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Use of Different Melon and Watermelon Fruit Extracts as a Carbon Source and Gelling Agents in Potato Micropropagation

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- *Solanum tuberosum*
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Abstract

The aim of the research was to determine the effects of melon and watermelon fruit extracts and low agar concentrations on *in vitro* propagated plants of potato. In this research, melon and watermelon fruits were used as experimental material. The plant traits such as leaf growth, root development, plant growth , plant vigour, plant height, number of nodes per plant, internode length and plant height were measured and evaluated after 30 days incubation period. One of the most important plant characteristic for *in vitro* potato propagation was number of nodes per plant which was obtained for the medium MS + 0.5% melon flesh with seeds + 20 g/l sucrose + 5 g/l agar. There was a significant relationship between number of nodes per plant and fresh weight and plant height. Potato plants grown on media with 20% melon flesh + 3 g/l agar and 0.5% melon flesh with seed and 2% sucrose + 5g/l agar as a carbon sources had equal plant growth characters compared to using medium with 3% sucrose. Melon extract media were well capable in performing optimum plant growth and development specially an increasing the number of nodes per plant (a fundamental of propagation rate). Hence, it was concluded that the combination of low concentration of agar and melon extract in the solid medium could offer a good supporting surface for potato micropropagation.

INTRODUCTION

Many techniques have been developed during the last decades for producing potato plants in aseptic environments. The propagation of potato by *in vitro* culture of single node cuttings and other plant tissues are commonly used in the propagation of high genetic and disease-free seed tubers, germplasm exchange and conservation [1-3]. The micropropagated plants were genetically stable, and did not show any morphological aberrations except for one variegated plant among several thousand produced [4]. *In vitro* propagated potato plants are commonly used in potato seed production programs for production of *in vitro* tubers, glasshouse production of transplants and minitubers, or field planting [5]. For the routine multiplication of *in vitro* plants, single node cuttings for example can be used to produce rooted plants *in vitro* during the rooting phase. These rooted plants are subsequently acclimatized *ex vitro* in a glasshouse to produce plants in the field to produce seed tubers or minitubers [5,6].

The main advantage of potato micropropagation technology is the production of high quality and uniform plants. However, production of low cost high value plants is an ultimate objective which could be achieved by appropriate choice of media components. Various brands and grades of agar, agarose, phytagel and gelrite were used for *in vitro* propagation. However, plants growth is strongly influenced by the physical consistency of the culture media [7]. Agar, the conventional gelling agent, has a number of drawbacks that negatively affect culture growth and differentiation in many cases [8]. Cheaper agar alternatives include various types of starch and gums which have been investigated in commercial micropropagation [9]. Other options include white flour, laundry starch, semolina, potato starch, rice powder and sago [10]. A mixture of laundry starch, potato starch and semolina in a ratio of (2:1:1) reduced the cost of gelling agent by 70 – 82 % [7].

Sucrose is frequently used as a carbon source in plant tissue culture media. Sucrose has been established as prime component for potato micropropagation [11]. There have been several reports comparing the effects of sucrose concentrations on propagation and established that 3% sucrose was the optimum level for *in vitro* potato propagation [12-14]. Media chemicals, account for less than 15 %, while the carbon sources such as grade sucrose contribute about 34 % of the production cost. Therefore, for most of developing countries to benefit from direct use of tissue cultured material, the cost of commercial micropropagation has to be drastically reduced without compromising on the quality of micropropagules [15]. These can be done through identifying cheap alternatives to expensive grade sucrose [16].

In potato, micropropagation using commercial grade sucrose and agar makes up approximately 80 % of the total medium cost [17]. The most important attempts during the investigation were taken to make *in vitro* propagation protocol, cost effective by using economically cheaper alternatives to MS salts, agar and sucrose [6]. Identification of cheap or low-cost alternative gelling and carbon sources will greatly reduce the cost of production (90%) especially in large-scale commercial potato micropropagation. The objective of this study was to evaluate the potential of melon and watermelon fruit extracts as sugar source and agar substitute in micropropagation of potato plants using single node cuttings.

MATERIALS AND METHODS

Evaluated products

In this study, melon and watermelon fruits were used as experimental material. Fruit flesh and fruit rind was taken and made to pieces by blender separately. Watermelon fruit rind, watermelon fruit juice, melon flesh and melon flesh with seed was prepared and added to media. Melon is a good source of niacin, vitamin B6 and folate, and a very good source of vitamin A, vitamin C and potassium [18]. Watermelon is a good source of potassium, vitamin A and vitamin C [19].

Plant material and culture conditions

In vitro plants of *Solanum tuberosum* L. cultivars PA99 (mid late) were multiplied routinely by sub-culturing single node cuttings every 3 weeks. Single node cuttings were propagated in [20]. MS basal medium with 3 % sucrose and 0.7 % agar (Sigma type A) in petri dishes (25x100mm).

In this study we tested MS0 and different media combinations containing melon and watermelon flesh and rind extracts (10 to 50 %, watermelon juice and 0.1 %, 0.3 %, 0.5 %, 0.7% agar; 5 %, 10 % watermelon rind waste and 0.1 %, 0.3 %, 0.5 % agar; 10 %, 20 % melon flesh and 0.1 %, 0.3 % agar; 0.5 %, 1 % melon flesh with seed and 0.1 %, 0.3 %, 0.5 % agar) as a preliminary study. Watermelon and melon flesh and rind containing media (MS1, MS2, MS3 and MS4) was selected. Medium selection was taken into consideration medium case (solid, semi-solid and liquid), low sugar content and low agar content (Table 1). MS1 and MS2 mediums were not contained sucrose, MS3 and MS4 mediums lower sucrose content compare to MS0 control medium. For agar, lower concentrations were used mediums MS2, MS3 and MS4.

Cultures were placed in tissue culture growth room at 16 hour photoperiod and 25±1 °C temperature regime for 3 weeks. Ten *in vitro* explants (0.5-0.8 cm long with single leaf) having one nodes were placed into 10 petri dishes (25x100 mm) containing 15 ml of five different growing medium (Medium MS0: MS + 30 g/l sucrose + 7 g/l agar; Medium 1: MS + 50 % WJ (watermelon juice) +7 g/l agar; Medium 2: MS + 20 % MF (melon flesh) + 3 g/l agar; Medium 3: MS + 10 % WR (watermelon rind) + 10 g/l sucrose + 5 g/l agar and Medium 4: MS + 0.5 % MFS (melon flesh with seeds) + 20 g/l sucrose + 5 g/l agar). They were then developed in controlled environment in culture room with light intensity (cool-white fluorescent lamps, ca. 4000 lux). The pH was adjusted to 5.7 prior to autoclaving for 20 min. at 121 °C. Petri dishes were closed with polypropylene closures and sealed with parafilm to reduce medium desiccation.

Parameters and visual evaluation of the potato plants

Cultures (10 plants) were incubated for 30 days and following plant traits were measured plant height (cm), number of nodes per plant, internode length (cm) and fresh weight (g). The data thus obtained represent repeated non-destructive measurements and the experiments were repeated three times. Least Significant Differences were used to compare the means [21]. These evaluations should be recorded in 2 weeks intervals from day 30 after incubating in petri dishes. Stem, leaf, root and general development of potato plants scale from 1 – 9, highest (best development of plants) = 9, lowest development of plants = 1 (in Table 2.)

RESULTS AND DISCUSSION

The potato plants grown in each medium was compared after 15 days (Figure 1) and 30 days period by 1-9 scale values (Table 3). All the tested mediums showed variable response to different sugar sources and agar concentrations. In general, scale values for root development, leaf growth, plant growth and plant vigour in MS0 medium were higher than melon and watermelon extract contained media. In the plant extracted media best scale values were found in MS2 (MS + %20 MF + 3 g/l Agar medium). The lowest scale values were obtained from MS1 (MS + 50 % WJ

Table 1: Melon and watermelon extract mediums composition.

Medium No	Medium	Result
MS0	MS + 30 g/l Sucrose + 7 g/l Agar	Solid
MS1	MS + 50 % Watermelon Juice + 7g/l Agar	Solid
MS2	MS + 20 % Melon Flesh + 3 g/l Agar	Solid
MS3	MS + 10 % Watermelon Rind + 10 g/l Sucrose + 5 g/ Agar	Solid
MS4	MS + 0.5 % Melon Flesh with Seed + 20 g/l Sucrose + 5 g/l Agar	Solid

Table 2: 1-9 scale values.

Scale No	Scale
1	Lowest Development
3	Low Development
5	Medium Development
7	Good Development
9	Best Development

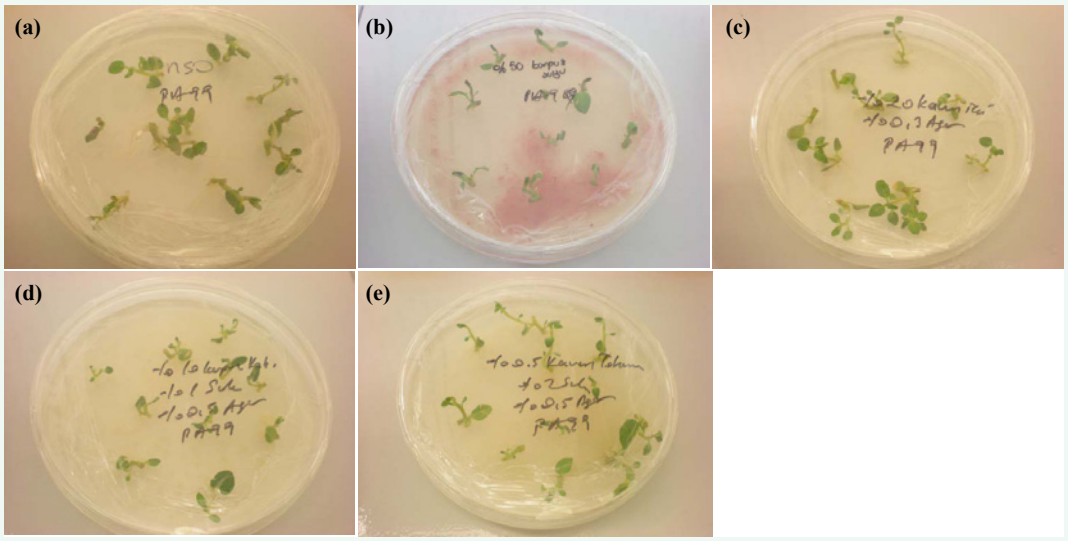

Figure 1 Potato plant growth after 15 days incubation period on media: a) MS0 b) MS1 c) MS2 d) MS3 e) MS4.

medium) for root development, leaf growth, plant growth and plant vigour. The overall results clearly indicated that especially in MS2 (MS 20 % MF), MS3 (10 % WR) and MS4 media, root development values screened were moderate value. In MS4 (MS + 0.5% MFS + 20 g/l Sucrose + 5 g/l Agar) medium, first 15 days scale values were low. After 15 days, leaf growth, plant growth and plant vigour scale values were increased clearly.

A number of low-cost alternatives can be used to simplify various operations and reduce the costs in a plant tissue culture facility. The physical components of a typical plant tissue culture facility include equipment and buildings with preparation room, transfer room, culture room, hardening and weaning area, soil-growing area, packaging and shipping area, and a store for chemicals, containers and supplies [22].

The composition of culture media used for proliferation has a tremendous influence on production costs. The replacement of expensive imported vessels with reusable glass jars and lids, alternatives to gelling agents, use of household sucrose, and some medium components can reduce costs of production. Bulk making of media and storage as deep frozen stocks also reduces labour costs [22]. Biologically active plant-derived components can be expected to play an increasingly significant role in commercial development on new products for regulating plant growth. Fruit juice extract, as one of plant-derived chemicals, is studied on its effects on plant growth in vitro in containing salt's MS media [23]. Melon sugar composition is: 7699 mg sucrose, 2726 mg glucose, 3310 mg fructose, 70.8mg maltose and 106mg galactose. In watermelon flesh the composition of sugar is 1863mg sucrose, 2433 mg glucose, 5174 mg fructose and 92.4 mg maltose. Melon flesh is higher and more different sugars composition than watermelon. In general, melon higher energy, carbohydrate, minerals and vitamins values were contained comparing to watermelon [24,25]. The comparative efficacy of gelling agents like starches from various sources as barley, corn, potato, rice and wheat; synthetic polymers and gel rite in comparison with agar on medium solidification for *in vitro* culture of plants have been widely studied but agar was found to be the best [26]. But Lalitha et al. (2014) [27] reported that using corn flour instead of agar as gelling agent is efficient for mulberry micropropagation from single node. The combination of low concentration of agar 0.35 % (w/v) with corn flour 2.2 % (w/v) could offer a good supporting surface for mulberry micropropagation. A significant cost reduction of 42.95 % is possible by replacing agar with corn flour and agar combination as experimented.

The present investigation was conducted to find out the effects of alternative sugar and agar sources on direct growth and development of potato. Data on seedling visible :(plant height, number of nodes per plant, internode length root development, leaf growth, plant growth, plant vigour) After 30 days, the discussions of the study have been presented below: All the tested media showed variable response to different sugar sources and agar concentrations. In general, 1-9 scale values for root development, leaf growth, plant growth and plant vigour, MS0 medium recorded higher values than those screened from melon and watermelon extract contained mediums. Sucrose containing MS media were well capable in performing optimum growth but in some cases glucose and maltose also performed well specially an increasing number of nodes per plant [28]. In the plant extracted media, best scale values were found in MS2 (MS + 20 % MF + 3 g/l Agar). MS4 (MS + 0.5 % MFS + 20 g/l Sucrose + 5 g/l Agar) medium after 15 days, leaf growth, plant growth and plant vigour scale values were increased clearly. Preece (2011) [29] reported that many plant species have reduced growth as agar levels increases; he concluded that eliminating agar or other gelling agent can improve micro-shoot proliferation and growth [30]. The selected plant derived alternative gelling and sugar agents are easily available in the market and can be added with ease, as inexpensive substitute of agar and sucrose [27]. For example, recommend the addition of lemon juice for MS media to an increase in growth, and cost less productivity for MS media in potato [23].

The potato plants grown in each medium was compared at the end of 30 days. All the tested media combinations showed variable response to melon and watermelon sucrose and gelling

Table 3: 1-9 Scale values for root development, leaf growth, plant growth, plant vigour in 15 and 30 days incubation in different media.

Medium No	Medium Content*	Root Development		Leaf Growth		Plant Growth		Plant Vigour	
		15D	30D	15D	30D	15D	30D	15D	30D
MS0	MS + 30 g/l Sucrose, 7 g/l Agar	7	9	9	9	9	9	7	9
MS1	MS + 50 % WJ + 7 g/l Agar	1	1	3	3	3	3	3	3
MS2	MS + 20 % MF + 3 g/l Agar	5	5	7	9	7	7	9	9
MS3	MS + 10 % WR +10 g/l Sucrose + 5 g/l Agar	5	5	7	7	5	7	7	7
MS4	MS + 0.5 % MFS + 20 g/l Sucrose + 5 g/l Agar	5	5	5	7	3	7	5	9

Note: 15D (15 days) and 30D (30days) incubation of potato plants.

Table 4: Means of various plant characteristics grown on different media.

Medium	Medium Composition	Number of nodes per plant	Internode length (cm)	Fresh weight (g)	Plant height (cm)
MS0	MS + 30 g/ Sucrose + 7 g/l Agar	15.0[a]	0.48[a]	0.78[a]	5.90[a]
MS1	MS+ 50% Watermelon Juice + 7g/l Agar	7.20[c]	0.28[b]	0.62[b]	2.44[c]
MS2	MS+ 20 % Melon Flesh + 3 g/l Agar	12.60[b]	0.46[a]	0.75[a]	6.90[a]
MS3	MS+ 10 % Watermelon Rind + 10 g/l Sucrose + 5 g/l Agar	9.20[c]	0.34[b]	0.56[b]	3.80[b]
MS4	MS + 0.5 % Melon Flesh with Seed + 20 g/l Sucrose + 5 g/l Agar	15.80[a]	0.20[b]	0.79[a]	5.02[a]
	Mean	11.96	0.35	0.70	4.81
	LSD (0.05):	2.23	0.11	0.10	1.08

Note: Within columns, means followed by the same letter are not significantly different by ANOVA protected LSD test ($p<0.05$).

Table 5: Correlation coefficients between various plant characters of *in vitro* raised potato plants.

	Internode length	Fresh weight	Plant height
Number of nodes per plant	0.165	0.739**	0.730**
Internode length		0.306	0.468*
Fresh weight			0.694**

sources. Table 4 shows that characters analyzed for growth of plants under *in vitro* conditions had statistically significant differences ($p<0.05$) among medium with respect to all plant characters. In general, the highest values were obtained from MS4 for number of nodes per plant and fresh weight (except internode length and plant height). The average values of number of nodes per plant, internode length, fresh weight and plant height in different media were as follows respectively: 11.96, 0.35 cm, 0.70 g and 4.81 cm. Plant height changed over 4 weeks-time courses revealed that significant differences existed among the different media. The highest plant height was obtained in MS2 medium which ranged between 2.44 cm to 6.90 cm. Plants grown on medium supplemented with 0.5% melon flesh (MS4), MS0 and MS2 had significantly higher fresh weights (0.79, 0.78, 0.75 g/ plant, respectively) compared to MS1 and MS3 (0.62 and 0.56 g/ plant). Number of nodes per plant is very important characteristic for *in vitro* potato propagation. The average number of nodes was found between 15.80 and 7.20 for melon and watermelon extract media respectively.

In general, the highest values were obtained from media supplemented with 0.5 % melon flesh (MS4) for important plant characteristics number of nodes per plant and fresh weight. The average number of nodes per plant was found approximately 12 in all over the mediums. Mediums responded to different sugar types were varied as far as plant height and number of nodes per plant is concerned. Potato plant (average 12 nodes per plant) can be utilized after four weeks in culture for minitubers production in glass house owing to high survival rates after transplanting.

Simple correlation coefficients between potato plant components are presented in Table 5. Highest correlations were found between node number per plant and fresh weight (r: 0.739**) and plant height (r: 0.730**). Significantly high correlations were also observed among internode length with plant weight and fresh weight with plant height.

Sucrose is a prime carbon source for potato micropropagation and influences the development of vigorous plants. Kubota et al. (2001) [31] reported that supply of sugar to the culture medium promoted the plant growth *in vitro* and compensate for the low or negative net photosynthetic rate and thus increasing the survival rates of tissue sections cultured *in vitro*. Therefore, potato plants require an initial source of carbon and hence energy from the medium until they are capable of using CO_2 as their main carbon source for efficient metabolism. Rahman et al. (2010) [28] reported that cultivar (Shilbilaty, Shepody, Atlanta, All Blue and Diamant) responded to sugar types were varied as far as plant height and plant weight is concerned. They were

also noted that media with 3 % fructose had deleterious effect to *in vitro* plant growth. They also suggested that maltose in the micropropagation media remained largely intact *i.e*. not hydrolysed (sucrose immediately hydrolysed). Potato plants grown on media 20 % melon flesh + 3 g/l agar and 0.5 % melon flesh with seed + 2 % sucrose + 5 g/l agar as a carbon sources had equal potato plant growth characters compared to using medium with 3 % sucrose. Because melon flesh was containing higher sucrose, glucose and fructose and lower maltose values comparing to watermelon flesh. Due to this reason, the melon containing media was better for potato plant growth compare to watermelon containing media. In low cost media, tapioca was used as substitute of agar and replacing sucrose with sugar cane, because of low cost and easy availability [32]. Calcium ammonium nitrate, single super phosphate, potash and sugar cane were used as low cost media in place of MS salts [22].

CONCLUSIONS

Sucrose is of prime importance for cell growth; however significant cost incurred by importing analytical sucrose presents economic obstacle in full exploitation of tissue culture for certified potato seed production. Plant tissue culture technology offers an alternative for enhanced rates of multiplication. The technology is, however costly resulting in low adoption rates in developing countries. Low cost options should lower the cost of production without compromising the quality of the micropropagules and plants. In low cost technology cost reduction is achieved by improving process efficiency and better utilization of resources [22]. The design of cost efficient tissue culture protocols is a prerequisite in the adoption of the low cost tissue culture technology in developing countries [27]. The cost of tissue culture can be brought down by 34 to 51 % utilizing locally available table sugar without compromising the quality of tissue cultured plants [16]. Like Demo et al. (2008) [16] results, this study also suggest that using very cheap melon and watermelon fruit extracts instead of sugar as carbon sources are efficient for potato micropropagation by single node. These fruit extracts in addition to sugars, they are sources of vitamins and inorganic ions required potato growth. The results of the present study offer new possibilities of using low cost raw materials as sugar alternatives which will reduce materials costs considerably and will help popularizing potato tissue culture. The combination of low concentration of agar (3 and 5 g/l respectively) and melon extract containing in the solid medium could offer a good supporting surface for potato micropropagation and could be used for other economically important species, when high levels of agar are suspected to have inhibitory effects.

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