EVALUATION OF GENETICALLY MODIFIED POTATO LINES RESISTANT TO POTATO VIRUS Y

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Potatoes are the second most important vegetable crop in Egypt in terms of crop value and total production. Potato production in Egypt is limited by both biotic and abiotic factors. Viral diseases such as Potato virus Y is major factors limiting potato production in Egypt accounting for substantial losses in production. Chemical insecticides are being used to reduce the negative impact of the insect transmission of the virus. Even with the application of chemical insecticides, losses due to viral transfer by insect are estimated to be 30%.

Potato is subject to many pathogenic agents such as viruses, bacteria and fungi. One of the most devastative diseases that causes great loss considerably affect potato exportation is the virus. One of these diseases is potato virus y (PVY), a type member of potyvirus (Hollings and Brunt, 1981). Potyvirus group is one of the most important virus groups allover the world. It represents 90% of the known RNA viruses with single positive strand. Members of this group have several features in common. They have filaments particles, a narrow host range and they transmitted mechanically or by the aphids in non-persistent manner. PVY is transmitted by *Myzus persicae* and reduce the crop by 80% depending on the viral strain and the presence of other pathogens. The viral spread in the potato fields is not easy to control due the vector activity and the way of transmission (Eastop, 1977 & 1983; Murant *et al.,* 1988). Different breeding programs were applied to potato in order to introduce resistance against PVY with a considerable success. However, these programs are time as well as money consuming and laying under license from the breeding companies. More recent, the application of the gene transfer to potato plants using genes isolated from the pathogens to introduce resistance against these pathogens was applied after the establishment of the regeneration and transformation techniques in potato (Tavazza *et al* 1988, lshida *et al.,* 1989; Trinca *et al.,* 1991).

Genetically modified plant expressing resistant to potato virus Y has been used as an alternative methods to conventional breeding programs (Robaglia *et al.,* 1989; De-Block, 1988; Tavazza *et al*., 1988; Wenz1er *et al*., 1989; lmai *et al.,* 1993).

On the other hand, field performance was assessed in 13 transgenic potato lines from three cultivars express-

ing a selectable marker gene conferring kanamycin resistance (Conner *et al.*, 1994). All transgenic lines developed unexpected changes in the phenotypic appearance of shoots, and/or poor tuber yield. Each independently selected transgenic line showed distinctly different changes in phenotypic appearance or yield performance. The observed changes were very uniform within each clonally propagated transgenic line, and were consistent in appearance over two seasons in the field. They attributed these changes to either epigenetic or genetic events occurring during the tissue culture phase of transformation.

The present work described micropropagation of transgenic potato lines through *in vitro* nodal cuttings techniques, adaptation and acclimatization of the transgenic potato lines under Bio-containment greenhouse and then evaluation under field conditions at Assiut region with the consideration of Egyptian bio-safety regulations.

MATERIALS AND METHOD

In vitro micropropagation of transgenic potato

Transgenic potato lines used in this study were developed at AGERI under the National Genetic Engineering Program (Metry *et al*., 2000).

Transgenic potato lines (PVY 1, 2, 4, 5, 15, 23, 24, 25 and 31) were micropropagated *in vitro* using nodal cutting technique as described by Roca *et al.* (1978). Nodal cuttings were routinely sub-cultured on a fresh medium every 3-4 weeks. MS salts medium (Murashige and Skoog, 1962) was used as a basal medium supplemented with 3% sucrose, 0.4 mg/l thiamin-HCl, 2 mg/l calcium pantothenate, 1 ml of silver thiosufate solution STS (0.1 M sodium thiosulfate and 0.1 M silver nitrate, the ratio between silver and thiosalfate was 1:4), 100 mg/l myo-insoitol and 1mg/l gibberellic acid (GA_3). The pH of the medium was adjusted to 5.6-5.7 by 1M NaOH and HCl. Two g/l Phytagel was added and the medium was cooked in a microwave for 6 min. The medium was poured into baby food jars and covered with transparent polypropylene film provided by a filter disc (6.0 mm in diameter) with pore size of 0.2 μm. Then the medium was autoclaved at 121°C (15 psi) for 20 min.

Adaptation of transgenic potato lines

Adaptation and acclimatization of transgenic potato (*Solanum tuberosum* L.) lines and control were carried out in the Bio-containment greenhouse facilities at AGERI according to Metry *et al.* (2003).

Observations on morphological characters of plants such as survival and plant height were recorded after 30 and 60 days from adaptation. The average weight of 10 tubers, diameter and number of tubers were calculated after harvesting the tubers and data was statistically analyzed.

Challenging with the virus and ELISA detection

Putative transgenic plants were inoculated using infected sap obtained from tobacco plants infected with strain of potato virus Y. Incidence and severity of PVY symptoms were recorded by visual inspection of inoculated transgenic plants over a period of time during growing season and compared to those of non-transgenic plants.

The indirect enzyme linked immunosorbant assay (Indirect ELISA) was used for virus detection as mentioned by Regenmortel and Burkard (1980), Koenig (1981) and Banttari and Goodwin (1985).

Dot-blot immunoassay (Dot-ELISA)

This method was used for virus detection according to Regenmortel and Burkard (1980) Koenig (1981) and Banttari and Goodwin (1985). Nitrocellulose membranes (NCM), 0.45 µm poer size were immersed in PBS- tween 20 (PBST) 0.1 % (v/v) and being assembled on to a Bio-Dot SF Micro filtration Apparatus. Nine transgenic and non transgenic potato lines were grinding with ratio 1 g /10 ml coating buffer containing 1% (BSA), pH 9.6. 100 µl / well of double fold dilution's of transgenic and non-transgenic potato plant samples were applied while the micro filtration was attached to vacuum pump. The membranes were prepared for detection using NPT and BCIP.

Field evaluation of transgenic potato lines

AGERI in collaboration with Assiut University supported by MUCIA project (USAID Grant) tested nine transgenic potato lines resistant to PVY in field trials at Assiut University after obtaining clearance from Egyptian National Biosafety Committee (NBC). The nine transgenic (~ 1-2 cm diameters) lines derived from mintubers stock Desiree cultivar were used. Potato mintubers obtained from Bio-containment greenhouse at AGERI (harvested 20-6-2005) were planted in a randomized complete block design with four replications. Commercial non-transgenic Lady Rosetta potatoes were cultivated as control in the borders. Additionally, Spunta 6A-3 line expressed both resistance for PVY and Bt was also used in this experiment. The infection was carried out using the mechanical inoculation with potato virus Y strain after one month from plantation. Germination and vegetative growth traits (plant height and numbers of stems and leaves per plant) were periodically scored. Viral infection was monitored visually and by ELISA test. Yield and yield components (tubers number and weight per plant and tubers grade) were also determined.

RESULTS AND DISCUSSION

In vitro propagation of transgenic potato lines

Nine transgenic potato lines resistant to potato virus Y were success-

fully micropropagated *in vitro* using nodal cuttings technique on MS salts medium supplemented with gibberellic acid (GA_3). Nodal cuttings were sub-cultured on a fresh medium after three weeks. The appearance of *in vitro* plantlets was normal.

Adaptation of transgenic potatoes

Adaptation and acclimatization of transgenic potato (Desiree cultivar) lines as well as control (non-transgenic line) were carried out in the Bio-containment greenhouse facilities at AGERI. The plantlets were grown in plastic pots (25 cm in diameter) at 25±2°C. The percentage of survival was 74 to 100% (Table 1).

In general, results are in agreement with findings of Heszky *et al.* (1983) and Tao *et al.* (1987).

Thirty days from culturing, the height of transgenic and non-transgenic plantlets was recorded. The average height of control, PVY 4 and PVY 5 lines were higher than the other lines. After 60 days, control, PVY 2 and PVY 4 were higher than the other lines (Table 2). These results may due to culture conditions rather than effect of viral coat protein gene.

After 130 days from culturing of plantlets, minitubers were harvested. The number, weight, and diameter of tubers per plant were recorded and data were statistically analyzed (Table 3). Significant increase in weight of tubers was recorded for three lines i.e. PVY 4, PVY 23 and PVY 31. In PVY 23 and PVY 31 the diameter of tubers was significantly higher than other lines. Whereas, PVY 2, PVY 23 and PVY 31 the number of tubers showed highly significant values than the other lines.

Evaluation of transgenic potato lines under greenhouse conditions

Adaptation and acclimatization of nine transgenic potato lines (Desiree cultivar) in addition to control (non-transgenic) line were carried out at AGERI bio-containment greenhouse facilities. Plantlets were challenged with strain of potato virus y for resistance evaluation of the lines under containment conditions.

Leaf samples were collected 15 days after challenged with strain of potato virus Y to detect the resistant lines (Table 4) using ELISA test (serological reaction). Dot blot test was carried out on the same samples to confirm ELISA results (Fig. 1).

Highly resistant lines for PVY infection were 23, 4, 5, 31 and 15 according to ELISA and dot blot tests. The resistance was expressed as low, moderate and high degree relatively according to ELISA reading values.

Minitubers were harvested, after 130 days from culturing of plantlets. The weight, volume and number of tubers per plant were recorded. This data was statistically analyzed using the analysis of

variance as outlined by Gomez and Gomez, (1984) using MSTATC program. The difference between means was compared using Duncan multiple test (Duncan, 1955).

In lines PVY 23, PVY 4, PVY 5 and PVY 31, weight and volume of tubers were significantly higher than other lines. Number of tubers was no-significant between transgenic and non-transgenic potato lines (Table 5).

Field evaluation of transgenic lines at Assiut University

Evaluation of transgenic lines under field conditions at Assiut included a) incidence of PVY symptoms as well as ELISA diagnosis, b) vegetative growth evaluation and c) yield of planted minitubers.

Incidence of PVY

Visual observation of viral infection showed some relatively high PVY incidence in lines 2 & 24; such observations are confirmed after ELISA diagnosis. However, PVY 23 line expressed the highest resistance to PVY (Fig 2 & Table 6) followed by lines 4, 5, 15, SP 6A-3 and 31. Therefore we have to depend on serological tests and not only on the symptoms.

Vegetative growth characteristics under field conditions at Assiut

Figures (3a, b and c) illustrate differences among transgenic lines after planting during Fall 2005 plantation at Assiut environment. Data indicated superiority of some lines in no. of stems per plant (lines 2, 4, 23 and 31), no. of leaves per plant (lines 2, 5, 23 and 31) and plant height (lines SP 6A-3, 2, 15 and 23). Such findings confirm visual and ELISA data on these lines where lines SP 6A-3, 4, 5, 15, 23 and 31 exhibited resistance to PVY and therefore had the best vegetative growth characteristics.

Yield and yield components of planted transgenic minitubers

Figure (4a and b) demonstrates yield of transgenic plants grown in Assiut**.** Yield study expressed as tubers weight and/or number per plant. Data confirmed the high performance of resistant transgenic lines from the standpoint of yield of plants grown from minitubers of transgenic lines, Transgenic lines SP 6A-3, 2, 4, 5, 24, 25 and 31were found to have the highest yield when expressed as number of tubers per plant and lines SP 6A-3, 4, 5, 23 and 31 had the highest yield when expressed as weight of produced tubers per plant.

Transgenic lines produced by AGERI in co-operation with Assiut University, and evaluated at greenhouse and under field environment of Upper Egypt proved high resistance to PVY and were highly productive from the standpoint of horticultural aspects (growth and yield). Meanwhile, ELISA evaluation confirmed the high resistance to PVY infection by transgenic lines 23, 4, 5, 31 and Sp-6a-3. Further evaluation and tests should be cared out according to Egyptian

Biosafety regulation in order to release the transgenic lines for commercialization.

SUMMARY

Nine putative transformed potato lines resistant to potato virus Y (PVY) were successfully micropropagated *in vitro* using nodal cutting technique on MS salts medium. Adaptation and acclimatization of the transgenic potato lines were carried out in the Bio-containment greenhouse facilities at AGERI. The percentage of survival was 74 to 100%. The plantlets were challenged artificially with a strain of potato virus Y. Five highly resistance lines to PVY infection were determined according to ELISA and Dot-blot analysis. Evaluation of transgenic and non-transgenic potato lines under field experiment was conducted at Assiut University. Such evaluation included vegetative growth characteristics (plant height, leaves and stems number per plant), yield and yield components (tubers number, size, weight and total yield/plant). Challenging with virus strain was also conducted mechanically in the field and the resistance was determined visually and by ELISA test. Field plantation confirmed data obtained at greenhouse experiments. It can be concluded that five of the putative transformed lines exhibited high resistance to PVY under field conditions.

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Table (1): The survival percentages for transgenic and non-transgenic potato lines under greenhouse conditions

Line	PVY 1	PVY 2	PVY 4	PVY 5	PVY 15	PVY 23	PVY 24	PVY 25	PVY 31	Control
Survival %	100	94	94	74	88	100	88	81	100	100

Table (2): Plant height of transgenic and non-transgenic potato lines after 30 and 60 days.

Line	Plant height after 30 days Means ± SE (cm)	Plant height after 60 days Means ± SE (cm)
PVY 1	11.1 ± 1.2	15.7 ± 1.3
PVY 2	11.0 ± 1.2	21.0 ± 1.7
PVY 4	13.0 ± 1.4	24.0 ± 1.8
PVY 5	12.0 ± 1.3	15.0 ± 1.3
PVY 15	7.6 ± 0.7	12.0 ± 1.2
PVY 23	8.9 ± 0.9	18.6 ± 1.5
PVY 24	9.9 ± 1.0	18.0 ± 1.5
PVY 25	10.0 ± 1.0	17.3 ± 1.4
PVY 31	9.0 ± 1.0	15.0 ± 1.3
Control	12.0 ± 1.2	20.0 ± 1.7

Table (3): Yield evaluation of nine transgenic and non-transgenic potato lines under greenhouse condition.

Line	Weight of tubers (g)	Diameter of tubers (cm)	Number of tubers
Control	12.19	1.99	5.40
PVY 1	9.13	2.30	2.40
PVY 2	6.50	1.40	11.90*
PVY 4	18.50*	2.10	4.90
PVY 5	8.90	1.70	8.70
PVY 15	9.30	1.90	4.50
PVY 23	23.10*	2.95*	13.30*
PVY 24	10.50	2.20	8.00
PVY 25	7.34	1.70	11.70*
PVY 31	19.20*	3.01*	15.10*
Means ± SE	12.48±1.52	2.13±0.13	8.59±0.85

* Significant at 5%

Table (4): ELISA readings for transgenic and non-transgenic potato lines after challenged with strain of potato virus Y.

Line	ELISA Value	Resistancelevels
Non-infected	0.188	Healthy (negative control)
PVY 1	0.535	Non-resistant
PVY 2	0.443	Non-resistant
PVY 4	0.123	Resistant**
PVY 5	0.207	Resistant**
PVY 15	0.384	Resistant*
PVY 23	0.041	Resistant***
PVY 24	0.618	Non-resistant
PVY 25	0.701	Non-resistant
PVY 31	0.351	Resistant*
Infected	0.674	Infected (positive control)

ELISA values are the average of two wells measured at 405 nm
*barely resistant **Moderately resistant ***Highly resistant

Table (5): Yield evaluation of nine transgenic and non-transgenic potato lines under greenhouse conditions.

Line	Weight of tubers (g)	Volume of tubers (cm^3)	Number of tubers
Control	26.40abc	4.520cd	5.600a
PVY 1	16.20bc	2.360d	5.200a
PVY 2	28.20abc	4.720cd	5.200a
PVY 4	35.40abc	6.080abc	8.600a
PVY 5	35.40ab	6.980ab	8.200a
PVY 15	28.10abc	5.420abc	6.400a
PVY 23	39.80a	7.580a	8.800a
PVY 24	17.50bc	4.520cd	6.600a
PVY 25	18.80bc	5.386abc	6.400a
PVY 31	32.40abc	6.040abc	5.800a

Table (6): ELISA readings for transgenic and non-transgenic potato lines after challenged with strain of potato virus Y under field conditions at Assiut University.

Line	ELISA Value	Resistance Level
Non-infected	0.188	Healthy (negative control)
PVY 1	0.359	Sensitive
PVY 2	0.386	Sensitive
PVY 4	0.162	Resistant**
PVY 5	0.199	Resistant**
PVY 15	0.307	Resistant*
PVY 23	0.123	Resistant***
PVY 24	0.423	Sensitive
PVY 25	0.487	Sensitive
PVY 31	0.246	Resistant*
Sp-6A-3	0.254	Resistant*
Infected	0.453	Infected (positive control)

ELISA values are the average of two wells measured at 405 nm
* barely resistant **Moderately resistant ***Highly resistant

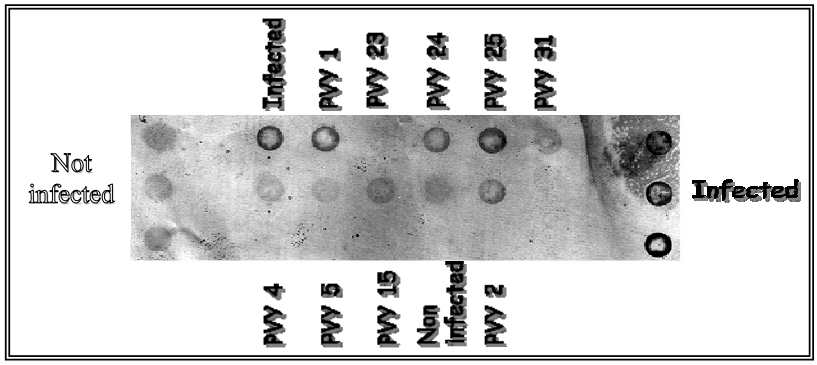


Fig. (1): Dot blot analysis for transgenic and non-transgenic potato lines after challenged with strain of potato.

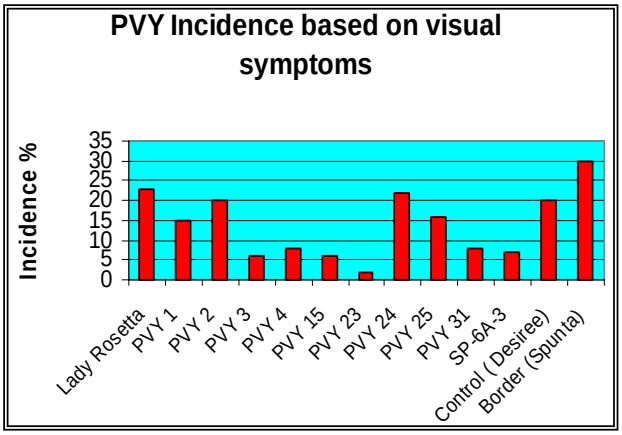
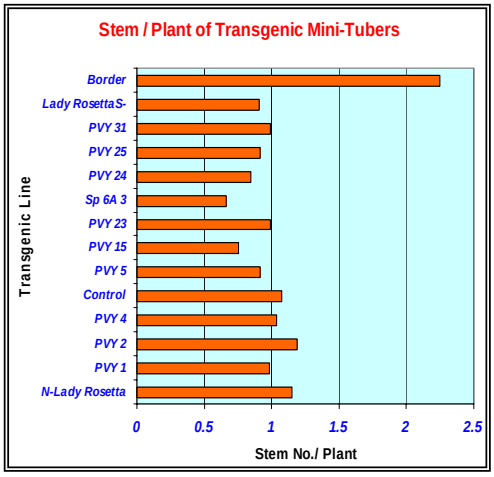
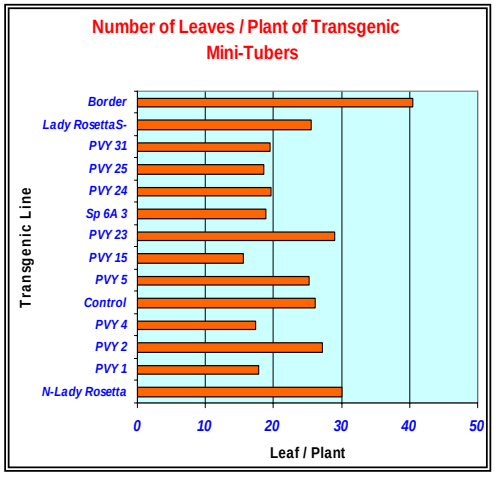


Fig. (2): Incidence of PVY based on visual symptoms.

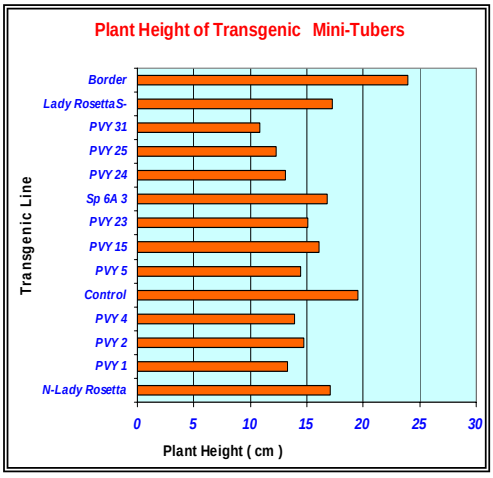


a: Stem Number/Plant



b: Leaves Number/Plant

Fig. (3): Growth Characteristics of Minitubers Grown in Assiut during Fall 2005.



c: Plant Height (cm)

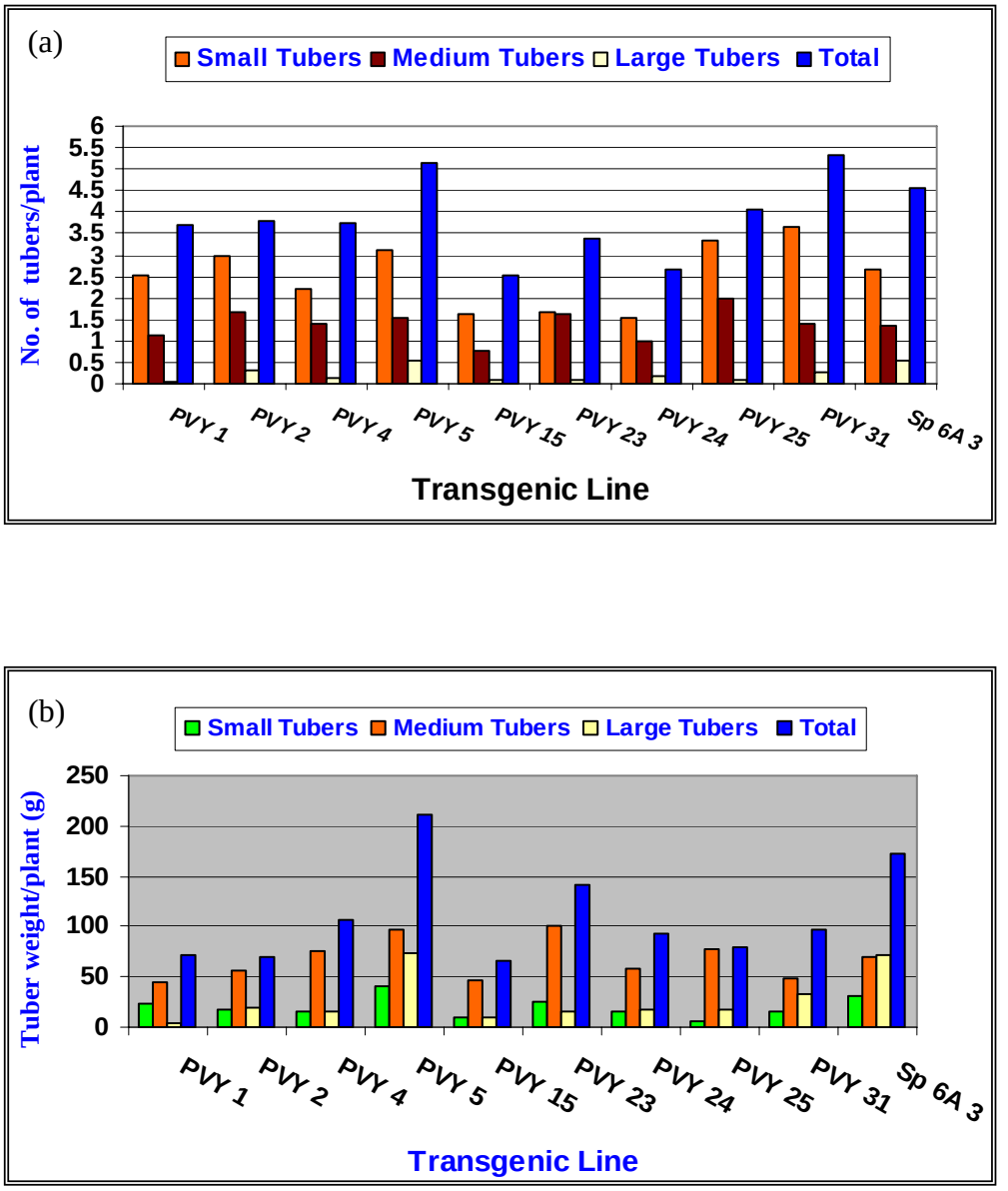


Fig. (4): Yield (expressed as number of tubers/plant (a) and tuber weight/plant (b)) of transgenic plants grown under field condition at Assiut environment.