

## Micro-Tuberization of Four Potato (*Solanum tuberosum* L.) Cultivars Through Tissue Culture

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#### ABSTRACT

An experiment for the micro-tuberization of four potato cultivars through tissue culture on different sucrose concentration in MS media was conducted at tissue culture lab Abbottabad, Pakistan. Four cultivars (Desiree, Cardinal, Ultimus and Astrix) and MS media supplemented with four levels of sucrose (20 g<sup>l</sup><sup>-1</sup>, 30 g<sup>l</sup><sup>-1</sup>, 40 g<sup>l</sup><sup>-1</sup> and 50 g<sup>l</sup><sup>-1</sup>) were used in the experiment. All cultivars showed best performance in MS media supplemented with 40 g<sup>l</sup><sup>-1</sup> of sucrose in respect of all parameters such as micro-tubers formation, days to micro-tubers harvest, number of micro-tubers plantlet<sup>-1</sup>, micro-tuber diameter (cm), micro-tuber weight (g) and number of nodes in micro-tuber. MS media containing 50 g<sup>l</sup><sup>-1</sup> sucrose showed less effect in all the underline parameters under *in vitro* condition. Among the four different potato cultivars, astrix performed better followed by desiree in all under study parameters under *in vitro* condition.

### INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important dicotyledonous tuber crops, ranking fourth in food crop after wheat, rice and maize<sup>[1]</sup>. It contributes more proteins and calories than other major crop plants<sup>[2]</sup>. Potato is an important crop of Pakistan, with an annual production of 3084.3 thousand tons, a dramatic increase in the yield hectare<sup>-1</sup> has also been recorded in the last few years due to improvement in seed and crop management practices<sup>[3]</sup>.

Potato is highly heterozygous and segregates on sexual reproduction. Therefore, elite parental lines and cultivars of potato are vegetatively propagated to maintain its genetic integrity. Potato tuberization is very complex developmental process, which may be changed in different ways. Therefore, tuberization can be obtained by using *in vitro* techniques. Moreover, different scientists and researchers have been used different media to obtain micro-tubers of potato in tissue culture<sup>[4]</sup>. Seed tuber is the most common asexual propagating technique in potato reproduction, but it may be frequently attacked by different pathogens and this method is also time consuming and laborious<sup>[5-7]</sup>. Therefore, throughout the world, potato gene banks prefer to conserve elite parental lines and clones as *in vitro* propagated micro-plants under disease-free tissue culture conditions<sup>[8-10]</sup>.

Plant tissue culture is the only technique of creating 100% viruses' free seeds and micro-tubers is an innovative approach in this prospect; micro-tubers have small size and weight, and can be easily handled, stored and transported. Therefore, it is important to establish a new method or protocol for potato micro-tuberization and rapid multiplication by *in vitro* process. In addition, their cultural and morphological characteristics also resembles to field produced tubers. Therefore, a number of researchers are trying on the bulk production of potato micro-tubers to revolutionize the world potato production<sup>[11-14]</sup>. Different researchers observed in

their experiments that micro-tuberization is controlled by so many factors, including different hormonal percentage, photo period ratio, temperature variations, sugars concentrations and nutrient compositions etc<sup>[15-17]</sup>. Furthermore, scientists have been given very little attentions to the different percentages of sucrose, kinetin (KIN) and different types of explants to develop suitable regeneration protocol by using tissue culture techniques<sup>[18]</sup>.

Micro-tubers (*in vitro* tubers) are miniature seed potatoes and can be considered as the intermediate stage between *in vitro* plantlets and miniature plants. Micro-tubers are the first generation of nuclear seed potato produced from tissue culture. These have been used to overcome the problems of transplanting of tender vegetative plantlets from *in vitro* condition to *in vivo* condition and to overcome the problems of storage. Potato micro-tubers produced *in vitro* can be used as a source of germplasm for conservation, transfer between countries, and seed certification schemes. Larger micro-tubers withstand adverse planting conditions and produce more vigorous plantlets in the succeeding generation than smaller micro-tubers.

Keeping in view the importance of this plant and micro-tubers production, the present study was designed to achieve the disease-free stock seeds through propagation and multiplication of potato micro-tubers, to evaluate the response of different potato cultivars towards micro-tubers production and to check the performance of different sucrose concentration in MS media for micro-tuberization.

## MATERIALS AND METHODS

The study reported in this manuscript was carried out at the tissue culture laboratory agriculture research station Abbottabad, Khyber Pakhtunkhwa, Pakistan. The experiment was laid out on two factorial Randomized Complete Block Design (RCBD) having four cultivars and four treatments replicated four times. Each of the treatment consisted of at least three flasks. Healthy and disease-free explants of all the cultivars of potato as mentioned below were obtained from the plantlets already under investigation at agriculture research station Abbottabad. The details of cultivars and media used in the experiment are given as under:

### Potato Cultivars Used

C1—Desiree

C2—Cardinal

C3—Ultimus

C4—Astrix

### Media Culture (Treatments)

T1—20 g<sup>l</sup><sup>-1</sup> Sucrose in MS Media

T2—30 g<sup>l</sup><sup>-1</sup> Sucrose in MS Media

T3—40 g<sup>l</sup><sup>-1</sup> Sucrose in MS Media

T4—50 g<sup>l</sup><sup>-1</sup> Sucrose in MS Media

No. of plantlets in a treatment=3

Total no. of cultured flasks=240

### Statistical Analysis

The data recorded on different parameters were subjected to the Analysis of Variance (ANOVA) to observe the difference between the different cultivars performance to different treatments as well as their interactions. In cases where the differences were significant, the means were further assessed for differences through Least Significant Difference (LSD) test. Statistical computer software, MSTATC was applied for computing both the ANOVA and LSD.

## RESULTS AND DISCUSSION

### Days to Micro-Tubers Formation

Micro-tuberization of different potato cultivars in the MS media took place differently. The cultivar Astrix took minimum number of days to micro-tuberization (67.82 days), while the cultivar Ultimus took maximum (71.07 days), however it was at par with cultivar Desiree in days taken to micro-tuberization (**Table 1**). The cultivar Cardinal was almost statistically similar to cultivar Desiree in micro-tuberization by taking nearly the same time such as 69.35 and 70.23 days. Though these variations in days to micro-tuberization were not much wider however, could be considered as the genotypic responses. The findings are almost alike, who observed variations in genotypic responses to micro-tuberization which occurred in 2 months to 3 months period in different genotypes<sup>[19]</sup>. Micro-tubers formation related results of this experiment are the agreement of the previous findings<sup>[18,20-22]</sup>.

**Table 1.** Days to micro-tubers formation of four potato cultivars with different concentrations of sucrose in MS media under *in vitro* condition.

Cultivars	g <sup>l</sup> <sup>-1</sup> Sucrose concentrations in MS Media				Means
	20 g <sup>l</sup> <sup>-1</sup>	30 g <sup>l</sup> <sup>-1</sup>	40 g <sup>l</sup> <sup>-1</sup>	50 g <sup>l</sup> <sup>-1</sup>	
Desiree	68.05 <sup>de</sup>	71.50 <sup>c</sup>	65.12 <sup>f</sup>	76.27 <sup>ab</sup>	70.23 <sup>ab</sup>
Cordinal	66.07 <sup>ef</sup>	70.10 <sup>cd</sup>	66.52 <sup>ef</sup>	74.72 <sup>ab</sup>	69.35 <sup>b</sup>
Ultimus	77.22 <sup>a</sup>	67.00 <sup>ef</sup>	65.77 <sup>ef</sup>	74.30 <sup>b</sup>	71.07 <sup>a</sup>
Astrix	67.37 <sup>ef</sup>	66.97 <sup>ef</sup>	65.80 <sup>ef</sup>	71.15 <sup>c</sup>	67.8 <sup>2c</sup>
Mean	69.68 <sup>b</sup>	68.89 <sup>b</sup>	65.80 <sup>c</sup>	74.11 <sup>a</sup>	--

LSD value for cultivar=1.302, LSD value for treatment=1.302, LSD value for interaction=2.603  
 (a-f) Means followed by the same letter are not significantly different using LSD test at 5% level of probability.

The addition of varying amounts of sucrose in the MS media also influenced the days to micro-tuberization significantly. Plantlets grown in 40% sucrose level in MS media took significantly less number of days to micro-tuberization (65.80 days) and more (74.11 days), when grown in 50% sucrose. The response of 20 g<sup>l</sup><sup>-1</sup> and 30 g<sup>l</sup><sup>-1</sup> sucrose for days to micro-tuberization was statistically similar (69.68 days, 68.89 days) respectively. These results showed that the sucrose level in the MS media beyond 40 g<sup>l</sup><sup>-1</sup> delayed micro-tuberization. The interactions of cultivars and sucrose levels were also significant. All the cultivars when grown in MS media having 40 g<sup>l</sup><sup>-1</sup> sucrose took significantly less number of days to micro-tuber formation these results are almost alike the findings of who reported sucrose, as an important factor for the formation of micro-tubers [23].

**Days to Micro-Tubers Harvest**

**Table 2** shows that minimum days to micro-tuber harvest (146.4) were taken by plantlets grown in 40 g<sup>l</sup><sup>-1</sup> sucrose in the MS media, while, maximum number of days to micro-tuber formation (157.6) were taken by the plantlets grown in 50 g<sup>l</sup><sup>-1</sup> sucrose in MS media. The other two treatments (20 g<sup>l</sup><sup>-1</sup>, 30 g<sup>l</sup><sup>-1</sup> sucrose in MS media) took almost same number of days to micro-tuber harvest (154.9 days and 154.5 days) respectively, which indicates that an appropriate sucrose concentration had a pronounced effect on the tuber maturation. The results are in accordance with the findings who did not observe the growth of micro-tubers on a media supplemented with 3% to 4% sucrose while the growth was maximum when the concentration was increased to 8% which indicates that the micro-tubers needs a suitable concentration of sucrose to grow [24]. As regards the response of cultivars to micro-tubers harvest, it was significant. Minimum days to micro-tuber harvest (150.6) were taken by cultivar Astrix followed by (153.5) days by cultivar Ultimus. The cultivar Desiree took maximum days to micro-tuber harvest (154.8), which shows genetic variability because all other requirements were same for all the cultivars. The influence of genetic variability on the growth of micro-tubers was also reported by Gopal et al. in 1998. The interaction between the cultivars and sucrose levels had also significantly affected the days to micro-tubers harvest. The cultivars Cardinal, Ultimus and Astrix showed the same results for micro-tuber harvest at 40 g<sup>l</sup><sup>-1</sup> sucrose in the MS media by taking 146.00 days, 145.77 days and 145.80 days respectively. While the cultivar Desiree was taken 147.87 days to micro-tubers harvest when grown in 40 g<sup>l</sup><sup>-1</sup> sucrose in the MS media. This could be referred to the genotypic variations and their performance in different sucrose concentration in MS media [25-27].

**Table 2.** Days taken to micro-tuber harvest of four potato cultivars with different concentrations of sucrose in MS media under *in vitro* condition.

Cultivars	g <sup>l</sup> <sup>-1</sup> Sucrose concentrations in MS Media				Means
	20 g <sup>l</sup> <sup>-1</sup>	30 g <sup>l</sup> <sup>-1</sup>	40 g <sup>l</sup> <sup>-1</sup>	50 g <sup>l</sup> <sup>-1</sup>	
Desiree	156.80 <sup>bc</sup>	156.25 <sup>bc</sup>	147.87 <sup>de</sup>	158.62 <sup>ac</sup>	154.8 <sup>a</sup>
Cordinal	158.70 <sup>ab</sup>	155.70 <sup>c</sup>	146.00 <sup>e</sup>	156.22 <sup>bc</sup>	154.2 <sup>ab</sup>
Ultimus	156.72 <sup>bc</sup>	157.00 <sup>bc</sup>	145.77 <sup>e</sup>	154.55 <sup>c</sup>	153.5 <sup>b</sup>
Astrix	147.37 <sup>de</sup>	148.97 <sup>d</sup>	145.80 <sup>e</sup>	161.15 <sup>a</sup>	150.8 <sup>c</sup>
Mean	154.9 <sup>b</sup>	154.5 <sup>b</sup>	146.4 <sup>c</sup>	157.6 <sup>a</sup>	--

LSD value for cultivar=1.289, LSD value for treatment=1.289, LSD value for interaction=2.579  
 (a-e) Means followed by the same letter are not significantly different using LSD test at 5% level of probability.

**Number of Micro-Tubers Plantlet<sup>-1</sup>**

The mean result for media shown in **Table 3** depicted that the minimum but same result were given in the 20 g<sup>l</sup><sup>-1</sup>, 30 g<sup>l</sup><sup>-1</sup> and 50 g<sup>l</sup><sup>-1</sup> sucrose containing MS media. The plants which were grown in the above medias had given (2.06), (2.56) and (2.06) number of micro-tubers respectively, while maximum number of micro-tuber (4.85) were given by plants grown in 40 g<sup>l</sup><sup>-1</sup> sucrose MS media. Due to the above result, it was observed that optimum and normal sucrose level is needed for the formation of micro-tubers otherwise the result will be not good.

The data noted for cultivars shown that minimum number of micro-tubers plantlet<sup>-1</sup> (2.17) were produced by cultivar Ultimus and the two cultivars i.e., Desiree and Cardinal behaved alike producing (2.80) and (2.89) number of micro-tubers plantlet<sup>-1</sup>, while maximum number of micro-tubers plantlet<sup>-1</sup> (3.68) were produced by cultivar Astrix. This fact may be attributed to the genetic potential of these cultivars.

Non-significant interaction was observed between media and cultivars indicating that the response of the cultivars was same in different medias; however minimum number of micro-tuber plantlet<sup>-1</sup> (1.62) were produced by cultivar Ultimus grown in 50 g<sup>-1</sup> sucrose level MS media and maximum number of micro-tuber plantlet<sup>-1</sup> (6.52) were given by cultivar Astrix grown in 40 g<sup>-1</sup> sucrose level MS media.

**Table 3.** Numbers of micro-tubers plantlet<sup>-1</sup> of four potato cultivars with different concentrations of sucrose in MS media under *in vitro* condition.

Cultivars	g <sup>-1</sup> Sucrose concentrations in MS Media				Means
	20 g <sup>-1</sup>	30 g <sup>-1</sup>	40 g <sup>-1</sup>	50 g <sup>-1</sup>	
Desiree	1.95	2.50	4.72	2.05	2.80 <sup>ab</sup>
Cordinal	2.17	2.65	4.57	2.17	2.89 <sup>ab</sup>
Ultimus	1.80	1.70	3.57	1.62	2.17 <sup>b</sup>
Astrix	2.35	3.42	6.52	2.42	3.68 <sup>a</sup>
Mean	2.06 <sup>b</sup>	2.56 <sup>b</sup>	4.85 <sup>a</sup>	2.06 <sup>b</sup>	--

LSD value for cultivar=0.9412, LSD value for treatment=0.9412  
(a, b) Means followed by the same letter are not significantly different using LSD test at 5% level of probability.

### Micro-Tubers Diameter (Cm)

The results for media presented in **Table 4** revealed that the plants grown in the given three media i.e., 20 g<sup>-1</sup>, 30 g<sup>-1</sup> and 50 g<sup>-1</sup> behaved quite similar giving (2.17 cm), (2.25 cm) and (2.13 cm) micro-tuber diameter respectively, while the plants grown in 40 g<sup>-1</sup> sucrose level MS media were given (2.82 cm) micro-tuber diameter. It means that 40 g<sup>-1</sup> sucrose gives good food to plant which resulted in more micro-tuber diameter.

**Table 4.** Micro-tubers diameter (cm) of four potato cultivars with different concentrations of sucrose in MS media under *in vitro* condition.

Cultivars	g <sup>-1</sup> Sucrose concentrations in MS Media				Means
	20 g <sup>-1</sup>	30 g <sup>-1</sup>	40 g <sup>-1</sup>	50 g <sup>-1</sup>	
Desiree	2.55	2.38	2.79	1.72	2.36
Cordinal	1.63	2.18	2.59	2.27	2.16
Ultimus	2.15	2.05	2.69	2.15	2.26
Astrix	2.34	2.38	3.22	2.40	2.59
Mean	2.17 <sup>b</sup>	2.25 <sup>b</sup>	2.82 <sup>a</sup>	2.13 <sup>b</sup>	--

LSD value for treatment=0.3652  
(a, b) Means followed by the same letter are not significantly different using LSD test at 5% level of probability.

All the four cultivars showed approximately similar effect for micro-tuber diameter; however minimum micro-tuber diameter (2.16 cm) was produced by cultivar Cardinal, while maximum micro-tuber diameter (2.59 cm) was in cultivar Astrix. Maximum micro-tuber diameter in cultivar Astrix may be attributed to the genetic potential of this cultivar to utilize the nutrients efficiency, synthesize more food and hence more diameter in this cultivar.

The non-significant interaction for media and cultivars shows that minimum micro-tuber diameter (1.63 cm) was given by cultivar Cardinal grown in 20 g<sup>-1</sup> sucrose level MS media and maximum micro-tuber diameter (3.22 cm) was achieved by cultivar Astrix in 40 g<sup>-1</sup> sucrose level MS media.

### Micro-Tubers Weight (G)

The means for media in **Table 5** shows that the three media i.e., 20 g<sup>-1</sup>, 30 g<sup>-1</sup> and 50 g<sup>-1</sup> sucrose concentration in MS media behaved alike producing (0.80 g), (0.93 g) and (0.95 g) micro-tuber weight respectively, while heavier micro-tuber produced by the plants grown in 40 g<sup>-1</sup> sucrose level MS media were given (1.92 g) micro-tuber weight.

**Table 5.** Micro-tubers weight (g) of four potato cultivars with different concentrations of sucrose in MS media under *in vitro* condition.

Cultivars	g <sup>-1</sup> Sucrose concentrations in MS Media				Means
	20 g <sup>-1</sup>	30 g <sup>-1</sup>	40 g <sup>-1</sup>	50 g <sup>-1</sup>	
Desiree	0.82	0.97	1.67	0.72	1.05
Cordinal	0.85	1.00	1.92	0.95	1.18
Ultimus	0.60	0.50	1.90	1.22	1.05
Astrix	0.95	1.25	2.20	0.92	1.33
Mean	0.80 <sup>b</sup>	0.93 <sup>b</sup>	1.92 <sup>a</sup>	0.95 <sup>b</sup>	--

LSD value for treatment=0.4390  
(a, b) Means followed by the same letter are not significantly different using LSD test at 5% level of probability.

Non-significant variation in micro-tuber weight produced by various potato cultivars indicate that two cultivars i.e., Desiree and Ultimus behaved similar and the rest two cultivars i.e., Cardinal and Astrix behaved alike, however minimum micro-tuber weight (1.05 g) was produced by Desiree as well as cultivar Ultimus, while maximum micro-tuber weight (1.33 g) was achieved by cultivar Astrix. Maximum micro-tuber weight in cultivar Astrix may also be attributed to maximum micro-tuber diameter and more micro-tuber length in this cultivar.

Non-significant interaction between media and cultivars indicating that the cultivars response to different media were same. Minimum micro-tuber weight (0.50 g) was given by cultivar Ultimus grown in 30 g<sup>l</sup><sup>-1</sup> sucrose level MS media and maximum micro-tuber weight (2.20 g) was produced by cultivar Astrix in 40 g<sup>l</sup><sup>-1</sup> sucrose level MS media.

### Number of Nodes in Micro-Tubers

The mean values recorded for treatment in **Table 6** revealed that the three treatments i.e., 20 g<sup>l</sup><sup>-1</sup>, 30 g<sup>l</sup><sup>-1</sup> and 50 g<sup>l</sup><sup>-1</sup> sucrose level in MS media were similar in number of nodes in micro-tuber, producing (5.00), (6.03) and (5.05) nodes micro-tuber<sup>-1</sup> in which minimum number of nodes were by plants grown in 20 g<sup>l</sup><sup>-1</sup> sucrose level MS media. While maximum number of nodes in tuber (8.40) were produced by potato plants grown in 40 g<sup>l</sup><sup>-1</sup> sucrose level MS media. It was observed during the experiment that maximum number of micro-tuber and maximum micro-tuber diameter were produced by 40 g<sup>l</sup><sup>-1</sup> sucrose level MS media therefore number of nodes in tuber also more in 40 g<sup>l</sup><sup>-1</sup> sucrose level MS media.

**Table 6.** Number of nodes in tubers plantlet<sup>-1</sup> of four potato cultivars with different concentrations of sucrose in MS media under *in vitro* condition.

Cultivars	g <sup>l</sup> <sup>-1</sup> Sucrose concentrations in MS Media				Means
	20 g <sup>l</sup> <sup>-1</sup>	30 g <sup>l</sup> <sup>-1</sup>	40 g <sup>l</sup> <sup>-1</sup>	50 g <sup>l</sup> <sup>-1</sup>	
Desiree	4.97	6.72	8.27	4.80	6.19
Cardinal	4.92	5.77	6.80	4.82	5.58
Ultimus	4.90	4.90	7.77	4.32	5.47
Astrix	5.20	6.72	10.75	6.22	7.22
Mean	5.00 <sup>b</sup>	6.03 <sup>b</sup>	8.40 <sup>a</sup>	5.04 <sup>b</sup>	–

LSD value for treatment=1.429

(a, b) Means followed by the same letter are not significantly different using LSD test at 5% level of probability.

Non-significant variation in number of nodes in tuber in various potato cultivars were found, however minimum number of nodes in tuber (5.47) was obtained by cultivar Ultimus, while maximum number of nodes in tuber (7.22) was produced by cultivar Astrix. This may be attributed to the genetic potential of these cultivars to utilize the nutrients efficiently and synthesized more food.

All the cultivars showed similar response to different treatments, however minimum numbers of nodes in tuber (4.32) were produced by cultivar Ultimus grown in 50 g<sup>l</sup><sup>-1</sup> sucrose level MS media while maximum numbers of nodes in tuber (10.75) were obtained by cultivar Astrix grown in 40 g<sup>l</sup><sup>-1</sup> sucrose level MS media.

## CONCLUSION

On the bases of the results obtained it is concluded that both cultured media and cultivars had significant effect on the formation of micro-tubers of potato through tissue culture under *in vitro* condition. In this research, better results were obtained by planting Astrix, Desiree and Cardinal cultivars grown in 40 g<sup>l</sup><sup>-1</sup> sucrose concentration MS media and then in 50 g<sup>l</sup><sup>-1</sup> sucrose concentration MS media.

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