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Effect of Plant Growth Regulators on Callus Formation in Potato

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ABSTRACT

This study was carried out to investigate the stimulating effects of plant growth regulators on callus formation. The nodal segments were cultured on different concentration of growth regulators viz. BA, NAA and GA_3 combinations to check their effect on callus formation. Plant growth regulators had significant results on callus formation. The results showed that the combination of BA and NAA at the concentration of 5 and 4 mg l^{-1}, respectively, produced maximum callus from nodal explants. Maximum brownish callus was observed in variety Desiree while maximum whitish callus was produced in cardinal variety.

Keywords: *Plant Growth, Callus, Potato*

INTRODUCTION

Potato (*Solanum tuberosum* L.) belongs to solanaceae family. It is one of the most widely distributed crops in the world. It is grown in the tropical, sub-tropical and temperate regions and forms an important food crop. In some countries, potatoes constitute the main daily food due to their low price and highly nutritive value (Xin, 1998). It produces more protein (524 kg/ha) as compared to wheat (254 kg/ha). It supplies at least 12 essential minerals including vitamins, proteins, carbohydrates and iron (Irfan, 1992).

Tissue culture techniques are used worldwide to produce pre-basic, virus-free seed potatoes known as microtubers. Because of their small size and weight, microtubers 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 microtuber is likely to revolutionize the world potato production. A number of research groups all over the world are trying to bring about this revolution (Sakha, 2004; Gopal, 2004; Zhijun, 2005 Rafique, 2012; Rafique, 2012; Rasheed, 2013).The recent advancement in tissue culture and the flexibility of organ development in potato allows for alternative methods of propagation through *in vitro* techniques. In the rapid multiplication of clean material *in vitro*, the use of single nodal cutting is the most preferred method of propagation since it ensures higher propagation rates with maximum genetic uniformity in potato (Chandra and Naik, 1993). There are also many factors that affect the microtuber induction and formation.

Plant Growth regulators play an important role in this progress and have been studied extensively but the interactions that take place between them are still being discovered (Ross and O'Neill, 2001; Zhang, 2002, Rafique, 2012; Rafique, 2012; Rasheed, 2013). One widely used method for microtuberization is to induce the tuber formation on a medium containing a

cytokinin and a growth retardant of gibberellin biosynthesis (Zhang, 2003). Other factors influencing microtuber production *in vitro* include potato genotypes, explants and culture media such as sucrose, light and temperature (Jackson, 2000).

Much work has been carried out on callus induction and growth in potatoes. Callus is used for most of these transformation methods such as particle gun and *Agrobacterium tumefaciens* mediated transformation (Stiekema, 1988) as well as initiation of cell culture. A callus from an explant tissue occurs as a result of dramatic changes in the appearance and metabolism of the cells (Aitchison, 1978). Induction of callus, physical disorganization of cultured cells, is thought as result of the breakdown of intercellular physical and chemical communication (Lindsey and Jones, 1992). It has been already an established fact from the earlier findings in which the callus culture showed higher multiplication rate in comparison to other methods of *in vitro* culture, as in nodal culture the major factors limiting the rates of multiplication, short height of the plantlets and the low number of nodes on the plantlets (Gebre and Sathyanarayana, 2001). Improvements have been made possible by callus culture and addition of growth regulators to the medium.

The purpose of this work was to investigate the stimulating effects of BA, NAA and GA_3 in different combinations to produce callus of potato explant under *in vitro* conditions.

MATERIALS AND METHODS

The present research was carried out in Plant Tissue Culture Cell, Institute of Horticultural Sciences, University of Agriculture, Faisalabad during the years 2008-2010. Potato varieties Cardinal, Diamant and Desiree were obtained from Plant Virology Section, Ayub Agricultural Research Institute, Faisalabad.

Following culture conditions were maintained artificially in growth room for growth and microtuber induction of potato.

1. Temperature 25±2 ºC
2. Photoperiod 16 hours
3. Light intensity 2500 lux

The cultures were kept in complete darkness at 25°C to induce callus.

MS basic medium **(**Murashige and Skoog, 1962) supplemented with 80 g l^{-1} sucrose, 7.5 g l^{-1}agar and different concentration of plant growth regulators i.e., BA (0, 5, 10, 15 mg l^{-1}), NAA (4 mg l^{-1}) and GA_3 (1 mg l^{-1}) were used in combinations shown in Table 1.

Table 1. MS media supplemented with different PGRs (mg/L) for callus formation

Treatment	MS medium + PGR's (mg/L) PGRs-1= BA + NAA	PGRs-2 = BA + GA_3	PGRs-3= BA+NAA+GA_3
T_1	0 + 0	0 + 1	0 + 0 + 1
T_2	5 + 4	5 + 1	5 + 4 + 1
T_3	10 + 4	10 + 1	10 + 4 + 1
T_4	15 + 4	15 + 1	15 + 4 + 1

Data was collected regarding the Percentage of explants induced callus, Callus formation, Kind of callus and callus colour.

Statistical Analysis

The experiment was laid out according to Completely Randomized Design (CRD) with five treatments and four replications (5 test tubes per replication) in first experiment and twelve treatments and four replications (3 test tubes per replication) in second experiment. Data was collected and analyzed using computer programme MSTAT-C.

RESULTS AND DISCUSSION

Percentage of explants induced callus

The statistical analysis of data regarding callus induction on different treatments showed highly significant results for different concentrations of growth regulators and their interaction. However, non significant results were observed for varieties and interaction between plant growth regulators, treatment and varieties as shown in Table 2.

Table 2. Analysis of variance for % of explants induced callus in potato

SOV	df	SS	MS	F.value
Plant growth regulator (PGR)	2	46285	23142.4	76.69 **
Treatment (TRE)	3	71719	23906.2	37.90 **
Varieties (VAR)	6	17187	2864.6	4.54 **
PRG x TRE	2	1806	902.8	1.43 ^{NS}
PGR x VAR	4	1215	303.8	0.48 ^{NS}
TRE x VAR	6	1250	208.3	0.33 ^{NS}
PRG x TRE x VAR	12	1262	130.2	0.21 ^{NS}
Error	108	68125	630.8	
Total	143			

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

The Table 3 shows different interaction of plant growth regulators, varieties and treatments. It is clear from the interaction of treatments and varieties that variety Desiree produced the highest (70.8%) percentage of callus at treatment T_2 followed by variety Cardinal and Diamant, which produced 62.5% and 54.2% callus induced from nodal explant, respectively, at T_2. The three varieties were statistically at par with one another at T_2. The minimum (29.2%) percentage of callus was produced in variety Diamant at T_4, followed by Cardinal (33.3%) and Desiree (37.5%) at the same treatment T_4. The data also shows that 0% of callus was formed at control treatment in all varieties.

Table 3. Effect of growth regulators on percentage of explants induced callus in different varieties of potato

	VARITIES												
	Diamant				Desiree				Cardinal				
	MS medium + PGRs (mg l^{-1})				MS medium + PGRs (mg l^{-1})				MS medium + PGRs (mg l^{-1})				
Treatment	PGRs-1	PGRs-2	PGRs-3	TxV	PGRs-1	PGRs-2	PGRs-3	TxV	PGRs-1	PGRs-2	PGRs-3	T xV	Treat. Mean
T1	0.0 e	0.0 e	0.0 e	0.0e	0.0e	0.0e	0.0e	0.0e	0.0e	0.0e	0.0e	0.0e	0.0c
T2	75.0 ab	25.0 de	62.5bc	54.2sbc	100.0a	375cd	75.0ab	70.8a	75.0ab	50.0bcd	62.5bc	62.5ab	62.5a
T3	62.5 bc	0.0 e	50.0bcd	37.5cd	75.0ab	0.0e	62.5bc	45.8bcd	62.5bc	0.0e	37.5cd	33.3d	38.8b
T4	50. bcd	0.0 e	37.5cd	29.2d	62.5bc	0.0e	50.0bcd	37.5cd	62.5	0.0e	37.5cd	33.3d	33.3b
Var. x PGRs	46.8 ab	6.2 c	37.5b		59.4a	9.4c	46.8ab		50.0ab	12.5c	34.4b		
Variety Mean	30.2				38.5				32.3				

PGRs-1= BA+NAA
PGRs-2= BA+GA_3
PGRs-3= BA+NAA+GA_3

Callus formation

Table 4. Effect of growth regulators on callus formation in different varieties of potato

	Diamant			Desiree			Cardinal		
	MS medium + PGRs			MS medium + PGRs			MS medium + PGRs		
Treatments	PGRs-1	PGRs-2	PGRs-3	PGRs-1	PGRs-2	PGRs-3	PGRs-1	PGRs-2	PGRs-3
T_1	-	-	-	-	-	-	-	-	-
T_2	+ + + +	+	+ + +	+ + + +	+	+ + +	+ + +	+	+ +
T_3	+ +	-	+ +	+ + +	-	+ +	+ +	-	+ +
T_4	+	-	+	+	-	++	+	-	+

(-) = No Callus, (+) =Normal, (+ +) = Good, (+ + +) = Very good, (+ + + +) =Excellent

The Table 3 also shows the interaction between plant growth regulators and varieties. It can easily be observed that variety Desiree produced the highest (59.4%) percentage of callus at PGR-1 followed by variety Cardinal and Diamant, which produced 50.0% and 46.9% callus, respectively on the same growth regulator combination. The three varieties were statistically similar with one another at PGR-1. The minimum (6.3%) percentage of callus was produced in variety Diamant at PGR-2, followed by Desiree (9.4 %) and Cardinal (12.5 %) on the same combination of plant growth regulators.

Table 3 also shows the overall means of varieties and treatments. Desiree developed maximum (38.5 %) percentage of callus from explant followed by Cardinal (32.3%) and Diamant (30.2%). All three varieties were statistically non significant with each other. It is also clear that T_2 produced maximum (62.5 %) percentage of callus from explant followed by T_3 (33.3%) and T_4 (33.3%). The last two treatments were significantly similar with each other. At control no callus was produced because of the absence of plant growth regulators.

The data regarding callus induction from nodal explant of potato on MS medium supplemented with various concentrations of BA (0, 5, 10 and 15 mg l^{-1}) + GA3 (1 mg l^{-1}), BA (0, 5, 10 and 15 mg l^{-1}) + NAA (4 mg l^{-1}) and BA (0, 5, 10

and 15 mg l^{-1}) + GA3 (1 mg l^{-1}) + NAA (4 mg l^{-1}) shows that variety Desiree produced excellent callus at PGR-1 combination at . Similarly variety Diamant also produced best callus at same media concentration, but very good callus was generated by Cardinal at MS media supplemented with BA (5 mg l^{-1}) and NAA (4 mg l^{-1}) as shown in Fig 1. No callus was formed at control and at PGR-2 at all treatments except T_1 in all varieties.

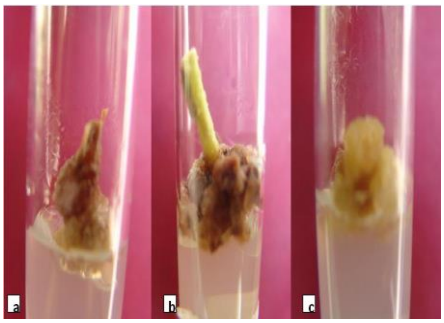

Figure 1. Callus formation in a) Diamant, b) Desiree and c) Cardinal on MS media supplimented with BA (5 mg/L) and NAA (4 mg/L)

The data regarding kind (quality) of callus from nodal explant of potato on MS medium supplemented with various concentrations of BA (0, 5, 10 and 15 mg l^{-1}) + GA3 (1 mg l^{-1}), BA (0, 5, 10 and 15 mg l^{-1}) + NAA (4 mg l^{-1}) and BA (0, 5, 10 and 15 mg l^{-1}) + GA3 (1 mg l^{-1}) + NAA (4 mg l^{-1}) shows that two kinds of callus was produced by the varieties (Diamant, Desiree and Cardinal) as shown in Table 5.

Table 5. Effect of growth regulators on kind of callus in different varieties of potato

	Diamant MS medium + PGRs			Desiree MS medium + PGRs			Cardinal MS medium + PGRs		
Treatments	PGRs-1	PGRs-2	PGRs-3	PGRs-1	PGRs-2	PGRs-3	PGRs-1	PGRs-2	PGRs-3
T_1	-	-	-	-	-	-	-	-	-
T_2	C	F	C	C	F	C	C	F	F
T_3	C	-	F	C	-	C	F	-	F
T_4	F	-	F	C	-	F	F	-	F

C= Compact, F= Friable

Table 5 shows that variety Desiree produced maximum compact callus on PGR-1 combination on all three treatments followed by Diamant. Maximum friable callus was produced in cardinal variety in PGR-3 combination at all three treatments except control followed by variety Diamant which produced friable callus in PGR-3 combination at T_3 and T_4. Compact callus is preferred for regeneration of plantlets in vitro.

The data regarding callus colour from nodal explant of potato on MS medium supplemented with various concentrations of BA (0, 5, 10 and 15 mg/L) + GA3 (1 mg l^{-1}), BA (0, 5, 10 and 15 mg l^{-1}) + NAA (4 mg l^{-1}) and BA (0, 5, 10 and 15 mg l^{-1}) + GA3 (1 mg l^{-1}) + NAA (4 mg l^{-1}) shows different colors of callus at different treatments in the varieties (Table 6).

Table 6. Effect of growth regulators on callus color in different varieties of potato

	Diamant MS medium + PGRs			Desiree MS medium + PGRs			Cardinal MS medium + PGRs		
Treat-meants	PGRs-1	PGRs-2	PGRs-3	PGRs-1	PGRs-2	PGRs-3	PGRs-1	PGRs-2	PGRs-3
T_1	-	-	-	-	-	-	-	-	-
T_2	B	LB	LB	B	LB	B	LB	W	W
T_3	B	-	LB	B	-	LB	W	-	W
T_4	LB	-	W	B	-	W	W	-	W

W= Whitish, B= Brownish, Lb= Light Brown

Table 6 expresses the interaction between treatment, variety and plant growth regulators. Variety Desiree produced maximum brownish callus in PGR-1 combination at all three treatments except control. Maximum whitish callus was produced in cardinal variety in PGR-3 combination at all three treatments except control. No callus was formed at control. and at PGR-2 at all treatments except T_1 in all varieties

DISCUSSION

Our results are in line with the findings of Shirin, (2007). It was also found that callusing percentage increase with increasing combination of BA and NAA until some limit, after that it inhibited the callus formation (Tican, 2008). Chai and Mariam (1998) found that cytokinins at low concentrations, in combination with auxins were often used in plant species to promote callus formation

The results regarding the Kind of callus are in accordance with the results of Badoni and Chauhan (2009), who find same kind of callus in their experiment. The brownish callus is seemed to good for regeneration, because it is also of compact type. So brownish callus can be used for further regeneration process.

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