تأثير المعالجة الكيميائية في فيروسي التفاف الأوراق والبطاطا واي

Utilizing Chemotherapy for Efficient Elimination of Potato Leaf Roll Virus and Potato Y Virus

Mouhamad Al Hoshan **Dr. Cyrus Ghobadi** **Dr. Amer masah**

Science Degree: MSc in Horticulture Science
Place of work: General Commission of Scientific Agriculture Research
Tel: 011 5743038
Mob: 0934840268
Email: hoshan77@yahoo.com
Place fulfilling this research: Iran- Isfahan- Isfahan University of Technology

الملخّص

أجري هذا البحث لدراسة مدى كفاءة بعض المضادات الفيروسية على استبعاد فيروسي التفاف الأوراق والبطاطا واي وذلك باستخدام خزعات (بأطوال 3 و 5 مم) من قمم البراعم النامية على درنات صنفين من البطاطا (جاندراموخ و مارفونا) مصابين بالفيروسين المذكورين. تم التحقق من إصابة البراعم بفيروسي التفاف الأوراق والبطاطا واي باستخدام تقنيات ELISA و RT-PCR . زرعت الخزعات على وسط النمو MS المضاف إليه الهرمون نفتلين اسيتيك اسيد بالتركيز 0.1 ملغ/ ل، مضافا إليه المضادات الفيروسية إما Ribavirin (RBV) أو(AZA) Azacitidine بالتراكيز 0، 20، 40 و 60 ملغ/ل. أظهرت نتائج اختبار الصنف شاندراموخ باستخدام تقنية RT–PCR قدرة Ribavirin وبالتراكيز المستخدمة كافة على تخليص جميع النباتات النامية من خزع بطول 3 و5 مم من فيروسي التفاف الأوراق والبطاطا واي، في حين لم يستطع RBV تخليص نباتات الصنف مارفونا من نوعي الفيروسات بشكل كامل كما هو الحال في الصنف شاندراموخ. أما تحليل النباتات المعاملة ب Azacitidine، فقد أظهر التركيزان 20 و 40 ملغ/ل كفاءتهما في تخليص نباتات الصنف شاندراموخ النامية من خزع بطول 3مم و 5 مم من فيروس البطاطا واي بالترتيب، وكذلك تخليص النباتات النامية من خزع 3 و5مم عند التركيز 60 ملغ/ل و3مم عند التركيز 20 ملغ/ل من فيروس التفاف الأوراق عند الصنف نفسه، في حين عمل Azacitidine على استبعاد نوعي الفيروسات من معظم نباتات الصنف مارفونا النامية من خزع 3 و 5 مم وبالتراكيز المستخدمةكافة من المضادات الفيروسية. أثبتت النتائج المتحصل عليها تبايناً في استجابة أصناف البطاطا المدروسة لمضادات الفيروسات المستخدمة، وتأتي أهمية هذه النتائج من قيمتها التطبيقية عند إنتاج نباتات خالية من الفيروسات وإكثارها نسيجياً.

الكلمات المفتاحية : Ribavirin، Azacitidine ، فيروس التفاف الأوراق، فيروس البطاطا واي.

Abstract

This research was carried out to study the efficiency of some antivirals for efficient elimination potato leaf roll virus and potato y virus. In this investigate the segments of sprout tips (3.0 and 5.0 mm in length) from infected mother

 ISSN:2305 - 5243

plant (indexed by ELISA and RT-PCR) were cultured on MS medium supplemented with NAA (0.1 mgl^{-1}) containing Ribavirin (RBV) and Azacitidine (AZA) each at 0, 20, 40 and 60 mgl^{-1}. Results of RT-PCR procedure showed that RBV, at all applied concentrations, eliminated PVY and PLRV viruses from Chandramukhi infected vitroplants grown from sprout tips length 3 and 5 mm. while PVY was destroyed by AZA in Chandramukhi infected vitroplants grown from length 3 and 5 mm sprout tips at concentrations of 20 and 40 mgl^{-1} respectively. PLRV in the same cultivar was eradicated from infected vitroplants developed of sprout tip of 3 and 5 mm length at 60 mgl^{-1} of AZA and also from sprout tips of 3 mm length at 20 mgl^{-1}. Elimination efficiency of Ribavirin in eradicating PVY and PLRV viruses from Marfona infected vitroplants grown from sprout tips of both sizes (3 and 5 mm in length) could not have positive outcome as compared to Chandramukhi cultivar. But those Marfona infected vitroplants treated with Azacitidine doses (20, 40 and 60 mgl^{-1}) showed negative reaction with RT-PCR. These results showed that the response of two potato cultivars to antivirals were different, and the importance of these results comes from its application value for producing virus free plants and propagating them by tissue culture technique.

Keywords: Azacitidine, Ribavirin, Chemotherapy, Potato Leaf Roll Virus, Potato Virus Y, Sprout tip.

Introduction

Potato (*Solanum tuberosum* L.) is one of the very important tuber crops for people feeding over the world. Potato produces high protein and calories after wheat and rice (Khawajeh pour, 2006; Roodbar Shojaei et ***al.,*** 2007; Mobli and Perasteh, 1994). As will as it is considered economically the fourth major crop after wheat, rise and maize (Anonymous, 2007; Pua and Davey, 2007; Singh, 2007; Singh and Kaur, 2009). In 2008 Potato was cultivated in 18.19 million hectares, produced 413.14 million tons with productivity 17.27 ton/ hectar (Rajabi, 2009; Anonymous, 2008). In 2006, Iran cultivated about 170 thousand hectares of potato and produced 4260 thousand tons (Anonymous, 2007). Potato is sensitive to 300 disease and pests, but most of these diseases can be controlled with suitable management (Pajohandeh, 2001). Fungi, bacteria and viruses causes high decrease in quality and quantity of potato yield (Pajohandeh, 2001; Akhtar et ***al.,*** 2006; Burhan et al., 2007; Gebhardt et ***al.***, 2006; John et ***al.,*** 2007; Nascimento et ***al.,*** 2003; Wang et ***al.,*** 2006). Potato is a host of 37 virus species which can move from plant to plant through vegetative propagation (Chikh Ali et ***al.,*** 2008; Salim Khan et ***al.,*** 2003). Potato leaf roll virus (PLRV) and Potato Y virus (PVY) are among the most damaging potato viruses and are prevalent in most potato growing areas (Awan et ***al.,*** 2007; John et ***al.,*** 2007; Manzer, 1959; Nascimento et ***al.,*** 2003; Wang et al., 2006). Both PLRV and PVY are transmitted by aphids where the most known species of them is Myzus persicae (Sulzer) (Roodbar Shojaei et ***al.,*** 2007; John et ***al.,*** 2007; Wang et ***al.,*** 2006). Experimental trails showed that PLRV-infected plants seed tubers produce only 60% of total yield and 88% less marketable yield (tubers >85 g) than plants from healthy seed tubers(Ehrenfeld et ***al.,*** 2004; Wang et ***al.,*** 2006). PVY infected tubers caused 49 and 65% reductions in total and marketable yield, respectively, compared to healthy plants (Mahmoud et ***al.,*** 2009; Novy et ***al.,*** 2007). Control of potato viruses based on the production of healthy plants and use of virus resistant cultivars (Pajohandeh, 2001; Roodbar Shojaei et ***al.,*** 2007; Wang et ***al.,*** 2006). Meristem culture, thermotherapy and thermotherapy followed by meristem culture are the most employed methods to obtain virus-free potato plants (Wang et ***al.,*** 2006).

Elimination of viruses from plants is achieved in chemical ways as Ribavirin (RBV) and 5-Azacitidine (AZA) which prevent the propagation or the movement of viruses inside and between plants (Pajohandeh, 2001). Ribavirin (Copegus; Rebetol; Ribasphere; Vilona,Virazole, also generics from Sandoz, Teva, Warrick) and other antivirals like 5-Azacitidine are an anti-viral drug which is active against a number of DNA and RNA viruses. It is a member of the nucleoside antimetabolite drugs that interfere with duplication of viral genetic material. Though not effective against all viruses, ribavirin is remarkable as a small molecule for its wide range of activity; including important activities against both DNA and RNA viruses. Ribavirin's carboxamide group can make the native nucleoside drug resemble adenosine or guanosine, depending on its rotation. For this reason, when ribavirin is incorporated into RNA, as a base analog of either adenine or guanine, it pairs equally well with either uracil or cytosine, inducing mutations in RNA-dependent

replication in RNA viruses. Such hypermutation can be lethal to RNA viruses (Oana et *al.,* 2009; Pajohandeh, 2001; Awan et *al.,* 2007; Bittner et *al.,* 1989; Nascimento et *al.,* 2003).

This study will investigate the effect of antivirals (Ribavirin or virazole (1-ß D- ribofuranosyl-1, 2, 4-triazole-3-carboxamide) and Azacitidine) in eliminating two potato viruses: Potato leaf roll virus and Potato y virus from two potato cultivars (Marfona and Chandramukhi).

Materials and methods

Plant material:

Tubers of two potato cultivars (Marfona and Chandramukhi) from Isfahan Institute for Scientific and Agriculture Research has been used, some of them had been stored in dark room at 25 °C for 30 days for breaking their dormancy then sprout tips began to grow, and some tubers of potato cultivars has been cultured in greenhouse. After growth and appearance of symptoms (leaves necrosis and mosaics), leaf samples have been taken to test existence or not existence potato viruses (PLRV and PVY) by using two techniques ELISA for infected plants which are used as control (data not shown) and PCR for samples.

Samples sterilization:

First, Sprouts were washed with water containing many drops of dishwashing liquid to remove soils and strange materials, then sprouts are gulped in the ethanol 70 % for 30s after that sprouts were washed with sterile water for three times and stocked in sodium hypochlorite 10% for 15 minutes, and sprouts are washed in sterile water 5-6 times under the laminar air flow (Akhtar et *al.,* 2006; Anura and Lanka, 1988; Sidaros et *al.,* 2004; Wambugu et *al.,* 1985).

Media culture:

MS medium (Murashige and Skooge, 1962) PH 5.8-6 supplemented with 7g/l agar has been used. Segments of sprout tip (3.0 and 5.0 mm length) from infected mother plants (indexed by ELISA and PCR) were cultured in MS medium (20 ml with 4 replications) supplemented with 0.1 mgl^{-1} Naphtalin acetic acid (NAA), containing Ribavirin (RBV) and Azacitidine (AZA) at 0.0, 20, 40 and 60 mgl^{-1}. Samples were stocked in growth room at temperature 22-25 °C, (L: D= 16:8), light intensity was 40 micro mol/ m^2/s for 168 days. After that the best plants from each treatment were chosen for extracting RAN of Potato leaf roll virus and Potato virus y.

Detection of virus by PCR:

Detection of Potato leaf roll virus (PLRV) by PCR, Two specific pairs (PLRV1F, PLRV1R and PLRV2F, PLRV2R) were used and one specific primer pair (PVY1F and PVY1R) for Potato Y virus (Shalaby et *al.,* 2002). Sequence of these primers and expected fragment length are shown in table 1.

Table 1. Names and sequences of used primers and size of expected PCR products.

Primer	Sequence ´3 -5 ´	Section size (bp)
PLRV2F PLRV2R	CGC GCT AAC AGA GTT CAG CC GCA ATG GGG GTC CAA CTC A	336
PLRV1F PLRV1R	AAT AGA ATT CTA ATG AGT ACG GTC GTG GTT ARA GG AAA ACC ATG GCT ATY TGG GGT TYT GCA RGA CYA C	546
PVY1F PVY1R	TCA AGG ATC CGC AAA TGA CAC AAT TGA TGC AGG AGA GAG AAT TCA CAT GTT CTT GAC TCC	801

m RNA virus from plantlet leaves of two c.v potato (Marfona and Chandramukhi) has been extracted using lithium chloride method (Rosner and Maslenin, 2006).

c DNA syntheses:

Synthesis of c DNA stock has been done from individual plants as follows: 5 µL of RNA extracted, 1µL of 10 pmol/µl primer reverse, 5 µL Di Ethy Pyro Carbonate (DEPC) water has been remained for 10 minutes in the temperature 70 °C. Final volume of Reverse Transcriptase (RT) mixture was prepared in 20 µL containing 5 µL RNA, 1µL of 10 pmol/µl primer reverse, 0.5mM dNTPs Mix, 0.3µl of40U/µl enzyme M-Malv, 4µl M-Malv and 8.7µl DEPC. This mixture was remained at 42 °C for 45 minutes in order to make c DNA.

PCR assay:

PCR Mixture was prepared in 20 µL containing 5 µL cDNA, 2 µL of 10x PCR buffer, 0.5mM dNTPs, 10pmol/µl of each primer, 0.5µl Taq DNA polymerase, 0.75µl Mgcl2 and 8.75µl H2O. PCR program for PVY1R/PVY1F and PLRV1F/PLRV1R was 1cycle for Denaturation at 95 C° for 3minutes. Followed by 30cycles each one composed of 1minute at 94 C°,1minute at 55 C° and1minute at 72 C° and final cycle at 72 C° for 10 minutes (Shalaby et ***al.,*** 2002). For completion Nested –PCR, in the sterile vial 1.5 ml, 1 µL of PCR product has been added to 49 µL of sterile water. Then after using the pair of primers PLRV2F and PLRV2R and 5 µL of Nested-PCR yield, PCR program was carried out as table 2, in Thermo cycler Techno model TC-512.

Table 2. Nested PCR program for primer pair PLRV2F/ PLRV2R.

Time	Stage of Completion	NO. Cycles	Temperature (C°)
30second	Denaturation	10cycle	92
30second	Connection		62
90second	Extension		72
30second	Denaturation	10cycle	92
30second	Connection		60
90second	Extension		72
30second	Denaturation	15cycle	92
30second	Connection		58
90second	Extension		72
10minutes	Final Extension	1cycle	72

Electrophoresis and staining:

Separation of PCR products was made on 1.2% of agarose gel in TBE buffer. 8 µL of PCR yield were added to 2 µL dye and put in well and the ladder 100 bp was used. Gel was stained in Ethedium bromide (5 µL /ml) for10 minutes.

Results and Discussion

Results of PCR showed that in the control treatment Potato y virus and Potato leaf roll virus were found in plantlets which have been grown in vitro conditions without using antivirals. RBV at all applied concentrations eliminated PVY and PLRV viruses from infected vitroplants which grown from 3 and 5 mm lengths of sprout tips in Chandramukhi. While PVY was destroyed by AZA from infected vitroplants in Chandramukhi which grown from 3 and 5 mm lengths of sprout tips at concentrations of 20 and 40 mgl^{-1} respectively. PLRV virus in the same cultivar was eradicated from infected vitroplants developed sprout tip of 3 and 5 mm length at 60 mgl^{-1} of AZA and also from 3 mm length of sprout tips at 20 mgl^{-1} (table 3).

Table 3. Results of the application of Ribavirin and Azacitidine in elimination of virus in c.v Chandramukhi.

virus	0		Ribavirin (mgl^{-1})						Azacitidine (mgl^{-1})					
			20		40		60		20		40		60	
	Samples length (mm)								Samples length (mm)					
	3	5	3	5	3	5	3	5	3	5	3	5	3	5
PVY	+	+	-	-	-	-	-	-	-	+	+	-	+	+
PLRV	+	+	-	-	-	-	-	-	-	+	+	+	-	-

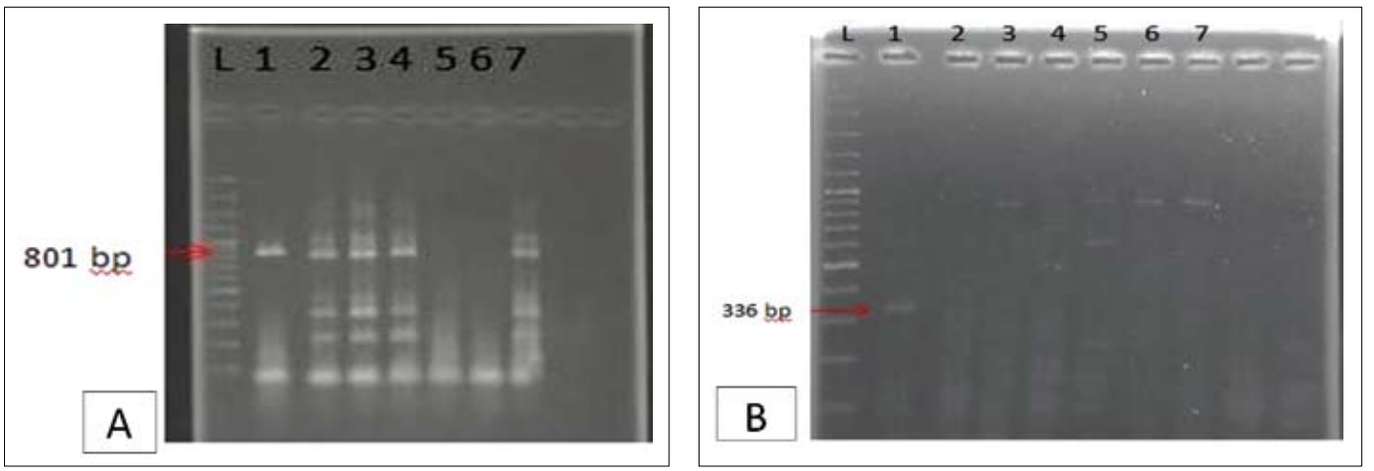

Fig1. PCR results for detection of PVY (A) from potato Sprout tips c.v Chandramukhi after treatment with: Azacitidine (AZA) and PLRV (B) by using Ribavirin (RBV): L: ladder. 1: control. 2, 4 and 6 Sprout lengths (3 mm) with concentrations 60, 40 and 20 mgl^{-1} respectively. 3, 5 and 7 Sprout lengths (5 mm) with concentrations 60, 40 and 20 mgl^{-1} respectively.

PVY was destroyed by RBV in Marfona infected vitroplants grown from sprout tips of 5 and 3 mm length at concentrations of 40 and 60 mgl^{-1} respectively. PLRV in the same cultivar was eradicated from infectd vitroplants developed from sprout tip of 3 and 5 mm length at 40 mgl^{-1} of RBV and also from sprout tips of 3 mm length at 20 and 60 mgl^{-1} of RBV respectively, these results because of choosing one plant from each treatment.

Marfona infected vitroplants treated with Azacitidine at most tested doses revealed negative in PCR except of PVY virus at concentration of 40 mgl^{-1} in sprout tips 3 and 5 mm length and PLRV from sprout tip of size 5 mm at 60 mgl^{-1} (table 4).

Table 4. Effect of Ribavirin and Azacytidine on elimination virus in c.v Marfona

virus	0		Ribavirin (mgl^{-1})						Azacitidine (mgl^{-1})					
			20		40		60		20		40		60	
	Samples length (mm)								Samples length (mm)					
	3	5	3	5	3	5	3	5	3	5	3	5	3	5
PVY	+	+	+	+	+	-	-	+	-	-	+	+	-	-
PLRV	+	+	-	+	-	-	-	+	-	-	-	-	-	+

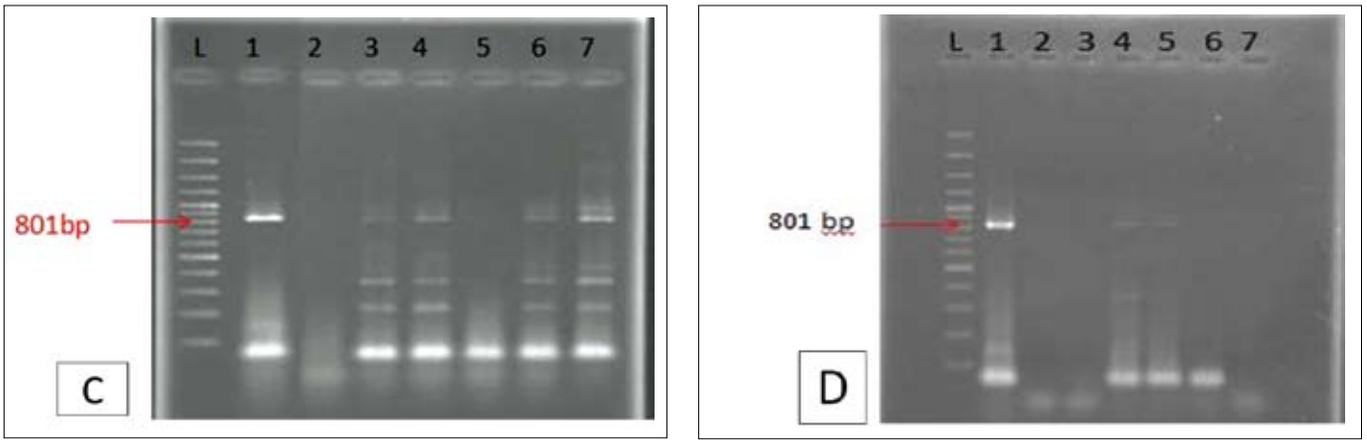

Fig 2. PCR results for detection of PVY (C) from potato Sprout tips c.v Marfona after treatment with Ribavirin (RBV) and (D) by using Azacitidine (AZA) : L: ladder. 1: control. 2, 4 and 6 Sprout lengths (3 mm) with concentrations 60, 40 and 20 mgl-1 respectively. 3, 5 and 7 Sprout lengths (5 mm) with concentrations 60, 40 and 20 mgl-1 respectively.

From this study we noticed that producing virus free plant depends on the genotype of plant, type and concentrations of antivirals and virus species. To producing virus free plant it needs high concentrations of Ribavirine in Marfona variety and Azacytidine in c.v Chandramukhi. Also researches noticed that size of samples is very important to produce virus free plants. Their experiments showed that chance of virus free plants by using small sizes of samples increased but their regeneration are decreasing (Oana et ***al.,*** 2009). On the contrary, the chance of virus free plants from big sizes of samples is decreasing. In the other side, probability the decrease of the effect of antivirals which was used in this study, unsuitable size of samples or did not answer the potato genotype when the use of Antivirals as (Ribavirine and Azacitidine) in chemotherapy must be considered (Oana et ***al.,*** 2009). For ether, the results in this investigate after utilization of antiviral concentrations in growth medium allowed to elimination PVY and PLRV, which was similar to what observed by many researchers. Awan et ***al.,*** (2007) was obtained PLRV virus free plants in cv. Cardinal after using 20 and 40 mgl^{-1} of Ribavirine and Azacitidine and the results showed that the percentage of virus free plants was 44.7 and 43.8 % respectively. Nascimento et al., (2003) indicated that viruses Potato S virus (PVS), Potato X virus (PVX) and Potato Y virus (PVY) could be removed from single node cuttings (1cm length) of potato plants cv. Baraka when using 20 mgl^{-1} of Ribavirine and Azacitidine and the percentages of virus free plants were 42.8 and 33.3 % respectively. Bittner et ***al.,*** (1989) Potato S virus (PVS) could be removed from potato plants cv. Lebana and lebora after using 0.003% of Ribavirine and the percentage of virus free plant was 95 and 83 % respectively. Cassells et ***al.,*** (1982) showed that Potato M virus (PVM) could be removed when using virasol at concentrations 41 and 205 µL after caltivate mirestems and pieces of stem (1cm length) of cv. Golden wonder and the percentages of virus free plants were 58 and 100 % respectively. In other plants like citrus, Indian citrus ringspot virus (ICRSV) virus could be removed after cultivation shoot tips and using Ribavirine at concentrations 20 and 25 mgl^{-1} and the percentages of virus free plants were 15.38 and 37 % respectively, (Sharma et ***al.,*** 2007).

Conclusions

From the results we notice that the response of two potato cultivars to antivirals were different. But in this investigate Ribavirine at all concentrations was able to elimination PLRV and PVY from infected plants which grown from 3 and 5mm lengths of sprout tips in cv. Chandramukhi. While the results is not the same by using Azacitidine. In cv. Marfona higher virus free plants noticed by using Azacitidine at all concentrations and all lengths of sprout tips. While results of using Ribavirine were not good.

Referances

- Akhtar, N., M. H. Munawwar, M. hussain and M. Mahmood. 2006. Steril shoot production and direct regeneration from the nodal explants of potato cultivars. Asian J. Plant Sci., 5: 885-889.
- Anonymous. 2007. Production of nuclear seed potatoes for the first time in tissue culture method in Khorasan Agriculture and Natural Resources Research Center. Newsletter Agriculture Organization of Razavi Khorasan. No. 29. (In. Persian).
- Anonymous, 2008. Crops. Available at: www.Faostat.Fao.org.
- Anura, H., and S. Lanka. 1988. Tissue culture and meristem culture in sweet potato (*Ipoaea batatas* (L.) Lam). Report . ARC Tra. J. Sweet Potato: 1-7.
- Awan, A. R., S. M.Mughal, Y. Iftikhar and H. Z. Khan. 2007. In Vitro Elimination of Potato Leaf Roll Polerovirus from Potato Varieties. Euro. J. Sci., 18: 155-164.
- Bittner, H., G. Schenk, G. Schuster and S. Kluge. 1989. Elimination by chemotherapy of potato virus S from potato plants grown *in vitro*. Potato Res., 32: 175-179.
- Burhan, M., M. Aslam Khan, M. Irfanullah, M. Riaz Jamil and M. Ishfaq. 2007. Incidence of Potato Virus X (PVX), Potato Virus Y (PVY), Potato Virus S (PVS) on Potato Cultivars in Potato Growing Areas. J. Agri. Soc. Sci., 3: 37-38.
- Cassells, A. C., and R. D. Long. 1982. The elimination of potato viruses X, Y, S and M in meristem and explant cultures of potato in the presence of Virazole. J. Potato Res., 25: 165-173.
- Chikh Ali, M., T. Maoka and K. T. Natsuaki. 2008. The Occurrence of Potato Viruses in Syria and the Molecular Detection and Characterization of Syrian Potato virus S Isolates. Potato Res., 51:151– 161.
- Ehrenfeld, N., E. Romano, C. Serrano and P. Arce-Johanson. 2004. Replicase mediated resistance against Potato Leafroll Virus in potato Desirée plants. Biol. Res., 37: 71-82.
- Gebhardt, C., D. Bellin, H. Henselewski, W. Lehmann, J. Schwarzfischer and J. P. T. Valkonen. 2006. Marker-assisted combination of major genes for pathogen resistance in potato. Theor. Appl. Gen., 112: 1458-1464.
- John, B., C. Gebhardt, F. Govers, D. K. L. Mackerron, M. Taylor and H. Ross. 2007. Potato biology and Biotechnology. Advances and perspectives. Wageningen, the Netherlands: 851-857.
- Khawajeh pour, M. R. 2006. Industrial plants. Publications Unit, Isfahan University Jihad. Page: 423-494. (In. Persian).
- Mahmoud, S. Y. M., M. H. Hosseny and M. H. Abbel-ghaffar. 2009. Evaluation of some therapies to eliminate potato Y potyvirus from potato plants. Inter. J. Virol., 5: 64-76.
- Manzer, F.E. 1959. Potato meristem culture and virus X. Amer. Potato J., 36: 191-195.
- Mobli, M., and B. Perasteh.1994. Vegetable production. (Translation), Isfahan Uni. Of Tech. (In. Persian).
- Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.
- Nascimento, L. C., G. P.Ribeiro, L. Willadino and G. P. Andrade. 2003. Stock indexing and potato virus y elimination from potato plants cultivated in vitro. Sci. Agri., 60: 525-530 .
- Novy. R. G., A. M. Gillen and J. L. Whitworth. 2007. Characterization of the expression and inheritance of potato leafroll virus (PLRV) and potato virus Y (PVY) resistance in three generations of germplasm derived from *Solanum tuberosum*. Theor Appl. Gen., 114: 1161–1172.

- Oana, D., L. Erdei, V. Livia, M. Danci, B. Anca, I. David and F. Berbentea. 2009. Influence of ribavirin on potato plants regeneration and virus eradication. J. Hort. For. Biot., 13: 421-425.
- Pajohandeh, M. 2001. Guidelines created virus-free potato of germplasm bank within the glass, plant pathology master's thesis, Faculty of Agriculture, Tarbiat Modarres University.
- Pua, E. C., and M. R. Davey. 2007. Biotechnology in Agriculture and Forestry. Transgenic Crops. Springer Verlag Berlin Heidelberg, 59. 1-498.
- Rajabi, M. A. 2009. *In vitro* plantlet formation and production of virus free potatoes (*Solanum tuberosum* "Istanbuli" employing chemotherapy and thermotherapy coupled with meristem culture). Master's thesis Horticulture, Faculty of Agriculture, Uni. of Tech. (In. Persian).
- Roodbar Shojaei, T., N. A. Sepahvand, M. Omidi, A. Mohammadi and H. R. Abdi. 2007. Response of four commercial potato cultivars to different combination of plant growth regulators in meristem culture and production of virus free plantlets. Irania J. of Crop Sciences, 9 (4): 332-344.(In.Persian).
- Rosner, A., and L. Maslenin. 2006. Nucleotide Sequence Variability at the 5 ' -UTR of Potato PVY Isolates. Potato Res., 49: 217– 224.
- Salim Khan, M., M. I. Hoque, R. H. Sarkerand and H. P. Muehlbach. 2003. Detection of Important Plant Viruses in In vitro Regenerated Potato Plants by Double Antibody Sandwich Method of ELISA. Plant Tiss. Cult., 13: 21-29.
- Shalaby, A. A., M. K. Nakhla, A. M. Soliman, H. M. Mazyad, A. Hadidi and D. P. Maxwell. 2002. Development of a highly sensitive multiplex reverse transcription polymerase chain reaction (mRT-PCR) method for detection of three potato viruses in a single reaction and nested PCR. Arab J. Biot., 5: 275-286.
- Sharma, S., B. Singh, G. Rani, A. A. Zaidi, V. Hallan, A. Nagpal and G. S. Virk. 2007. Production of Indian citrus ringspot virus free plants of kinnow employing chemotherapy coupled with shoot tip grafting. J. Gen. Euro. Agri., 8: 1-8.
- Sidaros, S. A., R. A. Omar, S. A. El-Kewey and S. Abd El-Khalik. 2004. Virus Elimination from Infected Garlic Plants Using Different Techniques. Egypt. J. Virol., 1: 333-341.
- Singh, R. J. 2007. Genetic resources, chromosome engineering, and crop improvement series. Vegetable Crops. CRC, Taylor and Francis Group, 3: 1-558.
- Singh, J., and L. Kaur. 2009. Advances in potato chemistry and technology. Academic Press is an imprint of Elsevier. USA, 67: 1-556.
- Wambugu, F. M., G. A. Secor and N. C. Gudmestad. 1985. Eradication of potato virus y and S from potato by chemotherapy of cultured axillary bud tips. Amer. J. Potato Res., 62: 667-672.
- Wang, Q., Y. Liu, Y. Xie and M. You. 2006. Cryotherapy of potato shoot tips for efficient elimination of potato leaf roll virus (PLRV) and potato virus y (PVY). Potato Res.,U 49: 119–129.

Ref : 296 / Accepted 7- 2013