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High frequency *in vitro* callus induction in potato (*Solanum tuberosum* L.) Genotypes

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Abstract
The present study was conducted to evaluate the effects of 2, 4-D on *in vitro* callus induction in two potato genotypes *i.e.* Kufri Bahar and Kufri Surya. Best callus induction with slightly lower contamination percent, number of days required for callusing and higher callus weights from leaf segments was reported in Kufri Bahar on full-strength basal MS media supplemented with 10.0 mg/L 2,4-D. The increasing concentrations of 2, 4-D exhibited an increase in callus induction percentage in both studied genotypes, but at the higher concentrations callus induction percentage reduced drastically. The protocol thus developed may be efficiently used to induce high frequency callus in potato genotypes for generating somaclonal variants possessing agronomically desirable traits.

Keywords: callus, auxins, leaf segments, potato, somaclonal variations.

Introduction
Potato (*Solanum tuberosum* L.), a member of family Solanaceae, is one of the most important cash crops that play major roles to strengthen Indian economy. It is cultivated in more than 100 countries belongs to the tropical, sub-tropical and temperate regions of the world (Iqbal *et al.*, 2014) [1]. Six countries including China, India, USA, Ukrain, Germany and Poland put together constitute more than 62% of the total potato production in the world. The area under potato crop in India is about 2.24 million hectares with the production of 46.40 million tonnes, whereas, it is 19.09 million hectares with the production of 394.46 million tonnes in the world (National Horticultural Board, 2015-16) [2]. Potato is the fourth most important field crop by the volume of production. Tissue culture techniques have been reported to generate disease-free quality planting materials in several crop plants including potato. The callus induction in potato was first time reported by Steward and Caplin (1951) [3]. However, the earlier studies shown that the protocols for *in vitro* callus induction in potato genotypes has been standardized by using various explants such as nodal segments (Khatun *et al.*, 2003) [4], internodal segments (Jelenic *et al.*, 2001; Nasrin *et al.*, 2003) [5, 6], leaf segments (Shirin *et al.*, 2007) [7], stem segment (Bordallo *et al.*, 2004; Turhan *et al.*, 2004) [8, 9] and leaf discs (Gavinlertvatana and Li, 1980; Dobranszki *et al.*, 1999) [10, 11]. It is also important to establish efficient and reliable protocols for *in vitro* callus induction in Indian potato genotypes. The development of efficient protocols for *in vitro* callus induction and plant regeneration is the first requisite of genetic transformation to generate transgenic plants with novel qualitative and quantitative traits. Therefore, the present study was undertaken to establish an efficient protocol for high frequency *in vitro* callus induction from leaf segments in two potato genotypes *i.e.* Kufri Bahar and Kufri Surya.

Materials & Methods
Plant Materials
For the present study, leaf segments were tested as explants for optimization of callus induction in potato genotypes. About three months old pot grown potato plants were used for excising leaves for *in vitro* experiments. Two potato cultivars *i.e.* Kufri Bahar and Kufri Surya were procured from Central Potato Research Institute (C.P.R.I.), Regional Centre, Modipuram, Meerut, Uttar Pradesh. The *in vitro* studies were conducted at Tissue Culture Laboratory, Department of Agricultural Biotechnology, Sardar Vallabhbhai Patel University of Agriculture & Technology (SVPUA&T), Modipuram, Meerut, Uttar Pradesh, India.

Callus Induction and Proliferation

Firstly, all the excised leaf explants were washed thoroughly under running tap water for 20-30 minutes and then treated with 5.0% Labolene (Qualigens, Mumbai, India) for 10 minutes followed by rinsing with sterilized distilled water for three times. After that, the leaf explants were surface sterilized with 0.1% (w/v) Bavistin (Biostadt India Limited, Mumbai, India) solution for 7 minutes and rinsed with autoclaved distilled water for 4-5 times. Then, the explants were further treated with 70% Ethanol (Jiangsu Huaxi International Ltd., China) for 45 seconds followed by rinsing with autoclaved distilled water for three times. Finally, the explants were sterilized with 0.1% (w/v) $HgCl_2$ (Qualigens, Mumbai, India) for 5 minutes under the hood of laminar airflow (Scientech Instruments, Delhi, India). After that, the explants were rinsed for 4-5 times with autoclaved distilled water to remove the traces of mercuric chloride and other surface sterilization agents. The leaf pieces of approximately 2.5 mm in size were excised aseptically and placed directly onto the full-strength basal MS (Murashige and Skoog, 1962) [12] media supplemented with 100 mg/L myo-inositol, 30 g/L sucrose (Hi-Media, Mumbai, India) and different concentrations of 2,4-diphenoxy acetic acid (2,4-D; 0.0, 2.5, 5.0, 7.5, 10.0 and 12.5 mg/L). The culture media pH was adjusted to 5.8 with either 1.0*N* HCl (Qualigens, Mumbai, India) or 1.0*N* NaOH (Merck, Mumbai, India) prior to add 0.8% (w/v) bacteriological grade agar powder (Hi-media, India) and autoclaving at 121ºC and 1.06 kg cm^{-2} for 20 min. All the culture tubes contained 15 mL of semi-solidified culture medium. After explant inoculation, all the culture tubes were incubated in a culture room at 25±2ºC with white fluorescent light of 40 μmol m^{-2} s^{-1} intensity provided by Philips tube lights (Philips, India) in under 16h/8h light/ dark photoperiods. For callus induction from leaf segments, the culture tubes were incubated in culture room under complete darkness for a minimum of two to three weeks. Callus was proliferated on best media combination of 2, 4-D. The effects of different media combinations were observed on various growth parameters of callus at regular time intervals.

Statistical Analysis

All the experiments were conducted in complete randomized design (CRD) with five replications and repeated thrice. The data on various parameters including contamination %, days for callus induction, callus induction %, callus texture, callus colour, degree of callus formation and callus weight (in grams) were recorded after 8-weeks of explants inoculation on culture medium. The recorded data were subjected to analyze as per the experimental design. Analysis of variance (ANOVA) of different data records was performed and means were compared after data analysis using OPSTAT software.

Results & Discussion

Present study deals with the investigation of the effects of different concentrations of 2, 4-D on *in vitro* callus induction in two potato genotypes *viz.* Kufri Bahar, Kufri Surya using leaf segments as explants. The culture contamination percent (43.6%) was highest in Kufri Bahar followed by Kufri Surya (41.5%). In both genotypes, callus induction percentage was significantly affected with the different concentrations of 2, 4-D (0.0, 2.5, 5.0, 7.5, 10.0 and 12.5 mg/L). Leaf segments showed highest (100.0%) callus induction in Kufri Bahar on full-strength basal MS media fortified with 10.0 mg/L 2,4-D, whereas, it was lowest (20.3%) in Kufri Surya at 12.5 mg/L 2,4-D (Table-1 & 2; Fig.- 1 & 2). The increasing concentrations of 2, 4-D was reported to be favourable for inducing rapid callus growth in both genotypes, but at the higher concentrations (12.5 mg/L) of 2, 4-D, callus growth was gradually reduced. Fastest callus induction was reported within 16.9 days in Kufri Bahar at 12.5 mg/L 2, 4-D followed by 18.4 days at 10.0 mg/L 2, 4-D in same genotype (Table-1 & 2). The lower concentrations of 2, 4-D took longer time for inducing callus in both genotypes of potato. Callus initiation was not reported on MS medium without the use of 2, 4-D. Both studied genotypes exhibited significant variability in callus texture and callus colour. The higher concentration (10.0 mg/L) of 2, 4-D showed maximum degree (from +++ to ++++) of callus formation in both genotypes of potato using leaf segments. At the higher concentration (10.0 mg/L) of 2, 4-D, callus weight was also higher. Maximum callus weight of 5.873 g was recorded in Kufri Bahar at 10.0 mg/L concentration of 2, 4-D followed by 5.478 g in Kufri Surya at 7.5 mg/L of 2, 4-D (Table-1 & 2; Fig - 1 & 2). A lot of works have been done by earlier researchers for improving the frequency of callus induction in potato by using auxins especially 2, 4-D. The present study also exhibited the potential and essentiality of auxins in the form of 2, 4-D for inducing callus in potato by using leaf explants. The results of the present study showed the conformity with the findings of Shirin *et al.* (2007) [7], AbdElaleem *et al.* (2009) [13], Huda *et al.* (2013) [14], Sherkar and Chavan (2014) [15] and Iqbal *et al.* (2016) [16].

Table 1: Effects of different concentration of 2, 4-D on *in-vitro* callus induction from leaf segments in potato genotype Kufri Bahar (Mean±SE)

2,4-D conc. (mg/L)	Contamination %	Days for callus induction	Callus induction %	Callus texture	Callus colour	Degree of callus formation*	Callus weight (in gms)
0.0	0.0±0.00	0.0±0.00	0.0±.00	NA	NA	NA	0.0±0.00
2.5	43.6±0.88	28.8±0.69	60.4±1.38	Watery	Greenish White	++	2.257±0.08
5.0	40.3±1.45	24.3±0.86	60.2±2.07	Friable	Greenish Brown	++	2.486±0.05
7.5	0.0±0.00	21.5±.0.98	90.7±4.21	Friable	Greenish Brown	++	1.548±0.03
10.0	10.5±0.33	18.4±0.34	100.0±4.73	Friable	Greenish White	++++	5.873±0.26
12.5	10.7±0.33	16.9±0.40	30.8±0.69	Friable	Greenish Yellow	+++	3.085±0.05
CD at 5.0%	2.243	2.002	6.668	---	---	---	0.367
SE(d)	1.018	0.909	3.027	---	---	---	0.167
SE(m)	0.720	0.642	2.140	---	---	---	0.118

* Here, ++=Poor; +++=Moderate; ++++=Good

Table 2: Effects of different concentration of 2, 4-D on *in-vitro* callus induction from leaf segments in potato genotype Kufri Surya (Mean±SE)

2,4-D conc. (mg/L)	Contamination %	Days for callus induction	Callus induction %	Callus texture	Callus colour	Degree of callus formation*	Callus weight (in gms)
0.0	0.0±0.00	0.0±0.00	0.0±0.00	NA	NA	NA	0.0±0.00
2.5	0.0±0.00	32.6±0.75	30.2±0.69	Watery	Greenish Yellow	++	1.128±0.03
5.0	41.5±1.38	27.3±0.92	60.5±2.07	Friable	Greenish Yellow	+++	2.814±0.05
7.5	30.8±1.44	24.5±1.15	40.6±1.84	Friable	Greenish Yellow	++	5.478±0.03
10.0	30.6±0.52	21.4±0.34	40.9±0.69	Friable	Greenish Yellow	++++	4.643±0.20
12.5	40.4±0.92	19.7±0.46	20.3±0.46	Friable	Greenish Brown	++++	4.477±0.08
CD at 5.0%	2.880	2.233	3.796	---	---	---	0.297
SE(d)	1.307	1.014	1.723	---	---	---	0.135
SE(m)	0.924	0.717	1.218	---	---	---	0.095

* Here, ++=Poor; +++=Moderate; ++++=Good

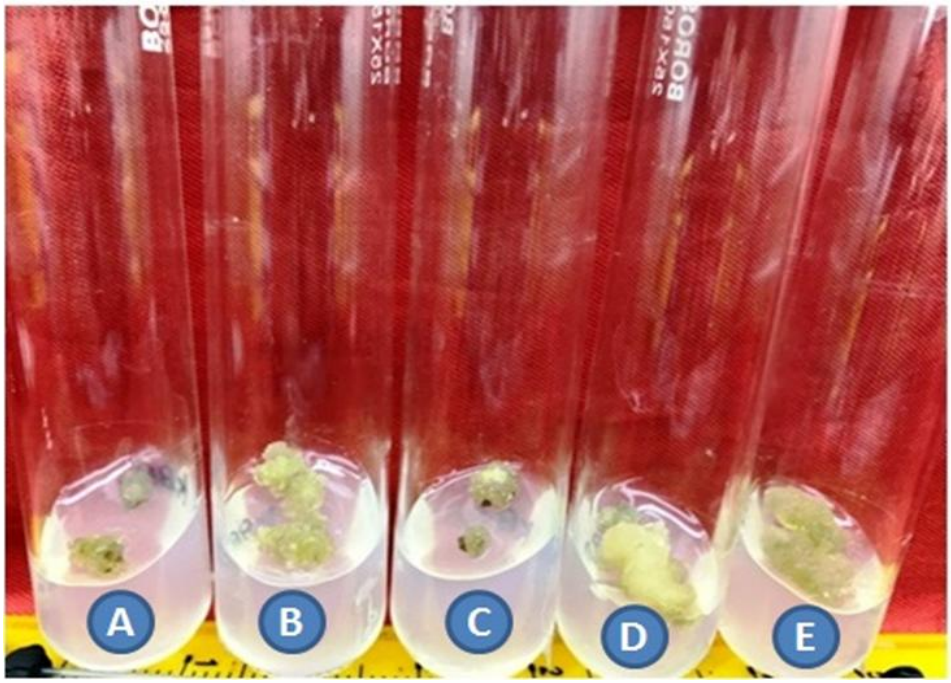

Fig 1: *In-Vitro* callus induction in potato genotype Kufri Bahar using leaf segments. Here, (A) 2.5 mg/L 2, 4-D, (B) 5.0 mg/L 2, 4-D, (C) 7.5 mg/L 2,4-D, (D) 10.0 mg/L 2,4-D, (E) 12.5 mg/L 2,4-D.

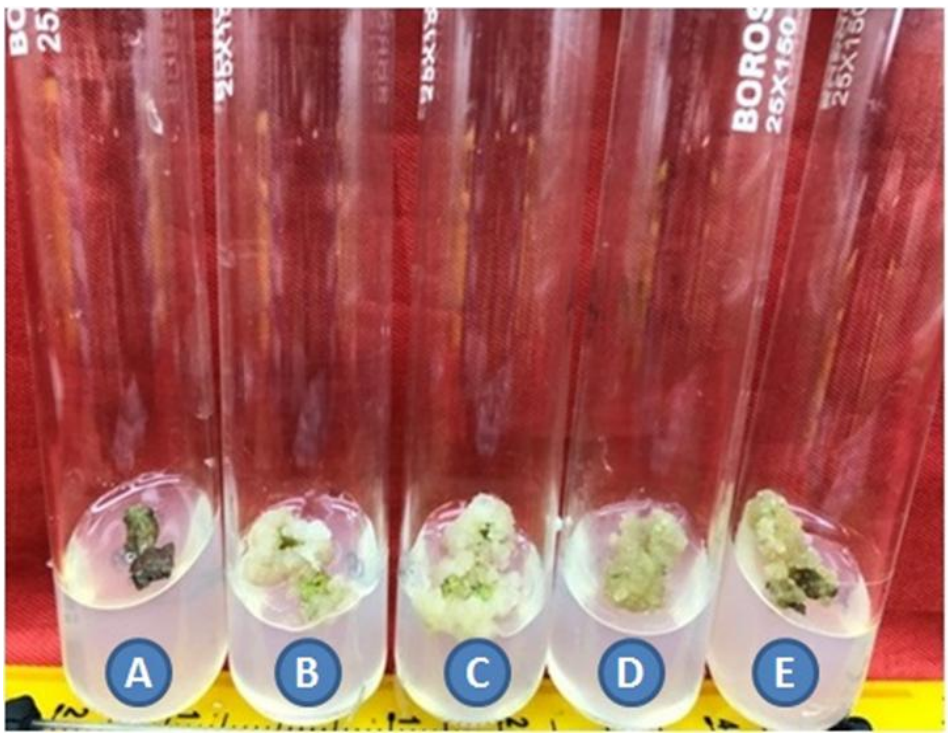

Fig 2: *In-Vitro* callus induction in potato genotype Kufri Surya using leaf segments. Here, (A) 2.5 mg/L 2, 4-D, (B) 5.0 mg/L 2, 4-D, (C) 7.5 mg/L 2,4-D, (D) 10.0 mg/L 2,4-D, (E) 12.5 mg/L 2,4-D.

Conclusion

Present study concluded that the presence of 2, 4-D (7.5-10.0 mg/L) in culture media was essential for high frequency *in vitro* callus induction in both genotypes of potato. Highest callus induction with slightly lower contamination percent, number of days required for callusing and higher callus weights from leaf segments was reported in Kufri Bahar on full-strength basal MS media supplemented with 10.0 mg/L 2,4-D. The protocol thus developed may be efficiently used to induce high frequency callus in potato genotypes for generating somaclonal variants possessing agronomically desirable traits.

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