Pak. J. Bot., 53(1): 89-96, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-1(22)

EFFECTIVE REGENERATION AND TRANSFORMATION OF POTATO VARIETY DESIREE WITH NUCLEOTIDE DIPHOSPHATE KINASE 2 USING IAA AND BAP

SAMIHA RASHID[1], MUHAMMAD SAAD[1], SOFIA BAIG[2], MOHAMMAD MAROOF SHAH[1], ISMAT NAWAZ[1] AND AYESHA BAIG[1*]

[1]Department of Biotechnology, COMSATS University Islamabad, Abbottabad Campus, Abbottabad 22060 Pakistan
[2]Department of Environmental Sciences, Institute of Environmental Sciences & Engineering, School of Civil & Environmental Engineering, National University of Sciences & Technology, Islamabad, 44000 Pakistan
**Corresponding author's email: ayeshabaig@cuiatd.edu.pk*

Abstract

The main objective of the present study was to investigate the effect of different combinations of two growth regulators IAA and BAP on the *In vitro* regeneration of potato for successful transformation. The best optimized concentrations for potato regeneration was used for *Agrobacterium* mediated transformation with Arabidopsis Nucleotide Diphosphate Kinase2 (*NDPK2*). Potato is vulnerable to a number of biotic and abiotic stresses which limit its production. The effective *In vitro* regeneration of potato was carried out using Indol acetic acid (IAA) and Benzyl aminopurine (BAP) for transformation with (*NDPK2*) known to be involved in various environmental stresses. The internode explants of potato Desiree variety were cultured on MS media augmented with different concentrations of IAA i.e. (1.2, 1.5, 1.7, 2.0, 2.5 $mg.L^{-1}$) in combination with BAP 1 $mg.L^{-1}$. Data was collected after 15, 30 and 45 days of culturing and analyzed for statistical significance using analysis of variance (ANOVA). Fisher least significance using R- program was used to compare means at $p = 0.05$ level of significance. Transformation with *NDPK2* was carried out using optimized regeneration protocol. The results indicated that high concentration of IAA i.e. 2.0 $mg.L^{-1}$ with 1 $mg.L^{-1}$ of BAP proved to be best for root regeneration. In all other parameters like shoot length, number of leaves, stem diameter and leaf area and potato regeneration, 1:1.5 $mg.L^{-1}$ of BAP: IAA produced best result and thus was transformed with *NDPK2* that generated 7 confirmed transgenic potato plants on PCR based sequencing. The findings demonstrate that the combination of BAP:IAA of 1:1.5 $mg.L^{-1}$ can be regarded as the best optimized concentration for regeneration for stress related *NDPK2 Agrobacterium* mediated transformation of Desiree variety in potato.

Key words: Potato, BAP, IAA, NDPK2, Transformation.

Introduction

Potato (*Solanum tuberosum* L.) is a tuber bearing tetraploid plant species that belongs to family Solanaceae. Potato crop under cultivation contains 4 sets of chromosomes; whereas there are about 150 species of potatoes or Solanum that include tuber-bearing tetraploids, triploids and diploids. Potato is highly profitable crop that is cultivated globally in the temperate, tropical and subtropical zones. It is the world's top most non-grain food source and is considered the fourth largest food crop after rice, wheat and maize (Armin *et al*., 2011). Potato is susceptible to several biotic and abiotic stresses which restrain its worldwide production. Biotechnology based approach can be effectively applied for enhancing it growth and production (Kumlay, 2014). Tissue culture method of regeneration and transformation can be used for mass production of high quality, salt tolerant and disease-free planting material (Birham & Chandra, 1994). *In vitro* propagation using node and internode cutting can be used for potato transformation (Liljana *et al*., 2012). Many studies show that regeneration of potatoes mainly depends on the type of explant such as leaf, node, shoot tip, culture medium, season, temperature, photoperiod, and a balanced combination of different plant growth regulators (PGRs) in the culture media (Akhtar *et al*., 2006; Danci & Danci, 2008; Dhital *et al*., 2012). The effective *In vitro* multiplication of potato is mainly dependent on the presence of appropriate combination of different PGRs in propagation medium such as auxins and cytokinins (SN Uddin, 2006; Badoni & Chauhan, 2009; Badoni & Chauhan, 2010; Hoque, 2010). Auxins and cytokinins play a vital role in regeneration of potato plant as the former are involved in the formation of roots, while the later are mainly concerned with the shoot formation and growth of plant buds (North *et al*., 2012). The combined effect of auxin and cytokinin is required for improved regeneration of potato plants (Hoque, 2010). The successful regeneration of potato plantlets is unrelentingly required for genetic manipulation of potato plant such as transformation. Genetic transformation of potato and various other crops claim a firm and vigorous system for regeneration of new plant from a single cell using tissue culture technique (Abbasi *et al*., 2016). Rate of transformation in potato is comparatively low and is dependent on factors including the bacterial strain, bacterial concentration, bacterial pre-culture and co-cultivation in addition to type of explant, Acetosyringone and growth regulators (Ahmad *et al*., 2012). Potato transformation was optimized by *NDPK2* gene, which is a housekeeping enzyme that upholds the intracellular levels of nucleoside triphosphates (NTPs). *NDPK2* was shown to be involved in plant development and stress response mechanism (Moon *et al*., 2003). Therefore, the current study was aimed at optimization of transformation of potato nodal explant with *NDPK2* for engineering plant for growth improvement and stress tolerance.

Materials and Method

Plant material: Sterile potato plants (cv. Desiree) were collected from Hazara Agriculture Research Centre, Abbottabad, Pakistan. Research work was conducted at Genetic Engineering lab of COMSATS University, Abbottabad.

Media composition and regeneration: 30 days old explants of potato cv. Desiree were inoculated on Murashige & Skoog (MS) (Murashige & Skoog, 1962) medium containing appropriate concentrations of BAP and IAA as shown in (Table 1). Sucrose (30 g/l) was used as a carbohydrate source and agar (8 g/l) was used for MS medium solidification. pH of the media was adjusted at 5.7 and media was autoclaved for 15 minutes at 121°C. After autoclaving 10 ml media was poured to each test tube. The culture conditions were 25 ± 2°C and 16 h photoperiod from cool white fluorescent tube lights (35 mmole/m.sec). Ten replicate test tubes were inoculated for each treatment.

Table 1. Different concentrations of IAA and BAP used in Media.

Treatments	Concentration of BAP: IAA
T1	1:1.2 mg/l
T2	1:1.5 mg/l
T3	1:1.7 mg/l
T4	1:2.0 mg/l
T5	1:2.5 mg/l

Data collection and analysis: Data was collected after 15, 30 and 45 days of culturing and the parameters which were measured included; number of roots, shoot length (cm), number of leaves, leaf area (cm^2) and stem diameter (mm). The collected data were analyzed for statistical significance using analysis of variance (ANOVA). These calculations were done by using a statistical software R-program (R Core Team, 2018). Fisher least significance was used to compare means at *p*-value of 0.05 level of significance. The best statistical result was used for potato transformation.

Agrobacterium infection and co-cultivation: The prepared explants were then transferred into a 50 ml falcon tube containing 40 ml of the *Agrobacterium* Infection Medium (AIM). The explants were then incubated for at least 20 minutes with *Agrobacterium* cells with continuous shaking. After 20 minutes the *Agrobacterium* suspension was removed, and explants were transferred to sterile filter paper and blot dried. The explants were then transferred onto new plates containing MS regeneration media supplemented with BAP and IAA (1:1.5 mg/l).

Transfer to selection media: After co-cultivation of two days, potato explants were transferred onto MS regeneration and selection media containing growth hormones BAP and IAA (1:1.5 mg/l), cefotaxime (250 mg/l) and kanamycin (50 mg/l). The plates were incubated in growth chamber at 25^0C, under fluorescent light with a photoperiod of 16/8 h light/dark day length for shooting and rooting.

Rooting and shooting of potato explant: 2-3 cm long potato shoots were successfully transferred to the test tubes containing low selection MS media with rooting and shooting hormones, cefotaxime (125 mg/l) and kanamycin (25 mg/l) and incubated as before.

Confirmation of putative transformants through PCR: Leaves of fully grown putative potato transformants were collected and grinded with pestle and mortal using CTAB extraction buffer method (Healey *et al*., 2014). PCR analysis was performed using 5X master mix (Thermo Fischer), *NDPK2* gene specific primers (1 µl), DNA leaf sample (1 µg) and ddH_2O in 20 µl reaction. The PCR reaction was performed at 94°C for 4 min for initial denaturation followed by 35 amplification cycles (30 sec at 94°C, 30 secs at 60°C and 1 min at 72°C) and final extension step of 72°C for 10 min. The products were visualized under UV light after gel electrophoresis using ethidium bromide.

Results

The establishment of potato *In vitro* cultures is required for genetic manipulation and *Agrobacterium* mediated transformation of potato explant. The effective *In vitro* multiplication of potatoes is dependent on the presence of an appropriate combination of auxin and cytokinin in the propagation medium. This study demonstrated the effect of different combinations of BAP: IAA mg/l 1:1.2, 1:1.5, 1:1.7, 1:2.0, and 1:2.5 for the regeneration of potato variety Desiree on MS media (Figs. 1-4). Data was recorded after the 15, 30 and 45 days of potato explant culturing (Supplementary Tables 1-3) was interpreted for *NDPK2* transformation.

The effect of different concentrations of BAP and IAA on number of roots: The use of BAP and IAA in combination showed significant effect on number of roots. Data recorded at all three intervals (after 15, 30 and 45 days) showed significantly highest results in T_4 treatment in which 1:2.0 mg/l concentration was used followed by T_5 having BAP and IAA concentrations of 1:2.5 mg/l (Figs. 2a-4a).

The effect of different concentrations of BAP and IAA on shoot length: In case of shoot length, the data recorded after 15 days, IAA and BAP combinations showed significant results with highest shoot length in cm in T_2, T_3 and T_1 where 1:1.5 mg/l, 1:1.7 mg/l and 1:1.2 mg/l concentrations of BAP and IAA were used respectively (Fig. 2b). Data recorded after 30 days gave highest shoot length at T_3 with concentration of BAP and IAA 1:1.7 mg/l while T_2 and T_1 gave intermediate results for shoot length (Fig. 3b). After 45 days, significant results were obtained with T_3, T_1 and T_2 with least difference amongst them which indicate that these treatments showed best result (Fig. 4b).

The effect of different concentrations of BAP and IAA on number of leaves: After 15 days, data analysis for number of leaves indicated significant result for T_2 (1:1.5 mg/l) followed by T_1 (1:1.2 mg/l) (Fig. 2c). Data recorded after 30 days also showed significant results in case of T_2 (1:1.5 mg/l) followed by T_1 (1:1.2 mg/l) then T_3 (1:1.7 mg/l) (Fig. 3c). Data analyzed after 45 days showed significant results in all the five treatments. This indicates that the best results were obtained when 1:1.5 mg/l and 1:1.2 mg/l concentrations of BAP and IAA were applied (Fig. 4c).

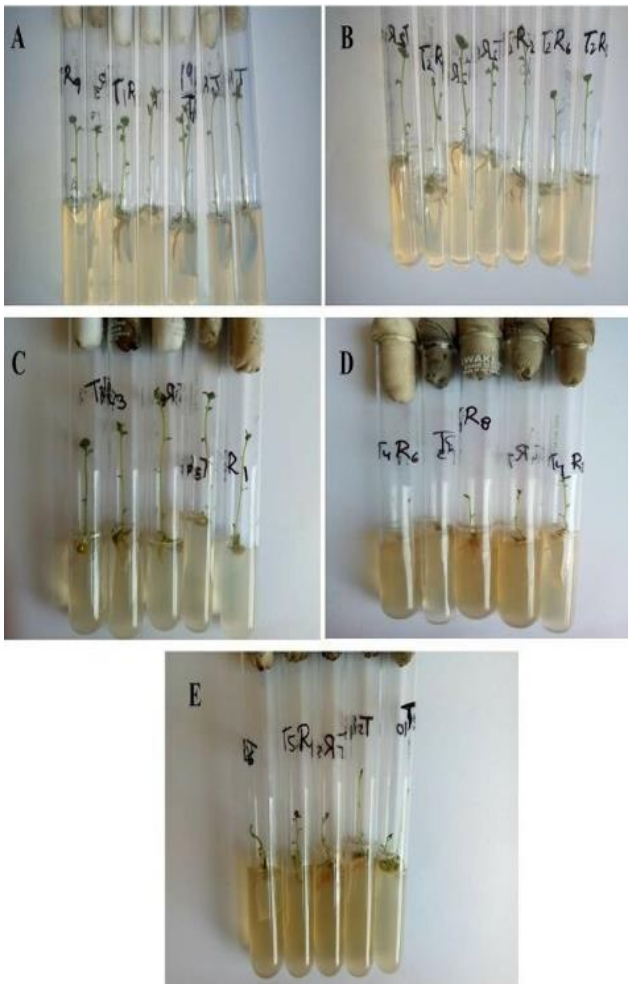


Fig. 1. *In vitro* grown potato plantlets showing root/shoot development at various concentrations of IAA and BAP. (A) T_1 having BAP: IAA concentrations 1:1.2 mg/l; (B) T_2 having concentrations 1:1.5 mg/l; (C) T_3 with concentrations 1:1.7 mg/l; (D) T_4 showing concentrations 1:2.0 mg/l; (E) T_5 showing BAP and IAA concentrations 1:2.5 mg/l.

Supplementary Table 1. Effect of different concentrations of IAA and BAP on growth parameters for regeneration of potato plantlets after 15 days.

Treatments	Conc. mg/l	No. of roots	No. of leaves	Shoot length (cm)
T1	1:1.2	1.3 b	5.7 a	2.46 a
T2	1:1.5	1.6 b	6.3 a	2.95 a
T3	1:1.7	2.2 ab	5.1 ab	2.70 a
T4	1:2.0	4.1 a	4.9 ab	1.41 b
T5	1:2.5	2.9 ab	3.6 b	0.68 b

Mean values followed by different letters along a column or row are significantly different at $p \leq 0.05$

Supplementary Table 2. Effect of different concentrations of IAA and BAP on growth parameters for regeneration of potato plantlets after 30 days.

Treatments	Conc. mg/l	No. of Roots	No. of Leaves	Shoot Length (cm)
T1	1:1.2	1.4 b	7.9 a	5.08 ab
T2	1:1.5	2.0 ab	8.0 a	5.32 ab
T3	1:1.7	2.7 ab	6.1 ab	5.71 a
T4	1:2.0	3.7 a	5.4 b	3.67 bc
T5	1:2.5	3.7 a	5.4 b	3.05 c

Mean values followed by different letters along a column or row are significantly different at $p \leq 0.05$

The effect of different concentrations of BAP and IAA on leaf area: The data of leaf area in cm^2 was recorded after 45 days just before transferring the MS cultured potato plants to sterilized soil. The most significant results were observed in case of T_1, T_3 and T_2 with BAP: IAA concentrations of 1:1.2 mg/l, 1:1.7 mg/l and 1:1.5 mg/l respectively followed by T_4 and T_5 that showed least significance then the preceding treatments (Fig. 4d).

The effect of different concentrations of BAP and IAA on stem diameter: Stem diameter was recorded for potato plantlets after 45 days, just before transferring them into the soil pots. The significantly highest results in cm were observed in three treatments T_1, T_3 and T_4 having concentrations 1:1.2 mg/l, 1:1.7 mg/l and 1:2.0 mg/l. The treatments T_2 and T_5 showed significantly lower values with concentrations 1:1.5 mg/l and 1:2.5 mg/l (Fig. 4e).

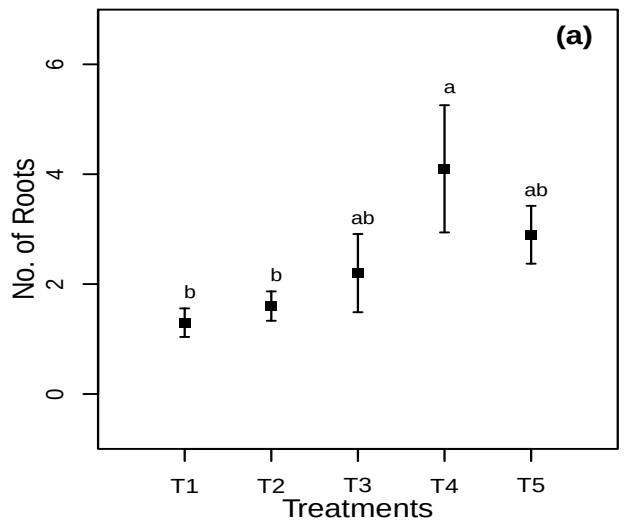
***Agrobacterium* mediated transformation of potato:** After protocol optimization for potato regeneration, potato node explants were infected with *Agrobacterium* containing *NDPK2*. Total 45 explants were co-cultivated with *Agrobacterium* strain *GV3101* containing *NDPK2* out of which 14 explants survived on selection media containing kanamycin. The numbers of shoots observed by these 14 nodal explants were around 27 and number of roots 24. The regeneration was observed almost 15-20 days after *Agrobacterium* infection containing selection media with IAA and BAP 1:1.5 mg/l as optimized previously (Fig. 5).

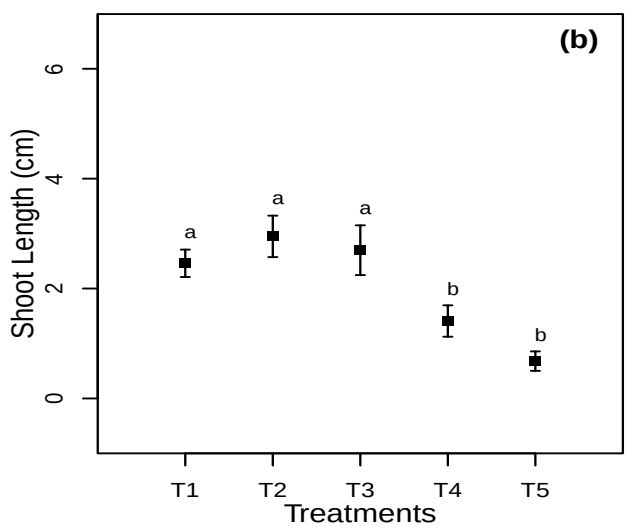
PCR analysis of putative transformants containing NDPK2 gene: PCR analysis performed on regenerated potato nodal explants revealed 6 positive transgenic potato plantlets containing *NDPK2* gene (Fig. 6). The PCR product of 200bp was further confirmed through sequencing.

Supplementary Table 3. Effect of different concentrations of IAA and BAP on growth parameters for regeneration of potato plantlets before transformation after 45 days.

Treatments	Conc. mg/l	No. of roots	No. of leaves	Shoot length (cm)	Stem diameter (mm)	Leaf area (cm^2)
T1	1:1.2	2.8 c	7.7 a	6.4 a	0.1746 a	0.27280 a
T2	1:1.5	3.0 bc	8.7 a	6.26 a	0.1562 ab	0.21526 a
T3	1:1.7	3.6 abc	6.67 a	6.69 a	0.1690 a	0.22860 a
T4	1:2.0	4.7 a	6.7 a	4.67 ab	0.1630 a	0.02454 b
T5	1:2.5	4.5 ab	6.1 a	3.19 b	0.1270 b	0.02203 b

Mean values followed by different letters along a column or row are significantly different at $p \leq 0.05$





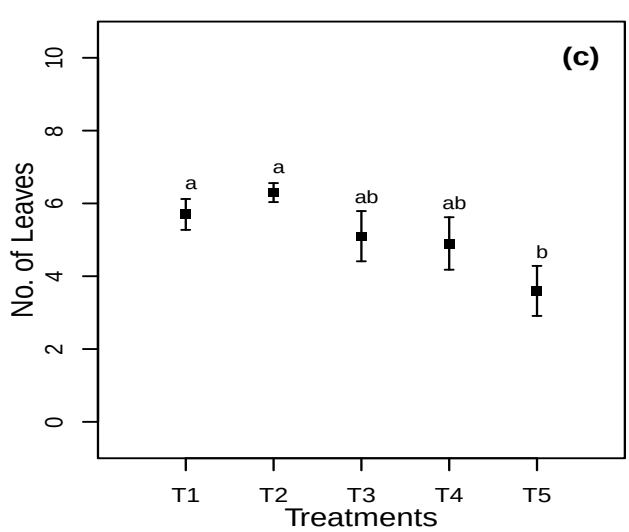
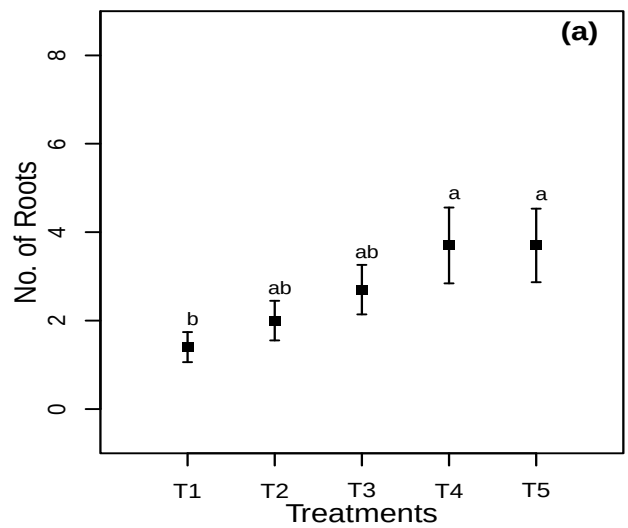
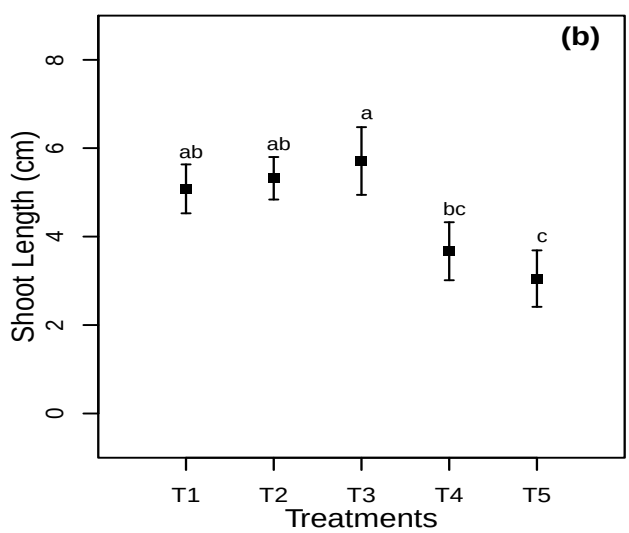


Fig. 2. Data recorded and analyzed after 15 days of culturing using different concentrations of BAP and IAA in combination (a) T_4 (1:2.0 mg/l) showing significantly highest results in case of no. of roots; (b) IAA and BAP combinations showed significant results with highest shoot length (cm) in T_2, T_3 and T_1 where 1:1.5 mg/l, 1:1.7 mg/l and 1:1.2 mg/l concentrations of BAP and IAA were used respectively; (c) Data analysis for number of leaves indicated significant result for T_2 (1:1.5 mg/l) followed by T_1 (1:1.2 mg/l). ■ represents mean values whereas vertical lines represent ±SE.





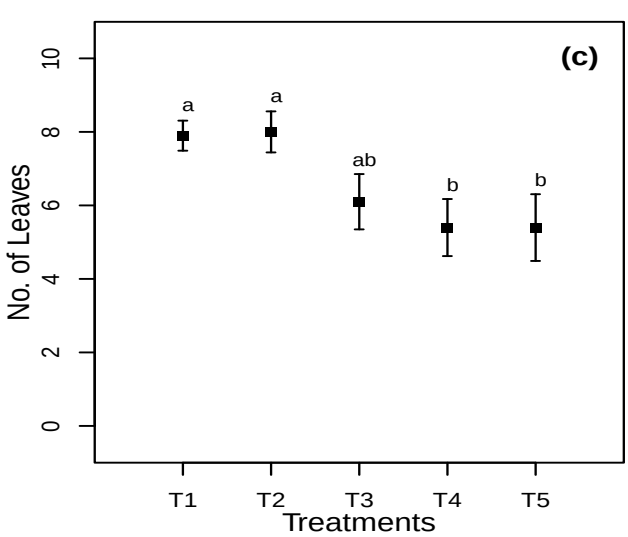


Fig. 3. Analysis of data after 30 days of culturing of potato plantlets. (a) In case of no. of roots T_4 (1:2.0 mg/l) and T_5 (1:2.5 mg/l) showing significantly highest results as compared to other treatments; (b) Highest shoot length (cm) was observed in case of T_3 with concentrations of BAP and IAA 1:1.7 mg/l while T_2 (1:1.5 mg/l) and T_1 (1;1.2 mg/l) gave intermediate results for shoot length; (c) In case of number of leaves significantly highest results were obtained in T_2 (1:1.5 mg/l) followed by T_1 (1:1.2 mg/l) while T_3 (1:1.7 mg/l) showed intermediate results. ■ represents mean values whereas vertical lines represent ±SE.

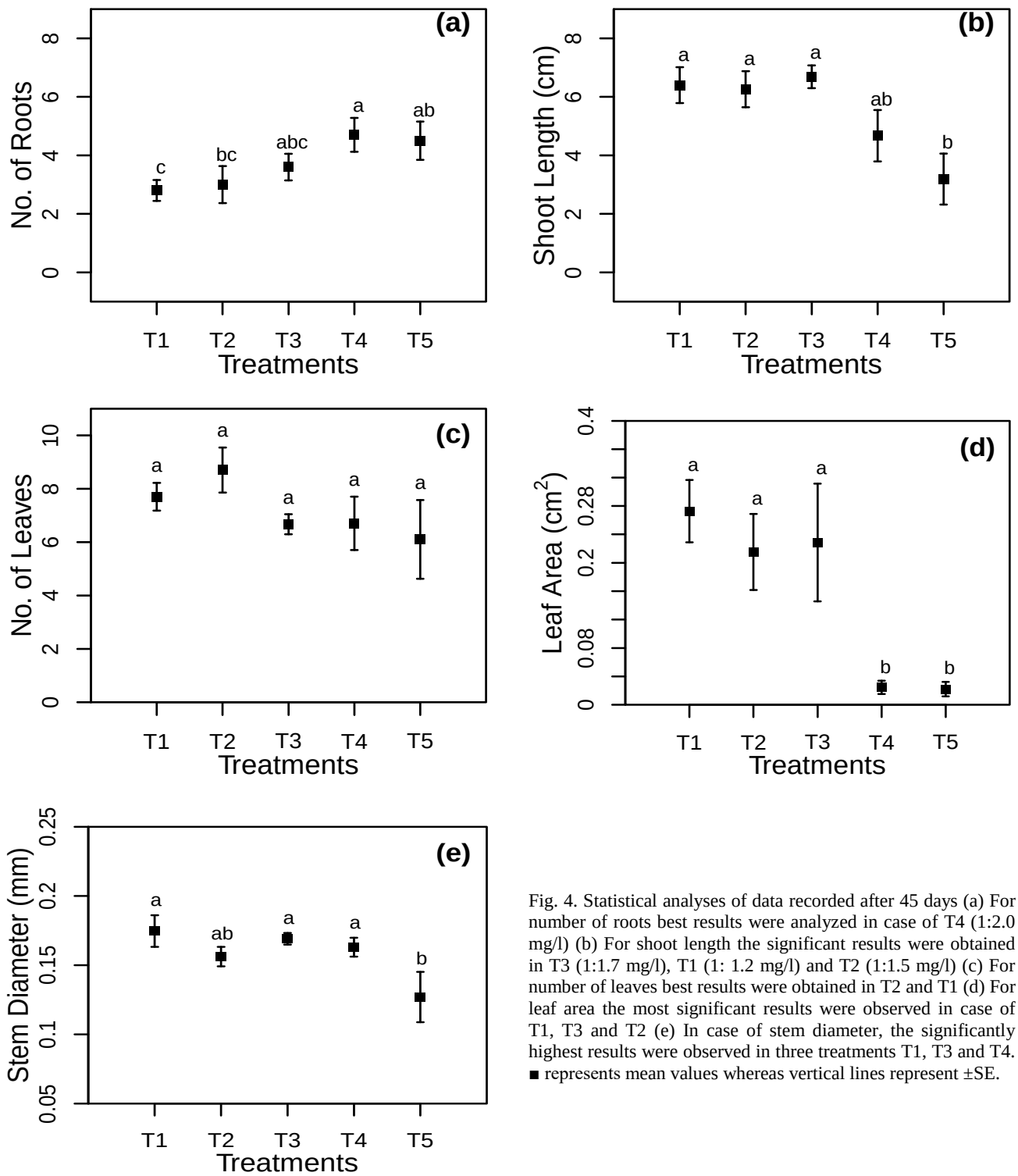


Fig. 4. Statistical analyses of data recorded after 45 days (a) For number of roots best results were analyzed in case of T4 (1:2.0 mg/l) (b) For shoot length the significant results were obtained in T3 (1:1.7 mg/l), T1 (1: 1.2 mg/l) and T2 (1:1.5 mg/l) (c) For number of leaves best results were obtained in T2 and T1 (d) For leaf area the most significant results were observed in case of T1, T3 and T2 (e) In case of stem diameter, the significantly highest results were observed in three treatments T1, T3 and T4. ■ represents mean values whereas vertical lines represent ±SE.

Discussion

Auxin and cytokinin are two important growth regulators that have a vital role in cell growth and cell division. It is also implicated that both cytokinins and auxins play different role in cell division (Sakakibara, 2006) where they are often used in different concentrations and combination. A high cytokinin to auxin ratio favors formation of shoot whereas a high auxin to cytokinin ratio supports root formation. The current study showed that cytokinin such as BAP was effective when combined with IAA used as auxin for potato nodal explant regeneration that was subsequently used for transformation with *NDPK2*. Ebad *et al*., 2015 in their study analyzed that after 21 days of potato culture, the growth parameters such as shoot length, numbers of leaves, the root phenotype and growth and the fresh matter of the plantlets showed numerous responses to the culture medium. In the same way Shah *et al*., 2003 also measured shoot length, number of roots, and number of nodes per plantlets and shoot relative growth rates of *In vitro* cultured potato plantlets after 3, 7, 10, 14, 17 days was measured. Their results revealed the significant results after 17 days of data analysis. Similarly, in the present study the effects of

culture media supplemented with different concentrations of BAP and IAA was analyzed statistically after 15 and 30 days of culturing in terms of shoot length, number of roots and leaves and also at transferrable plant stage after 45 days together with other parameters like stem diameter and leaf area. Statistical analysis revealed that parameters for shoot and root development produced significant results for potato regeneration.

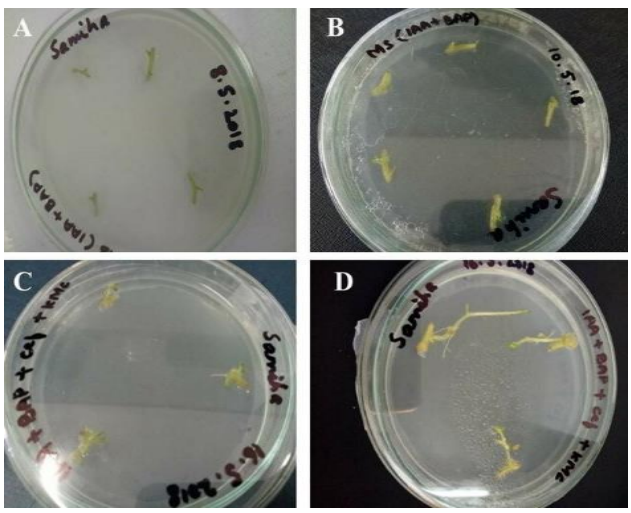


Fig. 5. *Agrobacterium* infected plants at different levels of development (A) Nodal explants were prepared on regeneration media for 2 days before infection (B) Transfer of explants again to regeneration media after co cultivation and *Agrobacterium* infection (C) Transfer of explants to selection media with kanamycin (50mg/l), cefotaxime (250 mg/l) along with regeneration hormones IAA and BAP (D) Shoots and roots development of nodal explants on selection media.

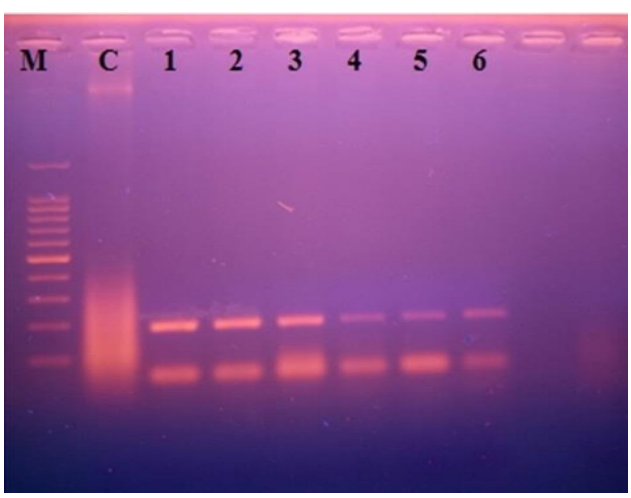


Fig. 6. PCR amplified bands of *NDPK2* in potato plants regenerated following *Agrobacterium* inoculation. Molecular weight markers (M) and expected PCR product are indicated. Lane 1(C) is showing control while Lanes 2-7 (1, 2, 3, 4, 5, and 6) are showing positive transformants potato plants exhibiting bands of 200bp.

The maximum numbers of roots were observed in T_4 followed by T_5. The maximum number of roots could be observed due to the presence of high concentration of IAA that plays a significant role in root emergence and root growth (Overvoorde *et al*., 2010).

In the present study, maximum shoot length was observed in case of T_2, T_3 and T_1. The reason was the presence of cytokinin that played a key role in cell division and growth of shoot meristem (Kwapata *et al*., 1999; Razdan, 2003). Previous studies indicated the role of BAP in enhancing the number of shoot development from explant (Hussain *et al*., 2005; Motallebi-Azar *et al*., 2011). It was reported that BAP gave better response for different parameters like shoot per explants, shoot length, number of nodes and leaves in different potato varieties (Mustafa & Sarker, 2002). Similar results were also reported for other potato varieties (Hoque *et al.*, 1996). Maximum regeneration ratio was achieved from nodal explants of potato on MS basal medium supplemented with 2.0 mg/l BAP and 0.5 mg/l IAA (Motallebi-Azar *et al*., 2011).

Our statistical analysis revealed the maximum number of leaves in T_2 and T_1 followed by T_3. Leaves originate from buds and cytokinins are generally known to promote buds' formation in many *In vitro* cultured organs (Hesar *et al*., 2011). Similarly, the effect of BAP and IAA on stem diameter showed the maximum stem diameter in case of T_1, T_3 and T_4. Application of IAA on cultured explant indicated an increase in stem diameter (Naeem *et al*., 2004), whereas cytokinin like BAP also plays an important role in cell division of lateral meristem and resulted in the stem and root thickness (Muhammad *et al*., 2015).

T_1, T_3 and T_2 showed significant results in terms of leaf area in cm^2. It was shown that the application of cytokinin encouraged the unfolding of leaf (Doerner, 2007). Leaf area development was promoted by the application of BAP that have an impact on cell size and cell number (Del Pozo *et al*., 2005; Doerner, 2007; Hamada *et al*., 2008; Benedetto *et al*., 2013).

Once the conditions for potato regeneration were significantly analyzed, transformation was performed with the significant best regeneration results on selection media. Out of 45 nodal explant that were co-cultivated with *Agrobacterium* containing *NDPK2* gene 14 explants survived on selection media containing Kanamycin and Acetosyringone that showed regeneration. Thus, the regeneration protocol was successfully used to transform *NDPK2* gene in potato. *NDPK2* is a housekeeping enzyme that is involved in maintaining nucleoside triphosphates (NTPs) intracellular level. Transgenic plants expressing Arabidopsis *NDPK2*, in poplar and sweet potato displayed considerably improved tolerance to numerous environmental stresses including salt and oxidative stress (Tang *et al*., 2008; Kim *et al*., 2009). Although *NDPK2* gene was previously transformed in potato but we were successful in transforming *NDPK2* in Desiree variety that is an important potato variety grown in Pakistan. *NDPK2* was found to be involved in plant stress tolerance so its transformation and successful regeneration in cultivated potato variety of Pakistan could be exploited for future engineering of plants for enhancing various stress tolerance. PCR analysis revealed 6 positive transgenic potato plants containing *NDPK2* gene that was confirmed via sequencing results.

It was observed during this study that *Agrobacterium* concentration played an important role in *NDPK2* gene transfer to potato explant. Too high or low *Agrobacterium* concentration did not yield positive result as also noted earlier for potato studies (Li *et al*., 2017). In this study, 0.6 OD at 600 nm was found optimum for *Agrobacterium* infection. It was also noted that other factors including potato explants, suspension medium for *Agrobacterium*, and co-cultivation time also played an important role in *Agrobacterium* mediated transformation of potato explant.

Conclusion

It is concluded from this study that MS media supplemented with BAP and IAA proved to be very effective for *In vitro* regeneration of potato plants where 1:1.5 mg/l of BAP and IAA proved to be the best optimized concentration for the regeneration of potato variety Desiree used for *Agrobacterium* mediated transformation of potato with *NDPK2*.

Acknowledgements

The authors are thankful to Sang Soo Kwak from Department of Environmental Biotechnology, KRIBB School of Biotechnology, Korea University of Science and Technology (UST), 217 Gajeong-ro, Yuseong-gu, Daejeon 34113, South Korea for providing Arabidopsis *NDPK2* for transformation into potato plant of Desiree variety.

References

Abbasi, A.Z., M. Bilal, J. Hussain, M.M. Shah, A. Hassan and S.Y. Kwon. 2016. Robust regeneration protocol for the *Agrobacterium tumefaciens* mediated transformation of *Solanum tuberosum*. *Pak. J. Bot.*, 48(2): 707-712.

Ahmad, M.Z., I. Hussain, A. Muhammad, S. Ali, G.M. Ali, S. Roomi and A. Ijaz. 2012. Factor affecting *Agrobacterium*-mediated transformation of rice chitinase gene in *Solanum tuberosum* L. *Afr. J. Biotechnol*., 11(41): 9716-9723.

Akhtar, N., M.H. Munawwar, M. Hussain and M. Mahmood. 2006. Sterile shoot production and direct regeneration from the nodal explants of potato cultivars. *Asian. J. Plant Sci*., 5(5): 885-9.

Armin, M.J., M.R. Asgharipour and S.K. Yazdi. 2011. Effects of different plant growth regulators and potting mixes on micro-propagation and mini-tuberization of potato plantlets. *Adv. Environ. Biol*., 5(4): 631-8.

Badoni, A. and J.S. Chauhan. 2009. Effect of growth regulators on meristem-tip development and *In vitro* multiplication of potato cultivar 'Kufri Himalini'. *Nature & Sci.*, 7(9): 31-4.

Badoni, A. and J.S. Chauhan. 2010. Potato seed production of Cultivar Kufri Himalini, *In vitro*. *Stem Cell*, 1(1): 7-10.

Benedetto, A. and C.J. Galmarini, Tognetti. 2013. Changes in leaf size and in the rate of leaf production contribute to cytokinin-mediated growth promotion in *Epipremnum aureum* L. cuttings. *J. Hort. Sci. Biotechnol.*, 88(2): 179-86.

Chandra, R. and R.K. Birhman. 1994. *In vitro* micro propagation in relation to pedigree in potato. *J. Ind. Potato Association*, 21(87): 8.

Danci, O. and M. Danci. 2008. The comparison between four potato cultivars multiple axillary bud micropropagation system efficiency. *Scientific Papers J. Anim. Sci. Biotechnol.*, 41(1): 64-9.

Del Pozo, J.C., M.A. Lopez-Matas, E. Ramirez-Parr and C. Gutierrez. 2005. Hormonal control of the plant cell cycle. *Physiol. Plant*. 123: 173-183.

Dhital, S.P., H.T. Lim and H.K. Manandhar. 2012. Direct and efficient plant regeneration from different explants sources of potato cultivars as influenced by plant growth regulators. *Nepal J. Sci. Technol*., 12: 1-6.

Doerner, P. 2007. Plant Meristems: Cytokinins the alpha and omega of the meristem. *Curr. Biol.* 17(9): 321-3.

Ebad, F.A.S., M.E.L.S.A. El-Sadek and A.A. El-Kazzaz. 2015. Micropropagation of four potato cultivars *In vitro*. *Academia J. Agric. Res.*, 3(9): 184-188.

Hamada, K., K. Hasegawa and T. Ogata. 2008. Strapping and a synthetic cytokinin promote cell enlargement in 'Hiratanenashi' Japanese persimmon. *Plant Growth Regul*., 54(3): 225.

Healey, A., A. Furtado, T. Cooper and R.J. Henry. 2014. *Protocol:* a simple method for extracting next-generation sequencing quality genomic DNA from recalcitrant plant species. *Plant Methods*. 10(1): 21.

Hesar, A.A., B. Kaviani, A. Tarang and S.B. Zanjani. 2011. Effect of different concentrations of kinetin on regeneration of 'Matthiola incana'. *Plant Omics*. 4(5): 236.

Hoque, M.E. 2010. *In vitro* regeneration potentiality of potato under different hormonal combination. *World J. Agric. Sci*., 6: 660-3.

Hoque, M.I., N.B. Mila, M.S. Khan and R.H. Sarker. 1996. Shoot regeneration and *In vitro* microtuber formation in potato *(Solanum tuberosum* L). *Bangladesh J. Bot*., 25(1): 87-93.

Hussain, I.Q., A.I. Muhammad, Z. Chaudhry, R.A. Naqvi and H. Rashid. 2005. Morphogenic potential of three potato (*Solanum tuberosum*) cultivars from diverse explants, a prerequisite in genetic manipulation. *Pak. J. Bot*., 37(4): 889.

Kim, Y.H., S. Lim, K.S. Yang, C.Y. Kim, S.Y. Kwon, H.S. Lee, X. Wang, Z. Zhou, D. Ma, D.J. Yun and S.S. Kwak. 2009. Expression of Arabidopsis *NDPK2* increases antioxidant enzyme activities and enhances tolerance to multiple environmental stresses in transgenic sweet potato plants. *Mol. Breed.*, 24: 233-244.

Kumlay, A.M. 2014. Combination of the auxins NAA, IBA, and IAA with GA3 improves the commercial seed-tuber production of potato (*Solanum tuberosum* L.) under *In vitro* conditions. *Biomed. Res. Int*., 439259: 1-7.

Kwapata, M.B., F. Kalengamaliro, J. Bakuwa and S. Manyela. 1999. *In vitro* rooting and axillary shoots proliferation of *Faidherbia albida* (Del.) A. Chev. under varying levels of plant growth regulators. *Afr. Crop Sci. J*., 7(4): 303-11.

Li, S., Y. Cong, Y. Liu, T. Wang, Q. Shuai, N. Chen and Y. Li. 2017. Optimization of *Agrobacterium*-mediated transformation in soybean. *Front. Plant Sci.*, 8.

Liljana, K.G., S. Mitrev, T. Fidanka and I. Mite. 2012. Micropropagation of Potato- *Solanum tuberosum* L. *Electr. J. Biol*., 8(3): 45-49.

Moon, H., B. Lee, G. Choi, D. Shin, D.T. Prasad, O. Lee, S.S. Kwak, D.H. Kim, J. Nam, J. Bahk, J.C. Hong, S.Y. Lee, M.J. Cho, C.O. Lim and D.J. Yun. 2003. NDP kinase 2 interacts with two oxidative stress-activated MAPKs to regulate cellular redox state and enhances multiple stress tolerance in transgenic plants. *Proc. Natl Acad. Sci., USA.* 100: 358-363.

Motallebi-Azar, A., S. Kazemiani, F. Kiumarsie and N. Mohaddes. 2011. Shoot proliferation from node explants of potato (*Solanum tuberosum* cv. Agria). II. Effect of different concentrations of NH4NO3, hydrolyzed casein and BAP. *Rom. Biotechnol. Lett.*, 16(3).

Muhammad, K., Z. Gul, Z. Jamal, M. Ahmed, A.R. Khan and Z.U. Khan. 2015. Effect of coconut water from different fruit maturity stages, as natural substitute for synthetic PGR in *In vitro* potato micro propagation. *Int. J. Biosci*., 6(2): 84-92.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant*. 15(3): 473-497.

Naeem, M., I.R. Bhatti, R.H. Ahmad and M.Y. Ashraf. 2004. Effect of some growth hormones (GA3, IAA and Kinetin) on the morphology and early or delayed initiation of bud of lentil (Lens culinaris medik). *Pak. J. Bot*., 36(4): 801-9.

North, J.J., P.A. Ndakidemi and C.P. Laubscher. 2012. Effects of antioxidants, plant growth regulators and wounding on phenolic compound excretion during micropropagation of Strelitzia reginae. *Int. J. Phys. Sci*., 7(4): 638-46.

Overvoorde, P., H. Fukaki and T. Beeckman. 2010. Auxin control of root development. *Cold Spring Harb. Perspect. Biol*., 2(6):

R Core Team. 2018. R: A language and environment for statistical computing Vienna, Austria.

Razdan, M.K. 2003. Introduction to Plant Tissue. *2nd Edition. Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi*, 27-29.

Sakakibara, H. 2006. Cytokinins: activity, biosynthesis, and translocation. *Ann. Rev. Plant Biol*., 57: 431-49.

Sarker, R.H. and B.M. Mustafa. 2002. Regeneration and Agrobacterium-mediated genetic transformation of two indigenous potato varieties of Bangladesh. *Plant Tissue Cult*., 12(1): 69-77.

Shah, A.H., S.H. Shah, Z.A. Swati and Z. Hussain. 2003. Cost effective micropropagation technology for potatoes. *Pak. J. Biol. Sci*., 6(4): 336-340.

Tang, L., M.D. Kim, K.S. Yang, S.Y. Kwon, S.H. Kim, J.S. Kim, D.J. Yun, S.S. Kwak and H.S. Lee. 2008. Enhanced tolerance of transgenic potato plants overexpressing nucleoside diphosphate kinase 2 against multiple environmental stresses. *Transgenic Res*., 17: 705-715.

Uddin, S.N. 2006. *In vitro* propagation of Elite indigenous potato (*Solanum tuberosum* L. var Indurkani) of Bangladesh. *J. Plant Sci*., 1(3): 212-6.

(Received for publication 28 January 2019)