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Protocol Optimization for *In Vitro* Regeneration System in Frost Sensitive Potato Genotypes: As a Basis for Genetic Transformation Studies in *Solanum tuberosum* L.

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Abstract

For genetic transformation, an efficient *in vitro* regeneration system in frost sensitive potato genotypes was established in this research study. Two frost sensitive genotypes of potato viz, Kuroda and FD-51-5 with good agronomic features but sensitive to frost were used. Six callus formation media were investigated for callus formation, in which three levels of 2, 4-D (3.5 mg/L, 4.5 mg/L and 5.5 mg/L) alone and in combination with one level of NAA (0.25 mg/L) were used. Of these, four callogenesis media viz, CM1, CM2, CM3 and CM4 were proved to be best for callus formation while genotype Kuroda gave best response. So these four CM and genotype kuroda was selected for *in vitro* regeneration study in which five regeneration media (RM) were investigated. In these media three levels of BAP (2.75 mg/L, 3.75 mg/L, and 4.75 mg/L) and one level of NAA and kinetin (0.25 mg/L) were used in different combinations. For *in vitro* regeneration, two age groups of calli viz, 28 days old and 35 days old were studies to check the effect of calli age *in vitro* regeneration. These calli of genotype kuroda were induced on selected CM and then were put on these RM. Thereby results showed that twenty eight days old calli of genotype Kuroda from CM2 when shifted on RM4 gave maximum number of shoots.

Keywords: Kuroda; Frost; Low temperature tolerance; Tissue culture

Introduction

Potato (*Solanum tuberosum* L.) is a food crop with great economic value. It is grown in temperate, tropical and sub-tropical regions [1]. This crop with multiple uses is attaining the status of staple crop. Potato is a staple food crop of forty countries around the world [2]. Potato is a tuberous crop plant which belongs to Solanacea family [3]. Potato is a source of complex carbohydrates and antioxidants that provide energy and immunity to our body. The kind and amount of antioxidants may differ in different potato cultivars, though carotenoids and anthocyanin are predominant in this crop. It also provides a significant amount of vitamin C and potassium. The skin of medium size potato (5.3 ounce) contains 110 calories per serving.

This crop, because of high nutritional value, meets the increasing demand of food in the world. Potato crop is being cultivated in more than hundred countries and ranks fifth globally due to high production [4]. Potato is most important dicotyledonous tuber crop in the world [5]. Pakistan ranks at 7th number worldwide, due to production of potato [6]. In Pakistan, potato is grown on an area of 104.5 thousand ha with 1684.7 thousand tons production per annum [7]. In Punjab, production of potato was 2767 thousand tons and yield was 24 thousand tons per hectare in 2007-2008. According to FAO report, potato production in 2010 was about 324 million tones in United Nations. Whereas in Pakistan its production was 2025 thousand tones and area under cultivation was 112 thousand hectare. However, during 2011-2012, potato production in Pakistan was 3392.5 thousand tons. This depicts that on an average, potato production in Pakistan is 2556.5 thousand tons from 2002-2012. In Pakistan current yield level of potato is 18 tons per hectare**.** In Pakistan three potato crops are achieved per year during autumn, summer and spring. Autumn crop is sown in October and harvested in January, summer crop is sown in January and harvested in May-June and spring crop is sown in April-May and harvested in August-September.

This crop has some environmental issues that severely restrict its production in terms of quality and quantity loss. Although plant growth and development are affected by both biotic as well as abiotic factors while abiotic stresses are the major cues that trigger various physiological and biochemical adaptations [8]. Biotic and abiotic factors adversely affect yield. Abiotic stress (temperature, chemical toxicity, drought, salinity and oxidative) are potential threats for potato crop [9]. Among these threats, frost is a major concern that is faced by potato crop. Frost causes different metabolic insults and yield deterioration in crop production [10].

At high latitudes frost damage is most important threat in potatoes [11]. Freezing temperatures restricted the length of the season suitable for the growth of potato crop. Frost damages potato crop when temperature becomes very low about -2°C. The lethal minimum temperature for the potato crop is 0°C. Intensity of frost injury depends on low temperature and duration of low temperature [12]. Ground frost was particularly severe during 2005-06 and 2007-08. Due to freezing, crystals form in tissues which lead to death of plant cell rapidly. Low temperature causes potato tuber injury and poor tuber germination.

During low temperature, a potato plant experiences three different stress conditions viz, dehydration, mechanical stress and osmotic stress i.e., due to the removal of water from vacuole. In dehydration stress, extracellular ice is formed while mechanical stress yields cell contraction as well as ice accumulation. Freezing injury of cell leads to ions leakage

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as well as turgidity loss. In addition to these panoramas, cell membrane disturbance, swelling of protoplasm and other plant organelles such as mitochondria and chloroplast are also the attributes of freezing injury [13]. Genetic engineering is becoming a useful technique and tissue culture is pre-requisite for this approach.

By considering frost as a major constraint of low production in potato crop, this study is designed that devoted exclusively to establish an efficient and expedite *in vitro* regeneration system in frost sensitive potato genotypes(s). Hence in this research study an *in vitro* regeneration system in selected genotypes of potato was established that would be used in genetic transformation studies in future. For developing frost tolerance through nonconventional ways, this would be a basis of genetic transformation strategies in potato.

Materials and Methods

Germplasm collection

Frost sensitive genotypes of potato viz, Kuroda and FD-51-5 were selected for this study. These genotypes were taken from Potato Research Institute, Sahiwal, Pakistan.

Media for callus induction

Six callus induction media containing different concentrations of plant growth regulators while basal MS salts and sucrose in common (Table 1) were investigated.

Explant sterilization and culturing on callogenesis media

Fresh and healthy potato tubers of studied genotypes were selected and surface sterilized by washing with water and dipped in laundry bleach containing 2 to 3 drops of tween 20, for 20 minutes. Then tubers were sprayed with 70% alcohol and cleaned with sterilized tissue paper. Thereafter under laminar flow hood tubers were sliced and then dipped into 70% ethanol for 1 minute following washing with ultrapure water. Thereafter these pieces were dipped into 25% bleach containing 2 to 3 drops of liquid soap for 20 minutes and then washed with ultrapure water. Subsequently in autoclaved distilled water these pieces were incised thereby small and thin sections were made and were cultured on petri plates containing callus induction media. All these steps were performed under axenic conditions. These petri plates were placed in growth room under dark condition. Sub culturing was done biweekly.

In vitro regeneration and root induction

For *in vitro* regeneration five regeneration media containing growth regulators (BAP, KIN, NAA) at different concentration along with Basal MS salt and sucrose in common were investigated (Table 1). But for the development of profuse rooting system, ½ MS media having no growth hormones was used. Proliferated calli of different ages viz, 28 days old and 35 days old were shifted to regeneration media, incubated for 6-8 hours in light/dark condition at 26 ± 1°C and were subcultured bi weekly. For root induction *in vitro* regenerated shoots were then shifted to ½ MS. Subsequent to rooting, plantlets were transferred into pots containing Belgium compost covered with polythene bags for acclimatization.

Collection and analysis of data

Data of callogenesis study were recorded in the form of rate of proliferation (see scale) and weight of calli for five weeks (Table 2). While *in vitro* regeneration data were collected as number of *in vitro* regenerated shoots/explants. Completely randomized design in factorial was used and Duncan's multiple range tests was calculated among various treatments.

Results

Potato (*Solanum tuberosum* L.) is an economically important vegetable crop but it is prone to various biotic and abiotic threats. Among these, frost is most important factor that limits its yield. Hence, this research study was designed to establish *in vitro* regeneration system in selected frost sensitive potato genotypes. Because *in vitro* regeneration system (Tissue culture) is the pre-requisite or / basis for genetic engineering of crop plant.

Callogenesis

The response of callus formation was investigated on six different callogenesis media (CM). For callus induction study, three levels of 2, 4-D (3.5, 4.5 and 5.5 mg/L) and one level of NAA (0.25 mg/L) were used for both genotypes. Data for callus formation response of both genotypes were scored on the basis of callus proliferation rate and weight of Calli and subjected to statistical analysis. The analysis of variance table revealed that, significant variation was present among CM and between genotypes (Tables 3 and 4). However interaction table revealed that excellent proliferation rate as well as weight of calli were observed on CM1, CM2, CM3 and CM4 (Plate 1; Figures 1 and 2; Tables 5 and 6).

In vitro regeneration studies

On the basis of callogenesis study, genotype Kuroda and four callogenesis media (CM1, CM2, CM3 and CM4) were selected for *in vitro* regeneration study. Thus calli of selected genotype (Kuroda) were induced on selected CM and then were shifted on five regeneration media containing different growth regulators viz, KIN, BAP and NAA in different combination at different level. However, data were collected in the form of number of shoots per explant and statistically analyzed. Analysis of variance table revealed that significant variation was present between age of calli, among CM and among RM as well as their interaction (Table 7).

Media composition	Callogenesis media (CM)						Regeneration media (RM)				
	CM1	CM2	CM3	CM4	CM5	CM6	RM1	RM2	RM3	RM4	RM5
Nutrients Supplement	MSN	MSN	MSN	MSN	MSN	MSN	MSN	MSN	MSN	MSN	MSN
Sucrose (g/L)	30	30	30	30	30	30	30	30	30	30	30
2,4-D (mg/L)	3.5	4.5	5.5	3.5	4.5	5.5					
NAA (mg/L)	0	0	0	0.25	0.25	0.25	0	0	0	0	0.25
BAP (mg/L)							2.75	3.75	4.75	4.75	4.75
Kinetin (mg/L)							0.25	0.25	0.25	0	0
Gellun gum powder (g/L)	2.66	2.66	2.66	2.66	2.66	2.66	2.66	2.66	2.66	2.66	2.66

MSN*(MS salt=4.33 g/L, Myo-inositol=0.1 g/L, 500 µL vitamins (Nicotinic acid, Pyridoxine HCl, Thymine HCl, Glycine) pH=5.7-5.8

Table 1: Tissue culture media to be used in this study.

+	Very low calli proliferation
+ +	Low calli proliferation
+ + +	Good calli proliferation
+ + + +	Very good calli proliferation
+ + + + +	Excellent calli proliferation

Table 2: Scale to score callus proliferation.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Week	4	11.0667	2.7667	5.50**
Genotype	1	50.4169	50.4169	100.23**
CFM	5	31.3500	6.2700	12.46**
Genotype x CFM	5	14.6833	2.9367	5.84**
Error	44	22.1333	0.5030	
Total	59	129.6500		

NS = Non-significant (P>0.05); *=Significant (P<0.05); **=Highly significant (P<0.0

Table 3: Analysis of variance table for Rate of proliferation.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Week	4	0.29437	0.07359	5.55**
Genotype	1	0.52547	0.52547	39.62**
CFM	5	0.28828	0.05766	4.35**
Genotype x CFM	5	0.65158	0.13032	9.83**
Error	44	0.58353	0.01326	
Total	59	2.34323		

**=Highly significant (P<0.01)

Table 4: Analysis of variance (ANOVA) table for Weight of calli.

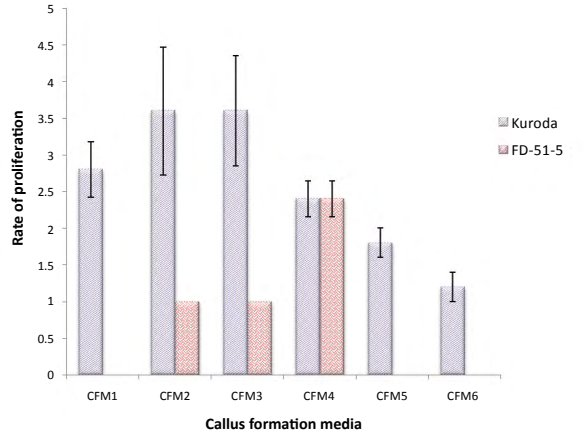

Figure 1: Rate of callus proliferation of potato genotypes to be studied, on different callus formation media.

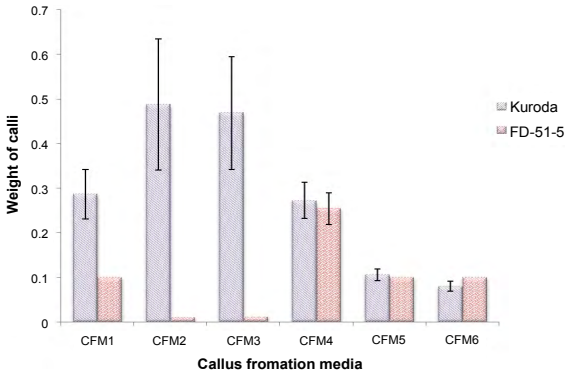

Figure 2: Weight of calli of potato genotypes to be studied on different callus formation media.

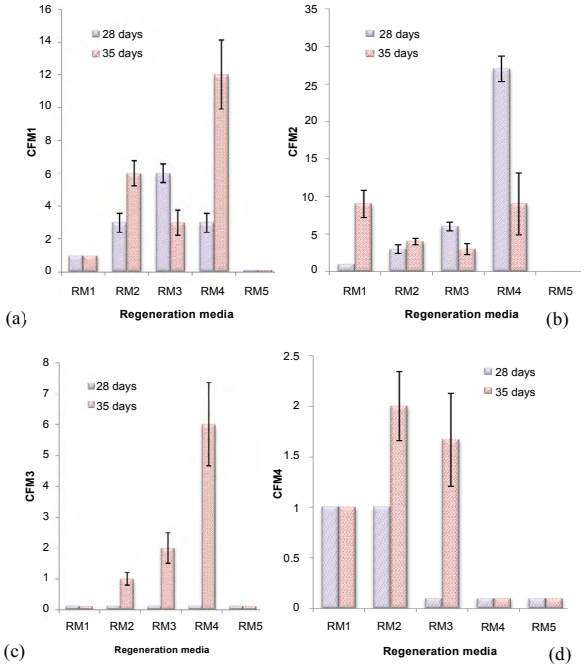

Figure 3: Responses of different callus formation media (CFM) on different regeneratio (RM): (a) Response of calli induced on CFM1 on regeneration media (b)Response of calli induced on CFM2 on regeneration media (c) Response of calli induced on CFM3 on regeneration media (d) Response of calli induced on CFM4 on regeneration media.

Similarly interaction table also depicts that when 28 days old calli induced on CM2 were shifted on RM4 showed an excellent response and gave maximum number of shoots per explant (Plates 2 and 3; Figure 3; Table 8), followed by 35 days old calli induced on CM1 were when shifted on RM4 (Figure 3; Table 8). However, 28 days old calli induced on CM3 when shifted on regeneration media, initially gave regeneration response and turned into greenish in color but latterly no shoot formation was observed (Plate 4). In this study calli of both age group induced on CM4 when shifted on RM4 and RM5, then just calli proliferation was achieved (Plate 5). Subsequently *in vitro* regenerated shoots were subcultured biweekly (Plate 6) and then were shifted on ½ MS media for getting profused rooting system (Plate 7). For hardening and acclimatization these *in vitro* regenerated plantlets were transferred into pots containing Belgium compost and then wrapped with polythene bags and were kept at 26 ± 1°C under 16/8 hr light-dark regime (Plate 8).

Discussion

In these study three levels of 2, 4-D (3.5 mg/L, 4.5 mg/L and 5.5 mg/L) and one level of NAA (0.25 mg/L) were studied for both genotypes (Kuroda and FD-51-5). Both genotypes showed different response on all CFM which depicts genotype based response. Kuroda genotype showed significant callus induction response on all CFM than genotype FD-51-5. Highest callus proliferation rate was observed by

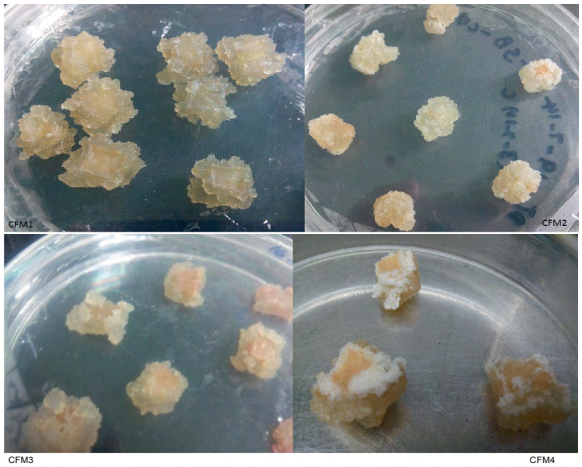

Plate 1: Callus induction response of different callus formation media (CFM).

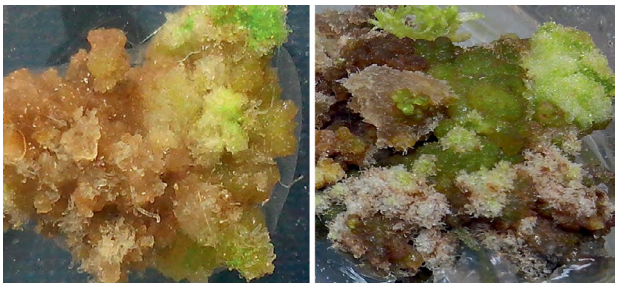

Plate 2: Regeneration response on RM4 of 28 days old calli (induced on CM2) of cv. Kuroda.

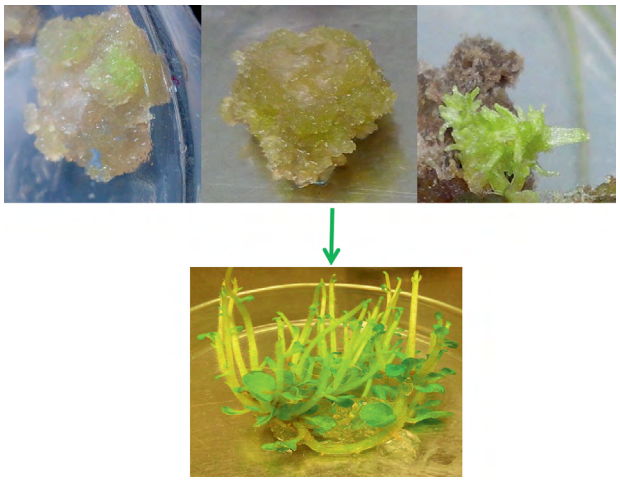

Plate 3: Different phases of *in vitro* regeneration in potato.

using 2,4-D alone. Data were analyzed after five weeks of culture and the results showed that there was a wide range of callus initiation, callus health and rate of callus proliferation.

Among all growth regulators used 2, 4-D was found to be the most effective growth regulator for potato callus induction when used alone. Castillo et al. [14] reported that auxin 2, 4-D by itself or in combination with cytokinins has been widely used to enhance callus induction and maintenance. Moreover many researchers observed 2,4-D as the best auxin for callus induction as common as in monocot and even in dicot

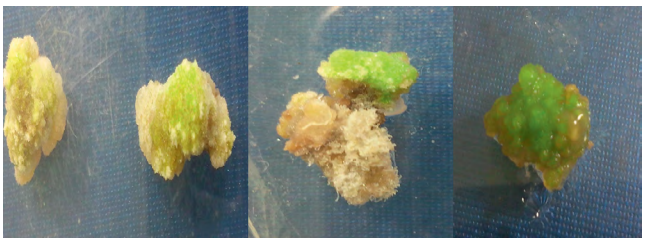

Plate 4: Regeneration response of 28 days old calli induced on CM3.

Plate 5: Regeneration response on RM4 and RM5 of calli of both age group (28 days and 35 days) induced on CM4.

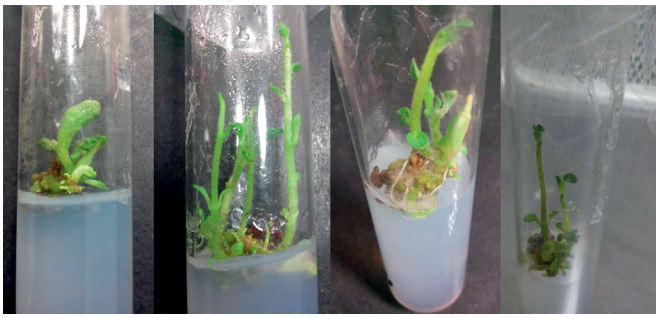

Plate 6: *In vitro* regeneration response of potato cv. Kuroda.

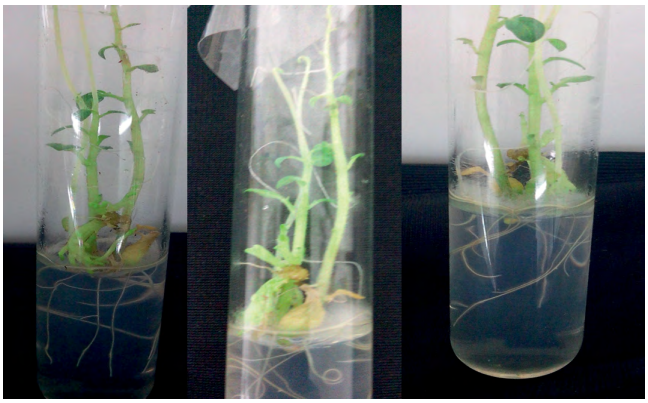

Plate 7: Root induction of *in vitro* regenerated shoots on ½ MS medium.

Plate 8: Acclimatization of *in vitro* regenerated plantlets of cv. Kuroda, in pots containing Belgium compost.

CFM	Genotype						Mean		
	Kuroda			FD-51-5					
CFM1	2.80	±	0.37	0.00	±	0.00	1.40	±	0.50
CFM2	3.60	±	0.87	1.00	±	0.00	2.30	±	0.60
CFM3	3.60	±	0.75	1.00	±	0.00	2.30	±	0.56
CFM4	2.40	±	0.24	2.40	±	0.24	2.40	±	0.16
CFM5	1.80	±	0.20	0.00	±	0.00	0.90	±	0.31
CFM6	1.20	±	0.20	0.00	±	0.00	0.60	±	0.22
Mean	2.57	±	0.25 A	0.73	±	0.17 B			

Table 5: Genotype x CFM interaction mean ± SE for rate of proliferation: Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

CFM	Genotype						Mean		
	Kuroda			FD-51-5					
CFM1	0.286	±	0.056	0.100	±	0.000	0.193	±	0.041
CFM2	0.487	±	0.147	0.010	±	0.000	0.249	±	0.105
CFM3	0.468	±	0.127	0.012	±	0.000	0.240	±	0.097
CFM4	0.272	±	0.040	0.254	±	0.036	0.263	±	0.026
CFM5	0.106	±	0.013	0.100	±	0.000	0.103	±	0.006
CFM6	0.080	±	0.011	0.100	±	0.000	0.090	±	0.006
Mean	0.283	±	0.043 A	0.096	±	0.016 B			

Table 6: Genotype × CFM interaction mean ± SE for weight of calli: Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Age of Calli	1	5.125	5.125	7.24**
CFMs	3	596.734	198.911	280.82**
RM	4	658.634	164.658	232.46**
Age*CFMs	3	87.058	29.019	40.97**
Age*RM	4	37.939	9.485	13.39**
CFMs*RM	12	729.653	60.804	85.84**
Age*CFMs*RM	12	679.405	56.617	79.93**
Error	80	56.667	0.708	
Total	119	2851.215		

NS=Non-significant (P>0.05); *=Significant (P<0.05); **=Highly significant (P<0.01)

Table 7: Analysis of variance table for *in vitro* Regeneration.

CFM	RM	Age of calli						Mean		
		28 days			35 days					
	1	1.00	±	0.00 fg	1.00	±	0.00 fg	1.00	±	0.00 F
	2	3.00	±	0.58 ef	6.00	±	0.58 d	4.50	±	0.76 CD
1	3	6.00	±	0.58 d	3.00	±	0.58 ef	4.50	±	0.76 CD
	4	3.00	±	0.58 ef	12.00	±	1.15 b	7.50	±	2.09 B
	5	0.10	±	0.00 g	0.10	±	0.00 g	0.10	±	0.00 F
	1	1.00	±	0.00 fg	9.00	±	0.58 c	5.00	±	1.81 C
2	2	3.00	±	0.58 ef	4.00	±	0.58 de	3.50	±	0.43 CD
	3	6.00	±	0.58 d	3.00	±	0.58 ef	4.50	±	0.76 CD
	4	27.00	±	1.73 a	9.00	±	0.58 c	18.00	±	4.11 A
	5	0.10	±	0.00 g	0.10	±	0.00 g	0.10	±	0.00 F
	1	0.10	±	0.00 g	0.10	±	0.00 g	0.10	±	0.00 F
3	2	0.10	±	0.00 g	1.00	±	0.00 fg	0.55	±	0.20 F
	3	0.10	±	0.00 g	2.00	±	0.58 efg	1.05	±	0.50 F
	4	0.10	±	0.00 g	6.00	±	0.58 d	3.05	±	1.34 DE
	5	0.10	±	0.00 g	0.10	±	0.00 g	0.10	±	0.00 F
	1	1.00	±	0.00 fg	1.00	±	0.00 fg	1.00	±	0.00 F
4	2	1.00	±	0.00 fg	2.00	±	0.58 efg	1.50	±	0.34 EF
	3	0.10	±	0.00 g	1.67	±	0.67 efg	0.88	±	0.46 F
	4	0.10	±	0.00 g	0.10	±	0.00 g	0.10	±	0.00 F
	5	0.10	±	0.00 g	0.10	±	0.00 g	0.10	±	0.00 F

Table 8: Age × CFM x RM interaction mean ± SE: Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

[15-20].

Many studies have been reported that *in vitro* callus induction was not only dependent on plant species but also on type of explants, light, temperature and explant age [5,21,22]. In potato, callus has been successfully induced from numerous explant, including leaf, stem segments including 1 or 2 node or without node (internode) and tuber [5,23-26]. According to the effect of hormones and their concentrationon callus induction and regeneration [27] founded the highest percentage of callus induction (100%) was at 2.0 -5.0 mg/L 2, 4-D alone.

It was investigated that 2, 4-D was best growth hormone among all other growth hormones (NAA, KIN and BAP) for rapid callus induction and for increasing rate of proliferation and weight of calli Different concentrations of 2,4-D were also tested by different scientist [5,23,27,28]. Potato cultivar granula was studied to test the effects of 2, 4-D on callus induction ability of potato. Highest callus formation (95%) was recorded on medium containing 2,4-D alone by Laboney et al. [28] and their results agree with the results of this study because the highest mass of callus was obtained at by using 2, 4-D.

Within different concentrations of 2, 4-D, when used alone highest degree of callus formation from tuber segment was recorded in MS medium. These results are agreement with our results. The necessity of cytokinin for shoot initiation is well documented by Ref. [15]. The success of callus induction and regeneration is dependent on genotype, the composition of the culture medium and the presence of appropriate combinations and concentration of hormones in the culture media. Many studies have been reported that *in vitro* callus induction was not only dependent on plant species but also on type of explants, light, temperature and explant age [5,21,22].

Genotype Kuroda showed highest callus proliferation rate on media containing 2, 4-D alone. Similar results were observed by Shirin et al. [5] when they used 2, 4-D alone. They also studied growth regulators 2, 4-D, NAA alone and combination of NAA with BAP to check the

callus formation response. 2, 4-D was found best growth regulator for callus induction than other growth regulators. They used potato tuber for callus formation. They used 2, 4-D, NAA and BAP to test the callus induction response in potato (*Solanum tuberosum* L.). They observed massive amount of callus, when 2, 4-D was used alone. They concluded that 2, 4-D was best option for callus induction than all other growth regulators studied. Their results are similar with this study. Forooghian et al. [29] studied three genotypes of potato viz, Santa, Agria and Savalan to observe the callus induction response. They used different concentrations of 2, 4-D and KIN for callus induction. They found 2, 4-D best growth regulator for callus induction. They concluded that higher concentration of 2, 4-D produced maximum callus. These results are similar with our results.

Kumar et al. [30] studied two potato genotypes (Kufri Chipsona 3 and MP- 97/644) for rapid callus induction. They found that 2, 4-D in combination with KIN produced best callus response in these two genotypes. They used BAP, KIN and $AdSO_4$ for shoot regeneration purpose. They observed that BAP in combination with $AdSO_4$ produced best shoots. Our results have no similarity with these results. Khalafalla et al. [27] used tuber as explant for callus formation in potato cultivar Almera. They found best callus response on media containing 2, 4-D alone. Their results agreed with our results. But for regeneration purpose, they used TDZ and BAP. They observed maximum regeneration on media containing TDZ hormone. In case of regeneration their results have no similarity with our results because in this study we observed maximum regeneration on media containing BAP alone. Elaleem et al. [23] used tuber explants for soot regeneration. They used BAP and TDZ growth hormones for regeneration purpose. They observed maximum shoots on media containing TDZ alone. Their results are not in support of our results. Maximum number of shoots were observed on media supplemented with BAP alone with concentration 4.75 mg/l. our results have no similarity with the results obtained by Hussaini et al. [31] because they used combination of different growth hormones viz, BAP, GA_3, NAA and TDZ. They found best regeneration on media supplemented with combinations of BAP, GA_3 and TDZ.

BAP alone produced maximum number of shoots per explants. Our results are not matched with Ref. [5]. They used BAP, NAA, KIN growth regulators for shoot regeneration. They found that media containing KIN in combination with NAA was best for shoot regeneration. Laboney et al. [28] used two different growth regulators (BAP, GA_3) for shoot regeneration. They observed maximum number of shoots by using 1.0 mg/L BAP and 0.5 mg/L GA_3 in combination. Maximum number of shoots was observed by using 1.0 mg/L IAA and 1.0 mg/L GA_3 in combination. Their results are not in support of our results because in this study maximum number of shoots per explants was obtained by using BAP alone with 4.75 mg/L concentration. For root induction ½ MS was used.

Haque et al. [32] studied different explants and concentrations of 2,4-D and kinetin for callus formation. Then they observed weight of calli. They used explants which gave maximum calli when 1.0 mg/L 2,4-D+0.25 mg/L kinetin concentration was used. Similarly, different explants versus different concentrations of BAP/GA_3/IAA showed significant differences for shoot length and leaf number per plantlet and also for root length. However, interaction term confirmed node and node/internode explants produced better results in shoot length and number of leaves per plantlet when concentrations 1.0 mg/L BAP+0.1 mg/L GA_3 and 1.0 mg/L BAP+0.2 mg/L GA_3, 1.0 mg/L BAP+0.4 mg/L GA_3, respectively, were used. Shoot tip explants also produced better results in root length after 28 days plantlet^{-1} when concentrations 1.0 mg/L IAA+0.25 mg/L GA_3 were used. These results have no similarity with our results.

References

1. Iqbal M, Jaskani MJ, Rafique R, Zia ul Hassan S, Iqbal MS, et al. (2014) Effect of Plant growth regulators on callus formation in potato. J Agri-Food and Appl Sci 2: 77-81.
2. Hoque ME (2010) In vitro tuberization in potato (Solanum tuberosum L.). Plant Omics J 3: 7-11.
3. Borna RS, Haque MI, Sarker RH (2010) Agrobacterium-mediated genetic transformation for local cultivars of Potato (Solanum tuberosum L.) using marker genes. Plant Tissue Cult and Biotech 20: 145-155.
4. Shekhar S, Agrawal L, Buragohain AK, Datta A, Chakraborty S, Chakraborty N (2013) Genotype independent regeneration and Agrobacterium-mediated genetic transformation of sweet potato (Ipomoea batatas L.). Plant Tissue Cult and Biotech 23: 87-100.
5. Shirin F, Hossain M, Kabir M, Roy MF, Sarker S (2007) Callus induction and plant regenration from internodal and leaf explants of four potato (Solanum tuberosum L.) Cultivars. World J Agric Sci 3: 1-6.
6. Sultan IN, Kakar MA, Yousafzai A, Ibrahim SM, Ahmmad F, et al. (2011) Effects of different concentrations and combinations of growth regulators on the callus formation of potato (Solanum tuberosum L) explants. Curr Res J Biol Sci 3: 499-503.
7. Akhtar N, Munawwar MH, Hussain M, Mahmood M (2006) Sterile shoot production and direct regeneration from the nodal explants of potato cultivars. Asian J Plant Sci 5: 885-889.
8. Bhatnagar-Mathur P, Vadez V, Sharma KK (2008) Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. Plant Cell Rep 27: 411-424.
9. Begam SN, Nasiruddin KM, Haque MS, Yasmin S (2008) Agrobacterium-mediated genetic transformation of potato for abiotic stress tolerance. Progress Agric 19: 37-43.
10. Onamu R, Legaria JP, Sahagun JC, Rodriguez JL, Perez JN (2012) In vitro Regeneration and Agrobacterium-mediated transformation of potato (Solanum tuberosum L.) Cultivars Grown in Mexico. Plant Tissue Cult and Biotech 22: 93-105.
11. Hijmans RJ, Jacobs M, Bamberg JB, Spooner DM (2003) Frost tolerance in wild potato species: Assessing the predictivity of taxonomic, geographic and ecological factors. Euphytica 130: 47-59.
12. Arrora RK, Singh RK, Gulato S (2010) Managing loss from ground frost- A major constraint to potato production in North-Western plains. Potato J 37: 73-74.
13. Levitt J, Palta JP (1972) Freezing stress in potato. Plant physiol 25: 398-401.
14. Castillo AM, Egana B, Sanz JM, Cistue L (1998) Somatic embryogenesis and plant regeneration from barley cultivars grown in Spain. Plant Cell Rep 17: 902-906.
15. Evans DA, Sharp WR, Filck CE (1981) Growth and behavior of cell culture: embryogenesis and organogenesis. In Plant Tissue Culture: Method and applications in Agriculture. Thrope TA (ed.), Academic Press, pp: 45-113.
16. Ho WO, Vasil IK (1983) Somatic embryogenesis in sugarcane (Saccharum officinarum L.) the morphology and physiology of callus formation and the ontogeny of somatic embryos. Protoplasm 118: 169-180.
17. Jaiswal VS, Narayan P (1985) Regeneration of plantlets from the callus of stem segments of adult plants of Ficus religiosa L. Plant Cell Rep 4: 256-258.
18. Chee PP (1990) High frequency of somatic embryogenesis and recovery of fertile cucumber plants. Hort Sci 25: 792-793.
19. Mamun ANK, Islam R, Reza MA, Joadar OJ (1996) In vitro differentiation of plantlet of tissue culture of Samonea saman. Plant Tissue Cult 6: 1-5.
20. Jayasree T, Pavan U, Ramesh M, Rao AV, Reddy KJM, et al. (2001) Somatic embryogenesis from leaf culture of potato. Plant Cell Tissue Organ Cult. 64: 13-17.
21. Salehi HM, Salehi, Ashiri F (2008) Some conditions for the best callus induction in common bermudagrass [Cynodon dactylon (L.) Pers. (California origin)]. Am-Euras J Agric & Environ Sci 3: 409-413.

22. Mohebodini M, Javaran M, Mahboudi F, Alizadeh H (2011) Effects of genotype, explant age and growth regulators on callus induction and direct shoot regeneration of lettuce (Lactuca sativa L.). AJCS. 5: 92-95.

23. Elaleem AKG, Modawi RS, Khalafalla MM (2009) Effect of plant growth regulators on callus induction and plant regeneration in tuber segment culture of potato (Solanum tuberosum L.) cultivar Diamant. Afr J Biotech 8: 2529-2534.

24. Omidi MA, Shahpiri, Yada RY (2003) Callus induction and plant regeneration in vitro in potato. Potatoes – Healthy Food for Humanity: International Developments in Breeding, Production, Protection and Utilization. A proceeding of the XXVI International Horticultural Congress. Acta Hortic 619: 315-322.

25. Sajid AS (2010) Biochemical characterization In vitro salt tolerant lies and regenerated plant of Potato (Solanum tuberosum L.). Department of Botany, University of the Punjab, Lahore, Pakistan.

26. Yasmin S, Nasiruddin KM, Begum R, Talukder SK (2003) Regeneration and establishment of potato plantlets through callus formation with BAP and NAA. Asian J Plant Sci 2: 936-940.

27. Khalafalla MM, Khadiga G, Elaleem A, Modawi RS (2010) Callus formation and organogenesis of potato (Solanum Tuberosum L.) cultivar almera. J phytol 2: 40-46.

28. Laboney UZ, Biswas GC, Shoeb MAA, MA Miah (2013) Callus induction and regeneration of potato from shoot tip culture. Int J Agr Sci 3: 40-45.

29. Forooghian S, Esfarayeni S (2013) An evaluation of effects of plant growth regulators and light on callus induction for varities of potato. American-Eurasian J Agric and Environ Sci 13: 1129-1134.

30. Kumar V, Rashmi D, Banerjee M (2014) Callus Induction and Plant Regeneration in Solanum tuberosum L. cultivars (Kufri Chipsona 3 and MP-97/644) via Leaf Explants. Int J Biol Sci 3: 66-72.

31. Hussaini AZA, Yousif SH, Al-Ajeely SA (2015) Effect of different medium on callus induction and regeneration in potato cultivars. Int J Curr Microbiol Appl Sci 4: 856-865.

32. Haque AU, Samad MA, Shapla TL (2009) In vitro callus initiation and regeneration of potato. Bangladesh J Agril Res 34: 449-456.