A DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

CAUSES OF LEAF YELLOWING SYMPTOMS IN MUSKMELONS AND CULTURAL CONTROL PRACTICES

머스크멜론의 황화엽 증상 원인 구명과 발생 경감을 위한 재배 기술 개발

BY

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JULY, 2015

DEPARTMENT OF HORTICULTURAL SCIENCE AND BIOTECHNOLOGY

THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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UNDER THE DIRECTION OF DR. CHANGHOO CHUN SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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APRIL, 2015

APPROVED AS A QUALIFIED DISSERTATION OF HEE JU LEE FOR THE DEGREE OF DOCTOR OF PHILOSOPHY BY THE COMMITTEE MEMBERS

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CAUSES OF LEAF YELLOWING SYMPTOMS IN MUSKMELONS AND CULTURAL CONTROL PRACTICES

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ABSTRACT

The leaf yellowing symptoms (LYS) of muskmelon (*Cucumis melo* L.) are a new epidemic that has spread throughout cultivation regions. The objective of this study was to determine the causal factors of LYS, to characterize symptoms, and to develop cultural control practices. In the first chapter, electron microscopic observation and reverse transcription-polymerase chain reaction (RT-PCR) assay revealed that all LYS affected plant samples were free of major melon viruses including CMV, MNSV, CGMMV, SqMV, WMV, KGMMV, PRSV, and ZYMV. Furthermore, the next-generation sequencing assay showed the LYS-affected plant samples contained *Cucurbit aphid-borne yellows virus* (CABYV), a member of the genus *Polerovirus* in the family Luteoviridae. The presence of CABYV in these leaf samples was also confirmed by RT-PCR using the CABYV-specific primers. The CABYV infection in muskmelon has not been

reported in Korea until now. The complete genome sequences of 22 isolates of CABYV collected in two years (2013-2014) were determined and analyzed comparatively along with previously reported CABYV genome sequences. They contained 5,680-5,684 nucleotides in length and encoded six open-reading frames which were separated into two regions by a non-coding internal region of 199 nucleotides. Their genomic organizations were typical for a member of the genus *Polerovirus*. When phylogenetic relationship was analyzed with four known groups of CABYV (Asian, Mediterranean, Taiwanese, and R), Korean CABYV isolates were clustered with the Asian group over 94% nucleotide sequence identity. The nucleotide sequence identities of the Korean CABYV isolates with other groups were 87-89% with Mediterranean, 88% with Taiwanese, 81-84% with CABYV-R group, and 72% with *Melon aphid-borne yellows virus*. This study confirmed that LYS in muskmelon was not merely a physiological disorder, but a viral disease caused by CABYV which was transmitted by aphids. In the second chapter, the physiological and morphological influences of the CABYV-infected muskmelon were characterized. Concentrations of glucose, fructose, and sucrose were greater in CABYV-infected leaves than in uninfected leaves. Sugar contents in stem, fruit, and root tissues were slightly lower in CABYV infected plants than in non-infected plants. The scanning electron microscope observation revealed that the sieve tubes of the leaf vessels were closed more frequently in the CABYV-infected plants

than in normal plants. The blockage of the sieve tubes could have restricted the translocation of free sugars from the leaf tissues to other organs in CABYV-infected plants that always produced smaller root masses. The average photosynthetic activity (4.09 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was measured by chlorophyll fluorescence yield in the leaves of CABYV-infected plants and it was about one third of that (12.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$) measured in normal plants. The average root function (0.28 $mg \cdot g^{-1}$) of CABYV-infected plants was about the half of that (0.48 $mg \cdot g^{-1}$) observed in uninfected plants. Cytological observations revealed that the infected and uninfected leaves showed similar morphological characteristics in palisade parenchyma and mesophyll spongy cells of leaves. However, leaf cells of the infected plants contained more starch granules compared to those of uninfected plants. And in the third chapter, various cultural practices were applied as means for reducing LYS in muskmelons. Root pruning before transplanting increased percent incidents of LYS lowering fruit quality, while showing little difference in plant growth. When the aged seedlings were transplanted and cultured, the fruit weight was reduced by LYS development, while plant growth, photosynthesis, and root activity were not significantly different compared to those of muskmelon plants grown from young seedlings. To minimize the occurrence of LYS, seedlings to be transplanted should have less than three fully-expanded leaves at transplanting. Minimizing the number of fruits per plant lowered the incidence of LYS, with 2.4, 6.7, and

13.3% of plants developing the symptoms. As number of leaves left on the vine increased, net photosynthesis and biomass yield increased, while showing less LYS and better fruit quality except the net index of the fruit skin. More than 25 leaves should be maintained on each plant to negate the deterioration of fruit quality influenced by LYS. The incidence of LYS development was reduced by increasing the number of leaves left on the vine above the fruit-bearing nodes.

Keywords: aphid, *Cucurbit aphid-borne yellows virus,* growth characteristic, mesophyll cell, net index, next generation sequencing, phylogenetic analyses

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ABBREVIATIONS

BPVY	*Beet pseudo-yellows virus*
CABYV	*Cucurbit aphid-borne yellows virus*
CCYV	*Cucurbit chlorotic yellow virus*
CGMMV	*Cucumber green mottle mosaic virus*
ChlFY	chlorophyll fluorescence yield
CYSDV	*Cucurbit yellow stunting disorder virus*
FBN	fruit-bearing node
LYS	leaf yellowing symptoms
MABY	*Melon aphid-borne yellows virus*
MNSV	*Melon necrotic spot virus*
MYSV	*Melon yellow spot virus*
NGS	next generation sequencing
nt	nucleotide
RT-PCR	reverse transcription-polymerase chain reaction
SEM	scanning electron microscope
WMV	*Watermelon mosaic virus*
ZYMV	*Zucchini yellow mosaic virus*

GENERAL INTRODUCTION

Severe leaf yellowing symptoms (LYS) of muskmelon (*Cucumis melo* L.) has recently been observed in several greenhouse cultivation areas in Korea, especially in summer and autumn. In Korea, the first LYS was observed in Chungyang, Damyang, and Uiryeong in 2004. Since the first report the distribution and incidence of LYS has increased steadily, and it has been reported from majority of greenhouse muskmelon cultivation areas such as Buyeo, Gurea, Hoengseong, Namwon, and Yanggu in Korea (Lee et al., 2015). LYS appears as light green spots on the surface of lower leaves (sometimes upper leaves) in the first stage and then eventually become yellow. Yellow spots are enlarged until a whole leaf is changed to yellow color and, finally, the LYS spread to upper leaves. In the LYS occurred, plants growth and fruit development are severely retarded, net on fruit surface are not developed, and sugar contents of fruit are lowered (Lee et al., 2009b; Park et al., 2011).

LYS has been reported to be caused primarily by several different viruses that are widespread in many countries: *Beet pseudo-yellows virus* (BPYV; Tomassoli et al., 2003); *Cucumber vein yellowing virus* (CVYV; Martinez-Garcia et al., 2004); *Cucurbit yellow stunting disorder virus* (CYSDV; Celix et al., 1996); *Cucurbit aphid-borne yellows virus* (CABYV; Guilley et al., 1994; Lecoq et al., 1992); *Melon aphid-borne yellows virus* (MABYV; Han

et al., 2010; Xiang et al., 2008); *Cucurbit chlorotic yellow virus* (CCYV; Gyoutoku et al., 2009; Okuda et al., 2009, 2013; Sugiyama, 2013); and *Melon yellow spot virus* (MYSV; Kuwabara and Sakai, 2008). LYS is due to physiological disorders or pesticide phytotoxicity. Copper fungicides often cause this symptom by leading a phytotoxic reaction. Foliar fertilizations can often worsen LYS by increasing salt levels on the leaves. At low pH, manganese toxicities occur so that plant available manganese increases greatly and plants take up quantities that become toxic. Magnesium deficiencies can also occur at low pH and older leaves will show interveinal chlorosis (Lemaire et al., 1993). LYS in muskmelon is attributed to the unbalance of sugar translocation when cultivars with fewer roots, larger leaves and faster fruit enlargement than others were cultivated under poor conditions (Takeshita, 2004). However, the exact cause of the LYS is still not identified.

In Korea, melon was introduced for the first time by Dr. Jang-Chun U (Nagaharu U) in 1954. Since then, melon is of the great importance among fruit vegetables in Korea where its cultivation area and production have increased from 17,000 metric tons in 659 ha in 2000 to 48,000 tons in 1,477 ha in 2013 (MIAFRA, 2014a). About 1,123 tons of melons are exported to Japan and countries in Southeast Asia in 2013 (MIAFRA, 2014b). All Korea melon is cultivated in greenhouses. Due to advances in melon cultivation technology, growers now produce high quality melon in greenhouses year-

round. However, melons produced from some areas have low sugar contents and poorly netted skin, which depreciate their commercial value. It is attributed to many diseases including gummy stem blight (Cho et al., 1997), fusarium wilt (Lee et al., 2015), *Melon necrotic spot virus* (MNSV; Choi et al., 2010), powdery mildew (Lee et al., 2014), and black root rot (Heo et al., 2001). In addition to these diseases, aphids, greenhouse whitefly, and root-knot nematodes are of great concern in melon cultivation. Recently, muskmelon crops are seriously affected by LYS which results in decreased fruit weight, poor net formation, and low sugar content.

This study was conducted to diagnose and identify LYS's cause in muskmelon cultivation areas of Korea during summer in 2013-2014. The molecular characteristics and genetic structure of Korean isolates of CABYV were analyzed to understand the evolutionary relationships among isolates. Growth and morphological characteristics, mineral concentrations of leaf and sap, and sugar contents were investigated to determine the causal factors of LYS of muskmelon. Cultural practices were developed to reduce the occurrence and damage of LYS.

LITERATURE REVIEW

Viruses of cucurbit crops

Cucurbit crops, which have a great of ecological and genetic characteristics, are infected by diverse viruses. Cucurbits production is affected by more than 59 different viruses of the major plant virus genera (Fauquet et al., 2005; Lecoq, 2003; Lecoq and Desbiez, 2012). Cucurbit viruses are categorized by typical symptoms as follow (Blancard et al., 1994): 1) mosaics on leaves sometimes associated with leaf size reduction, blister, laceration, or fruit enation that may develop a range of discolorations and deformations altering their quality; 2) yellowing of older and mature leaves and, reduction of fruit production without deterioration of fruit quality in general; 3) necrosis either as necrotic spots on leaves or as generalized necrosis or wilt.

Plants can show similar symptoms by different viruses; for example, mosaic symptoms of potyviruses and cucumoviruses, or leaf yellowing by poleroviruses, criniviruses, or begomoviruses (Pitrat, 2012). Cucurbits can show yellowing symptoms on leaves by several viruses such as CABYV (Guilley et al., 1994), MABYV (Han et al., 2010; Xiang et al., 2008), CCYV (Okuda et al., 2013), CYSDV (Boubourakas et al., 2006), BPYV (Tomassoli et al., 2003), and MYSV (Kuwabara and Sakai, 2008). CABYV is a member of genus *Polerovirus* in the family Luteoviridae and limited to

phloem tissues in infected plants. Viral particles are icosahedral approximately 25 nm in diameter and the genome consists of a single-stranded positive-sense RNA molecule of 5.7 kb (Guilley et al., 1994). CABYV infects cucumber, melon, squash, and watermelon. Typical symptoms of CABYV include yellowing and thickening of lower and older leaves. There is a wide range of symptom intensities according to the cultivars, varying from a yellowing limited to a few older leaves to a complete discoloration of the plants (Lecoq et al., 1992). CABYV incidence on fields varies greatly according to the plant cultivation conditions and cultivars. Yield reduction can reach up to 50% of the marketable production in cucumber, but in melon, losses are more generally in the range of 10-15% (Lecoq, 1999). In contrast to mosaic inducing viruses, CABYV does not affect fruit quality, but rather induces flower abortions and reduces the number of fruits per plant (Lecoq et al., 1992). CABYV is transmitted in a persistent, circulative nonpropagative manner by a few aphid species (*A. gossypii*, *M. persicae*, and *M. euphorbiae*) (Reinbold et al., 2003). CABYV now infects cucurbits (almost all Cucurbitaceae) widely throughout the world with economic importance (Chen et al., 2011). MABYV is a newly identified *Polerovirus* from China. MABYV often co-exists in plants with another member of this genus, CABYV, but little is known about the pathogenicity, serological specificity, or vector transmission of MABYV (Xiang et al., 2008). Like other *Polerovirus* members, MABYV comprises a

plus sense-RNA genome encapsidated in icosahedral virions that encodes 6 ORFs. MABYV and CABYV share 51-74% sequence identity to other *Polerovirus* (Xiang et al., 2008). CCYV has been reported to chlorotic yellows disease that usually observed in Japanese melon cultivars in summer and transmitted by *Bemisia tabaci* biotype Q (Okuda et al., 2009, 2013; Sugiyama, 2013). If plants were infected with CCYV, leaves initially show slight mottling symptoms which develop into severe yellowing after 2 to 3 weeks. The virus infection causes a significant decrease in the sugar content of melons, which reduces their market value (Gyoutoku, 2009). BPYV has been reported worldwide causing yellowing diseases mainly in glasshouse and open field-grown cucurbits (Wisler et al., 1998). The main symptoms are chlorotic angular spots on leaves that evolve to interveinal yellowing, beginning on lower leaves and gradually progressing to the upper part of the plant. A few weeks later the affected plants exhibit a generalized interveinal leaf chlorosis with dark green veins. Plants with symptoms are less vigorous, and severe yield loses (40-60%) have been observed because of the reduction of photosynthetic area (Abou-Jawdah et al., 2000; Wisler et al., 1998). In addition to BPYV, CYSDV, also a member of the family Closteroviridae, is associated with yellowing of cucurbits. CYSDV was first described in the United Arab Emirates (Hassan and Duffus, 1991) and later in Spain (Celix et al., 1996), where it eventually displaced BPYV (Berdiales et al., 1999). CYSDV has a bipartite genome and is transmitted

semipersistently by the tobacco whiteflies *Bemisia tabaci* (biotypes A and B) and *B. argentifolii* (Perring et al., 1993), and its host range is restricted to the family Cucurbitaceae.

Control of cucurbit viruses and cultural practices for reducing outbreak

Plant viruses can be controlled by planting healthy seeds or seedlings in a clean environment, interfering with vectors activity, and using resistant cultivars. Resistance to viruses can be obtained by grafting, cross-protection, and conventional breeding or genetic engineering (Lecoq and Nikolaos, 2014). Resistances to CABYV (Dogimont et al., 1996), CYSDV (Lopez-Sese and Gomez-Guillamon, 2000), *Cucumber mosaic virus* (CMV) Kawaide, 1975; Risser et al., 1977), *Melon necrotic spot virus* (MNSV) (Coudriet et al., 1981), *Cucumber green mottle mosaic virus* (CGMMV) (Rajamony et al., 1987; Sugiyama et al., 2006) have been described in accessions from India, Far-East, or Africa. A resistance to colonization by the cotton-melon aphid *Aphis gossypii* and to the transmission of viruses has been found (Bohn et al., 1973; Kishaba et al., 1971; Lecoq et al., 1979; Soria et al., 2003).

In addition to these methods, diverse cultural practices may be taken to limit virus spread and overcome growth reduction by virus infection. The prevention methods to viral diseases were normally removal and burning of

infected plants, and eradication of weeds. However, the cultural practices to reduce outbreak derived from virus should be developed for accomplishing sustainlable agriculture. Recently, many researches have focused on the devlopment of methods for controlling strategy or managing viral disease. Plant devense activator, acibenzolar-S-methyl-ester (ASM), was of great value to induce systemic resistances against a wide range of plant pathogens, including several plant viruses (Anfoka, 2000; Ishii et al., 1999). ASM treatment on melon plants greatly increased the expression levels of pathogenesis-related 1a gene, a marker gene for systemic acquired resistance. CCYV suppressed systemic symptoms and decreased CCYV accumulation. ASM treatment on melon plants before inoculation of CCYV suppressed systemic symptoms and decreased CCYV accumulation (Takeshita et al., 2013). The control of CYSDV is currently based on chemical treatments against its vector and preventive cultural practices, both with limited success. Possible sources for natural resistance or tolerance to CYSDV were reported (Lopez-Sese and Gomez-Guillamon, 2000). The resistance mechanism may involve a restriction of the virus movement in the vascular system of the plants and/or prevention of high levels of virus accumulation (Marco et al., 2003). Brown et al. (1993) and Summers et al. (1995) found silver (aluminum) plastic mulch superior to white in repelling aphids in squash (*Cucurbita pepo* L.). Silver or gray reflective mulches have been used successfully to delay and reduce the incidence of aphid-borne

virus diseases in squash and other crops (Stapleton and Summers, 1997; Summers and Stapleton, 1998; Webb and Linda, 1992). The full-coverage reflective mulches tested with both polyethylene and spray mulch could provide significant benefits to culture of late-season melon production when aphids and mosaic viruses are present (Stapleton and Summers, 2002).

Fruit quality improvement and yield increase in muskmelon

Cell size of plug tray and seedling age affected root morphology, growth characteristics, and fruit quality in muskmelon (Cho et al., 1998; Lee et al., 2009a). Melon seedlings grown in a hot season are likely to have aged root system resulting in declination both in yield and fruit quality that foliar application of 24-epibrassinolide alleviated high-temperature-induced inhibition of photosynthesis in seedlings of two melon cultivars (Zhang et al., 2013). The dry mass, net photosynthetic rate, and stomatal conductance of melon plants were greatest when the roots were exposed at 24ºC. Root temperature played an important role in the regulation of stomatal behavior by increaging amount of abscisic acid at inappropriate soil temperature (Zhang et al., 2008). Treatment of a cytokinin-based vegetative growth inhibitor at 2 weeks before harvest increased total soluble solids of melon fruits. In addition, the fruit thining reduced yield, but influenced soluble sugar content of melon fruits (Long et al., 2004). The major soluble solids of melon fruit are sucrose, glucose, and fructose and their accumulation was regulated by invertase and

α-galactosidase (Kim et al., 2007). Bagging at 20 days before harvest improved fruit quality of muskmelon (Shin et al., 2011). Changing the light intensity, planting density, and leaf pruning also affects source strength. Changing the temperature and the number and position of earlier formed fruits influences sink strength (Marcelis et al., 2004). To increase resistance to soil-borne disease and wilt incidence and tolerance to low temperature and soil salinity, grafting is an important practice for the production of fruit bearing vegetables (Caruso et al., 1996; Lee, 1994; Nisini et al., 2002; Oda, 1995; Yano et al., 2002). Muskmelon seedlings grafted onto the same species, pumpkin (*Cucurbita* spp.) and white gourd (*Benincasa hispida*) rootstocks have recently been used (Traka-Mavrona et al., 2000). The grafted muskmelon improved the net photosynthetic rate, stomatal conductance, transpiration rate, and sugar translocation (Liu et al., 2011).

Transpiration and photosynthetic rates of several crops were reported to be reduced by fruit removal (Jauoudi and Widders, 1993; Marcelis, 1991). Fruit removal from melon plants infected with *Monosporascus cannonballus* reduced leaf stomatal conductance and increased root growth to survive water stress and poor translocation imposed by the pathogen (Pivonia et al., 2002).

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CHAPTER 1

DIAGNOSIS AND IDENTIFICATION OF LEAF YELLOWING SYMPTOMS DERIVED FROM YELLOWING ASSOCIATED VIRUS IN MUSKMELON

ABSTRACT

The study was conducted to determine the cause of leaf yellowing symptoms (LYS) in muskmelon cultivation regions of Korea during the summer in 2013 to 2014. Muskmelon leaves with yellowing symptoms were observed using an electron microscopy and were analyzed for detecting major cucurbit-infecting viruses (CMV, MNSV, CGMMV, SqMV, WMV, KGMMV, PRSV, and ZYMV) reported in Korea using reverse transcription polymerase chain reaction (RT-PCR) assay. Those viruses have not been detected earlier. Through the next generation sequencing, the virus was identified as *Cucurbit aphid-borne yellows virus* (CABYV), which is a member of the genus *Polerovirus* in the family Luteoviridae, and then confirmed by RT-PCR using CABYV specific primers. Complete genome sequences of 22 isolates of CABYV, collected from the muskmelon plants showing LYS in Korea during the years 2013-2014, were determined and

compared to the previously reported CABYV genome sequences. The complete genomes were found to be 5,680-5,684 nucleotides (nt) in length and to encode six open reading frames that are separated into two regions by a non-coding internal region of 199 nt. Their genomic organization is typical of the genus *Polerovirus*. Based on phylogenetic analyses of complete nt sequences, CABYV isolates were divided into Asian, Mediterranean, Taiwanese, and R groups. The Korean CABYV isolates clustered with the Asian group with > 94% nt sequence identity. In contrast, the Korean CABYV isolates shared 87-89% nt sequence identities with the Mediterranean group, 88% with the Taiwanese group, 81-84% with the CABYV-R group, and 72% with another *Polerovirus*, *Melon aphid-borne yellows virus* . Results confirm that LYS of muskmelon is not merely a physiological disorder but a virus disease caused by CABYV which spreads by aphids.

Keywords: *Cucurbit aphid-borne yellows virus*, leaf yellowing symptoms, phylogenetic analyses, physiological disorder

INTRODUCTION

Cucurbit viruses have been reported to be at least 59 species of the major plant virus groups (Fauquet et al., 2005; Lecoq, 2003). Among them, several viruses such as *Zucchini yellow mosaic virus* (ZYMV; Dukic et al., 2002; Papayiannis et al., 2005; Vucurovic et al., 2009), *Cucurbit aphid-borne yellows virus* (CABYV; Guilley et al., 1994), *Melon aphid-borne yellows virus* (MABYV; Han et al., 2010; Xiang et al., 2008b), *Cucurbit chlorotic yellow virus* (CCYV; Okuda et al., 2013), *Cucurbit yellow stunting disorder virus* (CYSDV; Boubourakas et al., 2006), *Beet pseudo-yellows virus* (BPYV; Tomassoli et al., 2003), and *Melon yellow spot virus* (MYSV; Kuwabara and Sakai, 2008) are associated with leaf yellowing symptoms (LYS) mainly in cucurbit crops.

Typical symptoms of these viruses in cucurbits are yellowing and thickening of older and mature leaves, and their intensity was significantly different upon cultivar and season (Lecoq et al., 1992). The cucurbit viruses reduce significant amount of fruit production but does not affect the fruit quality in cucurbit crops. Yellowing viruses are all insect-borne virus, particularly aphid-borne viruses (Alonso-Prados et al., 2003; Grafton-Cardwell et al., 1996; Stapleton and Summers 2002), whitefly-borne viruses (Esteva and Nuez, 1992; Rubio et al. 1999; Yamashita et al., 1979), and thrips-borne viruses (Murai, 2001). These insect-borne viruses are known to

be difficult to control because they spread rapidly throughout the crops and develop vectors being highly resistant to insecticides (Perring et al., 1999).

LYS usually occurred randomly in greenhouse during summer or early autumn and has been reported since 1980 worldwide. The presence of early foci of some plants with symptoms near plastic house openings (doors and side roll-up windows), their subsequent random distribution, the rapid spread, the ineffective addition of nutrients, and the high densities of the aphid populations is supporting the hypothesis of a phytovirus-associated disease (Wisler et al., 1998).

In Korea, incidence and spread of LYS in muskmelon, which is of great economic importance, has increased rapidly since LYS was observed in 2005. LYS are the major cause of low quality fruits in muskmelon for 2 or 3 years. The cause of the LYS in muskmelon of Korea is still conflicted. In this study, a survey was performed to identify the causal agent of the LYS in muskmelon. Major viruses infecting melon were investigated using an electron microscopy, reverse transcription-polymerase chain reaction (RT-PCR) and next generation sequencing (NGS) methods and, as the result, CABYV was detected from muskmelon showing LYS. For molecular characterization of CABYV, the complete genome sequences of 22 CABYV isolates, collected from muskmelon plants showing LYS in their cultivation areas during the years 2013-2014, were determined and analyzed comparatively along with previously reported CABYV genome sequences.

MATERIALS AND METHODS

Virus survey and sample collection

Leaf samples of muskmelon (*Cucumis melo*) plants showing LYS were collected for 2013-2014 from the 48 greenhouses distributed in major geographical areas of approximately 33,600 m^2 in seven different provinces: Gyeonggi-do (Suwon), Gangwon-do (Hoengseong), Chungcheongnam-do (Cheongyang), Gyeongsangbuk-do (Gumi), Gyeonsangnam-do (Hadong), Jeollabuk-do (Namwon), and Jeollanam-do (Gokseong) (Fig. 1-1, Table 1-1). In total, 308 samples were collected, put in plastic bags, and maintained at -70°C or lyophilized until RT-PCR analysis was performed. A sample consisted of three symptomatic leaves per plant. Muskmelon cultivars included 'Earl's Elite' (Syngenta Korea Co., Seoul, Korea), 'Earl's Elysee' (Syngenta Korea Co.), 'Earl's Friend' (Nongwoobio Co. Ltd., Suwon, Korea), 'Earl's Tipani' (Hannil Seed Co., Gongju, Korea), and 'Summer Ace' (Daeyeon Seed Co., Hadong, Korea).

Of 245 CABYV-positive samples, the following 22 CABYV samples were selected to determine their full-length genome sequences: five samples (SW1, SW2, SW1(14), SW25, and SW64) selected from Suwon in 2014, three samples (CY3, CY6, and CY4) from Cheongyang in 2013 and 2014, five samples (NW2, NW5, NW18, NW1, and NW2(14)) from Namwon in 2013 and 2014, three samples (GS1, GS2, and GS6) from Gokseong in 2013

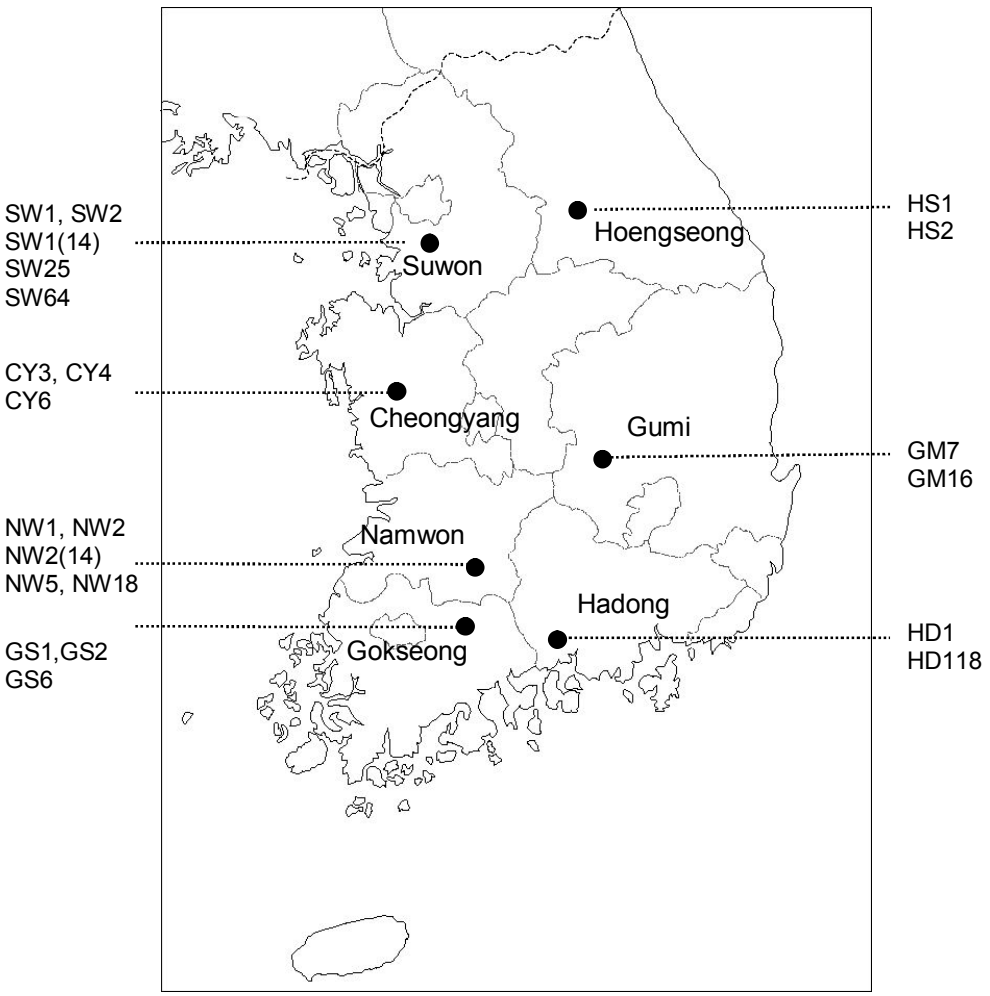


Fig. 1-1. Collection areas of CABYV isolates in Korea.

Table 1-1. Survey and isolation of CABYV from muskmelon samples in seven cultivation areas in Korea.

Area	Date	No. of plant			CABYV Isolate
		Survey	CABYV infection	Full sequencing	
Cheongyang	9/ 6/13	8	8	2	CY3, CY6
	7/2514	6	4	1	CY4
Gokseong	7/11/14	1	1	1	GS1
	8/14/14	8	7	2	GS2, GS6
Gumi	7/26/13	21	20	2	GM7, GM16
Hadong	9/18/14	146	123	2	HD1, HD118
Hoengseong	8/20/14	2	2	2	HS1, HS2
Namwon	7/26/13	8	8	2	NW2, NW5
	5/ 9/14	35	1	1	NW18
	8/14/14	2	2	2	NW1, NW2(14)
Suwon	6/ 9/14	2	2	2	SW1, SW2
	7/11/14	69	67	3	SW1(14), SW25, SW64
Total		308	245	22	

and 2014, two samples (GM7 and GM16) from Gumi in 2013, two isolates (HD1 and HD2) from Hadong in 2014, and two samples (HS1 and HS2) from Hoengseong in 2014 (Fig. 1-1, Table 1-1).

Electron microscopy

Virus particles were observed in leaf samples by using negatively stained leaf dips. The leaf dips prepared by grinding a small specimen (1 × 1 mm) of collected leaf tissues with 2-3 drops of 2% phosphotungstic acid. The extracts were mounted on a carbon-stabilized and Formvar coated grid (Kwak, 2013). Virus particles viewed under an electron microscope (LEO 912AB; Carl Zeiss, Oberkochen, Germany) at 80 kV.

NGS analyses

Leaf samples from muskmelon plants showing LYS were analyzed using NGS to detect unknown viruses in Korea. NGS and bioinformatics analysis were performed by C&K Company (Seoul, Korea). The total transcriptome of infected plants was extracted. In addition, the reduced rRNA, included in total transcriptome and construct library, was constructed using the Ribo-Zero kit (Epicentre Biotechnologies, Madison, WI, USA). Whole genome sequencing was performed on Illumina's HiSeq 2000 system (Illumina Inc., San Diego, CA, USA). About 16.8 Gbp data was obtained by paired-end sequencing with 101 bp read length. To analyze NGS data produced,

database for plant virus sequence were constructed from GenBank, National Center for Biotechnology Information (NCBI). After quality control (QC) of raw data, QC pass through reads were alignment by Bowtie 2 based plant virus sequence (Langmead and Salzberg, 2012). Alignment files were modification and coverage calculation by Samtools which implements various utilities for post-processing alignments in the sequence alignment/map (SAM) format and provides tool for processing read alignments (Li et al., 2009). The viruses with read mapping coverage > 90% and average mapping depth > 10 × of virus reference sequence were selected as candidates. For the reconstruction of virus sequence, relevant virus standard was extracted by reference genome re-sequencing analysis which record most alignment level (Carver et al., 2010; McKenna et al., 2010). By using extracted variation and reference virus genome sequence, the nucleotide (nt) sequence of plant virus was reconstructed.

Primer design

The primers for detection of major cucurbit viruses reported in Korea were obtained from plant virus laboratory of National Academy of Agricultural Science (NAAS), Korea (Table 1-2). Pairs of specific primers for the detection and full-length genome sequencing of CABYV were designed based on previously reported CABYV nt sequences and contig sequences determined by NGS (Lee et al., 2015) (Table 1-3). cDNA clones

Table 1-2. Cucurbit-infecting viruses reported in Korea.

Genus	Virus[z]	Primer name	Primer sequence (5'→3')	Product size (bp)	Virus particle
Carmovirus	MNSV	MNSVpu5	TGGAGGTAYATGAATGATAC	535	Isometric
		MNSV pd5	TAGGCGAGGTARGCRGTTTC		
Comovirus	SqMV	SqMV 1F	TGGAGAGTTTYCCCCACAAG	491	Isometric
		SqMV 1R	TTCTTCCAAGCAGCCACTTT		
Cucumovirus	CMV	CMV DP u1	CGTCGTGGTTCCCGCTCCG	473	Isometric
		CMV DP d2	AGCGCGCATCGCCGAAAGAT		
Potyvirus	PRSV	PRS-C10	AGACTCAGAGAACTCGAAT	610	Filamentous
		PRS-N60	CAATTTGAGAAGTGGTATGAG		
	WMV	WMV-UNI-1F	CAGTTTGAATCATGGTACAGCGC	392	Filamentous
		WMV-UNI-1R	TGTGCTATTGCTTCTCTTGCCC		
	ZYMV	ZYM-C10	AGGCTTGCAAACGGAGTCTAAT	510	Filamentous
		ZYM-N50	TATATAGAGATGAGAAATGCAGA		
Tobamovirus	CGMMV	CGMM-N30	ATGGAACGTACCGGAATC	609	Rod-shaped
		CGMM-C60	AATTAAGTAAAGTCCTGACG		
	KGMMV	KG-N60	AGTCGCGCATTGCTGCTTTGAT	403	Rod-shaped
		KG-C10	GAGAACTTACAGATAG		

[z]MNSV, *Melon necrotic spot virus*; SqMV, *Squash mosaic virus*; CMV, *Cucumber mosaic virus*; PRSV, *Papaya ringspot virus*; WMV, *Watermelon mosaic virus*; ZYMV, *Zucchini yellow mosaic virus*; CGMMV, *Cucumber green mottle mosaic virus*; KGMMV, *Kyuri green mottle mosaic virus*.

Table 1-3. Primer pairs for the detection and full-length sequencing of CABYV genomes.

Primer	Sequence (5'→3')[z]	Loci[y]	Size (nt)
Primers for detection			
CABYV-u4	ACACGAGTTGCAAGCATTGGAAGT	3341-3364	466
CABYV-d3806	AGTATTCCAGAGCTGAATGCTGGG	3806-3782	
Primers for full-length sequencing			
CABYV-1F-1	ACTATGTTTATACCCCTGGAGCCAG	214-238	736
CABYV-1R-1	AGTGGGATCTTGTTTCCATTCCTGG	950-926	
CABYV-2F	ATATGGTGAAGATGGCGGCTTGG	620-642	1051
CABYV-2R	GAAGCAYTGGTGGTGGGGGAT	1670-1650	
CABYV-3F	ACCACGGCACCCCAAGGACG	1330-1349	1025
CABYV-3R	CCGGTTGAAGGTGAGRCGAGC	2354-2334	
CABYV-4F	GCCCAGTCAGTTAAAATCCCCTC	2076-2098	1052
CABYV-4R	ACCGGAATGGCGAGGTCCTC	3127-3108	
CABYV-5F	GTCCCAGGCGTGCAGAAGAG	2892-2911	1031
CABYV-5R	AGCTAAGCTTGCAGTGGGGGTC	3922-3901	
CABYV-6F-1	GGAAGGAGCCCAGGCGAAAC	3679-3698	984
CABYV-6R-1	ATTCGAAGGAAGCGTACCAATCGAC	4663-4639	
CABYV-7F	ACGATGTTTCCCARAGAGGTTGGAA	4496-4520	1022
CABYV-7R	TTAYGAGGTTTTRTCAGCTAGCACC	5517-5493	
CABYV-5'RACE-R	GCGAGGAAAAATCGCGCAAC	352-333	
CABYV-3'RACE-F	ATGGATARYAGGAAGAAATGGGGA	5314-5337	

[z]Primers were designed based on the nucleotide sequences of CABYV isolates registered in GenBank and contig sequences determined by NGS.

[y]Reference sequence, accession No. GQ221224

containing the 5' end of the genomes were produced using a sense primer (5'-ACAAAAGATACGAGCGGGTGATGC-3') complementary to the conserved 24 nt at the 5' terminus and an antisense primer (5'-GCGAGGAAAAATCGCGCAAC-3') complementary to nt 352-333 in the CABYV genome. In addition, cDNA clones containing the 3' end of the genomes were produced using a sense primer (5'-ATGGATARYAG GAAGAAATGGGGA-3') complementary to nt 5314-5337 and an antisense primer (5'-ACACCGAAACGCCAGGGGG-3') complementary to the conserved 19 nt at the 3' terminus of the CABYV genome.

RT-PCR, cloning, and sequencing

Total RNA were extracted from infected leaf samples by easy-spin™ total RNA extraction kit (Intron Biotechnology, Seongnam, Korea) according to the manufacturer's instructions. RT-PCR was carried out as either one-step RT-PCR (Genet Bio, Nonsan, Korea) or two-step RT-PCR including RT using AMV reverse transcriptase (Promega Corp., Madison, WI, USA) and PCR using LA Taq polymerase (Takara Bio Inc., Otsu, Japan).

One step RT-PCR for CABYV detection was carried out in a total volume of 20 mL containing 0.5 mg of total RNA, 0.5 mL of forward and reverse primers (10 pmol), 10 mL of 2× RT-PCR premix (SR-8000; Genet Bio), and made up to volume with distilled H_2O. RT-PCR condition consisted of RT at for 30 min at 50°C and for 10 min at 95°C and PCR of 35

cycles of 30 s at 95°C, 30 s at 55°C, 1 min at 72°C and adds five min at 72°C at the end of extension.

In case of two-step RT-PCR for full-length genome sequence, RT reaction was carried out at 42°C for 30 min in a final 5 μL volume obtained by adding 1 μL total RNA (2.5 ng/μL), 10 pmole (1 μL) of the downstream primer, 1× RT buffer, 2 mM dNTP, and 0.5 U AMV reverse transcriptase (Promega) and made up to volume with distilled H_2O, and was terminated by heating at 95°C for 5 min. When RT was completed, total 25 μL of 10 pmole (1 μL) of the forward primer, 1 U LA-Taq DNA polymerase (Takara Bio), 1× PCR buffer and 1.5 mM $MgCl_2$, were added. Mixtures were then amplified in a thermal cycler (Bio-Rad, Hercules, CA, USA) for a total 35 cycles. Each cycle included a denaturing step at 94°C for 30 s, an annealing step at 55°C for 30 s, and extension step at 72°C for 90 s, and finally kept at 72°C for 10 min. PCR products were separated on 1% agarose gels in electrophoresis for 60 min and visualized following ethidium bromide staining under ultra violet lights.

RT-PCR amplified DNA fragments were purified using a MEGA Quick-spin™ Kit (Intron) and cloned into the pGEM-T easy vector (Promega) according to the manufacturer's instructions, followed by transformation into *Escherichia coli* DH5α. Adjacent regions of these PCR fragments were overlapped by about 200 bp to ensure that they were from the same genome.

At least three clones of each fragment were completely sequenced. To verify sequence discords among the clones and identify the dominant sequence in the viral population, direct sequencing of RT-PCR products of entire genomes was performed. Sequencing was performed by a commercial company (Genotech Co. Ltd., Daejeon, Korea). The resultant sequences were assembled using DNA Star Ver. 5.02 and submitted to GenBank database under the accession numbers listed in Table 1-4.

Sequence and phylogenetic analyses

The complete nt sequences and the deduced amino acid sequences were aligned using the Geneious and ClustalW methods in Geneious Pro 8 and compared with those of previously reported isolates; i.e., the JAN (Japan), CHN, FJ, Xinjiang, and CZ (China; Xiang et al., 2008b), R-TW82 and C-TW20 (Taiwan; Knierim et al., 2013b), N (France; Lecoq et al., 1992), Sq/2003/7.2, Sq/2004/1.9, and Sq/2005/9.2 (Spain; Kassem et al., 2013) isolates. The MABYV isolates, CHN and TW1, were used as outgroups (Table 1-6). Geneious Pro 8 software was used to calculate the percentage nucleotide and amino acid identities. Phylogenetic analyses were performed using maximum likelihood methods in MEGA 6 (Tamura et al., 2013). Maximum likelihood phylogenetic trees were constructed using best fit nucleotide substitution models (GTR+G+I for full-length genome, K2+G for 3' UTR and IR region) and best fit amino acid substitution models

Table 1-4. Database of the complete nucleotide sequence of CABYV and MABYV genomes.

Virus[z]	Host plant	Isolate[y]	Origin	Genome (nt)	Accession No.	Year collected
CABYV	Bitter melon	C-TW20	Kaohsiung, Taiwan	5670	JQ700305	2008
	Cantaloupe	Xinjiang	Xinjiang, China	5682	EU636992	-
	Cucumber	JAN	Okayama, Japan	5682	GQ221224	-
	Cushaw	CHN	Beijing, China	5682	EU000535	2006
	Melon	N	Nerac, France	5669	X76931	1989
		CY3	Cheongyang, Korea	5683		2013
		CY6	Cheongyang, Korea	5682		2013
		CY4	Cheongyang, Korea	5684		2014
		GS1	Gokseong, Korea	5682		2014
		GS2	Gokseong, Korea	5683		2014
		GS6	Gokseong, Korea	5682		2014
		GM7	Gumi, Korea	5683		2013
		GM16	Gumi, Korea	5681		2013
		HD1	Hadong, Korea	5680		2014
		HD118	Hadong, Korea	5683		2014
		HS1	Hoengseong, Korea	5682		2014
		HS2	Hoengseong, Korea	5682		2014
		NW2	Namwon, Korea	5682		2013
		NW5	Namwon, Korea	5683		2013
		NW18	Namwon, Korea	5683		2014
		NW1	Namwon, Korea	5683		2014
		NW2(14)	Namwon, Korea	5683		2014
		SW1	Suwon, Korea	5683		2014
		SW2	Suwon, Korea	5683		2014
		SW1(14)	Suwon, Korea	5682		2014
		SW25	Suwon, Korea	5682		2014
		SW64	Suwon, Korea	5681		2014
	Spong gourd	R-TW82	Tainan, Taiwan	5679	JQ700306	2009
	Squash	FJ	Fuziang, China	5682	GQ221223	-
		Sq/2003/7.2	Murcia, Spain	5672	JF939812	2003
		Sq/2004/1.9	Murcia, Spain	5672	JF939814	2004
		Sq/2005/9.2	Murcia, Spain	5675	JF939813	2005
	Zucchini	CZ	Beijing, China	5691	HQ439023	-
MABYV	Winter melon	CHN	Beijing, China	5674	EU000534	2006
	Watermelon	TW1	Yunlin, Taiwan	5676	JQ700307	2000

[z]CABYV, *Cucurbit aphid-borne yellows virus*; MABYV, *Melon aphid-borne yellows virus*.
[y]Isolates analyzed in this study are shown in bold.

(JTT+G for P0, P1, P1-P2, P3, P4, and P3-P5). Bootstrap values were calculated for 1,000 random replications.

Virus transmission and host range

To confirm virus transmission by aphid, CABYV was inoculated using the mass inoculation method described by Lecoq et al. (1992). Infected 7 weeks old muskmelon seedlings were planted in a 50 cm diameter plastic pot (approximately 7 L) as a virus source were placed in 30 mesh screen (The mesh size of the screen is the number of threads per linear inch) 54 days after transplanting (July 28, 2014). Between 30 and 50 non viruliferous larvae or young apterous adults (*Aphis gossypii* and *Myzus persicae*) were allowed to feed on leaves of the infected muskmelon plants. The inoculated plants were grown in a plant factory at controlling air temperature 25-28ºC with 14-h photoperiod (10-h dark period). After 5 days, ten healthy muskmelon seedlings at the second true leaf stage were transferred to infected muskmelon plants covered with a nylon bag for 7 days for viruliferous aphids transmission of CABYV to healthy seedlings. And then, each plant was tested individually for the presence of CABYV using RT-PCR.

To confirm mechanical transmission of CABYV, inoculation method was used followed by Sugiyama and Sakata (2004) and Sugiyama et al. (2009). Muskmelon seeds 'Earl's Talent' (Nongwoobio Co. Ltd., Suwon, Korea) were sown in 12 cm diameter plastic pots (0.8 L) and grown in a glasshouse

at National Institute of Horticultural & Herbal Science, Suwon, Korea. When the cotyledons were fully expanded 12 days after sowing, carborundum-dusted cotyledons of CABYV were rubbed with cotton pieces soaked in the inoculum. The inoculum had been prepared by grinding infected leaves of muskmelon in 1:10 (w/v) of 100 mM phosphate butter (pH 7.0) containing 0.1% (v/v) 2-mercaptoehanol. The inoculated plants were grown in a glasshouse with setting air temperature 25 to 30°C with 12-h photoperiod. Ten plants were inoculated and virus detection was performed by RT-PCR 16 days after inoculation.

To identify soil contamination of CABYV, seeds of 'Earl's Talent' were sown in 12 cm diameter plastic pots in July 31, 2014 and grown in a glasshouse. When the true leaves were fully expanded 12 days after sowing, inoculum prepared by grinding infected leaves of muskmelon was mixed with soil in the muskmelon seedling pot. The inoculated plants were grown in a glasshouse at 25-30°C with 12-h photoperiod. Ten plants were inoculated in September 12, 2014. Virus detection was performed by RT-PCR 16 days after inoculation.

In an experiment to study host range of CABYV infection, seedlings of major cucurbit cultivars in Korea; *Cucurbita moschata* cv. 'Jinhan Aehobak' (Nongwoobio Co. Ltd.) and 'Nongwoo Aehobak' (Nongwoobio Co. Ltd.), *Citrullus vulgaris* cv. 'Sambokkkul' (Dongbu Hannong Seeds Co. Ltd., Seoul, Korea), 'Speedkkul' (Nongwoobio Co. Ltd.) and 'Urikkul'

(Nongwoobio Co. Ltd.), *Cucurmis melo* L. var. *makuwa* cv. 'Joeundae' (Syngenta Korea Co. Ltd., Seoul, Korea), 'Obokkkul' (Nongwoobio Co. Ltd.), 'Obokpluskkul' (Nongwoobio Co. Ltd.), *Cucumis sativas* L. cv. 'JoeunBackdadaki' (Heungnong Seed Co. Ltd., Pyeongtaek, Korea), 'Nebakja' (Syngenta Korea Co. Ltd.), and 'Wellbingmatjjangoi' (Asia Seed Co. Ltd., Seoul, Korea) and *Cucumis melo* L. cv. 'Homerun star' (Syngenta Korea Co. Ltd.) and 'Yellow sun' (Nongwoobio Co. Ltd.) were cultivated in plastic pots in an insect-proof glasshouse until the second leaf stage. Each differential hosts were inoculated in at least two independent experiments with 20-30 viruliferous aphids on muskmelon plants infected with CABYV. Each plant was tested individually for the presence of CABYV using RT-PCR.

RESULTS AND DISCUSSION

Symptomatology

In the plants showing LYS, light green spots appeared on the surface of lower leaves (sometimes upper leaves) in the first stage and then eventually became yellow (Fig. 1-2A, B, C). Yellow spots were enlarged until a whole leaf was changed to yellow color and, finally, the yellowing symptom spread to upper leaves (Fig. 1-2C). This is consistent with Lecoq (1999) findings that chlorotic patches eventually were coalesced on the older leaves, the whole leaf taking on a light to bright yellow color after inoculation with CABYV. Although Lecoq (1999) found that LYS developed from older to younger leaves, it was found that the symptom appeared on the surface of lower leaves and sometimes upper leaves (Fig. 1-2). Sugiyama et al. (2009) found that *Melon yellow spot virus* (MYSV), a devastating thrips-transmitted virus of cucurbits in Japan, induces chlorotic spots, mosaic mottling, yellowing on leaves, and mosaic patches on fruits of melon, and sometimes causes mottling on fruits of cucumber. However, mosaic patches on the muskmelon fruit were not observed in this study.

NGS and virus detection

Thirty seven muskmelon plants showing LYS were taken in greenhouses during summer and autumn in Cheongyang, Gumi, and Namwon, and

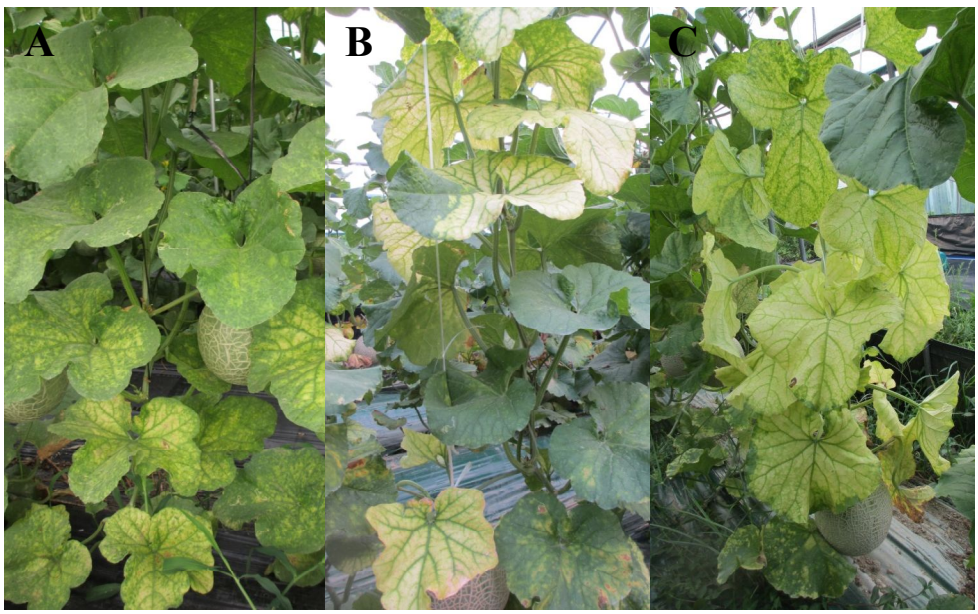


Fig. 1-2. Leaf yellowing symptoms in muskmelon plants at the end of growing season. A, yellowing of the basal leaves; B, yellowing symptoms from mid to top leaves; C, whole leaves showing on yellowing symptoms.

analyzed for major cucurbit infecting viruses by RT-PCR using specific primers and electron microscopy (Table 1-2). Almost 75% of muskmelon plants collected from Namwon were infected by only MNSV, while all the symptomatic muskmelon plants from Cheongyang and Gumi were negative for the eight viruses considered (Table 1-5). The NGS analysis was used to find out whether the cause of LYS is unknown virus in Korea. The viruses more than 90% of complete genome coverage and more than average 10 mapping depth of virus reference sequence were selected as candidates (Table 1-6 and Fig. 1-3). Assembled contig sequences were analyzed against the plant viral reference genome database. The complete contig sequence had about 5.682 bp with 97% nt identity with isolate JAN (GenBank Accession No. GQ221224) of CABYV, which was isolated from cucumber in Japan.

The NGS result was confirmed by RT-PCR using specific primers for CABYV genome. These tests revealed that 95% of muskmelon showing LYS collected from Gumi was infected by CABYV (Table 1-1). In addition, all the muskmelon samples with LYS collected from Namwon and Cheongyang were confirmed to have CABYV (Table 1-1).

From seven muskmelon cultivation areas of Korea, including the above three areas, a total 308 leaf specimens of muskmelon showing yellowing and mosaic symptoms were collected during the years 2013-2014 and analyzed CABYV using RT-PCR. Among them, 245 samples (80%) were positive for CABYV (Table 1-1).

Table 1-5. Incidence of most common cucurbit viruses detected by RT-PCR in muskmelon plants showing leaf yellowing symptoms collected from plastic houses in July 26 to August 10, 2013.

Virus	Incidence [z]		
	Cheongyang	Gumi	Namwon
CGMMV	0/8	0/21	0/8
CMV	0/8	0/21	0/8
KGMMV	0/8	0/21	0/8
MNSV	0/8	0/21	6/8
PRSV	0/8	0/21	0/8
SqMV	0/8	0/21	0/8
WMV	0/8	0/21	0/8
ZYMV	0/8	0/21	0/8

[z]Number of infected plants / number of sampling plants.

Table 1-6. List of candidate viruses selected using next generation sequencing (NGS) from muskmelon plants in Gumi.

ID	Description
gi|113460144|ref|NC_008310.1|	Hibiscus latent Singapore virus, complete genome
gi|157420211|gb|EU000535.1|	Cucurbit aphid-borne yellows virus, complete genome
gi|194320363|gb|EU636992.1|	Cucurbit aphid-borne yellows virus from China: Xinjiang complete genome
gi|20260786|ref|NC_003688.1|	Cucurbit aphid-borne yellows virus, complete genome
gi|226426425|gb|FJ751927.1|	Citrus exocortis Yucatan viroid isolate 9, complete genome
gi|226426428|gb|FJ751930.1|	Citrus exocortis Yucatan viroid isolate 12, complete genome
gi|226426431|gb|FJ751933.1|	Citrus exocortis Yucatan viroid isolate 15, complete genome
gi|293612867|gb|GQ221224.1|	Cucurbit aphid-borne yellows virus isolate CABYV-JA complete genome
gi|295675094|gb|GQ221223.1|	Cucurbit aphid-borne yellows virus isolate CABYV-FJ complete genome
gi|336444806|gb|HQ439023.1|	Cucurbit aphid-borne yellows virus strain CABYV-CZ complete genome
gi|388325674|gb|JF939812.1|	Cucurbit aphid-borne yellows virus strain Sq/2003/complete genome
gi|388325686|gb|JF939813.1|	Cucurbit aphid-borne yellows virus strain Sq/2005/complete genome
gi|388325696|gb|JF939814.1|	Cucurbit aphid-borne yellows virus strain Sq/2004/complete genome
gi|392328722|gb|JQ700305.1|	Cucurbit aphid-borne yellows virus isolate CABYV-C-complete genome
gi|392328729|gb|JQ700306.1|	Cucurbit aphid-borne yellows virus isolate CABYV-R-complete genome
gi|392718241|gb|JN606110.1|	Cacao swollen shoot virus isolate CI152-09complete genome
gi|41353207|gb|AY513268.1|	Citrus exocortis viroid, complete genome
gi|440496637|gb|JX997952.1|	Garlic virus A isolate WA7, complete genome
gi|540270988|gb|KC427105.1|	Citrus exocortis Yucatan viroid isolate CEYVd-WA complete genome
gi|564886468|gb|AF395898.3|	Hibiscus latent Singapore virus, complete genome
gi|58119512|gb|AY789137.1|	Dulcamar mottle virus, complete genome
gi|75905689|gb|AY147260.2|	Sclerotisclerotidebilit aRNA virus, complete genome
gi|79676039|emb|AM109896.1|	Pepino mosaic virus complete genome, isolate SM.74
gi|86651786|gb|DQ318792.1|	Citrus exocortis viroid clone 2/5, complete genome
gi|86651787|gb|DQ318793.1|	Citrus exocortis viroid clone 5/5, complete genome
gi|9629160|ref|NC_001747.1|	Potato leaf roll virus, complete genome

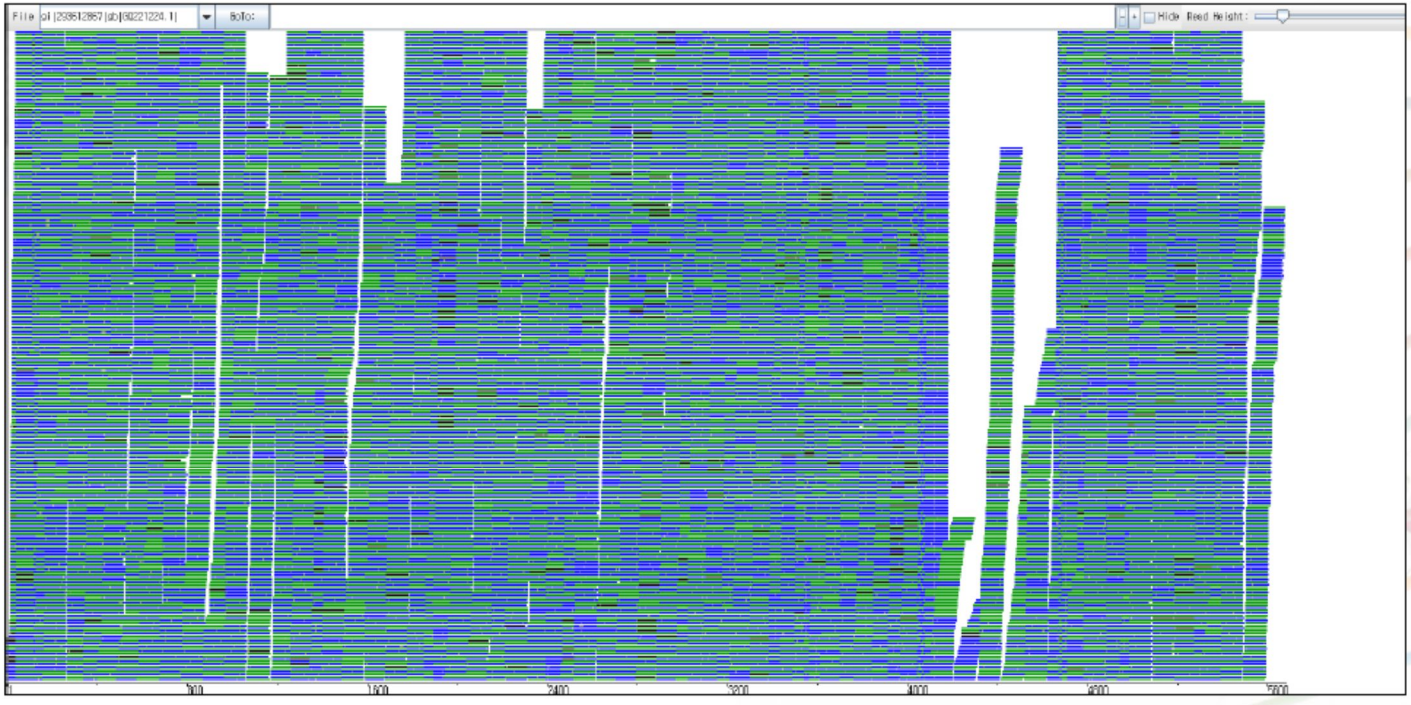


Fig. 1-3. Read stack visualization of alignment result in candidate virus complete genome.

The fact that cause of LYS in muskmelon is CABYV for several years have been overlooked, because its symptoms were attributed to nutrient deficiencies, ageing or infection by other pathogens. The difficulty in detecting or visualizing the virus by mechanical inoculation or by electron microscopy of leaf dips might be another reason for the failure to identify CABYV earlier. This might be the first report of muskmelon plants with LYS infected by CABYV in Korea.

CABYV has been ecologically adapted to a temperate environment (Lecoq, 1999). CABYV has been found in temperate, Mediterranean and subtropical climatic countries, including Italy, Lebanon, Spain, Iran, Turkey, Tunisia, Slovakia, USA, Nepal, and China, since its first observation in France (Abou-Jawdah et al., 1997; Bananej et al., 2009; Dahal et al., 1997; Juarez et al., 2004; Lecoq, 1999; Lemaire et al., 1993; Mnari et al., 2005; Omar and Bagdady, 2012; Tomassoli and Meneghini, 2007; Xiang et al., 2008a). Therefore, recently the high incidence of CABYV in Korea is associated with high temperature in summer and autumn. In addition, the frequent occurrence of the virus is probably favored by the large populations of the muskmelon aphid observed in recent years, in conjunction with the development of insecticide-resistant biotypes.

This virus may well become a major threat for cucurbit crops in Korea. Use of plastic covers for the controlling aphids during the early stages of plant development results in a delay of a few weeks in the establishment of

infections but does not confer efficient protection. An important option for disease control would be the use of virus-resistant cultivars.

Genome characterization of Korean CABYV isolates

Out of CABYV positive samples, 22 CABYV isolates were selected from seven cultivation locations, and their full-length genome sequences were determined in this study (Table 1-4). The representative symptoms include yellowing, chlorotic local, and mosaic on the infected leaves, and irregular net formation on fruits (Fig. 1-4). The complete genomes of CABYV Korean isolates were determined to be from 5,680 to 5,684 nt. They encode six open reading frames (ORFs) which are separated into two regions by a non-coding internal region (IR) of 199 nt. Their genomic organizations are typical for a member of the genus *Polerovirus* (Fig. 1-5). The 5' and 3' non-coding region are 20 nt and 164-167 nt in length, respectively. The 5'-proximal ORFs (ORF 0, 1, 2) encode P0, P1, and the ribosomal frameshift protein P1-P2 with the size of 239, 631, and 1,056 aa, respectively. The 3'-proximal ORFs (ORF 3, 4, 5) encode P3 (CP), P4 (MP), and read-through protein P3-P5 with the size of 199, 191, and 667-668 aa, respectively. The deduced amino acid sizes of five proteins, the exception being the P3-P5 protein, were identical to those of the Asian CABYV group, which includes Japanese and Chinese isolates. Most of Korean CABYV isolates had a P3-P5 protein of 668 aa, while some isolates comprised 667 aa due to the lack of one proline in the 5' terminal region of P5.

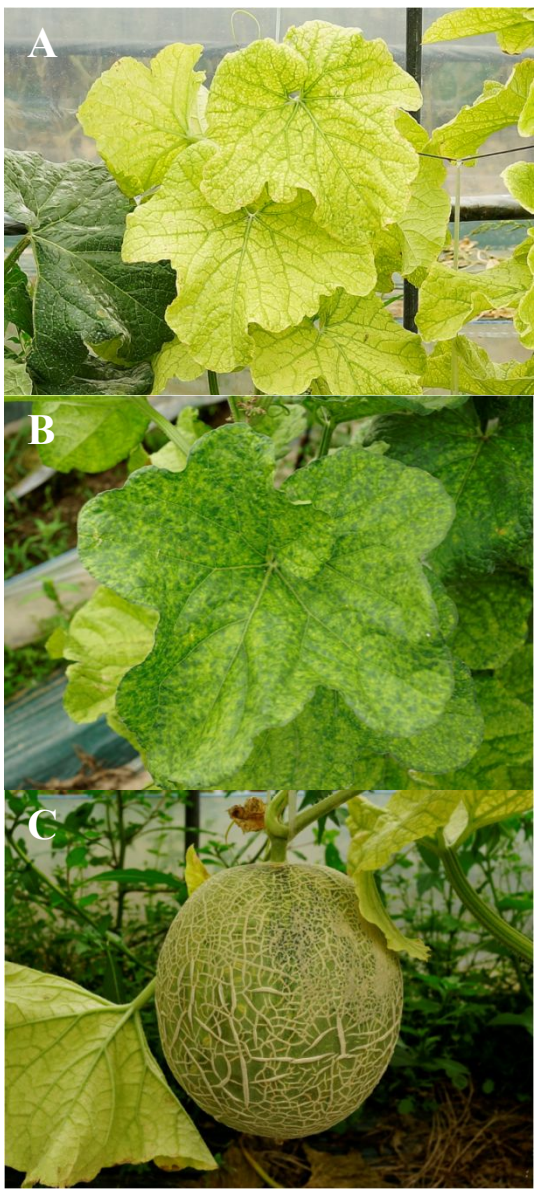


Fig. 1-4. Symptoms on CABYV-infected muskmelon plants in Korea. A, yellowing; B, mosaic on leaves; C, abnormal net on fruits.

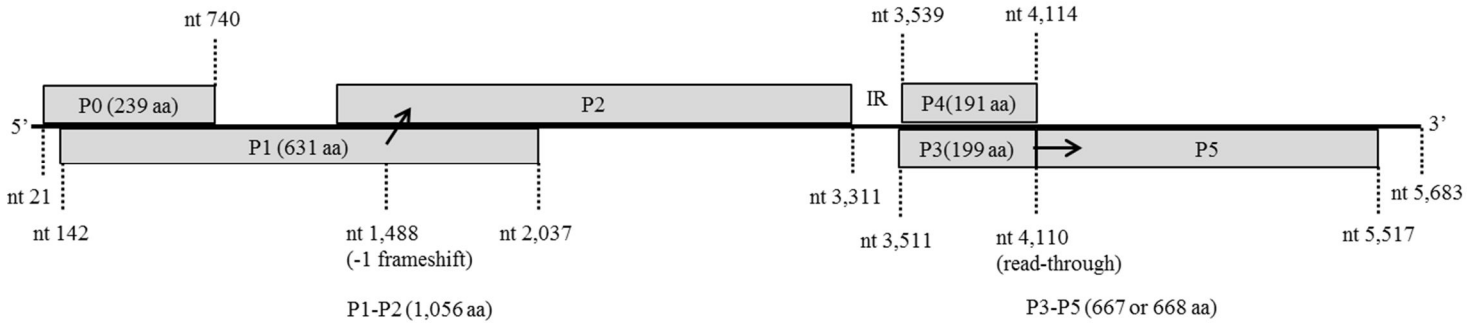


Fig. 1-5. Genome organization of Korean CABYV isolates. The six proteins are separated by IR into 5' and 3' proximal proteins. A ribosomal frame shift (-1) in the P1-P2 proteins is indicated at nt 1,488 and read-through of the P3-P5 proteins occurs at nt 4,110.

Analysis of phylogenetic relationships

The complete nt and deduced amino acid sequences of 22 Korean CABYV isolates were compared to those of 11 previously reported CABYV isolates. Two MABYV isolates were included as outgroup isolates in the phylogenetic analyses (Table 1-6). Full-length genome sequence based phylogenetic analyses revealed that the Korean CABYV isolates clustered with the Asian group, including Japanese and Chinese isolates (Fig. 1-6).

The reconstructed phylogenetic trees based on amino acid sequences of the six proteins (P0, P1, P1-P2, P3, P4, and P3-P5) and the nt sequences of two non-coding regions (IR and 3' UTR) showed that the Korean CABYV isolates clustered with the Asian group, similar to the tree based on nt sequences (Figs. 1-7). However, in the case of the 3' proximal proteins and 3' UTR, the Korean CABYV isolates were differentiated into two subgroups within the Asian group. In addition, the Chinese isolate CZ and Taiwanese isolate R-TW82, which belong to the CABYV-R group, grouped with MABYV based on full-length genome sequences and amino acids of 5' proximal ORFs, but grouped with CABYV based on 3' proximal ORFs. According to previous reports, CABYV isolates are divided geographically into Asian and Mediterranean groups (Shang et al., 2009). However, our phylogenetic results suggest that CABYV isolates are divided into Asian, Mediterranean, Taiwanese, and R groups. Phylogenetic trees reconstructed using the amino acid sequences of six individual proteins and nt sequences

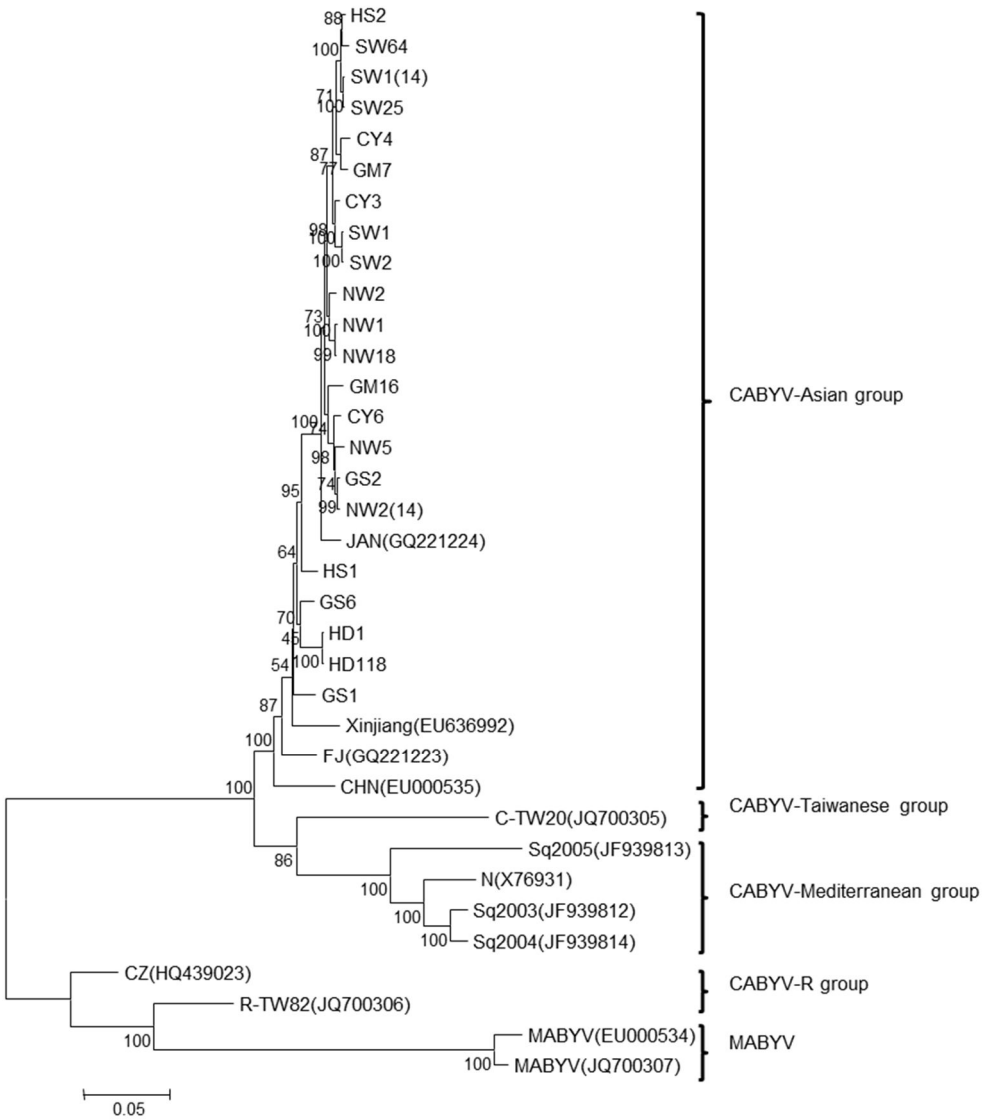
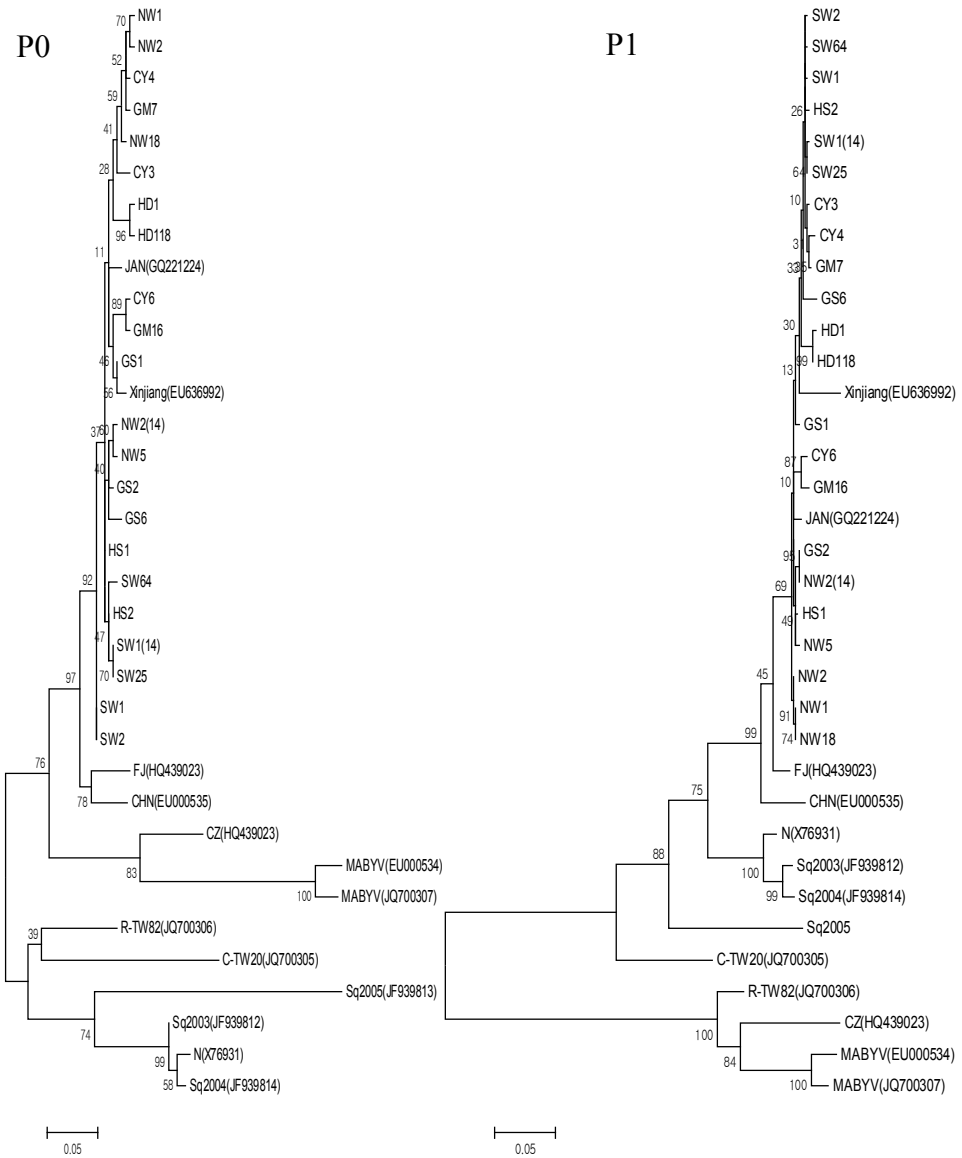
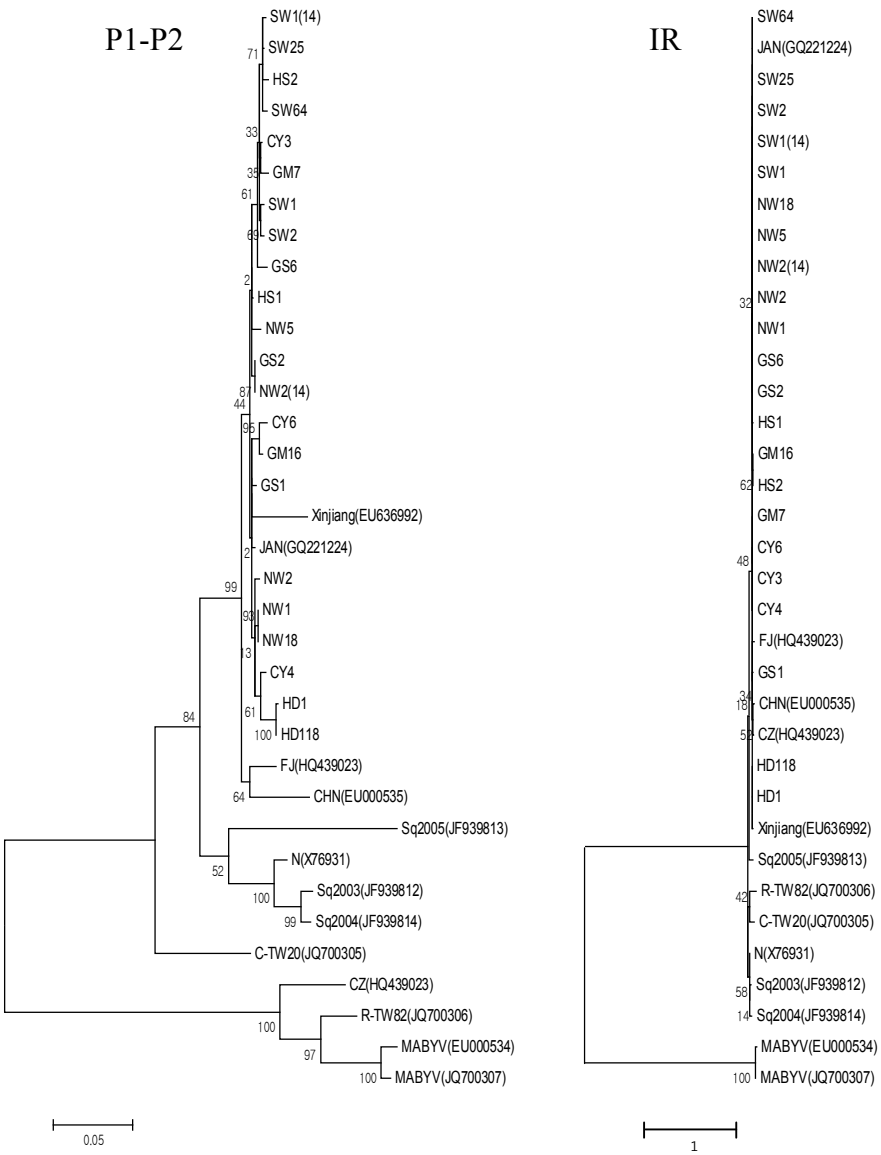


Fig. 1-6. Phylogenetic trees reconstructed using the complete nucleotide sequences of CABYV isolates. The maximum likelihood method implemented in MEGA 6 was used to reconstruct the phylogenetic trees.


P0
NW1
NW2
CY4
GM7
NW18
CY3
HD1
HD118
JAN(GQ221224)
CY6
GM16
GS1
Xinjiang(EU636992)
NW2(14)
NW5
GS2
GS6
HS1
SW64
HS2
SW1(14)
SW25
SW1
SW2
FJ(HQ439023)
CHN(EU000535)
CZ(HQ439023)
MABYV(EU000534)
MABYV(JQ700307)
R-TW82(JQ700306)
C-TW20(JQ700305)
Sq2005(JF939813)
Sq2003(JF939812)
N(X76931)
Sq2004(JF939814)
0.05
P1
SW2
SW64
SW1
HS2
SW1(14)
SW25
CY3
CY4
GM7
GS6
HD1
HD118
Xinjiang(EU636992)
GS1
CY6
GM16
JAN(GQ221224)
GS2
NW2(14)
HS1
NW5
NW2
NW1
NW18
FJ(HQ439023)
CHN(EU000535)
N(X76931)
Sq2003(JF939812)
Sq2004(JF939814)
Sq2005
C-TW20(JQ700305)
R-TW82(JQ700306)
CZ(HQ439023)
MABYV(EU000534)
MABYV(JQ700307)
0.05


P1-P2
SW1(14)
SW25
HS2
SW64
CY3
GM7
SW1
SW2
GS6
HS1
NW5
GS2
NW2(14)
CY6
GM16
GS1
Xinjiang(EU636992)
JAN(GQ221224)
NW2
NW1
NW18
CY4
HD1
HD118
FJ(HQ439023)
CHN(EU000535)
Sq2005(JF939813)
N(X76931)
Sq2003(JF939812)
Sq2004(JF939814)
C-TW20(JQ700305)
CZ(HQ439023)
R-TW82(JQ700306)
MABYV(EU000534)
MABYV(JQ700307)
0.05
IR
SW64
JAN(GQ221224)
SW25
SW2
SW1(14)
SW1
NW18
NW5
NW2(14)
NW2
NW1
GS6
GS2
HS1
GM16
HS2
GM7
CY6
CY3
CY4
FJ(HQ439023)
GS1
CHN(EU000535)
CZ(HQ439023)
HD118
HD1
Xinjiang(EU636992)
Sq2005(JF939813)
R-TW82(JQ700306)
C-TW20(JQ700305)
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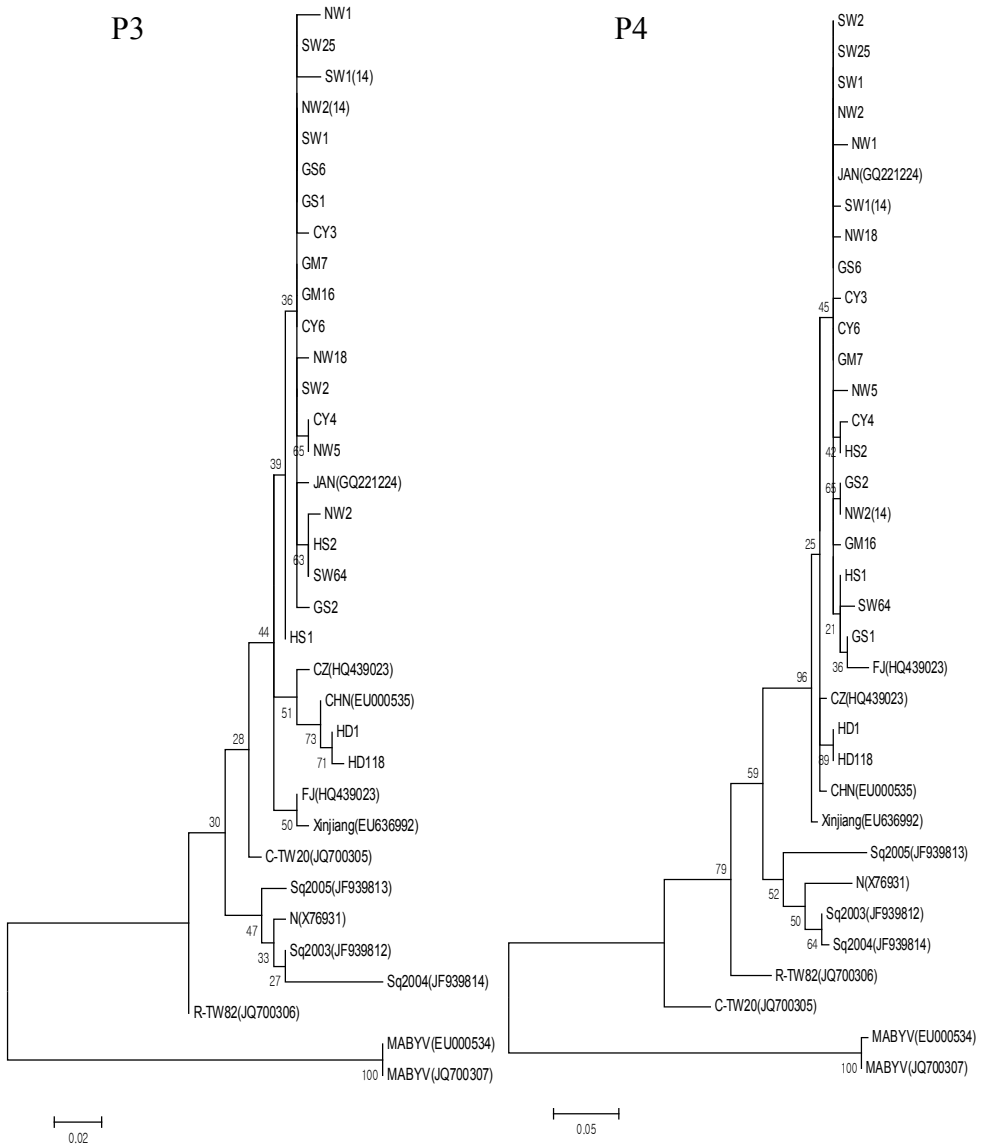
MABYV(EU000534)
MABYV(JQ700307)
1


P3
NW1
SW25
SW1(14)
NW2(14)
SW1
GS6
GS1
CY3
GM7
GM16
CY6
NW18
SW2
CY4
NW5
JAN(GQ221224)
NW2
HS2
SW64
GS2
HS1
CZ(HQ439023)
CHN(EU000535)
HD1
HD118
FJ(HQ439023)
Xinjiang(EU636992)
C-TW20(JQ700305)
Sq2005(JF939813)
N(X76931)
Sq2003(JF939812)
Sq2004(JF939814)
R-TW82(JQ700306)
MABYV(EU000534)
MABYV(JQ700307)
0.02
P4
SW2
SW25
SW1
NW2
NW1
JAN(GQ221224)
SW1(14)
NW18
GS6
CY3
CY6
GM7
NW5
CY4
HS2
GS2
NW2(14)
GM16
HS1
SW64
GS1
FJ(HQ439023)
CZ(HQ439023)
HD1
HD118
CHN(EU000535)
Xinjiang(EU636992)
Sq2005(JF939813)
N(X76931)
Sq2003(JF939812)
Sq2004(JF939814)
R-TW82(JQ700306)
C-TW20(JQ700305)
MABYV(EU000534)
MABYV(JQ700307)
0.05

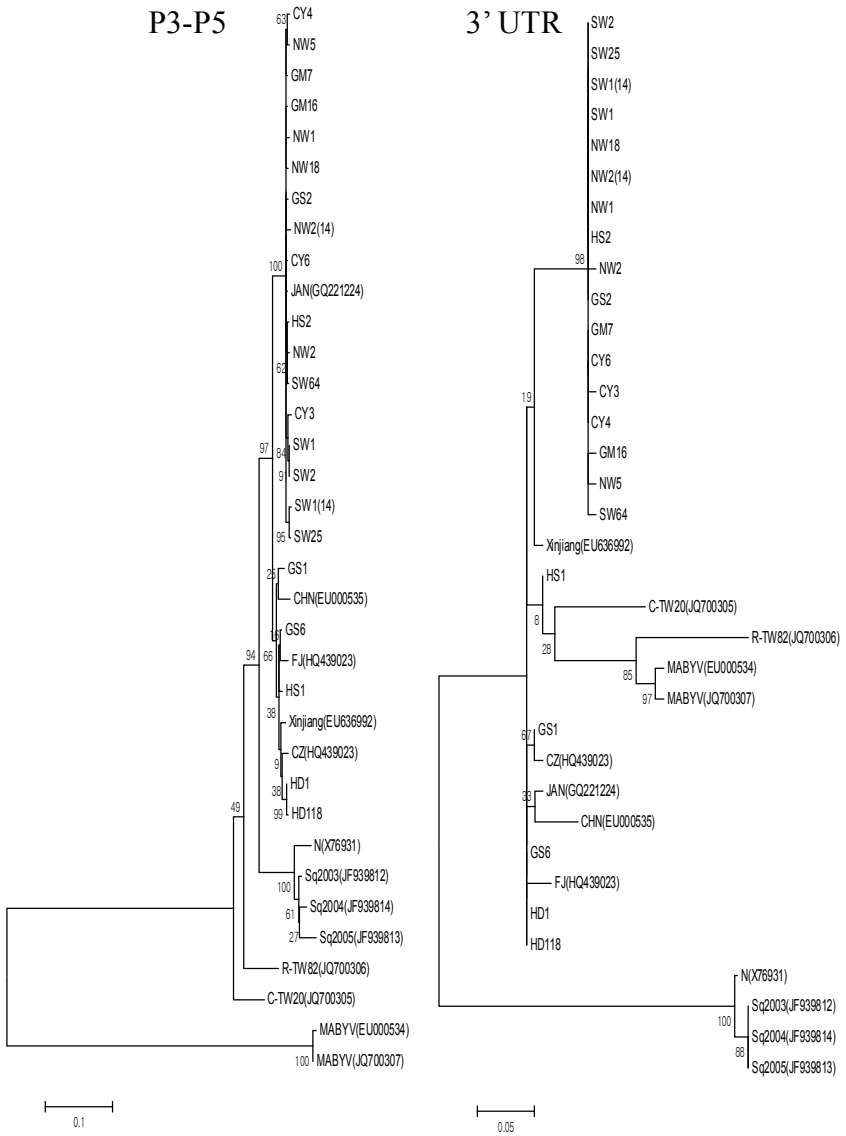


Fig. 1-7. Phylogenetic trees reconstructed using the amino acid sequences of 5' proximal proteins and nucleotide sequences of IR region. The maximum likelihood method implemented in MEGA 6 was used to reconstruct the phylogenetic trees.

of non-coding regions showed that Korean CABYV isolates consistently grouped into the Asian group (Figs. 1-7). However, using 3' proximal proteins and the 3' UTR, the Korean CABYV isolates were classified into two subgroups within the Asian group. This shows the possibility of recombination in the IR region between the 5' and 3' proximal proteins. Although the CABYV-R group belonged to CABYV, it grouped into MABYV in the 5' proximal protein and 3' UTR-based phylogenetic analyses, due to recombination between CABYV and MABYV isolates (Knierim et al., 2013a).

Sequence comparison

The nt and amino acid sequence identities between CABYV isolates are summarized in Table 1-7. For the full-length genome nt sequences, Korean CABYV isolates had 96-99% nt sequence similarity. CABYV-CY3, a Korean CABYV isolate, showed 94-98% nt sequence similarity with the Asian group including Japanese and Chinese isolates, 87-89% with the Mediterranean group, 88% with the Taiwanese group, 81-84% with the CABYV-R group, and 72% with the other *Polerovirus*, MABYV.

Regarding the deduced amino acid sequences of six individual proteins, CABYV-CY3 (as a representative Korean CABYV isolate) showed relatively high sequence identity of 92-100% with the Asian group. In contrast, aa sequence identities between CABYV-CY3 and the

Mediterranean group were 75-82% for P0, 83-88% for P1, 87-92% for P1-P2, 92-97% for P3 (CP), 87-91% for P4 (MP), and 89-91% for P3-P5. In comparison with CABYV-CZ and R-TW82, CABYV-CY3 had lower aa sequence identity of 65-75% for the 5' proximal proteins (P1 and P1-P2), but 89-98% for the 3' proximal proteins (P3, P4 and P3-P5). In addition, CABYV-CY3 shared only 62-82% aa sequence identity with MABYV isolates for each individual protein. The nt sequence identities of the IR region and 3' UTR were 92-100 and 69-92%, respectively, among the four CABYV groups, and were 70 and 84% with MABYV, respectively.

Sequence comparison revealed that the Korean CABYV isolates shared 95-98% nt sequence identity and 92-100% aa sequence identities for six individual proteins with the Asian group (Table 1-7). In addition, the Korean CABYV isolates showed 82-89% nt sequence identity and 75-98% aa sequence identity with three other CABYV groups. Of the individual proteins, the 5' proximal proteins were more variable than the 3' proximal proteins. In particular, P0 was most variable, while P3 (CP) was most conserved. These characteristics; i.e., highly variable P0 and conserved P3 (CP), have been reported for other *Polerovirus* species (Hauser et al., 2000; Huang et al., 2005; Xiang et al., 2010).

Transmission and host range

A series of experiments were carried out to identify whether LYS in

Table 1-7. Nucleotide and amino acid sequence identities of the other Korean CABYV and MABYV isolates compared to CY3.

Virus[z]	Isolate[y]	Sequence identity (%)[x]								
		Full genome	P0	P1	P1-P2	IR	P3(CP)	P4(MP)	P3-P5	3'UTR
CABYV	**CY4**	98.8	97.5	99.2	99.0	100	99.0	98.4	98.7	98.8
	CY6	98.5	95.8	97.6	98.1	100	99.5	99.5	99.0	98.8
	GM7	98.9	97.5	99.5	99.4	100	99.5	99.5	99.0	99.4
	GM16	98.3	95.4	97.6	98.4	99.5	99.5	99.0	98.8	98.2
	GS1	96.7	97.1	98.4	98.8	98.5	99.5	98.4	95.7	92.2
	GS2	98.7	97.5	98.6	99.1	100	99.0	99.0	98.8	99.4
	GS6	97.5	96.7	98.4	99.1	100	99.5	99.5	96.0	92.3
	HD1	96.8	96.2	98.1	98.2	99.5	97.0	97.4	95.2	92.2
	HD118	96.8	96.2	98.4	98.3	99.5	96.5	97.4	95.2	92.2
	HS1	97.4	98.3	98.7	99.1	99.5	99.0	99.0	95.8	91.0
	HS2	99.0	97.9	99.4	99.2	99.5	99.0	99.0	98.8	98.8
	NW1	98.9	97.1	98.9	99.1	100	98.5	98.4	98.7	99.4
	NW2	98.9	97.1	99.0	99.1	100	98.5	99.5	98.5	98.2
	NW2(14)	98.7	97.1	98.6	99.1	100	99.5	99.0	98.5	99.4
	NW5	98.4	97.1	98.6	98.7	100	99.0	98.4	98.5	98.8
	NW18	98.9	97.9	98.9	99.1	100	99.0	99.0	98.8	99.4
	SW1	99.3	97.5	99.5	99.5	100	99.5	99.5	99.3	99.4
	SW1(14)	99.0	97.5	99.4	99.4	100	98.5	99.0	98.4	98.8
	SW2	99.3	97.5	99.5	99.5	100	99.5	99.5	99.3	99.4
	SW25	99.0	97.5	99.5	99.5	100	99.5	99.5	98.5	98.8
	SW64	98.8	97.1	99.5	99.3	100	99.0	97.9	98.8	97.6
	JAN	97.9	96.7	97.9	98.9	100	99.0	99.5	99.0	92.2
	FJ	95.7	92.1	96.2	96.8	97.5	98.5	96.9	95.5	90.4
	CHN	94.7	91.6	93.7	95.4	97.5	97.5	97.9	95.2	91.0
	Xinjiang	95.6	96.2	95.7	95.7	98.5	98.0	97.4	96.0	92.2
	CZ	83.8	81.0	64.4	75.0	98.0	97.0	98.4	94.9	91.6
	R-TW82	81.4	82.4	66.2	75.0	92.0	95.0	88.5	90.4	81.4
	C-TW20	87.5	76.2	83.7	89.5	92.5	96.5	86.9	90.0	86.1
	N	89.0	80.3	88.0	91.9	94.5	94.5	89.0	90.4	69.0
	Sq/2003/7.2	88.8	81.6	87.3	90.9	94.0	95.0	89.6	91.0	68.5
	Sq/2004/1.9	89.1	81.6	87.0	91.2	95.0	92.0	90.6	90.9	68.5
	Sq/2005/9.2	87.1	74.9	83.4	87.3	94.0	95.5	86.9	89.4	68.5
MABYV	CHN	71.5	73.1	62.4	72.7	69.2	81.5	67.0	62.0	84.4
	TW1	71.8	73.6	62.6	72.5	70.1	81.5	66.5	62.5	83.8

[z] CABYV, *Cucurbit aphid-borne yellows virus*; MABYV, *Melon aphid-borne yellows virus*.

[y] Isolates analyzed in this study are shown in bold.

[x] Nucleotide sequence identity(%) for full genome, IR, and 3'UTR. Amino acid sequence identity (%) for P0, P1, P1-P2, P3, P4, and P3-P5.

muskmelon is affected by aphids feeding on leaves of infected plants. Test results for presence of CABYV using RT-PCR in 5 days after aphids transmission revealed that CABYV was not detected in control muskmelon seedlings, but in both of *Aphis gossypii* and *Myzus persicae* transmission seedlings (Table 1-8). CABYV was readily transmitted by *A. gossypii* and *M. persicae*. The results showed that casual agents of LYS in muskmelon plants were virus transmission by aphids. *A. gossypii.* is known to be an important vector of CABYV, requiring prolonged feeding times and direct contact with vascular tissues, spreading CABYV mainly by colonizing vectors (Fereres and Moreno, 2009). *A. gossypii* was not only the most abundant aphid species in the fields but also the aphid species with the greatest number of aphids carrying CABYV (Brault et al., 2005). This information indicates that *A. gossypii* is the main aphid species involved in the spread of CABYV in melon crops (Kassem et al., 2013). The test results of mechanical inoculation and soil transmission showed that the sap and soil transmission did not occur. Thus, the results revealed that the CABYV symptoms transmitted by virus-infected aphids (Table 1-8). The rapid spread of the virus in muskmelon is probably related to the efficient transmission by *A. gossypii* and *M. persicae* which are principal species colonizing cucurbit crops.

Host ranges of CABYV were compared using *A. gossypii*. The results showed that CABYV infected five cucurbit species such as non-netted

Table 1-8. Identification of CABYV in muskmelon plants showing leaf yellowing symptoms following transmission by various methods.

Transmission method		No. of positive seedlings/seedlings tested
Aphid[z]	*Aphis gossypii*	7/10
	Myzus persicae	2/10
Mechanical inoculation		0/36
Soil		0/10

[z]Non viruliferous aphids were allowed 5 days acquisition period before being transferred in groups of two to test plants for 7 days inoculation period. More than ten aphids were used per test plant for CABYV transmission.

melon, Korean melon, cucumber, watermelon, and squash, even though transmission rate differed depending on cultivars (Table 1-9). However, CABYV does not infect some other major differential hosts, belonging to the Solanaceae (eggplant, tomato, etc.) or Leguminosae (bean, pea, etc.) (Lecoq et al., 1992). CABYV is ecologically adapted to a temperate environment. Symptom severity of CABYV infected plants varied greatly by season with the summer being more severe than the winter; it also differed with cultivar (Lecoq et al., 1992).

In conclusion, our analyses showed that CABYV is widespread in most important muskmelon cultivation areas in Korea. CABYV infection was associated with the typical yellowing symptoms of older leaves and yield reductions. The CABYV population has a low diversity and seems to be genetically related to the Japan isolate. Thus, CABYV should be considered as an important pathogen of cucurbits and an important component of the group of viruses inducing yellowing disease of these crops. It is needed to find appropriate control measures, and to screen cucurbit gene banks for resistance to this virus. CABYV, an important pathogen that causes yellowing symptoms in cucurbit crops, has been reported to infect nine cucurbit crops in China (Xiang et al., 2008a). In Korea, many cucurbit species including watermelon, cucumber, and squash are widely cultivated. More studies on host range, pathogenicity, and vector transmission of Korean CABYV isolates are required to prevent the spread of CABYV.

Table 1-9. Comparative host range of CABYV detected by RT-PCR in muskmelon plants from October 8 to November 13, 2014.

Differential host[z]	Cultivar	CABYV incidence[y]
Citrullus vulgaris	'Sambokkkul'	11/17
	'Speedkkul'	4/17
	'Urikkul'	0/17
Cucumis melo	'Homerun star'	3/20
	'Yellow sun'	6/20
Cucumis melo L. var. makuwa	'Joeundae'	8/16
	'Obokkkul'	5/16
	'Obokpluskkul'	0/16
Cucurbita moschata	'Jinhan Aehobak'	1/20
	'Nongwoo Aehobak'	1/20

[z]Groups of 10-20 viruliferous aphids were deposited on each test plant and left for 7 days inoculation period.

[y]Number of infected plants / number of tested plants.

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CHAPTER 2

PHYSIOLOGICAL AND CYTOLOGICAL CHARACTERISTICS OF MUSKMELON PLANTS SHOWING LEAF YELLOWING SYMPTOMS

ABSTRACT

This study was conducted to investigate the causal factors of leaf yellowing symptoms (LYS) and to characterize sugar contents and morphology. When photosynthetic ability was measured by chlorophyll fluorescence yield, the leaves of the diseased plants showed 4.09 $\mu mol \cdot m^{-2} \cdot s^{-1}$ which was one third of the readings observed for the uninfected normal plants (12.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The root functions of the infected plants was 0.28 $mg \cdot g^{-1}$, about a half that measured for the normal uninfected plants (0.48 $mg \cdot g^{-1}$). No morphological differences were observed in the palisade parenchyma and mesophyll spongy cells of the leaves between the infected and normal uninfected plants. However, the same leaf cells of the infected plants contained more starch granules compared to those of the normal, uninfected plants. The contents of glucose, fructose, and sucrose in leaves were greater in the infected plants than in the normal plants and those in stems, fruits, and roots were also slightly smaller in the infected plants.

Scanning electron microscope observation revealed that the sieve tube of mesophyll was closed in the infected plants. This result suggests that the occurrence of LYS is closely related to shortage of root length and amount and the greater sugar contents in leaves with yellowing symptoms may be due to poor translocation of free sugars produced in leaves to sink organs by closed sieve tubes.

Keywords: mesophyll, parenchyma cell, scanning electron microscope, sieve tube, sugar content, vessel

INTRODUCTION

Leaf yellowing symptoms (LYS) are the major cause of low-quality muskmelons and have been steadily spreading during the past 2 to 3 years in Korea. LYS can be attributed to physiological factors such as high temperatures, nutritional deficiencies (mainly to Mg and Mn), or virus diseases (Park et al., 2011; Takeshita, 2004). However, the cause of LYS is still not identified.

Plant diseases and physiological disorders can be caused by biotic agents like pathogens and parasites or abiotic agents like nutritional deficiency, pollution, and heavy metals (Maiti et al., 2012). Developmental abnormalities such as yellowing, chlorosis, necrosis, and leaf curling represent common symptoms associated with many virus diseases (Walters et al., 2005). Plant viruses generally cause common physiological alterations such as decreases in photosynthetic activity, increases in respiration rate, the accumulation of nitrogen compounds, and expanded oxidase activities (Goodman et al., 1986; Zaitlin and Hull, 1987). Plant viruses such as *Watermelon mosaic virus* change chloroplast morphology and metabolism, resulting in decreased photosynthetic activity and plant growth (Roberts and Wood, 1982). Carbohydrate metabolisms in the source leaf were reported to be influenced by viral infections (Balachandran et al., 1997; Biemelt and Sonnewald, 2006; Tecsi et al., 1994a, b, 1996). Infected leaves were usually

characterized by reduced photosynthetic rate, a decrease in concentrations of soluble sugars and starch accumulations (Goodman et al., 1986). In this plant-virus interaction starch accumulation is an indirect consequence of virus infection associated with the removal of the physiological sink for photosynthates (Herbers et al., 2000; Shalitin and Wolf, 2000; Tecsi et al., 1996). The transgenic expression of virus-encoded movement proteins (MP) leads to accumulations of carbohydrates and starch in virus-infected sink leaves (Herbers et al., 1997; Hofius et al., 2001). The transgenic expression of *Tomato spotted wilt virus* MP results in callose deposition at plasmodesmata which potentially blocks the symplastic transport of sucrose and shows disease-like symptoms (Rinne et al., 2005). Therefore, the mechanisms through which this reallocation occurs are virus specific and likely the result of interactions between specific virus and host components (Culver and Padmanabhan, 2007).

To produce high quality muskmelons with high soluble sugar contents and good netted skins, environmental conditions such as temperature and nutrients should be provided appropriately with growth stages. In addition, carbohydrates from photosynthesis in leaves should to be transmitted to fruits. This study was conducted to investigate the relationship between LYS and physiological characteristics of muskmelon plants.

MATERIALS AND METHODS

Plant materials and growth conditions

Seeds of a muskmelon cultivar 'Earl's Tipani' (Hannil Seed Co., Gongju, Korea) were sown in 32-cell plug trays in a greenhouse. After germination, seedlings at two true-leaf stages were transplanted into raised soil beds with 40 cm of plant spacing in greenhouses in National Institute of Horticultural and Herbal Science, Suwon, Korea on May 29, 2014. Plants grew on trellis. When plants had 22-23 internodes, the shoot of the mother vines were pinched off to encourage lateral branches. After fruits were formed from three female flowers on the secondary vines developed from 11th to 13th internodes on the mother vines, only one developing fruit of a good shape was kept with the rest of the fruits removed 7 to 10 days after pollination.

Photosynthetic rate

Photosynthetic rate was measured using a portable photosynthesis system (LI-6400, LI-COR, Lincoln, NE, USA) for control plants (two plants with three replications) and for plants showing LYS. Leaves of the 5 to 7th nodes from the terminal bud were used for measurement 75 days after transplanting. Leaf chamber temperature, relative humidity, CO_2 concentration, and photosynthetic photon flux density were maintained at 25^{o}C, 60%, 400 $\mu mol \cdot mol^{-1}$, and 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively.

Root activity

Root activity of control plants (two plants with three replications) and of plants showing LYS was estimated from the absorbance values of formazan products 35 days after fruit set. Roots were sampled from transplanting positions at 50 cm in radius and 30 cm in depth, and washed in running water before measurement. Fine roots were cut into 0.5 cm and homogenized, and then 0.1 g of homogenized root sample was added in a test tube with 1 mL deionized water and mixed with 10 μL of WST-1 assay (Premix WST-1 cell proliferation assay system, Takara Bio Inc., Otsu, Japan). The mixed samples were placed in the dark at 25°C for 3 h, and analyzed by ELISA reader (Microplate Spectrophotometer, EonTM, BioTek Inc., Winooski, VT, USA) at 420 nm.

Analysis of mineral contents

Mineral ions such as K, Ca, Mg, and P were measured using an atomic absorption spectrophotometer (AA-6800, Shimadzu, Kyoto, Japan). Dried leaf samples (0.5 g) were decomposed with a 10 mL digest solution containing HNO_3 and $HClO_4$ (3:1, v/v) at 180°C for 12 to 16 h in 100 mL flasks. Decomposed samples were filtered through filter papers (No. 6, Advantec Co., Tokyo, Japan), and then distilled water was added to the filtrate to bring up to a final volume of 100 mL.

To measure total N content, 0.5 g of dried leaf samples was decomposed

with 1 g of catalyst (K_2SO_4:$CuSO_4$ = 9:1) and 10 mL of concentrated sulfuric acid in Kjeldahl flasks at 380°C for 3 to 4 h. Total N contents of the decomposed samples were analyzed using a Kjeldahl analyzer (Kjeltec 2300, Foss Tecator AB, Hoganas, Sweden).

Microscopic observation of cellular tissue

Leaf tissues of the plants showing LYS were anatomically observed a microscope. Fixation, dehydration, and the embedding of leaf materials were performed as described by Clement et al. (1994). Leaf samples from muskmelon plants were first fixed with 2.5% glutaraldehyde for 90 min at 4°C and then washed 4-5 times for 15 min intervals in 0.1 M phosphate buffer solution (pH 7.2). The samples were second fixed with 1% OsO_4 for 90 min and then immersed overnight after washing the same way. They were dehydrated through a graded ethanol series (40, 60, 80, 90, and 95%) for 5 min and then in 100% ethanol for 5, 15, 15, and 30 min before being embedded in Epon (EMS, Fort Washington, PA, USA) for 4 days at 60°C. The Epon blocks were sectioned at 1,500 nm-thick with a ultramicrotome (Ultracut R ultramicrotome, Leica Microsystems, North Ryde, NSW, Australia) and stained using the methods of periodic acids and Schiff's reagents, and then observed 100-fold under a microscope (Axioskop II, Carl Zeiss, Oberkochen, Germany).

Sugar contents

For measuring sugar contents, leaves, stems, roots, and fruits of plants showing no, mild, and severe LYS were collected 55 days after fruit set. Each tissue of 3 to 5 g in fresh weight was homogenized in 50 mL falcon tube containing 20 mL of deionized water. Homogenized tissues were vortex mixed, and centrifuged for 10 min in 11,077 ×g, and then filtered through filter paper (No. 2, Advantec Co.). HPLC grade water was added for filtrating to make a final volume of 100 mL and then 50 mL of that was transferred to 50 mL falcon tube. Sep-Pak cartridges (Plus C18, Waters, Milford, MA, USA) was washed with 4 mL of deionized water, activated with 4 mL of methanol (100%), and filtered through syringe filter having 0.45 μm pore size. Bio-LC (Biocompatible Liquid Chromatography, Dionex ICS-2500 system, Dionex, Sunnyvale, CA, USA) with column (CarboPac, PA10, Dionex) was used for analyses. The HPLC system was equipped with an eluent degas module, a GP 40 gradient pump, a guard column CarboPac PA10 (4 × 50 mm), and an analytical column CarboPac PA10 (4 × 250 mm). The mobile phase consisted of isocratic 40 mM NaOH eluant and 200 mM NaOH for 15 min at a flow rate of 1 mL/min at 24. A 15-min column wash with 200 mM NaOH followed by 15-min equilibration with 40 mM NaOH at a flow rate of 1 mL/min at ambient temperature was required to yield highly reproducible retention times for the monosaccharides. The total run time was about 50 min. Retention times were calibrated daily for

glucose, fructose, and sucrose (Sigma-Aldrich, St. Louis, MO, USA) by injecting 10 μL of a calibration mixture containing 5.50, 13.75, and 13.75 $mg \cdot kg^{-1}$ of these sugar contents, respectively.

Scanning electron microscopic observation of sieve tube

Leaves showing no and severe LYS were collected 57 days after transplanting. Fixation, dehydration, and embedding of leaf materials were performed as described by Maldonado-Amparo and Ibarra (2002). Leaves were excised in the length of 0.8 to 1.0 cm and fixed in 2.5% glutaraldehyde for 90 min and washed 4 to 5 times in 0.1 M phosphate buffer (pH 7.2) to conduct microscopic observation. Leaf tissues were then fixed in 1% OsO_4 for 90 min, washed 4 to 5 times in 0.1 M phosphate buffer (pH 7.2), and immersed overnight. Samples were dehydrated in a gradual series of ethanol concentrations of 40, 60, 80, 90, and 95% for 5 min each and in 100% ethanol for 5, 15, and 30 min. After dehydration, samples were transferred into isoamyl acetate twice for 40 min and dried in a critical point dryer. The specimens were then mounted and gold-coated using Emitech K450 sputter coater (Quorum Technologies Ltd., Lewes, UK) and examined with a scanning electron microscope (N-2460, Hitachi Ltd., Tokyo, Japan).

Statistical analyses

Experiments for each treatment were triplicated. Data of growth and root

activity were analyzed by using *t*-test, while root growth was applied to evaluate using Duncan's multiple range test at $P = 0.05$. In addition, influences of LYS on sugar contents of muskmelon fruits were assessed using one-way analysis of variance (ANOVA) in SAS 9.2 (SAS Inst. Inc., Cary, NC, USA) to identify least significant differences ($P < 0.05$).

RESULTS AND DISCUSSION

Growth and development

Muskmelon plants showing LYS had gradually decreased greenness in leaves. Leaf color was definitely distinguished by the intensity of LYS (Fig. 2-1).

Plant height, leaf length, internode length, and leaf area of muskmelon plants were not affected by LYS, whereas, root length, root fresh weight, and root dry weight were significantly smaller in the plants showing LYS (Table 2-1). There was no significant difference on above ground fresh weight, but plants showing LYS had significantly greater dry weight than normal plants. However, in root length, fresh weight, and dry weight there were significantly smaller in the plants showing LYS. Plants showing LYS exhibited healthy growth except for roots. These results are consistent with previous reports that root mass was affected by LYS in muskmelon plants (Takeshita, 2004). Because of poor root growth in the plants showing LYS, the muskmelon plants would not properly uptake nutrients, and thereby fruit growth and quality might be negatively affected.

Photosynthesis and root activity

Plants showing LYS had significantly smaller photosynthetic rate (4.09 $\mu mol \cdot m^{-2} \cdot s^{-1}$) than normal plants (12.36 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Table 2-2). The

Fig. 2-1. Typical leaf yellowing symptoms of the muskmelon plants. A, not affected; B, mildly affected; C, severely affected by LYS.

Table 2-1. Effect of leaf yellowing symptoms on growth and development of muskmelon plants.

Plant		Plant height (cm)	Leaf area (cm^2)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
Healthy		175.5 a[z]	9,504 a	44.3 a	21.0 a	2.08 a	767.0 a	145.0 b
LYS	Mild	175.2 a	10,054 a	38.8 b	17.4 ab	1.55 b	776.3 a	180.2 a
	Severe	169.7 a	9,076 a	38.8 b	14.3 b	1.42 b	695.7 a	176.5 a

[z]Mean separation within columns by LSD test at $P = 0.05$.

Table 2-2. Effect of leaf yellowing symptoms on photosynthetic rate and root activity in muskmelon plants.

Plant	Photosynthetic rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Root activity[z]
Healthy	12.36	0.484
LYS	4.09	0.284
Significance	***	***

***Significant at the $P = 0.001$ by t-test.

[z] Root activity was calculated by changing in WST-1 formazan absorbance at 420 nm measured after 3 h.

decrease in photosynthetic rate might be associated with reduced chlorophyll content due to chlorophyll breakdown by LYS. This result confirmed that photosynthetic activity was decreased in the muskmelon plants showing LYS (Park et al., 2011; Takeshita, 2004).

Root activities were 0.28 and 0.48 $mg \cdot g^{-1}$ in plants with and without LYS, respectively (Table 2-2). Lower root activity in the plants showing LYS might be critical for water and mineral uptake.

Mineral concentration in muskmelon

Mineral concentrations in leaves of the muskmelon plants showing LYS were significantly smaller than in those of normal plants (Table 2-3). Especially, Ca and Fe concentrations were significantly much smaller in leaves of the plants showing LYS. Marco nutrient concentrations in leaves of the plants showing LYS were 17.7, 35.8, 8.59, and 0.36 $g \cdot kg^{-1}$ in K, Ca, Mg, and Na, respectively. The ratios of macro nutrient concentration in leaves of plants showing LYS were almost half of those in normal leaves. Micro nutrient concentrations in leaves of plants showing LYS were 11, 5, 29, and 37 $mg \cdot kg^{-1}$ in Fe, Cu, Zn, and Mn, respectively, and were under the half of those in normal leaves. Especially, Fe concentration in leaves of the plants showing LYS was 4.2% of that in normal leaves. We are of the opinion that the smaller mineral concentrations in the leaves of the plants showing LYS than in normal leaves were not causes but effect of LYS (Takeshita, 2004).

Table 2-3. Effect of leaf yellowing symptoms on mineral concentration in muskmelon leaves.

	Macronutrient concentration ($g \cdot kg^{-1}$ dry wt.)				Micronutrient concentration ($mg \cdot kg^{-1}$ dry wt.)			
Plant	K	Ca	Mg	Na	Fe	Cu	Zn	Mn
Healthy	26.9	69.6	16.44	0.78	258	10	59	79
LYS	17.7	35.8	8.59	0.36	11	5	29	37
Significance[z]	**	***	**	**	***	**	**	**

[z]ns, *, **, *** non-significant or significant at $P = 0.05$, 0.01, or 0.001, respectively, by *t*-test.

Fruit growth and quality

Muskmelon plants showing severe LYS exhibited significantly smaller fruit growth than normal and the plants showing mild LYS. Fruit quality like sugar contents and net index were also affected by the extent of LYS (Table 2-4). In normal plants, maximum sugar content in fruit was 14.5°Bx and net index was 1.1. Fruits in the plants showing mild and severe LYS had maximum sugar content and net index at 13.2 and 11.2°Bx, respectively, and 5.4 and 6.7, respectively. The decreases of maximum sugar content and net index in fruits of the plants showing LYS were associated with lowered root growth (Table 2-1) due to reduction of nutrient uptake and low photosynthetic abilities of yellowing leaves.

Fruit enlargement

Fruit enlargement in the plants showing LYS was significantly smaller than that in normal plants (Fig. 2-2). The width and length enlargement of fruit were significantly different between the plant showing LYS and the normal plant. Changes of the fruit width and length on normal plant from July 19 to August 18 were about 2 cm each, while that on plants showing LYS showed about 1 cm each. The decreases in fruit size and enlargement might be associated with low photosynthetic abilities of yellowing leaves with small amount of chlorophylls due to LYS.

Table 2-4. Effect of leaf yellowing symptoms on fruit growth and quality in muskmelon plants.

Plant		Length (cm)	Width (cm)	Shape index[z]	Weight (g)	Net index[y]	Flesh width (cm)	Soluble solids (ºBx)
Healthy		16.7 a[x]	15.7 a	1.06 ab	2,211 a	1.1 a	4.2 a[y]	12.0 a
LYS	Mild	16.2 a	15.6 a	1.04 b	2,040 a	5.4 b	4.3 a	10.3 b
	Severe	15.3 b	14.1 b	1.09 a	1,535 b	6.7 c	3.7 b	9.0 b

[z]Length/width.

[y]1 (good) - 9 (poor).

[x]Mean separation within columns by LSD at $P = 0.05$.

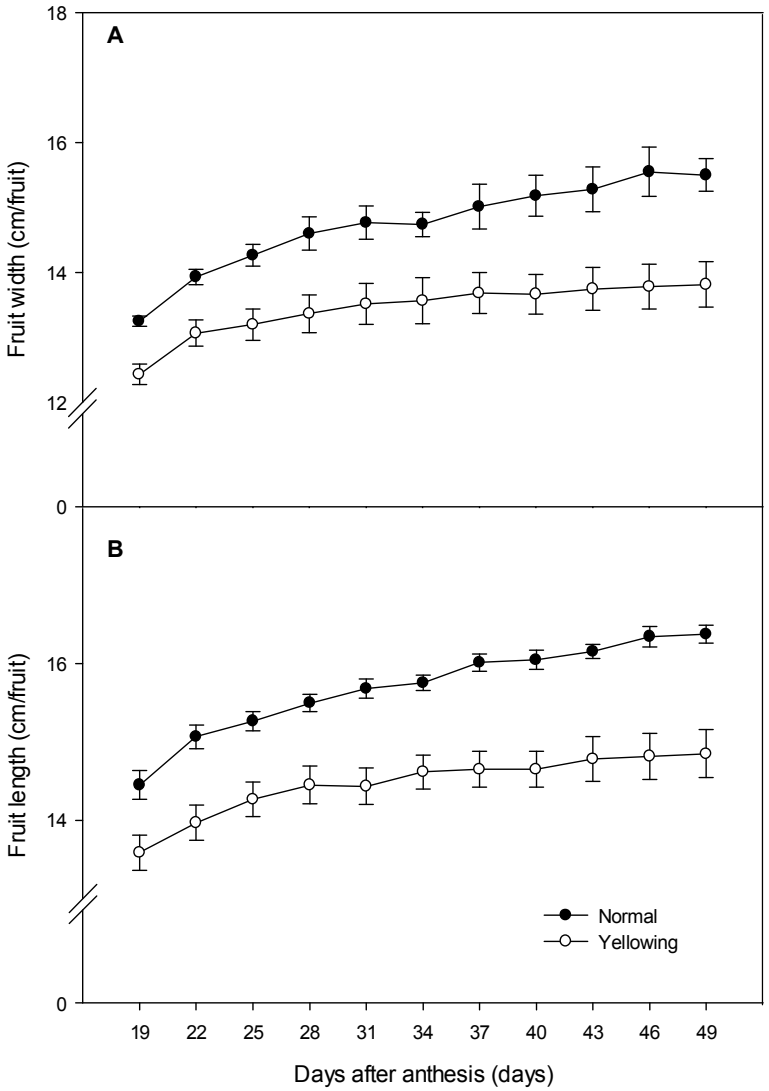


Fig. 2-2. Fruit development in muskmelon plants showing leaf yellowing symptoms. A, fruit width; B, fruit length. Bars represent standard errors of the means (n = 3).

Anatomical characteristics of leaf tissues

Microscopic observations revealed that there were no anatomical differences in the palisade parenchyma and mesophyll spongy cells of the leaves of the plants showing LYS from the leaves of the normal plants. However, the leaf cells of the plants showing LYS contained more starch granules compared to those of the normal plants (Fig. 2-3). Amount of photo-assimilates transmitted from the leaves of the plants showing LYS to the fruits might be smaller, because closed plasmodesmata in the leaf cells can block virus infections. These results are in agreement with those of Epel (2009), Lee et al., (2009), and Lee (2014), who showed that the control of plasmodesmata permeability was integrated into innate immune responses, which have varying effects on infection hindrance or spreading, depending on the virus.

Sugar translocation

The contents of glucose, fructose, and sucrose were higher in the leaves of the plants showing LYS than in those of normal plants, while the sugar contents of roots were lower in the plants showing LYS compared to those in normal plants (Fig. 2-4A, B, C). The higher and lower sugar contents in leaves and roots, respectively, are possibly due to poor translocation of free sugars from leaves to other organs. Similar result was observed in Guilley et al. (1994) who reported that sugar translocations of plants infected by

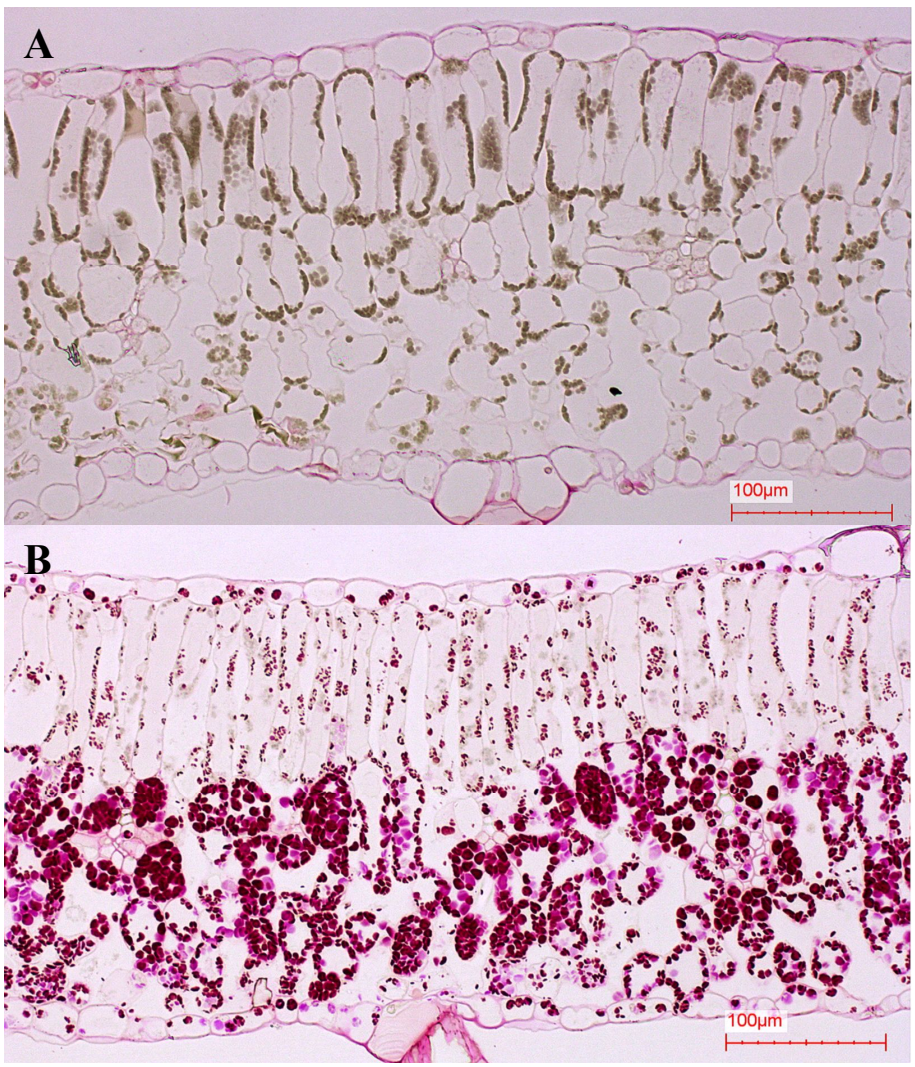
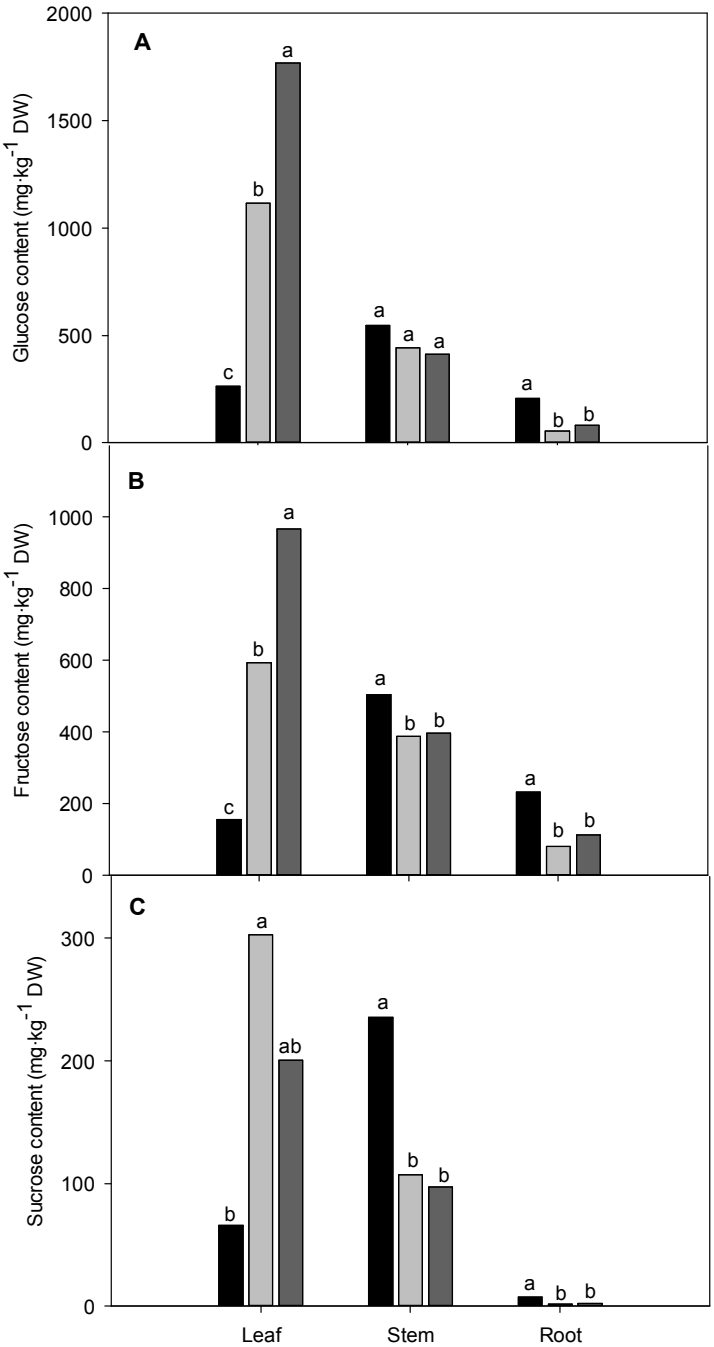


Fig. 2-3. Effect of leaf yellowing symptoms on leaf tissues of muskmelon plants examined under a light microscope (bar = 100 μm). A, the normal muskmelon plants; B, muskmelon plants showing LYS.


A
Glucose content (mg·kg-1 DW)
2000
1500
1000
500
0
c
b
a
a
a
a
a
b
b
B
Fructose content (mg·kg-1 DW)
1000
800
600
400
200
0
c
b
a
a
b
b
a
b
b
C
Sucrose content (mg·kg-1 DW)
300
200
100
0
b
a
ab
a
b
b
a
b
b
Leaf
Stem
Root

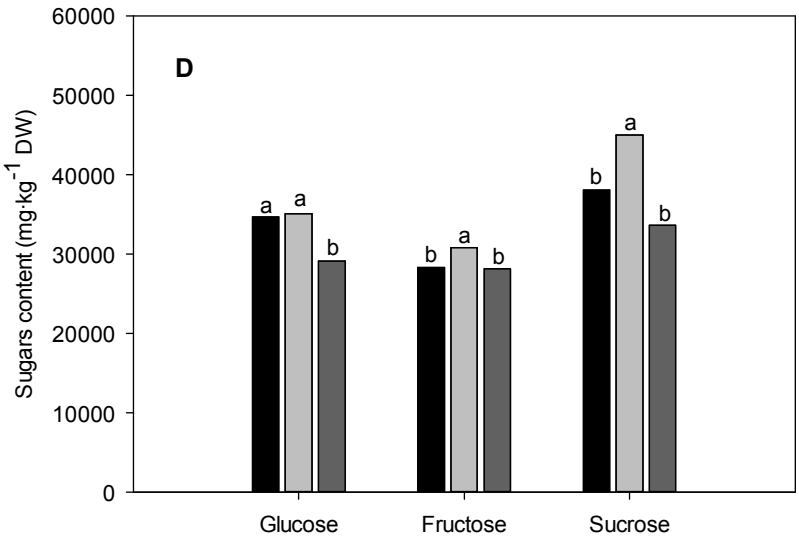


Fig. 2-4. Contents of glucose (A), fructose (B), and sucrose (C) in different organs of muskmelon plants and those of sugars of muskmelon fruits (D) showing little (black bar), mild (gray bar), and severe (dark gray bar) leaf yellowing symptoms 55 days after fruit set. Letters indicate significant differences at 0.05% LSD.

CABYV were interrupted due to abnormal sieve tubes. Sugar contents in fruit of the plants showing severe LYS were lower than those of normal plants and the plants showing mild LYS (Fig. 2-4D). Similarity, growth retardants and environmental stress conditions reduced sugar contents in muskmelon fruits (Matsumoto et al., 2012). In addition, fruits act as the strongest sink and, therefore, the highest sugar contents were observed in fruits than in other organs regardless of LYS. The fruits were the strongest sinks in watermelon and tomato (Lee et al., 2006) and pumpkin (Lee et al., 2009b), which were determined by analyzing electric current in different leaves using ^{14}C.

Anatomical characteristics of sieve tubes

Sieve tubes of leaves from normal plants and plants showing severe LYS were compared under a SEM 57 days after transplanting. Xylem and phloem in mesophyll tissues from normal plants appeared normal (Fig. 2-5A, 5B), but those from plants showing LYS were abnormal (Fig. 2-5C, 5D), and some sieve tubes were closed (Fig. 2-5C). High sugar contents in the leaves of the plants showing LYS might be due to some closed sieve tubes. Similarly, sugar translocation of plants infected by viruses was interrupted due to abnormal sieve tubes (Guilley et al., 1994) and flexuous filamentous structures were observed in phloem of melon plants infected by CCYV (Gyoutoku et al., 2009). This result suggests that the occurrences of

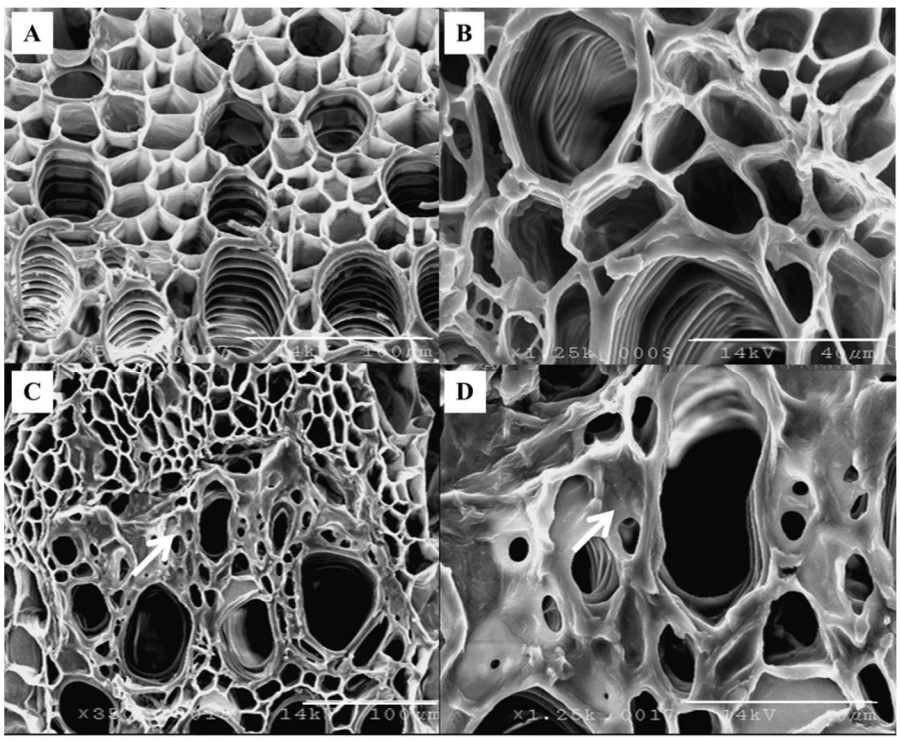


Fig. 2-5. Sieve tubes of muskmelon leaves observed under a SEM 57 days after transplanting. A, normal plant at 100 μm; B, normal plant at 40 μm; C, plant showing leaf yellowing symptoms at 100 μm; and D, plant showing leaf yellowing symptoms at 40 μm. White arrows show closed sieve tubes.

LYS and shortage of root length and amounts are closely related to each other. In addition, higher sugar contents in leaves of the plants showing LYS may be caused by poor translocation of free sugars from leaves to other organs by closed sieve tubes. Consequently, which has been spreading rapidly in domestic muskmelon cultivation areas are considered to be more associated with viruses from an aphid than physiological disorders. LYS adversely affects muskmelon fruit quality by physiological disorder of roots and inhibiting sugar translocations from leaves. Therefore, preventing aphid infections are recommended to reduce LYS on muskmelon plants. However, researches on root systems and sink-source relations are further needed to find out precise reasons for the stress by LYS.

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CHAPTER 3

DEVELOPMENT OF CULTURAL PRACTICES FOR REDUCING LEAF YELLOWING SYMPTOMS IN MUSKMELON

ABSTRACT

This study was conducted to evaluate the cultural practices for reducing the occurrence of leaf yellowing symptoms (LYS). Plant growth characteristics were not significantly different between normal plants (control) and the plants of which two-third of roots was removed (root pruned). Root length of control and root-pruned plants were 1,197 and 1,120 cm, respectively, while that of the plants showing LYS was only 696 cm. The root volume was greater in control (10.3 cm^3) than in the plants showing LYS (6.99 cm^3). The percentage of the plants showing LYS was 5 times greater in root-pruned plants than in the normal plants. The average fruit weight and sugar content of the root-pruned plants were about 70% below those of the normal plants. Root pruning increased LYS, but lowered fruit quality in the plants showing LYS. Seedling ages did not significantly affect growth, photosynthesis, and root activity. However, the incidence of LYS was twice as great in the plants grown from aged seedlings (13.7%)

than in those grown from younger seedlings (6.1%). Especially, the average weight of fruits harvested was greatly reduced when plants were grown from older seedling transplants. For reducing damages from LYS, therefore, seedlings used for transplanting must have a maximum number of three true leaves. When the influence of number of fruits left on vine on the growth of muskmelon plants was investigated 75 days after transplanting, plant height was greatest in one-fruit plants (180.8 cm), followed by two-fruit (168.5 cm) and three-fruit (165.2 cm) plants. Leaf area was greatest in one-fruit plants (10,117 cm^2), followed by two-fruit (7,577 cm^2) and three-fruit (6,247 cm^2) plants. Root activity was greater when plants had only one fruit than two or three fruits. Percentage of the plants developing LYS was smallest when only one fruit was left on the vine (2.4%) and increased as the number of fruits left increased to two fruits (6.7%) and three fruits (13.3%). Plants supporting only one fruit with healthy growth had the greatest net index. The infected plant supporting two or three fruits had fruit weights below 1,000 g not to be commercially suitable. Healthy plants with one fruit showed a net index value of 1.1, whereas greater fruit numbers per plant provided a greater than 6.0 net index. Increasing the number of fruits left in each plant is not a good strategy for reducing the occurrence of LYS. Plants with 35 leaves showed the greatest plant growth. Net photosynthetic rate was positively related to increasing leaf numbers with plants having over 25 leaves showing the greatest photosynthetic rates. The ratio of LYS

infection was also greater in plants having 25-30 leaves, than in those having leaf numbers. Plants with different leaf numbers and LYS infection showed a variation in fruit quality, although LYS did not significantly affect fruit quality except net index. Therefore, leaving more than 25 healthy leaves per plant was recommended for minimizing damage from LYS. The positions of the leaf nodes also influenced on the occurrence of LYS. The percentage of the plants with LYS decreased as the number of leaves borne on the nodes above the fruit bearing node increased. Hence, retaining the largest number of leaves on the nodes above the fruit-bearing node was a good cultural practice to reduce LYS.

Keywords: infected plant ratio, leaf number, net index, photosynthetic rate, root activity, seedling age, fruit-bearing node

INTRODUCTION

Muskmelon is one of the major fruit bearing vegetables in Korea where its cultivation area and production have increased from 17,000 metric tons (659 ha) in 2000 to 48,000 tons (1,477 ha) in 2013 (MIAFRA, 2014a). About 1,100 tons of muskmelons were exported to Japan and other countries in Southeast Asia in 2013 (MIAFRA, 2014b). Due to recent advances in muskmelon cultivation technology, growers now produce high quality muskmelons in greenhouses all year-round. Nevertheless, there are still low quality muskmelons with low sugar contents and poorly netted skins which depreciate their commercial values.

The major reason for low quality muskmelons in these days is the occurrence of LYS. In the past 2 to 3 years, LYS on muskmelon has steadily increased in all production areas in Korea. However, the cause of LYS has not been identified. Affected plants showed light green spots on the surface of the lower leaves (sometimes upper leaves) at the beginning and then gradually developed LYS. The yellow spots on the leaf were enlarged until a whole leaf blade was changed to yellow color and, finally, LYS spread to upper leaves. In the plants with LYS, normal fruit development were retarded with little netting on the fruit skin and low sugar contents (Lee et al., 2009b; Park et al., 2011).

Plants showing LYS were reported to have unbalanced sugar

translocation from the leaves, fewer roots, larger leaves, and faster fruit enlargement compared to uninfected plants especially when grown under poor environmental conditions (Takeshita, 2004). The objective of this study was to characterize the influence of fruit thinning, root pruning, leaf canopy management, and other cultural practices aimed at minimizing LYS in muskmelon plants.

MATERIALS AND METHODS

Plant materials and growth conditions

Seeds of a muskmelon cultivar 'Earl's Tipani' (Hannil Seed Co., Gongju, Korea) were sown in 32-cell plug trays (280 × 540 × 63 mm, W × L × H, Bumnong Co. Ltd., Seoul, Korea), respectively, filled with a commercial root substrate (BM2, Berger Group Ltd., Quebec, Canada) in a greenhouse. Seedlings at the two true-leaf stage were transplanted into plastic-mulched raised beds (plant spacing 40 cm) in greenhouses at National Institute of Horticultural and Herbal Science, Suwon, Korea on June 4, 2014. For potted culture, all plants were transplanted in pots of 45 cm in diameter and 50 cm in height, then filled with a mixture of substrate media consisting of rice straw, sand, spent mushroom, and fermented leaf compost at a rate of 3:3:2:2 (v/v/v/v). Each pot was hand irrigated twice a week with tap water and fertilized with a nutrient solution (EC 1.4 $dS \cdot m^{-1}$, 'Hanbang' for seedling, Coseal Co. Ltd., Seoul, Korea).

After seven to eight true leaves were unfolded, the main stems were trained on a trellis. The main (mother) vines were decapitated when they had 22 to 23 stem internodes to encourage secondary vine formation. Female flowers formed on the secondary vines were pollinated for fruit set. Out of three developing fruits from pollinated female flowers borne on the secondary vines growing from the 11th to 13th nodes, only one fruit with

good shape is kept with the rest of the developing fruits being removed after 7 to 10 days from pollination.

Root pruning, seedling age, fruit, and leaves left on the vine

Two-thirds of the lower portion of the root system was cut off from the seedling to examine if the reduction of root mass by pruning can influence the incidence of LYS.

To compare the influence seedling age at the time of transplanting on LYS development, performance of older seedlings with 4-5 true leaves open and younger seedlings with 2-3 true leaves was investigated. Young seedlings were transplanted on June 4, 2014, and older seedlings on June 11, 2014. Plant growth and LYS occurrence were determined on plants grown from these transplants.

Development of LYS was examined with the different numbers of leaves left on the vine. The growth and appearance of LYS on muskmelon plants having 25, 30, and 35 fully expanded leaves on the vine were compared to those of the control plant having 20 leaves.

Carbohydrate sink characteristic, fruit quality, and development of LYS were examined with different fruit loads on the vine. Performance of the plants having only one fruit on the vine (control) was compared to that of the plants having two and three fruits on the vine by removing extra fruits from the plants.

Plant growth, fruit yield and quality, and LYS development were examined with the influence of the total number and position of the leaves borne above and below the fruit-bearing node (FBN). Four treatments were used were BL-5 (five bottom leaves borne below FBN), BL=0 (no bottom leaves left below FBN), BL+5 (five leaves left above FBN), and BL+10 (ten leaves left at above FBN).

Photosynthetic rate

Net photosynthetic rates of the leaf were measured on six plants (two plants with three replications) on each treatment using a portable photosynthesis system (LI-6400, LI-COR, Lincoln, NE, USA). The fully expanded leaves borne on the 5th to 7th nodes from the terminal bud were used to measure net photosynthetic rate at 75 days from transplanting. During the measurements, the leaf chamber temperature, relative humidity, CO_2 concentration, and photosynthetic photon flux density were maintained at 25°C, 60%, 400 $\mu mol \cdot mol^{-1}$, and 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively.

Root activity

Root activities, as estimated by absorbance values of formazan products, were measured for six plants (two plants with three replications) at 35 days after fruit set. Roots were sampled from transplanting positions at 50 cm in radius and 30 cm in depth, and washed in running water before measuring.

Fine roots were cut into 0.5 cm and homogenized, and then 0.1 g of homogenized root sample was added in a test tube with 1 mL deionized water and mixed with 10 μL of WST-1 assay kit (Premix WST-1 cell proliferation assay system, Takara Bio Inc., Otsu, Japan). Mixed samples were placed in the dark at 25°C for 3 h, and analyzed by ELISA reader (Microplate Spectrophotometer, EonTM, BioTek Inc., Winooski, VT, USA) at 420 nm.

Root scanning

To investigate the relationship between root growth and the occurrence of LYS, the amount of roots produced by the plants grown in plastic pots was measured. Root growth was measured on the unaffected (control) plants, the plants grown after root pruning, and the plants showing LYS on August 26, 2014. After carefully washing the soil off from roots 87 days after transplanting, the amount of roots was measured with a flatbed scanner (Epson Expression 10000XL, Seiko Epson Corp., Tokyo, Japan). Before scanning, roots were spread not to overlap each other under water in a transparent water tank. Images were analyzed by WinRhizo root analysis software (2009 ver., Regent Instr., Quebec, Canada), which can analyze root image and measure root length, surface area, volume, and average diameter (Kim et al., 2010). This program can also modify and analyze overlapped roots, and measure root length to diameter ratio (Arsenault et al., 1995; Bouma et al., 2000; Wang and Zhang, 2009).

Measurements of growth and fruit quality parameters

Plant height, leaf area, fresh weight, dry weight, and chlorophyll content were measured 75 days after transplanting. Fruits were harvested 55 days after fruit set. After ripening 3 days, the rind roughness, the longitudinal and transversal fruit diameters, the fruit shape index, the mesocarp thickness, the average fruit weight, net index, and the sugar content per plant were measured.

Statistical analysis

Experiments for each treatment were triplicated. In root pruning and seedling age experiments, data of plant growth, photosynthetic rate, chlorophyll content, and root activity were analyzed by using *t*-test (P = 0.05). For other measurement, experimental data were analyzed by using Duncan's multiple range test at P = 0.05 in SAS 9.2 (SAS Inst. Inc., Cary, NC, USA) to identify least significant differences ($P < 0.05$).

RESULTS AND DISCUSSION

Effect of root pruning on growth

When determined after 75 days, pruning of the root system at the time of transplanting did not influence the growth of plants (Table 3-1). There was no significant difference in plant height with (174.5 cm) and without (173.3 cm) root pruning. Leaf area and fresh and dry weights of the roots and shoots were also unaffected by root pruning of the seedlings. No significant differences were observed in photosynthetic capacity between the plants with and without root pruning as the former was 13.9 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ and that of the latter was 13.1 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ (Table 3-2). No effect of root pruning on chlorophyll contents was observed. Photosynthetic rate increased when muskmelon plants were grown under the stress condition of continuously high salt (NaCl) concentration (Kwak et al., 2003). However, in this experiment, the stress condition from root pruning did not increase photosynthetic rate, which may be due to the recovery of volume and function during the cultivation process. There was no significant difference in root activity between the two different root pruning treatments.

However, root pruning resulted in increased rate of developing LYS on plants. The percentage of the plants showing LYS was 5 times greater in root-pruned plants (33.9%) than in the normal plants (6.1%). In spite of no differences in plant growth, fruit quality characteristics like length, weight,

Table 3-1. Effect of root pruning on growth characteristics of muskmelon plants 75 days after transplanting.

Treatment	Plant height (cm)	Leaf area (cm^2)	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
Control	174.5 a[z]	9,234 a	17.3 a	1.47 a	699.0 a	43.9 a
Root pruning	173.3 a	9,242 a	17.1 a	1.44 a	689.1 a	41.9 a

[z]Mean separation within columns by *t*-test at $P = 0.05$.

Table 3-2. Effect of root pruning on photosynthesis and root activity in muskmelon plants 75 days after transplanting.

Treatment	Net photosynthetic rate ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)	Chlorophyll contents (SPAD value)	Root activity[z] (Abs.)	Percentage of infected plant[y] (%)
Control	13.9 a[x]	51.1 a	0.27 a	6.1 ± 1.2
Root pruning	13.1 a	51.0 a	0.18 a	33.9 ± 12.2

[z]Root activity represents absorbance value of formazan product.

[y]Data are mean ± standard error.

[x]Mean separation within columns by *t*-test at $P = 0.05$.

net index, and sugar content were significantly different between the healthy and infected plants (Table 3-3). In addition, the root-pruned plants showing LYS had the smallest fruit size, weight, net index, and sugar content. The fruits formed on the root-pruned plants weighed 1,415 g with an 8.3°Bx reading. These values were below 70% of the normal muskmelon fruits. Fruits of root-pruned plants had 6.3 to 7.3 net indexes with fewer nets forming especially when infected with LYS. In healthy plants, net index and sugar content were significantly smaller in root-pruned plants than that of control plants. Consequently, root pruning increased LYS on muskmelon plants. Fruit quality also decreased when plants were infected with LYS.

There were significant differences between the control and root pruning for most of the root growth characteristics 15 days after transplanting (Table 3-4). The root-pruned plants developed roots with smaller length, diameter, surface area, and volume than the control. But root pruning did not influence negatively on root characteristics 87 days after transplanting (Table 3-5). Root lengths of the normal plant and root-pruned plants were 1,197 and 1,120 cm, respectively, but that of the plants showing LYS 695 cm. Root volume was greatest in the control plants (10.3 cm^3), followed by root pruning (8.28 cm^3) and LYS plants (6.99 cm^3). However, there were no differences in average diameter and surface area of roots among control, root pruning, and LYS plants. This result is similar to Takeshita (2004) who concluded that LYS was highly related to root developments, and small root volume was the cause of LYS. Although there

Table 3-3. Effect of root pruning on fruit quality in muskmelon plants 75 days after transplanting.

Plant	Treatment	Length (cm)	Width (cm)	Shape index	Weight (g)	Net index	Width of flesh (cm)	Sugar content (°Bx)
Healthy	Control	16.8 a[z]	15.7 a	1.07 a	2,246 a	1.1 a	4.2 a	11.9 a
	Root pruning	16.4 b	15.3 b	1.07 a	2,167 a	3.0 b	4.0 b	11.3 b
LYS	Control	15.7 c	15.0 b	1.05 a	1,869 b	6.3 c	4.1 ab	9.8 c
	Root pruning	15.1 c	13.9 c	1.09 a	1,415 c	7.3 c	3.9 b	8.3 d

[z]Mean separation within columns by LSD test at $P = 0.05$.

Table 3-4. Effect of root pruning on physical characteristics of root growth in muskmelon plants 15 days after transplanting.

Treatment	Length (cm)	Average diameter (mm)	Surface area (cm^2)	Volume (cm^3)
Control	6,408a	0.35a	700.6a	6.1a
Root pruning	4,095b	0.35a	452.3b	4.0b

[z]Mean separation within columns by LSD at $P = 0.05$.

Table 3-5. Effect of root pruning on physical characteristics of root growth in muskmelon plants 87 days after transplanting.

Treatment	Length (cm)	Average diameter (mm)	Surface area (cm^2)	Volume (cm^3)
Control	1,197 a[z]	1.10 a	362.9 a	10.3 a
Root pruning	1,120 a	0.96 a	324.7 a	8.3 ab
Control with yellowing symptoms	695 b	0.98 a	295.1 a	7.0 b

[z]Mean separation within columns by LSD at $P = 0.05$.

were no significant differences in surface area and average diameter of roots, root scanning data showed that the plants showing LYS had less lateral roots than the uninfected plants (Fig. 3-1).

The severity of plant collapse is influenced by the state of infested root systems (Crosby et al., 2000; Dias et al., 2002). More vigorous and branched root systems, regenerating new roots after infection, increase tolerance to vine decline (Walters and Wehner, 1994). Likewise, the reduction of lateral roots could have been associated with less fruit quality and severe LYS of muskmelon plants. Thus, root pruning is not recommended to prevent or overcome LYS on muskmelon plants.

Effect of seedling ages on growth

The influence of seedling ages at transplanting on the growth of muskmelon plants was investigated 75 days after transplanting (Table 3-6). Plant height, leaf area, and shoot fresh weight were significantly smaller when older seedlings with four to five true leaves were used than young seedlings with two to three true leaves. There were no differences in root fresh weight, root dry weight, and shoot dry weight between the young and older seedling treatments. The plants grown from young seedlings resulted in 9,234 cm^2 of leaf area and 699.0 g of shoot fresh weight, while the plants grown from old seedlings resulted in 8,093 cm^2 and 606.4 g, respectively, and the former showed the great numbers in other growth parameters.

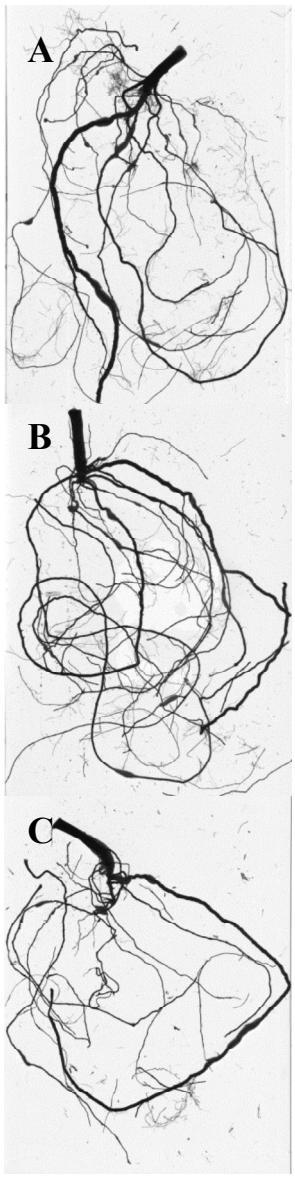

Fig. 3-1. Root scan pictures of the normal (A), root-pruned (B), and LYS-showing (C) plants of muskmelon.

Table 3-6. Effect of seedling ages on growth characteristics in muskmelon plants 75 days after transplanting.

Treatment	Plant height (cm)	Leaf area (cm^2)	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
2-3 leaves	174.5 a[z]	9,234 a	17.3 a	1.43 a	699.0 a	43.9 a
4-5 leaves	164.2 b	8,093 b	15.5 a	1.36 a	606.4 b	36.4 a

[z]Mean separation within columns by *t*-test at $P = 0.05$.

Seedling age had a significant effect on the top growth such as plant height, leaf area, and shoot fresh weight. These results are in line with those of other studies (Choi et al., 2002; Toshimitsu et al., 1979).

On the other hand, there were no significant differences in photosynthetic rate, chlorophyll, and root activity between the plants grown from young and older seedlings (Table 3-7). It was found that the root growth and activity was unaffected by seedling age, whereas Choi et al. (2002) found that the root growth of cucumber plants was affected by seedling age. However, the incidence of LYS increased when plants were grown from older seedlings (13.7%) compared to the young seedlings (6.1%). The plants showing LYS showed poor fruit quality than the healthy plants (Table 3-8). In fruit length, width, weight, and flesh width, the plants showing LYS that were grown from old seedlings had the smallest plant growth parameters compared to the unaffected plants. In heathy plants, there were no differences in fruit quality except net index. But, in the plants showing LYS that were grown from old seedlings had smaller fruit sizes, weight, net index, and sugar content. Muskmelon fruits harvested from the plants showing LYS had fruit weights less than 2,000 g. The greatest fruit weight of the muskmelon was 2,246 g in heathy plants grown from young seedlings, and the smallest was 1,297 g in the plants showing LYS that were grown from old seedlings. Net index of the healthy plants grown from young seedlings was 1.1, while net indexes of the healthy plants grown from old seedlings,

Table 3-7. Effect of seedling ages on photosynthesis and root activity in muskmelon plants 75 days after transplanting.

Treatment	Net photosynthetic rate ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)	Chlorophyll contents (SPAD value)	Root activity[z] (Abs.)	Percentage of infected plant[y] (%)
2-3 leaves	14.4 a[x]	42.6 a	0.27 a	6.1 ± 1.2
4-5 leaves	12.7 a	45.0 a	0.25 a	13.7 ± 3.2

[z]Root activity represents absorbance value of formazan product.

[y]Data are mean ± standard error.

[x]Mean separation within columns by *t*-test at $P = 0.05$.

Table 3-8. Effect of seedling ages on fruit quality in muskmelon plants 75 days after transplanting.

State	Treatment	Length (cm)	Width (cm)	Shape index	Weight (g)	Net index	Width of flesh (cm)	Sugar content (°Bx)
Healthy	Control	16.8 a[z]	15.7 a	1.07 a	2,246 a	1.1 a	4.2 a	11.9 b
	Aged	16.3 b	15.2 b	1.07 a	2,041 b	5.1 b	4.2 a	12.4 a
Infected	Control	15.7 c	15.0 b	1.05 a	1,869 c	6.3 c	4.1 a	9.8 c
	Aged	14.5 d	13.4 c	1.09 a	1,297 d	7.2 d	3.5 b	8.4 d

[z]Mean separation within columns by LSD test at $P = 0.05$.

the plants showing LYS that were grown from young seedlings, and the plants showing LYS that were grown from old seedlings were 5.1, 6.3, and 7.2, respectively. Except the healthy plants grown from young seedlings, all the other plants showed poor net formation.

Sugar contents of the healthy plants grown from young seedlings was 11.9°Bx, while that of the healthy plants grown from old seedlings was 12.4°Bx. The plants showing LYS showed the low sugar contents below 10°Bx regardless of seedlings ages. Although Seabra et al. (2004) found that seedling age had no influence on the commercial yields of cucumber when the seeds for were sown in large sized trays, we found that the plants grown from old seedlings had a much greater incidence of LYS and it reduced fruit size and quality in muskmelon. Hence, to reduce damages from LYS, the old seedlings that have more than three leaves at transplanting should not be used.

Effect of fruit number on growth

The effect of fruit numbers on the growth of muskmelon plants were investigated 75 days after transplanting (Table 3-9). Plant height, leaf area, shoot fresh weight, and shoot dry weight were significantly greater in the plants with one-fruit set. There were no differences on root fresh weight and root dry weight among all fruit number treatments. Plant height was greatest in the one-fruit plants (180.8 cm), followed by the two (168.5 cm) and three-fruit (165.2 cm) plants. Leaf area was greatest in the one-fruit plants

Table 3-9. Effect of number of fruits on growth characteristics in muskmelon plants 75 days after transplanting.

Treatment	Plant height (cm)	Leaf area (cm^2)	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
One	180.8 a[z]	10,117 a	17.4 a	1.45 a	800.8 a	67.6 a
Two	168.5 b	7,577 ab	17.1 a	1.54 a	586.8 b	52.4 b
Three	165.2 b	6,247 b	18.3 a	1.55 a	696.7 ab	58.6 ab

[z]Mean separation within columns by LSD test at $P = 0.05$.

(10,117 cm^2), followed by the two (7,577 cm^2) and three-fruit (6,247 cm^2) plants. Also, the one-fruit plants showed the greatest shoot fresh weight (800.8 g) and dry weight (67.6 g), followed by the three (696.7 and 58.6 g) and two-fruits (586.8 and 25.4 g) plants. Results are consistent with the previous ones that reported that the leaf area was reduced on condition of unrestricted fruit-load because competition among fruits had lowered the available pool of assimilates (Valantin-Morison et al., 1998, 2006).

In photosynthetic rate and chlorophyll contents, there were no significant differences among all treatments of varying the number of fruits left on vines (Table 3-10). This is in good agreement that reducing fruit load from one to five fruits per plant did not affect the net photosynthetic rate in cantaloupe (Valantin-Morison et al., 1998). Muskmelon plants having only one fruit per plant had a greater root activity (0.37) than those having two fruits (0.27) or three fruits (0.16). Plants with one fruit had a lower incidence of LYS (2.4%) compared to the plants having two (6.7%) and three fruits (13.3%). Moreover, a greater root activity of the plants with only one fruit resulted in lower incidence of LYS.

The plants showing LYS showed lower fruit quality (Table 3-11) compared to the plants not showing LYS. The plants with one fruit regardless of LYS provided greater fruit length and width and greater fruit weight compared to those of the plants having two or three fruits. There was no significant difference on fruit shape index among fruit number treatments.

Table 3-10. Effect of number of fruits on photosynthesis and root activity in muskmelon plants 75 days after transplanting.

Treatment	Net photosynthetic rate ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)	Chlorophyll contents (SPAD value)	Root activity[z] (Abs.)	Percentage of infected plant[y] (%)
One	14.3 a[x]	47.6 a	0.37 a	2.4 ± 1.5
Two	13.5 a	44.7 a	0.27 b	6.7 ± 6.6
Three	12.3 a	49.9 a	0.16 c	13.3 ± 6.6

[z]Root activity represents absorbance value of formazan product.

[y]Data are mean ± standard error.

[x]Mean separation within columns by LSD test at $P = 0.05$.

Table 3-11. Effect of number of fruits on fruit quality in muskmelon plants 75 days after transplanting.

State	Treatment	Length (cm)	Width (cm)	Shape index	Weight (g)	Net index	Width of flesh (cm)	Sugar content (°Bx)
Healthy	One	16.6 a[z]	15.5 a	1.07 a	2,132 a	1.1 a	4.3 a	12.4 a
	Two	15.1 b	14.0 b	1.07 a	1,564 c	6.2 b	3.8 b	11.6 a
	Three	14.9 b	13.9 b	1.08 a	1,485 c	6.0 b	3.7 b	11.6 a
LYS	One	16.1 a	15.1 a	1.07 a	1,904 a	6.6 b	4.1 a	10.1 b
	Two	12.4 c	11.7 c	1.06 a	813 d	9.0 c	NA[y]	NA
	Three	12.0 c	11.3 c	1.07 a	786 d	9.0 c	NA	NA

[z]Mean separation within columns by LSD test at $P = 0.05$.

[y]NA means non-available because of too small fruit size.

The net index was greatest when plants had only one fruit. The plants with one fruit that did not show LYS resulted in the greater mesocarp (flesh) thickness and higher sugar content compared to the plants having two or three fruits. The length of fruits of the plants having one fruit was greater whether they showed LYS (16.1 cm) or not (16.6 cm) compared to those of the plants having two (15.1 cm) or three fruits (14.9 cm).

The plants showing LYS that had two and three fruits left on vines resulted in 12.4 and 12.0 cm of fruit length, respectively. Similar results were found for fruit size and weights (Table 3-11). Especially, the greatest fruit weight was obtained when plants had one fruit whether they showed LYS (2,132 g) or not (1,904 g). The plants showing LYS with two or three-fruits left resulted in below 1,000 g of fruit weights which was not commercially suitable for market in Korea. The fruits harvested from the plants having one fruit left on vines, regardless of incidence of LYS, had the greater mesocarp thickness (4.1 and 4.3 cm), compared to those harvested from the plants having two or three fruits left (3.8 and 3.7 cm).

Number of fruits left on vines did not affect the sugar contents of fruits showing Brix readings of 11.6-12.4. As results, we can conclude that leaving more than two fruits per muskmelon plant is not a good in terms of LYS management and high-quality fruit production as well. Our results share with findings of McCollum et al. (1987) indicated that the first set fruit maintained a dominant position among the fruit sets and had specific

characteristics of reproductive development in cucurbits.

Effect of total leaf numbers on growth

The influence of the total number of leaves left per plants on the growth was investigated 75 days after transplanting (Table 3-12). Plant height, leaf area, root fresh weight, and root dry weight increased as the number of leaves increased. Shoot fresh and dry weights of the plants with 25 leaves were smallest among treatments. Plant height was greatest in the plants having 35 leaves (242.2 cm), followed by the plants having 30 leaves (203.2 cm), 25 leaves (166.8 cm), and 20 leaves (180.8 cm). Total leaf area of the plants having 35 leaves (11,877 cm^2) was greatest, followed by the plants having 30 leaves (11,222 cm^2), 20 leaves (10,117 cm^2), and 25 leaves (8,485 cm^2). The plants having 25 leaves resulted in the smallest plant height and leaf area. Shoot fresh weight of the plants having 20, 30, and 35 leaves was 800.8, 891.1, and 929.7 g, respectively, and there were no significant differences among treatments. The plants having 25 leaves resulted in the smallest shoot fresh (563.8 g) and dry (62.8 g) weights. Root fresh and dry weights in the plants having 20 leaves (17.4 and 1.45 g) were smaller than those observed in the plants having 25 leaves (20.5 and 1.80 g), 30 leaves (23.7 and 2.05 g), and 35 leaves (21.4 and 1.86 g).

Net photosynthetic rates increased as the number of leaves increased as well (Table 3-13). The plants having more than 25 leaves showed greater

Table 3-12. Effect of total number of leaves on growth characteristics of muskmelon plants 75 days after transplanting.

Treatment	Plant height (cm)	Leaf area (cm^2)	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
20 leaves	180.8 c[z]	10,117 b	17.4 b	1.45 b	800.8 ab	67.6 ab
25 leaves	166.8 c	8,485 c	20.5 ab	1.80 ab	676.6 b	62.8 b
30 leaves	203.2 b	11,222 ab	23.7 a	2.05 a	891.1 a	80.9 a
35 leaves	242.2 a	11,877 a	21.4 a	1.86 a	929.7 a	81.1 a

[z]Mean separation within columns by LSD test at $P = 0.05$.

Table 3-13. Effect of total numbers of leaves on photosynthesis and root activity of muskmelon plants 75 days after transplanting.

Treatment	Net photosynthetic rate ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)	Chlorophyll contents (SPAD value)	Root activity[z] (Abs.)	Percentage of infected plant[y] (%)
20 leaves	14.3 b[x]	47.6 a	0.38 a	6.1 ± 1.2
25 leaves	18.2 a	68.5 a	0.34 a	16.7 ± 16.6
30 leaves	17.1 ab	47.5 a	0.32 a	13.3 ± 6.6
35 leaves	17.1 ab	50.8 a	0.36 a	6.7 ± 6.6

[z]Root activity represents absorbance value of formazan product.

[y]Data are mean ± standard error.

[x]Mean separation within columns by LSD test at $P = 0.05$.

photosynthetic rates than those having the smaller numbers of leaves. It was greatest in the plants having 25 leaves (18.2 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), followed by plants having 30 and 35 leaves (17.1 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) and with 20 leaves (14.3 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$). On the other hand, there were no significant differences in chlorophyll content and root activity among treatments with different leaf numbers. The occurrence of LYS in the plants having 25 and 30 leaves (16.7 and 13.3%, respectively) was more than twice as great as in the plants having 20 leaves (6.1%).

Fruit quality was not significantly affected by the number of leaves left on vines (Table 3-14). The plants having a larger number of leaves produced longer and wider fruits resulting in the greater fruit weight compared to those than the plants having smaller number of leaves. There was no significant difference in fruit shape index as affected by number of leaves per plant. The mesocarp thickness and sugar content of the fruits harvested from plants having 20 leaves that showed LYS were significantly smaller than those of the plants having the larger numbers of leaves that did not show LYS. The length of fruits of the plants having 30 and 35 leaves that did not show LYS were 18.2 and 18.0 cm, respectively, while they were 18.6 and 17.5 cm, respectively when the plants showed LYS. The shortest fruit length was 16.1 cm in the plants having 20 leaves that showed LYS, followed by the healthy plants having 20 leaves (16.6 cm).

The same tendency was found in the width and weight of fruits. The plants

Table 3-14. Effect of total numbers of leaves on fruit quality of muskmelon plants 75 days after transplanting.

State	Treatment	Length (cm)	Width (cm)	Shape index	Weight (g)	Net index	Width of flesh (cm)	Sugar content (°Bx)
Healthy	20 leaves	16.6 cd[z]	15.6 de	1.07 a	2,140 c	1.1 a	4.3 ab	12.4 a
	25 leaves	17.2 bcd	16.0 cde	1.08 a	2,323 c	3.0 bc	4.3 ab	11.9 ab
	30 leaves	18.2 ab	17.0 abc	1.08 a	2,900 a	1.5 ab	4.4 ab	11.9 ab
	35 leaves	18.0 ab	16.9 abc	1.07 a	2,894 a	1.3 a	4.4 ab	12.5 a
LYS	20 leaves	16.1 d	15.1 e	1.07 a	1,904 c	6.6 e	4.1 b	10.1 c
	25 leaves	18.1 ab	17.2 ab	1.05 a	2,828 ab	4.0 cd	4.6 a	11.2 abc
	30 leaves	18.6 a	17.4 a	1.07 a	3,100 a	2.0 ab	4.7 a	10.5 bc
	35 leaves	17.5 abc	16.2 bcd	1.08 a	2,388 bc	5.0 d	4.5 ab	12.1 a

[z]Mean separation within columns by Duncan′s multiple range test at $P = 0.05$.

having 35 and 30 leaves that did not show LYS had 2,894 and 2,900 g fruit weights, respectively. When LYS was found, the plants having 30 and 25 leaves on the vine produced fruits weighing 3,100 and 2,828 g, respectively, as compared to 2,388 g of fruit weight of the plants having 35 leaves. Healthy plants having small numbers of leaves such as 20 and 25 leaves and the plants showing LYS, especially those having small numbers of leaves such as 20 and 25 leaves resulted in small fruit weights (2,140, 2,323, 1,904, and 2,828 g, respectively). All the plants showing LYS, except when it had 30 leaves, and the healthy plants having smaller numbers of leaves than 25 resulted in poor net formation on the fruit surface (net indexes greater than 3.0). The healthy plants having 20, 30, 35 leaves and the plants showing LYS having 30 leaves had net index of 1.1, 1.5, 1.3, and 2.0, respectively.

The plants having 20 leaves that showed LYS developed fruits that had significantly smaller flesh (mesocarp) thickness than, the plants having greater numbers of leaves. The plants having 20 leaves that did not show LYS resulted in the lowest sugar content (10.1°Bx), followed by the plants having 30 leaves showing LYS (10.5°Bx). The higher sugar contents of fruits were found in the plants having 35 leaves whether they showed LYS (12.1°Bx) or not (12.5°Bx). In production of fruit vegetables like muskmelon and watermelon, number of leaves per fruit should be maintained in an appropriate range to produce high quality fruits with high soluble solid content and/or good net formation (Hagiwara and Oware, 1942;

Hubbard et al., 1989; Takagi, 1940). Reduction of leaf area inhibited vegetative growth and decreased net photosynthesis of the plant resulting in decreased fruit quality in muskmelon (Kang, 2010; Long et al., 2004). It may be suggested that more than 25 leaves must remain to reduce LYS and other physiological disorders when one fruit is produced from a muskmelon plants.

Influence of feeding leaf positions on growth

The influence of the number and the position of the leaves on growth of muskmelon plants were investigated 75 days after transplanting (Table 3-15). More the number of feeding leaves above the fruit-bearing node on the vine, the greater the plant height, root fresh and dray weights, shoot fresh and dry weights. The greatest plant growth was found in the BL+10 treatment (ten more leaves were kept above the fruit-bearing node than below it), followed by the BL+5 treatment (five more leaves were kept above the fruit-bearing node than below it), the BL=0 treatment (same number of leaves left above and below the fruit-bearing node), and the BL-5 treatment (five leaves less above the fruit-bearing node than below it). The plant height and shoot fresh weight were greatest (234.5 cm and 904.9 g) in the BL+10 treatments, followed by the BL+5 (207.8 cm and 714.4 g), BL=0 (164.0 cm and 619.7 g), and BL-5 (123.8 cm and 473.8 g) treatments. Plants having five or ten more leaves above the fruit-bearing node produced the

Table 3-15. Effect of number of feeding leaves above fruit-bearing node on vine on growth characteristics in muskmelon plants 75 days after transplanting.

Treatment	Plant height (cm)	Leaf area (cm^2)	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
BL –5[z]	123.8 d[y]	6,241 a	14.5 bc	1.24 b	473.8 d	38.0 c
BL=0	164.0 c	8,000 a	12.9 c	1.22 b	579.6 c	46.5 bc
BL +5	207.8 b	8,426 a	16.9 ab	1.50 ab	734.8 b	52.3 b
BL +10	234.5 a	9,456 a	19.2 a	1.66 a	904.9 a	72.8 a

[z]BL (bottom leaves) means number of leaves under fruit set node.

[y]Mean separation within columns by LSD test at $P = 0.05$.

greater biomass with greater fresh and dry weights of shoots and roots than plants having equal or five less leaves above the fruit-bearing node. The greater the number of the feeding leaves placed above the fruit-bearing node, the heavier the shoot weights compared to the plants having more foliage below the fruit-bearing node.

The effect of leaf number above and below the fruit-bearing node on photosynthesis, root activity, and incidence of LYS was investigated 75 days after transplanting (Table 3-16). The plants in the BL+5 treatment resulted in significantly smaller photosynthetic rate and chlorophyll content (13.5 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ and 45.6 in SPAD value) compared to those in other treatments. But, the root activity was greatest in the plants in BL=0 and BL+5 treatments (0.38 and 0.40 in absorbance values of formazan products), followed by the BL-5 and BL+10 treatments (0.23 and 0.31). The incidence of LYS decreased as the number leaves above the fruit-bearing node increased, and percentage of the plants showing LYS in the BL-5 treatment (45.0%) was 1.4 times as great as that in the BL=0 treatment (33.1%). Plants in the BL+5 and BL+10 treatments showed the lower incidence of LYS (76 and 53%, respectively) compared to that in the BL=0 treatment. This result suggests that more than five more feeding leaves must be kept above the fruit-bearing node than below it to effectively reduce the incidence of LYS.

Number of leaves kept above and below the fruit-bearing node also influenced on the size and quality of fruits. Length, width, and weight of fruits

Table 3-16. Effect of number of feeding leaves above fruit-bearing node on vine on photosynthesis and root activity in muskmelon plants 75 days after transplanting.

Treatment	Net photosynthetic rate ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)	Chlorophyll contents (SPAD value)	Root activity[z] (Abs.)	Percentage of infected plant[y] (%)
BL –5[x]	15.1 a[w]	49.0 abc	0.23 b	45.0 ± 5.0
BL=0	15.6 a	51.4 ab	0.38 a	33.1 ± 13.1
BL +5	13.5 b	45.6 c	0.40 a	25.0 ± 15.0
BL +10	14.2 a	52.5 a	0.31 b	17.5 ± 7.5

[z]Root activity represents absorbance value of formazan product.

[y]Data are mean ± standard error.

[x]Mean separation within columns by Duncan′s multiple range test at $P = 0.05$.

[w]BL(bottom leaves) means number of leaves under fruit set node.

75 days after transplanting significantly decreased in the BL-5 treatment regardless of LYS (Table 3-17). The plants not showing LYS in the BL=0, BL+5, and BL+10 treatments had the greater fruit sizes than that in the BL-5 treatment. The plants showing LYS in the BL+10 and BL+5 treatments resulted in the greater fruit sizes compared to the BL-5 treatment. There was no significant difference in fruit shape index among treatments. Net formation of the fruits in the BL-5 treatment was the smallest regardless of LYS.

Fruit mesocarp thickness and sugar contents for the plants that showed LYS in the BL-5 treatment could not be measured due to too small size. It was the smallest in the plants that did not show LYS in the BL-5 treatment among other treatments. Sugar content of fruits was greater in the plants not showing LYS than that in the plants showing LYS. Among the plants showing LYS, those in the BL=0 treatment resulted in the lowest sugar content. The plants showing LYS in the BL-5 treatment resulted in the smallest fruit size and weight (length 12.9 cm; width 11.9 cm; weight 896 g). Fruit weights were significantly the smallest in the plants showing LYS in the BL-5 treatments, followed by the plants not showing LYS in the BL-5 treatment plants (1,497 g) and those showing LYS in the BL=0 treatment (1,596 g), and those in other treatments (over 2,000 g).

The greater net index of fruit was found in the plants not showing LYS in the BL=0 and BL+10 treatments (4.3 and 4.5), while that in other treatments was 5.7 regardless of LYS. The smaller numbers of leaves left above the

Table 3-17. Effect of number of feeding leaves above fruit-bearing node on vine on fruit quality in muskmelon plants 75 days after transplanting.

State	Treatment	Length (cm)	Width (cm)	Shape index	Weight (g)	Net index	Width of flesh (cm)	Sugar content (°Bx)
Healthy	BL –5[z]	14.7 d[y]	13.9 c	1.05 a	1,497 b	9.0 d	3.5 b	10.7 ab
	BL=0	16.5 ab	15.3 ab	1.08 a	2,082 a	4.3 a	4.0 a	11.6 a
	BL +5	16.4 ab	15.4 a	1.05 a	2,018 a	6.0 bc	3.9 a	11.4 a
	BL +10	17.2 a	16.1 a	1.08 a	2,281 a	4.5 ab	4.0 a	11.5 a
LYS	BL –5	12.9 e	11.9 d	1.08 a	896 c	8.8 d	NA[x]	NA
	BL=0	15.3 cd	14.4 bc	1.07 a	1,596 b	6.6 c	3.8 a	8.8 c
	BL +5	16.0 bc	15.3 ab	1.05 a	1,950 a	6.3 c	3.8 a	9.4 bc
	BL +10	17.0 ab	15.7 a	1.08 a	2,175 a	5.7 abc	4.1 a	9.4 bc

[z]BL (bottom leaves) means number of leaves under fruit set node.

[y]Mean separation within columns by Duncan′s multiple range test at $P = 0.05$.

[x]NA means non-available because of too small fruit size.

fruit-bearing node negatively influenced the mesocarp thickness of fruits. There was little difference in fruit mesocarp thickness when the plants had equal or the greater numbers of leaves above the fruit-bearing node than below it. The plants showing LYS in the BL-5 treatment resulted in the lowest sugar content (8.8°Bx), followed by the plants showing LYS in the BL+5 and BL+10 treatments (9.4°Bx both). The greater sugar contents (about 11°Bx) were measured in fruits of the plants not showing LYS (higher than 11°Bx). Fruit quality (length, diameter, weight, thickness of mesocarp, soluble solid content, and net index) was affected by the increased number of leaves above the fruit-bearing node in muskmelon plants (Hwang et al., 1998; Kamitani, 1967; Shishido et al., 1992). Hence, maintaining an enough number of feeding leaves above the fruit-bearing node, not below, is a good management strategy for reducing LYS in greenhouse muskmelon cultivation (Han and Park, 1993).

For *Cucurbit aphid borne yellowing virus* (CABYV) as an insect vector, control methods can include removal of weeds to eliminate virus or vector reservoirs before planting, protection of seedlings from the vectors, installing insect-proof nets on doors and ventilation windows (Janssen et al., 2003; Lecoq and Nikolaos, 2014). Use of virus resistant hybrids or commercial cultivar is easier, more efficient, and environmentally friendly than any other ways to control viral diseases. However, breeding the resistant variety to CABYV has been conducted mainly for germplasm

evaluation and introgression of the resistance gene into commercially acceptable melon cultivars (Lecoq et al., 2004; Moury et al., 2011).

In addition to controlling the insect vector, introduction and application of various cultural practices that may be effective in managing LYS are needed. To minimize the incidence of LYS, seedlings should have less than three fully-expanded leaves at transplanting and root pruning is not recommended. Producing only one fruit on a plant is an appropriate strategy both for managing LYS and maintain the high quality of fruits with good net formation. More than 25 leaves with an enough number of feeding leaves above the fruit-bearing node, not below, should be maintained on each plant to negate the deterioration of fruit quality influenced by LYS.

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CONCLUSIONS

The causal factors of leaf yellowing symptoms (LYS) as well as the developmental and physiological characteristics of LYS in muskmelon were investigated in three separate experiments in this study.

Results of the experiment in chapter 1 revealed that LYS in muskmelon is caused by *Cucurbit aphid-borne yellows virus* (CABYV) which has not been reported in Korea until now. The complete genome sequences of 22 isolates of CABYV, collected from muskmelons showing LYS in Korea during the years 2013-2014, were determined and analyzed comparatively along with previously reported CABYV genome sequences. The CABYV Korean isolates were clustered with the Asian group over 94% nucleotide sequence identity. The, nucleotide sequence identities of the CABYV Korean isolates with other groups were 87-89% with Mediterranean group, 88% with Taiwanese group, 81-84% with CABYV-R group, and 72% with the other *Polerovirus*, *Melon aphid-borne yellows virus*, respectively. It is concluded that LYS in muskmelon is not merely a physiological disorder but a virus disease caused by CABYV and spread by aphids.

Findings from the experiment in chapter 2 indicated that the tissue contents of glucose, fructose, and sucrose in leaves were greater in the plants showing LYS than in the healthy plants and those in stems, fruits, and roots were slightly smaller in the plants showing LYS compared to

unaffected plants. It was also found that the occurrence of LYS is concomitant with reduced root mass and size in affected plants. The elevated levels of free sugars in the leaves showing LYS in comparison to the leaves may have been due to poor translocation of photosynthates from the source leaf to other organs in affected plants. Some closed sieve elements found in the leaf veins might have restricted the movement of free sugars to other sink tissues in affected plants.

Results of chapter 3 showed that the partial removal (root-pruning) of the seedling root system during transplanting increased LYS and decreased fruit quality in plants showing LYS. Seedling ages did not significantly affect the growth, photosynthesis, and root activity. But the percentage of LYS incidence was 2 times greater when aged seedlings were transplanted (13.7%) with reduced fruit weights, compared to the young seedlings (6.1%). To minimize damage by LYS, muskmelon seedlings at the time of transplanting should have 3 or less fully expanded leaves. In order to reduce incidence of LYS, no more than one fruit should be kept on each plant. Muskmelon plants with over 25 leaves showed greater photosynthetic rates. Incidence of LYS was greater in plants with 25 and 30 leaves than plants with 35 leaves. Therefore, it is suggested that more than 25 leaves per plant are needed to minimize physiological damages from LYS. Incidence of LYS decreased as the number of feeding leaves above the fruit-bearing node increased.

Findings from various experiments detailed in the three chapters of this thesis revealed that the cause of the rapidly spreading LYS of muskmelon in Korea is not a physiological disorder but an infection by CABYV which has not been reported in the country, yet. Since aphids are an important vector for CABYV, in addition to controlling the insect vector, introduction and application of various cultural practices that are effective in managing LYS are needed. In this study, some of the cultural techniques and management strategies suitable for reducing the impact of potentially important LYS epidemic are suggested.

ABSTRACT IN KOREAN

본 연구는 최근 머스크멜론 재배 지역에서 확산되고 있는 잎 황화 증상(LYS)의 발생 원인을 구명하고 이를 억제하기 위한 재배 기술을 개발하기 위해 수행하였다. 제1장에서는 머스크멜론 LYS의 발생 원인을 구명하였다. 국내 박과 감염 8종의 바이러스(CMV, MNSV, CGMMV, SqMV, WMV, KGMMV, PRSV, ZYMV)에 대해 전자 현미경 검경 및 RT-PCR을 수행하였으나 바이러스가 진단되지 않았다. 이에 국내 보고되지 않은 새로운 바이러스로 의심되어 차세대 유전체 염기 서열 분석(next generation sequencing, NGS)을 이용하여 진단한 결과, 박과 진딧물 바이러스(*Cucurbit aphid-borne yellows virus*, CABYV)임을 확인하였다. 또한 CABYV 특이 프라이머를 선발하고 이를 이용하여 LYS를 보이는 잎을 대상으로 RT-PCR을 수행한 결과 국내 머스크멜론 LYS는 Luteoviridae과 *Polerovirus*속에 속하는 직경 23nm의 구형 바이러스로 진딧물에 의해서 영속 전염(순환형)되는 CABYV 감염에 의한 것임을 최초로 확인하였다.

2013-2014년 동안 국내에서 황화 증상을 보이는 머스크멜론 22점을 수집하여 이의 전체 유전자 염기 서열을 이미 보고되어 있는 CABYV의 유전자 염기 서열과 비교 분석하였다. 그 결과

전체 유전자는 길이가 5680-5684 뉴클레오타이드이고 6개의 오픈 리딩 프레임으로 암호화되어 있었으며, 199개 뉴클레오타이드의 비암호화 부분에 의해 2개의 영역으로 분리되어 있었다. 그들의 유전체 구성은 전형적인 *Polerovirus*속에 속하는 것으로 나타났다. 전체 염기 서열 분석을 토대로 하여 CABYV를 4개의 그룹 즉 아시아, 지중해, 타이완 그리고 R 그룹으로 구분하였다. 우리나라에서 분리한 CABYV 계통은 전체 염기 서열이 각각 아시아 그룹 94% 이상, 지중해 그룹 87-89%, 타이완 그룹 88%, CABYV-R 그룹 81-84%, MABYV와 72%의 상동성을 보였다. 우리나라의 CABYV는 일본 계통과 상동성이 높은 것으로 확인하였다. 이로써 우리나라 머스크멜론의 LYS는 생리 장해가 아니라 진딧물에 의해 매개되는 CABYV임을 최초로 규명하였다.

제2장에서는 CABYV 감염이 머스크멜론의 생육, 과실 비대 및 동화 양분의 전류에 미치는 영향을 알아보기 위하여 광합성 능력, 뿌리 활력, 무기 성분, 엽육 세포 및 동화 양분 전류 등을 조사하였다. 광합성 능력은 황화엽이 정상엽의 1/3 수준으로 낮았다. 뿌리 활력은 정상 개체와 비교하여 황화 증상을 보이는 개체가 1/2 수준으로 낮았다. 잎의 무기 성분은 정상엽의 모든 무기 성분 함량이 황화엽에서 보다 2배 이상 높았고, 특히 철분의

함량은 약 20배 높았다. 정상 개체와 황화 증상을 보이는 개체의 엽육 조직을 광학 현미경으로 관찰한 결과, 울타리 조직과 해면 조직의 형태는 차이를 보이지 않아 황화 증상은 잎의 조직에 영향을 미치지 않는 것으로 판단되었으나, LYS를 보인 개체의 잎은 통도 조직 주변을 중심으로 전분이 많이 축적되어 있었다. 착과 후 55일 경과한 식물체의 부위별 유리당 함량을 분석한 결과, LYS를 나타낸 식물체의 잎 내 유리당 함량과 정상 식물체 뿌리의 유리당 함량이 높았다. 과실의 유리당 함량은 LYS이 심한 식물체의 과실에서 정상이거나 증상이 약하게 나타난 식물체의 과실에서보다 낮게 나타났다. LYS이 발생하면 정상 생육을 보인 식물체와 비교하여 잎 조직 내 당 함량이 높았고, 뿌리와 과실의 당 함량이 낮게 나타났다. 그 원인을 알아보기 위하여 주사 전자 현미경으로 체관 조직을 관찰한 결과 황화 증상을 보인 잎의 사관 조직에서 막힘 현상이 나타났다. 즉, 당 함량이 정상엽에서보다 황화엽에서 더 높았던 것은 잎에서 광합성으로 생성된 동화 양분이 식물체 내 다른 기관으로 전류될 때 사관 조직의 막힘 현상이 장해 요인으로 작용하기 때문으로 판단되었다.

제3장에서는 양분 수용부와 공급부 크기에 대한 처리가 머스크멜론의 생육, LYS 발생 정도 및 과실 품질에 미치는 영향을 조사하였다. 정식 시 단근에 의한 뿌리의 양 감소, 정식 시기

지연에 따른 노화 묘 정식, 착과 수 조절, 주간 엽수, 결과지 상부 엽수가 머스크멜론의 지상부와 지하부의 생육, 광합성 능력, 그리고 LYS 발생에 미치는 영향을 조사하였다. 이러한 처리가 LYS 발생 시 과실의 품질에 미치는 영향을 분석한 결과, 정식 묘의 지하부 발육을 촉진시키고, 묘의 노화를 방지하며, 주당 1과를 착과 시키고 주당 엽수는 25매 이상, 결과지 위쪽의 잎 수를 아래쪽 보다 5매 이상 많게 유지하는 것이 황화 발생 및 피해를 경감할 수 있었다.

본 연구들의 결과를 종합해 보면, 최근 국내의 머스크멜론 재배지에서 급속하게 발생하고 있는 LYS 증상은 생리적 원인보다는 진딧물에 의한 바이러스 이병이 원인임을 국내 최초로 구명하였다. 이로써 LYS 피해와 이 증상의 확산을 줄이기 위해서는 일차적으로는 바이러스 매개충인 진딧물을 사전에 방제해야 할 것으로 판단된다. 또한 재배적 방법으로 LYS 발생 및 피해를 경감시킬 수 있는 기술을 개발하여 보급함으로써 우리나라 머스크멜론의 주 재배 작형인 봄부터 여름에 재배하는 멜론 재배자의 안정 생산과 소득 증대에 기여할 것으로 기대된다.