MINITUBERS PRODUCTION OF FOUR POTATO (*SOLANUM TUBEROSUM* L.) CULTIVARS BY TISSUE CULTURE TECHNIQUE

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ABSTRACT

This study was aimed to produce potato minitubers via tissue culture technique by testing four cultivars; Agria, Nicola, SM 12-124-15 and JIP 1600-1 under *in vitro*, greenhouse and open-air field conditions. Nicola cultivar was superior upon the other cultivars under *in vitro* and greenhouse conditions by producing the best shoot multiplication (1.89 shoots/ explant and 13..27 leaves/ explant) and the highest number of minitubers (8.87 minitubers/ plant) in greenhouse whereas, SM 12-124-15 was better in the open field conditions by producing 11.25 tubers per plant, recording the highest mean weight of tubers (321.33 g), the highest weight of tubers per plant (3.615 kg), the bigger sizes of tubers (length 8.22 cm: width 3.43 cm) and the highest total yield per hectare (49.70 Ton/ ha). An interesting microtubers formation was noticed on SM 12-124-15 explants grown on MS medium supplemented with 0.5 mg/l^{-1} BA. The main conclusion drown from the current study is MS medium with basic vitamins and solidified by agar without the need of adding exogenous plant growth regulators can be used for mass seed tubers production based on potato genotype *in vitro* under optimized culture conditions with a low cost propagation system and shorter period of time than normal minitubers production process.

Keywords: minitubers, potato cultivars, *Solanum tuberosum* L., plant tissue culture

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إنتاج الدرنات الدقيقة لأربعة أصناف من البطاطا (*Solanum tuberosum* L.) باستخدام تقانة الزراعة النسيجية

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المستخلص

استهدفت هذه الدراسة انتاج درنات دقيقة من البطاطا باستعمال تقنية الزراعة النسيجية من خلال اختبار أربعة أصناف وهي AGRIA، NICOLA، SM12-124-15 وJIP1600-1 تحت ظروف المختبر والبيت الزجاجي والحقل المكشوف. أظهرت النتائج بأن الصنف NICOLA كان متفوقاً على بقية الأصناف عند النمو في المختبر وكذلك في البيت الزجاجي من خلال تسجيل أفضل النتائج في مرحلة التضاعف الخضري (1.89 فرع/ جزء نباتي و13.27 ورقة/ جزء نباتي) فضلاً عن تسجيل أكبر عدد من الدرنات الدقيقة (8.87 درنة/ نبات) في البيت الزجاجي. بينما أظهر الصنف SM 12-124-15 تفوقاً ملحوظاً على بقية الأصناف عند النمو في الحقل المكشوف من خلال انتاج 11.25 درنة/ نبات وتسجيل أكبر معدل لوزن الدرنات (321.33 غم) وأكبر وزن للدرنات لكل نبات (3.615 كغم) وأكبر طول وعرض للدرنات المنتجة (8.22 سم طولاً و3.43 سم عرضاً) وكذلك تسجيل أكبر حاصل كلي من الدرنات (49.70 طم/ هكتار). تم ملاحظة انتاج درنات دقيقة مختبرياً على الأجزاء النباتية للصنف SM 12-124-15 النامية في وسط MS المزود بـ0.5 ملغم/ لتر من البنزيل أدنين. من أهم الإستنتاجات التي تم الحصول عليها من خلال هذه الدراسة هو أن استخدام وسط MS الحاوي على الفيتامينات الأساسية والمصلب بالآجار بدون الحاجة الى أي اضافات هورمونية ممكن للانتاج الكمي للدرنات الدقيقة وفقاً لصنف البطاطا المزروع تحت ظروف المختبر مع امكانية تقليل تكاليف نظام الاكثار خلال فترة زمنية قصيرة للحصول على نظام انتاج اقتصادي وغير مكلف.

كلمات مفتاحية: زراعة الأنسجة النباتية، أصناف البطاطا، الدرنات الدقيقة.

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INTRODUCTION

It is well known that potato (*Solanum tuberosum* L.) is one of the four most important crops all over the world. Iraq in general and Kurdistan Region in special, is increasingly developing and expanding potato production in recent years. The main source of potato seed tuber is Europe especially from Netherlands and France. The local farmers' demand for super potato varieties is dramatically increased year by year. To meet such urgent need, experimental trials are very necessary both *ex vitro* and *in vitro* to improve and select the best varieties for both table and processing uses (12, 22). In recent years, fast multiplication systems have been improved in the field of potato seed tubers production to produce huge quantities of high qualitative potato plantlets, microtubers and minitubers (9). They are high quality starting material that can be provided year round under *in vitro* conditions (potato Microtubers and plantlets) or under *ex vivo* conditions (minitubers) at a high quantity (11). Locally, there are various problems facing potato production like unavailability and high-cost of potato seed tubers, insufficient of well adopted varieties to the local climate and environment, bad agronomic practices, pests including disease and insects, bad storage servicers and of course inadequate transport and marketing (22). The conventional tuber seed production that had been applied for many decades, usually requires intensively controlled process and seed production programs basing on the clonal selection might take about ten years. *In vitro* propagation techniques have been developed in involve in potato seed tuber production systems, by which it could be reducing time needed for tuber seed multiplication. Such techniques will ensure the obtain of healthy stock materials through virus and virus-like infections eradication by using meristem culture technique, fast *in vitro* plantlets multiplication and producing huge numbers of plants of the first year clones through the process of minitubers production (16). The first published report on potato micropropagation was done by Steward and Caplin (6). After that, hundreds of investigation articles have been created and published (19). *In vitro* propagation is considered as an alternative means to classical potato propagation (4, 5) that specifically done vegetatively by seed tubers or segments of tubers and sometimes by seeds. On the other hand, *in vitro* propagation methods starting with meristem tips, nodal segments or microtubers are more reliable for maintaining germplasms of the multiplied genotypes (21). In previous potato micropropagation protocols, different culture media enriched with various plant growth regulators and other additives were investigated. For example, Bostan and Demirel (3) published that growth regulators-MS medium was the best medium for potato single node culture. The effect of various concentrations of GA_3 and BA on *in vitro* Desiree potato propagation was investigated by others (1). They indicated that maximum shoot length (8.96 cm) was achieved when using 4 $mg.l^{-1}$ GA_3. The highest number of shoots per explant (14 shoots/ explant) was recorded when 2 $mg.l^{-1}$ BA was added. A protocol for potato micropropagation was developed by Everson and Renan (7) in liquid culture media with different growth regulators combinations. They found that MS medium enriched with 0.25 $mg.l^{-1}$ GA_3, 5.0 $mg.l^{-1}$ vitamin pantothenic acid, 1.0 $mg.l^{-1}$ thiamine and 20 $g.l^{-1}$ and under constant agitation condition showed high efficiency of the potato micropropagation. Badoni and Chauhan (2) applied a low-cost alternative to MS salts for shoot multiplication in potato with different growth regulator combinations of kinetin at 0.04, 0.06 and 0.08 $mg.l^{-1}$ and 0.50 $mg.l^{-1}$ IAA. MS medium enriched with 4 $mg.l^{-1}$ of kinetin was shown to be the best in performance in respect of multiple potato shoot regeneration by (15). Some other investigators declared that various potato varieties perform variously to micropropagation protocols (10). The current study aimed to establish a comparison between the performance of four selected potato verities at *in vitro* conditions; by improving a reliable micropropagation protocol leading to minitubers production in greenhouse; and cultivars performance in the out-air field conditions.

MATERIALS AND METHODS

Potato seed tubers of four varieties including AGRIA, NICOLA, SM12-124-15 and JIP 16-001 were received from IBP Company in

Netherlands. The tubers were kept at room temperature and indirect light for about 21 days. When the new buds sprout out, the tips were cut off (around 0.1~0.3mm). In each tip, there was 1~2 primordium left and virus-free. Explants disinfestations were done by immersing the excised buds in 2.5% of sodium hypochlorite for 10 minutes and in 0.1% of $HgCl_2$ for 5 minutes. Disposable and reusable big growing containers were used in growing the explants by assigning 5 containers containing 16 explants for each treatment. At establishment (initiation stage), the tips were placed in corresponding propagation MS medium (13) with vitamins (Myo-Inositol 100 $mg.l^{-1}$, Nicotinic Acid 0.5 $mg.l^{-1}$, Pyridoxine-HCl 0.5 $mg.l^{-1}$ and Thiamine-HCl 1.0 $mg.l^{-1}$) supplemented with 30 $g.l^{-1}$ sucrose and solidified with 7 $g.l^{-1}$ agar. After four weeks, the survival rates of explants were recorded taking in consideration the levels of contamination. At shoot multiplication stage, single nodes were transferred to MS medium supplemented with 0.0, 1.0, 1.5 and 2.0 $mg.l^{-1}$ BA. After four weeks in culture, the number of shoots per explant, mean length of shoots, number of leaves per explant, rooting percentage, number of roots per explant and the mean length of roots were recorded. In these stages, temperature was controlled at 25±2°C, light exposure 16 hours per day and 1000 lux light intensity from cold white light (8). All materials including potato tubers, buds, tips, glassware and tools were thoroughly sterilized, making sure the newly developed plantlets are contamination-free. At acclimatization stage, peatmoss was used as culture medium. To enhance the plantlets growth, a foliar application of ¼ strength of MS salts was applied every two days during the first two weeks from acclimatization. The produced minitubers were harvested after 75 days from acclimatization and the number, mean weight, mean length and mean width of minitubers were recorded. The harvested minitubers were stored at 20 °C for 3 weeks as curing treatment and then transferred to cold stores at 5 °C till the date of field planting. Potato cultivars were planted in a trial field. The experiment was designed according to RCBD in three blocks. Thirty two tubers were planted from each variety in each block. This experiment aimed to compare the performance of the tested varieties in regard of number of tubers per plant, mean weight of each tuber, means of length and width of each tuber, yield of each plant and the total yield per hectare after 100 days from planting. The field grown plants received the ideal service operations that usually done in potato fields including cultivation, irrigation, fertilization, pest management, etc. The statistical analysis was done using analysis of variants according to Duncan multiple range test at 0.05 level (4).

RESULTS AND DISCUSSION

Explants disinfestation results were very successful by giving a 100% survival of cultures without any kind of contamination. So, the healthy cultures were directly moved to shoot multiplication stage. Table 1 reveals the multiplication parameters recorded after four weeks in culture medium enriched with different concentrations of BA (Figure, 1). Nicola cultivar was significantly superior upon other cultivars except JIP 16-001 by giving the highest number of shoots and leaves per explant (1.89 shoots/ explant and 13.27 leaves/ explant, respectively). On the other hand adding 0.5 $mg.l^{-1}$ BA gave the highest number of shoots (1.83 $mg.l^{-1}$ shoots/ explant) which was significantly higher than other of BA concentrations. The combined treatment of Nicola cultivar and 0.5 $mg.l^{-1}$ BA produced the highest number of shoots reached to 2.44 shoots/ explant and the highest number of leaves reached to 17.67 leaves/ explant as compared to other of combined treatments. The addition of different concentrations of BA to MS medium reduced the number of leaves and the mean length of shoots as compared to the control treatment. Agria cultivar produced the longest shoots (4.22 cm), which were significantly higher than other cultivars. The reason behind reducing the multiplication parameters as a result of BA addition might due to the sufficient endogenous hormonal content of cytokinins (18). It is well known that cytokinins especially BA has many positive roles in enhancing tissue cultures by increasing the levels of cell division, growth and differentiation (17). Such results lead to reduce propagation costs by applying micropropagation protocols without the need of highly coasted growth regulators.An

interesting Microtuberization process was noticed on SM 12-124-15 cultivar explants grown on MS medium supplemented with 0.5 $mg.l^{-1}$ BA (Fig. 1 B). This can be due to the positive role of cytokinins in enhancing cell division and bud formation (21). Table 2 shows the rooting response of the tested potato cultivars after four weeks of culture on growth regulator-free MS medium. A 100% rooting was recorded for the whole explants taken from the four cultivars. JIP 16-001 cultivar recorded the highest number of roots per explant (8.80 roots/ explant) which was significantly higher than other cultivars. Whereas, Agria cultivar produced the longest roots reached to 9.70 cm (Figure, 2). Potato plants are well known to be easy in rooting when nodal segments are cultured on MS media without the need of growth hormones addition (5). After a successful acclimatization stage, the potato plantlets were gradually moved from lab conditions into greenhouse where the atmosphere was totally controlled in regard of temperature and humidity. The plantlets were transferred in small pots containing peatmoss. (Figure 1: C and B). After 75 days, the produced minitubers were harvested (Figure 1: D) and the numbers of minitubers per a plant, mean weight of each minituber and their length and width were recorded and statistically analyzed. Results in Table 3 show that Nicola cultivar produced the highest number of minitubers (8.87 minitubers/ plant) which was significantly higher than other cultivars. On the other hand, Agria cultivar was significantly superior upon other cultivars in mean weight of minitubers by reaching 6.98 g/ minituber. Regarding the size of produced minitubers, Nicola minitubers were significantly shorter than other cultivars by reaching to 2.94 cm only. Whereas, no significant differences were seen among the four cultivars in regard of mean width of minitubers. In general, Agria minitubers were high in number with a deep yellow flesh color, a yellow skin and round shape, Nicola minitubers were medium in number with low weight and small in size, SM 12-124-15 minitubers were medium in number, elongated in shape with red color with a moderated size, JIP 16-001 minitubers were medium in number, with moderate sizes (Figure 3). The differences between the tested cultivars may be due to the genetic makeup of these genotypes which always reflect on their performance in *in vitro* culture as well as in greenhouse conditions (20). According to (4), 100 days is a normal production cycle for potato minitubers. Producing minitubers in the current investigation in only 75 days will highly raise the productivity per time and this will directly reflect on saving time, labor and cost in large-scale seed tuber production projects. Using glass containers in potato micropropagation is very costly but using bigger disposable and reusable plastic containers will reduce costs and raise the mass production accordingly (Figure 1 A). The produced minitubers were directly transferred to storage under 20°C for 21 days (Curing treatment) and then transferred to cold storage at 3-5°C. Before planting date, the minitubers were transferred to room temperature (20-25°C) for three weeks to enhance sprouting. A trial field experiment was arranged to evaluate the productivity of the four tested cultivars under *in vivo* conditions. Table 4 shows the results recorded after 100 days from growing in the field in regard of numbers of tubers per plant, mean weight of tubers, weight of tubers per plant, mean length and width of produced tubers and the total yield per hectare. The results reveal that SM 12-124-15 cultivar was the best among the cultivars in regard of producing 11.25 tubers per explant which was significantly higher than other cultivars except Nicola which gave 11.44 tubers/ explant. The good performance of SM 12-124-15 was very clear in other of parameters by showing highly significant differences as compared to the other cultivars by recording the highest mean weight of tubers per plant (321.33 g), the highest mean weight of tubers (3.615 kg), the bigger sizes of tubers (length 8.22 cm: width 3.43 cm) and the highest total yield per hectare (49.70 Ton/ ha). These results prove that the growth cultivars in the open-air field is totally different when compared with their growth and performance under *in vitro* or greenhouse conditions. The produced tubers from MS 12-124-15 cultivar were elongated in shape, no sprouting and were of good skin (Figure 4) as compared to the other cultivars. Where Agria tubers were low in numbers with second

growth on tubers and sprouting, Nicola tubers were elongated with no sprouting but with some second growth, JIP 16-001 tubes were with various sizes, no sprouting and good skin. The different response of potato varieties to the different growth conditions might be due to the genotypes tested that usually responses variously to the endogenous and exogenous affecting factors on their growth and development .These results are similar to those achieved by other researchers (10, 20).

Table 1. Response of shoot multiplication of AGRIA, NICOLA, SM12-124-15 and JIP 16-001 potato cultivars to different BA concentrations grown on MS medium after four weeks from inoculation

Number of shoots/ explant						
	BA (mg.l^{-1})					
Variety	**0.0**	**0.5**	**1.0**	**1.5**	**2.0**	**Means of cultivars**
AGRIA	**1.40 c**	**1.33 c**	**1.33 c**	**1.56 b**	**1.50 b**	**1.42 b**
NICOLA	**1.33 c**	**2.44 a**	**2.11 a**	**1.56 b**	**2.00 a**	**1.89 a**
SM 12-124-15	**1.44 c**	**1.73 b**	**1.44 c**	**1.11 c**	**1.18 c**	**1.38 b**
JIP 16-001	**1.78 b**	**1.80 b**	**1.80 b**	**1.54 b**	**2.10 a**	**1.80 a**
Means of BA	**1.49 c**	**1.83 a**	**1.67 b**	**1.44 c**	**1.70 b**	
Number of leave/ explant						
	BA (mg.l^{-1})					
Variety	**0.0**	**0.5**	**1.0**	**1.5**	**2.0**	**Means of cultivars**
AGRIA	**5.90 f**	**6.11 ef**	**6.00 ef**	**7.00 de**	**5.50 f**	**6.10 b**
NICOLA	**15.56 a**	**17.67 a**	**12.80 b**	**10.00 c**	**10.33 c**	**13.27 a**
SM 12-124-15	**11.20 b**	**5.30 f**	**4.78 g**	**5.44 f**	**6.11 b**	**6.57 b**
JIP 16-001	**10.78 bc**	**6.30 e**	**5.00 f**	**4.80 f**	**7.89 d**	**6.95 b**
Means of BA	**10.86 a**	**8.85 b**	**7.15 c**	**6.81 d**	**7.46 c**	
Mean length of shoots/ explant (cm)						
	BA (mg.l^{-1})					
Variety	**0.0**	**0.5**	**1.0**	**1.5**	**2.0**	**Means of cultivars**
AGRIA	**4.78 c**	**4.56 c**	**3.00 e**	**4.50 c**	**4.25 cd**	**4.22 a**
NICOLA	**7.06 a**	**4.00 d**	**3.11 e**	**2.83 ef**	**2.44 f**	**3.89 b**
SM 12-124-15	**7.10 a**	**2.50 f**	**2.44 f**	**2.61 f**	**4.11 d**	**3.75 b**
JIP 16-001	**5.85 b**	**3.33 e**	**3.30 e**	**2.80 ef**	**2.70 f**	**3.60 b**
Means of BA	**6.20 a**	**3.60 b**	**2.96 b**	**3.19 b**	**3.38 b**	

Table 2. Root formation response of AGRIA, NICOLA, SM12-124-15 and JIP 16-001 potato cultivars to growth regulator-free MS medium after four weeks from inoculation

Variety	**Rooting percentage (%)**	**Number of roots/ explant**	**Mean length of roots (cm)**
AGRIA	**100**	**7.90 b**	**9.70 a**
NICOLA	**100**	**7.50 b**	**3.95 d**
SM 12-124-15	**100**	**6.70 c**	**5.20 c**
JIP 16-001	**100**	**8.80 a**	**7.85 b**

Table 3. Potato minitubers production in greenhouse after 75 days of growing in peatmoss

Variety	**Number of minitubers per plant**	**Weight of each minituber (g)**	**Mean length of each minituber (cm)**	**Mean width of each minituber (cm)**
AGRIA	**5.78 b**	**6.98 a**	**3.60 a**	**1.80 a**
NICOLA	**8.87 a**	**5.55 c**	**2.94 b**	**1.36 a**
SM 12-124-15	**4.69 c**	**5.88 b**	**3.63 a**	**1.56 a**
JIP 16-001	**4.42 c**	**6.01 b**	**3.54 a**	**1.42 a**

Table 4. Potato Trial Field Experiment after 100 days from planting

Variety	Number of tubers per plant	Mean weight of tubers (g)	Weight of Tubers per plant (kg)	Mean length of tubers (cm)	Mean width of tubers (cm)	Yield per hectare (Ton)
AGRIA	8.13 b	212.18 d	1.725 d	5.34 c	2.23 b	23.70 d
NICOLA	11.44 a	263.55 c	3.015 b	6.86 b	3.11 a	41.50 b
SM 12-124-15	11.25 a	321.33 a	3.615 a	8.22 a	3.43 a	49.70 a
JIP 16-001	8.25 b	294.55 b	2.430 c	6.67 b	3.23 a	33.40 c

Figure 1. different stages of potato minitubers production:
A. shoot multiplication stage grown in bigger disposable growing containers under under in vitro condition.
B. microtubers initiated on SM 12-124-15 cultivar.
C. potato plantlets growing in green house after 10 weeks from acclimatization.
D. potato plantlets after 10 days ready for minitubers harvest.

Figure2. intact potato plantlets after 25 days growing in MS culture in primary growth area (ready for acclimatization). From left to right : agrai,jip 16001,SM 12-124-15 and Nicola cultivars.

Figure3. harvested potato minitubers after 100 days from growing in greenhouse.

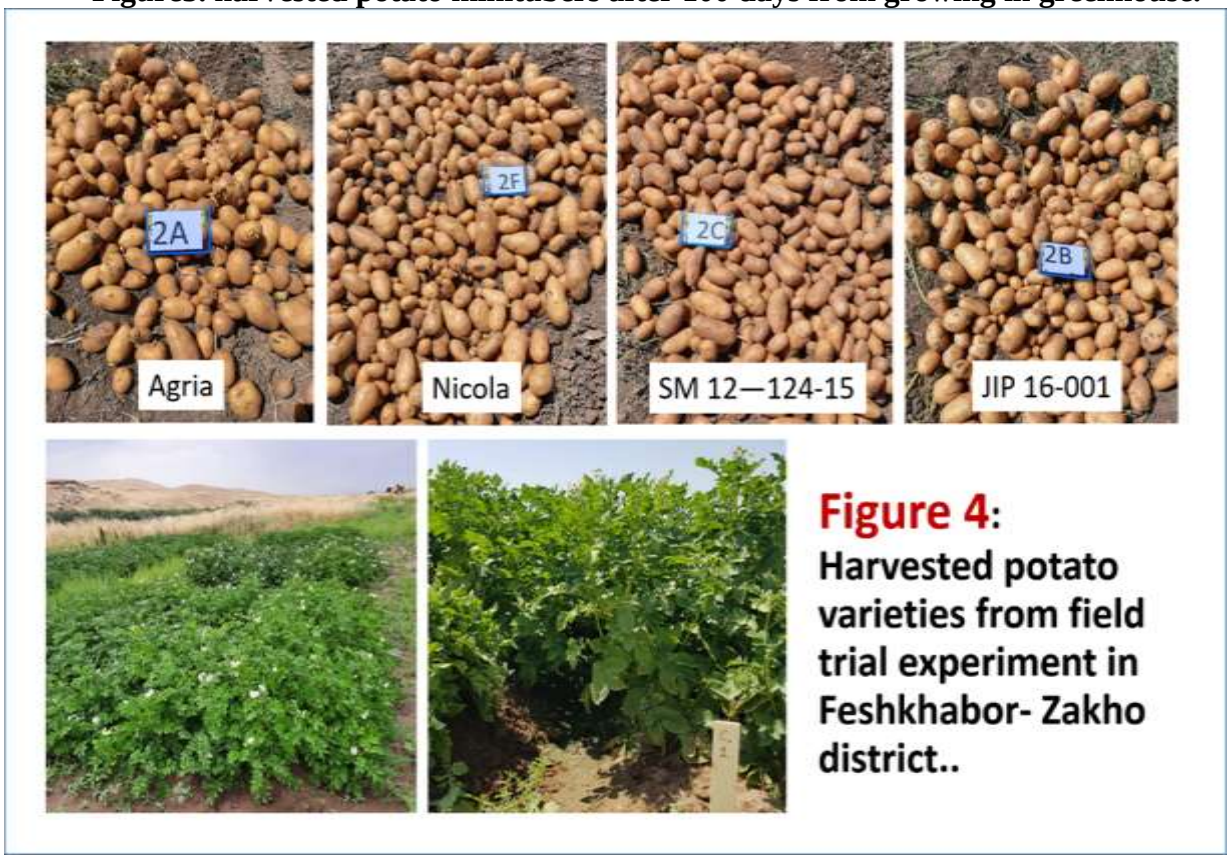

Figure4. harvested potato varieties from from field trial in feshabor- zakho district..

CONCLUSIONS

The results obtained in this study indicate that potato minitubers could be produced locally in Iraqi Kurdistan Region via tissue culture technique. It was found that Murashige and Skoog (MS) medium with basic vitamins and solidified by agar without the need of exogenous plant growth regulators addition

can be used for mass-propagation due to the kind of potato genotype *in vitro* under optimized culture conditions and conserving of money which is consumed for purchasing of the PGRs. The produced minitubers can perform well in the field to produce seed tubers within the standard characteristics. This reliable and successful micropropagation protocol can be applied in wider range to raise the production of well adopted potato cultivars locally and this will help in reducing the importing of foreign potato seed tubers annually. This will highly reflects on reducing production costs and raising the local farmer annually income. The interesting result was that Nicola cultivar was superior upon other cultivars under *in vitro* and greenhouse conditions whereas, SM 12-124-15 was better in the open field conditions.

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