were harvested on 25 September in 2022. Irrigation information was provided in (Table 2.16).

Date	2022	Date	2023
	Amount (mm)		Amount (mm)
27-Jun	51	20-Jun	28
6-Jul	25	27-Jun	28
8-Jul	25	5-Jul	33
13-Jul	38	11-Jul	25
18-Jul	38	18-Jul	33
1-Aug	38	24-Jul	41
5-Aug	25		
25-Aug	51		
Total	292		188

Table 2.16: Detailed information of irrigation supplied to black gram in 2022 and 2023 under irrigated conditions.

Note: mm=Millimeter.

A hailstorm occurred in Nesson on August 1, 2023, and wiped out the entire field trials of black gram shown in (Figure 2.7).



Figure 2.6: Black gram trial before August 1, 2023, under irrigated conditions.



Figure 2.7: Entire black gram trial wipeout by hailstorm on August 1, 2023.

# 2.8.1.3. Data Collection and Phenotypic Measurement

Plant stands were manually counted from all 7 rows of experimental plot 20 days after sowing to calculate percent seed emergence. The live seed emergence percentage was calculated as plant stand divided by number of seeds sown per plot multiplied by 100 (Carson & Clay, 2016). The pure live seed per experimental unit (Plot) was determined to be 228 for all accessions to achieve the desired in field plant population of 333333 plant ha<sup>-1</sup>.

The flowering date was recorded when the first flower appeared in the plot, and the number of days to flower was subsequently computed. Five plants were randomly selected and tagged after 1 month of sowing in each experimental plot, ensuring there were neighboring plants surrounding it. Chlorophyll content was measured using atLEAF handheld digital chlorophyll meters from tagged plants. One trifoliate leaf was tagged for repeated measurements of chlorophyll atLEAF value. The measurements were conducted on accessions at the flowering stage, with data collected weekly for three consecutive weeks in August. Chlorophyll content was not recorded because of hailstorm wiped out entire experiment in 2023. At maturity, several parameters were measured from five tagged plant, measurements include above-ground biomass weight, plant height, pod clusters per plant, primary branches, number of pods per plant, pod length, number of seeds per pod, yield per plant, thousand seed weight and harvest index. Theoretical grain yield in kg ha<sup>-1</sup> was computed based on grain yield per plant by considering the targeted plant population at harvest. This calculation was performed to enable comparison with yields reported by other researchers, who typically use kg ha<sup>-1</sup> as the unit of yield. Five pods from the sample were chosen for pod length. Plant height was measured from the ground base to the tip of the stem using a measuring scale.

#### 2.8.1.4. Data Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, North Carolina, USA) software package. Data were checked for normality and outliers using histograms, scatter plots, Q-Q Plots, and plots of residuals. Analysis of variance (ANOVA) was performed in SAS using

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the PROC GLIMMIX procedure. A combined analysis was conducted for plant stand, seed emergence, and days to 1<sup>st</sup> flowering, as these measurements were taken before hailstorm damage occurred. Accessions and years were treated as fixed effects, while replication, replication within year, and block within replication  $\times$  year were considered as random effects. Due to the hailstorm damage, we were unable to obtain yield data from the 2023 trial, so only the data from the 2022 trial were analyzed. In the model, accessions were treated as fixed effects, and replication and block within replication were considered as random effects. When significant, the means were separated using the least square mean test at a significance level of P<0.05. Correlation and multiple regression analysis were performed using PROC CORR and PROC REG procedures in SAS, respectively.

#### 2.9. Results and Discussion

#### 2.9.1. Analysis of Variance

Analysis of variance showed statistically significant effect of year, accessions, and interaction effect of year  $\times$  accessions on plant stand, and days to 1<sup>st</sup> flowering (Table 2.17). The significant effect of year was observed for live seed emergence. There was statistically significant difference for number of pods per plant, pod length, thousand seed weight, yield per plant and harvest index and leaf chlorophyll content among accessions. Pod clusters per plant, primary branches, and number of seeds per pod were statistically non-significant among accessions under irrigated conditions in 2022.

Table 2.17: Sources of variation, degrees of freedom, and levels of significance of the ANOVA measured parameters of black gram under irrigated condition in 2022 and 2023.

SOV	DF	PS	LSE	FD	NP	PCP	PB	SP	PL	TW	YPP	HI	PH
Year	1	***	***	***	-	-	-	-	-	-	-	-	-
Accessions	20	***	ns	***	***	ns	ns	ns	***	***	***	***	***
Year × Accessions	20	***	ns	***	-	-	-	-	-	-	-	-	-

Note: ns, \*, \*\*, \*\*\* = not significant, significant at (P $\leq$ 0.05), (P $\leq$ 0.01), and (P $\leq$ 0.001), respectively. PS, LSE, FD represent combined analysis of 2022 and 2023 and other parameters were analyzed from 2022 as accession as fixed effect due to hailstorm damage. SOV = Source of variation, DF = Degrees of freedom, PS = Plant stand, LSE = Live seed emergence, FD = Days to 1<sup>st</sup> flowering, NP = Number of pods per plant, PCP = Pod clusters per pod, PB = Primary branches, SP = seed per pod, PL= Pod length, TW = Thousand seed weigh, YPP= Yield per plant, HI=Harvest index, PH= Plant height.

#### 2.9.1.1. Germination, Plant Stand and Live Seed Emergence Percent

Analysis of variance showed statistically significant effect of year, accessions, and interaction effect of year  $\times$  accessions on plant stand (Table 2.17). In all accessions, a decline in plant stands was observed in 2022 compared to those in 2023 (Table 2.18).

When averaged across accessions, the plant stand was (73.9) in 2022, while it was (154) in 2023. There was a non-significant effect of accessions and interaction effect of year × accessions on live seed emergence. Only year was statistically significant for live seed emergence. It was observed seed emergence was lower in 2022 compared with 2023. When averaged across accessions, seed emergence was (27 %) in 2022 while it was (47 %) in 2023. Similar results were observed under rainfed conditions in Williston. The live seed emergence percent of black gram in 2022 under rainfed conditions was (18 %) while it was (54 %) in 2023. Use of older seeds in 2022 might be the reason for the low seed emergence percent and plant stand in both conditions.

Temperature is an important factor for seed emergence. The optimum temperature for black gram germination ranged between 28 °C to 30 °C (Lawn & Ahn, 1985). The average

maximum air temperature was 23 °C in 2022 whereas in 2023, it was 27 °C in June as shown in

(Table 2.15). Plant stand varied from (96.7-98.2) for PI377391 and PI377387 to (47.8) for

PI425189 in 2022, whereas plant stand ranged from (179.7) for PI377390 to (134.7) for

## PI377390 in 2023.

Table 2.18: Germination and plant stand of black gram accessions in 2022 and 2023 under irrigated conditions.

	Germi	ination	Plant s	tand
	()	%)	(no	.)
Accessions	2022	2023	2022	2023
PI164441	87	80	59.5 nop	158.7 bcde
PI164727	91	57	72.1 klm	175.3 ab
PI183462	91	68	77.1 jkl	149.7 cdefg
PI288602	91	59	74.2 kl	149.8 cdefg
PI298910	80	76	76.2 kl	159.4 bcde
PI308573	89	89	76 kl	148 cdefg
PI360949	80	44	93.8 hi	159.6 bcde
PI374134	93	78	70.4 klmn	151 cdefg
PI374135	93	77	57.7 opq	154.6 cdef
PI376871	87	61	60.1 mnop	149.4 cdefg
PI377387	78	55	98.2 h	162.1 abcd
PI377390	93	96	68.9 lmno	179.7 a
PI377391	82	73	96.7 h	134.7 g
PI377394	93	77	81.3 ijk	143.2 efg
PI377396	89	56	80.1 jkl	160.5 abcde
PI377397	93	71	76.6 jkl	149.1 cdefg
PI377406	87	67	55.2 pq	165.8 abc
PI383310	84	68	90 hij	137.7 fg
PI425187	89	75	71.5 klmn	146.7 defg
PI425189	93	94	47.8 q	148.2 cdefg
PI425190	98	46	68.6 lmno	150.3 cdefg
Year mean			73.9 B	154 A

Note: Same small letter in a column or a row indicates statistically non-significant at p=0.05. Same capital letter in a row indicates statistically non-significant at p=0.05.

# 2.9.1.2. Days to 1<sup>st</sup> Flowering

Analysis of variance revealed statistically significant effect of year, accessions, and interaction effect of year  $\times$  accessions for days to 1<sup>st</sup> flowering (Table 2.17). All accessions flowered 2-12 days earlier in 2023 compared to 2023 (Table 2.19). When averaged across accessions, days to 1<sup>st</sup> flowering was (56 days) in 2022 whereas it was (50 days) in 2023.

Table 2.19: Days to 1 <sup>st</sup> flowering of black gram accessions in 2022 and 2023 under irre	igated
conditions.	

	Days to 1 <sup>st</sup> flowering				
-	(days)				
Accessions	2022	2023			
PI164441	61.6 a	50.4 hijkl			
PI164727	53.9 efgi	49.1 hl			
PI183462	54.7 defg	50.7 ijkl			
PI288602	58.2 abcd	50.4 hijkl			
PI298910	59.9 ab	50.3 hijkl			
PI308573	61.2 a	50.2 hijkl			
PI360949	53.1 fgi	50.6 hijkl			
PI374134	58.9 abc	50.6 hijkl			
PI374135	56.7 bcdef	50.5 hijkl			
PI376871	61.5 a	49.3 hkl			
PI377387	55.7 cdefg	491			
PI377390	57.7 abcde	50.4 hijkl			
PI377391	52.7 gijk	50.7 ijkl			
PI377394	51.6 ijkl	52 gijkl			
PI377396	55 cdefg	49.3 hkl			
PI377397	49.4 hjkl	53 fgij			
PI377406	49.2 hkl	50.7 ijkl			
PI383310	56.7 bcdef	49.3 hkl			
PI425187	53.9 efgi	50.5 hijkl			
PI425189	57.7 abcde	49.2 hkl			
PI425190	61.1 a	50.6 hijkl			
Year mean	56 A	50.3 B			

Note: Same small letter in a column or a row indicates statistically non-significant at p=0.05. Same capital letter in a row indicates statistically non-significant at p=0.05. Under rainfed conditions, days to 1<sup>st</sup> flowering was (59 days) in 2022 while it was decreased to (47.7 days) in 2023. Temperature greatly influences flowering time and small change in temperature can shift flowering time of many species (Fitter & Fitter, 2002). In our research site, the average maximum air temperature was 23°C and 27°C in June and July in 2022 respectively, while in June and July 2023, it was 27°C and 27°C as shown (Table 2.15). Accessions PI308573, PI425190, PI376871 and PI164441 flowered late (61.1-61.6 days) while PI377406 flowered early (49 days). In 2023, PI377397 flowered late (53 days) while PI377387 flowered early (49 days).

## 2.9.1.3. Plant Height

Analysis of variance showed that plant height was statistically significant among accessions (Table 2.17 and Table 2.20). Accession PI308573 was found to be the tallest (33.31 cm), while PI377397 and PI377406 were observed as a dwarf accession (18.59 cm) and (16.58 cm) respectively, with a mean height of (27.40 cm).

#### 2.9.1.4. Number of Pods per Plant

Analysis of variance revealed that number of pods per plant was statistically significant among accessions (Table 2.17 and Table 2.20). Black gram did not produce a greater number of pods per plant under irrigated condition compared to rainfed conditions. Under rainfed conditions, the highest number of pods per plant was (40) pods with a mean (22). It was observed profuse vegetative growth, foliage, and less pod initiation among accessions. Aman and Singh (2022) found that pod per plant varied from (20 to 38) among 20 genotypes evaluated under irrigated condition in India. Kumar et al. (2018) observed that pods per plant ranged from (27-38) under different sprinkler irrigation level (50-100 %) pan evaporation among three black gram genotypes evaluated in India. They also observed more vegetative growth and branches and prolonged vegetative growth while flowering due to excess soil moisture, leading to lower number of pods per plant. Accession PI377406 produced a higher number of pods per plant (31.2), while accession PI308573 recorded the lowest (4.7), with a mean value of (12.8).

Table 2.20: Plant height and number of pods per plant of black gram accessions in 2022 under	ſ
irrigated conditions.	

	Plant height	Pods per plant
_	(cm)	(no.)
Accessions	2022	2022
PI164441	28.6 bcdef	5.9 ij
PI164727	30.6 abc	17.9 bc
PI183462	30 abcd	9.6 efghi
PI288602	27.4 cdef	12.1 defg
PI298910	28.7 bcde	8.7 fghi
PI308573	33.3 a	4.7 ј
PI360949	25.4 ef	14.1 cde
PI374134	30.2 abc	11.9 defg
PI374135	28.7 bcde	12.1 defg
PI376871	31.9 ab	7.2 hij
PI377387	30.4 abc	14 cde
PI377390	25.4 ef	14.6 cde
PI377391	25.3 f	16.5 cd
PI377394	25.5 ef	12.4 cdef
PI377396	26.8 def	11.3 defgh
PI377397	18.6 g	23.4 b
PI377406	16.6 g	31.2 a
PI383310	28.1 cdef	8.1 fghij
PI425187	28.6 bcde	13.6 cde
PI425189	26.8 def	8.7 fghi
PI425190	28.6 cdef	7.7 ghij
Mean	27.4	12.8

Note: Same small letter in a column indicates statistically non-significant at p=0.05.

# 2.9.1.5. Pod Length

There was statistically significant for pod length among accession (Table 2.21).

Accession PI183462 had longest pod length (5.5 cm), while the shortest pod was in PI288602

(4.0 cm), with a mean length of (4.7 cm). Aman and Singh (2022) revealed pod length differed

from (3.3 to 4.7 cm) among 20 genotypes evaluated under irrigated conditions in India.

Table 2.21: Pod length and thousand seed weight of black gram accessions in 2022 under irrigated conditions.

	Pod length	Thousand seed weight
	(cm)	(g)
Accessions	2022	2022
PI164441	4.3 gh	45.3 j
PI164727	4.6 ef	51 fgh
PI183462	5.5 a	60.4 a
PI288602	4 h	41.3 k
PI298910	4.9 bc	52.9 def
PI308573	4.5 fg	47 ij
PI360949	5 b	55 bcde
PI374134	4.9 bc	50.8 fgh
PI374135	4.8 cde	51.2 fg
PI376871	4.6 def	53.2 def
PI377387	4.8 bcd	49.1 ghij
PI377390	4.6 ef	56.4 bcd
PI377391	5 b	53.8 cdef
PI377394	4.9 bc	52.2 efg
PI377396	5 b	50.2 fghi
PI377397	4.8 bcd	48.7 ghij
PI377406	4.4 fg	46.6 ij
PI383310	4.6 def	52.3 efg
PI425187	4.6 def	47.5 hij
PI425189	5 bc	57.3 abc
PI425190	4.8 bcd	57.6 ab
Mean	4.7	51.5

Note: Same small letter in a column indicates statistically non-significant at p=0.05.

#### 2.9.1.6. Thousand Seed Weight

Analysis of variance showed thousand seed weight was statistically significant among accessions (Table 2.21). Accessions PI183462 recorded the highest thousand seed weights (60.4 g), where the lowest was in PI288602 (41.3 g) with a mean value of (51.5 g).

## 2.9.1.7. Yield per Plant

There was statistically significant effect of accessions on yield per plant under irrigated conditions (Table 2.17 and Table 2.22). Accession PI377397 and PI377406 recorded the highest yield per plant (6.4 and 6.7g respectively), while the lowest was recorded for PI308573 (1.2 g), with a mean value of (3.5 g). Aman and Singh (2022) showed yield per plant ranged from (2.61 g) to (4.18 g) among 20 black gram genotypes evaluated in India. Kumar et al. (2018) found that black gram genotypes yielded (1035 kg ha<sup>-1</sup>) under sprinkler-irrigated conditions. The theoretical yield, calculated in kg ha<sup>-1</sup>, indicated that in 2022, 12 accessions produced >1035 kg of grains per hectare with mean yield (1167.6 kg ha<sup>-1</sup>) among accessions.

Although the design of the study does not allow the statistical analysis of black gram considering irrigated and rainfed conditions as sources of variation, the results showed comparatively low black gram average yield per plant under irrigated conditions (3.5 g) than in rainfed (5.2 g) during 2022. We irrigated black gram continuously from planting to maturity. In 2023, the crop was completely damaged by a hailstorm at the flowering stage and yield could not be measured. Black gram is drought tolerant crops, and they do best when irrigation is provided during the critical stages such as flowering and early pod-filling (Nilanthi et al., 2015; Nair et al., 2024). However, excessive water can lead to prolonged vegetative growth even after flowering and delay seed formation. These cause a reduction in grain yield (Kumar et al., 2018). The

excessive vegetative growth and delayed seed formation noticed under our irrigated studies shall

be the reasons for lower yield under irrigation compared to rainfed conditions.

## 2.9.1.8. Harvest Index

Analysis of variance revealed harvest index was statistically significant among accessions

(Table 2.16 and 2.21). Accession PI377397 recorded higher harvest index (0.46) while the

lowest was PI164441 (0.04), with a mean value of (0.24).

Table 2.22: Yield per plant, grain yield and harvest index of black gram accessions in 2022 under irrigated conditions.

	Yield per plant	Grain yield	Harvest index
	(g)	$(kg ha^{-1})$	
Accessions	2022	2022	2022
PI164441	1.3 fg	440.5 gh	0.04 i
PI164727	4.9 ab	1634.6 abc	0.25 cde
PI183462	3.5 abcde	1159.6 bcdefg	0.27 abcde
PI288602	2.9 bcdefg	961.3 defgh	0.14 fg
PI298910	2.4 cdefg	811.9 efgh	0.23 cdef
PI308573	1.2 g	413 h	0.07 hi
PI360949	4.8 ab	1606.2 abcd	0.38 abc
PI374134	3.6 abcd	1185.7 bcdef	0.27 bcde
PI374135	3.5 abcde	1154.8 bcdefg	0.3 abcd
PI376871	2 efg	671.3 fgh	0.11 gh
PI377387	3.5 abcd	1174.5 bcdef	0.27 cde
PI377390	4.4 abc	1466.2 bcde	0.25 cde
PI377391	5.3 ab	1773.5 ab	0.33 abcd
PI377394	3.7 abcd	1234.6 bcdef	0.26 cde
PI377396	3 bcdef	1011.6 cdefgh	0.3 abcd
PI377397	6.4 a	2150.7 a	0.46 a
PI377406	6.7 a	2224.5 a	0.45 ab
PI383310	2.2 defg	722.7 fgh	0.16 fg
PI425187	3.3 bcdef	1107.7 cdefg	0.15 fg
PI425189	2.6 cdefg	854.8 efgh	0.17 efg
PI425190	2.3 cdefg	760.5 fgh	0.2 def
Mean	3.5	1167.6	0.24

Note: Same small letter in a column indicates statistically non-significant at p=0.05.

## 2.9.1.9. Pod Clusters per Plant, Primary Branches, and Number of Seeds per Pod

There was absence of genetic variability and effect of year, accession, and year  $\times$  accessions were differed statistically non-significant for pod clusters per plant, primary branches, and number of seeds per pod with mean value of (5.3), (4.4), and (5.4), respectively.

## 2.9.1.10. Leaf Chlorophyll Content

Analysis of variance showed statistically significant differences among accessions for chlorophyll atLEAF values (Figure 2.8). Time was also found to be statistically significant for chlorophyll atLEAF values (Figure 2.9). While interaction between accessions × time was non-significant.

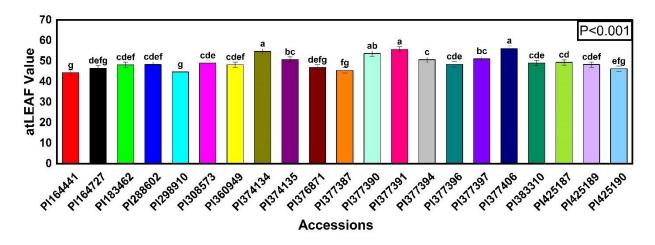


Figure 2.8: Leaf chlorophyll content at LEAF value of black gram accessions under irrigated conditions in 2022.

Accessions PI377391, PI377406, PI374134 exhibited the highest atLEAF values at (54.7-

56.1) respectively, while accession PI164441 and PI298910 had the lowest at LEAF value at

44.68 and 44.30.

On August 5, 2022, the atLEAF value was (50.7). By August 12, it had increased to

(51.5), but then decreased to (45.7) by August 19, 2022, among accessions.

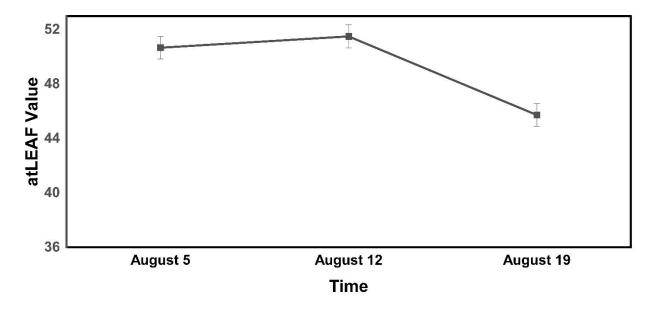


Figure 2.9: Trend of leaf chlorophyll atLEAF value of black gram accessions under irrigated conditions in 2022.

### 2.9.2. Correlation

Knowledge regarding the association of component characters with yield is of great importance to plant breeders, as it facilitates more precise and accurate selection. The degree of relationship and association of these components with yield can be measured by correlation coefficients. Yield per plant showed significant strong positive correlation with pod clusters per plant and number of pods per plant. This suggested that an increase in traits was associated with an increase in yield per plant (Table 2.23).

Aman and Singh (2022) found positive and significant correlation between yield per plant with number of pods per plant and pod clusters per plant. Pod clusters per plant was positive and statistically significant correlation with number of pods per plant. Yield per plant had no significant correlation with plant stand, primary branches, pod length, thousand seed weight and primary branches. The yield per plant had negatively statistically significant correlation with plant height, and days to 1<sup>st</sup> flowering. We observed higher yield from dwarf and early flowering accessions. Plant stand also had positive and statistically significant

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correlation with number of seeds per pod, but negative correlation with days to 1st flowering and

primary branches.

Table 2.23: Pearson correlation coefficients of black gram variables under irrigated condition in 2022.

	PS	PH	FD	PB	PC	NP	PL	SP	TW	YPP
PS										
PH	-0.05									
FD	-0.38 ***	0.58 ***								
PB	-0.44 ***	0.36 ***	0.24 *							
PC	-0.13	-0.37 ***	-0.43 ***	0.07						
NP	-0.21	-0.55 ***	-0.50 ***	0.07	0.91 ***					
PL	0.13	0.07	-0.13	-0.21	0.13	-0.01				
SP	0.25 *	0.13	-0.09	0.04	0.02	-0.07	0.35 **			
TW	-0.01	0.16	0.12	-0.07	-0.09	-0.16	0.63 ***	0.17		
YPP	-0.15	-0.46 ***	-0.46 ***	0.08	0.91 ***	0.94 ***	0.17	0.17	0.05	

'\*'- significant at 0.05, '\*\*'- significant at 0.01, '\*\*\*'- significant at 0.001 level of probability. Note: PS: Plant stand, PH: Plant height(cm), FD: Days to 1<sup>st</sup> flowering; PCP: Pod clusters per plant, PB: Primary branches, NP: Number of pods per plant, PL: Pod length(cm), SP: Number of seeds per pod, TW: Thousand seed weight(g), YPP: Yield per plant(g).

## 2.9.3. Multiple Regression

The multiple linear regressions were estimated to measure the relationship and the change

in magnitude of the dependent variable such as yield per plant due to other independent variables

like number of seeds per pod and thousand seed weight in black gram (Table 2.24)

The multiple linear regression equation could be written as

Yield per plant = -7.12 + 0.01 plant height(cm) + 0.02 primary branches + 0.15 pod

clusters + 0.23 number of pods per plant - 0.07 pod length (cm) + 0.69 number of seeds per pod

+ 0.07 thousand seed weight(g).

Variables	DF	Coefficients	Std. error	t-stat
Intercept	1	7.12 ***	0.59	-11.98
PH	1	0.01	0.01	0.31
PB	1	0.02	0.02	0.78
PCP	1	0.14 **	0.05	2.89
NP	1	0.23 ***	0.01	15.33
PL	1	-0.07	0.15	-0.46
SP	1	0.69 ***	0.07	10.39
TW	1	0.07 ***	0.01	7.56
R2			0.98	
Observation			84	

Table 2.24: Multiple regression studies between yield and other variables of black gram in 2022 under irrigated conditions.

'\*'- significant at 0.05, '\*\*'- significant at 0.01, '\*\*\*'- significant at 0.001 level of probability. Note: PH: Plant height(cm), PB: Primary branches, PCP: Pod clusters per plant, NP: Number of pods per plant, PL: Pod length(cm), SP: Number of seeds per pod, TW: Thousand seed weight(g).

The R<sup>2</sup> (0.98) depicts a good model fit, implying that the independent variables caused 98 percent of the yield per plant. Independent variables like plant height, primary branches, pod clusters per plant, number of pods per plant, number of seeds per pod and thousand seed weight were found statistically significant with yield per plant and other variables remain non-significant (Table 2.24). The number of seeds per pod had a significant impact on grain yield per plant. When there is one per cent increase in the number of seeds per pod, there would be a significant increase in the grain yield per plant of 0.69 percent, other variables being held constant. Similarly, when there is a one percent increase in the other variables like plant height, primary branches, pod clusters per plant, number of pods per plant, and thousand seed weight there would an increase in the grain yield by .01, 0.02, 0.15, 0.23 and 0.07 percent, respectively. Moreover, yield per plant was affected by pod length which showed a negative relationship with yield per plant. It means yield per plant decreased with increase with pod length.

#### 2.10. Summaries

Our study demonstrated the feasibility of growing black gram under irrigated conditions in the Northern Great Plains. We observed significant genetic variability among black gram accessions for various agronomic traits including plant height, flowering days, number of pods per plant, pod length, thousand seed weight, yield per plant, and harvest index. Among the accessions studied, PI377397 and PI377406 emerged as promising candidates due to its yield per plant (6.44 and 6.66 g respectively), early flowering (49 days), dwarf plant height (18 and 16 cm), and high harvest index (0.46 and 0.45). Additionally, the presence of considerable genetic variability among accessions suggested ample opportunity for further genetic and agronomic enhancements in black gram cultivation. Correlation studies revealed that primary pod clusters per plant, number of pods per plant, pod length, number of seeds per pod, and thousand seed weight significantly contribute to yield per plant of black gram. Regression analysis further indicated that pod clusters per plant, number of pods per plant, number of seeds per pod and thousand seed weight had a statistically significant impact on yield per plant variability. Therefore, focusing on these yield-contributing characteristics could prove beneficial for improving black gram yields and introducing superior varieties to this region.

The excessive and longer vegetative growth, few number of pods per plant and pod clusters per plant as well as delayed seed formation noticed under irrigated condition one of the reasons for lower yield under irrigated conditions compared to rainfed condition. Unfortunately, we were unable to corroborate our findings due to a hailstorm that destroyed the entire experiment in 2023. Nevertheless, our initial results suggest that black gram cultivation is feasible under irrigated conditions in the Northern Great Plains U.S. Further evaluations are recommended to identify high-yielding accessions suitable for growing in this region.

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# 3. GENETIC VARIATION IN GUAR FOR PHENOLOGY, PHYSIOLOGY, GROWTH, AND YIELD UNDER IRRIGATED AND RAINFED CONDITIONS OF NORTHERN GREAT PLAINS

#### **3.1.** Abstract

Guar (*Cyamopsis tetragonoloba* (*L.*) *Taub*) is drought tolerant and popular for guar gum. Field trials were conducted to assess the feasibility of growing guar and identify superior germplasms during 2022 and 2023 at the Williston Research Extension Center's rainfed and irrigated site. The experiment was laid out using an alpha lattice design with 18 accessions replicated twice. The results showed significant differences among accessions for number of pods per plant, thousand seed weight, and yield per plant, while genetic variation in days to 1<sup>st</sup> flowering and pod clusters per plant remained non-significant. Accession PI428572-2 and PI428572-3 had the highest yield per plant (7 g and 6 g respectively) under irrigated conditions while (7.9 and 8.3) under irrigated conditions in 2022. These yields were substantially lower than reported in Southern High Plain, U.S. Although, the accessions demonstrated adaptation to grow in Northern Great Plains. Further evaluation of additional accessions is necessary to identify economically high-yielding accessions suitable for this region.

#### **3.2. Introduction**

Guar (*Cyamopsis tetragonoloba* (*L.*) *Taub*), is known as cluster beans, is a drought and heat-tolerant summer nitrogen-fixing legume grown primarily in semi-arid and arid regions, particularly where hot temperatures and low precipitation prevail (MacMillan et al., 2021; Ravelombola et al., 2021; Shrestha et al., 2021). Guar can recover from drought, preserve a suitable seed output, and dry matter when the stress is relieved (Meftahizadeh et al., 2019). It requires minimal water due to its ability to deplete soil water deep within the profile and its thick foliar epidermis, which reduces transpiration losses (Alexander et al., 1988). However, research indicates that typical dryland yields for guar range from 600–1000 lb/a. With limited irrigation of 3"–6", the yield potential rises to about 1000–1500 lb/a. Recent guar contractor yield goals in the region are 900 lb/a dryland and 1500 lb/a irrigated (Abidi et al., 2015). Avola (2020) findings suggested that guar seed yields varied from 1.24 t ha<sup>-1</sup> at 25% of the ET (IL) irrigated to 3.28 t ha<sup>-1</sup> at (IH) 100% of the ET) in 2011, and from 0.98 t ha<sup>-1</sup> 25% of the ET (IL) irrigated to 2.88 t ha<sup>-1</sup> (IH, 100% of the ET) in 2012. Water stress induced plant growth suppression can be due to several metabolic disorders including an imbalance in macro/micro-nutrients and hormones, alteration in ultrastructure of vital proteins and enzyme activities, cellular membrane leakage, disturbance in water or turgor potentials, stomatal or non-stomatal changes in the gas exchange characteristics (Prakash & Singh, 2020; Seleiman et al., 2021).

Research conducted in Pakistan by Ali et al. (2015) showed that there was a significant reduction in the plant height, plant fresh and dry biomass of all 36 accessions of guar grown under water deficit conditions. Zubair et al. (2017) conducted to evaluate 5 guar accession under irrigated and drought conditions in Pakistan and observed statistically significant differences among studied traits such as plant height, clusters plant per plant, pod length, no of pod per plant, no of seed per pod, and grain yield. Stafford and McMichael (1990) showed that the number of pods per plant was most affected by water stress, while seed weight and seeds per pod had a progressively smaller effect on guar yield. Alexander et al. (1988) found that irrigation increased vegetative growth and resulted in reduced seed production per unit of water use. Guar growth halts until moisture becomes available, thereby prolonging the growing season and adequate available soil moisture condition ensures maximum production of forage and beans (Tripp et al., 1977). However, excessive irrigation water applied throughout the season can lead to decreased

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guar seed yields due to excessive vegetative growth and delayed seed formation (Garcia et al., 2023). For optimal seed yield production, guar requires 900 mm of rainfall, and an excess of irrigation water can reduce maximum seed yield (Garcia et al., 2023). A few studies showed that water stress during critical growth stages can significantly reduce guar seed yield. Alexander et al. (1988) research reported that water stress during mid-pod-filling stages decreased seed yield. Furthermore, Reddy (2001) found that moisture stress during flowering stages had a more pronounced negative effect on achieving higher pod yield.

In the U.S., Guar is primarily produced in the Southern High Plains, including Texas, Arizona, and Oklahoma, where hot, dry conditions and erratic rainfall are common (Abidi et al., 2015). All the guar germplasm evaluation research has been concentrated on warm dryland climatic conditions. The Northern Great Plains exhibit diverse climatic conditions; however, recent trends indicate a shift towards warmer and drier weather, characterized by high temperatures, and reduced precipitation, which significantly impacts agricultural management practices (Shafer et al., 2014). The NGP region requires the identification of alternative crops that are drought-tolerant, disease-resistant, in demand both domestically and internationally, and can be managed effectively with low input resources, particularly in terms of plant-available water and nitrogen (Hansen et al., 2012). Thus, evaluating germplasm or accessions is key for identifying adaptive and superior agronomic traits in guar genotypes suited for the NGP.

#### **3.3.** Objectives

The overarching objectives were to evaluate the feasibility of growing guar under rainfed and irrigated conditions of the Northern Great Plains of the U.S. and to identify the superior accessions. The secondary objective was to identify guar traits (phenological, growth, physiological, and yield components) responsible for adaptation and higher yield.

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### **3.4.** Plant Materials

Dr. Jerald Bergman, the emeritus director of the Williston Research Extension Center, facilitated the selection and evaluation process of guar accessions. In 2020, Dr. Bergman screened 32 guar germplasms obtained from the USDA-ARS Plant Genetic Resources Conservation Unit: Griffin, GA, USA U.S. National Plant Germplasm System (NPGS) in greenhouse conditions. Subsequently, he selected these 18 guar accessions (Table 3.1), which were evaluated in this study. We used 17 accessions for data analysis. One accession did not grow and produced 1-2 plants in row. We discarded that accession from the data analysis due to outlier.

Accessions	Country of origin
PI116034-1	India
PI116034-2	India
PI158119	India
PI254367	India
PI255928	India
PI263900	India
PI271546-1	India
PI271546-2	India
PI271546-3	India
PI288347	India
PI288425	India
PI288443	India
PI288742	India
PI288749	India
PI288759	India
PI428572-1	India
PI428572-2	India
PI428572-3	India

Table 3.1: Accession number and country of origin of 18 guar evaluated in the study.

Source: USDA-ARS Plant Genetic Resources Conservation Unit, Griffin, Georgia, USA.

### **3.5. Guar under Rainfed Conditions**

# 3.5.1. Methodologies

#### 3.5.1.1. Experimental Site

In 2022 and 2023, A field trial was conducted at the Williston Research Extension Center's dryland site, Williston, North Dakota. The elevation of the site was 640 m and is characterized by Williams-bowbell loam soil type. In 2022, we seeded guar in WREC field No. 10 (103.7374360°W 48.1261503°N), where the previous crop was oats. In 2023, we used WREC field No. 3 (103.7383104°W 48.1356701°N) where the previous crop was wheat. The monthly mean maximum air temperature, minimum air temperature and precipitation of this location was obtained from NDAWN located at WREC and presented in (Table 3.2).

Table 3.2: Mean monthly air temperatures and	l precipitation	during guar	growing season	n in 2022
and 2023 in Williston, ND.				

	Max Air Temp			Mi	Min Air Temp			Precipitation		
		°C			°C			mm		
Month	Norm.	2022	2023	Norm.	2022	2023	Nor	m.	2022	2023
June	25	24	27	11	11	14	72	2	46	32
July	29	29	28	14	15	14	64	1	49	46
August	29	30	28	13	15	14	44	1	15	69
September	24	25	24	8	8	11	35	5	10	46
Total							21	5	120	193

Note: Norm. = Normal, represents a 30-year average from 1990-2021. Data obtained from North Dakota Agricultural Weather Network.

### 3.5.1.2. Experimental Design

The experiment's layout was carried out in an alpha lattice design with single row plots and two replications. Within each replication, 18 accessions were randomly distributed across 3 blocks, with 6 experimental plots in each block. Each single row plot was 18 ft in length and 2ft width in 2022 while single row plot was 18 ft in length and 2.5 ft width in 2023.

### 3.5.1.3. Seeding and Crop Management

Guar seeds were inoculated with Exceed® Superior Legume Inoculant at a rate of 70.9 g per 22.68 kg of seed before sowing. In 2022, guar was seeded using a six-row seeder with individual cones, whereas in 2023 a GPS based autosteered SRES seeder was used. Each single row was considered one plot and there were two replications. The seeding depth was adjusted to place the seed approximately 1 inch deep in the soil. The targeted plant population at harvest was 1,22,588 plants ha<sup>-1</sup>. Planting dates were June 11, 2022, and June 6, 2023. Only starter fertilizer (N-P-K-S-Zn: 12-14-0-10-1) was applied during planting. In the 2022 experiment, a pre-emergence herbicide, Valor EZ @ 3 oz/a in October 2021, and Spartan Charge @ 3.5 oz/a on May 6, 2022, were used to keep the experimental plots weed-free. Similarly, for 2023 experiment, pre-emergence herbicides Valor EZ @ 88.7 ml/a in October 2022 and AIM eC @ 47.3 ml/a + Agsaver Glyphosate 41% PLUS @ 946.4 oz/a + MSO @ 2pt/a + Ammonium Sulphate @ 2 lb/a on June 1, 2023, were applied. The crop was harvested on the 14th and 15th of October in 2022 and 2023.

The hailstorm occurred on August 1, 2023, under rainfed conditions and caused significant damage to the guar crop in (Figure 3.1). It resulted in defoliation, the loss of flowers, damage to pods, broken stems, and torn-off branches. Despite this setback, the asynchronous flowering and pod-setting characteristic of guar, presence of green parts in the plants and its intact roots in the soil aided in its recovery, allowing for some yield and yield component production in 2023 (Figure 3.2).

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Figure 3.1: Hailstorm damage to guar under rainfed conditions in 2023.



Figure 3.2: Guar growth after hailstorm under rainfed conditions in 2023.

### 3.5.1.4. Data Collection and Phenotypic Measurement

Plant stand were manually counted from single row plot 20 days after sowing. The live seed emergence percentage was calculated as plant stand divided by number of seeds sown per plot multiplied by 100 (Carson & Clay, 2016). The pure live seed per experimental unit (Plot) was determined to be 41 for all accessions to achieve the desired in field plant population of 1,22,588 plants ha-1 in 2022. Due to poor germination percentage, poor seed quality obtained from 2022 guar harvested, and limitation of pure live seed in 2023, we sown 9 viable seed per ft in a single row plot to achieve the desired in plant population, suggested by (Undersander et al., 1991).

The days to 1<sup>st</sup> flowering was recorded when the first flower appeared in the plot, and the number of days to flower from sowing was recorded. Five plants were randomly selected and tagged after 1 month of sowing in each experimental plot, ensuring there were neighboring plants surrounding it. Leaf chlorophyll content was measured using atLEAF handheld digital chlorophyll meters from tagged plants. One trifoliate leaf was tagged for repeated measurements of leaf chlorophyll atLEAF value. The measurements were conducted on accessions at the flowering stage, with data collected weekly for three consecutive weeks in August. Leaf chlorophyll content was not recorded because of hailstorm-induced leaf destruction in 2023. At maturity, several parameters were measured from five tagged plant, measurements include above-ground biomass weight, plant height, primary branches, pod clusters per plant, number of pods per plant, pod length, number of seeds per pod, grain yield per plant, thousand seed weight and harvest index. Theoretical grain yield in kg ha<sup>-1</sup> was computed based on grain yield per plant by considering the targeted plant population at harvest. This calculation was performed to enable comparison with yields reported by other researchers, who typically use kg ha<sup>-1</sup> as the unit of

yield. Five pods from the sample were selected for pod length. Plant height was measured from the ground base to the tip of the stem using a measuring scale in 2022. However, canopy height was measured from the ground base to the top of the plant using a measuring scale because of hailstorm damage in 2023.

#### 3.5.1.5. Data Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, North Carolina, USA) software package. Data were checked for normality and outliers using histograms, scatter plots, Q-Q Plots, and plots of residuals. Analysis of variance (ANOVA) was performed in SAS using the PROC GLIMMIX procedure. In the SAS model, accessions and year were treated as fixed effects. Replication, replication within year, and block within replication × year were considered as random effects. When the source of variance was significant, the means were separated using the least square mean test at a significance level of P<0.05. Correlation analysis and multiple regression analysis were performed using PROC CORR and PROC REG procedures in SAS, respectively.

### **3.6. Results and Discussion**

#### **3.6.1.** Analysis of Variance

Analysis of variance showed significant effect of year, accessions, and interaction effect of year  $\times$  accessions on measured parameters (Table 3.3). The interaction effect of year  $\times$ accession was found statistically significant for plant stand, number of pods per plant and yield per plant. The analysis of variance showed that the effect of year was non-significant for all measured parameters. Analysis of variance showed significant effect of accessions for pod clusters per plant, thousand seed weight and harvest index. The number of seeds per pod, days to 1<sup>st</sup> flowering and plant height was statistically non-significant for all sources of variation. Leaf chlorophyll content at LEAF value and trend of chlorophyll content at LEAF value were also

found statistically non-significant among accessions in 2022. This analysis of variance showed

that guar accession had limited genetic variability for measured parameters.

Table 3.3: Sources of variation, degrees of freedom, and levels of significance for the ANOVA of measured parameters of guar in 2022 and 2023 under rainfed conditions.

SOV	DF	PS	LSE	FD	PH	NP	PCP	SP	PL	TW	YPP	HI
Year	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Accessions	16	**	ns	ns	ns	***	*	ns	**	***	***	***
Year $\times$ Accessions	16	***	ns	ns	ns	**	ns	ns	ns	ns	*	ns

Note: ns, \*, \*\*, \*\*\* = not significant, significant at (P $\leq$ 0.05), (P $\leq$ 0.01), and (P $\leq$ 0.001), respectively. SOV = Source of variation, DF = Degrees of freedom, PS = Plant Stands, LSE = Live seed emergence, FD = Days to 1<sup>st</sup> flowering, PH= plant height, NP = Number of pods per plant, PCP = Pod clusters per pod, PB = Primary branches, SP = Seeds per pod, PL= Pod length, TW = Thousand seed weigh, YPP= Yield per plant, HI=Harvest index.

# 3.6.1.1. Germination, Plant Stands and Live Seed Emergence Percent

Analysis of variance showed significant effect of accessions and interaction effect of year × accession on plant stand. The analysis revealed that the effect of year was non-significant for plant stand. All the sources of variance were statistically non-significant for percent live seed emergence (Table 3.3). When averaged across accessions and year, mean percent live seed emergence was (41 %). When averaged across year, percent live seed emergence was (44 %) in 2022 while it was (38 %) in 2023. Low live seed emergence percent was observed among guar accessions in both years. It indicated that guar may need high soil and air temperature for germination. In the lab, germination of all guar accessions was 100 percent in 2022. We used 2022 trials harvested seed in 2023. Due to limitation of quality seed and poor germination in lab, we sown 9 pure live seeds per foot in a single row plot to maintain desirable plant populations in plot row. This was the reason the plant stand was different in 2022 and 2023 (Table 3.4).

	Germ	ination	Plant	stand
	("	%)	(N	[0.)
Accessions	2022	2023*	2022	2023
PI116034-1	100	-	14.4 c	62.5 ab
PI116034-2	100	-	18.9 c	64.6 a
PI158119	100	-	14.8 c	73.8 a
PI254367	100	-	16.5 c	61.8 ab
PI255928	100	-	18.5 c	54.9 ab
PI263900	100	-	17.4 c	46.9 b
PI271546-1	100	-	15.3 c	70.3 a
PI271546-2	100	-	21.7 с	70.4 a
PI271546-3	100	-	19.3 c	73.7 a
PI288347	100	-	21.3 c	18.8 c
PI288425	100	-	16.0 c	73.6 a
PI288443	100	-	15.2 c	60.9 ab
PI288742	100	-	18.7 c	17.8 c
PI288749	100	-	21.6 c	72.5 a
PI428572-1	100	-	16.9 c	60.4 ab
PI428572-2	100	-	21.5 c	59.6 ab
PI428572-3	100	-	16.9 c	61.9 ab
Year mean			17.8 A	55.2 A

Table 3.4: Germination and plant stand of guar accessions in 2022 and 2023 under rainfed conditions.

Note: Same small letter in a column or a row indicates statistically non-significant at p=0.05. Same capital letter in a row indicates statistically non-significant at p=0.05.  $2023^* = 9$  pure live seeds were sown per foot in single row plot due to very poor germination and limitation of quality guar seed.

Analysis of variance showed that plant stand was non-significant among guar accessions

in 2022. Accession PI116034-2, PI158119, PI271546-1, PI271546-2, PI271546-3 and PI288425

and PI288742 produced (64.6-73.8) had highest plant stand while accession 288742 and

PI288347 had lowest plant stand (17.8) and (18.8) respectively. When averaged across year,

plant stand was (17.8) in 2022 whereas plant stand was (55.2) in 2023 under rainfed conditions.

# 3.6.1.2. Pod Length

Analysis of variance showed that accessions were statistically significant for pod length

(Table 3.5). The effect year and interaction effect of year × accessions were statistically non-

significant for pod length (Table 3.3).

Table 3.5: Pod length and pod clusters per plant of guar accessions in 2022 and 2023 under rainfed conditions.

	Pod length	Pod clusters per plant
Accessions	(cm)	(no.)
PI116034-1	4.9 bc	7.8 ab
PI116034-2	5.5 a	6.5 abcd
PI158119	5.2 ab	3.7 cd
PI254367	5.3 ab	8.1 ab
PI255928	5.5 a	5.3 bcd
PI263900	4.9 bc	6.6 abcd
PI271546-1	5.1 abc	3.5 d
PI271546-2	5.3 ab	4.8 bcd
PI271546-3	5.5 a	5.9 bcd
PI288347	4.7 c	8.3 ab
PI288425	5.2 ab	8.1 ab
PI288443	5.5 a	10.4 a
PI288742	5.0 bc	7.9 ab
PI288749	5.4 a	3.4 d
PI428572-1	5.2 ab	7.0 abc
PI428572-2	4.9 bc	7.6 ab
PI428572-3	4.9 bc	7.6 ab
Mean	5.2	6.6

Note: Same small letter indicates statistically non-significant at p=0.05.

Accession PI116034-2, PI255928, PI271546-3 and 288443 had longest pod length (5.5 cm) while PI288347 had shortest pod length (4.7 cm). Boghara et al. (2016) found that pod length varied from (4.29 cm) to (7.79 cm) among 31 genotypes evaluated in India. Santhosha et al. (2016) observed pod length ranged from (4.72 cm) to (11.16 cm) among 43 genotypes. This finding suggested the existence of genetic variability in pod length.

#### 3.6.1.3. Pod Clusters per Plant

Analysis of variance revealed that pod clusters per plant was statistically significant among accessions (Table 3.5). The effect of year and interaction effect of year × accessions were statistically non-significant for pod clusters per plant (Table 3.3). Accessions PI288443 had the highest pod clusters per plant (10.4) whereas accessions PI271546-1 had the lowest pod clusters per plant (3.5) with mean (6.6). Kgasudi et al. (2020) reported pod clusters per plant ranged from (10.9) to (21.8) among 56 guar genotypes. Santhostha et al. (2016) observed (11) to (38) among 43 genotypes evaluated in India. These results indicated that there was a presence of genetic variability for pod clusters per plant.

#### 3.6.1.4. Plant Height

Analysis of variance revealed that all the source of variation was non-significant for plant height (Table 3.3) When averaged across accessions and year, mean height was (41 cm).

# 3.6.1.5. Days to 1<sup>st</sup> Flowering

Analysis of variance demonstrated that all the source of variation was non-significant for plant height (Table 3.3). When averaged across accessions and year, mean days to 1<sup>st</sup> flowering was (45 days).

### 3.6.1.6. Number of Seeds per Pod

All the sources of variation were non-significant for seeds per pods (Table 3.3). When averaged across accessions and year, mean number of seeds per pod was (3.9).

### 3.6.1.7. Number of Pods per Plant

Analysis of variance showed significant effect of accessions and interaction effect of year × accession on number of pods per plant while effect of year was non-significant (Table 3.3 and Table 3.6). In all accessions, the number of pods per plant was higher in 2022 compared to 2023.

	Number of pods per plant						
	(no.)						
Accessions	2022	2023					
PI116034-1	59.1 a	22 efghi					
PI116034-2	46.5 ab	17.2 ghijk					
PI158119	37.8 bcd	10.7 k					
PI254367	48.2 ab	11.3 jk					
PI255928	34.1 bcde	24.1 defg					
PI263900	33.2 bcde	21.8 efghi					
PI271546-1	30.2 cdef	13.6 hijk					
PI271546-2	34.1 bcde	23.5 efgh					
PI271546-3	41.1 bc	24.5 defg					
PI288347	34.1 bcde	12.6 ijk					
PI288425	40.4 bc	19.4 fghij					
PI288443	40.7 bc	24.5 defg					
PI288742	48 ab	19.7 fghij					
PI288749	33.5 bcde	11.5 jk					
PI428572-1	63.3 a	22.4 efgh					
PI428572-2	41 bc	32.6 bcde					
PI428572-3	61.3 a	27.7 cdefg					
Year mean	41.7 A	18.9 A					

Table 3.6: Number of pods per plant of guar accessions in 2022 and 2023 under rainfed conditions.

Hailstorm reduced the number of pods per plant by breaking stem, loss of flowers and damage to pod initiation in guar in 2023. When averaged across accessions, number of pods per plant was (41.7) in 2023 while it was (18.9) in 2022. Accession PI116034-1 and PI428572-1 had higher number of pods per plant (59.1 and 63.3 respectively) while PI271546-1 had lower number of pods per plant (30.2) in 2022. In 2023, Accession PI428572-2 recorded a higher number of pods per plant (32.6) whereas PI158119 recorded lower number of pods (10.7) in 2023.

Note: Same small letter in a column or a row indicates statistically non-significant at p=0.05. Same capital letter in a row indicates statistically non-significant at p=0.05.

Santhosha et al. (2016) reported the number of pods per plant varying from 46 to 263 among 43 genotypes. Boghara et al. (2016) observed number of pods per plant ranging from (41.4) to (174.5) among 31 genotypes evaluated in India. Vir et al. (2013) recorded number of pods per plant differed from (8) to (102) among 44 accessions evaluated in India. These findings suggested the existence of genetic variability for the number of pods per plant among guar accessions.

#### 3.6.1.8. Thousand Seed Weight

Analysis of variance showed that thousand seed weight was statistically significant among accessions. The analysis revealed that the effect of year and interaction effect of year  $\times$ accessions was non- significant (Table 3.3). Accession PI263900 and PI288425 had highest thousand seed weight (36.6 and 36.3 g) whereas accession PI116034-1 had lowest thousand seed weight (24.3 g) with mean (31.5 g) (Table 3.7). Santhosha et al. (2016) reported thousand seed weight varied from (28 g) to (46.4 g) among 43 genotypes. Boghara et al. (2016) observed thousand seed weight ranged from (29.7) to (41.3) among 31 genotypes evaluated in India. Vir et al. (2013) recorded thousand seed weight differed from (21.2 g) to (39 g) among 44 accessions evaluated in India. These results suggested the presence of genetic variability for the thousand seed weight among guar accessions.

#### 3.6.1.9. Harvest Index

Analysis of variance revealed accessions were statistically significantly different for harvest index. The analysis showed that the effect of year and interaction effect of year  $\times$  accessions were non- significant (Table 3.3). Accession PI428572-2 had the highest harvest index (0.39) whereas PI158119 had lowest harvest index (0.18) with mean (0.27) (Table 3.7).

	Thousand seed weight	Harvest index
Accessions	(g)	
PI116034-1	24.3 i	0.33 abcde
PI116034-2	28.1 fgh	0.32 bcde
PI158119	32.8 bcde	0.18 g
PI254367	31.7 cde	0.19 fg
PI255928	30.5 defg	0.36 abc
PI263900	36.6 a	0.20 fg
PI271546-1	33.8 abcd	0.29 cde
PI271546-2	36.4 a	0.28 de
PI271546-3	35.0 ab	0.26 ef
PI288347	26.6 hi	0.15 g
PI288425	36.3 a	0.19 fg
PI288443	34.0 abc	0.26 ef
PI288742	27.5 ghi	0.20 fg
PI288749	32.6 bcde	0.31 bcde
PI428572-1	30.4 efg	0.36 abcd
PI428572-2	27.8 gh	0.39 a
PI428572-3	31.3 cdef	0.37 ab
Mean	31.5	0.27

Table 3.7: Thousand seed weight and harvest index of guar accessions in 2022 and 2023 under rainfed conditions.

Note: Same small letter indicates statistically non-significant at p=0.05.

# 3.6.1.10. Yield per Plant

Analysis of variance showed significant effect of accessions and interaction effect of year × accession on yield per plant. However, the analysis revealed that year was non-significant (Table 3.3 and Table 3.8). In all accession, yield per plant was lower in 2023 compared to 2022. This reduction was due to hailstorm damage which occurred during the growing season. Yield components, such as number of pods per plant and pod clusters per plant, were lower in 2023 compared to 2022. Accessions PI116034-1, PI428572-2 and PI428572-3 had the highest yield per plant (7.9 -8.3g) while PI263900 had the lowest yield per plant (3.8 g) in 2022. Accession

PI428572-2 had the highest yield (3.8 g) whereas PI158119 had the lowest yield per plant (0.8 g)

in 2023.

	Yield per	plant	Grain yie	ld
	(g)		(kg ha <sup>-1</sup> )	)
Accessions	Accessions 2022		2022	2023
PI116034-1	7.9 a	2.6 jklmn	963.2 a	321.2 hijkl
PI116034-2	5.9 bcde	1.8 lmn	715.3 bcde	218.6 jkl
PI158119	5.9 bcde	0.8 n	715.9 bcde	98.51
PI254367	5.7 cdef	1.3 mn	699.9 bcde	153.8 kl
PI255928	6.4 abcd	3.3 hijklm	784.9 abcd	396.6 ghijk
PI263900	3.8 fghijkl	2.8 jklmn	459.0 fghij	335.7 hijkl
PI271546-1	4.7 defghij	1.7 lmn	570.2 defgh	209.0 jkl
PI271546-2	6.7 abc	3.4 ghijkl	818.1 abc	420.5 fghij
PI271546-3	5.4 cdefg	3.1 ijklm	657.0 cdef	374.8 hijk
PI288347	4.0 efghijk	1.0 n	489.1 efghi	117.21
PI288425	4.1 efghijk	3.0 jklm	509.5 efghi	372.9 hijk
PI288443	6.3 abcd	3.7 fghijkl	779.7 abcd	444.0 fghij
PI288742	5.3 cdefgh	2.4 klmn	652.4 cdef	296.9 ijkl
PI288749	5.1 cdefghi	1.4 mn	633.1 cdefg	168.8 kl
PI428572-1	7.7 ab	2.3 klmn	944.6 ab	286.4 ijkl
PI428572-2	7.9 a	3.8 efghijk	976.3 a	474.4 efghi
PI428572-3	8.3 a	2.8 jklmn	1020.9 a	335.1 hijkl
Year mean	5.9 A	2.4 A	728.8 A	295.6 A

Table 3.8: Yield per plant and grain yield of guar accessions in 2022 and 2023 under rainfed conditions.

Note: Same small letter in a column or a row indicates statistically non-significant at p=0.05. Same capital letter in a row indicates statistically non-significant at p=0.05.

Santhosha et al. (2016) reported grain yield per plant varying from (4.67 g) to (18.47 g) among 43 genotypes. Boghara et al. (2016) recorded yield per plant ranged from 8.83 g to 41.80 among 31 genotypes evaluated in India. Vir et al. (2013) reported yield per plant differed from (3.89 g) to (27.62 g) among 44 accessions evaluated in India. These results confirmed genetic variability among guar accessions for grain yield. Among all guar accessions, delayed maturity and extended vegetative growth were noted. Additionally, the grain yield of all accessions was lower when compared with yields typically seen in the southern region of Texas. Singla et al. (2016) reported a seed yield of 1308 kg ha<sup>-1</sup> for guar in the southern high plains under semi-arid conditions.

# 3.6.1.11. Leaf Chlorophyll Content

Analysis of variance showed that accessions and time were statistically non-significant for leaf chlorophyll content with mean atLEAF value of (60.19). Time wise, it was (60.5) atLEAF value on 2 August, (60.9) value on 10 August and (59.1) value on 16 August 2022.

### 3.6.2. Correlation

In 2022, yield per plant showed no significant correlation with plant stand, plant height, days to 1<sup>st</sup> flowering, pod clusters per plant, number of pods per plant and thousand seed weight (Table 3.9).

Table 3.9: Pearson correlation coefficients of guar variables under rainfed condition in 2022.

	PS	PH	FD	PCP	NP	PL	SP	TW	YPP
PS									
PH	0.05								
FD	-0.11	-0.06							
PCP	-0.23	-0.49 **	-0.03						
NP	-0.37 *	-0.15	0.22	0.43 **					
PL	0.08	0.20	-0.29	-0.25	-0.15				
SP	0.22	-0.03	-0.34	-0.10	-0.19	0.26			
TW	-0.24	0.58 ***	0.11	-0.28	-0.28	0.02	-0.41 **		
YPP	-0.27	0.14	0.01	0.16	0.68 ***	0.14	0.42 *	-0.13	

'\*'- significant at 0.05, '\*\*'- significant at 0.01, '\*\*\*'- significant at 0.001 level of probability. Note: PS: Plant Stand, PH: Plant height(cm), FD: Days to 1<sup>st</sup> flowering; PCP: Pod clusters per plant, NP: Number of pods per plant, PL: Pod length(cm), SP: Number of seeds per pod, TW: Thousand seed weight(g), YPP: Yield per plant(g).

Yield per plant exhibited a statistically significant positive correlation with the number of pods per plant and seed per pod in 2022 and 2023. A similar finding was reported by (Singh et

al., 2021; Jukanti et al., 2014). (Boghara et al. (2016) also observed similar correlation between black gram yield and number of pods per plant. In 2023, yield per plant had significant correlation with plant stand, days to 1<sup>st</sup> flowering, pod clusters per plant, number of pods per plant, and number of seeds per pod, but no relation with other measured parameters. On the other hand, plant stand showed positive and statistically significant correlation with pod length and number of seeds per pod.

	PS	PH	FD	PCP	NP	PL	SP	TW	YPP
PS									
PH	0.02								
FD	-0.21	0.07							
PCP	-0.14	-0.04	-0.47 **						
NP	0.25	-0.07	-0.47 **	0.68 ***					
PL	0.39 *	-0.03	-0.28	-0.02	0.01				
SP	0.45 **	-0.37 *	-0.38 *	0.25	0.54 ***	0.52 **			
TW	0.20	0.61 ***	-0.04	0.01	-0.01	0.40	-0.07		
YPP	0.35 *	-0.02	-0.45 **	0.61 ***	0.94 ***	0.23	0.71 ***	0.18	

Table 3.10: Pearson correlation coefficients of guar variables under rainfed condition in 2023.

<sup>\*\*'-</sup> significant at 0.05, <sup>\*\*\*'-</sup> significant at 0.01, <sup>\*\*\*\*'-</sup> significant at 0.001 level of probability. Note: PS: Plant stand, PH: Plant height(cm), FD: Days to 1<sup>st</sup> flowering, PCP: Pod clusters per plant, NP: Number of pods per plant, PL: Pod length(cm), SP: Number of seeds per pod, TW: Thousand seed weight(g), YPP: Yield per plant(g).

# **3.6.3.** Multiple Regression

Multiple regression was done to predict how single dependent variable was influenced or

affected by independent variables.

In 2022 year, the multiple linear regression equation could be written as

Grain yield= -10.58 - 0.01 plant height(cm) - 0.02 primary pod clusters + 0.13 number of

pods per plant + 0.26 pod length (cm) + 0.96 number of seeds per pod + 0.16 thousand seed

weight(g).

The R<sup>2</sup> value of (0.94) indicates a good model fit, suggesting that the independent variables account for 94 percent of the variance in yield per plant in (Table 3.11). Independent variables such as the number of pods per plant, number of seeds per pod, and thousand seed weight were found to be statistically significant corelated with yield per plants variation (Table 3.11). The slope coefficient of seeds per pod suggests that a one percent increase in seeds per pod leads to a significant increase in grain yield by 0.96 percent, with other variables held constant. Similarly, a one percent increase in the variables such as number of pods per plant, pod length, seeds per pod, and thousand seed weight results in yield increases of 0.13, 0.26, 0.96 and 0.16, respectively. Pod clusters per plant showed negative impact in yield per plant.

Variables	DF	Coefficients	Std. error	t-stat
Intercept	1	-10.58 ***	1.46	-7.27
PH	1	0.01	0.02	0.32
PCP	1	-0.02	0.03	-0.56
NP	1	0.13 ***	0.01	16.87
PL	1	0.26	0.2	1.33
SP	1	0.96 ***	0.07	12.91
TW	1	0.16 ***	0.03	6.04
$\mathbb{R}^2$			0.94	
Observation			34	

Table 3.11: Multiple regression studies between yield and other variables of guar in 2022 under rainfed conditions.

'\*'- significant at 0.05, '\*\*'- significant at 0.01, '\*\*\*'- significant at 0.001 level of probability. Note: PH: Plant height(cm), PCP: Pod clusters per plant, NP: Number of pods per plant, PL: Pod length(cm), SP: Number of seeds per pod, TW: Thousand seed weight(g).

In 2023 year, the multiple linear regression equation could be written as

Yield per plant= -3.59 + 0.01 plant height(cm) + 0.01 primary pod clusters + 0.11 number

of pods per plant -0.11 pod length (cm) +0.53 number of seeds per pod + 0.07 thousand seed

weight(g).

The R<sup>2</sup> value of (0.94) depicts a good model fit, implying that the independent variables caused 94 percent of yield per plant (Table 3.12). A positive statistically significant was found between number of pods per plant, number of seeds per pod and thousand seed weight with yield per plant. Plant height, and pod clusters per plant had non-significant weak impact in yield per plant. Pod length showed negative variation on yield per plant.

Variables	DF	Coefficients	Std. error	t-stat
Intercept	1	-3.59 ***	0.47	-7.67
PH	1	0.01	0.01	0.71
PCP	1	0.01	0.02	0.38
NP	1	0.11 ***	0.01	14.37
PL	1	-0.11	0.12	-0.88
SP	1	0.53 ***	0.08	6.71
TW	1	0.07 ***	0.01	4.84
$\mathbb{R}^2$			0.98	
Observation			34	

Table 3.12: Multiple regression studies between yield and other variables of guar in 2023 under rainfed conditions.

'\*'- significant at 0.05, '\*\*'- significant at 0.01, '\*\*\*'- significant at 0.001 level of probability. Note: PH: Plant height(cm), PCP: Pod clusters per plant, NP: Number of pods per plant, PL: Pod length(cm), SP: Number of seeds per pod, TW: Thousand seed weight(g).

### 3.7. Summaries

Our findings provide valuable insights into the performance of guar accessions in the Northern Great Plains. All 18 guar accessions germinated, grew, and yielded under rainfed conditions. However, one accession produced only 2-3 plants in row plant in both years. Based on this finding, it can be noted that guar may adapt and grow to the Northern Great Plains U.S. We observed less genetic variability among accessions for growth and yield attributing traits in 2022 and 2023. Significant differences were observed in the number of pods per plant, pod length, thousand seed weight and harvest index across both years, with yield per plant differing only in 2022. Plant height, days to 1<sup>st</sup> flowering, primary pod clusters, number of seeds per pod were found to be non-significant.

Regression studies revealed a positive statistically significant relation between the number of pods per plant, number of seeds per pod and thousand seed weight with yield per plant. Correlation studies showed that number of pods per plant was strongly positively statistically significant associated with yield per plant. Accessions PI116034-1, PI428572-2 and PI428572-3 had the highest yield per plant (7.9 - 8.3g) while PI263900 had the lowest yield per plant (3.8 g) in 2022. Accession PI428572-2 had the highest yield (3.8 g) whereas PI158119 had the lowest yield per plant (0.8 g) in 2023.

Despite encountering challenges such as hailstorm damage during the 2023 growing season, guar plants recovered and produced yields and yield components but yield per plant remained non-significant among accessions. As yield obtained was significantly lower than in Southern Great Plains of the U.S. Further investigations are recommended with more accessions to identify germplasm with economic yield for NGP.

### **3.8.** Guar under Irrigated Conditions

#### **3.8.1.** Methodologies

#### 3.8.1.1. Experimental Site

A two-year field research trial was conducted at the Williston Research Extension Center's Nesson Valley Irrigation Research Site, located in Nesson Valley, Ray, North Dakota. The farm is situated approximately 31 miles east of Williston on Highway 1804 and has an elevation of 580 meters. The research site is characterized by lihen loamy fine sand soil type. In 2022, guar was seeded in field span no. 3 (103.0984430°W 48.1671920°N), and in 2023, the trial was conducted in field Span no. 4 (103.1082234°W 48.1669375°N). Spring wheat was the preceding year's crop in both locations. The monthly mean maximum air temperature, minimum air temperature and precipitation of this location was obtained from NDAWN located at Hofflund and presented in (Table 3.13).

Table 3.13: Mean monthly air temperatures and precipitation during guar growing season in 2022 and 2023 in Nesson, ND.

	Max Air Temp		Min	Min Air Temp			Precipitation			
		°C			°C				mm	
Month	Norm.	2022	2023	Norm.	2022	2023	No	rm.	2022	2023
June	24	23	27	11	10	13	8	1	59	51
July	28	27	27	14	14	13	7	1	68	42
August	28	28	28	14	14	13	3	8	4	66
September	72	23	24	7	8	9	3	3	10	38
Total							22	23	141	197

Note: Norm. = Normal, represents a 30-year average from 1990-2021. Data obtained from North Dakota Agricultural Weather Network.

### 3.8.1.2. Seeding and Crop Management

Guar seeds were then inoculated with Exceed® Superior Legume Inoculant at a rate of 70.9 g per 22.68 kg of seed which contains a bradyrhizobium strain of rhizobia. In 2022 and 2023, guar was seeded using a GPS-based autosteered six-row seeder (SRES). Each single row was considered one plot and there were two replications. The seeding depth was adjusted to place the seed approximately 1 inch deep in the soil. The targeted plant population at harvest was 1,22,588 plants ha<sup>-1</sup>. Planting dates were June 17, 2022, and June 6, 2023. The crop was harvested in the second week of October 2022. Starter fertilizer (N-P-K-S-Zn: 12-14-0-10-1) was applied during planting. In the 2022 and 2023 trial, Gramoxone @ 591 ml /a, Roundup @ 591 ml/and class act 2% on May 23, 2022, were used to keep the experimental plots weed-free. The crops were harvested on 13 October in 2022. Irrigation was provided using automated variable rate sprinkler irrigation technology and information was provided in (Table 3.14).

Date	2022	Date	2023
	Amount (mm)		Amount(mm)
27-Jun	51	20-Jun	28
6-Jul	25	27-Jun	28
8-Jul	25	5-Jul	33
13-Jul	38	11-Jul	25
18-Jul	38	18-Jul	33
1-Aug	38	24-Jul	41
5-Aug	25		
25-Aug	51		
Total	292		188

Table 3.14: Detailed information of irrigation supplied to guar in 2022 and 2023 under irrigated conditions.

Note: mm=Millimeter.

Unfortunately, the entire experiment was destroyed due to a hailstorm in Nesson on

August 1, 2023, as shown in (Figure 3.4).



Figure 3.3: Guar row plot before hailstorm under irrigated conditions in 2023



Figure 3.4: Guar plot after hailstorm under irrigated conditions in 2023.

#### 3.8.1.3. Data Collection and Phenotypic Measurement

Plant stands were manually counted from single row plot 20 days after sowing. The seed emergence percentage was calculated as plant stand divided by number of seeds sown per plot multiplied by 100 (Carson & Clay, 2016). Due to poor germination percentage, poor seed quality obtained from 2022 guar harvested, and limitation of pure live seed in 2023, we sown 9 viable seed per ft in a single row plot to achieve the desired in plant population, suggested by (Undersander et al., 1991).

The flowering date was recorded when the first flower appeared in the plot, and the number of days to flower from sowing was recorded. Five plants were randomly selected and tagged after 1 month of sowing in each experimental plot, ensuring there were neighboring plants surrounding it. Leaf chlorophyll content was measured using atLEAF handheld digital chlorophyll meters from tagged plants. One trifoliate leaf was tagged for repeated measurements of chlorophyll atLEAF value. The measurements were conducted on accessions at the flowering stage, with data collected weekly for three consecutive weeks in August 2022. At maturity, several parameters were measured from five tagged plant, measurements include above-ground biomass weight, plant height, pod clusters per plant, number of pods per plant, pod length, number of seeds per pod, grain yield per plant, thousand seed weight and harvest index.

Theoretical grain yield in kg ha<sup>-1</sup> was computed based on grain yield per plant by considering the targeted plant population at harvest. This calculation was performed to enable comparison with yields reported by other researchers, who typically use kg ha<sup>-1</sup> as the unit of yield. Five pods from the sample were chosen for pod length. Plant height was measured from the ground base to the tip of the stem using a measuring scale in 2023. In 2023, we took plant stand, live seed emergence and days to 1<sup>st</sup> flowering in guar trial due to hailstorm episodes in 2023.

#### 3.8.1.4. Data Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, North Carolina, USA) software package. Data were checked for normality and outliers using histograms, scatter plots, Q-Q Plots, and plots of residuals. Analysis of variance (ANOVA) was performed in SAS using the PROC GLIMMIX procedure. A combined analysis was conducted for plant stand, live seed emergence, and days to 1<sup>st</sup> flowering, as these measurements were taken before hailstorm damage occurred in 2023. In the SAS model, accessions and year were treated as fixed effects, while replication, replication within year, and block within replication × year were considered as random effects. Due to the hailstorm damage in 2023, we were unable to obtain yield and yield component data from the irrigated conditions. Hence, the data obtained from the 2022 trial were analyzed. In the SAS model, accessions were treated as fixed effects, and replication and block within replication were considered as random effects. When source of variance was significant, the means were separated using the least square mean test at a significance level of P<0.05. Correlation and multiple regression analysis were performed using PROC CORR and PROC REG procedures in SAS, respectively.

#### 3.9. Results and Discussion

### **3.9.1.** Analysis of Variance

Analysis of variance showed significant effect of year, accessions, and interaction effect

of year  $\times$  accessions on measured parameters (Table 3.15).

Table 3.15: Sources of variation, degrees of freedom, and levels of significance for the ANOVA of measured parameters of guar in 2022 and 2023 under irrigated conditions.

SOV	DF	PS	LSE	FD	PH	NP	PCP	SP	PL	TW	YPP	HI	LC
Year	1	ns	ns	ns									
Accessions	16	**	ns	ns	**	***	ns	ns	***	*	**	*	***
Year $\times$ Accessions	16	**	ns	ns									

Note: ns, \*, \*\*, \*\*\* = not significant, significant at (P $\leq$ 0.05), (P $\leq$ 0.01), and (P $\leq$ 0.001), respectively. PS, EM, FD represent combined analysis of 2022 and 2023 and other parameters were analyzed from 2022 as accession as fixed effect due to hailstorm damage. SOV = Source of variation, DF = Degrees of freedom, PS = Plant stands, LSE = Live seed emergence, PH= Plant height, FD = Days to 1<sup>st</sup> flowering, NP = Number of pods per plant, PCP = Pod clusters per pod, SP = Seed per pod, PL= Pod length, TW = Thousand seed weigh, YPP= Yield per plant, HI=Harvest index, LC= leaf chlorophyll.

There was a significant effect of accessions and interaction effect of year  $\times$  accessions on Plant stand. All the sources of variance were statistically significant for seed emergence and days to 1<sup>st</sup> flowering. There was statistically significant for plant height, number of pods per plant, pod length, thousand seed weight, yield per plant, harvest index and chlorophyll content.

#### 3.9.1.1. Germination, Plant Stand and Live Seed Emergence Percent

Analysis of variance showed significant effect of accessions and interaction effect of year  $\times$  accession on plant stand. The analysis showed the effect of year was non-significant. All the sources of variance were non-significant for seed emergence (Table 3.15). When averaged across accessions and year, mean percent live seed emergence was (31 %). Under rainfed conditions, the mean guar percent live seed emergence was (41 %). In all accession, there was lower plant stand in 2022 compared to 2023. Accessions PI271546-1 had the highest plant stand (19.9) while

PI428572-3 had the lowest plant stand (10.5) in 2022. Accession PI271546 had the highest plant stand (53.5) whereas PI288742 had the lowest plant stand (10) in 2023. The difference of plant stand is due to different seedling rate per plot in 2022 and 2023. In 2023, we sown 9 viable seeds per foot in a single row plot to achieve the desired in plant population due to very poor germination of guar in lab and limitation of quality seed.

	Germination		Plant stand	ł
-	(*	%)	(no.)	
Accessions	2022	2023*	2022	2023
PI116034-1	100	-	18.0 efg	24.7 de
PI116034-2	100	-	17.5 efg	45.5 ab
PI158119	100	-	19.1 efg	42.2 ab
PI254367	100	-	17.0 efg	14.3 efg
PI255928	100	-	19.1 efg	47.6 ab
PI263900	100	-	18.0 efg	41.1 ab
PI271546-1	100	-	19.9 ef	34.8 bcd
PI271546-2	100	-	16.5 efg	53.5 a
PI271546-3	100	-	13.5 efg	40.1 abc
PI288347	100	-	14.7 efg	19.9 ef
PI288425	100	-	18.4 efg	41.0 ab
PI288443	100	-	11.3 efg	45.5 ab
PI288742	100	-	12.6 efg	10.0 g
PI288749	100	-	14.4 efg	35.0 bcd
PI428572-1	100	-	16.5 efg	40.8 ab
PI428572-2	100	-	17.0 efg	26.4 cde
PI428572-3	100	-	10.5 fg	39.2 abc
Year mean			15.9 A	32.9 A

Table 3.16: Germination and plant stand in 2022 and 2023 of guar accessions under irrigated conditions.

Note: Same small letter in a column or a row indicates statistically non-significant at p=0.05. Same capital letter in a row indicates statistically non-significant at p=0.05.  $2023^* = 9$  pure live seeds were sown per foot in single row plot due to very poor germination and limitation of quality seed.

### 3.9.1.2. Plant Height

Analysis of variance revealed presence of genetic variability and statistically significant differences for plant height with mean height (60.7). Maximum growth was observed under

irrigated conditions compared to rainfed conditions.

Table 3.17: Plant height and number of pods per plant of guar accessions in 2022 under irrigation	ted
conditions.	

	Plant height	Pods per plant
-	(cm)	(no.)
Accessions	2022	2022
PI116034-1	57.5 efg	39 abc
PI116034-2	55.3 fg	41.9 abc
PI158119	71.1 ab	11.4 g
PI254367	57.1 efg	23.2 def
PI255928	53.7 fg	31 bcd
PI263900	61.2 cdef	22.3 def
PI271546-1	64.2 bcde	19.2 defg
PI271546-2	68.2 abc	17.3 efg
PI271546-3	74.3 a	28.2 cde
PI288347	57.5 efg	44.3 ab
PI288425	67.6 abcd	11.2 g
PI288443	59 cdef	17.6 efg
PI288742	58.7 def	24.9 def
PI288749	61.9 bcdef	16.7 fg
PI428572-1	59 def	48.6 a
PI428572-2	48.8 g	54.7 a
PI428572-3	58.7 def	51.7 a
Mean	60.7	29.7

Note: Same small letter indicates statistically non-significant at p=0.05.

The tallest accession was PI271546-3 (74.34 cm) while shortest accession was PI428572-2(48.82 cm) with mean height (60.7 cm). Meftahizade et al. (2017) reported mean height of guar was (73.1 cm) when four times irrigation supplied among 3 guar genotypes evaluated in Iran.

### 3.9.1.3. Number of Pods per Plant:

The number of pods per plant is quantitative character which greatly influenced grain yield. Analysis of variance showed that there was statistically significant difference among accessions under irrigated condition for number of pods per plant (3.15). Accession PI428572-2 had a higher number of pods per plant (54.68) and lower number of pods per plant was PI288425 (11.22) with mean value (29.70) (Table 3.17). Meftahizade et al. (2017) noted mean number of pods per plant was (75) when four times irrigation supplied among 3 guar genotypes evaluated in Iran.

# 3.9.1.4. Pods Clusters per Plant

Pod clusters per plant is genetic character, but absence of genetic variability was observed among guar accessions with mean pod clusters per plant (8.25) in 2022.

### 3.9.1.5. Number of Seeds per Pod

Non-significant difference was observed in the number of seeds per pod with mean seed (2.77) among accessions.

### 3.9.1.6. Days to 1<sup>st</sup> Flowering

Analysis of variance revealed that all the source of variation was non-significant for plant height (Table 3.15) When averaged across accessions and year, mean days to 1<sup>st</sup> flowering was (39 days).

### 3.9.1.7. Pod Length

Accessions were statistically significant differences for pod length (Table 3.15). The longest pod length was observed in accession PI255928 (7.7 cm) while shortest pod length was found in accession PI158119 (4.2 cm) with mean length (4.8 cm).

# 3.9.1.8. Thousand Seed Weight

Thousand seed weight is an important yield component which contributes to higher grain yield. There was statistically significant difference among accessions for thousand seed weight and confirmed genetic variability (3.2 and 3.2). Accession PI288742 had the highest thousand seed weight (46.4 g) while lowest was PI116034 (28 g) with mean weight (36.9 g). Gracia et al. (2023) reported mean thousands seed weight was (36.3 g) among 4 genotypes evaluated in New Mexico, U.S.

Table 3.18: Pod length and thousand seed weight of guar accessions in 2022 under irrigated conditions.

	Pod length	Thousand seed weight
	(cm)	(g)
Accessions	2022	2022
PI116034-1	4.6 bcde	28.0 f
PI116034-2	4.9 bcd	33.5 def
PI158119	4.2 e	39.5 abcd
PI254367	4.6 bcde	32.6 def
PI255928	7.7 a	44.8 ab
PI263900	5.0 bc	41.8 abc
PI271546-1	4.6 bcde	38.2 bcde
PI271546-2	4.3 de	35.7 cdef
PI271546-3	4.9 bcd	40.1 abcd
PI288347	4.6 bcde	34.6 cdef
PI288425	4.6 bcde	37.6 bcde
PI288443	4.4 cde	36.6 cde
PI288742	4.4 cde	46.4 a
PI288749	5.1 b	38.2 bcde
PI428572-1	4.6 bcde	34.3 cdef
PI428572-2	4.5 bcde	31.2 ef
PI428572-3	4.9 bcd	35.0 cdef
Mean	4.8	36.9

Note: Same small letter indicates statistically non-significant at p=0.05.

# 3.9.1.9. Yield per Plant

Analysis of variance showed that there was statistically significant difference among accessions for yield per plant (Table 3.15). The highest yield for plant was observed in PI428572-2 and PI428572-3 (7 g and 6 g respectively). Same these accessions also had higher yield per plant under rainfed condition (7.9 and 8.3) in 2022. The lowest yield per plant was observed in PI288425, PI254367 and PI158119 (1.0, 1.3 and 1 g respectively) with yield per plant (3.2 g). This result confirmed that there was existence of genetic variation among accession for grain yield under irrigated conditions.

Table 3.19: Yield per plant, grain yield and harvest index of guar accessions in 2022 under irrigated conditions.

	Yield per plant	Grain yield	Harvest index
	(g)	$(\text{kg ha}^{-1})$	
Accessions	2022	2022	2022
PI116034-1	4.9 abc	594.7 abcd	0.24 ab
PI116034-2	5.3 ab	648.2 abcd	0.22 bc
PI158119	0.9 e	106.4 g	0.07 de
PI254367	1.3 e	154.0 efg	0.02 e
PI255928	3.5 abcd	423.8 cdef	0.14 bcde
PI263900	2.0 bcde	242.8 efg	0.08 cde
PI271546-1	2.0 bcde	244.5 efg	0.16 bcd
PI271546-2	1.7 de	202.2 efg	0.13 bcde
PI271546-3	3.2 abcd	389.6 cdefg	0.15 bcde
PI288347	3.6 abcd	435.1 bcde	0.11 bcde
PI288425	1.0 e	123.5 fg	0.11 bcde
PI288443	1.4 de	166.7 efg	0.12 bcde
PI288742	2.9 abcd	347.1 defg	0.08 cde
PI288749	1.9 cde	228.4 efg	0.17 bcd
PI428572-1	5.7 ab	693.9 abc	0.19 bcd
PI428572-2	7.3 a	890.0 a	0.38 a
PI428572-3	6.0 a	731.0 ab	0.12 bcde
Mean	3.2	389.5	0.15

Note: Same small letter indicates statistically non-significant at p=0.05.

Gracia et al. (2023) found that grain yield under irrigated conditions was 2500-2600 kg ha<sup>-1</sup> in arid southwest U.S which was higher than our grain yield (extrapolated from yield per plant). The decrease in guar seed production may be attributed to inadequate growing degree days and low temperature. Alexander et al. (1988) found that while irrigation increased vegetative growth, it also led to a decrease in seed production per unit of water used. In this trial, mean height was (60 cm) while mean height under rainfed conditions was (41 cm). It suggested that guar had longer vegetative growth under irrigated conditions.

#### 3.9.1.10. Harvest Index

The highest harvest index was found in PI428572-2 (0.38) whereas lowest harvest index was observed in PI 254367 (0.02) with mean value (0.15).

### 3.9.1.11. Leaf Chlorophyll Content

Analysis of variance revealed that accession was statistically significant for chlorophyll atLEAF value under irrigated conditions. Accessions PI251546 had the highest chlorophyll content (76.6) value while lowest was observed in accession PI254367 (63.5). Time and interaction between time and accessions were non-significant.

#### **3.9.2.** Correlation

The correlation showed that yield per plant was strongly statistically significant association with number of pods per plant. Similarly, number of seeds per pod, number of pod clusters per plant, days to 1<sup>st</sup> flowering are positive statistically significant corelated with yield per plant. Pod length showed weak association with yield per plant. Plant height and thousand seed weight were weak negative statistically significant association with yield per plant under irrigated condition. Plant stand showed weak negative correlation with yield per plant and yield components such as pod clusters per plant, number of pods per plant, pod length and thousand seed weight. From these studies, characters such as higher number of pods per plant, pod clusters per plant, number of seeds per pod and shortest flowering days would increase yield per plant, selection of these characters along with yield would improve high yielding potential of guar. Table 3.20: Pearson correlation coefficients of guar variables under irrigated condition in 2022.

	PS	PH	FD	PC	NP	PL	SP	TW	YPP
PS									
PH	0.12								
FD	0.00	-0.10							
PC	-0.26	-0.59 ***	0.09						
NP	-0.12	-0.58 ***	0.37 *	0.62 ***					
PL	-0.01	-0.24	-0.24	0.10	0.06				
SP	0.06	-0.37 *	0.52 **	0.11	0.68 ***	0.07			
TW	-0.20	0.16	-0.41 *	-0.15	-0.43 *	0.40 *	-0.36 *		
YPP	-0.09	-0.55 ***	0.41 *	0.47 **	0.96 ***	0.11	0.82 ***	-0.36 *	

'\*'- significant at 0.05, '\*\*'- significant at 0.01, '\*\*\*'- significant at 0.001 level of probability. Note: PS: Plant stand, PH: Plant height(cm), FD: Days to 1<sup>st</sup> flowering; PCP: Pod clusters per plant, NP: Number of pods per plant, PL: Pod length(cm), SP: Number of seeds per pod, TW: Thousand seed weight(g), YPP: Yield per plant(g).

#### **3.9.3. Multiple Regression**

Multiple regression was done to predict how single dependent variable was influenced or

affected by independent variables (Table 3.21).

The multiple linear regression equation could be written as

Grain yield = -3.10 + 0.00 plant height(cm) - 0.05 primary pod clusters + 0.11 number of

pods - 0.02 pod length (cm) + 0.71 number of seeds per pod + 0.04 thousand seed weight(g).

The  $R^2$  value of (0.98) indicates a good model fit, suggesting that the independent

variables account for 98 percent of the variance in yield per plant. Variables such as the number

of pods per plant, number of seeds per pod and thousand seed weight were observed to be

statistically significant with yield per plant. The highest impact in the variation of yield per plant

was by seeds per pod followed by number of pods per plant and thousand seed weight. Pod

clusters per plant and pod length were negatively caused variation in yield per plant however,

both parameters were non-significant with yield per plant.

Variables	DF	Coefficients	Std. error	t stat
Intercept	1	-3.01 **	1.00	-3.02
PH	1	0.00	0.01	-0.41
PCP	1	-0.05	0.03	-1.86
NP	1	0.11 ***	0.01	15.11
PL	1	-0.02	0.08	-0.25
SP	1	0.71 ***	0.11	6.31
TW	1	0.04 **	0.01	3.26
$\mathbb{R}^2$			0.98	
Observation			34.00	

Table 3.21: Multiple regression studies between yield and other variables of guar in 2022 under irrigated conditions.

'\*'- significant at 0.05, '\*\*'- significant at 0.01, '\*\*\*'- significant at 0.001 level of probability. Note: PH: Plant height(cm), PCP: Pod clusters per plant, NP: Number of pods per plant, PL: Pod length(cm), SP: Number of seeds per pod, TW: Thousand seed weight.

### 3.10. Summaries

The results indicated the feasibility of growing guar under irrigated conditions. All guar accessions exhibited a non-significant plant stand per row plot, except for one accession which was omitted from the data analysis due to the germination of only a few plants in the row plot. Analysis of variance revealed statistically significant differences among accessions for plant height, pod length, number of pods per plant, thousand seed weight, yield per plant, and harvest index, while plant stand, pod clusters per plant, number of seeds per pod, and days to 1<sup>st</sup> flowering showed non-significance. There was a statistically significant variation among accessions for leaf chlorophyll atLEAF value; however, higher chlorophyll content was observed in all guar accessions under irrigated conditions, indicating good health.

The correlation study demonstrated a statistically significant positive association between the number of pods per plant, number of seeds per pod, number of pod clusters per plant, days to 1<sup>st</sup> flowering and yield per plant. Regression study predicted that the number of pods per plant, thousand seed weight and number of seeds per pod statistically significantly impacted grain yield. The highest yield for plant was observed in PI428572-2 and PI428572-3 (7 g and 6 g respectively). Same these accessions also had higher yield per plant under rainfed condition (7.9 g and 8.3 g) in 2022. The lowest yield per plant was observed in PI288425, PI254367 and PI158119 (1.0, 1.3 and 1 g respectively) with yield per plant (3.2 g).

However, guar grain yield remains considerably lower compared with average yield of irrigated conditions in the Southern High Great Plains. Delayed maturity, maximum and extended vegetative growth were noted under irrigated conditions. It indicated guar may not need more water for growth. Further studies are recommended to determine the optimum timing and amount of irrigation for black gram under semiarid conditions of NGP. Although guar cultivation is feasible in the Northern Great Plains, Further investigations are recommended with more guar accessions to identify superior guar accessions with economic yield for NGP. Unfortunately, a hailstorm on August 1, 2023, destroyed the entire experiment, preventing us from meeting our objectives and drawing conclusions from this study.

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