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Comparative Studies of Alternaria Leaf Spot on CMS, GMS and Conventional ISO- Hybrids in Upland Cotton (*G. hirsutum Linn*.)

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Abstract: ***Economic losses in cotton due to foliar diseases in cotton have been confirmed by many workers. In intra- hirsutum hybrid H4, losses in yield up to 68 percent have been reported in chemically unprotected crop in disease endemic areas of Akola district of Maharashtra (Shivankar and Wangikar, 1992). Screening of foliar disease has been done by several workers to identify resistant donors for disease management through host plant resistance. In present study, 49 genotypes comprising 36 iso-hybrids,10 parents and 3 commercial checks were evaluated for Alternaria leaf spot at Aurangabad. Thirty Six crosses were prepared by using cytoplasmic male sterility (CMS), genetic male sterility (GMS) and conventional methods were studied for Alternaria leaf spot (ALS) in natural field condition. After pooled analysis of two years data of PDI of Alternaria leaf spot at 120 days stage five crosses C10346B BGIIXR11, C10346G BGIIXR11, C10346A BGII XR11, C10026A BGIIXR14 & C10346B BGII XR14 found relatively resistant than commercial check Mallika BGII, Jai BGII and NHH44. Effect ofG.harknessii cytoplasm for Alternaria leaf spot reaction was studied at 120 days stage of crop growth in natural condition by comparing AXR crosses and BXR crosses. It was found that presence of crosses with G.harknessii cytoplasm & normal (G.hirsutum) cytoplasm in extreme classes of resistant and susceptible establish the fact that cytoplasm had no direct influence on reaction of genotypes to Alternaria leaf spot.***

Keywords: Male sterility, *G.herknessii* , CMS, GMS, Alternaria leaf spot, foliar diseases

1. Introduction

Cotton is one of the important commercial crops cultivated in India. In 2014-15, cotton acreage in India peaked to 12.8 M. ha average being 11 m ha. In early fifties when diploid cotton was being cultivated, fusarium wilt, root rot, seedling blight, grey mildew was major problem. With increase in cultivation of tetraploid cotton (*Gossypium hirsutum*) bacterial blight become the major problem to which indigenous cottons were highly resistant. After the introduction of Bt cotton hybrids during 2002 onwards and continuous increase in area under hybrids to around 95%, the disease scenario has also changed.

Leaf spot caused by *Alternaria macroscopra* and *Alternaria alternata* are common in cotton crop around the world. High leaf defoliation and yield loss found in Egyptian and Upland cotton if the crop is predisposed to infection by congenial environment or physiological stress or potassium deficiency(Hillock .R.J 2008). Leaf spot caused by *Alternaria macrospora* is found in Andra Pradesh and under favorable condition account for yield losses upto 26.59 % (Monga. et al. 2013) and 38.23% (Bhattiprolu and Prasad Rao, 2009) were noticed. During Surveyof foliar diseases in Karnataka on cotton it was concluded that Grey mildew was number one rank disease with PDI of 5 to 50 per cent and Alternaria Blight on second rank with 3 to 35 percent infestation in different district of Karnataka in 2006 (G. Hosagoudar.et al. 2006).

Today all cotton grown in the country is transgenic hybrid cotton. This has created tremendous competitiveness in hybrid seed production as the production area for hybrid cotton has remained stagnant. Due to increasing demand of hybrid cotton, cost effective hybrid seed production in the country has become a major challenge. Conventional hybrid seed production in cotton needs more labour and because of increasing labour wages the seed production is turning out to be an expensive affair. Use of male sterility can significantly reduce requirement of labour in hybrid seed production and can also contribute indirectly by improving the purity of the production lots (CICR Technical bulletin No. 24, 2002). Many workers studied and found that Cytoplasmic Male Sterility is most economical hybrid production system but it has deleterious effect on yield. As a commercial breeder one would like to see how CMS hybrids can be brought in use in to production system by addition value addition of imparting disease resistance in cotton cultivation which can compensate its 5- 8 % yield drag.

Keeping focus on important issues, present study was done to evaluate GMS, CMS and Conventional iso-hybrids for reaction to Alternaria leaf spot disease in natural field condition.

2. Materials & Method

Two BGII good combiner females consists of C10026 GMS BGII, C10026 CMS BGII, C10026BGII Conventional and C10346 GMS BGII, C10346 CMS BGII and C10346 BGII conventional female were crossed with six restorer parents DHY286-1, R14, AKH351-1 AKH355-1, AKH357 & R11 were used to prepare 36 iso-hybrids in summer 2015. In Kharif 2015 and Kharif 2016 trials were planted in 7 x 7 Simple Lattice Design in two replications that included 36 hybrids, 10 parents and 3 commercial checks. All hybrids with parents are evaluated for yield and yield contributing traits. All hybrids were evaluated for Alternaria leaf spot (ALS) in natural field condition for two seasons K 2015 and K 2016 at Bayer BioScienceResearch Farm, Aurangabad. Observations on disease severity were recorded on two leaves each from upper, middle and lower portion of plant.

Five plants were selected from each treatment for recording disease observations. The incidence of disease was recorded by using 0 – 4 scale (Sheo Raj, 1988) and these grades were converted into per cent disease indices (PDI) by using the formula given by Wheeler (1969).

$$\text{Per cent Disease Index (PDI)} = \frac{\text{Sum of numerical rating x 100}}{\text{(Total No. of leaves observed x Maximum disease Score)}}$$

Numerical	*Per cent of leaf area covered*	*Rating*
0	Immune	completely free from foliar diseases
1	Highly resistant, infection of	0 - 10%
2	Moderately resistant, infection of	11- 20%
3	Moderately susceptible, infection of	21- 40%
4	Highly susceptible, infection	more than 40%

ANOVA was used to test the differences between the genotypic means under study. Genotypic means were arranged in ascending order and classified using mean and standard deviation to classify hybrids and compare relative resistance / susceptibility among the genotypes using mean PDI. Diseases common in both years were analyzed using two factors (Year and Genotype) and disease appearing in one year using single factor analysis (Genotype).

3. Results and Discussion

ANOVA for Alternaria Leaf Spot disease observations taken at 120 days in 2015-16 and 2016-17 season and 150 days observation taken in season 2016 revealed that hybrids and parents differed significantly for ALS reaction indicating presence of genetic variability between the genotypes. (Table. No.1).

ANOVA also indicated significant F values for season meaning that there was a significant difference between years or seasons for disease reaction. This observation was obvious considering the fact that the observations were recorded under natural conditions and role of weather conditions in disease development in the experiment. Alternaria leaf spot was observed and recorded for all genotype in K-2015 and K-2016 season. The data was subjected for pooled analysis with two factors, years and genotype. Results indicated that there is significant impact of years over the Alternaria incidence although the genotypic differences within the year were significantly reflected in studies. Interaction effect between years and genotypes were significant indicating that genotypes reacted differently in different seasons (Table No.1).

The genotypes were classified on the basis of mean and SD for their relative resistance and susceptibility. Out of forty nine treatments, eight crosses were classified as resistant and their PDI ranges from 7.30 in cross C10346B BGII x R11 to 11.46 in cross C10346G BGII x DHY286-1R, nineteen treatments were classified as moderately resistant with their PDI ranging from 13.90 in cross C10346B BGII x DHY286-1R to 19.78 in cross C10026G BGII x AKH351-1, thirteen treatments were classified as moderately susceptible with PDI ranging from 20.83 in genotype C10026BGII GMS B parent to 27.08 in cross C10346G BGIIx AKH351-1 and nine genotype were classified as susceptible and their PDI ranged from 28.84 in cross C10346A BGII X DHY286-1R to 39.69 in cross C10346A BGII X AKH351-1.

At 120 days five crosses C10346BGII BXR11, C10346G BGIIXR11, C10346A BGIIXR11, C10026A BGIIXR14 & C10346B BGIIXR14 found resistant than commercial check Mallika BGII, Jai BGII and NHH44 (Table 02).

In 2016 data was recorded at 150 days stage on ALS disease and PDI was calculated. Analysis of variance was used to test the difference between the genotypic means. F test was found significant for genotypes indicating that genotypes differed significantly for ALS disease reaction. Genotypic means and SD of all genotypes including hybrids and parents were calculated. PDI mean were arranged in ascending order to classify the genotypes on the basis of Mean and SD. Genotype having PDI less than (mean + SD) was classified as Resistant (R), PDI more than (mean + SD) were classified as Susceptible (S), whereas genotype PDI above (mean – SD) and up to mean were classified as Moderately Resistant (MR) and genotype above mean up to (mean + SD) were classified as Moderately susceptible (MS).

ALS data of 150 days one year data taken 2016 was analyzed using single factor analysis result showed that out of forty-nine genotype only three crosses C10346BG2 B X AKH351-1, C10346BG2 A XDHY286-1R and C10346BG2 A X `R11 are resistant with PDI 0.0, 0.7 and 7.7 respectively out of two hybrids last two hybrids are CMS based on *herkensii* cytoplasm (Table No.4). Twenty genotype were classified in Moderately Resistant PDI range from 22.2 in cross C10346BG2 AXR14 to 32 in cross C10026BG2 GXAKH355-1. Whereas twenty genotype were classified as Moderately Susceptible to ALS PDI ranges from 33.3 in cross C10346BG2 AXAKH355-1 to 41.00 in hybrid C10026BG2 AXAKH351-1 and six genotype were classified as Susceptible PDI ranges from 44.5 in cross C10026BG2 AXR14 to 51.4 in cross C10026BG2 GXR14.

Effect of G. *harknessii* cytoplasm on Alternaria leaf spot reaction was studied at 120 days stage of crop growth under natural conditions in K 2015 & K 2016 by comparing AXR and BXR crosses. It was observed that out of twelve CMS hybrids, six CMS hybrids had higher PDI for ALS than their conventional iso-hybrids. In season 2016 data was recorded at 150 days stage out of twelve CMS hybrids seven CMS hybrids showed higher PDI for ALS than their conventional iso-hybrids under study. (Table No. 4).

Presence of crosses with G. *harknessii* cytoplasm & normal (G.*hirsutum*) cytoplasm in extreme classes of resistant and susceptible establish the fact that cytoplasm had no direct influence on reaction of genotypes to Alternaria leaf spot. At 120 days crosses with restorer male R11 in both the female irrespective of AXR and BXR crosses in all crosses PDI was less and disease reaction is relatively resistant confirming no role of cytoplasm in resistance reaction. We can also infer that the genes governing resistance alleles are nuclear genes as the genotypic reactions were different in a given year. The observations on resistance need to be validated by testing the entries further in artificial screening experiments.
Seed cotton yield per plant for all genotype under test was recorded and it was found that out of top ten high yielding crosses 5 crosses (were numerically higher yield than commercial check Mallika none of these crosses not come under susceptible category. It was concluded that for good yield though several yield contributing factors, traits are responsible and disease reaction also important factor with other yield contributing factor (Table No.5).

4. Conclusion

At 120 days five crosses C10346B BGIIXR11, C10346G BGIIXR11, C10346A BGII XR11, C10026A BGIIXR14 & C10346B BGII XR14 found resistant than commercial check Mallika BGII, Jai BGII and NHH44 (Table 02).

At 150 days data in 2016 it was found that crosses C10346B BGIIXAKH351-1, C10346A BGIIXDHY286-1R, C10346A BGIIXR11, C10346A BGIIXR14 and C10346B BGIIXDHY286-1R found with less PDI and relatively resistant than commercial check Mallika BGII, Jai and NHH44 (Table. 04).

On the basis of pooled mean yield per plant among 49 genotype, top ten high yielding crosses top 6 crosses C10026 BG2 GXR11, C10026 BG2 BXAKH357, C10346 BG2 GXAKH357, C10026 BG2 BXDHY286-1R, C10346 BG2 GXR11 & C10026 BG2 BXR14 have found at statistically at par with commercial check Mallika. Among these top ten crosses only commercial check and cross C10346 BG2 GXR11 has showed tolerant reaction to Alternaria leaf spot and none of the top crosses are in susceptible category it showed that good disease tolerance is important factor for good yield and disease reaction could not be ignore during commercial cotton hybrid development for good yield .

It was found that hybrids expresses disease reaction irrespective of impact of G.*herknessii* sterile cytoplasm and normal cytoplasm. Presence of crosses with G. *harknessii* cytoplasm & normal (G. *hirsutum*) cytoplasm in extreme classes of resistant and susceptible establish the fact that cytoplasm had no direct influence on reaction of genotypes to Alternaria leaf spot.

CMS can be effective utilized in cotton hybrids development by converting good female line in to CMS background and using disease resistant restorers for heterosis breeding for yield and yield contributing traits and to develop disease tolerant hybrids.

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Table 1: ANOVA for two factor analysis of Alternaria Leaf Spot over years (K15 & K16)

Source	DF	SS	MSS
Total treat SS	97	28303	292
Year SS	1	8366	8366*
Treat SS	48	12531	261*
Interaction	48	20929	436*
Er SS	97	15962	165
TSS	195	37099	

Table 2: Classification of Hybrids, Parents and Checks on the basis of Mean and SD Method at 120 days ALS observation 2015-16 & 2016-17

Hybrid	PDI	Relative Disease Reaction	Hybrid	PDI	Relative Disease Reaction
C10346B BGIIXR11	7.30	R	C10026G BGIIXDHY286-1R	19.46	MR
C10346G BGIIXR11	8.57	R	C10026G BGIIXAKH351-1	19.78	MR
C10346A BGIIXR11	9.01	R	C10026B GMS	20.83	MS
C10026A BGIIXR14	9.73	R	C10026B BGIIXAKH355-1	21.53	MS
C10346B BGIIXR14	10.06	R	C10346B BGIIXAKH357	21.88	MS
Mallika	10.76	R	C10026A BGIIXAKH355-1	21.88	MS
C10026G BGIIXR14	11.11	R	C10026G BGIIXAKH357	22.11	MS
C10346G BGIIXDHY286-1R	11.46	R	C10346G BGIIXAKH357	22.58	MS
C10346B BGIIXDHY286-1R	13.90	MR	C10346G BGIIXAKH355-1	23.95	MS
C10026A BGIIXAKH357	14.88	MR	C10346B BGIIXAKH355-1	24.39	MS
C10026G BGIIXAKH355-1	15.29	MR	C10026B BGIIXAKH357	24.64	MS
AKH351-1	15.63	MR	AKH357	24.65	MS
C10026B BGIIXR14	15.97	MR	C10026B BGIIXDHY286-1R	26.03	MS
C10346A BGIIXAKH355-1	16.31	MR	C10026G BGIIXR11	26.39	MS
C10346B BGIIXAKH351-1	16.31	MR	C10346G BGIIXAKH351-1	27.08	MS
C10026A BGIIXR11	16.31	MR	C10346A BGII XDHY286-1R	28.89	S
Jai	16.31	MR	C10346A BGIIXR14	31.26	S
R11	16.66	MR	DHY286-1R	31.93	S
NHH44	16.68	MR	AKH355-1	31.96	S
C10026B BGIIXAKH351-1	17.36	MR	C10026B BGII	32.99	S

C10026A BGIIXDHY286-1R	17.71	MR	C10346B BGII	35.41	S
C10346A BGIIXAKH357	18.06	MR	R14	36.8	S
C10346G BGIIXR14	18.06	MR	C10346 B GMS	37.52	S
C10026B BGIIXR11	18.40	MR	C10346A BGIIXAKH351-1	39.25	S
C10026A BGIIXAKH351-1	18.75	MR			
Mean	**20.69**		Mean +SD	**28.8**	**> S**
SD	**8.08**		Mean –SD	**12.6**	**< T**

Table 3: Classification of Hybrids, Parents and Checks on the basis of Mean and SD Method at 150 days in 2016 for ALS

Hybrid	PDI	Relative Disease Reaction	Hybrid	PDI	Relative Disease Reaction
C10346B BGIIXAKH351-1	0.00	R	C10026B BGIIXDHY286-1R	33.35	MS
C10346A BGIIXDHY286-1R	0.70	R	C10346G BGIIXDHY286-1R	33.35	MS
C10346A BGIIXR11	7.65	R	AKH357	34.05	MS
C10346A BGIIXR14	22.20	MR	Jai	34.05	MS
C10346B BGIIXDHY286-1R	23.60	MR	C10346A BGIIXAKH351-1	35.40	MS
C10026 BGII GMS B	23.65	MR	C10346B BGII	35.40	MS
Mallika	24.30	MR	C10026B BGII	35.40	MS
C10346 BGII GMS B	24.35	MR	C10026B BGIIXR14	36.10	MS
C10346B BGIIXR11	25.00	MR	R14	36.80	MS
C10346G BGIIXR14	26.35	MR	NHH44	36.85	MS
C10346G BGIIXAKH357	26.35	MR	C10346G BGIIXAKH355-1	37.50	MS
C10026B BGIIXAKH357	26.35	MR	C10026A BGIIXAKH357	37.50	MS
C10026G BGIIXR11	26.40	MR	C10026A BGIIXR11	38.20	MS
C10026B BGIIXAKH355-1	27.10	MR	R11	38.90	MS
C10026G BGIIXAKH357	27.75	MR	C10346G BGIIXR11	39.60	MS
DHY286-1R	27.80	MR	C10346G BGIIXAKH351-1	40.25	MS
C10346B BGIIXAKH355-1	29.85	MR	C10346B BGIIXR14	40.95	MS
C10346A BGIIXAKH357	30.55	MR	C10026A BGIIXAKH351-1	40.95	MS
C10346B BGIIXAKH357	31.25	MR	C10026A BGIIXR14	44.45	S
C10026G BGIIXAKH351-1	31.95	MR	C10026G BG2XDHY286-1R	45.10	S
C10026B BGIIXAKH351-1	31.95	MR	C10026A BGIIXAKH355-1	45.80	S
C10026B BGIIXR11	31.95	MR	AKH355-1	45.85	S
C10026G BGIIXAKH355-1	31.95	MR	AKH351-1	48.60	S
C10346A BGIIXAKH355-1	33.30	MS	C10026G BG2 XR14	51.40	S
C10026A BGIIXDHY286-1R	33.30	MS			
Mean	32.07		Mean +SD	42.38	> S
SD	10.31		Mean –SD	21.76	< T

Table 4: Effect of G. *herknessii* cytoplasm on ALS disease reaction

Hybrid	PDI Alternaria Leaf Spot Reaction 120 DAS Pooled 2015 & 2016		PDI Alternaria Leaf Spot Reaction 150 DAS 2016	
	CMS Version (AXR)	Conv. Version (BXR)	CMS Version (AXR)	Conv. Version (BXR)
C10346BGIIXDHY286-1R	28.8	13.90	0.70	23.60
C10346BGIIXR14	31.26	10.06	22.20	40.95
C10346BGIIXAKH351-1	39.25	16.32	35.40	0.00
C10346BGIIXAKH355-1	16.31	24.29	33.30	29.85
C10346BGIIXAKH357	18.06	21.88	30.55	31.25
C10346BGIIXR11	9.01	7.30	7.65	25.00
C10026BGIIXDHY286-1R	17.71	26.03	33.30	33.35
C10026BGIIXR14	9.73	15.97	44.45	36.10
C10026BGIIXAKH351-1	18.75	17.36	40.95	31.95
C10026BGIIXAKH355-1	21.88	21.53	45.80	27.10
C10026BGIIXAKH357	14.88	24.64	37.50	26.35
C10026BGIIXR11	16.32	18.40	38.20	31.95

Table 5: Top ten yielder crosses and their Alternaria leafd spot disease reaction

Cross	Aternaria leaf spot reaction 120 DAS Pooled 2015 & 2016				Alternaria leat spot reaction 150 DAS 2016				Pooled Mean Yld /Plant (g)
	T	MT	MS	S	T	MT	MS	S	
C10026 BG2 GXR11			√			√			172
C10026 BG2 BXAKH357			√			√			169
C10346 BG2 GXAKH357			√			√			165
C10026 BG2 BXDHY286-1R			√				√		164
C10346 BG2 GXR11	√						√		159

C10026 BG2 BXR14		√					√		157
Mallika	√					√			155
C10026 BG2 GXAKH355-1		√				√			154
C10026 BG2 BXR11		√				√			154
C10346 BG2 BXAKH357			√			√			154

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