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Assessment of resistance against bacterial wilt in marigold genotypes under humid tropics

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Abstract

Bacterial wilt of marigold caused by *Ralstonia solanacearum* is a highly devastating disease that limits successful cultivation of marigold under humid tropics. With the objective to screen genotypes of different *Tagetes* species for resistance to bacterial wilt, the present study was conducted at the Department of Floriculture & Landscaping, College of Horticulture, Thrissur, Kerala, India in two seasons *viz*., rainy and winter, during 2018-19. Thirty two marigold genotypes were subjected to field evaluation in a wilt sick plot. Among the genotypes, there were eight F_1 hybrids, eight varieties, seven local collections of *T. erecta,* eight genotypes of *T. patula* (including three varieties and five local collections) and one genotype of *T. tenuifolia.* The study revealed that two local collections of *T. erecta viz*., M-1 and M-2 were completely resistant to bacterial wilt. The resistance of these two local collections to bacterial wilt was also confirmed by artificial screening studies. It was also observed that bacterial wilt incidence was severe during winter season compared to monsoon. As per the result of screening conducted during rainy season, there were four resistant genotypes, five moderately resistant types, five moderately susceptible types, ten susceptible types and eight highly susceptible types while during winter season the number of highly susceptible types recorded was 20, along with eight susceptible types, two moderately susceptible types and two resistant types. During rainy season, flavonoid content in both leaves and petals was highest in M-1, M-2 and was on par with Bhagwati, which showed a PDI of 4.16% in field evaluation. Resistance to bacterial wilt was negatively correlated with flavonoid content in leaves and petals.

Keywords: Bacterial Wilt, Flavonoids, Marigold, PDI, *Ralstonia solanacearum*.

Introduction

Marigold *(Tagetes species* Linn.) is one of the most commercially viable flower crops belonging to family Asteraceae that can be grown throughout the year. In India, marigold (*Tagetes* spp.) is one of the most popular loose flowers owing to its wide utility for various purposes. Marigold flowers are used in preparation of high-grade perfumes and also as pest repellents. It is a crop which has the potential to keep nematodes away in the field due to presence of alpha-tertheinyl compound in the roots (Somasundaram, 2017). In Kerala, cultivation of marigold is gaining momentum. Now-a-days, most of the farmers are growing F_1 hybrids and improved varieties for cultivation, as the local collections used earlier were of low yield and with poor quality flowers (Umesh, 2017). However, many growers cultivating F_1 hybrids encounter heavy losses due to bacterial wilt incidence, which is very difficult to manage. The disease caused by *Ralstonia solanacearum* is the major limiting factor for successful cultivation of marigold in humid tropical conditions. Considering the importance of the disease, the present investigation was undertaken with the objective to evaluate marigold genotypes for high yield and resistance to bacterial wilt.

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Materials and Methods

The study was carried out during two seasons *viz*., June-September (rainy) and October-January (winter) in 2018-19, using thirty two genotypes of marigold in a bacterial wilt sick plot. Eight F_1 hybrids, eight varieties, seven local collections of *Tagetes erecta,* and eight genotypes of *Tagetes patula* (including three varieties and five local collections) and a genotype of *Tagetes tenuifolia* were used in the study. The experiment was laid out in Randomized Block Design (RBD) with three replications, a plot size of 2m x 1m and spacing of 0.5 x0.4 m. Before raising the crop, the soil was tested for pathogen load by serial dilution, which recorded an inoculum load of 5.01 x 10^5 cfu/ml in rainy season and 6.12 x 10^5 cfu/ml in winter. The details of the marigold genotypes used in the study and their sources are enlisted in Table 1. Plants were observed daily for bacterial wilt symptom which was confirmed by ooze test (Plate 1). The symptoms were further ascertained by applying Koch's postulates. Per cent disease incidence (PDI) and number of days to wilt were observed in the field. The genotypes were grouped into different categories based on the per cent disease incidence (PDI) and the reaction of the genotypes to bacterial wilt as described by Sinha et al. (1988).

Reaction	Per cent disease incidence
R (Resistant)	< 10
MR (Moderately resistant)	>10-20
MS (Moderately susceptible)	>20- 30
S (Susceptible)	>30 -70
HS (Highly susceptible)	>70 -100

Table 1. Marigold genotypes selected for evaluation

Species	Genotypes	Source
Tagetes erecta	Double Orange	Namdhari Seeds, Bengaluru
	Double Yellow	Namdhari Seeds, Bengaluru
	Pusa Narangi Gainda	ICAR-IARI, New Delhi
	Pusa Basanti Gainda	ICAR-IARI, New Delhi
	Suvarna Orange	Suvarna Hybrid seeds, Bengaluru
	Suvarna Yellow	Suvarna Hybrid Seeds, Bengaluru
	Arka Agni	ICAR-IIHR, Bengaluru
	Arka Bangara	ICAR-IIHR, Bengaluru
	Bhuvana	Keonics Seeds, Bengaluru
	Hawai Orange	Keonics Seeds, Bengaluru
	Rupa	Sakura Seeds, Bengaluru
	P-4	JYK-Seeds, China
	Bhagwati	Kalash Seeds, Maharashtra
	Royal Orange	Sagar Hybrid Seeds, Gujarat
	Sakura 031	Sakura Seeds, Bengaluru
	Maria 91	Sakura Seeds, Bengaluru
	M-1	Local collection from Kerala
	M-2	Local collection from Kerala
	Dharmapuri Local	TNAU, Coimbatore
	Coimbatore Local	TNAU, Coimbatore
	Madikeri Local	Local collection from Karnataka
	Nilakottai Local	TNAU, Coimbatore
	TNAU marigold	TNAU Coimbatore
Tagetes patula	Hissar Jafri 2	CCHAU, Hissar
	Pusa Arpita	ICAR-IARI, New Delhi
	Red brocade	Suttons Seeds
	TNAU Dwarf Marigold	TNAU, Coimbatore
	KDA-2	Local collection from Karnataka
	KDA-3	Local collection from Karnataka
	KDA-4	Local collection from Karnataka
	Chintamani Red	Local collection from Karnataka
Tagetes tenuifolia	Mexican Paprika	Red gem Seeds

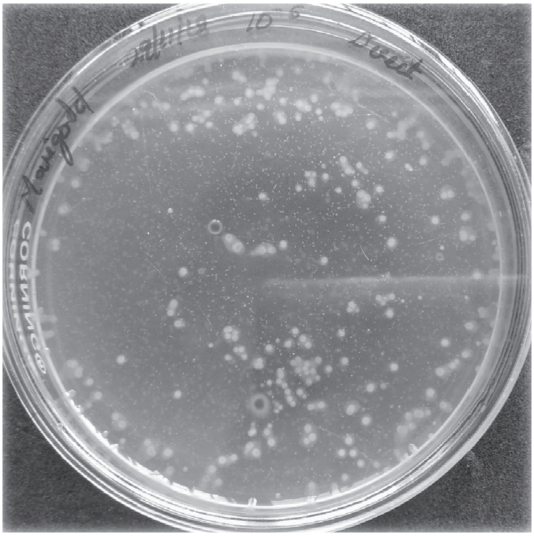

Plate1. Ralstonia solanacearum culture from Marigold

Twelve genotypes that were resistant and moderately resistant in field evaluation were subjected to artificial screening studies by inoculating the pathogen by root dip method. The bacterial isolate collected from the infected marigold plants was used for inoculation. Bacterial suspension containing an inoculum load of 3.01 x 10^5 cfu/ml was used for the study. The seedlings were raised in protrays in sterile soilless media comprised of cocopeat + vermiculite + perlite in 3:1:1 ratio. One-month old seedlings were transplanted to six-inch plastic pots filled with the same sterile soilless media as used for the nursery. The experiment was laid out in CRD with three replications, with five pots per genotype per replication. The pots were maintained in mist chamber ensuring 70-80 per cent humidity. Per cent disease incidence as well as days to wilt were observed.

Considering the importance of flavonoids in imparting resistance to diseases, total flavonoid content of both leaves and petals was estimated for the rainy season crop, following the method suggested by Mirecki and Teramura (1984). The relation between flavonoid content and disease incidence was analysed. A comparative analysis of the flavonoid content of both leaves and petals in different varieties in response to bacterial wilt incidence was also carried out by Z- scatter analysis.

Results and Discussion

The per cent disease incidence (PDI) and average number of days to wilt in all the genotypes were observed during rainy and winter season. Significant difference was recorded with regard to the PDI among the genotypes both in rainy and winter seasons (Table 2). Genotypes M-1 and M-2 did not show any incidence of bacterial wilt in both seasons. During rainy season, the maximum PDI was recorded in Coimbatore Local (87.50%) followed by Dharmapuri Local (83.33%) while during winter season, the highest PDI was recorded in Dharmapuri Local (100.00%), followed by Rupa (92.00%) and Pusa Narangi Gainda (91.70%). Based on PDI, genotypes were classified (Sinha et al., 1988) into resistant [M-1, M-2, Bhagwati and Maria 91], moderately resistant [Arka Agni, Arka Bangara, P4, KDA-2 and Madikeri Local], moderately susceptible (5), susceptible (10) and highly susceptible (8) types, during rainy season. In winter season, 20 genotypes showed highly susceptible reaction, whiile eight genotypes showed susceptible, two moderately susceptible, and two resistant type reactions. Except M-1 and M-2 (plate 2), the remaining genotypes had shown reasonably higher susceptibility during winter season when compared

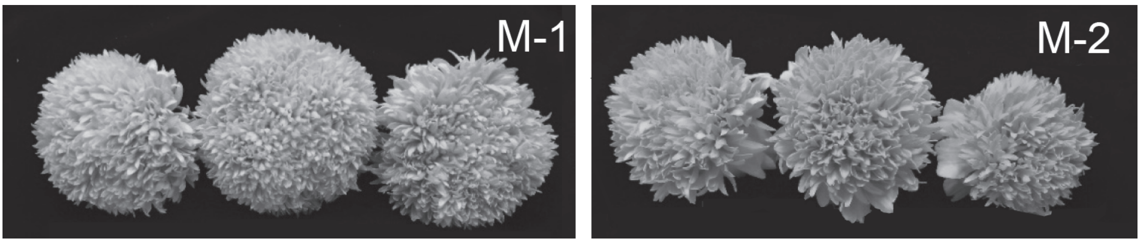

Plate 2. Resistant marigold genotypes

Table 2. Bacterial wilt incidence in marigold genotypes during different seasons

Genotypes (Factor A)	Seasons (Factor B)				Average PDI (Factor A)
	Rainy		Winter		
	PDI (%)	Reaction	PDI (%)	Reaction	
Double Orange	62.50 (7.9)	S	87.70(9.4)	HS	75.08
Double Yellow	79.16 (8.8)	HS	71.00(8.4)	HS	75.08
Pusa Narangi Gainda	54.16 (7.3)	S	91.70(9.6)	HS	72.92
Pusa Basanti Gainda	62.48 (7.7)	S	87.70(9.4)	HS	75.08
Suvarna Orange	41.66 (6.4)	S	79.70(9.0)	HS	60.67
Suvarna Yellow	70.83 (8.4)	HS	83.30(9.2)	HS	77.08
Arka Agni	12.50 (3.6)	MR	55.70(7.5)	S	34.08
ArkaBangara	20.00 (4.4)	MR	50.00(7.0)	S	35.00
Bhuvana	54.16 (7.5)	S	87.70(9.4)	HS	70.92
Hawai Orange	33.33 (5.1)	HS	79.30(9.0)	HS	56.33
Rupa	66.60 (8.9)	S	92.00(9.6)	HS	79.33
P-4	16.66 (3.7)	MR	83.70(9.2)	HS	50.17
Bhagwati	4.16 (1.8)	R	58.70(7.7)	S	31.42
Royal Orange	29.16 (5.4)	MS	58.30(6.6)	S	43.75
Sakura 031	41.66 (6.4)	MS	29.30(5.5)	MS	35.50
Maria 91	8.33 (2.7)	R	75.30(8.6)	HS	41.83
M-1	0.00 (1.0)	R	0.0(1.0)	R	0.00
M-2	0.00 (1.0)	R	0.0(1.0)	R	0.00
Dharmapuri Local	83.33 (9.9)	HS	100.0(10.1)	HS	91.67
Coimbatore Local	87.50 (9.3)	HS	83.70(9.2)	HS	85.58
Madikeri Local	20.83 (2.7)	MR	27.70(4.7)	MS	24.25
Nilakottai Local	77.77 (8.7)	HS	62.70(8.0)	S	70.22
TNAU Marigold	54.16 (7.4)	S	87.70(9.4)	HS	79.25
Hissar Jafri 2	70.83 (8.4)	HS	83.30(9.1)	HS	83.42
Pusa Arpita	79.16 (8.9)	HS	83.30(9.1)	HS	66.67
Red brocade	50.00 (6.9)	S	83.70(9.2)	HS	68.92
TNAU Dwarf Marigold	50.00 (6.9)	S	66.70(8.0)	S	58.33
KDA-2	16.66 (4.14)	MR	87.70(9.4)	HS	52.17
KDA-3	29.16 (5.10)	MS	68.00(8.3)	S	48.58
KDA-4	25.90 (4.9)	MS	62.70(7.8)	S	43.83
Chintamani Red	20.83 (4.6)	MS	79.30(8.8)	HS	50.08
Tagetes tenuifolia	45.83 (6.8)	S	75.30(8.7)	HS	60.58
Average (Factor B)	42.77		69.59		
Factors	C.D(0.01)	SE(m+/-)			
Factor (A)	22.67 (1.74)	0.6			
Factor (B)	5.67 (0.44)	0.2			
Factor (AXB)	32.07(2.47)	0.9			

(Values in the parentheses are square root transformed data)

to rainy season as evidenced in Table 2. Irrespective of the season, the average PDI was high in *T. erecta* genotypes when compared to genotypes of *T. patula* (except Hissar Jaffri) and *T. tenuifolia*. Significant variations among the genotypes to bacterial wilt incidence has been reported and this response of the genotypes has been attributed to its genetic makeup in marigold (Umesh et al., 2018; Singh and Singh, 2010) and in tomato (Gopal et al., 2005). The thickenings in the cell membrane and cell wall could have been a major factor that provided a stronger barrier against bacterial invasion (Kim et al., 2016; Lohar et al., 2018).

It is also evident from Table 2 that bacterial wilt incidence was more during winter (69.59%) season compared to rainy season (42.77%). This might have been due to the high inoculum present in the soil during winter season. Besides, compared to average humidity during rainy season (88 %), there was a

Table 3. Days to wilt in marigold genotypes

Genotypes (Factor A)	Days to wilt		
	Season (Factor B)		Average days to wilt (Factor A)
	Rainy	Winter	
Double Orange	51.71	28.63	40.17
Double Yellow	75.13	17.40	46.27
Pusa Narangi Gainda	81.07	28.07	54.57
Pusa Basanti Gainda	63.36	25.67	44.51
Suvarna Orange	72.72	27.57	50.14
Suvarna Yellow	64.08	32.47	48.28
Arka Agni	59.83	21.77	40.80
ArkaBangara	47.83	22.23	35.03
Bhuvana	68.50	26.33	47.42
Hawai Orange	80.83	19.80	50.32
Rupa	65.06	26.60	45.83
P-4	57.17	28.80	42.98
Bhagwati	25.67	27.77	26.72
Royal Orange	76.25	16.57	46.41
Sakura 031	75.80	10.17	42.99
Maria 91	42.00	30.23	36.12
Dharmapuri Local	65.98	27.43	46.71
Coimbatore Local	64.99	26.97	45.98
Madikeri Local	78.33	17.33	47.83
Nilakottai Local	55.66	20.83	38.25
TNAU Marigold	68.23	23.77	46.00
Hissar Jafri 2	61.07	31.07	46.07
Pusa Arpita	69.64	34.00	51.82
Red brocade	54.90	29.43	42.17
TNAU Dwarf Marigold	70.25	26.30	48.28
KDA-2	53.83	37.43	45.63
KDA-3	57.29	22.47	39.88
KDA-4	82.33	21.23	51.78
Chintamani Red	55.00	30.97	42.98
Tagetes tenuifolia	75.33	13.20	44.27
Average (Factor B)	64.00	25.08	
Factors	CD	SEm	
A	N/A	7.5	
B	5.4	1.9	
A x B	N/A	10.6	

sharp fall in humidity during winter (69%) and the dry climate might have favoured high bacterial wilt incidence. Mondal et al. (2014) observed a similar trend of increased wilt incidence in marigold in West Bengal just after rain during pre-winter period in October. Comparatively low incidence of bacterial wilt during rainy season might have been due to relatively low average temperature (26.08ºC) during heavy rainfall period from June to September compared to slightly higher temperature (27.87ºC) during winter. Irrespective of the season, there was no significant difference among the genotypes for days to wilt among the genotypes; but season had significant influence on days to wilt (Table 3). Very early wilting was observed during winter season (25.08 days) than rainy season (64.00 days). However, there was no interaction effect of genotypes with season with regard to days to wilt. Twelve genotypes which were rated as resistant and moderately resistant in the field evaluation were subjected to artificial inoculation by root dip method. PDI of sixty was recorded in genotypes Maria -91 and KDA-2 followed by KDA-4 and Chintamani Red with a PDI of 53.33. The two local collections *viz.,* M-1 and M-2 were completely wilt resistant even in the artificial screening studies (Table 4). The differential response of genotypes might have been due to the genetic makeup as reported in marigold by Umesh (2017). Similar findings were also noticed in brinjal by Bhanwar et al. (2019).

Santhosha et al. (2015) attributed stability of resistance against *Ralstonia solancearum* to various secondary metabolites. Among the secondary metabolites, flavonoids played an important role in imparting resistance to pathogenic bacteria and fungi. Results of the quantification of flavonoids

Table 4. Bacterial wilt incidence in marigold genotypes on artificial inoculation

Genotypes	PDI (%)	Days to wilt	Reaction
Bhagwati	6.70(2.2)	4.6	R
Royal orange	46.70(5.9)	9.9	S
Maria 91	60.00 (7.5)	7.8	S
P4	40.00 (6.3)	13.6	S
Madikeri Local	40.00 (6.3)	8.1	S
KDA-4	53.33 (7.3)	8.7	S
KDA-2	60.00(7.5)	9.6	S
Chintamani red	53.33 (7.3)	11.9	S
M-1	0.00 (1.0)	*	R
M-2	0.00 (1.0)	*	R
ArkaBangara	46.60(6.7)	6.4	S
Arka Agni	46.60(6.7)	12.6	S
C.D (0.01)	3.9	7.4	
SE(m+/-)	1.3	2.5	

*No wilt incidence
(Values in the parentheses are square root transformed data)

Table 5. Correlation of flavonoid content in marigold genotypes and with bacterial wilt incidence

Genotypes	PDI	Flavonoid content ($A_{300}\ g^{-1}$)	
		Leaves	Petals
Double Orange	62.50 (7.9)	2.2	1.5
Double Yellow	79.16 (8.8)	0.6	0.1
Pusa Narangi Gainda	54.16 (7.3)	1.8	1.6
Pusa Basanti Gainda	62.48 (7.7)	3.0	1.4
Suvarna Orange	41.66 (6.4)	1.5	1.0
Suvarna Yellow	70.83 (8.4)	1.6	1.4
Arka Agni	12.50 (3.6)	2.9	1.4
Arka Bangara	20.00 (4.4)	3.3	1.5
Bhuvana	54.16 (7.5)	2.1	0.9
Hawai Orange	33.33 (5.1)	2.4	1.0
Rupa	66.60 (8.9)	2.7	0.5
P-4	16.66 (3.7)	1.9	1.2
Bhagwati	4.16 (1.8)	3.7	1.6
Royal Orange	29.16 (5.4)	2.6	0.9
Sakura 031	41.66 (6.4)	2.6	1.0
Maria 91	8.33 (2.7)	2.2	1.4
M-1	0.00 (1.0)	4.3	1.7
M-2	0.00 (1.0)	3.8	2.0
Dharmapuri Local	83.33 (9.9)	2.8	0.4
Coimbatore Local	87.50 (9.3)	0.9	-
Madikeri Local	20.83 (2.7)	2.7	1.4
Nilakottai Local	77.77 (8.7)	2.8	0.4
TNAU Marigold	54.16 (7.4)	3.5	0.6
Hissar Jafri 2	70.83 (8.4)	2.0	-
Pusa Arpita	79.16 (8.9)	1.6	-
Red brocade	50.00 (6.9)	2.8	-
TNAU Dwarf Marigold	50.00 (6.9)	0.7	0.7
KDA-2	16.66 (4.2)	1.4	0.1
KDA-3	29.16 (5.1)	1.0	0.3
KDA-4	25.90 (4.9)	0.8	1.2
Chintamani Red	20.83 (4.6)	2.0	1.1
Tagetes tenuifolia	45.83 (6.8)	2.4	0.9
C.D (0.01)		0.78	0.65
Correlation		-0.423*	-0.587**

(Values in the parentheses are square root transformed data)

in both leaves and petals of marigold genotypes during rainy season are given in Table 5. The present study revealed that disease incidence was negatively correlated with flavonoid content of leaves and petals. The highest flavonoid content (Fig. 1 & 2) was recorded in M-1, M-2 which were on par with Bhagwati (Leaves-4.3, 3.8 and 3.7 $A_{300}\ g^{-1}$ leaf; Petals- 1.7, 2.0 and 1.6 $A_{300}\ g^{-1}$ petal respectively). The flavonoid content was highest in leaves as compared to petals, which corroborated the fact that flavonoids were deposited in epidermal cell layers in the cuticle of leaves (Merzylak et al., 2002). Flavonoid accumulation in the leaves and petals were significantly correlated with per cent disease incidence and the disease incidence was negatively correlated with flavonoid content (Fig 1 and Fig. 2). The resistance of the genotypes was possibly exhibited due to the secondary metabolism of polyphenols, and the presence of higher concentration of steroidal glycoalkaloids in resistant plants prevented bacterial movement into the vicinity of the plant system (Vasse et al., 2005).

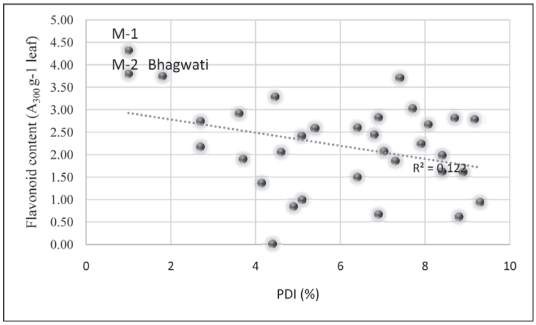

Figure 1. Correlation of disease incidence with flavonoid content in leaves

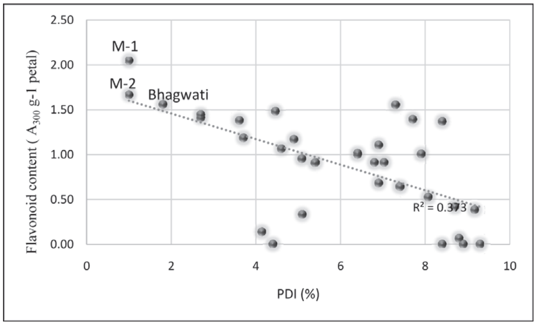

Figure 2. Correlation of disease incidence with flavonoid content in petals

In support to the correlation analysis, the comparative role of the flavonoid content of leaves and petal in different varieties and their response to bacterial wilt incidence was also analysed by Z-scatter analysis (Fig. 3 and Fig. 4), which indicated that increase in flavonoid content decreased disease incidence. This was indicative of the role of flavonoids in providing resistance to pathogen as reported in earlier studies (Mierziak et al., 2014). Mitigation of the bacterial wilt disease was a must

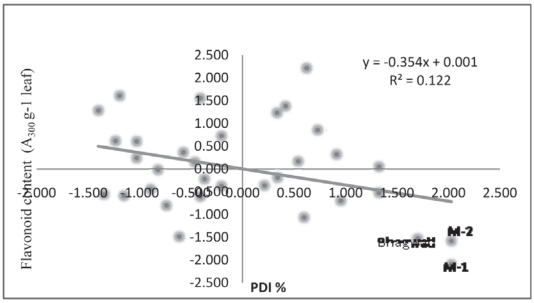

Figure 3. Z- Analysis of marigold genotypes in response to bacterial wilt incidence

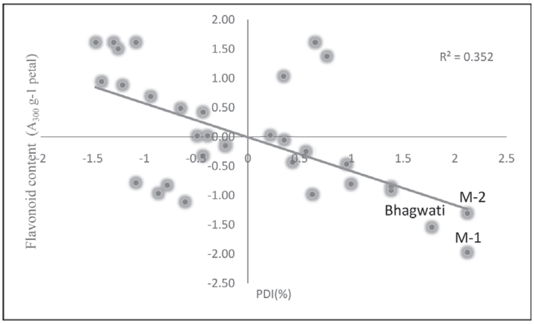

Figure 4. Z- Analysis of marigold genotypes in response to bacterial wilt incidence

for crop improvement in marigold (Grimault and Prior, 1990). Since the pathogen persisted in the soil for long periods, there was a need for adoption of resistant genotypes for successful cultivation.

The two local collections from Kerala, M-1 and M-2, showed complete resistance against bacterial wilt, but lacked the requisite quality with respect to floral parameters, which could be the possible reason for poor marketing and less consumer acceptance. However, the resistance trait of these varieties could be effectively used in crop improvement. The results of the present investigation also indicated that hybrids like Bhagwati, Maria, Arka Agni and Arka Bangara were promising genotypes and were superior with good flower characteristics and moderate resistance to bacterial wilt.

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