



## Determine of Isolation Distances for Seed Potato Production in Ardabil Region

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### ABSTRACT

This study was performed to determine the distance of isolation for the seed potato production from ware potato in three regions of Ardabil, Namin and Nir. The experiment was carried out in a randomized complete block design (RCBD) with five distances (100, 200, 300, 400 and 500 meters from ware potato) and three replications with Super Elite (SE) class of Agria potato cultivar from ware potato as the source of infection studied by ELISA during 2014 and 2015. According to standard of Seed and Plant Certification and Registration Institute (SPCRI), as many as 200 leaves and 200 tubers samples of each distance were collected and their virus infection was done by ELISA assay. Analysis of variance tuber yield showed that were significant differences between regions, years and distances of isolation. In terms of tuber yield, between distances 500, 400 and 300 meters, there was no significant difference and they were in one group. By reducing isolation distance from 500 meters to 100 meters from host of viral diseases vectors (edible potato of Agria cultivar) tuber yield reduced from 44.328 t/ha to 39.828 t/ha (about 4.5 t/ha, or 10.15 percent). Average infection of Rhizoctonia fungal diseases of tuber in isolation distances and three regions were about 5 percent. In three regions and five isolation distances, tubers were without infection of vascular rot fungus diseases, without infection of bacterial diseases of blackleg and brown rot and without infection of potato moth pest. In each three regions, the PVY virus was in distances 100, 200 and 300 meters from the host. Insects of collected from yellow pan were 1.1% green peach aphid, 1.78% thrips, 32.54% green cicada, 57.99% brown cicada, 1.18% hawk moth of potato and 5.41% other insects. To determine the exact isolation distance in different regions of potato cultivation in Ardabil province, additional projects, provided in the suggestions, need to be implemented.

**Key words:** Isolation distanc, Seed potato, Vectors, PVY

### Introduction

The cultivated potato (*Solanum tuberosum* L.) is one of the world's most important foods and vegetable crops, rating fourth in production. It contains 78% water, 18% starch, 2%

protein, 1% vitamins and several trace elements. It is the world's leading crop and grown in approximately 140 countries (Haase, 2008; Bhutta, 2008).

Considering moderate and suitable air condition, Ardebil province with a cultivated area of about 21,000 hectares and production of more than 800,000 tons of potatoes, is one of the favorite areas for cultivation of this crop (Hassanpanah, 2013). Isolation interval is important in potato seed production due to the importance of vectors in the transmission of viral and phytoplasma diseases. Determination of standards for production, reproduction and certification of seed potatoes should be done according to the conditions of the production area and its capabilities. In reproducing and producing seed tubers from certified seed, soil and preparation farm, soil health, crop health, water management, field health, seed tuber health, alternation Agronomic and observation of isolation distance were noted. Standards defined for potato mini-tuber production in India include mini-tuber weight of at least 1 g, germination power of 90%, purity of 99% and viral diseases 0.01% (Bostan *et al.*, 2006). In India, they also consider isolating the minimum required distance between commercial potato fields and five-meter seed production. Other standards for seed production in India include high altitude seed production due to low contamination with aphids and other carriers during the potato growing season (Anonymous, 2005).

Commercial and seed potato production are constrained by several viral diseases that reduce yields and lower tuber quality (Ragsdale *et al.*, 2001). Viral diseases are one of the most important inhibitors and contaminants of potatoes due to the coincidence of potato growth with the life span of viruses carrying aphids.

Potato viral diseases were first seen in Europe, but by 20th century their cause was unknown. To date, more than 35 different viruses have been reported from potatoes (Salazar, 1996). Some of the viruses are dependent on potato survival and spread and are of greater economic damage and importance. Hence, much research has been done on their identification, transmission and control worldwide (Matthews, 1991; Salazar, 1996; Buchen Osmond, 2002).

Potato virus damage varies from 10 to 90%, depending on virus type, potato cultivar and environmental conditions; since potatoes are vegetatively propagated, this creates exclusive opportunities for pathogens to establish and spread several diseases (Khurana, 2004). Among the plant pathogens, more than 37 viruses are reported to infect potato crops. Potato leaf roll virus is one of the most important potato viruses in the world. Potato leaf roll virus (PLRV) can reduce tuber yield and quality in potato (Mondal *et al.*, 2017). Potato virus Y (PVY) is the potato virus with the highest economic impact on seed potato production worldwide (Rolot, 2005; Valkonen, 2007). Depending on the breed, the damage is 10-70% in seasonal infections and up to 80% in primary infections (De Boks, 1987). Potato Virus X (PVX) and PLRV are considered more important due to major role they play in crop losses (Lung'aho *et al.*, 2007; Olubayo *et al.*, 2010).

Potato virus S (PVS) is a major plant pathogen that causes considerable losses in global potato production (Duan *et al.*, 2018). The A potato virus was first introduced in 1932 (Murphy and McKay, 1932). In Iran, only the distribution of specific potato viruses in some provinces has been studied, including PLRV, PVS and PVX viruses in Khorasan province (Beykzadeh *et al.*, 2001), PLRV, PVA, PVY, PVS, AMV, PVM and TSWV viruses in Firoozkooh region, PLRV, PVA, PVY, PVS, AMV, PVM and TSWV viruses in Damavand region and PLRV, PVY, PVM, PVS, PVX and PVA viruses in Firoozkouh, Karaj and Tajarak station of Hamadan (Pajouhandeh *et al.*, 2000). Ardebil's 95,000-hectare plain in terms of geographical location,

elevation of 1400 m above sea level, enclosure by natural insulators, windsurfing (usually wind speeds of 7 m/s at 10 m) and activity limitations Viral vector carriers is one of the most important centers of potato production in Iran. Since there is no report on the existence and spread of potato viruses in Ardabil, the aim of this project is determination of the most suitable isolation distance for seed potato production in areas such as Ardabil, Namin and Nir, investigation of tuber yield at the studied isolation distances studied in each region and determination of vectors and viral diseases.

**Experimental**

**Materials and methods**

This study was conducted in three regions of Ardabil province (Ardabil, Namin and Nair) with the main host plant of potato viral vectors based on a randomized complete block design with five intervals (100, 200, 300, 400 and 500 meters) of edible potato (Agria cultivar) and three replicates from seed of superalite during 2014 and 2015.

Each plot consisted of 10 stacks of 20 meters with a distance of 75 cm and two plants with a distance of 25 cm. Ammonium phosphate fertilizer at 150 kg twice (50% at planting time and 50% at tuber formation), urea at 300 kg at three times (25% at planting time, 50% at emergence and 25% immediately after tuber formed) and potassium sulfate fertilizer (100 kg) were applied on the basis of soil test. According to the guidelines of the Institute for Seed and Certification Research, 14 days before planting, 200 mid-stem leaf and 200 tuber samples were randomly prepared from each treatment and viral diseases of PVX, PVS, PVM, PVA, PVY and PLRV were evaluated by Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA) (Nasir *et al.*, 2012; Anonymous, 2015). Bacterial and fungal diseases (superficial and vascular) were evaluated and compared with Tables 1-3. For the detection and identification of carriers of viral diseases during the growing season, light yellow pan with 15×30 cm area with water content and 0.002% detergent was used. Analysis of variance of data was done and mean comparison was run based on LSD test using SAS 9.1 statistical software.

**Table 1.** Standard maximum tolerable pollution percentage in field and laboratory in pre basic class (S)

Disease contamination (%)	Seed class S		
	Primary	Final	Lab
Symptoms of leaf roll virus	1	0	0
Mosaic, Pisk Symptoms and Symptoms of PVY Symptoms	1	0	0
Symptoms of Phyto Plasma	1	0	0
Fungal diseases Rhizoctonia	0	0	0
Fungal diseases Vascular fungi	0	0	0
Bacterial diseases Black leg	0	0	0
Bacterial diseases Brown rot	0	0	0
Willow pest	0	0	0
Non green areas	0	2	0
Mixing cultivar	0	0	0

**Table 2.** Standard maximum tolerable pollution percentage in the field and laboratory in the basic class (SE and E)

Disease Infection (Percentage)	Class SE			Class E		
	Primary	Final Farm	Laboratory	Primary	Final Farm	Laboratory
Symptoms of leaf roll virus	1	0.5	0.5	2	1	1
Mosaic, Pisky Symptoms and Symptoms of PVY Symptoms	1	0.5	1	2	1	2
Other Viral Symptoms	1	0.5	0	2	1	0
Symptoms of Phyto Plasma	1	0.5	0	2	1	0
Fungal diseases						
Rhizoctonia	0	0	0	0	0	0
Vascular fungi	2	0	0	3	0	0
Bacterial diseases						
Black Leg	0.5	0	0	1	0	0
Brown rot	0	0	0	0	0	0
Willow pest	0	0	0	0	0	0
Non green Areas	0	4	0	0	5	0
Mixing cultivar	0	0	0	0.5	0	0

**Table 3.** Standard maximum tolerable pollution percentage in the field and laboratory in the certified class (A, B and C)

Disease Infection (Percentage)	Class A Farm		Class B Farm		Class C Farm	
	Primary	Final	Primary	Final	Primary	Final
Symptoms of leaf roll virus	4	2	6	4	8	5
Mosaic, Pisk Symptoms and Symptoms of PVY Symptoms	4	2	6	4	8	5
Symptoms of Phyto Plasma	8	4	10	5	14	∨
Symptoms of leaf roll virus	3	1.5	4	2	5	3
Fungal diseases						
Rhizoctonia	0	5	0	5	0	5
Vascular fungi	4	2	5	3	5	3
Bacterial diseases						
Black leg	3	2	4	3	5	4
Brown rot	0.5	0	0.5	0	1	0.5
Willow pest	0	0	0	0	0	0
Non green areas	0	5	0	5	0	5
Mixing cultivar	1	0.5	2	1	3	2

**ELISA test steps (Nasir *et al.*, 2012) (Anonymous, 2015)**

Agdia's ready-made kits were used for this test.

**1. Providing wash buffer PBST Phosphate Buffer Saline = PBS-T (NaCl, KH<sub>2</sub>PO<sub>4</sub>, KCl, Na<sub>3</sub>N, Tween 20):** To prepare the PBST wash buffer, the volume was dissolved in 1000 mL volume after dissolving the following substances in distilled water.

Sodium chloride	8.0 g
Sodium phosphate, dibasic (anhydrous)	1.15 g
Potassium phosphate, monobasic (anhydrous)	0.2 g

Potassium chloride	0.2 g
Tween-20	0.5 g
pH	7.4

50 mL of PBST wash buffer prepared with 950 mL of distilled water was diluted. This buffer was kept at room temperature when used and kept at 4 °C when not in use.

**2. Providing general extract buffer (GEB):** Method 1: to 16.5 g of buffer powder, 10 mL of water was added and after stirring, the clear slurry solution was obtained.

As it was stirred, it was slowly poured into 500 mL volume with water. Then, 10 g of Tween-20 was added to the solution and the pH of the buffer was adjusted between 7.2 - 7.8.

This buffer was stored at 4 °C when used at room temperature and when not in use.

#### General extract buffer compounds

Sodium sulfite (anhydrous)	0.65 g
Polyvinylpyrrolidone (PVP) MW 24 - 40,000	10.0 g
Sodium azide	0.1 g
Powdered egg (chicken) albumin, Grade II	1.0 g

**3. Buffer ECM provision:** To prepare 100 mL of ECM buffer, dissolve 0.4 g of lean milk in 100 mL of PBST wash buffer and stir for 30 minutes and adjust the pH of the buffer to 7.4.

This buffer was stored at 4 °C when used at room temperature and when not in use. This buffer requires 100 microliters for each well. The buffer was used for eight wells of 800 µl.

**4. Preparation of PNP substrate buffer:** To prepare 10 mL of PNP substrate buffer, 2 mL of PNP buffer (×5) was dissolved in 8 ml of distilled water. This buffer was provided when in use.

#### PNP buffer compounds

After dissolving the following substances in 800 mL of distilled water, the volume was brought to 1000 mL.

Magnesium chloride hexahydrate	0.1 g
Sodium azide	0.2 g
Diethanolamine	97.0 ml
pH (adjust pH to with hydrochloric acid)	9.8

**5. Providing positive control:** The bottle containing positive lyophilized control was dissolved in 2 mL of extraction buffer. Positive control was maintained at -20 °C when used at room temperature and when not in use. 100 mL of this solution was added to the well.

**6. Leaf or tuber extraction:** Before extraction, each plate was numbered from 1 to 96 according to sample number. The ratio of leaf or tuber sample to buffer was 10: 1 (g: mL). 0.1 g of each leaf or tuber sample was extracted in 1 mL of public extraction buffer inside a Chinese mortar.

## **Procedure**

### **Step One: Distributing the samples**

100 µl of leaf or tuber extract was added to each well. Instead of leaf or tuber extract, six wells of positive control solution with concentrations of 0, 5, 25, 50, 75 and 100% were added to three wells of negative control and to three wells of buffer extraction.

100 wells were added to each well. Three wells were considered as empty wells (without material).

### **Stage Two: Incubation**

After adding leaf or tuber extract, positive and negative controls and extraction buffer to the plate wells, they were placed in a box containing wet paper towels and kept at room temperature for 2 hours.

### **Step Three: Preparation of enzyme conjugate**

10 min before the end of the incubation phase, to prepare 10 mL of Enzyme conjugate, from bottle A (Detection antibody) and bottle B (Alk phos enzyme conjugate) (100: 1 dilution), each containing 100 mL of 10 mL. EC ECM buffer was added.

### **Step Four: Washing the plate**

After the end of the incubation phase, the plates were washed with PBST wash buffer seven times carefully without mixing the contents of the wells.

After filling the wells with 200 microliters of PBST wash buffer, wait about 5 minutes and then vacuum them and place the plate upside down on the paper towel to remove the wash buffer droplets. The wells should be without plant tissue.

### **Step Five: Adding enzyme conjugate**

At this stage, 100 µl of Enzyme conjugate was added to each well.

### **Stage Six: Incubation**

At this point, the plates were kept in a box containing wet paper towels for 2 h at room temperature.

### **Step 7: Preparation of PNP solution**

15 minutes before the end of the incubation phase, two PNP tablets weighing 5 mg were added to the 10 mL PNP buffer, stored at room temperature, and dissolved in the dark.

### **Step Eight: Plate wash**

After the end of the incubation phase, the plates were washed with PBST wash buffer eight times carefully without mixing the contents of the wells.

After filling the wells with the PBST wash buffer, quickly vacuum them and then place the plate upside down on the paper towel to remove the wash buffer droplets.

### **Step 9: Adding PNP solution**

At this stage, 100 µl of PNP solution was added to each well.

### **Stage 10: Incubation**

At this point, the plates were kept in the dark in a box containing a wet paper towel for 60 minutes at room temperature. At this point the plates were protected from direct and intense light.

### Step 11: Evaluating the results

**1. Observation:** Wells yellow in samples were infected with the virus.

**2. Using ELISA:** Remove any bubbles before reading plate with ELISA.

Potato virus was measured by BioTek ELISA according to the RIDAWin software at 405 nm.

The formula  $R = \bar{x} + 3SD$  was used to determine contamination and non-contamination of samples (Salazar, 1996; Ahmadvand and Hassanabadi, 2009).

$\bar{x}$  = Mean Absorption Rate of Negative Controls

SD = standard deviation of negative control absorbance values

If the amount of sample read from the ELISA machine was greater than the value of R, it was taken as the sample infected with the virus.

### Results and discussion

Analysis of variance showed that there was a significant difference between the studied areas and isolation interval at 1% probability level and 5% probability level between years (Table 4).

**Table 4.** Mean squares of tuber yield at isolation intervals of three and two years in potato cultivar Agria with superalite seed class

Sources of variation	degree of freedom	means of square
Areas	2	**336.51
Year	1	*92.41
Regions×Year	2	15.51 <sup>ns</sup>
Error	12	27.51
Isolation distance	4	**65.28
Areas× Isolation distance	8	4.34 <sup>ns</sup>
Year × isolation distance	4	1.42 <sup>ns</sup>
Areas × years×isolation distance	8	9.75 <sup>ns</sup>
Error	48	18.53
Coefficient of variation	-	10.31

\* and \*\* significant at 5 and 1% probability levels

Comparison of mean tuber yield in studied regions showed that Ardabil region had the highest value and was classified in group a. The lowest tuber yield was in the Nair region (Figure 1). Compared to other regions, Ardebil region had the most appropriate factors for altitude, total rainfall, minimum temperature, average daily temperature, absolute minimum temperature, frost days, sunny hours and air pressure for potato cultivation. Therefore, areas that are similar in character to the Ardabil region may be suggested for the production of potato mini-tubers.

The comparison of mean tuber yield at five isolation intervals showed no significant differences between 500, 400 and 300 m intervals and were in the a, ab and abc groups. The lowest tuber yield was in two distances of 200 and 100 m and was not significantly different (Figure 2). Yield of tuber differed between 500 and 100 about 4.5 t/ha. By reducing the isolation distance from 500 m to 100 m in potato seed production cultivar

Agria with superalite seed class from host of viral vectors (edible Agria potato), tuber yield increased from 44.328 t/ha to 39.828 t/ha decreased (about 4.5 t/ha or 10.15%).

Isolation distance is the minimum separation required between two or more varieties of the same species for the purpose of keeping seed pure. Transmission of viral viruses has decreased within 500 m between farms, but as isolation distance has decreased, transmission of viral viruses has increased.

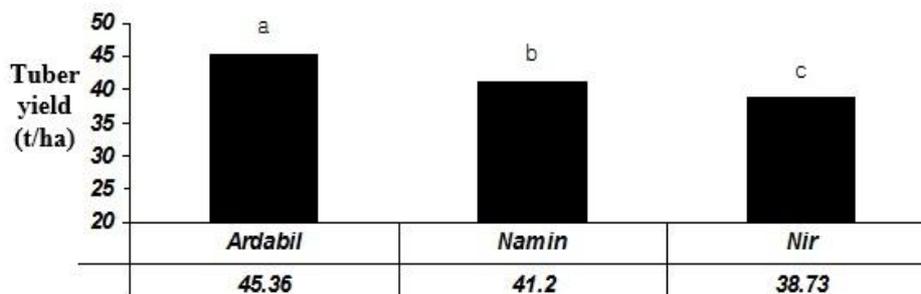


Figure 1. Mean tuber yield in the three study areas

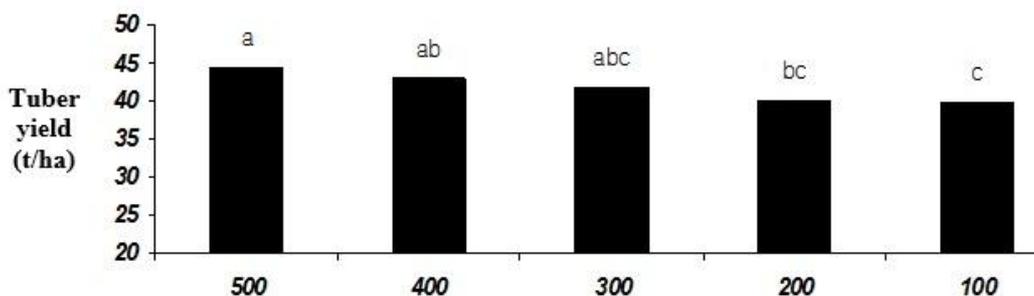


Figure 2. Mean tuber yield at the five isolates studied

The results of tuberculosis rhizoctonia showed that in all isolates and three study areas, about 5% of rhizoctonia was observed (Table 5). Ardebil region had the highest rate of infection (6.8%) and Nair area had the lowest rate (3.2%). In the three regions and 5 distances studied, the potato tubers were without vascular caries fungal disease and without bacterial diseases of the black leg and brown rot.

Table 5. Mean percentage of Rhizoctonia fungal disease in the isolation intervals studied in three regions

Areas	100	200	300	400	500	Average
Ardebil	8	6	8	6	6	6.8
Namin	5	4	5	7	6	5.4
Nair	3	4	3	3	3	3.2
Average	5.33	4.67	5.33	5.33	5.00	5.13

PVY virus was detected in leaf and tuber specimens prepared from isolation intervals at 100, 200 and 300 m intervals. In Ardabil region, PVY virus was 6% in 100 meters, 4% in 200 meters and 3% in 300 meters. In Namin region, the amount of PVY virus was 5% at 100 meters, 200% and 300 meters at 3% and in the Neyir area at 4% at 100 meters, 2% at 200

and 300 meters (Table 6). PVY virus was not observed in the three study areas at distances of 400 and 500 m from the host (Table 6). Potato viruses of PLRV, PVA, PVX, PVM, and PVS were not detected in field samples obtained from field samples.

**Table 6.** Mean PVY virus disease at the isolation intervals studied in three regions

Areas	100 meter	200 meter	300 meter	400 meter	500 meter	Average
Ardebil	6	4	3	-	-	4.30
Namin	5	3	3	-	-	3.70
Nair	4	2	2	-	-	2.67
Average	5	3	2.67	-	-	3.56

Insects collected from the yellow pan installed in Agria potato seed production farm, 1.18% green peach aphid, 1.78% trips, 32.54% green zebra, 57.99% brown zebra, 18.1% of moths were potatoes and 5.33% were insects (Table 7). The tubers produced in three regions at the studied isolation intervals were free from contamination with potato moth.

Potato is a vegetatively propagated crop; hence the crop vigour and yield largely depends upon the quality of seed material obtained. Usually the farmers are in practice of purchasing smaller tubers from cold stores without bothering much about quality as it is cheaper. But if the seed tubers are disease affected then the yield is found to be very poor. The practice is very obvious because cost of seed itself becomes more than half of the total cost of cultivation and non availability of seed at sowing time compels the farmers to purchase the tubers from the nearest convenient place.

**Table 7.** Percentage of pests identified at the isolation intervals studied in the three zones

Pests	Number	Percentage
Myzus persicae	2	1.18
Trips spp	3	1.78
Empoasca faba (Harris)	55	32.54
Agallia spp	98	57.99
Phthorimaea operculella	2	1.18
Other insects	9	5.33
Total	169	100

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