Tobacco Research 44(2) : 68 - 73, 2018

SCREENING OF FCV TOBACCO GERMPLASM (*N. TABACCUM*) FOR REACTION TO TMV INFECTION AND IDENTIFICATION OF RESISTANT DONORS

C. NANDA, K. SARALA, S. RAMAKRISHNAN AND S. S. SREENIVAS

ICAR-CTRI Research Station, Hunsur, Mysore District, Karnataka-571105

(Received on 27th September 2018 and accepted on 30th November, 2018)

Tobacco mosaic virus is one of the major virus diseases of Tobacco in Karnataka state. It causes severe to moderate yield losses depending on the stage of infection and early infection causes higher reduction in yield and quality. Incidence of TMV is increasing every year due to monoculture of susceptible varieties and also due its wide host range. Development and deployment of resistant cultivars is the most effective and sustainable approaches as chemical measures are ineffective in containing TMV. Identification of suitable resistance donor in FCV tobacco (*N. tobaccum* L.) is essential to hasten the speed of variety development without linkage drag. 272 germplasm accessions were artificially screened for their reaction to TMV infection. Twenty two lines were identified as resistant to TMV based on hypersensitive reaction. Among these 22, thirteen were the advanced breeding lines and were evaluated for their yield potential. Based on their yield performance and desirable plant type, three lines *viz.*, FCJ 34, FCJ 35 and FCJ 37 were identified as suitable donors for Karnataka Light Soil (KLS) region. Of these three donors, FCJ 34 was found to be homozygous for the *N* locus and thus stable TMV resistant source for breeding TMV resistant cultivar.

INTRODUCTION

Tobacco mosaic virus is one of the major virus diseases of Tobacco (*N. tobaccum*). It causes severe to moderate yield and quality losses depending on the stage of infection and early infection causes higher reduction. In recent years, incidence of TMV is increasing in Karnataka due to cultivation of susceptible varieties and availability of alternate hosts. As of now, chemical measures are found to be ineffective in managing this disease. The losses due to TMV could be successfully reduced with the cultivation of resistant varieties in Andhra Pradesh (Sarala *et al.*, 2012). This was facilitated with the identification of resistance source in diploid wild relative, *N. glutinosa* which is controlled by single dominant gene '*N* ' and its transfer to pre-breeding populations through inter-specific hybridization. Number of efforts was made to transfer '*N* 'gene into the cultivated *N. tobaccum* L in India and worldwide (Holmes 1938, Goodspeed 1942).Thus, number of FCV cultivars viz., Godavari Spl, CTRI Spl MR, JMR, VT-1158 and CTRI Sulakshna resistant to TMV were developed and released for cultivation to black soils in India. However, introgression of "*N*" gene often resulted in reduction yield and quality (mainly due to linkage drag) in flue-cured tobacco (Chaplin *et al.*, 1966; Chaplin and Mann, 1978) which has hampered the development of commercially viable TMV resistant cultivars. Thus, identification of suitable resistance donor in FCV tobacco (*N. tobaccum* L.) is an important task to hasten the speed of variety development by reducing the linkage drag. Hence an attempt was made to at CTRI RS, Hunsur, to identify the suitable resistant donor by screening the FCV germplasm for use in developing high yielding TMV resistant variety suitable to Karnataka light soils.

MATERIALS AND METHODS

Material

Two hundred and seventy two germplasm accessions were used to screen for TMV resistance under artificial inoculation during 2016-17 season at ICAR-CTRI RS, Hunsur. The germplasm accessions consisted of FCV, Burely and *Bidi* types maintained at CTRI, Hunsur and also entries received from ICAR-CTRI, Rajahmundry. The germplasm accessions were grown in glass house conditions to screen for TMV resistance (Table 1).

TMV Screeing

Key words: FCV Tobacco, TMV, Resistance, Screening

TMV infected tobacco leaves collected from the field were crushed and the sap filtered through cheese cloth.The inoculum thus prepared was stored in a glass bottle under refrigeration. Leaves of the individual plants of each germplasm line were inoculated with the TMV inoculums following the sap inoculation technique described by Reddy and Nagarajan (1981). Tip of the inoculated leaf was cut as an identification mark indicating inoculation. *N. glutinosa* was used as resistant check and Kanchan (*N. tabacum* L.) was used as susceptible check. Appearance of localized necrotic lesions due to hypersensitive (HS) reaction in resistant *N. glutinosa* L. described by Holmes (1929) was used as criterion to determine resistance reaction after 48-72 hours from sap inoculation.

Agronomic evaluation for yield:

Out of the twenty two lines which expressed hypersensitive reaction, thirteen lines *viz.*, FCR 41, FCR 42, FCR 43, FCR 44, FCR 47, FCR 48 FCR 49, FCR 50, FCK 7, FCJ 33, FCJ 34, FCJ 35 and FCJ 37 were the entries developed at ICAR-CTRI, Rajahmundry, in its efforts to breed high yielding and TMV resistant cultivars suitable to black soils and irrigated Alfisols. As the linkage drag was found to be less in these lines. All the 13 entries were forwarded to initial varietal trial (IVT) of AINPT conducted during 2016-17 and evaluated in RBD with three replications against checks Kanchan and FCH 222 for their yield potential along with reaction to TMV. Data on green leaf, cured leaf and bright leaf was recorded. Top Grade Equivalent (TGE) was derived as per standard and data was analyzed using design software developed at CTRI, Rajahmundry.

Inheritance study: Three resistant lines (FCJ 34, FCJ 35 and FCJ 37) selected based on their suitable plant type were crossed with susceptible check *Kanchan*. Ten plants of each F_1 hybrid was raised and screened artificially for TMV reaction.

RESULTS AND DISCUSSION

Screening of 272 germplasm lines for TMV resistance through artificial screening was carried out in order to identify potential resistance donors. Among the entries evaluated, twenty two lines *viz.*, FCH 162, FCH 165, FCH 196, COR 14, Bell no.110, SL 21, FCR 41, FCR 42, FCR 43, FCR 44, FCR 47, FCR 48 FCR 49, FCR 50, FCK 7, FCJ 33, FCJ 34, FCJ 35 and FCJ 37 exhibited hypersensitive reaction (Fig.1) indicating their resistance to TMV. Among these resistant lines, thirteen lines (FCR

Fig. 1: Hypersensitive reaction of some of the germplasm lines for TMV infection

41, FCR 42, FCR 43, FCR 44, FCR 47, FCR 48 FCR 49, FCR 50, FCK 7, FCJ 33, FCJ 34, FCJ 35 and FCJ 37) were the advanced breeding lines which have entered initial varietal trial of coordinated trials (AINPT). As these are high yielding lines with TMV resistance, they can be potential sources for TMV resistant breeding programs. However, in order for identifying suitable donor, these lines were evaluated for their agronomic performance as well as for the introgressed TMV resistant gene as enough studies indicated that TMV resistant gene is associated with reduced yield due to linkage drag associated with it (Chaplin *et al.*, 1966; Chaplin and Mann, 1978, Lewis *et al.*, 2005).

Agronomic evaluation:

Thirteen lines *viz.*, FCR 41, FCR 42, FCR 43, FCR 44, FCR 47, FCR 48 FCR 49, FCR 50, FCK 7, FCJ 33, FCJ 34, FCJ 35 and FCJ 37 which were part of IVT were evaluated along with other entries in IVT for their yield potential. Analysis of the data indicated that the lines tested were not significantly differing with respect to yield potential (Table 1). Though the tested entries didn't yield significantly

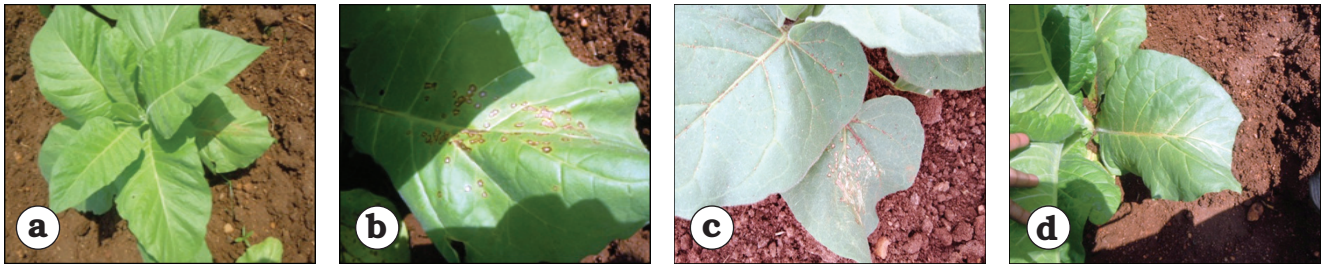

Fig. 2: a &b: Hypersensitive reaction of F_1 of the cross FCJ34 Kanchan; c: Hypersensitive reaction of resistant check *N. glutinosa*; d: absence of HS in susceptible crosses

Table 1: Evaluation of TMV resistant lines for their yield potential

Sl.No.	Treatments	YIELD (Kg/ha)			
		Green leaf	Cured leaf	Bright grade leaf	Top Grade Equivalent
1	FCR 41	7146	895	699	695
2	FCR 42	5505	705	538	546
3	FCR 43	5833	699	523	525
4	FCR 44	5177	611	490	470
5	FCR 47	6313	876	672	662
6	FCR 48	5177	641	460	492
7	FCR 49	6566	854	677	657
8	FCR 50	6490	977	785	737
9	FCJ 33	5227	601	447	452
10	FCJ 34	5985	753	551	561
11	FCJ 35	7879	1053	838	801
12	FCJ 37	7752	818	634	631
13	FCK 7	5429	662	477	487
14	Kanchan	7677	866	712	747
15	FCH222	8106	987	897	902
SEM±		924	126	126	119
CD @5%		NS	NS	NS	NS
CV%		25	27.7	36	33.62

Annexure 1: Reaction of germplasm lines for TMV inoculation

S.No	Name	S.No	Name	S.No	Name
1	A-23	36	DELCREST-66	71	FCH-222
2	A-119	37	EC-3	72	FCH-226
3	A-14-2	38	FCH-68	73	FCH-6002
4	ACR-34-5	39	FCH-145	74	FCH-6101
5	Adcock	40	FCH-148	75	FCH-6310
6	Burley-1	41	FCH-152	76	FCH-6313
7	Burley-3	42	FCH-156	77	FCH-6314
8	Burley-4	43	FCH-157	78	FCH-6513
9	Burley-5	44	FCH-158	79	GSH-2
10	Burley-6	45	FCH-159	80	GOLDEN CURE
11	Burley-8	46	FCH-161	81	GOLDEN WILT
12	Bell.No-2	47	FCH-162	82	HE-2
13	Bell.No-93	48	FCH-163	83	HICKS
14	Bell.No-110	49	FCH-165	84	HICKS-55
15	BY-104	50	FCH-166	85	HICKS-104
16	Bright capsule#2	51	FCH-167	86	HR-65-35
17	COR-3	52	FCH-168	87	HR-70-52
18	COR-14	53	FCH-196	88	HR-70-57
19	COR-16	54	FCH-197	89	HR-70-58
20	CY-136	55	FCH-198	90	HR-70-63
21	CY-137	56	F-207	91	HR-70-65
22	COKER	57	F-209	92	HR-70-74
23	COKER-347	58	F-210	93	HR-72-67
24	COKER-254C	59	F-212	94	J-514
25	COKER-298	60	F-220	95	JL-52 #36
26	COKER-76-51MM	61	FCH-200	96	JL-52 #78
27	COKER-258	62	FCH-201	97	JL-52 #56
28	COKER-411	63	FCH-202	98	JL-52 #96
29	CPR-1 #1	64	FCH-203	99	JL-52 #97
30	CY-142	65	FCH-204	100	JAYASHREE
31	BHAVYA	66	FCH-205	101	ILTD SPL
32	D-1	67	FCH-207	102	KST-23
33	DELHI-34	68	FCH-209	103	K-149
34	DELHI-34 CR	69	FCH-210	104	K-394
35	DAVIS SPL	70	FCH-221	105	K-326
106	K-346	141	NAMBIAR	176	Va-145
107	K-358	142	OLOR-10	177	V 3659
108	KUMKUMATHRI	143	PCT-5	178	VT 1158
109	KANCHAN	144	PCT-10	179	VAMMOR-407/2
110	LV-1	145	PCT-11	180	YELOW GOLD
111	Kanchan	146	PCT-14	181	ZZ-100
112	LV-5	147	PCT-17	182	WILD FIRE RESISTANT
113	L.1129	148	PBG-2	183	V-23 (COR 14)
114	L.1130	149	PMRR-2	184	V-38 (D.1)
115	L.1131	150	PMRR-3	185	V-49 (F.9.....87)
116	L.1132	151	PL-5	186	V-4 (AR 14-2)
117	L.1133	152	PD-4	187	V-33 (Candel)

118	L.1135	153	PK-10	188	13/5
119	L.1136	154	PK-70	189	56-3
120	L.1137	155	RT-13	190	95/5
121	L.30-26	156	RILA-9	191	135/9
122	L.34-34	157	RIWAKA-1	192	136/3
123	MC-1	158	REAMS-64	193	1099/2/1
124	MRS-4	159	RATHNA	194	1099/2/4
125	MDC-19	160	SL-15	195	1171/1
126	MDC-33	161	SL-17	196	2671-3
127	MDC-48	162	SL-21	197	269-8
128	MDC-54	163	STOLAC-17	198	2702-1
129	Mc.Nair-30	164	SBR-1	199	2703-1
130	Mc.Nair-1040	165	SBR-2	200	2691-8
131	NLS-3	166	SBS-1	201	2678-1
132	NLS-4	167	SPIGHT.G.28	202	16681/200
133	NLS-5	168	SPIGHT.G.33	203	2338
134	NLS-2	169	SIRONE	204	3158
135	NC-73	170	SWARNA	205	2359
136	NC-79	171	SUPER GOLD	206	3667
137	NC-95	172	TI. 163	207	3712(normal)
138	NC-98	173	TI.165	208	3127 Albamaculatus
139	NPN-190	174	TI.168	209	A1
140	N-301	175	Va-116	210	A2
S.No	Name	S.No	Name	S.No	Name
211	A4	231	FCJ 15	251	NLST-4
212	TMVRR-2	232	FCJ 16	252	NLST-5
213	TMVRR-3(a)	233	FCJ 17	253	NLST-6
214	TMVRR29	234	FCJ 18	254	TBST-11
215	TMVRR3	235	FCJ 19	255	TBST-16
216	TBST-2	236	FCJ 20	256	TBST-17
217	A-13	237	FCJ 21	257	FCR 41
218	FCJ 1	238	FCJ 22	257	FCR 42
219	FCJ-3(NLCR-7)	239	FCJ 23	259	FCR 43
220	FCJ 4	240	FCJ 24	260	FCR 44
221	FCJ 5	241	FCJ 25	261	FCR 45
222	FCJ 6	242	FCJ 26	262	FCR 46
223	FCJ 7	243	FCJ 27	263	FCR 47
224	FCJ 8	244	FCJ 28	264	FCR 48
225	FCJ 9	245	FCJ 29	265	FCR 49
226	FCJ 10	246	FCJ 30	266	FCR 50
227	FCJ 11	247	FCJ 31	267	FCJ 33
228	FCJ 12	248	FCJ 32	268	FCJ 34
229	FCJ 13	249	NLST 1	269	FCJ 35
230	FCJ 14	250	NLST-3	270	FCJ 36
				271	FCJ 37
				272	FCK 7

higher yield than checks, they were on par with the checks indicating that they can be good donors for yield as well as for TMV resistance. Lewis and Rose (2010) reported that they didn't observe the previously reported (Chaplin *et al.*, 1966; Chaplin and Mann, 1978) reductions in quality as reflected by grade index.

Inheritance study: Three resistant lines (FCJ 34, FCJ 35 and FCJ 37) selected based on their suitable plant type (they were having Kanchan plant type which is preferred in KLS) were crossed with susceptible check Kanchan F_1 hybrids were screened artificially for TMV resistance. F_1 of the cross FCJ34 Kanchan expressed hypersensitive reaction to TMV inoculation indicating its resistance while the other two crosses viz., FCJ35 Kanchan and FCJ37 Kanchan were susceptible (Figure 2). The results suggest that, FCJ34 is homozygous for the *N* locus and the other two lines *viz.*, FCH35 and FCJ37 are still segregating for the same.

From the present study FCJ 34 was identified as stable donor for TMV resistance and it can be used in either backcross breeding or in hybrid development programmes to develop high yielding TMV resistant cultivars. Application of molecular markers and sequencing technology in characterizing the resistant gene in the above identified resistant lines will aid in backcross breeding programs to select against undesirable *N. glutinosa* alleles and thus increase the probability of developing commercially acceptable TMV resistant FCV cultivars (Young and Tanksley, 1989).

REFERENCES

Holmes, F.O. 1938. Inheritance of resistance to tobacco-mosaic disease in tobacco. **Phytopathol.** 28:553–561.

Goodspeed, T.H. 1942. El tabaco y otras especies del genero Nicotiana. Bol. Bac. Agron. Vet. Buenos Aires No. 22.

Chaplin, J.F., and T.J. Mann. 1978. Evaluation of tobacco mosaic resistance factor transferred from burley to fl ue-cured tobacco**. J. Hered**. 69:175–178.

Chaplin, J.F., D.F. Matzinger, and T.J. Mann. 1966. Infl uence of the homozygous and heterozygous mosaic-resistance factor on quantitative character of fl ue-cured tobacco. **Tob. Sci.** 10:81–84.

Lewis, R.S., S.R. Milla, and J.S. Levin. 2005. Molecular and genetic characterization of *Nicotiana glutinosa L.* chromosome segments in tobacco mosaic virus-resistant tobacco accessions. **Crop Sci.** 45:2355–2362.

Lewis, R.S. and C. Rose. 2010. Agronomic Performance of Tobacco Mosaic Virus-Resistant Tobacco Lines and Hybrids Possessing the Resistance Gene N Introgressed on Different Chromosomes. **Crop Sci**. 50:1339-1347.

Young, N.D., and S.D. Tanksley. 1989. RFLP analysis of the size of chromosomal segments retained around the Tm-2 locus during backcross breeding. **Theor. Appl. Genet**. 77:353–359.