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Original Research Paper

Utilisation of GMS lines for production of heterotic bacterial wilt resistant hybrids in *Tagetes* spp.

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ABSTRACT

The study was conducted using six testers, of which two (KAU M-1 and KAU M-2) were completely bacterial wilt resistant, and four genetic male sterile lines (GMS), in a line x tester model and further the F_1 hybrids were evaluated in a bacterial wilt sick plot for various vegetative, floral and yield parameters along with their response to wilt incidence. Field evaluation for bacterial wilt resistance revealed that out of 24 hybrids, 13 hybrids involving KAU M-1 and KAU M-2 as testers exhibited bacterial wilt resistance. Analysis on heterosis for various characters *viz.*, vegetative, floral, yield and total carotenoids content showed significant heterosis for many of the hybrids over mid parent, better parent and the check variety. The highest standard heterosis for yield was observed for the interspecific hybrid KU Ms-6 xx KAU M-47 (190.84%) followed by an intraspecific hybrid KAU Ms-42 x KAU M-2 (70.93%). Based on the wilt resistance and heterotic performance, these two hybrids can be suggested for commercial cultivation in wilt affected areas.

Keywords: Bacterial wilt resistance, carotenoids, heterotic hybrids, male sterile lines, *Tagetes* species

INTRODUCTION

Marigold, a crop with various landscape uses and commercial importance, is one of the prominent loose flower crop ruling in the domestic as well as international markets. However, its cultivation in Kerala is still confined to small areas since bacterial wilt incidence by *Ralstonia* sp. is reported as a severe menace in Kerala (Umesh et al., 2018; Jeevan et al., 2019). Hence, the development of high yielding varieties/hybrids having bacterial wilt resistance is a pre-requisite for the commercial cultivation of marigold in the state. For development of such improved high yielding varieties/hybrids with desirable ornamental traits and bacterial wilt resistance, more focused breeding program is needed. The exploitation of male sterility of *Tagetes erecta* can be considered as one of the modern breeding techniques for the development of such elite hybrids.

The male sterility system in marigold helps to skip the tedious procedure of emasculation during hybridisation. Genetic male sterility (GMS) which is reported to be more stable compared to cytoplasmic male sterility (CMS) can be reliably utilised for the production of F_1s with desirable traits (Tejaswini et al., 2016). On the background of such facts, a study was conducted to develop bacterial wilt F_1s with desirable traits in *Tagetes* spp. utilising genetic male sterility.

MATERIALS AND METHODS

The primary evaluation of parental population which consisted of twenty genotypes (Table 1) was carried out during October 2019 to February 2020 in a wilt sick plot infected by *Ralstonia solanacearum* which was recorded with an inoculum load of 6.12 x 10^5cfu/ml. From the parental genotypes, six testers and four lines were selected based on the scoring system suggested by Arunachalam & Bandopadhyay (1984) and these were crossed in L x T model during October 2020 to February 2021. The male sterile flowers were pollinated with respective testers for three consecutive days at three different stages *viz.*, bud opening, half opening and full opening stages for male sterile flowers. The pollination was done from 8.30 am to 11.30 am.

The F_1 seeds collected from the male sterile lines were grown for the hybrid evaluation and testing of combining ability during October 2021 to February 2022 in the same wilt sick plot where the parental genotypes were evaluated (Fig. 1). The hybrids were

Table 1 : Marigold genotypes selected for evaluation

Species	Line/Tester	Genotype
Tagetes erecta	Lines	KAU-Ms5
		KAU-Ms6
		KAU-Ms16
		KAU-Ms18
		KAU-Ms22
		KAU-Ms23
		KAU-Ms24
		KAU-Ms42
	Testers	KAU-M1 (IC-630499)
		KAU-M2 (IC-630500)
		KAU-M4
		KA- M8
		KAU-M11
		KAU-M15
		KAU-M19
		KAU-M21
		KAU-M40
		KAU-M46
		(IC-637260)
Tagetes patula	Testers	KAU-M47
		KAU-M48

evaluated along with their parental genotypes and a commercial check (Arka Abhi), for various vegetative, floral, harvest and post-harvest, characters and also for bacterial wilt incidence. The incidence of bacterial wilt was analysed using the scoring method suggested by Sinha et al. (1988).

Estimation of heterosis

Mid-parent heterosis (relative heterosis, RH), better-parent heterosis (heterobeltiosis, HB) and standard heterosis (SH) for each cross were calculated as percent deviation from the mid-parent (MP), better-parent (BP) and commercial check (CC) values, respectively (Turner, 1953; Hays et al., 1955).

i. $\text{Relative heterosis (RH)} = \frac{F1-MP}{MP} x\ 100$

ii. $\text{Heterobeltiosis (HB)} = \frac{F1-BP}{BP} x\ 100$

iii. $\text{Standard heterosis (SH)} = \frac{F1-CC}{CC} x\ 100$

Where, MP, BP and CC are mid-parent, better parent and commercial check variety, respectively.

RESULT AND DISCUSSION

Performance of F_1s on bacterial wilt resistance

The response of parents as well as hybrids to bacterial wilt incidence in the field was also observed. Based on the per cent wilt incidence, the parents and hybrids are categorised into 5 groups as per the score suggested by Sinha et al. (1988). The per cent wilt incidence among the hybrids ranged from 0-75 (Table 2). Under the wilt resistant category, the parents *viz.*, KAU-M1, KAU-M2 and KAU-M8 (0% wilt) and most of their hybrids *viz.*, KAU Ms-6 x KAU M-1, KAU Ms-6 x KAU M-2, KAU Ms-18 x KAU M-8, KAU Ms-42 x KAU M-1 and KAU Ms-42 x KAU M-2 were recorded with zero percent wilt incidence. Apart from these, other hybrids recorded under wilt resistant category were KAU Ms-6 x KAU M-4 (10%), KAU Ms-6 x KAU M-8 (5%), the interspecific hybrid KAU Ms-6 x KAU M-47 (10%), KAU Ms-18 x KAU M-1 (10%), KAU Ms-18 x KAU M-2 (2%), KAU Ms-24 x KAU M-1 (10%), KAU Ms-24 x KAU M-2 (5%) and KAU Ms-24x KAU M-8 (10%). Looking into other categories, the parent KAU Ms-24 and other two hybrids fit in the group of moderately resistant (KAU Ms-24 x KAU M-4 and KAU Ms-42 x KAU M-8) with very low per cent wilt incidence (15%). Genotypes KAU M-4, KAU M-47 and KAU Ms-18 and the hybrids KAU Ms-18 x KAU M-4, KAU Ms-18 x KAU M-40, KAU Ms-42 x KAU M-4 and KAU Ms-42 x KAU M-47 were grouped into moderately susceptible, while Ms6 and Ms42 along with hybrids KAU Ms-6 x KAU M-40, KAU Ms-18 x KAU-M47, KAU Ms-24 x KAU M-40 and KAU Ms-24 x KAU M-47 were grouped into susceptible ones. The most susceptible category was recorded with the genotype KAU M-40 and its hybrid KAU Ms-42 x KAU M-40 with 75 per cent wilt incidence.

Based on the scoring, all the F_1s generated using the bacterial wilt resistant testers *viz.*, KAU M-1 and KAU M-2 have shown outstanding resistance (zero percent) to bacterial wilt disease even in a highly wilt sick plot. Hence, KAU M-1 and KAU M-2 can be considered as the best parents for the development of wilt resistant hybrids/varieties in breeding programs. Besides, the two high yielding hybrids *viz.*, KAU Ms-6 x KAU M-47 and KAU Ms-42 x KAU M-2, were also observed for its bacterial wilt resistance in the field. The study clearly indicated the scope of GMS lines for the production of the hybrids/ varieties with multiple traits.

Table 2 : Response of marigold genotypes and hybrids to bacterial wilt incidence

	Resistant (<10%)	**Moderately resistant (>10 - 20%)**		**Moderately susceptible (>20 - 30%)**		**Susceptible (>30 - 70%)**		**Highly susceptible (70 - 100%)**	
Parents/Hybrids	Wilt incidence (%)	Parents/ Hybrids	Wilt incidence (%)	Parents/ Hybrids	Wilt incidence (%)	Parents/ Hybrids	Wilt incidence (%)	Parents/ Hybrids	Wilt incidence (%)
KAU-M1	0	KAU-Ms24	15	KAU-M4	25	KAU-Ms6	48	KAU-M40	75
KAU-M2	0			KAU-M47	30	KAU-Ms42	60		
KAU-M8	0			KAU-Ms18	21				
KAU Ms6 x KAU M1	0	KAU Ms24 x KAU M4	15	KAU Ms18 x KAU M4	25	KAU Ms6 x KAU M40	65	KAU Ms42 x KAU M40	75
KAU Ms6 x KAU M2	0	KAU Ms42 x KAU M8	15	KAU Ms18 x M40	30	KAU Ms18 x KAU M47	35		
KAU Ms6 x KAU M4	10			KAU Ms42 x M-4	25	KAU Ms24 x KAU M40	50		
KAU Ms6 x KAU M8	5			KAU Ms42 x M47	30	KAU Ms24 x KAU M47	40		
KAU Ms6 x KAU M47	10								
KAU Ms18 x KAU M1	10								
KAU Ms18 x KAU M2	2								
KAU Ms18 x KAU M8	0								
KAU Ms24 x KAU M1	10								
KAU Ms24 x KAU M2	5								
KAU Ms24 x KAU M8	10								
KAU Ms42 x KAU M1	0								
KAU Ms42 x KAU M2	0								

From the data, we could clearly observe the contribution of testers on the response of hybrids against wilt resistance. Similar results of inheritance of the bacterial wilt resistance were also reported by Devi et al. (2015) in bell pepper, wherein, two bacterial wilt resistant genotypes were used in combination with moderately resistant or susceptible varieties of bell pepper for the development of bacterial wilt resistant F_1s, which showed the complete or near complete dominance of wilt resistance.

Magnitude of heterosis

The extent of magnitude of heterosis for number of flowers per plant and yield/plant are presented in Table 3. Since, the number of flowers/plant is directly correlated with the yield of the crop, the positive heterosis range of the parameter was considered. The interspecific hybrid KAU Ms-6 x KAU M-47 showed highest positive significant heterosis of 254.87%, 159.48% and 380.63% over mid-parent, better parent and check variety, respectively. Similarly, with regard to yield, the highest significant positive magnitude for average and standard heterosis was again exhibited by KAU Ms-6 x KAU M-47 (264.50% and 190.84%, respectively). The next hybrid which exhibited high standard heterosis for number of flowers as well as flower yield over the commercial check was KAU-Ms42 x KAU-M2 (73.46% and 70.93%, respectively). Similar results with high heterosis for interspecific hybrids in marigold over mid parent, better parent and standard check variety has been reported in marigold (Li et al., 2005; Ai et al., 2015). The results are also in conformity with Kumari et al (2018), Bhargav et al (2018), Santhosh et al (2018), Lahkar et al.(2020) and Zhang et al. (2019).

The F_1s expressed obvious heterosis over better parent, mid parent and standard variety for all the important traits which are pre-requisite for commercial cultivation as well as for the various landscape purposes. This outstanding performance of hybrids might be due to the high magnitude of heterozygosity which ultimately leads to the maximum heterosis in polyploids (Bonierbale et al., 1993). Also, with the support of the theory of Lundqvist (1966), the above results can be justified with the fact that the variety of allelic interaction can lead to the superiority of heterozygote and accounts for the progressive heterosis in polyploids.

From the overall analysis of above observations of hybrids on mean performance, resistance to bacterial wilt incidence and their heterotic potential over parents

Table 3 : Heterosis of marigold hybrids for yield characters

Cross	No. of flowers/plant			Yield		
	RH	HB	SH	RH	HB	SH
KAU Ms-6 x KAU M-1	-22.74*	-34.02*	-20.17	15.07	-15.95	9.35
KAU Ms6 x KAU M 2	-26.62**	-44.42**	-7.53	2.78	-28.37**	9.06
KAU Ms-6 x KAU M-4	62.73**	51.94*	30.13	116.29**	100.59**	20.26
KAU Ms-6 x KAU M-8	31.68*	26.94	17.16	94.23**	88.67**	13.12
KAU Ms-6 x KAU M-40	-38.34*	-57.45**	-63.55**	-11.86	-31.61	-59.00**
KAU Ms-6 x KAU M-47	254.87**	159.48**	380.63**	264.50**	191.92**	190.84**
KAU Ms-18 x KAU M-1	26.33*	1.95	23.34	88.78**	23.91*	61.22**
KAU Ms-18 x KAU M-2	-39.30**	-56.10**	-26.97	-34.22**	-58.32**	-36.54*
KAU Ms-18 x KAU M-4	60.74**	60.73*	19.41	102.87**	81.97**	-6.74
KAU Ms-18 x KAU M-8	66.40**	50.15**	38.59*	117.31**	86.87**	5.62
KAU Ms-18 x KAU M-40	122.56**	60.08*	18.91	228.89**	198.16**	21.32
KAU Ms-18 x KAU M-47	174.66**	92.40**	256.38**	129.12**	61.35**	60.74**
Cross	**No. of flowers/plant**			**Yield**		
	RH	HB	SH	RH	HB	SH
KAU Ms-24 x KAU M-1	41.47**	-1.72	18.91	76.61**	13.15	47.22**
KAU Ms-24 x KAU M-2	-22.10*	-50.02**	-16.85	-4.12	-40.53**	-9.45
KAU Ms-24 x KAU M-4	98.62**	62.30*	20.57	135.25**	101.64**	3.35
KAU Ms-24 x KAU M-8	117.49**	64.26**	51.61**	150.13**	106.07**	16.48
KAU Ms-24 x KAU M-40	71.75*	45.23	-31.56	89.07**	79.97*	-34.11*
KAU Ms-24 x KAU M-47	86.34**	16.87	116.47**	100.33**	36.98*	36.47*
KAU Ms-42 x KAU M-1	31.97*	-4.69	15.31	77.47**	10.47	43.73**
KAU Ms-42 x KAU M-2	57.59**	4.26	73.46**	85.66**	12.26	70.93**
KAU Ms-42 x KAU M-4	54.00**	32.73	-1.40	83.51**	48.82	-23.73
KAU Ms-42 x KAU M-8	100.67**	58.78**	46.56*	168.86**	110.23**	18.83
KAU Ms-42 x KAU M-40	89.73**	52.34	-18.09	179.11**	173.99**	-9.35
KAU Ms-42 x KAU M-47	112.47**	37.07**	153.89**	111.23**	39.40**	38.88**
SEd	6.36	10.4	10.4	18.04	29.47	29.47

RH-relative heterosis, HB-heterobeltiosis, SH-standard heterosis; * significant at 5% level, ** significant at 1% level

as well as commerical check, the two hybrids are to be highlighted. The interspecific hybrid KAU Ms-6 x KAU M-47 and intraspecific hybrid KAU Ms-42 x KAU M-2 are the two heterotic hybrids that could be suggested for commercial cultivation for their bacterial wilt resistance as well as potential yield in Kerala condition.

CONCLUSION

From the study, it could be concluded that the genetic male sterility in African marigold can be effectively utilized for bacterial development of wilt resistant potential heterotic hybrids in *Tagetes* spp. Also, it is evident that almost all the F_1s have superior ornamental traits making them suitable for commercial purpose as well as various landscape uses. Evaluation on the bacterial wilt incidence highlighted the testers KAU M-1 and KAU M-2 which can be used for development of wilt resistance hybrids. By studying genotypic and phenotypic correlation, gene interaction, heritability and gene linkage studies for various characters, the breeders can exploit the male sterility and bacterial wilt resistance of marigold genotypes for the heterosis/resistance breeding, developing superior hybrids resistant to bacterial wilt disease and with improved qualitative and quantitative traits.

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