Pak. J. Agri. Sci., Vol. 58(4), xxx-xxx; 2021
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/21.398
http://www.pakjas.com.pk

Interaction of genetic variability × phytohormonal composition and concentration may influence speed of *in vitro* micropropagation of potato (*Solanum tuberosum* L.)

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Potato (*Solanum tuberosum* L.) is a staple food for more than a billion people and is becoming increasingly popular in the developing world. To meet this progressive demand and sustained productivity a higher level of genetic diversity is required in this crop so that it could cope with new threats from pests, disease, heat, and drought. However, creating a new potato variety is slow and difficult process even by the patient standards of plant breeders. Tissue culture is a promising alternative technique to create rapid genetic variability and multiplication in asexually propagated crops like potato. Thus, reported study was planned to investigate regeneration response of nodal cuttings of two potato varieties i.e., Lal Moti and Lady Rosita to different concentrations of hormonal supplements. Under two concentrations (1mg/L and 2 mg/L) of 6-benzyl amino purine (BAP), four treatments each of kinetin and Indole-3-acetic acid (IAA) were used for shoot/root induction. The results exhibited that Lady Rosita performed better than Lal Moti in shoot formation and gained maximum shoot length (13.08 cm) at concentration of 1mg/L each for BAP and kinetin. Regarding root formation, Lal Moti performed better than Lady Rosita and attained maximum root length (8.34 cm) when 1 mg/L BAP was used with 1mg/L of IAA. Taken together our findings suggested that the above-mentioned combinations of reported phytohormones with BAP are excellent for shoot/ root induction in Lady Rosita and Lal Moti potato varieties. Furthermore, these protocols may be utilized for rapid *in vitro* multiplication of disease-free potato plantlets.

Keywords: BAP, Phytohormones, IAA, regeneration, lady Rosita, lal Moti.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important non cereal food crop of the world. It is a root vegetable used as a staple food. Potato feeds 80% of the world's population because it grows well in poor soils (FAO, 2019). It is a cool weather crop. It is the cheapest source of carbohydrates, protein and other essential nutrients. It contains 2% protein, 80% water and 18% starch (Kumar *et al*., 2015, Naumann *et al*., 2020). Potato tubers are used as food, feed and for industrial purpose (Khan *et al*., 2013, Javed *et al*., 2019). It is herbaceous short stature annual plant. In Pakistan, it is grown in all three seasons (spring, summer and autumn) across the country. China is the leading country in production of potato while Pakistan is included in top 25 potato producing countries and its total production of potato is about 4578.9 tonnes (FAO, 2019). It is the third most important crop that is more consumed after wheat and rice in the country (Anwar *et al*., 2015). Due to rapid increase in its consumption, it is difficult to sustain required level of its production especially with new threats from pests, diseases, heat, and drought. Production of potato has already decreased by 5.5% (FAO, 2019). There are many factors that are responsible for the low production of potato i.e., unavailability of registered genetically diverse good quality seed, attack of viral, bacterial and fungal diseases and insect pests triggered by climatic catastrophe.

Akhtar N., S. Shaukat, Z. Iqbal, A. Aziz, T. Mahmood, M. Asif, N. Raza, S. Fatima, R. Shukat, I. Kousar, A. Ali[2] and M. Ramzan. 2021. Interaction of genetic variability × phytohormonal composition and concentration may influence speed of *in vitro* micropropagation of potato (*Solanum tuberosum* L.)Pak. J. Agri. Sci., Vol. 58(4), xxx-xxx; 2021.
[Received 24 Mar 2021; Accepted 17 June 2021; Published (online) xx Xxx 2021]

Diseases are the major reason for low production of potato (Miassar *et al*., 2011). There are approximately 160 wild potato species present all over the world but cultivated species have low genetic potential (Hawkes, 1978). Commercially cultivated varieties of potato are tetraploid (2n=4×=48) and heterozygous in nature with inheritance (Aaaa) for many traits. Some cultivars do not give flower easily, these cultivars either have low fertility, or they have pollen sterility which cause reduction of number of crosses. Genetic variation is possible through conventional and non-conventional breeding methods in potato (Miassar *et al*., 2011). There are many methods to increase the production of potato among them micro-propagation is popular method for rapid potato reproduction. It is used to develop disease fee plantlets (Badoni, 2009). For multiplication and production of potato seed tubers are required but it is too much costly and slow method for multiplication. One of the most important and promising industrially important biotechnological tools is plant tissue culturing technique for plant improvement and propagation in relatively short time period (Hussain *et al*., 2012, Zheng *et al*., 2019). It provides better opportunity to prepare disease free plants in a short period of time if regeneration protocols are established by standardizing different steps including concentration of phytohormones. Regeneration potential of potato depends upon genotypic background (Mohapatra *et al*., 2017) of the cultivar and media interaction which is most probably controlled by phytohormone concentration (Bajaj, 2012). For *in vitro* regeneration of potato different types of protocol has been developed by different scientists (Badoni and Chauhan 2010, Koleva *et al*., 2012, Kaur *et al*., 2018 and Morais *et al*., 2018) which needs to be further standardized. Hence, the present study was designed to investigate *in vitro* micro-propagation response of two potato varieties under different concentrations of growth regulators and to develop a standard protocol for rapid multiplication of potato varieties.

MATERIALS AND METHODS

The experiment was conducted in tissue culture laboratory, College of Agriculture, University of Sargodha, Pakistan. Two promising potato varieties Lal Moti and Lady Rosita were chosen as ex-plants source from available potato germplasm in the Department of Plant Breeding and Genetics. Explants were taken from the field grown 3-4 weeks old potato plants when the plants were started to develop branches. Auxiliary buds, terminal buds and leaf internodes were cut from selected plants with the help of scissors and used as explants. Propagules were washed with distilled water to remove contamination. After washing explants were dipped in 10% Clorox for 3 minutes and rinsed three times with double distilled water to remove the traces of bleach. After this, explants were dipped in 99% ethanol for 5 minutes and washed buds three times with double distilled water in order to remove the traces of ethanol.

Culture media recipe as described by Murashiage and Skhoog (1962) was used in this experiment for potato auxiliary buds culturing. Different concentrations of studied growth regulators (BAP, Kinetin and IAA) were used along with MS medium to generate shoots/roots of potato plants. The combinations were as following:

1. Treatment combinations of BAP and kinetin for shoot regeneration

Dose 1 (BAP)= 1mg/L
T1=MS+1mg/L BAP+0.5mg/L KIN
T2=MS+1mg/L BAP+1mg/L KIN
T3=MS+1mg/L BAP+1.5mg/L KIN
T4=MS+1mg/L BAP+2mg/L KIN
Dose 2 (BAP)=2mg/L
T1=MS+2mg/L BAP+0.5mg/L KIN
T2=MS+2mg/L BAP+1mg/L KIN
T3=MS+2mg/L BAP+1.5mg/L KIN
T4=MS+2mg/L BAP+2mg/L KIN

2. Treatment combinations of BAP and IAA for root formation

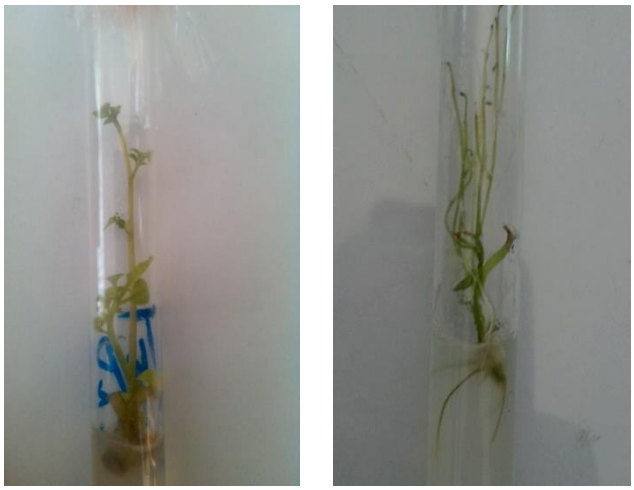
Dose 1 (BAP)= 1mg/L
T1=MS+1 mg/L BAP+0.5mg/L IAA
T2=MS+1 mg/L BAP+1mg/L IAA
T3=MS+1 mg/L BAP+1.5mg/L IAA
T4=MS+1mg/L BAP+2mg/L IAA
Dose 2 (BAP)=2mg/L
T1=MS+2mg/L BAP+0.5mg/L IAA
T2=MS+2mg/L BAP+1mg/L IAA
T3=MS+2mg/L BAP+1.5mg/L IAA
T4=MS+2mg/L BAP+2mg/L IAA

These growth conditions were controlled by keeping temperature range 22 ºC - 25 ºC and humidity 65%. Growth room was kept free from any type of contaminations either aerial or material borne by following standard operating procedures. The wrapped test tubes were placed in the growth room under light for 21 days and data on different growth parameters was recorded.

Regarding shoots development, potato plant axillary buds were used as explants in MS medium having different concentrations of BAP and kinetin in different combinations. After shoot formation sub-culturing was performed in MS media having different concentrations of IAA and BAP for initiation of roots. Data was recorded for Shoot length (cm), Number of shoots, Root length (cm) and Number of roots.

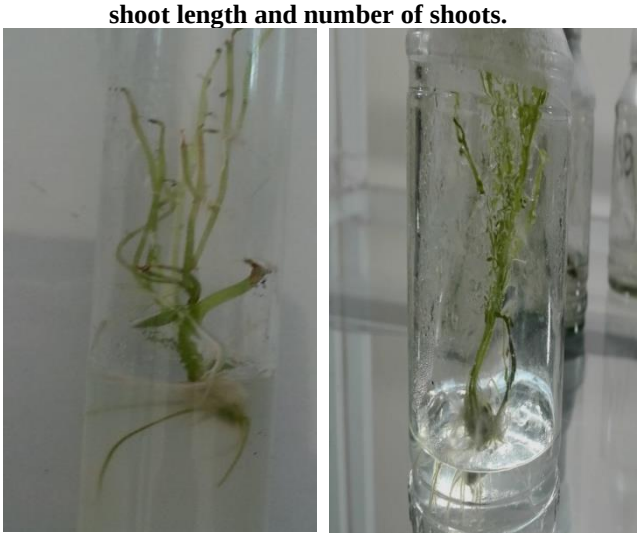
Experimental Layout and Statistical analysis: *In vitro* experiment was conducted by using completely randomized design (CRD) with three factors factorial arrangements having four treatments of each growth regulator for shoot and root induction separately. Each treatment was repeated three times with ten sub units in each repeat on Lady Rosita and Lal Moti potato varieties. The data were statistically analyzed using statistic 8.1. software. ANOVA was computed through

analysis of variance technique (Steel *et al*. 1997) and Tuckey's test was also applied for the comparisons of treatment means to observe significant difference between means at probability level of P<0.01.



Lady Rosita **Lal Moti**

Figure 1. MS media containing BAP and Kinetin for shoot length and number of shoots.



Lady Rosita **Lal moti**

Figure 2. MS media containing BAP and Indole acetic acid for root length and number of roots.

RESULTS

***Effect of BAP doses and kinetin treatments on potato varieties for shoot length (cm)*:** Analysis of variance for shoot length showed that potato varieties (Lal Moti and Lady Rosita) and 6-benzyl amino purine (BAP) doses showed highly significant differences (P<0.01) while effect of Kinetin treatments on shoot length formation was non-significant (P>0.05). The interactive effect of all these chemical combinations with potato varieties, it was observed that the variety × BAP and variety × kinetin showed highly significant results while BAP × kinetin and variety × BAP × kinetin interactions revealed non-significant differences for shoot length (Table 1). The statistical means comparison of data presented in (Table 1a) indicated that low concentration of BAP (1mg/L) gave excellent results for shoot length and Lady Rosita gained more shoot length (10.97 cm) than Lal Moti. The means comparison between potato varieties and different kinetin treatments showed that Lady Rosita gained maximum shoot length (11.53cm) at 0.5 mg/L of kinetin in MS media while Lal Moti attained only 10cm shoot length at this dose.

Table 1. Analysis of variance for shoot length in two potato varieties using BAP and Kinetin

SOV	DF	MS	F	P
Varieties	1	167.627	42.89	0.0000
BAP	1	75.752	19.38	0.0001
Kinetin	3	7.471	1.91	0.1476
Variety × BAP	1	69.360	17.75	0.0002
Variety × Kinetin	3	110.526	28.28	0.0000
BAP × Kinetin	3	3.401	0.87	0.4666
Variety × BAP × Kinetin	3	3.479	0.89	0.4567
Error	32	3.908		
Total	47			

Note: Significant (P<0.05) and highly significant (P<0.01)

Table 1a. Means comparison between potato varieties and BAP doses for shoot length (cm).

Potato varieties	6-benzylaminopurine (BAP) doses	
	Dose 1 (1 mg/L)	Dose 2 (2 mg/L)
Lal Moti	4.838c	4.729c
Lady Rosita	10.979a	6.063b

Note: Values sharing the same letters are non-significant.

Table 1b. Means comparison of potato varieties and kinetin treatments for shoot length (cm).

Potato varieties	Kinetin treatments			
	T_1 (0.5 mg/L)	T_2 (1 mg/L)	T_3 (1.5 mg/L)	T_4 (2 mg/L)
Lal Moti	2.63e	10.07ab	4.50cd	2.33e
Lady Rosita	11.54a	10.21ab	7.00bc	5.33cd

Note: Values sharing the same letters are non-significant.

Table 1c. Mean comparisons of BAP doses and kinetin treatments for shoot length (cm)

BAP doses	Kinetin treatments			
	T_1 (0.5 mg/L)	T_2 (1 mg/L)	T_3 (1.5 mg/L)	T_4 (2 mg/L)
D_1 (1 mg/L)	7.63abc	7.83ab	7.50abc	8.47a
D_2 (2 mg/L)	6.54abc	4.71bc	4.00c	6.33abc

Note: Values sharing the same letters are non-significant.

Table 1d. Means comparison of varieties × BAP × Kinetin treatments interactions for shoot length (cm)

Variety x BAP	Kinetin treatments			
	T_1 (0.5 mg/L)	T_2 (1 mg/L)	T_3 (1.5 mg/L)	T_4 (2 mg/L)
Lal Moti x D_1	2.77e	2.58e	4.67b-e	9.33abc
Lal Moti x D_2	2.50e	10.00abc	4.33cde	2.08e
Lady Rosita x D_1	12.50a	13.08a	10.33ab	8.00a-e
Lady Rosita x D_2	10.58ab	7.33a-e	3.67de	2.67e

Note: Values sharing the same letters are non-significant.

Results revealed that increase in kinetin concentration decreased the shoot length. (Table 1b). Pertaining to means comparison between BAP doses and kinetin treatments, it was found that BAP at 1mg/L concentration along with 2mg/L kinetin gave the best results for shoot length with over all mean value of 8.47cm (Table 1c). Murashige and Skoog (MS) medium containing different concentrations of growth regulators used to observe variation existing in shoot length. Two potato varieties revealed that Lady Rosita gained maximum shoot length (13.08 cm) at 1mg/L each of BAP and kinetin while Lal Moti attained maximum shoot length (10 cm) at 2 mg/L of BAP and 1mg/L of kinetin (Table 1d).

***Effect of BAP doses and kinetin treatments on potato varieties for number of shoots*:** Number of shoots is an important plant trait to observe the differences between potato varieties. Analysis of variance table for number of shoots exhibited that 6-benzyl amino purine doses had highly significant effect ($P<0.01$) on potato varieties for this trait.

Table 2. Analysis of variance for number of shoots in two potato varieties using BAP and Kinetin

SOV	DF	MS	F	P
Varieties	1	6.02083	17.00	0.0002
BAP	1	1.68750	4.76	0.0365
Kinetin	3	0.02083	0.06	0.9810
Variety × BAP	1	2.52083	7.12	0.0119
Variety × Kinetin	3	3.63194	10.25	0.0001
BAP × Kinetin	3	0.07639	0.22	0.8848
Variety × BAP × Kinetin	3	0.02083	0.02	0.9810
Error	32	0.35417		
Total	47			

Note: Significant ($P<0.05$) and highly significant ($P<0.01$)

Table 2a. Means comparison between potato varieties and BAP doses for number of shoots

Potato varieties	6-benzylaminopurine (BAP) doses	
	Dose 1 (1 mg/L)	Dose 2 (2 mg/L)
Lal Moti	2.25b	2.90b
Lady Rosita	9.95a	10.99a

Note: Values sharing the same letters are non-significant.

BAP doses also depicted significant results for varieties while kinetin treatments individually showed non-significant ($P>0.05$) effects on potato varieties (Table 2). It is evident from the data that interactions between variety × BAP and variety × kinetin treatments displayed significant effect on potato genotypes for induction of shoots.

Table 2b. Means comparison of potato varieties and kinetin treatments for number of shoots

Potato varieties	Kinetin treatments			
	T_1 (0.5 mg/L)	T_2 (1 mg/L)	T_3 (1.5 mg/L)	T_4 (2 mg/L)
Lal Moti	2.08d	1.99d	3.58cd	2.63d
Lady Rosita	9.83ab	7.38bc	13.67a	11.00ab

Note: Values sharing the same letters are non-significant.

Table 2c. Mean comparisons of BAP doses and kinetin treatments for number of shoots

BAP doses	Kinetin treatments			
	T_1 (0.5 mg/L)	T_2 (1 mg/L)	T_3 (1.5 mg/L)	T_4 (2 mg/L)
D_1 (1 mg/L)	2.33a	2.23a	2.17a	2.17a
D_2 (2 mg/L)	1.83a	1.83a	1.83a	2.00a

Note: Values sharing the same letters are non-significant.

Table 2d. Means comparison of varieties × BAP × Kinetin treatments interaction for number of shoots

Variety x BAP	Kinetin treatments			
	T_1 (0.5 mg/L)	T_2 (1 mg/L)	T_3 (1.5 mg/L)	T_4 (2 mg/L)
Lal Moti x D_1	2.77e	2.58e	4.67b-e	9.33abc
Lal Moti x D_2	2.50e	2.08e	4.33cde	10.00abc
Lady Rosita x D_1	12.50a	13.08a	10.33ab	8.00a-e
Lady Rosita x D_2	10.58ab	7.33a-e	3.67de	2.67e

Note: Values sharing the same letters are non-significant.

The statistical comparison of means between BAP and varieties indicated that potato variety Lady Rosita attained more number of shoots (2.83) than Lal Moti (1.75) when 1mg/L of BAP was used in MS media (Table 2a). The means comparison between potato varieties and kinetin treatments revealed that Lady Rosita produced more number of shoots (2.83) at 0.5 mg/L of Kinetin as compared to Lal Moti which gave rise to 1.75 shoots (Table 2b). Regarding interaction between BAP doses and kinetin treatments it was accomplished that 1mg/L BAP+1mg/L kinetin induce more shoots with mean value of 2.33 (Table 2c). At this combination Lady Rosita developed more number of shoots than Lal Moti. Therefore, it was depicted that low

concentration of kinetin and BAP was good for development of shoots in potato when axillary buds, terminal bud and leaf internodes were used as explant in tissue culture (Table 2d).

***Effect of BAP doses and IAA treatments on potato varieties for root length (cm)*:** Analysis of variance results for root length indicated that potato varieties showed highly significant differences (P<0.01) for 6-benzyl amino purine doses and interaction between them. Indole acetic acid (IAA) treatments and its interaction with varieties and BAP exhibited non-significant differences (Table 3).

Table 3.Analysis of variance for root length in two potato varieties using BAP and Indole acetic acid.

SOV	DF	MS	F	P
Varieties	1	381.151	53.94	0.0000
BAP	1	148.544	21.02	0.0001
Kinetin	3	1.428	0.20	0.8942
Variety × BAP	1	267.719	37.89	0.0000
Variety × Kinetin	3	8.356	1.18	0.3319
BAP × Kinetin	3	11.325	1.60	0.2081
Variety × BAP × Kinetin	3	5.324	0.75	0.5285
Error	32	7.066		
Total	47			

Note: Significant (P<0.05) and highly significant (P<0.01)

Table 3a. Means comparison between potato varieties and BAP doses for root length (cm)

Potato varieties	6-benzylaminopurine (BAP) doses	
	Dose 1 (1 mg/L)	Dose 2 (2 mg/L)
Lal Moti	12.48a	3.33b
Lady Rosita	2.14c	4.24b

Note: Values sharing the same letters are non-significant.

Table 3b. Means comparison of potato varieties and IAA treatments for root length (cm)

Potato varieties	IAA treatments			
	T_1 (0.5 mg/L)	T_2 (1 mg/L)	T_3 (1.5 mg/L)	T_4 (2 mg/L)
Lal Moti	8.33a	9.98a	7.25abc	7.88ab
Lady Rosita	1.96d	2.91cd	3.04bcd	3.00bcd

Note: Values sharing the same letters are non-significant.

Table 3c. Mean comparisons of BAP doses and IAA treatments for root length (cm).

BAP doses	IAA treatments			
	T_1 (0.5 mg/L)	T_2 (1 mg/L)	T_3 (1.5 mg/L)	T_4 (2 mg/L)
D_1 (1 mg/L)	6.16ab	8.35a	7.83a	6.88ab
D_2 (2 mg/L)	4.30ab	5.08ab	2.46b	4.00ab

Note: Values sharing the same letters are non-significant.

Table 3d. Means comparison of varieties × BAP × IAA treatments interaction for root length (cm)

Variety x BAP	IAA treatments			
	T_1 (0.5 mg/L)	T_2 (1 mg/L)	T_3 (1.5 mg/L)	T_4 (2 mg/L)
Lal Moti x D_1	10.33a-d	15.33a	12.67ab	11.60abc
Lal Moti x D_2	4.63b-e	6.33b-e	1.83e	4.17cde
Lady Rosita x D_1	1.35e	1.98e	3.00de	2.17e
Lady Rosita x D_2	2.57de	3.83cde	3.08de	3.83cde

Note: Values sharing the same letters are non-significant.

In case of interaction of growth phytohormones on potato varieties, it was reported that Lal Moti performed well and developed 12.348cm mean root length than Lady Rosita which developed only 2.14 cm root length at 1mg/L of BAP (Table 3a). The interaction between potato varieties and IAA treatments exhibited that variety Lal Moti performed good (9.98 cm) as compared to variety Lady Rosita (2.91) at 1mg/L of IAA. The variation for root length in varieties was statistically non-significant which indicated that low concentrations of IAA were enough to get desired root length in potato (Table 3b). Interaction between BAP doses and IAA treatments displayed that 1mg/L BAP along with 1 mg/L IAA gave best results for potato roots length (Table 3c).

Regarding the interaction among three factors on root length i.e., varieties × BAP × IAA it was observed that Lal Moti performed better than Lady Rosita having 15.33 cm and 1.98 cm mean root length, respectively at 1mg/L BAP and 1 mg/L IAA (Table 3d).

***Effect of BAP doses and IAA treatments on potato varieties for number of roots*:** Number of roots just like shoots number is also an important plant trait to observe the differences between potato varieties. ANOVA table for number of roots exhibited that potato varieties were highly significantly (P<0.01) influenced by 6-benzyl amino purine doses. While IAA treatments alone displayed non-significant effect on potato varieties regarding number of roots initiation. This depicts that potato varieties used in this experiment produced different number of roots with different concentrations of phytohormones and genetic variability existed between varieties. (Table 4). Regarding interaction between BAP and potato varieties for number of roots initiation, the statistical mean comparison indicated that potato varieties showed significant variation for root formation and Lal Moti performed better and developed maximum roots (3.33) than Lady Rosita which produced 2.33 roots at 1mg/L of BAP.

Table 4.Analysis of variance for number of roots in two potato varieties using BAP and Indole acetic acid.

SOV	DF	MS	F	P
Varieties	1	14.0833	21.13	0.0001
BAP	1	4.0833	6.13	0.0188

IAA	3	0.5833	0.87	0.4643
Variety × BAP	1	14.0833	21.12	0.0001
Variety × IAA	3	0.6944	1.04	0.3874
BAP × IAA	3	0.3611	0.54	0.6573
Variety × BAP × IAA	3	0.4722	0.71	0.5541
Error	32	0.6667		
Total	47			

Note: Significant (P<0.05) and highly significant (P<0.01)

Thus, it was observed that lower concentrations of BAP were more useful for production of more roots in potato varieties under study (Table 4a). The interaction between potato varieties and IAA treatments for number of root development exhibited that variety Lal Moti performed better by obtaining maximum number of roots (2.17) than Lady Rosita (1.33) at 1mg/L of IAA but means comparison indicated no statistical difference among different treatments application on studied potato varieties (Table 4b). Sarkar and Mustafa (2002) and Kaur *et al.* (2018) have also reported such types of results.

Table 4a. Means comparison between potato varieties and BAP doses for number of roots

Potato varieties	6-benzylaminopurine (BAP) doses	
	Dose 1 (1 mg/L)	Dose 2 (2 mg/L)
Lal Moti	3.33a	1.67b
Lady Rosita	1.17c	1.67b

Note: Values sharing the same letters are non-significant.

Table 4b. Means comparison of potato varieties and IAA treatments for number of roots

Potato varieties	IAA treatments			
	T_1 (0.5 mg/L)	T_2 (1 mg/L)	T_3 (1.5 mgLl)	T_4 (2 mg/L)
Lal Moti	2.67a	2.17abc	2.67a	2.33ab
Lady Rosita	1.17c	1.33bc	1.50bc	1.67bc

Note: Values sharing the same letters are non-significant.

Table 4c. Mean comparisons of BAP doses and IAA treatments for number of roots

BAP doses	IAA treatments			
	T_1 (0.5 mg/L)	T_2 (1 mg/L)	T_3 (1.5 mg/L)	T_4 (2 mg/L)
D_1 (1 mg/L)	2.50a	3.00a	2.17ab	2.33ab
D_2 (2 mg/L)	1.33b	1.33b	1.17b	1.83b

Note: Values sharing the same letters are non-significant.

Table 4d. Means comparison of varieties × BAP × IAA treatments interaction for number of roots.

Variety x BAP	IAA treatments			
	T_1 (0.5 mg/L)	T_2 (1 mg/L)	T_3 (1.5 mg/L)	T_4 (2 mg/L)
Lal Moti x D_1	3.33ab	4.00a	3.00ab	3.00ab
Lal Moti x D_2	2.00ab	2.00ab	1.00b	1.67ab
Lady Rosita x D_1	1.00b	1.33b	1.00b	1.33b
Lady Rosita x D_2	1.67ab	1.33b	1.33b	2.33ab

Note: Values sharing the same letters are non-significant.

In case of interaction between BAP doses and IAA treatments, it was observed that 1mg/L BAP + 1 mg/L IAA gave better results for root induction in potato variety Lal Moti with mean value of 3.00 (Table 4c). The interaction between potato varieties, BAP doses and IAA treatments showed that the variety Lal Moti gave superior results when the BAP (1mg/L) and IAA(1mg/L) were used with the mean root number value of 4.00. The protocol used in this experiment was only useful for potato variety Lal Moti which gave maximum number of roots (Table 4d).

DISCUSSION

The effect of 6-benzyl amino purine (BAP) doses and kinetin treatments on potato varieties for shoot length (cm) revealed that BAP doses had significant effect on potato varieties (Lal Moti and Lady Rosita) for shoot length while effect of Kinetin treatments on shoot length formation was non-significant (Table 1). This indicated that low concentration of BAP (1mg/L) gave excellent results for shoot length and Lady Rosita gained more shoot length (10.97 cm) than Lal Moti. Thus, it is suggested that use of 1mg/L BAP in MS media is enough to get maximum shoot length in potato (Bhuiyan, 2013). But in literature use of high BAP concentration was also reported by few researchers for shoot length formation in potato (Kumlay, 2014). In case of kinetin effect on shoot length formation, it was observed that the use of different concentrations of kinetin alone in MS media did not show any significant effect on potato shoot length. Therefore, results indicated that use of low concentration of kinetin in MS media is comparatively better for shoot length than high doses. Regarding interactive effects of these chemical combinations with potato varieties indicated that variety × BAP and variety × kinetin showed highly significant results while variety × BAP × kinetin interaction showed non-significant differences for shoot length in studied potato varieties. (Table 1). Therefore, based on statistical means comparisons (Tables 1a, 1b, 1c,1d) the protocol is validated as low concentrations of kinetin and BAP are more effective for shoot length formation in potato (Goppi *et al*., 2006, Bhuiyan, 2013, Kaur *et al*., 2018 and Morais *et al*., 2018).

Number of shoots is an important plant trait to observe the differences between potato varieties. ANOVA table for number of shoots exhibited that 6-benzyl amino purine doses had highly significant effect (P<0.01) on potato varieties number of shoot. This revealed that clear genetic variability exists between potato varieties (Lal Moti and Lady Rosita) for

this trait while kinetin treatments individually showed non-significant (P>0.05) effects on potato varieties (Table 2). But interaction between variety × BAP and variety × kinetin treatments displayed significant effect on potato genotypes for number of shoots. The means comparisons (Tables 2a, 2b, 2c, 2d) indicated that potato variety Lady Rosita performed better than Lal Moti to attain a greater number of shoots in different chemical combinations. Thus, based on interaction between BAP doses and kinetin treatments it was accomplished that 1mg/L BAP+1mg/L kinetin in MS media induced more shoots in Lady Rosita. Therefore, it is suggested that low concentration of kinetin and BAP is good for development of shoots in potato genotypes when axillary buds, terminal bud and leaf internodes are used as explant in tissue culture (Karadag *et al*., 2013, Bhuiyan, 2013 and Kaur *et al*., 2018).

The effects of BAP doses and IAA treatments on potato varieties for root length (cm) were also studied and found that potato varieties showed highly significant differences (P<0.01) for BAP doses and interaction between them. While Indole acetic acid (IAA) treatments and its interaction with varieties and BAP exhibited non-significant differences (Table 3). This indicated that BAP doses had more effect on root length formation. It was also noticed that Lal Moti performed well and developed 12.348 cm mean root length than Lady Rosita which developed only 2.14 cm root length at 1mg/L of BAP. This revealed that the difference in root length between two varieties was mainly due to their genetic background differences and less due to BAP doses which could be observed at 2 mg/L BAP dose where Lady Rosita gained 4.24 cm root length. Thus, based on obtained results it is suggested that BAP doses 1mg/L for Lal Moti and 2 mg/L for Lady Rosita are ideal to attain good root length (Table 3a). Furthermore, interaction between potato varieties and IAA treatments exhibited that variety Lal Moti performed better than variety Lady Rosita at 1mg/L of IAA when compared with other IAA treatments (Table 3b). As variation for root length in varieties using different concentrations of IAA was observed non-significant, therefore it is suggested that low concentration (1 mg/L) of IAA along with 1mg/L BAP are enough to get desired root length in potato (Sarkar and Mustafa, 2002, Laboney, 2013 and Kaur *et al*., 2018). However, our results are not in conformity with some researchers' findings who reported that higher concentration of auxins were required for development of root formation (Bhuiyan, 2013). This difference may be due to genetic variation in studied potato genotypes.

In case of number of roots, ANOVA table exhibited that potato varieties were highly significantly (P<0.01) influenced by BAP doses. While IAA treatments alone displayed non-significant effect on potato varieties for number of roots initiation. This depicted that genetic variability existed between potato varieties for number of roots and responded prominently at different concentrations of phytohormones (Table 4).

Statistical means comparison indicated that Lal Moti performed better and developed maximum roots than Lady Rosita at 1mg/L of BAP and 1mg/L of IAA (Tables 4a,4b, 4c & 4d). Thus, it is suggested that lower concentrations of BAP and IAA are more useful for production of more roots in potato varieties (Sarkar and Mustafa, 2002, Morais *et al*., 2018 and Kaur *et al*., 2018). However, in literature, high concentrations of growth regulators are also reported to produce a greater number of roots in potato (Bhuiyan, 2013).

***Conclusion*:** High level of genetic variability for root/shoot induction was present in potato genotypes and it was concluded that Lady Rosita performed better than Lal Moti for shoot formation by using Kinetin +BAP while Lal Moti gave better results for root formation by using Indole acetic acid +BAP each at 1mg/L. Therefore, it is suggested that low concentrations (1mg/L) of phytohormones should be used for better root/shoot induction in potato to get rapid *in vitro* multiplication of disease-free plantlets.

***Acknowledgement*:** We are highly thankful to Incharge tissue culture lab, College of Agriculture, University of Sargodha for providing opportunity to complete this study.

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