Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 4672-4677
Received: 07-03-2019
Accepted: 09-04-2019

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Genetic manipulation through gamma irradiation for high gum content and heat tolerance in clusterbean genotypes (*Cyamopsis tetragonoloba* (L.) Taub.)

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Abstract
The present investigation was carried out in three seasons during *summer*-2016 to Kharif-2017 at Research Farm, College of Agriculture, Gwalior. On the basis of per se performance four genotypes were selected by screening sixteen genotypes which were collected from two region (ARS, Durgapura, Jaipur & Dept of Agronomy, COA, RVSKVV, Gwalior). Four selected genotypes were irradiated by using 10 doses of physical mutagen (gamma rays). The irradiated seeds along with controls were sown to raise M1 generation without replication. Progeny of individual selected mutant plants from M_1 generation were grown using compact family block design with two replications. Mutant lines *viz.*, 42-10-RGC-1038 followed by 133-35-RGC-1038, 133-35kR-RGC-1038,144-40kR-RGC-1038, 139-40kR-RGC-1038, 48-10kR-RGC-1038, 140-40kR-RGC-1038, 141-40kR-RGC-1038, 166-50kR-GST-15-204, and 74-15kR-GST-15-204 were identified for high gum content than best control RGC-1066 (26.85) and these mutant lines were also recorded for high yield. High Proline content was recorded in mutant line *viz.*, 161-50-GST-15-204 (199.50) followed by 159-50kR-RGC-1066, 165-50kR-GST-15- 204, 153-45kR-GST-15-204, 154-45kR-GST-15-204, 130-35kR-RGC-1038, 128-35kR-RGC-1038132-35kR-RGC-1038 and these mutant lines was also found for high yield. Thus, we can say that mutant lines which show high proline content can survive in stress condition and can give high yield. Proline content was found lowest at 5 kR and highest at 50kR. High gum content was also found at higher dose (40kR) over the control. Thus, by this result it can say that higher dose can beneficial to increase proline content and gum content. Recently novel hydrogels were synthesized from guar gum. Hydrogels helps combating problem of water scarcity in arid regions by being super absorbent (ie. Nearly it can absorb 600ml water/gm of hydrogel) and are being evaluated as soil conditions. At last we can concluded that Mutant lines which has high proline content and high gum content can boon for Arid and semi-arid zones.

Keywords: Genetic manipulation, gamma irradiation, high gum content, heat tolerance

Introduction
Clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.) (2n=14) is a member of leguminosae and subfamily papilionaceae. It is an important multipurpose crop specially grown for feed, green fodder, vegetable and green manuring. Initially it was used as green vegetable, fodder and green manure, as it is a rich source of protein. However, today Guar is highly commercialized for its economic importance due to World War II and modern chemistry. Guar was not known for its industrial application until the times of World War II, when there was shortage of locust bean crop and the paper and textile industry of the world was searching for a substitute. They found efficient alternative in the form of guar gum and since then, this derivative of guar ruled out locust bean from this scenario and it was readily accepted for application in many other industries. Guar gum is used as a binder and drug-release control agent (Eherton *et al.*, 1955). The tobacco industry uses guar gum for moisture retention in tobacco and for making reconstituted tobacco preparations from tobacco leaves, which are broken or powdered during processing. During the past several years, the use of clusterbean in paper making has been reduced in favour of cationic starch, which also reduces the cost, for cheaper grades of paper in medicinal tablets, guar gum is used as a binder and drug-release control agent. In soups, ice-cream, sauces, and ketchup guar gum is used as a thickener and stabilizer.
Although guar is drought-tolerant and sun-loving crop, but frequent drought periods can lead to delayed maturation. There is an increasing need for guar gum in the food industry in recent years. High gum content variety development is required to meet this demand. Understanding the inheritance of gum content is key to its successful genetic improvement. To breed stress tolerant genotypes genetic variation is a prerequisite for any crop & for its genetic improvement. Therefore, in any breeding programme, this is always the first step to generate

unless variation it does not pre- exists. Genetic variation can be created by using various breeding methods eg. hybridization, domestication and plant introduction etc. In the clusterbean to create genetic variability 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 buds. Due to these reasons, not much desirable genetic variability has been generated through conventional breeding approaches.

So, mutation breeding can be important tool for enriching genetic variation in a cluster bean and hence looking at this limitation, efforts were initiated to create variability in clusterbean by using the tool of induced mutations. Many desirable varieties of crops have been developed through mutation breeding for improving plant yield. We can find the variations due to spontaneous mutation in various plants. However, the frequency is very low and not induces the full range of variations. Therefore, the induced mutation through physical and chemical mutagen is very effective tool to induce variations for significant characters. A number of chemical and physical mutagens are widely used to induce genetic variability in plants. But according to (Gual H. 1964) gamma radiation is most widely used as physical mutagen in crop improvement. Induced mutation may bring changes in the overall morphological and physiological and genetic traits of the crop.

Therefore the present investigation is being undertaken to study the response of clusterbean genotypes to the physical mutagen gamma rays with the following objectives:

1. To identify/select mutant lines for high proline in promising mutant lines for heat stress in M2 generation.
2. To identify/Select mutant lines for high gum content in M2 generation.

Materials and Method

The present investigation was carried out in three seasons during *summer*-2016 to Kharif-2017 at Research Farm of Genetics and plant breeding, College of Agriculture, Gwalior. On the basis of per se performance sixteen genotypes were screened during *Summer*-2016 which were collected from two regions (ARS, Durgapura, Jaipur & Dept of Agronomy, COA, RVSKVV, Gwalior). Four genotypes were chosen from 16 genotypes for irradiation.

Sample of 100 hundred dry, healthy and uniform size seeds of selected cluster bean genotypes *viz.* RGC-1066, RGC-1055, RGC-1038 & GST-15-204 were treated with 5kR, 10kR, 15kR, 20kR, 25kR, 30kR, 35kR, 40kR, 45kR and 50kR doses of gamma rays using 60Co gamma source at the National Botanical Research Institute, Lucknow, U.P, India. The irradiated seeds along with controls were sown to raise M1 generation without replication during *kharif*-2016. Individually plants were selected from the M_1 generation & seeds of these plants were used to raise M_2 generation.

During *kharif-2017,* selected 163 mutant lines were evaluated using compact family block design with two replication for high gum content and high proline content against heat stress during *kharif*-2017. Four controls *i.e.* RGC-1066, RGC-1038, RGC -1055 & GST-15-204 were repeated at every replication of M2 progenies.

Proline content

Proline is a basic amino acid found in high percentage in basic protein. Free proline is said to play a role in plants under stress conditions. Though the molecular mechanism has not yet been established for the increased level of proline, one of the hypotheses refers to breakdown of protein into amino acids and conversion to proline for storage.

Proline in maintaining osmolitic adjustment and adaptation stress and protect membranes proteins from adverse environmental stress increase.

Estimation of total proline was done according to the method of Bates *et al*., (1973) [4].

Fresh 250 mg leaf sample was taken from each mutant lines and controls. Leaf tissue was homogenized using 10ml of 3% sulfo-salicylic acid and centrifuged at 3000 rpm for 10min. To 2 ml of the supernatant, 2ml of 6 molar orthophosphoric acid, 2 ml of acid ninhydrin reagent and glacial acetic acid in 1:1:1:1 ratio was added, the tubes were heated in a water bath at 100°C for 1h and subsequently cooled on ice for 10 min. To the resultant mixture, 4ml of toluene was added and incubated at room temperature for 30 min. The tubes were shaken for 15s and allowed to stand for 10 min to separate the phases. The upper phase (pink in colour) was separated and the absorbance was measured at 520 nm using toluene as a blank. L- Proline at the concentration of 60 μg ml^{-1} was taken as a standard.

Calculation

Express the proline content on fresh-weight-basis as follows:

$$\mu moles\ per\ g\ tissue = \frac{\mu g\ proline \times ml\ toulene/ml}{115.5} \times \frac{5}{g} sample$$

Where,

115.5 is the molecular weight of proline.

Gum content

Guar gum also called as guaran or is a galactomannan. It is primarily the ground endosperm of guar beans. The guar seed are dehusked, milled and screened to obtain the guar gum. It is typically produced as a free-flowing, off-white powder.

The most reliable and accurate method of gum estimation involve extraction and purification of the galactomannan (Figure – 3.5). Gum can be estimated by a rapid and accurate method developed by Das *et al.* (1977) [5] and Joshi (2004).

- ✓ Grind seed sample by using Cyclotec Grinding Mill (0.2 mm screen).
- ✓ Weigh 0.1 g ground sample and transfer in 100 ml conical flask and add 40 ml of 0.01 M HgCl2 solution.
- ✓ Place cotton plug and aluminium foil on the mouth of flask and keep in autoclave at 15 psi for one hour.
- ✓ Cool the samples and make 100 ml volume using 0.01 M HgCl2 solution.
- ✓ After shaking the samples, take 30 ml for centrifuge it at 5000 rpm for 20 minutes.
- ✓ Take 0.5 ml of supernatant in a centrifuge tube and add 4.5 ml ethyl alcohol to make 90 per cent alcohol. Keep the solution for overnight.
- ✓ Next day, centrifuge it at 5000 rpm for 20 minutes and discard supernatant.

Result and discussion

Mutant lines *viz.*, 42-10-RGC-1038 and 133-35-RGC-1038, 133-35kR-RGC- 1038, 144-40kR-RGC-1038, 139-40kR-RGC-1038, 48-10kR-RGC-1038, 140-40kR-RGC-1038, 141-40kR-RGC-1038, 166-50kR-GST-15-204, 74-15kR-GST-15-204 were identified for high gum content than best control RGC-1066 (26.85) (Table-1) and these mutant lines were also recorded for high yield.

The mutant line *viz.*, 161-50-GST-15-204 (199.50) showed higher proline content and mutant lines 5-5-RGC-1066 (12.65), 8-5-RGC-1066 (12.95), 7-5-RGC-1066 (14.85) showed lowest significant (Table-1).

High proline content was recorded in mutant line *viz.*, 161-50-GST-15-204 (199.50), 159- 50kR-RGC-1066, 165-50kR-GST-15-204, 153-45kR-GST-15-204, 154-45kR-GST-15-204, 130-35kR-RGC-1038, 128-35kR-RGC-1038132-35kR-RGC-1038 and these mutant lines was also found for high yield (Table-1).

Table 1: Mean performance of 167 mutant lines for three characters

S. No.	Mutant lines/Controls	Mean performance		
		Seed yield/ Plant (gm)	Gum Content	Proline content
1	RGC-1066	3.45	26.85	94.55
2	RGC-1038	3.50	23.65	119.50
3	RGC-1055	3.40	24.55	82.00
4	GST-15-204	3.55	27.95	76.10
5	5-5kR-RGC-1066	3.20	22.65	12.65
6	6-5kR-RGC-1066	3.10	22.95	16.75
7	7-5kR-RGC-1066	3.45	21.95	14.85
8	8-5kR-RGC-1066	3.70	22.65	12.95
9	9-5kR-RGC-1066	3.20	21.25	15.65
10	10-5kR- RGC-1066	3.55	22.65	17.65
11	11-5kR-RGC-1066	3.75	21.65	15.45
12	12-5kR-RGC-1066	3.15	22.85	16.35
13	13-5kR-RGC-1066	3.65	23.95	15.05
14	14-5kR-RGC-1038	3.45	27.75	70.70
15	15-5kR-RGC-1038	3.40	26.95	66.00
16	16-5kR-RGC-1038	3.50	27.75	63.70
17	17-5kR-RGC-1038	3.05	27.75	68.90
18	18-5kR-RGC-1038	3.30	20.75	68.00
19	19-5kR-RGC-1038	3.95	22.85	69.90
20	20-5kR-RGC-1055	3.55	25.45	47.65
21	21-5kR-RGC-1055	3.35	24.75	40.25
22	22-5kR-RGC-1055	3.35	25.35	39.25
23	23-5kR-RGC-1055	3.45	24.15	45.25
24	24-5kR-RGC-1055	3.25	25.45	43.25
25	25-5kR-RGC-1055	3.45	23.85	63.20
26	26-5kR-GST-15-204	3.60	26.55	58.65
27	27-5kR-GST-15-204	3.55	26.55	55.95
28	28-5kR-GST-15-204	3.15	27.55	60.75
29	29-5kR-GST-15-204	3.30	28.25	53.95
30	30-5kR-GST-15-204	3.45	27.55	62.05
31	31-5kR-GST-15-204	3.30	26.75	55.55
32	32-5kR-GST-15-204	4.20	27.55	27.55
33	33-10kR-RGC-1066	3.45	21.95	18.95
34	34-10kR-RGC-1066	2.95	22.85	23.65
35	35-10kR-RGC-1066	3.30	23.45	19.85
36	36-10kR-RGC-1066	3.35	22.25	25.65
37	37-10kR-RGC-1066	3.55	23.55	28.45
38	38-10kR-RGC-1066	3.30	21.95	32.65
39	39-10kR-RGC-1038	3.35	26.65	95.50
40	40-10kR-RGC-1038	3.25	24.45	85.10
41	41-10kR-RGC-1038	3.90	24.45	91.20
42	42-10kR-RGC-1038	3.10	36.15	87.10
43	43-10kR-RGC-1038	3.25	33.35	89.20
44	44-10kR-RGC-1038	3.60	32.35	90.40
45	45-10kR-RGC-1038	3.50	31.35	88.30
46	46-10kR-RGC-1038	3.05	29.35	92.00
47	47-10kR-RGC-1038	3.60	29.35	95.10
48	48-10kR-RGC-1038	3.60	30.35	94.10
49	49-10kR-RGC-1055	3.40	23.85	60.45
50	50-10kR-RGC-1055	3.30	25.55	57.35
51	51-10kR-RGC-1055	3.45	26.25	54.75
52	52-10kR-RGC-1055	3.35	21.15	59.35
53	53-10kR-RGC-1055	3.65	25.35	60.35
54	54-10kR-GST-15-204	3.45	27.55	77.45
55	55-10kR-GST-15-204	3.60	20.55	66.35
56	56-10kR-GST-15-204	3.00	22.65	68.75
57	57-10kR-GST-15-204	3.80	26.45	56.55

S. No.	Mutant lines/Controls	Mean performance		
		Seed yield/ Plant (gm)	Gum Content	Proline content
58	58-10kR-GST-15-204	4.20	20.05	72.65
59	59-10kR-GST-15-204	3.80	20.05	65.55
60	60-15kR-RGC-1066	4.35	21.95	39.15
61	61-15kR-RGC-1066	4.40	23.65	42.85
62	62-15kR-RGC-1066	3.45	24.35	40.95
63	63-15kR-RGC-1066	3.55	19.25	39.45
64	64-15kR-RGC-1066	3.45	23.45	41.05
65	65-15kR-RGC-1066	3.70	24.85	39.25
66	66-15kR-RGC-1038	3.40	27.35	98.00
67	67-15kR-RGC-1038	3.30	25.85	86.60
68	68-15kR-RGC-1038	3.45	26.45	97.60
69	69-15kR-RGC-1038	3.15	23.65	92.30
70	70-15kR-RGC-1038	3.45	24.95	90.30
71	71-15kR-RGC-1038	3.10	24.55	84.40
72	72-15kR-RGC-1038	4.25	20.75	82.60
73	73-15kR-GST-15-204	4.75	31.75	79.35
74	74-15kR-GST-15-204	4.05	28.95	67.65
75	75-15kR-GST-15-204	4.35	27.95	69.75
76	76-15kR-GST-15-204	4.20	26.95	73.75
77	77-15kR-GST-15-204	4.10	24.95	73.55
78	78-15kR-GST-15-204	4.35	24.95	70.75
79	89-15kR-GST-15-204	3.65	25.95	67.35
80	80-15kR-GST-15-204	3.20	22.95	72.35
81	81-20kR-RGC-1066	4.13	24.45	93.10
82	82-20kR-RGC-1066	4.05	20.65	94.60
83	83-20kR-RGC-1038	4.45	18.05	91.60
84	84-20kR-RGC-1038	4.15	15.65	92.90
85	85-20kR-RGC-1038	3.90	17.55	91.50
86	86-20kR-RGC-1038	4.10	18.45	91.55
87	87-20kR-RGC-1038	4.05	15.65	93.40
88	88-20kR-RGC-1038	3.45	14.65	66.15
89	89-20kR-RGC-1055	3.24	26.75	64.65
90	90-20kR-RGC-1055	3.15	26.35	67.35
91	91-20kR-RGC-1055	3.50	22.55	63.25
92	92-20kR-RGC-1055	4.10	19.85	81.35
93	93-20kR-GST-15-204	3.05	21.45	78.15
94	94-20kR-GST-15-204	3.65	22.05	67.92
95	95-25kR-RGC-1066	3.65	22.55	47.05
96	96-25kR-RGC-1066	3.35	20.15	48.85
97	97-25kR-RGC-1066	3.40	22.05	46.15
98	98-25kR-RGC-1038	3.65	15.85	100.50
99	99-25kR-RGC-1038	3.30	21.45	104.90
100	100-25kR-RGC-1038	3.30	22.65	106.90
101	101-25kR-RGC-1055	7.04	17.45	77.25
102	102-25kR-RGC-1055	5.97	19.35	75.65
103	103-25kR-RGC-1055	5.75	20.25	72.25
104	104-25kR-RGC-1055	5.16	17.55	72.70
105	105-25kR-RGC-1055	5.00	16.55	70.30
106	106-25kR-RGC-1055	4.90	17.75	74.10
107	107-25kR-GST-15-204	5.09	21.35	90.65
108	108-25kR-GST-15-204	4.10	22.65	80.85
109	109-25kR-GST-15-204	4.00	22.25	79.95
110	110-25kR-GST-15-204	3.40	18.45	87.35
111	111-25kR-GST-15-204	3.80	15.75	81.45
112	112-25kR-GST-15-204	4.05	13.35	85.15
113	113-25kR-GST-15-204	3.80	15.25	89.35
114	114-30kR-RGC-1066	3.60	22.95	50.85
115	115-30kR-RGC-1066	3.45	20.15	57.95
116	116-30kR-RGC-1038	3.35	24.65	130.80
117	117-30kR-RGC-1038	3.50	24.65	136.80
118	118-30kR-RGC-1038	3.40	25.65	128.90
119	119-30kR-RGC-1038	3.30	26.35	133.70
120	120-30kR-RGC-1038	3.10	25.65	129.30
121	121-30kR-RGC-1038	3.70	24.85	135.80
122	122-30kR-RGC-1038	3.35	25.65	132.80
123	123-30kR-RGC-1038	3.45	25.65	130.30
124	124-30kR-GST-15-204	3.35	16.15	95.85

S. No.	Mutant lines/Controls		Mean performance		
			Seed yield/ Plant (gm)	Gum Content	Proline content
125	125-30kR-GST-15-204		3.95	13.35	91.75
126	126-30kR-GST-15-204		3.30	12.35	88.45
127	127-30kR-GST-15-204		4.70	13.55	92.35
128	128-35kR-RGC-1038		3.60	19.15	135.90
129	129-35kR-RGC-1038		3.40	20.75	137.80
130	130-35kR-RGC-1038		3.60	24.55	138.70
131	131-35kR-RGC-1038		3.50	22.35	134.70
132	132-35kR-RGC-1038		3.70	22.33	131.20
133	133-35kR-RGC-1038		3.65	34.05	132.40
134	134-35kR-GST-15-204		4.40	19.15	99.15
135	135-35kR-GST-15-204		4.05	20.35	104.05
136	136-35kR-GST-15-204		4.40	22.35	99.45
137	137-35kR-GST-15-204		4.45	24.12	102.35
138	138-35kR-GST-15-204		3.20	23.35	96.55
139	139-40kR-RGC-1038		3.55	31.80	135.20
140	140-40kR-RGC-1038		3.55	30.25	150.70
141	141-40kR-RGC-1038		3.45	29.25	157.40
142	142-40kR-RGC-1038		3.25	27.25	151.10
143	143-40kR-RGC-1038		3.60	27.25	149.90
144	144-40kR-RGC-1038		4.05	28.25	152.80
145	145-45kR-RGC-1066		5.20	19.15	169.15
146	146-45kR-RGC-1066		4.18	16.35	171.65
147	147-45kR-RGC-1066		3.47	21.95	164.55
148	148-45kR-RGC-1066		4.23	23.15	170.75
149	149-45kR-RGC-1066		3.45	25.15	169.25
150	150-45kR-RGC-1038		4.15	25.25	185.00
151	151-45kR-GST-15-204		4.15	24.05	151.65
152	152-45kR-GST-15-204		3.70	23.35	152.45
153	153-45kR-GST-15-204		3.80	22.55	151.05
154	154-45kR-GST-15-204		4.20	23.35	149.45
155	155-45kR--GST-15-204		3.50	23.35	156.55
156	156-45kR--GST-15-204		3.55	16.35	154.15
157	157-45kR--GST-15-204		3.20	18.45	156.10
158	158-45kR-GST-15-204		3.45	22.05	149.60
159	159-50kR-RGC-1066		4.15	25.15	193.85
160	160-50kR-RGC-1066		3.50	26.15	189.15
161	161-50kR-RGC-1038		4.85	23.75	199.50
162	162-50kR-RGC-1038		4.56	24.35	196.30
163	163-50kR-RGC-1055		3.30	23.35	172.20
164	164-50kR-GST-15-204		4.25	19.85	196.70
165	165-50kR-GST-15-204		4.16	19.85	191.70
166	166-50kR-GST-15-204		3.15	29.05	193.10
167	167-50kR-GST-15-204		3.25	26.25	189.00
	Total mean		3.72	23.44	90.55
	Range	Max	7.04	36.15	9.50
		Min	2.95	12.35	4.50
	SE(m)		0.14	0.70	0.61
	CD 5%		0.38	1.95	1.69
	CV%		1.43	2.91	1.28

Proline content was found lowest at 5 kR and highest at 50kR (Table-2). High gum content was also found at higher dose 40kR over the control (Table-2). High yield was found with high proline content. Thus, by this result it proves that higher dose can beneficial to increase proline content and gum content. Mutant lines which show high proline content can survive in stress condition and can give high yield

Table 2: Mean performance of Kr doses for gum and proline.

Doses (Kr)	Gum Content	Proline content	Seed yield/ Plant (gm)
	Mean	**Mean**	**Mean**
Control	25.75	93.04	3.48
5	24.86	42.78	3.44
10	25.68	65.06	3.45
15	25.02	69.00	3.79
20	20.29	81.25	3.78
25	19.09	78.49	4.35
30	21.54	109.69	3.54
35	22.95	119.30	3.81

40	29.01	149.52	3.58
45	21.75	160.81	3.87
50	24.19	191.28	3.91

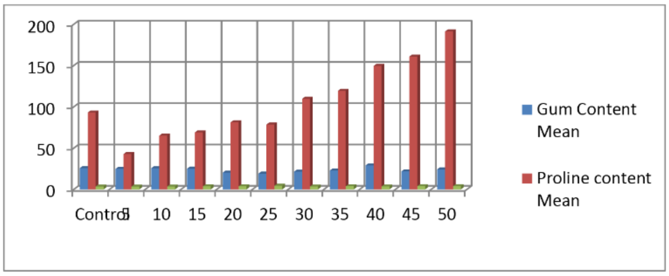

Fig 1: Effect of Kr doses for gum and proline.

Conclusion

The present study was undertaken to study the response of clusterbean genotypes to the physical mutagen gamma rays. Recently novel hydrogels were synthesized from guar gum. Hydrogels helps combating problem of water scarcity in arid regions by being super absorbent (ie. Nearly it can absorb 600ml water/gm of hydrogel) and are being evaluated as soil conditions. Thus, by this novel research it can concluded that mutant lines which has high gum content can boon for arid zones means hydrogels which is synthesized from guar gum can used in other crops to prevent water scarcity.

Future importance of this research

Recently novel hydrogels were synthesized from guar gum. Hydrogels helps combating problem of water scarcity in arid regions by being super absorbent (ie. Nearly it can absorb 600ml water/gm of hydrogel) and are being evaluated as soil conditions. Thus, by this novel research it can concluded that mutant lines which has high gum content can boon for arid zones means hydrogels which is synthesized from guar gum can used in other crops to prevent water scarcity.

References

1. Anjum S, Kalhoro MA, Afza N, Abdul Hai SM. Guar meal in poultry feed. J Chem. Soc. Pak. 2001; 23:175-7.
2. Arora RN, Pahuja SK. Mutagenesis in guar [*Cyamopsis tetragonoloba* (L.) Taub. Plant Mutat. Rep. 2008; 2(1):7-9.
3. Amrita KR, Jain UK. Induction of variability through gamma irradiation in guar (*Cyamposis tetragonoloba* L. Taub.), Progressive Agriculture. 2003; 3:121-122
4. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies. Plant Soil. 1973; 39:205-207.
5. Das B, Arora SK, Luthra YP. A rapid method for determination of gum in guar (*Cyamopsis tetragonoloba* (L.) Taub.). In: Proc. First ICAR Guar Res. Workshop. CAZRI, Jodhpur. 1977, 117-123.
6. Devi S, Punya Sheela. Drought-Induced Accumulation of Soluble Sugars and Proline in two Pigeon Pea (*Cajanus Cajan* L. Millsp.) Cultivars, 2014, ISSN 2278 – 0211.
7. Gaul H. Mutagenic effects observed in first generation. In Manual on Mutation Breeding. Technical Report. FAO/IAEA, Vienna. 1964; 119:85-89.
8. Lather BPS, Chowdhury JB. Studies on irradiated guar, Nucleus 1972; 15:16-22.
9. Mahla HR. A Study on EMS and Gamma Mutagenesis of Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub], Plant Mutation Reports, 2010, 2(2).
10. Mahla. Mutagenesis of Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub], Genet.Resour. Crop Evol. 2018; 58:961-96.