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EFFECTS OF SUCROSE AND GROWTH REGULATORS ON THE MICROTUBERIZATION OFCIP POTATO (*SOLANUM TUBEROSUM*) GERMPLASM

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Abstract

The present experiment was aimed to study the effect of sucrose and growth regulators (kinetin and 6-benzylaminopurine) on potato microtuber formation. For this purpose the Murashige and Skoog (MS) media, supplemented with different levels of sucrose concentration and growth regulators had been used. The result showed that MS medium with double level (60g) of sucrose without any growth regulator had significantly enhanced the tuber formation but T1 treatment (i.e.100g of sucrose) had no significant effect on tuber formation. It was revealed that sucrose had positive impacts on the microtuberization up to some extent but at very high concentration reduced the microtuber formation. The kinetin and 6-benzylaminopurine (BAP) also enhanced the microtuber formation. Early tuber initiation was observed in kinetin treated plants as compared to other treatments and BAP treated plants showed increase in weight of microtubers. The study also revealed that increase in density of plantlets had negatively affected the number of microtuber formation but the kinetin and BAP had positive impacts to enhance the microtuber formation. Dark condition provides better response as compared to light condition. The two explants source (nodal and sprout explants) the nodal explants showed best results as compared sprout explants as tuber initiation and weight of microtuber.

Key words: Growth regulators, MS medium, Sucrose, Micro-tuber, Kinetin, 6-benzylaminopurine (BAP), Potato.

Introduction

Potato (*Solanum tuberosum* L.) is an important food crop and well known for economical purposes. The potato production is very low and cannot full fill the human's requirement due to many reasons, one of them is potato infected tubers. After harvesting the infected seeds (tuber) are used for next crop production (Hirpa, 2010). Potato can be asexually propagated from seed tubers. The micro-propagation technique is used universally to achieve disease free tubers for potato producers. In *In vitro* condition through micropropagation and tuberization disease free micro-plantlets and microtubers can produce. The *In vitro* plantlets have a lot of risk during transplantation and acclimatization as compared to microtuber. The microtuber is an intermediary stage between micro-plantlets and mini-tubers. The microtuber is used to produce the mini-tuber so the size of the tuber increases gradually. The microtuber size and shape is under the control of many factors like sucrose level, growth regulators, light duration etc. The high sucrose level up to some concentration enhanced the microtuber production and cytokinins alone or in association with growth regulator also enhanced microtuber productivity. The initiation of microtuber was earlier in plants grown in dark as compared to light, because in light GA_3 is produced and in dark condition tuberonic acid is synthesized.

Microtubers can be produced *In vitro* from explant as a single node cutting or from layered shoots under tuber inducing medium in small stationary containers (Wang & Hu, 1982) bioreactors and fermentors (Akita & Ohta, 1998). Microtubers are recently used for a number of purposes throughout the world. They can be used to produce minitubers or used for screening genotypes for the majority of the vital tuber qualities for example shape, color, average weight and yield (Gopal & Minocha, 1997; G opal, & Minocha, 1998). Microtubers are less delicate and simpler to exchange and handle than plantlets, so require less care, when planted in a screen house (Hoque, 2010). Nowadays, potato microtubers have been considered as effective alternative propagule to plantlets and mini tubers for direct plantation in field. Haverkort *et al.*, 1991; Ranalli *et al.*, (1994) harvested tubers from field planted microtuber (MT) plants and recorded less yield 50% less than those of conventional tubers (CT) whereas recently, the results from 3 years experiment comparing microtuber (1-3 g) with CT (50 g) to produce tuber yield, uncovered that yield from MT was 84% of CT, suggesting a great potential for using MT for field planting (Kawakami, 2003). Hence, the use of microtuber technology in seed tuber production, germplasm conservation, breeding programs and research has huge advantages including, production of microtuber in pathogen free condition, its easy handling, storage and distribution.

The main objective of the present study was to optimize protocol for microtuberization of 31 genotypes of CIP potato germplasm grown in Pakistan. It was also performed to identify the effects of growth regulators and sucrose on micro-tuberiztion. Our objectives were to develop an economically practicable diseases-free planting material and micro tuber production system through nodal stem culture in different CIP cultivars.

Materials and Methods

Plant materials: The germplasm of exotic potato (potato CIP) was acquired from *In vitro* conservation laboratory PGRI at National Agriculture Research Center (NARC),

Islamabad. Thirty-one CIP genotypes used as an explants source which were routinely maintained on MS media.

Disease free plantlets were multiplied with MS medium containing different concentration of GA_3. Plants with maximum number of shoots (1 thousands) were shifted to microtuber induction medium. The response of explants was recorded on the basis of tuber production (number of tuber/plant and weight of tube/plant). The explants were cultured in MS medium supplemented with different concentrations of hormone and sucrose.

Experimental work plan for the present study

T_0	MS Media without any hormone
T_1	MS/2+ KIN 2.5mg/l+ BAP 1mg/l+ 10% Sucrose
T_2	MS+6% Sucrose +KIN 4mg/l
T_3	MS Media + 16 %Sucrose

Microtuberization: For microtuberization, four different MS media supplemented with different concentrations of sucrose and growth regulators were used as described above.

The microtuberization is similar to micropropagation but differ only in medium and incubation condition. *In vitro* cultures were removed under aseptic condition from their vessels and manipulated in a sterile Petri plate with the help of forceps and scalpels. The *In vitro* culture was free from all contamination and the individual plantlet was collected from the culture and the roots were removed. The leaves were removed from the shoots of the plantlets and cut into 1.5 to 2 cm long segments. After inoculation the culture was transferred into a growth room at 22 ± 25°C and light of 1000lux (Nistor, 2010).

Statistical analysis: Harvested microtubers were analyzed for number and weight per plant. The weight of microtubers was taken with the help of GSM (gram per square meter) weight machine. The collected data were analyzed through microsoft excel to calculate descriptive statistics and their mean and standard error value were plotted in graph. The mean value of the number and weight of microtubers were also analyzed through one-way analysis of variance (ANOVA).

Results

Number of micro tuber: The microtuber per plantlets were counted and separated from the plantlets and stored at 4ºC (Figs. 1 and 2).The results revealed that the highest number of microtuber were produced (mean 3.33) with T3 (double sucrose treatment) as compared to control in CIP 3 cultivar (Figs. 3 and 4). The minimum (0.3333 mean) number of micro tubers/plants were recorded in T1 treatment in CIP 22 cultivar.

Weight of micro tuber: The separated microtubers were weighed with the help of digital balance. The results showed that the maximum weight (1.0867g) of microtuber per plants was obtained in T3 treated explants as compared to other treatments in CIP 1 genotype. The minimum weight (0.5060g) of microtubers was produced in plant treated with T2 treatment. Shoot formation and plant growth was also recorded in T1 (100g sucrose) treatment as compared to other treatments.

Effect of photoperiods on micro tuber: The MS medium with double level of sucrose produced maximum number of microtuber per plant. Hence only this parameter was selected to study the effect of photoperiods in microtuber formation. The photoperiods influence the CIP potato tuberization as shown in the table1. It was proved that the plant grown in dark condition produce early microtuber initiation response (34 days) as compared to the same concentration of medium and species grown in light condition (46 days). The number of microtuber (0.93) and the average weight of microtuber (1.04 g) in dark condition are higher than light condition. The light conditions prolong the tuber initiation. The color of microtuber has a direct correlation with photoperiods, the explants grown in light condition produced green microtuber, in light condition alkaloid salonin is produced.

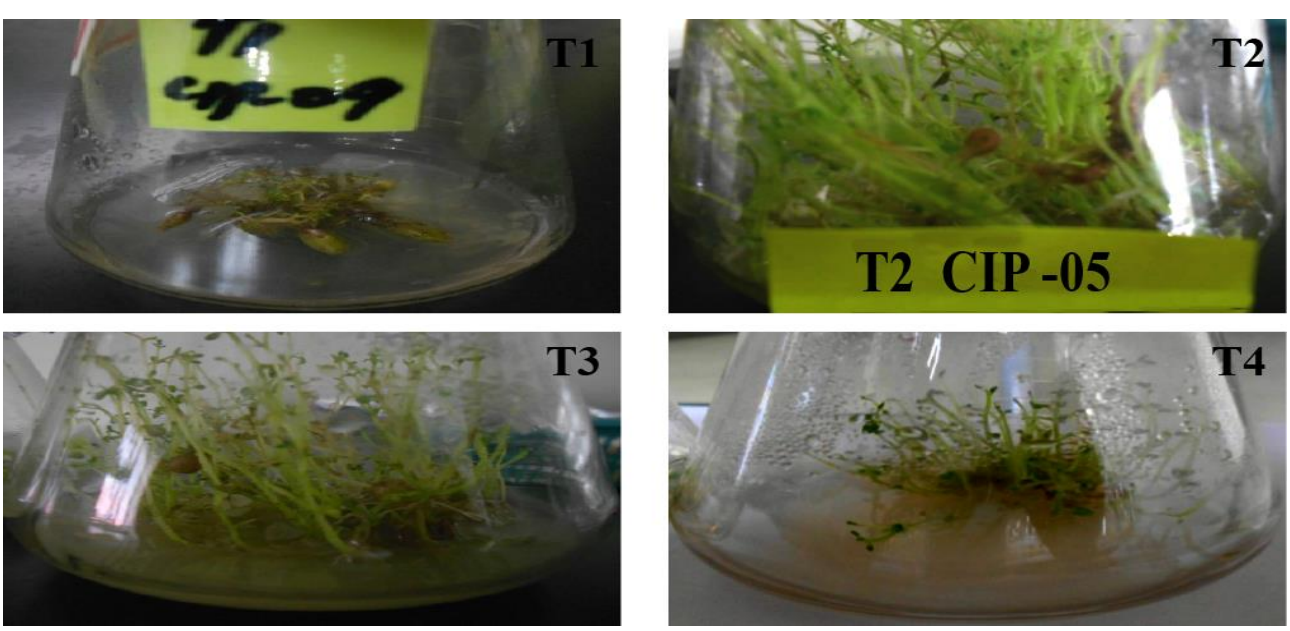

Fig. 1. Effects of hormone and sucrose on plant growth and microtuber.

Fig. 2. Morphological variation among CIP potato germplasm.

Fig. 3. Number of micotuber in T3 treatment of CIP3 potato germplasm.

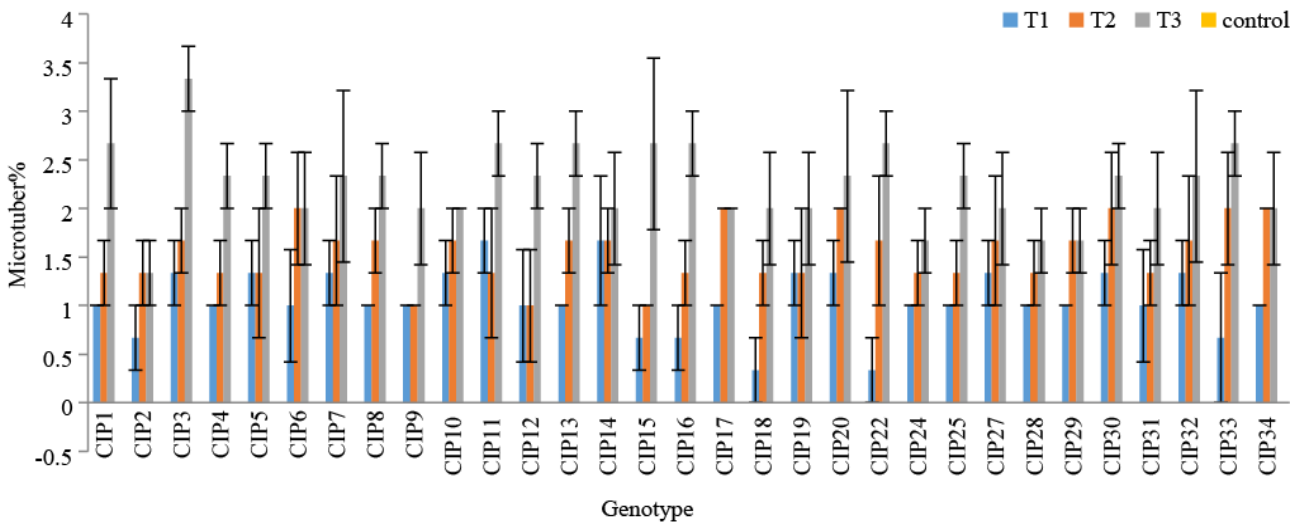

Fig. 4. Effects of different hormonal concentrations on number of microtuber/ plant.

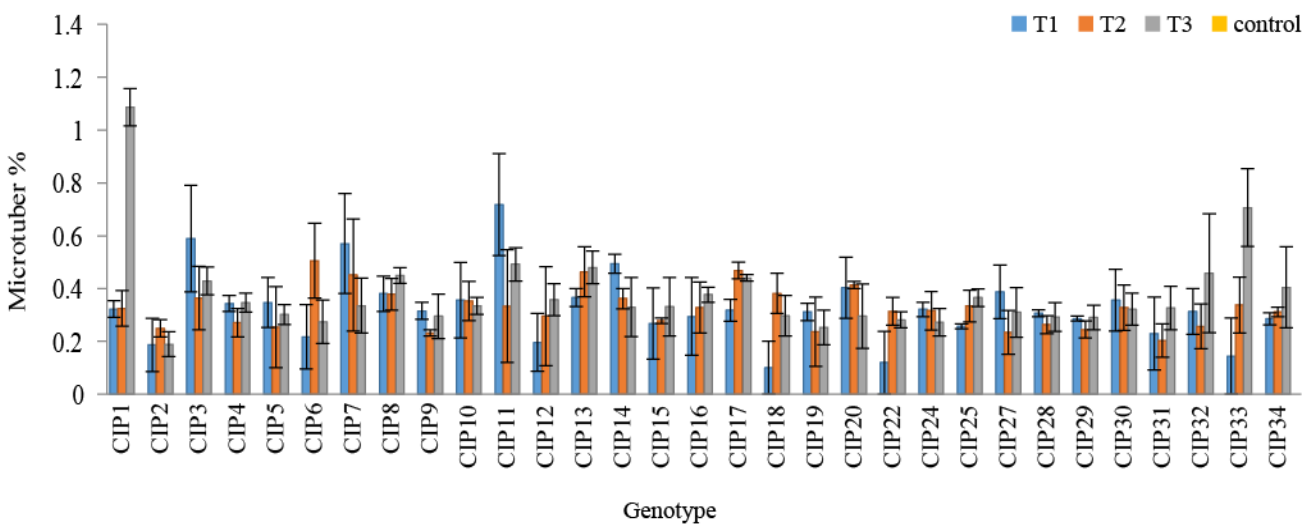

Fig. 5.Effects of different hormonal concentration on weight of microtube/ plant.

Table 1. Effect of photoperiods on *In vitro* microtuberization.

Treatment	Media	explant	Days of tuberization	No of microtuber / explant	Ag.wt (g)of microtuber	Color of microtuber
Light condition	Double sucrose	Nodal explants	46	0.93	0.61	Green
Dark condition	Double sucrose	Nodal explants	34	3.91	1.04	Brown

Table 2. Explants response to microtuberization.

Explants type	Media	Days of tuberization	No of microtuber / explant	Ag.wt (g)of microtuber	Percentage of explants formed microtuber
Sprout explants	Double sucrose	41	0.87	0.93	74
Nodal explant	Double sucrose	33	3.54	1.02	94

Table 3. Analysis of variance for weight of Microtuber in T1 treatment.

Source of variance	Degree of freedom	Sum of squares	Mean of squares	F-value	Probability
Replication	2	0.05	0.02		
Genotypes	30	1.58	0.05**	1.54	0.0773
Error	60	2.58	0.03		
Total	92	3.69			

Explants response to microtuberization: The explants sources have direct effects on microtuberization as shown in the table 2. It was shown that the nodal segment explants produce maximum percentage (94) of microtuber as compared to sprout explants (74). The nodal explants took lowest days (33) on tuber initiation as compared to sprout apex (41) and having maximum number (3.54) and average weight of microtuber (1.02 g). All the parameters of the nodal segments responded well as compared to sprout apex.

Discussion

Microtubers are the final products of micropropagation (Dobranszki, 2008). In the present work, tuberization were performed under *In vitro* condition by culturing nodal cuttings from *In vitro* plantlets, the explants were cultured on liquid MS medium (pH 5.8) supplemented with different concentrations of hormone and sucrose in each treatment (T1, T2, T3, T0). Our results revealed that, *In vitro* tuberization in potato was influenced by many factors but hormone played a dominant role in this process (Agud, 2010; Altindal & Karadogan, 2010; Hoque, 2010; Khan & Bano, 2016). Much attention has so far been focused on the use of cytokinins (Lentini & Earle, 1991). It was observed that kinetin (KIN) was responsible for early tuberization in comparison to hormone treated plants. Similarly it was reported by (Aryakia & Hamidoghli, 2010) that KIN induced early tuberization but BAP had incremental effect on weight and size of micro tuber. Prat, (2004) reported that KIN played a significant role in creating sink during plant growth, and through regulating the expression of a gene involved in the partition of assimilates towards the stolon as observed in potato. The highest numbers of microtubers were produced in CIP3 cultivar due to their genetic diversity in same culture medium then other. Similar findings were reported by many researchers that potato cultivars showed different responses to various growth regulators (Prat, 2004; Zhang *et al*., 2005; Ghavidel *et al*., 2012).

Plants cultivated in double sucrose medium, enhanced the number of microtubers as compared to other medium. Similarly it was reported by Donnelly *et al*., (2003) that sucrose was essential for *In vitro* tuberization as an energy source and at high concentration it served as a signal for microtuber formation. The addition of 8% sucrose in MS medium can increase number and weight of microtubers and can initiate tuber formation even in the absence of growth regulators (Alix, 2001; Gopal, 2004; Sani, 2008). It was observed that initiation of microtubers was affected by plantlet densities. Tabori *et al*., (2000a); Dobranszki *et al.* (2008) conducted experiments and found out that the culture densities of micro-plantlets could affect the tuber initiation.

The germination and growth rates of the plantlets were significantly enhanced in T3 (double sucrose) treated plants followed by T2 (6% sucrose + KIN 4mg) whereas, the minimum growth of the plantlets were recorded in T1 (100 gm sucrose). Similarly it was reported by Dieme *et al*., (2013), that high concentration of sucrose had reduced the germination rate up to 55%. Present study suggested that BAP increased the weight of microtubers as reported by Aryakia & Hamidoghli, (2010). Significantly higher number of microtubers was induced by using 6% sucrose in the MS medium, while further increase in sucrose concentration reduced the number of microtubers whereas, no induction occurred in Santa. These results were in accordance with Zaag & Zaag, 1990; Khan, 2003).

Tábori & Dobránszki (2002) reported that the increase in explant size resulted in the increase in the number of microtubers with large diameter (up to 16 mm) and fresh weight of tubers. The results suggested that explants with two nodes should be used to produce microtubers with enhanced fresh weight (250 mg) and also to increase the number of large microtubers (79% was larger than 6 mm and 53% was larger than 8 mm) according to Rafique *et al.*, (2012). Plant growth was observed in simple medium, that was not supplemented with any growth regulator but no production of mircrotuber was recorded (Iqbal *et al.*, 2014). It was reported that 30g/l of sucrose produced the healthy shoots without tuber formation, indicating that lower concentration of sucrose was responsible for vegetative growth and shoot development. Hoque, (2010) also reported that simple MS was not able to produce any microtubers under *In vitro* conditions. Wattimena, (1983) reported that the explants growing in dark condition provided maximum number of microtuber. Wany & Hu, (1982) analyzed that in dark condition the microtubers were brown and with no spout on them. Myeong *et al*., (1990) and knawel *et al*., (2006) showed that several factors affected the microtuber production one of them was explants source and concluded or resolved? that the nodal explants were best for microtuber production.

Table 4. Analysis of variance for weight of Microtuber in T2 treatment.

Source of variance	Degree of freedom	Sum of squares	Mean of squares	F-value	Probability
Replication	2	0.05 3	0.01		
Genotypes	30	1.53	0.01**	0.61	0.9283
Error	60	1.74	0.02		
Total	92	2.31			

Table 5. Analysis of variance for weight of Microtuber in T3 treatment.

Source of variance	Degree of freedom	Sum of squares	Mean of squares	F-value	Probability
Replication	2	0.085	0.04		
Genotypes	30	2.38	0.07**	3.74	0.000
Error	60	1.27	0.02		
Total	92	3.37			

Table 6. Analysis of variance for number of Microtuber in T1 treatment.

Source of variance	Degree of freedom	Sum of squares	Mean of squares	F-value	Probability
Replication	2	1.11	0.55		
Genotypes	30	9.39	0.31**	0.93	0.5764
Error	60	20.21	0.33		
Total	92	30.73			

Table 7. Analysis of variance for number of Microtuber in T2 treatment.

Source of variance	Degree of freedom	Sum of squares	Mean of squares	F-value	Probability
Replication	2	1.63	0.81		
Genotypes	30	8.45	0.28**	0.48	0.9841
Error	60	35.03	0.58		
Total	92	45.11			

Table 8. Analysis of variance for number of Microtuber in T3 treatment.

Source of variance	Degree of freedom	Sum of squares	Mean of squares	F-value	Probability
Replication	2	4.47	2.23		
Genotypes	30	14.79	0.41**	0.68	0.8748
Error	60	43.52	0.72		
Total	92	62.79			

Conclusion

The sucrose and kinetin play a critical role in microtuber formation. Increase in sucrose concentration in medium can enhance the microtuber production up to some extent whereas high concentrations (100g) had negative impacts on the germination rate of the plantlets. The dark condition and nodal explants show well response to microtuberization. It was also concluded that the influence of BAP on the size of microtubers was more than Kinetin.

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