## **Development of Tobacco Mosaic Virus (TMV)** resistant Flue-cured F<sub>1</sub> Hybrid, KF118

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Abstract: Tobacco mosaic virus (TMV), TMV-common strain, causes severe damage at growth, yield and leaf quality on flue-cured tobacco in Korea. Controlling TMV outbreak with cultivating practices and chemicals are limited, thus, the best way would be developing TMV resistant variety. A new flue-cured hybrid, KF118, was developed from a cross of maternal parent MSTC175 and TC853 at the Agro-tech. Research Group, KT&G Central Research Institute. KF118 exhibited more vigorous growth characteristics in early stage than that of NC82, standard variety in Korea, while other characters were favorable to those of NC82, It is highly resistant to TMV and bacterial wilt(Ralstonia solanacerum), and black shank(Phytophthora parasitica var. nicotianae) is comparable to that of NC82. It should adapt well to the flue-cured production area, and can reduce premature flowering under unfavorable weather conditions. Yield of KF118 is 1 to 2% higher, and leaf quality is 4% higher than NC82. F1 hybrid, KF118, met acceptable standards for chemical and physical characteristics of cured leaf, and ranked high in good smoking taste by panel members in KT&G.

**Keywords:** TMV resistance,  $F_1$  hybrid. cytoplasmic male sterility, premature flowering.

Tobacco mosaic virus (TMV) is worldwide in its distribution and probably occurs in every country where susceptible cultivars are grown. TMV caused significant reduction in yield(Gooding, 1969, 1981) value and change in chemical constituents in tobacco. *Nicotiana tabacum* L. TMV is difficult to control because of the exceptional ease by which it is mechanically transmitted. Planting resistant cultivar is the most practical means for controlling tobacco virus. Holmes(1938) transferred resistance to tobacco mosaic from *N. glutinosa* L. into a breeding line, numerous efforts have been made to develop mosaic resistant breeding lines (NN and nn) and varieties of tobacco. These efforts have been successful in burley and other tobacco types but not in fluecured.

Work by Chaplin *et al.*, (1961) has shown that yield depression, and possibly poor quality, are associated with the *glutinosa* type of resistance in flue-cured lines. In later work Chaplin & Ford(1965) showed that resistance breeding lines (F<sub>1</sub> hybrids) could be produced that were equal to their TMV susceptible recurrent parent for characteristics measured. In 1995, the Korea Ginseng and Tobacco Research Institute (KT&G) released TMV resistant flue-cured F<sub>1</sub> hybrid KF114 (Chung *et al.*, 1995). This variety appeared to be adapted to the flue-cured growing area, it have been grown widely.

The KF114 lack resistance to Granville wilt and has difficult curing process in yellowing stage. The objectives of this study were conducted to investigate the probability of commercial use of TMV resistant F1 hybrid variety (KF118) and to test the agronomic, chemical, and other important characteristics between KF118 and NC82 (Standard Variety in Korea).

## MATERIALS AND METHODS

Cytoplasmic male sterility was transferred to flue-cued variety, cultivar 'TC175' by the backcross method. Male sterile NC82 (*N. tabacum* genome-N. *debneyi* cytoplasm) was used as the nonrecurrent parent and source of male sterility TC175 was used as recurrent pollen parent. In 1986, a plant of NC82 male sterile line was crossed by one plant of TC175. The first generation hybrid male-sterile was grown in 1987. A single plant of male sterile hybrid was backcrossed by a plant produced from selfed seed of TC175 male plant. Parallel series of hybridization were continued until the BC<sub>5</sub> generation, F<sub>1</sub> hybrid (KF118) was developed from a cross of male-sterile TC175 line×TC853 (Fig. 1).

Using the hybrid breeding method, a new cultivar 'KF118' have been tested in official testing programs in Korea. This experiment conducted at the Agro-tech. Research Group, KT&G Central Research Institute, using the randomized block design with 3 replications in 1999 to 2002. Cultural practices including fertilization, cultivation, sucker and pest control were those normally recommended for flue-cured

Year	Breeding Procedures	Remarks
	MSNC82(N.debneyi)×TC175	
1986	$\stackrel{\downarrow}{MS}F_1$ hybrid	
1987	MS F <sub>1</sub> hybrid×TC175 The source of the TMV resistance was TC175 and MSTC175 was made by backcrossing method from BC <sub>1</sub> to BC <sub>5</sub> .	Backcrossing
1991	MSTC175	Cytoplasmic male sterile, TMV resistant variety
1998	MSTC175×TC853 ↓ KF118	Named as KF118
1999	Performance Test	
2000	Performance Test /Regional Farm Test	
2001	Performance Test /Regional Farm Test	
2002	Regional Farm Test, released	

Fig. 1. Breeding procedures of a new TMV resistant flue-cured tobacco variety KF118.

Table 1. Reaction of parents or F<sub>1</sub> hybrid to the inoculation of TMV

Cultivar or Line	Reaction to TMV	Genotypes
MSTC175 (P <sub>1</sub> )	Local lesions on inoculated leaves without systemic symptom.	NN
TC853 (P <sub>2</sub> )	Systemic mosaic	nn
KF118 (F <sub>1</sub> )	Local lesions on inoculated leaves without systemic symptom	Nn

**Table 2.** Occurrence of mosaic disease(TMV) of tobacco plants NC82 and KF118 when transplanted in farmers' field having chronic problem with the virus.

	Location		Infection(%)	
_	Hongcheon	Chungju		
NC82	67/317*	107/315	27.5	
KF118	0/300	0/300	0.0	

<sup>\*</sup>No. of plants appeared visual mosaic symptom/ no. of total investigated plants at flowering stage.

tobacco production at the Agro-tech. Research Group. Entries were grown in two rows, 20 competitive plant plots with spacing of 45 cm between plant and 110 cm between rows. Treatments were compared for agronomic performance by days to flowering rate of premature flowering, bacterial wilt, black shank, and value (price) per kg. Chemical composition of the treatments was characterized by determining percent nicotine, percent reducing sugar and percent total nitrogen in cured leaf samples.

## RESULTS AND DISCUSSION

The genotype of each variety was investigated about a week after TMV common strain inoculation. Since the hypersensitive reaction to TMV was dominant, plants carry-

ing the N factor was local lesion. Thus, MSTC175 [NN] of homozygous hypersensitive and KF118 [Nn] of heterozygous variety were local lesion, and the TC853 [nn] of homozygous susceptible variety was shown to no symptoms (Table 1). In the naturally TMV infected field conditions, TMV outbreak of KF118 and NC82 at early flowering stage of plant development reaction was shown to Table 2. The KF118 was high resistance to TMV, but the TMV incidence of NC82 was 27.5 percent.

The average performance for certain agronomic characteristics of KF118 and NC82 were shown in Table 3. The KF118 flowered later, taller in stalk height, and more leaves per plant than check variety. And also, it can reduce premature flowering under unfavorable conditions compared with NC82. Essentially, no differences were found for the chemi-

**Table 3.** Comparison of agronomic characteristics and chemical compositions between NC82 and KF118 in the performance test, 1999-2002

Variety	Stalk height	Harvested leaves	Days to flowering	Rate of PF*	Nicotine (%)	Reducing sugar (%)	Total Nitrogen (%)
NC82	113 a	17.0 a	62 a	40 b	3.31 a	15.4 a	2.49 a
KF118	119 b	19.5 b	64 b	13 a	3.02 a	16.2 a	2.33 a

\*PF: Premature flowering

Means with same letter in the same column are not significantly different at p=0.05 (DMRT).

Table 4. Comparison of disease resistance between KF118 and NC82, 1999-2002.

Variety	Bacterial wilt* (%)	Black shank** (%)	TMV
NC82	54 b	37 a	Susceptible
KF118	40 a	34 a	Resistant

\*Resistance was screened under the natural field conditions infested with the pathogen.

The resistance was evaluated by percent of infested plants.

Means with same letter in the same column are not significantly different at p=0.05 (DMRT).

Table 5. Comparison of a new variety KF118 and NC82 in performance tests across four years, 1999-2002.

	Price index	Price index *	Yield index	Remark	
NC82	100 a	100 a	100 a	Based on estimates of the 0 percent of TMV	
KF118	102 a	104 b	102 a	infected plants in field.	

<sup>\*</sup>Regional farm test at six locations in 2002. Price is based on the 2001-2002 purchasing prices of KT&G. Means with same letter in the same column are not significantly different at p=0.05 (DMRT).

cal constituents, and all were within an acceptable range.

Comparison of disease resistance between KF118 and NC82 was shown in Table 4. The KF118 was highly resistant to TMV and bacterial wilt, but black shank were comparable to those of NC82. And also, the KF118 produced yield and value per kg higher to those of NC82 in performance test and regional farm test (Table 5).

Commercial F<sub>1</sub> hybrids were adopted slowly however, first in burley, then in flue-cured tobacco, because expressions of hybrids vigor in inbred line crosses were low. The availability of cytoplasmic male-sterility made F<sub>1</sub> hybrid seed production possible(Chaplin, 1964; Nielsen & Weiss, 1999), yet most of these alien cytoplasm were found to have deleterious effects on plant phenotype. TMV resistance was transferred from N. glutinosa to N. tabacum(Gray et al., 1974; Holmes, 1938) and the N gene has been available to flue-cured tobacco breeders for 50 years. The gene provides a very high level of resistance to this virus disease, but TMV-resistant cultivars have never been popular with growers, even those with severe TMV problems on their farms. This problem has never been resolved using the interspecific N gene source, but the advantages of using the gene in a heterozygous condition as shown in KF118 is evident. The N gene has been used extensively in burley tobacco, however, without yield or quality problems(Legg et al., 1974, 1979).

These genes express complete dominance; only one parent of an  $F_1$  hybrid needs to posses the gene for the trait to be expressed in the hybrid. Consequently, deleterious agronomic effects associated with these genes are reduced in the heterozygous condition (Linger, 1999; Wernsman, 1999).

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