TEMPERATURE AND GELLING AGENT EFFECTS ON *IN VITRO* MICROTUBERIZATION OF POTATO (*SOLANUM TUBEROSUM* L.)

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ABSTRACT

*It was found that **in vitro** microtuberization of potato (**Solanum tuberosum** L.) was affected considerably by temperature and gelling agent. The best results were obtained in cultures maintained at 20-22° C in dark. Moreover, considerable increases in number, average weight and yield of microtubers was obtained on media solidified with gelrite compared to agar, when used as gelling agent. Number of microtuber formation and their weight had significant positive relationship (r = 0.630 and r = 0.771 respectively) with microtuber yield per plantlet.*

Introduction

Potato (*Solanum tuberosum* L.) is an important food source in many parts of the world. Considerable efforts are continuously being made to breed potato for improvement both through conventional breeding and *in vitro* techniques. *In vitro* techniques are being applied to improve potato production through micropropagation, pathogen elimination and germplasm conservation etc. The use of *in vitro* techniques for virus elimination and clonal mass propagation are the most advanced applications to this respect.

In vitro manipulations in potato are mainly carried out to maintain genetic stability and achieve some level of genetic modifications through gene transfer, gene inhibition and microtuberization. *In vitro* mass tuberization in potato and successful integration of this technology for virus-free seed potato production was reported before (1).

In vitro micropropagation in potato is generally used to bulk up new cultivars or breeding lines, for germplasm storage, transport and production of microtubers or minitubers which thus are easy to store, transfer and distribute. Moreover, they are free of pests, diseases and virus, which helps in transport of certified virus-free shoot cultures as well. Production of disease-free potato clones combined with *in vitro* multiplication methods have become an integral part of seed production in many countries with several advantages helping to reduce time taken to produce seed tubers and number of field generations, resulting in higher quality seed tubers (2).

Molecular analysis of *in vitro* propagated and stored potatoes indicate that micropropogation results in production of genetically stable plants (3). In recent years genetic, transformation has been achieved using *in vitro* microtubers via *Agrobacterium tumefaciens* in potato. Many researchers have developed new transformation methods using *in vitro* microtubers (4, 5, 6). It was emphasized that microtubers are good source of explant material for developing genetic transformants (6).

In vivo and *in vitro* tuberization of potato is influenced by genetic, physiological and

environmental factors including photoperiod, temperature, irradiance, mineral elements, physiological age and hormone concentrations. This suggests that culture conditions must be optimized for optimal *in vitro* microtuberization. Thus, the aim of this study was to find out effect of gelrite and agar on *in vitro* microtuberization of potato under different temperature regimes.

Materials and Methods

Tubers of Lady Rosetta, Tomensa and Monalisa potato cultivars were obtained from Agriculture Credit Cooperatives and private companies in Turkey. Preliminary studies in our laboratory indicated that these cultivars had higher micrutoberization capacity. Tubers of Lady Rosetta, Tomensa and Monalisa potato cultivars were surface-sterilized by shaking for 20 min in 50 % commercial bleach (Axion) and rinsed four times in sterile water. Then, the tubers were sprouted in dark at 20-22 °C. After six weeks, sprouts were excised from the tubers and subjected to surface sterilization again for 20 min in 25 % commercial bleach and rinsed three times in sterile water.

The sprouts (approximately 2 cm long) were placed on MS (7) 10 ml basal medium supplemented with 30 g/l sucrose and 7 g/l agar in glass tubes (2 x 10 cm) at 24 ºC. After 5 weeks of growth period, single nodes from plantlets were excised and subcultured on the same medium twice. Finally, single node segments were cultured on microtuber induction medium described before (8) at pH 5.7 in a dark growth chamber at temperatures of 18, 20, 22 and 24 °C for 20 days. This induction medium consisted of MS medium, 60 g/l sucrose, 2.25 mg/l kinetin and 8 g/l agar or 2.5 g/l gelrite. After 20 days, the cultures were transferred to 16-h-day length photoperiod of 38 µMol 38 m^{-2} s^{-1} under day and night temperature regime of 24 ± 2 °C. After three weeks, microtubers were harvested, scored and weighted to determine the number of microtubers per plantlet, average weight of microtubers and microtubers yield per plantlet. Dry matter content (%) was determined after drying microtubers at 80 °C for 26 h in ventilated oven. The experiment was conducted using randomized complete block design (4 temperatures x 2 gelling agent) with four replications. Each replication consisted of 5 nodal segments per Magenta GA-7 vessels. Analysis of variance was performed using standard techniques and the differences among the means were compared through Duncan's multiple range test using MSTAT-C computer programme (Michigan State University). Correlation coefficient among microtuber yield, number of microtuber, mean microtuber weight and dry matter content were also determined.

Results and Discussion

It was observed that micotubers formed in all treatments (all temperatures and gelling agents) and cultivars. Microtubers formed under dark conditions were white, however they turned green when transferred to light. First microtubers appeared after three weeks of culture. It was seen that tuberization began earlier on medium containing gelrite than agar medium along with increase of temperature. Analysis of variance showed that effect of temperature and gelling agent were highly significant ($p < 0.01$) on number of microtuber per plantlet, mean microtuber weight and microtuber yield per plantlet. Analysis of variance further indicated that interactions between temperature and gelling agents were not significant in three cultivars. Different temperature regimes and gelling agents resulted in significant variations for microtuber yield per plantlet, number of microtuber per plantlet and mean microtuber weight in all cultivars (**Table 1**).

TABLE 1

Effect of different temperatures and gelling agents on microtuber yield, number of microtuber, mean microtuber weight and dry matter content

	Mean microtuber weight (mg)			Number of microtuber (per plantlet)			Microtuber yield (mg/plantlet)			Dry matter content (%)		
Temperatures	L. Rosetta	Tomensa	Monalisa	L. Rosetta	Tomensa	Monalisa	L. Rosetta	Tomensa	Monalisa	L. Rosetta	Tomensa	Monalisa
18 °C	104.12 c[1]	89.12 c	104.55 c	1.38 b	0.72 a	0.66 b	153.87 c	65.12 bc	68.45 c	15.8 [ns]	17.6 [ns]	15.9 [ns]
20 °C	243.75 a	127.75 ab	145.62 a	1.46 b	0.67 a	0.78 a	304.37 a	87.03 a	113.12 a	16.1	17.3	16.3
22 °C	210.87 ab	139.25 a	159.22 a	1.62 a	0.68 a	0.55 c	331.12 a	95.52 a	89.50 b	15.7	17.4	16.4
24 °C	191.25 b	124.87 b	131.65 b	1.47 b	0.56 b	0.61 bc	243.87 b	72.32 b	82.44 bc	16.3	16.9	15.8
Gelling agents												
Agar	167.06 b[2]	102.13 b	121.27 b	1.26 b	0.56 b	0.59 b	214.68 b	72.53 b	78.58 b	15.7 [ns]	17.4 [ns]	15.9 [ns]
Gelrite	207.93 a	138.36 a	149.25 a	1.72 a	0.75 a	0.71 a	300.44 a	87.46 a	98.17 a	16.2	17.2	16.2

Each value is the mean of 4 replications with 5 nodal segments

[1,2]) Values within a column followed by the different letters are significantly different at the 0.01 probability level using Duncan's multiple range test.

[ns]) not significant

Mean microtuber weight was influenced by the different temperature regimes. For all tested cultivars, the highest mean microtuber weight was obtained under 20 and 22 °C temperature regimes, the lowest mean microtuber weight was found at 18 °C. These results were statistically different from aforementioned in terms of mean microtuber weight for each of the cultivars. Comparison of mean microtuber weight between agar and gelrite indicated that the use of gelrite led to greater mean microtuber weight in all cultivars. Lady Rosetta resulted in higher mean microtuber weight than the other two cultivars in most treatments (Table 1).

A comparison among temperatures indicated that Lady Rosetta had the highest number of microtubers per plantlet (1.62) at 22 °C. However, Monalisa produced the maximum number of microtubers per plantlet (0.78) at 20 °C. Tomensa had the highest number of microtuber (0.72) at 18 °C, however, there were no significant differences among 18, 20 and 22 °C for this cultivar. Among temperature regimes, 24 °C was the least effective in all cultivars. The highest numbers of microtubers per plantlet were also obtained on medium solidified with gelrite in all cultivars (Table 1). Similar to the microtuber weight results, the highest microtuber number per plantlet was achieved from cultivar Lady Rosetta in all treatments tested (Table 1).

The results related to the effect of various temperatures on microtuber weight were similar to that for microtuber yield. Monalisa had the highest microtuber yield per plantlet (113.12 mg) at 20 °C, however the best results in Lady

TABLE 2

Correlations among microtuber weight, number of microtuber per plantlet, microtuber yield per plantlet and dry matter content

Microtuber traits	Microtuber weight	Microtuber number	Microtuber yield	Dry matter content
Microtuber weight	1.000			
Microtuber number	0.047	1.000		
Microtuber yield	0.771**	0.630**	1.000	
Dry matter content	-0.169	0.045	0.031	1.000

Rosetta (331.12 mg) and Tomensa (95.52 mg/plantlet) were obtained at 22 °C, but there was no significant difference between 20 and 22 °C temperature regimes on both cultivars. As the case for mean microtuber weight and number of microtubers per plantlet the best results were achieved on gelrite solidified medium and Lady Rosetta produced much higher microtuber yield per plantlet than Tomensa and Monalisa in all treatments.

In general, the rate of tuberization increased at 20-22 °C in all cultivars. In contrast, there were dramatic decreases in mean microtuber weight, microtuber yield and number of microtubers at 18 and 24 °C in all cultivars. Type of gelling agent also influenced the aforementioned parameters. The highest mean microtuber weights, number of microtubers and microtuber yield in all cultivars were obtained on gelrite solidified medium. The effects of gelling agent did not vary in different cultivars. Different temperature regimes and gelling agents did not significantly affect dry matter content of the cultivars.

Correlation analysis indicated significant positive relationship for microtuber yield and number of microtuber (r = 0.630**) and microtuber yield and mean microtuber weight (r = 0.771 **).The other parameters did not show significant correlation (Table 2).

Environmental factors, especially photoperiod and temperature, play a major role in the initiation of tubers (9,10). Unfavorable conditions such as high temperature and low light intensity inhibit shoot and root growth along with tuberization. It was reported that microtuberization is positively affected by temperature up to certain temperature degrees.

The optimum temperature for *in vitro* tuberization was reported to be 20° C with a constant temperature being more effective than alternating day-night temperature (1). Temperatures below 12 °C and above 28 °C have been found to be inhibitory for potato microtuber production (1). It has also been shown that in the absence of growth regulating substances, especially cytokinins, lower temperatures enhance tuber-induction. It was stated that cultures maintained under a short photoperiod (day 20 ± 2 °C; night 18 ± 2 °C) led to both higher microtuber yield and a greater number of microtubers than those maintained under long days (16 h 38-50 µmol m^{-2} s^{-1}) combined with high tem-

peratures (day 28 ± 2 °C and night 25 ± 2 °C (12). It was reported that high temperatures (33/25 °C day/night) increased length of internodes, whereas either no effect or slightly decreased node number, the highest number of tubers was obtained at 20/15 °C day/night temperatures (13). Size of microtubers produced within two months depends on temperature (1, 14). The results of our study indicated that temperature was an important factor for *in vitro* microtuberization of potato. The highest microtuber production was recorded at 20 and 22 °C, however it was decreased considerably at 18 and 24 °C temperature regimes. Our findings with regard to effect of temperature on microtuberization agree with other literatures (13, 15, 16). Moreover, our data indicated that 24 °C and higher temperatures reduced tuberization. Similar observations were also reported in the literatures (1, 17, 18).

Gelling agent used *in vitro* may also affect microtuberization of potato. It was stated that *g*elrite is an alternative to agar for micropropagation and microtuberization of potato (19). *In vitro* tuberization of four potato cultivars under heat stress yielded more uniform microtubers on medium solidified with gelrite than agar (14). It was also found that tuberization was earlier and more uniform on gelrite than agar solidified medium in dark compared to light. Our observations confirmed the findings in the literature (20). In our study, the best results also were obtained on media containing gelrite in all cultivars. Microtuber yield per plantlet, number of microtuber per plantlet and mean microtuber weight increased with the use of gelrite under all temperature regimes. Although there was no interaction between temperature and gelling agent, the highest microtuberization was achieved on a medium solidified with gelrite at 20-22 °C temperature regimes. Gelling agents and temperature regimes did not influence the dry matter content of microtubers. However, it may be considired that 24 °C and higher temperatures may affect dry matter content of tuber and microtuber as well.

The highest correlation coefficients between mean microtuber weight and microtuber yield suggested that microtuber weight or size is an important factor in determining microtuber yield potential *in vitro*. By comparison, it may be said that microtuber weight is more important than microtuber number in determining microtuber yield potential.

It was concluded that the most suitable temperatures for microtuberization were 20-22 °C. Considerable improvements in microtuberization of potato can be induced by use of gelrite as a gelling agent under *in vitro* conditions. Our results when combined with a number of *in vitro* manipulations (photoperiod, irradiance, mineral elements, physiological age and hormone) will lead to efficient tuberization in potato. These results may be highly useful in production of transgenic potato plants carrying resistance genes against viruses, pathogens, herbicides etc. in our laboratory.

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