Effect of explant age and applied IBA on growth and rooting of apical stem cuttings of potato for early generation seed potato production

Abstract

The aim of this work is to study the effect of explant age and exogenously applied IBA on the growth and rooting potential of potato apical stem cuttings for production of first-generation seed potatoes. The experimental protocol consists in taking apical stem cuttings from potato plantlets at different physiological ages (30 and 60 days old) in interaction with the growth regulator indole-3-butyric acid (IBA) at different concentrations (100, 200, and 300 ppm) to study the production of potato minitubers. The results of the present study showed that mini-cuttings from young potato plants (30 days old) have a higher rooting capacity than mini-cuttings from mature plants. In addition, IBA concentrations, especially at 300 ppm, have a significant effect on most of the characteristics studied, e.g. rooting %, root number, root length, shoot length, and number of leaves. Regarding yield attributes, application of IBA at 300ppm gave the highest average tuber number (6.66) and tuber yield (142.97g) was higher in 30days old explant cuttings. The data presented in this study may encourage potato growers to use potato stem cuttings as a new, cheaper method of growing potatoes instead of expensive imported seed tubers. It is suggested that the use of apical stem cuttings can be a quick and effective method for mass propagation and to increase the yield of minitubers in the seed potato production cycle.

Key-words: Rooting response, shoot tip cuttings, mother plant age, Indole butyric acid, potato minitubers, Rapid Multiplication Technique (RMT)

Introduction

"As a major food crop, potato ranks fourth in terms of production in the world. It is stapled food crop in some countries and others used it as a vegetable" (Mahmood 2005; Zamil *et al* 2010). Potato tubers provide vitamin C, potassium, and dietary fiber in the diet (McGill et al. 2013) and potato flesh is a good source of essential nutrients like B1, B2, B6, B9, some antioxidants and several trace elements (Khalid et al., 2020). It is an important food and cash crop in Pakistan. In Pakistan, potato crops covered 234.4 thousand hectares of area and gave 4,681.0 thousand tons of production (Pakistan Economic Survey 2020-21). "Although potato production in Pakistan has increased many folds, its per acre yield is far less than in other parts of the world. Among the various factors responsible for low per-acre potato production, the unavailability of certified seed is the most important one" (Qasim *et al*., 2013).

Usually, three potato crops i.e spring, summer, and autumn are grown in different agro-ecological zones of Pakistan ranging from plains to hilly areas (Khan & Akhtar, 2006). Potato seed production in Pakistan is informal and usually recycled from previous crop harvests. Currently, the common method for potato propagation is through tubers. Seed selection is basically on size with small ones being preferred due to their less market demand. Seed potato production by using a conventional system has not been effective in eluding or reducing the buildup of diseases which consequently led to reduced seed quality and lower tuber yields

(Chindi et al., 2014). Gildemacher et al. (2009); Nyende et al. (2005) reported that seed recycling and monoculture contributes to the accumulation of seed-borne diseases in potato in subsequent seasons, resulting in losses in both tuber quality and yield over seasons. The use of seed potatoes infected by fungi, bacteria, and/or viruses reduces the potential yield of the crop as a result of the progressive degeneration caused by such pathogens during successive field generations which contributes to disease build-up. This requires the regular renewal of the seed potatoes (Bisognin et al., 2015). A critical constraint in meeting increasing demand is the non-availability of the high-quality seed of most adapted varieties with considerable resistance to pests and diseases.

Certified seed production is an essential factor for higher and disease-free seed potato production. One of the attempts to multiply potato seeds quickly and in larger quantities is by rapid propagation techniques. Harahagazwe et al. (2018) illustrated that for seed potato production, Rapid Multiplication Techniques (RMT) can be carried out with in vitro plantlets and micro-tubers production, or can also be practiced with in vivo production of cuttings and minitubers. Yasmin and Zakaria (2019) stated that potato propagation materials like sprout and shoot tip cuttings or nodal segments with at least one bud and true potato seeds can be used.

Rooted apical stem cuttings in the greenhouse are the easiest and cheapest means of propagating potatoes (Struik and Wiersema, 1999) for minitubers production as an initial step in certified seed production. They have faster regeneration potential and are true to type. Their ability to regenerate rapidly gives them great potential for the conservation of potato clones and the production of potato seed. If rooted apical stem cuttings originate from true-to-type and pathogen-free planting material, they serve as an efficient means of producing basic seed. Dahshan, et al. (2018) studied the possibility to propagate potatoes through stem cuttings and enhance the growth capabilities of these cutting by using plant growth regulators (PGR) e.g., IBA. Each physiological process in plants is usually regulated by a number of defined hormones, some of them often exerting opposite effects. In potatoes, for example, distinct hormones (IAA, cytokinins, and abscisic acid stimulate some tuberization stages (Kolachevskaya et al., 2018).

Ishartati and Rehan (2019) reported that potato seeds can be procured through cuttings by treating the cuttings with plant hormones that encourage and accelerate the formation of roots, and new shoots, and enhance the quality and number of shoots and roots.

Plant growth of apical stem cuttings of white potato derived from disease-free G_0 plants for mass production of potato G1 seed tubers was demonstrated by Nikmatullah et al. (2018). Different cutting lengths, Indole Acetic Acid (IAA) concentrations, and age of mother plants were evaluated for cutting's growth and survival rate. The highest survival rate and cutting growth were obtained from apical cuttings taken from two to three weeks old mother plants treated with 1 ppm IAA.

"Cuttings obtained from tissue-cultured plantlets are seen as an alternative to reduce the number of plantlets that are cultivated *in vitro* and then used in the production of potato mini-tubers. This technique consists of rooting the mini-cuttings taken from newly acclimatized plants. The nutritional status of the plant and the degree of juvenility of propagules may influence the adventitious rooting of potato mini-cuttings" (Silva et al., 2011). "The degree of juvenility of propagules can also contribute to rhizogenesis, as a result of the morphological and physiological changes that occur during plant development" (Hackett, 1987).

Searching for other means to propagate potatoes is very crucible nowadays because of the very high prices of seed tubers. The present research work has been conducted to assess the effect of the physiological age of the mother plant on the rooting capability of apical mini cuttings of potato clones under Greenhouse conditions and to evaluate the effect of various concentrations of IBA on their rooting performance.

Materials and Methods

Experimental Site/location:

This study was conducted in the Green House at Hazara Agriculture Research Station, Abbottabad.

Experimental layout:

In the experiment, the propagated material consisted of potato plants' apical mini-cuttings (2-2.5cm). The mini-cuttings were taken from mother explants obtained from *in vitro* micro-propagated plantlets and were transplanted in the greenhouse.

The cuttings were planted in sterilized peat moss soil under greenhouse conditions (Fig. 1). For rooting, IBA was used at different concentrations(100 ppm, 200 ppm, and 300 ppm). The experiment was conducted in a 3 × 2 factorial arrangement with factor one was IBA concentrations; and factor two was the physiological age of the explant counted from the time of transplantation in the greenhouse (30 and 60 days old) having three replications with fifteen mini-cuttings in each replication.

Data on the number of days for root initiation, root number, root length, shoot length, the number of leaves, and the average number of tubers/plant and tuber yield were recorded. The data were subjected to analysis of variance for the F test and the means were compared by the Least significant difference test (LSD) at a 5% probability of error with the aid of computer software Statistix 8 Version 8.1 (Analytical Software, 2005).

Results and Discussion

Root Initiation

For recording data on root initiation, the apical stem cuttings were uprooted and observed. No roots were recorded in cuttings of both 30 days and 60 days old explant after 10 days of plantation (Table 1). The uprooted cuttings were replanted for the next time observation. All the cuttings were watered regularly to keep the soil moist for rapid root initiation.

Figure 1: Apical mini cuttings planted in peat moss soil in the greenhouse after treatment with different IBA concentrations.

Table 1: Percent means root initiation in potato apical cuttings from 30 and 60 Days old mother plants treated with different IBA concentrations.

IBA conc.	% Root Initiation					
	After 10 days		After 15 days		After 20 days	
	30 Days old	60 Days old	30 Days old	60Days old	30Days old	60 Days old
100ppm	0	0	53.33bc	33.33c	83.33a	66.67b
200ppm	0	0	86.67a	60b	100a	93.33a
300ppm	0	0	100 a	86.67a	100a	100a

Means followed by different letters are significantly different at $P \leq 0.05$

After 15 days of the plantation, cuttings from 30 days old mother plants showed 100% root initiation at 300ppm IBA concentration (Fig. 2) followed by 86.67% at 200ppm while significantly lower rooting % was recorded in the IBA treatment of 100 ppm (Table 1). Similarly, in the 60 days old explant cuttings the highest mean rooting % after 15 days of the plantation was recorded at 300 ppm IBA i.e 86.67 %. While the lowest 33.33% was found at the lowest IBA concentration (Table 1). The results here are consistent with those of Hossain et al. (1998) who observed that root induction in the potato stem cuttings was quicker at a higher IBA concentration than at lower IBA concentrations (« 50 g L^{-1}).

Nizam Uddin *et al.*, (2005) also reported similar findings that the maximum (81.66 %; 81.67 %) number of cuttings of MF-I and LT-8 potato lines, respectively induced roots at higher IBA concentrations which were significantly higher than the percentage of roots produced by cuttings treated with 100 ppm IBA.

After 20 days of transplantation, an increase in rooting percentage has been observed in both age groups at all three IBA concentrations (Table 1). However, the rooting % was comparatively higher in cuttings taken from plants at younger growth stages i.e 30 days old. Regarding IBA concentrations at 300ppm 100% rooting was recorded in both 30 days old and 60 days old plant cuttings while at 100ppm 83.33% and 66.67% rooting were found in 30 days old and 60 days old plant cuttings respectively (Table 1). Rasmussen *et al.* (2015) stated that apical cuttings of potato plants derived from 2 and 3 week old mother plants had faster root and shoot growth and a higher survival rate. The age of the mother plants determines the maturity of the stem from which the cuttings are taken: maturity increases as the physiological age of the mother plant increases. Juvenile cuttings have been shown to root better than mature cuttings possibly due increase of lignification in older cutting and the production of a rooting inhibitor as the stem age increases (Milborrow, 1994)

Figure 2: Root initiation in the apical cuttings.

Root number:

Eight cuttings were randomly selected in each replication of every treatment combination. Regarding root number, after 15 days it was observed that cuttings of younger age mother plants i.e 30 days old had more root numbers as compared to 60 days old mother plant at all three IBA concentrations (Table 2). Further, the different concentrations of IBA also have a significant effect on root number and at 300 ppm concentration, more roots were recorded i.e. 7.53 and 5.50 mean number of roots in 30 days and 60 days old mother plant cuttings, respectively, while at the lowest concentration i.e 100 ppm only 2.50; 2.33 mean root number was recorded in both 30 days and 60 days old age group cuttings (Table 2). A similar trend in the increase in mean root number was observed after 30 days of plantation in cuttings of both age groups at all three IBA concentrations (Table 2). According to Davis and Hassig (1990), the production of adventitious roots in plants through cell division, multiplication, and differentiation is also controlled by plant growth substances, especially auxins. This implies that treating stem cuttings with IBA can not only increase the rooting percentage but also root initiation and the number of roots. Various research experiments have confirmed that the application of optimal hormone concentration is very important for the successful rooting of cuttings (Leakey et al., 1982; Ezzat, 2016). IBA treatment of stem cuttings may cause cambial activity that resulted in root induction and ultimately an increase in the number of roots per cutting. Nymora and Mnzwa (1983) illustrated that IBA increased the elasticity of cambium and thus accelerated cell division and produced more roots in treated cuttings. However, regarding the physiological age of mother plants comparatively, no significant difference in mean root number was seen after 30 days of transplantation (Table 2).

Table 2: Mean number of roots and root length in potato apical cuttings of 30 and 60 Days old mother plants treated with different IBA concentrations after 15 and 30 days of the plantation.

IBA conc.	Root number				Root length (cm)			
	After 15 days		After 30 days		After 15 days		After 30 Days	
	30 Days old	60Days old	30 Days old	60 Days old	30 Days old	60 Days old	30Days old	60Days old
100 ppm	2.50d	2.33d	8.46b	8.46b	0.93b	0.21c	4.05a	2.86b
200 ppm	5.05b	3.58c	10.73b	8.80b	0.96b	0.73bc	4.03a	3.76ab
300 ppm	7.53a	5.50b	15.8a	15a	1.93a	1.66a	4,26a	3.60ab

Means followed by different letters are significantly different at P≤0.05

Root length:

Data showed that root length recorded in 30 days old explant cuttings after 15 days of plantation is significantly higher than 60 days old explant cuttings (Table 2). After 15 days of plantation regarding IBA concentrations, it is stated that higher IBA concentrations caused an increase in root length in both age groups with the highest 1.93cm and 1.66cm length recorded at 300ppm in 30days old and 60 days old explant cuttings, respectively (Table 2). In apple (Badshah at al., 1995) and Apricot (Ishtiaq at al., 1988) it was reported that root length greatly increased with increasing IBA concentration. The increase in root length may be due to the effect of IBA on metabolic translocation and increased plasticity of the cell wall of cambium cells as reported by Nymora and Mnzwa 1983; Ishtiaq et al., 1989. The results are also supported by the findings of El-Gamal (1992) who dipped stem cuttings of potato in potassium of indole butyric acid solution at 6000 ppm for one minute and demonstrated that this treatment succeeded to stimulate adventitious roots and affected the production and growth parameters of potato e.g., fresh and dry weights of roots. After 30 days of transplantation, a considerable increase in average root length has been found in all the IBA treatments for both age group cuttings (Table 2).

Table 3: Mean numbers of new fresh leaves and shoot length in potato apical cuttings of 30 and 60 Days old mother plants treated with different IBA concentrations.

IBA conc.	Shoot length (cm)				Leaf numbers			
	After 30 days		After 50 days		After 30 days		After 50 Days	
	30 Days old	60 Days old	30 Days old	60 Days old	30 Days old	60 Days old	30 Days old	60 Days old
100	1.17c	1.2c	8.63c	8.1c	2.1b	1.3a	10.6bc	9.3c

ppm								
200 ppm	1.96ab	0.85c	9.16bc	8.2c	2.6ab	2.4ab	11.5b	9.8bc
300 ppm	2.45a	1.38bc	11.5a	10.6ab	2.9ab	2.4ab	14.3a	11.3b

Means followed by different letters are significantly different at $P \leq 0.05$

Shoot length:

Vegetative data presented in Table 3 revealed that plantlets developed from cuttings taken from 30 days old explants have greater shoot length as compared to plantlets developed from 60 days old explant cuttings. After 30 days of plantation of cuttings, an increase in shoot length was observed in both age groups and the growth rate was comparatively higher in the cuttings taken from younger age explants i.e 30 days old. This may be due to the fact that the mother plant age determines the maturity of the stem from which the cuttings are taken. The observations reported by Nikmatullah et al. (2018) also support our results who found that apical cuttings of potato plants derived from 2 and 3 week old mother plants had faster root and shoot growth and a higher survival rate. The maximum mean shoot length (2.45cm; 1.38cm) was obtained at the highest IBA concentration i.e. at 300ppm in both the age group cuttings i.e. 30 days old and 60 days old, respectively (Table 3). A similar trend in the increase in the growth rate of the cuttings was recorded after 50 days of plantation (Table 3). The results are in agreement with the findings of Dahshan et al. (2018) who showed that treated potato cv Lady Rosetta with 6000 ppm IBA significantly increased the stem cuttings length compared with other treatments. Tsoka et al. (2012) also reported higher mean plant height and root length in potato plants derived from apical stem cuttings under the aeroponic system.

Leaf number:

Data representing mean leaf number showed that at 100ppm IBA concentration the average mean leaf number was lower in both age group cuttings and a considerable increase in mean leaf number has been recorded with the increase in IBA concentration after 30 days and 50 days of plantation (Table 3). Regarding the age of the mother plant, it was found that the mean leaf number was higher in cuttings taken from 30 days old explant as compared to 60 days old explant cuttings (Table 3). The results are consistent with the findings of Bhatia et al., 1992 who recorded the superiority of the application of IBA over other plant growth regulators for plant height, the number of branches and the number of leaves in potatoes and gave the highest dry weight of foliage. Similarly Zaman et al., (2001) concluded from an *in vitro* study that higher the concentration of auxin (IBA) level will results in production of highest number of leaves per plant.

Tuber number and average yield:

Regarding yield data minitubers were first categorized into three categories based on weight i.e large > 20g; medium 10-20g; small < 10g (Fig. 3). Statistical analysis of the recorded data revealed that the average mean tuber number was higher in cuttings of 30 days old mother plant as compared to 60 days old (Table 4) (Fig. 4) at all the three concentrations of IBA, however, among the three IBA concentrations the average tuber number was higher at 300ppm IBA concentration i.e 6.66 and 4.33 followed by 5.00 and 3.33mean tuber numbers at 200ppm in cuttings of 30 days old and 60 days old age groups, respectively (Table 4). Moreover, it was observed that there was a greater number of small tubers produced at 300ppm concentration (Table 4). The results of our study are supported by the findings of Dahshan et al. (2018) who declared that the use of growth regulators especially in high concentration resulted in higher values of the mostly studied potato plant characteristics e.g plant height, plant fresh and dry weights, the number of tubers per plant and total tuber yield per plant as compared to the control. The average tuber yield per plant was higher (142.97g) in 30 days old age group at 300ppm IBA concentration. The lowest average tuber yield i.e 71.54g was recorded at 100ppm IBA concentration in 60 days old mother plant age group. Sillu et al. (2012) sprayed IBA at concentrations of 100 and 200ppm on potato foliage and found the maximum number of tubers (6.87) per plant, average weight of tuber (69.14 g) and yield of tuber (258.02 g) at higher IBA concentration (200 ppm).

Table 4: Mean tuber number and average yield per plant in potato apical cuttings taken from 30 and 60 Days old mother plants treated with different IBA concentrations.

IBA conc.	Mean Tuber number								Av. Yield per plant (g)	
	30 Days old				60 Days old				30 Days old	60Days old
	large	medium	small	Total	large	medium	small	Total		
100 ppm	1.00a	0.33b	2.66b	4.00bcd	0.33ab	0.33b	2.66b	3.00d	119.67ab	71.54c
200 ppm	0.33ab	1.00ab	3.67ab	5.00b	0.66ab	0.00b	2.66b	3.33cd	101.86bc	78.04c
300 ppm	0.00b	1.66a	5.00a	6.66a	0.00b	0.66ab	3.66ab	4.33bc	142.97a	87.17bc

Means followed by different letters are significantly different at $P \leq 0.05$

Figure 3: The three tuber categories based on weight i.e large > 20g; medium 10-20g; small < 10g (left to right).

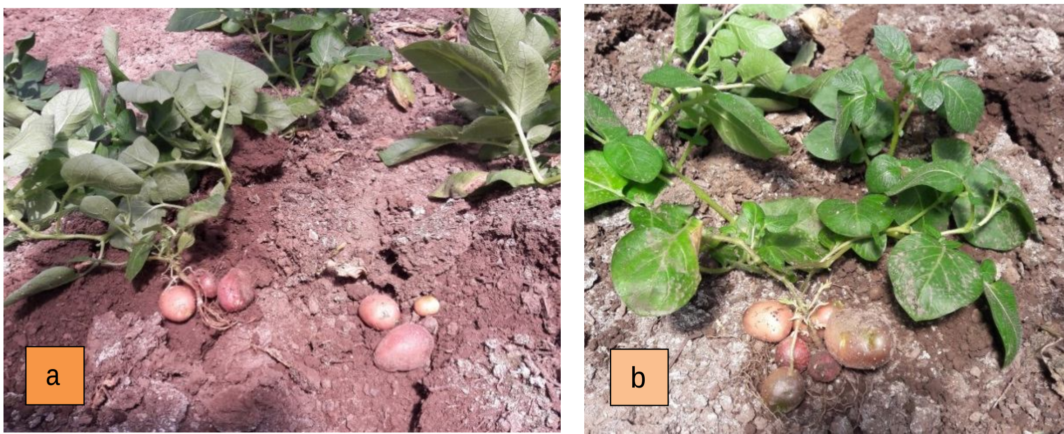

Figure 4: Tubers harvested in 60 days old mother plant cuttings (a); tubers from 30 days old age group (b).

Conclusion and recommendation:

Results of the present study declared that mini-cuttings from young *in vitro* grown potato plants have higher rooting capacity compared to mini-cuttings from mature plants. Moreover, IBA concentrations especially at higher level i.e 300 ppm has a significant effect on most studied characteristics e.g., root number, shoot length and tubers yield.

The findings of this study may encourage potato growers to use potato stem tip cuttings as a new and cheaper method to grow potato instead of expensive imported seed tubers. It is suggested that the use of apical stem cuttings can be a fast and efficient method for mass propagation and to increase the yield of minitubers in the seed potato production cycle.

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