

With best compliments
+ regards

K. Linn
24/4/66.

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3. Project title. Development of a micro-chemical method for determining gum content in Guar (Cyamopsis tetragonoloha L. Taub.) and the collection and isolation of superior genotypes.
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5. Dates of research Period covered by report. January, 1965 to 31st December, 1965.

Summary

Guar or clusterbean plant has been grown, from ancient times in the Indian sub-continent for vegetable and forage purposes. The seeds are highly valued as a cattle feed. It was introduced into U.S.A. in 1903 by the U.S. Department of Agriculture as green manure and cover crop. Recently the use of guar seeds as a mucilage has increased many folds, as it appeared to be a promising substitute for carob bean gum (Ceratonia siliqua L.).

Guar seed powder on dispersion in water, yields a milky viscous solution, due to the presence of polysaccharide galacto-mannan, popularly known as guar gum. Due to extraordinary properties of the gum, it has found extensive applications in textile, paper, food, mining, explosives, drugs, pharmaceuticals and several other industries.

In view of its growing importance as a source of gum and because of facts that it grows satisfactorily even under drought conditions in large areas of Rajasthan, Punjab, Delhi, U.P. and into the southern region of India, there is a great scope for selecting some varieties richer in gum for each of the growing area. The main aim of the project is to find a micro chemical method for the estimation of gum, to be useful in isolating a variety for recommending to the breeder to breed that variety.

(b) Breeding programme was initiated at the Division of Plant Introduction and about 300 accessions are being processed for Agrobotanical characters. Some 150 cultures with high yielding potentialities and colour of seeds have been selected.

The observation collected about length, height and number of pods varies from variety to variety. The range is as follow:

Height of branches	-	60 cms to 215 cms.
No. of branches	-	13.3 to 20.3
No. of pods per plant	-	30.3 to 32.4.

It has been found that collections from Kutch region gave a high number of pods and the seeds are pink in colour. The highest yield of 6 - 6.4 quintals was obtained in case of I.C.9229/P2 and I.C.9208/P2 respectively.

Some 41 single plant selections grown under arid conditions were made from Plant Introduction sub-station, Jodhpur, Rajasthan.

Chemical:-

A method for the estimation of guar gum has been standardised and it has been observed that the gum content varies from variety to variety. The quality of gum (viscosity), is nearly of the same order, whereas colour of gum varies from white to greyish and brownish depending upon the varieties and their seed's colour.

It is interesting to note that light and pink coloured seeds gave a whiter coloured gum than grey or dark grey seeds. The quantity of gum was also higher in pink or light coloured varieties.

These selected varieties have been analysed for their major constituents, Protein, Ash, Calcium, Iron and Phosphorus.

Another method for the estimation of galactose and manrose quantitatively using chromatographic technique for developing spots and then elution of dark brown colour and measuring its intensity in a colorimeter has been standardised. The method can be applied for estimation of gum in guar, which on hydrolysis give galactose and manrose in the ratio of 1 part to 2 parts respectively.

Significance of the Research:-

Botanical:- The significance of breeding work initiated, when completed will help in evolving varieties suitable for different regions giving a maximum yield, secondly a variety having a maximum quantity of gum of good quality.

Chemical:- The method developed for chemical estimation of guar gum, will ultimately help a plant breeder to breed a variety richer in gum of superior quality. The method can be applied for estimation of gum in a very small quantity of seed available. A little modification and improvement in the method, can make its application for extraction of gum industrially. The Industrial method is also being developed.

Data on the chemical composition of major constituent will help to breed a variety richer in Proteins, Calcium, Phosphorus and other nutrients, as Guar is used both as fodder and vegetable.

The second method developed can be successfully applied for the quantitative estimation of sugars (galactose and maniose), in plants. The method is very useful also for estimation of gum in guar in a very small quantity.

Detailed Report

Botanical Portion:

Sowing of guar material was done in July. Due to weak monsoonic conditions the growth of the crop was not as could be desired.

Agrobotanical assessment of the guar collections comprising 300 accessions is being processed. It has been possible to select out more than 150 cultures with high yielding potentialities, besides possessing various shades in seed colour and other desirable characters. One important consideration has been for the addressed type of branching, a character that will facilitate intercultural operations. Observations on height, number of branches, number of Pods, Pod length, yield and seed colour are being taken. Height in fully branched types ranged from 60 cms. to 150 cms., while in sparsely branched types it reached 175 cms. and in single-stemmed ones it touched new height of 215 cms. The average number of branches per plant varied from 13.3 to 20.3 and the number of Pods averaged from 30.3 to 324.0 per plant. It is interesting to mention that the collections originating from the Kutch region in North-Western India appeared to be superior in pod formation and also in having pink seed colour. As for example, two accessions from this area, viz., I.C.11427 and I.C.11425 produced 223.3 and 324.0 Pods per plant respectively besides being pink seeded.

It may be stated that pink colour of the seeds seem to be of importance since preliminary gum extraction studies have indicated that the gum obtained from light or pink coloured seeds is whiter in comparison to that obtained from grey or dark grey seeds. Assessment for seed size and yield is in progress.

Of the 70 single plant progenies grown in 4-row observation plots, 38 selections have been retained. The highest calculated seed yield of 6.4 quintals (above 17 mds.) per acre was obtained from the selection I.C.9208/P2 followed by I.C. 9229/P2 which gave 6 quintals.

The F₂ and F₃ progenies of the crosses made earlier were grown for further studies, single plant observations were taken in respect of height, branching, Pod formation, seed yield and size. The data is being analysed.

Further collection of guar was made from the chief guar growing areas viz., Western Rajasthan and parts of Gujrat and Punjab. The material is being accessioned.

At the plant Introduction sub-station, Jodhpur, Rajasthan, also a duplicate lot of guar collections was grown for assessment of the material under semi-arid conditions. 41 single plant selections were made on the basis of their agronomic qualities and botanical characters.

According to the comments on the previous report, it was desired to describe the technique followed in hybridisation work. Much difficulty was experienced in effecting emasculation and subsequent pollination due to the small size of flowers. However, with the help of watch-maker's eye glass, handling of flowers could be made easier, the percentage of success being low i.e., only about

2% . Emasculation was done in the afternoons for pollination in the following mornings. Pollen used to be scooped out on the tip of the forcep and then transferred to the stigma.

Chemical Portion:

Introduction

Galactomannans are common constituents of the ungerminated seeds of leguminous plants. They occur as mucilages in the endosperms of the seeds, from which they may be isolated by extraction with water. As mucilage and endosperm disappears during germination it serves as a reserve food^{1,2}.

An extensive investigation of the seeds of more than 150 species of legumes has revealed that many of them contains galactomannans, in some cases more than 40% Wise and Appling³ and Anderson⁴.

Locust bean gum was being widely used in the textile, paper, food and other Industries Andrews et al⁵, but during the second World War when carob gum was not available, a suitable substitute was searched for gum source. The galactomannoglycans of Guar (Cyamopsis tetragonolobus L.) usually known as 'Guaran' was found to be a suitable source for gum. Guar Gum has taken an important place, in paper, textile, food, mining, explosives drugs, Pharmaceuticals and several other Industries. The highest yield of mucilage in guar was reported to be 35% Andrews et al⁵. The composition of galactomannan's is also variable, although many workers have reported that 'Guaran' contains D-Galactose and D-mannose units in the molar ratio 1:2 and known as D-galactose - D-mannoglycan (1:2).

In view of its growing importance as a source of gum and because of facts that it grows satisfactory even under drought conditions in large areas of India, there is a greater scope for selecting some varieties rich ~~in a greater scope for selecting some varieties rich~~ in gum for each of the growing area. The main aim of the project is to find a microchemical method for the estimation of gum, to be useful in isolating a variety for recommending to the breeder to breed that variety. Secondly to breed a variety richer in nutrient for feeding cattles.

Experimental

(a) Guar seeds were cleaned and a portion of it was ground in a willy grinding Mill using mesh No.40.

(b) The second portion of weighed seeds was ground in a grinder "Multimix MX3 (BRAUN)" for 1 minute and the endosperm was separated out. The separated endosperm was weighed and ground in a willy grinding Mill.

Extraction of Galactomannan.

First Method: 0.1 gm to 0.2 gm of the ground endosperm was extracted with 10 ml M20 for 1/2 an hour with occassional shaking at 70° to 80° in a boiling water bath. The cooled mixture was centrifuged and the solid was collected and again extracted with 10 ml water as described above. This

extraction + centrifugation process was repeated 3 times. To the supernatant liquor about 20 ml of Fehling solution was added and the resultant insoluble copper complex was separated by centrifugation, washed with water and again centrifuged. The copper was removed by the addition of N. Hydrochloric acid 8 ml., with vigorous stirring to its suspension in ice cold water Daond⁶. The resultant viscous solution was filtered and the filtrate poured into alcohol Brown et al⁷ and Vladimir Podrazby⁸ and Jaroslov Fantik. The precipitates were washed with acetone and then with ether and dried, weighed and percentage of gum determined.

Second Method:- 0.1 gm to 0.2 gm of the ground Endosperm was extracted with 10 ml H₂O for 1/2 an hour with occasional shaking at 70° to 80° in a boiling water bath. The cooled mixture was centrifuged and the solid collected and again extracted with 10 ml water as described above. This extraction and centrifugation process was repeated 3 times. The supernatant liquor was poured into 50 to 60 ml of ethyl alcohol. White amorphous precipitates were centrifuged washed with acetone and Petroleum ether, dried and weighed. Percentage of gum obtained calculated. To see the efficiency of this method a recovery experiment was conducted by adding Guar Gum obtained from (Nutritional Biochemical Corporation, Cleveland, OH¹⁰). The recovery was found to be 100%.

Third Method:- 2.0 gms of guar atta was boiled with 50% (ethanol) (5 fold) for 15 minutes to inactivate the flours. The inactivated flours were extracted with 10 ml H₂O. The supernatant liquid was centrifuged. The process of extraction was repeated 4 times to be sure of complete extraction. The extracts were deproteinised Vladimir Podrazky and Jaroslov Fantik⁸). Centrifuged and dialyzed overnight against distilled water, and Gum was precipitated with ethanol, washed with acetone and petroleum ether and dried on a water bath or electric oven at 70° to 80°C.

General analysis:- Protein, ash, calcium, Iron were determined according to A.O.A.C.⁹ and Phosphorus was determined by King's¹⁰ method.

Results and Discussions:

The following selected varieties received ^{from} for the Division of Plant Introduction were analyzed for their gum content, data is presented in Table I.

Table I

Results expressed on sample basis
(Mean value of duplicates).

S.No.	Name of variety	Percentage of Gum by Ist Method	Percentage of Gum by Ind method	Percentage of Gum by Ird method	Colour of Gum	Viscosity
1.	I.C.9007	11.3	23.9	22.13	Greyish white	++
2.	I.C.9230	-	33.5	33.34	White	++
3.	I.C.9233	-	19.3	-	Greyish	++
4.	I.C.9054	-	16.2	-	White	++
5.	I.C.439	-	18.0	-	White	++
6.	I.C.9026	-	17.4	-	Brown	++
7.	I.C.8926	-	16.9	11.13	White	++
8.	Sada Bahar	-	2.13	3.17	Brown	++

From the data presented in Table I, it appears that I.C.9230, and I.C.9007 contains 33.5 & 23.9 percent of gum on sample basis, which agrees with the results reported by Andrews et al⁴. As regards the method of analysis. It appears that the results obtained by the second method and the 3rd Method used by Vladinir Podrasky and Jaroslov Fantik⁸ in rye are quite agreeable. Recovery test carried with the second method evolved here showed a 100% recovery as shown in Table 2. The method developed is a quick and can be applied for a small amount of material available. It is also less time consuming.

Table 2

Recovery Table

S.No.	Name of variety	Amount of gum added	Amount of gum recovered or extracted	% of recovery
1	I.C.9233	nil	0.0162	..
2.	"	0.0283	0.0448	100%

The quality characteristic as judged from the colour of the gum obtained as given in Table I shows that the variety I.C.9230 which has yielded highest amount of gum has given the whitest amorphous gum and as such is better than any other variety. The vegetable variety (grown for vegetable purposes) has yielded brown gum with lowest percentage of gum in it.

The viscosness on (weight/volume) shows that they have the same order of viscosity.

The major constituents present in different selected varieties are presented in Table 3.

Table 3

On oven dry basis

S.No.	Name of variety	Protein %	Ash %	Calcium mg/100 gms	Iron mg/100 gms	Phosphorus mg/100 gms
1.	I.C.9007	31.85	3.03	268.74	39.20	339.15
2.	I.C.9230	27.17	2.63	303.96	26.60	299.80
3.	I.C.9026	29.04	3.44	339.17	34.48	282.0
4.	I.C.9004	28.24	3.07	296.54	34.12	296.31
5.	I.C.8926	24.35	3.14	294.69	32.93	321.30
6.	I.C.439	29.35	3.06	337.32	35.00	264.18
7.	I.C.9233	31.60	2.85	294.69	26.77	264.18

It appears from the data in Table 3, that major constituents vary from variety to variety. Protein and ash content varies from (24.35 to 31.85) percent and (2.63 to 3.44) percent respectively. Whereas calcium, Phosphorus and iron content varies from (268.84 - 339.17); (264.18 - 339.15); and (26.60 - 39.20) mg/100 gm respectively. No correlation appears to be in the gum content and the major constituents.

More data will be required to establish some correlation between Major constituents and the gum content. Results obtained in the Major constituents agrees very well with the data reported in wealth of India¹¹.

Conclusions:

The present status of the project is to continue the analysis work both in the gum content and in the Major constituents. The Method No.(2) developed (sent for publication) will be followed for the gum analysis and any further improvements in it (method) if possible will be carried out.

The variety No. I.C.9230 and I.C.9007 which contains maximum amount of gum respectively have been recommended for multiplication.

Plan for future work.

The work of analysis will be continued in the new crop for this year also. The varieties containing maximum amount of gum will be recommended for multiplication. The correlation between gum content and the major constituents if any, will be worked out. The varieties containing maximum amount of nutrients will also be recommended for multiplication.

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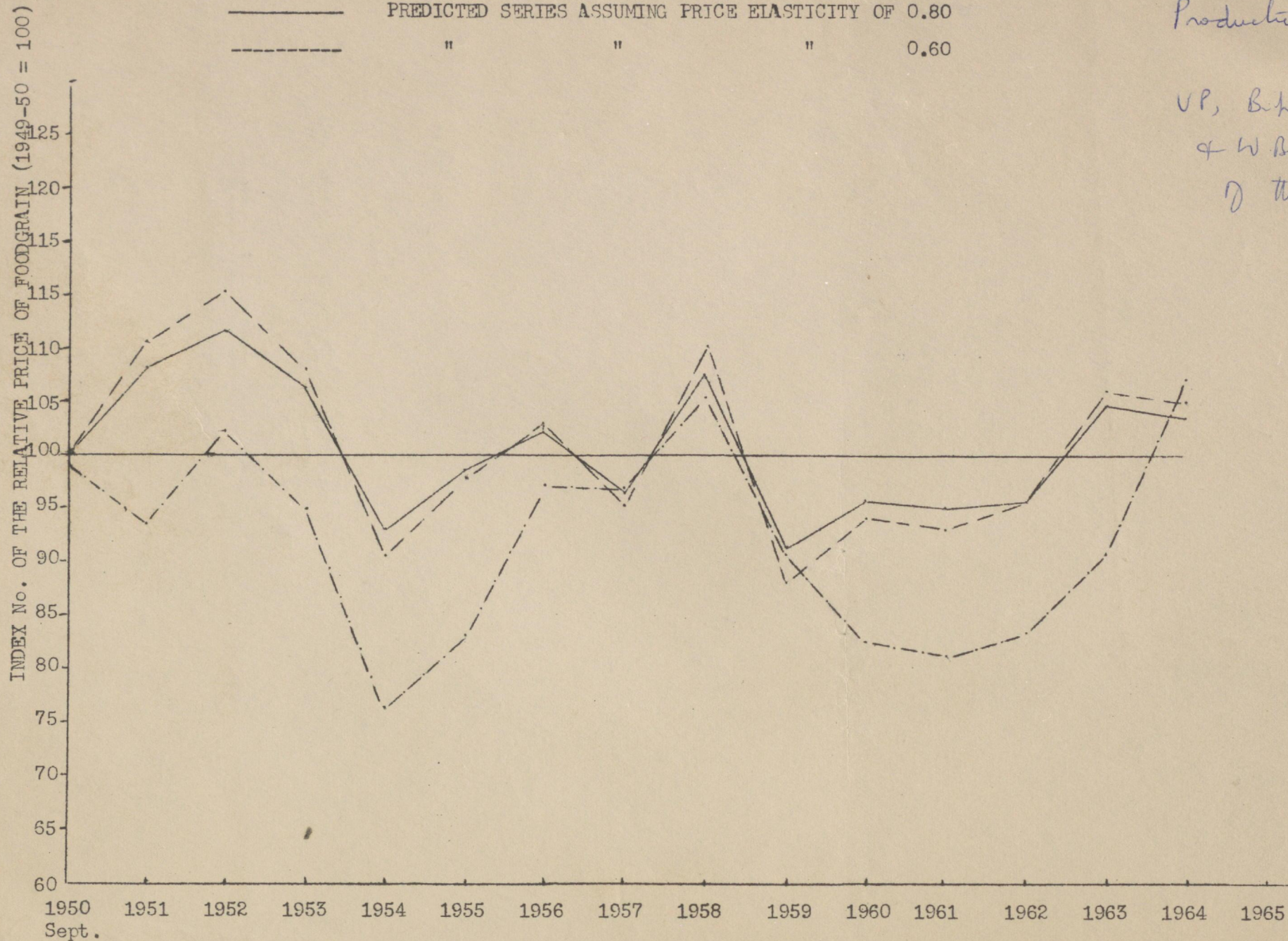
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CHAPTER II

- COMPUTED FROM OFFICIAL INDEX Nos. OF WHOLESALE PRICES
 _____ PREDICTED SERIES ASSUMING PRICE ELASTICITY OF 0.80
 - - - - - " " " " 0.60



Production in 1949-50 - 60.4 million tons

UP, Bihar, Gujarat, Maharashtra & W Bengal consume most of the imported food.