BIOLOGICAL CHARACTERIZATION OF BUD NECROSIS VIRUS DISEASE IN WATERMELON

(*Citrullus lanatus* T.)

By

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(2012-11-202)

THESIS
Submitted in partial fulfillment of the requirement
for the degree of

Master of Science in Agriculture

(PLANT PATHOLOGY)

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2014

DECLARATION

I hereby declare that the thesis entitled **"Biological characterization of bud necrosis virus disease in watermelon (*Citrullus lanatus T*)."** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Vellanikkara
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DECLARATION

Certified that the thesis entitled **"Biological characterization of bud necrosis virus disease in watermelon (*Citrullus lanatus* T)."** is a bonafide record of research work done independently by Ms. ASWATHI K.K. (2012-11-202) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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ACKNOWLEDGEMENT

First and foremost, I bow my head to the GOD ALMIGHTY who enabled me to successfully complete the thesis work.

I place on record my deep sense of gratitude and indebtedness to Dr. Vimi louis, Associate Professor, Department of Plant Pathology as Chairperson of my advisory committe. I am extremely delighted to place on record my profound sense of gratitude for her guidance, critical and valuable suggestions, constant encouragement and unfailing patience during the course of investigation

I avail this opportunity to express my deep sense of gratitude and sincere thanks to Dr. Koshy Abraham, Professor and Head, Department of Plant Pathology and member of my advisory committee for the valuable suggestions and whole hearted support extended throughout the course of study.

Words are inadequate to express my sincere gratitude to Dr. Anita Cherian, Professor, Banana research station, Kannara for her constant support, expert and valuable advice and whole hearted co-operation and guidance extended throughout the investigation.

I consider it as my privilege to express my deep felt gratitude to Dr. Pradeep Kumar, Associate Professor, Kerala Agricultural University and member of my advisory committee for his invaluable help, unbounded support, suggestions and constant encouragement throughout the period of the work.

I take this opportunity to thank Dr. Madhu Subramanian, Asstistant Professor, Department of Agricultural Entomology, Kerala Agricultural University and member of my advisory committee for the critical comments, motivation and encouragement. I thank him for all the help and co-operation he has extended to me.

I wish to express my grateful thanks to Dr. Haseena Bhaskar, Asssociate Professor, Department of Agricultural Entomology, College of Horticulture for the timely help in identification of thrips.

I would like to express this opportunity to extend my profound sense of gratitude and sincere thanks to Dr. S. Krishnan, Professor & Head, Department of Agriculture Statistics, College of Horticulture for his immense help extended for the statistical analysis of the data.

My sincere obligations are due to Dr. Sally K. Mathew, Professor; Dr. S. Beena, Professor; Dr. T. Sheela Paul, Professor, Department of Plant Pathology, for their valuable suggestions and co- operation.

I take this opportunity to extend my gratitude to all the non- teaching staff members, research assistants and labourers of the Department of Plant Pathology, College of Horticulture for the help offered at different stages of study.

I express my deep sense of gratitude to all my beloved seniors, Mrs. Deepa James, Mrs. Gleena Mary, Mrs. Jyothi Sara Jacob, Ms. Remya J.S., Mrs. Hima, Ms. Asha v. Pillai, Ms. Sreelakshmi., Mrs. Roshin Varghese and Ms. Vaishakhi, for their valuable assistance and guidance during the entire period of my research. Special word of thanks to, all juniors for their prompt help and co-operation during the entire period of study.

I duly acknowledge the encouragement, moral support and timely persuations by my dear friends, Aswini Haridasan, Suchithra B. Shenoy, Athira P. S., Aparna V.s., Rekha K.G., Anshee Antony, Preethy T.T., Radhika, Arun Paul, Prasad, Sandhya P.T., Maheswari R.S. Nair and Deepika not only in my research but also throughout my course programme.

My special thanks go to Mr. Aravind of student's computer club, COH for rendering necessary help whenever needed.

The award of KAU Junior Research Fellowship is thankfully acknowledged

I offer my salutation at the feet of my Father and Mother, whose blessings enabled me to undertake this venture successfully. Iam deeply indebted to my brother Anoop for his unbound love, moral support and unfailing inspiration throughout this research work.

Aswathi K. K.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ABBREVIATIONS

CaCV	- *Capsicum chlorosis virus*
cm	- Centimeter
CI	- Coefficient of Infection
CP	- Coat Protein
DAC-ELISA	- Direct Antigen Coating ELISA
DAS	- Days After Sowing
ELISA	- Enzyme Linked Immuno Sorbent Assay
g	- Gram
GBNV	- *Groundnut bud necrosis virus*
GYSV	- *Groundnut yellow spot virus*
h	- Hour
IYSV	- *Iris yellow spot virus*
KAU	- Kerala Agricultural University
l	- Litre
m	- Metre
ml	- Millilitre
µg	- Microgram
µl	- Microlitre
min	- Minute(s)
M	- Molar
nm	- Nanometer
PBNV	- *Peanut bud necrosis virus*
PBS	- Phosphate Buffered Saline
PBS-T	- PBS Tween
PDI	- Per cent Disease Incidence
PDS	- Per cent Disease Severity
PVP	- Poly Vinyl Pyrrolidone
rpm	- Rotations per minute
sec	- Second(s)
v/v	- Volume by volume
WBND	- Watermelon Bud Necrosis Disease
WBNV	- Watermelon *bud necrosis virus*
w/v	- Weight by volume

Introduction

1. INTRODUCTION

Watermelon, *Citrullus lanatus* (Thumb.) is an annual trailing creeper belonging to the family Cucurbitaceae. The crop is considered to be a native of Africa. Its cultivation dates back to at least 4000 years by the Egyptians, whose artistic records remain even today. World over watermelon is grown in an area of 3.69 million ha with an estimated annual production of 97.43 million tonnes and average productivity of 26.37 tonnes per ha. Leading producers in the world are China, Turkey, Iran, Brazil, USA, Egypt, Russian federation and Mexico. It is a major crop of various river beds in Uttar Pradesh, Punjab, Haryana, Rajasthan, Bihar, Gujarat, Maharashtra, Andhra Pradesh and Karnataka. It occupies an area of about 20,000 ha with an annual production of 2.55 lakh tonnes and productivity of 12.75 tonnes per ha (FAO, 2014).

The major watermelon cultivars in our country comprises several introductions, *viz.*, Asahi Yamato, New Hampshire Midget, Improved Shipper, Dexielce and released varieties such as Durgapura Meetha, Sugar Baby, Arka Manik and a hybrid Arka Jyoti (Rai *et al.*, 2008). In recent days hybrid varieties developed by private companies are gaining popularity in India.

The major limiting factor in watermelon cultivation is low productivity due to diseases of diverse etiology. Among the diseases affecting watermelon, those caused by viruses are difficult to control and can be very destructive. A total of about 14 viruses have been reported as naturally infecting watermelon (Caciagli, 2008). Among them, *Papaya ring spot virus watermelon strain W* (PRSV-W), *Watermelon mosaic virus 2* (WMV2) and *Cucumber mosaic virus* (CMV) (Dikova, 2005), *Cucumber green mottle mosaic virus* (CGMMV) (Vani and Verma, 1993) and *Watermelon bud necrosis virus* (WBNV) (Singh and Krishnareddy, 1996) are reported in India.

Bud necrosis is an emerging disease causing significant yield reduction in watermelon cultivation. In Karnataka, one of the major watermelon growing states, cultivation sustained severe setback due to bud necrosis disease. It was very severe and resulted in huge yield loss ranging from 60 to100 per cent in the state (Singh and Krishnareddy, 1996).

Recently, in Kerala this disease is posing a serious threat to watermelon growers, but it was not received any attention so far. The information is scanty on the incidence and severity of WBNV in different locations of Kerala. Hence the investigation was carried out with the following objectives.

- Assessment of incidence and severity of bud necrosis disease in Kerala
- Biological assay and maintenance of virus culture
- Study on symptomatology
- Transmission study of the virus through sap and vector
- Study on physical properties of virus
- Host range study of the virus
- Immunological detection of WBNV in infected samples
- Varietal evaluation

Review of literature

2. REVIEW OF LITERATURE

Watermelon is an important summer vegetable crop of India. The cultivation of watermelon is often hindered by many biotic constraints such as diseases. Viral disease is a serious problem in cultivation of cucurbits in India. A total of 14 viruses have been reported to infect watermelon naturally (Caciagli, 2008). Among them, *Cucumber green mottle mosaic virus* (Vani and Verma, 1993), *Watermelon bud necrosis virus* (Singh and Krishnareddy, 1996), *Papaya ring spot virus watermelon strain W*, *Watermelon mosaic virus 2* and *Cucumber mosaic virus* (Dikova, 2005) were reported from India.

2.1 Tospoviruses

In India, tospoviruses are arising as serious pathogens and affecting the cultivation of a variety of crops. Five *Tospovirus* species, *viz., groundnut bud necrosis virus* (GBNV) (Reddy *et al.,* 1992) and *groundnut yellow spot virus* (GYSV) (Satyanarayana *et al.,* 1998) from groundnut, *watermelon bud necrosis virus* (WBNV) from watermelon (Singh and Krishnareddy, 1996), *iris yellow spot virus* (IYSV) from onion (Ravi *et al.,* 2005) and *capsicum chlorosis virus* (CaCV) from tomato (Kunkalikar *et al.*, 2007) have been identified on the basis of host range, vector specificity, serological properties and amino acid sequence identity of NP gene.

Among *Tospovirus* diseases, watermelon bud necrosis disease (WBND) has emerged as a serious threat to watermelon cultivation in Kerala recently. The literatures related to the studies done so far on the disease in India and abroad are reviewed in this chapter.

2.2 Occurrence

The occurrence of this disease in India has been reported by many of the workers. The disease was first reported in India by Singh and Krishnareddy (1996). According to this report the extent of WBND was 39 to 100 per cent with an estimated yield loss of 60 to 100 per cent in Karnataka. The overall incidence of bud necrosis disease in Bangalore, Kolar, Chitradurga and Mandya districts of Karnataka ranged between 10 to 100 per cent at 60 to 100 days after sowing (Krupashankar, 1998). Pandey and Pandey (2001) also reported a very high incidence of the disease up to 71 per cent in the watermelon experimental field at Indian Institute of Vegetable Research, Varanasi. Forty per cent incidence was reported from Kalegaon area in Jalna district of Maharashtra (Bhanupriya, 2006). According to the report of Krishnakumar *et al.* (2006), the WBND incidence on watermelon was 100 per cent by eleventh week after sowing in the experimental field of IIHR. A recent report by Suresh *et al.* (2013) showed high incidence of WBND in Aurangabad and Paithan regions of Maharashtra.

2.3 Symptomatology

The symptoms induced by *tospoviruses* are highly varied and include ring spots, line patterns, wilting, stunting, silvering, mottling, bronzing, chlorosis, necrosis and a range of leaf and stem lesions (German *et al.*, 1992). Yeh *et al.* (1992) reported that the diseased plant shows symptoms such as mosaic, crinkling and yellow spotting on leaves, narrowing of leaf lamina and severe stunting of plant, upright growth of younger branches, tip necrosis and necrotic spots on fruits. Singh and Krishnareddy (1996) reported the disease first time in India and the symptoms described were crinkling, mottling and yellowing of leaves, necrotic streaks on vines, shortened internodes, upright branches and necrosis and dieback of the buds. Bhanupriya (2006), recorded symptoms such as mosaic on leaves, stunted growth and

shortened internodes on stem, un-opening of flower buds, bud necrosis, longitudinal brown necrotic streaks on vines, tendrils, petioles and fruit stalks.

2.4 Causal agent

WBNV belongs to genus *Tospovirus* and family *Bunyaviridae* (German *et al.*, 1992). The virions are quasi-spherically shaped and enveloped particles of diameter varying from 80-120 nm (Moyer *et al.*, 1999) and sediment at 520-530s (Ullman *et al.*, 1992). Further, in electron microscopic studies revealed that *Tospovirus* infected watermelon in India was, WBNV particles of roughly spherical in shape with a diameter ranging from 80 to 110 nm (Singh and Krishnareddy, 1996).

The nucleocapsid protein gene of a *Tospovirus* infecting watermelon in India was cloned and sequenced. Sequence analysis showed that the gene was most closely related to those of *Watermelon silver mottle tospovirus* (WSMV) from Taiwan and *Peanut bud necrosis tospovirus* (PBNV) from India, the two definitive species of serogroup IV (Jain *et al.*, 1998).

2.5 Transmission

2.5. 1 Mechanical transmission

Yeh *et al.* (1992) reported the sap transmission of WBNV using phosphate buffer (pH 7.0) to *Chenopodium quinoa* and *Nicotiana benthamiana* and observed local lesions on *C. quinoa* and systemic symptoms as mottling and wilting on *N. benthamiana*. Singh and Krishnareddy (1996) extracted tospovirus from leaves of watermelon showing bud necrosis symptom using 0.1M phosphate buffer, pH 7.0, containing 0.02 M 2 mercaptoethanol. On mechanical inoculation of the extract local lesions were produced in cowpea cv. C-152 and systemic infection was produced on watermelon. Krupashankar (1998) reported 20 to 80 per cent transmission of WBNV among different plant species. The virus produced chlorotic or necrotic lesions on *Vigna unguiculata, Chenopodium amaranticolor* and *Nicotiana rustica.*

2.5.2 Insect transmission

Pittman (1927) first reported the transmission of *Tospoviruses* by thrips (Thysanoptera; Thripidae). The transmission by infective adults while the acquisition of virus occurred during the larval stage of the insect was reported first by Linford (1932). Singh and Krishnareddy (1995) reported transmission of WBNV in watermelon by *Thrips flavus* Schrank (Thysanoptera: Thripidae). According to this report about 10-15 nymphs of *T. flavus* require 3-4 days acquisition access period and 15-20 days of inoculation access period to transmit the virus successfully.

Krupashankar (1998) recorded 30 per cent transmission of WBNV in watermelon plants by *Thrips palmi* Karny. According to the report, after the thrips inoculation 30-40 days was taken for the expression of symptoms. Kendre *et al.* (2000) reported *T. palmi* as the vector of bud necrosis virus of groundnut in Marathwada region of Maharashtra. Singh *et al.* (2006) found that potato stem necrosis disease caused by PBNV was readily transmitted by thrips, mainly *T. palmi* and inefficiently by *Scirtothrips* spp. Sreekanth *et al.* (2006) reported that adults of *T. palmi*, could not acquire the virus, while their larvae could aquire. On the other hand, acquired larvae could not transmit the virus, and only freshly emerged adults from these acquired larvae could transmit the virus. According to this report *T. palmi* required two days acquisition access period during larval stage and two days inoculation access period in the adult stage for the virus transmission.

2.6 Physical properties

Physical properties of the virus include dilution end point (DEP), thermal inactivation point (TIP) and longevity *in vitro* (LIV).

Ghanekar *et al.* (1979) determined the physical properties of the virus from the extracts of groundnut leaves prepared in phosphate buffer. According to this report DEP of PBNV in the extract was ranging from 10^{-2} to 10^{-3} and LIV was not

more than four hours at a temperature of 30°C. Bhanupriya (2006) reported the DEP of PBNV as 10^{-1} and TIP was ranging between 45°C to 50°C as the virus lost its infectivity when heated for ten minutes above 45°C. With regards to the LIV, the virus was found to be infectious at room temperature up to three hours.

2.7 Host range

Among the plant-infecting viruses, *Tospoviruses* have a very broad host range that spans more than 1100 different species from within more than 80 families, which include both monocots as well as dicots. Many of these plants serve as reservoirs of infection that contribute to epidemics on crop plants (Cho *et al.*, 1986).

Moyer *et al.* (1999) reported that *Tospoviruses* belonging to family *Bunyaviridae* were causing substantial losses worldwide to crops such as groundnut, potato, tobacco, vegetables and ornamental plants. *Tospovirus* infect important vegetables and other crops in tropical, subtropical and temperate regions worldwide (Whitfield *et al.*, 2005). Ghotbi and Shahraeen (2005) reported the occurrence of *Tospoviruses* in many ornamentals and weed species.

Singh and Krishnareddy (1996) reported that the watermelon *Tospovirus* isolate from India systemically infected watermelon, muskmelon and other cucurbits. Thirty three plant species belonging to seven families were evaluated for the host range studies of WBNV. Among them only 17 produced different types of symptoms. Chlorotic local lesions were observed on *V. unguiculata* cv. C-152, *Vigna mungo* and *Sesamum indicum*, whereas chlorotic or necrotic spots followed by systemic infection was observed on *Citrullus lanatus* cv. Arka Manik , *C. lanatus* cv. Madhu, *Cucumis melo, Cucumis sativus, Lagenaria siceraria, Arachis hypogaea* cv. JL-24, *Cassia tora, Dolichos lablab, Glycine* max cv. Bragg, *Capsicum annuum* cv. Yellow Wax, *Datura stromonium, Solanum lycopersicon* cv. Pusa Ruby, *S. lycopersicon* cv. Arka Sourabh, *Nicotiana glutinosa, Nicotiana rustica, Nicotiana tabaccum* cv. Samsun, *Physalis floridana, Gomphrena globosa* and *Emilia sonchifolia.*

Mandal *et al.* (2003) reported the natural infection of WBNV in *Luffa acutangula* for the first time in India. The characteristic symptom noticed on field was yellowing of leaves. Jain *et al.* (2006) reported natural infection of *Tospoviruses* on three cucurbitaceous (*C. sativus, L. acutangula* and *C. lanatus*) and three fabaceous (*V. unguiculata, Phaseolus vulgaris* and *D. lablab*) vegetable crops in India.

Experimental host range studies of WBNV isolate was done on 21 host plants belonging to six families, out of which 13 hosts were successfully infected. Out of thirteen, eight hosts exhibited local chlorotic or necrotic lesions, while four hosts, *viz., N. rustica, N. glutinosa, D. stramonium* and *C. lanatus* showed systemic infection (Bhanupriya, 2006).

Krishnaveni *et al.* (2004) tested 31 plant species by mechanical inoculations with six different PBNV isolates collected from different crops species, *viz.*, tomato, groundnut, black gram, peas, chilli and brinjal. Out of them, visible symptoms were expressed by all the six isolates on nine plant species such as lima bean, green gram, soybean, black gram, groundnut, tomato, brinjal, chilli and tobacco.

A study was conducted to determine the host range of PBNV in Bhapatla, Andrapradesh. Fourteen various crops and weed species belonging to different families were tested by mechanical inoculation for their susceptibility to PBNV. Among these selected hosts *C. amaranticolor, V. radiata, V. mungo, V. unguiculata, A. hypogaea, P. vulgaris, D. lablab, S. lycopersicon, C. annuum, D. stramonium, Physalis minima, N. glutinosa, N. tabaccum, Cucumis melo* and *Euphorbia geniculata* showed different types of symptoms after sap inoculation (Kumar *et al.*, 2006).

2.8 Immunodetection and diagnosis

Plant viruses are immunogenic and were used as antigens for the production of antibodies and utilized in serological detection of viruses. The identification of the virus is best carried out by serological assays like immnuo-diffusion and ELISA (Clark and Adams, 1977).

Serological tests by ELISA and western blotting with polyclonal and monoclonal antibodies demonstrated that the virus infecting watermelon in Taiwan was serologically related to *Watermelon silver mottle virus* (Yeh *et al.,* 1992; Adam *et al.,* 1993). WBNV from India was detected by ELISA using PBNV or WSMoV specific antisera. According to the results, WBNV in India was found to be similar to watermelon *Tospovirus* from Japan and Taiwan and closely related to Indian PBNV (Singh and Krishnareddy, 1996). Pandey *et al.* (2004) raised polyclonal antiserum against N protein of WBNV and detected WBNV as well as PBNV isolates of wide range of hosts from different locations in India.

According to the report of Jain *et al.* (2005) the antiserum to the N protein of PBNV at 1:4000 dilution successfully detected the natural infection of PBNV and WBNV in a wide range of cucurbitaceous, leguminous and solanaceous hosts from different locations of India. Reddy *et al.* (1992) reported that the virus causing peanut bud necrosis in India was found to be distinct and is restricted to Asia. Bhat *et al.* (2001) confirmed the association of PBNV with crops like black gram, cowpea, green gram and soybean based on the DAC- ELISA.

The weed plants *Achyranthes aspera, Acanthospermum hispidum, Commelina benghalensis, Ageratum conyzoides, Borreria hispida, Euphorbia geniculata* and *Datura stramonium* showed strong reaction to PBNV antisera in DAC-ELISA (Nagaraja and Murthy, 2005). Raja and Jain (2006) and Anjaneyareddy *et al.* (2008) diagnosed bud blight of tomato using polyclonal antiserum (N protein) of PBNV in

DAC-ELISA and DIBA and suggested that the tomato samples were infected with *Peanut bud necrosis virus*.

2.9 Varietal evaluation

Limited progress has been made in breeding crops for increased resistance to *Tospoviruses*. Polygenic nature of resistance in many cases makes incorporation of resistance a difficult task (Goldbach and Peters, 1996).

A total of 16 watermelon hybrids and selection lines were screened for resistance against WBNV in a field experiment conducted in Uttar Pradesh, India during 1998. Among the hybrids and lines tested, four were moderately resistant (EC-393240, Durgapur Meetha, MHW-6 and Ashey Questo) and three were resistant (EC-393243, Durgapur Selection and RHRWH-2) to the pathogen (Pandey and Pandey, 2001).

Among the interspecific cross (Arka Manik x *Citrullus colocynthis*) a total of 146 individual plants were raised and these plants were screened against WBNV under natural field condition. Of the germplasm screened, eight germplasm accessions namely, IIHR-81, IIHR-83, IIHR-85, IIHR-90, IIHR- 102, IIHR-110, IIHR-114 and IIHR-118 were found free from WBNV under natural field condition, whereas, Arka Manik susceptible check showed 85 per cent WBNV incidence (IIHR, 2007).

In India, despite intensive efforts over a number of years to find resistance, none of the 7000 groundnut (*A. hypogaea*) genotypes evaluated by researchers exhibited resistance against PBNV. However, tolerant lines with good agronomic characteristics included TCGV- 86029, TCGV- 86030, ICGV- 8603I, TCGV- 86033 and ICGV- 86538. But this has not been yet incorporated into commercially attractive groundnut cultivars (Reddy *et al.*, 1983).

Venkataramana *et al.* (2006) screened 63 tomato entries for resistance to PBNV under field condition during Kharif 2003. Among them only one entry EC5888 showed highly resistant reaction while EC 8630 and EC 26512 were resistant. Pusa Uphar, EC 251709, EC 165700, LE 23, IIHR 2187, IIHR 2272, IIHR 2273 and IIHR 2274 were moderately resistant. These field promising genotypes were further tested by sap inoculation for confirmation of resistance. Two genotypes, *viz.*, EC 8630 and EC 5888 were highly resistant, LE 23 and EC 526512 were resistant and EC 165700 displayed a moderately resistant reaction.

Field experiments were conducted in 2007 and 2008 at AVRDC in Hyderabad, India to evaluate 30 improved lines of tomato for yield performance and field tolerance against *tomato leaf curl virus* and *peanut bud necrosis virus*. Peanut bud necrosis disease severity recorded was minimum in lines DR2-1 and NC 3220 (Sain and Chadha , 2012).

Materials and Methods

3. MATERIALS AND METHODS

The present investigation "Biological characterization of bud necrosis virus disease in watermelon (*Citrullus lanatus* T.)" was carried out during 2012 to 2014. The experiments were conducted at the Department of Plant Pathology, College of Horticulture, Vellanikkara except the serology work which was conducted at Banana Research Station, Kannara. The details of the materials used and the techniques adopted for the investigation are described below.

3.1 SURVEY

3.1.1 Assessment of incidence and severity of watermelon bud necrosis disease

A roving survey was undertaken to study the per cent incidence and severity of watermelon bud necrosis disease (WBND) in major watermelon growing areas of Kerala. First survey was conducted during March and April 2013 in watermelon growing areas of Attappady in Palakkad district, Kuttippuram and Thavannur in Malappuram district and Kanhangad and Chattamchal in Kasaragod district. Second survey was conducted during March 2014 at Pattambi in Palakkad district, Parappanangadi, Kuttippuram, Parappur, Vettom and Vengara in Malappuram district and Kanhangad and Thikkadappuram in Kasaragod district. Simultaneously, survey was also conducted in the research plots of College of Horticulture, Vellanikkara and Krishi Vigyan Kendra, Thrissur during the above periods (Plate 1). The disease incidence, severity and different type of symptoms were recorded from the surveyed areas. Thirty plants were selected from each plot for recording the incidence. The details of survey are presented in Table 1.

A. Vellanikkara

B. Kanhangad

C. Vettom

Plate 1. Surveyed locations

Table 1. Details of survey on WBND at different locations of Kerala

Sl. No.	District	Location	No of plots surveyed
1.	Thrissur	Department of Olericulture, College of Horticulture, Vellanikkara	4
		Krishi Vigyan Kendra, Thrissur	2
2.	Palakkad	Farmer's field, Attappady	2
		Farmer's field, Pattambi	2
3.	Malappuram	Farmer's field, Kuttippuram	5
		Farmer's field, Thavannur	2
		Farmer's field, Parappur	3
		Farmer's field, Vettom	4
		Farmer's field, Vengara	2
		Farmer's field, Parappanangadi	2
4.	Kasaragod	Farmer's field, Chattamchal	2
		Farmer's field, Kanhangad	4
		Farmer's field, Thikkadappuram	2
5.	Kollam	Farmer's field, Ummannur	2

The per cent disease incidence (PDI) was calculated as,

$$\text{Per cent disease incidence (PDI)} = \frac{\text{No. of plants infected}}{\text{Total no. of plants observed}} \times 100$$

For the assessment of severity of disease, 25 plants were selected randomly and scored as per the 0 to 4 scale of Sain and Chadha (2012) with a slight modification (Table 2).

Table 2. Scoring scale adopted for the assessment of WBND

Score	Description
0	No symptom
1	Slight upward curling and crinkling of leaves
2	Upward curling and crinkling of leaves with mosaic symptom
3	Crinkled leaves with brown necrotic spots and necrotic patches on the stem
4	Severe stunting of the plant with bud necrosis symptom

The per cent disease severity (PDS) was calculated using the following formula suggested by Wheeler (1969).

$$\text{PDS} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of plants observed} \times \text{maximum disease grade}} \times 100$$

3.1.2 Influence of seasons on the incidence and severity of WBND

Survey was conducted in the experimental plot of Department of Olericulture, Vellanikkara to assess the seasonal influence on the incidence and severity of watermelon bud necrosis disease. The observations were taken during October to December in the Rabi season crop of 2013 and January to March in the summer season crop of 2014, at monthly intervals in the variety Sugar Baby. Fifty plants were selected randomly for recording the disease incidence and 25 plants were selected randomly for recording the disease severity.

3.1.2.1 Meteorological data of Vellanikkara

Weather parameters such as mean temperature, relative humidity and rainfall during the survey period in Vellanikkara were recorded.

3.2 BIOLOGICAL INDEXING OF VIRUS

Plants showing WBND symptoms, collected from different locations were mechanically inoculated to the local lesion host *Amaranthus viridis* using 0.1 M potassium phosphate buffer pH (7.0).

3.3 MAINTENANCE OF VIRUS CULTURE

The culture of WBNV was maintained in watermelon plants by periodical mechanical transmission under insect proof condition. These plants were used as source of inoculum for various transmission studies.

3.4 SYMPTOMATOLOGY

Symptomatology was studied by observing the development of symptoms on naturally infected as well as artificially inoculated watermelon plants at different stages of plant growth. Symptoms on leaves, stem, buds, tendrils, flowers as well as

on fruits were observed for studying the symptomatology of the disease. Based on observations, symptoms were documented.

3.5 TRANSMISSION STUDIES

The mode of transmission of WBNV was studied by mechanical and vector transmission.

3.5.1 Mechanical transmission

The mechanical transmission of WBNV was standardized with different buffers at four pH level (7.0, 7.2, 7.4 and 7.6) (Table 3). The compositions of the buffers used are given in Appendix I.

Table 3. Buffers used for mechanical transmission

Sl. No.	Buffers	Concentration	pH
1.	Citrate phosphate buffer	0.1 M	7.0, 7.2, 7.4 and 7.6
2.	Potassium phosphate buffer	0.1 M	
3.	Sodium phosphate buffer	0.1 M	
4.	Acetate buffer	0.1 M	
5.	Borate buffer	0.1 M	

For the sap transmission, young leaves of watermelon plants with bud necrosis symptoms were selected, washed with tap water, dried with blotting sheet and weighed separately for preparation of standard extract. Buffer volume equal to the weight of the leaves was added into a chilled mortar and ground with the pestle.

After thorough grinding, the homogenized leaf pulp was filtered through double layered muslin cloth to get filtered standard extract of the leaves. A pinch of carborandum powder (600 mesh) was added to the extracted sap. Cotton pad soaked in standard extract was rubbed on the primary leaves of test plants, in one direction only that was from the petiole to the apex of the leaf by supporting the leaf from the centre with a cardboard. After five minutes of inoculation, test plants were washed with distilled water to remove excessive inoculum and extraneous particles. The numbers of treatments were twenty and each treatment was replicated thrice. Plants inoculated with buffer without the infected sap served as control. The inoculated plants were kept in insect proof net house and observed daily for the development of symptoms.

3.5.2 Vector transmission

The vector transmission was conducted using the thrips maintained in the rearing cages.

3.5.2.1 Vector identification

Thrips were collected in Alcohol - Glycerin- Acetic acid (AGA) solution (Mixture of 10 parts of 60% ethyl alcohol with one part of glycerin and one part of acetic acid) from watermelon fields for the prolonged safe storage.

For microscopic examination, the thrips were mounted as follows,

1. Collected thrips were treated in 10% NaOH solution for 30 minutes
2. Given two washes in distilled water.
3. Kept the specimens in different grades of alcohol (50%, 70% and 90%) for 5 minutes.
4. Given two changes in 100% ethanol for 10 minutes each.
5. Placed the specimen in the centre of a slide with little glycerol. Arranged the specimen in the desired position and covered the specimen by a cover slip.

Thrips mounted on microscopic slides were identified from the Department of Entomology, College of Horticulture, Vellanikkara.

3.5.2.2 Maintenance of thrips culture

The thrips were collected by plucking the leaves with high population of adult thrips in polyethylene bags, from the watermelon field of Department of Olericulture, COH, Vellanikkara. The thrips from the collected leaves were transferred to healthy watermelon plants using a delicate painting brush. These collected thrips were reared on healthy watermelon plants raised in polyethylene bags, kept inside the insect proof rearing cages of size 30 x 60 x 60 cm (Plate 2.a.). Old plants were replaced periodically with healthy young plants for the proper maintenance.

3.5.2.3 Source of inoculum

WBND infected samples collected from the maintained culture in the insect proof net house, served as the source of virus inoculum. The acquisition access period was given for the nymphs on the infected watermelon twig floated on water in a Petri dish (Plate 2.b.).

3.5.2.4 Test plants

Twelve day old watermelon seedlings raised in polyethylene bags were kept in inoculation cage @ one plant/ cage. Fifteen plants were kept for each inoculation access period to determine the minimum time period (Plate 2.c.).

Plate 2. a. Rearing cage

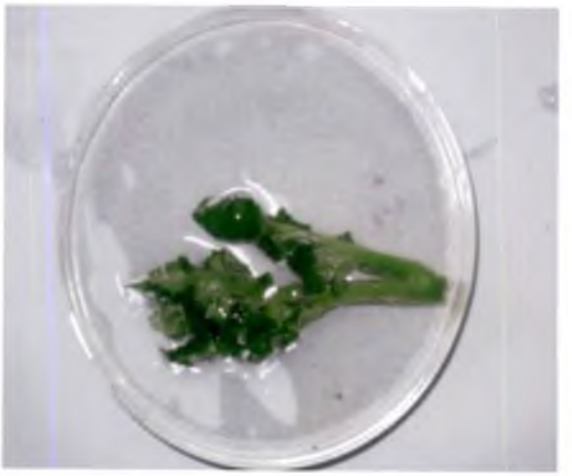


Plate 2. b. Acquisition on infected twig

Plate 2. c. Inoculation cage

Plate 2. Thrips transmission studies

3.5.2.5 Virus vector relationship

3.5.2.5.1 Effect of different durations of acquisition access period on the transmission of WBNV

To determine the minimum acquisition access period for transmission, groups of non- viruliferous nymphs were allowed to feed on the twig of infected plant for different periods, *viz.*, 24, 48, 72 and 96 hours. At the end of each acquisition access period, the nymphs were allowed to feed on healthy watermelon plants @ 10 / plant, for a period of 48 h after six days of latent period. After 48 h of inoculation access period, insects were killed by spraying 0.1 per cent quinalphos and the plants were kept for observation for two months in insect proof net house. Fifteen plants were kept for each treatment. Per cent transmission and time taken for symptom expression were recorded. Uninoculated plants were kept as control.

3.5.2.5.2 Effect of different durations of inoculation access period on the transmission of WBNV

Non viruliferous nymphs were given with an acquisition access period of 48 h. Ten nymphs were released to each test plant to feed for a inoculation access period of 24, 48, 72 and 96 hours after six days of latent period . Fifteen seedlings were kept for each experiment. Per cent transmission and incubation period in host were recorded. Uninoculated plants served as control.

3.6 PHYSICAL PROPERTIES

Dilution end point (DEP), Thermal inactivation point (TIP), and Longevity *in vitro* (LIV) of the virus was studied for its characterization.

3.6.1 Dilution end point (DEP)

Five gram of infected watermelon leaves was homogenized in chilled mortar and pestle by adding five ml of 0.1M citrate phosphate buffer (pH 7.2). The

homogenate was strained through a thin layer of cotton. Serial dilution of the sap, *viz.*, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were made as follows. Six test tubes were kept in a raw in a test tube rack. Nine ml of buffer was dispersed into each of five test tubes starting from the second test tube using a clean Pipette. The extract prepared was transferred to the second test tube with nine ml buffer to get a dilution of 10^{-1}. It was thoroughly mixed and 1ml of 10^{-1} dilution was transferred to the next test tube to obtain a dilution of 10^{-2}. This process was continued till a dilution of 10^{-6} was obtained. These dilutions were inoculated on primary leaves of young healthy watermelon seedlings. Twenty replications were maintained for each treatment. The inoculated plants were labeled and kept under insect proof condition and observed for the development of symptoms. Plants treated with buffer were kept as control.

3.6.2 Thermal inactivation point (TIP)

Sap was extracted from infected leaves of watermelon as mentioned in the experiment (3.6.1). Three ml of sap was pipetted out into a thin walled glass test tube. Care was taken not to smear on the upper part of the test tube. This was placed in a water bath with thermostat arrangement. The level of water was maintained three cm above the level of sap in the test tubes. Three ml aliquots were treated for ten minutes each at 35, 40, 45, 50, 55 and 60°C. After each treatment the test tubes were removed and cooled immediately. The samples were inoculated on primary leaves of young watermelon seedlings and observed for the symptom development. Twenty replications were maintained for each treatment. The plants inoculated with infected sap at room temperature (28 ± 2) °C were maintained as control.

3.6.3 Longevity *in vitro* (LIV)

Sap was extracted from infected leaves of watermelon as mentioned in the experiment (3.6.1). Ten ml each of the sap was pipetted out into two test tubes and closed with the cotton wool. One tube was incubated at room temperature (28±2) °C and the other in a refrigerated condition. The sap was pipetted into a beaker for

inoculating primary leaves of young healthy watermelon seedlings and used for inoculation at specific periods of 2, 4, 6, 24, 48 and 72 hours. Twenty replications were maintained for each treatment. The plants inoculated immediately after incubation was treated as control. The inoculated plants were observed for the development of symptoms.

3.7 HOST RANGE STUDIES

In order to find out the host range of WBNV, different species of plants belonging to the Cucurbitaceae, Fabaceae, Solanaceae and Amaranthaceae families were artificially inoculated with infected sap containing virus inoculum using citrate phosphate buffer pH(7.2) as mentioned in 3.6.1. Ten seedlings of each species were used for the experiment. The inoculated plants were examined for the development of symptoms for four weeks. Respective host plants without inoculation served as control. The plants were observed daily for the development of symptoms. The details of the plants used for host range studies are furnished in Table 4.

3.8 IMMUNODETECTION OF WBNV IN INFECTED SAMPLES

Serological experiments were done utilizing the facility of Banana Research Station, Kannara. Direct Antigen Coating - Enzyme Linked Immuno Sorbent Assay (DAC-ELISA) suggested by Barbara and Clark (1982) was used for the serological studies. The polyclonal antiserum directed against nucleocapsid (N) protein of PBNV (Collected from IISc, Bangalore) was used for the detection. The assay was carried out in 96-well polystyrene microtiter plate.

Four samples of WBNV infected plants with distinct symptoms as curling and crinkling on leaves, mosaic symptoms on leaves, necrotic spots on leaves, shoots with bud necrosis symptom were collected from the culture maintained in the insect proof net house. The four infected samples and one healthy sample were used each in two replications.

Table 4. List of plants used for host range study

SI.No.	Family	Common name	Scientific name
1.	Cucurbitaceae	Watermelon	*Citrullus lanatus*
		Oriental prickling melon	*Cucumis melo* cv. *Conomon*
		Bottlegourd	*Lagenaria siceraria*
		Ridge gourd	*Luffa acutangula*
		Bitter gourd	*Momordica charantia*
		Cucumber	*Cucumis sativus*
2.	Fabaceae	Cowpea	*Vigna unguiculata*
		Dolichos bean	*Dolichos lablab*
		Groundnut	*Arachis hypogaea*
3.	Solanaceae	Chilly	*Capsicum annuum*
		Tomato	*Solanum lycopersicon*
		Brinjal	*Solanum melongena*
4.	Amaranthaceae	Amaranthus	*Amaranthus viridis*
		Gomphrena	*Gomphrena globosa*

3.8.1 Reagents used

1. Stock buffer (phosphate buffer saline 1x PBS, pH-7.4) (Appendix- II)
2. Coating buffer: carbonate buffer, pH 9.6 (Appendix- II)
3. Wash buffer (PBS-Tween, PBS-T)
4. Enzyme conjugate diluent buffer (PBS-T poly vinyl pyrrolidone ovalbumin, PBS- TPO). Added 20.0 g poly vinyl pyrrolidone (PVP-MW 44,000) and 2g egg ovalbumin to one litre PBS-T
5. Antibody diluents /buffer- same as PBS-TPO
6. Substrate buffer (diethanol amine buffer, pH- 9.8) Diluted 97 ml diethanol amine in 800 ml distilled water, pH adjusted to 9.8 using 6N hydrochloric acid and made upto one litre.
7. Blocking solution (Added 5g Bovine serum albumin to one litre PBS-T)
8. Stop solution (Sodium hydroxide 12g was dissolved in distilled water and made upto one litre).

3.8.2 Methodology

1. Leaf samples of 1g were weighed and the four infected samples and one healthy sample were ground separately in pre chilled pestle and mortar with 2.5 ml buffer (0.4g PVP was dissolved in 20 ml of the buffer).
2. The ground samples were transferred to Eppendorf tubes and labelled. It was subjected to centrifugation at 5000 rpm for 15 min.
3. Added 200µl supernatant to the wells of labelled microtitre plate. The plates were covered and incubated at 37^0 C for 1 h.
4. Washed the plate in PBS-T three times. Finally removed the remaining drops of washing buffer by gently tapping plates upside down on a tissue paper.
5. Added 200µl of blocking solution to each well and incubated at 37^0 C for one hour to block the unoccupied sites.
6. Washed the plates with PBS-T as before.
7. Added 200µl crude antiserum (primary antibody) diluted in PBS-TPO to each well. Incubated at 4^0 C overnight.

8. Washed the plates with PBS-T as before.
9. Added 200µl enzyme labelled (alkaline phosphatase) antirabbit IgG (secondary antibody) diluted in PBS-TPO to the wells which were coated with antigen. Incubated at 37^0 C for 2 h.
10. Washed the plates three times as before.
11. Added 100µl freshly prepared substrate, para nitrophenyl phosphate (0.5mg/ml) to each well. Incubated at room temperature for 30 min. in dark.
12. The reaction was stopped by adding 50µl of 3M sodium hydroxide to each well.
13. Measured absorbance at 405 nm in an ELISA reader (Microplate Manager Bio-Rad, Laboratories, Inc.)

3.8.3 Standardization of dilution of antiserum

The crude antiserum of PBNV was diluted in PBS-TPO (Antibody buffer), *viz.*, 1:10000, 1:5000, 1:2000 and 1:500 for the standardization of antiserum dilution. The enzyme labelled (alkaline phosphatase) antirabbit IgG (secondary antibody) diluted in PBS-TPO as 1:500 dilution was used for coating the antigen.

3.9 VARIETAL EVALUATION

Twenty two genotypes of watermelon maintained in the varietal screening experiment plot of Department of Olericulture were evaluated for disease reaction. The genotypes were categorized using disease reaction scale described by Pandey and Pandey (2001).

The disease incidence was recorded in the vegetative and fruiting stage of the crop. The pathogen reaction was categorized between resistant to highly susceptible in five categories based on PDI value as follows.

Per cent disease incidence	Disease reaction
0-5	Resistant
6-10	Moderately resistant
11-20	Moderately susceptible
21-40	Susceptible
> 40	Highly susceptible

3.10 STATISTICAL ANALYSIS

Analysis of variance was performed on the data collected in mechanical transmission experiment using the statistical package, MSTAT (Freed, 1986).

Results

4. RESULTS

The results of the investigation "Biological characterization of bud necrosis virus disease in watermelon (*Citrullus lanatus* T.)" are presented in this chapter under the following heads.

4.1 SURVEY

4.1.1 Assessment of incidence and severity of watermelon bud necrosis disease

The survey on watermelon bud necrosis disease (WBND) incidence was conducted in 2013 and 2014. The roving survey was carried out in watermelon growing locations of five districts of Kerala, *viz.*, Thrissur, Palakkad, Malappuram, Kasaragod and Kollam. A total of 38 plots including farmers and experimental plots were surveyed to assess the incidence and severity of WBND. The major varieties cultivated in the surveyed fields were Sugar Baby, NS-295 hybrid, Mahyco hybrid, Durgapura Lal and Triploid accessions.

First survey was conducted during March and April 2013 in watermelon growing areas of Attappady in Palakkad district, Kuttippuram and Thavannur in Malappuram district, Kanhangad and Chattamchal in Kasaragod district and research plots of Olericulture Department and Krishi Vigyan Kendra in Thrissur. The disease incidence recorded from the surveyed locations was 35-95 per cent during 2013. Among the surveyed locations, Kanhangad and Chattamchal locations of Kasaragod district, cultivated variety was NS-295 hybrid recorded the highest PDI (95.00 %). The PDS recorded was highest for kanhangad area (60.00 %) followed by Chattamchal area (59.5%). The minimum PDI (35.00 %) and PDS (15.50 %) were observed in Attappady location of Palakkad district where Sugar Baby was the cultivated variety (Table 5).

Table 5. Survey on the occurrence of WBND in different locations of Kerala - 2013

Sl. No.	Season	Location	Variety	Stage of the crop	Mean per cent disease incidence	Mean per cent disease severity
		Thrissur				
1.	March 2013	Department Of Olericulture (2)	Durgapura Lal	Fruiting	93.33	51.50
2.	March 2013	KVK, Thrissur (1)	Sugar Baby	Fruiting	36.00	20.00
		Palakkad				
3.	March 2013	Attappady (2)	Sugar Baby	Fruiting	35.00	15.50
		Malappuram				
4.	March 2013	Kuttippuram (2)	Mahyco hybrid	Fruiting	92.66	52.00
5.	April 2013	Thavannur (2)	Sugar Baby	Fruiting	37.00	16.50
		Kasaragod				
6.	April 2013	Kanhangad(2)	NS- 295	Fruiting	95.00	60.00
7.	April 2013	Chattamchal (2)	NS- 295	Fruiting	95.00	59.50

Number of plots surveyed in each location is given in paranthesis

Second survey was conducted during March and April 2014 at Pattambi in Palakkad district, Parappanangadi, Kuttippuram, Parappur, Vettom and Vengara in Malappuram district, Kanhangad and Thikkadappuram in Kasaragod district and research plots of Olericulture Department and Krishi Vigyan Kendra in Thrissur. The disease incidence recorded from the surveyed locations ranged from 30 to 98 per cent during 2014. Among the different locations surveyed, Ummannur area of Kollam district, cultivated variety was triploid watermelon accession had the maximum PDI (98.34 %) and PDS (68.00 %) followed by Kanhangad area of Kasaragod district where NS-295 hybrid was the cultivated variety. The PDI and PDS recorded from Kanhangad was (96.34 %) and (64.5 %) respectively. The minimum PDI (30.00 %) and PDS (15.50 %) was recorded in Pattambi area of Palakkad district and Kuttippuram area of Malappuram district, where Sugar Baby was the cultivated variety (Table 6).

Thus, from the survey it was observed that, the disease incidence recorded in the selected areas was 30 to 98 per cent. WBND was severe in the locations cultivated with Triploid accessions and NS-295. It was also observed that, the variety Sugar Baby had minimum incidence compared to the hybrid varieties. Symptoms such as curling, mosaic, yellowing and necrotic spots on leaves, necrotic streaks on vines, shortened internodes and necrosis of fruits and buds were observed in different locations.

4.1.2 Influence of seasons on the incidence and severity of watermelon bud necrosis disease

Survey was conducted in the experimental plot of Department of Olericulture, Vellanikkara to assess the influence of seasons on the incidence and severity of WBND. The observations were taken during October to December in the Rabi season of 2013 and January to March in summer season of 2014.

Table 6. Survey on the occurrence of WBND in different locations of Kerala- 2014

SI. No.	Season	Location	Variety	Stage of the crop	Mean per cent disease incidence	Mean per cent disease severity
		Thrissur				
1.	March 2014	Department of Olericulture (2)	Durgapura Lal	Fruiting	90.00	49.00
2.	March 2014	KVK, Thrissur (2)	Sugar Baby	Fruiting	32.00	19.50
		Palakkad				
3.	March 2014	Pattambi (1)	Sugar Baby	Fruiting	30.00	15.50
		Malappuram				
4.	March 2014	Parappanangadi (2)	Sugar Baby	Fruiting	33.00	16.50
5.	March 2014	Kuttippuram (3)	Sugar Baby	Fruiting	30.00	15.50
6.	March 2014	Parappur (3)	Mahyco hybrid	Fruting	90.67	55.00
7.	March 2014	Vettom (4)	NS- 295	Fruiting	95.67	60.25
8.	March 2014	Vengara (2)	Mahyco hybrid	Fruiting	87.33	51.50
		Kasaragod				
9.	March 2014	Kanhangad (2)	NS- 295	Fruiting	96.34	64.5
10.	April 2014	Thikkadappuram(2)	NS- 295	Fruting	95.00	60.00
11.		Kollam				
12.	April 2014	Ummannur(2)	Triploid accession	Fruiting	98.34	68.00

Number of plots surveyed in each location is given in paranthesis

The crop grown during 2013 Rabi season recorded a disease incidence (10, 16 and 24 %), severity (6, 11 and 21 %) and the coefficient of infection (0.60, 1.76 and 5.04 %) in the months of October, November and December respectively. The crop grown during 2014 summer season recorded incidence of (16, 30 and 38 %), severity of (11, 25 and 34 %) with the coefficient of infection (1.76, 7.50 and 12.92 %) in the months of January, February and March respectively. The survey data shows comparatively less infection in Vellanikkara during October to December, recording 10 to 24 per cent disease incidence and 6 to 21 per cent disease severity. It increased to 16 to 38 per cent incidence and 11 to 34 per cent severity in the crop grown during January- March, in the variety Sugar Baby (Table 7).

The meteorological data presented in Table 8 for the period from October 2013 to March 2014 showed that, the mean temperature varied from 27.3 to 28.2^0 C during Rabi and 27.9 to 30.4^0 C during summer season with maximum (30.4^0 C) in the month of March. The mean relative humidity was also high (61 – 83 per cent) during the Rabi season as compared to summer (51 – 55 per cent). It is also observed that there was no rainfall during summer months.

Thus the data showed that, the high temperature, less humidity and lack of rainfall in summer months favoured the high incidence of WBND.

4.2 BIOLOGICAL INDEXING OF VIRUS

The local lesion host *Amaranthus viridis* was used for the bioassay of the virus. The symptom developed as necrotic local lesions on seventh day after the inoculation of the infected sap using potassium phosphate buffer pH (7.0) (Plate 3).

Table 7. Influence of seasons on the occurrence of WBND in variety Sugar Baby at Vellanikkara

SI. No.	Parameter	Season					
		Rabi season 2013			Summer season 2014		
		October	November	December	January	February	March
1.	Mean per cent disease incidence	10.00	16.00	24.00	16.00	30.00	38.00
2.	Mean per cent disease severity	6.00	11.00	21.00	11.00	25.00	34.00
3.	Coefficient of infection	0.60	1.76	5.04	1.76	7.5	12.92

Table 8. Meteorological data of Vellanikkara during the period October 2013 to March 2014

Parameter	October 2013	November 2013	December 2013	January 2014	February 2014	March 2014
Mean temperature (^{0}C)	27.3	28.2	27.6	27.9	28.8	30.4
Mean Relative Humidity (%)	83	73	61	51	56	55
Rainfall (mm)	369.8	82.0	0.5	0	0	0

Necrotic local lesions developed on *Amaranthus viridis*

Plate 3. Symptom developed on local lesion host

4.3 MAINTENANCE OF VIRUS CULTURES

The WBNV infected watermelon samples were collected from Olericulture department field of College of Horticulture, Vellanikkara. The cultures were maintained in the insect proof net house which served as the source of inoculum.

4.4 SYMPTOMATOLOGY

Symptoms of bud necrosis disease on watermelon were studied under natural and artificial conditions.

4.4.1 Symptoms observed under natural conditions

4.4.1.1 Symptoms on leaves

The symptom initially appeared as curling of leaves which later turned to mosaic and crinkling. Yellowing of leaves was also noticed in the severely infected plants. The leaves showing yellowing were later found with dark brown coloured necrotic spots on the surface. In the severely infected plants, narrowing of leaf lamina was also observed (Plate 4).

4.4.1.2 Symptoms on branches

Small dark brown necrotic streaks were seen on the stem, vines, tendrils and petioles of WBNV infected plants. As the disease progressed, the stem split was observed and the growing branches started drying from the tip. In the severely infected plants, shortened internodes and upright growth of young branches were also observed (Plate 5).

4.4.1.3 Symptoms on flower buds

Un-opening of flower buds and their necrosis were the symptoms observed in the flowering stage (Plate 6.a).

a. Crinkling and mosaic

b. Yellowing

c. Dark brown necrotic spots

d. Narrowing of leaf lamina

Plate 4. Symptoms on watermelon leaves

A) Necrotic streaks on stem

C) Stem splitting

B) Necrosis on tendrils

D) Upright growth of branches

Plate 5. Symptoms on watermelon branches

4.4.1.4 Symptoms on fruits

The fruits developed on the infected plants appeared small and malformed. Brown necrotic rings were developed on the fruits in case of severely infected plants (Plate 6.b, c and d).

4.4.2 Symptoms observed under artificial conditions

In mechanically inoculated plants, symptoms initiated as curling of younger leaves in about 8 days after inoculation which later spread to other leaves. Later, the leaves showed crinkling and mottling, leaf size was reduced, became malformed and was rough and brittle. Flowering started 40 days after sowing and flowers were less in number. Un-opening of flower buds and bud necrosis was also observed in the flowering stage (Plate 7).

4.5 TRANSMISSION STUDIES

The transmission of WBNV was carried out using the infected sap and vector. The details of the transmission studies are described below.

4.5.1 Mechanical transmission

The standardization of mechanical transmission was carried out with variety Durgapura Lal using five different buffers at pH range, *viz.*, 7.0, 7.2, 7.4 and 7.6. The result presented showed significant difference in the disease transmission with variation in buffer and pH level. Of the different buffers tested, citrate phosphate buffer at pH 7.2 showed maximum disease incidence (72.2%) compared to others at all pH levels and was on par with the potassium phosphate buffer. Acetate buffer (pH 7.6) showed minimum disease incidence (5.55 per cent). The absolute control plants without the inoculation of infected sap remained healthy (Table 9).

a. Necrosis on buds

b. Necrosis on fruit stalk

c. Necrotic lesion on fruits

d. Malformed fruit

Plate 6. Symptoms on watermelon buds and fruits

b. Symptom developed on sap Inoculated leaf

a. Control without inoculation

c. Yellowing and necrosis in flowering stage

Plate 7. Symptoms developed under artificial condition

Table 9. Standardization of buffers at different pH level based on the per cent disease incidence

Sl .No.	Buffer/pH	Mean per cent disease incidence				Mean
		7.00	7.2	7.4	7.6	
1	Citrate phosphate	66.67	72.22	44.44	38.89	54.17
2	Potassium phosphate	61.11	66.67	38.89	38.89	51.38
3	Sodium phosphate	27.78	38.89	22.22	11.11	25.00
4	Acetate	11.11	22.22	5.55	5.55	11.11
5	Boric acid borax	38.89	44.44	27.78	22.22	33.33
Mean		40.00	48.89	27.78	23.33	

CD (0.05) buffers- 8.76, CD (0.05) pH- 7.83, CD (0.05) interaction- NS

Incubation period was the other factor involved in selection of suitable buffer. Taking into consideration of the incubation period for the symptom appearance, significant difference was observed between the selected buffers and the four pH level used for each buffer. Among the selected buffers, citrate phosphate buffer (pH 7.2) showed minimum incubation period (8 days) for the symptom appearance followed by potassium phosphate buffer (pH 7.2) (9 days). Acetate buffer (pH 7.6) showed the maximum incubation period (20 days). According to the results, citrate phosphate buffer (0.1 M, pH 7.2) was selected as the best buffer for further studies (Table 10).

4.5.2 Vector transmission

4.5.2.1 Vector identification

Thrips collected from watermelon plots was identified as *Thrips palmi* Karny from Department of Agricultural Entomology, College of Horticulture, Vellanikkara (Plate 8).

4.5.2.2 Maintenance of thrips culture

Pale yellowish adult thrips were collected from healthy watermelon plants in field. Adults collected from the field were reared on the healthy watermelon plants in the insect proof cages. After 15 days, creamy white coloured nymphs were observed on the leaves. Nymphs were found in groups feeding along the leaf midrib and veins of older leaves and took 6 days for the adult emergence.

Thrips palmi - **Nymph**

Thrips palmi - **Adult**

Plate 8. *Thrips palmi* nymph and adult stage

Table. 10. Standardization of buffers at different pH level based on incubation period

SI .No.	Buffer/pH	Incubation period (Days)				Mean
		7.0	7.2	7.4	7.6	
I	Citrate phosphate	9.0	8.0	11.0	14.0	10.50
2	Potassium phosphate	10.0	9.0	11.0	14.0	11.00
3	Sodium phosphate	13.0	12.0	14.0	16.0	13.75
4	Acetate	15.0	15.0	18.0	20.0	17.00
5	Boric acid borax	13.0	11.0	14.0	17.0	13.75
Mean		12.00	11.00	13.60	16.20	

CD (0.05) buffers-0.70, CD (0.05) pH- 0.63, CD (0.05) interaction- NS

4.5.2.3 Virus vector relationship

4.5.2.3.1 Effect of different durations of acquisition access period on the transmission of WBNV

Thrips nymphs were given with acquisition access periods of 24, 48, 72 and 96 hours in the infected watermelon plant floated on water in a Petri dish. The nymphs were then allowed to feed on healthy watermelon plants for 48 h after six days of latent period. Result of the experiment is presented in Table 11. It was observed that a minimum acquisition access period of 24 h required for the acquisition of WBNV from the infected plants as evident from the 20 per cent transmission obtained. However, the per cent transmission increased from 20 to 47 with increase in acquisition access period from 24 to 96 h. It was also noticed that the incubation period of WBNV in host decreased with increase in acquisition access period.

4.5.2.3.2 Effect of different durations of inoculation access period on the transmission of WBNV

Viruliferous insects were given inoculation access periods of 24, 48, 72 and 96 hours after six days of latent period on healthy test plants. From the data presented in Table 12, it was observed that a minimum inoculation access period of 48 h was enough for the successful transmission of WBNV as evidenced from 20 per cent transmission obtained. However, the per cent transmission increased from 20 to 40 per cent with increase in inoculation access period from 48 to 96 h. It was noted that the incubation period of virus in host plant also decreased with increase in inoculation access period.

Table 11. Effect of different durations of acquisition access period on the transmission of WBNV

Sl.No.	Acquisition access period (h)	Number of plants inoculated	Number of plants infected	Per cent transmission	Incubation period in plant (Days)
1.	24	15	3	20	28
2.	48	15	4	27	26
3.	72	15	6	40	24
4.	96	15	7	47	22

Inoculation access period- 48 h

Number of thrips used- 10 per plant

Table 12. Effect of different durations of inoculation access period on the transmission of WBNV

Sl.No.	Inoculation access period (h)	Number of plants inoculated	Number of plants infected	Per cent transmission	Incubation period in plant (Days)
1.	24	15	0	0	0
2.	48	15	3	20	27
3.	72	15	4	27	23
4.	96	15	6	40	21

Acquisition access period- 48 h

Number of thrips used- 10 per plant

4.6 PHYSICAL PROPERTIES

4.6.1 Dilution end point (DEP)

The undiluted sap recorded maximum incidence of 70 per cent. However, no infection was observed in dilution 10^{-3}. The results showed that the dilution end point of the virus is between 10^{-2} and 10^{-3} (Table 13 and Fig. 1).

4.6.2 Thermal inactivation point (TIP)

The studies on thermal inactivation point recorded maximum disease incidence of 75 per cent at (28±2^{0}C). The virus was infective up to 45^{0}C treated sap. The results indicated that the virus was inactivated at a temperature range between 45^{0}C and 50^{0}C (Table 14 and Fig.2).

4.6.3 Longevity *in vitro* (LIV)

The maximum infection (70 %) was observed when inoculated immediately after sap extraction. A gradual decline in infectivity was observed by keeping the extract at room temperature and refrigerated condition and the virus was infective for 24 h at refrigerated condition and up to four h at room temperature (Table 15 and Fig.3).

4.7 HOST RANGE STUDIES

Host range studies were conducted with 14 plant species belonging to four families. The result showed that 11 species belonging to 3 families, *viz.*, Cucurbitaceae, Fabaceae, and Amaranthaceae produced symptoms of virus disease (Table 16).

Table 13. Assessment of dilution end point of WBNV

SI. No.	Dilution	PDI (%)
1.	Undiluted sap	70
2.	10^{-1}	25
3.	10^{-2}	10
4.	10^{-3}	0
5.	10^{-4}	0
6.	10^{-5}	0
7.	10^{-6}	0

Table 14. Assessment of thermal inactivation point of WBNV

SI. No.	Temperature (^{0}C)	PDI (%)
1.	Control (28± 2)	75
2.	35	60
3.	40	15
4.	45	5
5.	50	0
6.	55	0
7.	60	0

Fig.1. Assessment of dilution end point of WBNV

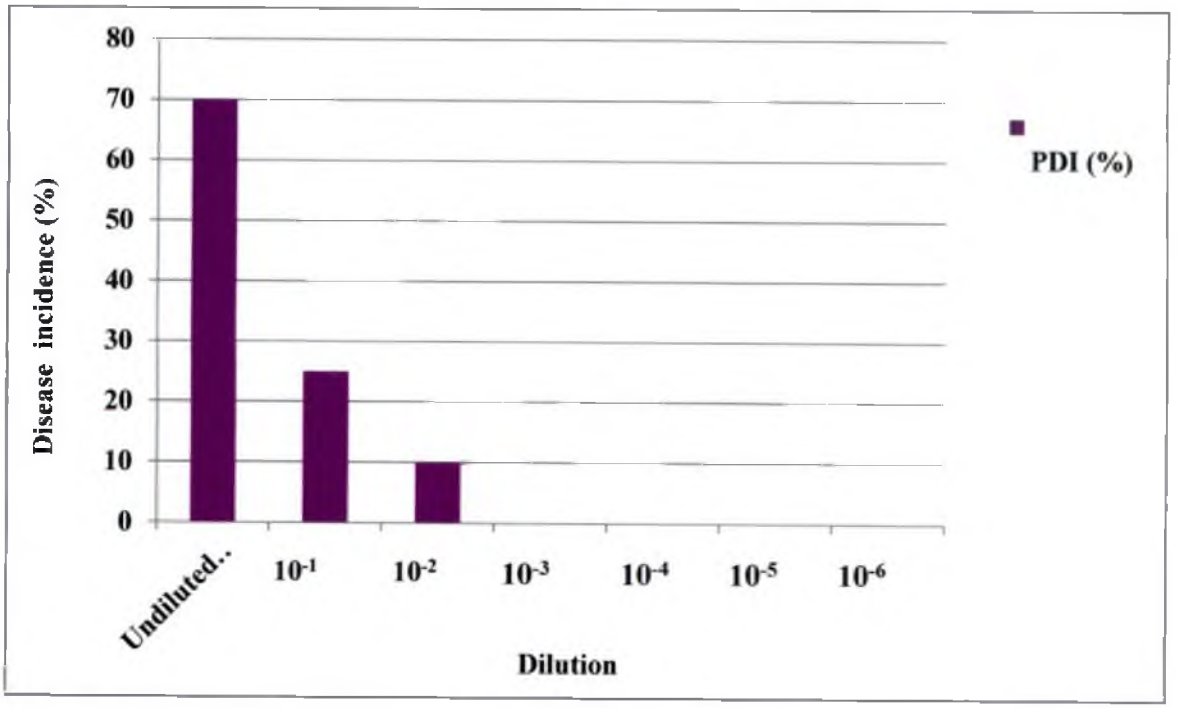


Fig. 2. Assessment of thermal inactivation point of WBNV

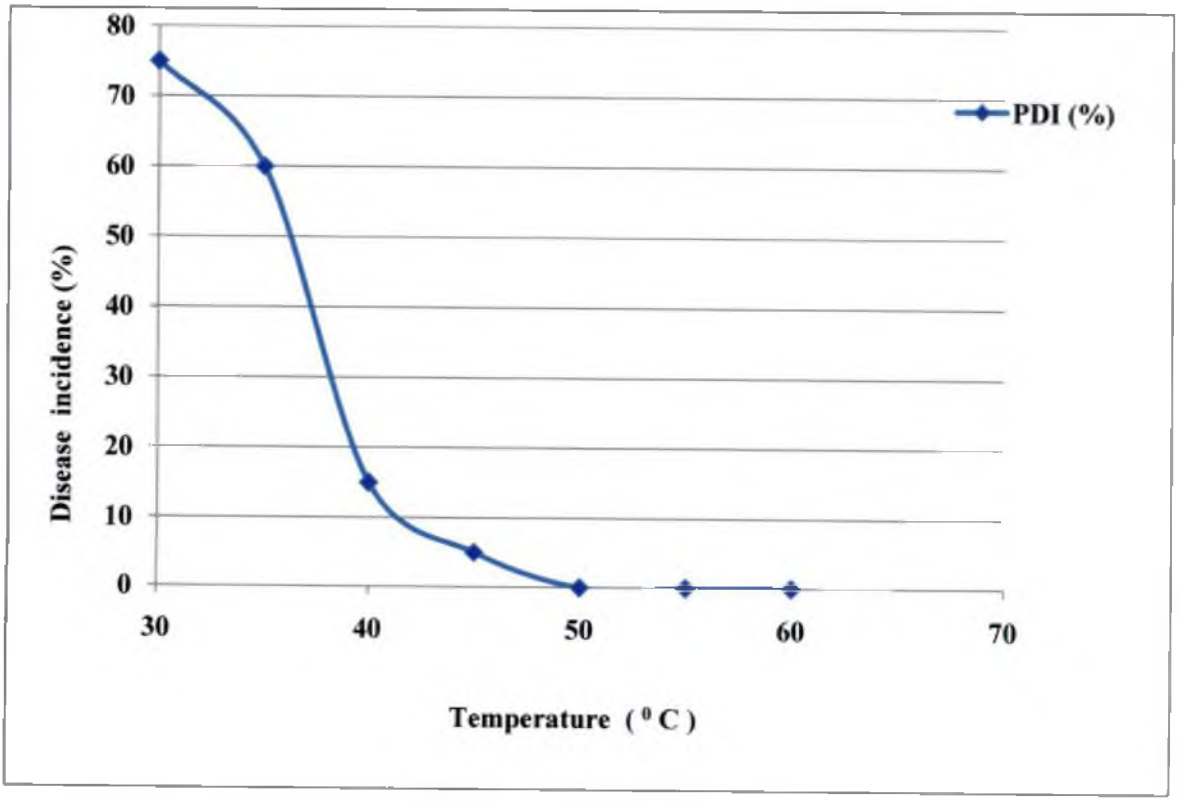


Table 15. Assessment of longevity *in vitro* of WBNV

SI .No.	Time (h)	PDI (Refrigerated)	PDI (Room temperature)
1.	0	60	70
2.	2	20	10
3.	4	15	5
4.	6	10	0
5.	24	5	0
6.	48	0	0
7.	72	0	0

Fig. 3. Assessment of longevity *in vitro* of WBNV

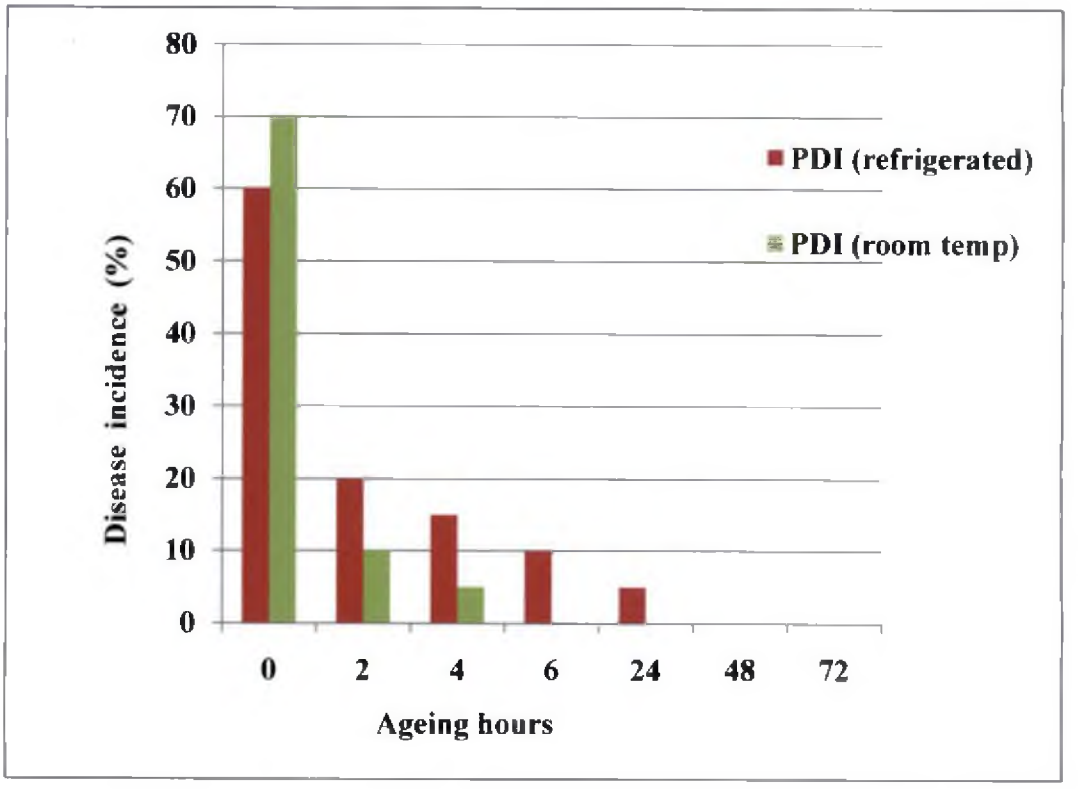


Table 16. Symptoms of WBNV infection in different host plants

Sl. No.	Scientific name	Transmission (%)	Symptoms		Incubation period in plant (Days)
			Local	Systemic	
	Cucurbitaceae				
1.	*Citrullus lanatus*	70		Mo, Ld, N	8
2.	*Cucumis melo* cv. *Conomon*	0	NS	-	0
3.	*Lagenaria siceraria*	50		Mo, Ld	12
4.	*Luffa acutangula*	70	Cs	Mo, Y	10
5.	*Momordica charantia*	10		Pk , Ld	12
6.	*Cucumis sativus*	20		Pk, Ld	14
	Fabaceae				
7.	*Vigna unguiculata*	40	Cs	Ld	7
8.	*Dolichos lablab*	20	N	NS	11
9.	*Arachis hypogaea*	20	N	Mo, Y	13
	Solanaceae				
10.	*Capsicum annuum*	0	NS	-	0
11.	*Solanum lycopersicon*	0	NS	-	0
12.	*Solanum melongena*	0	NS	-	0
	Amaranthaceae				
13.	*Amaranthus viridis*	10	N	-	7
14.	*Gomphrena globosa*	60	Cs, N	Vc, Ld	14

NS- No symptom, Mo- Mosaic, N- Necrosis, Y- Yellowing, Cs- Chlorotic spots, Pk- puckering, Ld - Leaf distortion, Vc-Vein clearing

Six cucurbitaceous hosts including the host plant watermelon were studied. *C. lanatus* and *L. acutangula* recorded the maximum infection of 70 per cent. *L. acutangula* showed symptom such as chlorotic spots, mosaic and yellowing. Whereas mosaic and puckering were the symptoms observed on *L. siceraria, M .charantia* and *C. sativus* (Plate 9).

The symptoms developed on other host plants are given in Plate 10. In the fabaceous hosts, necrotic spots were observed on *D. lablab*. *A. hypogaea* and *V. unguiculata* showed local and systemic infection. A maximum of 40 per cent infection was achieved through sap inoculation in *V. unguiculata.*

Among the amaranthaceous hosts, *G. globosa* recorded a maximum of 60 per cent incidence. The symptoms, *viz.*, necrotic spots and chlorotic spots, vein clearing and leaf distortion were observed in *G. globosa.*

None of the selected solanaceous hosts produced symptoms on sap inoculation with WBNV.

4.8 IMMUNODETECTION OF WBNV IN INFECTED SAMPLES

DAC- ELISA was carried out for the detection of *watermelon bud necrosis virus* in the infected field samples using polyclonal antiserum of *Peanut bud necrosis virus* (PBNV). Four dilutions of PBNV polyclonal antiserum, *viz.,* 1:10000 , 1:5000 , 1:2000 and 1:500 was used with 1:500 dilution of Alkaline Phosphatase (ALP) labeled-goat anti-rabbit secondary antibody. The absorbance was measured at 405 nm in ELISA reader. The results of the experiment revealed that samples with WBND showed positive reaction with the antiserum of closely related PBNV.

The absorbance values of the diseased samples at 1:500 dilution of the antiserum were always more than 1:2000, 1:5000 and 1:10000 dilutions. The absorbance value more than twice of the healthy sample was recorded in the 1:10000

a. Mottling and leaf distortion on bottle gourd

b. Mosaic and yellowing on ridge gourd

c. Puckering and leaf distortion on salad cucumber

Plate 9. Symptoms developed on cucurbitaceous host plants

a. Leaf distortion on cowpea

b. chlorotic spot on cowpea

c. Mosaic and yellowing on ground nut

d. Mosaic and vein clearing on gomphrena

Plate10. Symptoms developed on other host plants

dilution. This result revealed that 1:10000 dilution of the antiserum is enough for the detection of WBND.

Among the different samples assayed, young leaves with necrotic spots showed the maximum reactivity in all dilutions with highest absorbance value in 1:500 dilution (1.661) and least in 1:10000 dilution (0.395). The minimum reactivity was observed in shoot samples with bud necrosis symptom and absorbance value was highest in 1:500 dilution (0.866) and least (0.262) in 1:10000 dilution (Table 17 and Plate 11).

4.9 VARIETAL EVALUATION

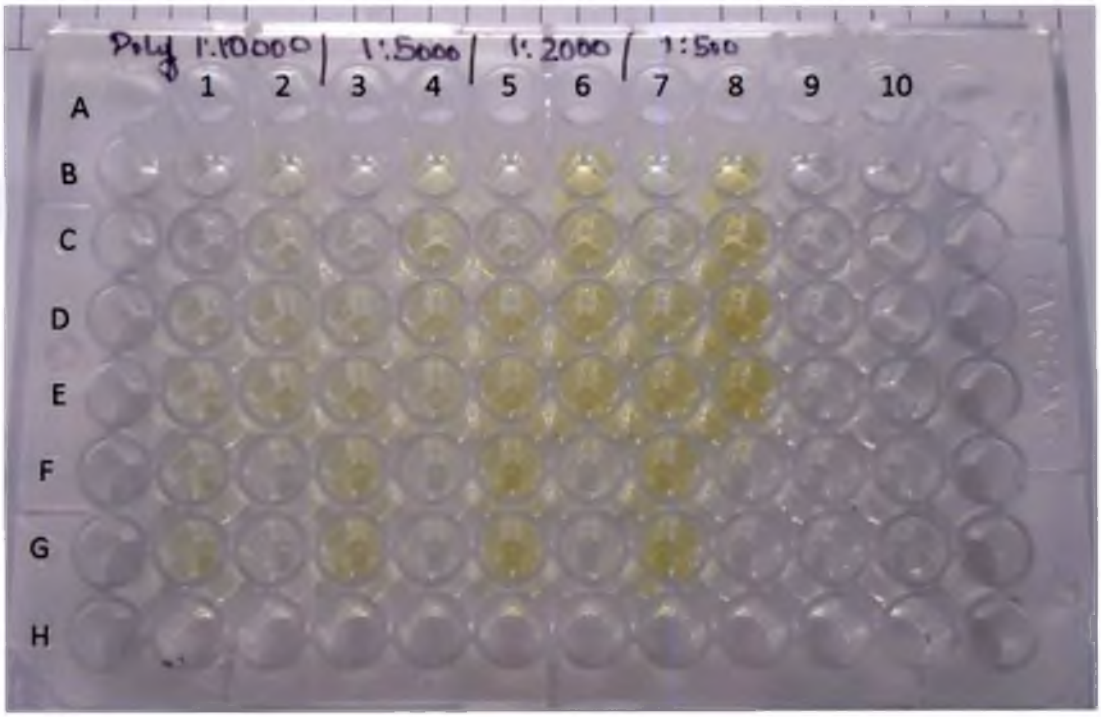
A total of 22 genotypes were evaluated for resistance against watermelon bud necrosis virus from the varietal screening plot of Department of Olericulture, College of Horticulture, Vellanikkara.

Out of the twenty two genotypes screened, none recorded resistance against the WBNV. The per cent disease incidence was minimum for WMH 12031 (8.33 per cent) and highest for NS 295 (96.00 per cent) in the fruiting stage of the crop.

Based on the disease reaction scale (0 to 5) described by Pandey and Pandey (2001), WMH 531 and WMH 12031 were recorded as the moderately resistant genotypes with disease incidence of six to ten per cent. The genotypes WMH 12123, Shoueing 175, WMH 351, IBH-23 and H-20 were moderately susceptible and recorded incidence of 11 to 20 per cent. Sugar Baby, Cl-6 and Indam Pantnagar were the susceptible genotypes and NS-295, Cl-4, Cl-5, Cl-7, Cl-8, WMH 12031, Arka Manik, Indam Sumo, Arka Muthu, Sindari, Yamuna, and Durgapura Lal were the highly susceptible genotypes. The per cent disease incidence and disease reaction of different genotypes are presented in Table 18.

Table 17. Immuno detection of WBNV infected samples using PBNV polyclonal antiserum

SI.No.	Type of symptoms	Mean absorbance values at 405 nm			
		Dilutions (ml)			
		1:10000	1:5000	1:2000	1:500
1.	Control (Buffer)	0.00	0.00	0.00	0.00
2.	Healthy	0.076	0.094	0.172	0.288
3.	Curling and crinkling symptoms on leaves	0.282	0.421	0.739	0.963
4.	Leaves with mosaic symptoms	0.343	0.483	0.931	1.278
5.	Young leaves with necrotic spots	0.395	0.595	0.997	1.661
6.	Shoot with bud necrosis symptom	0.262	0.389	0.579	0.866



B- Healthy samples (1-8)

C- Curling and crinkling symptoms on leaves (1-8)

D- Leaves with mosaic symptoms (1-8)

E- Young leaves with necrotic spots (1-8)

F and G 1, 3, 5 and 7- Shoot with bud necrosis symptom (1-8)

F and G 2,4, 6 and 8- Buffer (blank) (1-8)

Plate 11. DAC ELISA reaction

Table 18. Varietal evaluation under field condition

SI. No.	Genotypes	Per cent disease incidence		Disease reaction
		Vegetative stage	Fruiting stage	
1.	WMH 531	0.00	10.00	MR
2.	WMH 12031	0.00	8.33	MR
3.	WMH 12123	0.00	11.12	MS
4.	Shoueing 175	0.00	16.67	MS
5.	WMH 351	0.00	12.50	MS
6.	H-20	0.00	16.67	MS
7.	IBH-23	0.00	14.29	MS
8.	Sugar Baby	8.00	36.00	S
9.	Cl-6	8.33	33.33	S
10.	Indam Pantnagar	0.00	22.22	S
11.	NS 295	27.78	96.00	HS
12.	Indam Sumo	16.67	83.33	HS
13.	Sindari	8.33	83.33	HS
14.	Yamuna	16.67	91.67	HS
15.	Cl-7	20.00	92.00	HS
16.	Cl-8	20.00	90.00	HS
17.	WMH 12029	11.11	88.89	HS
18.	Arka Manik	20.00	85.00	HS
19.	Durgapura Lal	24.00	92.00	HS
20.	Cl-4	16.00	80.00	HS
21.	Cl-5	20.00	90.00	HS
22.	Arka Muthu	20.00	86.67	HS

PDI – Per cent disease incidence, [0 to 5% (Resistant); 6 to 10% (Moderately resistant); 11 to 20% (Moderately susceptible); 21 to 40% (Susceptible); more than 40 % (Highly susceptible)]

Discussion

5. DISCUSSION

Watermelon, *Citrullus lanatus* is one of the important summer fruit- vegetable crops in India. It is popular, especially during summer, for its sweet, cool, juice. Diseases and pests are the major constraints for increasing its productivity. Recently, bud necrosis disease of watermelon is becoming a serious threat to the cultivation of watermelon in Kerala and is causing severe damage to the crop. Hence, in the present project attempts were made to study the symptomatology, mode of transmission, physical properties and serodiagnosis of the watermelon bud necrosis disease.

Survey was conducted to assess the incidence, severity and to record the symptoms of watermelon bud necrosis disease in major watermelon growing locations of five districts of Kerala, *viz.*, Palakkad, Thrissur, Malappuram, Kasaragod and Kollam. The results showed that the disease incidence in these locations ranged from 30 to 98 per cent. Among the varieties observed, Sugar Baby recorded minimum disease incidence (30 to 37 per cent) and the triploid watermelon recorded the maximum disease incidence 98.34 per cent. Hence, the survey results revealed that, the disease incidence and severity varied with location and the varieties grown. The present results on the disease incidence and severity were found to be in line with the earlier reports. Pandey and Pandey (2001) reported up to 71 per cent incidence of bud necrosis disease in the experimental field at Indian Institute of Vegetable Research, Varanasi. Krishnakumar *et al.* (2006) reported that WBND incidence was 100 per cent by eleventh week after sowing at Indian Institute of Horticulture Research, Bangalore.

The influence of seasonal variation on disease incidence was also assessed. The results revealed that at Vellanikkara, the disease incidence was least during October to December, recording 10 to 24 per cent. But during January - March, the incidence increased to 16 to 38 per cent in the variety Sugar Baby. Other parameters such as disease severity and coefficient of infection were also higher during summer

months. This indicates that occurrence of bud necrosis disease incidence is higher during summer months. Reddy *et al.* (1992) also opined that peanut bud necrosis *Tospovirus* infection was common on crops grown in dry summer seasons in India.

The meteorological data recorded during the survey period at Vellanikkara showed that, during summer, the mean temperature recorded was 27.9 to 30.4^{0}C with less relative humidity of 51 to 56 percent and there was no rainfall, which favoured the increase of thrips population. This increase in vector population might be one of the reasons for the high incidence of bud necrosis disease during summer months. Kawai (1985) also reported that increase in temperature showed high population of *Thrips palmi* during summer months.

Symptoms are the visible effects that the pathogen causes on the growth, development and metabolism of an infected host plant. Study on symptomatology was undertaken under natural and artificial conditions. The symptoms observed were curling, mosaic, yellowing and dark brown to black coloured necrotic spots on leaves and narrowing of leaf lamina, stunting, shortened internodes in young branches, stem splitting, unopening of flower buds and their necrosis, necrotic streaks on vines, tendrils, fruit stalk and necrotic rings of corky texture on fruits. These types of symptoms on infected plants were also reported by other workers (Singh and Krishnareddy, 1996; Krupashankar, 1998 and Bhanupriya, 2006).

Under artificial condition the major symptoms noticed were curling and mottling of leaves in the initial stage followed by necrosis in the flowering stage. Under artificial inoculation, the severity of the symptoms was less as compared to those observed under field conditions.

Biological indexing is important for the characterization of the virus. In the present study the virus was isolated from the symptomatic watermelon plants by mechanical transmission on a local lesion host *Amaranthus viridis* using potassium phosphate buffer pH 7.0. The earlier report on biological assay was by Singh and

Krishnareddy (1996), isolated tospovirus from leaves of watermelon showing bud necrosis using 0.1 M phosphate buffer pH 7.0 containing 0.02 M 2. Mercaptoethanol. Extract from infected plants were inoculated on cowpea produced chlorotic local lesions.

Transmission is an important experimental tool to establish the etiology of viral diseases and plays vital role in disease spread. With this view, an attempt was made to understand the mode of transmission of the watermelon bud necrosis disease through sap and vector. In the present study, the mechanical transmission was standardized using five different buffers each at four pH range, *viz.*, 7.0, 7.2, 7.4 and 7.6. The result indicates that the different buffers and the varied pH level used for each buffer shows significant difference in per cent transmission of the disease. Of the different buffers used, citrate phosphate buffer 0.1 M (pH 7.2) showed maximum disease incidence of 72.22 per cent with minimum incubation period of 8 days and was selected as the best buffer for further studies. The early report on the successful transmission of WBNV was by Singh and Krishnareddy (1996). According to this report, the leaves of watermelon showing bud necrosis disease was extracted using 0.1 M phosphate buffer pH 7.0 and on mechanical inoculation it produced localized followed by systemic infection on watermelon and localized chlorotic local lesions on cowpea. Hence, this is the first report on the efficacy of citrate phosphate buffer (pH 7.2), in the transmission of WBNV.

As the studies on virus-vector relationship are necessary for the better understanding of the virus, the next approach was to find out the role of vectors. In the present study thrips collected from watermelon plots was identified as *T. palmi* Karny from Department of Entomology, College of Horticulture, Vellanikkara which was in line with the reports of (Krupashankar, 1998 and Bhanupriya, 2006). But shows variation from the report of Singh and Krishnareddy (1995), as he reported *Thrips flavus* shrank (Thysanoptera: Thripidae), as a new insect vector of a tospovirus infecting watermelon in India.

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Transmission efficiency of thrips nymphs and adults were compared first with and without the latent period. The results proved that, the nymphs without latent period shows five per cent transmission, whereas with the latent period shows 21.5 per cent transmission. The adult thrips failed to transmit the virus without the latent period. Similar observations were also reported by earlier workers (Sakimura, 1963; Amin *et al.,* 1981 and Ullman *et al.*, 1992).

The next importance was given to study the virus vector relationship which is very essential for the proper management of the disease. The first approach was on the basic aspects of transmission such as acquisition access period, inoculation access period and the incubation period of the virus in the host. During these basic studies, it was found that 24 h was the minimum period required by *T. palmi* to aquire WBNV from source plant and per cent of infective insects increased with an increase in the length of acquisition access period. In the experiment on inoculation access period, the minimum period require to transmit the virus to healthy plant was 48 h and the per cent of transmission increased with an increase of inoculation access period. According to Sreekanth *et al.* (2006) *T. palmi* , require two days acquisition access period in the nymph stage and two days inoculation access period in the adult stage for the transmission of PBNV which is the closely related tospovirus of WBNV. The inoculation access period obtained in the present study shows similarity with earlier report. But a variation was observed in case of the acquisition access period which found to be 24 h.

In the general transmission studies, symptoms were expressed by host plants within 28 to 30 days of inoculation which shows variation from the early report by Krupashankar (1998). According to this report, 30 to 40 days was taken for the expression of symptoms on inoculation of *T. palmi*.

Physical properties are characteristic for a virus. Dilution end point (DEP), thermal inactivation point (TIP) and longevity *in vitro* (LIV) are the studies carried

out to find out the physical properties of the virus. The virus recorded a DEP in the range of 10^{-2} and 10^{-3}, TIP of 45^0C to 50^0C and LIV of four hour at room temperature (28±2) and 24 h at refrigerated condition (8^0C). The result of the study shows similarity with physical properties of PBNV of groundnut reported by Ghanekar *et al.* (1979). Bhanupriya (2006) also reported the physical properties of PBNV from groundnut. DEP and TIP were found to be in line with those obtained in the present study, but a difference was observed in the case of LIV which was found to be three hours.

Collateral hosts play an important role in the perpetuation of the pathogen and the vectors. The knowledge on this aspect is very useful for the successful management of viral diseases. It was observed that *Vigna unguiculata, Arachis hypogaea, Luffa acutangula* and *Gomphrena globosa* produced localized infection followed by systemic infection. The systemic infection alone was noticed in *Lagenaria siceraria, Cucumis sativus* and *Momordica charantia.* These results support the fact that *Tospoviruses* have broad host range (Moyer *et al.*, 1999). Further, these plant species could serve as potential reservoirs of *Watermelon bud necrosis virus.* Singh and Krishnareddy (1996) reported that the watermelon tospovirus isolate from India systemically infected watermelon, muskmelon and other cucurbits. According to this report chlorotic or necrotic spots followed by systemic infection was observed on *C. lanatus* cv. Arka Manik, *C. lanatus* cv. Madhu, *Cucumis melo, C. sativus, Cucurbita maxima, Cucurbita pepo, L. siceraria, A. hypogaea* cv. JL-24, *Cassia tora, Dolichos lablab, Glycine* max cv Bragg, *Phaseolus lanatus, Capsicum annuum* cv. Yellow Wax, *Solanum lycopersicon* cv. Pusa Ruby, *Physalis floridana, Gomphrena globosa* and *Emilia sonchifolia.* In the present study localized infection followed by systemic infection was observed in *L. acutangula* and *G. globosa* and *A. hypogaea.* Only systemic infection was observed in *C. lanatus* cv. Durgapura Lal, *C. lanatus* cv. Sugar Baby, *L. siceraria, C. sativus* and *M. charantia.*

The earlier reports by Mandal *et al.* (2003) Jain *et al.* (2006) also showed the WBNV infection on vegetable crops such as *C. melo, L. acutangula, L. siceraria, C. sativus, Cucrbita pepo* and *V. unguiculata.* The results of the present study and earlier reports revealed that vegetable crops grown round the year near watermelon fields, helps in the perpetuation of the virus in the crop free period.

The results of the immunodiagnostic study revealed that WBNV infected samples showed high reactivity with the polyclonal antiserum of *Peanut bud necrosis virus* received from IISc, Bangalore and confirmed that the bud necrosis virus infecting watermelon is a tospovirus, serologically related to PBNV. The absorbance values of the diseased samples were inversely proportional to the dilution of the antiserum. The 1:10000 dilution itself was more than two times of the healthy sample which revealed that this is enough for the detection of WBNV. According to the results the polyclonal antiserum used in this study could be used for the serodiagnosis of WBNV. The positive reaction of WBNV samples with PBNV antiserum was also repoted earlier. Singh and Krishnareddy (1996) detected the WBNV samples using PBNV and *Watermelon silver mottle virus* (WSMoV) specific antisera and reported that it is closely related to PBNV. According to Jain *et al.* (2005) the antiserum against N protein of PBNV at 1:4000 dilution successfully detected the natural infection of PBNV and WBNV in a wide range of cucurbitaceous, leguminous and solanaceous hosts from different locations.

In the present investigation, 22 genotypes of watermelon maintained in the varietal evaluation trial of Department of Olericulture, College of Horticulture were screened against WBND. It was found that WMH 531 and WMH 12031 were moderately resistant genotypes and WMH 12123, Shoueing 175, WMH 351, IBH-23 and H-20 were moderately susceptible. Sugar Baby, Cl-6 and Indam Pantnagar were the susceptible genotypes and NS-295, Cl-4, Cl-5, Cl-7, Cl-8, WMH 12031, Arka Manik, Indam Sumo, Arka Muthu, Sindari, Yamuna, and Durgapura Lal were found to be highly susceptible. There are some earlier reports showing the screening of

watermelon cultivars against WBNV. Pandey and Pandey (2001) screened sixteen watermelon hybrids and selection lines for resistance against WBNV in a field experiment conducted in Uttar Pradesh. Among the hybrids and lines tested, four were moderately resistant (EC-393240, Durgapur Meetha, MHW-6 and Ashey Questo) and three were resistant (EC-393243, Durgapur Selection and RHRWH-2) to the pathogen. A report from IIHR during 2007 showed that, among the interspecific cross derivatives of (Arka Manik x *Citrullus colocynthis*) a total of 146 individual plants were raised and these plants were screened against WBNV under natural condition. Among the plants screened, eight germplasm accessions namely, IIHR-81, IIHR-83, IIHR-85, IIHR-90, IIHR- 102, IIHR-110, IIHR-114 and IIHR-118 were found free from WBND.

In conclusion, the study provided better understanding on the incidence and severity of watermelon bud necrosis disease in Kerala. The study on symptomatology, mode of transmission, host range and varietal evaluation will be helpful for evolving crucial management practices in near future.

Summary

6. SUMMARY

The study entitled "Biological characterization of bud necrosis virus disease in watermelon, (*Citrullus lanatus* T.), was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2012 to 2014.

A survey was undertaken to assess the incidence of the WBND in watermelon growing areas of Thrissur, Palakkad, Malappuram, Kasaragod and Kollam districts of Kerala. During 2013 and 2014, the disease incidence from the surveyed locations ranged from 30 to 98 per cent. While the Survey data on Rabi and summer season on the variety Sugar Baby shows less infection in Vellanikkara during October to December, recording 10 to 24 per cent disease incidence and 6 to 21 per cent disease severity. It increased to 16 to 38 per cent incidence and 11 to 34 per cent severity in the crop grown during January - March. Among the varieties, Sugar Baby showed minimum disease incidence (30 to 37 per cent) and triploid watermelon recorded the maximum disease incidence 98.34 per cent. This revealed that the disease incidence varied from location to location, season to season and it also depends on the genotype grown.

The virus was isolated from symptomatic watermelon plants by biological indexing through a local lesion host *Amaranthus viridis* using potassium phosphate buffer pH 7.0.

The characteristic symptoms of WBND at each growth stages of the plant were documented. The symptoms expressed on different parts of the plant like leaves, branches, flower buds and fruits were also recorded. Major symptoms of WBND observed in the infected plants were curling, mosaic, yellowing , dark brown coloured necrotic spots on leaves, narrowing of leaf lamina, stunting, shortened internodes in the young branches, un-opening of flower buds and their necrosis, necrotic streaks on vines, necrotic rings on fruits and malformation of fruits.

Under artificial condition symptom initiated as curling of younger leaves which later spread to other leaves. The leaves showed crinkling and mottling in the later stages. The leaf size was reduced, became malformed and was rough and brittle. Un- opening of flower buds and bud necrosis was observed in the flowering stage.

The mechanical transmission studies were standardized using five different buffers at four pH level (*i.e.*, 7.0, 7.2, 7.4 and 7.6). The citrate phosphate buffer 0.1M citrate phosphate buffer (pH 7.2) showed maximum mean per cent transmission (72.22 per cent) with minimum incubation period (8 days) and selected as the best buffer for further studies.

Insect transmission studies were conducted with thrips maintained in the insect proof cages. Collected thrips were identified as *Thrips palmi* Karny. Studies on virus- vector relationship showed that acquisition access period and inoculation access period were 24 h and 48 h respectively. Increase in acquisition access period from 24 to 96 h increased per cent transmission and decreased incubation period in the host. The above trend was also noticed with increase in inoculation access period from 48 to 96 h.

Physical properties of virus *i.e.*, dilution end point, thermal inactivation point and longevity *in vitro* were investigated and it was found that the virus was inactivated at dilutions between 10^{-2} and 10^{-3} and at temperatures between 45 and 50^{0}C. Longevity in *in vitro* recorded as four h at room temperature (28± 2) and 24 h at refrigerated condition (8^{0} C).

The virus found to have its host range in the members of the families Cucurbitaceae, Fabaceae and Amaranthaceae. It was observed that *Vigna unguiculata* , *Arachis hypogaea, Luffa acutangula* and *Gomphrena globosa* produced localized infection followed by systemic infection. The systemic infection alone was noticed in *Lagenaria siceraria, Cucumis sativus* and *Momordica charantia.*

The virus causing necrosis disease in watermelon was identified as *Tospovirus* by serodiagnosis with DAC ELISA method. The virus isolates of watermelon samples showed close relationship with PBNV. Standardisation of PBNV antiserum dilution showed 1:10000 is enough for the serological detection of WBNV.

Twenty two genotypes of watermelon maintained in the varietal evaluation plot of Department of Olericulture were screened against WBND. It was found that WMH 531 and WMH 12031 were the moderately resistant genotypes and WMH 12123, Shoueing 175, WMH 351, IBH- 23 and H-20 were moderately susceptible. Sugar Baby, Cl-6 and Indam Pantnagar were recorded as susceptible genotypes and NS-295, Cl-4, Cl-5, Cl-7, Cl-8, WMH 12031, Arka Manik, Indam Sumo, Arka Muthu, Sindari, Yamuna, and Durgapura Lal were found to be highly susceptible genotypes.

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Appendices

APPENDIX – I

Composition of buffers used in mechanical transmission

1. **0.1 M sodium borate buffer**

   A. Boric acid (0.2 M) - 1.273g/100ml

   B. Borax $Na_2B_4O_7.10H_2O$ - 1.907g/ 100 ml

   3 ml of A mixed with 7 ml of B, diluted to a total of 20 ml.

2. **0.1 M Potassium phosphate buffer**

   A. 0.1 M Potassium dihydrogen phosphate - 6.084g/500ml

   B. 0.1 M Dipotassium hydrogen phosphate – 8.079g/500ml

   28 ml of A mixed with 72 ml of B

3. **0.1 M sodium phosphate buffer**

   A. 0.1 M Sodium phosphate monobasic anhydrous – 5.999g/500ml

   B. 0.1 M Sodium phosphate dibasic dehydrate – 8.899g/500ml

   28 ml of A mixed with 72 ml of B

4. **0.1 M Citrate phosphate buffer**

   A. 0.1 M Citric acid – 2.101g/100ml

   B. 0.1 M Sodium phosphate dibasic dehydrate – 1.77g/100ml

   1.6 ml of A mixed with 18.4 ml of B

APPENDIX – II

Composition of buffers used in DAC- ELISA

1) Phosphate buffer saline (PBS- pH 7.4)

Sodium chloride	-	8.0g
Potassium dihydrogen phosphate	-	0.2g
Disodium hydrogen phosphate	-	1.1g
Potassium chloride	-	0.2g
Sodium azide	-	0.2g
Water	-	1000ml
Tween 20 (0.05%)	-	0.5ml

2) Coating buffer (pH 9.6)

Sodium carbonate	-	1.59g
Sodium bicarbonate	-	2.93g
Sodium azide	-	0.2g
Water	-	1000ml

Abstract

BIOLOGICAL CHARACTERIZATION OF BUD NECROSIS VIRUS DISEASE IN WATERMELON

(*Citrullus lanatus* T.)

By

ASWATHI K.K.
(2012-11-202)

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement

for the degree of

Master of Science in Agriculture

(PLANT PATHOLOGY)

Faculty of Agriculture

Kerala Agricultural University, Thrissur

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VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2014

ABSTRACT

Watermelon, *Citrullus lanatus* (Thunb.), is an annual trailing creeper belonging to the family Cucurbitaceae. It occupies a pivotal position among fruit vegetables. Among the diseases affecting watermelon, those caused by viruses are difficult to control and can be very destructive.

Recently, bud necrosis, a *Tospovirus* disease, has emerged as a serious problem of watermelon cultivation in Kerala. Not much work on the various aspects of the disease has been carried out. Further, little is known on disease incidence, severity and mode of transmission of the disease which are crucial for evolving appropriate management practices. Hence, the present project was undertaken to study the symptomatology, transmission, physical properties, host range and serological reaction of *Watermelon bud necrosis virus* (WBNV).

Survey undertaken on the incidence and severity of the disease in major watermelon growing locations of Thrissur, Palakkad, Malappuram, Kasaragod and Kollam districts revealed the variations, based on the genotype, season and location.

Under natural conditions, symptoms observed were curling, mottling, narrowing of leaf lamina, stunting, necrotic streaks on stem, unopening of flower buds and their necrosis, necrotic rings and malformation of fruits. Under artificial inoculation, symptom initiated as curling, crinkling and mottling of leaves, which later spread to young leaves and resulted in brittleness.

The virus was isolated from symptomatic watermelon plants by mechanical transmission through a local lesion host *Amaranthus viridis* using potassium phosphate buffer pH 7.0.

Transmission studies proved that WBNV transmitted both by sap and vector. Citrate phosphate buffer 0.1M (pH 7.2) showed the maximum transmission (72.22 %) with

the minimum incubation period (8 days) and was used for further studies. The insect vector of WBNV was identified as *Thrips palmi* Karny. Studies on virus vector relationships showed that the minimum acquisition access period and inoculation access period were 24 h and 48 h respectively.

Physical properties of virus *i.e.*, dilution end point, thermal inactivation point and longevity in *in vitro* were investigated and it was found that the virus was inactivated at dilutions between 10^{-2} and 10^{-3} and at temperatures between 45^0 and 50^0C. Longevity in *in vitro* was recorded as four h at room temperature (28± 2) and 24 h under refrigerated condition (8^0 C).

Host range studies revealed that ridge gourd, bottle gourd, salad cucumber, bitter gourd, cowpea, groundnut and gomphrena could serve as collateral hosts of the virus.

Serological tests using DAC – ELISA of WBNV revealed close relation of the virus with *Peanut bud necrosis virus*.

Among the 22 genotypes of watermelon screened for response to the virus none of them showed resistance. However, WMH531 and WMH 12031 recorded the lowest incidence of 10 and 8.33 per cent respectively, while the highest (96 per cent) was in NS 295 hybrid.

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