In Vitro Micro Tuber Formation in Potato (Solanum tuberosum L.): is there any Relation between Methyl Jasmonate, Sugars, and Explants?

Gülsün E. Vural[1], Tugce Ozsan[1], Volkan Gozen[2], Ahmet N. Onus[1*]
[1]*Department of Horticulture, Faculty of Agriculture, Akdeniz University, Antalya, Turkey*
[2]*Bati Akdeniz Agricultural Research Institute, Antalya, Turkey*

Abstract

Studies on potato (Solanum tuberosum L.) plants have been carried out to plant tissue culture laboratories because of the disease and virulent free seed tubers in field conditions. Since generative production in commercial potato production is not economically massive, production is made more economically with tubers. Jasmonic acid (JA) and its related compounds are newly recognized plant growth regulators and they are known to have effects on many physiological processes. In this study, it was aimed to determine the effects of methyl jasmonate and sucrose on micro tuber formation in potato micropropagation. To serve the purpose the relationships between twelve media combinations (including control medium), varying concentrations of sucrose (3, 6 and 9 %) either alone or in combination with 1.0 ppm or 2.0 ppm methyl jasmonate were investigated. Three different plant parts such as node, shoot eye and primordial tissue wereused as explant sources. Nitsch&Nitsch was used as the basic nutrient medium and plant growth regulators that used in media were constant at 2.5 mg/L BAP + 0.5 mg/L NAA except for control medium. Obtained micro tubers, plantlets and callus formations were recorded and analyzed. Results revealed that, when methyl jasmonate used at 1.0 ppm concentration in media, it had a positive effect on micro tuber formation and can be recommended to use commercially in mass production.

Keywords — *Solanum tuberosumL., micro tuber, methyl jasmonate, sucrose, in vitro*

I. INTRODUCTION

Thanks to the high nutritional value of the potato plant (*Solanum tuberosum* L.) belonging to *Solanaceae* family, it is cultivated and consumed in many countries. The total potato production was 376.8 million tonnes (19.2 million ha) in the world [1]. It is tolerant in terms of climatic conditions and is a perennial temperate climatic plant. It's tubers and vegetative parts are damaged at temperatures of 0 °C and below. The production of potato plant is usually made by asexual routes, especially with tubers and micro tubers. The uses of healthy and certified high quality seeds are very important for potato production and for high yield. The production of the thousands of genotypes in field conditions are more difficult than the production through tissue culture method sinceit is more costly, takes long time and largely dependents on climate.

Tissue culture methods are utilized in rapid propagation and breeding trials in addition to protecting gene sources in potato. These techniques are also used to obtain disease and virus free plant materials. In this way, irrespective of climatic conditions, diseases and virus-free starting materials can be obtained and studies with controlled conditions may bring increased yield with higher reproduction rate. Due to population growth in the world, the rapid growth of the industry, the increase in the construction and decrease in the amount of the fertile lands have led to search for different alternatives in seed production.Among these different alternatives, micro tuber production with tissue culture methods has gained importance in recent years.

There are two simple and reliable methods among micropropagation techniques in potato. The first method is the development of offshoots by cultivating single or multiple nodes, called single or multiple node cultures, specially designated for artificial nutrient media. Single node explants have been usually used for potato micropropagation [2, 3]. The second and possibly more interesting micropropagation technique is producing micro tubers from potato offshoots. Micro tuber production is a commercial production method and suitable for large-scale cultivation with machines [4-6]. It has been reported that *in vitro*micro tuber development in potato is promoted by adding especially cytokine and high-level sucrose [6-8]. Micropropagation can be easily performed on potato and disease free clones under controlled environment can be obtainedin a short time [9-14].

Among the most important components of the nutrient medium used in plant tissue culture are sugars. Carbohydrates are at the forefront as a source of energy for plants to continue their

development under aseptic conditions. Since plants cannot synthesize enough carbohydrates when they are *in vitro*cultured, sugar is used at different concentrations as a basic energy source. The most common used sugars are sucrose, glucose, maltose, fructose and raffinose. Sugar ratios used in studies conducted for different purposes in tissue culturestudies show variations. According to Ovono et al. [15] 2009, since sucrose supply a stable osmotic pressure in a medium, it is most widely used as carbohydrate sourcein*in vitro*. Due to its lower cost and easy accessibility, sucrose is more preferred than other types.

Nowadays, the jasmonates have been recognized as one of the plant growth regulators in many studies. Jasmonates gained importance as they support plant resistance against biotic and abiotic stress. The storage proteins found in bulbs, tubers and seeds are very important in enabling plants to regenerate life cycles and to activate genes that control vegetative storage proteins. Jasmonates, moreover, promote the accumulation of storage proteins at high levels. Although it is known that jasmonic acid (JA) has potential plant growth inhibitor in several plant species [16], *in vitro* micro tuber induction and formation are stimulated via jasmonates [17, 18]. When plants are exposed to stress, the level of jasmonate they contain increases. Jasmonic acid and its derivatives have been found to have positive effects on young bulbs, tubers and micro tubers formations in plants such as potatoes, onions and daffodils in *in vivo* conditions [19]. Many previous studies have reported that jasmonic acid has positive effects on the formation and development of micro tubers in potato plants [20-22] and reported that it could stimulate vegetative development if applied in low quantities [20, 22]. Due to the positive effects of jasmonates on *in vitro* micro tuber formation, 2 different doses of methyl jasmonate were used to serve the purpose. Present study was also conducted with three different explant types (primordial tissue, shoot eyes and nodes) and three different amounts of sucrose (30, 60 and 90 g/L) to study the effects of sucrose and methyl jasmonate on micro tuber formation in potato.

II. MATERIALSAND METHODS

The study was carried out in the Tissue Culture Laboratory of the Department of Horticulture, Faculty of Agriculture, Akdeniz University by using the vegetative components of the potato plants of Agata variety, grown in the field and in the greenhouses and obtained from AR Agriculture Seed Company.

Three different explant types were investigated, namely, tuber primordial tissue, shoot eyes and nodes, which were taken from the potatoes used as an explant. In the experiment there were 2 replicates, 10 explants for each explant type and 120 explants for each repeat. Total number of explants used during the experimet was 720. Surface sterilizations of these explants were performed as multi-stage and different from each other. Sterilization of tuber primordial tissue; explants were kept in flowing tap water for 2 hours and treated with antibacterial soap (900 mL purified water + 100 mL antibacterial soap), rinsed 3 times with distilled pure water, they were soaked inthe 30% sodium hypochlorite solution for 30 minutes, and rinsed again 3 times with distilled pure water before taken to the laminar air flow cabinet and were subjected to sterilization in there one more time. The second stage of sterilization was performed in laminar flow. Firstly they were incubated in 70% ethyl alcohol for 1 minute, then in 20% sodium hypochlorite solution for 15 minutes. After these procedures they were rinsed with sterile distilled pure water 3 times and placed in nutrient media. For surface sterilization of shoot eyes; explants were first treated with antibacterial soap (95 mL of purified water + 5 mL of antibacterial soap), then rinsed 3 times with distilled pure water and taken into a sterile cabinet and finally re-sterilized. Shoot-eye explants were more delicate structures; therefore they were kept 30 seconds in 70% ethyl alcohol, then in 20% sodium hypochlorite solution for 20 minutes and rinsed 3 times with sterile distilled water. After sterilization, the dead tissues in all explants were removed with the help of forceps and scalpel, and the remaining healthy plant parts were cultured. All steps of sterilization of the node explants from the vegetative parts of the potato plants were carried out in the laminar air flow cabinet. Firstly they were treated with antibacterial soap (95 mL purified water + 5 mL antibacterial soap) and then rinsed 3 times with distilled pure water. They were kept in 70% ethyl alcohol for 1 minute, then in 15% sodium hypochlorite solution for 15 minutes and rinsed 3 times with sterile distilled water to complete the sterilization steps and planting was done in the prepared nutrient media. The explant pieces cultured were transferred to the growth chamber which has 8 hours photoperiod and 3000 lux illumination at 24 ± 2 °C.

Nitsch&Nitsch [23] basal medium were used in the study and 12 different nutrient media combinations including control medium were tested as shown in Table I.

TABLE I
THE NUTRIENT MEDIA USED IN THE STUDY

	Media Code	Basal Nutrient Composition	Plant Growth Regulators (mg/L)	Sucrose (g/L)	Methyl Jasmonate (ppm)
1	N_{30} J.0	N	-	30	-
2	N.1 J.0	N	2.5 BAP + 0.5 NAA	30	-
3	N.1 J.1	N	2.5 BAP + 0.5 NAA	30	1.0
4	N.1 J.2	N	2.5 BAP + 0.5 NAA	30	2.0
5	N_{60} J.0	N	-	60	-
6	N.2 J.0	N	2.5 BAP + 0.5 NAA	60	-
7	N.2 J.1	N	2.5 BAP + 0.5 NAA	60	1.0
8	N.2 J.2	N	2.5 BAP + 0.5 NAA	60	2.0
9	N_{90} J.0	N	-	90	-
10	N.3 J.0	N	2.5 BAP + 0.5 NAA	90	-
11	N.3 J.1	N	2.5 BAP + 0.5 NAA	90	1.0
12	N.3 J.2	N	2.5 BAP + 0.5 NAA	90	2.0

After the measurements on obtained micro tubers were completed, a mixture of peat and perlite (1:1) was prepared in pots in *in vivo* conditions. The planting was done separately and each pot was given its own number. The potatoes planted into the pots were given sap, then regular irrigation and general controls were conducted weekly. After the initial development of the transferred micro tubers, fertilization was started and conducted out twice in total. In the fertilization program, 15:15:15 N:P:K was mixed with the mixture of peat and perlite as a base fertilizer. Urea and ammonium nitrate dehydrates were given with the irrigation. The water levels of the pots have not been lowered below the field capacity. After 45 days from planting, obtained tubers harvested and measured in terms of the length (mm), diameter (mm) and weights (g).

The experiment was carried out in two replications, for the different media and explants. Our data were not fit in a normal distribution curve. Therefore the data transformation was done by using square root. After that, the data obtained in the trial were subjected to a one-way ANOVA test using the JMP-SAS 8.1 packet program. Means were compared with the LSD test to check significant differences.

III. RESULTS AND DISCUSSIONS

In the present study, micro tubers, plantlets and callus formation of the node, primordial and shoot eye explants used were investigated. Improvements were observed in the media of the different contents prepared, and both observation results (Table II) and statistical results obtained were given in the following charts (Tables III and IV). According to the observations, in terms of explant types; shoot eye had the greatest response and it was the best explant, regardingmicrotuber formation (18 pieces). The primordial tissue explant was the second among the explants used by forming 12 micro tubers, and the node explant had the lowest level (8 pieces) in terms of micro tuber formation (Fig. 1-5).

In terms of evalutaion of explant types, plantlets were recorded in all media combinations for node explants, while plantlets were recorded in 5 different media combinations for shoot eye explants. Only one medium for primordial tissue explants resulted with plantlet formation. Regarding the callus formation, it took place in 6 different media for shoot eye explants while it was 5 different media for primordial tissue explants. On the other hand there was no callus formation in node explants (Table II).

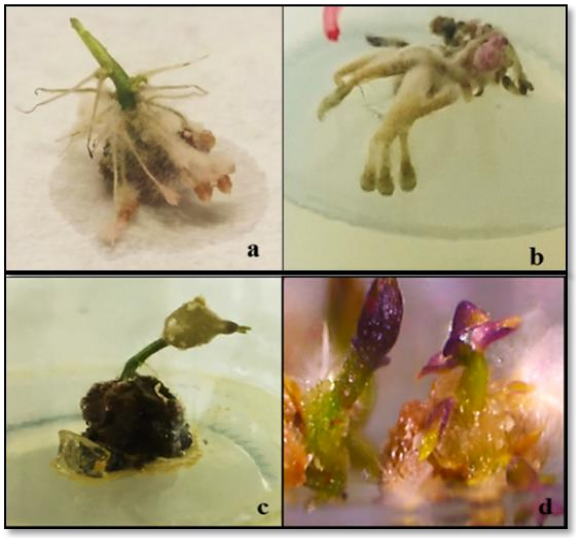


Fig. 1. Observation photographs related to some micro tuber formations in different media compositions and different explant types; (a): primordial tissue, N2 J1; (b): shoot eye, N1 J1; (c): primordial tissue, N2 J1; (d): shoot eye, N1 J2.

Fig. 2. Observation photographs related to some micro tuber formations in different media compositions and different explant types; (a): N1 J1 – primordial tissue; (b): N2 J1 – primordial tissue; (c): N1 J1 – shoot eye; (d): N1 J1 – shoot eye; (e): N2 J1 – node; (f, g, h): N2 J1 – primordial tissue.

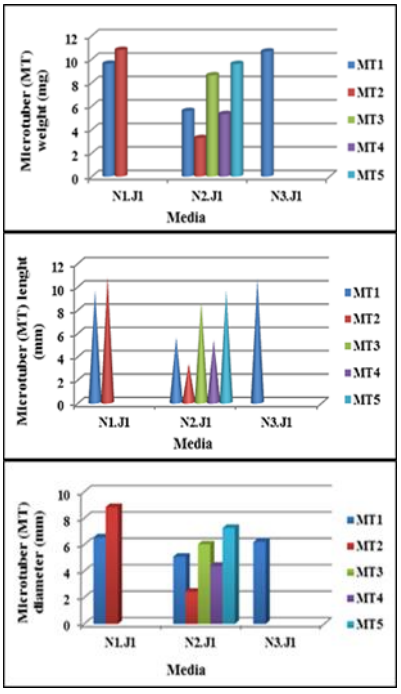
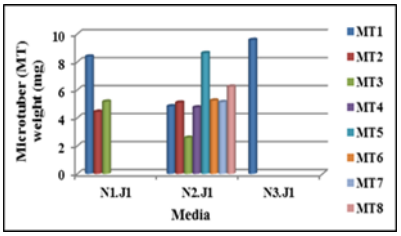


Fig. 3. The diagrams about weight (a), lenght (b) and diameter (c) of microtubers obtained from node explants



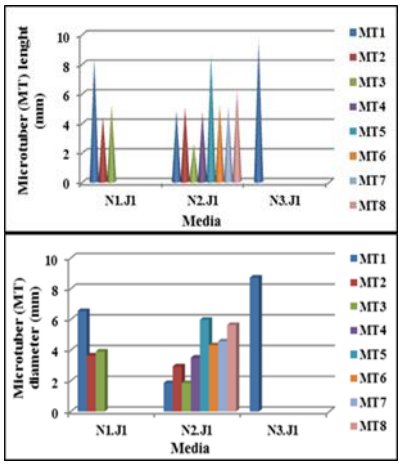


Fig. 4. The diagrams about weight (a), lenght (b) and diameter (c) of microtubers obtained from primordial tissue explants

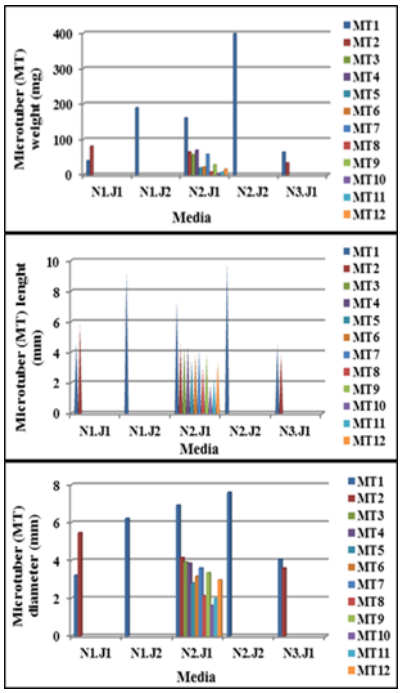


Fig. 5. The diagrams about weight (a), lenght (b) and diameter (c) of microtubers obtained from shoot eye explants

TABLE II
THE DATA ABOUT THE REACTIONS OF MEDIA CONTENTS AND EXPLANT TYPES

Media Codes	Explants											
	Node			Primordial Tissue				Shoot Eye				
	Micro tuber Formation		Plant Formation	Callus Formation	Micro tuber Formation		Plant Formation	Callus Formation	Micro tuber Formation		Plant Formation	Callus Formation
	No.	%			No.	%			No.	%		
N_{30} J.0	-	-	+	-	-	-	-	-	-	-	-	-
N.1 J.0	-	-	+	-	-	-	-	+	-	-	+	-
N.1 J.1	2	10	+	-	3	15	-	-	2	10	+	+
N.1 J.2	-	-	+	-	-	-	+	-	1	5	-	+
N_{60} J.0	-	-	+	-	-	-	-	-	-	-	-	-
N.2 J.0	-	-	+	-	-	-	-	+	-	-	+	-
N.2 J.1	5	25	+	-	8	40	-	+	12	60	-	+
N.2 J.2	-	-	+	-	-	-	-	+	1	5	-	+
N_{90} J.0	-	-	+	-	-	-	-	-	-	-	-	-
N.3 J.0	-	-	+	-	-	-	-	-	-	-	+	-
N.3 J.1	1	5	+	-	1	5	-	-	2	10	-	+
N.3 J.2	-	-	+	-	-	-	-	+	-	-	+	+

Observations were made weekly intervals following culture of different explant types in different media in *in vitro* conditions. The obtained micro tubers were measured in terms of length (mm), diameter (mm) and weights (g) recorded. While micro tubers were obtainedfromallnode and primordial explants, in media supplemented with 1.0 ppm methyl jasmonate, microtubers were obtained from some shoot eye explants in the same medium.As reported earlier, many researchers assume that bulb and tuber formations are kept under control by jasmonate levels through the stimulation of cell division and support of cell expansion [24-26]. Considering the sucrose ratios, it was determined that 60 g/L sucrose stimulated the micro tuber formation more than 30 g/L sucrose. In addition, when 2.0 ppm of methyl jasmonate concentration was used in 2 different nutrient media with sucrose of 30 g/L and 60 g/L, an increase in the size, diameter and weight of micro tubers formed were observed.

*In vitro*microtuber formation can be effected separately or interactively by several factors such as plant growth regulators, different sucrose levels, pre-treatments, photoperiods [7, 27, 28, 29]. In the present study obtained results are consistent with previous studies.Statistically significant differences were observed for media combinations used in present study (Tables III and IV). On the other hand there were no statistically significant differences among the explant types and media*explant interactions. According to the results obtained from statistical data, medium number 3 (N.1 J.1) was the most successful in response to evaluated parameters, followed by media number 7 (N.2 J.1) and number 11 (N.3 J.1), respectively (Table IV).

In this study, the effect of the basic composition on plant and micro tuber formation from different explants of potato plant such as node, shoot eye and primordial tissues were investigated by using Nitsch&Nitsch [23] medium instead of Murashige& Skoog [30]. Cultivars showed different sensitivity reactions to nitrogen ratio,added to medium [5, 31, 32]. Many researchers agree that with low nitrogen media have the best positive effects on microtuberisation [5, 33].

In some studies, it has been reported that lowering the nitrogen content in the nutrient medium generally increases the amount of chlorophyll in all potato varieties and leads to the early formation of micro tubers [34]. As shown in Table V in these two basic nutrient compositions, which differ in their macro- and micro- elements and their concentrations, the major difference is that the MS medium has a high salt content and the N medium has a lower salt content than MS. It is also thought that the presence of folic acid and biotin in nutrient medium promotes tuber formation.

Sucrose is one of the key factors regarding *in vitro* potato micro tuber formation as it has an osmotic effect [5, 35, 36]. For increasing micro tuber induction, sucrose levels are commonly used at 8% or 9% [5, 37]. In *in vitro* studies for potato micro tuber formation, the use of sucrose as a source of carbohydrate is quite common and there are many studies used in different proportions. Abbot and Belcher [37], in their studies in which they added 3%, 6%, 9%, and 12% sucrose doses into nutrient media, reported that the best amount of the dose was 6% to promote micro tubers. Hussey & Stacey [13], also reported that 6% sucrose use was good enough in their study of micro tuber

TABLE III
STATISTICAL RELATIONSHIPS AMONG PARAMETERS AND REPLICATES, EXPLANTS, MEDIA AND MEDIA*EXPLANTSINTERACTIONS

	PARAMETERS					
	Micro tuber Length (mm)	Micro tuber Diameter (mm)	Micro tuber Weight (g)	Tuber Length (mm)	Tuber Diameter (mm)	Tuber Weight (g)
Replicates	N.S	N.S	N.S	N.S	N.S	N.S
Explants	N.S	N.S	N.S	N.S	N.S	N.S
Media	***	***	***	***	***	***
Media*Explants	N.S	N.S	N.S	N.S	N.S	N.S

Rows indicate a statistically significant difference at * $P \leq 0.05$; ** $P \leq 0.01$ and *** $P \leq 0.001$, respectively. NS, not significant

TABLE IV
PARAMETERS OF MICRO TUBER AND TUBER FORMATION IN RESPONSE TO MEDIA COMBINATIONS

Media		PARAMETERS					
		Micro tuber Length (mm)	Micro tuber Diameter (mm)	Micro tuber Weight (g)	Tuber Length (mm)	Tuber Diameter (mm)	Tuber Weight (g)
(1)	**N_{30} J.0**	0,70 ^{c}	0,70 ^{c}	0,70 ^{d}	0,70 ^{c}	0,70 ^{c}	0,70 ^{c}
(2)	**N.1 J.0**	0,70 ^{c}	0,70 ^{c}	0,70 ^{d}	0,70 ^{c}	0,70 ^{c}	0,70 ^{c}
(3)	**N.1 J.1**	2,76 ^{a}	2,46 ^{a}	12,10 ^{a}	5,16 ^{a}	4,00 ^{a}	3,15 ^{a}
(4)	**N.1 J.2**	1,11 ^{c}	1,02 ^{c}	2,88 ^{cd}	1,28 ^{c}	1,08 ^{c}	0,91 ^{c}
(5)	**N_{60} J.0**	0,70 ^{c}	0,70 ^{c}	0,70 ^{d}	0,70 ^{c}	0,70 ^{c}	0,70 ^{c}
(6)	**N.2 J.0**	0,70 ^{c}	0,70 ^{c}	0,70 ^{d}	0,70 ^{c}	0,70 ^{c}	0,70 ^{c}
(7)	**N.2 J.1**	2,38 ^{ab}	2,13 ^{ab}	7,82 ^{ab}	4,29 ^{ab}	3,53 ^{ab}	2,36 ^{ab}
(8)	**N.2 J.2**	1,12 ^{c}	1,06 ^{c}	3,91 ^{bcd}	1,41 ^{c}	1,26 ^{c}	1,04 ^{c}
(9)	**N_{90} J.0**	0,70 ^{c}	0,70 ^{c}	0,70 ^{d}	0,70 ^{c}	0,70 ^{c}	0,70 ^{c}
(10)	**N.3 J.0**	0,70 ^{c}	0,70 ^{c}	0,70 ^{d}	0,70 ^{c}	0,70 ^{c}	0,70 ^{c}
(11)	**N.3 J.1**	2,04 ^{b}	1,86 ^{b}	7,35 ^{bc}	3,62 ^{b}	2,97 ^{b}	2,18 ^{b}
(12)	**N.3 J.2**	0,70 ^{c}	0,70 ^{c}	0,70 ^{d}	0,70 ^{c}	0,70 ^{c}	0,70 ^{c}
LSD (0,05)		0,706	0,598	4,599	1,376	1,029	0,899

Means sharing common English letters are statistically similar

formation*in vitro*.In the present study when 3% sucrose was used, not far more notable results were obtained than 6%, but results were a slightly better than other concentrations (Table IV). Vegetative occurrences were observed in the media and these were found to be in media without methyl jasmonate.

Jasmonates (JA and its related compounds) are newly recognised plant growth regulators and they have effects on many physiological processes regarding to plant growth and development, such as prevent of germination of seeds, impulsion of root, floral and fruit parts, bulb and tuber formation, self defense of plants [17, 38-42]. Many studies especially have shown that jasmonic acid, just like tuberonic acid, promotes *in vitro* tuber formation [17, 28, 43]. Recently jasmonic acid's effects on potato micro tuber formation are observed by many researchers [17, 40, 44, 45] and this was shown in the present study. When the effects of methyl jasmonate on micro tuber formation were evaluated, medium containing 1.0 ppm methyl jasmonate was better than other media combining different amount of methyl jasmonate (Table IV). It was reported that methyl jasmonate, known to accelerate senescence, had a negative effect on plant growth and micro tuber formation when used at a rate of 2.0 ppm. In this study 2.0 ppm methyl jasmonate was effective only when it was used with 60 g/L sucrose concentration (Table IV).

TABLE V
NITSCH & NITSCH [23] AND MURASHIGE & SKOOG [30] NUTRIENT MEDIA CONTENTS

Nitsch & Nitsch [23]		Murashige & Skoog [30]	
Micro Elements	**mg/L**	**Micro Elements**	**mg/L**
$CuSO_4.5H_2O$	0.025	$CuSO_4.5H_2O$	0.025
FeNaEDTA	36.70	FeNaEDTA	36.70
H_3BO_3	10.00	H_3BO_3	6.20
$MnSO_4.H_2O$	18.94	$MnSO_4.H_2O$	16.90
$Na_2MoO_4.2H_2O$	0.25	$Na_2MoO_4.2H_2O$	0.25
$ZnSO_4.7H_2O$	10.00	$ZnSO_4.7H_2O$	8.60
		$CoCl_2.6H_2O$	0.025
		KI	0.83
Macro Elements	**mg/L**	**Macro Elements**	**mg/L**
$CaCl_2$	166.00	$CaCl_2$	332.02
KH_2PO_4	68.00	KH_2PO_4	170.00
KNO_3	950.00	KNO_3	1900.00
$MgSO_4$	90.27	$MgSO_4$	180.54
NH_4NO_3	720.00	NH_4NO_3	1650.00
Vitamins	**mg/L**	**Vitamins**	**mg/L**
Glycine	2.00	Glycine	2.00
myo-Inositol	100.00	myo-Inositol	100.00
Nicotinic acid	5.00	Nicotinic acid	0.50
Pyridoxine HCl	0.50	Pyridoxine HCl	0.50
Thiamine HCl	0.50	Thiamine HCl	0.10
Biotin	0.05		
Folic acid	0.50		

Obtained micro tubers from different explant types and different nutrient media were transferred to the pots in *in vivo* conditions and the measurement data and statistical analyses data of the obtained tubers were done. When the responses of the explant types were examined, node explants formed 28 tubers out of 8 micro tubers; primordial tissue explants formed 32 tubers out of 12 micro tubers and the shoot eye explants formed 40 tubers out of 18 micro tubers. When the connection between the micro tubers and the tubers the explants formed were examined, it was found that although shoot eye explants formed the highest number of the micro tubers and tubers, the node explants were found to be more efficient (3,5 fold) since they formed 28 tubers out of 8 micro tubers. Primordial tissue yielded a value between the yields of the other two explants (2.66 fold), while the least yield was observed in shoot eye explants (2.22 fold). When examining tuber formation, while there were statistically significant differences among media combinations, there were no differences among explant types and media*explants interactions in terms of tuber formation (Tables III and IV). According to the results obtained medium number 3 (N.1 J.1) was the most successful in terms of forming tubers from micro tubers, followed by media number 7 (N.2 J.1) and number 11 (N.3 J.1), respectively (Table IV).

Examining the effects of methyl jasmonate on tuber yield, micro tubers transferredto*invivo*conditionsfrom the nutrient medium, containing 1.0 ppm methyl jasmonate, was observed to have high tuber formation potentials and yields. It has been observed that the micro tuber formation leads to an increase in tuber formation in order to maintain their life cycles under the conditions where methyl jasmonateis used at a rate of 1.0 ppm in the process of micro tuber formation. Methyl jasmonate is thought to induce more internal micro tuber formation qualitatively and quantitatively by triggering internal stress.

IV.CONCLUSION

As labor, time and space are scaled down, micropropagated micro tubers are an efficient way to stock germplasm [7, 29, 46]. In the present study, it was aimed to obtain potato micro tubersin*in vitro* conditions by using different explants, sucrose doses and methyl jasmonate and then to obtain tubers by transferring the micro tubers to *in vivo* conditions. Experimental results revealed that, the most successful explant type was the shoot eye when the explant types were evaluated in terms of their reaction to the nutrient media. The most successful nutrient medium was found to be number 3 (N.1 J.1; 2.5 mg/L BAP+ 0.5 mg/L NAA + 30 g/L sucrose + 1.0 ppm methyl jasmonate) according to the different sucrose and methyl jasmonate ratios used in nutrient media. It is anticipated that in*in vitro* selection in potato plant breeding studies, this can be used effectively and integrated into long-term potato breeding programs to achieve desired results in a short time.

REFERENCES

[1] (2016) The FAO website. [Online]. Available: http://www.fao.org/

[2] G. Hussey, & N. J. Stacey, "In vitro propagation of potato (Solanum tuberosum L.)," Ann. Bot., vol. 48, pp. 787-796, 1981.

[3] S. Khuri and J. Moorby, "Nodal segments or microtubers as explants for in vitromicrotuber production of potato," Plant Cell, Tissue and Organ Culture, vol. 45, pp. 215-222, 1996.

[4] B. H. McCown and P. J. Joyce, "Automated propagation of microtubers of potato," In Scale-Up and Automation in Plant Propagation, I.K. Vasil, Ed., pp. 95-109. Academic Press. San Diego, CA. 1991.

[5] D. J. Donnelly, W. K. Coleman & S. E. Coleman, "Potato microtuber production and performance: A review," Amer. J. Potato Res., vol. 80, pp. 103-115, 2003.

[6] M. Kane, "Micropropagation of Potato by Node Culture and Microtuber Production," In: Plant Tissue Culture, Development, and Biotechnology, Robert N. Trigiano, Dennis J. Gray Ed., pp. 207-212. CRC Press.USA, 2011.

[7] J. E. A. Seabrook, S. Coleman, and D. Levy, "Effects of photoperiod on in vitrotuberization of potato (Solanum tuberosum L.)," Plant Cell Tiss. Organ Cult.vol. 34, pp. 43-51, 1993.

[8] J. Gopal, J. L. Minocha& H. S. Dhaliwal, "Microtuberization in potato (Solanum tuberosum L.)," Plant Cell Rep., vol. 17, pp. 794-798, 1998.

[9] W. M. Roca, N. O. Espinoza, M. R. Roca & J. E. Bryan, "A tissue culture method for the rapid propagation of potatoes," Amer. Potato J.,vol 55, pp. 691-701, 1978.

[10] R. B. Copeland, "Micropropagation of potatoes," Agric. Northern Ireland, vol. 57, pp. 250-253, 1982.

[11] N. O. Espinoza, R. Estrada, D. Silva-Rodriguez, P. Tovar, R. Lizarraga& J. H. Dodds, "The potato: A model crop plant for tissue culture," Outlook on Agriculture, vol. 15, pp. 21-26, 1986.

[12] B. S. Ahloowalia, "Production and performance of potato mini-tubers," Euphytica, vol. 75, pp. 163-172, 1994.

[13] G. Hussey & N. J. Stacey, "Factors affecting the formation of in vitro tubers of potato (Solanum tuberosum L.)," Annals of Botany, vol. 53, pp. 565-578, 1984.

[14] R. Estrada, P. Tovar & J. H. Dodds, "Induction of in vitro tubers in a broad range of potato genotypes," Plant, Cell, Tissue and Organ Culture, vol. 7, pp. 3-10, 1986.

[15] P. O. Ovono, C. Kevers, J. Dommes, "Effects of reducing sugar concentration on in vitro tuber formation and sprouting in yam (Dioscoreacayenensis – D. rotundata complex)," Plant Cell Tiss Organ Cult., vol. 99, pp. 55–59, 2009. DOI 10.1007/s11240-009-9575-1.

[16] C. Wasternack, "Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development," Annals of Botany, vol. 100, pp. 681–697, 2007.

[17] Y. Koda, Y. Kikuta, H. Tazaki, Y. Tsujino, S. Sakamura, T. Yoshihara, "Potato tuber-inducing activities of jasmonic acid and related compounds," Phytochernistry, vol. 30, pp. 1435-1438, 1991.

[18] Jeffrey C. Suttle& Linda L. Huckle& Edward C. Lula, "The Effects of Dormancy Status on the Endogenous Contents and Biological Activities of Jasmonic Acid, N-(jasmonoyl)-Isoleucine, and Tuberonic Acid in Potato Tubers," Am. J. Pot Res. Vol. 88, pp. 283–293, 2011. DOI 10.1007/s12230-011-9192-5.

[19] İ. Baktır, "Jasmonateler (YaseminAsitleri)," Hormonlar, BitkiGelişimDüzenleyicileri, ÖzelliklerveTarımdaKullanımları, sf. 90-92, HasadYayıncılık, İstanbul, Türkiye, 2015.

[20] L. I. Martin-Closas, S. Sol and A. M. Pelacho, "Potential application of jasmonic acid for Solanum tuberosummicropropagation," XXV. International Horticultural Congress, Part 10: Application of Biotechnology and Molecular Biology and Breeding-In vitro Culture, Brussels, Belgium (Eds. L. H. W. van der Plas and G. J. de Klerk). ISHS ActaHorticulturae vol. 520, pp. 127-134, 2000.

[21] Y. Koda and Y. Kikuta, "Effects of jasmonates on in vitrotuberization in several potato cultivars that differ greatly in maturity," Plant Prod.Sci., vol 4, pp. 66–70, 2001.

[22] A. M. Kumlay, "The Effect of Jasmonic Acid on the Micropropagation of Potato (Solanum tuberosum L.)Under Long Days Conditions," YYÜ TAR BİL DERG (YYU J AGR SCI), vol. 26, pp. 79-88, 2016.

[23] J. P. Nitsch and C. Nitsch, "Haploid plants from pollen grains," Science, vol. 163, pp. 85- 87, 1969.

[24] Y. Koda, "Possible involvement of jasmonates in various morphogenic events," Physiol Plant, vol. 100, pp. 639–646, 1997.

[25] D. Sarkar, S. K. Pandey, S. Sharma, "Cytokinins antagonize the jasmonate action on the regulation of potato (Solanum tuberosum) tuber formation in vitro," Plant Cell Tissue Organ Cult., vol. 87, pp. 285–295, 2006. DOI: 10.1007/s11240-006-9166-3.

[26] P. O. Ovono, C. Kevers, J. Dommes, "Tuber formation and growth of Dioscoreacayenensis-D. rotundata complex: interactions between exogenous and endogenous jasmonic acid and polyamines," Plant Growth Regul, vol. 60, pp. 247–253, 2010. DOI 10.1007/s10725-009-9441-5.

[27] K. Pruski, T. Astatkie, J. Nowak, "Jasmonate on in vitrotuberization and tuber bulking in two potato cultivars (Solanum tuberosum L.) under different media photoperiod conditions," In vitro Cell Dev. Biol. Plant., vol. 38, pp. 203–209, 2002.

[28] A. M. Pelacho, A. M. Mingo-Castel, "Jasmonic acid induces tuberisation of potato stolons cultured in vitro," Plant Physiol. Vol. 97, pp. 1253-1255 1991.

[29] E. A. Janet, L. Seabrook, K. Douglass, & D. A. Arnold, "Effect of Leaves on Microtubers Produced from Potato Single-Node Cuttings In Vitro," Amer J of Potato Res., vol. 81, pp. 1-5, 2004.

[30] T. Murashige, F. Skoog, "A revised medium for rapid growth and bioassays with tobacco tissue culture," Physiol. Plant.vol. 15, pp. 473-497, 1962.

[31] N. Garner & J. Blake, "The induction and development of potato microtubersin vitro on media free of growth regulating substances," Ann Bot, vol. 63, pp. 663-674, 1989.

[32] D. Sarkar and P. S. Nalk, "Effect of Inorganic nitrogen nutrition on cytokinin-induced potato microtuber production in vitro," Potato Res, vol. 41, pp. 211-217, 1998.

[33] G. F. Stallknecht and S. Farusworth, "The effect of nitrogen on the coumarin-induced tuberization of potato axlllary shoots cultured in vitro," Am Potato J, vol. 56, pp. 523-530, 1979.

[34] A. Zarrabeitia, X. Lejarcegui, J. Veramendi, A. M. Mingo Castel, "Influence of nitrogen supply on micropropagation and subsequent microtuberization of four potato cultivars," Am. Pot. J.,vol. 74, pp. 369-378, 1997.

[35] P. Wang and C. Hu, "In vitro mass tuberization and virus-free seed potato production in Taiwan," Am Potato J., vol. 59, pp. 33-37, 1982.

[36] S. Khuri and J. Moorby, "Investigations into the role of sucrose in potato cvEstimamicrotuber production in vitro," Ann Bot, vol. 75, pp. 295-303, 1995.

[37] A. J. Abbott & A. R. Belcher, "Potato tuber formation in vitro" In: LA Withers, PG Alderson (Eds.). Plant Tissue Culture and its Agricultural Applications, pp. 113-122, Butterworths, London, 1986.

[38] F. Corbineau, R. M. Rudnicki& D. Come, "The effects of methyl jasmonate on sunflower seeds germination and seedling development," Plant Growth Regul., vol. 7:, pp. 157-169, 1988.

[39] S. Q. Huang, J. H. Bin & Z. P. Li, "Effects of methyl jasmonate and ABA on the growth of root and hypocotyls of peanut seedling," J. Plant Physiol. mol. Biol., vol. 28, pp. 351-356, 2002.

[40] M. Ravnikar, B. Vilhar and N. Gogala, "Stimulatory effects of jasmonic acid on potato node and protoplast culture," J Plant Growth Reg., vol. 11, pp. 29-33, 1992.

[41] V. Repka, I. Fischerová, K. Šilhárová, "Methyl jasmonate is a potent elicitor of multiple defense responses in grapevine leaves and cell-suspension cultures," Biol. Plant, vol. 48, pp. 273- 283, 2004.

[42] Z. J. Zhang, W. J. Zhou, H. Z Li, G. Q. Zhang, K. Subrahmaniyan and J. Q. Yu, "Effect of jasmonic acid on in vitro explant growth and microtuberization in potato," BiologiaPlantarum, vol. 50, pp. 453 - 456, 2006.

[43] S. D. Jackson & L. Willmitzer, "Jasmonic acid spraying does not induce tuberisation in short-day-requiring potato species kept in non-inducing," Planta, vol. 194, pp. 155-159, 1994.

[44] S. Biondi, S. Fornale, K. M. Oksman-Caldentey, M. Eeva, S. Agostani& N. Bagni, "Jasmonates induce over-accumulation of methylputrescine and conjugated polyanfines in Hyoscyamusmuticus L. root cultures," Plant Cell Rep, vol. 19, pp. 691-697, 2000.

[45] K. Pruski, T. Astatkie, P. Duplessis, T. Lewis, J. Nowak and R. C. Struik, "Use of Jasmonate for Conditioning of Potato Plantlets and Microtubers in Greenhouse Production of Minitubers," Amer J of Potato Res, vol. 80, pp. 183-193, 2003.

[46] P. Tovar, R. Estrada, L. Schilde-Rentschler and J. H. Dodds, "Induction and use of in vitro potato tubers," International Potato Centre, CIP Circular, vol. 13, pp. 1-5, 1985.