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• Name of Candidate: Mohamed Atef Abdel-Rahman Nasr-EldinB.Sc. (Microb.&Chem.), Zagazig Univ., Benha Branch, 2003M.Sc. (Microbiology), Benha Univ., 2007• Degree: Ph.D.• Title of thesis: Molecular Studies on Some Viroids Infecting Grapevinein Egypt• Department: Botany Branch: Microbiology, VirologyFaculty of Science, University of Benha, Egypt, 2013Hop stunt viroid (HSVd), Citrus exocortis viroid (CEVd), Potatospindle tuber viroid (PSTVd) and Grapevine yellow speckle viroid-1(GYSVd-1) were characterized from naturally infected grapevine Vitisvinifera cv. Balady/banaty during two summer seasons 2009/2010,2010/2011 in Egypt. One hundred sticks of grapevine trees were collected outof three thousand grapevine trees showing symptoms such as stem cracking,torsion, leaf deformation, mosaic vein-banding, yellow batches, Also someleaves showing distinctive viroid and virus like symptoms involved ”yellowspeckle, vein clearing and wavy leaflets margin”.Nucleic acid hybridization using digoxygenin-labelled riboprobes forHSVd, CEVd and PSTVd was used to detect the viroid-infected samples. Thetotal percentage of positive infection was 88.88%. Viroid-infected samplesgave negative results in DAS-ELISA using specific polyclonal antibodiesagainst ToRSV and GFLV.The frequency of HSVd, CEVd and PSTVd naturally infectedgrapevine trees recorded different percentages as in single (8.33, 8.33 and11.11%) respectively, double (11.11, 11.11 and 19.44%) for CEVd+HSVd,CEVd+PSTVd and HSVd+PSTVd respectively and mixed infection(19.44%) with different disease symptoms.Grapevine viroids (HSVd, CEVd, PSTVd, and may be GYSVd) gavedifferent disease symptoms on some host range plants (indicator plants).HSVd, gave characteristic symptoms as mosaic with chlorotic spots, veinclearing, rugosity and stunting on Cucumis sativus L. cv. alpha, CEVd gavemosaic and vein banding on Gynura aurantiaca, and PSTVd gave leafdeformation and epinasity on Lycopersicon esculantum L. cv. Castle rock).GYSVd did not give any symptoms on indicator hosts but gave yellowspeckle and yellowing spots on the main host V. vinifera cv. Balady/banaty.Grapevine viroids isolates were transmitted through grafting by eyebuds from infected grapevine cv. Balady/ banaty, to healthy one and thesymptoms appeared after 6-8 weeks.CF-11 cellulose column chromatography was utilized to removecontaminating host RNAs from viroid preparations as well as to separateindividual viroids with selective elusion by different ethanol

concentrations.Serial elusion with 25 % ethanol-STE, 15 % ethanol-STE and 0%ethanol-STE buffer eluant by CF-11 cellulose column chromatography wasdone, and grapevine viroids-RNA yield was determined by UVSpectrophotometer.Sequential PAGE confirmed the presence of GYSVd-1, CEVd andHSVd. In the 15 % ethanol-STE buffer eluant was highly enriched ingrapevine viroids. This manipulation greatly improved the recovery ofviroids, the four fractionated infected samples appears different band patternsas mixed and single infection whereas, the mixed infection with HSVd,GYSVd, and CEVd, appeared in two samples, while another two samplesrevealed single infection with single band for HSVd. In the 0% ethanol-STEbuffer eluant did not appears any bands.Total RNA was extracted from four group infected grapevine plantleaves. The integrity and quantity of the purified RNA were confirmed byUV Spectrophotometer and gel -electrophoresis. About 368 bp DNA fragmentwas amplified from the samples by RT PCR using specific GYSVd-1 (+) andGYSVd-1 (-) primers.The nucleotide sequence of the PCR-amplified fragment for theGYSVd-1 genome (Egyptian isolate) was done to determine the relationshipwith other recommended GYSVd-1 registered in Gen Bank.Comparison between bases composition of complete genome sequencefor GYSVd-1 (Egyptian isolate) and seven GYSVd-1–isolates was done todetermine the relationship with other recommended GYSVd-1. A Similarityprecentage index of GYSVd-1(EG) revealed 99.55% a high degree ofsimilarity to the other isolates sequences of GYSVd-1 in GenbankThe minimum free energy of a secondary structure for RNA-GYSVd-1(EG) isolate was determined from its primary sequence by summing theenergy contribution of all base pairs, interior loops, hairpin loop, bugle loopsand external loop at 37 °C using MFOLD program was -173.33 kcal -mol-1. Itis rod-shaped structure composed of alternating single- and double strandedregions.Grapevine viroids reduce vegetative growth, fruit yield quality -andquantity. The reduction rate in total pigment content nearly to 50 % forviroids infected grapevine plants comparing with healthy ones.The rate of reduction in viroids-infected grapevine berries juice totalsoluble solids comparing with healthy ones was 27.77%. While the rate ofincrease in acidity of the berries juice of viroids infected grapevine was33.33%. Total soluble sugar content differs greatly in viroids infectedgrapevine yielding than healthy ones. The reduction percentage of totalsoluble sugar content in viroids infected grapevine yielding was 11.11%. andthe rate of reduction in viroids-infected grapevine berries total insolublesugars was 22.99%. The rate of reduction in total carbohydrate content ofviroids-infected -grapevine yielding was 14.26%. The rate of reduction inSugar:acid ratio of viroids infected grapevine yielding comparing withhealthy ones was 35.68%.Shoot tip cultures coupled with cold-therapy were successfully usedto eliminate grapevine viroids from infected mother plants, cold therapy at 4°C for 1, 2 and 3 months, treated plantlets on M.S. media showing thepercentage of survival was 73, 64, 45 % and viroids-free plants were 18, 27and 40 %, and thus reduces the risk of introducing these viroids to Egypt.Key words: Hop stunt viroid (HSVd), Citrus exocortis viroid (CEVd),Potato spindle tuber viroid (PSTVd) and Grapevine yellow speckle viroid-1(GYSVd-1), Nucleic acid hybridization, DAS-ELISA, CF-11 cellulosecolumn chromatography, S-PAGE, RT-PCR, Sequencing, Tissue culture,Cold therapy.