The introduction of the new control method of plant viruses infection for organic farming

S.B. Kwon[1] and J.S. Chung[2]

[1]*Gangwon Agricultural Research and Extension Services, Chunchon, Korea* (snbkwon@korea.kr)
[2]*B.I.G Co., Ltd, Daejeon, Korea*

Implications

Pepper mild mosaic virus (PMMoV) and cucumber mosaic virus (CMV) are economically important viruses, which cause enormous losses by infecting various vegetable crops. worldwide. Various strategies based on the avoidance of sources of infection, control of vectors have been conventionally employed to minimize the losses caused by these viruses. These strategies, however have not been effective as control tools. We have found that the extracts of gallnuts from *Quercus dentate* and *Rhus javanica* strongly inhibit PMMoV and CMV infection. The gallnuts are plant excretion produced when irritants are released by the larvae of gall insects. They contain high amounts of tannic acid such as gallic acid and ellagic acid. Also the gallnut extracts are widely used in pharmaceuticals, food and feed additives, it is safe natural material which can be used in organic farming. Our results indicate that they are potent plant viruses inhibitors that maybe used to prevent the spread of viruses infections in the cultivating farm.

Background and objectives

This study was undertaken to develop environmental friendly new anti plant-viral agents using natural materials of plant resources, several substances have been reported as plant viral inhibitors such as milk, polysaccarides (Sano 1999). Also many plant resources have been reported to have potent antiviral activity and some of them have already been used to treat animal and human who suffer from viral infection (Hudson 1990), because they virtually constitute a rich source of bioactive. However, little work has been done to control plant viruses by using these natural products in spite of their excellent pharmacologies signification. We found the extracts from gallnuts of *R. javanica* and *Q. dentate* which strongly inhibited the infection of PMMoV and CMV. Here, we introduce several properties of the antiviral activities by the gallnut extracts.

Key results and discussion

The mixed treatment effect of Qbyrus-1 against infections of each virus (PMMoV and CMV) to local infection plant was measured to be 100% to PMMoV and 100% to CMV in 1% conc. As shown in Tab. 1, the pre-treatment effects against infection of each virus to local host plants were estimated to be 75 to 97.5% for PMMoV and 70.6 to 99.0% for CMV in 0.1 to 1% conc.

Tab.1: Inhibitory effects of Qbyrus-1 against local infection of PMMoV and CMV

Treatment	Concentration (mg mL^{-1})	Inhibition (%)	
		PMMoV	CMV
Qbyrus-1	10	97.5±1.5	99.0±1.0
	5	93.0±1.2	93.3±0.6
	2	80.2±2.4	84.0±0.5
	1	75.1±0.5	70.6±2.2
Water(control)	-	0.0±0.0	0.0±0.0

To assay the absorption of the antiviral composition of extracts to the inside of the leaf tissue, the extracts (10mg/ml) were applied on the backside of the half leaves of host plants *Nicotiana glutinosa* or *Chenopodium amaranticolor*. Virus infection on the upper surface was inhibited by 55.7% for PMMoV and 63.8% for CMV. These results indicated that the inhibitory effect of the extract was induced not only by barrier effect, but also by another unclear antiviral effects.

When the Qbyrus-1 was sprayed before virus inoculation, PMMoV or CMV infection ratio through the leaves of their systemic host was remarkably reduced in greenhouse conditions (Tab.2). This result showed that the inhibitory activity of Qbyrus-1 was superior to the effects of the known viral inhibitors such as skim-milk or Lentemin (Oka 2008). The Qbyrus-1 was apparently harmless to the tobacco seedlings, judging from the fact that there's no change of leaf colours and there's no symptoms of growth inhibition.

Tab. 2 : Inhibitory effects of Qbyrus-1 against systemic infection of PMMoV or CMV

Treatment	No. plants infected / inoculated			
	PMMoV		CMV	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Qbyrus-1	2/20	4/20	8/20	5/20
Skim milk	7/20	12/20	9/20	5/20
Water(control)	20/20	20/20	20/20	20/20

The PMMoV particles were almost segmented by mixing Qbyrus-1, but not affected in the absence of Qbyrus-1. It's thought that one mode of action of Qbyrus-1 is inactivation of the virus due to the destruction of viral particles.

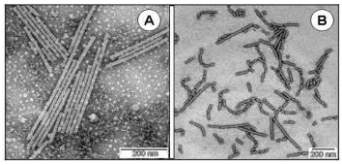

Fig. 1 : Electron micrographs of PMMoV particles in the absence(A) or presence of Qbyrus-1 (B), Bar=200 nm.

How work was carried out?

The fresh Gallnuts of *Q. Dentata* and *R. javanica* were sampled in Korea. The dried samples were ground using a blender, extracted and filtered. The filtrate was concentrated *in vacuo* at 40℃ and freeze-dried. Furthermore, the inhibitor named "Qbyrus-1" formulated from these gall extracts was tested for its inhibitory effects on PMMoV and CMV infection to each local lesion host plant. *N. glutinosa* was used for local lesion assay of PMMoV infection, while *N. tabacum* cv. samsun was used for systemic assay. *C. amaranticolor* was used for local lesion assay of CMV infection, and *N. tabacum* cv. Samsun NN was used to systemic infection assay of CMV. Antiviral activities in local lesion assay plants were tested using the half-leaf method (Kwon et al. 2010). For the assay of the systemic host, Qbyrus-1 was sprayed onto the leaves of assay plants and the viruses were inoculated onto the leves of each assay plants. The inhibition ratio was confirmed 4 weeks after inoculation by ELISA. 1% Qbyrus-1 was mixed with the purified PMMoV in 10mM phos. buffer (pH7), observed with transmission electron microscopy.

References

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