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# Individuation of Mediterranean ideotypes of guar (*Cyamopsis tetragoloba* (L.) Taub.) based on agronomic and qualitative characterization and cross pollination

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## Short Abstract

This research project aims to individuate guar (*Cyamopsis tetragonoloba* (L.) Taub.) genotypes suited to the Mediterranean environment and to mechanical harvest with valuable productive and qualitative traits. The identification and evaluation of guar genotypes has been carried out in order to explore the possibility of overcoming the main obstacles that hinder the cultivation of this species: the long biological cycle, the branching habitus, the low height of the first fertile node and the indeterminate growth. To reach this aim a collection of 68 guar genotypes, obtained from different seed banks and grown in a Mediterranean environment, has been characterized for the main morphological, biological, productive and qualitative traits. As a result, among the 68 accessions, 14 genotypes were selected on the basis of their morphological, biological, productive and qualitative traits (elevated height of the first fertile node, single stem, high seed production, short biological cycle, high galactomannan and protein content) and, in the second year, were evaluated (together with 11 genotypes of recognised agronomic value coming from a collection owned by Mediterranean University of Reggio Calabria, Department of Agraria) for the same characteristics, with a considerable improvement of the studied parameters. An interesting negative relation between galactomannan and protein content was found on the 68 genotypes. In addition, seed galactomannan accumulation was also determined at six stages during seed development linking morphological and phenological traits with the galactomannan content. Moreover, during the last two years, a forced cross pollination trial has been carried out following the procedures reported in the literature, and a total of five pods has been obtained, which seeds still need to be tested. The results of this trial lay down the foundation to start a breeding program from which to obtain an ideotype that could be a profitable crop in a sustainable cropping system within irrigated areas of the Mediterranean environment.

**Keywords:** guar, *Cyamopsis tetragonoloba*, morpho-productive characterization, galactomannan and protein content, cross pollination.

# Extended abstract

## Introduction

Guar is mainly cultivated in areas of the word such as India, Pakistan and Texas with climate characteristics quite different from the Mediterranean one, but recently its valuable performance in irrigated areas in this environment has been widely demonstrated (Sortino and Gresta, 2007; Gresta *et al*., 2013; Gresta *et al*., 2016a, Gresta *et al*., 2016b). However, appropriate genotypes suitable for this environment, able to maximize seed production, are still missing. In fact the main problems of guar are an excessive length of the crop cycle, a low insertion of the first fertile node, branching habitus, the indeterminate growth and a yield that rarely exceed 2.0-2.5 t $ha^{-1}$. A too long crop cycle create problems at harvest, since in Mediterranean environment it takes place after the first rains of late summer, which implies the blackening of the seeds and the consequent lowering of their commercial value (Liu *et al*., 2007). The low insertion of the first fertile node, together with the branching habitus, represent an obstacle for wheat combine harvester, which implies a loss of seeds when the branches are not intercepted by the cutter bar (basal branched genotypes). Same problems create the indeterminate growth with the contemporary presence of mature and immature pods. With this in mind, this research project aims to individuate guar (*Cyamopsis tetragonoloba* (L.) Taub.) genotypes suited to the Mediterranean environment and to mechanical harvest with valuable productive and qualitative traits.

## Materials and methods

**Trial 1:** Morphological, biological and productive characterization of 68 genotypes guar

The trial was carried out in Botricello (CZ) in 2014, on a sand-silt soil. Sowing of 68 genotypes coming from different seed bank was performed manually on the 10 of May on rows 1.0 m long, adopting an intra-row distance of 0.10 m and an inter-row distance of 0.50 m; therefore it has been used a density of 20 plants of $m^2$. A completely randomized design was adopted with plots of 1.5 x 1.0 m three time replicated. Harvest was carried out stepwise following the maturation of different genotypes, between 14 October and 3 November. Meteorological data were also recorded. For each genotype, at the end of the cycle, were detected: plant height, height of first fertile node, branch number, stem diameter, number of fertile nodes, number of pods per plant, number of seeds per pod, 1000 seeds weight, seed production per plant, degree of earliness. Data were submitted to ANOVA. As multiple comparison test, Scott-Knott test was applied (this data are reported as supplementary material). In order to assess simultaneously all the variables studied and identify any correlations among them, a Pearson correlation was performed. PCA analysis was also performed adopting the software StatistiXL (Roberts e Withers, Broadway-Nedlands, Western Australia).

**Trial 2**: Qualitative characterization of 68 genotypes guar

In order to evaluate the galactomannans content of guar seeds, the enzymatic determination was set up following the information reported in literature (Gresta *et al*., 2013; McCleary, 1981). Seeds of the 68 guar genotypes were milled into guar flour using a laboratory mill (Retsch Model ZM100) with a sieve of 0.5 mm diameter. The dry weight of guar flour obtained after milling was determined in a IR drier where the samples were exposed to temperature of 110 °C. The method for galactomannans determination was based on McCleary (1981) as adapted by Megazyme method "Galactomannan assay procedure" (www.megazyme.com, 20/04/2016) with the following modifications. After elimination of the raffinose series oligosaccharides by repeated ethanol precipitation, the samples of guar seed flour were resuspended in 50 mM acetate buffer, pH 4.5, and incubated for 30 min at 95–100 °C with vigorous stirring on vortex every 10 min and further incubated for another 30 min at 50 °C. This procedure is necessary to ensure complete solubilization of the galactomannans. Analyses were carried out in duplicate on each sample of seeds. Guar seeds were also analysed for their total nitrogen content (Kjeldahl method).

Seed galactomannan accumulation was also determined at six stages during the seed development of India2 genotype.

**Trial 3**: Morphological, biological, and productive evaluation of 25 guar genotypes

The trial was carried out in Bovalino (RC) in 2015 on 25 selected genotypes 14 of which selected among the original 68 grown in Botricello (CZ) for morpho-productive traits and the remaining 11 genotypes of recognised agronomic value coming from a collection owned by Department of Agraria. Sowing was performed manually on a medium textured soil, the 6 of May in rows of 1.0 m long, 0.50 m apart with an a intra-row distance of 0.10 m, it has been used a density of 20 plants of $m^2$. Seed were placed at 2-3 cm depth. A completely randomized design was adopted with plot of 1.5 x 1.0 m with three replications. Water supplies were assured with a drip system supplying a total volume of about 3.000 $m^3$ $ha^{-1}$. For each genotype, at the end of the cycle, were detected the same parameters the previous year's test: height, height of first fertile node, branch number, stem diameter, number of fertile nodes, number of pods per plant, number of seeds per pod, 1000 seeds weight, seed production per plant, degree of earliness.

**Trial 4**: Cross pollination trial of a selection of guar genotypes

A two years trial on forced cross pollination was carried out. In the first year, the cross pollination trial was carried out in greenhouse on the 25 genotypes of guar used in trial 3. Sowing was performed manually on the 19 February 2015 in styrofoam plateau to avoid errors or seed loss. The plateau were watered, and transferred to germination chamber for 48 hours at controlled temperature and humidity (30°C and 90% R.H., respectively). Once the plants germinated, the plateau were transferred into the greenhouse. The seedlings after reaching the development stage of 3-5 true leaves were transferred in pots; the transplantation was carried out manually in different dates, based on the different development of each genotype. The substrate used for the filling of pots was prepared with a mixture of sandy soil and sphagnum peat in the ratio 5: 3. Water supplies were assured with a mechanical bar sprayer. As soon as the plant reached the flowering stage we performed the cross-pollination adopting the methods reported in the literature by Chaudhary et al. (1974). Since guar is a strictly self-pollinated plant (cleistogamous), the methods is articulated into two steps: 1) emasculation and 2) pollination. Artificial hybridization in guar requires a pair of forceps, short glassine bags, a hand stapler and label tags.

In the second year the cross-pollination trial was carried out in greenhouse on 13 genotypes. Sowing was performed manually the 23 of April, 2016. Moreover, the seeds obtained from the crossings of the previous year were planted in pots, as well. For the second year of the cross-pollination trial were used the same parameters of the first year.

## Results

**Trial 1:** Morphological, biological and productive characterization of 68 guar genotypes

Biological traits

The studied genotypes completed the whole crop cycle between the half of October and the first week of November in 181.6 days, on average. From the value of the median (190 days) emerges that the great part of the genotypes have a long biological cycle. Eleven genotypes can be considered “early”, with a biological cycle between 155 and 163 days; “medium-early” genotypes (163-175 days) were 12, “medium-late” genotypes (175-184 days) were 6 and 39 were “late” genotypes, with a crop cycle that goes beyond 185 days. Earliness is probably the most important trait for the introduction of this species in the Mediterranean environment, allowing for early harvest. Contrarily, a long crop cycle does not allow mechanized harvest, since the crop is still in field when the rains of late summer season fall (September and beyond).

Biometric traits

Biometric traits were studied in order to find valuable genotypes able to reduce seed loss during mechanical harvest and increase sowing density to obtain a greater yield. Average plant height of the 68 genotypes was 59.6 cm, ranging from 34.0 cm to 97.3 cm, with a CV of 20.9%. As far as the branch number, it must be said that it is one of the most important traits to increase the sowing density and improve the mechanical harvest. Among the 68 studied genotypes, 17 genotypes resulted non-branching, while the other genotypes ranged between 2 and 11.7 branches per plant. This trait showed a very wide CV (72.9%). This trait, in fact, shows a very large variability, representing a valuable base for breeding purposes looking for non-branching or fine-branching genotypes. As far as the height of the first fertile node, which is another very important trait to reduce seed loss during mechanical harvest, it was on average 1.8 cm, with value included between 1 cm and 4 cm.

Productive traits

The cluster number (fertile nodes) on the main stem was on average 22.4 with values included between 6 and 60 and with a wide CV of 51.8%. The number of pods per plant resulted on average 122.5 with values ranging between 19.7 and 290.7 and CV of 51.7%. The number of seeds per pod, together with the 1000 seed weight, was one of the studied traits with the lowest variability (CV 12.1% and 9.7%, respectively). Plants showed an average number of seeds per pod of 6.6, with a maximum value of 8.1. The 1000 seed weight resulted on average 40.1 g with values included between 31.6 g and 48.3 g. Plant seed production was on average 29.0 g with a wide variability among genotypes (CV 53.6%) starting from 3.6 g up to 73.7 g.

**Trial 2:** Qualitative characterization of 68 guar genotypes and correlation with morpho-productive traits

Galactomannan content ranged from 19.6% up to 32.3% with an average value of 26.5%, while protein content ranged from 26.0% to 33.5% with an average value of 30.2% comparable with those reported in literature (Gresta *et al*., 2013; Kumar and Singh, 2002). As expected, coefficient of variation for this qualitative traits was very low (9.9% for galactomannan content and 4.9% for protein content) compared to those of the morphological and productive traits. An interesting association was found, for the first time, between galactomannan and protein content. The two qualitative traits show a highly significative negative relation (p=0.0002). From a physiological point of view, it suggests that seed galactomannans increase at the expense of protein content, causing competition of carbon source, as well as it has been demonstrated for carbohydrates and protein in faba bean (Gasim *et al*., 2015), for oil and protein in rapeseed (Grami *et al*., 1977; Kennedy *et al*., 2011) and in soybean (Wilcox and Shibles, 2001; Charron *et al*., 2005). This association highlights that breeding process looking for guar genotypes improved for galactomannans content will have as a result genotypes with a lower protein content.
The seed galactomannan accumulation passed from 12% at 48 days after sowing (DAS) to 26.1% at 150 DAS. Several morphological indicators (i.e. pod length, pod width, dry seed diameter and pod colour) have been identified and put in relation with galactomannan content.

Correlation

Morpho-productive characterization highlights the negative correlation between the branch number and the height of the insertion of the first fertile node. This means that branching genotypes showed a lower height of the first node with a worse harvestability. The cluster number was positively associated with branch number and negatively with the height of the first node. Obviously branching genotypes have a greater number of clusters, while the higher is the insertion of the first node, the lesser is the cluster number. Likewise, the number of pods per plant was higher in the branching genotypes and increases with the growth of cluster number. The number of seeds per pod was significantly and positively related to the number of pods per plant. The 1000 seed weight resulted negatively related with cluster number, pods per plant and with seeds per pod. Therefore, these parameters may represent the yield components to be taken into consideration as discriminatory for a breeding program to increase the seed production per plant.

**Trial 3:** Morphological, biological, and productive evaluation of 25 genotypes selected of guar

As far as the morpho-productive traits of 25 selected genotypes, the average plant height was 80.7 cm, ranging from 30.5 cm to 161.0 cm with a coefficient of variation of 32.5%. Height of the 1st node was on average 3.3 cm with a very large CV (74.1%), with values generally included between 1 cm and 5.5 cm. In these trial the majority of the studied genotypes showed a non-branching habitus (branch number =0), the most desirable habitus to increase sowing density and facilitate harvest. At harvest, the largest number of genotypes showed a degree of earliness with green stem and dried pods for less than half of the stem. The fertile nodes per plant (cluster number) on the main stem was on average 25.4 with values included between 4 and 98 with a CV of 77.9 %. Pods per plant were on average 63.8, showing a high CV (88.5%), the maximum value of 260. The average number of seeds per pod was 5.6 with a CV of 20.4% indicating a quite low amount of variability, with the highest value of 7.3. Seed production ranged between 0.5 and 56.3 g plant$^{-1}$ with the highest CV (99.7%). The 1000 seed weight was on average 36.4 g with a CV of 19.2% indicating a lower variation compared to the other parameters. Among the 25 genotypes, the 14 genotypes deriving from the original 68, showed a considerable improvement compared to these last. On average the height of the first node passed from 1.8 cm to 3.1 cm, this parameter in very important to reduce the loss of pods at the combine harvest. Number of branched passed from 5.3 to 0.1, that means that almost all the 14 genotypes selected were single-stem, a very important result to increase sowing density and facilitate harvest. Degree of earliness passed from 0.95 to 2.6 showing a better maturation at harvest. On the contrary the productive parameters, seed per pod, pods per plant and consequently also the total production per plant decreased, because the selected plants were almost all single-stem. This productive result obviously does not take in account the greater sowing density that can be obtained with single stem genotype.

**Trial 4:** Cross pollination trial of a selection of guar genotypes

As regards the first test of the cross-pollination on 25 genotypes, more than 100 attempts were carried out crossing all plants, as the flowers were ready. In the literature, it is reported a percentage of success of the hand-cross of 6-8% with pods that show number of seeds per pod below the average and shape and size of the pod smaller than usual. As far as is possible to understand from the above mentioned information, three cross-pollinations have been successful in our first year. The second year of cross-pollinations (made on 13 selected genotypes) has been used the same procedure and about the same number of attempts (100) of the first year has been performed. Two cross-pollinations have been successful. In this second year, the seeds obtained from cross-pollination of the first year were sowed to evaluate their morpho-biological characteristics. At the moment in which I am writing this thesis, the trial is still on-going and we have not quantitative results, however, from a visual point of view, the first two genotypes showed pods with intermediated traits between their parent plants, while the third cross showed a clearly determinate growth. This last genotype, if this trait will be confirmed from the future trials, can be an useful tool for breeding program looking for high-yielding determinate-growth genotypes.

# 1. Introduction

Guar (*Cyamopsis tetragonoloba* (L.) Taub.) is a summer, annual, legume crop that exhibits excellent drought resistance. It needs a hot climate, takes advantage of irrigation and fertilization, has a high tolerance to salinity and a good capability to fix atmospheric nitrogen. Guar is primarily grown as an industrial crop, but its by-product is used as ingredient for animal feeding, due to its high protein content. Its origin is not completely clear: it is commonly considered an Indian species, however, the most accepted scientific hypothesis reveals that it probably developed in Africa from a wild species named *Cyamopsis senegalensis* and was brought to India by Arab traders between the $9^{th}$ and the $13^{th}$ centuries where it has been cultivated and improved.

Guar, considered as a minor crop until the recent past, is now assuming an increasing role among industrial crops, due to the galactomannans, named gum, contained in the endosperm of its seed. Guar gum (long branching polymers of mannose and galactose) is used as thickening, gelling and suspending agent, viscosifier and emulsion stabilizer, and is used in a variety of fields such as textile, paper, painting, oil operation, drilling, civil engineering, agro-chemistry, food, cosmetics and pharmaceuticals.

Guar is best adapted to tropical and subtropical regions. Even though official international statistical data for guar is missing, nowadays, it is mainly cultivated in India (80%), Pakistan (15%) and USA (Texas and Oklahoma) (5%). In Europe, it has been grown in the past in Italy, where details were reported for late sowing in Sicily (Whyte *et al.*, 1953), but it is no longer grown in this region. Due to high drought and salinity tolerance, guar could be an alternative crop for the exploitation of the semiarid environments, and a valuable crop for Mediterranean areas, where limited water availability does not allow the cultivation of many summer crops. The adaptability of this species to the Mediterranean environments has been widely demonstrated.

Guar is an almost fully self-pollinating plant, with a 2n = 14 chromosome number. It shows an indeterminate growth, producing flowers and setting pods scalarly from about 4-6 weeks following seedling emergence until the death of the plant. Guar genotypes are characterized by a large variability, ranging from 0.4 to 3.0 m in plant height, with a growing season from 70-80 (determinate varieties) to 170-190 days (indeterminate varieties), from single stem to basal branching, etc.

In the following pages, a deepening of the main agronomic and chemical traits are reported to supply the main knowledge to the readers for a better comprehension of the experimental trials carried out in this study.

# 2. History

## 2.1 Introduction

The early history of the Old World legume *Cyamopsis tetragonoloba* (L.) Taub., now generally known as guar, is unknown. However, established records and circumstantial evidence indicate man cultivated guar in the Indo-Pakistan subcontinent for numerous generations. Until recently, guar has remained a minor crop. Now it seems destined to assume a larger role among the domesticated plants that supply the food and needs of man.

The discovery that guar seed endosperm could be source of a useful industrial gum brought this little-known crop world recognition and started it on its way to major crop prominence. Among the exigencies of World War II was a strident demand for a starch replacement in paper manufacture. The finding that guar endosperm is an even better beater additive than starch led to the use of guar in industry and initiated further study for uses of guar gum in a multitude of food and non-food applications.

Growth in the recognition of guar as a new crop for Western agriculture (Hymowitz and Matlock, 1963) has brought the legume to the point where it now can legitimately be considered a commercial cash crop (Stein and Hall, 1968) supplying industrial gums, animal feed, and, at the same time, providing a potential new source of protein to meet the staggering nutritional demands of an increasing population.

## 2.2 Common names

The vernacular name *guar* probably is derived from the Sanskrit word *go* or *gav*, meaning “cow” (Anon, 1896). Hence in northern and central India, in the languages derived from the Sanskrit such as Hindi, Marathi, Gujarati, and Punjabi, the plant is known as gavar, gawar, goor, gowaree, gouree, govar, guar, guara, guwar, and gwar (Anon, 1896; Baden-Powell, 1868; Beames, 1869; Birdwood, 1862; Dalzerll and Gibson, 1861; Dymock and Gadgil, 1883; Patil, 1921; Patwardhan and Ranganathan, 1951; Roberts and Faulkner, 1921).

In southern India, guar was known to the British as cluster bean. This vernacular name was taken directly from the translation of the Tamil name for the plant, *cottaveraykai* (Ainslie, 1813) or *kothaverai* (Rama Rao, 1941). Less common English names for C. *tetragonoloba are aconite* *bean* (Huprikar and Sohonie 1961), Calcutta lucerne (Burkill, 1935), field vetch (Ozanne, 1894), four-angled (Voelcker, 1953), and Siam bean (Burkill, 1935).

It is interesting to note that in Swahili the plant is known as *Mgwaru* (Greenway, 1940). The Swahili language is spoken by the inhabitants of the central east coast of Africa. The language was compounded from several African dialects and contains an admixture of Portuguese, English, French, German, and Indian words. The plant most probably was introduced into East Africa by the British or North Indian tradesmen in the early part of the twentieth century (Greenway, 1945).

## 2.3 Early history

The search for the time and place of domestication of guar is complicated by the fact that the plant has not been found in any Neolithic site in India (Vishnu-Mittre, 1968) and by the lack of descriptive botanical or agricultural information in old records. To further complicate the domestication issue, the species has been assumed by many writers to have been domesticated in ancient India. However, such statement which have been appeared in the literature are made without reference to earlier works. Thus, one must reconstruct the early history of *Cyamopsis tetragonoloba* by tracing the origin of the plant's common names and by attempting to correlate the origin of the uses of the plant in indigenous system of medicine and in religious and ceremonial customs with specific time periods.

The main evidence for the antiquity of *Cyamopsis tetragonoloba* in ancient India is based on the alleged Sanskrit names for the cultigen. The earliest surviving form of Sanskrit, that of the *Rg Veda*, probably was composed between 1500 and 1000 B. C. (Basham, 1959). Sanskrit names ascribed to guar and transliterated into English by Kirtikar and Basu (1933) are as follows:

| **Sanskrit** | **English translation (Monier-Williams 1956)** |
|---|---|
| Dridhabija | Having hard seed |
| Nishandhyaghni | Cures night blindness |
| Sushaka | Is a good vegetable |
| Varktashimbi | Twisted pod |
| Gorani | Beans white |
| Gorakshaphalini | Cattle beans |
| Bakuchi | No translation |

A plausible answer to the perplexing problem of why *Cyamopsis tetragonoloba* has Sanskrit names which can only be traced back to the nineteenth century was suggested by Leslie (1969).

According to him, symbolic traditionalism, defined as the effort to garb the new and foreign in the guise of the antique and indigenous, is intimately entwined in nationalistic passion. The revival of the Ayurvedic indigenous system of medicine in the nineteenth century was, in a sense, a backlash to the British system of modern medicine. Perhaps it was in this cultural milieu that Sanskrit names were ascribed to *Cyamopsis tetragonoloba.*

**2.4 Eighteen and nineteenth century history**

According to Roxburgh (1814), *Dolichos fabeaformis* (now *Cyamopsis tetragonoloba*) was first introduced to the Botanical Garden at Calcutta in 1797. Seeds were sent to Calcutta by a Dr. John from Coromandel under the Telgu name goor-c*hikurkai*. Dr. John probably was Dr. Johan Gerhard Koenig, a pupil of Linnaeus, who joined the Tranquebar Mission as a surgeon and naturalist in 1768 (Burkill, 1953; Santapau, 1958). The Tranquebar Mission was located about 250 Kilometers south of Madras. In *Flora Indica*, Roxburgh (1874) states that the plant is much cultivated in the gardens of the native on the coast of Coromandel. The young, tender legumes are used in curries and stews. Cattle are also fond of the plant.

In 1813 Ainslie (1813) recorded that in southern India the legume *D. fabaeformis* (now *Cyamopsis tetragonoloba*) was much prized by the natives as a vegetable. Chevalier (1939) noted that Commerson observed the cultigen growing in Pondecherry (south of Madras) in the eighteenth century. Curiously, neither Garcia da Orta (1913) nor Commelin's index to Rheede Tot Draakenstein's *Flora Malaborica* (Commelin, 1718) mention the cultigen in their pre-Linnaean publications on the plants of southern India.

In his *Mantissa Plantarum* (1767), Linnaeus established that *Psoralea tetragonoloba* (now *Cyamopsis tetragonoloba*) is a legume whose habitat was Arabia (Linnaeus, 1961). Linnaeus must have received seed of the cultigen rather than a plant specimen from his pupil Pehr Forsskal, who collected in Arabia from 1761 to 1763. Christensen's index to Forsskal's Flora *Aegyptiaco-Arabica* makes no reference to the cultigen (Christensen, 1922) However, the symbol "H. U." in the Linnaean manuscript indicates that seeds were grown out in the Uppsala Botanical Garden. Candolle (1825) noted that guar was from Arabia. Subsequent publications by Blatter (1919), Schwartz (1939), and Schweinfurth (1912) point to Aden where guar was cultivated. Most probably guar was brought to Aden by Indian merchants and traders from the Bombay area. Schweinfurth (1912) observed that in Arabic the vernacular name of the cultigen is *hindia*. The earliest citation seen for guar in the western part of the Indo-Pakistan subcontinent is by Linnaeus. In his *Mantissa Plantarum*, Linnaeus (1961) cites a second specimen of *P. tetragonoloba* (now *Cyamopsis tetragonoloba*) from Surat, India. The specimen most probably was collected by Henrik Braad. The first introduction of guar in the United States, came in 1903 from the Government Farm in Surat (Hymowitz and Matlock, 1963).

## 2.5 Traditional use of guar

The traditional uses of guar in the Indo-Pakistan subcontinent are as follows:

<u>Human Consumption</u>

- Immature pods are dried, salted, and preserved for future use (Huprikar and Sohonie 1961; Wiser 1955).
- Immature pods are dried and fried like potato chips.
- Green pods are cooked like French beans (Watt, 1908).
- Mature seeds are used as an emergency pulse in time of drought.

<u>Cattle Feed</u>

- Plants are cut and fed as green forage (Watt, 1908).
- Beans are boiled in a large kettle and fed to cattle as a high protein source (Watt, 1889).

<u>Medical purposes</u>

- Plants are ashed, then mixed with oil and used as a poultice on cattle boils (Indraji, 1910).
- Leaves are eaten to cure night blindness (Indraji, 1910).
- Seeds are used as a chemotherapeutic agent against smallpox.
- Boiled guar seeds, are used as poultices for the plague, enlarged livers, head swellings, amd on swellings due to broken bones (Roxburgh, 1814).
- Seeds are used as a laxative (Chopra *et al.,* 1956).

<u>Crop and soil improvement</u>

- Plants are used as shade for ginger (Watt, 1908).
- Guar commonly is used as a cover crop and green manure (Watt, 1908).

Religious Use

- Guar is not considered a sacred plant in the Bombay area (Lisboa, 1883). However, same individuals omit immature pods and seeds from their diet during their pilgrimage to the holy city, Varanasi. Only those plants believed by oral tradition and legend to be native to India are eaten by the pilgrims in Varanasi.

## 2.6 Recent history

When guar was first introduced into the United States in 1903 (Hymowitz and Matlock, 1963), the centre of crops production was located in the provinces of Bombay Presidency and Sind of British India. These areas today form a major portion of the western half of the Indo-Pakistan subcontinent. Even in its centre of crop production, guar was never planted on as large an area as the most commonly sown pulses, pigeon peas (*Cajanus cajan*) and chick peas (*Cicer arietinum*).
Mollison (1901) pointed out that since guar usually was sown as a mixed crop it actually was grown over a much wider area than the statistical figures indicate. Statistical information for guar from other areas is lacking or unreliable, in part because large regions of the subcontinent were never consolidated under British rule and remained semiautonomous until independence.
In the Unites States, the early introduction of guar were sent to federal and state experimental stations located in the Southwest, where it was believed to be suited to the soils, hot climate, and long growing season of the area (Wiser, 1955). Emphasis was placed on its use as a soil-improving legume and as an emergency forage for cattle. Since guar was not found to be outstanding, it soon fell into oblivion and remained in the United States as a crop curiosity until World War II.
The year of introduction of guar into various countries is presented in table 2.1. In some cases, the year of introduction is only an approximation, since the crop could easily have been introduced earlier by individual or government agencies without proper documentation of the date of introduction. The dates given in table 2.1 are the earliest references cited in abstract journals or in publications received by U.S. National Agricultural Library.
With the advent of World War II, the importation of locust bean (*Ceratonia siliqua*) gum from the Mediterranean was cut off, and a shortage developed. Of necessity, the Institute of Paper Chemistry at Appleton, Wisconsin, searched for domestic replacements that could be used as a wet-end additive in paper making. This is a material which, on addition to the paper beater or the

head box of a paper-making machine, will adhere to the surface of the wood fibres and cause then to stick together more firmly when they are felted together in formation of the paper sheet.

Galactomannan gums have an amazing adherence to cellulose and hence to wood fibres, and by forming a coating on the fibres, aid in cementing then together as they touch and cross each other in the laying down of the fibres in paper sheet formation. These facts were observed by Prof. Ernest Anderson (1949) who worked at the Institute of Paper Chemistry each summer when he was on leave from the University of Arizona. His main finding was that the endosperm of guar contained a useful galactomannan, present in the seed to the extent of about 50% usable material. In 1943, General Mills, Inc. undertook the milling of guar in order to use the endosperm gum in paper manufacture. Discussion of the excellent quality of guar gum in paper manufacture is described by Rowland (1945).

Examining the properties of guar gum, Whistler (1948) visualized its wide industrial potential and, among other, recommended its continuation as an industrial gum and protein source and as a new crop for the United States. Since World War II, the industrial uses of guar have increased and will continue to do so. This prospect emphasizes the need for improved genetic breeding of guar as well efforts to use the seed's protein for human nutrition.

Table 2.1 "Language, geographic site, and earliest reference to common names of *Cyamopsis tetragonoloba*"

| Country | Year | Reference |
|---|---|---|
| Argentina | 1932 | Memmler, 1932 |
| Australia | 1910 | Anon, 1911 |
| Brazil | 1950 | Costa, 1950 |
| Egypt | 1956 | Saber *et al.*, 1956 |
| Fiji | 1936 | Sampson, 1936 |
| France | 1790 | Lamarck, 1786 |
| Indonesia | 1912 | Burkill, 1935 |
| Israel | 1955 | Lachover and Plaut, 1955 |
| Italy | 1948 | Calvino, 1948 |
| Kenya | 1891 | Sacleux, 1891 |
| Malawi | 1936 | Sampson, 1936 |
| Malaysia | 1919 | Spring and Milsum, 1919 |
| Marocco | 1954 | Foury, 1954 |
| New Guinea | 1936 | Sampson, 1936 |
| People's Republic of China | 1951 | Overholt, 1951 |
| Philippines | 1911 | Piper, 1911 |
| Sierra Leone | 1958 | Hutchinson and Dalziel, 1958 |
| Singapore | 1917 | Burkill, 1935 |
| Somali Republic | 1939 | Schwartz, 1939 |
| South Africa | 1952 | Doidge, 1952 |
| Spain | 1959 | Casares and Lopez-Herrerra, 1959 |
| Sudan | 1936 | Sampson, 1936 |
| Tanzania | 1891 | Sacleux, 1891 |
| United Kingdom | 1790 | Aiton, 1812 |
| United States | 1903 | Hymowitz and Matlock, 1963 |
| Zaire | 1947 | Anon, 1948 |

# 3. Taxonomy

*Cyamopsis* DC (Candolle, 1825) (*kyamos* translates “bean” and *opsis* “resemblance to”) is an old world genus of four species. In his treatment of *Cyamopsis* for the *Flora of Tropical East Africa*, Gillett (1958) divided the genus into three species: *Cyamopsis tetragonoloba* (L.) Taub., *Cyamopsis senegalensis* Guill. And Perr., and *Cyamopsis serrata* Schinz. However, he left unsettled the question of what to do with the intermediate forms between *C. senegalensis* and *C. serrata*.

In 1960 Torre (1960) proposed that intermediate forms be recognized as a distinct species, which he named *Cyamopsis dentata* (N. E. Br.) Torre. On the other hand, Merxmuller (1970) recognizes only three species in the genus, i.e., *C. tetragonoloba, C. senegalensis, and C. serrata*. In this chapter we shall follow Gillet’s taxonomic of Cyamopsis (Table 3.1).

From a cytological point of view, Senn (1938) suggested there was a close relationship between the genus *Cyamopsis* and the genus *Indigofera.* He postulated that *Cyamopsis* (n=7) is a derived aneuploidy from *Indigofera* (n=8). Nevertheless, Gillett (1958) concluded that although *Cyamopsis* indeed was closely related to *Indigofera*, it was preferable to retain *Cyamopsis* as a distinct genus.

Table 3.1 “Chromosome number and geographic distribution of species in the genus *Cyamopsis”*

| Species | Diploid chromosome number | Distribution |
|---|---|---|
| *C. senegalensis* Guill. and Perr. | 14 | Saudi Araba, Ethiopia, Sudan, Mali, Senegal, Tanzana |
| *C. serrata* Schinz | 14 | Southwestern Africa, Botswana South Africa |
| *C. dentata* (N. E. Br.) Torre | ? | Rhodesia, Angola, Southwestern Africa, South Africa |
| *C. tetragonoloba* (L.) Taub. | 14 | Cultigen |

Cyamopsis senegalensis

The species *Cyamopsis senegalensis* (Figure 3.1) is an erect herb about 30 cm tall with profuse branching toward the base. The pink, grey, or black seeds, rhomboid in shape, weigh about 12 g per 1000 seeds. Plant of *Cyamopsis senegalensis* have been found in the semiarid savannah zone south of the Sahara from Senegal to Saudi Arabia (Gillett, 1958) on alluvium after inundation (Hutchinson and Dalziel, 1927), and on settled sandy dune during the rainy season (Chevalier, 1939). Trochain (1957) reports that 5% of the natural forage by weight near the village of Sabsabre, Senegal, was *Cyamopsis senegalensis*. The samples were taken during the month of October in 1946. Miège (1960) first reported that *Cyamopsis senegalensis* has 2*n* = 14 chromosomes. Jardin's (1967) compilation of foods used in Africa is the only publication seen that lists *Cyamopsis senegalensis* has having an economic use. According to Jardin, leaves of *Cyamopsis senegalensis* are eaten in Chad during periods of food shortage.

An extremely interesting specimen of *Cyamopsis senegalensis* was collected by Polhill and Paulo near the Ruaha River in Tanzania in 1962 (Roy and Theodore, 1979). Seeds of *Cyamopsis senegalensis* could have been carried to the region by migratory birds or the specimen may represent the remains of an isolated population.

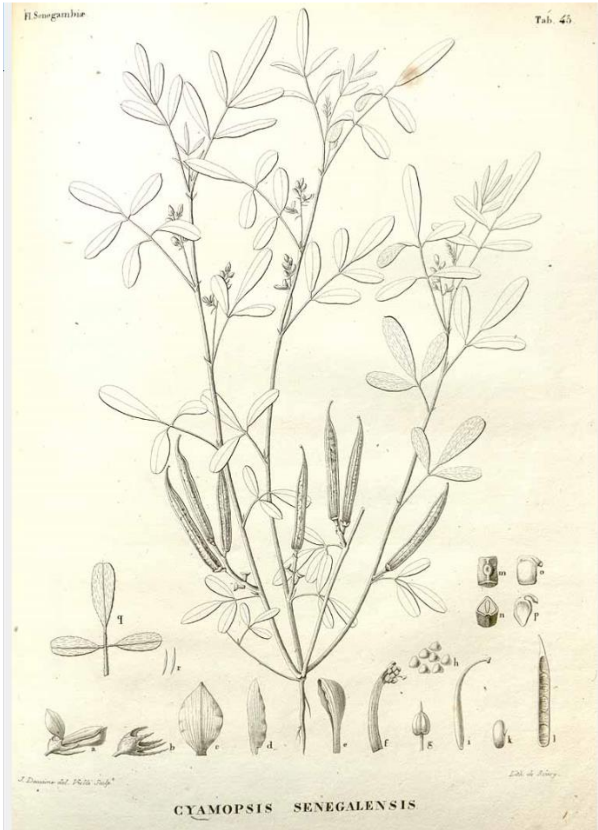


Figure 3.1 "*Cyamopsis senegalensis"*

Cyamopsis serrata and Cyamopsis dentata

Little is known about the ecology and geographical distribution of the small annual herbs *Cyamopsis serrata* and *Cyamopsis dentata*. Apparently both species are found in semiarid habitats similar to those of *Cyamopsis senegalensis.* The pink, rhomboid-shaped seeds of *Cyamopsis serrata* weight about 6 g per 1000 seeds. Plants of *Cyamopsis serrata* have been reported in the Republic of South Africa, southwest Africa, and Botswana (Boker, 1926; Frahm-

Leliveld, 1966; Gillett, 1958; Merxmuller, 1970), while *Cyamopsis dentata* has been reported in Rhodesia, Angela, and southwestern Africa (Drummond, 1972; Gillett, 1958; Torre, 1960).

Hymowitz and Upadhya (1963) demonstrated that *Cyamopsis serrata* had 2*n* = 14 chromosomes, a count confirmed by Frahm-Leliveld (1966). In addition, Frahm-Leliveld observed that there were chromosome size differences between her two *Cyamopsis serrata* specimens. The specimen from Botswana had larger chromosome dimensions that the specimen from south-western Africa. The chromosome number for *Cyamopsis dentata* is unknown. No common name or uses have been seen for either of the species.

Cyamopsis tetragonoloba

The species *Cyamopsis tetragonoloba* (Figure 3.2) is a summer annual herb that has never been found in the wild (Gillett, 1958). Ayyangar and Krishnaswamy (1933) report 2*n* = 14 chromosomes for *Cyamopsis psoralioides* (now *Cyamopsis tetragonoloba*). Other investigator have confirmed this count (Frahm-Leliveld, 1953; Sen and Vidyabhushan, 1960; Vig, 1963). No hybrids between species of Cyamopsis have been reported in the literature.

Extensive cytotaxonomic studies by Frahm-Leliveld (1960; 1962; 1966) established that 2*n* = 16 is the most common chromosome number in *Indigofera*. However, several species have chromosome number 2*n* = 14 and one of these, *Indigofera ischnoclada* (section Latestipulatae), resembles *Cyamopsis tetragonoloba* in total chromosomes length (27.3 to 31.7 µ) and shape (Frahm-Leliveld 1962). The investigations by Frahm-Leliveld reinforced the beliefs of Gillet (1958) and Seen (1938) that *Cyamopsis* is closely related to *Indigofera*.

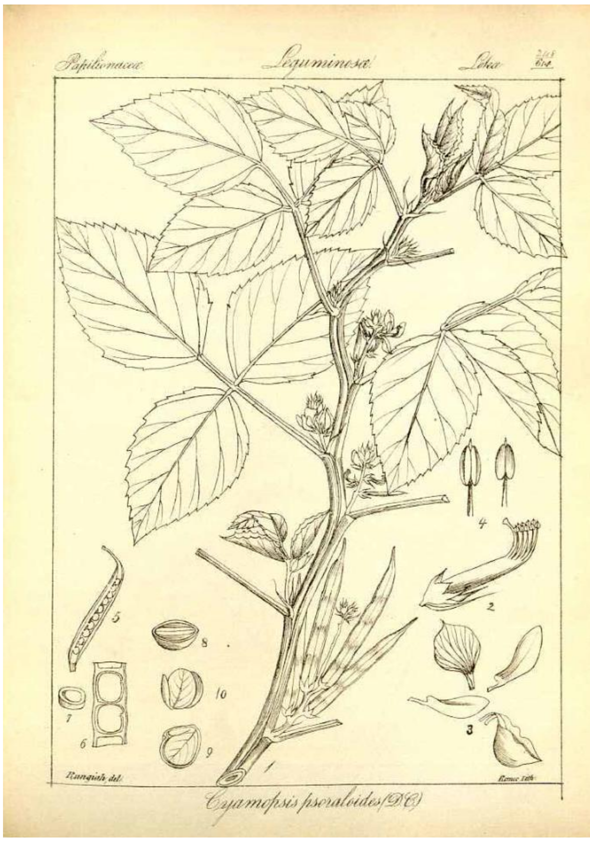

Figure 3.2 "*Cyamopsis psoralioides*"

# 4. Origin of *Cyamopsis tetragonoloba*

The history of guar has been considered in the previous chapter, but a brief discussion of the origin of *Cyamopsis tetragonoloba* seems pertinent here. Vavilov (1951) attempted to determine the centre of origin of domesticated crops by locating the area in which the greatest diversity occurred for a particular crop. One of his independent geographical regions in which plants were first domesticated is the Indian Centre of Origin.

Among the pulses that Vavilov considered to be of Indian origin was *Cyamopsis psoralioides* (now *Cyamopsis tetragonoloba*), as well as *Cajanus cajan*, *Delichos lablab* (now *Lablab purpureus* [L.] Sweet), *Vigna sinensis* (now *Vigna unguiculata* [L.] Walp.), and *Trigonella foenum-groecum.* However, the latter three pulses mentioned appear to be of African origin. Therefore, the major criticism of Vavilov's concept is that minimized time, space, and cultural diffusion.

For the pulses, Vavilov overestimated the contribution of the Indian flora to the development of Indian agriculture and highly underestimated the importance of the African flora. For example, Chablani (1951) comments that Sind was the first province to receive the onset of Islam where the Arabic invaded it in 712 A.D. The Arabs introduced into the region several important crops, such as the date palm, fig, and pomegranate.

Even prior to the Arab invasion of India, early trade routes between north-western India and the eastern coast of Africa area mentioned in the *Periplus of the Erythraean Sea*, which was probably written around 100 A.D. (Freeman-Grenville, 1962). Historically, it appears that the dry regions of the Indo-Pakistan subcontinent developed a derivative agriculture and that either by trade or through conquest its food stuffs came from elsewhere.

It is unlikely the cultigen is indigenous to the north-eastern area of the Indian subcontinent (Long, 1859). As late as 1905, Prain (1903; 1905) noted that the cultigen occasionally was cultivated in the Bengal region and that the most common name for the plant was guar. Today, in Bengal, guar pods are eaten mainly by Indians, who trace their ancestry to western or southern India. In addition, a vernacular name of the plant in the Calcutta area is *bilati sim*, which translated into English means “foreign bean” (Mukerji, 1915).

The ecological conditions under which guar thrives suggest that southern India is not the native area for the cultigen. Guar, like the other three species in the genus *Cyamopsis*, flourishes in hot, dry climates such as in the south-western United States and north-western India. Under high soil moisture conditions or drizzly weather with high humidity, *Alternaria brassicae, Rhizoctonia solani*, and *Fusarium coereleum* cause appreciable damage to the cultigen (Singh,1953; Streets, 1948). It seems reasonable, then, that the focus on the domestication of guar should be concentrated toward the drier regions of western Asia.

From an economic and social viewpoint, Chablani (1951) suggests that Sind does not appear to have been particularly distinguished for the cultivation of fruits, vegetable, or flowers. According to Chablani, there is not authoritative account of the agricultural life and produce of the country during the days if the Moghuls and Kalhoras. These people were not interested in agriculture but rather in industry and trade. Therefore, it is most unfortunate that until the arrival of the British in the nineteenth century, little is known about the agriculture of Sind.

After analysing the flora of British India, Hooker and Thomson (1855) roughly estimated that 90% of the Sind vegetation consist of plants indigenous to Africa, of which about 50% are common to Nubian or Egyptian plants. Biwas (1943) concurs with Hooker and Thomson that Sind is poor in endemic floral content. He goes one step further, however, in stating that this is true also for the desert regions of Rajputana and the dry regions of Baluchistan. Concerning the flora a little further southward, Gray (1886) speculates that most of the cultivated Papilionaceae in the Bombay Presidency appear to be indigenous to Egypt and Abyssinia.

In Chapter I it was pointed out that guar has not been found in any Neolithic sites in India, that the use of Sanskrit names to ascribe antiquity to the cultigen is questioned, and that a Tamil name for the cultigen perhaps can be traced back to the thirteenth to fifteen century A. D. In addition, guar has an unusual acreage and use distribution throughout the subcontinent. It is cultivated as a field crop in the dry regions of north-western India and the adjacent areas of western Pakistan and as a kitchen garden crop in peninsular southern India. Occasionally it is cultivated along the coastal regions connecting the two area.

The dilemma of when and where guar was adapted to the needs of man; how, when, and by whom it was disseminated; and lastly, whether or not the distribution of the cultigen took place in prehistory or within the modern era cannot be answered definitively due to the absence of concrete evidence.

It is possible that wild *Cyamopsis tetragonoloba* forms still exist in the dry regions of Africa, Arabia, and or the Indo-Pakistan subcontinent but have not been discovered or that, if such forms did exist, they are now extinct. A report wild *Cyamopsis tetragonoloba* in Tanzania appears to be nothing more than an escape from cultivation (Roy and Theodore, 1979).

The probability that guar was domesticated very early in Africa or Arabia and then made its way to the Indo-Pakistan subcontinent is remote. Guar is not used in tribal or ceremonial customs in Africa. The name of the cultigen in Arabic is *hindia,* suggesting an Indian origin. As indicated in table 2.1, guar is a fairly recent import to Africa.

The use of the trans-domestication concept to explain the origin of guar was proposed by Hymowitz (1972). The trans-domestication concept is defined as the movement by man of a wild species from its indigenous area to another region where it subsequently is domesticated. Guar is given as an example of trans-domesticate.

According to Hymowitz, a drought-tolerant legume, *Cyamopsis senegalensis*, found in the semiarid savannah zone south of the Sahara from Senegal to Saudi Arabia, arrived on the Indo-Pakistan subcontinent as flotsam in Arab-India trade. One the major items of trade between the Arabs and Indians were horses. Basham (1959) states that horses would not breed well in the Deccan and "were regularly imported by sea from Sind, Persia and Arabia to the ports of western India". Sastri (1966) points out that Marco Polo noted that ships came to India from Hormos, Aden, and all Arabia, laden with horses for trade. The unfavourable climate of southern India and ignorance of Indian horse-keepers necessitated large annual imports of fresh animals.

The Arabs must have taken on board their ships huge quantities of fodder to feed the horses while on the seas and perhaps sold any remaining fodder to the Indians. Plants of *Cyamopsis senegalensis* probably were cut and taken along on the ships as fodder. Since the climatic conditions in the dry regions of the Indo-Pakistan subcontinent are favourable to *Cyamopsis senegalnsis*, any seed brought along by the Arabs which germinated could have easily formed the basis for the development through selection of what is today *Cyamopsis tetragonoloba*. The arrival of *Cyamopsis senegalensis* on the subcontinent probably was sometime between the ninth to thirteenth century A. D. Extensive trade took place between the Arabs and the Indians during this period.

The unusual distribution and use of guar in north-western India and southern India, according to Hymowitz, also is due to the influence of the Arabs. Maqbul Ahmed (1960) states that "the majority of Arab accounts of India up to the time of al-Biruni (1048 A. D.) dealt with either the north-western regions or the southern peninsula. The reason for this is probably that the Arabs of Sind were always at loggerheads with the neighbouring princes belonging to the Gujara-Pratihara dynasty. Hence, they had little opportunity for social or cultural contact with northern or central India. On the other hand, the Arab's contact with the South and with the coastal regions was very ancient and was based on trade and commerce".

The above hypothesis presented for the origin of guar is very speculative. Biosystematic studies are urgently needed to critically analyse the relationship between the cultigen *Cyamopsis tetragonoloba* and the other species in the genus *Cyamopsis.*

# 5. Importance of guar

### 5.1 Agriculture economic importance

Guar or cluster-bean (*Cyamopsis tetragonoloba* (L.) Taub.) is an important agricultural product widely cultivated in  arid and semi-arid areas in north and northwest parts of India and east and south-eastern part of Pakistan (Santosh, 2014).

Nearly 75-80% of world guar seed production is contributed by India (Figure 5.1). Rajasthan is the major guar producing state in India followed by Haryana and Gujarat and small contributions come from the states of Uttar Pradesh, Punjab and Madhya Pradesh (Figure 5.2)

In Pakistan, guar seed is mainly produced in Punjab and Sindh province with about 80% of total guar acreage under irrigation. Pakistan contributes nearly 15% of world guar seed production (Figure 5.1). Bhakkar district of Punjab province contributes about 43.2% of total guar production in Pakistan followed by Layyad (15.8%), Khusab (4.8%), Mianwali (4.7%), and rest by other districts. Sudan, Australia and parts of USA are the other guar growing countries (Purushottam, 2010).

Total production in India of guar seed has crossed 3.000.000 ha in the early 2000s and has remained in the range of 3.000.000-3.500.000 hectares over a decade (Figure 5.4). The production fluctuates highly and depending upon the monsoon condition in India particularly western districts of Rajasthan.

Total production of guar seed in India is estimated to have crossed 2.7 million tons during the agricultural year 2013-14 due to good weather conditions in the major guar producing areas in India (Figure 5.4). With a moderate production of 250,000 tons in Pakistan, which is another important guar producing area, the total global production of guar bean is estimated to have crossed 3 million tons during 2013-14.

Nearly 75% of the guar gum or other derivatives of guar seed are being produced in India and are exported mainly to USA, China and European countries. Italy imports about 3% of Indian guar , the amount of guar imported in 2013-2104 amounted to 21.297 tons, with a value of 24.678.435,00 USD (Table 5.1., Figure 5.3). The value added derivatives of guar powder are used by the various industries in India as well as abroad. Taking the US, Australian, African crop the total world supply of Guar seed is around 10-16 lakh tons (Santosh 2014).

Table 5.1 “Guar gum Exports Data From India 2013 - 2014”

| Country | Quantity (t) | Value (USD) |
|---|---|---|
| U S A | 332.247 | 1.279.307.715,00 |
| China P Rp | 56.968 | 145.184.055,00 |
| Germany | 16.957 | 49.833.180,00 |
| Russia | 10.971 | 45.100.710,00 |
| Canada | 8.736 | 33.198.705,00 |
| Italy | 21.297 | 24.678.435,00 |
| Netherland | 3.732 | 12.975.000,00 |
| Brazil | 3.096 | 12.852.330,00 |
| Egypt A Rp | 29.454 | 10.887.855,00 |
| Indonesia | 12.681 | 10.216.575,00 |
| U K | 3.211 | 9.783.075,00 |
| Argentina | 2.328 | 9.077.700,00 |
| Japan | 2.842 | 8.600.865,00 |
| U Arab Emts | 2.301 | 7.735.245,00 |
| Latvia | 10.906 | 6.587.925,00 |
| Australia | 1.722 | 6.204.270,00 |
| Malaya | 12.913 | 5.939.970,00 |
| Turkey | 8.657 | 5.833.395,00 |
| Switzerland | 1.621 | 5.104.995,00 |
| South Africa | 1.593 | 5.074.095,00 |
| Iran | 1.128 | 4.341.615,00 |
| Mexico | 1.376 | 4.340.595,00 |
| Greece | 8.263 | 4.086.240,00 |
| Belgium | 2.052 | 3.923.595,00 |
| Kore Rp | 4.120 | 3.813.885,00 |
| Thailand | 2.722 | 3.718.920,00 |
| Poland | 4.755 | 3.539.280,00 |
| Denmark | 1.352 | 3.288.120,00 |
| Singapore | 571 | 2.994.060,00 |
| Chile | 848 | 2.558.745,00 |
| Sri Lanka Dsr | 7.627 | 2.531.040,00 |
| France | 1.123 | 2.265.660,00 |
| Taiwan | 4.980 | 2.103.045,00 |
| Philippines | 2.918 | 2.076.075,00 |
| Spain | 698 | 1.849.380,00 |
| Lithuania | 3.438 | 1.459.860,00 |
| Saudi Arab | 264 | 1.406.265,00 |
| Jordan | 384 | 1.139.145,00 |
| Israel | 361 | 1.009.875,00 |
| Vietnam Soc Rep | 497 | 943.695,00 |
| Ukraine | 281 | 940.410,00 |
| Syria | 238 | 866.745,00 |
| Bulgaria | 1.523 | 864.810,00 |
| Oman | 263 | 704.760,00 |
| Colombia | 219 | 684.030,00 |
| Nigeria | 166 | 650.685,00 |
| Afghanistan Tis | 90 | 512.205,00 |
| Ecuador | 1.169 | 505.755,00 |
| Guatemala | 148 | 487.440,00 |
| New Zealand | 142 | 462.615,00 |
| Korea Dp Rp | 116 | 384.735,00 |
| Algeria | 90 | 323.010,00 |
| Peru | 115 | 319.035,00 |
| Finland | 100 | 318.465,00 |
| Cyprus | 898 | 300.060,00 |
| Uruguay | 77 | 276.510,00 |

| | | |
|---|---|---|
| Pakistan Ir | 100 | 261.135,00 |
| Sweden | 51 | 241.230,00 |
| Yemen Republic | 71 | 234.660,00 |
| Czech Republic | 98 | 233.490,00 |
| Bangladesh Pr | 748 | 222.585,00 |
| Kazakhstan | 80 | 218.730,00 |
| Slovenia | 98 | 201.075,00 |
| Venezuela | 30 | 184.395,00 |
| Morocco | 78 | 181.380,00 |
| Lebanon | 394 | 175.500,00 |
| Belarus | 50 | 170.730,00 |
| Romania | 61 | 147.915,00 |
| Iraq | 50 | 116.730,00 |
| Panama Republic | 35 | 110.190,00 |
| Swaziland | 20 | 95.430,00 |
| Dominic Rep | 25 | 94.875,00 |
| Hong Kong | 19 | 83.910,00 |
| Congo P Rep | 15 | 80.865,00 |
| Zimbabwe | 20 | 75.405,00 |
| Chad | 20 | 71.775,00 |
| Croatia | 23 | 66.645,00 |
| Mali | 31 | 66.345,00 |
| Myanmar | 55 | 65.925,00 |
| Liechtenstein | 120 | 52.410,00 |
| Hungary | 20 | 52.035,00 |
| Tanzania Rep | 14 | 50.625,00 |
| Estonia | 18 | 43.545,00 |
| Mongolia | 10 | 43.500,00 |
| Costa Rica | 12 | 40.965,00 |
| Portugal | 9 | 28.395,00 |
| Bahrain Is | 5 | 28.275,00 |
| Mozambique | 10 | 27.360,00 |
| Norway | 9 | 25.650,00 |
| Kuwait | 8 | 25.020,00 |
| Unspecified | 10 | 23.640,00 |
| Albania | 5 | 23.625,00 |
| Nepal | 30 | 22.320,00 |
| Senegal | 9 | 22.305,00 |
| Turkmenistan | 4 | 22.110,00 |
| Kenya | 3 | 17.565,00 |
| Malta | 40 | 14.535,00 |
| Georgia | 44 | 13.125,00 |
| Chana | 4 | 9.735,00 |
| Macedonia | 20 | 7.515,00 |
| Zambia | 2 | 6.435,00 |
| Madagascar | 21 | 6.180,00 |
| Gambia | 1 | 2.385,00 |
| Congo D Rep | 0 | 1.950,00 |
| Sudan | 0 | 225,00 |
| Total: | 601.945 | 1.760.178.810 |

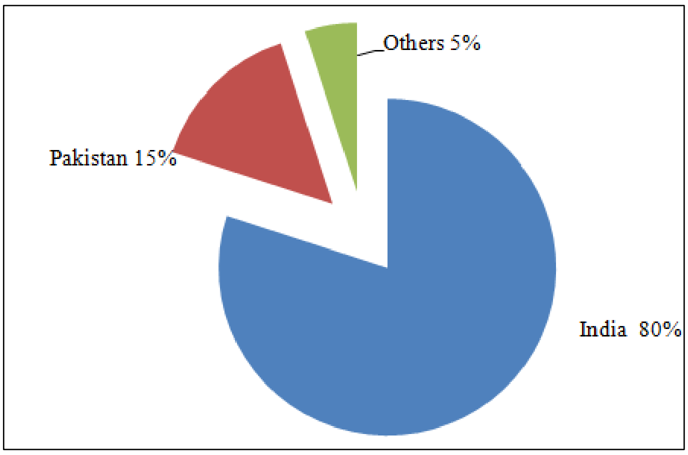


Fig. 5.1 “Major producing countries”

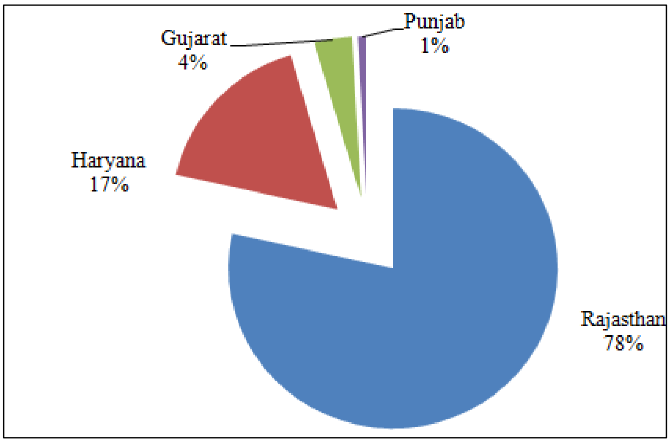


Fig. 5.2 “Major producing States of India countries”

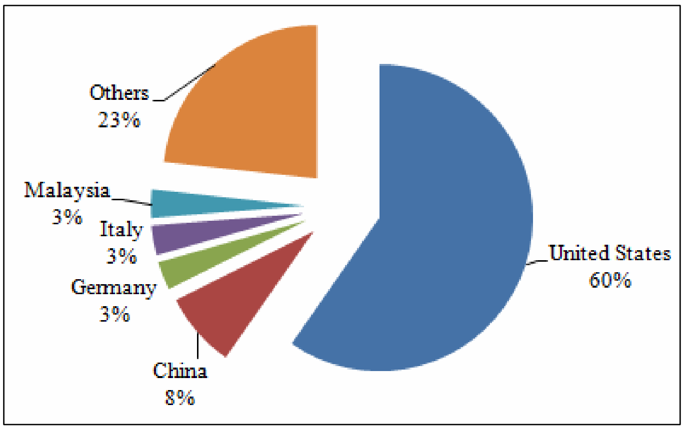


Fig. 5.3 “Major export destination countries”

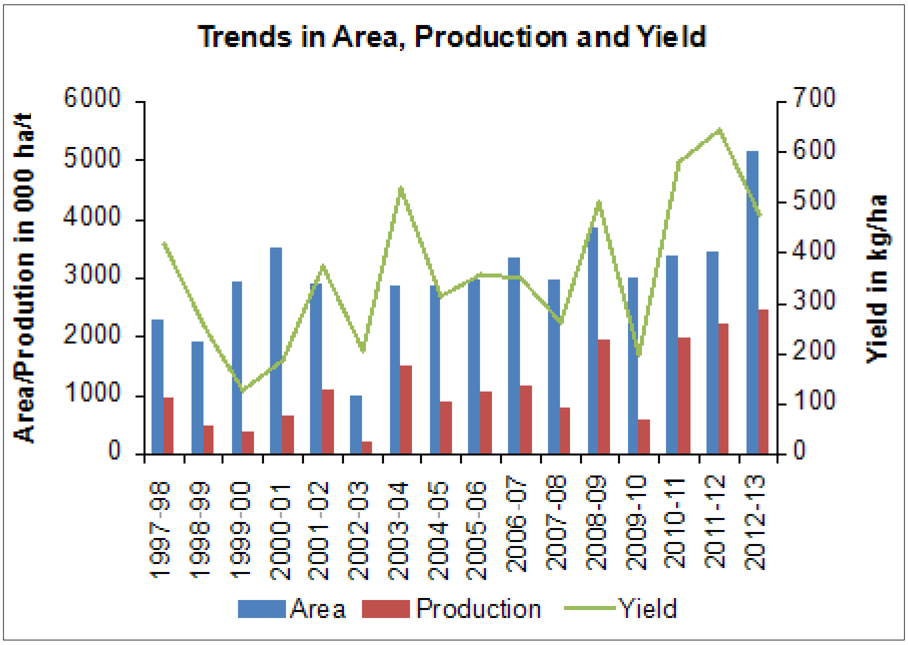


Fig. 5.4 “Trends in area, production and yield of India”

The trend of the price of the splits guar has increased sharply from March 2011, starting from less than 2.20 USD/kg until 19.8 USD/kg in the period between March 2012 and June 2012 and then gradually return to the initial price at the beginning of the 2014 year (Figure 5.5).


Fig. 5.5 “Price trend of splits guar”

Guar production in India

The production of guar in India has seen wide variation during in recent past ranging from 0.2 to 2.7 million tons due to the over dependence of the crop on monsoon precipitation (Table 5.2). Only 0.2 million tons of guar bean was produced from an area of nearly 1 million hectares during 2002-03 due to severe drought. A harvest of 2.7 million tons from an area of 5.6 million hectares was achieved in 2013-14 due to good monsoon. In spite of the fluctuation in the area and production of guar, an increasing trend has been observed during last decade in acreage under the crop and production of guar bean in India (Figure 5.6). It is apparent from the figure that production is increasing at a faster rate than the area under cultivation of guar due to increase in the productivity, it is expected to increase of price in coming years on account based on increasing demand for factors like use of guar gum in shale gas industry, low-input requirement and relative profitability of crop even at lower prices.

The cultivation of guar in India may be grouped into three categories. The traditional farmers taking guar under rain-fed conditions, this category broadly covers an area of 3 million hectares. Traditional farmers taking guar under irrigated condition are moderately likely to shift to other crops. Lastly, non-traditional irrigated resource rich farmers from state like Haryana and Gujarat (Santosh, 2014).

Table 5.2 “Area, production and yield of guar in recent past in India”

| Year | Area (000 ha) | Production (000 tons) | Yield (Kg/ha) |
|---|---|---|---|
| 2000-01 | 3497 | 659 | 188 |
| 2001-02 | 2903 | 1090 | 375 |
| 2002-03 | 975 | 199 | 204 |
| 2003-04 | 2854 | 1513 | 530 |
| 2004-05 | 2867 | 903 | 315 |
| 2005-06 | 2956 | 1059 | 358 |
| 2006-07 | 3344 | 1169 | 350 |
| 2007-08 | 3472 | 1789 | 515 |
| 2008-09 | 3863 | 1936 | 501 |
| 2009-10 | 2996 | 595 | 199 |
| 2010-11 | 3382 | 1965 | 581 |
| 2011-12 | 3444 | 2218 | 644 |
| 2012-13 | 5152 | 2461 | 478 |
| 2013-14# | 5603 | 2715 | 485 |
| 2014-15* | 4255 | 2415 | 567 |

*Source: Ministry of Agriculture, GOI*

*#Figures for 2013-14 at all India level are not available and hence rough estimates have been worked out on the basis of area and production in Rajasthan and its relative position in guar in India.*

**Author (Santosh, 2014) estimate based on regression analysis for area and previous three-year average for productivity.*

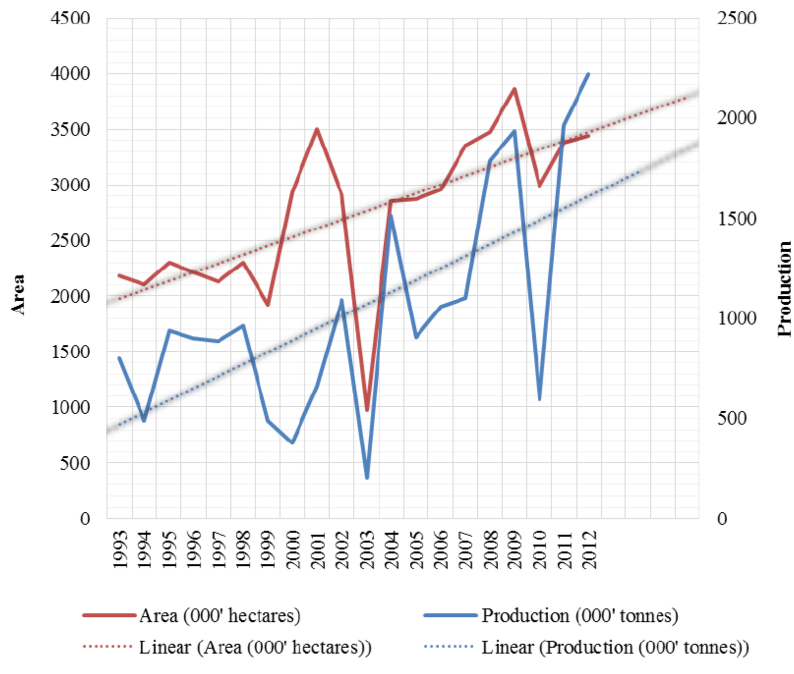


Fig. 5.6 "Area and production of guar in India"

<u>Guar production in Rajasthan and in other states of India</u>

Rajasthan is the largest guar bean producing state in India. The crop is cultivated in Kharif season and sowing starts with the onset of monsoon in the month of June/July. Guar is being grown mainly in arid districts of western part of Rajasthan (Santosh, 2014). It is grown mainly as rain-fed crop and thus, production and productivity of crop is highly dependent on the intensity, spread and level of monsoon rainfall in the state. In Rajasthan, presently Hanumangarh is the leading guar producing district contributing about one-fifth of total guar production and about 10% of area under guar cultivation in the state (Purushottam, 2010).

The details of the leading guar producing districts in Rajasthan state is given in table 5.3. The table reveals that Bikaner is the leading district both in terms of area and production. The districts of Hanumangarh, Sriganganagar and Churu are the other three major producers. These four districts together contribute nearly three-fourth of the total guar production in the state.

Area and production of guar-bean in Rajasthan for the period 1991-2011 is shown in figure 5.7. The lowest production of guar-bean in Rajasthan was recorded at 28,000 tons during 2002-03 (which was a drought year) while the production has increased in recent years. The figure reveals the high degree of fluctuation over year is observed in the production of guar-bean in Rajasthan. This may be due to overdependence of crop on rainfall (Santosh 2014).

Table 5.3 "Leading Guar producing districts in Rajasthan (TE 2011-12)"

| Districts | Area (%) | Production (%) |
|---|---|---|
| Bikaner | 29.1 | 28.5 |
| Hanumangarh | 9.6 | 16.1 |
| Sri Ganganagar | 5.2 | 10.6 |
| Churu | 10.6 | 9.1 |
| Jaisalmer | 13.9 | 7.5 |
| Barmer | 13.8 | 6.0 |
| Jodhpur | 4.6 | 5.0 |
| Sikar | 2.5 | 4.0 |
| Jhunjhunu | 2.0 | 2.9 |
| Nagaur | 2.8 | 2.5 |
| Jaipur | 1.2 | 2.0 |
| Jalore | 1.4 | 1.5 |
| Alwar | 0.6 | 1.2 |
| Pali | 1.0 | 1.1 |
| Bhilwara | 0.2 | 0.2 |

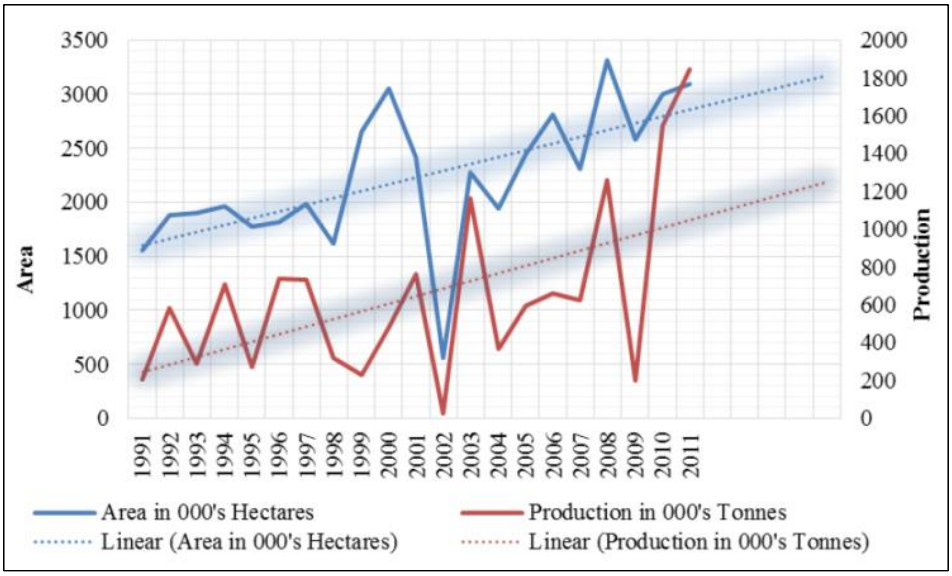


Fig. 5.7 "Area and production of guar in India in Rajasthan"

In addition to Rajasthan, guar is also cultivated in Haryana, Gujarat, Uttar Pradesh and Punjab. In terms of area, Haryana and Gujarat are comparable but Haryana has made significant growth in production in recent years due to higher productivity. The latest available official statistics reveal that the production of guar-bean in Haryana has ranged from 91,000 tons in the year 2002-03 to 602,000 tons in 2008-09 (Table 5.4; Santosh, 2014).

The high yielding and short duration varieties developed by Haryana Agricultural University, Hissar viz.; HG 365, and HG 563 and its extensive use by farmers has supported higher productivity in the state. Increasing area under cultivation of Guar in Haryana will help in augmenting the overall production of Guar from India (Purushottam, 2010).

The growth in area, production and yield of guar seed in Haryana during 1991-2011 is presented in figure 5.8. The figure reveals an increasing trend in area and production over years though declining trends are observed during recent years. This may be due to the fact that guar may not find itself competitive at lower prices in area like Haryana and Gujarat with availability of resources mainly irrigation and availability of more remunerative options like cotton in these areas.

The crop considered generally for marginalised land has developed a lot of curiosity among farmers mainly from the non-conventional states after sudden rise in the prices owing to its demand in international market mainly USA. The analysis of the relative share of different states in total production and area shows that Rajasthan is the leading producer but suffers from high fluctuation in production. On the other hand Haryana has significant contribution in terms of production based on high productivity. This has been achieved by using high yielding short duration varieties by farmers and assured irrigation. The Rajasthan has shown the lead in production but with high fluctuation in its share. On the other hand the share of different states has been observed to be almost uniform over years. It is also revealed that production of guar in Rajasthan is relying heavily on monsoon while in other state the production is not that much affected by rainfall due to assured irrigation (Santosh, 2014).

Table 5.4 "Area, production and yield in recent past in Haryana and Gujarat"

| **Year** | **Haryana** | | | **Gujarat** | | |
|---|---|---|---|---|---|---|
| | **Area (000 ha)** | **Production (000 tons)** | **Yield (Kg/ha)** | **Area (000 ha)** | **Production (000 tons)** | **Yield (Kg/ha)** |
| 2000-01 | 148 | 102 | 689 | 273 | 60.9 | 223 |
| 2001-02 | 196 | 127 | 648 | 263 | 112 | 424 |
| 2002-03 | 205 | 91 | 444 | 213 | 65 | 306 |
| 2003-04 | 269 | 117 | 435 | 266 | 204 | 766 |
| 2004-05 | 217 | 254 | 1171 | 214 | 157 | 733 |
| 2005-06 | 270 | 289 | 1070 | 188 | 108 | 575 |
| 2006-07 | 295 | 334 | 1132 | 205 | 83 | 404 |
| 2007-08 | 341 | 395 | 1200 | 196 | 130 | 662 |
| 2008-09 | 370 | 602 | 1627 | 150 | 53 | 353 |
| 2009-10 | 252 | 329 | 1305 | 133 | 45 | 337 |
| 2010-11 | 256 | 333 | 1300 | 125 | 73 | 586 |
| 2011-12 | 215 | 290 | 1350 | 37 | 33 | 892 |

*Source: Department of Agriculture, Haryana & Department of Agriculture, Gujarat*

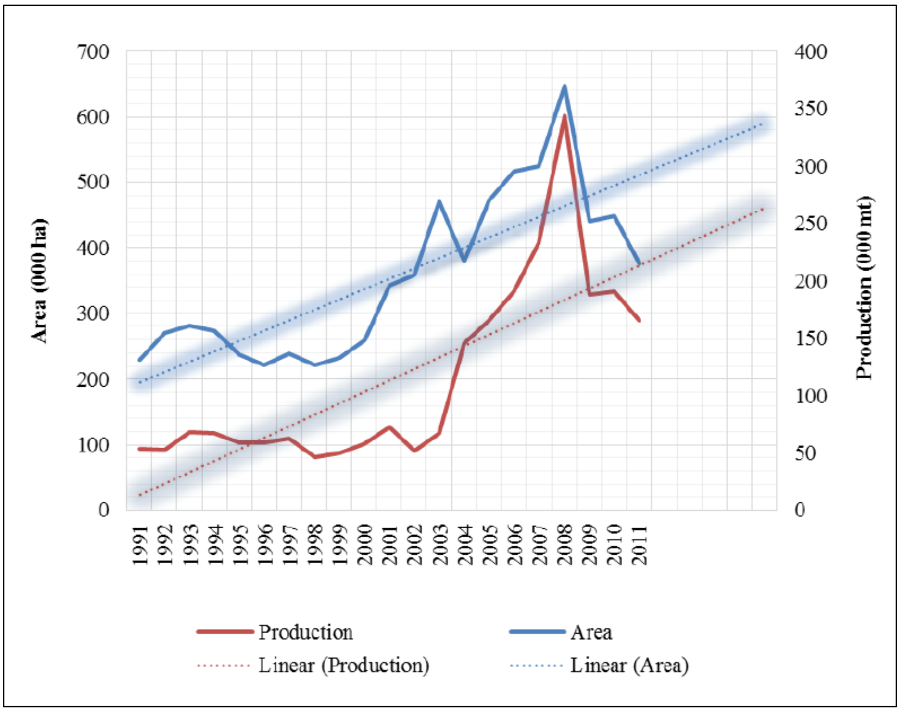


Fig. 5.8 “Area and production of guar in Haryana”

## 5.2 Uses and production of guar and its derivatives

Guar is used for extracting of gum from seeds, as animal fodder from vegetative part, and is also used as green manure. Moreover the tender green guar is an important source of nutrition to both human being and animals, which is consumed as a vegetable and cattle feed, respectively. Like other legumes, guar is an excellent soil-building crop with respect to availability of nitrogen. Root nodules contain nitrogen-fixing bacteria and crop residues, when ploughed under, improves yields of succeeding crops. Guar constitutes about 0.27% share in India's agricultural GDP. Guar gum, extracted from guar seeds, is one of important item of export, which constitutes about 0.23% of Indian total exports under the agriculture and allied products.

The guar seed consists of three parts: the seed coat (14-17%), the endosperm (35-42%), and the germ (43-47%). the endosperm that guar gum is derived, which is the prime marketable product of the plant. *Cyamopsis tetragonoloba* is used worldwide for food stabilization, fiber source, food, and industry (Morris, 2010). This spherical-shaped endosperm contains significant amounts of galactomannan gum (19 to 43% of the whole seed), which forms a viscous gel in cold water. The by-products of guar processing, "*guar meal"* are used as cattle feed (mainly cattle and poultry). It can be used up to 10% in poultry diet and can replace up to 100% protein supplements such as ground nut oil cakes in ruminants (Purushottam, 2010).

Guar flour is the ground endosperm of guar seeds. Its industrial value rest upon the gum, which constitutes 78-82% of the endosperm and which is a natural, water-dispersable, hydrocolloid that has great thickening power when dissolved in water. The hydrocolloid commercially known as guar gum is chemically a galactomannan-designated guaran. Galactomannans are polysaccharides consisting of long chains of (1→4)-β-D-mannopyranosyl units to which single unit side chains of (α-D-galactopyranosyl units are joined by 1→6 linkages. Galactomannans are found in a variety of plants but differ in molecular size and in the number of D-galactosyl side chains. The galactomannans are commonly found in the endosperms of the leguminosae. Of 1633 species of legume seeds examined by Anderson (Anderson, 1949) three-fourths contained gum-yielding endosperms (Table 5.5). Galactomannans are present in relatively small amounts in such important agricultural clovers as lespedeza, birdsfoot trefoil (*Lotus corniculatus*), and sweet clover (*Melilotus*). They are more abundant in alfalfa (*Lucerne*, *Medicago*) seeds (Andrews *et al*., 1952).

They are also abundant in fenugreek (*Trigonella foenum-graecum*), koa hoale (*Leucaena glauca*) of Hewaiian Islands (Unrau, 1961), *Cassia* (Tookey and Clark, 1965) and coffee beans (Larson and Smith, 1955).

Table 5.5 "Estimated endosperm content of leguminous seeds"

| Family | Endosperm % | Family | Endosperm % |
|---|---|---|---|
| *Acacia* | 1-15 | *Glottidium* | 2 |
| *Astragalus* | 2-3 | *Glymnoclaudus* | 15 |
| *Baryxylum* | 30 | *Indigofera* | 20 |
| *Caesalpinia* | 8-40 | *Lespedeza* | 1-4 |
| *Cassia* | 10-60 | *Leucaena* | 15 |
| *Cercidium* | 20 | *Lotus* | 2-4 |
| *Ceratonia* | 50 | *Lysiloma* | 4 |
| *Chamaecrista* | 8-15 | *Melitotus* | 8-12 |
| *Colvillea* | 30 | *Mimosa* | 3-30 |
| *Crotalaria* | 8-25 | *Onomis* | 25 |
| *Cyamopsis* | 50 | *Parkinsonia* | 25 |
| *Cytisus* | 15 | *Parryella* | 20 |
| *Dalea* | 20 | *Prosopis* | 15 |
| *Daubentonia* | 10-15 | *Schrankia* | 12 |
| *Delonix* | 25 | *Sesbania* | 20 |
| *Desmanthus* | 15 | *Sophora* | 20-25 |
| *Desmodium* | 2 | *Trifolium* | 3-10 |
| *Gleditsia* | 30 | *Virgilia* | 20 |

*Source: Anderson (1949)*

Guar gum recovery normally constitutes around 31% of total guar seed processed, whereas "*guar meal*" account for 29% and 37% respectively. Guar gum is an important ingredient in producing food emulsifier, food additive, food thickener and other guar gum products. India is the largest producer of guar gum products. Guar gum is purely an export oriented commodity with about 80% of total output exported from the country. Industrially it is used in mining, petroleum drilling and textile industry. In food it is used as a thickener and as a mean of preventing ice crystal formation in frozen desserts. Guar gum is produced from the seed and this is turned into powder. The powder is used in a host of industries, ranging from bakery, dairy, meat, dressing and sausages, beverages, pharmaceuticals and cosmetics, textile printing, mining, water treatment and paper industry. Detailed information on guar application of guar gum are reported in table 5.6 and 5.7.

Table 5.6 "Application-wise global consumption of guar derivatives"

| Type of application | Target industries | Global consumption |
|---|---|---|
| Food grade | Bakeries (bread), dairy (Ice cream, sherbets, cheese etc.), dressing sauces, ketchup's) beverages (chocolate drinks), pet food (thickener). | 50-55% |
| Pharmacy grade | Cosmetics and medicines (as binder and thickener), slimming (Reducing weight and laxative). | 5-10% |
| Industrial grade | Oil drilling (as a well stimulant and fraction reducer), mining (increased yield, filter aid), explosives (gelling agent), coal mining (fraction reducer, binding). | 25-30% |
| Other | Textile printing (thickening agent for dyes), paper (increase strength and decrease porosity), photography (gelling and hardening). | 10-15% |

Guar gum is one of the most cost effective and functional ingredients available for formulating food products. Soluble in cold water, guar imparts a high viscosity and exhibits superior water-binding capacity at low usage levels. These characteristics make it suitable for use in applications as diverse as cottage cheese, sauces, soups, and frozen desserts. The non-ionic nature of guar makes it tolerant to extreme salt and electrolyte levels, important criteria when selecting a thickener for nutraceutical beverages. Guar is an all-natural ingredient, unlike other common thickeners such as modified food starch and cellulose gum (CMC), and has been shown to provide important health benefits. Numerous studies have shown that the consumption of Guar Gum lowers serum LDL cholesterol and triglycerides and increases glucose tolerance. Guar is also high in soluble dietary fiber (Purushottam, 2010).The gum is a polysaccharide with a straight chain of mannose units and galactose in the ratio of 2:1. The gum contains about 6% protein. Guar is more than 6 times as effective as starch in thickening power and is used for upgrading starches. Various derivatives of Guar Gum are available that will stiffen gels even up to a water content of 99%. Commercially important derivatives of Guar gum are:

a) Hydroxy Alkylated Guar gum
b) Carboxy Methylated Guar gum
c) Oxidised Guar gum
d) Acetates of Guar gum
e) Cationic derivatives of Guar gum
f) Sulphated Guar gum
g) Guar gum formate
h) Guar gum acryl amide
i) Borate cross linked Guar gum
j) Reticulated Guar gum
k) Carboxy methyl hydroxy propyl Guar gum
1) Depolymerised Guar gum

Table 5.7 "Commercial application of guar gum powder"

| Product | Form of gum | Function | Product ref. |
|---|---|---|---|
| **Food** | | | |
| Ice Cream | Blended Guar Gum | Thickening, gelling, stabilizing agent | RG-IC201CM |
| Liquid egg | Blended Guar Gum | Improves consistency | RG-LE201GX |
| Acidified dairy products | Blended Guar Gum | Improved thickness and aroma texture | RG-135, RG-150 |
| Fat containing whips cream | Blended Guar Gum | Pudding and cream dressing of cakes | RG-LF201CGM |
| Instant corn grits | Guar Gum | Binder | RG-250 |
| Water ices | Guar Gum | Stabilizer | RG-250 |
| Bakery fillings | Guar Gum | Freeze thaw and heat stabilizer | RG-235, 250 |
| Tomato sauces, mayonnaise | Blended Guar Gum | Thickening agent | RG-KS82 |
| Protein low bread | Blended Guar Gum | Binder | RG-BR135,BR150 |
| Icing stabilizers | Guar Gum | Stabilizer | RG-250 |
| Cheese | Blended Guar Gum | Stabilizer | RG-CS55GMX |
| Salad dressing | Blended Guar Gum | Stabilizer, thickener | RG-SD245 |
| Noodles (like Maggi) | Guar Gum | Binder, thickener, stabilizer | RG-250, 260 |
| **Pharmaceuticals** | | | |
| Vitamin B12 prep. | Treated Guar Gum | Stability, Synergistic activity +bismuth salt | RG-PH235 |
| Sustained or time release drugs | Treated Guar Gum | Binder | RG-PH245 |
| Microencapsulation of drugs | Treated Guar Gum | High resorpitivity | RG-PH235PS |
| Gastrointestinal disorders | Treated Guar Gum | Laxative Property | RG-PH460LX |
| Comp. tablets prep. | Guar Gum | Dry binder | RG-PH250 |
| Dietetic compositions | Guar Gum | Malnutrition treatment | RG-PH235 |
| Ant inflammatory drugs | Guar Gum | Suspending agent | RG-PH235 |
| **Medicinal** | | | |
| Dietary fibre | PHP Guar Gum | Protective effect | RG-DF100 |
| Mixed in diet | Guar Gum | Reduces urine sugar , cholesterol | RG-MD46 |
| Mixed in diet | Guar Gum | Antidiabetic agent , effects bacterial counts in faeces | RG-460LX |
| Dietary fibre | PHP Guar Gum | Hypocholesterolemic agent | RG-DF100 |
| **Cosmetics** | | | |
| Shampoo prep. | Cationic Guar Gum | Thickener agent | RG- CO 235 |
| Tooth paste perp. | Guar Gum | Stabilizer and thickener | RG-TP250 |
| Hair setting | Guar Gum | Thickener agent | RG-C0235 |
| **Petroleum well drilling** | | | |
| Oil bore | Cross-Linked Guar Gum | Stable super elastic | TG-CD250 |
| Oil, gas water bores | Guar Gum | For plugging leaks, stabilization of cross -linkage | TG-GX2100 |
| Rotary drilling | Guar Gum | Indicator of fluid life | TG-270BL |
| Fracturing solution | Guar Gum | Reduce friction and increase permeability | TG-240HPG |
| Drilling | Guar Gum | Thickener | TG-270 |
| Petroleum wells | Guar Gum | Water barrier gelling agent | TG-GA260 |
| Fracturing fluids | Guar Gum | Provides viscosity stability | TG-25BAMO |
| Well stimulator | Guar Gum | Gelling agent | TG-35CMHP |
| Drilling mud | Guar Gum | Thickening agent | TG-HPG |
| Fracturing well | Guar Gum | Gelling agent | TG-270KM |
| Petroleum well fracturing | Guar Gum | Thickening agent | TG-275 |
| Petroleum well blow out | Guar Gum | Thickening agent | TG-HPG |
| **Paper** | | | |
| Additive sizing | Guar Gum | Improves wet strength, increase dry strength | RG-230PG,-230PGSD |
| Paper/paper board | Modified Guar Gum | Retention aid, imparts dry strength | RG-230AN |
| Paper making | Cationic Guar Gum | Improves dry strength | RG-235PGCO |
| Paper making | Guar Gum | Flocculent &sizing agent | RG-230SS |
| **Explosives** | | | |
| Explosives gel | Guar Gum | Improved resistance to water & aging | RG-260EX |
| Blasting agent | Guar Gum | Increases viscosity | RG-260EX |
| Blasting slurry | Guar Gum | Thickening agent | RG-260EX |
| Explosives mixture | Derivative Guar Gum | Thickening agent | RG-EX240CN |
| Gel explosives | Guar Gum | Gelling agent | RG-260EX |

| | | | |
|---|---|---|---|
| Gel explosives | ANFO Guar Gum | Gelling agent | RG-255EXPO |
| Slurry explosives | Guar Gum | Thickening agent | RG-260EX |
| Nitroalkane explosives | Derivate Guar Gum | Thickening agent | RG-260EXN |
| **Building** | | | |
| Plaster | Guar Gum | Thickening agent | TG-250 |
| Concrete | Guar Gum | Water proofing | TG-260 |
| Foamed cement material | Guar Gum | Foam stabilizer | TG-250 |
| Fire fighting | | | |
| Air drop , forest- fire control | Treated Guar Gum | Provides viscosity stability | FF-240FR |
| Forest fire fighting | Treated Guar Gum | Dispersions | FF-250MEG |
| **Photography** | | | |
| Processing solution | Treated Guar Gum | Binder and thickener | RG-PHGG |
| Coal mining | Treated Guar Gum | For shock impregnation of coal seams | TG-250CD |
| **Ore mining** | | | |
| Iron scrap | Modified Guar Gum | In settling fine particles colloidal flocculent | TG-104FF |
| Copper electro refining | Guar Gum | | TG-104FF |
| **Textiles** | | | |
| Textile print | Treated Guar Gum | Stabilizer | RG-206AA |
| Polyester printing | Treated Guar Gum | Thickener | RG-206DV |
| Printing fluid | Treated Guar Gum | Printing paste thickener | RG-DPL RANGE |
| Textile printing | Treated Guar Gum | Thickener | RG-DPL RANGE |
| **Agriculture** | | | |
| Insecticidal comp. | Guar Gum | | RG-250 |
| Coating for fertilizer | Guar Gum | Prevention of granules | RG-AGMS |
| Poultry | Guar Gum | Decrease cholesterol levels in chicks | RG-46 |
| Shoe industry | Guar Gum | Good fibrillation & reaggregation of the collagen fibres | TG-235 |
| **Ceramics** | | | |
| Ceramics articles | Guar Gum | Plaster for moulded articles | TG-250 |
| Tobacco products | Guar Gum | Adhesive | RG-250TO |
| Tobacco sheet | Guar Gum | Reduces irritation properties, Strengthening agent | RG-235TO |
| **Analytical** | | | |
| Selective resins | Treated Guar Gum | Resin for separation of boron | RG-250CD |
| Beads | Treated Guar Gum | Purification of lectins | RG-250 |
| Gel filtration | Treated Guar Gum | Chromatographic separation | RG-250CD |
| **Paints and Distempers** | | | |
| Oil Base Paints | Modified Guar Gum | Rheology controller, thickener | TG-240HPG |
| Water Base Paints | Modified Guar Gum | Rheology controller, thickener | TG-220CMG |
| Distempers | Guar Gum | Binder, thickener | TG-250 |
| **Miscellaneous** | | | |
| Production of sodium carbonate | Guar Gum | Clarifier | TG-220CMG |
| Aluminium reflectors | Guar Gum | In increasing reflecting power | TG-335 |
| Turbulent flow between pipe walls | Guar Gum | Reduction of resistance | TG-335 |
| Civil disorder control | Guar Gum | Lubricant for concrete surface | TG-335 |
| Mosquito coil | Guar Gum | Saw dust binder | TG-235AGM |
| Flocculation or Water Treatment | Modified Guar Gum | Flocculating Agent | TG-WT230 |

### 5.3 Processing of guar seed

The general outline of the manufacturing process of guar gum is shown in figure 5.9. When guar seed are removed from their pods these are spherical in shape, brownish in colour, smaller than pea seeds in size.

The gum is commercially extracted from seed essentially by a mechanical process of roasting, differential attrition, sieving polishing. The seed are broken and the germ is separated from the endosperm. Two halves of the endosperm are obtained from each seed and are known as undehusked *guar splits*. When the fine layer of fibrous material, which forms the husk, is removed and separated from the endosperm halves by polishing, refined guar splits are obtained. The hull (husk) and germ portion of guar seed are termed as *guar meal* which is a major by-product of guar gum power processing and is utilized as cattle feed (Figure 5.10).The refined guar splits are then treated and finished in to powders (known as *guar gum*) by a variety of routes and processing techniques depending upon the end product desired. The pre hydrated guar splits are crushed in flacker mill and then uniformly to ultra-fine grinder, which grinds the spits without producing too much heat. The grinded material is dried and passed through screens for grading of the material according to the particle size. Various grades are available depending upon color, mesh size, viscosity potential and rate of hydration (Chudzikowski, 1971). In industrial processing of guar gum extrusion is also included before hydration and flaking. After these steps grinding and drying are done. Inclusion of extrusion gives guar gum powder with improved rate (Chowdhary, 2002). The by-products of guar gum industry are Churi and Korma which are utilized for cattle feed.

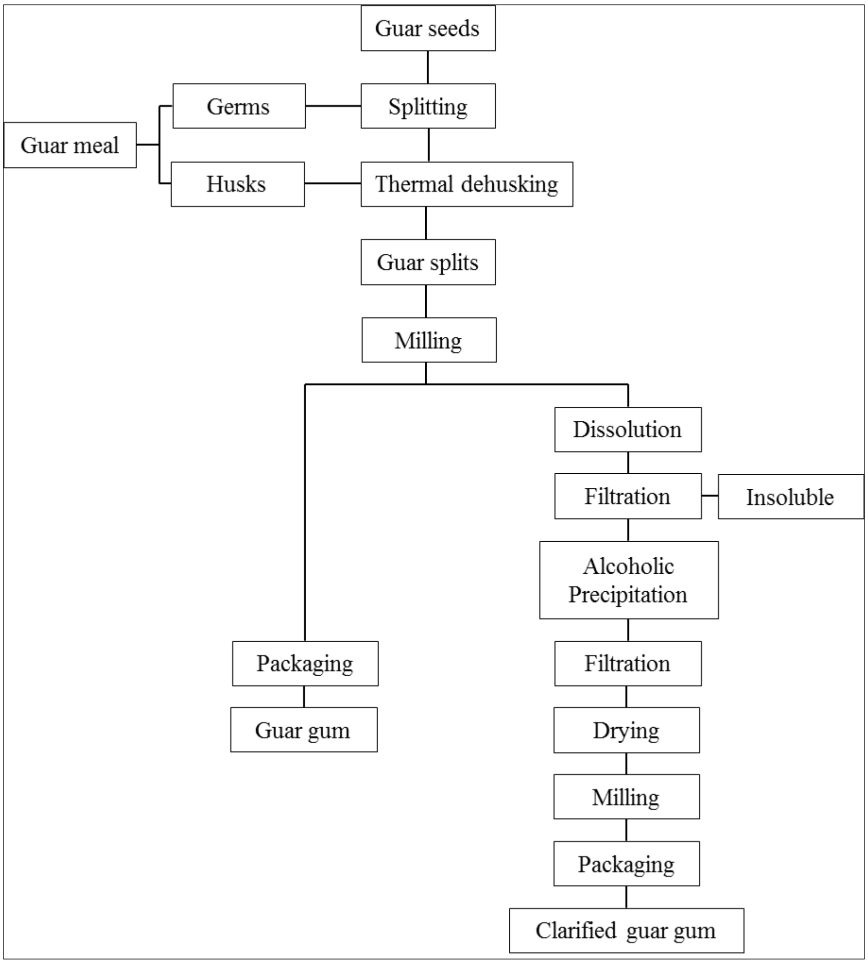


Fig. 5.9 “Flow diagram for industrial manufacturing of guar gum”

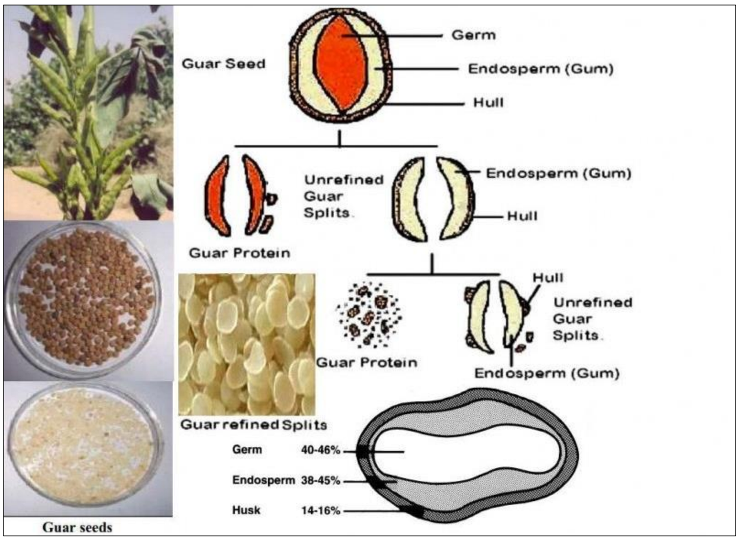


Fig. 5.10 “Processing of guar seed”

### 5.4 Chemical structure and composition of galactomannans

Galactomannan are formed in the matured pods of guar, and the seed are ready for galactomannan extraction when the matured pods are dry (Mathur, 2012). The guar kernel is composed of several layers, namely the outer husk (16-18%), the germ (43-46%) and the endosperm (34-40%). The germ portion of its seed is predominantly protein and the endosperm predominantly galactomannan.

Guar gum mainly consist of the high molecular weight polysaccharides of galactomannans which are linear chain of (1→4)-linked β-D-mannopyranosyl units with (1→6)-linked α-D-galactopyranosyl residues as side chains as shown in figure 5.11. These galactose and mannose groups constitute the galactomannan portion of seed endosperm. General composition of guar gum is given in table 5.8.

It was first believed that the side-groups were substitute at regular intervals along the manna backbone (Whistler and Hymowitz, 1979).

However, experiment using enzyme degradation of guar (McCleary, 1979), spectroscopic methods (Grasdalen and Painter, 1980) and computer simulation (McCleary *et al*., 1985), indicate more random distribution of galactose side groups as given in figure 5.12.

One such model proposes a guar galactomannan in which the galactosyl units are randomly arranged mainly in pairs and triplets (Hoffman and Svensson, 1978). The ratio of mannose to galactose units has historically been reported as 2:1 (Garti and Laser, 2001). Various research studies support ratios in the range of 1.6:1 to 1.8:1 (Grasdalen and Painter, 1980; McCleary *et al*., 1985; Hoffinan and Svensson 1978; Barth and Smith 1981; Vijayendran and Bone 1984; McCleary 1981; Mathur and Mathur 2005). Current data also suggest that galactomannans from different guar varieties have the same galactose/mannose arrangement (McCleary *et al*., 1985). The greater branching of guar is believed to be responsible for its easier hydration properties as well as its greater hydrogen bonding activity (Whistler 1954). It is also reported that aggregates are prominent in guar systems and may have important role in viscoelastic behaviour of solution, depending on how they are interlinked (Gittings *et al*., 2000).

Guar is a polysaccharide with one of the highest molecular weights of all naturally occurring water soluble polymers. The viscosifying effect of commercial guar gum preparations can vary normously depending on the molecular weight of the galactomannan. Early publications reported that average molecular weight of guar gum vary enormously, depending on what method is used, but these are typically in the range of 0.25-5.0 million.

Absolute methods have also been used to determine molecular weight, including light scattering techniques, which are also useful for providing structural information on the polysaccharide (Robinson *et al.*, 1982; Burchard, 1994; Ross-Murphy *et al.*, 1998). One relatively simple and reliable way of estimating molecular weight is to use intrinsic viscosity measurements, calibrated by light scattering or some other absolute method using the Mark- Houwink equation. But more recent results obtained with size exclusion chromatography and low angle laser light scattering show the average molecular weight in the range of 106 to 2 x 106 (Barth and Smith 1981; Vijayendran and Bone 1984).

Table 5.8 “General composition of guar gum”

| Constituent | Percentage |
|---|---|
| Galactomannan | 75-85 |
| Moisture | 8.0-14 |
| Protein (N x 6.25) | 5.0-6.0 |
| Fiber | 2.0-3.0 |
| Ash | 0.5-1.0 |

*Source: Chudzikowski (1971)*

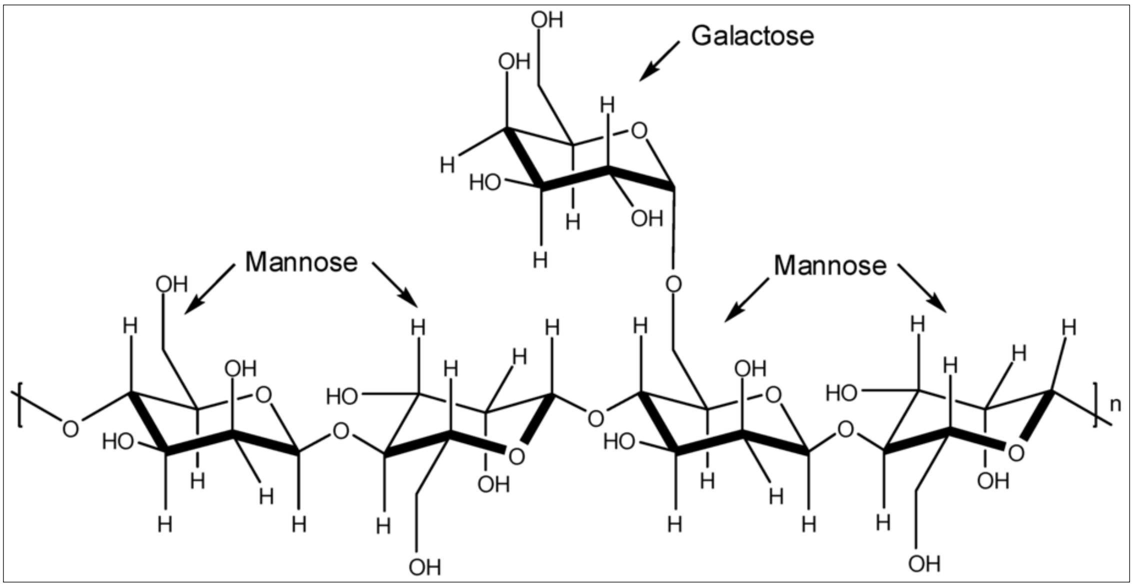


Fig. 5.11 “Structure of guar gum molecule”

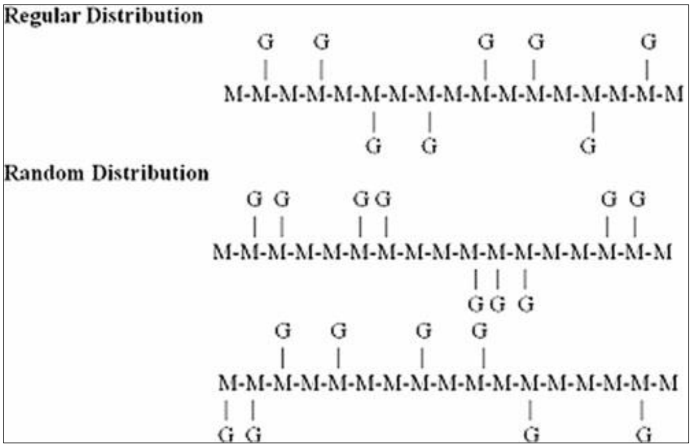


Fig. 5.12 “Sequence of galactose and mannose in guar”

### 5.5 Guar meal

The germ and outer seed coat of guar seed together constitute guar meal. Removal of guar gum from guar seed increased the protein content of the residual by-product i.e. guar meal. It is light greyish tab material with beany flavours. The guar seed results in 62-68% of meal having a protein content of 35-46% (Kumar and Singh, 2002).

Guar meal is a rich source of protein. It contents about one and half times more protein than guar seed and compares well other vegetable protein source like oilseed cake used in poultry and cattle diets. Chemical composition of guar meal is given in table 5.9.

Table 5.9 “Average percentage composition of Guar meal”

| Components | % range |
|---|---|
| Oil | 5-6 |
| Protein | 35-55 |
| Fiber | 10-12 |
| Carbohydrates | 28-30 |
| Mineral matter | 5-8 |
| Digestibility of protein | 96-97 |

*Source: Arora SK (1981) Modified*

Nagar *et al*. (1981) found that autoclaving of toasted guar meal did not change its crude protein content, however, acid treatment increased the crude protein content. Rajput *et al*. (1998) observed that the total protein of defatted guar meal consisted of 4 fractions, two of high and other two of low molecular weight proteins and detoxification of guar meal did not cause any significant change in the protein while autoclaving resulted in drastic changes.

Joshi *et al*. (1990) compared the chemical composition of *Cyamopsis tetragonoloba* (L.) Taub. with *Cyamopsis serrata* and *Cyamopsis senegalensis* and found that *Cyamopsis Tetragonol* oba containd maximum protein and oil contest (Table 5.10).

Table 5.10 "Chemical composition of seed of *Cyamopsis*"

| Species | Gum % | Protein % | Oil % |
|---|---|---|---|
| *Cyamopsis tetragonoloba* | 31.30 | 32.58 | 6.1-7.5 |
| *Cyamopsis serrata* | 30.25 | 27.12 | 4.40 |
| *Cyamopsis senegalensis* | 30.60 | 26.25 | 3.06 |

*Source: Joshi et al. (1990)*

# 6. Description of the crop

Guar belongs to the family *Leguminosae* and sub-family *Papilionaceae* (Table 6.1). Is an important leguminous annual crop also called as "clusterbean" for its pattern of pod arrangements in clusters.

Table 6.1 "Botanical classification of guar"

| Order | *Fabales* |
|---|---|
| Family | *Fabaceae* |
| Subfamily | *Faboideae* |
| Tribe | *Indigofereae* |
| Genus | *Cyamopsis* |
| Species | *Cyamopsis tetragonoloba* |

It is generally 50 to 100 cm tall, but it can be up to 2-3 metres. It bears 4-10 branches (branched types), however, unbranched type varieties having main stem only which is heavily clustered with pods, have been selected (Figure 6.1). Guar plant may bear on an average basis 30-90 pods per plant, but even many more. The developing pods are rather flat and slim (Figure 6.2) containing 5 to 12 small oval seeds of 5 mm length (TSW = 25-40 g). Usually, mature seeds are white or grey, but in case of excess of soil moisture just before seed harvest they can turn black and lose germination capacity.

Guar has well developed tap root system, in fact the plant can access soil moisture in low soil depths (Undersander *et al*., 1991) and has a high tolerance to salinity (Ashraf *et al*., 2002; Ashraf *et al*., 2005; Francois *et al*., 1990). Additionally, this legume develops root nodules with nitrogen-fixing soil bacteria rhizobia in the surface part of its rooting system and a good capability to fix atmospheric nitrogen (Wetselaar, 1967; Elsheikh and Ibrahim, 1999). Its leaves and stems are mostly hairy, dependent on the cultivar. Its fine leaves have an elongated oval shape (5 to 10 cm length) and of alternate position.


Fig. 6.1 (a) unbranched type; (b) branched type

Fig. 6.2 Development pods

The inflorescence of guar is a raceme (Figure 6.3), about 9 to 13 cm long in the branched types and 15 to 20 cm long in the erect sparsely branched types. Normally, 40 to 60 flowers are present in an inflorescence of the branched types and 50 to 70 in the erect and sparsely branched types (Menon, 1973). Fruiting pedicels are stiffly erect in the leaf axils. Flowers are bisexuals, about 9 mm long, and almost sessile. The calyx has five sepals and the corolla has five petals the standard is circular, the wing petals are oblong, and the keel petals are as long and broad as the wing petals. The keel is blunt and slightly incurved, the pistil consist of one carpel. The ovary is linear, sessile and one-celled containing 6 to 10 ovules. The style is short and incurved with a head-shaped stigma (Menon, 1973; Gillet, 1958; Jafri, 1966). There are 10 stamens enclosed in the keel, the anthers are uniform, and the pollen grains are spherical in shape, averaging 40 to 43µ in diameter (Menon *et al*., 1968; Menon, 1973).

The pods hairy are rather flat and slim 4-12 cm length, containing 5 to 12 small oval seeds of 5 mm length (TGW = 25-40 g). Usually, mature seeds are white or grey, but in case of excess moisture they can turn black and lose germination capacity. The chromosome number of guar seeds is 2n=14 (Guarbohne, 2012). The seeds of guar beans have a very remarkable characteristic. Its kernel consists of a protein-rich germ (43-46%) and a relatively large endosperm (34-40 %), containing big amounts of the galactomannan (Mudgil, *et al.*, 2011).

Fig. 6.3 "Inflorescence of guar"

# 7. Biological cycle

Guar is a three-four months crop. In literature is reported that from sowing to harvesting it takes about 110 to 180 days, but in Mediterranean environment it can take up to 190 days. The figure 7.1 shows the major stages of guar crop cycle. Cycle starts with sowing in May. Germination takes place in 4 to 6 days of sowing in relation to temperature. Guar is an indeterminate plant showing continuous flowering and pods from the first weeks after germination up to the end of the biological cycle, if soil moisture is available. The pod formation takes place scalarly after flowering. The harvesting of the crop begins when 90 percent pods are matured roughly 90 to 110 days after germination, but it can be extended also over (depending on the variety, soil and climatic conditions; Santosh, 2014)

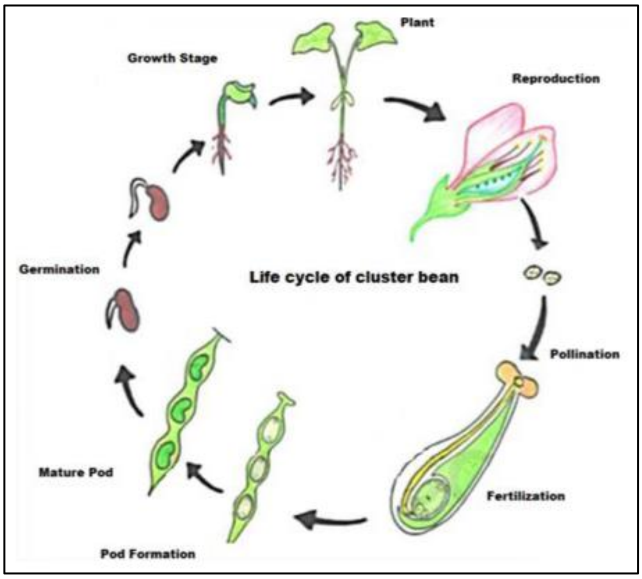


Figure 7.1 Crop cycle of guar

### 7.1 Germination

The constant temperature of 30°C appeared to be optimum for the germination of guar seed (Musil 1946). The lowest temperature for germination is considered 21°C, anyway it is able to germinate at lower temperatures, but with a greater time of germination and with a lower germination capability. However, storage temperature exceeding 16-35 °C coupled with high relative humidity can lead to the complete loss of viability (Doijode, 1989). Yadav and Pant (1979) also reported that the stockholding negative effects and high relative humidity are further compounded at temperatures elevate. Storage of guar seeds for six months significantly reduced the germination percentage (Kalavathi and Ramamoorthy, 1992).

Like many legumes, guar is not able to germinate at very low soil water potentials. Likewise, high salinity and submergence significantly reduced seed germination in guar (Yadava *et al*., 1975; Datta and Dayal, 1988).

Therefore guar seeds are known to germinate only after sufficient imbibition, approximately twice their original weight. A constant temperature of 30°C, seed treatment with dilute sulphuric acid (Musil, 1946), scarification for five minutes (Hymowitz and Matlock, 1964) and sowing depth of 1.5 to 3.0 cm (Zheng *et al*. 1980) are some of the practical means for obtaining prompt and uniform germination.

Musil (1946) suggested treating hard seed with dilute sulfuric acid or delaying planting until the soil is warm as a practical means of obtaining prompt and uniform germination. Hymowitz and Matlock (1964) obtained a significant difference in germination at 15.6, 21.1, 26.7, and 32.2°C. The greatest increase in germination occurred between 15.6°C and 21.1°C. At 15.6°C, the guar germination percentage was 23, while at 21.1°C the germination percentage was 81. When seed were scarified for 5 min and germinated at 21.1°C, the investigator obtained a 17% increase in germination in the scarified seed over the un-scarified seed. Their data suggest that in order to obtain good stands o guar, the crop should be planted in the field when the soil temperature is at least 21°C.

Mehta and Desai (1958) studied the effect of soil salinity on the germination of seed of several crop species. They added sodium chloride and calcium chloride at eight salinity levels (from 0-2.5% of soil solution at field capacity) to a fine sandy loam soil. Guar seed exhibited the greatest salt tolerance, followed by pearl millet, cotton, and hyacinth bean. Salt tolerance of guar also was reported by Thomas (1936). Yadava *et al*. (1975) observed significant difference in germination of seed from nine varieties of guar when they were germinated in petri dishes containing solution of NaCl, $Na_2CO_3$, $NaHCO_3$, and $CaCl_2$ each at 11 difference electrical conductivity levels. They found that the relative toxicity of the salts to seed germination was in the ascending order of $NaHCO_3$, $Na_2CO_3$, $CaCl_2$ and NaCl.

The endosperm of guar contains about 41% dry matter and 45% acetone insoluble solids (75% of this comprises galactomannan and 3-11% N and P), McClendon *et al*. (1976) in a five days germination study observed that galactomannan and almost 95% dry matter was translocated from endosperm to cotyledons. In addition, N and P were also translocated.

Pre-soaking of seeds at 25-30°C, reversed the low temperature mediated inhibition due to increase in the activity of certain isoenzymes peroxidase (Zheng *et al*., 1980). Pre-soaking in in NaCl or solution of trace elements also increased germination of guar (Singh *et al*., 1976). This has been attributed to mobilization of material into growing seedlings due to hydration-dehydration (Doijode and Raturi, 1987).

### 7.2 Seedling growth

Kathju *et al*. (1971) studied the effect of cycocel (2-chloroethyl trimethyl-ammonium chloride) and gibberellic acid and their interaction on the growth behaving of germinating seedling of guar. Gibberellic acid completely reversed the hypocotyl inhibition induced by cycocel and partially that of the radicle. Gibberellic acid increased the activities of β-glycerophosphatase, acid pyrophosphatase, and alkaline pyrophosphatase. Cycocel inhibited the activities of these enzymes.

When the two chemicals were applied simultaneously, gibberellic acid could not reverse the inhibitory effect of cycocel. Additional studies by Kathju *et al*. (1972) revealed that gibberellic acid caused an increase in the activity of α-amylase in the cotyledons, while cycocel depressed the α-amylase activity in both the cotyledons and seedling axes. Cycocel inhibited the activity of starch phosphorylase, while gibberellic acid did not influence the activity of starch phosphorylase.

### 7.3 Vegetative growth

In the early 1900s, Leather (1910) conducted investigations on the water requirement or transpiration ratio of crops grown in India. The transpiration ratio in the ratio between the total water lost during the growing season and the total dry matter accumulation in the plant. Leather found that unfertilized guar had a transpiration ration of 1,100:1, while fertilized guar had a transpiration ratio of 600:1. For comparison, fertilized maize, sorghum, and pigeon peas had transpiration ration of 300:1, 400:1, and 600:1, respectively. Leather concluded that fertilization od crops increased their efficient use of water; that crops which matured rapidly had lower transpiration rations; and that, in general, cereals had lower transpiration ratios than the legumes. Hymowitz and Matlock (1964) measured the growth of Groehler guar grown under natural rainfall conditions at Stillwater, Oklahoma. When planted in early June, Groehler reached its peak height of 89 cm in the second week of September. The growth equation for Groehler, as measured by plant height during the growing season, followed the standard sigmoid curve. The greatest increase in growth took place during the fourth week in July.

### 7.4 Nodulation

Guar shows a good capability to fix atmospheric nitrogen (Wetselaar, 1967; Elsheikh and Ibrahim, 1999). The symbiotic relationship between *Rhizobium* bacteria and legumes is well known. The bacteria live in nodules formed on legume roots. The legume plant furnishes carbohydrates to the bacteria, while the bacteria change atmospheric nitrogen into a form that the legume plant can assimilate and use to build protein.

Strains of legume bacteria are selective in terms of which crop species they will nodulate. Cross-inoculation groups have been developed to account for these differences. Any plant within a cross inoculation group con be inoculated with a culture of the right kind of bacterial strains. The main cross-inoculation group are: alfalfa, clover, cowpea, bean, lupine and soybean (Erdman, 1967).

Rochmond (1926) established that guar belonged to the cowpea cross-inoculation group. He found that rhizobia strains that formed nodules on cowpeas also formed nodules on guar roots. Pure cultures of bacteria taken from guar nodules formed nodules on guar roots; pure cultures of bacteria taken from guar nodules formed nodules on cowpea roots. Erdman (1948) found two strains of *Rhizobium*, one from *Crotalaria sagittalis* and another from *Erythrina indica*, that were highly effective in promoting vegetative growth and nitrogen fixation in guar plants.

Narayana (1963) observed that in guar, rhizobia enter through the root hairs and from a single unbranched infection thread. On the sixth day of seedling growth, he found that a nodule had formed on the primary root close to the surface of the soil. Subsequently, additional nodules were formed on the primary root and then on roots of other orders. According to Narayana, the effective nodules are slightly pink in colour, while the ineffective nodule are white or brown. The nodules wrinkle with age and change colour from pink to black or green.

Oke (1967) found that fixation reached a maximum of 5 mg nitrogen per day per guar plant and that over 90% of the nitrogen was transferred from the nodules to other parts of the plant throughout the life of the experiment. Oke noted that younger guar plants seemed to be more efficient fixers of nitrogen than older ones.

Wetselaar (1967) observed that The amount of nitrogen fixed by guar on Tippera clay loam it was 88.9 Kg nitrogen an acre.

In a laboratory experiment, Taylor and Gardener (1960) studied the penetrating ability of roods of various plant species. Waxes provided nonporous substrates of different rigidity. They found that the penetrating ability of guar roots was not significantly greater than non-legumes such as cotton and sesame. Further studies by Taylor (1962) and Parker and Tayler (1965) revealed that seedling emergence of guar was affected by hardness and thickness of crusts. In addition, increasing planting depths drastically reduced emergence of guar through compressed soil cover.

**7.5 Flowering**

Guar is completely self-fertile and is highly self-pollinated. Flowers pass through an array of colours from white to deep blue from the bud stage to petal drop, and a mature bud is creamy white in colour (Figure 7.2). Petals develop a pink colour just prior to opening. Anthers dehisce about 1.5 to 2 hours before flower opening, and pollen was found to be capable of germination 2 hours before and 11 hours after flower opening. Buds change from a cream colour to light pink between 8:00 and 10:00 am and petals started opening about 9:00 am (Menon, 1973). Petals continue to open until 16:00 pm, with a few flowers opening as late as 13:00 pm. Flowers that open early in the morning shed petals the same day, between 17:00 and 20:00 pm. Late opening flowering shed petals the following morning (Menon, 1973). The longevity of pollen at room temperature was reported by Menon *et al.* (1968) to be 11 to 13 hours and maximum pollen germination of 46% was obtained between 8:00 and 9:00 am. Each pollen cell normally produced one tube, although two tubes occasionally were observed. The average pollen tube length recorded was 1.6 mm.

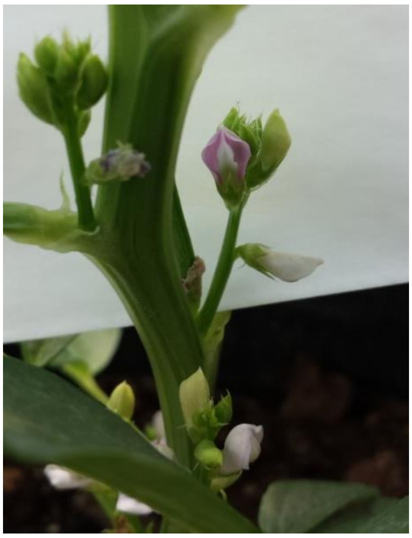

Figure 7.2 Guar flowers at different stages

## 7.6 Leaf, seed and pod development

Guar has two leaf forms: a simple leaf and a trifoliate leaf. According to Sparks and Postlethwait (1967), both short daylengths and high temperatures delay the production of the trifoliate form. Treatments using gibberellic acid, reduced light, and the removal of cotyledons of young seedlings favour the production of the single leaf form. The investigators attempted to determinate the precise point at which the leaf primordium become destined to produce either a simple or trifoliate leaf. They observed that up to 140µ in length, the leaf primordia are morphologically indistinguishable. If a simple leaf is to be formed, the marginal meristem gives rise to only lamina. If a trifoliate leaf is to be formed, the marginal meristem is interrupted by a pocket cell area dividing it into an upper lamina meristem and a basal portion, which gives rise to lateral leaflets (Sparks, 1967).

In the young flower bud, the ovule, consist of nucellar tissue surrounded by two integuments, supported on a funicular stalk. Within the ovule, a single megaspore grows into the embryo sac. The megaspore undergoes mitotic divisions to form a monosporic, 8-nucleate female gametophyte structure. After fertilization, the zygote divides by a transverse wall to form a basal cell and a terminal cell. Ultimately, the terminal cell develops into the embryo proper and the basal cell forms the suspensor supporting the embryo.

At the time of fertilization, a nuclear type of endosperm is initiated by the fusing of a sperm nucleus and two polar nuclei. As the endosperm develops, the nucellus is broken down and at seed maturity the nucellar tissue is no longer present. The endosperm, which in most legumes is commonly digested and absorbed during embryo development, persist in the mature guar seed. Galactomannan gum extracted from guar seed is located in the endosperm. The outer integument is modified to form parts of the seed coat, while the inner integument in the mature seed has been broken down or resorbed (Anataswamy 1954; Green *et al.* 1964; Saber *et al.* 1956).

Menon *et al.* (1971) studied the effect of removal of floral buds from different portions of the inflorescence on total pod. They found that all flowers in an inflorescence of guar have the potential of developing into pods. In addition, they observed that removal of floral buds from different portions of an inflorescence did not increase or decrease pod set. The control treatment of on floral bud removal from an inflorescence produced the same number of pods as those inflorescences that had floral buds removed. The average number of pods per cluster was seven, and about 12.5% of the flowers developed into pods.

# 8. Cultivation techniques

## 8.1 Land preparation

Guar crop requires a well prepared field, with adequate soil moisture for its seed germination. During early growth period, soil aeration encourages root development and bacterial growth. Therefore, field should be ploughed by giving two or three deep ploughing with soil-turning plough, followed by milling and planking. At the time of sowing field should be well drained and free from weed to ensure good germination. Arrangements for drainage channel-cum-water channel for heavy rainfall areas or irrigated areas should be made while preparing the field. The farmyard manure should be mixed with soil at the time of last ploughing.

## 8.2 Sowing requirements

Sowing method:

In India, farmers sow guar mainly by traditional method of broadcasting. Broadcasting is done manually spreading seed over the soil surface. After broadcasting the seeds, one ploughing is required for proper seed incorporation in the soil. In this method inter and intra row spacing is not followed which creates problem in intercultural operations like hoeing, weeding and removing excessive water in the field. Strategy for proper line and row spacing may help in enhancing the productivity of the crop. Line method normally is done by seed drill which ensures sowing with proper spacing and depth. This method results in good germination and proper weed management and drainage of excess water in the field (Santosh, 2014).

Sowing time:

The sowing times is one of the important variables bringing about dramatic changes in growth environment of the crop, and consequently the yield variables. There are numerous reports which indicate that yield is considerably reduced when sowing is delayed to last week of July or first week of August. Similarly, early-sown clusterbean makes luxuriant grown and the crops is damaged due to lodging. Bains and Dhillon (1977) conducted experiments on various sowing dates of guar at Ludhiana and observed that first week of July was the best sowing-period compared to third week of July and first week of August, giving 19.4 and 76.6% higher seed-yield over the later respective dates. Maximum seed yield of guar could be realized with 5 July sowing in comparison to that sowing on 20 June, 20 July and 5 August in a study at Hisar

(Sharma BD *et al*., 1984). In another trial conducted at Hisar, 15 July was found optimum sowing time. Too early (1 June, 15 June, and 30 June) and too late (30 July) sowing resulted in lower seed yield as compared to 15-Jule sowing, for instance, respective reductions in yield were to the magnitude of 41.77, 19.73, 6.80 and, 38.40% (Sigh *et al*., 1979). Similar results on seed yield were also reported by Das *et al*. (1977), Singh *et al.* (1980) and Tomer *et al*., (1976). Study conducted at Jhansi, revealed that sowing done between 15 and 25 July, gave significantly higher grain yield that early (25 June - 5 July) or late (5 August) sowing. It was therefore, concluded that for higher seed yield on drylands of Bundelkhand region (UP), guar could be sown on 15 to 25 July (Ali *et al*., 1978). At Jodhpur, the seed yield of guar declined with delayed in sowing beyond 1 July, and the reduction was to the extent of 32.5 and 63.7%, when sowing was delayed up to 30 July and 16 August, respectively (Singh and Singh, 1977). Based on work conducted at Hisar, Jain *et al*. (1987) recorded maximum value of yield (16.75 q/ha), protein (5.65 q/ha) and gum yield (4.85 q/ha) at 10 July sowing. Similarly, Teneja *et al*. (1995) reported 10 July as the optimum sowing time which gave maximum seed yield (21.7 q/ha), whereas further delay in sowing to 30 July caused drastic reduction in seed yield 12.6 q/ha). Bhadoria and Chauhan (1994) also got the similar trends relating to sowing time on yield and quality.

Dhukia and Singh (1988) indicated high crude-protein content, protein yield and gum yield with 10 July sowing. The gum content in seed, however, increased with delay in sowing. Dry matter accumulation, leaf-area index and seed yield were higher with 10 July sowing in comparison to delayed sowing to 25 July and 9 August (Dhurkia *et al*., 1990).

Spacing:

Spacing is required to ensure proper utilisation of inputs like nutrients, moisture and light resulting in better production performance of the plant. The sowing strategy involves inter and intra row spacing. The spacing depend on the optimum plant stand/population required for different regions having varied rainfall intensities. Cultivars of guar are reported to show variation as regards their growth habit and yield potential and respond differentially to plant population (Mittal *at al*., 1977; Singh and Tomer, 1975). Trials on sowing methodologies conducted under the aegis of *National Network Research Projects on Arid Legumes* reveal that the recommended spacing should be 10-15 cm plant to plant distance in a row and 35-60 cm row to row distance on the basis of rainfall, soil type and inputs availability.

Trials on sowing methodologies conducted under the aegis of *National Network Research Projects on Arid Legumes* reveal that the recommended spacing should be 10-15 cm plant to plant distance in a row and 35-60 cm row to row distance on the basis of rainfall, soil type and inputs availability. For instance, for low rainfall (200-350 mm) zones like Jaisalmer, Barmer, Bhuj, Jodhpur, etc., optimum plant population is 110,000 plants/ha, the same can be obtained with sowing strategy of 0.60 x 0.10 m (inter and intra row spacing). Similarly, for the regions particularly semi-arid, having moderate rains (450-500 mm), the optimum sowing strategy has been found to be 0.45 x 0.10 m, which may give required plant population of 150,000 plant/ha. For those regions having still higher rainfalls (550-600 mm) planting pattern of 0.30 x 0.10 m has been observed optimum and maximum grain yield can be harvested with 200,000 plants/ha. All these strategies are for branching varieties. However, for the varieties having un-branched growth, the optimum plant spacing having been observed to be 0.25 x 0.10 m. Under late sown conditions, close planting from more seeds and closer interspacing have proved optimum. Under normal sown conditions, invariably a plant population of 125,000-150,000 plants per hectare is optimum at 45 cm spacing for branched type varieties. In late sown condition, inter row spacing of 0.30 m was better than 0.45 m (Santosh, 2014).

Inter row spacing (30, 45, 60, 90 cm) effect on the growth and yield of guar studied under rain-fed condition at CAZRI, Jodhpur revealed that grain yield decreased with increasing row spacing, while yield per plant increased with increasing row spacing (Singh 1977). A combination of 45 inter and 15 to 20 cm intra row-spacing was found to be optimum for variety FS 277 when planed at normal sowing time (middle of July). For late sown crop (14 August), a row spacing of 30 cm, instead of 45 cm, was found optimum. Sharma *et al*. (1984) and Taneja *et al*., (1984) obtained higher seed yield at 30 cm row spacing for variety FS 277. Similar studies at Hisar of India indicated that intra-row spacing of 15 cm gave significantly higher grain yield (18.03 q/ha) and maximum gum content over 7.5 cm (15.91 q/ha) and 22.5 cm (15.0 q/ha) sowing (Das *et al*., 1977).

In AICRP trials conducted during Kharif 1977 at five location (Jodhpur, Bikaner, Durgapura, Junagarth and Agra), it was observed that higher yield of variety FS 277, 2470, Durgapur Safed and KVS-1 could be achieved with inter row spacing of 60, 45 and 30 cm, respectively (Singh, 1978). At Hisar, 45 cm row spacing was found suitable for HG 75, Dergapus Safed (Taneja *et al*., 1982) and Hg 182 (Jain *et al*., 1987), whereas 30 cm row spacing was optimum for FS 277 (Taneja *et al*., 1982, Yadav *et al*., 1989).

Bains and Dhillon (1977) recommended a spacing patter of 30 x 22.5 and 45 x 15 cm at Ludhiana. However, 45 x 15 cm con be adopted safely. At Jhansi, row spacing of 60 cm (1.1 lakh plants/ha) was optimum for Durgapur Safed, 45 cm (1.5 lakh plants/ha) for B19-55 and 30 cm (2.22 lakh plants/ha) for FS 277 (Ali, 1982). However, Singh (1982) suggested a row spacing of 45 cm (1.25 to 1.50 lakh plants/ha) for optimum seed yield for branched variety HG 75 at Dryland Research Station, Bawal (Haryanna).

Rana *et al*., (1991) obtained higher seed yield with 30 cm row spacing than 45 cm under late sowing condition. An intra-row spacing of 15 cm was more suitable for branched and 10 cm for unbranched varieties. Higher LAI, LAD and CGR were observed with 30 cm row spacing, but seed yield was more with 45 cm row spacing (Yadav *et al*., 1990). In further studies carried out at Hisar, Yadav *et al*., (1991) found that total dry-matter, N and P content in seed and straw were not influenced by different row spacing. However, total uptake of N and P was significantly higher in 45 cm row spacing than 30 cm row spacing.

<u>Seed treatment:</u>

Seed treatment is essential for checking early seedlings mortality and development of diseases at later stages.

a) For killing the spores of dry root rot fungus, seed is treated with Ceresan or Thiram at the rate of 3 gm/kg seed.

b) Seeds can be treated with Imidachlorpid at the rate of 6 ml/kg seed to control sucking pest like Jassids and Aphids.

c) Immersion of seeds in hot water at 50°C for 10 minutes followed by drying at room temperature before sowing helps in killing fungus mycelium and inactivating their spores (Santosh, 2014).

## 8.3 Inoculation

Guar is a legume crop and its roots bear nodules with special kind of bacteria that converts atmospheric free nitrogen to fertilizer available form. This symbiotic relation between bacteria and root nodules helps to reduce the cost of nitrogen fertilizer for crop. Therefore, to maintain the population of bacteria in the soil with respect to growth of plant seeds of Guar are to be inoculated with these bacteria before sowing. This is done by preparing a 10 percent sugar or guar solution in boiling water. This sugar solution is allowed to cool. After cooling, 3-4 packets of Guar bacterial culture (*Bradyrhizobium spp.*) are mixed to make a thin paste. This paste is coated over to the seed. Seed is dried under shade for 30-40 minutes before sowing (Santosh, 2014).

Elsheikh and Ibrahim (1999), studied the effect of inoculation on yield of guar. Bradyrhizobium strains TAL 169 and TAL 1371 (introduced) and strains ENRRI 16A and ENRRI 16C (local) were used to inoculate five guar cultivars, namely, HFG-75, HFG-182, HFG-363, HFG-408 and WB-195. Most of the *Bradyrhizobium* strains significantly increased yield, protein, crude fibre, and mineral content. The locally-isolated strains affected the parameters more than the introduced ones.

## 8.4 Irrigation

Guar is a drought-tolerant summer legume that requires only 300-400 mm annual rainfall. Adequate moisture ensures maximum production of forage and grain. Areas with low humidity are best for growing Guar as grain crop. Farmers historically use irrigation first on high-yield, high-value crops. At most, only a few percent of U.S. guar hectares are irrigated. Farmers historically use irrigation first on high-yield, high-value crops (Trostle *et al*, 2012), though the crop responds well to supplementary irrigation (Santosh, 2014). One lifesaving irrigation at 60 days after sowing, increases the grain yield by 32.1% over no-irrigation on loamy-sad soil at Jobner (Rajasthan), (Meena *et al*., 1991). One post-sowing irrigation scheduled at 275 mm CPE, at 80 days of sowing It is not particularly significant to increase in seed yield, dry-matter accumulation and leaf-area index (Dhukia *et al*., 1990). The seed protein content result significantly improved when the crop is irrigated at 55 days of sowing, but irrigation beyond third week of September decrease the seed quality (Singh *et al*., 1980).

Nevertheless, guar can be effectively grown with limited irrigation though it is not essential, which is one advantage that guar frequently has versus other crops in hotter, drier environments. Farmer records suggest that West Texas guar yields approximately 0.11-0.17 t $ha^{-1}$ per 1 of irrigation. Individual fields that have received higher levels of irrigation, more than 12, often have higher disease incidence. Field observations suggest that, after initial irrigation and when guar starts flowering in 30–40 days after planting, over-the-top sprinkler irrigation should be avoided. In cases of heavy irrigation, the plants turn nearly black with disease and yields are even reduced relative to adjacent non-irrigated land (Abidi N *et al*., 2015).

## 8.5 Fertilization

<u>Effect of nitrogen</u>

Being a leguminous crop, it does not respond much to nitrogen, yet a small starter dose (20 kg N/ha) stimulates growth of the plants in early stages. Application of small dose of nitrogen improves the nodulation and nitrogen fixation in guar (Atwal and Sidhu, 1964). Application of 20 kg N/ha results in higher grain and straw yield, nodulation plant and root dry-weight (Singh and Singh 1989). On sandy loam soil application of 20 kg N/ha it leads to significant increase in physiological parameters, crop growth rate, leaf-area index, leaf-area duration, dry matter accumulation, uptake of N and P in seed and yield of protein and gum (Yadav *et al*., 1989; 1990; 1991).

Studies carried out at Hisar prove that 30 kg N/ha increase the seed yield, N-uptake and concentration of N in seed and straw (Singh *et al*., 1993).

Effect of phosphorus

The application of phosphoric fertilizer is important for effective nodulation, bolder seed size and consequently improved yield (Misra, 1961; Singh and Singh 1989). Application of phosphorus helps in better plant growth lead by better root system (elongate) and increased number of seeds per pod and per 1000-seed weight. Application of 40 kg $P_2O_5$/ha increase the seed and stalk yield, dry matter accumulation, crop growth rate, leaf area index, leaf-area duration, number of pods per clusters, number of pods per plant and seed yield per plant (Yadav *et al*., 1989; 1990; 1991). On loamy-sand soil a dose of 30 kg $P_2O_5$/ha is the most economic dose (Dahiya *et al*., 1986).

Therefore, application 40 30 kg $P_2O_5$/ha was found superior to 20 kg with regard to increase in grown, yield attributes, seed yield and stover yield (Shivran *et al*., 1996), whereas, in earlier study at the same location, Meena *et al*. (1991) reported 30 kg $P_2O_5$/ha as optimum dose. Similar response of phosphorus was observed at Bhatinda (Singh and Tiwana, 1995). Significantly higher response on yield od seed and straw with the application of 40 kg $P_2O_5$/ha over 20 kg and control (Solanki *et al*., 1998).

Singh and Singh (1989) observed a significant increase in the content and yield of protein and gum with the application of 60 kg $P_2O_5$/ha over 30 kg $P_2O_5$/ha and control. Yadav *et al*. (1989) observed that contend of protein and gum remained uninfluenced with phosphorus application, but yield of protein and gum increased with 40 kg $P_2O_5$/ha over control. Jan *et al*. (1988) revealed that gum content increased with the application of phosphorus of 20 kg $P_2O_5$/ha (29.41%) over control (27.95%). Higher doses of phosphorus (40 and 60 kg $P_2O_5$/ha) resulted in lowering of gum content, though it was higher (29.04 and 28.87%) than the control value. The extent of increase in protein content was recorded as 1.17, 2.50 and 2.81% over control, with 20, 40 and 60 kg $P_2O_5$/ha, respectively.

P in combination with N, increased the seed yield significantly over control, whereas, the response in kg/ha of applied P was 9.0 and 8.2 at 20 and 40 kg $P_2O_5$/ha, respectively (Dahiya *et al*., 1986).

Nodulation in guar was found positively correlated to phosphorus uptake leading to higher nitrogen fixation (Rewari *et al*., 1965). Gill (1979) observed that P content and its uptake improved in plant by phosphorus fertilization. Yadav *et al*. (1991) found significantly higher uptake of N and P in both seed and stalk of guar, with the application of 40 kg $P_2O_5$/ha, over control. Singh and Singh (1990) also found increase in both contents and uptake of N, P and K with increasing rates of $P_2O_5$ (0, 30, 60 kg/ha).

<u>Effect of sulphur</u>

Sulphur is known to increase growth, yield attributes, straw yield, seed yield and harvest index up to a dose of 60 kg S/ha on loamy-sand, S-deficient soil under rain-fed condition (Shivran *et al*., 1996). However, application of 40 kg S/ha on S-deficient soil, increases grain yield as well as quality characters, phosphorus, sulphur, gum and protein content in grain (Bhadoria *et al*., 1997). Application of 20 kg S/ha it is insignificantly, with higher seed yield (12.12 q/ha) and gum content (29.98%) over control (10.82 q/ha, 29.10%) at Bawal (Kumar *et al*., 1999). Studies carried out on the effect of varying S-sources on S-deficient sandy-loam soil showed that gypsum was better than pyrite. The number of seeds/pod and branches were higher with gypsum than pyrite (Shekhawat *et al*., 1996).

<u>Effect of zinc</u>

Guar responds very well to $ZnSO_4$ up to 25-30 kg/ha. Hence it is recommended to supply basal dose of 25 kg $ZnSO_4$/ha. Soil application of 50 kg $ZnSO_4$/ha increases guar yield under acute Zn-deficiency. Zn applied to soil, was more effective in increasing yield than that applied as foliar spray (Takkar *et al*., 1973). Application of zinc of 10 kg/ha produces significantly higher primary branches, pods per plant and grain yield (Maliwal *et al*., 1987).

Studies carried out at Durgapura showed that foliar spray of 1 kg Zn/ha (0.2%) at 45 days of sowing was better than soil application of 5 or 10 kg Zn/ha. Pot-culture studies on the effect of source ($ZnSO_4$), dose (5 and 10 kg/ha) and mode (soil application, seed coating, foliar spray) of Zn application revealed that 3 sprays of 0.5% $ZnSO_4$ at 15 day interval proved very effective, which increased proteins as well as carbohydrate content of both leaves and nodules, and significantly improved the number of pods, seed weight, leaf area, nitrogen content and nitrogenase activity per plant (Nandwal *et al*., 1990). Studies on the effect of micro-nutrients conducted at Hissar, Bawal, Durgapura and Gwalior revealed that at all the locations one spray of 0.5 percent $ZnSO_4$ either at 25 or 45 DAS (days after sowing) gave significantly higher seed yield than control, but statistically equivalent to soil application of $ZnSO_4$ 25 kg/ha except at Hissar where 0.5 percent $ZnSO_4$ spray proved better than soil application.

Micronutrients Zn, Cu, Ma and Fe, applied individually or collectively, revealed that Zn alone or in combination with other nutrients, significantly increased the dry-matter yield, its concentration and uptake. FeSO4 had no effect on the seed yield of Guar at Hissar and Bawal but significant response was observed at Durgapura and Gwalior, where one spray of 0.5 percent of FeSO4 at 45 DAS gave significantly higher seed yield than control (Kumar, 2009).

### 8.6 Crop rotation

The guar plant is characterized by a spring-summer cycle that fits well into a crop rotation (Tucker and Foraker, 1975) that can be profitably introduced into crop rotation systems since it has been demonstrated to increase yields in succeeding crops (Whistler and Hymowitz, 1979; Rao *et al.*, 1995; Saxena *et al.*, 1997).

### 8.7 Guar maturity and harvest management

Guar maturity depends on the indeterminate nature of the plant (it will keep growing if possible), but current varieties can reach suitable maturity in about 90–120 days (Trostle *et al*., 2012).

Even after a moderate freeze, the guar stem may remain green and tough for up to a month. This makes combine harvest and threshing more difficult. Thus, much guar is not harvested until well into November and December. Prolonged field exposure to environmental factors makes the guar pod prone to shattering off the plant. Some custom harvesters have added air reels to their combine to shoot a blast of air at the cutter bar to push shattering pods onto the header and reduce harvest losses as much as 56.04 Kg/ha (Trostle *et al*., 2012). Excessive rain after maturity may increase the proportion of black seed that is perceived to have lower quality (either reduce gum content or lower molecular weight gum), (Liu *et al*., 2007).

Current research in the USA is investigating the use of harvest aids, similar to how cotton is managed for earlier harvest (Kelley, 2013), to hasten harvest and potentially preserve higher quality seed and gum. Currently, paraquat dichloride and sodium chlorate are labelled for harvest aid use in guar. A particular advantage of harvest aids is their application when the bulk of the guar seed in a field is mature with little additional yield potential present. Harvest aid applications will terminate any further growth and could potentially move the harvest forward a month or more relative to natural drying.

# 9. Genetic and breeding

Guar, until recently considered a minor crop, seems destined to assume a larger role among the domesticated plants. However, it has so far received practically little attention by the researchers as far its genetic improvement is concerned. Here I report a synthetic frame on cross-pollination and breeding.

### 9.1 Crossing techniques

Guar is a strictly self-pollinated crop because of its cleistogamous nature. The extent of outcrossing has been found to vary from 0.3 to 7.92 per cent (Menon *et al*., 1973; Saini *et al*., 1981; Chaudhary and Singh, 1986).

<u>Artificial Hybridization</u>

Chaudhary *et al*. (1974) developed a crossing technique which is two to three times more effective than the usual direct emasculation and pollination procedure. By using this techniques, the investigators could get a 7% pod. The methodology is clearly described in materials and methods, since it has been adopted in one of the experimental trial.

<u>Natural Hybridization</u>

Another reasonably successful method has been developed for using controlled, natural hybridization to generate new material for selection and evaluation. By utilizing the genetic marker glabrous-pubescent leaf type, hybrid plants can be easily identified and utilized for further evaluation. In this procedure, a pubescent breeding line (male parent) and several glabrous breeding lines (female parents) are planted in a block, well isolated from other guar. Isolation requirements equal to or greater than that for Foundation seed production (200m) are followed. A common design is to plant every third row with the pubescent parent and plant glabrous lines in the remaining rows. Rows in the crossing blocks are generally 5 m long and spaced 1 m apart. Bees or other insect pollinators are needed to achieve maximum crossing between the pubescent and glabrous line.

No special treatment of plants is required for natural crossing. Natural crossing between early maturity breeding lines and medium to late maturity lines has been found to be only slightly less than crossing within a given maturity class. Altering flowering dates by date of planting does not seem to be necessary in obtaining natural crosses in guar.

Seed harvested from the glabrous lines on a single row basis. The following season, several rows 100 m in length are planted form seed of each harvested row. Pubescent plants (hybrids) are tagged during the growing season and harvested in a single plant basis. Detectable natural crossing (pubescent x glabrous) using only will insect pollinators has ranged from less than 1 to 4.4% at Chillicothe, Texas (Stafford and Lewis, 1975). Other natural crossing (globrose x glabrous)is less easily detected and has not been utilized.

Genetic male sterility was reported by Mital *et al*. (1968), but has not been exploited for hybrid seed production. Semisterility caused by reciprocal translocations has been reported in the United States (Kinman *et al*.,1969) and Indian (Vig, 1965). It has been of little utility from the standpoint of hybridization techniques.

### 9.2 Germplasm collection and evaluation

Enormous variability in guar germplasm is represent by branched or unbranched, hairy or smooth or sickle shaped pod, pubescent or glabrus, determinate or indeterminate, regular or irregular pod bearing types. A wide range of genetic variability for plant height (78-226.6 cm), branching behaviour (0-22), pod length (9.0-12.5 cm), 1000-seed weight (38.0-40.0 g), gum content (22.5-39.8%) was reported by Mittal *et al*. (1977) from a collection of more than 1500 accessions of guar. Moderate to high estimates of genotypic coefficient of variability for seed yield and most of its component characters of guar have also been reported by Sanghi *et al*. (1964), Mittal *et al*. (1969), Dass *et al*. (1973), Tikka et al. (1974), Tripathi and Lal (1975), Paroda et al. (1977), Dabas et al. (1982), Arora and Lodhi (1995) and Satyaparkash and Singh (2000). The selections made from land races have also resulted in the release of improved cultivars of this crop (Singh and Sikka, 1955; Mittal et al., 1963; Singh and Sharma, 1965; Saini et al., 1982; Henry et al., 1992; Bharodia et al., 1993, Singh et al., 1995).

# 10. Aim and scope

Guar genotypes are characterized by a large variability, ranging from 0.4 to 3.0 m in plant height, with a growing season from 70 (determinate genotypes) to 190 (indeterminate genotypes) days, from single stem to basal branching, etc. Moreover, differences in guar galactomannan content and its properties have been recently observed among varieties (Liu *et al*., 2009; Liyanage *et al*., 2015). Obviously, the high yield genotypes are those with indeterminate growth, but this last trait creates many problems at harvest. Guar is mainly cultivated in areas of the word such as India, Pakistan and Texas with climate characteristics quite different from the Mediterranean one, but recently its valuable performance in irrigated areas in this environment has been widely demonstrated (Sortino and Gresta, 2007; Gresta *et al*., 2013; Gresta *et al*., 2016a, Gresta *et al*., 2016b). In any case, appropriate genotypes suitable for this environment, able to maximize seed production, are still missing. In fact the main problems of guar are an excessive length of the crop cycle, a low insertion of the first fertile node and a yield that rarely exceed the 2.5 t $ha^{-1}$. A too long crop cycle create problems at harvest, since in Mediterranean environment it takes place after the first rains of late summer, which implies the blackening of the seeds and the consequent lowering of their commercial value (Liu *et al*., 2007). In fact, galactomannans are extracted with more difficult and with a lower quality in darker seeds The low insertion of the first fertile node represents an obstacle for wheat combine harvester for a possible loss of seeds, when the branches are not intercepted by the cutter bar (basal branched genotypes). This problem is absent in single stem genotypes, that also allows for a higher sowing density and a consequently higher seed yield. Although the widespread importance in industrial use, limited interest from the research community was granted to guar, especially for worldwide germplasm characterization (Raghuprakash *et al*., 2009; Morris, 2010, Sultan *et al*. 2012). Conversely, a successful crop improvement program starts from the evaluation of germplasm variability (Poehlman and Sleper, 1995). The variability among germplasm can be assessed by using morphological, biological and productive traits.

Therefore, the aim of the present research was to look for a Mediterranean ideotype of guar through: *i*) Characterization of 68 guar genotypes coming from different areas of origin, focusing our attention on the morphological traits that have a role in seed yield expression in Mediterranean-type environments; *ii*) Evaluation of galactomannans and protein content of those genotypes; *iii*) Characterization of 25 selected genotypes ; *iiii*) Cross pollination of valuable guar genotypes.

# 11. Materials and Methods

**Trial 1** - Morphological, biological and productive characterization of a guar seed collection coming from international seed banks, in order to select genotypes showing characteristics suited to the Mediterranean environment and to an easy mechanical harvesting (elevated height of the first fertile node, single stem and high seed production).

**Trial 2** - Determination of galactomannan and protein content of the above mentioned guar seed collection.

**Trial 3** - Morphological, biological, and productive evaluation of 25 genotypes selected on the basis of their morpho-bio-productive traits, among the 68 genotypes already characterized and from other trials previously performed on this species.

**Trial 4** – Cross pollination trial carried out between guar genotypes conducted over two years: a first test was conducted in the second year of PhD on 25 selected genotypes and the second test was conducted the third year on 13 selected genotypes.

<u>**Trial 1**: Morphological, biological and productive characterization of 68 guar genotypes.</u>

The trial was carried out in Botricello, southern Calabria, Italy (CZ, 19 m a.s.l., 38° 56′ 00″ North, 16° 51′ 00″ East) in 2014, on a sand-silt soil, which main parameters are reported in table 11.1. It consisted of a morphological, biological and agronomic characterization of 68 accessions of guar (*Cyamopsis tetragonoloba* L.) provided by different seed banks, all listed in the table below (Table 11.2).

In April, a rotary hoeing was performed in order to bury the crop remains of the common wheat adopted as previous crop. At the end of April a 30-40 cm ploughing was executed followed by a rotary harrowing. At the seedbed tillage, a fertilization using 200 kg $ha^{-1}$ of 11 22 16 and 130 kg $ha^{-1}$ of mineral phosphate (20%) for a total amount of 22 kg $ha^{-1}$ di N, 70 kg $ha^{-1}$ of P2O5 e 32 kg $ha^{-1}$ of $K_2O$ was supplied. As a consequence of the lack of nodulation, during the crop cycle, 100 kg $ha^{-1}$ of N as ammonium nitrate were also applied.

Sowing was performed manually on the 10 of May on row 1.0 m long, adopting an intra-row distance of 0.10 m and an inter-row distance of 0.50 m, therefore it has been used a density of 20 plants of $m^2$.We were obliged to a reduced experimental design due to the little availability of seed supplied by the seed banks. Seed were placed at 2/3 cm depth. The rows were north-south oriented. A completely randomized design was adopted with plot of 1.5 x 1.0 m three time replicated. Around the experimental field, two guar rows were sowed, to avoid any edge effect.

Just after sowing, a chemical weed management has been adopted, using a mix of Stomp aqua (a.p. Pendimetalin) and Afalon (a.p. Linuron), at the dose of 2+1 l $ha^{-1}$ of c. c. (chemical compound), respectively. Subsequently, the rows were irrigated with a sprinkling system that, in addition to the normal function humectant, also had the purpose of trigger the active ingredients of herbicides. A second irrigation was performed five days after the first one. From now on, water supplies were assured with a drip system. As a whole, a total volume of about 3.000 $m^3$ $ha^{-1}$ was applied.

The irrigations were carried out on the following dates:

- 26.05.2014;
- 10.06.2014;
- 20.06.2014;
- 08.07.2014;
- 25.07.2014.

Weed control, born during the test, was assured by manual weeding.

Harvest was carried out stepwise following the maturation of different genotypes, between 14 October and 3 November. The air temperature and rainfall data were recorded using a meteorological station located near the experimental field.

For each genotype, at the end of the cycle, were detected:

plant height,

height of first fertile node,

branch number,

stem diameter,

number of fertile nodes on the main stem,

number of pods per plant,

number of seeds per pod,

1000 seeds weight,

seed production per plant,

degree of earliness.

Data were submitted to ANOVA. As multiple comparison test, Scott-Knott test was applied. Data are reported as supplementary material. In order to assess simultaneously all the variables studied and identify any correlations among them, a Pearson correlation was performed. Moreover, to evaluate different variables, among multivariate statistical analysis, PCA (Principal Component Analysis) was chosen for its ability to reduce the dimensionality of data (Jolliffe, 2002), by building linear combination of the original variables and producing a reduced set of variables, retaining most of the variability of the original set. PCA analysis was performed on the correlation matrix, adopting the software StatistiXL (Roberts e Withers, Broadway—Nedlands, Western Australia).

Table 11.1 "Chemical, physical and chemical-physical properties of the soil"

| Property | Analytical method | Value |
|---|---|---|
| Skeleton (>0.2 mm) (%) | D.M. 13/09/99 - Met.II.3 | 3.6 |
| Total sand (0.1-0.05 mm) (%) | D.M. 13/09/99 - Met.II.6 | 63.2 |
| Silt (0.05-0.02 mm) (%) | D.M. 13/09/99 - Met.II.6 | 27.4 |
| Clay (<0.002 mm) (%) | D.M. 13/09/99 - Met.II.6 | 9.4 |
| Total limestone ($CaCO_3$) (g $kg^{-1}$) | D.M. 13/09/99 - Met.V.1 | 89 |
| Active limestone (CaCO3) (g $kg^{-1}$) | D.M. 13/09/99 - Met.V.2 | 14 |
| Total nitrogen (N) (g $kg^{-1}$) | D.M. 13/09/99 - Met.XIV.2 | 0.5 |
| Organic substance (g $kg^{-1}$) | D.M. 13/09/99 - Met.VII.3 | 9.0 |
| C/N | | 10.3 |
| Phosphorus assimilable ($P_2O_5$) (mg $kg^{-1}$) | D.M. 13/09/99 - Met.XV.3 | 31 |
| Exchangeable potassium ($K_2O$) (mg $kg^{-1}$) | D.M. 13/09/99 - Met.XIII.5 | 208 |
| pH | pH indicator | 7.93 |
| Conductivity (saturated extract) (dS $m^{-1}$) | D.M. 13/09/99 - Met.IV.1 | 0.356 |
| Cation exchange capacity (meq 100 $g^{-1}$) | D.M. 13/09/99 - Met.XIII | 12.9 |
| Degree of saturation of bases (%) | D.M. 13/09/99 - Met.XIII | 100 |
| Exchangeable calcium (CaO) (mg $kg^{-1}$) | D.M. 13/09/99 - Met.XIII.5 | 3016 |
| Exchangeable magnesium (MgO) (mg $kg^{-1}$) | D.M. 13/09/99 - Met.XIII.5 | 266 |
| Exchangeable sodium (Na) (mg $kg^{-1}$) | D.M. 13/09/99 - Met.XIII.5 | 90 |

Table 11.2 "Country of origin and seed bank supplier of the genotypes used for the characterization"

| Row number | Accessions | Origin | Seed bank |
|---|---|---|---|
| 1 | PI 323302 | India | ARS-USDA, Georgia |
| 2 | PI 340509 | India | ARS-USDA, Georgia |
| 3 | PI 288762 | India | ARS-USDA, Georgia |
| 4 | PI 340253 | India | ARS-USDA, Georgia |
| 5 | PI 288760 | India | ARS-USDA, Georgia |
| 6 | PI 164420 | India | ARS-USDA, Georgia |
| 7 | PI 288392 | India | ARS-USDA, Georgia |
| 8 | PI 340346 | India | ARS-USDA, Georgia |
| 9 | PI 288377 | India | ARS-USDA, Georgia |
| 10 | PI 268228 | India | ARS-USDA, Georgia |
| 11 | PI 271542 | India | ARS-USDA, Georgia |
| 12 | PI 288757 | India | ARS-USDA, Georgia |
| 13 | PI 288759 | India | ARS-USDA, Georgia |
| 14 | PI 212986 | India | ARS-USDA, Georgia |
| 15 | PI 288745 | India | ARS-USDA, Georgia |
| 16 | PI 288742 | India | ARS-USDA, Georgia |
| 17 | PI 288738 | India | ARS-USDA, Georgia |
| 18 | PI 288362 | India | ARS-USDA, Georgia |
| 19 | PI 288435 | India | ARS-USDA, Georgia |

| | | | |
|---|---|---|---|
| 20 | PI 288384 | India | ARS-USDA, Georgia |
| 21 | PI 288394 | India | ARS-USDA, Georgia |
| 22 | PI 288347 | India | ARS-USDA, Georgia |
| 23 | PI 426639 | Pakistan | ARS-USDA, Georgia |
| 24 | PI 116034 | India | ARS-USDA, Georgia |
| 25 | PI 288763 | India | ARS-USDA, Georgia |
| 26 | PI 236479 | India, Delhi | ARS-USDA, Georgia |
| 27 | PI 288758 | India | ARS-USDA, Georgia |
| 28 | PI 288748 | India | ARS-USDA, Georgia |
| 29 | PI 271646 | India | ARS-USDA, Georgia |
| 30 | PI 288381 | India | ARS-USDA, Georgia |
| 31 | PI 288385 | India | ARS-USDA, Georgia |
| 32 | PI 288749 | India | ARS-USDA, Georgia |
| 33 | PI 255928 | India, Delhi | ARS-USDA, Georgia |
| 34 | PI 254368 | India, Delhi | ARS-USDA, Georgia |
| 35 | PI 198297 | India, Delhi | ARS-USDA, Georgia |
| 36 | PI 340601 | India | ARS-USDA, Georgia |
| 37 | PI 426635 | Pakistan | ARS-USDA, Georgia |
| 38 | PI 426631 | Pakistan | ARS-USDA, Georgia |
| 39 | PI 426633 | Pakistan | ARS-USDA, Georgia |
| 40 | PI 275322 | India | ARS-USDA, Georgia |
| 41 | PI 547070 | Texas | ARS-USDA, Georgia |
| 42 | PI 340263 | India | ARS-USDA, Georgia |
| 43 | HALL 78000 | Australia | Australian Grains Genebank, Canberra |
| 44 | CP31 61055 | Australia | Australian Grains Genebank, Canberra |
| 45 | PUSA MAUSMI 300537 | Australia | Australian Grains Genebank, Canberra |
| 46 | NAWABSHAR 300528 | Australia | Australian Grains Genebank, Canberra |
| 47 | LASBELLA 95042 | Australia | Australian Grains Genebank, Canberra |
| 48 | BROOKS 77998 | Australia | Australian Grains Genebank, Canberra |
| 49 | KATHSEL 300538 | Australia | Australian Grains Genebank, Canberra |
| 50 | TARI 300529 | Australia | Australian Grains Genebank, Canberra |
| 51 | NC70 300525 | Australia | Australian Grains Genebank, Canberra |
| 52 | 95078 (NA 444 X Texse) | Australia | Australian Grains Genebank, Canberra |
| 53 | Q20023 95327 | Australia | Australian Grains Genebank, Canberra |
| 54 | FINE BRACHING1 95046 | Australia | Australian Grains Genebank, Canberra |
| 55 | S - 47 - 2 95069 | Australia | Australian Grains Genebank, Canberra |
| 56 | MA20SAN 68794 | Australia | Australian Grains Genebank, Canberra |
| 57 | FSSRQ 77999 | Australia | Australian Grains Genebank, Canberra |
| 58 | HF118 61104 | Australia | Australian Grains Genebank, Canberra |
| 59 | PUSA MAUSMI 61043 | Australia | Australian Grains Genebank, Canberra |
| 60 | IC9229/P3 62437 | Australia | Australian Grains Genebank, Canberra |
| 61 | CP380 61051 | Australia | Australian Grains Genebank, Canberra |
| 62 | CP66 61044 | Australia | Australian Grains Genebank, Canberra |
| 63 | VADAVALLI 61050 | Australia | Australian Grains Genebank, Canberra |
| 64 | KINMAN | Texas | Dipartimento di Agraria UNIRC |
| 65 | MONUMENT | Texas | Dipartimento di Agraria UNIRC |
| 66 | LEWIS | Texas | Dipartimento di Agraria UNIRC |
| 67 | INDIA 2 | India | Dipartimento di Agraria UNIRC |

| | | | |
|---|---|---|---|
| 68 | INDIA 3 | India | Dipartimento di Agraria UNIRC |

**Trial 2**: Qualitative determination: galactomannan and protein contents

In order to evaluate the galactomannans content of guar seeds, the enzymatic determination was set up following the information reported in the literature (Gresta *et al*., 2013; McCleary, 1981). Seeds of the 68 guar genotypes were milled into guar flour using a laboratory mill (Retsch Model ZM100) with a sieve of 0.5 mm diameter. The dry weight of guar flour obtained after milling was determined in a IR drier where the samples were exposed to temperature of 110 °C. From the weight difference determined at the end of the drying process, the water content percentage and the dry weight of the samples were calculated. The method for galactomannans determination was based on McCleary (1981) as adapted by Megazyme method "Galactomannan assay procedure" (www.megazyme.com). The method consists in the determination of the released D-galactose, using a mixture of β-galactose dehydrogenase. Calculation of galactomannan content is based on a prior knowledge of the D-galactose: D-mannose ratio of the galactomannan being analysed. In broad terms, a sample of test material (approx. 100 mg) is extracted with aqueous ethanol (80% v/v) to remove galactosyl sucrose oligosaccharides (which, like galactomannan, contain α-linked D-galactose) and is then suspended in acetate buffer and incubated in a boiling water bath to obtain complete hydration of the galactomannan. The viscous slurry is then cooled to 40°C and treated with β-mannanase to effect depolymerisation and complete solubilisation of the galactomannan, along with a dramatic viscosity decrease. Following centrifugation, aliquots of the supernatant solution are treated with a mixture of pure α-galactosidase and pure β-mannanase to effect complete hydrolysis of the galactomannan to D-galactose and manno-oligosaccharides. This method can be applied to the measurement of galactomannan in whole milled seed, milling fractions, pure galactomannan samples or food products containing galactomannan.

The D-galactose: D-mannose contents of some commercially available, or otherwise commonly studied, galactomannans are as follows:

| **Common name** | **Botanical name** | **Gal : Man** |
|---|---|---|
| Guar | *Cyamopsis tetragonoloba* | 38 : 62 |

The following modifications have been applied suggested by McCleary (1981). After elimination of the raffinose series oligosaccharides by repeated ethanol precipitation, the samples of guar seed flour were resuspended in 50 mM acetate buffer, pH 4.5, and incubated for 30 min at 95-100 °C with vigorous stirring on vortex every 10 min and further incubated for another 30 min at

50 °C. This procedure is necessary to ensure complete solubilization of the galactomannans. Analyses were carried out in duplicate on each sample of seeds.
Guar seeds were also analysed for their total nitrogen content (Kjeldahl method).

It was also evaluated the seed galactomannan content in six subsequent various stages of development of the seed and pod. The trial was carried out in Sant'Ilario dello Jonio southern Calabria, Italy (RC, 130 m a.s.l., 38° 13′ 00″ North, 16° 12′ 00″ East) in 2016, on a sand-silt soil. On the 20 of March it was executed a mechanical hoeing (25-30 cm deep), while two following tillages with rotary tiller were carried out on the 17 of April and on the 24 of May. After the last tillage, a soil fertilization using 460 kg $ha^{-1}$ of 11 22 16 was applied. As a consequence of the lack of nodulation, during the crop cycle, 100 kg $ha^{-1}$ of N was top-dressed. India 2 was used as test variety. Sowing was performed manually on the 27 of May in row 2.0 m long and 0.50 m apart, adopting a density of 45 plants of $m^2$. Seeds were placed at 2/3 cm depth. The rows were north-south oriented. A completely randomized design was adopted with plot of 2.0 x 2.5 m with three replications. Around the experimental field, two extra guar rows were sowed, to avoid any edge effect. Water supplies were assured with a drip system. As a whole, a total volume of about 3.000 $m^3$ $ha^{-1}$ was applied. The pod samples were collected at different stages of pod development. On the seeds of these pods the content of galactomannans was analysed. They were identified six different stages of pod development (and consequently of the seed) included between seed germination and final maturity. Since guar has an indeterminate growth and shows a scalar pod maturity, pods were taken from several plants at half height of the plant to have homogeneous samples. The size of the and the colour pods and the diameter of seeds were recorded in the different stages of growth. It was applied the Megazyme method (above mentioned) for the determination of the content of galactomannans.

**Trial 3**: Morphological, biological, and productive evaluation of 25 guar genotypes

The trial was carried out in Bovalino (RC) in 2015 on 25 selected genotypes (Table 11.3), 14 of which selected among the original 68 grown in Botricello (CZ) for morpho-productive traits and the remaining 11 genotypes of recognised agronomic value coming from a collection owned by Mediterranean University of Reggio Calabria, Department of Agraria.
Sowing was performed manually on a medium textured soil, the 6th of May in rows of 1.0 m long, 0.50 m apart with an a intra-row distance of 0.10 m, it has been used a density of 20 plants of $m^2$. Seed were placed at 2-3 cm depth. The rows were North-South oriented. A completely

randomized design was adopted with plot of 1.5 x 1.0 m with three replications. Around the experimental field, two guar rows were sowed, to avoid any edge effect. Water supplies were assured with a drip system. As a whole, a total volume of about 3.000 $m^3$ $ha^{-1}$ was applied.

For each genotype, at the end of the cycle, were detected the same parameters of the previous year's test:

- plant height,
- height of first fertile node,
- branch number,
- stem diameter,
- number of fertile nodes,
- number of pods per plant,
- number of seeds per pod,
- 1000 seeds weight,
- seed production per plant,
- degree of earliness.

Table 11.3 "Origin of 25 genotypes selected"

| N° | Genotype | Year | Origin |
|---|---|---|---|
| 1 | PI288757 | 2014 | Botricello (CZ); p 1 |
| 2 | PI288759 | 2014 | Botricello (CZ); p 3 |
| 3 | PI212986 | 2014 | Botricello (CZ); p 1 |
| 4 | PI288745 | 2014 | Botricello (CZ); p 1 |
| 5 | PI288738 | 2014 | Botricello (CZ); p 1 |
| 6 | PI288758 | 2014 | Botricello (CZ); p 1 |
| 7 | PI255928 | 2014 | Botricello (CZ); p 3 |
| 8 | PI254368 | 2014 | Botricello (CZ); p 3 |
| 9 | PI340263 | 2014 | Botricello (CZ); p 1 |
| 10 | NC70300525 | 2014 | Botricello (CZ); p 3 |
| 11 | Q2002395327 | 2014 | Botricello (CZ); p 3 |
| 12 | Monument | 2014 | Botricello (CZ); p 2 |
| 13 | Lewis | 2014 | Botricello (CZ); p 3 |
| 14 | India 2 | 2014 | Botricello (CZ); p 1 |
| 15 | India 1 | 2011 | India; Orig. |
| 16 | India 2 | 2011 | India; Orig. |
| 17 | India 3 | 2011 | India; Orig. |
| 18 | India 2 | 2013 | Gela; Bev. |
| 19 | India | 2013 | Gela; p 39 |
| 20 | Johannesburg | 2012 | Sud Africa; Orig. |
| 21 | Lewis | 2012 | Gela; p 1 |
| 22 | Lewis | 2012 | Gela; p 5 |
| 23 | Monument | 2013 | Gela; p 42 |
| 24 | Monument | 2012 | Gela; p 3 |
| 25 | Monument | 2012 | Gela; p 4 |

**Trial 4**: Cross pollination trial of a selection of guar genotypes

A two years trial on forced cross pollination was carried out. In the first year, the cross pollination trial was carried out in greenhouse on the 25 genotypes of guar used in trial 3 (Table 11.3). Sowing was performed manually on the 19 February 2015 in styrofoam plateau to avoid errors or seed loss. The plateau were watered, and transferred to germination chamber for 48 hours at controlled temperature and humidity (30°C and 90% R.H., respectively). Once the plants germinated, the plateau were transferred into the greenhouse.

The seedlings after reaching the development stage of 3-5 true leaves were transferred in pots; the transplantation was carried out manually in different dates, based on the different development of each genotype. The substrate used for the filling of pots was prepared with a mixture of sandy soil and sphagnum peat in the ratio 5: 3. Water supplies were assured with a mechanical bar sprayer.

In the second year the cross-pollination trial was carried out in greenhouse on 13 genotypes (Table 11.4). Sowing was performed manually the 23 of April, 2016. Moreover, the seeds obtained from the crossings of the previous year were planted in pots, as well. For the second year of the cross-pollination trial were used the same parameters of the first year.

As soon as the plant reached the flowering stage we performed the cross-pollination by adopting the methods reported in the literature by Chaudhary *et al*. (1974). Since guar is a strictly self-pollinated plant (cleistogamous), the methods is articulated into two steps: 1) emasculation and 2) pollination. Artificial hybridization in guar requires a pair of forceps, short glassine bags, a hand stapler and label tags.

*Emasculation (preparation of the female)*

Mature buds are selected for emasculation in the late afternoon between the hours of 16:00 to 18:00 pm. Buds are selected whose stigmas will be receptive to pollen for at last 2 days. All self-pollinated flowers below the selected buds are removed, thus, the lowest buds on the raceme are always the emasculated ones. To avoid damage to the raceme, upper buds are not removed until 3 days after emasculation; however, upper buds should be removed if flowers open during this period. No more than two buds usually are emasculated on a raceme at on time.

The buds should be supported by the finger during emasculation to prevent damage to the stigma or style. The front sepal is removed with a forceps by gently pulling upward. Petals are removed in like manner. Many of the anthers are removed with the petals. Remaining anthers should be removed and visually checked to insure that they are undehisced. The whole inflorescence, including emasculated buds, is bagged with a glassine bag and stapled at the base of the raceme.

*Pollination*

Buds are pollinated the morning following emasculation, preferably between the hours of 8:00 and 9:00. To obtain better seed set, buds should be pollinated twice, on succeeding mornings. Newly opened flowers are selected for the pollen source. Pollination is achieved by gently brushing the anthers across the stigma of the emasculated bud. Mature anthers generally dehisce on contact.

Following pollination, the raceme is labelled with a tag and rebagged. The bag should be removed after pod formation takes place, about 3 to 5 days after pollination. Generally, pods resulting from hybridization contain only two to three seeds and are smaller the those resulting from self-pollination. The shape and size of the pod insure easy identification of pods obtained from hybridization. Chaudhary et al. (Chaudhary et al., 1974) achieved a 6% to 8% effective pod set using the hand emasculation and crossing technique.

Table 11.4 "Origin of the 13 genotypes selected"

| N° | Genotype | Year | Origin |
|---|---|---|---|
| 1 | PI340263 | 2015 | Pellaro (RC); p. 3c |
| 2 | NC70300525 | 2015 | Pellaro (RC); p. 4a |
| 3 | Lewis | 2015 | Pellaro (RC); p. 6b |
| 4 | Johannesburg | 2015 | Pellaro (RC); p. 8a |
| 5 | Monument | 2015 | Pellaro (RC); p. 11a |
| 6 | Monument | 2015 | Pellaro (RC); p. 12a |
| 7 | India | 2013 | Gela (CT); p. 39 |
| 8 | PI 340253 | 2014 | Botricello (CZ); p. 4 |
| 9 | Lasbella 95042 | 2014 | Botricello (CZ); p. 1 |
| 10 | S - 47 - 2 95069 | 2014 | Botricello (CZ); p. 3 |
| 11 | Pusa mausmi 61043 | 2015 | Botricello (CZ); p. 3 |
| 12 | PI 263883 | 2015 | USDA, Georgia |
| 13 | PI 288761 | 2015 | USDA, Georgia |

# 12. Results and discussion

**Trial 1**: Morphological, biological and productive characterization of 68 guar genotypes.

*Meteorological data*

During the trial rainfalls were 328 mm, most of which in September, at the end of the crop cycle. The minimum and maximum temperatures started to increase gradually from the middle of February to the first ten day of August. During the trial, minimum temperature was 15.8°C at the end of May, while the highest temperature was 34.6°C at the end of July. After that, temperatures went down to an average temperature of 21°C (Figure 12.1).

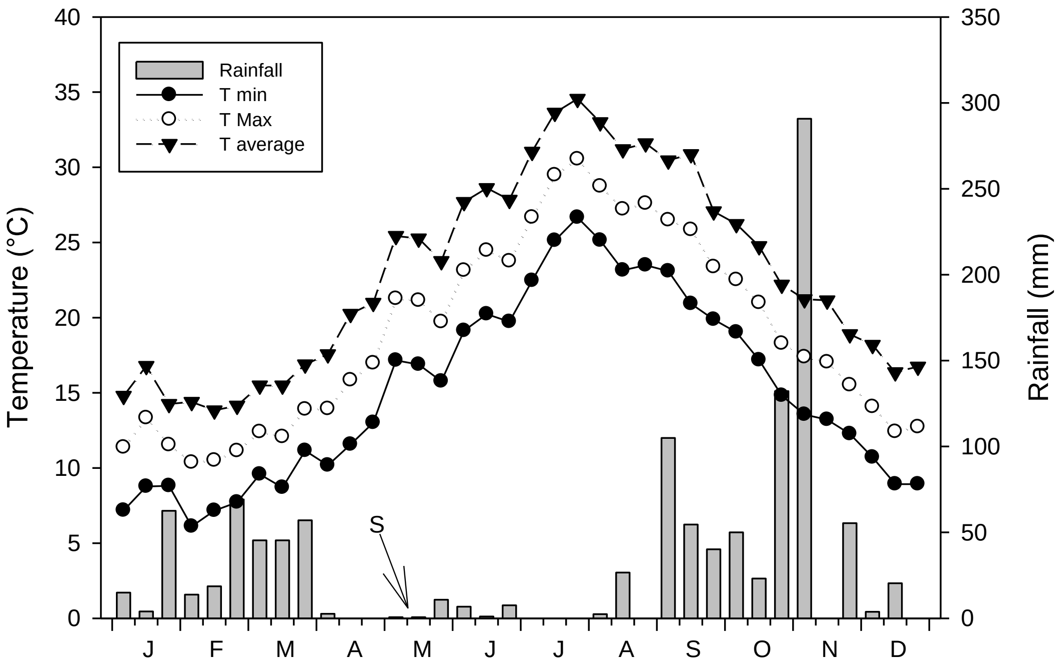


Figure 12.1 Thermopluviometric diagram of Botricello

*Biometric traits*

Biometric traits were studied in order to find valuable genotypes able to reduce seed loss during mechanical harvest and increase sowing density to obtain a greater yield.

Average plant height of the 68 genotypes was 59.6 cm, ranging from 34.0 cm of HF118 61104 to 97.3 cm of PI 547070, with a total CV of 20.9% (Table 12.1 and Table 12.2).

As far as the branch number, it must be said that it is one of the most important traits to increase the sowing density and improve the mechanized harvest. In fact even though branching genotypes show a higher seed yield per plant, single stem genotypes allow for a more dense sowing rate and minor loss of seed at harvest. Among the 68 studied genotypes, 17 genotypes resulted non-branching (PI288762, PI 288757, PI 212986, PI 288745, PI 288742, PI 288738, PI 116034, PI 236479, PI 255928, PI 254368, PI 198297, PI 547070, NC70 300525, Q20023 95327, Monument, India 2, India 3), while the other genotypes ranged between 2 and 11.7 branches per plant. This trait showed a very wide CV (72.9%), representing a valuable base for breeding purposes looking for non-branching or fine-branching genotypes.

As far as the height of the first fertile node, which is another very important trait to reduce seed loss during mechanical harvest, it was on average 1.8 cm, with value included between 1 cm and 4 cm.

Genotypes with height greater of 3 cm were PI 288762 (4 cm), PI 340263 (3.7 cm), PI 288759 (3.3 cm), Pusa Mausmi 300537 (3.3 cm), PI 255928 (3.3 cm) and PI 288742 (3.2 cm), while the remaining genotypes showed lower values included between 1 cm and 3 cm.

The stem diameter was an average of 7.9 mm, the maximum value was showed by PI288381 with 12.5 mm and the minimum value was 5 mm, with a CV of 21.5%.

Table 12.1 "Main biometric parameters of the 68 guar genotypes"

| | Plant height (cm) | Branch number (n) | Height 1° node (cm) | Stem diameter (mm) | Length of biological cycle (days) |
|---|---|---|---|---|---|
| Mean | 59.6 | 5.3 | 1.8 | 7.9 | 181.6 |
| Median | 59.5 | 7.2 | 1.3 | 7.7 | 190 |
| Std. Dev. | 12.4 | 3.9 | 0.9 | 1.7 | 11.1 |
| Std. Err. | 1.51 | 0.47 | 0.11 | 0.21 | 1.34 |
| Min | 34.0 | 0.0 | 1.0 | 5.0 | 163 |
| Max | 97.3 | 11.7 | 4.0 | 12.5 | 190 |
| CV | 20.9 | 72.9 | 51.3 | 21.5 | 6.11 |

*Biological traits*

As previously reported, the length of the biological cycle represent the main obstacle to the introduction of guar in the Mediterranean area. In fact, guar has a quite long crop cycle compared to the species generally cultivated in the Mediterranean area. For this reason, we were looking for genotypes with short crop cycle, able to better fit into the Southern Italy cropping systems.

The studied genotypes completed the whole crop cycle between the half of October and the first week of November in 181.6 days, on average (Table 12.1 and Table 12.2). From the value of the median (190 days) emerges that the great part of the genotypes have a long biological cycle.

Eleven genotypes were more precocious with a biological cycle between 155 and 163 days, medium-early genotypes (163-175 days) were 12, medium-late genotypes (175-184 days) were 6 and 39 were late genotypes, beyond 185 days. Specifically, the genotypes with the shortest biological cycle were: PI 288757, PI 288759, PI 212986, PI 288745, PI 426639, PI 116034, PI 236479, PI 288758, PI 426633, PI 340263, NC70 300525 (Table 12.2). Earliness is probably the most important trait for the introduction of this species in the Mediterranean environment, allowing for an early harvest. Contrarily, a long crop cycle does not allow mechanized harvest, since the crop is still in field when the rains of late summer season fall (September and beyond).

Table 12.2 “Biometric traits of the 68 guar genotypes ± s.d.”

| N° row | Accessions | Plant height (mm) | Branch number (n) | Height 1st node (cm) | Stem diameter (mm) | Average length of biological cycle (days) |
|---|---|---|---|---|---|---|
| 1 | PI 323302 | 457 ± 45 | 10 ± 4.6 | 2.3 ± 0.6 | 5.3 ± 0.3 | 175-184 |
| 2 | PI 340509 | 603 ± 85 | 7 ± 2.6 | 2.3 ± 1.5 | 6.7 ± 0.6 | 188-195 |
| 3 | PI 288762 | 720 ± 82 | 0 ± 0.5 | 4.0 ± 1.7 | 8.0 ± 1.0 | 188-195 |
| 4 | PI 340253 | 623 ± 93 | 11 ± 5.1 | 1.0 ± 0 | 7.8 ± 1.0 | 188-195 |
| 5 | PI 288760 | 712 ± 67 | 2 ± 0.6 | 2.2 ± 0.6 | 8.7 ± 0.6 | 175-184 |
| 6 | PI 164420 | 537 ± 32 | 10 ± 3.1 | 1.0 ± 0 | 6.3 ± 0.6 | 188-195 |
| 7 | PI 288392 | 570 ± 36 | 8 ± 1.0 | 1.0 ± 0 | 9.8 ± 0.3 | 175-184 |
| 8 | PI 340346 | 493 ± 60 | 7 ± 0 | 1.0 ± 0 | 7.0 ± 0 | 163-170 |
| 9 | PI 288377 | 520 ± 26 | 9 ± 4.6 | 2.3 ± 0.6 | 6.8 ± 1.3 | 188-195 |
| 10 | PI 268228 | 533 ± 65 | 10 ± 3.5 | 1.3 ± 0.6 | 7.0 ± 0 | 188-195 |
| 11 | PI 271542 | 540 ± 101 | 8 ± 2.3 | 1.0 ± 0 | 6.2 ± 1.2 | 188-195 |
| 12 | PI 288757 | 703 ± 25 | 0 ± 0.6 | 3.0 ± 0 | 8.5 ± 0 | 155-163 |
| 13 | PI 288759 | 413 ± 195 | 1 ± 1 | 3.3 ± 0.6 | 5.5 ± 0.5 | 155-163 |
| 14 | PI 212986 | 653 ± 57 | 0 ± 0 | 2.7 ± 0.6 | 6.7 ± 0.3 | 155-163 |
| 15 | PI 288745 | 710 ± 147 | 0 ± 0.6 | 2.7 ± 0.6 | 8.3 ± 2.9 | 155-163 |
| 16 | PI 288742 | 722 ± 61 | 0 ± 0.6 | 3.2 ± 1.5 | 7.9 ± 0.3 | 188-195 |
| 17 | PI 288738 | 777 ± 55 | 0 ± 0 | 3.0 ± 1.7 | 9.7 ± 0.3 | 163-175 |
| 18 | PI 288362 | 443 ± 25 | 9 ± 0.6 | 1.0 ± 0 | 8.0 ± 0 | 188-195 |
| 19 | PI 288435 | 590 ± 26 | 9 ± 1.0 | 1.0 ± 0 | 6.8 ± 0.8 | 188-195 |
| 20 | PI 288384 | 583 ± 76 | 8 ± 3 | 1.3 ± 0.6 | 12.2 ± 0.6 | 188-195 |
| 21 | PI 288394 | 623 ± 68 | 9 ± 1.5 | 1.0 ± 0 | 10.3 ± 0.6 | 188-195 |
| 22 | PI 288347 | 587 ± 81 | 7 ± 2.1 | 1.7 ± 0.6 | 11.5 ± 0 | 188-195 |
| 23 | PI 426639 | 550 ± 44 | 8 ± 0.6 | 1.0 ± 0 | 6.2 ± 0.3 | 155-163 |
| 24 | PI 116034 | 567 ± 12 | 0 ± 0 | 3.0 ± 0 | 6.7 ± 0.6 | 155-163 |
| 25 | PI 288763 | 453 ± 50 | 10 ± 2 | 1.0 ± 0 | 5.7 ± 0.3 | 188-195 |
| 26 | PI 236479 | 650 ± 151 | 0 ± 0 | 2.0 ± 0 | 7.5 ± 0.9 | 155-163 |
| 27 | PI 288758 | 413 ± 51 | 2 ± 3.5 | 1.0 ± 0 | 6.0 ± 0.9 | 155-163 |
| 28 | PI 288748 | 677 ± 40 | 1 ± 2.3 | 3.0 ± 0 | 7.5 ± 0 | 175-184 |
| 29 | PI 271646 | 573 ± 25 | 9 ± 2.6 | 1.0 ± 0 | 7.0 ± 0.9 | 188-195 |
| 30 | PI 288381 | 633 ± 58 | 8 ± 1.5 | 1.0 ± 0 | 12.5 ± 0 | 188-195 |
| 31 | PI 288385 | 603 ± 45 | 9 ± 1.7 | 1.3 ± 0.6 | 11.0 ± 0 | 188-195 |
| 32 | PI 288749 | 680 ± 193 | 4 ± 5.5 | 2.0 ± 1.7 | 8.3 ± 0.6 | 188-195 |
| 33 | PI 255928 | 653 ± 70 | 0 ± 0 | 3.3 ± 1.5 | 7.0 ± 0.9 | 163-175 |
| 34 | PI 254368 | 763 ± 43 | 0 ± 0 | 1.7 ± 0.6 | 7.8 ± 0.8 | 163-175 |
| 35 | PI 198297 | 540 ± 35 | 0 ± 0.6 | 2.0 ± 2.0 | 9.3 ± 0.3 | 188-195 |
| 36 | PI 340601 | 467 ± 25 | 7 ± 1.5 | 1.3 ± 0.6 | 7.3 ± 1.2 | 188-195 |
| 37 | PI 426635 | 407 ± 40 | 5 ± 1.7 | 1.0 ± 0 | 5.0 ± 0.9 | 175-184 |
| 38 | PI 426631 | 450 ± 62 | 8 ± 2.1 | 1.3 ± 0.6 | 6.7 ± 0.6 | 163-175 |
| 39 | PI 426633 | 410 ± 36 | 6 ± 0.6 | 1.3 ± 0.6 | 5.3 ± 0.6 | 155-163 |
| 40 | PI 275322 | 413 ± 31 | 6 ± 1.2 | 1.0 ± 0 | 6.0 ± 0 | 188-195 |
| 41 | PI 547070 | 973 ± 68 | 0 ± 0.6 | 3.0 ± 1.0 | 9.0 ± 1 | 188-195 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 42 | PI 340263 | 853 ± 38 | 1 ± 0.6 | 3.7 ± 2.1 | 9.5 ± 0 | 155-163 |
| 43 | HALL 78000 | 587 ± 635 | 8 ± 1.5 | 1.0 ± 0 | 7.5 ± 0.5 | 188-195 |
| 44 | CP31 61055 | 647 ± 47 | 8 ± 1.6 | 1.0 ± 0 | 8.7 ± 0.6 | 188-195 |
| 45 | PUSA MAUSMI 300537 | 740 ± 87 | 1 ± 0.6 | 3.3 ± 0.6 | 11.3 ± 1.2 | 188-195 |
| 46 | NAWABSHAR 300528 | 700 ± 20 | 9 ± 2.1 | 1.3 ± 0.6 | 8.7 ± 0.6 | 188-195 |
| 47 | LASBELLA 95042 | 473 ± 35 | 10 ± 2.1 | 1.0 ± 0 | 7.3 ± 0.3 | 175-184 |
| 48 | BROOKS 77998 | 600 ± 35 | 8 ± 2.0 | 1.3 ± 0.6 | 7.2 ± 0.8 | 188-195 |
| 49 | KATHSEL 300538 | 573 ± 32 | 8 ± 1.2 | 1.0 ± 0 | 7.0 ± 0 | 163-175 |
| 50 | TARI 300529 | 530 ± 56 | 9 ± 1.5 | 1.0 ± 0 | 7.3 ± 0.6 | 188-195 |
| 51 | NC70 300525 | 660 ± 26 | 0 ± 0.6 | 3.0 ± 0 | 8.0 ± 0 | 155-163 |
| 52 | 95078 (NA 444 X Texse) | 647 ± 45 | 8 ± 0.6 | 1.3 ± 0.6 | 7.2 ± 0.3 | 188-195 |
| 53 | Q20023 95327 | 777 ± 16 | 0 ± 0 | 2.0 ± 0 | 8.5 ± 0.9 | 163-175 |
| 54 | FINE BRACHING1 95046 | 590 ± 10 | 7 ± 1.0 | 1.3 ± 0.6 | 7.3 ± 0.3 | 188-195 |
| 55 | S - 47 - 2 95069 | 677 ± 31 | 12 ± 1.5 | 1.0 ± 0 | 8.2 ± 0.8 | 188-195 |
| 56 | MA20SAN 68794 | 653 ± 50 | 8 ± 0 | 1.0 ± 0 | 8.0 ± 0 | 188-195 |
| 57 | FSSRQ 77999 | 663 ± 66 | 10 ± 1.6 | 1.0 ± 0 | 6.0 ± 0 | 188-195 |
| 58 | HF118 61104 | 340 ± 10 | 8 ± 1.2 | 1.0 ± 0 | 7.2 ± 0.6 | 163-175 |
| 59 | PUSA MAUSMI 61043 | 450 ± 50 | 9 ± 3.2 | 1.0 ± 0 | 7.7 ± 0.3 | 188-195 |
| 60 | IC9229/P3 62437 | 523 ± 63 | 9 ± 1.5 | 1.3 ± 0 | 9.3 ± 0.6 | 188-195 |
| 61 | CP380 61051 | 553 ± 145 | 2 ± 2.3 | 4.0 ± 1 | 10.0 ± 1.7 | 188-195 |
| 62 | CP66 61044 | 600 ± 73 | 6 ± 1.6 | 1.3 ± 0.6 | 9.0 ± 0 | 188-195 |
| 63 | VADAVALLI 61050 | 677 ± 100 | 2 ± 1.5 | 3.3 ± 1.2 | 11.7 ± 0.8 | 188-195 |
| 64 | KINMAN | 377 ± 38 | 5 ± 0.6 | 1.0 ± 0 | 5.3 ± 0.6 | 188-195 |
| 65 | MONUMENT | 647 ± 42 | 0 ± 0 | 2.7 ± 0.6 | 8.3 ± 0.3 | 163-175 |
| 66 | LEWIS | 407 ± 16 | 7 ± 7.1 | 1.3 ± 0.6 | 7.7 ± 0.3 | 163-175 |
| 67 | INDIA 2 | 773 ± 25 | 0 ± 0 | 2.3 ± 0.6 | 9.7 ± 1.2 | 163-175 |
| 68 | INDIA 3 | 800 ± 44 | 0 ± 0 | 1.3 ± 0.6 | 8.5 ± 0 | 163-175 |

*Productive traits*

The cluster number (fertile nodes) on the main stem was on average 22.4 with values included between 6 and 60 and with a wide CV of 51.8% (Table 12.3 and 12.4). The genotype Lewis showed the highest value for this parameter. Pods per plant resulted on average 122.5 with values ranging between 19.7 for PI 288347 and 290.7 for PI 340253 and a total CV of 51.7%.

Table 12.3 “Main productive traits”

| | Cluster number (n) | Pods per plant (n) | Seeds per pod (n) | 1000 seeds weight (g) | Seeds per plant (g) |
|---|---|---|---|---|---|
| Mean | 22.4 | 122.5 | 6.6 | 40.1 | 29.0 |
| Median | 19.5 | 116.2 | 6.7 | 40.0 | 27.0 |
| Std. Dev. | 11.6 | 63.4 | 8.0 | 3.9 | 15.5 |
| Std. Err. | 1.41 | 7.68 | 0.1 | 0.5 | 1.88 |
| Min | 6.0 | 19.7 | 3.5 | 31.6 | 3.6 |
| Max | 60.0 | 290.7 | 8.1 | 48.3 | 73.7 |
| CV | 51.8 | 51.7 | 12.1 | 9.7 | 53.6 |

The number of seeds per pod, together with the 1000 seed weight, was one of the studied traits with the lowest variability (CV 12.1% and 9.7%, respectively). Plants showed an average number of seeds per pod of 6.6, with a maximum value of 8.1 in the genotypes Kathsel 300538 and CP 380 61051. High number of seeds per pod was also recorded on NC70 300525 (7.9), PI 288384 (7.6), PI 116034, HF118 61104 and CP66 61044 (7.5).

The 1000 seed weight resulted on average 40.1 g with values included between 31.6 g and 48.3 g, PI254368 and India 2 genotypes were those who showed the highest seed weight. High thousand seed weight was also recorded on PI 255928 (47.7 g), PI 212986 (47.2 g), PI 288384 (47.1 g), PI 340253 (46.6 g), PI 288381 (46.3 g), PI 340263 (45.9 g), PI 340346 (45.5), PI 288758 (45.1 g) and PI 288762 (45.1 g).

Finally plant seed production was on average 29.0 g with a wide variability among genotypes (CV 53.6%) starting from 3.6 g up to 73.7 g, PI 340253 has been the most productive genotype. Namely, genotypes with a plant production greater than 50 g were: PI 271646 (59.7 g); Nawabshar 300528 (55,5 g); PI 288749, Lewis (54.4 g); Lasbella 95042 (53.3 g) and S - 47 - 2 95069 (52.1 g).

Table 12.4 “Average of the productive traits of the 68 guar genotypes ± s.d.”

| N° row | Accessions | Cluster number (n) | Pods per plant (n) | Seeds per pod (n) | 1000 seeds weight (g) | Seeds per plant (g) |
|---|---|---|---|---|---|---|
| 1 | PI 323302 | 20.0 ± 2.6 | 206.0 ± 101.6 | 7.3 ± 1.1 | 38.3 ± 2.0 | 49.6 ± 21.9 |
| 2 | PI 340509 | 14.0 ± 2.6 | 95.3 ± 30.4 | 6.2 ± 0.6 | 43.5 ± 1.8 | 25.7 ± 8.5 |
| 3 | PI 288762 | 12.3 ± 2.3 | 124.3 ± 33.9 | 6.5 ± 0.7 | 45.1 ± 1.6 | 29.2 ± 11.7 |
| 4 | PI 340253 | 21.0 ± 7.8 | 290.7 ± 162.3 | 6.5 ± 0.1 | 46.6 ± 2.3 | 73.7 ± 42.5 |
| 5 | PI 288760 | 9.3 ± 1.5 | 80 ± 3.0 | 6.7 ± 0.5 | 43.9 ± 2.4 | 29.4 ± 3.1 |
| 6 | PI 164420 | 28.0 ± 14.1 | 149.0 ± 49.6 | 7.1 ± 0.2 | 37.6 ± 1.6 | 38.3 ± 21.1 |
| 7 | PI 288392 | 17.0 ± 9.5 | 133.7 ± 103.6 | 6.6 ± 0.2 | 39.9 ± 1.0 | 27.4 ± 25.5 |
| 8 | PI 340346 | 20.3 ± 6.5 | 61.3 ± 12.9 | 4.7 ± 1.7 | 45.5 ± 1.8 | 9.2 ± 3.0 |
| 9 | PI 288377 | 12.0 ± 3.6 | 74.3 ± 60.3 | 6.5 ± 0.5 | 40.8 ± 2.8 | 18.7 ± 16.8 |
| 10 | PI 268228 | 9.3 ± 4.9 | 78.7 ± 92.1 | 7.3 ± 0.6 | 40.9 ± 0.5 | 17.0 ± 20.1 |
| 11 | PI 271542 | 18.3 ±4.9 | 162.7 ± 141.5 | 6.4 ± 0.3 | 42.9 ± 0.6 | 37.7 ± 33.4 |
| 12 | PI 288757 | 18.3 ±17.0 | 186.0 ± 146.4 | 7.1 ± 0.6 | 43.2 ± 0.7 | 43.4 ± 36.2 |
| 13 | PI 288759 | 9.0 ± 1.0 | 75.3 ± 4.2 | 7.1 ± 0.4 | 39.0 ± 2.5 | 17.7 ± 2.6 |
| 14 | PI 212986 | 15.0 ± 2.0 | 68.3 ± 31.6 | 6.4 ± 0.5 | 47.2 ± 0.8 | 19.6 ± 9.9 |
| 15 | PI 288745 | 12.0 ± 7.8 | 113.7 ± 93.4 | 6.7 ± 0.4 | 42.8 ± 4.0 | 30.6 ± 27.5 |
| 16 | PI 288742 | 12.3 ± 0.6 | 56.7 ± 33.8 | 6.7 ± 0.3 | 44.3 ± 1.1 | 20.8 ± 6.5 |
| 17 | PI 288738 | 8.7 ± 0.6 | 115.0 ± 28.6 | 6.2 ± 0.8 | 43.7 ± 0.9 | 26.9 ± 11.0 |
| 18 | PI 288362 | 17.3 ± 8.0 | 77.0 ± 56.0 | 5.0 ± 1.1 | 39.9 ± 2.5 | 12.7 ± 9.5 |
| 19 | PI 288435 | 21.3 ± 11.6 | 109.3 ± 64.9 | 6.0 ± 1.0 | 37.8 ± 2.5 | 19.0 ± 4.1 |
| 20 | PI 288384 | 20.3 ± 8.6 | 99.3 ± 66.0 | 7.6 ± 0.1 | 47.1 ± 1.0 | 33.0 ± 21.4 |
| 21 | PI 288394 | 19.3 ± 6.7 | 153.3 ± 80.2 | 6.2 ± 0.6 | 40.1 ± 3.6 | 35.6 ± 21.6 |
| 22 | PI 288347 | 6.0 ± 1.0 | 19.7 ± 10.0 | 5.8 ± 0.8 | 43.9 ± 2.0 | 4.6 ± 2.2 |
| 23 | PI 426639 | 17.0 ± 3.6 | 59.3 ± 27.2 | 6.2 ± 0.2 | 38.4 ± 1.2 | 12.5 ± 5.2 |
| 24 | PI 116034 | 9.0 ± 1.0 | 52.7 ± 3.8 | 7.5 ± 0.4 | 33.6 ± 1.4 | 11.5 ± 1.2 |
| 25 | PI 288763 | 37.7 ±13.5 | 186.7 ± 67.5 | 7.0 ± 0.4 | 43.6 ± 1.3 | 49.3 ± 18.6 |
| 26 | PI 236479 | 9.0 ± 2.6 | 36.0 ± 10.0 | 6.2 ± 0.3 | 41.0 ± 7.6 | 8.1 ± 2.8 |
| 27 | PI 288758 | 15.7 ± 7.2 | 79.7 ± 27.8 | 6.6 ± 0.2 | 45.1 ± 5.5 | 17.6 ± 6.2 |
| 28 | PI 288748 | 10.7 ± 2.5 | 82.0 ± 23.8 | 6.9 ± 0.8 | 44.3 ± 2.3 | 20.7 ± 5.8 |
| 29 | PI 271646 | 36.3 ± 13.7 | 276.7 ± 146.1 | 7.0 ± 0.6 | 38.1 ± 0.2 | 59.7 ± 34.3 |
| 30 | PI 288381 | 17.7 ± 2.3 | 74.7 ± 12.9 | 7.0 ± 0.1 | 46.3 ± 1.5 | 21.5 ± 3.2 |
| 31 | PI 288385 | 15.3 ± 10.0 | 60.7 ± 35.5 | 6.2 ± 0.5 | 44.4 ± 1.5 | 14.3 ± 8.9 |
| 32 | PI 288749 | 30.0 ± 20.0 | 194.3 ± 108.8 | 7.0 ± 0.2 | 41.8 ± 2.1 | 55.4 ± 37.1 |
| 33 | PI 255928 | 13.7 ±1.2 | 50.7 ± 31.6 | 5.7 ± 0.4 | 47.7 ± 1.9 | 11.1 ± 4.9 |
| 34 | PI 254368 | 18.0 ± 2.0 | 68.7 ± 8.8 | 5.3 ± 0.7 | 48.3 ± 1.5 | 13.9 ± 3.1 |
| 35 | PI 198297 | 13.7 ± 2.3 | 110.3 ± 24.4 | 6.7 ± 0.8 | 39.9 ± 4.8 | 27.1 ± 12.7 |
| 36 | PI 340601 | 32.7 ± 2.5 | 127.7 ± 15.4 | 7.0 ± 0.4 | 36.2 ± 2.8 | 30.1 ± 3.9 |
| 37 | PI 426635 | 16.3 ± 4.0 | 31.7 ± 10.1 | 6.8 ± 0.5 | 37.5 ± 2.0 | 7.7 ± 2.7 |
| 38 | PI 426631 | 18.7 ± 4.5 | 41.3 ± 16.3 | 7.3 ± 0.9 | 36.3 ± 1.4 | 10.4 ± 4.4 |
| 39 | PI 426633 | 19.7 ± 4.7 | 49.3 ± 16.0 | 6.2 ± 0.9 | 41.8 ± 2.9 | 11.9 ± 5.4 |
| 40 | PI 275322 | 29.0 ± 10.6 | 134.3 ± 70.0 | 5.5 ± 0.4 | 43.4 ± 1.7 | 24.2 ± 13.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 41 | PI 547070 | 21.0 ± 1.7 | 89.0 ± 12.2 | 6.0 ± 0.2 | 39.0 ± 0.1 | 16.0 ± 3.1 |
| 42 | PI 340263 | 11.0 ± 1.0 | 109.0 ± 25.6 | 7.1 ± 0.7 | 45.9 ± 1.0 | 32.7 ± 11.0 |
| 43 | HALL 78000 | 28.0 ± 4.0 | 140.0 ± 55.6 | 6.5 ± 0.8 | 36.3 ± 1.1 | 25.9 ± 5.6 |
| 44 | CP31 61055 | 24.0 ± 2.6 | 95.7 ± 31.0 | 7.1 ± 0.7 | 37.4 ± 0.1 | 20.2 ± 8.7 |
| 45 | PUSA MAUSMI 300537 | 11.3 ± 3.2 | 32.7 ± 12.6 | 4.7 ± 1.0 | 35.6 ± 2.3 | 3.6 ± 1.4 |
| 46 | NAWABSHAR 300528 | 45.0 ± 3.0 | 178.7 ± 196.2 | 6.8 ± 0.2 | 39.5 ± 3.3 | 55.5 ± 36.1 |
| 47 | LASBELLA 95042 | 58.3 ± 25.7 | 178.3 ± 85.3 | 6.7 ± 0.6 | 40.6 ± 1.6 | 53.3 ± 37.8 |
| 48 | BROOKS 77998 | 24.3 ± 13.6 | 180.0 ± 120.0 | 7.1 ± 0.3 | 36.0 ± 0.9 | 42.1 ± 29.0 |
| 49 | KATHSEL 300538 | 32.3 ± 11.0 | 97.7 ± 28.2 | 8.1 ± 0.1 | 39.4 ± 1.9 | 24.6 ± 11.4 |
| 50 | TARI 300529 | 24.3 ± 18.6 | 167.0 ± 214.1 | 6.5 ± 0.1 | 40.1 ± 1.8 | 34.5 ± 43.3 |
| 51 | NC70 300525 | 16.0 ± 5.3 | 148.0 ± 56.5 | 7.9 ± 0.3 | 31.6 ± 1.2 | 31.0 ± 12.8 |
| 52 | 95078 (NA 444 X Texse) | 32.7 ± 8.0 | 139.0 ± 53.6 | 6.9 ± 0.6 | 36.4 ± 0.5 | 34.2 ± 15.8 |
| 53 | Q20023 95327 | 18.7 ± 1.15 | 124.7 ± 9.3 | 6.8 ± 0.5 | 36.7 ± 0.4 | 23.6 ± 2.6 |
| 54 | FINE BRACHING1 95046 | 25.3 ± 6.7 | 188.0 ± 77.1 | 7.2 ± 1.1 | 38.4 ± 0.5 | 45.4 ± 19.4 |
| 55 | S - 47 - 2 95069 | 40.0 ± 20 | 278.7 ± 280.7 | 6.6 ± 0.4 | 37.6 ± 0.5 | 52.1 ± 51.4 |
| 56 | MA20SAN 68794 | 34.0 ± 8.2 | 117.3 ± 25.6 | 6.3 ± 1.0 | 38.4 ± 5.6 | 25.6 ± 15.1 |
| 57 | FSSRQ 77999 | 20.0 ± 13.7 | 185.0 ± 120.3 | 6.4 ± 1.2 | 36.5 ± 0.8 | 39.0 ± 28.0 |
| 58 | HF118 61104 | 39.3 ± 10.0 | 140.0 ± 81.5 | 7.5 ± 0.6 | 41.1 ±1.8 | 35.3 ± 24.6 |
| 59 | PUSA MAUSMI 61043 | 37.7 ± 22.4 | 143.3 ± 57.8 | 6.9 ± 0.8 | 39.9 ± 3.3 | 30.4 ± 14.2 |
| 60 | IC9229/P3 62437 | 24.7 ± 5.1 | 186.3 ± 125.3 | 5.6 ± 1.2 | 37.6 ± 1.1 | 49.7 ± 36.4 |
| 61 | CP380 61051 | 29.7 ± 11.7 | 160.0 ± 119.1 | 8.1 ± 0.4 | 38.6 ± 0.4 | 46.3 ± 38.6 |
| 62 | CP66 61044 | 50.0 ± 8.0 | 206.3 ± 35.8 | 7.5 ± 1.0 | 38.8 ± 1.0 | 48.6 ± 8.3 |
| 63 | VADAVALLI 61050 | 20.3 ± 5.5 | 139.7 ± 49.4 | 7.2 ± 0.70 | 44.0 ± 6.3 | 38.2 ± 12.4 |
| 64 | KINMAN | 33.7 ± 12.1 | 126.0 ± 30.1 | 6.7 ± 0.5 | 39.1 ± 0.1 | 36.0 ± 21.2 |
| 65 | MONUMENT | 12.3 ± 0.6 | 89.0 ± 34.7 | 7.4 ± 0.1 | 32.4 ± 0.6 | 23.6 ± 1.0 |
| 66 | LEWIS | 60.0 ± 17.4 | 287.7 ± 138.4 | 6.8 ± 0.5 | 35.3 ± 1.4 | 54.4 ± 27.2 |
| 67 | INDIA 2 | 25.0 ± 4.4 | 67.0 ± 28.2 | 6.0 ± 0.4 | 48.1 ± 2.1 | 15.9 ± 4.9 |
| 68 | INDIA 3 | 14.0 ± 1 | 34.7 ± 9.1 | 3.5 ± 0.4 | 44.0 ± 2.0 | 4.3 ± 0.9 |

Limited studies similar to this one have been conducted on guar. Among these, our findings are in agreement with Morris (2010), who reports values of 100 seed weight ranging from 2.3 g to 4.8 g with a mean value of 3.3 g on 73 guar genotypes, and Raghuprakash *et al*. (2008) who, on fifty genotypes, found the number of branches ranging from 0 to 8.3, the number of pods per plant from 17 to 87, the number of seeds per pod from 6.53 to 8.53, with an average of 7.77, and the 100 seed weight from 2.89 to 4.45 g, with an average of 3.6 g, similar to our results (6.6 seeds per pod and 40.1g as 1000 seed weight). Morris (2010) found CV of 28% and 15% for plant height and 100 seed weight, respectively. Our results are also in agreement with Sultan *et al*. (2012) and Manivannan *et al*. (2015), who found significantly lower variation for seeds per pod, seed weight and plant height compared to pods per plant. Namely, Sultan *et al*. (2012) in a research carried out in Pakistan on 101 genotypes, found a slightly higher number of seeds per pod (8.1 on average), with a lighter 100 seed weight (3.0 g). Also Gresta *et al*. (2013), in a trial carried out in irrigated plots in a Mediterranean environment, found 1000 seed weight included between 28.2 g and 31.3 g and an average number of seeds per pod of 7.2. Several researches (Alexander *et al*., 1988; Undersander et al., 1991) carried out when Monument was not yet released (2008), have considered Lewis as the best variety of guar. In our study, Monument compared to Lewis showed a preferable growth habit with higher first insertion node and an absence of branches allowing for a more dense sowing rate and for minor loss during combine harvest.

Seed yield of guar reported in literature are highly variable, ranging from less than 1,000 kg $ha^{-1}$ without irrigation up to 5,000 kg $ha^{-1}$ as potential yield in irrigated row plots (Tucker and Foraker, 1975, Beech *et al*., 1989, Jackson and Doughton, 1982; Kumar and Singh, 2002). Undersander *et al*. (1991) in a trial carried out in Texas on five American varieties found that Lewis was the most productive. On the other hand, Sortino and Gresta (2007) found no difference between five American varieties including Lewis and Kinman, with an average yield of 2.4 t $ha^{-1}$. Higher yields (3-4 t $ha^{-1}$) were obtained in Australia by Beech *et al*. (1989) testing some other American varieties. Yield obviously vary in relation to soil, climate, varieties and management techniques and it is the results of the single yield components. In this trial we did not assess yield, but the most important yield components have been evaluated, laying the foundations for future breeding work.

In previous studies (Sultan *et al*., 2012) the main yield components correlated to grain yield are the number of pods per plant and the seed weight. On the contrary, Raghuprakash *et al*. (2009) and Ibrahim *et al*. (2012) in two different researches, adopting path coefficient analysis, stated that the main yield components were pods per plant and seeds per pod, indicating that these productive traits are positively associated and that these are the main selection criteria for improving seed yield in guar.

Obviously our results of seeds per plant is strictly related to the sowing density adopted (20 plants $m^{-2}$) since a higher sowing density would have favoured the single stem genotypes, while a lower density would have favoured branching genotypes.

*PCA (Principal Component Analysis) and correlation analysis of the 68 genotypes*

To identify patterns in our dataset and to express the data in such a way to highlight its similarities and differences, we applied the multivariate statistical technique of the Principal Components Analysis. The eigenvalues, the percentage of variance and aggregation are shown in table 12.5, while the correlation matrix is shown in table 12.6. PCA showed seven principal components, the first two of which account, as a whole, for 68.8% of the total data variability. This means that a quote of 31.2% of the variability has been lost in the simplification of the PCA. The first component, which explain 47.4% of the variation, was mainly associated with the number of branches, the number of clusters, pods per plant and seeds per plant.

Component two, responsible for 21.4% of the variation, was mainly represented by height of the 1° node and seeds per pod. Component three contributed for 14.4% of the variation and it is exclusively associated with 1000 seeds weight.

Table 12.5 "Percentage of variance explained by the different components of the PCA"

| Explained Variance (Eigenvalues) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Value** | **PC 1** | **PC 2** | **PC 3** | **PC 4** | **PC 5** | **PC 6** | **PC 7** |
| Eigenvalue | 3.317 | 1.499 | 1.012 | 0.580 | 0.385 | 0.175 | 0.033 |
| % of Var. | 47.390 | 21.408 | 14.450 | 8.290 | 5.497 | 2.496 | 0.468 |
| Cum. % | 47.390 | 68.798 | 83.248 | 91.538 | 97.035 | 99.532 | 100.000 |

The graphical representation of the relationships among varieties and parameters shows an evident distinction among the genotypes (Fig. 12.2). Two main groups can be individuated. A smaller group that clearly stand out for a lower value of PC1 and higher value of PC2, corresponding mainly to a plant with a high height of the first node and low branch number. The other bigger group showed exactly the opposite traits. All the other parameters were not clearly separated.

Table 12.6 "Components loading of the studied parameters"

| Component Loadings<br>(correlations between initial variables and principal components) | | | |
|---|---|---|---|
| **Variable** | **PC 1** | **PC 2** | **PC 3** |
| N° branched | 0,718 | -0,535 | -0,083 |
| Height 1° node | -0,542 | 0,767 | 0,103 |
| Cluster number | 0,826 | -0,061 | 0,078 |
| N° pods per plant | 0,876 | 0,257 | 0,252 |
| N° seed per pods | 0,432 | 0,644 | -0,298 |
| 1000 seeds weight | -0,372 | -0,123 | 0,855 |
| Seeds per plant | 0,856 | 0,352 | 0,323 |

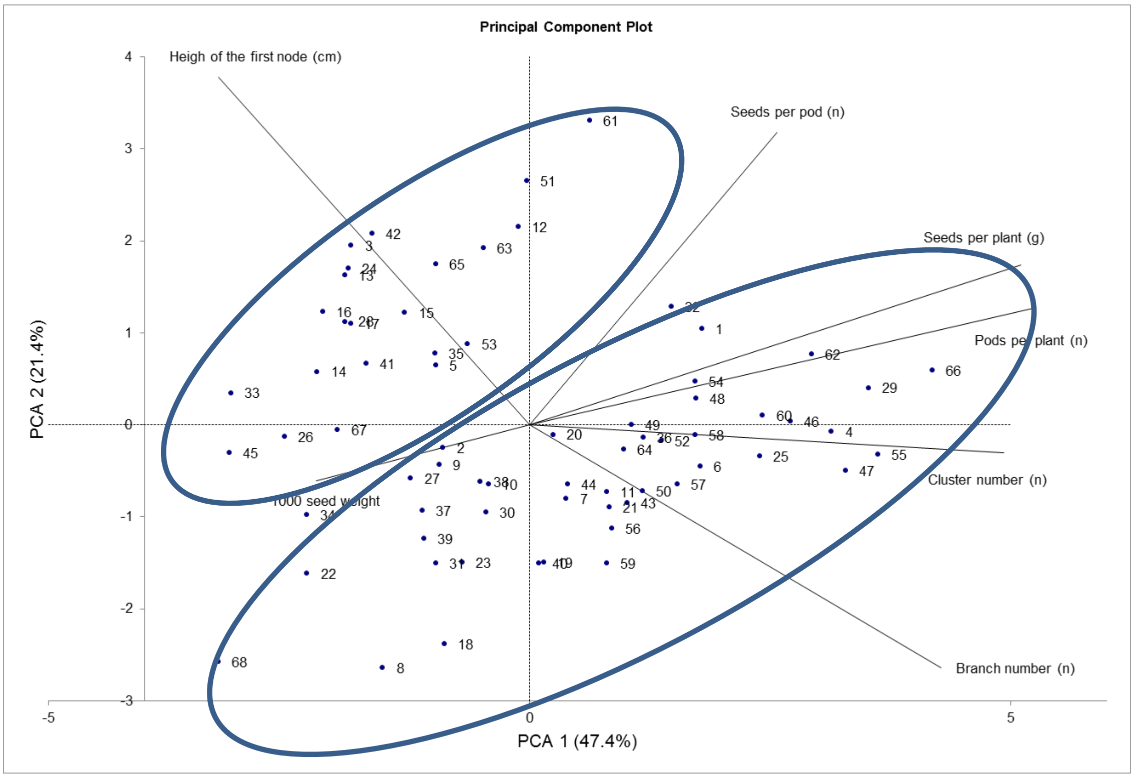


Figure 12.2 Correlation based biplot of the main components

**Trial 2:** Qualitative characterization of the guar seed collection

The seed collection of 68 genotypes, previously studied for the morphological, biological and productive traits, was characterised from a qualitative point of view, taking into account the main traits useful for its destination: galactomannans (for industrial use) and protein (for animal feeding).

Among the 68 genotypes, galactomannan content ranged from 19.6% up to 32.3% with an average value of 26.5%, while protein content ranged from 26.0% to 33.5% with an average value of 30.2% (Table 12.7), comparable with those reported in literature (Gresta *et al*., 2013; Kumar and Singh, 2002). As expected, coefficient of variation was very low (9.9% and 4.9%, respectively) compared to those of the morphological and productive traits, indicating that the qualitative parameters have a narrow range of variability upon which start a breeding program.

The genotype with the highest content of galactomannans was PI 323302 (32.3%); high content of galactomannans was also recorded on PI 255928 (31.1%), Monument (31.1%), PI 426635 (30.8%), PI 426633 (30.8%), FSSRQ 77999 (30.7%), PI 340253 (30.4%) and Tari 300529 (30.3%) (Table 12.8).

The genotypes with the highest content of protein were PI 288385 (33.5%) and PI 288347 (33.1%), high content of protein was also recorded on, PI 288435 (32.8%), PI 288381 (32.7%), PI 288362 (32.0%), CP66 61044 (32.0%), PI 288384 (31.9%), PI 426639 (31.8%), CP31 61055 (31.7%), S - 47 - 2 95069 (31.7%), India 3 (31.7%) and PI 254368 (31.6%) (Table 12.8).

Regarding galactomannan content, our results are in agreement with Gresta *et al*. (2013) who, in a similar environment, obtained values of 30.2% for Kinman and 28.8% for Lewis. Yadav *et al*. (2003) in India in a sowing density trial (from 10 to 25 kg $ha^{-1}$), reported a galactomannan value ranging from 29.9% to 31.2%.

Compared to reports from Batthi and Sial (1971) and Elsheikh *et al.* (1999), a greater seed protein content was measured in the present experiment. Similar protein content was detected in guar by Jain *et al*. (1987). On average, the chemical composition of guar seeds showed similar values to those reported by Badr *et al*. (2014). Eldaw (1998) observed in three genotypes a similar content of crude protein (29.1%). Kays *et al*. (2006), in guar seeds of nine different accessions, reported a lower level of crude protein (26.4%). As reported in the literature, the high protein content of guar seeds is one of the most important nutritional factors, which allows their use as excellent protein supplements to cereal grains for animal feeding (Bressani, 1993).

Table 12.7 “Main parameters of qualitative traits”

| | Galactomannans content % | Protein content % |
|---|---|---|
| Mean | 26.5 | 30.2 |
| Median | 26.3 | 30.4 |
| Std. Dev. | 2.6 | 1.5 |
| Std. Err. | 0.3 | 0.2 |
| Min | 19.6 | 26.0 |
| Max | 32.3 | 33.5 |
| CV | 9.9 | 4.9 |

Table 12.8 “Galactomannans and protein content of 68 guar genotypes ± s.d.”

| N° row | Accessions | Galactomannans content % | Protein content % |
|---|---|---|---|
| 1 | PI 323302 | 32.3 ± 1.0 | 30.3 ± 0.2 |
| 2 | PI 340509 | 29.2 ± 0.4 | 30.7 ± 0.4 |
| 3 | PI 288762 | 27.4 ± 0.7 | 30.8 ± 0.0 |
| 4 | PI 340253 | 30.4 ± 1.1 | 28.9 ± 0.0 |
| 5 | PI 288760 | 28.1 ± 2.3 | 30.5 ± 0.3 |
| 6 | PI 164420 | 27.2 ± 0.4 | 30.8 ± 0.6 |
| 7 | PI 288392 | 19.9 ± 0.7 | 31.3 ± 0.1 |
| 8 | PI 340346 | 26.2 ± 0.7 | 31.2 ± 0.2 |
| 9 | PI 288377 | 26.3 ± 1.4 | 30.4 ± 0.3 |
| 10 | PI 268228 | 28.0 ± 0.5 | 29.9 ± 0.3 |
| 11 | PI 271542 | 21.7 ± 2.5 | 30.9 ± 0.0 |
| 12 | PI 288757 | 24.9 ± 0.6 | 30.9 ± 0.3 |
| 13 | PI 288759 | 24.1 ± 0.2 | 29.2 ± 0.1 |
| 14 | PI 212986 | 29.6 ± 3.1 | 31.1 ± 0.6 |
| 15 | PI 288745 | 24.4 ± 1.8 | 29.2 ± 0.2 |
| 16 | PI 288742 | 27.8 ± 1.3 | 29.9 ± 0.0 |
| 17 | PI 288738 | 24.6 ± 0.3 | 31.0 ± 0.0 |
| 18 | PI 288362 | 24.5 ± 0.6 | 32.0 ± 0.0 |
| 19 | PI 288435 | 23.1 ± 1.4 | 32.8 ± 0.1 |
| 20 | PI 288384 | 25.6 ± 0.5 | 31.9 ± 0.1 |
| 21 | PI 288394 | 23.1 ± 0.9 | 29.7 ± 0.4 |
| 22 | PI 288347 | 24.5 ± 0.4 | 33.1 ± 0.0 |
| 23 | PI 426639 | 23.9 ± 0.2 | 31.8 ± 0.2 |
| 24 | PI 116034 | 28.8 ± 1.0 | 27.7 ± 0.2 |
| 25 | PI 288763 | 26.2 ± 0.9 | 28.5 ± 0.2 |
| 26 | PI 236479 | 27.7 ± 0.4 | 31.2 ± 0.9 |
| 27 | PI 288758 | 26.0 ± 0.8 | 29.5 ± 0.0 |
| 28 | PI 288748 | 26.4 ± 0.5 | 30.3 ± 0.2 |
| 29 | PI 271646 | 19.6 ± 1.0 | 30.5 ± 0.1 |

| | | | |
|---|---|---|---|
| 30 | PI 288381 | 26.3 ± 0.2 | 32.7 ± 0.3 |
| 31 | PI 288385 | 21.4 ± 0.0 | 33.5 ± 0.0 |
| 32 | PI 288749 | 24.7 ± 0.5 | 30.4 ± 0.2 |
| 33 | PI 255928 | 31.1 ± 2.0 | 31.3 ± 0.2 |
| 34 | PI 254368 | 25.3 ± 1.2 | 31.6 ± 0.2 |
| 35 | PI 198297 | 28.1 ± 0.3 | 30.0 ± 0.0 |
| 36 | PI 340601 | 29.7 ± 0.1 | 28.2 ± 0.0 |
| 37 | PI 426635 | 30.8 ± 0.3 | 28.8 ± 0.2 |
| 38 | PI 426631 | 28.3 ± 0.0 | 28.1 ± 0.1 |
| 39 | PI 426633 | 30.8 ± 0.8 | 29.2 ± 0.2 |
| 40 | PI 275322 | 27.0 ± 0.2 | 28.5 ± 0.1 |
| 41 | PI 547070 | 25.4 ± 0.6 | 30.5 ± 0.0 |
| 42 | PI 340263 | 27.1 ± 0.8 | 30.5 ± 0.0 |
| 43 | HALL 78000 | 25.8 ± 0.1 | 31.4 ± 0.1 |
| 44 | CP31 61055 | 26.3 ± 0.2 | 31.7 ± 0.1 |
| 45 | PUSA MAUSMI 300537 | 27.5 ± 2.0 | 30.2 ± 0.1 |
| 46 | NAWABSHAR 300528 | 28.1 ± 0.0 | 30.2 ± 0.1 |
| 47 | LASBELLA 95042 | 29.2 ± 1.2 | 27.0 ± 0.2 |
| 48 | BROOKS 77998 | 28.3 ± 0.2 | 28.1 ± 0.0 |
| 49 | KATHSEL 300538 | 25.2 ± 0.8 | 29.4 ± 0.5 |
| 50 | TARI 300529 | 30.3 ± 0.5 | 29.7 ± 0.2 |
| 51 | NC70 300525 | 25.0 ± 0.6 | 26.0 ± 0.2 |
| 52 | 95078 (NA 444 X Texse) | 26.3 ± 0.3 | 30.7 ± 0.3 |
| 53 | Q20023 95327 | 28.3 ± 0.4 | 28.3 ± 0.6 |
| 54 | FINE BRACHING1 95046 | 24.9 ± 0.3 | 30.5 ± 0.2 |
| 55 | S - 47 - 2 95069 | 26.5 ± 0.6 | 31.7 ± 0.5 |
| 56 | MA20SAN 68794 | 26.4 ± 0.1 | 30.4 ± 0.0 |
| 57 | FSSRQ 77999 | 30.7 ± 0.1 | 29.6 ± 0.1 |
| 58 | HF118 61104 | 25.6 ± 0.1 | 29.5 ± 0.0 |
| 59 | PUSA MAUSMI 61043 | 27.5 ± 0.0 | 29.1 ± 0.0 |
| 60 | IC9229/P3 62437 | 24.6 ± 0.6 | 29.1 ± 0.0 |
| 61 | CP380 61051 | 24.6 ± 0.2 | 30.8 ± 0.0 |
| 62 | CP66 61044 | 24.1 ± 0.9 | 32.0 ± 0.2 |
| 63 | VADAVALLI 61050 | 27.2 ± 0.5 | 30.4 ± 0.0 |
| 64 | KINMAN | 25.9 ± 0.6 | 29.1 ± 0.1 |
| 65 | MONUMENT | 31.1 ± 0.0 | 26.8 ± 0.2 |
| 66 | LEWIS | 27.3 ± 0.5 | 28.6 ± 0.0 |
| 67 | INDIA 2 | 26.1 ± 0.1 | 31.1 ± 0.0 |
| 68 | INDIA 3 | 23.7 ± 0.8 | 31.7 ± 0.0 |

An interesting association was found, for the first time, between galactomannans and protein content (Figure 12.3). The two qualitative traits show a highly significative negative relation (p=0.0002). From a physiological point of view, it suggests that seed galactomannans increase at the expense of protein content, causing competition of carbon source, as well as it has been demonstrated for carbohydrates and protein in faba bean (Gasim *et al*., 2015), for oil and protein in rapeseed (Grami *et al*., 1977; Kennedy *et al*., 2011) and in soybean (Wilcox and Shibles, 2001; Charron *et al*., 2005). This association highlights that breeding process looking for guar genotypes improved for galactomannans content will have as a result genotypes with a lower protein content.

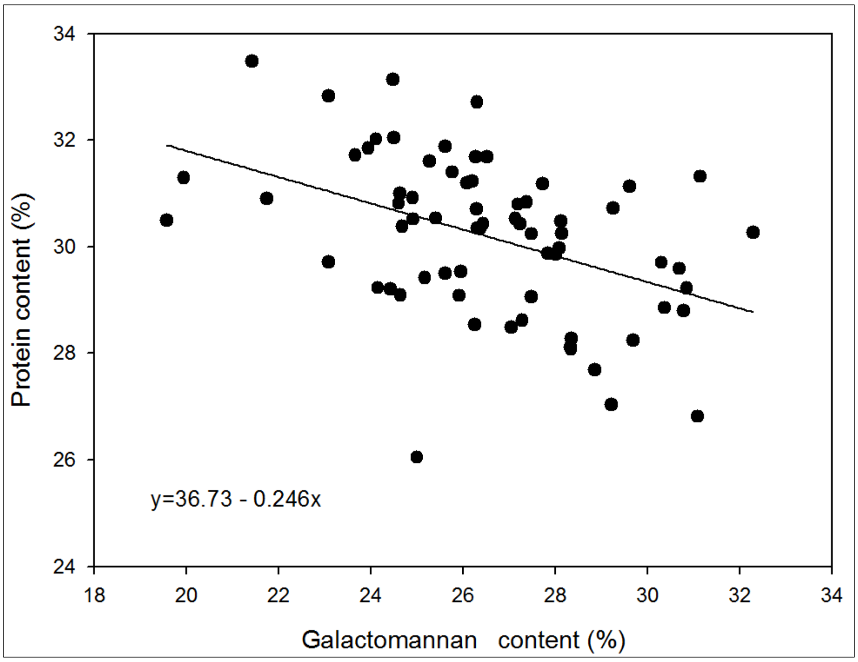


Figure 12.3 Relation between galactomannans and protein content (F test = 15.301, p=0.0002) in guar genotypes

It was also determined the increase of seed galactomannan content in six different stages of pod development. The stages were identified as days after sowing (DAS) (48, 80, 110, 130, 140 and 150 days). The size and colour of the pods and the galactomannan content of the seeds in the different stages of development are shown in table 12.9, while in Figure 12.4 is indicated the seed galactomannan accumulation during the growth of crop. The galactomannan content increased from 0% (assumed, not determined) at emergence to 12.0% (± 0.4) at 48 DAS when the pod was 70 mm long and light green coloured. At 80 DAS the pod was still light green, long 80-90 cm with a galactomannan content of 18.4% (± 0.4). This was the period in which the galactomannan content showed the greatest increase, around 50% passing from 12.0% to 18.4%.

In the following four steps at 110, 130, 140 and 150 DAS, pod length remained the same, while changed the pod width from 8-9 mm to 10-12 mm, and the colour from dark green to light brown and dark brown, clear indications that the pod was approaching to the maturation. In this period the galactomannan content passed from 20.3% (± 0.1), to 21.2% (± 0.7), to 23.5% (± 0.9) and 26.1% (± 0.1). The increase of galactomannan content of each step greatly decrease compared to the previous one. At the final stage above mentioned (150 DAS) the harvest was carried out. This shows that the content of galactomannans also increases in the final stages of growth of the pod and maturation of the seed, even though with a lesser extent.

Table 12.9 "Size, color and galactomannans content (± standard error) of seeds and pods at different stages of development of India 2"

| Number of days after sowing | Pod length (mm) | Pod width (mm) | Dry seed diameter (mm) | Pod colour | Galactomannan content % |
|---|---|---|---|---|---|
| 48 | 70 | 6 | 2 - 2.5 | Light-green | 12.0 ± 0.4 |
| 80 | 80 - 90 | 7 | 3 | Light-green | 18.4 ± 0.4 |
| 110 | 120 | 8 - 9 | 4.5 - 5 | Dark-green | 20.3 ± 0.1 |
| 130 | 120 | 9 - 10 | 5.5 | Light-brown | 21.2 ± 0.7 |
| 140 | 120 | 10 - 12 | 5.5 - 6 | Brown | 23.5 ± 0.9 |
| 150 | 120 | 10 - 12 | 5.5 - 6 | Dark-brown | 26.1 ± 0.1 |

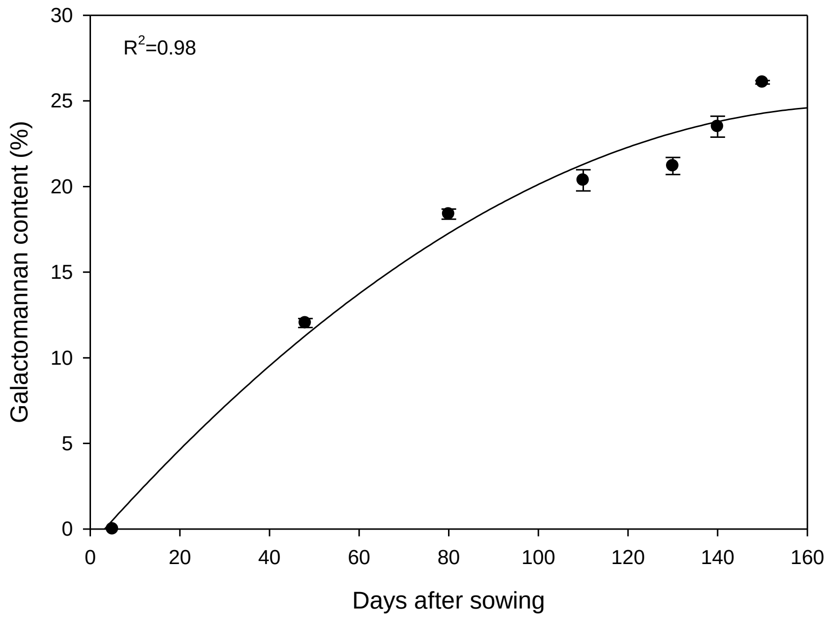


Figure 12.4 Accumulation curve of the galactomannan content (%) in India 2 guar seed

*Correlations*

In the present study, the morpho-productive characterization highlights the negative correlation between branch number and the height of the insertion of the first fertile node (-0.758) (Table 12.10). This means that branching genotypes showed a lower height of the first node with a worse harvestability. The cluster number was positively associated with branch number (0.480) and negatively with the height of the first node (-0.455). Obviously branching genotypes have a greater number of clusters, while the higher is the insertion of the first node, the lesser is the cluster number. Likewise, the number of pods per plant was higher in the branching genotypes (0.444) and increased with the growth of cluster number (0.657). The number of seeds per pod was significantly and positively related to the number of pods per plant (0.348). The 1000 seed weight resulted negatively related with cluster number (-0.256), pods per plant (-0.222) and with seeds per pod (−0.307). Manivannan *et al*. (2015) reported similar results in a study on 42 guar genotypes. Since pods per plant is known as one of the most important yield components, the best plant-ideotype should have a high number of pods per plant and of seeds per pod. These data are also in agreement with those reported by Sultan *et al*. (2012).

In the light of the relations among studied productive parameters, we can affirm that the plant production of the 68 guar genotypes is strictly linked to the number of pods per plant (0.947), consequently to the cluster number (0.647) and to the seeds per pod (0.459), while it resulted not significantly related to the 1000 seed weight. Therefore, these parameters may represent the yield components to be taken into consideration as discriminatory factors for a breeding program to increase the seed production per plant.

Our data are in agreement with Raghuprakash *et al*. (2009) and Ibrahim *et al*. (2012) who in two different researches found the same correlations between morphological and productive traits.

As far as the qualitative traits, protein content resulted negatively related to number of seeds per pod (-0.353) the number of seeds per plant (-0.234) and galactomannan content (-0.434), that have been discussed in the previous figure. Positive correlation was also found between protein content and 1000 seed weight (0.465), indicating that bigger seeds have higher protein content.

Table 12.10 “Correlation analysis among studied factor of the 68 guar genotypes with p-level underneath”

| | Height 1° node | Cluster number | Pods per plant | Seeds per pod | 1000 seed weight | Seeds per plant | Galatt. | Protein |
|---|---|---|---|---|---|---|---|---|
| Branch N. | -0.758 | 0.48 | 0.444 | 0.0947 | -0.206 | 0.407 | -0.0685 | 0.087 |
| | 6.97E-14 | 3.44E-05 | 0.000148 | 0.442 | 0.0927 | 0.000571 | 0.579 | 0.48 |
| Height 1° | | -0.455 | -0.233 | 0.126 | 0.142 | -0.158 | 0.154 | -0.0603 |
| | | 9.75E-05 | 0.0561 | 0.305 | 0.25 | 0.199 | 0.21 | 0.625 |
| Cluster number | | | 0.657 | 0.228 | -0.256 | 0.647 | -0.0325 | -0.189 |
| | | | 1.19E-09 | 0.0614 | 0.0348 | 2.46E-09 | 0.792 | 0.123 |
| Pods per plant | | | | 0.348 | -0.222 | 0.947 | -0.0587 | -0.208 |
| | | | | 0.00359 | 0.0692 | 3.51E-34 | 0.635 | 0.088 |
| Seeds per pod | | | | | -0.307 | 0.459 | 0.107 | -0.353 |
| | | | | | 0.0108 | 8.24E-05 | 0.384 | 0.00318 |
| 1000 seed weight | | | | | | -0.116 | -0.0617 | 0.465 |
| | | | | | | 0.348 | 0.617 | 6.34E-05 |
| Seeds per plant (g) | | | | | | | -0.0021 | -0.234 |
| | | | | | | | 0.986 | 0.0448 |
| Galattomannanns (%) | | | | | | | | -0.434 |
| | | | | | | | | 0.000219 |

**Trial 3**: Morphological biological and productive evaluation of 25 selected genotypes of guar

*Meteorological data*

Total natural water supplies were 599 mm, typical of the Mediterranean environment. The greatest rainfalls were in the period between January and March (41%) and between November and December (42%). During the growing season, from May to October, the amount of rainfall was 76 mm. The minimum and maximum temperatures started to increase gradually from the middle of February to the first ten day of August. During the trial, minimum temperature was 14.1°C at the end of May while the highest temperature was 36.3 at the beginning of August. After that, temperatures began to decline reaching average temperature around 20°C (Figure 12.5).

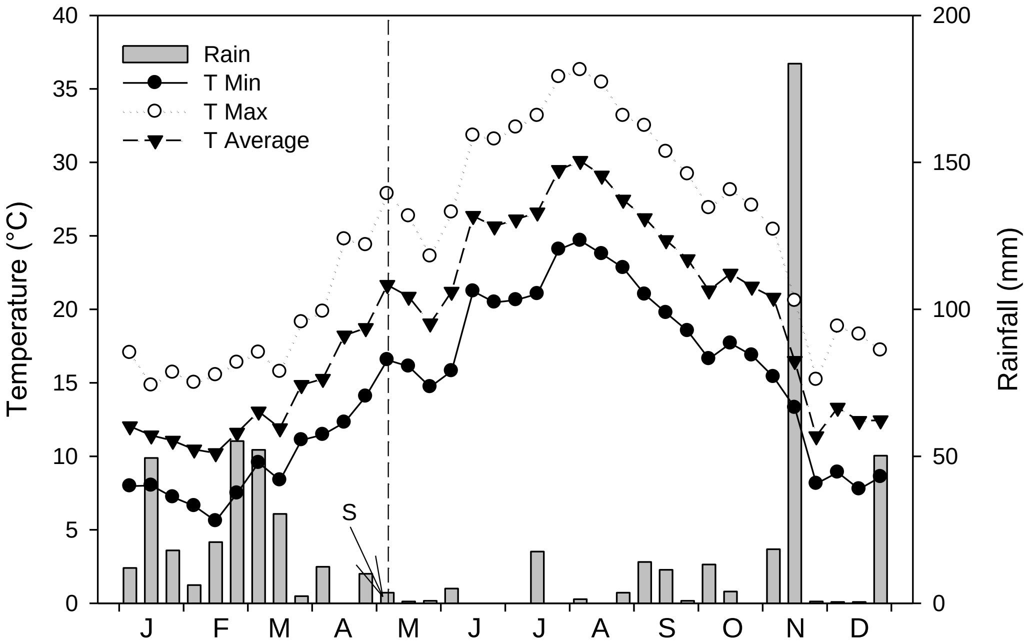

Figure 12.5 Thermopluviometric diagram of Bovalino

As far as the morpho-productive traits of the 25 selected genotypes, the average plant height was 80.7 cm, ranging from 30.5 cm (India 2-Botr.) to 161.0 cm (Q2002395327) with a coefficient of variation of 32.5% (Table 12.11).

Height of the 1st node was on average 3.3 cm with a very large CV (74.1%), with values generally included between 1 cm and 5.5 cm, even though a peak of 13.5 cm on India 1 was recorded. Genotypes with height of the 1st node greater than 3 cm were India 2-Vac. (5.8 cm), PI288745 (4.8 cm), PI288738 (4.0 cm), Q2002395327 (4.0 cm), India 3 (4.0 cm), PI212986 (3.3 cm), PI255928 (3.3 cm), India 2-Bev. (3.3 cm) and Monument-p3 (3.3 cm), (Table 12.12).

In this trial the majority of the studied genotypes showed a non-branching habitus (branch number =0), the most desirable habitus to increase sowing density and facilitate harvest. At harvest, the largest number of genotypes showed a degree of earliness of 3 (green stem and dried pods for less than half of the stem); specifically genotypes India 2-p39 and PI340263 exhibit a more early cycle with value of 3.5.

The fertile nodes per plant (cluster number) on the main stem was on average 25.4 with values included between 4 (India 2-Botr.) and 98 (Lewis-p5) and with a wide CV of 77.9 %. A high cluster number was also recorded in Johannesburg (55), Lewis-p1 (50.5), Q2002395327 (39), Monument-p42 (34.5), Monument-p3 (32.5), NC70300525 (32) and Monument-2 (31.5).

Pods per plant were on average 63.8, showing a high CV (88.5%), the maximum value of 260.0 was ascertained in Lewis-p5. High values of this trait were also recorded in the following genotypes: Q2002395327 (174), Lewis-p1(105.5), Johannesburg (98), Monument-p42 (94.5), Monument-p3 (90.5), NC70300525 (87.5) and PI288759 (82).

The average number of seeds per pod was 5.6 with a CV of 20.4% indicating a quite low amount of variability, with the highest value of 7.3 recorded in Lewis-p5 and PI212986; high number of seeds per pod was also recorded in PI288759 (6.8), Monument-p3 (6.7), PI288745 (6.6), PI288738 (6.6), Q2002395327 (6.5) and Monument-p4 (6.2).

Table 12.11 “Main parameters of the 25 selected genotypes”

| | Plant height (cm) | Height I° node (cm) | N° branched | Degree of earliness | Fertile nods per plant (n) | Pod number | N° seeds per pod | Seed per plant (g) | 1000 seeds weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 80.7 | 3.3 | 0.2 | 2.7 | 25.4 | 63.8 | 5.6 | 13.1 | 36.4 |
| Median | 76.0 | 2.8 | 0.0 | 3.0 | 18.5 | 49.5 | 5.6 | 9.2 | 36.5 |
| Std. Dev. | 26.2 | 2.5 | 0.5 | 0.6 | 19.8 | 56.5 | 1.1 | 13.0 | 7.0 |
| Std. Err. | 5.2 | 0.5 | 0.1 | 0.1 | 4.0 | 11.3 | 0.2 | 2.6 | 1.4 |
| Min | 30.5 | 1.0 | 0.0 | 1.0 | 4.0 | 4.0 | 2.4 | 0.5 | 25.3 |
| Max | 161.0 | 13.5 | 2.0 | 3.5 | 98.0 | 260.0 | 7.3 | 56.3 | 49.6 |
| CV | 32.5 | 74.1 | ---- | 22.3 | 77.9 | 88.5 | 20.4 | 99.7 | 19.2 |

The most productive genotype was Lewis-p5 with 56.3 g plant$^{-1}$, compared to an average of 13.1 g plant$^{-1}$ with an extremely high CV (99.7%). Genotypes that showed a high seed production per plant were Q2002395327 (40.5g plant$^{-1}$), PI340263 (28.8g plant$^{-1}$), PI288759 (20.8 g plant$^{-1}$), Johannesburg (18.3g plant$^{-1}$), Lewis-p1 (18.1g plant$^{-1}$), Monument-p3 (17 g plant$^{-1}$), Monument-p42 (15.6 g plant$^{-1}$) and NC70300525 (15.5 g plant$^{-1}$).

The 1000 seed weight was on average 36.4 g with a CV of 19.2% indicating a lower variation compared to the other parameters. PI254368 exhibited the highest seed weight (49.6 g). High values were also recorded in PI340263 (48.5 g), PI212986 (45.8 g), India 3 (45.5 g), PI288757 (45.2 g) and PI288735 (45.0 g).

Table 12.12 “Main morphological and productive traits of the 25 selected genotypes ± s.d.”

| Genotype | Plant height (cm) | Height I° node (cm) | N° branched | Degree of earliness | Fertile nods per plant (n) | Pods number | N° seeds per pod | Seed production (g) | 1000 seeds weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| PI288757 Botr. | 62.0 ± 7.0 | 2.5 ± 0.3 | 0,5±0.5 | 2 ± 0 | 9.5 ± 4.5 | 27 ± 18 | 4.4 ± 1.1 | 5.8 ± 4.4 | 45.2 ± 1.4 |
| PI288759 Botr. | 124 ± 29.4 | 2 ± 0.2 | 0 | 3 ± 0.3 | 12 ± 3.1 | 82± 26.4 | 6.8 ±0.9 | 20.8 ± 12.6 | 38.3 ± 0.9 |
| PI212986 Botr. | 84 ± 11.0 | 3.3 ± 1.3 | 0 | 3 ± 0.6 | 16 ± 3.5 | 36 ± 20 | 7.3 ± 0.8 | 10.3 ± 4.7 | 45.8 ± 1.1 |
| PI288745 Botr. | 94 ±16.7 | 4.8 ± 0.8 | 0,5±0.5 | 3 ± 0.4 | 18.5 ± 4.5 | 49.5 ± 25.5 | 6.6 ± 0.3 | 9.2 ± 5.4 | 27.9 ± 1.3 |
| PI288738 Botr. | 74.3 ± 8.3 | 4 ± 1.5 | 0 | 3 ± 0.9 | 11.5 ± 1.5 | 27.5 ± 12.5 | 6.6 ± 0.8 | 6.7 ± 1.9 | 45.0 ± 0.6 |
| PI288758 Botr. | 50.5 ± 1.5 | 3 ± 0.5 | 0,5±0.5 | 2 ± 0.4 | 15 ± 3 | 26 ± 8 | 5.6 ± 0.5 | 3.6 ± 0.1 | 39.8 ± 1.7 |
| PI255928 Botr. | 49.5 ± 3.5 | 3.3 ± 0.3 | 0 | 2 ± 1.0 | 14 ± 2 | 23.5 ± 7.5 | 5.5 ± 0.8 | 4.6 ± 2.0 | 41.7 ± 2.2 |
| PI254368 Botr. | 63.5 ± 15.5 | 2.3 ± 1.3 | 0 | 3 ± 1.1 | 13 ± 3 | 19 ± 3 | 5.5 ± 0.5 | 4.7 ± 1.2 | 49.6 ± 0.7 |
| PI340263 Botr. | 100.5 ± 25.5 | 2 ± 0.4 | 0 | 3.5 ± 1.5 | 13 ± 2.5 | 51.5 ± 42.5 | 6.4 ± 1.1 | 28.8 ± 14.1 | 48.5 ± 1.4 |
| NC70300525 Botr. | 101 ± 28.2 | 2.8 ± 0.3 | 0 | 3 ± 0.9 | 32 ± 4 | 87.5 ± 28.5 | 5.2 ± 0.9 | 15.5 ± 7.6 | 35.4 ± 2.1 |
| Q2002395327 Botr. | 161 ± 34.3 | 4 ± 0.6 | 0 | 2 ± 0.6 | 39 ± 8.8 | 174 ± 72.2 | 6.5 ± 1.2 | 40.5 ± 18.7 | 36.5 ± 0.4 |
| Monument 2 Botr. | 69 ± 14.0 | 2.3 ± 0.3 | 0,5±0.5 | 2.5 ± 0.5 | 31.5 ± 11.5 | 68 ± 17.0 | 4.8 ± 0.2 | 8.1 ± 2.5 | 35.4 ± 0.2 |
| Lewis, Botr. | 74 ± 16.8 | 2.5 ± 0.4 | 0 | 3 ± 0.6 | 22 ± 5.5 | 66 ± 18.4 | 6.1 ± 0.8 | 13.6 ± 8.4 | 37.9 ± 0.8 |
| India 2, Botr. | 30.5 ± 6.4 | 5.5 ± 0.8 | 0 | 1 ± 0.2 | 4 ± 1.5 | 4 ± 0.5 | 3.8 ± 0.4 | 0.5 ± 0.1 | 30.9 ± 0.6 |
| India 1 | 78.5 ± 8.5 | 13.5 ± 2.5 | 0 | 2 ± 0.3 | 14 ± 3 | 15 ± 2.0 | 4.3 ± 0.8 | 1.5 ± 1.1 | 35.6 ± 0.9 |
| India 2 | 68.5 ± 23.6 | 2.8 ± 1.8 | 0 | 2.5 ± 0.5 | 13 ± 4 | 15 ± 5.0 | 5.4 ± 0.4 | 1.8 ± 1.0 | 30.8 ± 0.9 |
| India 3 | 85.7 ± 8.8 | 4 ± 0.8 | 0 | 2.3 ± 0.3 | 20.7 ± 3.2 | 40 ± 13.3 | 6.0 ± 0.2 | 7.4 ± 3.8 | 45.5 ± 1.8 |
| India 2, Bev. | 87 ± 17.0 | 3.3 ± 0.3 | 0 | 3 ± 0.4 | 21 ± 5 | 36.5 ± 9.5 | 2.4 ± 0.1 | 2.1 ± 0.5 | 38.5 ± 0.6 |
| India 2, Vac. p39 | 68 ± 7.0 | 5.8 ± 1.3 | 0 | 3.5 ± 1.5 | 15 ± 1 | 23 ± 1.0 | 4.6 ± 0.1 | 2.4 ± 1.3 | 38.4 ± 1.4 |
| Johannesburg | 71 ± 16.1 | 1 ± 0.5 | 0,5±0.5 | 3 ± 0.6 | 55 ± 22.4 | 98 ± 32.4 | 5.7 ± 0.6 | 18.3 ± 11.1 | 38.5 ± 0.8 |
| Lewis, p1 | 77 ± 25.0 | 1 ± 1.0 | 1±1 | 3 ± 1.0 | 50.5 ± 28.5 | 105.5 ± 66.5 | 5.5 ± 0.4 | 18.1 ± 11.2 | 31.9 ± 0.6 |
| Lewis, p5 | 110 ± 26.4 | 1 ± 0.2 | 0,5±0.5 | 2 ± 0.2 | 98 ± 34.8 | 260 ± 124.1 | 7.3 ± 1.3 | 56.3 ± 19.8 | 32.3 ± 0.6 |
| Monument, p42 | 63 ± 2.9 | 1.5 ± 0.2 | 2±2 | 3 ± 0.8 | 34.5 ± 12.5 | 94.5 ± 24.5 | 5.2 ± 0.9 | 15.6 ± 1.7 | 38.3 ± 0.9 |
| Monument, p3 | 94.5 ± 6.5 | 3.3 ± 0.3 | 0 | 3 ± 0.6 | 32.5 ± 3.5 | 90.5 ± 22.5 | 6.7 ± 0.1 | 17 ± 2.7 | 30.1 ± 2.4 |
| Monument, p4 | 76 ± 8.4 | 2.3 ± 0.3 | 0 | 3 ± 0.9 | 30 ± 3.8 | 77.5 ± 6.5 | 6.2 ± 0.3 | 13.2 ± 2.0 | 33.2 ± 1.0 |
| *Average* | *80.7* | *3.3* | *0.2* | *2.7* | *25.4* | *63.8* | *5.6* | *13.1* | *36.4* |
| CV % | 32.5 | 74.1 | 191.3 | 22.3 | 77.9 | 88.5 | 20.4 | 99.7 | 19.2 |

Degree of earliness at harvest**:** 1= vegetative state; 2= green stem with dried pods for less than half of the stem; 3= green stem with dried pods for more than half of the stem, 4= green stem with dried pods.

The results of the 14 genotypes showed a considerable improvement compared to the values of the 68 genotypes from which they were selected. On average the height of the first node passed from 1.8 cm to 3.1 cm, this parameter is very important to reduce the loss of pods at the combine harvest. Number of branched passed from 5.3 to 0.1, that means that almost all the 14 genotypes selected were single-stem, a very important result to increase sowing density and facilitate harvest. Degree of earliness passed from 0.95 to 2.6 showing a better maturation at harvest (Table 12.13).

On the contrary the productive parameters, seeds per pod, pods per plant and consequently also the total production of seeds per plant decreased, because the selected plants were almost all single-stem. This productive results obviously can not take in account the greater sowing density that can be obtained with single stem genotype.

Table 12.13 “Comparison of the main traits between the 68 original genotypes and 14 selected ones”

| Genotype | Plant height (cm) | Height I° node (cm) | Branch number | Degree of earliness | Fertile nods per plant (n) | N° Pods per plant | N° seeds per pod | Seed production (g) | 1000 seeds weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| **Average 68 genot.** | 59.6 | 1.8 | 5.3 | 0.95 | 22.4 | 122.5 | 6.6 | 29.0 | 40.1 |
| **CV %** | 20.9 | 51.3 | 72.9 | 56.2 | 51.8 | 51.7 | 12.1 | 53.6 | 9.7 |
| **Average 14 sel. genot.** | 81.3 | 3.1 | 0.1 | 2.6 | 17.9 | 53.0 | 5.8 | 12.3 | 39.8 |
| **CV %** | 41.2 | 34.0 | 164.1 | 26.3 | 54.9 | 80.9 | 17.2 | 89.5 | 16.2 |

Degree of earliness at harvest**:** 1= vegetative state; 2= green stem with dried pods for less than half of the stem; 3= green stem with dried pods for more than half of the stem, 4= green stem with dried pods.

**Trial 4:** Cross-pollination of guar genotypes

The two-year cross-pollination tests were performed according to the method of Chaudhary *et al*. (1974). In the first cross-pollination test, carried out on 25 genotypes, more than 100 attempts were executed crossing all plants as the flowers were ready. For what we may know from the morphological characteristics of the pods obtained, three cross-pollinations have been successful. We were not far from the percentage of success reported in the literature (6-8%), indicating that pods show number of seeds per pod below the average and shape and size of the pod different from usually (Fig. 12.6). In table 12.14 are reported the cross-pollinations obtained.

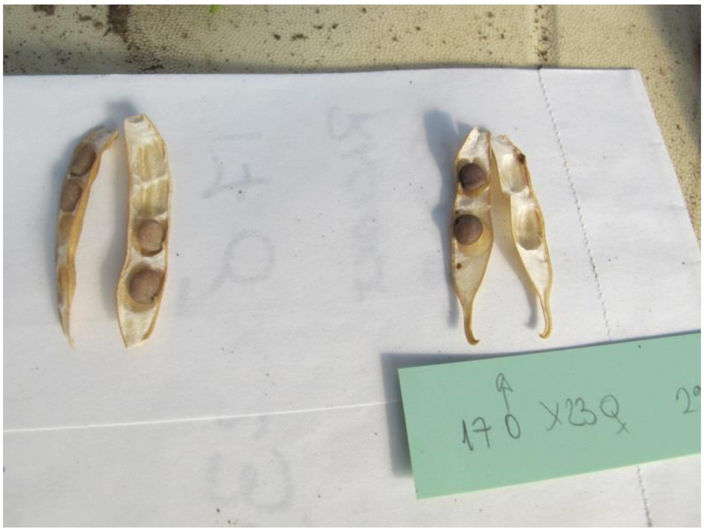

Fig. 12.6 Pod and seeds result of cross pollination (right) compared to a small self-pollinated pod (left)

Table 12.14 “Cross-pollinations obtained in the first year”

| Genotypes receiving (♀) | Genotypes pollen donor (♂) | Seeds number per pod |
|---|---|---|
| Lewis; 2012; Gela; p 1 | India 2; 2011; Orig. | 1 |
| Monument; 2013; Gela; p 42 | India 3; 2011; Orig. | 2 |
| Monument 2013 Gela; p 42 | India 2; 2011; Orig. | 5 |

The second year of cross-pollinations (carried out on 13 selected genotypes) it has been used the same procedure and about the same number of attempts of the first year has been performed. Two cross-pollinations have been successful. In table 12.15 are reported the cross-pollinations obtained.

Table 12.15 “Cross-pollinations obtained in the second year”

| Genotypes receiving (♀) | Genotypes pollen donor (♂) | Seeds number per pod |
|---|---|---|
| Lewis; 2015; Pellaro (RC); p. 6b | PI 340253; 2014; Botricello (CZ); p. 4 | 2 |
| NC70300525; 2015; Pellaro (RC); p. 4a | India; 2013; Gela (CT); p. 39 | 6 |

In this second year, the seeds obtained from cross-pollination of the first year were sowed to evaluate their characteristics. At the moment in which I am writing this thesis the trial is still on-going and we have not quantitative results, however, from a visual point of view, the first two genotypes (Lewis x India e Monument x India3) showed pods with intermediated traits between their parent plants, while the third cross (Monument x India2) showed a clearly determinate growth. This genotype, if this traits will be confirmed in the next years, can be an useful tool for future breeding program looking for determinate growth genotypes.

# 13. Conclusions and Future Perspectives

As general consideration we can affirm that interesting seed production, galactomannan and protein contents were obtained in these trials.

Many traits have been characterized on 68 genotypes looking for genotypes with appropriate traits for the Mediterranean environment (short crop cycle), with an easy harvestability (non-branching and elevated height of the first node) and with valuable productive and qualitative traits. Among the most interesting traits: 17 genotypes resulted non-branching (PI288762, PI 288757, PI 212986, PI 288745, PI 288742, PI 288738, PI 116034, PI 236479, PI 255928, PI 254368, PI 198297, PI 547070, NC70 300525, Q20023 95327, Monument, India 2, India 3), 6 genotypes showed a height of the first fertile node greater than 3 cm (PI 288762, PI 340263, PI 288759, Pusa Mausmi 300537, PI 255928 and PI 288742), 11 genotypes showed a short crop cycle, between 155 and 163 days (PI 288757, PI 288759, PI 212986, PI 288745, PI 426639, PI 116034, PI 236479, PI 288758, PI 426633, PI 340263, and NC70 300525).

From a productive point of view, genotypes with a plant seed production greater than 50 g were: PI 271646, Nawabshar 300528, PI 288749, Lewis, Lasbella 95042 and S-47–2-95069.

As far as the qualitative characterization, genotypes with galactomannan content higher than 30% (PI255928, Monument, PI426635, PI426633, FSSRQ77999, PI340253 and Tari300529) and other with high protein content (PI288385, PI288347, PI288435, PI288381, PI288362, CP6661044, PI288384, PI 426639, CP3161055, S-47-295069, India 3 and PI254368) were individuated.

As a whole, three of the studied genotypes emerged as they exhibit more than one desirable trait. Among these, PI 340263 combines short crop cycle, elevated high of the first fertile node, high seed production and fine branching; NC70300525 was single stem, short cycle and with a medium high production; PI 288759 combines short crop cycle, elevated high of the first fertile node, fine branching and high seed production.

An interesting association was found, for the first time, between galactomannans and protein content, with a highly significative negative relation. This association highlights that breeding process looking for guar genotypes improved for galactomannans content will have as a result genotypes with a lower protein content.

Moreover, for the first time the accumulation of seed galactomannan content during growth has been measured and put in relation with the pod length, pod width, dry seed diameter and pod colour. This information will help to perform even a rough assessment of the accumulation of galactomannan content from a non-destructive morphologic evaluation.

In addition, interesting a negative correlation was found between the branch number and height of the insertion of the first fertile node (-0.758), indicating that branching genotypes show a worst harvestability due to the lower height of the first node. However, the height of the first node resulted negatively related to the cluster number (-0.455).

The results of the 14 genotypes selected showed significantly improvements compared to the values of the 68 genotypes from which they were selected. On average, the height of the first branch passed from 1.8 cm to 3.1 cm; this parameter is very important to reduce the loss of pods at the combine harvest. Branch number passed from 5.3 to 0.1; that means that almost all the 14 genotypes selected are single-stem, a very important result to increase sowing density and facilitate harvest. The degree of earliness passed from 0.9 to 2.6 showing a better maturation at harvest time. On the contrary, the productive parameters, pods per plant and seeds per pod, decreased for two reasons: first of all because we did not select the 14 genotypes for productive traits, and secondly because we selected non-branching genotypes, which are less productive. This implies, in fact, a lower number of pods per plant, but also the possibility of a higher plant density in the field, increasing yield per hectare.

Finally, with the cross-pollination trials, in the two years a total of 5 crosses seem to have been successful. Tests are on-going to evaluated if they show traits of the parent plants. From a first visual observation some of the plants obtained in the first year show intermediated traits between their parent plants. However, all the crosses require a further detailed morphological and also genetic characterization to confirm if they are the result of the cross-pollination carried out.

The results of the present research provide new information on guar characteristics, useful for future breeding programs and that can contribute to the extension of guar cultivation in the Mediterranean environment, able to provide an important contribution in sustainable agricultural systems. Moreover it represents a valuable contribution to the improvement of knowledge on agronomic (productive and qualitative) performance of guar, representing a preliminary step towards the selection of a ‘Mediterranean’ guar ideotype.

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**Supplementary material**

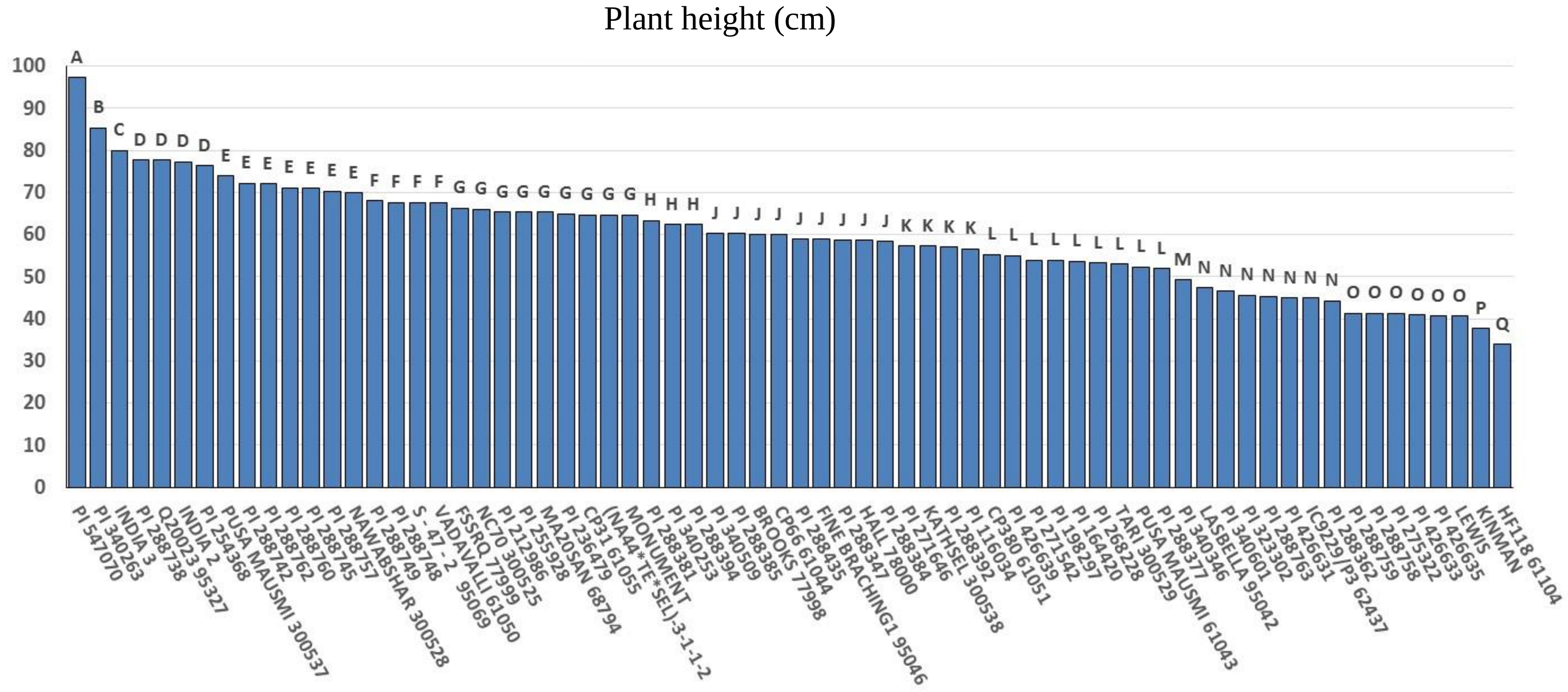


Figure 1S. Plant height of 68 studied genotypes

**Supplementary material**

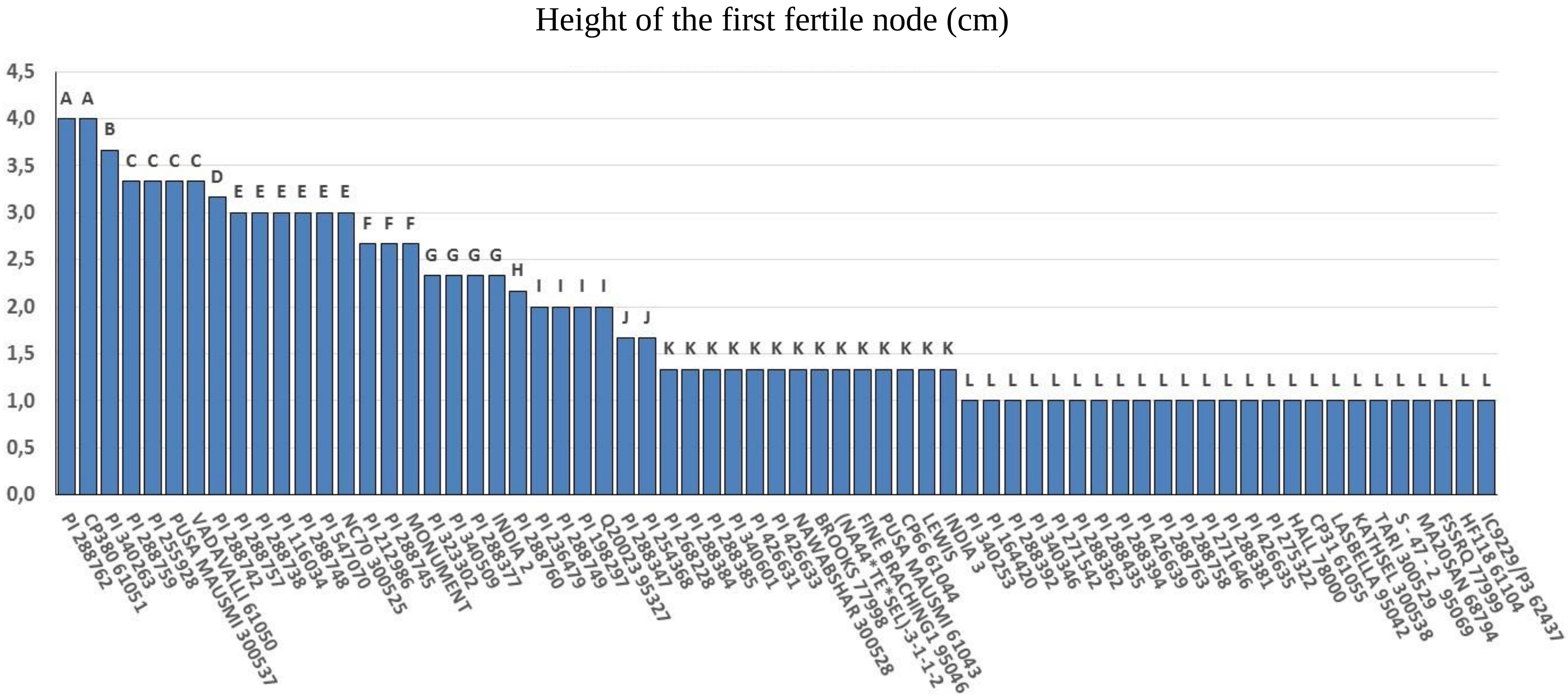


Figure 2S. Height of first fertile node of the 68 studied genotypes

**Supplementary material**

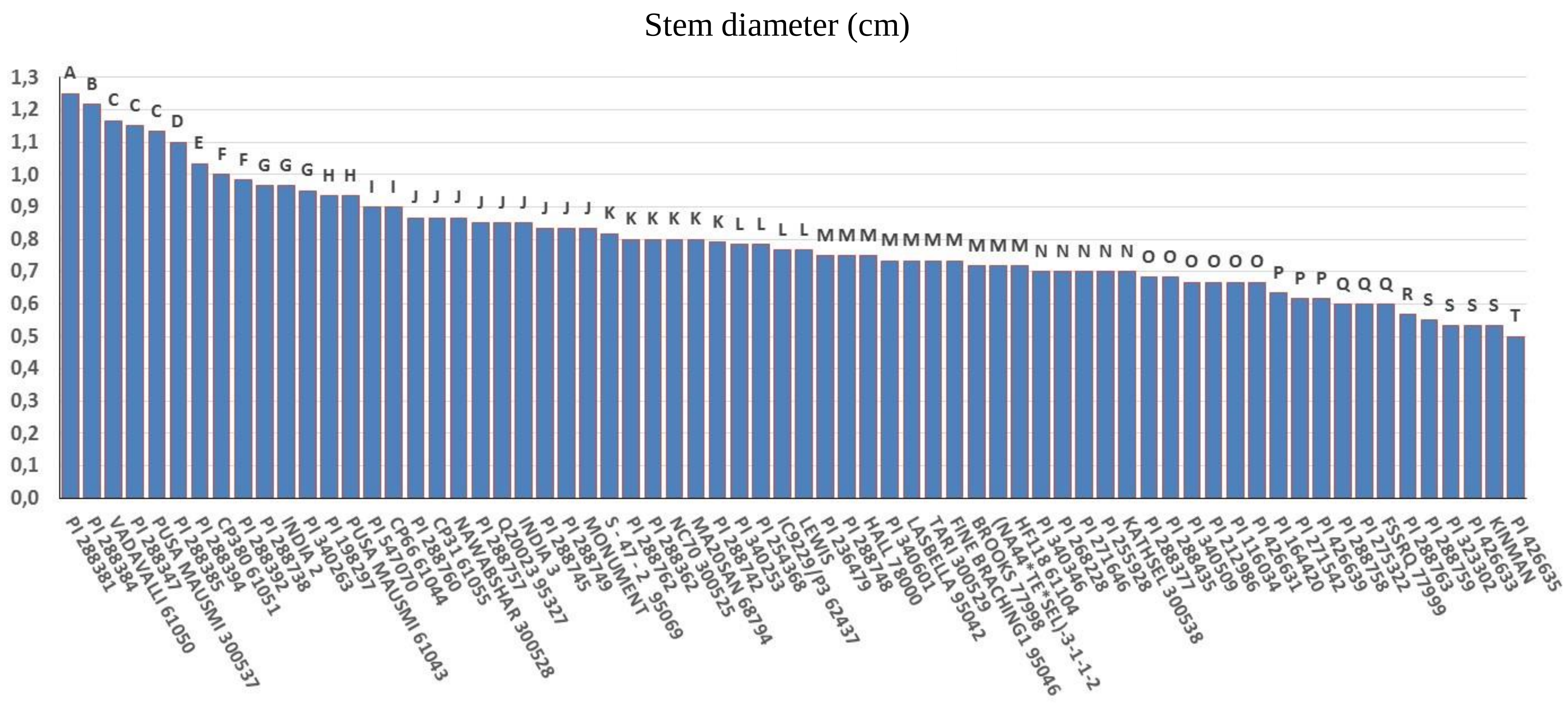


Figure 3S. Stem diameter of the 68 studied genotypes

**Supplementary material**

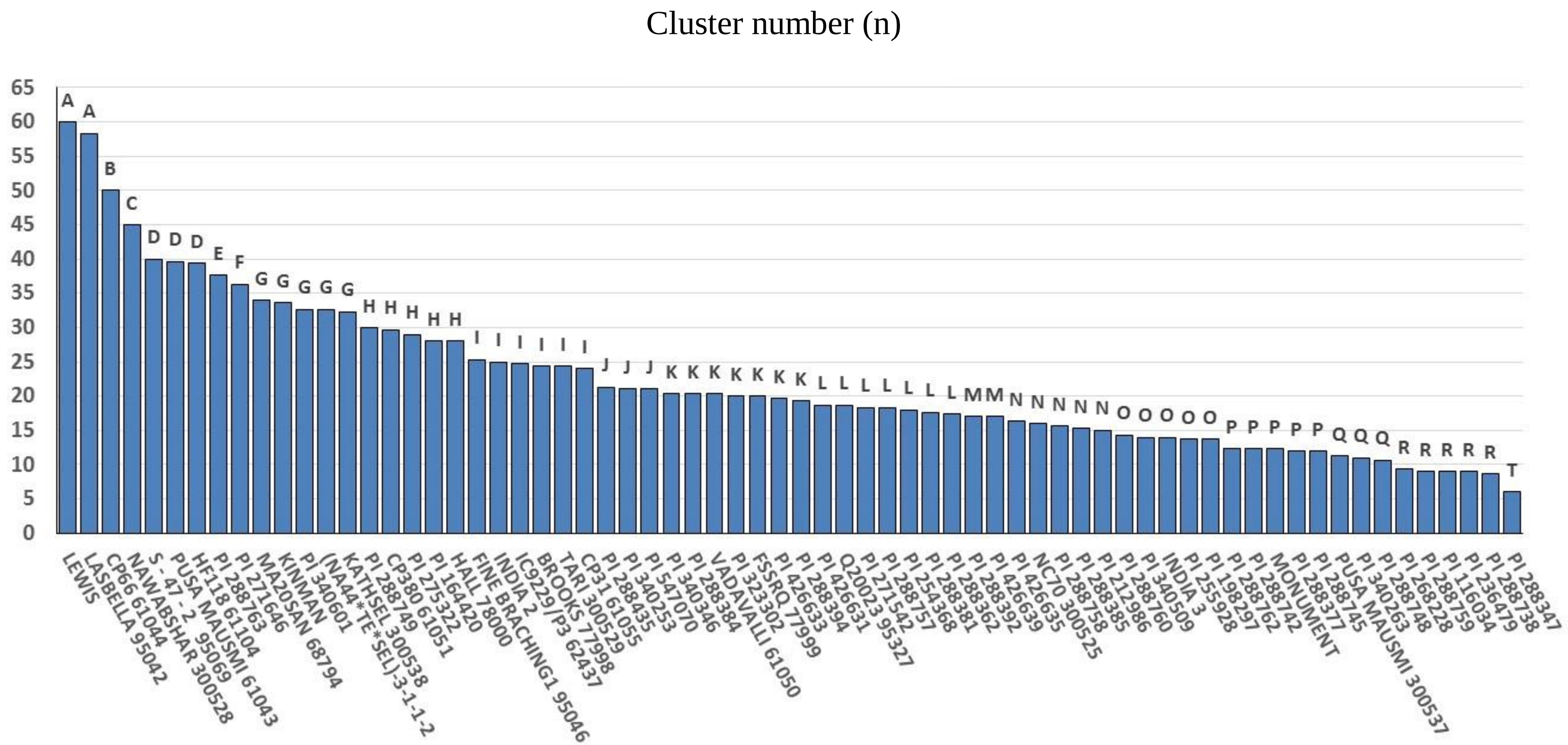


Figure 4S. Cluster number of the 68 studied genotypes

**Supplementary material**

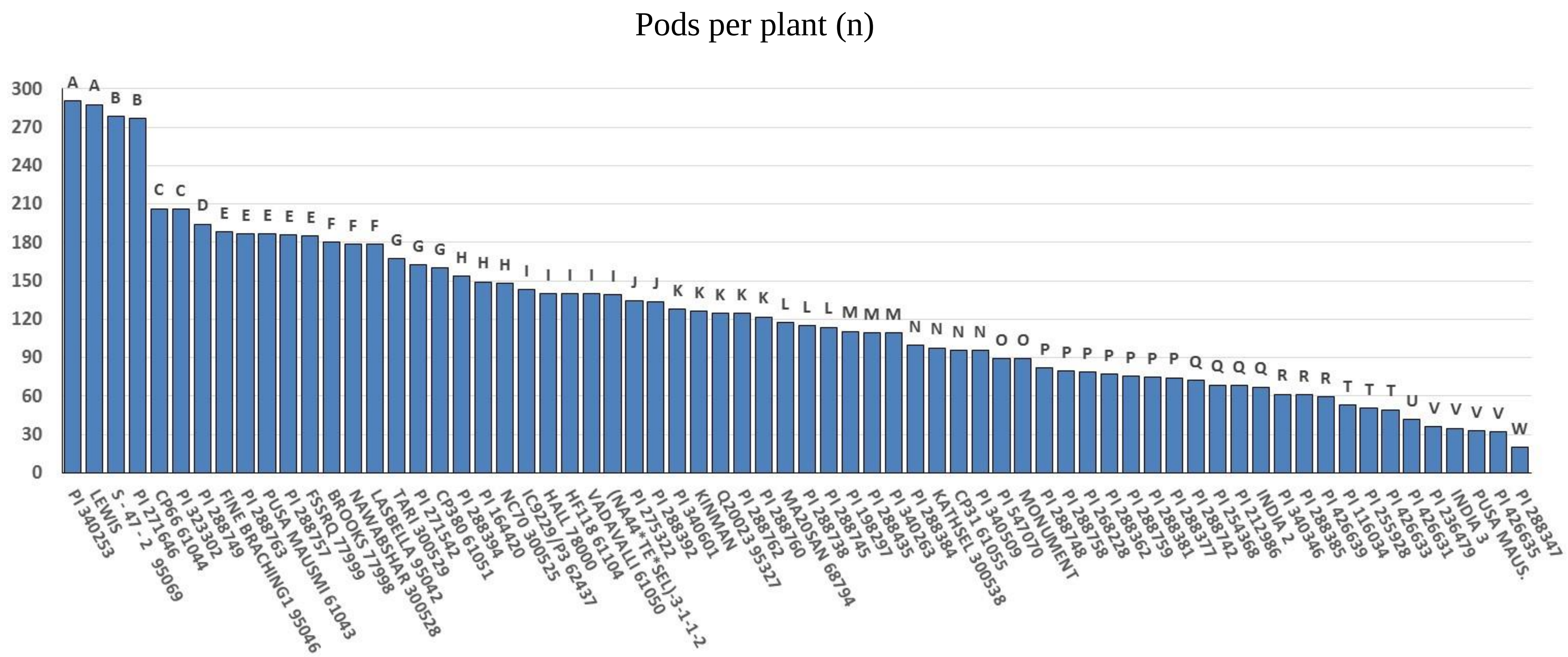


Figure 5S. Number of pods per plant in the 68 studied genotypes

**Supplementary material**

Seeds per pod (n)

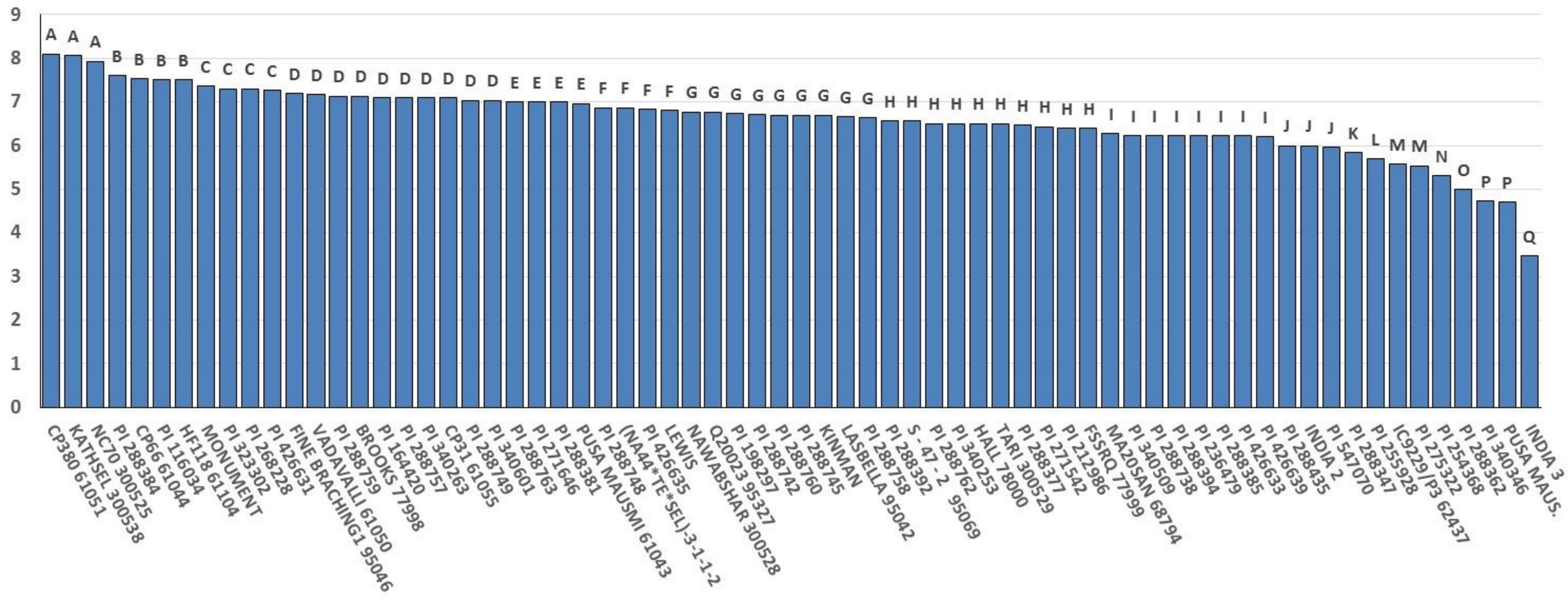


Figure 6S. Number of seeds per pod in the 68 studied genotypes

**Supplementary material**

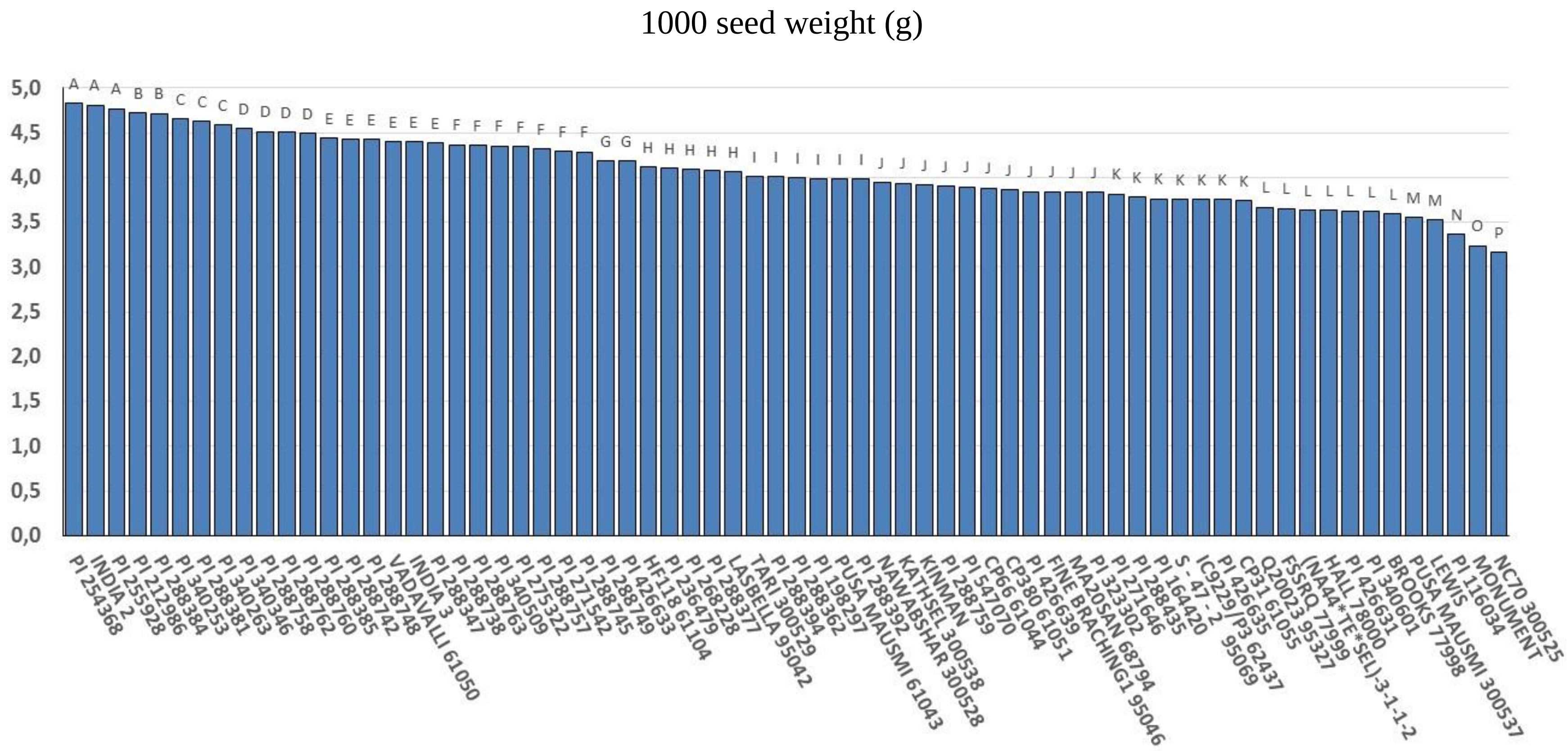


Figure 7S. 1000 seed weight of the 68 studied genotypes

**Supplementary material**

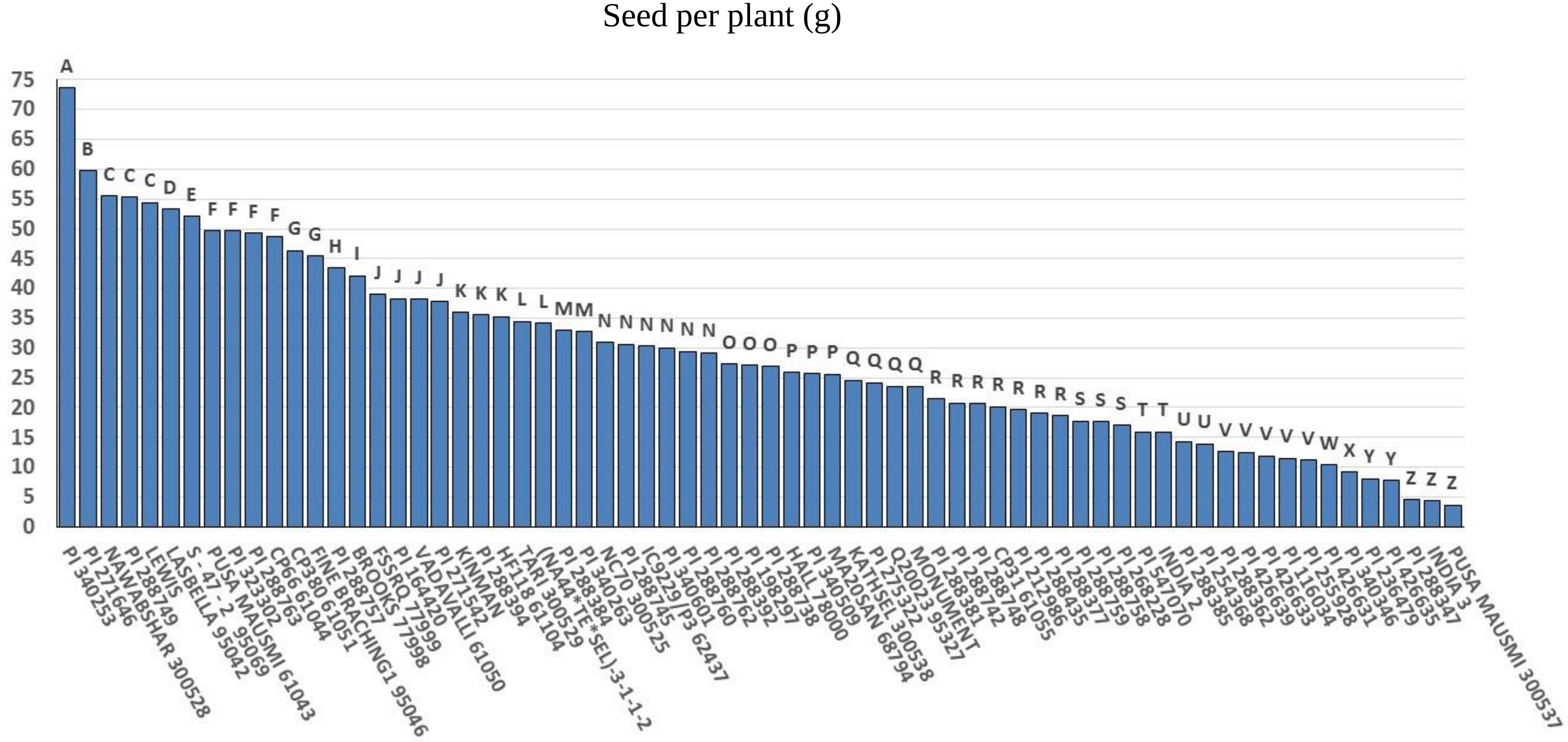


Figure 8S. Seed per plant of the 68 studied genotypes

Average of the length of crop cycle (days)

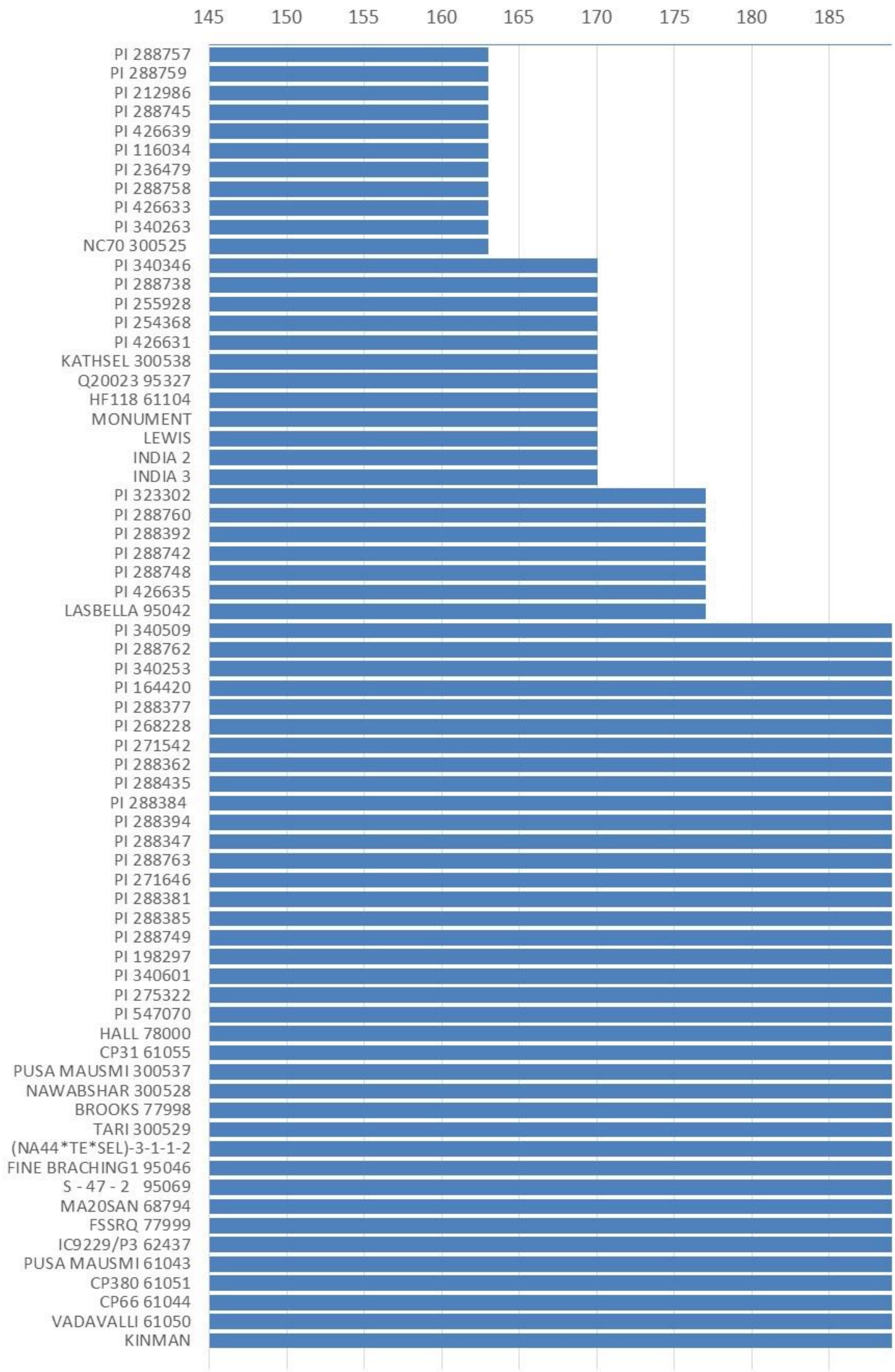


Figure 9S. Average of the length of crop cycle of 68 studied genotypes

# List of papers

1. Gresta F., Mercati F., Santonoceto C., Abenavoli M.R., **Ceravolo** G., Araniti F., Anastasi U., Sunseri F. 2016. Morpho-agronomic and AFLP characterization to explore guar (*Cyamopsis tetragonoloba* L.) genotypes for the Mediterranean environment. *Industrial Crops and Products*, 86, 23-30. DOI 10.1016/j.indcrop.2016.03.038. (I.F.: 3.449)

2. Gresta F., Santonoceto C., **Ceravolo** G., Formantici C., Grillo O., Ravalli C., Venora G. 2016. Productive, qualitative and seed image analysis traits of guar (*Cyamopsis tetragonoloba* (L.) Taub). *Australian Journal of Crop Science*, 10 (7), 1052-1060. DOI: 10.21475/ajcs.2016.10.07.p7810 (I.F.: 1.632).

3. Chiofalo B., Lo Presti V., D’Agata A., Raso R., **Ceravolo** G., Gresta F.(ACCEPTED, IN PRESS). Qualitative profile of degummed guar (*Cyamopsis tetragonoloba* L.) seeds grown in a Mediterranean area for use as animal feed. *Journal of Animal Physiology and Animal Nutrition*. (I.F. 2015: 1.212).

4. Gresta F., Napoli E., **Ceravolo** G., Santonoceto C., Strano T., Ruberto G. (ACCEPTED IN PRESS) Stigmas yield and volatile compounds of saffron (*Crocus sativus* L.) in a late sowing under greenhouse with two nitrogen rates. Saffron Symposium, *Acta Horticulturae*.

5. Chiera E., Gimma G., **Ceravolo** G., Monti M., Santonoceto C., Gresta F. 2014. Guar (*Cyamopsis tetragonoloba* L.): una coltura alternativa per agricoltura biologica degli ambienti meridionali del nostro paese. RIRAB, Roma, 11-13 giugno (Presentazione orale).

6. **Ceravolo** G., Santonoceto C., Monti M., Gresta F. 2015. Valutazione delle caratteristiche produttive e qualitative di specie diverse di lupino (*Lupinus spp.*). Atti del XLIV convegno della Società Italiana di Agronomia, Bologna, 14-16 settembre.

7. **Ceravolo** G., Caracciolo M., Sortino O., Santonoceto C., Gresta F. 2015 Caratterizzazione bio-agronomica e qualitativa di specie di amaranto da granella (*Amaranthus spp.*) in ambiente mediterraneo. Atti del XLIV convegno della Società Italiana di Agronomia, Bologna, 14-16 settembre.

ACCEPTED

8. Gresta F., Guerrini A., Sacchetti G., Maietti S., Sortino O., **Ceravolo** G., Onofri A. Agronomic, chemical and antioxidant characterization of grain amaranths grown in a Mediterranean environment. *Crop Science.*