PRODUCTION AND PROPAGATION OF VIRUS-FREE PLANTING MATERIALS OF STRAWBERRY

By

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B.Sc. Agric. Sc. (Agric. Microbiology), Ain Shams University, 2003

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Approval Sheet

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ABSTRACT

Yasmer Sayed Hussein Muhammad: Production and Propagation of Virus-Free Planting Materials of Strawberry. Unpublished M.Sc. Thesis, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, 2010.

About 250 samples from strawberry plants developing symptomless and distinct virus-like symptoms (stunting, mottling, yellowing, vein clearing, vein necrosis, vein banding, leaf curling downward and leaf deformation) were collected from strawberry nurseries and production fields. Plants were subjected to dot blot immunoassay (DBIA) tests using IgG polyclonal antibody specific for *strawberry latent ring spot virus* (SLRSV), *raspberry ring spot virus* (RpRSV), *strawberry mottle virus* (SMoV) and *strawberry vein banding virus* (SVBV). In addition plants were subjected to RT-PCR and PCR as a molecular detection test and identification some virus isolates for further confirmation. RT-PCR and PCR were used to amplify a fragment of about 497 bp, 490 bp , 384 bp and 472 bp with specific four primer sets from genome of viruses SLRSV, RpRSV , SMoV and SVBV respectively. RpRSV was the most incidence virus. The RpRSV was isolated and identified based on biological, serological and molecular tests. Experiments proved that the virus could be transmitted mechanically to some herbaceous plants, by grafting to sensitive strawberry indicator plants “Alpine” and transmitted by nematode to healthy strawberry. RpRSV isolate was identified by serological reaction with specific polyclonal antibodies by DBIA. The virus isolate was identified also by molecular characters where total RNA was extracted from infected strawberry plants with RpRSV. Production of strawberry virus-free plants was done through elimination of RpRSV from infected plants by chemotherapy, thermotherapy and combination between them using tissue culture. Based on the field inspection, positive serological,

RT-PCR and/or PCR detection results, we concluded that these viruses belong to strawberry viruses which cause diseases in strawberry.

Key Words: Strawberry virus, DBIA, RT-PCR, PCR and Tissue culture.

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LIST OF ABBREVIATIONS

	(A)
ACLSV	*Apple chlorotic leaf spot virus*
Agri.	Agriculture
ApMV	*Apple mosaic virus*
ArMV	*Arabis mosaic virus*
ASPV	*Apple stem pitting virus*
	(B)
BA	6-Benzyl amino purine
bp	Base pair
BPYV	*Beet pseudo yellows virus*
	(C)
°C	Centigrade
$CaCo_3$	Calcium Carbonate
cDNA	Complementary DNA
cm	Centimeter
cp	Coat protein
CsCL	Cesium Chloride
cv.	Cultivar
	(D)
2, 4-D	Dichloro phenoxy acetic acid
DAS	Double antibody sandwich
DBIA	Dot blot immunoassay
DEPC	Diethylpyrocarbonate
DHT	2,4 – dioxohexahydro - 1,3,5- triazine
DNA	Deoxy ribonucleic acid
ds	Double strand
	(E)
EDTA	Ethylene diamine tetra acetate
ELISA	Enzyme linked immunosorbent assay
EM	Electron microscopy
EMC	East Malling clone of *F. vesca* infected with *strawberry latent A virus* (mild strain of SCV)
EMK	EMC clone free of crinkle
EtoH	Ethanol

(F)

FCILV	*Fragaria chiloensis latent virus*
Fig.	Figure
FV- 72	*F. vesca*

(G)

g	Gram
GA_3	Gibberellic acid
GFkV	*Grapevine fleck virus*
GFLV	*Grapevine fanleaf virus*
GLRaV	*Grapevine leafroll-associated virus*
GVA	*Grapevine virus A*

(H)

HCL	Hydrochloric acid
$HgCl_2$	Mercuric chloride
H_2O	Water
hr.	Hour

(I)

IAA	Indol acetic acid
IBA	Indol butyric acid
IgG	Immunoglobin
Inst.	Institute

(K)

KAc	Potassium acetate
Kin	Kinetin

(L)

L.	Liter
Lab.	Laboratory

(M)

M	Molar
µg	Microgram
mg	Milligram
min.	Minute
µl	Microliter
ml	Milliliter(s)
µM	Micrometer
mm	Millimeter(s)
mM	Millimolar

MS	Murashige and Skoog
MW	Molecular weight
	(N)
NAA	Naphthalene acetic acid
NaCL	Sodium Chloride
NaOH	Sodium Hydroxide
ng	Nanogram
nm	Nanometer
	(P)
PBS	Phosphate buffer saline
PBST	Phosphate buffer saline tris
PCR	Polymerase chain reaction
PDV	*Prune dwarf virus*
pH	Potentiometric hydrogen ion concentration
PNPSV	*Prunus necrotic ring spot virus*
PPV	*Plum pox virus*
PVP	Polyvinylpyrrolidone
	(R)
RNA	Ribonucleic acid
rpm	Revolutions per minute
RpRSV	*Raspberry ring spot virus*
RT	Reverse transcriptase
	(S)
SCV	*Strawberry crinkle virus*
sec.	Second(s)
SLCV	*Strawberry leaf curl virus*
SLRSV	*Strawberry latent ring spot virus*
SMoV	*Strawberry mottle virus*
SMYEV	*Strawberry mild yellow edge virus*
SNSV	*Strawberry necrotic shock virus*
SPaV	*Strawberry pallidosis virus*
SPFMV	*Sweet potato feathery mottle virus*
SPMYEV	*Strawberry pseudo mild yellow edge virus*
SVBV	*Strawberry vein banding virus*