STUDIES ON AGRO-ECOLOGICAL PERFORMANCE AND CROP PHYSIOLOGY OF GUAR

A Dissertation

by

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ABSTRACT

Guar [*Cyamopsis tetragonoloba* (L.) Taub] is a legume grown worldwide with low production inputs and costs, tolerant of hot, dry, and saline conditions, with the potential to provide several ecosystem services. Guar production in the U.S. is centered in the Southern Great Plains (SGP). But regional cultivation of guar is challenged by a lack of scientific understanding and limited agronomic advances in several areas, including guar germplasm improvement, biological N fixation (BNF) functions, and alternative cropping systems. To address these issues, three distinct studies were conducted: [1] an evaluation of phenotypic variation and relationships among 50 guar germplasms; [2] a test of drought tolerance on growth and nodulation of guar at various stress levels and recovery functions; and [3] a systems study evaluating integration of guar into regional winter wheat system at different cropping intensities. Results of Study 1 showed phenotypic links between plant capacity to support nodulation, N_2 assimilation, and plant biomass production, with genotypic differences in nodule mass, branch numbers, and stem diameter with moderate to high heritability. In Study 2, drought or water stress generally had the greatest negative impact on nodule weight, followed by biomass and reproductive parameters. However, except at extreme water stress levels, drought had little effect on nodule number, indicating that the basic machinery for BNF (nodules) remained largely intact. Both nodule and biomass growth exhibited strong and rapid recovery upon relief of water stress. In Study 3, dry and hot environmental conditions were detrimental to the growth, development, and yield of field-grown guar. Differences among the tested cropping systems were most pronounced in the driest year of the study with the most intensive cropping system exhibiting negative effects of soil water deficit. Among integrated wheat-guar systems, the double-cropping (WG2) system showcased high system productivity over time, but the seasonal likelihood for a productive crop was greater and the risk of crop failure was lower for WG1.3 (1.3 crop/year) under the regional climate. Overall, these studies revealed useful insights for improvement of guar production in the SGP and other guar production regions of the world.

DEDICATION

This dissertation is dedicated with genuine gratitude and warm regards to my beloved parents Mr. Sher Bahadur Shrestha and Ms. Kamala Shrestha who have always been patient, loving, and supportive all my life.

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1. INTRODUCTION

The history of legumes as an important component of crop production systems dates to ancient times. In modern agricultural production worldwide, however, monocrop systems are common, substantially affecting soil properties, crop development, and environmental quality (Negash et al., 2018). Diversifying monocrop systems with rotation of legume species can benefit the subsequent crop (Zou et al., 2015) and has been long been recommended to improve production and environmental sustainability. Introduced to the U.S. in the early 19th century by the U.S. Department of Agriculture as a drought-tolerant and soil-improving forage crop, guar (*Cyamopsis tetragonoloba* L.) is a legume that remains a minor crop to this day. However, the U.S. ranks as the largest importer of guar products for a multitude of uses, often as a lubricant, thickener, binder, and stabilizer in diverse industrial products. The Southern Great Plain (SGP) is the center of U.S. guar production. At present, U.S. guar production is minimal and unstable for several reasons, including slow agronomic advances and the relatively high priority regional cotton and wheat mono-crop systems. So, the production of guar is often restricted to a catch crop following cotton failures or when conditions do not favor the latter crop. Studies are notably limited on cropping systems for guar, as well as studies on guar germplasm and ecosystem benefits of the crop like biological nitrogen fixation (BNF), which is a primary motivating factor for producers to produce this crop. Underpinning these constraints, the research proposed herein will explore phenotypic/nodulation/BNF traits of guar and the fit of the crop in existing wheat-based winter cropping systems under dryland conditions of the SGP region. The following literature review covers the history of guar, uses and markets of the crop, its production and challenges, cultivation practices, nodulation, and BNF functions, as well as an overview of the SGP cropping systems.

1.1. LITERATURE REVIEW

The name “guar” derived its name from a Sanskrit word ‘Gauaahar,’ meaning cow/cattle food (Singh et al., 2016). Guar is commonly known as a cluster bean, especially in India. Guar is an annual summer crop, well adapted to drought and high temperature (Shockey, 2016; Hasan and Abdel-Raouf, 2018), and highly tolerant to saline conditions (Teolis et al., 2009; Suthar et al., 2018a, b). The cultivation of guar requires few production inputs (Gresta et al., 2018a; Santhosha et al., 2017) and is commonly grown under resource-constraint or marginal lands of arid- and semi-arid regions around the world (Bhatt et al., 2017). Historically, guar was utilized as food, feed, and a green manure crop. But the discovery and use of guar gum as a substitute for locust bean gum in the paper industry following World War II initiated a shift in the production of guar towards industrial uses (Abidi et al., 2015). Today, demand for guar is driven largely by numerous industrial applications of guar gum.

1.1.1. Morphology and Biology

Botanically, guar is a self-pollinated crop with a tiny proportion of cross-pollination, it bears chromosome number 2n = 14, and belongs to the family *Fabaceae* (Bhatt et al., 2017; CABI, 2019). The common name “cluster-bean” is derived from the reproductive growth habit of the plant in which the formation of pods occurs in clusters (Solangi et al., 2016). Guar is an upright, bushy, and deep tap-rooted crop (Singh et al., 2016; Bhatt et al., 2017). Most guar species are indeterminate in growth habits, but some are released as relatively determinate types (Gresta et al., 2016). Guar cultivars may be branching or non-branching, glabrous or pubescent, alternate or serrated, and simple or trifoliate leaves (Husman, 1985). The plant bears raceme inflorescence with 40-60 and 50-70 flowers in branched and non/sparsely branched types, respectively. The flowers are white, pink, or bluish, with 1st flower set four to six weeks after emergence and asynchronous pod setting for 4 to 5 months in indeterminate types or 2 to 3 months in determinate types (Husman, 1985; Gresta et al., 2016; Adams et al., 2020). Typically, flowering occurs first on the main stem of the plant, then progresses upward and outward together on the branches and main-stem reproductive clusters (Adams et al., 2020). The same pattern is observed for the pod set, and yet a reproductive cluster can simultaneously bear flowers, new pods, maturing pods, and fully mature pods at a single node. Guar genotypes have wide trait variation, ranging in plant height from 0.15 to 3.0 m (Husman, 1985; Gresta et al., 2018a; Gresta et al., 2016; Bhatt et al., 2017) and growing season from 70 days for determinate types to 195 days for indeterminate types (Gresta et al., 2016, Gresta et al., 2018a). The seeds of guar are the primary economic produce, used to derive a compound “galactomannan gum” or guar gum from its endosperm. The composition of guar seeds unlike other legumes with little or no endosperm bears a large endosperm (35-42%), husk (14-17%), and embryo, also known as germ (43-47%) (Thombare et al., 2016). Seeds are light tan/whitish to dark in color and dicotyledonous surrounding the embryo. The embryo is mainly protein, while the endosperm is galactomannan content (Thombare et al., 2016; Mudgil et al., 2014). The chemical composition of guar seeds contains 28.3-

35.0% crude protein, 3.5-6.0% ash, 1.8-5.2 % fat, 38.8-59.1% carbohydrate, 4.1-8.0% crude fiber, and 23.9-34.2% gum content (Bhatt et al., 2017).

1.1.2. History, Origin, and Distribution

The origin of guar lacks a universal consensus. Some reports suggest India and Pakistan as the place of origin (Solangi et al., 2016; Singh et al., 2017; Husman, 1985), while others report as Africa (Singh et al., 2017; Singh et al., 2016; Shockey, 2016). The trans-domestication theory is the most widely accepted explanation on the origin of guar, which states that guar is derived from *Cyamopsis senegalensis* with an African origin and domesticated in India (Gresta et al., 2018a). It was introduced in India as feed along with the horse trade by Arabian traders between the 9th and 13th centuries (Gresta et al., 2018a; Mudgil et al., 2014). The cultivated guar species *Cyamopsis tetragonoloba* is reported to be developed from the introduced wild species (Mudgil et al., 2014; Bhatt et al., 2017).

1.1.3. Guar Gum Products & Properties, Uses, and Demand in the U.S.

There are four major sources of commercial galactomannans: locust bean (*Ceratonia silique* (L.); guar (*Cyamopsis tetragonoloba)*; tara (*Caesalpinia spinose* (Molina) Kuntze); and fenugreek (*Trigonella foenum-graecum* (L.)). Each of these varies in mannose to galactose ratio and molecular weight (Prajapati et al., 2013). Guar gum is one of the cheapest sources of galactomannan (Sharma et al., 2018), composed of a linear chain of (1→4)-linked β-D-mannopyranosyl units with (1→6)-linked α-D-galactopyranosyl residues as side chains (Mudgil et al., 2014). The ratio of mannose to galactose units has been commonly reported as 2:1 (Mudgil et al., 2014), although other reports the range to be 1.6:1 to 1.8:1 and 1.8:1 to 2:1 (Prajapati et al., 2013; Bhatt et al., 2017). It is a water-soluble non-ionic natural polymer with a high molecular weight of 100,000 to 300,000 daltons (Hasan and Abdel-Raouf, 2018; Bhatt et al., 2017) or $10^{\wedge 6}$ to $2\times10^{\wedge 6}$ g mol^{-1} (Sharma et al., 2018). Unlike many other gums, guar gum has a high-water solubility, even at low temperatures (Gresta et al., 2016). Of all the natural gums, guar gum has the highest solution viscosity at low concentrations (5,000-10,000 cps for 1% aqueous dispersion) and thickening power of 5-8 times higher than corn starch (Thombare et al., 2016; Mudgil et al., 2014; Singh et al., 2016). The physical and chemical alteration of guar gum through various procedures and technologies (etherification, esterification, and crosslinking reactions of hydroxyl groups) has resulted in many guar gum derivatives that have widened its applications (Hasan and Abdel-Raouf, 2018). The intrinsic properties of guar gum and its derivatives present diverse application uses.

The uses of guar and guar-gum products can be classified into 1) Traditional uses and 2) industrial uses. Traditional uses of guar include consumption of green pods as a vegetable, plants as forage, and green manuring in parts of South-East Asia (Singla et al., 2016; Bhatt et al., 2017). Guar is also used as a shade crop for ginger, as a cover crop, and as a high-protein feed (32.5%) for livestock, poultry, and fish (Bhatt et al., 2017; Gresta et al., 2016). The germ and hull portions of guar seed after extraction of guar gum from the endosperm, collectively make guar meal. Guar seeds are also used for medicinal uses (Bhatt et al., 2017), as the presence of antioxidants imparts medicinal properties (Singh et al., 2016). The seeds have been used for treating diabetes, blood cholesterol, cholera, diarrhea, constipation, and related chronic functional bowel ailments like irritable bowel syndrome (Singh et al., 2016; Thombare et al., 2016).

In the 1940s and 1950s, guar gum found its first industrial application in paper manufacturing as the Institute of Paper Chemistry was seeking an alternative for locust bean gum, which was scarce at that time (Mudgil et al., 2014). Since then, guar gum products have seen diverse applications in food, paper, textiles, pharmaceuticals, cosmetics, mining, and petroleum industries, among many others (Bhatt et al., 2017). In the food industry, guar gum products are used for their high-moisture retention capability, reduced evaporation rate, alteration in freezing rate, modification in ice crystallization, and transformation of chemical properties (Sharma et al., 2018). Guar gum is used in many food products like ice cream, sauces, cake mixes, cheese spreads, fruit drinks, and dressings (Mudgil et al., 2014). The food industries use a highly refined grade guar gum in small amounts (Tripp et al., 1982), with a permissible concentration at < 1-2% (Sharma et al., 2018; Mudgil et al., 2014). Greater amounts of guar gum are used in textile, paper, drilling, mining oil and gas industries. The use of guar gum in the petroleum industry dates to the mid-1950s and its use in the hydraulic fracking process in the early 1960s (Abidi et al., 2015). Guar gum, when added to fracking fluid with the sand and water, thickens the fracking fluid and acts as an excellent carrier of the sand into the fracked cracks allowing the oil or gas to flow into the wells (Thombare et al., 2016; Mudgil et al., 2014). Guar gum also functions as a green corrosion inhibitor and dispersant (Hasan and Abdel-Raouf, 2018).

The demands for guar products peaked in the recent past, primarily due to extensive oil and gas exploration. Hydroxypropyl guar and carboxymethyl hydroxypropyl guar are popularly used in formulating fracturing fluids for a hydraulic fracking process by oil and gas industries (Thombare et al., 2016; Mudgil et al., 2014). Hydraulic fracking now constitutes a significant fraction of total crude oil and natural gas production, and it has also increased the level of production (Cook and Perrin, 2016). For example, in 2008 total U.S. crude oil production was about 5 million barrels per day with about 10% coming from fracking, but the production surged to over 9 million barrels per day with 51% coming from fracking by 2015 (Cook and Perrin, 2016). Further, the newer horizontal drilling technologies became increasingly common, which utilizes ten times more fracking fluid than the earlier vertical drilling technique (Mudgil et al., 2014; Kalha and Anand, 2012). For instance, the U.S. had 1,155 horizontal and 547 vertical rigs in August 2012, as compared to 100 horizontal rigs and 927 vertical rigs in 1991. Guar gum occupied 30% of total fracking costs in 2012 (Kalha and Anand, 2012), though this may be shifting over time as the oil and gas industry explores alternatives. The global hydraulic fracturing market was expected to grow from $31 billion in 2011 to $64 billion by 2017 led by the U.S. (Kalha and Anand, 2012).

The U.S. is the largest consumer of guar products worldwide. Also, because the domestic supply of guar products is very low, it is the largest importer in the world. Before 2010, U.S. guar gum imports remained relatively stable at approximately 11,000 Mg per month (Figure 1.1), and the average price of $1,200 per ton (Biermacher, 2012). The substantial increase in oil and natural gas exploration by the process of hydraulic fracking led to a tremendous increase in the import of guar gum products. For instance, fracking a single well requires 9 Mg of guar gum (Grover et al., 2016). An estimate of >170,000 Mg of guar gum would have been required for a total of 19000 wells drilled in the single year of 2012. The U.S. imports of guar gum from India recorded the highest with US$ 3.18 billion value in 2012/2013 and with 0.43 million Mg quantity in 2011/2012 (Figure 1.1).

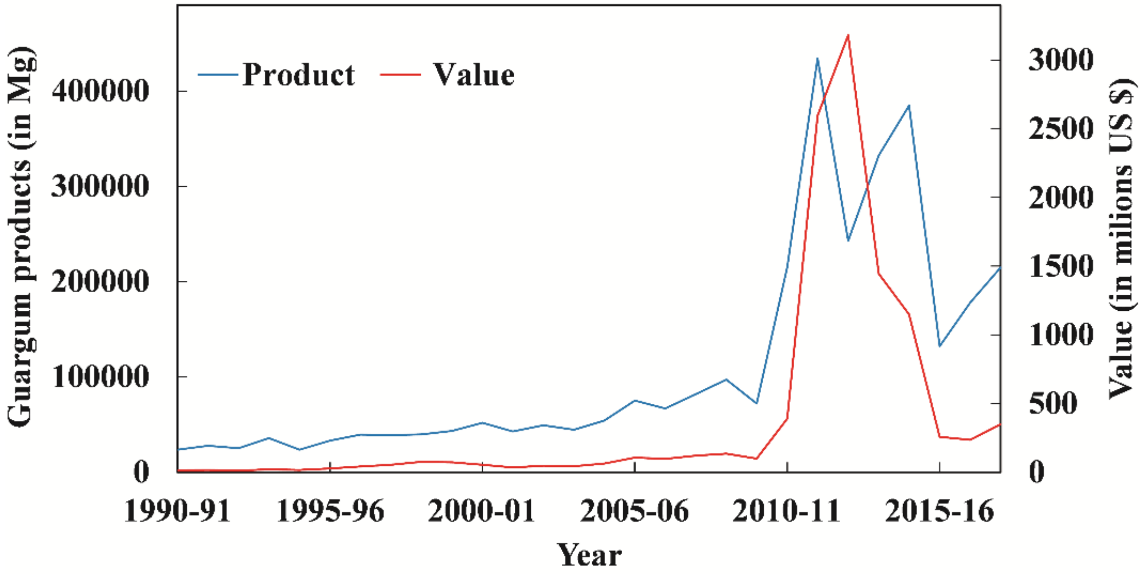

Figure 1.1 U.S. imports of Guar gum products from India from 1990-1991 to 2017-2018 (APEDA, 2019).

The U.S. ranks top in import of guar-gum products from India with 55% and 73% share in cumulative quantity and value since 1990/1991, respectively among the top 15 importing countries in the world, followed by China and Germany (Figure 1.2). Data on demand and import of guar products in the U.S. indicates a great economic opportunity for guar production in the SGP region.

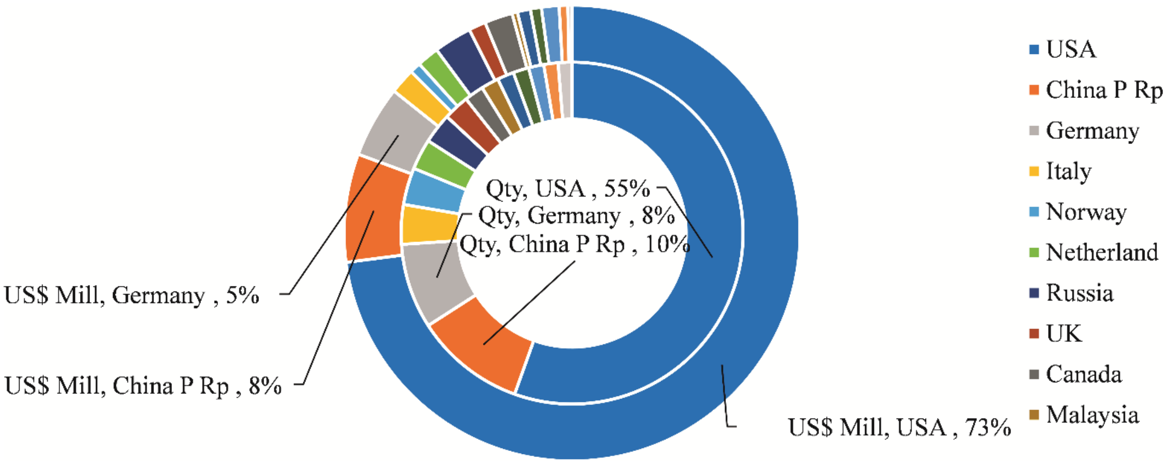

Figure 1.2 Top 15 countries importing guar gum products (from India) based on cumulative import quantities from 1990-1991 to 2018-2019 (APEDA, 2019).

1.1.4. Guar Production in the U.S.

The history of the guar crop in the U.S. dates to 1903 when a single guar line was introduced from India (Poats, 1960; Hymowitz and Matlock, 1963). Guar was first produced in New Mexico and Arizona, mostly the mesa types with irrigation supplements, but failed in making a profit (Poats, 1960). It was widely limited within research studies, primarily as green manure, and forage crop at that time, before the production of guar for industrial uses following the discovery of the utility of guar gum in the paper and textile industries in 1943 (Hymowitz and Matlock, 1963; Mudgil et al., 2014). A wider application of guar in different industries was realized after an intensive investigation of the physio-chemical properties of guar (Whistler, 1982). The production of guar shifted from Arizona and California to North Central Texas and Southwestern Oklahoma as a rotation crop with flax and cotton in 1948. Although the commercial guar production started in the early 1950s in South Texas, the production center quickly shifted to the Rolling Plains of Texas and Oklahoma, characterized by the sandy soils (Tripp et al., 1982; Abidi et al., 2015; Biermacher, 2012). The guar plant advanced as a multipurpose crop for N-fixation and grain yield (Poats, 1960). But the expansion of guar was poor due to several cultivation and harvesting difficulties. The production acreage and yield of guar had witnessed wide yearly fluctuations since then. Guar production is limited by late plantings, heavy soil types, shorter growing seasons, and potentially detrimental humid conditions from a stable and reliable production in other U.S. states (Abidi et al., 2015). Guar production is predominantly grown under dryland conditions after the end of World War II (Poats, 1960).

At present, the commercial production of U.S. guar lies primarily in Central/West Texas and Southwestern Oklahoma in the High Plains and Rolling Plains regions (Husman, 1985; Shockey, 2016). The region accounts for 95-100% of guar produced in the U.S., but the crop is also occasionally grown in New Mexico and Arizona (Abidi et al., 2015). The production acreage in Rolling Plains peaked at 100,000 acres in the early 1970s, but cheaper imports of guar products resulted in a gradual decrease (Olson et al., 2001). A recent agricultural census by USDA (2017) reported a very small total of 5,844 hectares of guar harvests in the U.S. (Table 1.1). Only the states of Texas and Oklahoma had an accountable scale of guar production from a total of 58 operations, mostly in Texas. The irrigated guar operations (3) remained nominal with a harvest of 376 hectares (Table 1.1). Growers had historically prioritized the use of irrigation water to high-yielding and high-value primary crops like cotton. In the U.S. southwest, guar remains more as a catch crop than a primary crop (Shockey, 2016). Guar acreage in U.S. Southwest fluctuates depending on the demand, market price, and drought severity.

Since 2000, guar production in the region ranged from 2023 hectares in the record-breaking year of drought in 2011 to 44515 hectares following a significant failure of cotton in 2013 (Abidi et al., 2015). However, considering the estimated requirement of 17000 Mg guar gum for total oil wells drilled in 2012, guar production would need approximately 0.61 million cultivation hectares although demands are likely to fluctuate over the years. There is a tremendous deficit in domestic production of guar compared to demand. Commercial guar production in the U.S. relies on production contracts, contract prices, discounts, delivery points, and acceptable grades.

Table 1.1 Area, operations, and production of guar in the U.S. by Agricultural Census, USDA.

	Year				
	1997	**2002**	**2007**	**2012**	**2017**
Total - Hectares harvested					
Texas	1,817	8,269	2,406	2,454	5,733
Oklahoma		(D)		(D)	273
National	1,817	(D)	2,406	(D)	5,844
Operations with area harvested					
Texas	38	75	35	44	53
Oklahoma		3		1	5
National	38	78	35	45	58
Irrigated - Hectares harvested					
Texas	50	1,465	176	690	376
Oklahoma	0	0	0	0	(D)
National	50	1,465	176	690	(D)
Irrigated - Operations with area harvested					
Texas	3	28	6	18	3
Oklahoma	0	0	0	0	2
National	3	28	6	18	5
Production (in Mg)					
Texas	2,298	6,334	1,974	1,654	4,664
Oklahoma		(D)		(D)	96
National	2,298	(D)	1,974	(D)	4,760

*(D) Withheld to avoid data disclosure for individual operations. (USDA Quick stats, 2019)

Advances in agronomics of guar lack a satisfactory pace and limited information are available to the producers. As a minor crop in the U.S., currently, guar taps very limited funding resources and interests within the research community. There is a lack of adequate infrastructure for the processing of guar seeds. Since 2016, Guar Resources LLC, located in Brownfield, Texas was the only active company with an annual production of 7030 Mg guar-gum and a seed storage facility of 22680 Mg capacity (“Guar Resources”, 2020). But the company suspended the operations for the 2021 cropping season and do not appear to resume processing anytime soon. There is no federally funded crop insurance coverage program for guar (Abidi et al., 2015), however, USDA is undertaking a feasibility evaluation for such program. Moreover, U.S. guar production faces huge competition by cheaper imports from India and Pakistan, despite the overhead costs for guar cultivation in the U.S. being considerably low than most other crops (Abidi et al., 2015; Olson et al., 2001). These factors had restricted guar to being a catch crop following a failed cotton crop or during the times of low prices for cotton (Trostle and Byrd, 2016) and cultivation in occasional drought years. Cotton is among the top-rank crops in Texas with 3.16 million hectares in 2018 (USDA Quick Stats, 2019), and is preferred by most regional producers due to relatively high economic return potential. Therefore, a deliberate rotation of cotton with guar is not very common despite the potential ecosystem and market benefits by diversifying the existing cropping system (Abidi et al., 2015). These prevailing constraints, render existing highly unreliable and volatile guar production system, failing the U.S. guar production to meet the domestic needs and compete with the global market.

1.1.5. World Guar Production

The global production of guar seed was estimated at 1-1.6 million Mg annually, which fluctuates widely, depending foremost on the monsoon conditions in India (Sharma and Gummagolmath, 2012). World guar production is dominated by India and Pakistan with about 80 and 15% share, respectively (Gresta et al., 2014). The remaining 5% of global guar is produced mostly in the U.S., Australia, South Africa, and Brazil, with an estimated annual production of 15,000 Mg (Olson et al., 2001; Gresta et al., 2014). The production and adaptation of guar in the Mediterranean region had been successful as the crop fits well in high temperatures, poor or erratic rainfall, and saline conditions (Gresta et al., 2018a). Besides, guar had been reported in many other countries like Italy, Morocco, Germany, Spain, Sudan, Argentina, Zimbabwe, Nigeria, Israel, and Greece (Singh et al., 2016; Solangi et al., 2016; Bhatt et al., 2017).

The "Thar Desert", a region across India and Pakistan, is center for the global guar production (Abidi et al., 2015). In India, the states of Rajasthan, Gujrat, Haryana, and Punjab are major commercial guar producing regions, although, in some other parts, guar is grown for its use as vegetables (Singh et al., 2016). The state of Rajasthan alone accounts for 80% of the guar cultivation acreage and production in India (Bhatt et al., 2017). India's guar also faces large fluctuations in area and production, primarily driven by global market demand and monsoon precipitation (Sharma and Gummagolmath, 2012). A severe drought in 2002/2003 followed the lowest cultivation acreage and production since 2000/2001 (Sharma and Gummagolmath, 2012; Table 1.2). Between 2000 to 2017, the total production of guar ranged from 0.1 to 3.2 million Mg from 0.8 and 5.6 million cultivated hectares, respectively (Table 1.2). Guar production increases in India is primarily through the expansion of the cultivation acreage. Guar yields ranged from 0.10 – 0.76 Mg ha^{-1} during 2000-2017, with little improvement on yield trend (Table 1.2). The lack of high-yielding, drought-resistant, and/or short-duration varieties and limited production technology restricts desirable improvement on guar production (Sharma and Gummagolmath, 2012). In Pakistan, guar is grown mainly in Punjab and Sindh provinces. Although before the 1990s, about 80% of guar cultivation area was under irrigated conditions, the production system is mostly under dryland conditions in recent years (Sharma and Gummagolmath, 2012; Mudgil et al., 2014).

Guar in India is an important export commodity. The cumulative value of guar gum export from 2000/2001 to 2018/2019 was $15.5 billion (Table 1.3).

Table 1.2 Guar production status in India since 2000/2001.

Year	**Area (in ‘000,000 ha)**	**Production (in ‘000,000 Mg)**	**Yield (Mg ha^{-1})**
2000/2001	3.3	0.5	0.16
2001/2002	2.7	0.9	0.35
2002/2003	0.8	0.1	0.14
2003/2004	2.6	0.2	0.76
2004/2005	2.2	0.5	0.24
2005/2006	2.7	0.7	0.27
2006/2007	3.0	0.8	0.25
2007/2008	3.1	1.4	0.45
2008/2009	3.5	1.3	0.38
2009/2010	2.7	0.3	0.10
2010/2011	3.1	1.6	0.52
2011/2012	3.2	1.9	0.60
2012/2013	4.9	2.3	0.47
2013/2014	5.6	3.2	0.58
2014/2015	4.9	3.0	0.61
2015/2016	5.2	2.4	0.48
2016/2017	3.6	1.4	0.40

(APEDA, 2019)

In 2012/2013, guar exports accounted for about 18% of India’s total agricultural exports (Bhatt et al., 2017). India is the largest exporter of several guar products in the world, with guar gum (refined split, treated, and pulverized) as the chief form of guar export item in terms of both quantity and value.

Table 1.3 Export status of guar and its derivates for India since 2000/2001.

Year	Seed (Mg)	Guar meal	Guar gum refined split	Guar gum treated & pulverized	Guar Gum	Guar gum value (in $ Millions)
2000/2001	-	-	-	-	130	132
2001/2002	-	-	-	-	118	86
2002/2003	-	-	-	-	112	100
2003/2004	-	-	-	-	121	113
2004/2005	225	5	50	77	131	154
2005/2006	360	3	49	134	187	237
2006/2007	1286	0	41	148	189	250
2007/2008	8	7	64	140	211	278
2008/2009	15	32	55	171	259	291
2009/2010	-	42	32	145	218	239
2010/2011	0.3	41	83	317	442	645
2011/2012	-	80	102	525	707	3446
2012/2013	0.4	75	71	261	406	3919
2013/2014	2	132	83	387	602	1980
2014/2015	0	144	85	437	665	1552
2015/2016	43	69	46	211	325	497
2016/2017	1	102	41	277	420	464
2017/2018	21	127	45	322	494	647
2018/2019	-	-	-	-	374	495

*Units for quantities are in ‘000 metric tons if not specified. (APEDA, 2019)

1.1.6. Guar Cultivation Practices

In recent times, guar is primarily cultivated as an industrial crop for its extensive use in a wide range of food and non-food applications due to the unique properties of guar gum extracted from the guar seed endosperm. Typically, guar is grown with very few external production inputs and has low production costs.

1.1.6.1. Climatic and Edaphic Requirements

Guar prefers a high temperature of about 40℃ in the day and is very susceptible to frost, with a base temperature of 8ºC (Mudgil et al., 2014; Rao et al., 2000). The ambient temperature range of 25-35ºC and soil temperature of 25-30℃ are desirable for optimal shoot and root development (Biermacher, 2012; Mudgil et al., 2014; Beckwith, 2012). Soil temperature of at least 21ºC is required for guar seed germination (Forbes and Beck, 2013) and the rapid establishment of plant stand (Tripp et al., 1982). Recent studies on the effects of temperature ranging from 5-35℃, showed large variations among guar genotypes on germination time, germination percentage, and seedling vigor, but such variability was narrowed at the higher temperatures (Gresta et al., 2018b; Singh et al., 2021). Sing et al., (2021), reported an optimum germination percentage at 22℃, however it differed among genotypes such as Kinman (16℃) and Matador (26℃). Gresta et al. (2018b) reported the optimal temperature for germination of at least 75% to be 30-35℃ with germination time of 1-6.5 days. Both the germination time and percentage were greatly affected at temperatures below 20℃.

Cultivation of guar is reported to be suitable in arid regions receiving 250-1020 mm of annual precipitation (Undersander et al., 1991). The optimum annual precipitation ranges from about 510-760 mm for the best growth of guar (Tripp et al., 1982). Guar performs well in a dry environment with light but frequent precipitations (Mudgil et al., 2014). In India, guar is grown in the regions of 18-42ºC temperature and 200-600 mm precipitation (Rao et al., 2000).

Guar grows well on light to medium textured soil types (Abidi et al., 2015) which associates with high susceptibility of plants to waterlogging conditions (Gresta et al., 2018a). But guar can also be grown in heavy soils with adequate-drainage facilities (Husman, 1985; Gresta et al., 2018a). Guar does the best in the slightly alkaline soil of pH 7.5-8 (Husman, 1985). A study reported a threshold EC value of about 8.8 dS m^{-1} for guar (Bhatt et al., 2017).

1.1.6.2. Planting Requirements

Guar performs better with 4-8 inches high uniform seedbed under conventional till according to the reports by the producers (Forbes and Beck, 2013). The raised seedbed allows the easier harvest of extremely low setting pods on the basal branches and improves soil drainage. But guar can also be grown on the flatbed under adequate moisture and drainage facilities. A warm seedbed with adequate soil moisture is critical for the growth and development of guar. Planting should be avoided in weedy fields, as in-season weed control options are limited for guar (Olson et al., 2007).

The planting time for guar in the U.S. can range from April to July, depending on the planting region (Singla et al., 2016a, b). The optimal sowing dates in Rolling Plains and Southern Plains of Texas and southwest Oklahoma lies between mid-May to the late-June (Abidi et al., 2015) and between mid-April to late-May in south Texas (Abidi et al., 2015; Singla et al., 2016a). Historically, Texas guar producers have planted guar in late June following a cotton failure (Abidi et al., 2015). Studies have indicated mid-June planting to be the best in the U.S. southwest (Singla et al., 2016a,b). Although guar is considered drought-tolerant, drought may prevent the planting of guar, depending on the severity, because adequate soil moisture is key for crop establishment (Abidi et al., 2015). Sowing time influences the guar phenological timings, growing season length, pests, and disease incidences, and ultimately seed yield and yield quality (Kumar et al., 2017).

The most common recommended seeding rate for guar ranges from 5.6 to 9.0 kg ha^{-1}, though rates up to about 11 kg ha^{-1} have also been recommended (Abidi et al., 2015). Relatively high seeding rates are favorable for more upright and higher basal branching growth above the soil surface, which allows for an easier mechanical harvest of low-setting pods. Guar producers in the Texas region identify an optimum seeding rate at 9 kg ha^{-1} (Forbes and Beck, 2013).

A seeding depth of 2.5 to 4 cm is considered optimum (Abidi et al., 2015; Tripp et al., 1982) and would depend on the availability of moisture and soil types.

Row spacing in guar may vary from 0.30-0.45 m and plant spacing within each row from 0.10-0.15 m, depending on planting time, method, and branching habit (Kumar et al., 2017; Abidi et al., 2015; Jagtap et al., 2017). In the U.S., plant spacing vary across the guar growing regions depending on the precipitation availability and/or use of planting equipment types. For instance, guar is commonly sown using planter at a row spacing of 0.76 -1.01 m in relatively drier Texas High Plain region. But guar seeds are drilled at the row spacing of 0.25-0.38 m in a comparatively wetter Rolling Plains region.

Guar planting can be done with a grain drill or vacuum planter depending on seedbed preparation and desired row spacing (Shockey, 2016). Planting by air-vacuum planter is desirable to prevent the crushing and clogging of guar seeds (Tripp et al., 1982).

1.1.6.3. Varieties

The early guar varieties include Mesa, Groehler, Texsel, Hall, and Mills, and the first improved variety “Brooks” was released in 1964 (Tripp et al., 1982; Rogers and Partridge, 1973). The development of disease-resistant varieties during the 1960s improved the cultivation and production of guar in the U.S. more than any other factor. All major varietal releases occurred during 1970 to 1980s like Kinman (1975), Esser (1975), Santa Cruz (1982), and Lewis (1986) with the only exception of Matador (2005) and Monument (2008) in the recent past (Forbes and Beck, 2013). The common guar varieties grown in the SGP region are Kinman, Matador, Santa Cruz, and Lewis (Singla et al., 2016b; Tripp et al., 1982). Kinman is widely adapted and commercially grown with a maturity period of 120 days and has moderate branching, high disease resistance, and higher-yield potential (Tripp et al., 1982). The variety Lewis has a basal-branching and relatively determinate growth with mid-season maturity (Alexander et al., 1988). Many studies showed the highest yield potential for Lewis variety among several guar genotypes (Alexander et al., 1988; Gresta et al., 2016; Olson et al., 2001). The Germplasm Resource Information Network (GRIN-Global) has a collection of 414 guar accessions from 11 countries around the world: India, Pakistan, Senegal, Sudan, Iran, South Africa, Brazil, Colombia, Congo, Thailand, and the USA.

1.1.6.4. Soil Moisture and Nutrient Management

Guar has low water needs of about 216 mm per growing season (Grover et al., 2016). It is well adapted to poor and erratic precipitation, associated with plant ability of its deep rooting system enabling efficient access and use of soil water (Santhosha et al., 2017). Precipitation amounts totaling 203-254 mm across 3-4 intervals before sowing, during budding and flowering, were shown to be desirable (Kalha and Anand, 2012). The water use efficiency of guar ranged from 1.5-4.1 kg ha^{-1} mm^{-1} in a study by Rao et al. (2000). Successful production of seed yield was achieved with an average water supply of 268 mm in the semi-arid Mediterranean environment in Southern Italy (Gresta et al., 2013). Conversely, guar is very intolerant to waterlogging (Gresta et al., 2018a). Excessive soil moisture may lead to diseases and/or pest incidences and extensive vegetative growth, resulting in a decline in seed yield and quality (Husman, 1985; Abidi et al., 2015; Rogers and Partridge, 1973; Tripp et al., 1988).

Guar tolerates low soil fertility (Ashraf et al., 2005; Gresta et al., 2018a) and is grown mostly under rainfed conditions, therefore, mostly the production system do not justify external fertilizer inputs, particularly nitrogen applications. Nitrogen fertility trials by West Texas Guar revealed no or little impact on guar productivity from added nitrogen (Forbes and Beck, 2013). But phosphorus and potassium supplementation had benefits of yield improvement (Bhatt et al., 2017; Forbes and Beck, 2013; Garg et al., 2005).

1.1.6.5. Disease, Pest, and Weed Management

Damage by insects on guar is not common. The most important insect pest is guar midge (*Contarinia texana*) also, known as alfalfa midge (Abidi et al., 2015; Tripp et al., 1982), and yield loss of up to 30% was recorded in West Texas (Forbes and Beck, 2013; Tripp et al., 1982). Some other insect pests include gall midge, three-cornered alfalfa hopper, cotton bollworms, loopers whitefly, pea/cowpea aphids, white grub, and thrips (Forbes and Beck, 2013; Tripp et al., 1982).

Diseases on guar do not reach severities to economic damage levels, either because of unfavorable dry environments or mostly, late development later towards maturity with minimal impacts on yield (Abidi et al., 2015). Nevertheless, Alternaria leaf spot and Bacterial blight (seed-borne) are two important diseases on guar (Forbes and Beck, 2013; Shockey, 2016). Other diseases are occasional including Myrothecium blight, Bacterial wilt (seed-borne), cotton root rot, top necrosis virus, Alternaria, and Fusarium (Tripp et al., 1982; Abidi et al., 2015).

Guar is a poor competitor with weeds (Brar, 2018) and effective pre-planting weed control is essential to maximize the yield. Guar production is not suitable on weedy fields or those prone to weed infestation (Olson et al., 2007) due to the slow early growth of the crop (Husman, 1985; Forbes and Beck, 2013; Brar, 2018). The first month of growth and development of guar is the most crucial period for weed control (Bhatt et al., 2017). Weed infestation was maximum at 30-40 days after planting over two years under different rainfall conditions and removal of weeds between 20-30 days of planting resulted in increased water use efficiency and seed yield compared to other timings in one study (Yadav, 1998). Late June planting of guar in the Texas Rolling Plains reduced weed competition (Tripp et al., 1982). Plant spacing may affect the weed situation, a study showed a better weed control with 30×10 cm spacing compared to 45×6.5 cm under rainfed conditions (Gupta et al., 2019). In the past, there were no labeled post-emergence broadleaf herbicides for guar (Olson et al., 2001). Currently, there are three labeled active herbicide ingredients for guar: (a) trifluralin; pre-planting incorporated in the soil for grasses and small-seeded broadleaves, (b) clethodium; post-emergence type for grass control, and (c) carfentrazone; post-emergence application for broadleaf weeds using hooded sprayer safeguarding the guar (Abidi et al., 2015; Olson et al., 2007).

1.1.6.6. Maturity, Harvest, and Yield

Guar has mostly an indeterminate growth habit, though varieties differ in maturity lengths. The growing season for guar ranges from 80-195 days or more depending on the genotype and the environment (Gresta et al., 2018a). A long crop cycle and low basal branching traits of guar are drawbacks to the easy and effective harvest operation (Gresta et al., 2016). The difficulty in the harvesting of basal pods by mechanical or combine harvest leads to yield losses. Historically, the guar crop is left out in the field well after physiological and/or harvest maturity due to high moisture content. Growers usually wait until the natural frost event(s) and killing of guar plants before harvest, which can take up to a month before it dries completely (Husman, 1985). But such practice often leads to reduced yield due to shattering loss (Abidi et al., 2015) and deterioration of the seed coat along with blackening of seeds (Shockey, 2016), though this does not necessarily reduce seed quality (Liu et al., 2007). Yield and quality can be preserved using harvest aids. Chemical harvest aids like paraquat dichloride and sodium chlorate are labeled for guar (Abidi et al., 2015).

Harvesting can occur from October-December when the pods are brown and dry at < 14% moisture content (Tripp et al., 1982; Kalha and Anand, 2012; Abidi et al., 2015). Harvesting has been primarily done with a conventional grain header in West Texas for decades (Forbes and Beck, 2013). A custom harvester with air reels to catch shattering pods onto the header can reduce harvest losses up to 56 kg ha^{-1} (Abidi et al., 2015).

The yield of guar may fluctuate greatly depending on several factors, including crop establishment, precipitation, and planting time, among others. Forbes and Beck (2013) of West Texas Guar Inc., who have extensive experience cultivating and contracting guar in the SGP region, stated that a typical yield range for dryland guar is 0.4-1.9 Mg ha^{-1}. They stated that greater yields can be expected with irrigation, up to about 5 Mg ha^{-1} under “ideal” conditions. Guar yield data reported in the scientific literature supports these statements. In the U.S. Southwest, Abidi et al. (2015) described that when the crop is properly established and is not subjected to severe drought, seed yield may vary between 0.4-1.3 Mg ha^{-1}. Stafford (1987) reported yield differences among 12 guar genotypes, ranging from 0.5-0.9 Mg ha^{-1} under dryland and 0.7 -1.3 Mg ha^{-1} under irrigated conditions. Two studies testing the effect of planting date on guar under irrigated conditions reported yields ranging from 0.9 -1.7 Mg ha^{-1} (Singla et al., 2016a, b).

Although, yield data reported by Adams et al. (2020) indicated that yields as great as 5 Mg ha^{-1} are possible under irrigated conditions. In that study, late-season precipitation promoted abundant pod setting and most of the seeds matured before frost, greatly enhancing yield. Interestingly, in the same report, a greatly reduced yield of 0.6 Mg ha^{-1} was recorded for another guar variety at a nearby site in dryland conditions when planting was delayed by about 19 days. Several other studies worldwide have reported greater guar yields up to 2.9 Mg ha^{-1} (Meftahizadeh and Hatami, 2021); 3.0 Mg ha^{-1} (Mahdipour-Afra et al., 2021); 3.3 Mg ha^{-1} (Gresta et al., 2019; Avola et al., 2020); and 4.1 Mg ha^{-1} (Ibrahim et al., 2016) under irrigated and/or more favorable growing conditions depending on genotypes and management factors including planting dates, plant density, *Rhizobium* inoculation and external nutrient inputs.

1.1.7. Biological Nitrogen Fixation

Producers who historically and currently grow guar have hoped to take advantage of its capacity for biological nitrogen fixation (BNF), which occurs through association with guar-specific *Rhizobium* bacteria that infect the roots and form root nodules. According to the classic model of rhizobia-legume association, host-plant roots release flavonoid compounds that trigger the secretion of nod factors by rhizobia leading towards root tissue penetration. The infection thread grows inward into the root cortex where it differentiates into a bacteroid capable of fixing atmospheric nitrogen for plant use in exchange for photosynthates from the host plant (Sachs et al., 2018). In legume species, nodule development can be determinate or indeterminate forms. Guar produces meristematic nodules, which refer to the indeterminate nodule type (Rao and Venkateswarlu, 1986). Unlike determinate nodules that lack meristem and growth ceases after a short developmental period, indeterminate nodules hold active meristems and nodule growth (Sachs et al., 2018). The BNF requires actual nodulation of the roots (Abidi et al., 2015) and active or effective nodules appeared as pink or reddish-purple inside the nodules (Thapa et al., 2018). Findings on guar BNF rates ranged widely in the literature. Substituting a traditional summer fallow period with guar in a winter wheat system contributed 92 kg N ha^{-1} and a maximum of 135 kg N ha^{-1} in some years (Rao and Northup, 2009a). Also, a three-year guar-wheat system in Sudan found BNF of 34-54 kg N ha^{-1} contributing 38-65% of total plant nitrogen (Mubarak et al., 2015).

Nodulation on guar is commonly perceived to be poor in the field conditions by U.S. growers and scientific studies back similar findings in many countries. In the Texas region, nodulation is reported to be sporadic at the best (Abidi et al., 2015). A poor nodulation status was reported with only 5-10 nodules per plant with compatible rhizobia species in the experiments conducted in India (Khandelwal and Sindhu, 2012; Brar and Singh, 2017). Only 32% of guar plants were nodulated by a native *Rhizobium* species in the saline-alkaline soil (Bhardwaj, 1974). Under the controlled conditions, abundant nodulation was found without any inoculant with a high number of low mass nodules in clay loam vs. a low number of high mass nodules to sandy loam soils (Thapa et al., 2018). In a similar greenhouse study, all 4 USDA rhizobial strains except USDA 3595 increased the nodule weight, while only USDA 3089 and USDA 3386 resulted in higher biomass productivity and total assimilated nitrogen compared to the uninoculated guar (MacMillan et al., 2021). Also, inoculated guar in the field conditions on average produced 36% more nodules than non-inoculated guar, and more nodules were found after 8 weeks than either at 4 or 12 weeks of planting (Stafford and Lewis, 1980). Inoculation of guar with the

Rhizobium plus phosphorous solubilizing bacteria together with 15 kg N ha^{-1} and 35.6 kg P ha^{-1} application improved nodulation and uptake of phosphorus and nitrogen (Brar and Singh, 2017). Despite these differences in nodulations, guar is noted for its ability to nodulate in hot and dry conditions than most other legumes (Zahran, 1999).

The cultivation of legume crops seeks inoculation of planting seeds with a compatible *Rhizobium*, particularly if the production goal is for N-credits. There is no commercial inoculant for guar in the U.S. at present. Although recently in Australia, an inoculant CB3035 was tested effective for nodulation on guar (Gresta et al., 2019). The inoculant is no longer commercially available at present, though the inoculant culture can be available upon request. Several studies have evaluated different rhizobia strains for effective nodulation on guar in different countries. (Khandelwal and Sindhu, 2012; Elsheikh and Ibrahim, 1999; Thapa et al., 2018, MacMillan et al., 2021).

Root nodulation and its functions in legume species can be affected by environmental and management factors, including the interaction between rhizobia bacteria and crop species itself (Zahran, 1999). The occurrence, growth, and symbiosis of rhizobia with guar and other legumes are affected by high temperature and drought (Mondal et al., 2017). For instance, results from a lab study on temperature tolerance among 63 *Rhizobium* isolates from guar root nodules showed that 68% of isolates were tolerant to temperatures of 40°C and only 32% at 45°C (Mondal et al., 2017). Also, only 27% of rhizobial isolates were tolerant to induced drought stress at a 40% concentration of polyethylene glycol 6000 (PEG 6000). Moreover, only 10% of isolates were tolerant to the combined stress of temperature (40°C) and drought (PEG 40%). Similarly, a short period of water stress during the vegetative and flowering stage reduced the nodule fresh weight (for instance, 64.5 and 54.5% decline following 8-days water stress, respectively) but not nodule numbers (Venkateswarlu et al., 1983). The author also observed reduced nitrogenase activity (> 95% lower) with the loss of moisture in nodules by 10% or more and a permanent loss when moisture loss exceeded 20%. The short-term and intermittent dry spells in arid and semi-arid environments can result in a considerable loss of BNF (Mondal et al., 2017). In another study, a decrease in nodule weight (≥ 50%) and N-fixation (≥ 80%) was noted at 40°C of root temperature (Arayangkoon et al., 1990). The author explained the root temperature of 37-40°C to be critical for guar-N-fixation (Arayangkcon et al., 1990). Others reported that the soil temperature of 27.8-44.4°C had no impact on guar root nodulation (Stafford and Lewis, 1980).

Other factors affecting root nodulation and N-fixation include soil pH, soil nutrient deficits, and low concentration of *Rhizobium* population (Bell et al., 1989, Hungria and Vargas, 2000; Ashraf et al., 2005). The added mineral N-supplement may increase the rhizobial tolerance and N-fixation of certain strains at higher temperatures on guar (Arayangkoon et al., 1990) but, many studies found the inhibitory effects of mineral-N on guar nodulation, much more pronounced at high or adequate N-supply (Alexander et al., 1988; Hinson and Adams, 2020). Phosphorus and calcium have a positive role in guar root nodulation (Bell et al., 1989; Barwa et al., 2017). Although guar has high salinity tolerance, salinity stress can decrease nodule numbers, inhibit the nodulation and nitrogen fixation process due to ion toxicity and osmotic drought stress (Ashraf et al., 2005). These earlier findings provide some understanding of nodulation and BNF on guar, but more insights and validation should be sought for guar under the U.S. Southern Plains conditions.

1.1.8. Southern Plains Cropping Systems: Suitability of Guar Production

The SGP region predominately features monocropping systems: cotton in summer cropping systems and winter wheat in winter cropping systems. A total of 2.47 million hectares was planted with cotton in the Texas plains in 2018, 36% of which was irrigated (USDA Quick Stats, 2019). Similarly, a total of 1.41 million hectares were planted with winter wheat in 2018, mostly under dryland conditions, with 14% irrigated. The characteristic low precipitation and high temperature of the region is the foremost limiting factor in diversifying cropping systems. Winter wheat grain yield has averaged 1.8 Mg ha^{-1} since 2000 (USDA Quick Stats, 2019), but the trend in winter wheat yields has been in decline since the 1990s (Figure 1.3). The declining trend in yield is associated with reduced growing season precipitation, plant shift towards earlier maturity, and the rising temperature since the 1980s (Shrestha et al., 2020; Stewart et al., 2018).

Irrigated agriculture in the region depends primarily on water from aquifers, including the Ogallala and Seymour aquifers, which are declining because of the high pumping rates and a lower rate of recharge (Colaizzi et al., 2009). Therefore, both the cotton and wheat systems are mostly grown continuously with a fallow period during the off-season to conserve and/or replenish soil water for the following season crop. The practices of summer fallow are widespread as the soil water accumulation strategy to stabilize wheat yields (Hasbullah et al., 2011). However, the fallow system had been reported to have negative effects, mostly a decline in soil organic matter, reduced soil microbial diversity, and soil erosion (Hasbullah et al., 2011). Moreover, a single-crop system offers limited land use and agroecosystem services.

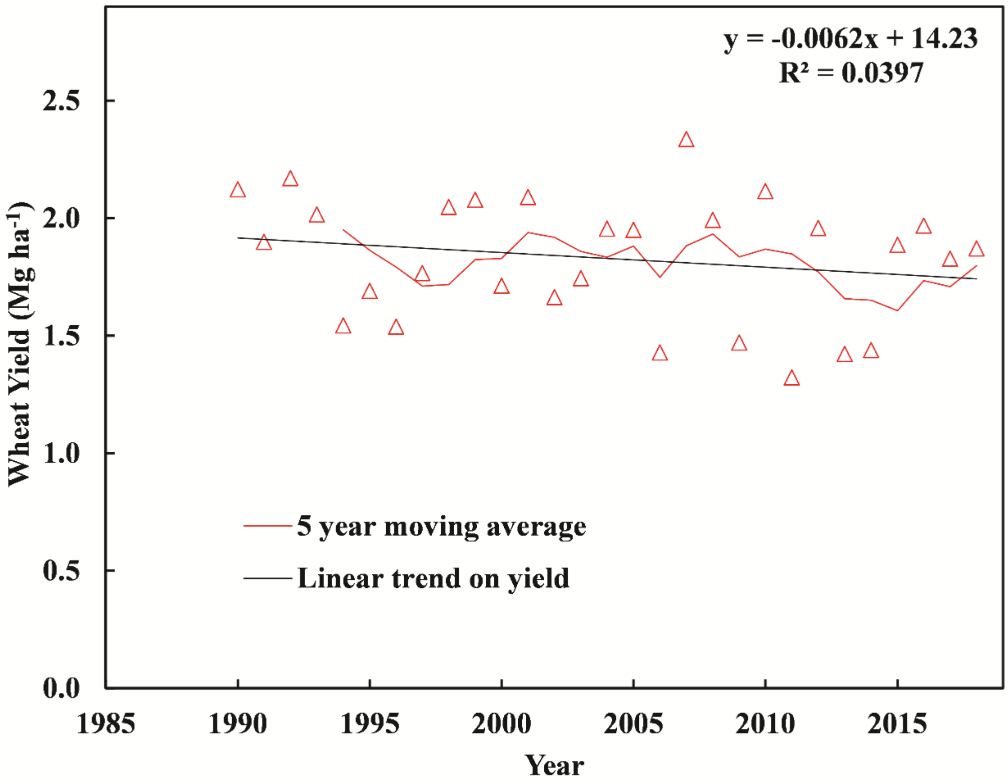

Figure 1.3 Winter wheat yields in the Great Plain Region, TX since 1990-2018 (USDA Quick Stats, 2019).

The climatic conditions that feature high temperatures and low precipitation in the SGP region correspond well to the drought and heat tolerance of guar. This may enable replacing a summer fallow with guar into the existing winter wheat cropping system. Legumes that use water efficiently have been shown as promising summer fallow replacements in other crop systems, allowing intensification and diversification of cropping systems (Miller et al., 2002; Hasbullah et al., 2011; O'Dea et al., 2013). However, Brown (1967) and Rao and Northup (2009b) described that double-crop systems of legume-cereal are uncommon under dryland conditions due to soil water limitation in the SGP region. Unpublished, ongoing research is investigating the expansion of canola as a winter rotation crop with wheat, but little research has focused on alternate ways to diversify the winter wheat system, like integrating a suitable summer crop, such as guar, into rotation (Baumhardt and Jones, 2002; Potter et al., 1997). Integrating guar into the wheat system may be an alternative approach to enhance ecosystem services in the existing wheat system and to increase the production of guar, but such a system is yet to be tested.

1.1.9. Suitability of Guar Integration into Winter Wheat Cropping Systems

Several important considerations may factor in whether guar could be successfully integrated into wheat cropping systems of the SGP region. The foremost factors include 1) Compatibility between the crops for growing season or timing; 2) Balance of crop water use requirements relative to average annual precipitation amounts, timing, and distribution patterns; and 3) Impacts on the soil environment and other ecosystem services that may affect productivity and sustainability of the system.

Growing season compatibility - Winter wheat in the SGP region has a very broad planting window where it can be sown successfully between September and November to achieve a high crop yield in any typical year (Winter and Musick, 1993). Early planted wheat produced higher biomass and faster reproductive maturity compared to late-planted wheat, however, eventual harvest dates varied little compared to late-planted crops (Musick and Dusek, 1980). Typically, wheat gets harvested from mid-May to early-June.

Guar, on the other hand, has a narrow timeframe for planting compared to wheat, primarily due to the high-temperature requirement of at least 21°C for planting (Abidi et al., 2015). Planting during mid-May to late-June or early July is possible for the Texas Rolling and lower South Plains and southwest Oklahoma region (Abidi et al., 2015; Tripp et al., 1982; Singla et al., 2016a, b). Earlier planting than mid-May is not feasible due to the lack of temperature requirement for germination. In delaying planting from early June to early July, yield declined approximately by 50% in Vernon, TX in two studies (Rogers, 1973; Husman, 1985). Similar results were found in recent planting date studies in Clovis, NM, with better establishment and yield for mid-June planting than July or May plantings (Singla et al., 2016a, b). Predominantly, indeterminate in growth habit, the harvest date for guar is influenced by the environmental and management factors. Under the typical weather conditions during fall, the plant usually defoliates and begins to dry. Indeterminate plant types commence defoliation upon increasing moisture stress and low-temperature conditions (Husman, 1985). But if conditions are wet, guar will continue or resume vegetative growth and not be fully defoliated and desiccated for natural harvest. When conditions do not favor the timely senescence and maturity, the use of chemical harvest aids is common (Abidi et al., 2015).

The timing of harvest-aid applications will be critical to ensure quality harvest and permit timely planting of the following wheat. Guar can be planted in June to achieve the best production following wheat harvest in May and harvested by October, allowing wheat to follow, likely in early November. Given the use of harvest aids, the growing season of wheat and guar will typically be compatible, though late-planted guar may not be highly productive if the harvest is also early and or premature.

Crop water demand and precipitation balance - Although guar uses relatively little water and is drought-resilient, water is the most important limiting factor for guar production in such a semi-arid environment (Olson et al., 2007). So, enacting the best use of the limited water supply will have to come from the improved production system. If guar is to be integrated into the SGP’s winter wheat cropping system, the system could be designed and tested with varying levels of crop intensities with periodic fallow seasons in the cropping sequence or by omitting fallow periods altogether. Any of such scenarios would need to be viable and sustainable under the regional dryland conditions as above 90% of the wheat system are managed in dryland conditions (USDA-NASS, 2019). A high-intensity cropping system would tax stored soil water to at least some extent, making the crop productivity more dependent on growing season precipitation compared to the winter wheat-summer fallow system, which is largely popular in the region. Rao and Northup (2009a, b) reported guar with low water use and soil water depletion compared to fallow conditions and suggested guar as a potential summer rotation crop with wheat in the Southern Great Plains.

The Southern Great Plain region is characterized by high evapotranspiration demand under the average maximum and mean temperature of 22.8 and15.2 °C in the High Plains and 24.4 and17.3 °C in the Rolling Plains of Texas since 1980, respectively (NOAA, 2020). The water use by the dryland wheat is reported to be 203 mm (8’’) to 381 mm (15’’) in the regional climate (Baumhardt and Jones, 2002; Musick et al., 1994; Winter and Musick, 1993). Similarly, guar requires about 203 mm (8’’) to 254 mm (10’’) of precipitation (Stafford and McMichael, 1991; Grover et al., 2016; Kalha and Anand, 2012) and grows well in the region of 20-30’’ annual precipitation (Grover et al., 2016; Shockey, 2016; Forbes and Beck, 2013). Based on such water requirements, a fully integrated dryland wheat-guar double cropping system would require about 16-25’’ of annual precipitation. Long term annual precipitation average since 1980 for Texas High plains is 19’’ and Rolling Plains is 24’’ (NOAA, 2019). Thus, the most intensive cropping systems may be feasible in the Rolling Plains, depending on precipitation distribution, but unlikely to be feasible in the High Plains region.

The timing and distribution of precipitation are other determinants for the feasibility of the guar-wheat cropping system. The precipitation is characterized by bimodal distribution in the SGP region, where the wettest conditions are expected in the late spring to early summer and in the fall periods. The wet periods synchronize with plantings times in the summer (guar) and winter (wheat) crops making it suitable for the critical establishment of the crops under the dryland system.

Soil environmental impacts and other ecosystem services - Although guar is classified as an industrial crop in recent years, historically it is well known for its use as a green manure crop. Guar being a legume can fix atmospheric nitrogen and credit free nitrogen to the soil. Besides this primary function that makes guar a soil-enriching crop, it offers several other ecosystem services. Guar is a very good summer cover crop (Hodges et al., 1970; Bhatt et al., 2017; Sachs et al., 2018) and could reduce erosion and increase soil organic matter, improving the overall soil quality (Biederbeck et al., 1993; Rao and Northup, 2009b). It can be successfully grown in marginal and poor lands under resource-constrained environments as guar requires very little or no external production inputs (Bhatt et al., 2017; Santhosha et al., 2017; Gresta et al., 2018a). Guar is well adapted to harsh environmental conditions due to its low water use and drought tolerance traits (Rao et al., 2000; Stafford and McMichael, 1991). So, guar can be a low-risk bearing crop from a viewpoint of crop failures even in the extreme environment and yet capable of attaining relatively high crop productivity and/or improving sustainability and productivity when incorporated into the cropping system. Guar is a low CO_2 emission crop (Gresta et al.,

2014) and the deep taproot system enables guar plant to utilize limited soil moisture efficiently (Santhosha et al., 2017). Such capabilities put guar on the top as a highly potential climate-resilient crop. The rising temperature trend since the 1980s under the recent climate change and fifteen of the sixteen hottest years recorded in the history registered between 2001-2015 (GISS, NASA) substantiates the proposed alternative ways of guar production. An increase in the temperature was linked to a decline in winter wheat yield in North Central Texas and Oklahoma since 1990 (Stewart et al., 2018). Irrespective of management practices (e.g., with or without the use of inoculants) carried, guar improved soil conditions and increased yields of subsequent crops are commonly reported from the Southern Plains producers to their Extension agencies (Abidi et al., 2015). The guar plant is resilient to additional stressors, high winds and blowing sands, soil salinity according to the reports by regional producers. The toughness of the guar plant allows high chances of substantial vegetative cover on the ground and sustained ecosystem services even in the extreme growing conditions where many crops fail, including cotton.

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2. EXPLORING PHENOTYPIC VARIATION AND ASSOCIATIONS IN ROOT NODULATION, MORPHOLOGICAL, AND GROWTH CHARACTER TRAITS AMONG 50 GUAR GENOTYPES*

2.1. ABSTRACT

Guar [*Cyamopsis tetragonoloba* (L.) Taub] is a legume grown worldwide, tolerant of hot, dry, and saline conditions. Phenotyping is a key bottleneck in guar improvement, with belowground root nodule phenes being largely overlooked. To address this bottleneck, a greenhouse study was conducted to evaluate phenotypic variability and associations in 14 plant and nodule growth parameters in 50 diverse guar genotypes over 48 days. Nodule mass per plant differed among genotypes and had moderate heritability (P=0.011, H^2=0.40), while nodule number did not differ and had low heritability (P=0.247, H^2=0.14), and the parameters were not correlated. Nodule mass was correlated with total plant N (r=0.56), plus most biomass parameters, indicating phenotypic links between plant capacity to support nodulation, N_2 accumulation, and plant biomass production. Stem diameter may be useful as an indirect, high-throughput selection trait for increased nodulation in guar, as there was a positive association between stem diameter and nodule mass. Both branch number and stem diameter had wide phenotypic variability and were

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highly heritable, confirming their utility in breeding selection due to their agronomic significance. Reproductive development was delayed in branched genotypes, which may have implications in breeding guar for environments that differ in growing season length. Despite low heritability, the wide range in SLA may be exploited by breeders to optimize drought tolerance and N use efficiency traits. These analyses give novel and confirmatory insights on the nature and associations of phenotypic character traits in guar, particularly nodule traits, providing added tools for breeders in developing desired and beneficial plant ideotypes.

2.2. INTRODUCTION

Guar (*Cyamopsis tetragonoloba* (L.) Taub) is a well-established industrial crop, grown worldwide predominantly in regions of high temperature and low precipitation (Abidi et al., 2015; Avola et al., 2020). Guar belongs to the family *Fabaceae* and bears chromosome number 2n=14 (Bhatt et al., 2017; CABI, 2019). Domestication of guar occurred in India and Pakistan (Singh et al., 2017), where the cultivated species was developed from *Cyamopsis senegalensis* which originated in Africa (Gresta et al., 2018a). The guar plant is an upright, bushy, deep tap-rooted, and mostly self-pollinated crop (Gresta et al., 2016; Bhatt et al., 2017). The historical uses of guar as food, feed, and a green manure crop are now largely supplanted by diverse industrial uses of "galactomannan gum" extracted from its seed endosperm (Kalha and Anand, 2012; Hasan and Abdel-Raouf, 2018). Guar production has experienced wide yearly fluctuations, depending primarily on growing season precipitation and demand for galactomannan gum.

Worldwide annual production of guar was estimated to range from 0.25-2.25 MT between 2005 and 2013 (NCDEX, 2013).

Renewed focus is needed on guar germplasm improvement for many guar cultivation regions around the world. For example, most guar variety releases in the U.S. occurred more than 30 years ago (Abidi et al., 2015). India is the most active country in guar variety development, as several variety releases have occurred since 2000, at least as late as 2017 (ICAR-IGFRI, 2018; Zubair et al., 2017). No information on guar variety releases was found for other countries. In making needed guar germplasm improvements, plant phenotyping is a key bottleneck, and more than just yield should be considered. Guar can provide biological N_2-fixation, but poor root nodulation in field-grown guar is often noted by producers and researchers (Abidi et al., 2015; Khandelwal and Sindhu, 2012; Rao and Northup, 2009). Considering both above- and belowground (root nodule) plant parameters in guar germplasm development could result in enhanced seed yield and quality, forage yield and quality, and soil fertility (Gresta et al., 2019). Understanding the phenotypic variability and associations among important plant character traits is fundamental to achieving this. Following are overviews of studies in which such analyses were conducted in collections of guar genotypes.

Several studies focused on aboveground guar germplasm responses to specific cultural or environmental conditions, such as salinity (Suthar et al., 2018a, b; Teolis et al., 2009), low temperature (Singh et al., 2021; Gresta et al., 2018b), and planting dates (Singla et al., 2016; Meftahizadeh and Hatami, 2021). Other studies have focused on evaluating phenotypic differences in aboveground growth and yield-related factors among guar germplasms. Plant character traits such as pods plant^{-1} (Gresta et al., 2016; Goudar et al., 2017; Jukanti et al., 2015; Stafford and McMichael, 1991), pod length and weight (Goudar et al., 2017; Jukanti et al., 2015), seed weight (Gresta et al., 2016; Gresta et al., 2018a), and plant biomass productivity (Seiler and Stafford, 1985) were found to be closely associated with seed yield. Stafford (1987) and Avola et al. (2020) reported differences in water use efficiency and plant dry matter among guar genotypes.

Notably, studies on the belowground phenome of guar are uncommon, with evaluations including multiple genotypes being exceedingly rare. MacMillan et al. (2021b) tracked temporal and spatial trends in guar root system development, revealing that guar has predominantly very fine roots (average root diameter of 0.38-0.44 mm along > 130 cm of soil depth) and that nodules are concentrated toward the soil surface. Rao and Venkateswarlu (1987) and Sachs et al. (2018) reported that guar forms indeterminate nodules that bear meristematic activity for a prolonged period. Several studies have documented changes in nodule traits in response to various exogenous factors, including moisture, nitrogen/phosphorus, *Rhizobium* inoculation, and soil type (Thapa et al., 2018; Gresta et al., 2019; Hinson and Adams, 2020; Shrestha et al., 2021). Only two studies were found in the literature that evaluated the belowground phenome of guar in more than two genotypes. Ashraf et al. (2005) reported variation in nodule number, dry weight, and root length parameters in 15 guar genotypes, noting a direct relationship between these parameters and seed yield in saline conditions. Rao et al. (1984) observed genotypic variation in nodule numbers, but not nodule dry weight or nitrogenase activity in 17 guar genotypes. The authors found no relationships among nodulation parameters, nitrogenase activity, and seed yield. Reports quantifying rates of N_2 fixation in guar are also rare. Mubarak et al. (2015) reported N_2 fixation rates ranging from 34-54 kg N ha^{-1} per year in a

3-year guar-wheat cropping system study. Buttar et al. (2009) reported that external N input to a wheat crop following guar could be reduced by up to 40 kg ha^{-1}.

Previous studies evaluating phenotypic character traits among many guar genotypes have focused mostly on aboveground yield and yield components, including some morphological parameters. Very limited analysis has been conducted on belowground character traits. Above all, studies that include a large set of guar genotypes with simultaneous assessment of both shoot and root system parameters have not been conducted. We hypothesized there are positive phenotypic relationships between nodule weight and plant N and plant growth parameters, indicating a link between nodule weight and N_2 fixation in guar. To test this hypothesis and more broadly evaluate guar phenes, the objective of this study was to assess phenotypic variability of above- and belowground morpho-physiological characters in 50 guar genotypes and establish pertinent relationships among measured parameters. This research is targeted primarily at plant breeders seeking to develop improved guar germplasm, as well as researchers seeking to interpret data collected on guar nodulation.

2.3. MATERIALS & METHODS

2.3.1. Experimental Design and Treatments

A greenhouse study was conducted at the Texas A&M AgriLife Research and Extension Center, Vernon, TX, USA in the summer of 2020. The experimental units were 7.6-L polyethylene pots, which were laid out in a completely randomized design on greenhouse benches with ~45 cm spacing. The treatments were 50 distinct guar genotypes, replicated four times, totaling 200 experimental units (Table 2.1). The seeds for all genotypes were obtained from the Plant Genetic Resources Conservation Unit (GRIFFIN-Global, GA, USA) and were selected to be early- to mid-season maturity, according to notes in the GRIN (Germplasm Resources Information Network) database. Early- to mid-season maturity lines were selected for their application in relatively short-season growing environments, such as the U.S. Southern Great Plains. The experiment period was 48 days because earlier studies indicate there is active nodule growth and development in guar around this timeframe (Venkateswarlu et al., 1983; Shrestha et al., 2021), including studies at the same research location.

Table 2.1 Identification number, name, and origin of the guar genotypes used in the study.

ID	PI	Description	Origin	ID	PI	Description	Origin
1	253184	B49821	Maryland	26	426635	K-149	Pakistan
2	271542	COL NO 1369	India	27	183400	NO 11397	India
3	271544	COL NO 1371	India	28	288384	NO 114	India
4	271545	COL NO 1372	India	29	288389	NO 119	India
5	271548	COL NO 1375	India	30	288408	NO 137	India
6	271549	COL NO 1376	India	31	288409	NO 138	India
7	275322	COL NO 1379	India	32	271159	NO 1495 PUSA SADAB	India
8	275323	COL NO 1380	India	33	288418	NO 209	India
9	288743	COL NO 456	India	34	288425	NO 280	India
10	288744	COL NO 459	India	35	288431	NO 320	India
11	288748	COL NO 479	India	36	288444	NO 379	India
12	288749	COL NO 488	India	37	164429	NO 8722 GAWAR	India
13	288750	COL NO 496	India	38	164592	NO 9016 KOTAVERI	India
14	288752	COL NO 511	India	39	164765	NO 9193 GUAR	India
15	288754	COL NO 517	India	40	338796	PLG 202	India
16	288757	COL NO 547	India	41	338811	PLG 241	India
17	288758	COL NO 551	India	42	338863	PLG 335	India
18	288759	COL NO 557	India	43	338865	PLG 337	India
19	288761	COL NO 570	India	44	338870	PLG 349	India
20	288763	COL NO 582	India	45	340511	PLG 782	India
21	250212	COL NO K635	Pakistan	46	340513	PLG 784	India
22	323002	IC 11350	India	47	340514	PLG 785	India
23	263887	IC 3289	India	48	340515	PLG 786	India
24	322775	IC 57A-1	India	49	340516	PLG 787	India
25	426634	K-107	Pakistan	50	158119	Sirsa 56	India

2.3.2. Experimental Procedures

A Miles loamy fine sand soil (fine-loamy, mixed, super-active, thermic Typic Paleustalfs) was used as the growing medium. The soil was collected from an area with no recent cultivation history near Lockett, TX. The chemical properties of the soil were analyzed in a commercial lab using the Mehlich III test method (Water's Agricultural Lab, Camilla, Georgia, USA) and presented in Table 2.2. Before filling the pots, the soil was thoroughly mixed and homogenized. Initially, pots were filled to a uniform level with the soil, simultaneously adding some water to facilitate uniform packing of the soil, then watered thoroughly and allowed to drain for 24 h. The pots were then weighed individually and adjusted to a uniform weight by adding or removing small amounts of soil, as needed. The soil water holding capacity of the pots was determined gravimetrically, which was found to be 23% by weight.

Table 2.2 Analysis of chemical and nutritional properties of the soil used in the study.

Parameter	Amount (mg kg^{-1})
NO3-N	5.5
NH4-N	0.38
P	65
K	407
Mg	287
Ca	1830
S	18
B	1.0
Zn	6.4
Mn	108
Fe	103
Cu	2.3
pH	7.95
SOM	0.21%
Texture	Sandy loam

Irrigation water was administered manually using a gravimetric approach based on the pot water-holding capacity. First, an upper weight set point (upper baseline) was established for the pots, which was the pot weight at 85% water-holding capacity. A water-holding capacity of 85% was used, rather than the full water-holding capacity, to prevent waterlogging in the root zone, while still allowing 100% ET replacement. Visual observations of soil moisture depletion in the top 2.5 cm of the soil guided the timing of watering. At each watering, the irrigation amount to replenish ET water loss was determined as a difference between the baseline pot weight and the average weight of 10 pots following a period of water loss. The 10 pots were flagged randomly at the start of the experiment and consistently used as water-use reference pots. The first watering was one week after planting. Subsequently, the intervals between watering events varied from 3-5 days during early plant growth, then reduced to 2-3 days as plant water demand increased.

Planting was done on 21 May 2020. Four seeds were planted per pot at 10-12 mm soil-depth, then thinned to a single plant 5-7 days after germination. Each pot was inoculated using 200 ml of an aqueous mixture of a custom *Rhizobium* inoculant, applied at a rate of 100 mg g^{-1} seed (0.8 g of inoculant per pot). The guar-specific, peat-based inoculant was composed of four *Rhizobium* strains (USDA 3089, USDA 3385, USDA 3386, and USDA 3595) obtained from the USDA-ARS National *Rhizobium* Germplasm Collection and developed in a microbiology lab using standard techniques (MacMillan et al., 2021a). A mixed-strain culture was used to increase the chances of root infection by rhizobia compatible with the various guar genotypes, especially given the background of native rhizobia in the study soil (Shrestha et al., 2021). The guar plants were harvested and processed on 7 July 2020, as described in the next section.

The greenhouse was cooled with an evaporative cooling system, which maintained outdoor ambient temperature levels. Soil temperature in the black pots was prevented from radiant heating above ambient temperature by wrapping the pots in aluminum foil to reflect incident solar radiation. This approach was successful in similar studies conducted in the same greenhouse (e.g., Shrestha et al., 2021). The ambient air temperature was measured using an OM-92 temperature sensor and data-logger package (OMEGA Engineering, Inc., Norwalk, CT, USA) (Figure 2.1).

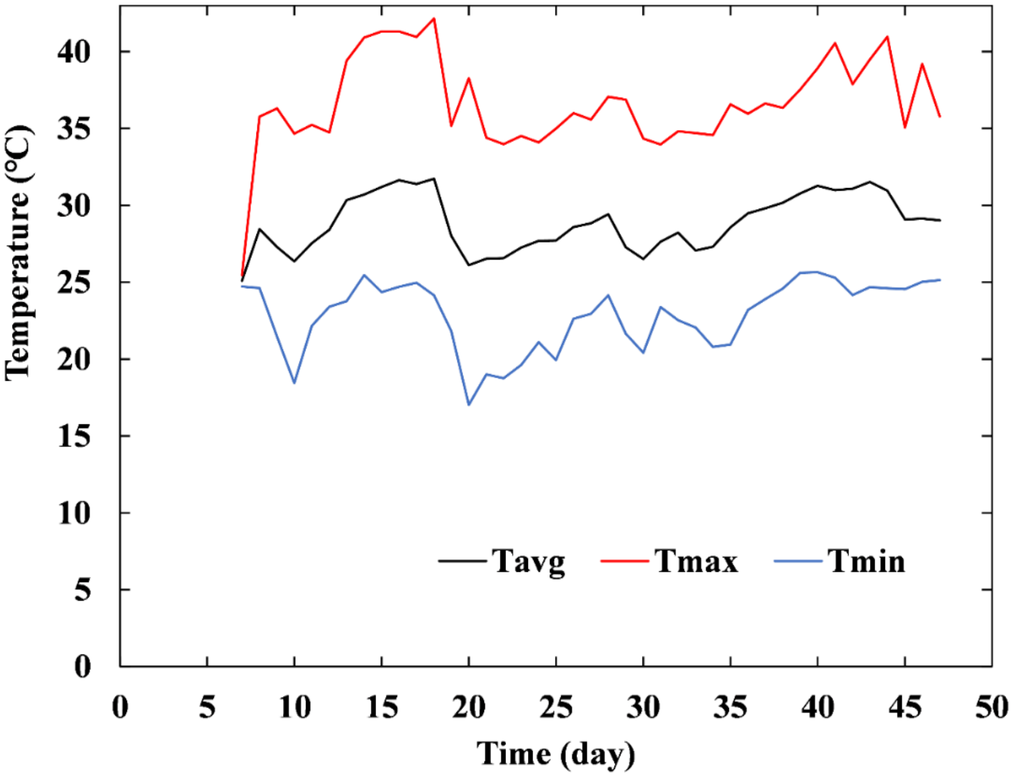

Figure 2.1 Maximum (T_{max}), average (T_{avg}), and minimum (T_{min}) daily ambient temperature inside the greenhouse during the study.

2.3.3. Measurements and Data Collection

The collection of aboveground plant data included qualitative and quantitative parameters, all obtained 48 days after planting (DAP). Data collection occurred in the R1-

R3 growth stage (First flower to first seed), depending on genotype phenology/maturity (Adams et al., 2020).

The evaluated qualitative parameters included leaf foliation pattern, margin type, and surface character, as well as plant branching habits. Leaf foliation pattern was recorded by observing whole-plant leaf distribution of simple, bifoliate, and trifoliate leaves. The leaf margins were characterized as smooth (even edge) or serrated (saw-toothed edge). Leaf surfaces were characterized as pubescent in the presence of hair-like projections and glabrous when lacking this character trait. Branching habits were evaluated by counting branches on the main stem.

The evaluated quantitative parameters included plant height, stem diameter, total main-stem nodes, and biomass components, including leaf, stem, and reproductive biomass. Plant height measurements were made from the soil surface to the topmost node on the main stem with fully opened leaves. Nodes on the main stem were counted, including the cotyledonary node, as branches on this node were common, and every subsequent node with fully opened leaves. Stem diameter was measured using digital calipers at 2 cm above the soil surface. For biomass measurements, whole plants were clipped just above the soil surface and partitioned into leaves, stem, and reproductive portions. Fresh leaves were immediately run through a LI-COR-3100 leaf area meter (LI-COR Biosciences, Lincoln, NE, USA) to determine the leaf area plant^{-1}. Each biomass component was placed into paper bags and dried to a constant weight at 55°C in a forced-air oven. After dry weight measurements, the biomass components for each plant were recombined and analyzed for N content using a combustion analyzer (Waters Agricultural

Lab, Camilla, GA, USA). The specific leaf area (SLA) parameter was derived mathematically, as a ratio of leaf area to total leaf dry mass.

The collection of belowground data included nodule number and nodule mass plant^{-1}. The root system of each pot was deconstructed immediately following the harvest of the aboveground portion of the plant. The soil of each pot was individually emptied into bins for the separation of nodules from the soil by hand sifting. The collected nodules were counted, washed, and dried to a constant weight in a forced-air oven at 55°C.

2.3.4. Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, North Carolina, USA) software package. Summary statistics [mean, minimum, maximum, quartiles, standard deviation (SD), and coefficient of variation (CV)] of each measured parameter were computed using the PROC MEANS procedure in SAS. Analysis of variance (ANOVA) was performed in SAS using the PROC MIXED procedure, testing the significance of differences among genotypes for measured parameters. Data were checked for normality and outliers using histograms, scatter plots, Q-Q Plots, and plots of residuals. Correlation analysis was performed using the PROC CORR procedure in SAS. A probability threshold of $P<0.05$ was used for statistical inferences.

Broad-sense heritability (H^2) was calculated using an approach suitable for a completely randomized design in a single environment. For n_g genotypes with n_r replicates, observed data were modeled using $y_{ik} = \mu + g_i + \varepsilon_{ik}$, where y_{ik} is the k^{th} observation of the i^{th} genotype, μ is the intercept, g_i is the effect for the i^{th} genotype and ε_{ik} is the error term (Schmidt et al., 2019). Based on the entry means unit method, the formula $H^2 =$

$\sigma_g^2 / [\sigma_g^2 + \sigma_e^2 / n_r]$ was used to compute H^2 (Fehr, 1991; Schmidt et al., 2019). In the formula, σ_g^2 is a total genetic variance and σ_e^2 is a residual variance. Estimates for σ_g^2 and σ_e^2 were $[\mathrm{MSGenotype} - \mathrm{Var(Residual)}] / n_r$ and Var (Residual), respectively. MSGenotype and Var (Residual) were obtained from the ANOVA table. Cluster analysis was conducted using the Ward method in JMP® Pro 15.2.0 Genomics 9 (SAS Institute Inc., Cary, NC) (Sahu, 2013), including all response variables.

2.4. RESULTS

2.4.1. Descriptive Statistical Analysis, ANOVA, and Heritability

Metrics describing data distribution [mean, quartiles (Q1, Q2, Q3), minimum, and maximum] and variability [SD, and CV] for all measured parameters are included in Table 2.3 (per plant basis). All measured plant parameters were normally distributed across guar genotypes to a greater extent, which was evident with mean and median values being similar for most parameters. The genotypes statistically differed for most measured parameters (Table 2.4), except nodule number (P=0.247) and SLA (P=0.107). There was wide phenotypic diversity for various biometric, productive, and nodulation characters (Table 2.3), indicating substantial genetic variations that can be utilized by guar breeders for introgression of desired traits. The H^2 for these parameters ranged from 0.14-0.86. The parameters for which heritability values were ≥0.70 (highly heritable) included branch number (H^2=0.86), reproductive mass (H^2=0.76), stem diameter (H^2=0.74), and main-stem nodes (H^2=0.70). All other parameters showed intermediate heritability ($0.30 < H^2 < 0.70$), except nodule number and SLA which had a low heritability (H^2<0.24).

Table 2.3 Summary statistics of 14 morphological, growth, and root nodule characters in a collection of 50 diverse guar genotypes.

Variable	Mean	Min.	Q1	Q2	Q3	Max.	SD	CV
Biometric Parameters								
Node number	20.3	18.0	19.0	20.0	21.0	23.0	1.4	6.7
Height (cm)	58.4	45.8	56.6	59.0	61.1	66.1	4.3	7.4
Branch number	8.4	0.0	4.0	10.0	12.0	13.0	4.3	50.9
Stem diameter (mm)	6.1	5.0	5.8	6.0	6.3	9.0	0.7	11.1
Productivity Parameters								
Total biomass (g)	21.5	16.1	20.3	21.9	22.7	26.2	2.0	9.5
Stem (g)	8.8	5.1	8.0	8.9	9.8	11.6	1.4	15.8
Reproductive (g)	1.6	0.0	1.0	1.5	2.2	6.0	1.0	62.4
Leaf (g)	11.2	8.6	10.4	11.3	12.0	13.6	1.0	9.3
Leaf area (cm^2)	2338	1782	2154	2324	2472	2965	252.8	10.8
Specific leaf area (cm^2 g^{-1})	211	171	198	214	224	240	17.0	8.1
Plant N Analysis and Nodulation Parameters								
Nodule number	17	10	16	17	20	27	3.6	20.5
Nodule mass (mg)	891.5	602.0	775.0	875.0	1000	1150	144.2	16.2
Plant N conc. (%)	3.1	2.7	3.0	3.1	3.2	3.6	0.2	6.5
Total plant N (g)	0.67	0.47	0.64	0.68	0.70	0.79	0.07	10.1

**Q1 =1st quartile, Q2 Median, Q3 = 3rd quartile, SLA = Specific Leaf Area*

Total biomass production among genotypes averaged 21.5 g, ranging from 16.1-26.2 g plant^{-1}, with relatively low variability (9.5% CV). The average total biomass was allocated to leaf, stem, and reproductive portions in percentages of 51.9, 40.7, and 7.4%, respectively. The variability was far higher for reproductive mass (62.4% CV) than leaf (9.3% CV) and stem mass (15.8% CV). The wide variability in reproductive mass was strongly influenced by genotypic differences in development (ontogeny) and the timing of data collection. Some genotypes did not produce measurable quantities of reproductive mass at the time of data collection.

Table 2.4 ANOVA and heritability results for 14 plant characters among 50 guar genotypes.

Variable	Source	DF	Sum of Squares	Mean Square	Error DF	*F* Value	*Pr* > *F*	Heritability (H^2)
Nodule mass	Genotype	49	4072097	83104	147	1.66	0.011	0.40
	Residual	147	7343870	49958	-	-	-	-
Nodule number	Genotype	49	2328.8	47.5	138	1.16	0.247	0.14
	Residual	138	5640.8	40.9	-	-	-	-
Biomass	Genotype	49	820.2	16.7	148	1.64	0.012	0.39
	Residual	148	1508.5	10.2	-	-	-	-
Leaf mass	Genotype	49	213.5	4.4	148	1.66	0.011	0.40
	Residual	148	389.2	2.6	-	-	-	-
Stem mass	Genotype	49	371.6	7.6	148	3.06	<.0001	0.67
	Residual	148	366.9	2.5	-	-	-	-
Reproductive mass	Genotype	49	175.7	3.6	147	4.16	<.0001	0.76
	Residual	147	126.8	0.9	-	-	-	-
Leaf area	Genotype	49	12430994	253694	148	2.53	<.0001	0.61
	Residual	148	14826621	100180	-	-	-	-
Height	Genotype	49	3637.9	74.2	148	1.91	0.002	0.48
	Residual	148	5763.0	38.9	-	-	-	-
Branch number	Genotype	49	3472.4	70.9	148	7.1	<.0001	0.86
	Residual	148	1477.8	10.0	-	-	-	-
Node number	Genotype	49	373.9	7.6	148	3.34	<.0001	0.70
	Residual	148	338.4	2.3	-	-	-	-
Plant N concentration	Genotype	49	7.7	0.2	145	1.58	0.020	0.37
	Residual	145	14.5	0.1	-	-	-	-
Total plant N	Genotype	49	0.8	0.0	145	1.74	0.006	0.42
	Residual	145	1.4	0.0	-	-	-	-
Stem diameter	Genotype	49	87.1	1.8	148	3.85	<.0001	0.74
	Residual	148	68.3	0.5	-	-	-	-
Specific leaf area	Genotype	49	56347	1149.9	148	1.32	0.107	0.24
	Residual	148	129292	873.6	-	-	-	-

Branch number varied from non-branching to 13 branches plant^{-1}. Branch number had high variability (50.9% CV), which averaged 8.4 branches plant^{-1} with a median of 10, indicating a larger proportion of genotypes were highly branching in the collection. Based on branching habits, genotypes were categorized into four groups: non-branched (0 branches plant^{-1}), sparse (1-3), medium (4-8), and highly branched (9-13). Only three genotypes were non-branching, seven sparsely branching, seven medium branching, and the remaining 33 were highly branching types. At harvest time (48 DAP), the non-branching and sparsely branching genotypes generally had the greatest reproductive biomass (>Q3), while the least reproductive biomass (<Q1) was observed primarily in highly branching genotypes (Tables 2.3, 2.5 and 2.6).

Plant height was relatively uniform among genotypes (7.4% CV), ranging from 46-66 cm (Table 2.3). Stem diameter measured from 5-9 mm, with a somewhat higher CV of 11.1%. Leaf area and SLA parameters averaged 2338 cm^2 and 211 cm^2 g^{-1}, respectively. These parameters had relatively low overall variability (10.8% CV for leaf area and 8.1% CV for SLA), though the ranges were great. Leaf area ranged from 1782-2965 cm^2 and SLA varied from 171-240 cm^{2}g^{-1}.

Belowground root nodule parameters were variable among genotypes (Table 2.7). Nodule number averaged 17.4 plant^{-1} and ranged from 10-27 plant^{-1}. Likewise, nodule mass averaged 891.5 mg and ranged from 602-1150 mg plant^{-1}. Overall, nodule number was more variable than nodule mass (20.5% *vs* 16.2% CV). The N concentration in whole plant tissue was homogeneous among genotypes (6.5% CV), ranging from 2.7-3.6%. The total plant N ranged from 0.47-0.79 g with a comparatively high CV (10.1%).

Table 2.5 Genotype-specific data on aboveground biometric and morphological characters.

ID	Nodes		Height		Stem		Branch		Leaf Character	
	#	SD	cm	SD	cm	SD	#	SD	Type*	Surface
1	19	±2.1	60.9	±8.0	6.0	±0.4	0.0	±0.5	Simple (S/B), Trifoliate	Pubescent
2	20	±1.5	59.1	±2.1	6.4	±1.0	10	±1.7	Simple (S/B), Bifoliate (S/B), Trifoliate	Pubescent
3	20	±1.0	62.5	±1.3	5.8	±0.8	12	±0.5	Simple (S/B), Bifoliate (S), Trifoliate	Mix
4	19	±1.0	58.0	±2.3	5.5	±1.0	12	±1.6	Simple (S/B), Bifoliate (B), Trifoliate	Pubescent
5	21	±0.6	62.4	±6.6	6.0	±0.4	13	±0.5	Simple (S/B), Bifoliate (S/B), Trifoliate	Mix
6	20	±1.3	56.4	±6.6	5.9	±0.4	11	±2.6	Simple (S/B), Trifoliate	Mix
7	21	±1.3	62.9	±8.4	5.9	±0.2	12	±1.7	Simple (S/B), Bifoliate (S/B), Trifoliate	Pubescent
8	21	±0.6	66.1	±3.0	5.9	±0.3	13	±1.0	Simple (S/B), Trifoliate	Mix
9	20	±1.9	56.9	±5.7	6.0	±0.9	1.0	±1.0	Simple (S/B), Bifoliate (B), Trifoliate	Mix
10	23	±1.3	58.0	±10	6.1	±0.2	3.0	±6.5	Simple (S/B), Bifoliate (B), Trifoliate	Pubescent
11	23	±1.9	65.9	±7.7	6.4	±0.6	4.0	±6.7	Simple (S/B), Bifoliate (S/B), Trifoliate	Pubescent
12	22	±0.5	61.1	±7.1	6.8	±1.2	1.0	±0.8	Simple (S/B), Trifoliate	Pubescent
13	22	±1.5	61.0	±7.4	6.2	±0.3	2.0	±1.5	Simple (S/B), Trifoliate	Pubescent
14	22	±3.0	58.3	±6.1	6.7	±0.5	3.0	±5.9	Simple (S/B), Bifoliate (S), Trifoliate	Pubescent
15	22	±0.5	60.3	±1.5	6.3	±0.6	13	±1.0	Simple (S/B), Bifoliate (B), Trifoliate	Mix
16	22	±1.5	57.9	±7.6	6.8	±0.6	4.0	±6.4	Simple (S/B), Bifoliate (S), Trifoliate	Pubescent
17	21	±1.7	56.8	±5.6	5.8	±0.4	10	±6.4	Simple (S/B), Bifoliate (S/B), Trifoliate	Pubescent
18	22	±1.5	58.8	±7.0	6.1	±0.4	0.0	±0.5	Simple (S/B), Bifoliate (B), Trifoliate	Pubescent
19	22	±1.7	60.8	±5.6	6.3	±1.2	3.0	±5.4	Simple (S/B), Bifoliate (S), Trifoliate	Pubescent
20	20	±0.6	47.3	±4.3	6.2	±0.7	13	±1.0	Simple (S/B), Trifoliate	Pubescent
21	21	±1.2	62.8	±6.9	6.0	±0.7	9.0	±4.1	Simple (S/B), Trifoliate	Pubescent
22	19	±1.0	52.3	±1.2	6.3	±0.4	12	±2.1	Simple (S/B), Bifoliate (B), Trifoliate	Pubescent
23	18	±1.0	58.6	±4.2	6.5	±0.5	0.0	±0.0	Simple (S/B), Trifoliate	Pubescent
24	19	±0.8	55.1	±8.1	5.8	±0.4	10	±2.4	Simple (S/B), Bifoliate (S), Trifoliate	Pubescent
25	20	±1.3	58.0	±5.5	6.1	±1.1	13	±0.8	Simple (S/B), Trifoliate	Mix
26	19	±0.5	61.5	±5.9	6.6	±0.6	12	±1.0	Simple (S/B), Bifoliate (B), Trifoliate	Mix
27	22	±2.1	59.4	±4.9	6.9	±0.6	4.0	±5.9	Simple (S/B), Trifoliate	Pubescent
28	21	±1.7	63.0	±4.2	8.0	±0.6	9.0	±2.2	Simple (S/B), Trifoliate	Mix
29	21	±1.5	59.8	±2.9	9.0	±0.4	8.0	±0.8	Simple (S/B), Trifoliate	Pubescent
30	19	±0.8	59.9	±7.7	7.2	±0.4	10	±2.2	Simple (S/B), Trifoliate	Pubescent
31	18	±1.8	49.5	±5.2	6.2	±1.0	10	±2.9	Simple (S/B), Bifoliate (S), Trifoliate	Pubescent
32	22	±1.5	56.6	±5.9	6.7	±0.5	4.0	±6.2	Simple (S/B), Trifoliate	Pubescent
33	22	±2.1	58.9	±10	6.0	±0.5	5.0	±5.4	Simple (S/B), Bifoliate (S), Trifoliate	Pubescent
34	19	±0.8	59.4	±4.8	5.3	±0.9	11	±1.5	Simple (S/B), Bifoliate (B), Trifoliate	Mix
35	19	±1.4	55.6	±7.6	5.9	±0.5	9.0	±2.1	Simple (S/B), Bifoliate (S/B), Trifoliate	Mix
36	20	±1.0	60.4	±3.4	6.3	±0.7	10	±2.9	Simple (S/B), Bifoliate (S), Trifoliate	Mix
37	18	±1.3	45.8	±1.0	5.6	±0.2	12	±1.0	Simple (S/B), Bifoliate (S/B), Trifoliate	Pubescent
38	21	±0.8	63.5	±4.3	6.0	±0.3	2.0	±1.5	Simple (S), Bifoliate (S/B), Trifoliate	Mix
39	19	±1.0	56.6	±4.3	5.6	±1.0	11	±1.0	Simple (S/B), Bifoliate (S/B), Trifoliate	Pubescent
40	21	±2.2	57.4	±10	5.6	±0.7	9.0	±4.7	Simple (S/B), Trifoliate	Pubescent
41	19	±1.7	49.3	±4.3	5.0	±0.6	11	±2.2	Simple (S/B), Trifoliate	Pubescent
42	20	±0.8	61.3	±6.4	5.4	±0.6	11	±1.0	Simple (S/B), Bifoliate (S), Trifoliate	Mix
43	21	±3.4	55.4	±9.7	5.5	±1.0	13	±3.5	Simple (S/B), Trifoliate	Pubescent
44	20	±1.0	59.8	±6.7	5.6	±1.0	12	±1.2	Simple (S/B), Bifoliate (S), Trifoliate	Mix
45	18	±3.1	53.4	±4.5	5.8	±0.5	12	±1.0	Simple (S/B), Bifoliate (S/B), Trifoliate	Pubescent
46	20	±1.2	63.2	±1.9	5.0	±0.3	11	±2.1	Simple (S/B), Bifoliate (B), Trifoliate	Pubescent
47	20	±0.5	62.0	±3.4	5.8	±0.5	11	±1.5	Simple (S/B), Bifoliate (S), Trifoliate	Pubescent
48	21	±1.0	54.8	±10	5.8	±1.4	13	±0.6	Simple (S/B), Bifoliate (S), Trifoliate	Mix
49	19	±1.7	60.5	±6.8	5.6	±0.7	11	±1.4	Simple (S/B), Bifoliate (S), Trifoliate	Pubescent
50	19	±1.2	54.8	±8.5	6.0	±0.2	7.0	±4.6	Simple (S/B), Bifoliate (S/B), Trifoliate	Pubescent

**Indicates all leaf types observed on the plant. Letters within parenthesis (S = Main-stem, B = Branch), implies position(s) of leaf type. Simple and bifoliate leaves occurred only within the 5th main-stem basal nodes or within the 3rd basal branches.*

Table 2.6 Genotype-specific data on biomass and leaf parameters.

ID	Total biomass		Biomass components						Leaf area		Specific leaf area	
			Stem		Reproductive		Leaf					
	g	SD	g	SD	g	SD	g	SD	cm^2	SD	cm^2 g^{-1}	SD
1	19.9	±3.7	6.8	±2.3	2.8	±2.5	10.2	±2.8	2121	±417	216	±50.3
2	22.0	±5.9	9.4	±2.7	1.5	±0.7	11.2	±2.7	2330	±583	211	±26.7
3	23.0	±1.5	9.3	±1.0	1.7	±1.1	12.1	±1.5	2238	±245	187	±18.5
4	22.4	±1.9	9.4	±0.7	1.0	±0.5	12.0	±1.0	2372	±236	198	±24.7
5	22.0	±2.1	9.4	±1.3	0.6	±0.4	12.1	±1.1	2578	±73.2	215	±16.3
6	18.3	±3.8	7.7	±1.7	1.0	±0.7	9.70	±1.4	2208	±287	230	±25.9
7	23.2	±3.7	10	±1.9	0.6	±0.5	12.6	±2.0	2599	±195	210	±29.9
8	26.2	±2.3	12	±1.2	1.0	±0.6	13.6	±1.8	2657	±187	198	±28.9
9	16.1	±3.4	5.7	±1.4	1.2	±0.9	9.20	±1.2	1782	±159	197	±35.8
10	20.7	±2.2	7.1	±1.0	2.6	±1.9	11.0	±1.4	2224	±133	205	±23.1
11	21.6	±0.7	8.2	±0.6	2.2	±0.7	11.2	±0.7	2231	±243	199	±18.9
12	22.7	±2.0	8.3	±0.9	2.5	±1.1	11.9	±0.6	2164	±292	182	±22.5
13	21.9	±3.7	8.9	±1.7	1.5	±0.8	11.6	±1.6	2038	±348	178	±33.7
14	20.4	±3.9	7.0	±1.4	2.3	±1.3	11.1	±1.7	2154	±231	196	±31.4
15	24.8	±3.1	11	±1.6	1.1	±0.3	12.4	±1.7	2900	±370	235	±5.30
16	22.0	±3.1	8.3	±1.5	1.6	±1.0	12.1	±2.2	2397	±468	199	±24.8
17	22.2	±1.5	8.8	±1.1	2.0	±1.4	11.4	±0.9	2431	±470	213	±36.7
18	19.4	±4.4	6.6	±1.4	3.2	±1.7	9.70	±1.8	1960	±179	205	±23.6
19	18.5	±2.9	6.5	±0.4	2.0	±1.1	10.0	±1.7	2061	±149	211	±38.1
20	22.3	±3.5	9.8	±1.7	0.5	±0.5	12.0	±1.6	2498	±273	212	±35.3
21	23.3	±6.3	9.6	±2.8	1.2	±1.3	12.5	±3.1	2441	±240	202	±43.8
22	23.6	±2.6	9.7	±1.5	1.8	±0.6	12.2	±1.1	2907	±211	240	±8.10
23	18.9	±1.3	5.1	±0.4	6.0	±0.5	9.30	±1.7	2061	±202	228	±49.8
24	19.7	±2.5	7.9	±1.2	1.9	±0.4	10.0	±1.2	2258	±317	226	±19.9
25	24.8	±3.4	11	±2.1	1.2	±0.3	13.1	±1.5	2965	±400	226	±7.30
26	23.7	±3.7	10	±2.1	1.4	±0.5	12.1	±1.8	2557	±170	216	±43.0
27	22.3	±2.8	8.5	±1.4	2.4	±0.7	11.4	±1.4	2413	±422	215	±52.7
28	20.4	±2.1	10	±1.6	0.0	±0.0	10.4	±0.5	2447	±210	235	±9.30
29	22.1	±2.8	11	±1.5	0.0	±0.0	11.4	±1.4	2472	±158	218	±21.4
30	21.0	±1.0	9.9	±0.6	0.3	±0.2	10.9	±0.7	2493	±293	229	±23.8
31	18.6	±5.3	8.0	±3.1	0.1	±0.1	10.5	±2.1	2314	±575	221	±34.8
32	21.8	±4.1	8.3	±2.6	2.9	±1.8	10.6	±2.3	2464	±587	233	±31.4
33	24.3	±3.5	9.1	±2.4	2.6	±1.6	12.6	±1.8	2777	±552	220	±25.1
34	20.3	±2.6	8.5	±0.8	1.0	±0.5	10.8	±1.4	2345	±119	220	±26.0
35	19.3	±2.1	8.2	±1.1	0.7	±0.1	10.4	±0.9	2276	±480	217	±27.9
36	24.6	±3.6	10	±1.2	2.5	±1.2	12.1	±1.3	2312	±220	191	±14.6
37	19.4	±3.4	7.9	±1.2	1.1	±0.8	10.4	±1.7	2317	±33.0	228	±37.7
38	21.6	±1.3	8.1	±0.7	2.0	±1.2	11.6	±0.7	1969	±173	171	±21.1
39	21.8	±1.5	8.4	±0.6	1.6	±0.3	11.8	±1.0	2091	±103	178	±17.0
40	22.7	±3.4	9.1	±1.7	2.0	±0.6	11.7	±1.1	2243	±338	192	±10.6
41	16.7	±1.4	6.7	±0.6	1.4	±0.9	8.60	±0.9	2000	±182	232	±7.00
42	20.9	±1.5	8.7	±0.8	2.2	±0.7	10.1	±0.8	2164	±156	215	±15.4
43	19.8	±4.5	8.5	±1.7	1.4	±0.7	10.0	±2.7	2141	±318	224	±48.9
44	22.3	±1.9	9.9	±1.0	0.8	±0.3	11.6	±0.6	2584	±277	223	±17.8
45	22.3	±3.4	9.2	±1.8	1.6	±0.3	11.5	±1.8	2404	±372	209	±22.9
46	21.2	±3.6	9.4	±1.8	1.0	±1.0	10.9	±1.4	2144	±282	197	±4.10
47	21.6	±2.0	9.6	±1.3	1.4	±0.5	10.6	±0.6	2349	±337	220	±21.6
48	23.2	±4.1	10	±1.6	1.8	±0.7	11.2	±1.8	2603	±351	240	±58.8
49	22.3	±3.5	9.9	±2.1	0.8	±0.5	11.6	±1.2	2356	±413	203	±25.1
50	21.0	±3.1	7.5	±1.3	2.3	±0.8	11.2	±2.5	2069	±289	189	±28.6

Table 2.7 Genotype-specific data on root nodules characters and plant-N analysis.

ID	Nodulation Parameters				N-analysis			
	Nodule number		Nodule mass		Plant N concentration		Total plant N	
	#	SD	mg	SD	%	SD	g	SD
1	14	±1.2	602	±343	3.33	±0.4	0.66	±0.1
2	17	±8.8	1025	±378	3.31	±0.3	0.73	±0.2
3	14	±3.3	850	±173	2.77	±0.3	0.64	±0.0
4	18	±2.1	875	±95.7	2.68	±0.2	0.60	±0.0
5	17	±9.0	850	±129	3.12	±0.2	0.69	±0.0
6	18	±3.9	725	±150	3.02	±0.4	0.55	±0.2
7	17	±4.9	1075	±222	3.03	±0.4	0.70	±0.1
8	18	±9.6	1075	±386	2.84	±0.4	0.74	±0.1
9	16	±6.7	700	±141	2.91	±0.3	0.47	±0.1
10	11	±1.8	1050	±192	3.20	±0.2	0.66	±0.0
11	19	±8.1	850	±129	3.17	±0.3	0.68	±0.0
12	16	±2.1	1075	±275	2.97	±0.2	0.67	±0.1
13	16	±2.7	1075	±330	3.02	±0.3	0.66	±0.1
14	18	±7.5	1150	±265	3.14	±0.4	0.64	±0.1
15	21	±5.5	1125	±95.7	3.18	±0.2	0.79	±0.1
16	20	±11	950	±129	3.17	±0.4	0.70	±0.1
17	19	±2.1	850	±173	3.04	±0.1	0.68	±0.1
18	24	±9.2	1100	±392	3.48	±0.4	0.68	±0.1
19	18	±11	825	±171	3.31	±0.2	0.61	±0.1
20	16	±5.7	1150	±252	3.02	±0.3	0.67	±0.1
21	21	±10	875	±350	2.87	±0.2	0.67	±0.1
22	18	±0.8	1000	±81.6	3.12	±0.2	0.73	±0.1
23	20	±8.0	775	±222	3.61	±0.3	0.68	±0.1
24	16	±2.5	875	±228	3.30	±0.2	0.65	±0.1
25	13	±4.8	900	±424	3.10	±0.1	0.77	±0.1
26	12	±3.5	800	±81.6	2.99	±0.3	0.71	±0.1
27	19	±5.7	950	±129	3.54	±0.6	0.79	±0.1
28	27	±11	900	±163	3.31	±0.1	0.68	±0.0
29	24	±6.7	950	±129	3.23	±0.3	0.72	±0.0
30	21	±4.0	900	±216	3.26	±0.3	0.69	±0.1
31	17	±5.2	725	±340	2.71	±0.3	0.50	±0.2
32	23	±5.7	1100	±294	3.49	±0.3	0.76	±0.1
33	19	±5.4	900	±141	3.13	±0.2	0.76	±0.1
34	21	±4.1	725	±95.7	2.95	±0.2	0.60	±0.0
35	15	±8.1	675	±222	2.86	±0.4	0.55	±0.1
36	11	±4.1	975	±150	2.89	±0.2	0.71	±0.1
37	21	±8.3	775	±222	3.28	±0.3	0.64	±0.1
38	17	±5.9	750	±265	3.04	±0.6	0.66	±0.1
39	13	±3.0	775	±150	2.90	±0.4	0.63	±0.1
40	17	±4.4	1100	±245	3.09	±0.2	0.70	±0.1
41	11	±3.7	650	±57.7	3.27	±0.1	0.55	±0.0
42	20	±9.4	800	±141	3.10	±0.4	0.65	±0.1
43	17	±11	675	±222	3.07	±0.6	0.61	±0.1
44	16	±8.1	833	±153	3.05	±0.3	0.68	±0.1
45	21	±7.4	900	±173	3.09	±0.2	0.69	±0.1
46	10	±0.0	867	±57.7	2.92	±0.2	0.62	±0.1
47	16	±4.9	975	±171	3.06	±0.2	0.66	±0.1
48	15	±3.2	950	±238	3.09	±0.3	0.72	±0.1
49	15	±2.5	750	±192	3.11	±0.4	0.69	±0.2
50	18	±5.8	775	±150	2.89	±0.6	0.60	±0.1

2.4.2. Plant Character Relationships

Correlation analysis of the biometric, productive, plant N, and nodule characters revealed interesting associations (Table 2.8). Total biomass was positively correlated with all biometric parameters ($0.24 \leq r \leq 0.34$), biomass components ($0.14 \leq r \leq 0.92$), total plant N, and nodule mass (r =0.54), but negatively correlated with plant N concentration (r= -0.31) and SLA (r= -0.36). The relationships between leaf mass, stem mass, leaf area, and all biometric parameters were positive ($0.15 \leq r \leq 0.78$), except there was no relationship between leaf area and node number. There was a positive relationship between leaf mass and leaf area (r=0.59). Leaf area had a positive relationship with SLA (r=0.41), while there was a mathematically derived inverse relationship between SLA and leaf mass (r= -0.48).

Plant height was positively correlated with total biomass and the biomass components, excluding reproductive mass. Total main-stem nodes had a positive relationship with stem diameter, total biomass, and biomass components. Branch number had a positive relationship with total biomass and biomass components, except for reproductive mass. A negative relationship between branch number and reproductive mass (r= -0.49) largely represented an ontogenic effect. Also, branch number was negatively correlated with total main-stem nodes and plant height, which may have also been influenced by ontogeny. Stem diameter had an inverse relationship with branch number (r= -0.17), indicating that more sparsely branching genotypes generally had larger stem sizes.

There was no relationship between root nodule number and nodule mass. Nodule mass was positively related to total biomass, the biomass components, and many other plant parameters, including total nodes, stem diameter, and total plant N. Nodule mass was positively related to leaf area, but negatively related to SLA.

Table 2.8 Correlation matrix quantifying relationships in plant morphological, growth, and root nodule characters of 50 guar genotypes (SLA, specific leaf area).

Variables	Nodule mass	Plant N concentration	Total plant N	Total Biomass	Stem mass	Reproductive mass	Leaf mass	Leaf area	SLA	Node number	Height	Branch number	Stem diameter
Nodule number	0.13 ns	0.19 **	0.12 ns	0 ns	0.05 ns	-0.04 ns	-0.02 ns	0.08 ns	0.11 ns	0.05 ns	-0.05 ns	-0.09 ns	0.24 **
Nodule mass	1	0.04 ns	0.56 ***	0.54 ***	0.43 ***	0.14 *	0.48 ***	0.35 ***	-0.18 *	0.32 ***	0.09 ns	0.02 ns	0.31 ***
Plant N concentration		1	0.33 ***	-0.31 ***	-0.26 ***	0.21 **	-0.47 ***	0.08 ns	0.61 ***	0.09 ns	-0.1 ns	-0.19 **	0.18 *
Total plant N			1	0.78 ***	0.67 ***	0.27 ***	0.6 ***	0.66 ***	0.04 ns	0.29 ***	0.26 ***	0.13 ns	0.38 ***
Total biomass				1	0.85 ***	0.14 *	0.92 ***	0.62 ***	-0.36 ***	0.26 ***	0.34 ***	0.24 ***	0.28 ***
Stem mass					1	-0.32 ***	0.78 ***	0.72 ***	-0.09 ns	0.15 *	0.3 ***	0.53 ***	0.28 ***
Reproductive mass						1	-0.08 ns	-0.26 ***	-0.18 *	0.17 *	0.07 ns	-0.49 ***	0.01 ns
Leaf mass							1	0.59 ***	-0.48 ***	0.22 **	0.3 ***	0.23 **	0.23 ***
Leaf area								1	0.41 ***	0.11 ns	0.15 *	0.45 ***	0.24 ***
SLA									1	-0.11 ns	-0.16 *	0.24 ***	0 ns
Node number										1	0.43 ***	-0.32 ***	0.32 ***
Height											1	-0.22 **	0.18 *
Branch number												1	-0.17 *

Statistical significance at *P<0.05, **P<0.01, and ***P<0.001, non-significant (ns) at P>0.05

None of these parameter relationships were significant for nodule numbers, except plant N concentration and stem diameter. Both nodule number (*r*=0.24) and nodule mass (*r*=0.31) were positively correlated with stem diameter. There was an interesting contrast in that nodule mass was correlated to total plant N (*r*=0.56), whereas nodule number was correlated with plant N concentration (*r*=0.19), which was also strongly correlated with SLA (*r*=0.61).

2.4.3. Genotype Cluster Analysis

Cluster analysis illustrated differences and similarities of the individual genotypes in association with measured parameters (Figure 2.2). Genotypes were classified into three broad clusters, each containing two narrower clusters (six total), highlighting meaningful similarities in plant characters among genotypes in each cluster and genetic diversity across the clusters. For instance, the 11 genotypes in Cluster 2 (from the top in Figure 2.2) were characterized by low branching and biomass productivity, but generally higher values for the number of nodes, height, stem diameter, reproductive mass, plant N concentration, nodule number, and nodule mass.

Contrarily, Cluster 5, composed of 19 genotypes, was distinguished by generally high values for parameters like branch number, leaf area, SLA, vegetative biomass productivity, and total plant N, but generally lower values for the remaining parameters. Clusters 3 and 4, consisting of 15 genotypes, had generally low values for most parameters, except relatively high branch numbers, as well as high SLA in Cluster 3. Cluster 6, having only three genotypes, had moderate to high values for most parameters, except reproductive mass.

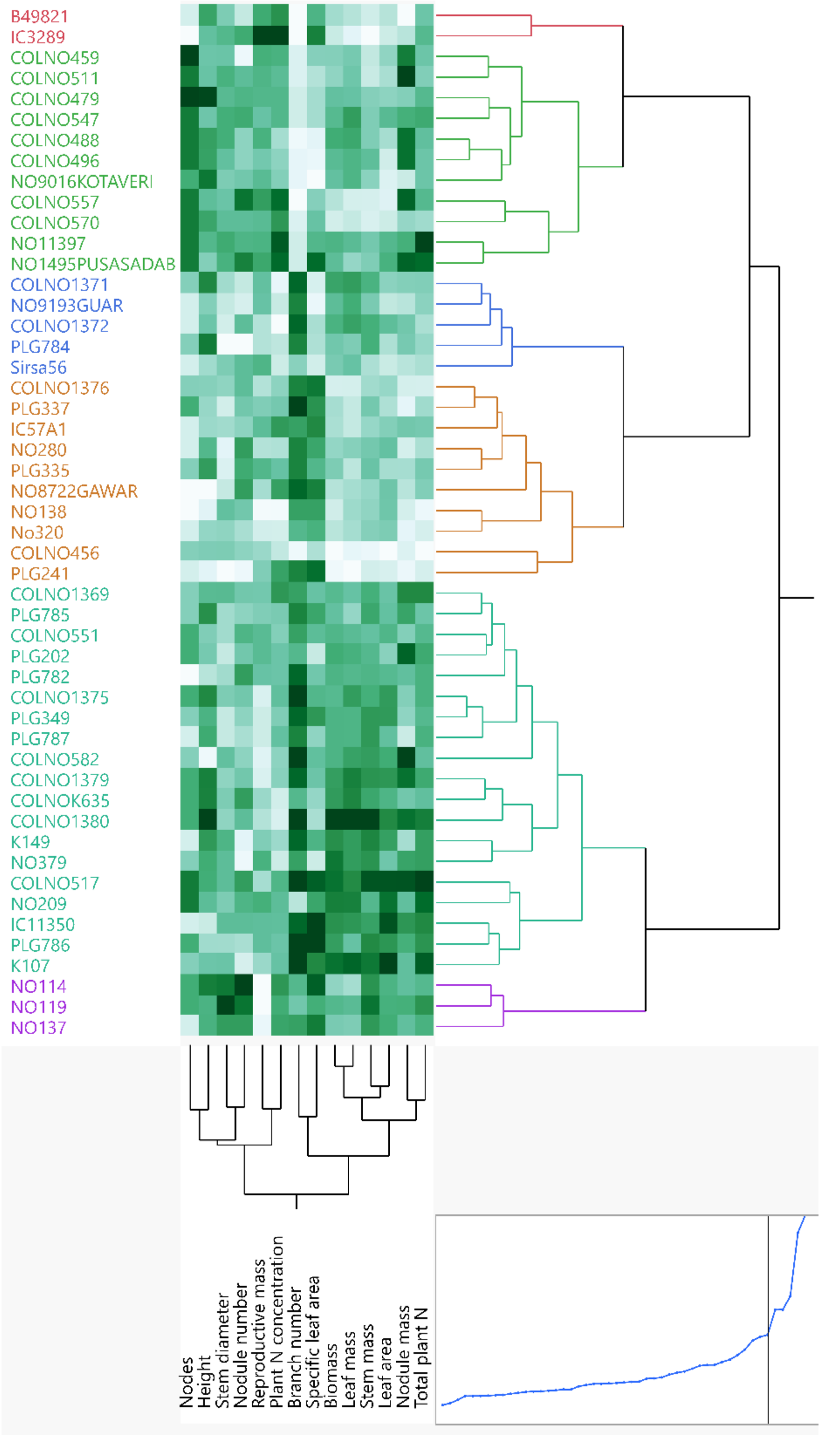

Figure 2.2 Figure 2.2 Dendrogram illustrating two-way cluster analysis of guar genotypes (left) and measured variables (bottom). The color map shows a relative ranking of genotypes for each variable from high (dark green) to low values (light green). The six genotype clusters are represented by varying color codes and classified from the top (Cluster 1) to the bottom (Cluster 6).

The measured variables themselves were grouped into two broad clusters and four narrower clusters, with subsets within each cluster. The clusters illustrate relationships among all 14 measured parameters. Cluster A (from the left in Figure 2.2) included nodes, plant height, stem diameter, and nodule number, indicating a close relationship among these parameters. Cluster B included reproductive mass and plant N concentration. Branch number and SLA was grouped into Cluster C. Total biomass, leaf mass, stem mass, leaf area, nodule mass, and total plant N made up Cluster D.

2.5. DISCUSSION

At the time of data collection (48 DAP), the plants were in the R1-R3 growth stage (First flower to first seed), depending on individual guar genotype phenology/maturity. This is a period of rapid change in reproductive development (Adams et al., 2020; Stafford, 1987), which is reflected in the wide variability observed in reproductive mass (Table 2.3), but also a period of active root nodule growth, suitable for evaluating nodules phenes in the species (MacMillan et al., 2021a; Thapa et al., 2018). The dataset must be interpreted in the context of that timing, as it is a snapshot in the process of plant development.

The most novel contributions of this research are insights into phenotypic differences in root nodule parameters among diverse guar germplasms. Nodule mass differed among genotypes, but there were no differences for nodule number, and the two parameters were not correlated (Tables 2 – 4). Similar findings have been reported for other legumes like peanut [*Arachis hypogaea* L.] (Wunna et al., 2009), moth bean [*Vigna aconitifolia* (Jacq.) Maréchal] (Rao et al., 1984), and soybean [*Glycine max* (L.) Merr.] (Sinclair et al., 1991). Broad-sense heritability for nodule mass (H^2=0.40) was predictably higher than nodule number (H^2=0.14). This indicates there are moderate amounts of both genetic and environmental influence on nodule mass in guar, while nodule numbers plant^{-1} are overwhelmingly influenced by non-genetic factors and prone to variability. Practically, this means that nodule mass is a more robust indicator of plant nodule character/performance than nodule number for guar researchers and producers. Environmental and cultural factors like soil N, soil type, soil moisture, and *Rhizobium* inoculation are well documented to exert substantial influence on nodule development in guar and cumulative nodule weight consistently provides a better indication of these impacts (Hinson et al., 2020; MacMillan et al., 2021a; Shrestha et al., 2021). Positive relationships of nodule mass with biomass production and total plant N indicate phenotypic links between the capacity of guar genotypes to support N_2 fixation, accumulate N_2, and enhance plant biomass production in the conditions of this study.

Wide diversity was measured in plant branching, which is an important character trait affecting planting density requirements, ground cover, and mechanical harvest efficiency. Abidi et al. (2015) described difficulty in mechanical harvest and yield losses associated with low pod setting (near the ground) on more highly branching varieties, though branched varieties may compete more effectively with weeds. Plant branching habits can be readily customized in guar breeding to meet stakeholder needs because heritability for branching was high in this study (H^2=0.86) and others (Gourdar et al., 2017; Santhosha et al., 2017; Singh et al., 2016). Branch number was positively related to most biomass production parameters, excluding reproductive biomass, which was inversely correlated. This inverse relationship largely reflected varietal differences in maturity under the relatively short growing period of this study and would likely weaken or reverse if the plants were permitted to grow to full maturity. For example, positive correlations (*r*=0.4) were reported between branch number and seed yield plant^{-1} in guar grown to maturity in the field (Gresta et al., 2018a; Jukanti et al., 2015). The authors also reported a positive relationship (*r*=0.55) between branch number and days to maturity, consistent with the later reproductive development in more highly branched genotypes in the current study. This dynamic may have implications on guar breeding for areas differing in the length of the growing season.

Total main stem nodes, plant height, and stem diameter had relatively low variation among genotypes, though the ranges for these parameters differed to a greater extent. Given the timeframe of measurement, the results for stem diameter may be most meaningfully extrapolated to fully mature plants. In the field studies, Gresta et al. (2018a) reported a mean stem diameter of 7.9 mm, ranging from 5.0-12.5 mm, and Santhosha et al. (2017) reported a mean stem diameter of 8.0 mm, ranging from 5.2 to 10.6 mm, similar to the findings of the current study. Thicker stems are associated with non-branching varieties in guar (Gresta et al., 2018a) and there was a weak negative relationship between stem diameter and branch number (*r* =-0.17) in this study. A thicker, stronger stem may better support aboveground growth and increase lodging resistance, though the negative relationship between stem diameter and branch number may create a genetic hindrance to breeding for increased stem diameter in highly branching genotypes. Relationships between stem diameter and lodging are well-established among many crop species (Kashiwagi, et al., 2008; Kujur et al., 2014; Zuber et al., 1999).

Interestingly, stem diameter in the current study was positively correlated with both the nodule number and nodule mass. This suggests there is a relationship between the size of the stem and qualities of the root system of guar that better support nodulation. Most nodule numbers and weight are found on the primary root structure of guar, near the soil surface (MacMillan et al., 2021b). Similar relationships between stem diameter and nodulation have been reported for other legumes, lending credence to this idea. Kasperbauer et al. (1984) reported the development of more nodules in plants with a larger root system in soybean. In cowpea, Dhakal et al. (2019) reported that stem diameter was positively correlated with nodule number (r=0.45, P<0.01), as well as plant height and yield. The high broad-sense heritability for stem diameter (H^2=0.74) in the current study shows stem diameter to be a readily heritable character to achieve desired breeding outcomes. Santhosha et al. (2017) reported a similar heritability for stem diameter (H^2=0.65) in guar in field conditions.

Although SLA did not statistically differ among guar genotypes in this study, the data showed how the genotypes differed in the allocation of leaf mass to leaf area and density (thickness). The SLA varied greatly (>49 cm^2 g^{-1}), was negatively correlated with total plant biomass productivity, and positively correlated with plant N concentration. A combined field and meta-analysis study involving >162 plant species found thicker/denser leaves (lower SLA) generally had lower N concentration and reported a positive linear relationship between nitrogen content and photosynthesis (Reich et al., 1998). These findings provide support to the current results. This relationship may be exploited by plant breeders to optimize potential SLA tradeoffs between leaf area production efficiency, relating to photosynthesis/light interception and N use efficiency, and leaf thickness, relating to drought tolerance. Chakraborty et al. (2011) reported that lower SLA and higher chlorophyll values were associated with drought tolerance in a screening of 10 guar genotypes. Similar relationships between SLA and drought tolerance have been reported in other crops such as peanut [*Arachis hypogaea* L.] (Falke et al., 2019), stylo species [*Stylosanthes scabra* Vogel, *Stylosanthes viscosa* (L.) Sw., *Stylosanthes hamata* (L.) Taub.] (Chandra and Bhatt, 2007), and other legumes (Lozano et al., 2019). However, the broad-sense heritability for SLA in the current study was low (H^2=0.24), indicating that breeding selection for SLA traits will require more effort.

2.6. CONCLUSIONS

This study documented substantial phenotypic variation and diverse interrelationships among measured parameters, highlighting potential avenues for breeding desired plant character traits related to N_2 fixation, morphology, and productivity. Nodule weight per plant is a useful indicator of N_2 fixation in guar, as it was moderately heritable and related to increased N_2 assimilation and plant biomass productivity. Conversely, nodule number is not a useful selection parameter for this purpose. Stem diameter may be useful as an indirect, high-throughput selection tool for increased N_2 fixation in guar, as there was a positive association between stem diameter and nodule mass. Branch number and stem diameter had wide phenotypic diversity and were highly heritable, confirming their utility in breeding selection for stakeholder needs. Reproductive development was delayed in branched genotypes, which may have implications in breeding guar for environments that differ in growing season length. Though SLA had low heritability, the wide range in SLA may be exploited by breeders to optimize drought tolerance and N use efficiency traits.

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3. DOES THE DROUGHT TOLERANCE OF GUAR [*Cyamopsis tetragonoloba* (L.) TAUB.] EXTEND BELOWGROUND TO ROOT NODULES?[*]

3.1. ABSTRACT

In cultivating guar [*Cyamopsis tetragonoloba* (L.) Taub], a drought-tolerant legume, producers anticipate soil N credits through biological N fixation (BNF), but it has been speculated that root nodulation is poor under dry conditions. To provide better insight, two greenhouse studies were conducted to investigate static (25, 50, 75, and 100% ET replacement in two soils) and dynamic (50, 75, and 100%, plus 50 and 75% relieved to 100% at 20 and 30 days after planting) water regimes on guar over 50 days. Water stress generally had the greatest negative impact on nodule weight, followed by aboveground biomass, reproductive nodes, height, then nodule number. Parameter responses were either linear or quadratic with ET replacement and response slopes differed between soils for nodule weight and biomass. The relatively minor effect on nodule number indicated the basic machinery for BNF (nodules) remained largely intact until water stress was extreme. This was likely a key factor that enabled strong and rapid recovery of nodule growth following relief of water stress. Biomass recovery was likewise robust, with production equal to the stress-free control, regardless of the intensity and duration of the stress. These

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results show that nodule growth in guar is sensitive to water stress, but the plant is resilient in maintaining nodules under water stress and recovering nodule growth upon moisture restoration.

3.2. INTRODUCTION

Commonly known as cluster bean, guar (*Cyamopsis tetragonoloba* L) is an annual warm-season legume well-known for drought, heat, and salt tolerance properties (Abidi et al., 2015; Singla et al., 2016a; Suthar et al., 2018). Guar is grown in semi-arid and arid regions around the world (Bhatt et al., 2017), with India and Pakistan being leading producers with a combined 95% global share (Gresta et al., 2014). Historically, guar was grown for food, forage, and as a green manure crop (Abidi et al., 2015). Today the primary driver of guar production is diverse uses of the ‘galactomannan’ gum or guar gum in the seed endosperm (Kalha and Anand, 2012). Among several gum products, guar gum has the advantages of being inexpensive, non-toxic, biodegradable, and renewable (Thombare et al., 2016). It is used in numerous applications as a thickener, binder, stabilizer, emulsifier, and gelling agent in many industries, including food, paper, textile, mining, pharmaceutical, cosmetic, petroleum, and others (Bhatt et al., 2017; Hasan and Abdel-Raouf, 2018; Suthar et al., 2018).

Guar is widely considered by producers to be an excellent soil-builder due to its N-fixing capacity (Singla et al., 2016b). But few studies have been reported on the biological nitrogen fixation (BNF) of guar in the literature and both producers and researchers have expressed the perception that nodulation of guar is often poor in field conditions (Abidi et al., 2015; Khandelwal and Sindhu, 2012). One report showed a variable rate of BNF in guar, with the average annual rate of BNF ranging from 34-54 kg N ha^{-1} over three years in a guar-wheat cropping system (Mubarak et al., 2015). A similar study by Buttar et al. (2009) showed that N input to a wheat crop following guar could be reduced up to 40 kg ha^{-1} while maintaining yield with inorganic N inputs. On nodulation, Brar and Singh (2017) reported observing an average of 8-10 nodules per plant in a comparison of four guar varieties in a field study. In a study by Bhardwaj (1974), only 32% of guar plants examined were nodulated (average of 4.6 nodules plant^{-1}) in a saline-alkali soil. Thapa et al. (2018) reported abundant nodulation in greenhouse-grown guar plants, with nodule physical characteristics and numbers greatly varying between two soils. The authors observed a high number of nodules with low mass per plant (497 mg/24.3 nodules) in a clay loam soil and a low number of nodules with high mass (583 mg/8.64 nodules) in a sandy soil. BNF-related functions in legumes are affected by biological, environmental, and management factors, including drought stress (Hungria and Vargas, 2000), and such factors likely played a large role in the wide variation reported in BNF and nodulation in guar.

Moisture deficits can affect any phase of the legume-*Rhizobium* symbiosis, including Rhizobia bacteria survival and multiplication in the soil; rhizobial mobility, root infection, and development; function of root nodules; and growth of the host legume (Subbarao et al., 1995). Hungria and Vargas (2000) found that water stress is a primary cause of nodulation failure, shortened nodule longevity, and lower BNF rates in legumes overall (Hungria and Vargas, 2000). Severe water stress may cause irreversible cessation of BNF (Sprent, 1971; Hungria and Vargas, 2000), although many legumes, including fababean, cowpea, groundnut, guar, and soybean, exhibit a greater ability to recover from drought than less drought-adapted species (Venkateswarlu et al., 1990; Hungrai and Vargas, 2000; Subbarao et al., 1995). In one study, guar was reported to have greater nodulation in hot and dry conditions than other legumes (Zahran, 1999). Venkateswarlu et al. (1983) reported that nodule fresh weight in guar was reduced by 64.5 and 54.4% during the vegetative and flowering periods, respectively, caused by water stress following 8 days of withholding water at 30 DAP, but the stress had no impact on nodule numbers. The authors also found a sharp decline in nitrogenase activity ($<1\mu$ C_2H_4 g^{-1} nodules) when the loss of water content in nodules exceeded 10% under water stress. An in-vitro culture study testing rhizobial isolates from guar nodules showed only 27% bacterial survival under water stress induced by a 40% polyethylene glycol solution (Mondal et al., 2017). In a pot experiment on guar including mung bean and moth bean, Rao and Venkateswarlu (1987) reported a considerable decrease in nitrogenase activity even with a slight drop of plant water potential (-0.4 MPa). Further, water stress imposed for a week-long period at 30 DAP caused a decline in nodule numbers and nodule fresh weight by 45% and 80% compared to non-stress conditions, respectively.

Reports in the literature demonstrate that nodulation, nodule function, and ultimately, BNF, in guar are negatively impacted by drought stress. Although BNF and its associated functions have been reported to be more resilient in guar than in less drought-adapted legumes, there is a perception that nodulation in guar is poor in the semi-arid conditions in which it is commonly grown. The lack of studies on the effects of water stress on nodulation/BNF in guar gives us an incomplete understanding of guar as a N-fixer, especially in dry conditions and under future projections of climate change. Our objectives in the two sets of studies reported here were to, 1) investigate the impacts of water stress on growth and nodulation parameters in guar over a broad range of static soil water regimes and, concomitantly, 2) to evaluate the post-water deficit recovery of these parameters following water restoration.

3.3. MATERIALS & METHODS

3.3.1. Experimental Design and Treatments

Two separate studies were conducted and repeated in a greenhouse at the Texas A&M AgriLife Research and Extension Center in Vernon, TX, USA. Experimental units were polythene pots of 7.6 L volume, with experimental treatments replicated four times and arranged in a completely randomized design (CRD). The first study was an evaluation of static water regimes (25, 50, 75, and 100% evapotranspiration (ET) replacement) in two soils (sandy loam and loam), including 32 pots per iteration of the study (Table 3.1). The second study was an evaluation of dynamic water conditions, in which static water regimes (50, 75, and 100% ET replacement) were compared to treatments in which water stress was relieved at different time scales (50 and 75% ET replacement treatments, relieved to 100% ET at 20 and 30 days after planting (DAP) in the sandy loam soil (Table 3.1).

Table 3.1 Treatments tested in the two studies, which were conducted in a greenhouse near Vernon, TX USA. (DAP, days after planting).

Study 1: Static Moisture	**Study 2: Dynamic Moisture**
Irrigation Regimes	***Irrigation Regimes***
25% ET Replacement	50% ET Replacement
50% ET	75% ET
75% ET	100% ET
100% ET	50% ET, relieved to 100% ET at 20 DAP
	50% ET, relieved to 100% ET at 30 DAP
Soil Types	75% ET, relieved to 100% ET at 20 DAP
Sandy loam soil	75% ET, relieved to 100% ET at 30 DAP
Loam soil	

There was a total of 28 pots per iteration of this study. The total growing period for each study was approximately 50 days. A 50-day growing period for Lewis guar falls within the First Pod or R2 growth stage (Adams et al., 2020), which has been shown in earlier studies to be a time of active nodule growth and development in guar (Venkateswarlu et al., 1983; Hinson and Adams, 2020; Thapa et al., 2018). No differences in guar phenology were observed among iterations of these studies.

3.3.2. Experimental Procedures

For both sets of studies, soils were collected from fence-line areas that had no known recent agricultural history. A Miles loamy fine sand (fine-loamy, mixed, superactive, thermic Typic Paleustalfs) was collected near Lockett, TX and was used in both studies. A Tipton loam (fine-loamy, mixed, superactive, thermic Pachic Argiustolls) was collected near Chillicothe, TX and was only used in the first study. The chemical properties of the two soils were measured by the Mehlich-III method in a commercial lab (Water's Agricultural Lab, Camilla, Georgia, USA) (Table 3.2). Fresh soil was collected one to two days before the beginning of each study.

At the start of each experiment, the 7.6 L pots were filled to a uniform level and weight of soil and the pot water-holding capacity was determined for each soil type. This was done by adding soil to the pots, thoroughly watering them, allowing them to drain for 24 hours (drainage stopped by this time), weighing them to determine differences in weight among pots, then making minor adjustments by adding or taking away soil to make them even in weight. The full water-holding capacity of the pots for the loam and sandy loam soils were approximately 27% and 23%, respectively, on a weight basis. The water treatments were administered manually using a gravimetric approach based on these water-holding capacities. First, upper weight set points were established for the 100% ET replacement pots for each soil, which was the pot weight at 85% water-holding capacity. A water-holding capacity of 85% was used, rather than at the full water-holding capacity of the pots, to prevent waterlogging in the root zone, while still allowing 100% ET replacement. Calculation of soil-specific ET replacement values for the 100% treatments was done by taking the difference between the weight set point and the average weight of the 100% ET replacement pots for each soil following periods of water use. The ET replacement in the other water treatments was calculated by multiplying treatment-specific coefficients to the water-use values determined in the 100% ET replacement treatments for each soil (e.g., 0.75 for 75% ET replacement). Watering times were based on visual observations of soil moisture loss in the upper 2.5 cm of soil in the 100% ET pots. The frequency of water application was approximately every 4 to 6 days during the early growing period, which increased over time to every 2 to 3 days as plant water demand increased. Water deficit treatments were started one week after planting.

The guar cultivar "Lewis" was used in the studies because it has been widely adopted by U.S. guar producers and is cited in international literature also (Abidi et al., 2015; Gresta et al., 2016). Four seeds were planted per pot at a depth of 13 mm, then thinned to a single plant per pot a few days after germination. Seeds were obtained from the Texas Foundation Seed Service (Vernon, TX, USA). Inoculation of the seeds was not performed, as the soils used in these studies were known from previous studies to contain native Rhizobia compatible with guar (Hinson and Adams, 2020; Thapa et al., 2018). Pots were arranged in three rows, spaced approximately 45 cm apart on greenhouse benches. For the first study (static water regimes), planting/harvesting was done on 14 May 2018 /

29 June 2018 and 18 April 2019 / 6 June 2019 for each study iteration. For the iterations of the second study (dynamic water regimes), guar was planted / harvested on 18 April 2019 / 6 June 2019 and 25 June 2019 / 12 August 2019. Phosphorus fertilizer was added to each pot as 0.3 g of Triple Super Phosphate (0-46-0) in a liquid suspension to wash it into the soil. This rate was based on a preliminary assumption of biomass production and a plant tissue P content of 0.3% (Amasaib et al., 2016). Phosphorus application was done to minimize potential P-limiting effects on root growth and nodulation.

Table 3.2 Analysis of chemical and nutritional properties for the soils used in these studies in 2018 and 2019. The soils were collected from two sites near Vernon, TX, USA. (SOM, soil organic matter).

	2018		**2019**	
Parameter	**Loam (mg kg^{-1})**	**Loamy Sand (mg kg^{-1})**	**Loam (mg kg^{-1})**	**Loamy Sand (mg kg^{-1})**
NO_3-N	2.68	4.77	0.750	10.0
NH_4-N	9.08	11.4	1.58	2.05
P	52.0	21.0	61.5	28.0
K	371	205	422	228
Mg	158	139.5	235	213
Ca	2608	874	2373	1166
S	7.50	6.50	14.5	22.0
B	0.790	0.450	0.650	0.450
Zn	1.75	1.00	1.85	2.20
Mn	154	60.5	148	64.0
Fe	41.5	57.0	88.0	60.5
Cu	1.65	0.850	3.40	2.95
pH	8.2	7.5	7.2	7.2
SOM	0.50%	0.91%	1.1%	0.68%

The cooling system in the greenhouse kept the temperature from rising above outdoor ambient levels, but was not capable of lowering the temperature below ambient levels. Soil temperature in the black pots was prevented from radiant heating above ambient temperature levels by wrapping the pots in aluminum foil, as a reflective medium for the incident solar radiation. This approach was successful in similar studies conducted in the same greenhouse (e.g., Thapa et al., 2018). The ambient air temperature was measured using an OM-92 temperature sensor and data-logger package (OMEGA Engineering, Inc., Norwalk, CT, USA) (Figure 3.1). There was no supplemental lighting in the greenhouse.

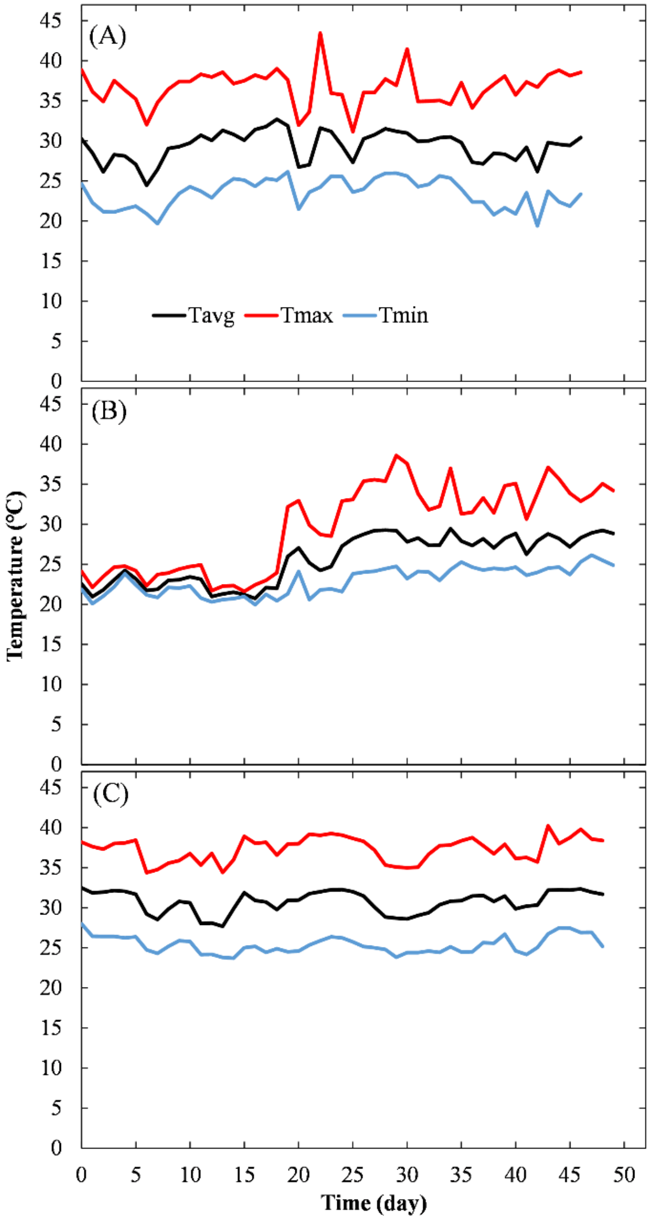

Figure 3.1 Maximum (T_{max}), average (T_{avg}), and minimum (T_{min}) daily ambient temperature inside the greenhouse near Vernon, TX USA during the studies. Data is provided for three growing periods (iterations of Study 1 and Study 2 occurred concurrently in the second period): (A) 14 May 2018 – 29 June 2018, (B) 18 April 2019 – 6 June 2019, and (C) 25 June 2019 – 12 August 2019.

3.3.3. Measurements and Data Collection

Destructive harvesting and processing of the pots occurred 50 DAP. Before harvest, the total number of main-stem branches containing flowers and/or pods were noted, and plant height measurements were taken. Guar plants were cut above the soil surface and placed into paper bags to be dried at 55ºC in an air-forced oven. Total aboveground biomass (plant productivity) was recorded after ensuring that the samples had attained a constant dry weight. The soil of each pot was then individually emptied into bins for the separation of nodules from the soil by hand sifting. Nodules were inspected at random to determine if they were active by cutting them open to observe the presence of red/pink color. Collected nodules were cleaned under running water, then dried to a constant weight in an air-forced oven at 55ºC.

3.3.4. Statistical Analysis

The data were analyzed using SAS 9.4 software (SAS Institute Inc., Cary, North Carolina, USA). For each study, the data from both study iterations (repetitions) were analyzed together. Analysis of variance (ANOVA) was carried out using the PROC GLIMMIX procedure. In the statistical model, all treatment factors (water and soil treatments, as applicable to the respective study) were considered fixed effects and iterations for the study were considered a random effect. An interaction term between the water and soil treatments was included in the model for the first study. The data were checked to ensure they satisfied the assumption of normality and equal variances using histograms, Q-Q Plots, and plots of residuals. Mean separation tests for pairwise comparison of treatments were accomplished using the Tukey method. Tests for polynomial relationships (regression analysis) on the response variables were performed using the PROC REG procedure. A probability threshold of 0.05 was used to determine statistical differences.

3.4. RESULTS

3.4.1. Static Water Regimes in Two Soils

The rates of ET over time for all ET replacement treatments (25, 50, 75, and 100%) are given in Figure 3.2, showing distinct differences in water loss and stress levels. Measured aboveground plant parameters, including the biomass, plant height, and the number of reproductive nodes per plant were negatively affected by water stress (Figure 3.3). Total shoot biomass production increased quadratically with increasing ET replacement (Figure 3.3A and Table 3.3). In comparing the soils, biomass production was greater in the loam compared to the sandy loam (11 g vs 7.8 g plant^{-1}, $P < 0.0001$). An interaction was observed between the soil and water regimes for biomass ($P = 0.0060$; Figure 3.3). The interaction occurred because biomass production was similar in both soils with severe water stress (25% ET replacement), but productivity became substantially higher in the loam soil relative to the sandy loam as the rate of ET replacement increased. The plant height increased linearly with increasing ET replacement in both soil types (Table 3.3), and there was no difference in plant height observed between the soils (Figure 3.3B). For reproductive nodes, there was a positive linear relationship with increasing water availability (P <0.0001, Table 3.2) in both soil types. The number of reproductive nodes decreased substantially under water stress, with statistical differences observed at all levels, except between 50 and 75% ET replacement for the loam soil (P <0.0001, Figure 3.3C). Reproductive nodes were greater in the loam soil compared to the sandy loam (P =

0.0118), consistent with biomass production, but there was no interaction between treatment factors.

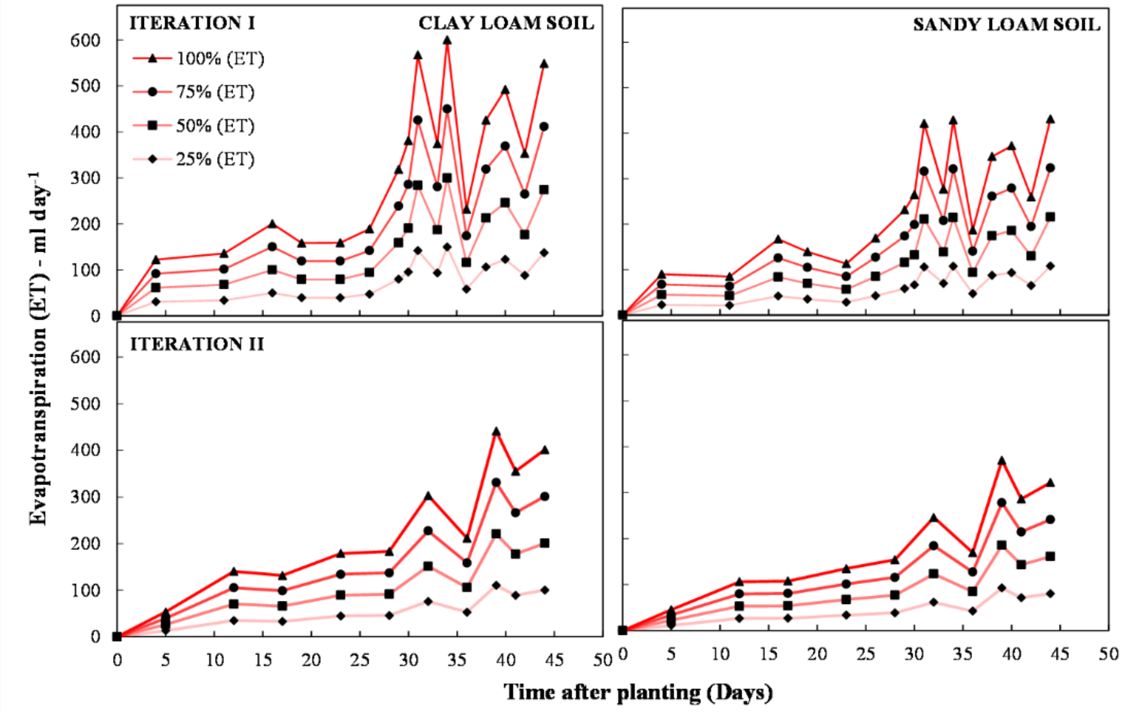

Figure 3.2 Evapotranspiration (ET) in the static moisture experiment (Study 1) at four rates of ET replacement as determined by weight measurements of the 100% ET pots.

The number of root nodules and nodule weight per plant were affected by both water and soil treatment factors. The number of nodules per plant varied little across water regimes, with the only difference being lower nodule numbers with 25% ET replacement (*P* < 0.0001, Figure 3.3D) and a quadratic relationship overall in both soils (Table 3.3). In contrast to nodule numbers, the water regimes exerted a strong impact on nodule weight per plant, with statistical differences observed at all levels of ET replacement (Figure 3.3E). The relationship between nodule weight and increasing ET replacement was positive and linear (*P* < 0.0001, in Table 3.3).

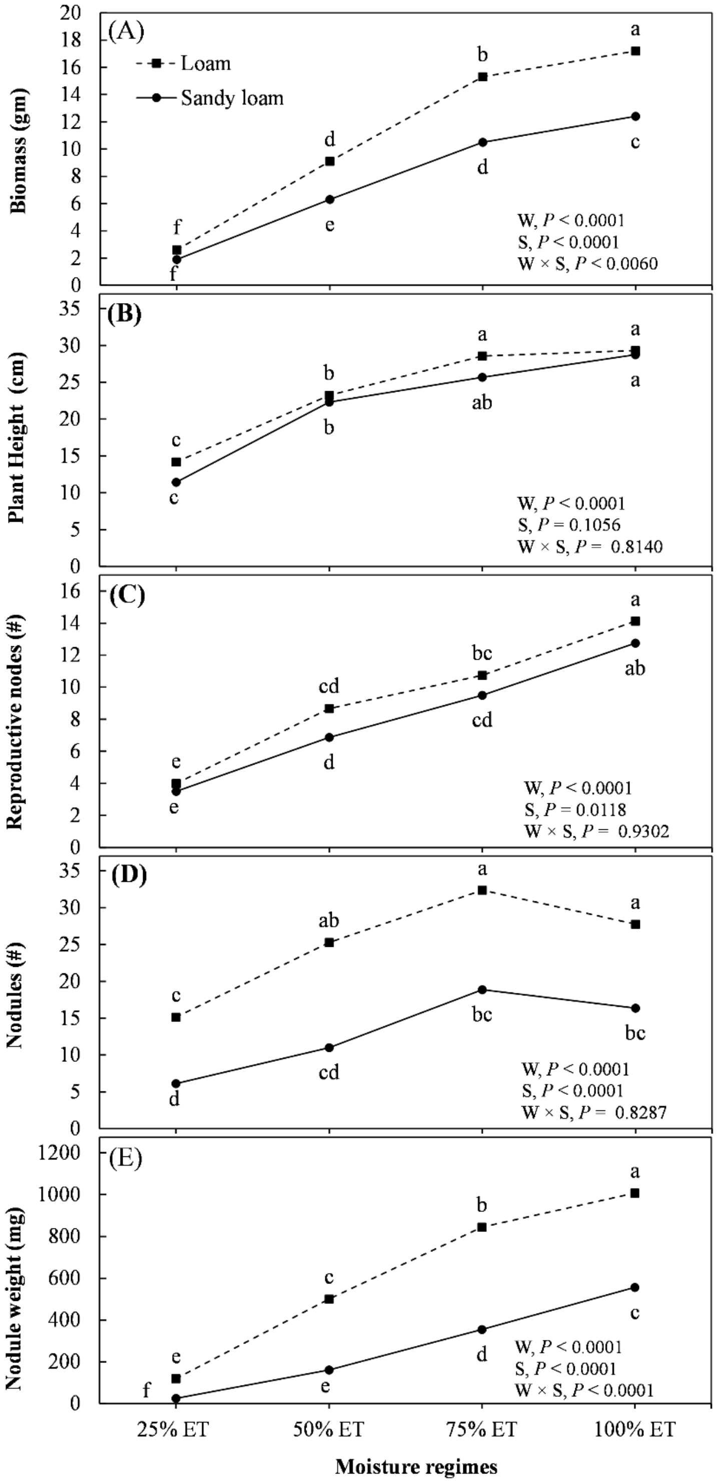

Figure 3.3 Graphical representation of effects of soil (S) and water (W) on biomass, plant height, reproductive nodes, nodule number, and nodule weight in the static moisture experiment (Study 1). For each parameter, means labeled with the same lowercase letter are not statistically different (0.05).

Both the nodule number and nodule weight were substantially higher in the loam soil than in the sandy loam (25 vs 13 nodules plant^{-1} and 618 mg vs 274 mg plant^{-1}, respectively). A strong interaction was observed between the soils and water regimes for nodule weight ($P < 0.0001$), as shown in Figure 3.3E. The nature of this interaction was consistent with the soil water interaction on plant biomass: nodule weight per plant was similar in both soils with 25% ET replacement, but nodule weight became relatively great in the loam soil as the rate of ET replacement increased.

Table 3.3 Regression analysis of data from the static water regimes experiment (Study 1).

Variable	Soil	Intercept	Coefficient (a, b)	Relationship	R^2	*P*-value
Biomass (g)	Loam	-7.25	0.43, -0.002	Quadratic	0.88	<0.0001
	Sandy loam	-4.38	0.27, -0.001	Quadratic	0.92	<0.0001
Height (cm)	Loam	10.26	0.21	Linear	0.19	0.0133
	Sandy loam	8.22	0.22	Linear	0.19	0.0118
Nodes (#)	Loam	0.53	0.15	Linear	0.71	<0.0001
	Sandy loam	0.56	0.12	Linear	0.76	<0.0001
Nodule wt. (mg)	Loam	-134.61	12.04	Linear	0.90	<0.0001
	Sandy loam	-172.75	7.155	Linear	0.89	<0.0001
Nodule no. (#)	Loam	-4.42	0.91, -0.006	Quadratic	0.25	0.0177
	Sandy loam	-5.78	0.52, -0.003	Quadratic	0.39	0.0008

Water stress negatively impacted the measured plant parameters to different extents relative to well-watered plants. Percent reduction in each parameter at each ET replacement level are given in Figure 3.4. Water stress had the greatest negative percent impact on nodule weight, followed by biomass production, reproductive nodes, plant height, and nodule number (Figure 3.4). The relative rankings for the impact of water stress on these plant parameters were generally consistent across all water stress levels tested. Interestingly, the number of root nodules per plant increased by 16% with the relatively mild water stress of 75% ET replacement, which was the only instance of a positive impact of water stress recorded.

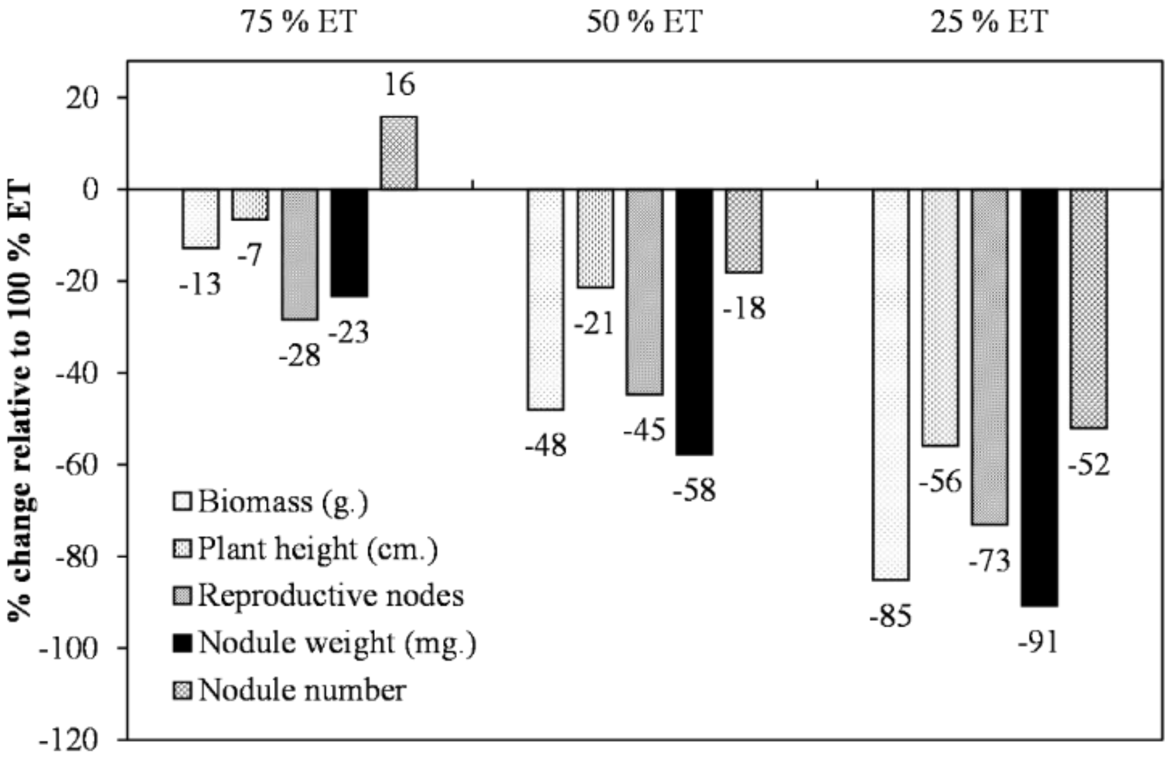

Figure 3.4 Impacts of water stress on plant biomass, height, reproductive nodes, nodule weight, and nodule number for 25, 50, and 75% ET replacement, relative to 100% ET replacement, in Study 1.

3.4.2. Dynamic Water Regimes

In this study, the effects of water stress imposed at two levels (50 and 75% ET replacement), followed by complete relief of stress at two timings, were compared to respective static water regimes. The static water regimes (50, 75, and 100% ET replacement) tested here had effects on all measured parameters consistent with those observed in the static water study described earlier (Figure 3.5; Figure 3.3). In this study, 25% ET replacement was not evaluated.

When the relatively severe water stress imposed at 50% ET replacement was relieved to 100% ET replacement, biomass production and plant height exceeded the 50% static control, irrespective of the timing of stress relief. In the relief treatments, biomass and plant height were equal to 100% ET replacement, except for plant height at 50% ET replacement relieved at 20 DAP, which exceeded the 100% control. When the relatively mild water stress imposed at 75% ET replacement was relieved, biomass again exceeded the 75% static control irrespective of the timing of relief, but plant height either equaled (relief at 20 DAP) or exceeded (relief at 30 DAP) the 75% static control, depending on the timing. In the relief treatments, biomass and plant height were equal to plants consistently given 100% ET replacement.

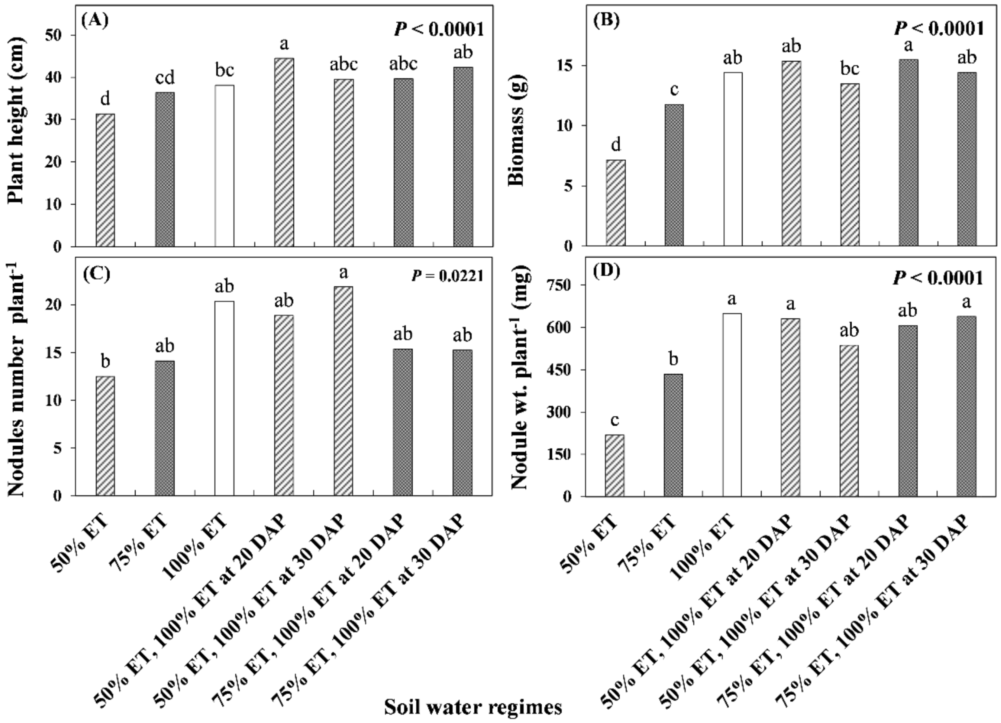

Figure 3.5 Effects of dynamic soil water regimes (Study 2) on guar, including height, biomass, nodule number, and nodule weight. The water treatments were 50%, 75%, 100% ET replacement, and 50% and 75% ET levels relieved to 100% ET replacement at 20 DAP and 30 DAP. For each parameter, means labeled with the same lowercase letter are not statistically different ($P \leq 0.05$).

Similar to the biomass parameters, when relieving the relatively severe water stress imposed at 50% ET replacement to 100% ET replacement, nodule weight exceeded the 50% static water control, irrespective of when the water stress relief occurred. Under the same conditions, nodule number recovery varied by the timing of water relief, which either equaled (relief at 20 DAP) or exceeded (relief at 30 DAP) the 50% static control. In all the water stress relief treatments, nodule weights and numbers equaled the 100% ET replacement control. When the relatively mild water stress imposed at 75% ET replacement was relieved, nodule weight either equaled (relief at 20 DAP) or exceed (relief at 30 DAP) the 75% static control depending on the relief timing. In the same conditions, nodule numbers were equal to the 75% static control, regardless of the timing of water stress relief. In the 75% relief treatments, like the 50% relief treatments, nodule weights and numbers were equal to the plants consistently given 100% ET replacement.

3.5. DISCUSSION

The stress caused by sustained deficits in soil water had significant detrimental effects on root nodulation and shoot growth responses in guar, with distinct responses among measured parameters (Study 1). Nevertheless, such effects were nullified following the relief of the water stress, showcasing the way guar can recover from water stress of various intensities and timings (Study 2). These findings both corroborate and extend the findings of previous studies on guar, as discussed below.

Among all evaluated above- and belowground plant parameters, the relative negative impact of increasing water stress was most severe on nodule weight per plant and the least severe on the number of nodules per plant (Figure 3.4; Table 3.2). There were sharp reductions in nodule weight with increasing water stress, while nodule numbers largely remained stable except with the most extreme stress. Venkateswarlu et al. (1983) and Rao and Venkateswarlu (1987) reported similar results in guar. In a comparison of several legumes (not including guar), Venkateswarlu et al. (1989) reported reduced nodule weight and a lack of change in nodule numbers in peanut under water stress, but both nodule weights and numbers declined under the same conditions in cowpea, mung bean, moth bean (Rao and Venkateswarlu, 1987). In another study, Venkateswarlu et al. (1989) reported shedding of nodules in drought conditions in cowpea. Our findings and those of others consistently show decreases in legume nodule weight with water stress, irrespective of the species, but impacts on nodule numbers seem to vary more by species, with the number of guar nodules being relatively resilient to water stress.

These results indicate that the basic machinery for BNF (nodules) largely remains intact under water stress in guar, excluding extreme stress conditions, though plant investment in nodules (nodule weight) is lowered in proportion to the level of stress. It has been reported that guar has indeterminate-type nodules (Rao and Venkateswarlu, 1987; Subbarao et al., 1995), which are more resilient and efficient in recovery from water stress than determinate types (Venkateswarlu et al., 1990, Serraj et al., 1999). Indeterminate nodules, also known as meristematic nodules, bear meristematic activity that allows prolonged growth and recovery (Sachs et al., 2018). It has also been reported that nodules formed close to the root (endodermis), as opposed to the root hairs (superficial), were better able to cope with moisture stress (Venkateswarlu et al., 1990; Subbarro et al., 1995). The bulk of nodules and nodule weight in guar are formed on the primary structural roots near the soil surface, with fewer and smaller nodules formed on lateral roots and roots hairs (unpublished data). All these findings, including the current study results showing rapid recovery of nodule weight following relief of water stress, support the conclusion that guar nodules are relatively resilient to water stress. Following relief of water stress, all plants fully recovered and produced nodule weights equal to the non-stressed 100% ET replacement control, without variation among the various intensities or timings of water stress relief tested.

In addition to physical maintenance of nodules in water stress conditions and robust recovery of nodule growth following water stress, reports in the literature indicate that nodule function in guar is also relatively resilient during water stress. Many studies have linked moisture deficits with reduced nitrogenase activity and N fixation in legumes. These studies found these functions to be more sensitive to water stress than other plant functions, such as leaf gaseous exchange/transpiration, soil N uptake, and photosynthesis (Kunert et al., 2016; Rao and Venkateswarlu, 1987; Serraj and Sinclair, 1998). Negative effects occur even with mild stress, though severe stress may cause impaired or permanent loss of nitrogenase activity, including in guar (Venkateswarlu et al., 1983; Venkateswarlu and Rao, 1987; Rao and Venkateswarlu, 1987; Venkateswarlu et al., 1989). These negative effects may be caused by a poor supply of photosynthate to the nodules, loss of leghemoglobin and regulation of nodule oxygen concentration, or accumulation of N-fixation products like ureides and amides (Venkateswarlu and Rao, 1987; Serraj and Sinclair, 1998; Kunert et al., 2016). However, Rao and Venkateswarlu (1987) reported less sensitivity of nitrogenase activity in guar compared to other legumes like mung bean and moth bean.

In the current studies, a robust recovery of nodule growth following relief of water stress was accompanied by a robust recovery of aboveground plant productivity. Relatively few studies have been conducted on the impacts of water stress on guar productivity, but even fewer have investigated guar recovery from water stress (Rao and Venkateswarlu, 1987; Venkateswarlu et al., 1983). Results from the current study show that guar rapidly and completely recovered from early-season water stress, with biomass production equal to the stress-free control, regardless of the intensity and duration of the stress. Others have reported links between biomass production, reproductive fitness, and ultimately seed yield in guar (Ibrahim et al., 2013; Loggale, 2018; Stafford and McMichael, 1991), suggesting that the observed recovery of biomass would be followed by a recovery of yield potential. Recovery of aboveground production and reproductive fitness following water stress in guar would likely depend on the phenological timing of the stress, however, as it does in other crops (Adams and Erickson, 2017; Gan et al., 2004; Liang et al., 2017).

The production of nodules, in terms of weight and number, does not perfectly reflect the potential of legumes for BNF, but nodule weight is a particularly strong indicator of plant and rhizobial fitness for hosting and conducting BNF (Heath and Tiffin, 2009; Pimratch et al., 2008; Provorov and Tikhonovich, 2003; Hardarson and Danso, 1993). Among the plant parameters measured in these studies, nodule weight was the most negatively affected by water stress and biomass production was the second most affected. Comparing these parameters in each of the static water stress treatments, the relative reduction in nodule weight was larger than the reduction in biomass production by 6%, 10%, and 10% at 25, 50, and 75% ET replacement, respectively. Rao and Venkateswarlu, (1987) also found that nodule weight was more negatively impacted by water stress than plant biomass, but the disparity between the two was drastically greater in their study (80.3% vs 37.4%, respectively), which may be explained by the approach and timing of imposing water stress. In the same study, the authors observed similar relative effects of this water stress on nodule weight and biomass in other legumes, namely mung bean (73.3% vs 46.9%, respectively) and moth bean (78.1% vs 54.0%, respectively). Studies on soybean have likewise shown plant capacity for BNF to be more sensitive to drought stress than plant growth and photosynthesis (Kirda et al., 1989; Djekoun and Planchon, 1991). The management decisions of guar producers can be informed by understanding that water stress has a greater negative influence on the capacity for BNF in guar than on biomass production, as they balance their goals for BNF and yield.

Between the two soils, measured plant parameters, including biomass, number of reproductive nodes, nodule number, and nodule weight were distinctly higher in the loam soil compared to the sandy loam (Figure 3.3). The rate of ET was likewise higher in the loam than in the sandy loam (Figure 3.2). Nutrient analysis for the soils provides a possible underlying reason for the differences observed (Table 3.2). Both soils had low levels of plant-available N that were not anticipated to suppress nodulation in guar (Hinson and Adams, 2020). But the loam soil had considerably greater levels of nearly all non-N macro- and micronutrients compared to the loamy sand, which would have allowed for greater plant productivity when combined with N inputs from BNF. The relatively high nodule numbers and weights per plant in the loam soil are consistent with this. Non-N-nutrient limitations may have been a primary factor in reducing nodule numbers and weights in the sandy loamy soil. The availability of nutrients has been reported to impact both nodulation and plant productivity in guar. Bell et al. (1989) reported the inability of the guar plant to form nodules at a Ca level below 50 µM. Barwa et al. (2017) reported that the application of P increased nodule weight up to 36% in guar. The authors also described improved uptake of other nutrients with P application, including N (12-18%) and K (8-25%). Phosphorus fertilizer was applied in the current studies, suggesting that one or more other nutrients may have been limiting in this case. There is also the possibility that another, non-nutrient soil factor was responsible for the differences.

3.6. CONCLUSIONS

Guar productivity and nodule parameters increased linearly or quadratically with an increasing rate of ET replacement. Differences in the impact of water on these parameters have implications on crop management. Because the relative reduction in nodule weight with water stress was greater than the impact on biomass, producers will need to manage their guar appropriately to meet their goals for BNF and productivity/yield. In contrast to nodule weight, water stress had mostly minor and insignificant effects on nodule numbers, until the stress was extreme. Thus, the basic machinery for BNF (nodules) remained largely intact under water stress conditions, which is likely a key factor enabling guar to recover nodule development and function following relief of water stress. Results demonstrated that, upon relief of water stress, both nodule development and biomass production strongly and rapidly recovered. Maintenance of nodules in water-stressed conditions and rapidly recovering nodule growth following relief of water stress are indicators of drought tolerance and resilience in the N fixation apparatus of guar.

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4. INTEGRATING GUAR INTO WHEAT CROPPING SYSTEMS: ASSESSMENT OF SYSTEM SUSTAINABILITY IN THE U.S. SOUTHERN GREAT PLAINS

4.1. ABSTRACT

Guar production in the U.S. is centered in the Southern Great Plains (SGP). However, the region features cotton as the predominant summer crop and wheat as the predominant winter crop, typically in monocropping systems. Guar, as a summer legume with heat and drought tolerant properties, is often restricted to a catch crop or substitute crop following cotton failures. This is despite the huge domestic market for guar gum products and the opportunity for diversifying regional cropping systems with guar, bringing ecosystem benefits like biological N fixation (BNF). To overcome the challenge, a field study (2018-2021) was conducted at Chillicothe, TX to evaluate the integration of guar into winter wheat systems under no-till dryland conditions. Treatments included integrated guar-wheat systems at 133% (WG1.3) and 200% cropping intensity (WG2), plus guar (G1) and wheat (W1) monocrops. Cumulative yield for the systems across all crops over the three-year study period was the greatest for WG2, followed by W1/WG1.3, then G1. But crop rotation sequence was critical in determining seasonal crop yields, as there were significant negative yield impacts on guar when immediately following wheat. As a result, the average seasonal yield for guar was greatest in G1/WG1.3 (1.01 /0.96 Mg ha^{-1}), followed by WG2 (0.64 Mg ha^{-1}). The seasonal yield of wheat averaged 2.31 Mg ha^{-1} across all three years, with no differences among systems. The relatively hot and dry conditions of the 2019 summer growing period were key in shaping differences among

systems. Guar growth was substantially reduced in 2019, moreso in the WG2 system, but the plant exhibited the capacity to recover from severe drought stress upon receiving timely late-season precipitation. There was no clear indirect evidence of positive impacts of guar BNF on subsequent wheat. Overall, the findings indicate that between the integrated wheat-guar systems, the intensive WG2 system has the potential for greater yield production over time, but the seasonal risk of crop failure is lower and the likelihood for a productive crop is greater for the WG1.3 system under the regional climate.

4.2. INTRODUCTION

The U.S. Southern Great Plains (SGP) region features cotton and wheat as the most important and widely grown field crops, but the region is also the primary domain for domestic cultivation of guar (USDA Quick stats, 2019). Commercial production of guar in this region dates to the early 1950s, decades-long after its initial introduction to the country in 1903 (Tripp et al., 1982). Today, U.S. guar production is centered in the Rolling Plains and High Plains of Texas, southwestern Oklahoma, and eastern New Mexico and is generally unstable. For instance, between 1997 and 2017, guar production fluctuated greatly, ranging from 1,817 to 8,269 hectares of harvested guar in Texas (USDA Quick stats, 2019). Because the U.S. is the largest consumer of guar gum products in the world, the lack of domestic guar production represents a variety of missed opportunities.

Guar is a multi-purpose summer legume with heat, drought, and salt-tolerant properties, and requires few or no cultivation inputs like irrigation, fertilizers, and other chemical inputs (Gresta et al., 2014, Abidi et al., 2015). Apart from guar seeds as major economic produce, the crop offers ecosystem benefits such as biological nitrogen fixation

(BNF), resiliency to extreme climate, and cropping system diversification. But increased guar production is largely set back by the existing regional cotton and winter wheat-based cropping systems, among several other reasons. Guar is restricted as a catch crop or substitute crop within a well-established regional cotton system following failure of cotton crop or during extensive droughts spells (Abidi et al., 2015). Cotton is the most produced crop in the region with a high potential for economic return compared to the guar. In the Great Plain region of Texas alone, cotton was planted in 1.5 million hectares in 2019 (USDA Quick Stats, 2021). Similarly, the region features winter wheat as the second-most-produced crop. In 2019, winter wheat was planted on 0.44 million hectares in the Great Plains of Texas (USDA Quick Stats, 2021). Mostly, the winter wheat system is practiced with summer fallow for replenishment of soil water (Dhuyvetter et al., 1996; Baumhardt and Salinas-Garcia, 2015), which delivers few ecosystem services.

Alternatively, guar is considered a soil-improving crop (Kalha and Anand, 2012; Mubarak et al., 2015) and fits well into rotations with other crops (Abidi et al., 2015), possibly including wheat (Rao and Northup, 2009). In the SGP region, guar and wheat would be expected to have growing season compatibility, enabling crop rotation. Guar breeders have developed relatively short-season guar cultivars (Burrow, undated). Planting of guar may be done from May to late-June and harvested by October (Abidi et al., 2015). For wheat, there is wide flexibility in planting times in this region, starting September to as late as December (NASS-USDA, 1997), which would allow guar plants to fully mature before wheat must be planted. The precipitation pattern in the region is characterized by bimodal distribution, with the wettest periods in the late spring and fall in the SGP, coinciding with the planting times of each crop (Keables, 1989; Singla et al., 2016; NASS-

USDA, 1997). Guar has relatively low water and high-temperature requirements, suited to the regional hot and dry semi-arid environment (Avola et al., 2020; Bath et al., 2020; Singh et al., 2021), that may enable it to tolerate rotation with a winter crop. For instance, guar has been reported to require about 203-254 mm of water during the growing season (Stafford and McMichael, 1991; Kalha and Anand, 2012) and relatively high optimum temperatures of 25 and 34.1℃ for reproductive and vegetative development, respectively (Baath et al., 2020). Moreover, BNF in guar has been shown to be relatively resilient to drought, with nodule growth being similar in drought sensitivity to biomass growth and with an exceptional capacity to recover nodule growth upon moisture restoration (Shrestha et al., 2021). All this information suggests that rotating wheat and guar may be feasible in dryland conditions in the SGP. Integrating guar into the winter wheat monocrop systems of the region could boost domestic guar production, reducing domestic supply deficits. Integrating the crops could also enhance provision of ecosystem services like BNF and climate change resilience, improving sustainability of the winter wheat systems that have trended downward in yield since the 1990s (Stewart et al., 2018; Shrestha et al., 2020).

Previous research related to integrating guar into wheat cropping systems is very limited worldwide and no such research has been conducted in the U.S.. Only one study involving wheat-guar rotation was found in the literature, which was conducted under irrigated conditions in the dry topics of Sudan (Mubarak et al., 2015). The authors found increased N-use efficiency and reduced soil N losses of applied inorganic fertilizers with continuous incorporation of crop residues into subsequent crops. They also reported rates of BNF ranging from 34-54 kg ha^{-1} by guar across the study period. Acharya (2000) conducted production simulations and budget analysis of guar as a rotation crop in cotton systems and reported that integration of guar has the potential to increase profitability, minimize production risk, and enhance long-term production sustainability compared to sorghum-cotton and continuous cotton systems. Studies addressing the impacts of integrating other summer legumes into wheat cropping systems in the SGP region also provide some insights, although those legumes differ from guar in multiple traits. For instance, Crabtree et al. (1987) reported reduced yields in both crops in a soybean-wheat rotation compared to the respective monocrop systems under the dryland conditions, though the double-cropped system resulted in the highest economic return. Likewise, Baath et al. (2021) reported a reduction in wheat biomass and yield in a forage soybean and winter wheat double-cropping system, which developed soil water deficits of 77-132 mm compared to a standard wheat-summer fallow system. But the authors found the yield trade-offs to be negated when summer precipitation was > 180 mm.

Previously reported research suggested that guar and wheat may be compatible as rotational crops in the SGP. Research on double cropping wheat with other summer legumes suggests that limited water availability may be the key challenge in implementing a legume-wheat cropping system of high intensity in the semi-arid dryland conditions of the region. Nonetheless, no previous studies have tested a wheat-guar system in the area. If guar is to be introduced into winter wheat cropping systems, enacting the best use of the limited water supply will have to come from an improved production system with careful considerations on crop selection, crop rotation sequence, and cropping intensity. We hypothesize that integrating guar into regional winter wheat cropping systems will increase system productivity, leading to a more sustainable system. The objective of the study was to test guar integrated into wheat cropping systems at different cropping intensities and rotational schemes, plus evaluate the impacts on individual crop and overall system yields.

4.3. MATERIALS & METHODS

4.3.1. Experimental Design and Treatments

A three-year field study was conducted at the Texas A&M AgriLife Research Center at Vernon, TX from November 2018 to November 2021. The soil at the field site is classified as a Tipton loam (fine-loamy, mixed, superactive, thermic Pachic Argiustolls) (USDA-NRCS, 2019). The experimental plots (8 × 9 m) were laid out in a randomized complete block design with four treatment replicates, giving a total of 20 plots. The treatments were cropping systems, including monocrops of guar (G1) and wheat (W1) at 100% cropping intensity, plus integrated wheat-guar systems at 133% (WG1.3) and 200% cropping intensity (WG2) (Table 4.1). There were two iterations of the WG1.3 cropping system, offset in timing, allowing guar and wheat to be represented in the system in each respective growing season. The wheat and guar monocrop systems were control treatments, acting as the baselines for comparison with the integrated systems.

Table 4.1 Cropping systems treatments tested in a three-year study period from 2018 to 2021 near Chillicothe, TX. There were two iterations of the WG1.3 treatment that were offset in time, represented by '(1)' and '(2)'. The cropping intensity, represented in terms of crops per year ("Crop/Yr."), indicates extent of land use per year relative to the wheat and guar monocrop treatments. Fallow seasons are represented by '-'.

System Description & Intensity	System ID	†W 2018	††S 2019	W 2019	S 2020	W 2020	S 2021
Wheat: 1 Crop/Yr.	W1	W	-	W	-	W	-
Guar: 1 Crop/Yr.	G1	-	G	-	G	-	G
Wheat-Guar: 1.33 Crops/Yr. (1)	WG1.3	W	-	W	G	-	G
Wheat- Guar: 1.33 Crops/Yr. (2)	WG1.3	W	G	-	G	W	-
Wheat- Guar: 2 Crops/Yr.	WG2	W	G	W	G	W	G

†W = winter season, ††S = summer season

4.3.2. Crop Management Practices

Most crop management information for all systems, including planting dates and rates, weed control measures, fertilizer application, and harvest dates, are included in Table 4.2. The table is separated into wheat, guar, summer fallow, and winter fallow subsections, each organized as a function of time. This was done because crop management was uniform across systems containing the same component (i.e., wheat or guar or summer/winter fallow) for any given growing season. All cropping systems were managed with no soil tillage and dryland moisture conditions. The row spacing was 0.25 m for wheat and 0.51 m for guar. The wheat variety “TAM 114” and guar variety “Lewis” were planted consistently in all systems, as applicable, over the course of the study. A conservative approach was taken to N fertilizer management in wheat to prevent masking effects of biological nitrogen fixation (BNF) from guar on the subsequent wheat crop. Similarly, N fertilizer was not applied to guar to maximize potential BNF activity (Hinson and Adams, 2019). Other fertilizers such as triple superphosphate (46% P), zinc sulfate (35% Zn, 16.5% S), and elemental sulfur (90% S) were applied at 126 kg ha^{-1}, 73 kg ha^{-1}, 12.5 kg ha^{-1} to all experimental plots at the start of guar growing period in 2019, respectively, based on the soil tests recommendations (Water’s Agricultural Lab, Camilla, GA, USA). All fertilizer applications were made using a hand-pushed fertilizer spreader (Model 75902, The Scotts Company LLC, CA, USA) after calibration to the desired rates. Fertilizer was applied just prior to predicted precipitation events. Herbicide applications were each done by using a backpack sprayer in all crop seasons.

Table 4.2 Management practice information during the three-year wheat-guar study period at Chillicothe, TX.

Crop / Year	Seeding rate, planting date	Fertilizer applied, rate, date	Herbicide applied, rate, date	Harvest date
Wheat				
2018/2019	67 kg ha^{-1}, planted 11/8/2018	Urea (46-0-0) 129 kg ha^{-1}, applied 2/18/2019	Pre-plant: Paraquat 1.5 L ha^{-1}, applied 10/22/2019. MCPA Ester 2.1 L ha^{-1}, applied 3/26/2019	6/11/2019
2019/2020	67 kg ha^{-1}, planted 10/26/2019	Urea (46-0-0) 73 kg ha^{-1}, applied 3/12/2020	Pre-Plant: Paraquat 1.5 L ha^{-1}, applied 10/22/2019	5/28/2020
2020/2021	67 kg ha^{-1}, planted 11/16/2020	Urea (46-0-0) 132 kg ha^{-1}, applied 3/6/2020	-	6/4/2021
Winter fallow				
2018/2019	-	-	Paraquat 1.5 L ha^{-1}, applied 10/22/2019 Glyphosate 1.8 L ha^{-1}, applied 2/18/2019 MCPA Ester 2.1 L ha^{-1}, applied 3/26/2019 Note: Spot spraying.	-
2019/2020	-	-	Paraquat, 1.5 L ha^{-1}, applied 10/22/2019	-
2020/2021	-	-	Glyphosate 2.1 L ha^{-1}, applied early-winter period Date: N/A	-
Guar				
2019	9 kg ha^{-1}, planted 6/25/2019		Pre-plant: Paraquat 3.7 L ha^{-1} and Glyphosate, 2.3 L ha^{-1}, applied 6/20/2019	10/14/2019
2020	9 kg ha^{-1}, planted 6/25/2020	-	Pre-plant: Paraquat 4.7 L ha^{-1} applied 6/4/2020. Paraquat 3.4 L ha^{-1}, Glyphosate, 2.3 L ha^{-1} applied 6/12/2020	Shredded 11/16/2020
2021	9 kg ha^{-1}, planted 6/4/2021	-	Pre-plant: Carfentrazone-ethyl 0.1 L ha^{-1}, Glyphosate, 2.3 L ha^{-1} applied 6/4/2021	Shredded 11/1/2021
Summer fallow				
2019	-	-	Paraquat 3.7 L ha^{-1} and Glyphosate 2.3 L ha^{-1}, applied 6/20/2019	-
2020	-	-	Paraquat 4.7 L ha^{-1} applied 6/4/2020. Paraquat 3.4 L ha^{-1}, Glyphosate, 2.3 L ha^{-1} applied 6/12/2020 Paraquat 2.3 L ha^{-1}, applied late summer/early fall, Date: N/A	-
2021	-	-	Carfentrazone-ethyl 0.1 L ha^{-1}, Glyphosate 2.3 L ha^{-1} applied 6/4/2021	-

4.3.3. Measurements and Data Collection

Data on weather parameters (maximum and minimum air temperatures, and precipitation) were obtained from a weather station (Campbell Scientific, Logan, UT) located < 500 m from the study site (Figure 4.1).

In-season data on plant growth parameters were collected for guar through regular observations and measurements over each growing period and at physiological maturity. Data were recorded on approximately 2-week intervals from emergence to leaf senescence for total aboveground plant biomass (Mg ha^{-1}), biomass components (leaf, stem, and reproductive mass, each in Mg ha^{-1}), plant height (cm), and plant or stand density (millions of plants ha^{-1}).

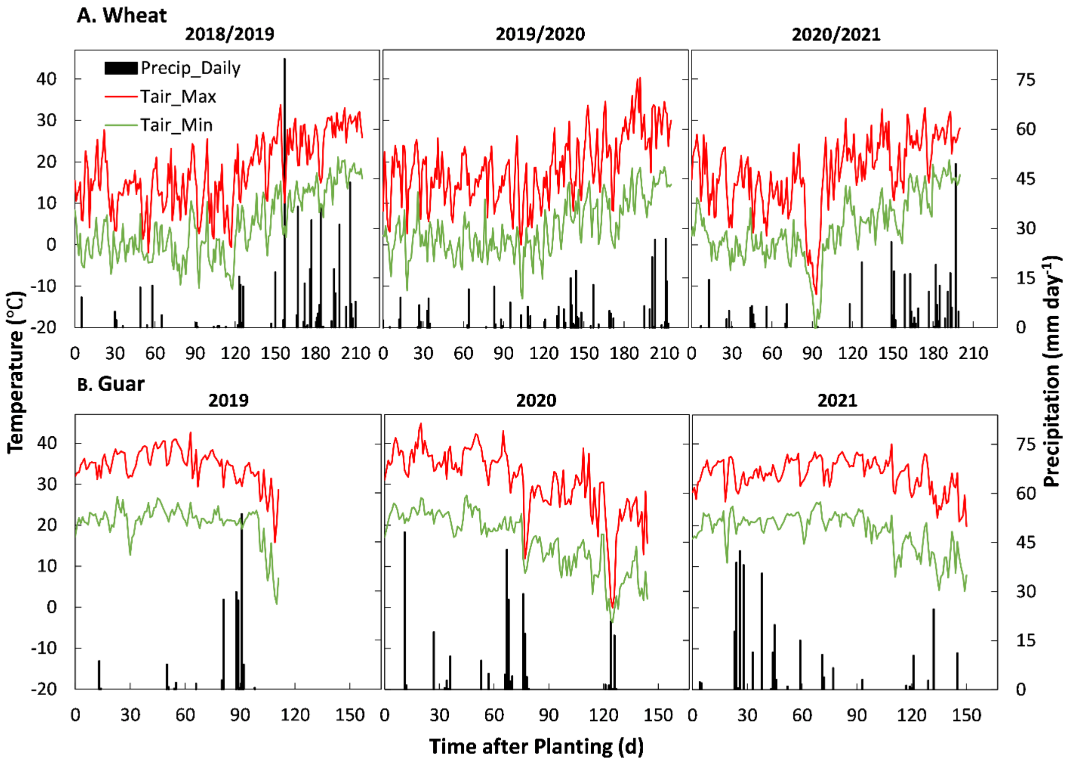

Figure 4.1 Daily air temperature (maximum and minimum) and daily precipitation during the study period, at Chillicothe, TX. from Fall 2018 through Summer 2021, separated into wheat (A) and guar (B) cropping seasons.

A 1-m length (which averaged 6 plants) of a representative row of guar was collected at each sampling date and partitioned into biomass components, which were placed into separate paper bags and dried to constant weight in a forced-air oven at 65°C. For the plant height measurements, three representative plants were tagged early in each growing period and were consistently measured over time. The crop growth rates for total biomass and the biomass components were computed by taking the maximum parameter values per unit area for each growing season period, divided by the time (days after planting) taken to reach the peak. Qualitative observations, such as the timing of critical developmental stages (i.e., R1, First Flower; R3, First Seed or Full Pod; R5, First Maturity; and R7, Harvest Maturity) were recorded according to the growth staging system established by Adams et al. (2020).

Grain yield measurements were made at physiological maturity by sampling a representative 1-m row length for guar and a single plot-long sweep (1.3 m × 9 m) of a small-plot combine for wheat. The pods were removed from the guar plants, then dried at 65°C to a constant weight, and threshed for seed yield and 1000-grain weight measurements. For wheat, five representative plants were collected from the plots to determine harvest index (HI), 1000-grain weight, grain N, and plant N content. The plants from each plot were combined into a single sample and dried at 65°C to a constant weight. Total sample weights were collected, then the heads were clipped, threshed, and grain weight was determined. The HI was calculated as the ratio of grain weight to total plant biomass. The 1000-grain weight was determined by manual counting of 250 seeds, then weighing and multiplying by four. Lastly, the grain and dry matter samples from the five-plant samples were ground to 1-mm particle size using Thomas-Wiley Laboratory Mill

(Arthur H. Thomas Company, Philadelphia, PA, USA) for determining grain and plant N content. The analysis was done by combustion analysis in a commercial laboratory (Water's Agricultural Lab, Camilla, GA, USA).

4.3.4. Statistical Analysis

Most data were analyzed using the SAS 9.4 software (SAS Institute Inc., Cary, North Carolina, USA). Additionally, the in-season time-series data (i.e., plant height, biomass partitioning, and plant density) was presented in graphical format with error bars representing the standard deviation. In all statistical models, cropping system treatments were considered as fixed effects, while year and/or block were considered random effects, as applicable to each analysis done. A probability threshold of $\alpha = 0.05$ was used to determine statistical differences. Pairwise comparison of treatment means for statistical differences was accomplished using Tukey's method. Data were checked to ensure they satisfied the assumption of normality and equal variances using histograms, Q-Q Plots, and plots of residuals. The data were analyzed in four ways: 1) statistical comparison of the performance of individual crops (i.e. guar or wheat) by cropping system in each growing season; 2) comparison of the average seasonal performance of individual crops by cropping system over the entire three-year study period; 3) comparison of cumulative harvested yield (wheat + guar) for each cropping system over the three-year study period; and 4) a focused comparison of the effect of crop rotation sequence, irrespective of cropping system, on subsequent crop yield. Regarding the WG1.3 treatment in analyses 1 and 2, seasonal analysis of both crops was accomplished by selecting data from the treatment iteration [i.e., WG1.3 (1) or WG1.3 (2)] that contained each crop during each growing season. In growing seasons where both treatment iterations contained the crop, the data were averaged. Detailed information for each analysis is given below.

Statistical comparisons of measured parameters for each crop were made by ANOVA using the PROC GLM procedure. Analysis of the performance of individual crops by cropping system in each growing season (analysis #1) was done using a simple one-way ANOVA (Schillinger, 2016). A split-plot in time ANOVA was used to analyze the average seasonal performance of individual crops by cropping system over the entire three-year study period (analysis #2) (Schillinger, 2016). This analysis was conceptualized with year as a subplot factor to partition the variability associated with the year effect in the ANOVA model, enabling a test of effects of the cropping system only. In the statistical model, appropriate error terms were used to test the main factor and interaction effects. For analysis of cumulative harvested yield for each cropping system over the three-year study period (analysis #3), a simple one-way ANOVA was used. The cumulative harvested yield was calculated simply as the sum of yield biomass for all crop production in each system. Data from the two iterations of the WG1.3 treatment were averaged at the block level before analysis. The analysis of the effect of crop rotation sequence, irrespective of cropping system, on subsequent crop yield (analysis #4) was done by one-way ANOVA. This included analysis of wheat after summer fallow (WAF), wheat after guar (WAG), guar after winter fallow (GAF), and guar after wheat (GAW). For this analysis, data were pooled together from all system treatments and assigned with appropriate variable terms. The size (entry numbers) of treatments were unbalanced, nonetheless, the PROC GLM procedure used for the ANOVA can handle imbalanced datasets.

4.4. RESULTS

4.4.1. Growth Characteristics of Guar

The average mean temperature and total precipitation during the guar growing periods were 27.9°C and 171 mm in 2019, 23.0°C and 266 mm in 2020, and 24.1°C and 318 mm in 2021 (Figure 4.1). The length of guar growing periods varied widely across the three-year study period (2019-2021), from 111 days in 2019, 144 days in 2020, and 150 days in 2021. The differences occurred primarily due to environmental conditions that affected the time of planting and the timing of growth-terminating frosts at the end of each growing period. For instance, in 2019, planting was delayed until June 25 due to the lack of adequate soil moisture to support germination. Relatively hot and dry conditions prevailed during the growing period. An early freeze (Oct. 13) terminated guar earlier (approximately 2-4 weeks) than in the latter two growing periods. Guar was also planted late in summer 2020 (June 25), but relatively early in 2021 (Jun. 4), however, rainfall was 1.6-1.9 times greater during those two growing periods, and there were later frosts (Oct. 25/Nov. 15).

Guar phenological timings differed among the cropping systems in 2020 and 2021, particularly during the early reproductive phases (Tables 4.3 and 4.4). The R7 stage was the only phenology data collected in 2019. The R1 and R3 growth stages generally occurred earlier for G1 than for WG1.3 and WG2. The only exception was R1, which did not differ between G1 and WG1.3 in 2020. There were no differences in the timing of either R1 or R3 between WG1.3 and WG2 in any year. The occurrence of R1 ranged between 35-46 DAP and R3 ranged between 49-73 DAP across the growing periods, with

R1 and R3 occurring about 6 to 12 days earlier in G1 than in WG1.3 and WG2. There were no differences among systems in phenological timings of R5 and R7. The occurrence of the R5 stage ranged between 76 and 105 DAP, earlier in summer 2021 and later in 2020. The range in the occurrence of the R7 stage was even wider, falling between 111 and 150 DAP across the three summer growing periods. The timeframe to R7 depended on the growing period length, with a terminal frost defining the growing period ends each year. The R7 stage occurred at fewer DAP in 2019 compared to 2020 and 2021.

The maximum plant height averaged 42, 75, and 71 cm across the cropping systems during three consecutive growing periods, respectively. There were no differences among the cropping systems, but differences occurred between the years (Tables 4.3 and 4.4). The maximum plant height was lower in 2019 than in 2020 and 2021. Plant height increased at a relatively low rate over a short period in the relatively dry 2019 growing period, but at higher rates for a longer duration in the subsequent growing season periods (Figure 4.2). The maximum total biomass averaged 3.4, 5.7, and 6.1 Mg ha^{-1} across the cropping systems in 2019, 2020, and 2021, respectively (Table 4.4). There were few differences among cropping systems in biomass production. There was a difference only between G1 and WG1.3/WG2 in 2019, with higher biomass production for G1 (Tables 4.3 and 4.4). The biomass production for G1 was higher by 59 and 77% compared to WG1.3 and WG2, respectively. The rate of biomass growth averaged 3.2, 4.9, and 6.0 g m^{-2} d^{-1} during the three consecutive growing periods (Table 4.4). Among the systems, the biomass growth rate differed only between G1 and WG2 in 2019. The biomass growth rate was 2.0 times higher for G1 than WG2 in that year (Tables 4.3 and 4.4). But there were no differences among cropping systems in biomass growth rates in 2020 and 2021.

Table 4.3 Guar growth characteristics - ANOVA results on significance tests for each growing season period and across the three-year study period conducted at Chillicothe, TX.

Parameter	**Treatment factors**	***P-value***			
		2019	**2020**	**2021**	**2019-2021**
Max. Height	System (S)	*0.930*	*0.462*	*0.145*	*0.489*
	Year (Y)	-	-	-	*<.0001*
	S×Y	-	-	-	*0.080*
Max. Leaf mass	S	0.296	0.361	0.777	*0.577*
	Y	-	-	-	*0.001*
	S×Y	-	-	-	*0.309*
Max. Stem mass	S	0.132	0.189	0.411	*0.604*
	Y	-	-	-	*<.0001*
	S×Y	-	-	-	*0.048*
Max. Reproductive mass	S	*0.009*	*0.288*	*0.056*	*0.007*
	Y	-	-	-	*0.001*
	S×Y	-	-	-	*0.240*
Max. Total Biomass	S	*0.021*	*0.217*	*0.209*	*0.056*
	Y	-	-	-	*0.0003*
	S×Y	-	-	-	*0.098*
Leaf Growth Rate	S	*0.076*	*0.088*	*0.170*	*0.116*
	Y	-	-	-	*<.0001*
	S×Y	-	-	-	*0.063*
Stem Growth Rate	S	*0.180*	*0.409*	*0.688*	*0.941*
	Y	-	-	-	*<.0001*
	S×Y	-	-	-	*0.342*
Reproductive Growth Rate	S	*0.007*	*0.347*	*0.434*	*0.026*
	Y	-	-	-	*0.002*
	S×Y	-	-	-	*0.433*
Biomass Growth Rate	S	*0.035*	*0.162*	*0.681*	*0.348*
	Y	-	-	-	*<.0001*
	S×Y	-	-	-	*0.112*
R1 Growth Stage	S	-	*0.018*	*0.005*	*0.001*
	Y	-	-	-	*0.036*
	S×Y	-	-	-	*0.262*
R3 Growth Stage	S	-	*0.005*	*0.009*	*0.003*
	Y	-	-	-	*<.0001*
	S×Y	-	-	-	*0.463*
R5 Growth Stage	S	-	*0.068*	*0.448*	*0.050*
	Y	-	-	-	*0.003*
	S×Y	-	-	-	*0.344*
R7 Growth Stage	S	-	-	-	-
	Y	-	-	-	*<.0001*
	S×Y	-	-	-	-

Note: Harvest maturity (i.e., R7 growth stage) was determined either by the natural maturation process or the growth-terminating frost occurring late in the guar growing period.

Overall, biomass production and the rate of growth were the lowest in 2019, greater in 2020, and the greatest in 2021, though there was no difference in biomass production between 2020 and 2021.

Table 4.4 Guar growth characteristics– ANOVA results with lsmeans and cropping system differences for each year and across all three years in a wheat-guar study conducted at Chillicothe, TX.

Variable	†System	Year			
		2019	2020	2021	2019-2021
Max. Leaf mass (Mg ha^{-1})	G1	1.0a*	1.3a	1.3a	1.2a
	WG1.3	0.8a	1.4a	1.4a	1.2a
	WG2	0.7a	1.2a	1.4a	1.1a
	††*Mean*	*0.8B***	*1.3A*	*1.4A*	*1.2*
Leaf Growth Rate (g m^{-2} d^{-1})	G1	1.4a	1.4a	2.3a	1.7a
	WG1.3	0.9a	1.4a	2.6a	1.6a
	WG2	0.8a	1.2a	1.9a	1.3a
	Mean	*1.0C*	*1.3B*	*2.3A*	*1.5*
Max. Stem mass (Mg ha^{-1})	G1	1.7a	2.1a	2.6a	2.1a
	WG1.3	1.0a	2.6a	3.1a	2.2a
	WG2	1.1a	2.0a	2.8a	2.0a
	Mean	*1.3C*	*2.2B*	*2.8A*	*2.1*
Stem Growth Rate (g m^{-2} d^{-1})	G1	1.8a	2.0a	2.8a	2.2a
	WG1.3	0.9a	2.4a	3.0a	2.1a
	WG2	1.1a	1.9a	3.6a	2.2a
	Mean	*1.3C*	*2.1B*	*3.1A*	*2.2*
Max. Reproductive mass (Mg ha^{-1})	G1	2.2a	3.6a	3.4a	3.1a
	WG1.3	1.1b	3.7a	3.7a	2.9a
	WG2	0.9b	2.9a	2.5a	2.1b
	Mean	*1.4B*	*3.4A*	*3.2A*	*2.7*
Reproductive Growth Rate (g m^{-2} d^{-1})	G1	2.0a	2.9a	2.8a	2.5a
	WG1.3	1.0b	3.1a	2.8a	2.3ab
	WG2	0.8b	2.3a	1.9a	1.7b
	Mean	*1.3B*	*2.7A*	*2.5A*	*2.2*
Max. Total Biomass (Mg ha^{-1})	G1	4.6a	5.7a	6.0a	5.4a
	WG1.3	2.9b	6.7a	6.8a	5.4a
	WG2	2.6b	4.8a	5.7a	4.4a
	Mean	*3.4B*	*5.7A*	*6.1A*	*5.1*
Biomass Growth Rate (g m^{-2} d^{-1})	G1	4.7a	4.8a	5.9a	5.1a
	WG1.3	2.6ab	6.0a	5.5a	4.7a
	WG2	2.3b	3.9a	6.7a	4.3a
	Mean	*3.2C*	*4.9B*	*6.0A*	*4.7*
R1 Growth Stage (DAP)	G1	-	41.0b	34.5b	37.8c
	WG1.3	-	44.5ab	40.3a	42.4b
	WG2	-	46.3a	44.5a	45.4a
	Mean	-	*43.9A*	*39.8A*	*41.8*
R3 Growth Stage (DAP)	G1	-	58.5b	48.8b	53.6b
	WG1.3	-	69.5a	57.0a	63.3a
	WG2	-	73.3a	60.3a	66.8a
	Mean	-	*67.1A*	*55.3B*	*61.2*

Variable	**†System**	**Year**			
		2019	**2020**	**2021**	**2019-2021**
R5 Growth Stage (DAP)	G1	-	90.0a	75.5a	82.8a
	WG1.3	-	102.8a	79.5a	91.1a
	WG2	-	104.8a	80.5a	92.6a
	Mean	-	*99.2A*	*78.5B*	*88.8*
R7 Growth Stage (DAP)	G1	111.0	144.0	150.0	136.0
	WG1.3	111.0	144.0	150.0	136.0
	WG2	111.0	144.0	150.0	136.0
	Mean	*111.0*	*144.0*	*150.0*	*147.0*

†G1/W1=guar/wheat mono-crop system at 100% cropping intensity, WG1.3=wheat-guar system at 133% cropping intensity, WG2 = wheat-guar system at 200% cropping intensity. ††The mean represents the average value for a given parameter across all systems in any given year. *The lowercase letters within each column for each parameter signify statistical differences among systems (α = 5%). **The uppercase letters within the rows signify statistical differences among years (α = 5%) Note: The calculations on growth rates were based on the time (DAP; days after planting) taken to reach the peak stage of each parameter, and not related to harvest time.

Among the biomass components, the maximum reproductive mass showed a trend similar to that for the maximum total biomass. The maximum reproductive mass was the greatest for G1 and lower for WG1.3 and WG2 in 2019, while there were no such differences among the cropping systems in 2020 and 2021 (Tables 4.3 and 4.4). The maximum reproductive mass measured 2.2 Mg ha^{-1} for G1 in 2019, which was 2 to 2.5 times higher than WG1.3 and WG2, respectively (Figure 4.2). Averaged across all cropping systems, the maximum reproductive mass was the lowest at 1.4 Mg ha^{-1} in 2019, and greater at 3.4 and 3.2 Mg ha^{-1} in 2020 and 2021, respectively. The reproductive growth rate among cropping systems followed the same statistical trend to that of maximum reproductive mass in each growing period and across all three growing periods. The rate of reproductive growth was the greatest at 2.0 g m^{-2} d^{-1} for G1 and lower for WG1.3 and WG2 in 2019. Contrarily, leaf and stem showed no differences in growth rate and production among the cropping systems in any of the growing season periods. However, overall growth rates and production of stem and leaf differed among the growing season periods, being the least in 2019, intermediate in 2020, and the greatest in 2021. The only exception to this was the lack of difference in leaf production in 2020 and 2021 (Tables 4.3 and 4.4).

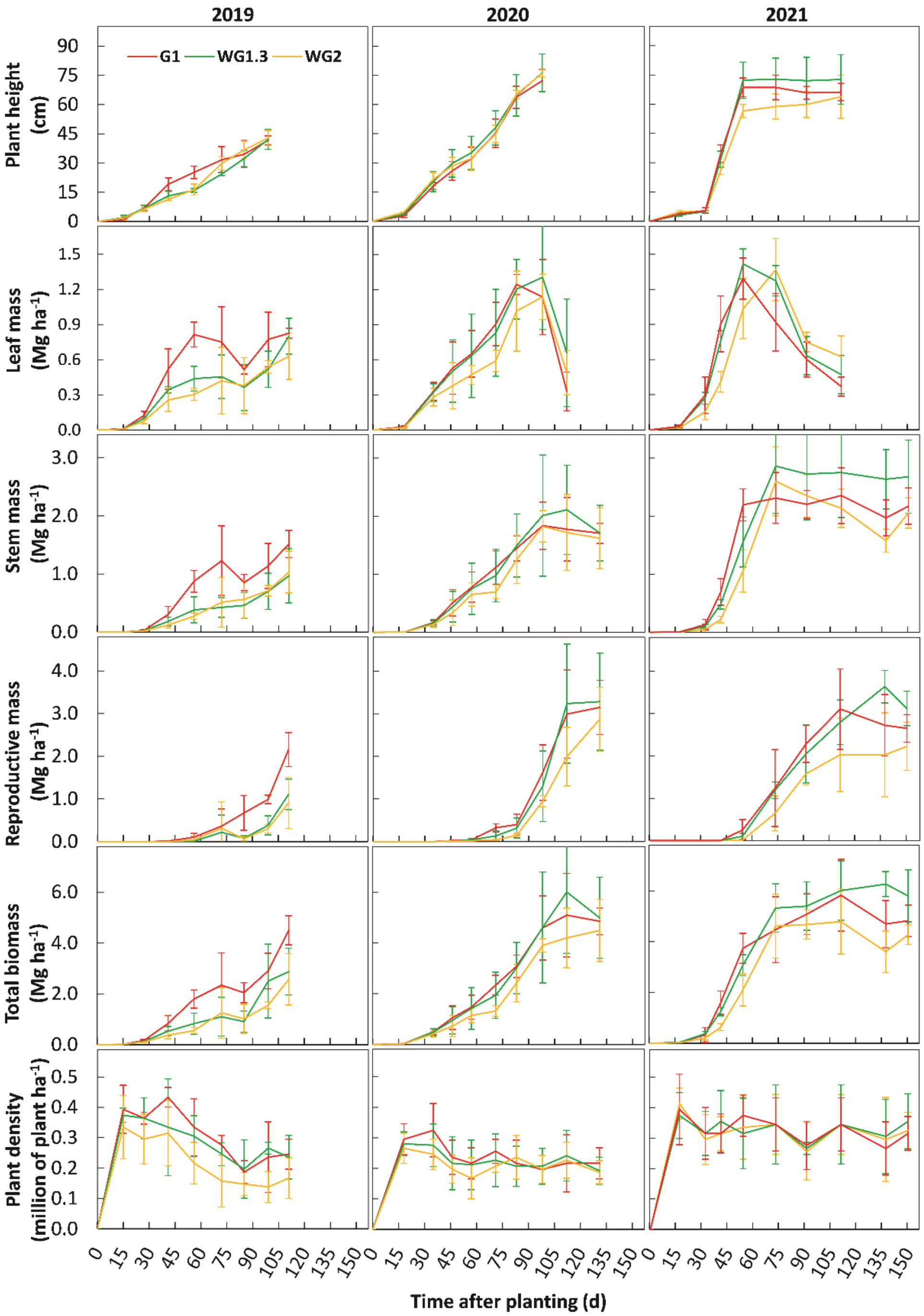

Figure 4.2 Time-series data showing the growth trends of guar in three cropping systems over three sequential summer growing season periods from 2019 to 2021 at Chillicothe, TX. The error bars represent the standard deviation of the mean.

Plant density averaged 0.27, 0.23, and 0.33 million plants ha^{-1} in 2019, 2020, and 2021, respectively. Plant density was relatively constant over the growing period in 2020 and 2021, but decreased over time in 2019 (Figure 4.2). The final plant density in 2019 was 0.22 million plants ha^{-1}, although a plant density as great as 0.37 million plants ha^{-1} was recorded earlier in the growing period.

4.4.2. Cropping Systems Effect on Growth and Yield Parameters in each Growing Season Period and across all Years

Guar yield and 1000-grain weight showed limited seasonal differences among the cropping systems, across the three growing season periods (2019 to 2021). Yield differed among systems in 2019 only with the highest yield for G1, which differed from WG2 with the lowest yield (Tables 4.5 and 4.6). The WG1.3 system had no differences from other systems, with relatively moderate yield levels. Meanwhile, the average guar yield across the three growing season periods did differ among systems. The highest average seasonal yield occurred in G1 and WG1.3, followed by WG2. Data on 1000-grain weight was not available for the 2019 growing period. The 1000-grain weight was the least in WG2 and greater in G1 and WG1.3 in 2020 (Tables 4.5 and 4.6). Overall, the average seasonal 1000-grain weight showed no differences among the systems across all growing season periods.

For wheat, measured parameters included yield, biomass, crop residue, 1000-grain weight, harvest index, plant N, and grain N content. There were few differences in the measured parameters among the cropping systems across all three growing season periods. The only parameters that differed among the systems included yield in 2019/2020, 1000-grain weight in 2020/2021, and grain N content in 2018/2019 and 2020/2021 (Tables 4.5 and 4.6). The difference in wheat yield in 2019/2020 was found between WG1.3 and

WG2, which had the greatest and the least yield, respectively. However, there was no difference in seasonal wheat yield among the systems when analyzed across all three growing season periods. The yield was relatively great in the 2018/2019 growing period compared to the two subsequent growing season periods (3.10 *vs* 2.08 *vs* 1.74 Mg ha^{-1}, respectively).

Table 4.5 Yield and associated parameters for guar and wheat – ANOVA results on significance tests of main factors for each growing period and across the three-year study period conducted at Chillicothe, TX.

Parameter	**Treatment factors**	***P*-value**			
Guar		**2019**	**2020**	**2021**	**2019-2021**
Yield	System (S)	*0.025*	*0.369*	*0.098*	*0.016*
	Year (Y)	-	-	-	*0.002*
	S×Y	-	-	-	*0.755*
1000-Grain Wt.	S		*0.017*	*0.149*	*0.091*
	Y	-	-	-	*0.008*
	S×Y	-	-	-	*0.022*
Wheat		**2018/2019**	**2019/2020**	**2020/2021**	**2018-2021**
Yield	S	*0.530*	*0.037*	*0.518*	*0.888*
	Y	-	-	-	*0.008*
	S×Y	-	-	-	*0.224*
1000-Grain Wt.	S	*0.248*	*0.082*	*0.003*	*0.001*
	Y	-	-	-	*0.001*
	S×Y	-	-	-	*0.027*
Biomass Residue	S	*0.601*	*0.944*	*0.305*	*0.722*
	Y	-	-	-	*0.637*
	S×Y	-	-	-	*0.193*
Harvest Index	S	*0.917*	*0.369*	*0.096*	*0.559*
	Y	-	-	-	*0.001*
	S×Y	-	-	-	*0.093*
Plant N	S	*0.906*	*0.837*	*0.690*	*0.953*
	Y	-	-	-	*0.002*
	S×Y	-	-	-	*0.879*
Grain N	S	*0.059*	*0.269*	*0.027*	*0.001*
	Y	-	-	-	*0.003*
	S×Y	-	-	-	*0.011*

The 1000-grain weight was lower for WG1 than WG1.3/WG2 in 2020/2021. This parameter also showed a similar statistical trend for system averages across the three growing season periods. Likewise, the grain N content was the least for W1 in 2020/2021, which differed only from WG1.3. On the contrary, the grain N content was the greatest in W1 in 2018/2019, which differed from WG2. Overall, the average seasonal grain N content was the greatest for WG1.3, followed by W1, and the least for the WG2 across the growing season periods. Other wheat parameters, including total biomass production, plant N content, residue biomass, and HI showed no differences among the systems in any growing period or across the three-year study period.

Table 4.6 System performances on individual crop yields- ANOVA results showing lsmeans and cropping system differences for each growing season period and across all three years study period conducted at Chillicothe, TX.

Crop - Variables	†System	Year			
Guar		**2019**	**2020**	**2021**	**2019-2021**
Yield (Mg ha^{-1})	G1	0.61a**	1.06a	1.36a	1.01a
	WG1.3	0.35ab	0.98a	1.56a	0.96a
	WG2	0.16b	0.67a	1.09a	0.64b
	††*Mean*	*0.37C****	*0.90B*	*1.34A*	*0.87*
1000-Grain Wt. (g)	G1	-	29.70a	35.00a	32.35a
	WG1.3	-	29.20a	36.50a	32.85a
	WG2	-	25.80b	36.25a	31.03a
	Mean		*28.23B*	*35.92A*	*32.10*
Wheat		**2018/2019**	**2019/2020**	**2020/2021**	**2018-2021**
Yield (Mg ha^{-1})	W1	3.04a	2.19ab	1.51a	2.25a
	WG1.3	2.93a	2.25a	1.76a	2.31a
	WG2	3.35a	1.81b	1.94a	2.37a
	Mean	*3.10A*	*2.08B*	*1.74B*	*2.31*
1000-Grain Wt. (g)	W1	23.30a	28.13a	31.13b	27.52b
	WG1.3	25.25a	30.00a	37.25a	30.83a
	WG2	26.20a	26.38a	37.25a	29.94a
	Mean	*24.92B*	*28.17B*	*35.21A*	*29.43*
Biomass Residue (Mg ha^{-1})	W1	4.32a	4.38a	3.29a	4.00a
	WG1.3	4.16a	4.27a	4.75a	4.39a
	WG2	4.78a	4.23a	4.14a	4.38a
	Mean	*4.42A*	*4.29A*	*4.06A*	*4.26*

Crop - Variables	**†System**	**Year**			
Wheat		**2018/2019**	**2019/2020**	**2020/2021**	**2018-2021**
Harvest Index (HI)	W1	0.41a	0.34a	0.32a	0.35a
	WG1.3	0.41a	0.35a	0.27a	0.34a
	WG2	0.41a	0.31a	0.32a	0.35a
	Mean	*0.41A*	*0.33B*	*0.30B*	*0.30*
Plant N (%)	W1	0.83a	0.64a	0.80a	0.75a
	WG1.3	0.80a	0.60a	0.87a	0.76a
	WG2	0.82a	0.62a	0.80a	0.75a
	Mean	*0.81A*	*0.62B*	*0.82A*	*0.75*
Grain N (%)	W1	2.14a	1.82a	1.88b	1.95b
	WG1.3	2.00ab	1.73a	2.31a	2.01a
	WG2	1.88b	1.72a	2.02ab	1.87c
	Mean	*2.00A*	*1.75B*	*2.07A*	*1.94*

†G1/W1=guar/wheat mono-crop system at 100% cropping intensity, WG1.3=wheat-guar system at 133% cropping intensity, WG2 = wheat-guar system at 200% cropping intensity. ††The mean represents the average value for a given parameter across all systems in any given year. **The lowercase letters within each column for each parameter signify statistical differences among systems ($\alpha = 5\%$). ***The uppercase letters within the rows signify statistical differences among years ($\alpha = 5\%$).

4.4.3. Effects of Crop Rotation Sequence

Crop rotation sequence had a marked impact on the yield of guar. The yield of guar after wheat (or GAW) declined substantially compared to guar yield after winter fallow (or GAF). This difference was consistently observed in all three summer growing season periods (Table 4.7). The relative reduction in guar yields for GAW compared to GAF was 59% in 2019, 41% in 2020, and 25% in 2021. The average seasonal yield was 1.08 Mg ha^{-1} for GAF and 0.67 Mg ha^{-1} for GAW, amounting to a 38% reduction in yield for GAW overall. There was less effect of crop rotation sequence on wheat yield. Across the growing season periods, there was no overall difference in yield for wheat after guar (or WAG) and wheat after summer fallow or WAF (Table 4.7). The average seasonal yield was 1.86 and 1.83 Mg ha^{-1} for WAF and WAG, respectively. There was, however, a seasonal reduction in yield of 19% for WAG relative to WAF in the 2019/2020 growing period, but no difference in 2020/2021.

Table 4.7 Crop sequence effects on yield for each crop (wheat/guar) in a wheat-guar study conducted at Chillicothe, TX (Fall 2018 – Summer 2021). Different letters within each column for each crop signify statistical differences between cropping sequences ($\alpha = 5\%$).

Crop - Variables	†Sequence	Yield (Mg ha^{-1})			
Guar		**2019**	**2020**	**2021**	**2019-2021**
	GAF	0.61a	1.16a	1.46a	1.08a
	GAW	0.25b	0.68b	1.09b	0.67b
	P-value	*0.017*	*0.007*	*0.049*	*0.022*
Wheat		***2018/2019**	**2019/2020**	**2020/2021**	**2018-2021**
	WAF	-	2.22a	1.51a	1.86a
	WAG	-	1.81b	1.85a	1.83a
	P-value	-	*0.008*	*0.282*	*0.814*

† GAF = guar after winter fallow, GAW= guar after wheat, WAF= wheat after summer fallow, WAG = wheat after guar. *Data analysis excludes the wheat-growing period (2019) referring to the starting phase of the study.

4.4.4. Cumulative Yield by Cropping Systems

Cumulative harvested yield differed among the cropping systems for each crop over the three-year study period (Table 4.8). There were up to three harvests for each crop, as applicable to treatments systems, and as few as two harvests for each crop. The cumulative yield for guar was the highest for G1 and the lowest in WG2, both with an equal number of harvests. Between WG1.3 and WG2, despite having two and three harvests, respectively, the cumulative guar yields were not different. Overall, these results indicate a positive effect of soil water stored over the winter on guar yields in G1 and even for WG1.3, while WG2 likely confronted greater water deficits. As described earlier, guar yields suffered when immediately following wheat. For wheat, however, the highest cumulative yield was found for WG2 and W1, each with three harvests. The cumulative wheat yield for WG1.3, with just two harvests, was equivalent to the W1 system. Overall, these results showed no negative impact of guar integrated into the cropping systems on subsequent wheat yield, irrespective of the cropping intensity. The combined yield of both crops (wheat + guar) was the greatest for WG2 [guar = 1.92 Mg ha^{-1} + wheat = 7.10 Mg ha^{-1}], followed by WG1.3 [guar = 1.92 Mg ha^{-1} + wheat = 4.94 Mg ha^{-1}] and W1 [wheat = 6.74 Mg ha^{-1}], then G1 [guar = 3.02 Mg ha^{-1}].

Table 4.8 Cumulative yield for each crop by cropping systems across the three-year study (2018-2021) conducted at Chillicothe, TX. Different lowercase letters within each column signify statistical differences between systems (α = 5%).

†System	**Cumulative yield (Mg ha^{-1})**	
	Guar	**Wheat**
G1 (3 crops)	3.02a	-
W1 (3 crops)	-	6.74ab
WG1.3 (2 crops each)	1.92b	4.94b
WG2 (3 crops each)	1.92b	7.10a
P-value	*0.0036*	*0.0366*

†G1/W1=guar/wheat mono-crop system at 100% cropping intensity, WG1.3=wheat-guar system at 133% cropping intensity, WG2 = wheat-guar system at 200% cropping intensity.

4.5. DISCUSSION

The results of this study shed light on the agronomic and environmental feasibility of integrated wheat-guar cropping systems in the SGP region. The cropping system effects on each crop plus system level, including crop rotation sequence and guar-legume effects in the system, showcased underlying benefits and limitations of the tested systems. These topics are discussed in detail below.

Environmental conditions during the growing periods greatly influenced the growth and phenology of guar, particularly limited precipitation, and high temperatures, which are common climatic features of the SGP region. The duration of the growing period and phenological development in guar were strongly impacted by prevailing environmental conditions ranging widely among the years from 111-150 days. The hot and dry conditions in summer 2019 (Figure 1B) delayed guar planting until late June, followed by the limited precipitation (171 mm) distributed unevenly over the growing period, which resulted in a short growing period (111 days) with a reduced yield. Singla et al. (2016b) reported similar findings of relatively short growing periods (92 days) with early maturity for guar planted in mid-June under relatively high early-growing period temperatures compared to late April/mid-May plantings (98-105 days) in southern New Mexico. The duration of the growing period in 2019 was also shortened by a relatively early frost that year compared to the subsequent guar growing season periods. Comparatively, extended growth periods of 144 to 150 days occurred in the following two summer growing season periods, with cooler temperatures and higher rainfall. However, wet growing conditions delayed planting until June 25 in the 2020 growing period, which resulted in late phenological developments and lower yields compared to the 2021 growing period (Table 4; Figure 1). In the Rolling Plains of Texas, Rogers (1973) reported faster reproductive growth but delayed developmental timings in the late-planted guar (July 5), with a decline in yield up to 54% compared to guar planted four weeks earlier. The findings indicated that planting times and water availability greatly influenced the developmental timings and duration of growing periods on guar.

As discussed previously, the summer of 2019 presented the harshest environmental conditions for guar production. The overall growth rates and other measured growth parameters declined in those relatively dry and hot conditions (Table 4). The in-season trends in plant growth were largely driven by total precipitation and precipitation distribution patterns in each growing period (Figures 1 and 2). The biomass productivity in 2019 decreased 60 and 56% compared to the subsequent two growing season periods, respectively (Table 4). Likewise, yield in 2019 declined to 41 and 28% compared to 2020 and 2021. Singla et al. (2016a) found similar results on late-planted (early/late July) guar with yield declined 73 and 46% compared to early planted (mid-June) guar yield, characterized with higher precipitation during the growing period. These findings indicated that prolonged dry and hot periods, which can occur in established guar production regions like the SGP, are detrimental to guar growth and yield functions, despite the drought and heat tolerance of the crop (Abidi et al., 2015; Gresta et al., 2014).

The dry and hot environmental conditions negatively affected guar growth and yield, but there was also evidence of the capacity of guar to recover from drought in the early-growing period. For instance, despite extended hot and water-limited conditions during summer 2019, biomass growth (especially reproductive growth) spiked following precipitation events late (~90 DAP) in the growing period (Figures 1 and 2). Consistent with these observations, guar has been reported to have excellent growth recovery potential upon restoration in soil moisture, irrespective of the moisture stress intensity and recovery timings during the first 50 days of growth (Shrestha et al., 2021). In a wetter growing period of the current study (2020 and 2021), reproductive and total biomass growth increased at more gradual and steady rates (Figures 1 and 2). The timing of precipitation, including the amount of precipitation occurring late in the growing period, may be the critical factor in overcoming early growing period drought and/or a successful harvest of improved yield. For instance, in the 2020 summer growing period, late growing period precipitation occurred later (~120 DAP), which promoted plant growth with an outburst in new pod settings, but these pods failed to reach physiological maturity before the onset of frost. Adams et al. (2021), reported a positive impact on yield when late-growing period precipitation occurred early enough (~100 DAP) for the pods to be set and attain physiological maturity before frost. The authors reported dryland guar yields ranging from 65-91% of irrigated yields, depending on the variety, following timely late-growing period precipitation despite minimal rainfall early in the growing period.

There were few differences in guar growth and yield parameters among cropping systems during the relatively wet 2020 and 2021 growing periods, though there were differences among systems in the dry and hot growing conditions of 2019. In 2019, there were differences in total biomass, reproductive biomass, and yield. The values for these parameters were greater for the G1 system compared to guar in rotation with wheat (i.e., WG1.3 and WG2). However, guar yields in WG1.3 were statistically equivalent to both G1 and WG2 systems. These results indicate there were positive impacts of stored soil moisture from the preceding winter fallow period in G1 on guar yield and biomass productivity. There may have also been less depletion of soil water in the less intensive WG1.3, positively impacting guar yield, but it was not enough to differentiate the yield from WG2. Stafford and McMichael (1991) and Kalha and Anand (2012) reported that guar needs about 203-254 mm of precipitation for production under dryland conditions. Divergence of the cropping systems in growth and yield in 2019 likely occurred because of the low precipitation in that growing period (171 mm). Nevertheless, averaged across all three growing season periods of the trial, guar yields were equivalent for the G1 (1.01 Mg ha^{-1}) and WG1.3 (0.96 Mg ha^{-1}) systems, while the yields were greatly reduced in WG2

(0.64 Mg ha^{-1}). For the intensive WG2 system, consistently high summer precipitation would likely be needed to overcome soil water deficits and produce seasonal yields equivalent to the other systems.

The seasonal guar yields ranged from as low as 0.16 Mg ha^{-1} for WG2 in 2019 to as high as 1.56 Mg ha^{-1} for WG1.3 in 2021. This is relatively close to the yield range (0.4 – 1.9 Mg ha^{-1}) stated as typical for guar in dryland conditions by Forbes and Beck (2013) of West Texas Guar Inc., who have extensive experience cultivating and contracting guar in the SGP region. They stated that greater yields can be expected with irrigation, up to about 5 Mg ha^{-1} under "ideal" conditions. Guar yield data reported in the scientific literature supports these statements. In dryland conditions of SGP, Abidi et al. (2015) reported yield ranges from 0.4 -1.5 Mg ha^{-1}, which is close to the findings in this study. Relatively low yields ranging from 0.10-0.76 Mg ha^{-1} were reported for guar mostly grown under dryland condition in India between 2000 to 2017 (APEDA, 2019). Studies under irrigated conditions, have also reported relatively low average guar yield of 0.9 Mg ha^{-1} (Stafford, 1987), 1.4 Mg ha^{-1} (Trostle, 2001), and 1.5 Mg ha^{-1} (Abidi et al., 2015). However, other studies worldwide have shown much greater guar yields up to 2.9-5.3 Mg ha^{-1} under irrigated and/or more favorable growing environments depending on genotypes and other management factors. (Meftahizadeh and Hatami, 2021; Mahdipour-Afra et al., 2021; Gresta et al., 2019; Avola et al., 2020; Ibrahim et al., 2016; Adams et al., 2020).

Compared to guar, there were few differences among cropping systems in the growth and yield of wheat. Among the two wheat-growing periods following rotational guar (2019/2020 and 2020/2021), differences in yield among the cropping systems occurred in 2019/2020 only. Wheat yield was the least in the WG2 system. This may be associated with the dry and hot summer conditions preceding wheat in 2020, which compounded the soil water deficits in this more intensive system. Additionally, the relatively low precipitation amounts (116 mm) from the boot stage to maturity in 2019/2020 compared to 210 mm in 2020/2021 may have exacerbated the yield differences linked to the WG2 system, despite no differences in the total biomass productivity (Figure 1; Table 6). However, wheat yields were still similar among the cropping systems in 2020/2021 and, across the three growing periods overall, there were no differences among systems in wheat yields. These results contrast with the results of other wheat-legume cropping systems studies in the SGP region. Using other legumes like forage soybean, these studies have generally shown reduced wheat yields in the integrated systems, frequently associated with moisture deficits (Bath et al., 2021; Nielsen and Vigil, 2005; Crabtree et al., 1987). The results suggest that, relative to other legumes, guar has little or no negative effects on the subsequent wheat associated with water use, except in the drier growing periods. This is supported by the result of a study by Rao and Northup (2009b) who tested water use by five warm-season legumes in the SGP. They reported a smaller water deficit for guar at the end of guar season than other legumes (i.e., soybean and pigeon pea) compared to soil water in a traditional summer fallow in three out of four years. The authors concluded that guar was a promising legume as a rotational crop with wheat in the SGP.

Moreover, crop rotational sequence was revealed to have substantial impacts on crop yield, depending on the sequence (Table 8). Guar yields after wheat or GAW were greatly reduced compared to guar following winter fallow or GAF each year. However, wheat yields were less affected by crop rotation sequence, whether following summer fallow (WAF) or guar (WAG). These results highlight the practical challenges of integrating and intensifying wheat and guar in cropping systems, concerned with the transition from wheat to guar. These results further indicate that the WG2 system comes with greater production risks than WG1.3, which confronted the wheat-to-guar transition less frequently.

In addition to seasonal crop yields, cumulative yield (Wheat + Guar) was investigated across all growing periods (2018-2021). The greatest cumulative yield was recorded for the intensive WG2 system, followed by WG1.3 and W1, then G1 (Table 4.8). A fundamental basis for these differences lies in differences between wheat and guar in germplasm improvement, plus the cropping intensity of each system. There has been only a limited guar breeding effort in the U.S. and worldwide. In the U.S., for example, most guar cultivars were released >30 years ago (Abidi et al., 2015). In contrast, wheat has received tremendous breeding effort in the SGP region and worldwide that has improved water use efficiency (WUE) and yield (Sadras and Lawson, 2013; Condon, 2004; Xue et al., 2014). Sadras and Lawson (2013) reported that WUE in wheat has increased at a linear rate of 0.12 to 24 kg ha^{-1} mm^{-1} from early 1990 to 2010. The results of the current study indicate a much greater WUE and yield for wheat compared to the guar. The WG2 system had greater production risks in the long-run, particularly for guar. Average seasonal guar yields were lower in WG2 and persistent water deficits would pose a greater risk of crop failure in the system in drier growing periods. The WG1.3 system produced seasonal yields equivalent to the respective monocrop systems, as discussed earlier.

Beyond the effects of integrating guar and wheat on system yield, there was no clear evidence of the positive effects of guar BNF on subsequent wheat. For instance, the grain N content across all wheat growing periods for WG2 was low compared to the W1 system. Grain N content in the WG1.3 system was greater overall than in the W1 system, but this was likely an artifact of troubled germination and poor growth establishment of the monocrop wheat in 2020/2021 (Table 6). The plant N content averaged 0.75% overall, with no differences among the cropping systems in each growing period or across all years. A similar observation was found for the 1000-grain weight of wheat, except for low values for W1 in 2020/2021, which again was likely an artifact of delayed germination. The results generally indicate there were no marked BNF benefits of guar on subsequent wheat yield and quality in the conditions of this study. Nevertheless, such benefits are possible in wheat-guar systems. Mubarak et al. (2015) reported rates of BNF in guar ranging from 17-54 kg ha^{-1} over six growing seasons in an irrigated guar-wheat system in the dry tropics of Sudan characterized by a low mean annual rainfall of 160 mm and high summer temperature (40°C). The reported BNF benefits in that study likely corresponded to the use of inoculated guar seeds (ENRI 16 inoculum) and favorable moisture conditions, since the system was irrigated. Likewise, Buttar et al. (2009) reported that the use of fertilizer N for wheat could be reduced up to 40 kg N ha^{-1} by including guar in the system. In that study, N and P fertilizer application at the time of sowing guar, plus irrigation supplement, likely promoted N fixation. The conditions in these studies are known to promote nodulation and BNF in guar and other legumes (Barwa et al., 2017; Arayangkoon et al., 1990; Shrestha et al., 2021). In contrast, poor nodulation and BNF functions can be caused by water stress, high temperatures, and lack of compatible *Rhizobium* strains, among others (Venkateswarlu et al., 1983; Mondal et al., 2017; Thapa et al., 2018; Khandelwal and Sindhu, 2012).

Water stress was likely a dominant factor inhibiting guar BNF in this study, especially in more intensive systems and/or when guar followed the wheat immediately. Studies on legumes, including guar, have highlighted the negative impacts of water stress on nodule formation (Subbarao et al., 1995; Mondal et al., 2017; Hungria and Vargas, 2000). Reductions in nodule weight and BNF in guar were reported under water stress conditions, with a sharp decline in nitrogenase activity (Venkateswarlu et al., 1983; Rao and Venkateswarlu, 1987). In a recent study evaluating the drought tolerance of guar in growth and nodulation, water stress showed the greatest negative impact on nodule growth, closely followed by biomass production (Shrestha et al., 2021). The authors reported declines in nodule weight of 58-91% with moderate to severe stress levels compared to non-stressed conditions. In various writings, researchers and producers have expressed the perception that nodulation in guar is poor under field conditions (Abidi et al., 2015; Khandelwal and Sindhu, 2012). For example, a study (Brar and Singh; 2017) in India found an average of 12 nodules and nodule weight of 43 mg per plant across four guar varieties with the application of *Rhizobium* and phosphorus solubilizing bacteria, but they found fewer nodules and lower nodule weight without such applications. MacMillan et al. (2021) revealed that there are almost no commercial guar rhizobia inoculants available in the world, including the U.S., which is a roadblock to getting BNF from guar in production settings. MacMillan et al. (2021) did identify some publically available rhizobia strains that were effective in increasing nodule weight in guar. The lack of a commercially available *Rhizobium* inoculant to use in this trial potentially constrained the establishment of the symbiotic relationships and BNF functions, although the local soil was known to have native rhizobia that can nodulate guar (Thapa et al., 2018).

4.6. CONCLUSIONS

Environmental conditions during the growing periods, particularly precipitation and distribution patterns, largely determined the plant stand, growth, length of the growing period, and yield of guar. Negative impacts on these parameters were observed, especially under extended dry and hot growing conditions. But guar also exhibited drought recovery capacity with timely late-growing season precipitation. If late-growing season precipitation comes too late, however, new pods may fail to reach physiological maturity before the onset of frost. Average seasonal guar yields were the least in the intensive WG2 system, and greater in the WG1.3 and G1 systems, indicative of water limitation and increased production risk in the WG2 system. Crop rotational sequence was critical in determining seasonal crop yields, as there were significant negative yield impacts on guar when immediately following the wheat, but little such impact occurred on wheat immediately after guar. Although the WG2 system had the greatest three-year cumulative yield (wheat + guar), the seasonal risk of poor production or crop failure must be considered before adoption of a system of this intensity in the regional climate. The WG1.3 system showcased lower risk, with relative ease in crop sequence transitions, and production equal to the conventional W1 system under the SGP regional climate. There was no clear evidence of the positive BNF effect of guar on subsequent wheat, potentially related to greater water stress in more intensive systems and/or lack of rhizobia inoculant application. The lack of commercially available inoculants for guar is a roadblock to guar production worldwide.

4.7. REFERENCES

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5. CONCLUSIONS

The findings of these studies revealed novel and confirmatory insights on the potential for guar in crop improvement, cropping system diversification, and provisions of ecosystem services, such as climate resilience and production sustainability. Although the plant is negatively affected by harsh environmental conditions, guar largely exhibited a resilient growth characteristic even under the harshest environmental conditions of high temperatures and low precipitation. But the BNF benefits of guar as a legume on a subsequent crop will vary depending on the growing period conditions, management practices, and availability of effective *Rhizobium* inoculants. Nevertheless, findings indicated that guar can be successfully integrated into the winter wheat systems of the SGP region, replacing the traditional summer fallow and increasing cumulative yield for each crop and system gross yield. Thereupon, an integrated wheat-guar system could boost and stabilize U.S. guar production. Thus, in the SGP and other suitable environments worldwide, guar may be an integral component of crop production systems, imparting resilience. This may be even moreso important in the future concerning the rising temperatures and increasingly erratic precipitation events associated with climate change.