

KENYA PLANT HEALTH INSPECTORATE SERVICE Quarantine and Plant Health Laboratory-Muguga			Page 1 of 6
TITLE AND ID.	SOP FOR DETECTION OF SWEET POTATO VIRUSES USING GRAFTING METHOD ON INDICATOR PLANTS-TE/01		
DEPARTMENT	Virology		
EFFECTIVE DATE	2 nd Feb 2023	Version Number: 03	

Approval and authorizations.

Completion of the below signature blocks signifies the review and approval of this document.

Name	Job title/role	Signature	Date
Authored by	Head Virology	<i>Approved</i>	2 nd Feb 2023
Checked by	Laboratory Manager	<i>Approved</i>	2 nd Feb 2023
Approved by	Deputy director-PQBS	<i>Approved</i>	2 nd Feb 2023

Periodic reviews, approvals and authorizations

Required by: 1st March 2024

Name	Job title/role	Signature	Date
Checked by	Head Virology		
Approved by	Deputy director-PQBS		

Periodic reviews, approvals and authorizations

Required by: 1st March 2027

Name	Job title/role	Signature	Date
Checked by			
Approved by			

KENYA PLANT HEALTH INSPECTORATE SERVICE Quarantine and Plant Health Laboratory-Muguga		 Page 2 of 6
TITLE AND ID.	SOP FOR DETECTION OF SWEET POTATO VIRUSES USING GRAFTING METHOD ON INDICATOR PLANTS-TE/01	
DEPARTMENT	Virology	
EFFECTIVE DATE	2 nd Feb 2023	Version Number: 03

Table of Contents

1.0 INTRODUCTION	3
2.0 SCOPE	3
3.0 SAFETY	3
4.0 MATERIALS	4
5.0 PROCEDURE	4
8.ANNEXES	Error! Bookmark not defined.
9.RECORDS	5
10.REVISION LIST	6

KENYA PLANT HEALTH INSPECTORATE SERVICE Quarantine and Plant Health Laboratory-Muguga		 Page 3 of 6
TITLE AND ID.	SOP FOR DETECTION OF SWEET POTATO VIRUSES USING GRAFTING METHOD ON INDICATOR PLANTS-TE/01	
DEPARTMENT	Virology	
EFFECTIVE DATE	2 nd Feb 2023	Version Number: 03

1.0 INTRODUCTION

Grafting is a technique whereby cut tissue surfaces of different plants are placed in close contact to effect a union. Grafting is considered to be a universal method for transmitting viruses, because grafting can transmit systemic viruses. Some grafts, however, are easier to do than others. Grafting is particularly useful for transmission of phloem-restricted viruses that cannot be transmitted mechanically and viruses whose vectors remain unknown, and for detecting viruses found in low concentrations. Virus transmission by grafting may not be 100%-effective if the virus is unable to cross the graft union, or if the virus source plant was not totally invaded and the portion used was virus-free due to irregular virus distribution. Most of the sweetpotato viruses infect *Ipomea setosa* causing visible symptoms. Successful virus transmission depends on the characteristics of the virus and on the union achieved through grafting. A virus such as Sweetpotato chlorotic stunt virus (SPCSV), which is restricted to the vascular system, can be transmitted only when vascular tissues are united. On the other hand, viruses affecting the parenchyma e. g Sweetpotato feathery mottle virus, SPFMV can be transmitted more easily because the transmission depends solely on the union of the cortex or the medula.

2.0 SCOPE

This SOP outline procedures followed during grafting to test for sweet potato viruses using indicator plant (*Ipomoea setosa*).

3.0 SAFETY

- I. A laboratory coat should be worn at all times when working in the greenhouse
- II. New scalpel blade should be used when grafting per sample.

KENYA PLANT HEALTH INSPECTORATE SERVICE Quarantine and Plant Health Laboratory-Muguga			Page 4 of 6
TITLE AND ID.	SOP FOR DETECTION OF SWEET POTATO VIRUSES USING GRAFTING METHOD ON INDICATOR PLANTS-TE/01		
DEPARTMENT	Virology		
EFFECTIVE DATE	2 nd Feb 2023	Version Number: 03	

4.0 MATERIALS

- I. Parafilm strips
- II. Sticks for supporting the plants
- III. Plastic bags (polystyrene) bags 14x14x2(wide long and thickness, respectively).
- IV. Ipomoea setosa indicator plants (recipient host, 'stock')
- V. Sweetpotato plant materials (donor host, 'scions')

5.0 PROCEDURE

5.1.1. Germination of indicator plants

Seeds will be germinated on a blotter paper in a plastic petri dishes of 10 cm in diameter. Cover seeds with an additional layer of a wet blotter paper or paper towel. Maintain seeds at 25± 4°C during germination. Add water to wet the blotter paper or towel when necessary.

N/B: In case of seed dormancy, breaking of the dormancy will be done using scarification as described in (Annex 1 of this SOP)

Do not use more than 30 seeds per plate.

5.1.2. Raising plants.

Three or four days after emergence of hypocotyl and radicle, remove the blotter paper/paper towel covering the petridish.

Transplant the seedlings to jiffy pots for 4 days and later transfer seedlings to individual pots. Grafting is done after about 4 weeks when the plants have 2-3 fully expanded true leaves. The seedlings will be maintained in an insect proof greenhouse at 25±4°C

KENYA PLANT HEALTH INSPECTORATE SERVICE Quarantine and Plant Health Laboratory-Muguga			Page 5 of 6
TITLE AND ID.	SOP FOR DETECTION OF SWEET POTATO VIRUSES USING GRAFTING METHOD ON INDICATOR PLANTS-TE/01		
DEPARTMENT	Virology		
EFFECTIVE DATE	2 nd Feb 2023	Version Number: 03	

5.1.3 Grafting

1. Remove most of the older leaves from the 4 week old indicator plant (stock), with about 2-3 fully expanded true leaves.
2. Cut an incision downwards into the stem.
3. From the donor host cut a scion which has a node with a fully expanded leaf
4. Trim the scion to a 0.5-1cm long. Insert the scion into the incision and fix (wrap) the graft with parafilm.
5. Wet the wound with water
6. Cover the grafted plant with a plastic bag for 3 days.
7. Maintain the plants in a screen house at 25±4°C, for 4 weeks.

5.1.4 Data collection

Viral disease symptoms are observed and recorded on the sample worksheet (Record/SOP/TE/01/01) once in a week for a period of 3 to 6 weeks

5.1.5 Internal quality control

- Periodic testing of indicator plants to ascertain freedom from viruses
- Assessing seed dormancy of indicator plants
- Having a control where setosa plant is used as a scion
- Wear and maintain separate protective clothing before entry to the screenhouse

6. RECORDS

- i. Sample worksheet for grafting
- ii. Screenhouse temperature records

KENYA PLANT HEALTH INSPECTORATE SERVICE Quarantine and Plant Health Laboratory-Muguga			Page 6 of 6
TITLE AND ID.	SOP FOR DETECTION OF SWEET POTATO VIRUSES USING GRAFTING METHOD ON INDICATOR PLANTS-TE/01		
DEPARTMENT	Virology		
EFFECTIVE DATE	2 nd Feb 2023	Version Number: 03	

ANNEXES

1. Preparation of Ipomoea setosa indicator plants

a) Scarification

- Put I. setosa seeds in a glass beaker.
- Add concentrated sulphuric acid (98%) up to slightly immerse the seeds
- Keep the seeds immersed in the acid for 50 min (or 20 min for I. nil seeds),
- Pour off the acid into another container for disposal.
- Wash off the acid from the seeds in water by stirring for a few minutes with a rod (you may use a wide bottomed bucket or a plastic tray). Carefully pour off the water and repeat the washing 4 times
- Place the seeds on blotter paper for germination

7. REVISION LIST

Current Revision number	Supersedes revision number	Reason for review	Reviewers name
01	00	New based on ISO170125:2017 standard	Maureen Mwangangi Avedi Edith
02	01	Inclusion of need for change of PPE on entry to the screen house	E. Avedi
03	02	Removed scarification since it is no longer no longer done Receiving of samples at the sample reception was missing	E.Avedi