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Changes in crude fiber in mutants of Cluster bean {*Cyamopsis tetragonoloba* L. (Taub.) induced by chemical and physical mutagens.

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Abstract

In the present study two varieties of Cluster bean, namely, Golden Early – 36 (GE-36) and Harit Rani (HR) were used to induce genetic variability. For present study physical mutagen like Gamma rays (5kR, 10kR and 15kR) and two chemical mutagens, namely, ethyl methanesulphonate (EMS) of different concentrations such as 0.05%, 0.10% and 0.15% and sodium azide (SA) of concentrations such as 0.01%, 0.02% and 0.03% were tried. Seeds from each treatment were sown in field following randomized block design (RBD) with three replications along with control as the M1 generation. Further two consequent generations were taken, M2 and M3 generations respectively. Different mutants were screened from both the varieties and they were further biochemically estimated. Crude fiber was estimated by Maynard (1970) method in all the eight mutants of two varieties. In variety GE-36, the highest value (61.31%) for crude fiber content was observed in large leaf, and the lowest value (58.00 %) for crude fiber content could be seen in the high yielding mutant. In variety HR, the highest value (70.20%) for the same parameter could be noted in long pod mutant and the lowest value (52.31%) has been observed in the dwarf mutant, which shows gradual difference in the content of fiber in both the control varieties ie in GE.36 it is 59.21% and in Harit Rani is 58.31%. Harit Rani mutant showed most promising result in increase in crude fiber content. It can be concluded that mutation breeding is one of important tool for enriching the biochemical properties.

Key words: Mutation breeding, Cluster breeding, Mutants, Crude fiber, Ethyl methyl sulphonate and sodium azide.

Introduction

The **guar** or **Lond bean**, with the botanical name ***Cyamopsis tetragonoloba***, is an annual legume and the source of guar gum. It is also known as gavar, guwar, or guvar bean.The origin of *Cyamopsis tetragonoloba* is unknown, since it has never been found in the wild (Whistler R.L. and Hymowitz T. 1979). It is assumed to have developed from the African species *Cyamopsis senegalensis*. It was further domesticated in India and Pakistan, where it has been cultivated for centuries (Undersander D.J. et al 2012). Guar grows well in semiarid areas, but frequent rainfall is necessary.This legume is a valuable plant in a crop rotation cycle, as it lives in symbiosis with nitrogen-fixing bacteria(Undersander D.J. et al 2012) Agriculturists in semi-arid regions of Rajasthan follow crop-rotation and use guar to replenish the soil with essential fertilizers and nitrogen fixation, before the next crop. Guar has many functions for human and animal nutrition, but the gelling agent in its seeds (guar gum) are the most important use(Mudgil D.et.al.2011).Demand is rising due to the use of guar gum in hydraulic fracturing (oil shale gas)(Mudgil.D.et.al. 2011) About 80% of world production occurs in India and Pakistan, but due to strong demand, the plant is being introduced elsewhere. Guar is grown principally in north-western India and Pakistan (Guar gum online 4) with smaller crops grown in the semiarid areas of the high plains of Texas in the US (Gaur Production 2006). Australia and Africa. The most important growing area centres on Jodhpur in Rajasthan, India where demand for guar for fractionation produced an agricultural boom as in 2012. (Gardiner Harris July 16, 2012). Currently, India and Pakistan are the main producers of cluster bean, accounting for 80% production of the world's total, while Thar, Punjab Dry Areas in Pakistan and Rajasthan, Gujarat, Kutch region occupies the largest area (82.1%) under guar cultivation in India. In addition to its cultivation in India and Pakistan, the crop is also grown as a cash crop in other parts of the world. (Pathak, R. et.al.2010).Several commercial growers have

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Volume: I

converted their crops to guar production to support the increasing demand for guar and other organic crops (Pathak R. 2011) in the United States.

Mutation breeding is built on mutation induction and mutation detection. Mutation induction coupled with selection remains the "cleanest" and most inexpensive way to create varieties by changing single characters without affecting the overall phenotype. Mutation induction involves the treatment of plant propagules with mutagens (chemical or physical). This is followed by selection for desirable changes in the resulting mutants. Breeders use mutation induction to broaden the genetic base of germplasm, and use the mutant lines directly as new varieties or as sources of new variation in breeding programs.Mutation breeding has many comparative advantages. It is cost effective, quick, proven and robust. In addition, mutation breeding is transferrable, ubiquitously applicable, non-hazardous and environmentally friendly. There are more than 3200 mutant varieties officially released for commercial use in more than 210 plant species from more than 70 countries, as referenced in the Mutant Varieties Database. The vast majority of released mutant varieties consist of cereals, followed by flowers and legumes.

Dietary Fiber

As indigestible complex carbohydrates, dietary fibers perform important biological functions, though they supply no calories or nutrients and are resistant to digestive enzymes. Dietary fiber comes from the walls of plant cells and includes cellulose, hemicellulose, lignin, pectin, mucilage and gum. Many food products list total fiber content in grams, which includes both soluble and insoluble fiber.

Crude Fiber

Crude fiber refers to one type of dietary fiber, the type that remains as residue after food receives a standardized laboratory treatment with dilute acid and alkali. The treatment dissolves all the soluble fiber and some of the insoluble fiber in a food. The residue or crude fiber is primarily composed of cellulose and lignin. Crude fiber is a nutritionally obsolete term, according to the National Research Council's Commission on Life Sciences. Crude fiber measurements, the result of lab analysis, may underestimate the actual dietary fiber in a food item by 50 percent or more.

Materials and methods

The seed material of two varieties of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.) namely, Golden Early 36 and Harit Rani obtained from Golden Seeds Pvt. Ltd, Bangalore, Karnataka and Navalakha Seeds Pvt. Ltd, Pune have been used in the present study.

Mutagens Used:

The chemical mutagens namely ethyl methane sulphonate (EMS) and sodium azide (SA) and physical mutagen Gamma rays were used in the present study.

Details of Mutagenic Treatments:-

To begin with the pilot experiments were conducted for determining the suitable concentrations/doses for further studies.

Preparation of mutagenic solution:-

The chemical mutagenic treatments were prepared at room temperature of 25±2°C. The fresh aqueous solutions of the mutagens were prepared prior to treatment.

Treatment:

Prior to chemical mutagenic treatments, seeds were immersed in distilled water for 6hrs. The presoaking enhances the rate of uptake of the mutagen through increase in cell permeability and also initiates metabolism in the seeds. Such presoaked seeds were later immersed in the mutagenic solution for 6hrs with regular shaking. Seeds soaked in distilled water for 6hrs served as control.

Three consequent generations were taken and observed M1, M2 and M3. Also during these three generations different morphological characters and biochemical parameters were observed and studied.

Estimation of crude fibre:-

The crude fibre estimation was carried out by the method given by Maynard (1970).

Extraction of pod fibre:-

Fine powder of oven dried pods was made and used as sample. 2g of dried sample was boiled with 200ml of 1.25% H_2SO_4 for 3minutes. After boiling the solution was filtered through Whatman filter paper and washed with 200ml of 2.5% NaOH for 30

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Volume: I

minutes. Again it was filtered through preweighed Whatman filter paper by washing the residue. It was again boiled with 100ml distilled water for 30minutes. The residue was filtered with preweighed Whatman filter paper with several washings with distilled water and lastly with 70% ethyl alcohol. At last the residue was dried overnight at 100°c in oven. Crude fibre content was calculated by formula.

$$\% \text{ Crude fibre content} = \frac{\text{On ignition (gm)}}{\text{Weight of sample (gm)}} \times 100.$$

Results

The crude fibre content was estimated in the mutants of cluster bean varieties GE-36 and HR. In control of variety GE-36, the crude fibre content was 59.21% while in variety HR it was 58.31%. In GE-36, crude fibre content was increased in the large leaf mutant (61.39%) and dropped (56.5%) in small pod mutant. In variety HR the highest crude fibre content (70.20%) was demonstrated by the long pod mutant and the least (52.41%) by the dwarf mutant.

Table 1: Crude fibre content in mutants of M3 generation in cluster bean variety GE-36.

Sr, No.	Mutants	Crude fibre %
1	GE-36(Control)	59.21
2	Tall	59.25
3	Dwarf	60.25
4	Long pod	60.32
5	Small pod	56.5
6	Branched	61.28
7	Large leaf	61.39
8	Early flowering	58.06
9	High yielding	58.00

Statistical analysis:
Mean : 59.37
S.E: 0.53
S.D: 1.610
CD at 0.05= 1.22
CD at 0.01= 1.72

Table 2: Crude fibre content in mutants of M3 generation in cluster bean variety HR.

Sr, No.	Mutants	Crude fibre %
1	HR(Control)	58.31
2	Tall	54.23
3	Dwarf	52.41
4	Long pod	70.20

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5	**Small pod**	64.31
6	**Branched**	69.33
7	**Large leaf**	62.20
8	**Early flowering**	58.31
9	**High yielding**	56.21

Statistical analysis:
Mean : 60.61
S.E: 20.5
S.D: 61.6
CD at 0.05= 22.8
CD at 0.01= 0.01

Discussion and Conclusion

Cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.) (2n=14) is a drought tolerant annual multipurpose legume that was introduced for feed, green fodder, vegetable, green manuring and grain purposes. The endosperm of cluster bean contains galactomannan gum, which has several diversified industrial uses. India earns huge amount as foreign exchange from the export of its gum and its derivatives. Cluster bean left after the extraction of the gum contains about 45% proteins and forms a valuable proteinaceous raw material (Subramanian and Parpia, 1975).

Mutation breeding is an important tool for enriching variation in a crop like cluster bean where exploitable and useful genetic variability is very meager. The creation of genetic variability in this crop through the recombination of genes by hybridization is very difficult and cumbersome owing to small, delicate flower structures resulting in low percentage of crossed seed setting in the manually hybridized bud. Due to these reasons, not much desirable and usable genetic variability has been generated through conventional breeding approaches in cluster bean. Looking at the above limitation, efforts have been made during the present work to create more and purposeful variability in cluster bean by induced mutations.

The survey of related literature indicated that very little attention has been paid by the scientists of our country in regard to creation of useful variability through the established method of induced mutation in cluster bean.

In the present study two varieties of cluster bean namely Golden Early – 36 (GE-36) and Harit Rani (HR) were used to induce genetic variability. For this study physical mutagen like Gamma rays (5kR, 10kR and 15kR doses) and two chemical mutagens, namely, ethyl methanesulphonate (EMS) of different concentrations such as 0.05%, 0.10% and 0.15% and sodium azide (SA) of concentrations such as 0.01%, 0.02% and 0.03% were tried. The chemical mutagenic treatments were given at room temperature 25±2°C. Immediately after the completion of treatment, the seeds were washed thoroughly under tap water. Later on seeds with chemical mutagenic treatment were kept for post soaking in distilled water. The seeds which were given physical mutagenic treatment were sown in field immediately. For each treatment a batch of 300 presoaked seeds was used. 50 seeds from each treatment were dried between the folds of filter paper and germinated in petridishes to record germination percentage. The remaining 250 seeds from each treatment were sown in field following randomized block design (RBD) with three replications along with control as the M1 generation. The seeds were sown at a distance of 40cm between the plants and 60cm between the rows. Studies pertaining to mutation breeding of cluster bean were spread over three generations.

Discussion

Mutagenic Studies:

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Volume: I

Mutation breeding has been employed as a tool to induce mutations at loci controlling economically important traits or for eliminating undesirable genes from the elite breeding lines (Lippert et al., 1964). In present investigation both physical and chemical mutagens were used to induce mutation. Observations recorded in present study revealed induction of broad genetic variability in case of cluster bean. Many crops with improved economic value have been obtained by using induced mutation (Broetzes 1988, BEAS 1995 and IAEA 1995). Besides the economic benefits, some mutants have also played important role in the study of genetics and plant development (Vanden et al., 1990 and Bretagne-Sgnard et al., 1996).

Biochemical Studies:

A relatively new aspect in applied mutagenesis has been the quantitative and qualitative
alteration of seed storage substances like proteins, carbohydrates, fibers and other
specific substances deposited in various parts of plants.

Crude fiber content:

In present study viable mutants have shown variation in content of crude fibre in both the varieties of cluster bean. The highest crude fiber was recorded in large leaf (61.39%) and long pod mutant (70.20 %) in the two varieties of cluster bean. Significant variation in TDF (Total dietary fibre) and SDF (Soluble dietary fibre) in cluster bean seeds indicated the possibility of genetic manipulation for these components. Although galactomannans, the predominant form of the soluble fibre in cluster bean, have most of the beneficial health effects in humans as the ß-glucan soluble fibre found in oats and barley (Okubo et al., 1994, Golay et al., 1995, Anderson and Hanna 1999, Brown et al., 1999, Chandalia et al., 2000, Battilana et al., 2001, Meyer and Tungland 2001), the oats and barley generally contain less than 25% total fiber (da Silva and Ciocca 2005). Thus, cluster bean has a distinct advantage over cereal crops as a source of TDF and SDF due to the large amount of such factors present in its seeds. Significant variability in TDF and SDF content among *C tetragonoloba* accessions has been observed earlier. The magnitude of variability indicates the possibility of improving the pertinent traits through plant breeding approaches (Kays et al., 2006).

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ISBN: 978-93-94819-16-0
Pub. Date: 30 Nov. 2022
Volume: I

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