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Development of PVY resistant flue-cured F_1 hybrid variety 'KF120'

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ABSTRACT : *Potato Virus Y* (PVY), PVY-vein necrosis strain, causes severe damage at growth, yield and leaf quality on flue-cured tobacco in Korea. The development of PVY resistant flue-cured varieties without quality deterioration is therefore urgently desired. The flue-cured tobacco, KF120 (Korea Flue-cured 120), was a male-sterile (ms) F_1 hybrid derived from the cross between msKF117 and KF0007-7. msKF117 was developed from the cross of NC82 with *N. africana* and KF0007-7 was developed from the cross of KF117 with NC82. The agronomic characteristics and disease resistance of KF120 was evaluated during 2006-2007 field performance test. It showed better growth characteristics and yield performance than standard cultivar KF109. It had 2 more leaves per plant, flowered 2 days later than KF109. The yield of cured leaf of KF120 was increased by about 5% compared to KF109. The chemical composition and physical properties of the cured leaf of KF120 were as much acceptable as those of KF109. KF120 showed high resistance to PVY compared to KF109. It showed a similar mode of resistance to bacterial wilt and black shank as was found in KF109.

***Key word* : *N. africana*, F_1 hybrid, Potato virus Y resistance**

Necrotic strains of *Potato virus Y*(PVY) is found worldwide and causes severe damages and economic losses in leaf tobacco production. PVY incidence has been increasing both flue-cured tobacco growing areas and burley growing areas in Korea. The disease can be mechanically transmitted, but dissemination is usually by aphids under nature conditions. Control practices of infection or spread of a virus disease in a tobacco usually rely on the elimination of aphids and prevention of their movement from field to field (Lucas, 1975). However, various cultural and chemical methods are ineffective in controlling the disease (Burk and Chaplin, 1980; Pirone, 1988; Chae *et al*., 1994; Chae *et al*., 2001). The best means to control PVY is to use resistant varieties in the regions where the virus is present.

Resistance to PVY is conferred by the irradiation-induced recessive gene *va* from cv. Virgin A Mutant (Koelle, 1958) and by its allelic forms found in several other cultivars (Wernsman, 1992a). Even though these sources of resistance still appears to be effective, no one source provides complete resistance to all strains of PVY, and additional sources of resistance would be valuable for increasing the range and level of resistance in *N. tabacum.*

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Lucas *et al.* (1980) found an accession of *N. africana* (2n=46)(Merxmuller and Buttler, 1975) that is immune to three strains of PVY. Wernsman (1992b) subsequently developed a chromosome addition line, NC152 (2n=50), which possesses a pair of homologous chromosomes from *N. africana* that conferred resistance to PVY.

Hybrids between *N. africana* and *N. tabacum* also can be easily produced (Lucas *et al.*, 1980). The introduction of this resistance into *N. tabacum* is the best method to increase breeding efficiency as compared to use of VAM (Virgin A Mutant) source and it is possible to use F_1 hybrids production.

The study describe the development of a New Flue-cured Tobacco Variety 'KF120'. The agronomic characteristics and chemical composition of 'KF120' in were evaluated in related to the 'KF109' (Standard Variety in Korea).

MATERIALS AND METHODS

Plants materials

Cytoplasmic male sterility was transferred to PVY resistance flue-cured variety 'KF117' through backcross method. Male sterile 'NC82' (*N. tabacum* genome - *N. debneyi* cytoplasm) was used as the nonrecurrent parent and source of the male sterility (ms) 'KF117' was used as recurrent pollen parent.

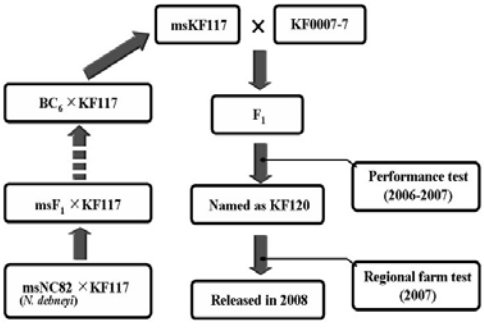

Fig. 1. Breeding strategy of a new flue-cured tobacco variety 'KF120' with potato virus Y resistance. KF0007-7 is a potato virus Y resistant line developed from a cross of KF117 × NC82.

Parallel series of hybridization were continued until the BC_6 generation. 'KF120' (F_1 hybrid) was developed from a cross between 'msKF117' and potato virus Y resistant line 'KF0007-7' that was developed from 'KF117' / 'NC82' (Fig. 1).

Field trials and trait evaluation

'KF120' has been evaluated at the Tobacco Research Group, KT&G Central Research Institute, using the randomized block design with 3 replications in 2006 to 2007. All the testing procedures were followed as official testing programs provided by KT&G Central Research Institute. Cultural practices including fertilization, cultivation, sucker and pest control were followed those normally recommended for flue-cured tobacco production at Tobacco Research Group. Entries were grown in two rows, 20 competitive plant plots with spacing of 42 cm between plant and 120 cm between rows. Agronomic characteristics of days to flowering, bacterial wilt, black shank, PVY and value (price) per kg were evaluated. Chemical compositions such as percent of nicotine, total sugar and total nitrogen in cured leaf sample of 'KF120' were analyzed.

Virus sources and resistance of 'KF120' to PVY

PVY isolates PVY-TOJC37 (classified as ntn-strain) and PVY-SM (classified as resistance-breaking isolate) have been maintained by the Tobacco Research Group, KT&G Central Research Institute (Kim *et al*, 2006). Inoculum was prepared by grinding PVY-infected *Nicotiana tabacum cv.* Xanthi nc leaves 1:10 (w/v) with 0.1 M phosphate buffer, pH 7.0. The largest leaves of 6 week-old 'KF120' and 'KF109' were rub-inoculated. The plants were grown at 23~27℃ under natural light conditions in the glasshouse. Symptom development was monitored on a daily

basis after inoculation.

Tissue print

Tissue print was prepared as described by Holt (1992). For the tissue prints of stem section stem sections of 10 or 14 days after inoculation were cut perpendicularly to the plant axis and pressed onto a nitrocellulose membrane. The prepared membranes were incubated with anti-PVY-N primary monoclonal antibody. After washing unbounded primary monoclonal antibody the membranes were incubated with rabbit anti-mouse immunoglobulin alkaline phosphatase conjugate. After unbound enzyme-labelled antibodies were removed by washing, 100 ㎕ of 50 mg of nitro-blue-tetrazolium per ml of 70 % dimethyl formamide and 50 ㎕ of 25 mg of 5-bromo-4-chloro-3-indolyl phosphate per ml of dimethyl formamide were added for the color development.

RESULTS AND DISCUSSION

The average performance for certain agronomic characteristics of 'KF120' and 'KF109' were shown in Table 1. The 'KF120' flowered later, lower in stalk height, and 2 more leaves per plant than check variety.

Comparison of chemical constituents between 'KF120' and 'KF109' was shown in Table 2. The 'KF120' showed higher nicotine and total nitrogen contents than 'KF109', but it showed remarkably lower total sugar content and ratio of total sugar and nicotine.

The 'KF120' was almost similar to 'KF109' in resistance to bacterial wilt and black shank, but it was resistance to PVY (Table 3). 'KF120' produced higher yield and price index per kg than that of 'KF109' in performance test and regional farm test (Table 4).

Table 1. Comparison of agronomic characteristics between 'KF109' and 'KF120' in the performance test, 2006-2007

Varieties	Stalk height (cm)	No. of leaves per plant	Largest leaf		Flowering date*	Midrib ratio (%)
			Length (cm)	Width (cm)		
KF109	100^{b**}	17.1^{a}	65^{a}	28^{a}	66^{a}	26.2^{a}
KF120	95^{a}	19.1^{a}	66^{a}	27^{a}	68^{a}	26.0^{a}

Days after transplanting

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

Table 2. Comparison of chemical constituents between 'KF109' and 'KF120', 2006-2007

Varieties	Nicotine (%)	Total Nitrogen (%)	Total Sugar (%)	Total sugar/ Nicotine	Total nitrogen/ Nicotine
KF109	2.88^{a*}	2.83^{a}	24.3^{b}	8.44^{b}	0.98^{b}
KF120	3.32^{b}	3.00^{b}	20.8^{a}	6.27^{a}	0.90^{a}

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

Table 3. Comparison of disease resistances and rate of PVY diseased plant at regional farm test between 'KF109' and 'KF120', 2006-2007

Varieties	Bacterial wilt*	Black shank**	PVY
KF109	MR***	MR	S(39.1%)
KF120	MR	MR	R(0.8%)

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

** Transplants were inoculated with *P. parasitica* var. *nicotianae* and were kept in environmental chamber at 28 ℃ for 3 weeks.

***MR, moderate resistance; R, resistance; S, susceptible

To investigate the resistance to PVY, 'KF120' and control variety 'KF109' were evaluated in response to PVY-TOJC37 and PVY-SWM. PVY-TOJC37 is classified as ntn-strain, which induced vein necrosis on 'KF109', whereas 'KF120' was

Table 4. Comparison of yield, price and value between 'KF109' and 'KF120'

Varieties	Yield index	Value index	Price index*	Remark
KF109	100[a]	100[a]	100[a]	Based on estimates of 0 percent of TMV and PVY infected plants in the field.
KF120	105 [b]	102[a]	107[b]	

*Means with the same letter in the same column are not significantly different at p=0.05.
Price is based on the 2006-2007 purchasing prices of KT&G

highly resistance to the isolate. PVY-SWM is classified as resistance-breaking isolate, which induced more severe vein necrosis on the 'KF109' than PVY-TOJC37 isolate. 'KF120' plants that were infected with PVY-SWM showed a 7 days delay in symptom development, and the amount of virus in plants was significantly lowered than check variety (Fig. 2).

Virus localization print were prepared from cross sections of tobacco plants that were 10 and 14 days after inoculation with PVY-SWM. Viral antigen was detected in the inner and outer phloem ring in all the stem section prepared from 'KF109'. In contrast, the stem section from 'KF120' did not contain viral antigen after 10 days, and relatively low of viral antigen were detected after 14 days (Fig. 3).

'KF120' is highly resistant to PVY-TOJC37 (ntn-strain / resistance- nonbreaking isolate) and tolerant to PVY-SWM (resistance breaking isolate), as described by Masuta *et al.* (1999) and Xiong (2005). The 'KF120' did not display any of the unfavorable characters, such as early-flowering, short plant height, small number of leaves, low leaf length and low yield of cured leaves, that were observed in other PVY resistant varieties (Yamamoto, 1992).

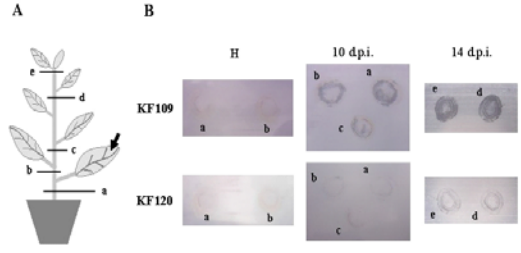

Fig. 3. Detection and localization ofPVY in a cross section of the main stem of *N. tabacum* cvs. KF109 and KF120. A, Relative location of stem sections; arrow indicates the inoculated leaf. B, Blot prepared 10 and 14 days post inoculation (d.p.i.) with PVY-SWM. Locations of the cross section are shown by the labeled 'a' to 'e'.

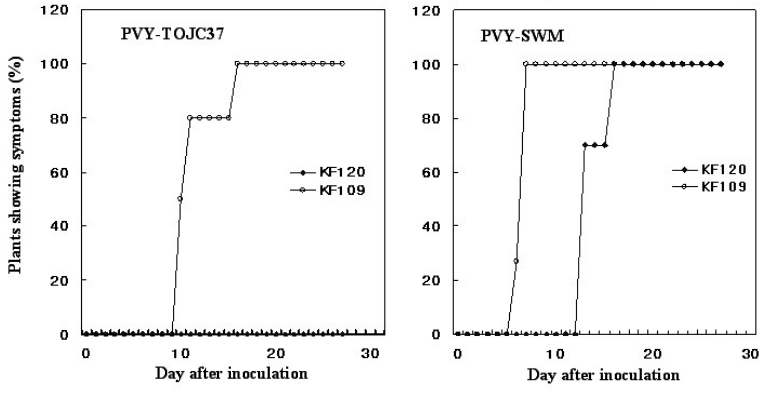

Fig. 2. Development of systemic symptoms in *Nicotiana tabacum*cv. KF109 and KF120 after inoculation of PVY-TOJC37 and PVY-SWM.

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요 약

감자바이러스 Y(PVY)는 국내 황색종 산지에서 잎담배의 생육, 수량 및 품질에 있어서 매우 심한 피해를 일으키는 병으로서 이를 방제하기 위해서는 PVY 저항성 품종의 육성이 시급히 요구된다. KF120은 msKF117과 KF0007-7를 교배하여 육성된 F_1 hybrid 품종이다. msKF117은 NC82와 *N. Africana*를 교배하여 육성하였고, KF0007-7은 KF117과 NC82를 교배하여 육성하였다. KF120의 농경적 특성과 병저항성을 평가하기 위하여 2006 ~ 2007년 동안 생산력 검정시험을 수

행한 결과 KF120은 KF109에 비하여 생육특성 및 수량성이 양호하였으며 수확엽수는 2매 정도 많았고 개화기는 2일정도 늦었다. KF120의 건조엽 수량은 KF109에 비하여 5% 정도 증수 되었으며 화학성분 및 물리성은 대등한 수준이었다.

KF120은 KF109에 비하여 세균성 마름병과 역병에 대해서는 유사한 저항성을 가지고 있었으며, PVY에 대해서는 높은 저항성을 보였다.