Screening of cluster bean genotypes/varieties for resistance to *Xanthomonas axonopodis* pv. *cyamopsidis* using two methods of Inoculation

Abstract

Clusterbean [*Cyamopsis tetragonaloba* (L.) Taub is an arid and semi-arid legume crop belonging to the family *leguminaceae*. It is a drought enduring leguminous crop because of its profound tap rooting system and has high capability to get well from water stress. Total fifty-nine varieties/genotypes of clusterbean were screened under artificial Inoculation field conditions at College Research Farm, College of Agriculture, Bikaner against bacterial blight disease during the *Kharif* season of 2018 and 2019. Two alternative inoculation techniques-seed inoculation and foliar sprays of bacterial suspension were employed to test for resistance to *Xanthomonas axonopodis* pv. *cyamopsidis* bacterial blight infection. ($2.5x10^8$cfu/ml). Out of 59 varieties, only one variety RGC-1066 found resistant against bacterial blight disease, 17 were found moderately resistant, 17 were moderately susceptible and 24 varieties were found susceptible. No one variety found highly susceptible against bacterial blight.

Keywords:- *Clusterbean, Bacterial blight, Resistant, Xanthomonas axonopodis pv. cyamopsidis*

1. Introduction

Clusterbean [*Cyamopsis tetragonaloba* (L.) Taub.] (2n=14) commonly known as "guar" means "cow food" is an arid and semi-arid legume crop belonging to the family *leguminaceae*. It is a drought enduring leguminous crop because of its profound tap rooting system and has high capability to get well from water stress. The green pods are a healthy vegetable with 82.5% water, 3.7% protein, 9.9% carbohydrates, 0.2% fat, 2.3% fiber, and 1.4% other minerals, including 0.13 % calcium, 0.25 % phosphorus, 5.8 mg/100 g iron, and 49 mg/100 g vitamin. In India, there are 39.36 million hectares of clusterbean crops, which produce 16.24 million tons, with a yield of 428 kg per hectare. Clusterbean cultivation spans a total of 28.41 lakh hectares in Rajasthan, producing 12.84 lakh tons and yielding 452 kg per hectare. Rajasthan is the top-producing state for cluster beans, with more than 70% of the country's total production.

Bacterial blight of clusterbean caused by *Xanthomonas axonopodis pv. cyamopsidis* has been reported from Arizona (Mihail and Alcorn, 1985), Madison (Undersander *et al.,* 1991) and Brazil (Almeida *et al.,*1992). In India, disease has been reported in Rajasthan (Patel *et al.,* 1953), Haryana (Gandhi and Chand, 1985) and Karnataka (Patel and Patel, 1958, Chakravarthy *et al.,* 2004a) (Jain and Agrawal, 2011). The leaf blight pathogen *Xanthomonas a.* pv. *cyamopsidis* causes drastic reduction in plant stand and yield as high as 58% in cultivar. The pathogen is seed-borne and has a

one-year lifespan in seeds. Pathogen is seed transmitted; thus, it can cause infection from pre appearance phase to growing stages of the plant in favourable environmental situation for the duration of the crop season. The disease appears both as leaf spot and blight simultaneously (Sain and Gour, 2009). Cultural, chemical or biological strategies for disease management are not adequate to control the efficient or economic. Identification of germplasm with resistance to pathogen appears promising for reducing damage and yield losses caused by pathogen. In this study, two inoculation procedures were used to assess how fifty-nine cultivars of cluster beans responded to Xanthomonas a. pv. cyamopsidis infection.

2. Materials and methods

Fifty-nine clusterbean germplasms/varieties of indigenous as well as exotic origin cultivars were screened for identification of resistance sources against artificially inoculated *X. a.* pv. *cyamopsidis*. The experiments were conducted at Research farm, College of Agriculture, Bikaner during the *Kharif* seasons of 2018 and 2019. The test entries were planted during mid-July and harvested during the November.

Seeds of different cultivars were artificially inoculated with *X. a.* pv. *cyamopsidis* by soaking in bacterial cell suspension($2.5x10^8$cfu/ml) (Rathore, 2006). Seeds were sown in rows, each of 5 m length and maintaining row to row and plant to plant distance as 30 x10 cm, with three replications in randomized block design (R.B.D.). After every two test entries, one row of susceptible check (local) was planted, as well as around the entire experiment. A fresh 72 hrs old bacterial culture, grown on Nutrient Agar media was used for inoculations on the plants. The culture was harvested in 10 ml sterile water diluted to a concentration of $2.5x10^8$cfu /ml and used immediately. The suspension was sprayed on plants with hand atomizer twice at 24 hrs interval. After the 7 days of inoculation when symptoms appear, the disease intensity was quite serious during the season due to favourable conditions for the development of the disease.

Table: 1 List of clusterbean germplasms / varieties

S.NO.	germplasms / varieties	Source
40	RGr-16-3, RGr-16-3-1, RGr-16-3-2, RGr-16-3-3, RGr-16-3-4, RGr-16-3-5, RGr-16-3-6, RGr-16-3-7, RGr-16-4, RGr-16-5, RGr-16-5-1, RGr-16-5-2, RGr-16-5-3, RGr-16-5-4, RGr-16-5-5, RGr-16-8, RGr-16-9, RGr-16-9-1, RGr-16-9-2, RGr-16-9-3, RGr-16-9-4, RGr-16-9-5, RGr-16-9-6, RGr-16-11, RGr-16-11-1, RGr-16-11-2, RGr-16-11-3, RGr-17-1, RGr-17-2, RGr-17-4-7-2, RGr-17-4-3-2, RGr-17-4-4-1, RGr-19-1-1, RGr-19-12-1, RGr-19-12-2, RGr-19-11-1, RGr-19-16-1, RGr-19-18-1, RGr-19-19-1, RGr-19-19-2	RARI, Rajasthan Agricultural Research Institute, Durgapura, Jaipur
19	RGC-563, RGC-936, RGC-963, RGC-986, RGC-1002, RGC-1003, RGC-1017, RGC-1031, GAUG 1104, CAZG 15-2, GAUG-1502, CAZG- 15-5, GAUG-1501, GAUG 1304, CAZG-15-7, RGC-1033 (ch), HG 2-20(ch), X-	Agricultural Research Station,

10, RGC-1066, HG-563(ch)		Bikaner

Observations for disease severity were recorded by visual scoring as per the standard continuous rating 0-5 scale (Rathore, 2006). Disease severity on each genotype was recorded at pre-flowering (30 DAS) and maturity stage using a 0-5 disease rating scale. On the basis of disease per cent disease index was calculated using formula described earlier and varieties were categorized on basis of per cent disease index range.

Table: -2 Category of varieties/lines based on per cent disease index

Rating	PDI	Category
0	0 or less than 1.0	Free from disease (Immune)
1	1-10%	Resistant (R)
2	10 - 25%	Moderately resistant (MR)
3	25 -50%	Moderately susceptible (MS)
4	50 - 75%	Susceptible (S)
5	More than 75%	Highly susceptible (HS)

Disease index on foliage was calculated using the formula of McKinney (1923):

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves observed x Maximum rating scale}} \times 100$$

3. Result and discussion

Total fifty-nine varieties/genotypes of clusterbean were screened under artificial Inoculation field conditions at College Research Farm, College of Agriculture, Bikaner against bacterial blight disease during the *Kharif* season of 2018 and 2019. The observations on disease intensity on various genotypes/varieties were recorded and categorized as per their disease reaction. The rating scale 0-5 was used for recording the observation.

Table:-3 Disease reaction of clusterbean varieties/genotypes to *X. a.* pv. *cyamopsidis* under artificial inoculation conditions.

S.N.	Germplasm/varieties	*Kharif*2018		*Kharif*2019		Pooled	
		Disease intensity (%)	Disease reaction	Disease intensity (%)	Disease reaction	Disease intensity (%)	Disease reaction

S.N.	Germplasm/varieties	*Kharif*2018		*Kharif*2019		Pooled	
		Disease intensity (%)	Disease reaction	Disease intensity (%)	Disease reaction	Disease intensity (%)	Disease reaction
1	RGC-563	13.25	MR	17.43	MR	15.34	MR
2	RGC-936	37.56	MS	29.13	MS	33.34	MS
3	RGC-963	52.55	S	47.10	MS	49.82	MS
4	RGC-986	58.50	S	64.33	S	61.41	S
5	RGC-1002	12.40	MR	19.56	MR	15.98	MR
6	RGC-1003	36.80	MS	29.45	MS	33.12	MS
7	RGC-1017	17.15	MR	13.67	MR	15.41	MR
8	RGC-1031	19.70	MR	24.54	MR	22.12	MR
9	RGC-1055	41.67	MS	36.50	MS	39.08	MS
10	GAUG-1502	53.70	S	55.45	S	54.57	S
11	CAZG- 15-5	65.30	S	48.30	MS	56.80	MS
12	GAUG-1501	56.84	S	61.45	S	59.14	S
13	GAUG 1304	17.50	MR	21.67	MR	19.58	MR
14	CAZG-15-7	13.67	MR	16.33	MR	15.00	MR
15	RGC-1033 (ch)	23.34	MR	27.33	MS	25.33	MS
16	HG 2-20(ch)	67.84	S	61.67	S	64.75	S
17	X-10	53.50	S	51.30	S	52.40	S
18	RGC-1066(ch)	6.40	R	7.50	R	6.95	R
19	HG-563(ch)	63.27	S	52.50	S	57.88	S
20	RGr-16-3	47.44	MS	53.25	S	50.34	S
21	RGr-16-3-1	27.50	MS	34.67	MS	31.08	MS
22	RGr-16-3-2	34.37	MS	29.50	MS	31.93	MS
23	RGr-16-3-3	23.67	MR	18.45	MR	21.06	MR
24	RGr-16-3-4	16.90	MR	23.25	MR	20.07	MR
25	RGr-16-3-5	68.70	S	61.30	S	65.00	S
26	RGr-16-3-6	53.32	S	57.45	S	55.38	S
27	RGr-16-3-7	69.67	S	64.50	S	67.08	S
28	RGr-16-4	52.50	S	46.45	MS	49.47	MS
29	RGr-16-5	56.00	S	52.25	S	54.12	S
30	RGr-16-5-1	24.30	MR	13.67	MR	18.98	MR
31	RGr-16-5-2	21.10	MR	24.40	MR	22.75	MR
32	RGr-16-5-3	29.45	MS	36.45	MS	32.95	MS
33	RGr-16-5-4	54.17	S	57.20	S	55.68	S
34	RGr-16-5-5	46.12	MS	39.50	MS	42.81	MS
35	RGr-16-8	67.55	S	64.33	S	65.94	S
36	RGr-16-9	45.17	MS	36.40	MS	41.78	MS
37	RGr-16-9-1	42.84	MS	33.45	MS	38.14	MS
38	RGr-16-9-2	29.60	MS	31.00	MS	30.30	MS

S.N.	Germplasm/varieties	*Kharif*2018		*Kharif*2019		Pooled	
		Disease intensity (%)	Disease reaction	Disease intensity (%)	Disease reaction	Disease intensity (%)	Disease reaction
39	RGr-16-9-3	19.78	MR	24.40	MR	22.09	MR
40	RGr-16-9-4	22.50	MR	17.30	MR	19.90	MR
41	RGr-16-9-5	38.17	MS	31.50	MS	34.83	MS
42	RGr-16-9-6	52.50	S	58.60	S	55.55	S
43	RGr-16-11	63.50	S	53.30	S	58.40	S
44	RGr-16-11-1	33.78	MS	27.50	MS	30.64	MS
45	RGr-16-11-2	47.00	MS	51.67	S	49.33	S
46	RGr-16-11-3	62.83	S	59.33	S	61.08	S
47	RGr-17-1	57.61	S	61.45	S	59.53	S
48	RGr-17-2	64.50	S	57.25	S	60.87	S
49	RGr-17-4-7-2	21.06	MR	15.67	MR	18.36	MR
50	RGr-17-4-3-2	18.06	MR	24.67	MR	21.36	MR
51	RGr-17-4-4-1	65.29	S	61.30	S	63.29	S
52	RGr-19-1-1	54.50	S	57.50	S	56.00	S
53	RGr-19-12-1	17.83	MR	26.30	MS	22.06	MS
54	RGr-19-12-2	70.50	S	66.45	S	68.47	S
55	RGr-19-11-1	38.44	MS	43.25	MS	40.84	MS
56	RGr-19-16-1	47.00	MS	46.45	MS	46.72	MS
57	RGr-19-18-1	30.67	MS	37.50	MS	34.08	MS
58	RGr-19-19-1	18.32	MR	24.30	MR	21.31	MR
59	RGr-19-19-2	55.33	S	52.40	S	53.86	S
	C.D (P=0.05)	**7.41**		**7.61**		**8.69**	
	SEm(±)	**2.61**		**2.68**		**3.06**	
	C.V.	**9.00**		**9.47**		**10.90**	

The results on pooled basis of both the *kharif* seasons 2018 and 2019 one variety RGC-1066 was found resistant against bacterial blight disease. Fifteen genotypes / varieties *viz.,*RGC-563, RGC-1002, RGC-1017, RGC-1031, GAUG-1304, CAZG-15-7, RGr-16-3-3, RGr-16-3-4, RGr-16-5-1, RGr-16-5-2, RGr-16-9-3, RGr-16-9-4, RGr-17-4-7-2, RGr-17-4-3-2, RGr-19-19-1 were moderately resistant (MR), while nineteen genotypes / varieties namely RGC-936, RGC-1003, RGC-1055, RGC-1033(ch), CAZG-15-5, RGr-16-3-1, RGr-16-3-2, RGr-16-4, RGr-16-5-3, RGr-16-5-5, RGr-16-9, RGr-16-9-1, RGr-16-9-2, RGr-16-9-5, RGr-16-11-1, RGr-19-11-1, RGr-19-16-1, RGr-19-18-1, RGr-19-12-1 were observed moderately susceptible (MS) reaction against bacterial blight of clusterbean. Twenty four genotypes / varieties *i.e.* RGC-963, RGC-986, GAUG-1502, GAUG-1501, HG-2-20(ch), X-10, HG-563(ch), RGr-16-3 RGr-16-3-5, RGr-16-3-6, RGr-16-3-7, RGr-16-5, RGr-16-5-4, RGr-16-8, RGr-16-9-6, RGr-16-11, RGr-16-11-2, RGr-16-11-3, RGr-17-1, RGr-17-2, RGr-17-4-4-1, RGr-19-1-1, RGr-19-12-2, RGr-19-19-2 were found reaction susceptible (S) and no single genotypes / varieties was found highly susceptible. Similar finding reported by Karwasra and Chand (1982) they conducted a field experiment at Hisar on 590 cultivars and strains, under conditions of natural infection during 1976,

none was immune, 35 were graded as resistant, 41 moderately resistant and remaining were susceptible to bacterial blight. When the resistant lines were further tested by inoculation in 1977, only GP-380, GP-508B and GP-590B were still resistant. Gandhi and Chand (1987) revealed the absence of immune of resistance source against bacterial blight in clusterbean lines with moderate degree of resistance identified from genetic stock include. GP-380, GP-508 B and GP-590 HG-75, HG-258, RGC-990, HGC-365, HGS-502, HGS-504 D-39-1, HG-75, HG-441, HG-182, PLG-851C-9065, HG-765, HG-464, HD-312 and HG-513.

Similarly results also reported at S K Nagar, Gujarat several promising lines with enhanced disease resistance against bacterial leaf blight (GAUG 9406, GG 1, RGC 1027), *Alternaria* blight (GAUG 9406, GAUG 9005, GAUG 9003 and GC 1) and root rot (GAUG 9406, GG 1 and HGS 844) have been identified (Kumar, 2005).

Similarly finding also reported by Lesly (2005) screened 169 genotypes to bacterial blight during *Kharif* 2004 in Dharwad with 56, 27, 47, 22 and 17 resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible reaction, respectively. Resistant genotypes such as IC202823, IC257420 and IC97767 showed high seed yield, harvest index and number of pods per plant.

Table: 4. Categorization of clusterbean genotypes/varieties according to disease reaction against bacterial blight (pooled) under artificial conditions.

Genotypes/varieties	Category of infection	Per cent leaf area affected	Host reaction
Nil	0	0 or less than 1.0	Immune (I)
RGC-1066 (01)	1	1-10%	Resistant (R)
RGC-563, RGC-1002, RGC-1017, RGC-1031, GAUG-1304, CAZG-15-7, RGr-16-3-3, RGr-16-3-4, RGr-16-5-1, RGr-16-5-2, RGr-16-9-3, RGr-16-9-4, RGr-17-4-7-2, RGr-17-4-3-2, RGr-19-19-1 (15)	2	10 - 25%	Moderately Resistant (MR)
RGC-936. RGC-1003, RGC-1055, RGC-1033(ch), CAZG-15-5, , RGr-16-3-1, RGr-16-3-2, RGr-16-4, RGr-16-5-3, RGr-16-5-5, RGr-16-9, RGr-16-9-1, RGr-16-9-2, RGr-16-9-5, RGr-16-11-1, RGr-19-11-1, RGr-19-16-1, RGr-19-18-1, RGr-19-12-1 (19)	3	25 -50%	Moderately Susceptible (MS)
RGC-963, RGC-986, GAUG-1502, GAUG-1501, HG-2-20(ch), X-10, HG-563(ch), RGr-16-3 RGr-16-3-5, RGr-16-3-6, RGr-16-3-7, RGr-16-5, RGr-16-5-4, RGr-16-8, RGr-16-9-6, RGr-16-11, RGr-16-11-2, RGr-16-11-3, RGr-17-1, RGr-17-2, RGr-17-4-4-1, RGr-19-1-1, RGr-19-12-2, RGr-19-19-2 (24)	4	50 - 75%	Susceptible (S)
Nil	5	More than 75%	Highly Susceptible (HS)

4. CONCLUSION

This study represents of two inoculation methods and fifty-nine clusterbean cultivars. further testing of these inoculation methods as well as comparison with others on more clusterbean cultivars will be necessary to identify clusterbean cultivars with resistance to *X. a.* pv. *cyamopsidis* inoculation load and both methods combine gave highest disease severity index. In screening out of fifty-nine varieties, only 1 variety were found completely resistant, 17 moderately resistant,17 moderately susceptible, 24 susceptible against bacterial blight disease.

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