Araştırma Makalesi
(Research Article)

Ege Üniv. Ziraat Fak. Derg., 2013, 50 (3):291-297
ISSN 1018 – 8851

Comparison of RNA extraction methods for the molecular detection of Artichoke latent virus in globe artichoke

Enginarda Artichoke Latent Virus (ArLV)'ün Moleküler Tanısında Kullanılan RNA Ekstraksiyon Yöntemlerinin Karşılaştırılması

Müge ERGÜN
İsmail Can PAYLAN
Semih ERKAN

Ege University, Faculty of Agricultural Engineering, Department of Plant Protection, 35100, İzmir, Turkey.
Corresponding Author: muge_ergun@hotmail.com

Alınış (Received): 05.06.2013 Kabul tarihi (Accepted): 19.06.2013

KeyWords:

Artichoke latent potyvirus, Lithium-chloride, RNA, Extraction, Silica-capture

ABSTRACT

Globe artichoke is very beneficial vegetable for human health because of involve vitamins and minerals within its structure. This vegetable which is intensively cultivated in the Mediterranean basin, are consumed in the form of meals, soups and salads with its own flavor in Mediterranean cuisine. In recent years, its production and consuming are increasing and spreading all over the world. As other cultivated plants, globe artichoke has been affected by many plant pathogens. In this study, the success of some RNA extraction methods that is very important step for detecting *Artichoke latent potyvirus* which cause yield and quality loss were investigated. RT-PCR is a reliable method and commonly used for detection and identification of viruses. Globe artichoke is a plant with high content of phenolic acids which make difficult isolation of RNA. Thereby the results of RT-PCR are affected. In this study, our aim is investigation of three widely used extraction methods and a commercial isolation kit's effectiveness on artichoke with high content of phenolic compounds. The results of this study showed substantially parallel to the literature and also the commercial kit and silica capture methods were found effective to obtain high purity RNA with high concentration.

Anahtar Sözcükler:

Artichoke latent potyvirus, Lithium Chloride, RNA Ekstraksiyonu, Silica Capture

ÖZET

Enginar, içeriğindeki vitamin ve mineraller ile insan sağlığı için oldukça yararlı bir sebzedir. Yoğun olarak Akdeniz havzasında yetiştirilen bu ürün kendine has tadıyla Akdeniz mutfağında, çorba, salata ve yemek şeklinde tüketilmektedir. Son yıllarda yetiştiriciliği artmakta ve dünyaya yayılmaktadır. Tarımı yapılan her bitki gibi enginar da birçok patojenden etkilenmektedir. Bu çalışmada enginarlarda verim düşüşlerine neden olan *Artichoke latent virus* tespitinde önemli bir aşama olan RNA ekstraksiyon yöntemleri araştırılmıştır. Virüslerin tespiti ve tanılanmasında yaygın olarak kullanılan RT-PCR'ın temelini oluşturan hastalıklı bitki dokularından RNA ekstraksiyonu testin güvenilirliği açısından en önemli aşamadır. Enginar fenolik asit içeriği yüksek bir bitkidir. Bu maddeler RNA ekstraksiyonunu zorlaştırmakta dolayısıyla RT-PCR sonuçlarını etkilemektedir. Bu çalışmada yaygın kullanılan üç ekstraksiyon yöntemi ve ticari RNA izolasyon kitinin fenolik madde içeriği yüksek olan enginar bitkilerinde RNA ekstraksiyon etkinliklerinin araştırılması amaçlanmıştır. Yapılan çalışma sonuçları büyük ölçüde literatürle paralellik göstermiş, silica capture yöntemi ve ticari kitin yüksek saflıkta ve yoğunlukta RNA elde edilmesinde etkili olduğu saptanmıştır.

INTRODUCTION

Globe artichoke (*Cynara cardunculus* L. subsp. *scolymus* (L) Hayek) is highly nutritious vegetable which is growing intensively in the countries bordering the Mediterranean basin. In recent years, its cultivation has been spreading over China, USA, South America and North Africa. Artichoke cultivation covers approximately 133.000 hectares over the word and annually yield value is about 1.500.000 tons. The leader of globe artichoke production is Italy with its share of 32% in all production and it is followed by Spain, Egypt and Peru respectively. In Turkey, globe artichoke is grown in an area of approximately 2900 hectares in size and according to the last 5-year data the average yield is about 35.000-36.500 tons in a year (Erkan ve Ergün, 2012; FAO, 2012). Globe artichoke is grown widely in Bursa (37%), Izmir (27.9%) and Aydın (10.8%) provinces in Turkey. In recent years, production of artichoke is also spreading to Antalya (4.8%), Adana (4.5%) and Muğla (4.2%) provinces (FAO, 2012; TUİK, 2012).

It is estimated that the globe artichoke is Mediterranean origin and has spread from the north-west African countries to Spain, then from Spain to European, American and Asian countries (FAO, 2012). Globe artichoke is very important vegetable for human health. It is consumed in Mediterranean basin as the form of food, salads and soups due to have its own flavor. Artichoke contains 85% water, 10% carbohydrate, 3% protein, 1-2% fat, B,C vitamins, riboflavin and thiamine and minerals (K, P, Ca, Na, Mg, Mn, Zn and Fe) (Vural ve ark., 2000; Eser ve ark., 2006). Besides these nutritious effects, it has good effects in terms of health such as lowering triglyceride levels, increasing the protective cholesterol (HDL) (Bayraktar, 1981).

As other cultivated plant, artichoke has also been affected by many pathogens. Among them the viral agents cause heavy yield loses. It was recorded that approximately 24 viral agents belonging to 15 different genus can affect artichokes. The most common viral agents are *Artichoke Italian latent nepovirus* (AILV), *Artichoke mottled crinckle tombusvirus* (AMCV), *Artichoke vein banding cheravirus* (AVBV), *Artichoke yellow ringspot nepovirus* (AYRSV), *Cucumber mosaic cucumovirus* (CMV), *Tobacco mosaic tobamovirus* (TMV), *Artichoke latent potyvirus* (ArLV) and *Tomato spotted wilt tospovirus* (TSWV) (Gallitelli et al., 2004; ICTVDB, 2012).

Artichoke latent potyvirus (ArLV) which has approximately 730 nm filamentous particles contains ssRNA (single-stranded ribonucleic acid) (Gallitelli et al., 2004, van Regenmortel, 2000). ArLV is transmitted by *Myzus persicae*, *Brachycaudus cardui,* and *Aphis fabae* in non-persistent manner (Rana et al., 1982). It was firstly isolated in Tunisia (Marrou and Mehani, 1964), Moracco (Fischer and Lockhart, 1974) and Italy (Foddai et al., 1977) from symptomless globe artichokes.

RT-PCR (Reverse transcriptase polymerase chain reaction) which is based on replication of specific nucleic acids is a very susceptible method commonly used for determination of viruses and viroids. Accuracy and reliability of RT-PCR test depends on the quality and quantity of total nucleic acids which are used in PCR (Sipahioğlu et al., 2006). RT-PCR is highly sensitive detection method which necessitates the nucleic acids highly purified.

Phenols, polyphenols and polysaccharides in plants can affect the sensitivity of PCR and may lead to false negative results (Wilde et al., 1990, Cieślińska, 2004). These substances in tissues prevent reverse transcriptase especially in RNA extraction phase and reduce RT-PCR's reliability (Sipahioğlu et al., 2006). Recent research studies show that, artichoke contains high levels of flavonoids, phenolic acids, sesquiterpene lactone and anthocyanin derivative substances in its structure (Hausler et al., 2002; Mulinacci et al., 2004; Curadi et al., 2005; Katırcıoğlu-Atabek, 2008). The substances known as phenolic compounds facilitate the inactivation of viruses. Thus, false negative results can occur at PCR stage.

Although many studies were carried out with RNA extraction methods, a standard method couldn't be found for all plants or viruses (Duman, 2008). Generally, nucleic acid extraction protocols can't eliminate the phenolic compounds and polysaccharides thus, reduce PCR's effectiveness (Demeke and Adams, 1992). In 2004, Cieślińska (2004) performed a study about effective RNA extraction methods on strawberries and found the lithium chloride method the most effective of all tried methods. MacKenzie et al. (1997) reported that high amounts of nucleic acids can be obtained with silica capture method.

In the present study, four RNA extraction methods including a commercial RNA extraction kit were compared and evaluated for the preparation of high quality RNA in order to detect *Artichoke latent potyvirus* in artichoke plants.

MATERIAL AND METHODS

In autumn 2011, 80 artichoke samples were collected from around Izmir province. The collected samples were stored in -20°C until extracted. In this study, our goal was determination of effectiveness of the extraction methods in artichoke. The extraction methods which are used for comparison purposes: Silica capture method (Foissac et al., 2001), Hughes and Galau's lithium chloride method (1988), Citric buffer method (Wetzel et al., 1992) and a commercial plant RNA extraction kit (Fermantas GeneJet™ Plant RNA Purification Mini Kit).

In this study firstly, silica capture protocol was applied according to Foissac et al. (2001). For this purpose, 100 mg of each leaf sample was used. They were grinded in sterile sample plastic bags with grinding buffer (Table 1), including 1% mercapto-ethanol. After homogenization, each tube was incubated at 70°C for 10 minutes. Then, they chilled on ice for 5 minutes. Each tube was centrifuged at 14.000 rpm for 10 minutes. 300 µl supernatant was transferred to sterilized microcentrifuge tubes. 150 µl ethanol, 300 µl 6 M NaI (sodium iodide), 25 µl silica suspension was added to each tube and incubated at room temperature for 10 minutes. After centrifugation at 6.000 rpm for 1 minute, supernatant was discarded. 500 µl washing buffer was added to each tube in order to wash silica particles. Washing step was applied twice. Afterwards washing steps, 150 µl RNase-free water was added to each tube which contained silica particles. After incubation at 70°C for 4 minutes, tubes were centrifuged at 14000 rpm for 3 minutes. 100 µl supernatant was transferred to sterile tubes. Each tube was stored at -20°C until used.

Table 1. The chemicals used in silica capture method and their usage rates

Buffer	Chemical	Rates
Grinding Buffer	Guanidine thiocyanate 4 M	23.64 g/50 ml
	NaOAc 0.2 N (pH:5.2)	1.36 g/50 ml
	EDTA 25 mM	0.465 g/50 ml
	KOAc 1M	4.9 g/50 ml
	PVP-40 2.5% (w/v)	1.25 g/50 ml
	Mercoptoethanol1% is added just before extraction.	
NaI 6M	Na_2SO_3	0.75 g/50 ml
	NaI	36 g/50 ml
Washing Buffer	Tris-HCl 110mM (pH:7.5)	1 ml/100 ml
	EDTA 5M (0.5 mM)	100 µl /100 ml
	NaCl 50mM (0.5M)	1 ml/100 ml
	Ethanol 50%	50 ml/100 ml
Silica	Silicon dioxide	6 g/50 ml

Lithium chloride extraction method was used according to Hughes and Galau's (1988). 10 mg frozen plant material from each sample was weighted and added 0.5% 2-mercaptoethanol and 1 mg extraction buffer which was mentioned at Table 2. Each sample was grinded in a mortar and homogenized. Then, 500 µl extract was transferred to 1.5 ml microcentrifuge tubes. Tubes were incubated at 65°C for 15 minutes. After that 500 µl 5M potassium acetate (pH:6.5) was added to the tubes and chill on ice for 10 minutes. Each tube was centrifuged at 14000 rpm for 15 minutes. 600 µl supernatant was transferred to sterilized microcentrifuge tubes. After that, 600 µl isopropanol was added to each tube and tubes were incubated at -20°C overnight. The mixture which has become pellets was centrifuged at 14000 rpm for 15 minutes and washed with 70% ethanol. Tubes were stored at -20°C, until used.

Table 2. Chemicals used in lithium chloride method

Buffer	Chemical
Extraction buffer	200mM Tris-HCl (pH:8.5)
	1.5% Sodium dodecysulphate
	300mM Lithium chloride
	10 mM EDTA
	1% Sodium deoxycholate
	2-mercaptoethanol 0.5%
	5M potassium acetate (pH:6.5)
	Isopropanol
	Ethanol

Citric buffer method was used as indicated by Wetzel et al. (1992). In our study, this method was applied with minor modifications. 500 mg leaf sample was homogenized in quartz sand including 1 ml citric buffer (50 mM sodium citrate, 2% PVP, 20 mM DIECA). Homogenized samples were centrifuged at 8000 rpm for 3 min. 450 µl citric buffer was added to 50 µl

supernatant. Isolated TNA's was stored at -20°C until used.

As a commercial RNA purification kit GeneJet™ Plant RNA Purification Mini Kit was used acording to manufacturer protocol. The contents of the kit were given in the Table 3. 100 mg frozen sample was grinded in mortar with 500 µl Plant RNA Lysis Solution. The extracts of each sample were transferred to 1.5 ml microcentifuge tubes. Then, each tube was incubated at 56°C for 3 minutes and was centrifuged at 14000 rpm for 5 minutes. Approximately 450-500 µl supernatant was transferred to sterile microcentifuge tube. Then, 250 µl of 96% ethanol was added to each tube and they were mixed by pipetting. That prepared mixture was transferred to column inserted collection tube. The column was centrifuged at 11.000 rpm for 1 minute. The flow-flough solution was discarded and the column and the collection tube were reassembled. 700 µl of Wash Buffer 1-WB1 solution involving ethanol (Table 3) was added to each tube and they were centrifuged at 11000 rpm for 1 minute. The flow-flough solution and collection tube were discarded and the purification column was placed into sterile 2 ml collection tube. 500 µl Wash Buffer 2-WB2 solution containing ethanol (Table 3) was added to each tube and they were centrifuged at 11000 rpm for 1 minute. The flow-flough solution was discarded and the column and the collection tube were reassembled. 500 µl WB-2 solution was added to the purification column again and they were centrifuged at 11000 rpm for 2 minutes. The collection tube was emptied out and the purification column was respinned at 14000 rpm for 1 minute. The collection tube and the flow-flough solution were discarded and the purification column was transferred to RNase-free 1,5 ml microcentrifuge tube. 50 µl of nuclease-free water was added to purification column and they were centrifuged at 11000 rpm for 1 minute. Floating part was used in this study. Each tube was stored at -20°C, until used.

Table 3. Chemicals from the Fermantas GeneJet™ Plant RNA Purification Mini Kit

Buffer	Usage
Plant RNA Lysis Solution	Add 10 µl DTT to each 500 µl Plant RNA Lysis Solution
Washing Buffer 1 Solution –WB1	Add 10.5 ml ethanol to each 200 ml WB-1
Washing Buffer 2 Solution –WB2	Add 100 ml ethanol to each 100 ml WB-2

80 artichoke samples were extracted with three methods separately, then each sample was visualized by 1,5% agarose gel electrophoresis. For this purpose, 1,5% agarose gel was prepared with 1XTAE (Tris Acetate EDTA) buffer. Electrophoresis process was applied 100 V for 60 minutes with 1XTAE buffer at horizontal mechanism. Before loading samples to gel, 6X Loading Buffer (LB) was added to each sample (2 µl LB-10 µl sample-total 12 µl to each well). After electrophoresis process, gel was stained with ethidium bromide and was visualized with gel imaging system (Sambrook et al., 1989).

After TNA extraction all the samples with four extraction methods, randomly selected 5 samples were analyzed for the presence and quality of RNA. In order to determine RNA quantity and quality, each sample were analyzed with Thermo Scientific NanoDrop 1000 spectrophotometer. Analyses were conducted at 260 nm and 280 nm absorbance wavelength (Sambrook et al., 1989; Reid et al., 2006; Yang et al., 2011; Yu et al., 2012).

cDNA synthesis of 80 aritchoke samples was performed by Fermentas First Strand cDNA Synthesis Kit according to the manufacturers procedure (Fermentas, USA). Synthesized cDNAs were subjected to PCR with genome specific primers of *Artichoke latent potyvirus* ArLV5BL: 5'-GAT CTA GCG ATA CAC ATG CAC AAC C-3' and ArLV3BL: 5'- CGC TCA AGC TCT CGA ACT AAC TGA AC-3' (Lumia et al., 2003).

RESULTS

80 artichoke plant samples, collected from Izmir were tested with all extraction methods. The prepared TNA extracts were subjected to gel electrophoresis and the results were assessed according to gel images. After gel electrophoresis randomly selected 5 samples were analyzed with Thermo Scientific NanoDrop 1000 spectrophotometer in order to determine the presence and quality of RNAs. Besides, all extracted samples with four extraction methods were subjected to RT-PCR tests in order to evaluate the efficiency of ArLV detection on artichoke plants.

Conducted extraction protocols and screening gel with gel imaging system showed that highly purified and high density RNAs were isolated with silica capture method and GeneJet™ Plant RNA Purification Mini Kit (Figure 1). It was observed that Lithium chloride and citric buffer extraction methods could not detect RNA afterwards extraction and electrophoresis (Figure 1). In addition spectrophotometrical analysis (A_{260}/A_{280}) results showed that concentrations of RNAs in 5 samples varied between 0.000-1.679 (Table 4).

Conducted RT-PCR results showed that expected size of amplicons (282 bp) was obtained on 10 samples that were extracted with GeneJet™ Plant RNA Purification Mini Kit and on 35 samples extracted with silica capture method (Foissac et al., 2001) (Figure 2). There were no amplicon on the samples extracted with lithium chloride method and citric buffer method.

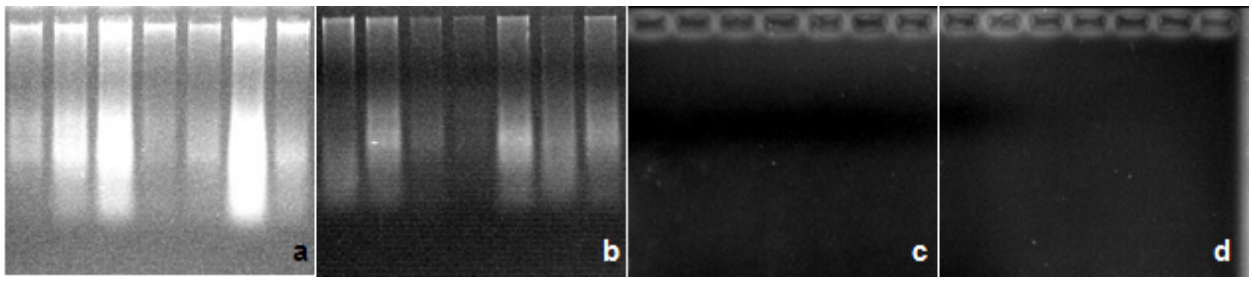

Figure 1. Image of RNAs isolated by four extraction methods after gel electrophoresis, a) Silica capture method b) GeneJet™ Plant RNA Purification Mini Kit c) Lithium chloride method d) Citric buffer method.

Table 4. Spectrophotometric measurement results at 260 nm absorbance wavelength.

Sample	Silica Capture		GeneJet™ Plant RNA Purification Mini Kit		Lithium Chloride		Citric Buffer	
	A_{260}/A_{280}	Consantration (µg µl^{-1})	A_{260}/A_{280}	Consantration (µg µl^{-1})	A_{260}/A_{280}	Consantration (µg µl^{-1})	A_{260}/A_{280}	Consantration (µg µl^{-1})
13	1.578	0.053	1.028	0.028	0.000	-	0.000	-
21	1.675	0.045	1.034	0.030	0.000	-	0.000	-
36	1.679	0.054	1.030	0.028	0.000	-	0.000	-
42	1.579	0.061	1.055	0.029	0.000	-	0.000	-
55	1.602	0.049	1.047	0.029	0.000	-	0.000	-

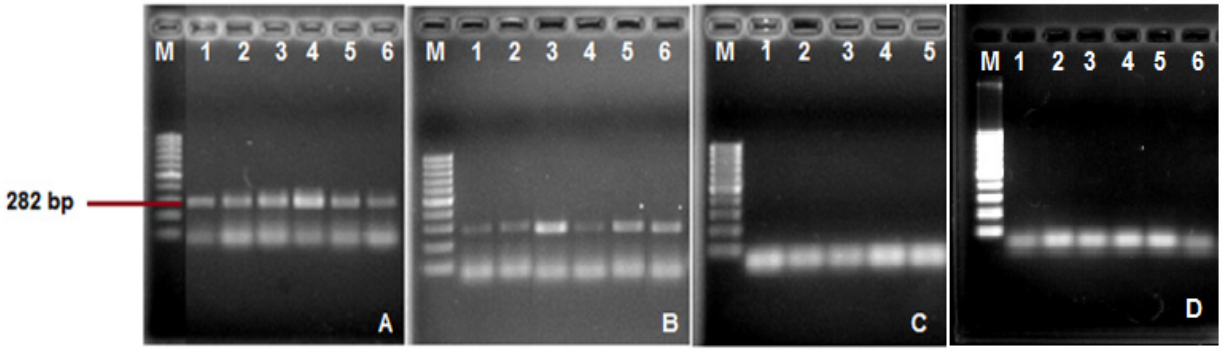

Figure 2. Image of some samples that conducted RT-PCR tests to ArLV with three extraction method, a) Silica capture method b) GeneJet™ Plant RNA Purification Mini Kit c) Lithium chloride method d) Citric buffer method

DISCUSSION

The most influential method of tested 4 methods was silica capture. Highly purified and high density RNA was isolated by silica capture . The data obtained from this study were paralleled with studies done by Cieślińska (2004) and Sipahioğlu et al. (2007). Mercaptoethanol which reduces the effectiveness of polyphenols existing in plant tissues, were used in silica capture method. Besides the buffer used in question method, contains Guanidine thiocynate which is essential substance for RNA extraction (Sipahioğlu et al., 2007). Silica capture method because of these substances makes it easier to obtain high-purity RNA from different plants (Cieślińska, 2004; Sipahioğlu et al., 2006). However, RNAs isolated by silica capture method can be stored for a long time and the method also reduces degradation of PCR products, similarly to study done by Sipahioğlu et al. (2006).

Lithium chloride and citric buffer extraction methods which are used in this study, showed different results from many studies in literature (Loreti et al., 1999; Shamloul et al., 2002; Ragozzino et al. 2003; Sipahioğlu et al., 2007) as they gave negative results with artichoke plants. Furthermore, the data showed accordance with Duman's studies (2008) on stone fruit trees. This situation suggests that the effectiveness of this method depends on the plant species. In addition, the amounts of chemicals required in the implementation of the method, were used at different densities in several studies appearing in literature. Thus, it is suggested that the effectiveness of the method can change according to the chemicals and plant species. Furthermore, lithium chloride method is required high chemical usage and application takes two days. For these reasons, the prevalence of usage is decreasing in recent years (Shamloul et al., 2002; Cieślińska, 2004; Hassen et al., 2005; Sipahioğlu et al., 2007; Duman, 2008).

The last method used in the study is Fermantas's commercial RNA extraction kit. RNAs isolated from artichoke plants with kit, were found to be close purity and density with silica capture method. In addition, isolation is carried out more quickly than silica capture and less chemical usage is required.

RNA isolation is a very important stage in order to conduct accurate and reliable RT-PCR. Purity and intensity of RNAs used in RT-PCR, affect PCR steps and can cause false negative results. Through this study, it was proved once again that the extraction method should be chosen according to plant species. In addition, it should be considered that the chemicals used during the extraction, affect the suitable RNA isolation at least as effective as the chosen extraction method.

REFERENCES

Bayraktar, K., 1981. Sebze Yetiştirme Cilt III. Kültür Sebzeleri. E.Ü.Z.F. Yayınları, No: 169.

Cieślińska, M., 2004. Detection of strawberry mottle virus (SMoV) using RT-PCR-comparison of two RNA extraction methods. *Journal of Fruit and Ornamental Plant Research,* 12, 17-22.

Curadi, M., Picciarelli, P., Lorenzi, R., Graifenberg, A. and Ceccarelli, N., 2005. Antioxidant activity and phenolic compounds in the edible parts of early and late Italian artichoke (*Cynara scolymus* L.) varieties. *Italian. Journal of Food Science.*, 17, 33-44.

Demeke, T. and Adams, R.P., 1992. The effects of plant polysaccharides and buffer additives on PCR. *Biotechniques*, 12 (3), 332-334.

Duman, K., 2008. Sert çekirdekli meyve ağaçlarında görülen Peach latent mosaic viroid (PLMVd) ve Hop stunt viroid (HSVd)'lerin RT-PCR yöntemi ile tanılanmasına yönelik çalışmalar. Ege Üniversites Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi. Bornova-İzmir, 2008.

Erkan, S. ve Ergün, M., 2012. Enginarda Görülen Virüs Hastalıkları. *Hasad Bitkisel Üretim*, 27 (320), 82-89.

Eser, B., İlbi, H. ve Uğur, A., 2006. Enginar Yetiştiriciliği. Hasad Yayıncılık Ltd.Şti., İstanbul, 2006.

FAO, 2012. Food and Agriculture Organization of the United Nations. http://faostat.fao.org/ (Date of Access: April, 2012).

Fischer, H.V. and Lockhart, B.E., 1974. A Moroccan isolate of Artichoke latent virus. *Plant Disease Reporter*, 58, 1123-1126.

Foddai, A., Marras, F. and Idini, G., 1977. Presenza di un "*Potyvirus"* sul Carciofo (*Cynara scolymus* L.) in Sardegna. *Annali della Facoltà di Agraria dell'Università di Sassari*, 25, 398-407.

Foissac, X., Savelle-Dumas, L., Genfit, P., Duluck, M.J. and Candresse, T., 2001. Polvalent detection ıf fruit tree Tricho, Capillo. *Research Number 45*, 2001, 33-35.

Gallitelli, D., Rana, G.L., Vovlas, C. and Martelli, G.P., 2004. Viruses of Globe Artichoke: An Overview. *Journal of Plant Pathology*, 86 (4, Special Issue), 267-281.

Hassen, I.F., Roussel, S., Kummert, J., Fakhfakh, H., Marrakchi, M. and Jijakli, M.H., 2005. Detection and epidemiological characteristics of peach latent mosaic viroid in Tunisia. *Communications in Agricultural and Applied Biological Sciences*, 70 (3), 115-128.

Hausler, M., Ganzera, M., Abel, G., Popp, M. and Stuppner, H., 2002. Determination of caffeoylquinic acids and flavonoids in *Cynara scolymus* L. by high performance liquid chromatography. *Chromatographia*, 56, 407-411.

Hughes, D.W. and Galau, G., 1988. Preparation of RNA from cotton leaves and pollen. *Plant Molecular Biology Reporter*, 6, 253-257.

ICTVDB,2012.http://ictvonline.org/virusTaxonomy.asp?version=2009&bhcp=1 (Date of Access: July, 2012).

Katırcıoğlu-Atabek, A., 2008. *Cynara scolymus* L.'un fitoterapideki kullanımı üzerine araştırmalar. Gazi Üniversitesi Sağlık Bilimleri Enstitüsü, Yüksek Lisans Tezi, 71 sayfa, Ankara.

Loreti, S., Faggioli, F., Cardoni, M., Mordenti, G., Babini, A.R., Poggi Pollini, C. and Barba, M., 1999. Comparison of different diagnostic methods for detection of peach latent mosaic viroid. *EPPO Bulletin*, 29 (4), 433-438.

Lumia, V., Pasquini, G. and Barba, M., 2003. Sensitive detection of artichoke latent virus in globe artichoke field samples by one-step RT-PCR or tissue imprint hybridization. *Journal of Phytopathology*, 151, 477-479.

MacKenzie, D.J, Mclean, M.A., Mukerji, S. and Green, M., 1997. Improved RNA extraction from woody plants for detection of viral pathogens by reverse transcription-polymerase chain reaction. *Plant Disease*, 81 (2), 222-226.

Marrou, J. and Mehani, S., 1964. Etude d'un virus parasite de l'artichaut. *Comptes Rendues de l'Académie d' Agriculture de France*, 50, 1051-1064.

Mulinacci, N., Prucher, D., Peruzzi, M., Romani, A., Pinelli, P. and Giaccherini, C., 2004. Commercial and laboratory extracts from artichoke leaves estimation of caffwoyl esters and flavonoidic compounds content. *Journal of Pharmaceutical and Biomedical Analysis,* 43, 349-357.

Ragozzino, E., Faggioli, F., Alioto, D. and Barba, M., 2003. Detection and differentiation of Peach latent mosaic viroid and Hop stunt viroid in stone fruit trees in Italy using multiplex RT-PCR. *Phytopathologia Mediterranea*, 42 (1), 79-84.

Rana, G.L., Russo, M., Gallitelli, D. and Martelli, G.P., 1982. Artichoke latent virus: characterization, ultrastructure, and geographical distribution. *Annals of Applied Biology*, 101, 279-289.

Reid, K.E., Olsson, N., Schlosser, J., Peng, F., and Lund, S.T., 2006. An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. *BMC Plant Biology,* 6 (27), 1-11.

Sambrook, J., Fritsch, E.F. and Maniatis, T., 1989. Molecular cloning. Laboratory Manual. Cold Spring Laboratory Press, New York, 1989 (2nd ed).

Shamloul A.M., Faggioli, F., Keith, J.M. and Hadidi, A., 2002. A novel multiplex RT-PCR probe capture hybridization (RT-PCR-ELISA) for simultaneous detection of six viroids in four genera: Apscaviroid, Hostuviroid, Pelamoviroid and Pospiviroid. *Journal of Virological Methods*, 105 (1), 115-121.

Sipahioğlu, H.M., Ocak, M. and Usta, M., 2007. Comparison of three conventional extraction methods for the detection of plant virus/viroid RNAs from heat dried high-phenolic host leaves. *Asian Journal of Plant Sciences*, 6 (1), 102:107.

Sipahioğlu, H.M., Usta, M. and Ocak, M., 2006. Use of dried high-phenolic laden host leaves for virus and viroid preservation and detection by PCR methods. *Journal of Virological Methods*, 137 (1), 120-124.

TUİK, Türkiye İstatistik Kurumu, 2012. Bitkisel Üretim İstatistikleri. http://www.tuik.gov.tr/bitkiselapp/bitkisel.zul (Date of Access: April, 2012).

van Regenmortel, M.H.V., 2000. On the relative merits of italics, Latin and binomial nomenclature in virus taxonomy. *Archives of Virology*, 145, 433–441.

Vural, H., Eşiyok D. ve Duman, İ., 2000. Kültür Sebzeleri (Sebze Yetiştirme). E.Ü. Basım evi, Bornova-İzmir, 2000.

Wetzel, T., Candresse, T., Macquaire, G., Ravelon, M. and Dunez, J., 1992. A highly sensitive immunocapture polymerase chain reaction from plum pox potyvirus detection. *Journal of Virological Methods*, 1992, 39, 27-37.

Wilde, J., Eiden, J. and Yolken, R., 1990. Removal inhibitory substances from human fecal specimen for detection of group A rotaviruses by reverse transcriptase and polymerase chain reactions. *Journal of Clinical Microbiolgy.*, 28, 1300-1307.

Yang, C., Li, F., Ji, X. and Wang, J., 2011. An effective method for RNA extraction from grapevine berry skins. *African Journal of Biotechnology.*, 10(45), 9032-9035.

Yu, D., Tang, H., Zhang, Y., Du, Z., Yu, H. and Chen, Q., 2012. Comparison and improvement of different methods of RNA isolation from strawberry (*Fragria ananassa*). *Journal of Agricultural Science*, 4 (7), 51-56.