

The Effects of Different Concentrations and Combinations of Growth Regulators on the Micro propagation Of Potato (*Solanum Tuberosum*)

Bhawna Chaudhary

Research Scholar
Mewar University
Chittorgarh, Rajasthan.

Pallavi Mittal

ITS Paramedical college
Department of Biotechnology
Muradnagar, Ghaziabad

ABSTRACT:

In this investigation, a high frequency single step and a simple method of direct plant regeneration has been developed for two potato cultivars K.CH3(Kufri. CHIPSONA 3) and K.Jyoti. (Kufri Jyoti) internodes were excised from field grown plants and cultured on MS media supplemented with different concentrations and combinations of auxins (NAA and IAA), cytokinins (BAP and Zeatin) and GA3. This study was aimed to evaluate the effect of different growth regulators (GRs) in seven different combinations named as T1, T2, T3, T4, T5, T6 and T7 along with control T0 (no growth regulators) on mass propagation of two potato cultivars K.CH3 and K.Jyoti. The best regeneration of internodes was obtained when MS medium was supplemented with 1.0 mgL^{-1} GA3, 0.01 mgL^{-1} IAA and 2.0 mgL^{-1} Zeatin (T2). This combination took minimum time for regeneration of multiple shoots and roots on internodes of cultivar K.CH3. Further, with either decrease, increase or change the combination of growth regulators from the optimum level, a significant decline in percent plant regeneration as well as number of shoots per explant was recorded.

Keywords: GA3, Zeatin, Potato and Internode

INTRODUCTION

Potato (*Solanum tuberosum* L.) belongs to the family Solanaceae, is the fourth most cultivated food crop after wheat, rice and maize (Moeinil et al., 2011). It is a native of South America; in sixteenth century Spanish explorers introduced it in Europe and later it became an important food crop of the world (Khoso, 1988). Potato is usually propagated asexually by means of tubers. However, pests, diseases and environmental factors prevent the crop from reaching its maximum agricultural potential. Viral diseases have been attributed as the main cause of low yield productivity (Wambugu, 1991) and the major cause of cultivar decline (Carey et al., 1999; Gibson et al., 1998). In potato, several studies indicated that potato chlorotic stunt virus (spcsv) and potato feathery mottle virus (spfmv) drastically reduced potato yields; losses may often reach 65 to 90% (Karyeija et al., 1998). Alternatively, micropropagation methods are ideal for rapid multiplication for diseases free good quality seeds and pathogen free planting materials (Rabbani et al., 2001). Tissue culture techniques are used worldwide to produce pre-basic, virus-free seed potatoes known as micro tubers. In addition, in vitro methods can be used for conservation, storage and easy distribution of potato germplasm in the form of breeding lines, new varieties and micro tubers. Because of their small size and weight, micro tubers have tremendous advantages in terms of storage, transportation and mechanization. They can be directly sown into the soil and can be produced in bulk in any season. They have the similar morphological and biochemical characteristics to field produced tubers. Therefore, mass production of potato micro tuber is likely to revolutionize the world potato production (Majid et al., 2014)

The provision of a steady supply of indexed planting materials through in vitro culture appears economically and technologically feasible. Although, there are many reports on potato micropropagation (Yousef et al., 2001; Badoni and Chauhan, 2009; Rahman et al., 2010). It is a well known fact that the regeneration potential of micropropagated plants is genotype dependent (Abe and Futsuhara, 1986). However, the chemical composition of the culture medium is perhaps the most studied aspect of potato tissue culture; it plays an important role in success of micropropagation. A sub-optimal culture medium may cause

physiological disorders or death of tissue (Nas and Read, 2000 and Preece, 1995). Numerous studies aiming to optimize the culture medium constituents have been conducted (George, 1993).

Thus, the present investigation was carried out to optimize the best combination of growth regulators (GRs) for the multiplication of local potato cultivars K.CH3 and K.Jyoti using stem cutting, internode as explants; the most regenerative variety could be then efficiently micropropagated for commercial purposes and molecular studies.

MATERIALS AND METHODS

Plant material

Virus-free potato plant material K.CH3 and K.Jyoti were procured from CPRI, Shimla used as the explants source. K.CH3 and K.Jyoti cultivar was micropropagated in vitro using the nodal cutting as described by Roca et al., (1978) and were routinely sub-cultured every 4 weeks on a fresh MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose. The cultures were maintained at 23 ± 2 °C under 300 footcandle for 16 h lights per day.

Initiation of Internode-Culture

After raising the plantlets for two weeks, the internode measuring 1-3 cm were excised and cultured on fresh MS solid medium containing seven different combinations (T1-T7) of GRs i.e. Benzylamino Purine (BAP), Naphthalene Acetic Acid (NAA), gibberellic acid (GA3), Zeatin and Indole Acetic Acid (IAA). Details of all the treatments are given in Table 1.

Cultures were kept at 22 ± 2 °C with a photoperiod of 16 hours light. This experiment was performed in three replicates and each replicate had 20 explants per treatment. Data was collected during 4-weeks after internode culture initiation. To evaluate the effect of different combinations of GRs on potato internode culture, different in vitro growth traits plantlet height, number of shoots per explant and number of roots per explant were studied.

Table 1: Different concentrations and combinations of auxins (NAA and IAA) , cytokinins (BAP and Zeatin) and GA3 used for the direct plant regeneration in different cultivars of potato

Cultivas	Experiment No.		Growth Regulator (mgL^{-1})				
			GA3	IAA	Zeatin	BAP	NAA
K.CH-3	T0	MS	–	–	–	–	–
	T1	MS	0.5	0.005	1	–	–
	T2	MS	1	0.01	2	–	–
	T3	MS	1.5	0.015	2.5	–	–
	T4	MS	2	0.02	3	–	–
	T5	MS	0.5	–	–	1	0.005
	T6	MS	1	–	–	2	0.01
	T7	MS	1.5	–	–	3	0.02
K.Jyoti	T0	MS	–	–	–	–	–
	T1	MS	0.5	0.005	1	–	–
	T2	MS	1	0.01	2	–	–
	T3	MS	1.5	0.015	2.5	–	–
	T4	MS	2	0.02	3	–	–
	T5	MS	0.5	–	–	1	0.005
	T6	MS	1	–	–	2	0.01
	T7	MS	1.5	–	–	3	0.02

Results and Discussion

Present investigation was carried out to evaluate the effect of different combinations of GRs on the in vitro regeneration potential of two important potato cultivars. The two cultivars K.CH3 and K.Jyoti were propagated in vitro initially through shoot tips which were later used to start internode culture. The best regeneration potential of the internode of variety K.CH3 was observed when MS medium was supplemented with 1.0 mgL^{-1} GA3, 0.01 mgL^{-1} IAA and 2.0 mgL^{-1} Zeatin (Table 1). This supplement of GRs took minimum time for regeneration of multiple shoots and roots on internode of variety K.CH3 potato cultivar.

Table 2: Effect of different treatments on mean performance of in vitro plant charecterstics.

Cultivars	Treatments (mgL^{-1})						Mean No. of Shoots	Mean No. of Roots	Mean Planlet Height (cm)
	#	GA3	IAA	Zeatin	BAP	NAA			
K.CH-3	T0	–	–	–	–	–	2	0	5
	T1	0.5	0.005	1	–	–	10	11	6.2
	T2	1	0.01	2	–	–	18	28	8.4
	T3	1.5	0.015	2.5	–	–	15	19	7.3
	T4	2	0.02	3	–	–	9	15	6.9
	T5	0.5	–	–	1	0.005	7	17	6.4
	T6	1	–	–	2	0.01	14	23	7.3
K.Jyoti	T7	1.5	–	–	3	0.02	8	20	5.9
	T0	–	–	–	–	–	1	0	4.4
	T1	0.5	0.005	1	–	–	6	10	5.8
	T2	1	0.01	2	–	–	16	26	7.9
	T3	1.5	0.015	2.5	–	–	13	17	6.8
	T4	2	0.02	3	–	–	8	15	6.1
	T5	0.5	–	–	1	0.005	6	21	5.4
	T6	1	–	–	2	0.01	12	22	7.1
	T7	1.5	–	–	3	0.02	7	19	6.2

Effect of Growth Regulators on Number of Shoots per Explants

Initially potato in vitro culture was started from nodal cuttings and maintained on a hormone free media at $23 \pm 2^\circ \text{C}$ for 2-weeks. It is clearly evident from the data that direct shoot regeneration was remarkably influenced by type and concentrations of the auxins, cytokinins and GA3 used and no organogenesis was recorded in the basal MS media i.e., T₀ media. Further Table 2 indicated that both the varieties exhibited fairly high direct plantlet regeneration, when internodes explants were cultured on T2 medium i.e. MS+GA3 (1.0 mgL^{-1}) + IAA (0.01 mgL^{-1})+Zeatin (2.0 mgL^{-1}). Maximum number of shoots per explants after 27 days were recorded in cultivar K.CH3 followed by K.Jyoti i.e. 18 and 16, respectively in comparison to control (T₀) i.e. 2 and 1. Moreover, either increase or decrease in the concentrations of growth regulators declined number of shoots. The replacement of IAA with another auxin i.e. NAA and replacement of zeatin with another cytokinin i.e. BAP increased the number of shoots considerably in both the cultivars in comparison to control. After replacement the growth regulators maximum number of shoot was observed in T6 treatment but the number was less than T2 treatment. Although there are several reports for the use of hormone free MS medium during potato proliferation (Ahsan et al., 2003; Yasmin et al., 2011c). However, the growth of explants is slow in such hormones free, cost effective media. Otherwise, the growth rate of explant can be

improved by supplementing medium with growth regulators (Yousef et al., 2001; Hoque, 2010). This data shows that for direct shoot regeneration IAA and Zeatin was more effective than NAA and BAP.

Further, increase in the concentration of IAA i.e. up to 0.015 mgL^{-1} and zeatin up to 3.0 mgL^{-1} was not effective for direct shoot regeneration from internodes. Cytokinins at high concentrations increased chlorophyll content and compactness of the tissues. The higher levels of cytokinins might have resulted in chlorophyll development rather than shoot regeneration.

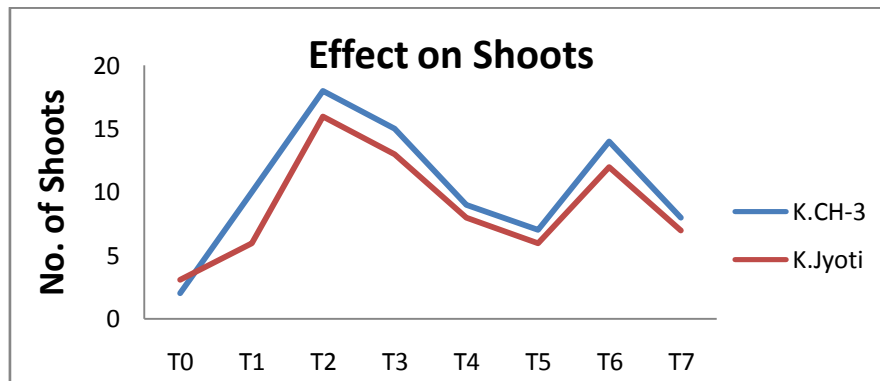


Fig1: Effect of different treatments on mean performance of in vitro induction of total number of shoots per explant for two potato cultivars.

Effect of Growth Regulators on Number of Roots per Explant

The data presented in Fig. 2 represent the number of roots per explant were obtained on all tested media compositions except in T_0 which lacked plant growth hormones. The highest number of roots i.e., 28 per explant was recorded in variety on media composition in which MS media was supplemented with GA3 (1.0 mgL^{-1})+IAA (0.01 mgL^{-1})+Zeatin (2.0 mgL^{-1}). Variety K.CH3 was followed by K.Jyoti (26 average number of roots). A decline was recorded in average number of roots with either increase or decrease from these hormonal concentrations in both the varieties. The number of roots was the lowest in T_0 as compared to other treatments. Genotypes were found detrimental for in vitro growth responses. The genotypic differences in the ability to regenerate roots were also reported by various workers (Yousef et al., 2011 and Pereira et al., 2003).

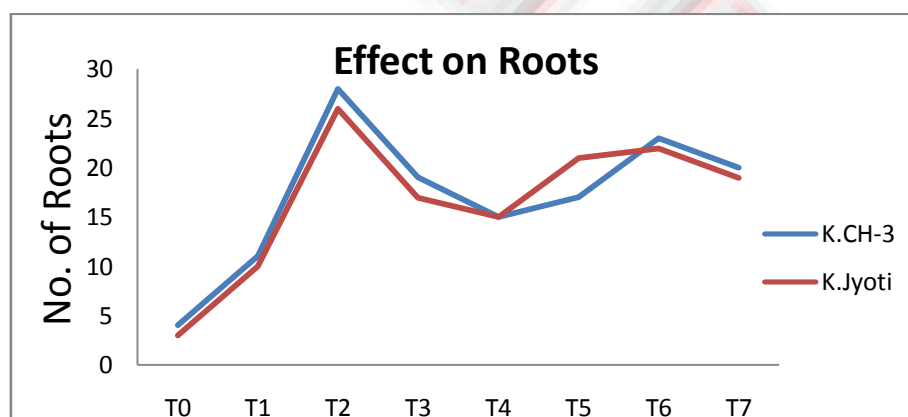


Fig2: Effect of different treatments on mean performance of in vitro induction of total number of roots per explant for two potato cultivars.

Effect of Growth Regulators on Plantlet Height

It is very obvious from the results shown in Table 2 and Fig. 3 that the treatment (T2) i.e. MS+GA3 (1.0 mgL^{-1})+IAA (0.01 mgL^{-1})+Zeatin (2.0 mgL^{-1}) induced the highest number of shoots and roots in studied cultivars. Similarly, plantlet height was observed maximum on T2 medium i.e 8.4 in K.CH3 Cultivar and 7.9 in K.Jyoti. K.CH3 consistently responded the best to the all studied growth traits on T2. Although K.Jyoti also revealed same pattern to growth parameters but lesser than K.CH3. Genotypes were found detrimental for in vitro growth responses. Pereira and Fortes, (2003) reported MS liquid medium supplemented with 0.25 mgL^{-1} gibberellic acid and 5.0 mgL^{-1} pantothenic acid as the most suitable regime for potato micro propagation. Águila et al., (2001) cultured potato explants on MS media supplemented with 1 mg gibberellic acid per litre in solid MS media. In agreement to above studies cultivars Desiree and Patrones also responded well when solid MS medium was supplemented with gibberellic acid (0.5 mgL^{-1}) and pantothenic acid (1.0 mgL^{-1}). However, the optimized concentration of GA3 and pantothenic acid is different in this study as compared to reported studies possibly due to varietal differences. Moeinil et al., (2011) reported the maximum growth of potato plantlets on MS solid medium with 0.25 mgL^{-1} GA3 and 2 mgL^{-1} calcium pantothenate. Nevertheless, Hoque, (2010) showed the best shoot and root regeneration on MS medium with 2 mgL^{-1} KIN and IAA, whereas Badoni and Chauhan, (2009) detected lower concentration of NAA (0.01 mgL^{-1}) with Gibberellic Acid (0.25 mgL^{-1}) as the best combination for the regeneration of complete plantlets from meristem tips.

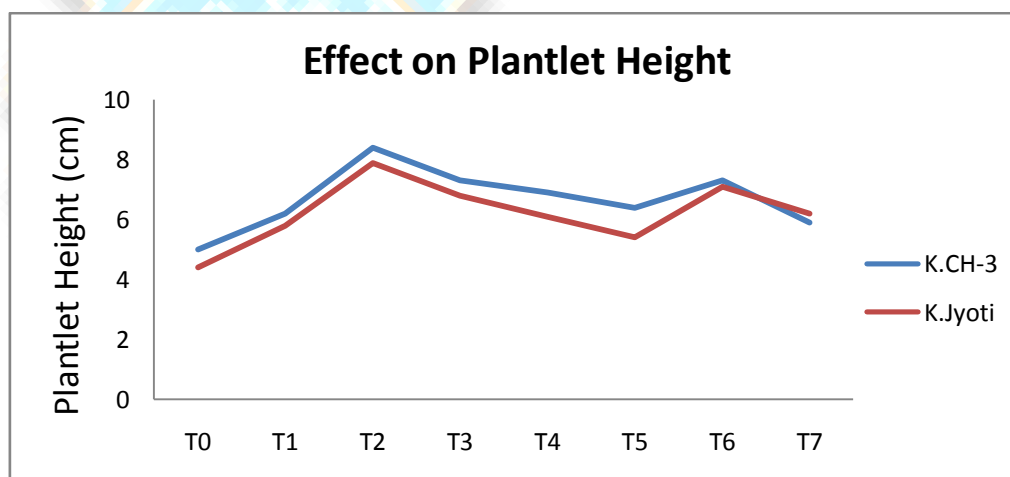


Fig 3: Effect of different treatments on mean performance of in vitro plantlet height for two potato cultivars.

CONCLUSION

From the above discussion, it is revealed that a genotype dependent In vitro protocol for the micro propagation was optimized from internodes explants of K.CH3 and K. Jyoti potato. We assume that the findings from the study presented here provide new insights for propagation of tuberous plants. Furthermore, the results could be used to produce large scale production of healthy and disease free planting materials commercially. The authors would like to extend this study by investigating the factors inducing micro tuberization in micro propagated potato plantlets and their virus indexing for the production of virus free potato plantlets and mini tubers.

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