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Horticultural and Viral Studies for Improving Local Potato Seeds Production

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ABSTRACT

Two experiments were carried out to study the possibility of production of improved potato seeds under Egyptian conditions. The first experiment was conducted in fall season to compare between improved seeds produced from three imported varieties (Diamant, Spunta and Lady Rosetta) in summer seasons (2012 and 2013) under control in Kaha research station and seeds produced by farmers in Kafr El-Zayat (KZ). The second experiment was performed in summer season (2013 and 2014) to compare the improved seeds produced from three varieties in previous summer seasons (2012 and 2013) with imported seeds. Detection of the most important potato viruses (*potato leafroll virus*; PLRV, *potato virus Y*; PVY, *potato virus X*; PVX and *alfalfa mosaic virus*; AMV) were carried out on potato leaves and tubers using ELISA and RT-PCR for the detection of these viruses. Specific primer pairs designed to amplify the coat protein gene of each virus (627 bp for PLRV, 801 bp for PVY, 714 bp for PVX and 666 bp for AMV) were successfully applied. The obtained data indicated that virus-infection incidence percentage was increased in KZ potato seeds. Most vegetative growth and yield characteristics were significantly higher in Spunta cultivar. Meanwhile, Diamant cultivar gave the highest value for leaf number per plant, number of tuber/plant, number of main stems at 75 DAP and number of tubers per plant at harvest time. Conversely, Lady Rosetta gave the highest dry matter percentage of tubers for both fall seasons. Improved seeds which produced under Kaha Research Station were significantly superior in emergence percentage, leaves number, leaf area per plant, total dry weight, tuber fresh and number of tubers/plant in both seasons. In summer season, the potato seeds produced from seeds produced in previous summer season under control (Kaha, local) showed increase in viral infection percentage which aggravated by using the cut seeds in plantation compared to the imported seeds. Spunta gave the lowest significant average tubers number per plant, on the contrary, produced the highest average tuber fresh weight, total yield per plant and feddan comparing with Diamant and Lady Rosetta. Imported potato seed tubers which were planted as complete tubers yielded the highest significant total tuber yield per plant and feddan compared to other seed sources. As well as, using whole local tubers seed increased yield per plant and feddan compared to all divided tubers seed. On the other hand, divided local seeds gave the lowest value of total yield per plant and feddan in the two growing seasons.

KEYWORDS: Potato - Diamant - Lady Rosetta - Spunta - Imported seeds - local seeds - Potato virus Y- Potato virus X - Potato leafroll virus - alfalfa mosaic virus.

INTRODUCTION

Potato is considered the second vegetable crop in terms of area in Egypt after tomato, the cultivated area in 2013 was about 423809.5 feddan (feddan = 4200 m^2) which cultivated in three seasons (Summer, Fall and Winter) produced 4.8 million tons, also occupies the second place in exported crops. Egypt exported about 679 thousand tons in 2014 for different markets such as Russia, England, West European countries and some Arab countries [1]. Furthermore, Egyptian potatoes globally ranked the fifth in terms of exported quantity [2]. Egypt annually imports large quantities of potato seeds for the cultivation in summer season (spring

plantation). Most of the summer season crop, roughly 35% of annual production, is kept as seeds for the fall and winter planting. Seeds represent about 50% of variable costs in potato production. Spring plantation produce higher tuber yield than autumn and winter plantation although the temperature in autumn and winter plantation is more favorable for potato production which could be attributed to the quality of imported seeds is better than locally produced (diseases - physiological age) or because of the advantageous lag time in spring between solar radiation and temperature [3]. Under Egyptian conditions, Tawfik [4] found that during spring planting of cv. Alpha total yield obtained from local seed (produced from the previous spring planting) sprouted for 2 weeks before planting was comparable to their respective imported seed. In Tunisia Benz and Fahem [5] studied the store of local seed from one main season to the next, a period of approximately 230 days. Also, Fahem and Haverkort [3] studied the crop components affecting tuber dry matter production in the two contrasting seasons as influenced by cultivar and seed age; seed tubers for the spring season were either imported (young seeds) or locally produced in the previous spring and stored in the cold store (old seeds). The most common viruses affecting potato crops throughout the world are potato virus Y (PVY), potato virus X (PVX) and potato leafroll virus (PLRV) [6]. Alfalfa mosaic virus (AMV) has a very wide host range and it can naturally infect many herbaceous and some woody plant hosts [7]. Standard methods for the detection of viruses counting mechanical inoculation to indicator hosts, electron microscopy, ELISA and visual examination are not sufficient for virus detection in potato tubers [8]. Increased specificity and sensitivity of detection presented by RT-PCR is an active method to overwhelm these problems [9]. Ahmed *et al.* [10] reported that productivity of potato cultivar Spunta to infect with virus strains was conducted in the farm of Benha, Kalyoubia governorate decreased by 22.6% to 50.5% according to the strain as compared with control. Li *et al.* [11] demonstrated that significantly lower contents of chlorophyll a, chlorophyll b and total chlorophyll were found in virus infected shoots than in healthy ones. Mugoro [12] indicated that tubers of the local cultivar Batte of farmers source was generally low in its yield and quality, whereas tubers of the released Bubu cultivar obtained from Kersa Local Seed Business Project and Haramaya University was superior in growth, yield, and quality to Batte. Although the advantage of producing local seeds source, there are not enough researches have been done to cover this area for the varieties of importance to the Egyptian producer in the recent years. This study aimed to investigate the validity of planting summer season with potato seeds produced in the previous summer season. Also, to compare between seeds produced under controlled conditions (rouging) with those produced by farmers in fall and summer seasons. Also, is to detect viruses in potato leaves and tubers. In this study three important cultivars for the Egyptian potato industry were used i.e., Diamant, Lady Rosetta and Spunta which represent 62.9 % of cultivated potato area in 2011/2012 [13].

MATERIALS AND METHODS

The horticultural experiments were carried out in the Experimental Research Farm of Kaha, Kalyoubia Governorate, Egypt during the period from 2012 to 2014. The site is located at an altitude of 21.1 m above sea level, latitude 30°16' N and longitude 31°12' E. with clay loam soil in texture. Chemical and physical properties of the experimental soil are shown in Table (1).

Table 1: The physical and chemical properties of the experimental soil.

Properties	Seasons	
	2012/2013	2013/2014
Physical (%)		
Clay	60.70	60.40
Silt	21.10	22.00
Sand	18.20	17.60
Texture	Clay loam	
Chemical (ppm)		
N	98.90	99.60
P	5.90	4.66
K	216.20	215.60
pH (1-2.5 suspension)	7.70	7.60

The aim of this study is to produce seed potato tubers improved from three varieties of potatoes imported (Diamant, Spunta and Lady Rosetta) in the summer season (2012 and 2013). This study included: The first experiment was performed in fall season (2012 and 2013) to compare two sources of potato seeds; the first produced under control in Kaha research station and the other produced by farmers in their private farm in Kafr El-Zayat. The second experiment was accomplished in summer season (2013 and 2014) to compare the potato seeds produced from summer season (2012 and 2013) under control (Kaha Research Station; local) with imported seeds. Imported potato seeds of three varieties (Diamant, Spunta and Lady Rosetta) were cultured in the summer season of 2012 for production of improved potatoes seed. Potato seed tubers were stored in a refrigerator at a temperature of 4°C. Extracting the amount of tubers for comparison between them

and those potato seeds produced by Kafr El-Zaya farmers (KZ) in the fall season of 2012 and remaining amount were reserved for comparison with imported potato seeds in the summer season 2013. The planting dates were on January 15th 2012 and January 17th 2013 in the summer seasons for improved seed production. In the first experiment (fall seasons) the planting dates were October 13rd 2012 and October 6th 2013. The planting dates were January 17th 2013 and January 23rd 2014 for the second experiment.

The experimental sub plot area was 17.75 square meters consisting of 5 ridges five meters in length and 0.71 meter in width whereas; one row was left without planting as a guard ridge between sub plots. In addition, 35 m^3 farm yard manure plus 90 kg P_2O_5/feddan were mixed together and added during soil preparation. Potato plants were supplied with ammonium sulphate (20.6% N) as a source of nitrogen fertilizer was added at a rate of 150 kg N/fed. The total amount of N-fertilizer was divided into two equal portions added at 40 and 60 days after planting. Potassium fertilizer was added as potassium sulphate (48-52% K_2O) at a rate of 96 kg K_2O/feddan, half of the total potassium amount was applied before planting (during soil preparation), whereas the rest of potassium was added at complete plant emergence (40 days after planting). Three plants were selected randomly from each plot at 75 days after planting (DAP) to take the following data:

Plant growth measurements:

1- Number of leave/plant.
2- Number of tubers/plant.
3- Stem length (cm).
4- Number of main stems/plant.
5- Leaf area per plant (cm^2/Plant) was determined by cutting out 20 leaf discs from each plant using a cork borer and dried them in an oven at 75°C (until constant weight). The rest of the leaves were similarly dried. Based on the known dry weight of a known surface area of leaves, i.e., leaf discs, and the total weight of leaves, leaves surface area was determined.
6- Tubers fresh weight/plant (g).
7- Total dry weight/plant (g).
8- Dry matter percentage was determined in 100 grams of a random tuber by drying the tuber slices at 70°C for 72 hours according to the method of Dogras *et al*. [14].

Yield and its components:

1- Tuber yield (ton/fed.).
2- Tuber number/plant.
3- Average tuber weight (g).
4- Tubers yield/plant (g).
5- Dry matter percentage.
6- Specific gravity of potato tubers was determined using the method of [15]. The weight in air/weight in water method is one of the common methods of specific gravity determinations. Selected sample units are first weighed in air and then the same unit is re-weighed suspended in water. Specific gravity can then be calculated using the following formula:

Specific gravity = Weight in air / (Weight in air - Weight in water).

7- Starch content was determined using the method of A.O.A.C. [16].

The obtained data were subjected to analyses of variance by statistics 10 statistical software. Means were separated by L.S.D. testing at 5% level. The field inspection procedures were done four times according to the ministerial decree 1550 published in 1994, i.e., 30-35 DAP verification of the acreage of the seed farm and isolation distance, 50-60 DAP for verification of plant density and inspection of pathogens and pests, 70-80 DAP for insurance of rouging plants and 85 DAP field inspection by randomized counting.

Viruses detection:

The detection experiments were started at Dept. of Virus & Phytoplasma Res., Plant Pathol. Res. Institute, Agricultural Research Center, Egypt and continued at Dept. of Arid Land Agric., College of Agriculture & Food Sciences, King Faisal Uni., Saudi Arabia.

Source of samples:

Leaves of collected potato samples were subjected directly to ELISA tests and RT-PCR to detect potato viruses PLRV, PVY, PVX and AMV while pieces of tubers, each bearing one eye, were planted in pots in a greenhouse with an ambient temperature of 20 to 25ºC. At 10 DAP, the young leaves of the developed sprouts were taken to the virus detection, as the same as the leaves.

ELISA:

ELISA tests for the detection of potato viruses (PLRV, PVY, PVX and AMV) in the collected potato samples (leaves and tubers) were carried out following the method described by ELISA kits (Bioreba AG).

Primers for PLRV, PVY, PVX and AMV:

Specific primers for coat protein gene of PLRV (PLRV-CP), coat protein gene of PVY (PVY-CP) and coat protein gene of PVX (PVX-CP) was used for the CP genes according to Shalaby *et al.* [17]. While specific primers for coat protein gene of AMV (AMV-CP) was designed by Xu and Nie [7]. The forward primers and the complementary primers used for the detection of potato viruses are illustrated in Table 2. The increase in size (bp) in the table for each virus comparing to the actual size of coat protein for each virus is due to the extra nucleotides present in the forward and reverse primers.

Extraction of total RNA from plant tissues:

Total RNA was isolated from the infected potato plants using RNeasy® Plant Mini Kit obtained from QIAGEN as manufacturer's instruction.

Table 2: The sequences of the specific primers used to detect the coat protein genes of Potato viruses PLRV, PVY, PVX and AMV respectively.

Viruses	Primers' Name	Nucleotide Sequences	Size (bp)
PLRV	PLRVCPv*Eco*RI	5`-AATAGAATTCTAATGAGTACGGTCGTGGTTARAGG-3`	650
	PLRVCPc*Nco*I	5`-AAAACCATGGCTATYTGGGGTTYTGCARAGCTAC-3`	
PVY	PVYCPv*Bam*HI	5`-TCAAGGATCCGCAAATGACACAATTGATGCAGG-3`	825
	PVYCPc*Eco*RI	5`-AGAGAGAATTCATCACATGTTCTTGACTCC-3`	
PVX	PVXCPv*Eco*RI	5`-GATAGAATTCAGATGACTACACCAGCCAACACC-3`	750
	PVXCPc*Nco*I	5`-TACGCGTCGGTTCCATGGACGTAGTTATGGTGG-3`	
AMV	AMVF2	5`-ATCATGAGTTCTTCACAAAAGAA-3`	669
	AMVR2	5`-TCAATGACGATCAAGATCGTC-3`	

One step RT-PCR:

One step Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was done by "Platinum Quantitative RT-PCR Thermo Script One Step System" got from Invitrogen Company (USA) on the potato samples (leaves and tubers). Total RNAs were used as templates for one-tube RT-PCR amplification reactions. RT-PCR mixture prepared by mixing 12.5 µL of 2X ThermoScript Reaction Mix, 5 µL of total RNA, 1 µL of 10 µM of each primer (forward and reverse primers, Table 2), 0.5 µL of ThermoScriptTaq Enzyme Mix and the reaction was finalized to 25 µL with double distilled water. Reverse transcription reaction started with incubation at 50°C for 30 min, followed by denaturation at 95°C for 5 min. PCR amplification was performed by 35 cycles in a thermal cycler starting with denaturation at 95°C for 1 min, primer annealing at 57°C for 1 min, and extension at 72°C for 1 min with final extension at 72°C for 10 min. Five microliters aliquots of RT-PCR products were analyzed on 1% agarose gels in 0.5X TBE buffer (Fig.1).

RESULTS AND DISCUSSION

1. First experimental: Fall season plantation:

1.1. Viruses detection:

1.1.1- ELISA test:

In this experiment we compared the seeds of potato produced under control in Kaha Research Station (Kaha) with those produced by farmers in their private Farm Kafr El-Zayat (KZ) in the fall season. Potato plants, which have virus-like symptoms, were selected out of the total of 3000 potato plants represented the 3 potato varieties, i.e. Diamant, Spunta and Lady Rosetta equally as stand grown plants. Those selected plants were tested for viruses using ELISA. Field collected plants expressing symptoms of each variety were tested and the results shown in Table (3). From Table 3 we can conclude that, 5 samples were positive with PLRV (2 Diamant, 1 Lady Rosetta and 2 Spunta) from the potato seeds produced under control (Kaha Research Station) compared to 12 samples were positive with the same virus (4 Diamant, 5 Lady Rosetta and 3 Spunta) from potato seeds produced by farmers Kafr El-Zayat (KZ) in the fall season.

Table 3: Virus-infection incidence in potato fields of seed tubers produced under control (Kaha Research Station) with those seed tubers produced by farmers (Kafr El-Zayat; KZ) in the fall season.

Potato leafroll virus (PLRV)				
Varieties	Sources	Nº of samples	Nº of infected samples	% of infection
Diamant	Kaha	90	2	2.22
	KZ	86	4	4.65
Lady Rosetta	Kaha	94	1	1.06
	KZ	92	5	5.43
Spunta	Kaha	88	2	2.27
	KZ	84	3	3.57
Potato virus X (PVX)				
Diamant	Kaha	90	0	0
	KZ	86	3	3.48
Lady Rosetta	Kaha	94	0	0
	KZ	92	2	2.17
Spunta	Kaha	88	1	1.13
	KZ	84	4	4.76
Potato virus Y (PVY)				
Diamant	Kaha	90	1	1.11
	KZ	86	3	3.48
Lady Rosetta	Kaha	94	2	2.17
	KZ	92	4	4.34
Spunta	Kaha	88	1	1.13
	KZ	84	5	5.95
Alfalfa mosaic virus (AMV)				
Diamant	Kaha	90	1	1.11
	KZ	86	5	5.81
Lady Rosetta	Kaha	94	2	2.17
	KZ	92	4	4.34
Spunta	Kaha	88	2	2.27
	KZ	84	4	4.76

Also, we can conclude that, only one sample was positive with PVX (Spunta) from the seed tubers produced under control (Kaha Research Station) compared to 9 samples were positive with PVX (3 Diamant, 2 Lady Rosetta and 4 Spunta) from seed tubers produced by farmers (Kafr El-Zayat; KZ) in the fall season. Four samples were positive with PVY (1 Diamant, 2 Lady Rosetta and 1 Spunta) from the potato seeds produced under control (Kaha Research Station) compared to 12 samples were positive with the same virus (3 Diamant, 4 Lady Rosetta and 5 Spunta) from potato seeds produced by farmers (Kafr El-Zayat; KZ) in the fall season. Finally, 5 samples were positive with AMV (1 Diamant, 2 Lady Rosetta and 2 Spunta) from the potato seeds produced under control (Kaha Research Station) compared to 13 samples were positive with the same virus (5 Diamant, 4 Lady Rosetta and 4 Spunta) from potato seeds produced by farmers (Kafr El-Zayat; KZ) in the fall season.

1.1.2- RT-PCR test:

RT-PCR amplification of viral RNAs were carried out on the total RNAs isolated from infected plants using specific primers designed to amplify the coat protein genes. Figure (1) showed the electrophoresis analysis of RT-PCR products where single amplified fragments of 650 bp for PLRV, 825 bp for PVY, 750 bp for PVX and 669 bp for AMV potato samples (leaves and tubers) were obtained while no fragments were amplified from the RNAs extracted from healthy samples. We found that, all the positive samples in ELISA test were also positive in RT-PCR test and RT-PCR is more accurate and sensitive than ELISA test and could detect some samples infected with viruses and did not appear in ELISA test. The following figure (Fig. 1) was illustrated as an example of positive samples in RT-PCR test.

1.2. Plant growth measurements at 75 days after planting:

Data illustrated in Table 4 show the effect of the studied factors (cultivars, seed sources and their interactions) on emergence percentage, number of leaves/plant, number of tubers/plant, stem length and number of main stems per plant at 75 days after planting (DAP) in 2012 and 2013, fall seasons. Significant differences were observed between cultivars in emergence percentage, leaf number, tuber number and stem length. In this concern, the obtained results show clearly that, emergence percentage was significantly increased in Spunta cultivar in two seasons 2012 and 2013. Diamant cultivar gave the highest value for leaf number per plant, number of tuber/plant and number of main stems compared with other cultivars; while, Spunta cultivar recorded the highest stem length for both fall seasons at 75 DAP.

The same results indicated that seed source caused significant effects on emergence percentage in two seasons, as well as, leaf number and tubers number per plant in the first season and stem length in the second season. Improved seeds which produced under Kaha Research Station (Kaha) significantly increased emergence

percentage and leaves number per plant in both seasons and gave the taller plants in the second season. Furthermore, number of tubers/plant was increased in improved seeds which produced under Kaha Research Station. On the other hand, number of main stems was not significantly affected by source of seed potato at 75 DAP in the two growing seasons. Regarding the effect of the interactions between cultivars and seed source, the obtained data in Table 4 showed that spunta seeds which produced under (Kaha Research Station) gave the highest values of emergence percent and stem length compared to the other interactions. Whereas, improved potato tuber seeds of cv. Diamant significantly increased in number of leaves and number of tubers per plant in two seasons, however, number of main stems significantly increased in first season 2012.

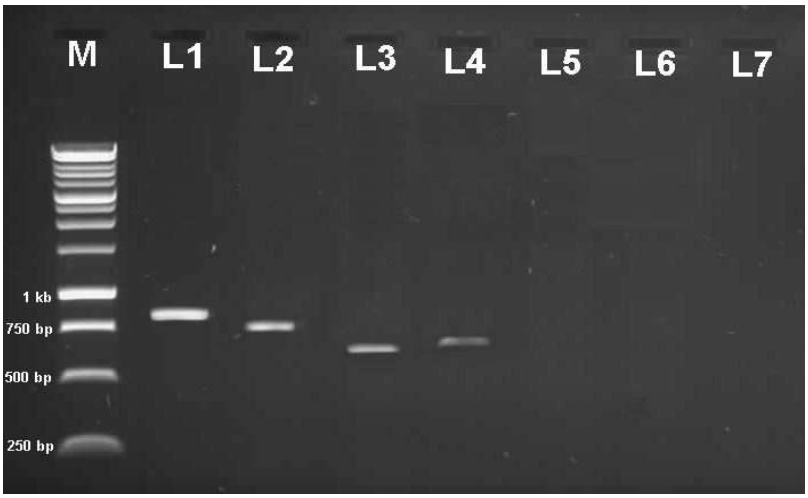

Fig. 1: RT- PCR products of potato viruses using primers for coat protein genes. M: 1 kb DNA Ladder; L1: sample infected with PVY (825 bp); L2: sample infected with PVX (750 bp); L3: sample infected with PLRV (650 bp); L4: sample infected with AMV (669 bp) while L5, L6 and L7: healthy samples.

Table 4: Effect of seed sources on emergence percent and vegetative growth of three varieties in fall plantation 75 days after planting.

Varieties	Seed sources	Emergence percent		Number of leaves/plant		Number of tubers/plant		Stem length (cm)		Number of main stems	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Diamant		88.50	91.33	58.17	53.67	7.67	8.00	40.33	47.67	3.67	3.33
Spunta		93.00	91.83	43.00	43.00	6.50	7.33	47.34	48.17	3.17	2.67
Lady Rosetta		86.00	85.50	32.83	46.17	5.33	6.67	22.00	29.33	2.17	2.50
L.S.D. at 5% significant level		1.31	1.10	6.14	4.36	2.07	1.67	5.77	6.62	N.S	N.S
	Kaha	90.89	91.33	50.11	50.11	7.55	7.55	37.44	44.33	3.11	3.00
	KZ	87.44	87.78	32.56	45.11	5.44	7.11	35.67	39.11	2.89	2.67
L.S.D. at 5% significant level		0.98	0.86	8.01	N.S	1.36	N.S	N.S	3.72	N.S	N.S
Diamant	Kaha	90.67	94.33	68.33	59.33	9.00	8.33	39.33	50.00	4.00	3.67
	KZ	86.33	88.33	48.00	48.00	6.33	7.67	41.33	45.33	3.33	3.00
Spunta	Kaha	94.00	93.33	48.00	45.67	7.33	8.33	44.00	52.67	2.67	2.67
	KZ	92.00	90.33	38.00	40.33	5.67	6.33	50.67	43.67	3.67	2.67
Lady Rosetta	Kaha	88.00	86.33	34.00	45.33	6.33	6.00	29.00	30.33	2.67	2.67
	KZ	84.00	84.67	11.67	47.00	4.33	7.33	15.00	28.33	1.67	2.33
L.S.D. at 5% significant level		1.70	1.49	13.88	16.72	2.35	2.54	9.91	6.44	1.49	N.S

Data presented in Table 5 show clearly that, Spunta variety significantly increased in leaf area, tuber fresh weight and total dry weight per plant at 75 day after planting, meanwhile Lady Rosetta produced the lower values in this respect in both seasons. Conversely, Lady Rosetta gave the highest dry matter percentage of tubers in both seasons compared to Diamant and Spunta varieties. These differences among cultivars are attributed to genetically factors. The improved seeds led to a significant increase in leaf area, total dry weight and tuber fresh weight per plant during both seasons of this study, although potato seeds which produced by farmers (Kafr El-Zayat) gave a significant increase in number of main stems in the first season only.

Furthermore, the interactions between varieties and seed sources were observed also in Table 5. The highest tubers fresh weight and total dry weight per plant was found in Spunta when improved seeds were used. On the other hand, dry matter of tubers was decreased by growing the improved seed production in Kaha Research Station from cv. Spunta compared with potato seeds produced by farmers (Kafr El-Zayat; KZ). The reduction in the percentage of emergence and vegetative growth from plants produced by growing potato seeds from KZ farm may be due to increase disease infections compared with the other source of seeds from Kaha Research Station (Table 3). These results was in agreement with Petrovic *et al.* [18] who found that virus infection PVY

decreased both fresh and dry weights of infected shoots and roots compared to the healthy shoots and roots. In addition, Fargette *et al.* [19], Hooks *et al*. [20] and Kassim *et al*. [21] stated that there is a significant difference in the size of leaf area between healthy and seed born viral infected plants. They showed that certain aspects of plant growth may be affected by virus infection. Li *et al.* [11] reported that PLRV and PVY had significant effects on vegetative growth, while the chlorophyll levels were the highest in healthy segments and the lowest in PLRV and PVY co-infected segments.

Table 5: Effect of seed sources on vegetative growth of three potato varieties in fall plantation, 75 days after planting.

Varieties	Seed sources	Leaf area (cm^2)/plant		Total DW/plant (g)		Tubers FW /plant (g)		Dry matter (%)	
		2012	2013	2012	2013	2012	2013	2012	2013
Diamant		2907.60	2762.60	135.79	123.10	615.50	511.32	18.07	19.55
Spunta		4625.80	4192.80	136.21	130.58	658.67	547.67	14.81	15.03
Lady Rosetta		2026.80	2347.00	107.63	109.40	444.67	471.50	19.53	20.09
L.S.D. at 5% significant level		106.41	255.81	2.80	8.56	18.71	16.37	0.46	2.06
	Kaha	3481.20	3382.50	132.66	129.23	583.11	515.85	17.24	18.00
	KZ	2892.30	2819.10	120.43	112.82	562.78	504.48	17.69	18.45
L.S.D. at 5% significant level		62.46	128.17	7.41	5.92	5.38	5.28	0.37	N.S
Diamant	Kaha	3026.50	2911.10	140.84	125.26	624.67	512.02	17.77	19.54
	KZ	2788.70	2614.00	130.73	120.93	606.33	510.62	18.37	19.56
Spunta	Kaha	5239.80	4761.00	146.35	148.92	678.00	553.86	14.16	14.12
	KZ	4011.80	3624.60	126.08	112.24	639.33	541.48	15.46	15.94
Lady Rosetta	Kaha	2177.20	2475.20	110.79	113.51	446.67	481.66	19.81	20.34
	KZ	1876.30	2218.80	104.47	105.29	442.67	461.33	19.25	19.84
L.S.D. at 5% significant level		108.19	222.00	12.83	10.26	9.33	9.15	0.64	1.74

1.3. Yield and its components at harvest time:

Data presented in Table 6 showed that there were significant differences in total yield per plant and feddan, average tuber weight and number of tuber per plant among the tested cultivars during the two seasons of study. In this connection, Spunta variety gave the heaviest significant tubers weight per plant, per feddan and average tuber weight compared to Diamant and Lady Rosetta varieties. On the contrary, the Diamant variety recorded the highest number of tubers per plant in both seasons. This difference in plant productivity and total yield of the varieties is probably due to the difference in the genotype. Regarding the effect of seed sources, it was shown from the results that average of tuber weight was not affected by source of potato seeds in the two seasons at harvest time. Whereas, tubers weight per plant (g), number of tubers per plant, yield per plant, total yield per plot and feddan were high significant from improved potato seed in Kaha farm in the two seasons. In this regard, Mugoro [12] indicated that tubers of the local cultivar Batte of farmers source was generally low in its yield and quality, whereas tubers of the released Bubu cultivar obtained from Kersa Local Seed Business Project and Haramaya University was superior in growth, yield, and quality to Batte.

Table 6: Effect of varieties and seed sources on yield and its components of potato plants at harvest time in fall seasons.

Varieties	Seed sources	Tubers weight per plant (g)		Average tuber weight (g)		Tuber Number/plant		Tuber yield (Kg/ plot)		Tuber yield (ton /fed.)	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Diamant		807.60	798.40	118.42	111.35	6.82	7.17	63.85	58.01	14.389	13.073
Spunta		1012.70	964.61	128.52	128.61	7.88	7.50	78.55	58.35	17.701	13.149
Lady Rosetta		722.10	701.69	88.28	75.21	8.18	9.33	45.84	42.84	10.330	9.654
L.S.D. at 5% significant level		13.44	2.84	7.24	21.55	0.42	1.46	5.25	2.57	1.18	0.58
	Kaha	891.78	848.12	116.12	101.82	7.68	8.33	67.70	56.57	15.256	12.748
	KZ	803.17	795.00	105.96	103.65	7.58	7.67	57.78	49.57	13.021	11.171
L.S.D. at 5% significant level		12.34	6.823	N.S	N.S	0.79	0.47	3.08	2.56	0.69	0.58
Diamant	Kaha	834.10	856.39	117.98	107.05	7.07	8.00	66.85	62.01	15.065	13.974
	KZ	781.00	740.40	118.87	116.97	6.57	6.33	60.85	54.01	13.713	12.171
Spunta	Kaha	1048.60	962.54	145.03	131.32	7.23	7.33	83.75	62.35	18.873	14.051
	KZ	976.80	966.68	114.51	126.03	8.53	7.67	73.34	54.35	16.527	12.248
Lady Rosetta	Kaha	792.50	725.44	90.78	75.02	8.73	9.67	52.50	45.34	11.831	10.217
	KZ	651.70	677.93	85.41	75.33	7.63	9.00	39.17	40.34	8.827	9.091
L.S.D. at 5% significant level		21.38	11.82	20.98	11.08	1.37	0.82	5.33	4.43	1.20	1.00

Data in Table 6 presented the effect of the interactions between varieties and seed sources on total yield per plant, per feddan, average tuber weight and number of tuber per plant. The highest values of total yield per plant, per feddan and average tuber weight were obtained from the improved potato seeds of Spunta during both seasons of this study.

On the contrary, the highest number of tubers per plant was produced from cv. Lady Rosetta regardless the source of seeds in both growing seasons. Lady Rosetta produced the topmost dry matter content, specific gravity and starch percentage followed by Diamant then Spunta (Table 7). Also, improved seeds (Kaha) enhanced tuber

dry matter percentage. The improved seeds (Kaha) produced greater quantity of large tubers (over 55 mm). On the other hand, conventionally used seeds (KZ) produced larger portion of medium size tubers (35-55 mm). Decreasing yield and its components at harvest time of seeds produced by farmers (Kafr El-Zayat) may be due to decline in percent emergence or weakness of vegetative growth (Table 4 and 5) or the increase in infection with virus strains displayed in the Table (3). The infection caused potato yield reduction reaches 50-90% [22], 33-77% [23] and 10-100% [24]. Yield reduction of an infected plant with a virus was greater when plants were infected from the vegetative propagation materials than later by the vector [19].

Virus infection has negative effects on plants by limiting their growth [25]. As well as, Ahmed *et al.* [10] reported that productivity of infected potato cultivar Spunta with virus strains was conducted in the farm of Benha, Kalyoubia decreased by 22.6% to 50.5% according to the strain as compared with control. Li *et al.* [11] demonstrated that significantly lower contents of chlorophyll A, chlorophyll B and total chlorophyll were found in virus infected shoots than in healthy ones.

Table 7: Effect of varieties and seed sources on dry matter, specific gravity, starch content and tuber size in fall plantation of potato at harvest time.

Varieties	Seed sources	Dry matter (%)		Specific gravity		Starch (%)		Tuber yield					
								Over 55 mm		35-55 mm		0-35 mm	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Diamant		21.03	20.89	1.08	1.08	14.68	14.62	65.75	65.31	31.29	31.06	2.96	3.63
Spunta		18.42	18.73	1.07	1.07	12.41	12.69	65.47	69.49	32.40	28.86	2.13	1.65
Lady Rosetta		22.86	23.19	1.09	1.09	16.37	16.67	57.30	59.69	41.36	37.15	1.34	3.17
	Kaha	20.84	20.95	1.08	1.08	14.41	14.67	66.78	65.77	36.38	31.64	2.45	2.59
	KZ	20.70	20.92	1.08	1.08	14.57	14.65	58.90	63.98	39.16	33.77	1.95	2.25
Diamant	Kaha	21.04	20.87	1.08	1.08	14.63	14.60	68.18	66.14	28.97	30.54	2.85	3.32
	KZ	21.02	20.90	1.08	1.08	14.73	14.63	63.32	64.48	33.61	31.58	3.07	3.94
Spunta	Kaha	18.61	18.78	1.07	1.07	12.24	12.74	69.64	70.14	28.44	28.14	1.92	1.72
	KZ	18.22	18.67	1.07	1.07	12.59	12.64	61.29	68.84	36.37	29.58	2.34	1.58
Lady Rosetta	Kaha	22.86	23.20	1.09	1.09	16.37	16.68	62.52	60.75	35.22	34.15	2.26	5.10
	KZ	22.86	23.19	1.09	1.09	16.37	16.66	52.07	58.62	47.50	40.14	0.43	1.24

2. Second experiment: Summer season (spring plantation):

2.1. Virus detection:

2.1.1- ELISA test:

In this experiment a comparison of potato seeds produced from the plantation of potatoes of the summer season under control (Kaha Research Station, local) with imported seeds in the next summer season. Imported potato seeds of three varieties (Diamant, Spunta and Lady Rosetta) as shown in Table 8. It is obvious from Table 8 that the potato seeds produced from the plantation of potatoes in summer season under control (Kaha Research Station, local) showed increase in the percentage of viral infection when using full seed and the percentage increased more by using the cut seeds in plantation compared to the imported seeds.

2.1.2- RT-PCR test:

The RT-PCR test was done as described before and the results were the same results obtained by ELISA test with little differences which confirm that the RT-PCR is more accurate and sensitive.

2.2. Yield components:

Recorded data in Table 9 illustrate that no significant effects were obtained for the studied cultivars on average tubers number per plant in the first season, whereas, cv. Spunta gave the lowest significant average tubers number per plant compared with Diamant and Lady Rosetta cultivars, in the second season. On the other hand, average tuber fresh weight was superior in Spunta cultivar comparing with Diamant and Lady Rosetta cultivars in the two seasons. Regarding the impact of different potato seed sources on tubers number per plant and average weight of tuber, the results in Table 9 emphasize that complete potato seeds give the highest significant value of number of tubers per plant compared with divided potato seeds in the two seasons. On the other hand, divided imported potato seeds caused a significant increase in average tuber weight in the two summer seasons. In agreement with these results, Hossain *et al.* [26] stated that whole tubers produced higher number of stems per hill, number of tubers per hill and per hectare than cut tubers.

Also, Tawfik [4] mentioned that whole tubers produced higher number of tubers per plant than their respective cut ones. Similarly, Nassar *et al.* [25], El-Baz *et al.* [7] pointed out that greater yield was obtained from whole than cut seed tubers. As for the effect of the interaction between cultivars and potato seed sources on the number of tubers per plant and the average weight of tubers data in Table 9 show clearly that average tuber number per plant was significantly increased with using undivided potato seeds from the three cultivars (Diamant, Lady Rosetta and Spunta) compared to divided potato seeds from the same cultivars in 2012 and 2013 seasons. While, the maximum increment of average tuber weight was obtained by dividing the imported seeds from cv. Spunta compared to other interactions in the two seasons.

Table 8: Virus-infection incidence in potato fields of potato seeds produced under control (Kaha Research Station) with imported potato seeds in the summer season.

		No. of samples	No. of infected samples	% of infection
Varieties	Seed sources	Potato leafroll virus (PLRV)		
Diamant	Imported, Full	87	3	3.45
	Imported, Cut	84	5	5.95
	Local, Full	88	4	4.54
	Local , Cut	86	7	8.14
Lady Rosetta	Imported, Full	91	2	2.91
	Imported, Cut	87	4	4.95
	Local, Full	90	2	2.22
	Local , Cut	89	3	3.37
Spunta	Imported, Full	89	2	2.25
	Imported, Cut	84	3	3.57
	Local, Full	88	3	3.41
	Local , Cut	86	6	6.98
		Potato virus X (PVX)		
Diamant	Imported, Full	87	3	3.45
	Imported, Cut	84	7	8.33
	Local, Full	88	3	3.41
	Local , Cut	86	5	5.81
Lady Rosetta	Imported, Full	91	2	2.19
	Imported, Cut	87	4	4.59
	Local, Full	90	2	2.22
	Local , Cut	89	5	5.62
Spunta	Imported, Full	89	2	2.25
	Imported, Cut	84	5	5.59
	Local, Full	88	3	3.41
	Local , Cut	86	5	5.81
		Potato virus Y (PVY)		
Diamant	Imported, Full	87	2	2.29
	Imported, Cut	84	6	7.14
	Local, Full	88	3	3.41
	Local , Cut	86	7	8.14
Lady Rosetta	Imported, Full	91	3	3.29
	Imported, Cut	87	5	5.47
	Local, Full	90	2	2.22
	Local , Cut	89	4	4.49
Spunta	Imported, Full	89	3	3.37
	Imported, Cut	84	5	5.95
	Local, Full	88	3	3.41
	Local , Cut	86	4	4.65
		Alfalfa mosaic virus (AMV)		
Diamant	Imported, Full	87	3	3.45
	Imported, Cut	84	5	5.95
	Local, Full	88	2	2.27
	Local , Cut	86	4	4.65
Lady Rosetta	Imported, Full	91	2	2.19
	Imported, Cut	87	4	4.95
	Local, Full	90	3	3.33
	Local , Cut	89	6	6.74
Spunta	Imported, Full	89	2	2.25
	Imported, Cut	84	4	4.76
	Local, Full	88	3	3.41
	Local , Cut	86	5	5.81

2.3. Total yield:

Data presented in Table 9 illustrated the differences between the three tested cultivars in yield characters were affected by seed source (imported complete seeds or divided and local improved seeds). There was a significant difference in total yield per plant and feddan among the three used cultivars in the two tested seasons. In this concern, Spunta cultivar achieved the highest value for total yield per plant and feddan comparing with the two other cultivars in the two growing summer seasons. The lowest total tuber yield per plant and feddan was obtained from Lady Rosetta cultivar. These differences among cultivars are attributed to the differences in genotype. The same data presented in Table 9 show the influence of different sources of tubers seed on total yield per plant and feddan. In this concern, imported potato seed tubers which were planted as complete tubers (not divided) yielded the highest significant total tuber yield per plant and feddan compared to other seed

sources (imported divided seeds and local improved seeds). As well as, using whole local tubers seed increased yield per plant and feddan compared to all divided tubers seed. On the other hand, divided local seeds gave the lowest value of total yield per plant and feddan compared to complete or divided imported seeds and complete local seeds in the two growing seasons. Generally, the results showed that the imported tubers seed gave the highest yield compared to the local seed. These results are in line with the previous findings of Van der Zaag [29], Sikka *et al.* [30] and Asamenew and Bahru [31] who reported that the performance of imported seed was better than the local check. Also Getachew and Mela [32] reported that the productivity of the imported seeds was higher ranging between 27-46 t/ha compared to the local seed which produced 16-18 t/ha. Mohamed [33] reported similar results. Concerning the interaction effect between the different varieties and tubers seed source, the same aforementioned data show that cv. Spunta whole imported seeds recorded a significant increment on total yield per plant and feddan compared with the other interactions. While, Lady Rosetta from local potato divided seeds gave the lowest total yield per plant and feddan in the two seasons. The reduction in yield of divided tuber seeds owing little number of eyes on a piece of seeds. Thornton and Conlon [34] and Nolte *et al.* [35] reported that whole tubers seed had the highest number of stems compared to cut tuber seeds. The reduction in yield of divided seeds may be attributed to increase the virus-infection which was shown in Table 8. Franc and Banttari [36] reported that PVS infectious was recovered from various surfaces contacted by tubers or potato plants during seed cutting The infection caused yield reduction of potato reach 50-90% [22], 33-77% [23] and 10- 100% [24]. In addition, Hossain *et al.* [26] found that the highest germination index was found when large size whole tubers were used compared to cut seeds.

In this connection, Tawfik (4) indicated that in spring plantation total yield of local Alpha seed (stored at 4°C for 6-7 months sprouted two weeks before planting) was comparable to their respective imported ones (Class A) and cut seeds resulted in significantly lower yield than whole tubers for both imported and local seeds. Also, Sharara [37] reported that locally produced cold stored potato seeds from preceding spring crop yielded significantly similar to the corresponding imported seeds. Ali [38] reported that repeated use of seed tubers lead to degeneration of seed tubers which result in reduction in the yield. He also added that use of potato tubers leads to high degeneration rate, due to the viral diseases, also encounters hazards of virus disease multiplication in the long run specially in the absence of rouging and proper certification. The productivity was very low because farmers grow the local varieties which are low yielding and are susceptible to late blight [32]. For instance, McKeown [39] reported that whole seed performed better than cut seed when cv. Superior was planted. In addition, Platt [40] and Nolte *et al.* [35] indicated that the use of whole seed resulted in significantly higher total yield than divided seeds and treated seeds. Mohamed [33] reported that the imported seeds produced higher number of stems per hill, plant height, and number of tubers per plant, number of leaves per plant, fresh and dry weight of tubers per plant, fresh weight of vine/plant, leaf area index and yield compared to the local seeds in the two seasons.

Table 9: Effect of varieties and seed sources on number of tubers per plant, average weight of tuber and yield / plant and feddan in the summer season.

Varieties	Seed sources	Average tuber number per plant		Average tuber weight (g)		Average tuber yield per plant (g)		Total yield per feddan (ton)	
		2013	2014	2013	2014	2013	2014	2013	2014
Diamant		8.78	9.53	58.91	56.27	499.163	522.450	9.835	10.715
Lady Rosetta		8.00	8.45	40.49	45.79	337.505	395.875	9.102	10.370
Spunta		7.33	6.88	72.93	103.03	539.193	704.330	11.726	13.112
L.S.D. at 5% significant level		N.S	N.S	1.13	16.15	16.97	49.11	0.365	0.606
	Imported full	9.11	9.09	62.96	69.52	563.493	608.077	11.33	12.626
	Imported cut	7.44	7.92	65.75	73.64	393.653	534.38	10.355	11.409
	Local full	7.78	7.92	51.95	71.47	520.45	577.533	10.746	11.953
	Local cut	7.33	7.48	48.38	59.85	356.883	443.55	8.453	9.608
L.S.D. at 5% significant level		0.57	0.57	0.53	9.68	9.04	42.73	0.491	0.524
Diamant	Imported full	9.67	10.19	60.08	56.14	576.190	557.840	11.086	11.634
	Imported cut	7.67	8.64	61.71	58.08	466.670	500.000	9.333	10.192
	Local full	9.00	9.77	54.95	54.61	494.190	533.500	9.937	11.141
	Local cut	8.00	8.38	57.60	59.60	459.600	498.460	8.982	9.893
Lady Rosetta	Imported full	9.33	10.10	41.90	45.48	390.480	459.810	9.841	11.670
	Imported cut	7.33	7.86	39.97	46.37	295.240	361.310	8.921	9.894
	Local full	9.00	9.70	41.17	43.95	371.920	426.190	9.540	10.474
	Local cut	7.33	7.38	39.61	45.54	292.380	336.190	8.106	9.441
Spunta	Imported full	8.33	6.98	86.90	106.94	723.810	806.580	13.063	14.573
	Imported cut	7.33	7.25	95.58	116.48	419.050	741.830	12.810	14.142
	Local full	7.00	6.60	61.28	114.28	695.240	772.910	12.762	14.243
	Local cut	6.67	6.67	47.94	74.43	318.670	496.000	8.270	9.490
L.S.D. at 5% significant level		0.99	0.99	0.91	16.76	15.67	90.80	74.01	0.850

Conclusion:

The production of potato seeds in the summer season under rouging and good inspection for diseases can improve local production moreover it could replace or reduce the large quantity of potato seeds imported by Egypt yearly for next summer season. The obtained results suggest using the imported seeds for two years or importing the half quantity imported each year with the same current cultivated area. At the same time this method could reduce the costs of production per area unit through the use of improved local seeds in summer and fall seasons by lessening the quantity imported annually of potato seeds.

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