SUMMARY

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A- Potato virus survey and identification:

1. The present work was undertaken to making survey about incidence of the aforementioned viruses and obtaining virus-free potato plants from original infected tubers of two potato cvs. (Spunta and Diamont), by using tissue culture technique alone or in combination with chemotherapy or irradiation treatments. In addition, the harmful effects of these viruses on potato yield production under in vitro conditions was also investigated. The yield of the virus-free plants that produced through different tested experiments was also compared under greenhouse conditions.
2. The obtained results revealed that the incidence of virus diseases. in general, was greater in Diamont cultivar than Spunta in different examined locations i.e. Giza, Qalubia and Gharbia Govrnorates. In different locations, the incidence of PVX-infection was ranged between 7.4-33.3% on Spunta and 19.2-26.1% on Diamont.. Infection with PVY was 10.0-74.0% and 34.4-46.1% on Spunta and Diamont, respectively. For PLRV-infection, it was ranged between 11.1-65.0% on Spunta cv. and 10.3-21.7% on Diamont cv. The mixed infection with more than one virus was ranged between 5.0-22.2% in Spunta cv. and 3.8-11.5% in Diamont cv. Infections with PVY (74.1%), PLRV (65.0%), and PVY (33.3%) were most common on potato cv. Spunta in locations of Giza, Qalubia and Ghrbia, respectively. While, the highest occurrence of PVX, PVY and PLRV were 24.1, 46.15 and 21.7 %, in the same locations, respectively. Among these three viruses, infection with PVY was the most common on Diamont cv. in all locations, however, on Spunta cv., PVX-infection in Qalubia, PVY-infection in Giza, and PLRV-infection in Gharbia were the most common.

B- Effect of size of meristem tips

1. The present results revealed survived plantlets produced from 0.2 mm long explants were greatly higher (70.8%) in Spunta than Diamont (58.3%) cultivars but no clear differences in survived plantlets developed from 0.5 or 0.7 mm long explants in both cultivars. Explant with 0.7 mm long resulted in 83.3% survivals in both Spunta and Diamont cvs. The present data noticed also that the *in vitro* relative growth rate measured by growth rating scale described by **Klien** and **Livingstone (1982)** was somewhat slower in Diamont cv. (average 38.02%) than that of Spunta cv. (average 50.16 %) at all tested incubation periods.
2. Number of virus-free plantlets from infected sprouted tubers by tissue culture technique alone was higher in Diamont cultivar than in Spunta one. Out of 15 plantltes (for the three sizes of explant for each cultivar) 7 plantlets in Diamont and 3 plantlets in Spunta were completely freedom from the three viruses. As for explants with 0.2 and 0.5 mm lengths, the same data proved that, 3/5 (60%) and 4/5 (80%) of the plantltes assessed for Diamont cultivar. and 2/5 (40%) and 1/5 (20%) of the plantltes assessed for Spunta cultivar were virus-free, respectively. In fact, incidence of PVX-infection was higher in the source plant materials of Diamont cv. than Spunta cvs.
3. The present data showed that PVY-infection was more difficult to eliminated by using tissue culture technique alone as it was detected in 5/15 and 6/15 of plantletes of Spunta and Diamont cvs., respectively while infection with PVX and PLRV each alone were detected in only 1/15 of the tested plantltes of both cultivars, respectively. In general, percentage of virus-free plantlets produced was inversed proportionally with size of meristem tips cultured.
4. The mixed infections with PVX + PLRV as well as PVX + PVY + PLRV were never detected in plantlets developed from all tested sizes of explant in

both tested potato cvs. Using 0.2 mm long explant, PVY + PLRV-infection was 20% in Diamont cv., compared with 0.0% in Spunta cv. With 0.5 mm long , PVY+PLRV-infection was 60% in Spunta cv. only. It is interest to state that all plantlets of Diamont cv., that developed from the larger size of explant i.e. 0.7 mm long were infected with PVY alone.

5. The present results indicated that the number of microtubers produced by Spunta plantlets healthy (virus-free) and infected PVX alone or PVX + PVY were significantly equal. However, total weight, total volume of microtubers, average weight and volume of individual microtuber were significantly higher in virus-free plantlets compared with those infected with PVX or PVX+ PVY. In plantlets infected with PVY alone, the average weight of single microtuber was significantly higher than that of virus-free plantlets and this could be attributed to lower number of microtubers produced by these plantlets compared with the virus-free ones. On other hand, the highest significant reduction in all above mentioned characters was observed in infection with PVY alone or combined with PLRV, while, in plantlets still infected by PVX, PVY, PVX+PVY and PVY+PLRV, the total weight of microtubers was reduced by 8.5, 26.9, 25.4 and 40.7%, respectively compared with virus-free plantlets. The same infections reduced total volume of the resulted microtubers by 45.0, 60.8, 53.0, and ,65.8%, respectively. The reduction in average of a single microtuber in infected plantlets was ranged between 22.5-50.0% compared with those virus-free.

6. Compared with virus-free plantlets of Diamont cv., PVY-, PLRV-, and PVY+PLRV-infection, number of microtubers was reduced by 11.1, 30.0, and 37.8%, and total weight of microtubers was reduced by 29.0, 16.6, and 22.6%, respectively. However, total volume of microtubers, average weight and volume of a single microtuber was responded differently against infection by these viruses.

Effect of Chemotherapy

1. In the present studies the meristem culture technique combined with different concentrations of Virazole or Salicylic acid was undertaken to testing their effect on the *in vitro* development as well as on elimination of PVX-, PVY- and PLRV-infection. The presented results indicated that both survived percentage and the relative growth of the *in vitro* plantlets, especially in potato cv. Spunta, were gradually decreased with increasing concentration of either Virazole or Salicylic acid in culture-medium up to 40 ppm compared with control treatments.
2. Production of virus-free plantlets by using Virazole-tissue culture combination was not satisfactory in most cases when 0,2 or 0.5 mm long explants were involved. The most beneficial effect for applying Virazole was exhibited only when it was used with the larger size of explant (0.7 mm long) compared with control (without Virazole). The latter combination resulted in 60, 60, and 10% virus-free plantlets in case of Spunta cv., and 80. 60, and 80% virus-free plantlets in case of Diamont cv., at 10, 20, and 40 ppm, in respective compared with 0.0% in control treatment of both cultivars. In Virazole treatment 60% of developed plantlets of Diamont cv., were virus-free compared with only 20% in Spunta cv. Therefor, it could be concluded that the potato genotype (cultivar) and the relation between chemical agent and plant cell might be involved in the process of virus elimination.
3. As for effect of Virazole on microtubers production of Spunta cv., *in vitro*:

a) At 10 ppm of Virazole, number of microtuber in PVX-infected plantlets of Spunta cv., was significantly increased while it was significantly decreased in PVY- and PVY+PLRV-infected plantlets compared with virus-free ones. PVY-infection alone reduced total weight of harvested microtubers significantly (38.3%) while mixed infection with PVX+PVY reduced it by 43.7 %. Average weight of the individual microtuber was significantly decreased (39.1 %) in

PVX+PVY-infection. Both total volume of harvested microtubers and average volume of the single microtuber were significantly decreased by infection with PVX and PVY each alone or in combination. In general, mixed infection with PVX+PVY caused the highest reduction in both total volume of harvested microtubers (68.7 %) and average volume of a single microtuber (66.7 %).

b) At concentration of 20 ppm of Virazole, the number of microtubers was reduced significantly by infections with PVX (57.0 %), PVY (62.9 %), PLRV (32.4 %), PVX + PVY (23.5 %), and PVY + PLRV (55.9 %). The highest significant reduction in total weight of microtubers per plantlet was caused by mixed infection with PVY + PLRV (60.3 %) followed by infection with PVY alone (55.4), while mixed infection with PVY + PLRV and PVX + PVY caused the highest significant reductions in total volume of microtubers per plant and average volume of a single microtuber i.e. 72.8 % and 58.1 %, respectively.

c) At 40 ppm of Virazole, the total number of microtubers per plantlet was significantly higher in most detected cases of viral-infected plantlets (except PVX + PVY) compared with virus-free plantlets. It could be observed also that total weight of microtubers per plantlet was significantly equal in both virus-free and virus-infected plantlets. The average weight of single microtuber was, however, significantly reduced by 46.3 %, 32.7 and 50.8 % in case of infection with PVY, PVY+PLRV and PVX + PVY+PLRV, respectively. Total volume of microtubers per plantlets was significantly reduced by 49.9 % in PVY-infection, 39.4 % in PVX+PVY-infection, 31.6 % in PVY+PLRV-infection and 49.9 % in case of infection with the three viruses i.e. PVX+PVY+PLRV. These virus-infection reduced average volume of a single microtuber by 69.8, 37.2, 53.5 and 67.4 %, in respective.

4. As for effect of Virazole on microtubers production of Diamont cv., *in vitro*, the obtained results proved that, both total number of microtuber and average volume of a single microtuber at both 20 and 40 ppm of Virazole, and total

volume of microtubers were significantly affected by variety of virus-infections. At 20 and 40 ppm for PVY-infection and 20 ppm for PLRV-infection, the total number of microtuber was significantly higher than the corresponding number of virus-free plantlets. However at 40 ppm, PVY+PLRV-infection significantly reduced total number of microtuber by 40.0 %. At 40 ppm, PVY-infection alone reduced total volume of microtubers and average volume of individual microtuber by 46 % and 56.7 %, while PVX+PLRV-infection deduced them by 61 % and 33.3 %, in respective.

Effect of Salicylic acid on survival percentage and rate of growth:

1. The percentage of survivals was relatively higher at concentration of 10 ppm. than 20 or 40 ppm. of acid. At all tested concentrations of acid, percentage of survived plantlets developed from explants with length of 0.7 mm was greatly higher, especially in Diamont cv., than those developed from 0.2 or 0.5 mm explants. acid seems to be inhibited the *in vitro* growth of both cultivars when compared with control (without acid treatment). This inhibition was increased with increasing concentrations of acid.
2. In Spunta cv., the present results showed that, acid treatment combined with 0.2 mm long explants had no clear effect on production of virus-free plantlets in Spunta cv. compared with control treatment. When 0.5 mm long explants were used with acid at 10 ppm, 40% virus-free plantlets were produced compared with only 20% in control treatment. Using the larger size of explant i.e. 0.7 mm long, the same results proved that 40 and 20% were virus-free at 10 and 40 ppm of acid, respectively compared with 0.0% in control treatment.
3. In Diamont cv., the acid treatment seems to be more effective in elimination of potato viruses from its cultured explants if compared with Spunta cv. No effect of acid on production of virus-free when 0.2 mm long explants were used. With 0.5 mm long explants, 100% virus-freedom was produced at 20

ppm of acid compared with only 80 % in both control treatment. Using 0.7 mm long explants 80, 60 and 80% of plantlets developed at 10, 20 and 40 ppm of acid, respectively compared with 0% in control.

4. About the *in vitro* tuberization of Spunta cv., the present results showed that, at 10 or 40 ppm of Salicylic acid, number, total weight and total volume of microtubers per plantlet was significantly higher in virus-free plantlets than in plants infected with PVX, PVY either singly or in combination. -- number of microtuber per plantlets was reduced by 42.9, 32.6 and 48.6 % and total weight of microtubers per plantlets was reduced by 12.9, 13.2, and 52.1 % in plantlets still infected with PVX, PVY or PVX + PVY, respectively compared with virus-free plantlests developed at 10 ppm of Salicylic aci. However, at 40 ppm, the number of microtubers per plantlets was reduced by 46.7 and 40.6 % and total weight of microtubers was reduced by 44.9 and 65.2 % in plantlets still infected with PVY alone PVX+PVY, respectively. Mixed infection with PVX + PVY only reduced average weight of individual microtuber by 41.3 compared with virus-freedom.

5. In potato cv. Diamont, it was observed that, the total number of microtubers in virus-infected plantlets developed at different concentrations of Salicylic acid was significantly equal or higher than virus-free plantlets. This was in contrast with total weight of microtubers per plantlet, which was significantly higher in virus-free plantlets compared with those infected with different viruses. -- In plantlets developed at 10 ppm of Salicylic acid but still infected with PVX, PVX+PLRV and PVY+PLRV, the total weight of microtubers per plantlets was reduced by 59.4, 39.8 and 55.9 %, and average weight of single microtuber was reduced by 50.5, 52.4 and 55.0%, respectively. However, PVX-infection only reduced total volume of microtubers per plantlet by 60.6 % compared with virus-free plantlets. Infection with PVX alone and mixed infection with PVX+PLRV reduced average volume of single microtuber

by 54.2 and 41.7 %, respectively. --- At 20 ppm of Salicylic acid, PVX-, PVX+PLRV-, PVY+PLRV-, and PVX+PVY+PLRV-infections caused 35.6, 45.3, 48.3 and 41.2% reduction in total weight of microtubers per plantlet, 32.2, 14.7, 34.1 and 32.2 % in total volume of microtubers per plantlet, and 46.2, 38.5, 26.9, and 42.3 % in average volume of a single microtuber, respectively. At concentration of 40 ppm, detected infections i.e. PVX and PVY each alone had no significant effects on total weight of microtubers per plantlets but they reduced average weight of single microtuber by 35.6 and 34.6 %, respectively.

Effect of irradiation on survival percentage and growth rate:

1. The presented results indicated that percentages of survived plantlets of both potato cvs. Spunta and Diamont were affected negatively by irradiation treatment. The dangerous effect of gamma irradiation on survivals, in general, was higher in Spunta than in Diamont cvs. It was increased with increasing irradiation doses but slightly decreased with increasing length of the excised explants. Using 0.2 mm long explants, the *in vitro* relative growth was inhibited at all tested doses of irradiation, while, applying 30 Gy irradiation dose resulted in excellent relative growth of 0.5 and 0.7 mm long explants in case of Spunta cv. only compared with control (no irradiation). From these results , it could be concluded that gamma irradiation at dose of 30 Gray has some promoting effect on growth rate of 0.5 and 0.7 mm explants of Spunta but not Diamont potato cvs.

Effect of radiation on elimination of potato viruses :

2. Using 0.5 mm long explant of Diamont cv. virus-free plantlets were increased to 80% when combined with 30 Gy irradiation compared with 60% in control. All other combinations between 0.2 or 0.5 mm long explants of both tested cultivars and different doses of Irradiation had no any improvement in production of virus-free plantlets compared with their controls. The number of

virus-free plantlets obtained through irradiation treatment was higher in Diamont than Spunta cultivar. This may related to genotype response.

Effect of pretreatment with irradiation on in vitro microtuber production:

3. In Spunta cv., microtuber productivity of plantlets, which were still infected (after irradiation) with different potato viruses, was significantly less than those were virus-free. When 15 Gy was used, The total weight of microtubers per plantlet, was reduced by 8.5, 44.6, 26.3, and 73.4 %, the average weight of single microtuber was reduced by 26.1, 43.6, 18.5, and 41.3 %, the total volume of microtubers per plantlet was reduced by 21.8, 56.3, 46.8, and 72.5 %, and the average volume of single microtuber was reduced by 36.8, 55.3, 42.1, and 39.5 % in plantlets still infected with PVX, PVY, PVX+ PLRV, or PVX+ PVY+PLRV, respectively compared with virus-free plantlets.
4. When 45 Gy was used, the total weight of microtubers per plantlet was reduced by 34.5, 15.3, 64.6, and 37.4 %, the average weight of single microtuber was reduced by 7.4, 28.0, 69.2, and 47.6 %, the total volume of microtubers per plantlet was reduced by 68.0, 60.0, 76.0 and 61.6 %, and the average volume of single microtuber was reduced by 52.8, 64.2, 77.4 and 66.0 % in plantlets still infected with PVY, PLRV, PVX+PVY or PVY+ OLRV, respectively. Mixed infection with PVX+PVY+PLRV (after 15 Gy treatment) and PVY alone (after 45 Gy treatment) reduced total number of microtubers per plantlet was reduced by 54.8 and 32.2 %, respectively compared with healthy virus-free plantlets.
5. After 15 Gy treatment in Diamont cv., the obtained results viewed that, the total number of microtubers per plantlets which still infected with PVX, PVY, or PLRV, each alone, was reduced by 42.4, 25.4, and 28.0 %, in respective compared with virus-free plantlet. At same dose of irradiation and same viral infections, both total weight of microtubers per plantlet and average volume of a single microtuber were not significantly differed, if compared with those of

virus-free plantlets. However, both total volume of microtubers per plantlet and average volume of a single microtuber were significantly higher especially in plantlets infected with PVX alone if compared with those of virus-free plantlets. The latter trend was noticed also in case of 30 and 45 Gy irradiation treatments.

Effect of irradiation on protein pattern:

6. the obtained results indicated that, in control treatments (without irradiation), the five bands with molecular weight (MW) of 38,36 34 and 14 Kda were detected in both healthy and infected plants of Spunta cv. only but not in Diamont cv. The band with MW of 41 Kda was found on healthy and infected plans of Spunta and Diamont cvs., respectively. On the other hand, the band with MW of 27 and 23 Kda were the common bands in both healthy and infected plants of both cultivars. In irradiation treatments, the same results showed that, the band with MW of 23 Kda found in all irradiation treatments of Diamont but not in Spunta cvs. However, the band with MW of 41 Kda was disappeared from all irradiation treatments in Spunta cv. but detected in 15 Gy treatment only in Diamont cv. In Spunta cv., the band with MW of 14 Kda was absent in 15 Gy but detected in 30 and 45 Gy treatments. The later observation was reversed in Diamont cv. Respecting with bands of 38, 36, 34, and 27 Kda, the all were detected in all irradiation treatments of Spunta cv. and in 15 Gy treatment only in Diamont cv. In the later cv., the bands with 36 and 27 Kda in both 30 and 45 Gy treatments and 34 Kda in 45 Gy treatment only were detected.

Minitubers productivity of different virus-free plants under greenhouse conditions as affected by different pre-treatments:

a) In Spunta cv., total fresh weight of minitubers/plant, total volume of minitubers/plant, average weight and volume of individual minitubers were relatively or significantly higher when 10 and 40 ppm of Virazole and 15 Gy

irradiation treatments were used, but 40 ppm of Virazole caused also significant increase in both fresh and dry weight of vegetative growth compared with virus-free in control treatments. On the other hand, all tested treatments caused significant reduction in number of minitubers /plant. The fresh weight of vegetative growth in 10 ppm of Virazole or Salicylic acid and 45 Gy irradiation, the dry weight of vegetative growth/plant in 30 and 45 Gy irradiation, the total fresh weight of minitubers /plant in 20 ppm of Virazole, 10 and 40 ppm of Salicylic acid and 45 Gy irradiation treatments were significantly lower than those of virus-free plants in control treatment.

b) In Diamont cv., the fresh weight of vegetative growth of virus-free plants developed through the treatments of 40 ppm of Virazole, 10 and 40 ppm of Salicylic acid and 45 Gy irradiation and the total volume of minitubers/plant in the treatments of 40 ppm of Virazole and 45 Gy irradiation were significantly higher than those of virus-free plants from control treatment. In contrast, the dry weight of vegetative growth in treatments of 20 and 40 ppm of Salicylic acid, the total weight of minitubers/plant in 10 and 20 ppm of Verazole and 40 ppm of Salicylic acid, and the total volume of minitubers/plant in 20 ppm of Verazole and 30 Gy irradiation treatments were significantly lower than those of control treatment. No significant differences in number of minitubers/plant, average weight and volume of single minitubers were observed between different treatments. These results emphasized differences between the tow tested potato cvs. Among the different examined treatments, 40 ppm of Verazole and 15 Gy irradiatin were significantly improved minituber production compared with tissue culture technique alone in case of Spunta cv., while in Diamont cv., the fresh weight of vegetative growth and total volume of minitubers only were improved significantly by using treatments of 40 ppm of Verazole or 45 Gy irradiation combined with tissue culture compared with tissue culture alone.