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Effect of BAP and soil type on production of Virus-free potato plant (*Solanum tuberosum* L.) regenerated via shoot tip meristem

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Abstract:

Potatoes are one of the major vegetable crops that are grown worldwide. Therefore, evaluation of varieties in production of virus free mini-tubers is critical.

In this study, the effect of BAP at 0, 1, 1.5 and 2 mg l^{-1} on shoot number explants^{-1}, plant height and number of root plantlets^{-1} for production of virus free plantlets was evaluated on two potato cultivars Spunta and Draga.

Spunta gave the highest number of shoots (2.4 shoots plant^{-1}) at BAP of 2 mg l^{-1}, whereas, Draga gave the longest plant (6 cm) at BAP of 1.5 mg l^{-1}. MS medium without hormones had the highest number of roots (2.3 and 2) in Draga and Spunta cultivars, respectively.

In both cultivars, plants grown in soil mixed with sand gave the highest tuber number of tubers plant^{-1} in Spunta (15) and Draga (14), respectively. At the same time, the highest tuber weight was 75 and 65 gm in Spunta and Draga plant respectively that were grown in perlite alone.

Using ElISA based detection method, proved that the potato viruses (PVY, PVM, PVS) and potato leafroll virus (PLRV) were not detected either in plantlets or formed tubers in both cultivars. Well rooted plants were transferred to green house for tuber production and the rate of survival was 100%.

In conclusion, virus free plant was obtained using shoot tip culture and BAP as growth hormones in both cultivars. Soil and sand was the best bed for potato cultivation.

Keywords: Shoot tip, diseases free tubers, potato, soil, sand.

Introduction:

Potato has been used as food for thousands of years. Today, the potato is the fourth largest food crop in the world and is grown in almost every country in the world, with annual worldwide production exceeding 300 million tons [8]. Potato is an important source of energy, protein, vitamins and minerals and is part of the diet of billions of consumers throughout the world.

Potato is a vital component of the crop production system in Palestine. According to the Ministry of Agriculture, 10,835 dunums of agricultural land has been utilized for the cultivation of potatoes in the West Bank in the year 2012/2013 with total production of potatoes is to be estimated at 37,552 tons in the West Bank. In West Bank, potatoes are grown mainly in Tubas and Nablus area. The major production of potato is in Gaza strip where production reaches to 46,262 metric tons. As there is absence of local organized seed potato supply system in Palestine at present, much of the tuber need of the country has been met by import that amounted to 4000 tons [18]. Till to date the external source is the only way to have a virus-free tuber potato primarily imported from the European countries. If the national potato tuber sector could be supported with capacity building, there is potentiality of Palestine in meeting its tuber demand with local production.

Potato is normally vegetative propagated by means of tubers [3]. Tubers are shortened and thickened underground stems with auxiliary buds.

Among the most important factors influencing potato yield are the physiological status and health of seed tubers [31]. Traditionally potato tubers are being used for multiplication and production [26]. High risk of infection with many diseases (fungal, viral, and bacterial diseases) and different pests could be happened [3, 31]. On the other hand, many pests and diseases can be transferred via potato tubers and these cause of less production of these seed tubers. To overcome these infection scientists usually use shoot tip culture for developing a virus free potato plants [7].

At least 37 viruses naturally infect cultivated potatoes [2, 23, 10]. Some of these viruses are economically important and can cause a major loss in potato production. Potato virus Y (PVY), potato virus A (PVA), potato virus V (PVV), potato virus M (PVM), potato virus X (PVX), potato virus S (PVS) and Potato leaf roll (PLRV),), potato

mop top virus (PMTV) and potato aucuba mosaic virus (PAMV) occur worldwide [4].
Meristem culture along with thermotherapy has become powerful and successful tool for virus elimination. Meristem culture has been applied in potato [17, 1, 16, 29,6].
There are a number of methods available for detection of viruses [25]. Serological procedures are one of the reliable methods for identification and quantitative assay of viruses. Direct double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) is commonly used for virus detection [5].
Therefore, the objectives of this study were to develop a reproducible meristem culture protocol for the production of virus free potato plants.

Material and methods

Media composition and culture condition

MS basal salts plus vitamins [13] with without growth regulators were used in this study. The medium is supplied with 30 g of sucrose and solidified with 8 g of agar. The pH of medium was adjusted to 5.8 before sterilization. It was autoclaved at 15 psi and 121ºC for 15 minutes. *In vivo*, grown cultures were kept in growth chamber at 23 ºC ± ºC under 16 hour light and 8 hours dark.

Plant materials

Two economically important potato cultivars, namely Draga and Spunta were used in this study. Tubers were obtained from certified local distributors, sterilized in 0.5-1% hypochlorite, washed again three times with sterile distilled water and kept under normal light (2000 lux) for 21-28 days till small shoots appeared from tubers eyes. These shoots were finally used as a source of shoot tip.

Establishment of shoot tip culture

Shoots with shoot tips were excised from tubers, washed with running tap water for 10 minutes and then soaked in hypochlorite 30% (V/V) for 30 minutes. Under sterile condition, shoots were washed three times with sterile distilled water. Shoot tips of both cultivars (0.2-0.3 mm in length) were inoculated on MS basal medium with different concentration of BAP (0. 1, 1.5 and 2 mg l^{-1}) in growth chamber at 23 ± 2 ºC for 4 weeks.

Shoot and root development:

After 4 weeks of culture, shoot formation was recorded and were refreshed every 2 weeks on MS fresh medium without growth regulator for shoot development and elongation.

DAS- ELISA Test:

Five grams of leaf tissue were taken from each 50 randomly selected regenerated plantlets and used for detection of Potato viruses, namely Potato virus Y, M, S and Potato leafroll virus (PLRV) which is present in Palestine using DAS- ELISA kits (BIOREBA, Switzerland). The procedure was according to manufacturer instruction.

After transferred to the soil, plants were tested again for viruses' presence

Plantlets hardening and tuber formation

Shoots with well developed roots were transferred to sterile perlite, covered with plastic bag and kept back in growth chamber for another 1 weeks. After that, 36 plants were transferred into bigger pot (2 liter capacity) containing either perlite or soil mixed with sand with 1:1 ratio, arranged in Complete Randomized block Design (CRD) and kept in green house for further growth.

Statistical Analysis:

Data were subjected to statistical analysis where, Duncan's Multiple Range Test (DMRT) was used to compare between means at $p \leq 0.05$ [22].

Results and discussion:

Potato is vegetative propagated through tubers and through subsequent planting of tubers obtained from first generation, infection could be increased. The most effective approaches to control viruses in potato include the production of virus free potato tuber.

Shoot tip, plantlet regeneration and multiplication

The result on shoot tip regeneration and plantlet recovery are presented in table (1). Potato tuber produced shoots 2-3 cm long with shoot tips (Fig. 1 A) Shoot tips were successfully isolated (Fig. 1 b). There was no significant difference between two cultivars in term of shoot tip formation, number of plantlets formed and number of tubers formed. Shoots with roots (plantlets) formed within 3-4 weeks from shoot tips (Fig. 1 C) and many plantlets were successfully multiplied using bud culture (Fig. 1 D & E).

Table 1. Plants regeneration and tuber formation from shoot tip of two potato cultivars.

Cultivar	Explants	% Shoot tips formed	% plantlets formed	% of plant formed tuber
Spunta	150	75 a	100	100
Draga	145	74 a	100	100

Means in a column with same letter(s) do not differ significantly according to DMRT at level of $p \leq 0.5$.

Number of shoots per explant

Cultivar Spunta responded significantly at all BAP level than Draga, where the average mean number of shoot was 1.46 compared to 1.02 in Draga (table 2). At BAP level of 2 mg/l, the highest average shoot number in both cultivars was observed (2.25) followed by 1.5 mg l^{-1} BAP. The interaction showed that Spunta x BAP at level of 2 mg l^{-1} gave the highest average shoot number per explant (2.4) where 1.5 mg came to the second rank (1.8) in cultivar Draga. This may be attributed to genetic variety as being supported by other findings that showed that genetic makeup gave differential responses of different potato varieties towards *in vitro* shoot multiplication [15].

These results also reported by other researcher. He found that different potato cultivar responded differently to different level of BAP concentration and Cardinal with 1 mg l-1 6-benzyl aminopurine gave maximum number of shoots (2.43 cm) per explant, whereas, Dheera with 1.5 mg l^{-1}, 6-benzyl aminopurine gave the tallest plantlet (5.23 cm) [1].

cultivars.

Cultivar	BAP level (mg l^{-1})				Mean
	0	1	1.5	2	
Spunta	1d	1.2 bc	1.25 b	2.4 a	1.46 a
Draga	1d	1.2 bc	1.8 ab	1.1 d	1.02 b
Mean	1c	1.2 bc	1.5 b	2.25 a	

Means in a column with same letter(s) do not differ significantly according to DMRT at level of p≤ 0.5 .

Number of roots

The cultivar Spunta gave insignificant higher average of roots than Draga plant at all BAP level (table 3). MS medium without any hormone gave significant higher average in root number in both cultivars (2.15) when compared with other concentrations.

Interaction between cultivars and MS revealed that in both cultivars, MS medium without hormone gave significant higher average shoot number (2.3 in Draga and 2 in Spunta) than that of the other BAP levels. This suggests that in our study, to enhance root formation, plantlets should be transformed to free MS medium.

Similar results were observed by others, where he found that maximum roots number was observed at MS medium without hormone [1] and BAP and NAA reduced root formation and plant formed root with these hormones [28].

Table 3. Effect of BAP levels on average number of roots plant^{-1} of two potato cultivars.

Cultivar	BAP level (mg l^{-1})				Mean
	0	1	1.5	2	
Spunta	2.0 ab	0.4 b	0.2 c	0.0 d	0.65 ab
Draga	2.3 a	0.6 b	0.4 b	0.05 d	0.83 a
Mean	2.15 a	0.5 b	0.3 bc	0.025 c	

Means in a column with same letter(s) do not differ significantly according to DMRT at level of p≤ 0.5 .

Plant height:

The difference in plant height between cultivars had slight insignificant difference, where the average of plant height was 3.6 in Draga and 3.25 in Spunta (table 4). The BAP at 1.5 mg l^{-1} produced the significant longest plant height (4.75) and at BAP level 2 mg l^{-1} came to the second rank (3.8). Interaction showed that the significant longest plants were observed in Draga cultivar (6 cm) at BAP level of 1.5 mg l^{-1}, while in Spunta it reached 3.5 cm at the same BAP level. In addition, Spunta at BAP level of 2 mg l^{-1} had the second rank of plant height (4.5 cm).

The same result was found by other researchers where BAP responded differently with cultivars and increasing BAP level decreased plant height [1, 28].

Table 4. Effect of BAP levels on average of plant height of two potato cultivars.

Cultivar	BAP level (mg l^{-1})				Mean
	0	1	1.5	2	
Spunta	2.0 d	3.0 cd	3.5 c	4.5 b	3.3 ab
Draga	2.5 d	2.9 cd	6.0 a	3.1 cd	3.6 a
Mean	2.3 c	3.0 bc	4.8 a	3.8 b	

Means in a column with same letter(s) do not differ significantly according to DMRT at level of $p \leq 0.5$.

Plantlets hardening and tuber formation

Invitro grown plantlets were hardened in perlite (Fig. 1 F& G) and transferred into green house for tuber production. One hundred percent of hardened plant formed tuber (Fig. 1 H & I).

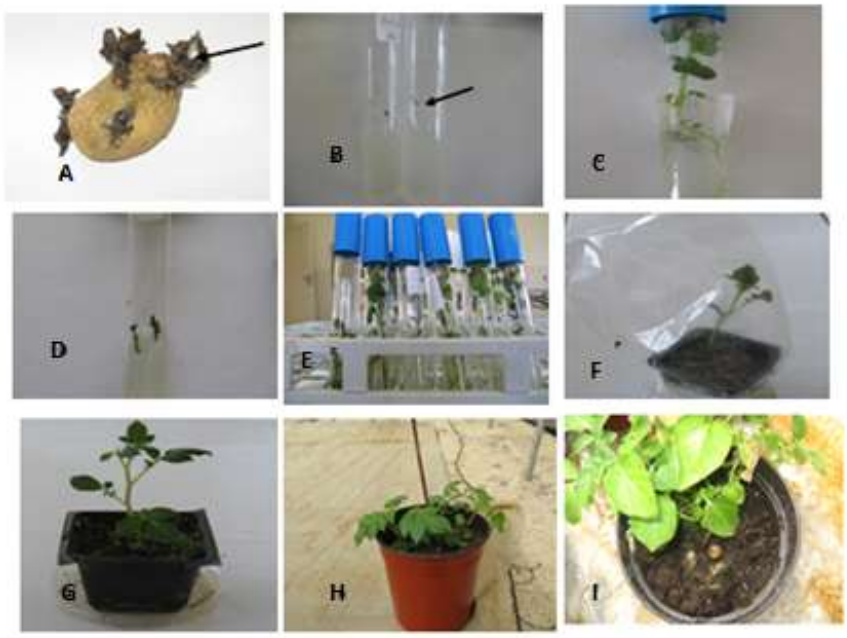

Fig.1. Stages of shoot tuber formation: **A** mother explant with shoot, **B** shot tip (arrow), **C** developed plantlets, **D** plant development through lateral bud, **E** clonal propagation of plantlets from lateral bud, **F** and **G** hardened plan **H** and I plants for tuber formation in a greenhouse.

Effect of soil type on number and weight of tuber formed

Data in table (5) shows insignificant difference between the two cultivars in number of tuber plant^{-1}. Soil and sand produced higher significant number (14.5) of tubers than perlite. The interaction between cultivars and soil type was significantly the highest in Draga x soil and sand (15) and same trend was also significantly true for Spunat x soil and sand (14).

Studies on the effect of different bed on number of minituber were conducted by [9], where she found that cocopeat and sand (3:1) gave the maximum minituber number (6.33). Planting on bed peat : perlite in ratio of 1: 1 in large pots (19 cm) showed highest yield (15-minituber per pot and yield of 95 grams per plant) [30]. Combination of peat moss and soil in volumetric ratio of 1:1, gave a significant

difference among the varieties in terms of mini-tubers number and yield when compared with Peat Moss and perlite (3:1) [20].

Table 5. Effect of soil type on average number of tubers plant^{-1} of two potato cultivars.

Cultivar	Soil type		Mean
	Perlite	Soil and sand	
Draga	8 a	15 a	11.5 a
Spunta	9 a	14 a	11.5 a
Mean	8.5 b	14.5 a	

Means in a column with same letter(s) do not differ significantly according to DMRT at level of $p \leq 0.5$.

Results in table (6) clear that weight of tubers^{-1}plant was significantly the highest for Spunta (55). The highest significant weight of tubers plant^{-1} was produced by perlite (70). The interaction was significantly the highest for Spunta x perlite (75) followed by Draga x perlite (65).

There is no report testing perlite alone or mixing sand and soil for production of min-tuber, but different planting beds were used by other researcher. For example, Researcher found that the average weight of mini-tuber was significantly different between Desiree and Marphona when planted in rice hull and turf (1:1) compared to peat and perlite (3:1) [21]. Peat mass amended culture bed as the best out of the various planting-beds [11], while bed of 2 parts of forest litter plus a part of soil as the best bed for the production of potato mini-tubers [24]. Comparing two beds including peat mass and sand, and vermiculite and sand. Obradovic and Sukha [19] found that bed with vermiculite is the best for longer survival of in vitro plantlets and the production of mini-tubers.

Table 6. Effect of soil type on the average weight of tubers plant^{-1} of the two potato cultivars.

Cultivar	Soil type		Mean
	Perlite	Soil and sand	
Draga	75 a	35 a	55 a
Spunta	65 b	19 b	42 b
Mean	70 a	27 b	

Means in a column with same letter(s) do not differ significantly according to DMRT at level of p≤ 0.5 .

DAS- ELISA:

EIISA method was widely used by researchers for detection of Potato viruses [12, 27]. In our study, these viruses PVY, PVM, PVY and Potato leafroll virus (PLRV) was detected in both cultivars wither in *vitro* grown plantlets or tuber. Our results are not different from those obtained by [14], who used *in vitro* culture using less than 0.3 mm meristem in variety Kelopetra obtained and obtained PVA and PVY free potato plants.

Our results are in conformation with other workers who regenerated potato through meristem culture for virus free production and found that regenerated plant are virus free mainly for PVY [1], PVX and PVY [4.17] and PVX, PVY, PVS and PLRV [29].

Cost effectiveness

The cost of all stages till tuber production has been carefully calculated. The total cost of 1 kg of microtuber is 0.5 USA $ where the cost of imported minituber reaches to 1.2-1.5 USA$ per Kg.

These results clearly show that minituber production in Palestine is very much possible and the total cost of minituber reduced by 40-50 %.

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