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Effects of Using Gelling Agent Guar Gum and Different Sugar Sources on Potato Micropropagation

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Abstract

This manuscript is concerned with reducing the production cost of potato in micropropagation through increasing the propagation rate and/or substituting expensive chemicals for cheaper widely available ones. Replacing the most widespread gelling agent, agar, was a priority due to cost as well as using white and brown sugars in place of analytical grade sucrose. The aim of the research was to determine the effects of different guar gum concentrations on *in vitro* propagated plants of potato in first time. Plant traits such as plant height, number of nodes per plant, internode length and fresh weight were measured and evaluated after 30 days incubation period. In general, the optimum values were obtained from MS1 (MS + 30 gl^{-1} sucrose + 18 gl^{-1} guar gum), MS5 (MS + 40 gl^{-1} white sugar+ 20 gl^{-1} guar gum), MS8 (MS + 40 gl^{-1} white sugar+ 10 gl^{-1} corn flour + 15 gl^{-1} guar gum), MS10 (MS + 30 gl^{-1} brown sugar + 20 gl^{-1} guar gum) and MS11 (MS + 30 gl^{-1} brown sugar + 10 gl^{-1} rice flour + 18 gl^{-1} guar gum) for all plant traits evaluated. As a result of the study, a medium containing 15-20 gl^{-1}concentration of guar gum and white (40 gl^{-1}) and/or brown sugar (30 gl^{-1}) would be suitable for micropropagation.

Key words: Guar gum, micropropagation, sugar, *Solanum tuberosum* L.

Patateste Microçoğaltımda Katılaştırma Ajanı Guar Gam Ve Farklı Şeker Kaynaklarının Etkisi

Özet

Araştırma, patateste mikroçoğaltmada üretim maliyetini azaltmak için; üretim oranını artırmak ve üretimde kullanılan pahalı kimyasalların yerine geniş ölçekli kullanılabilecek daha ucuz alternatif ürünleri belirlemek amacıyla yapılmıştır. En yaygın katılaştırıcı olarak kullanılan agarın yerine kullanılabilecek ürünler ve analitik sukroz yerine beyaz ve esmer şekerin kullanılabilme durumu değerlendirilmiştir. Araştırmanın amacı ilk defa patateste in vitro çoğaltmada farklı guar gum konsantrasyonlarının etkilerini belirlemektir. 30 günlük kültür süresi sonunda bitki boyu, bitki başına boğum sayısı, boğum arası uzunluğu ve bitki yaş ağırlığı gibi bitki özellikleri ölçülmüş ve değerlendirilmiştir. Genel olarak tüm bitki karakterlerinde optimum değerler MS1 (MS + 30 gl^{-1} sukroz + 18 gl^{-1} guar gum), MS5 (MS + 40 gl^{-1} beyaz şeker + 20 gl^{-1} guar gum), MS8 (MS + 40 gl^{-1} beyaz şeker + 10 gl^{-1} mısır unu + 15 gl^{-1} guar gum), MS10 (MS + 30 gl^{-1} kahverengi şeker + 20 gl^{-1} guar gum) ve MS11 (MS + 30 gl^{-1} kahverengi şeker + 10 gl^{-1} pirinç unu) ortamlarında saptanmıştır. Araştırma sonucuna göre 15-20 gl^{-1} konsantrasyonunda guar gum ve beyaz (40 gl^{-1}) veya kahverengi (30 gl^{-1}) şeker içeren katı besin ortamı kombinasyonları patateste mikroçoğaltımda iyi bir destekleyici ortam olarak önerilebilir.

Anahtar kelimeler: Guar gum, mikroçoğaltma, *Solanum tuberosum* L. şeker

Introduction

The main advantage of potato micropropagation is the fast production of high quality and uniform plants (Mohamed et al., 2009). The design of cost efficient tissue culture protocols is a prerequisite in the adoption of the low cost tissue culture technology in developing countries (Lalitha et al., 2014). Agar, the traditional gelling

agent, has a number of drawbacks that negatively affect culture growth and differentiation in many cases (Babbar et al., 2005; Ozel et al., 2008; Fakhreldin et al., 2014). Conventionally, agar is used, which is a polysaccharide extracted from seaweeds. The main differences among various agar-products are level and composition of the impurities, which can change according to producer. Cheaper agar alternatives include various types of starch and gums which have been researched in commercial propagation (Nagamori et al., 2001). Other options include flour, laundry starch, semolina, potato starch, rice powder, and sago (Prakash et al., 2003). 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% (Mohamed et al., 2009). Starch is the cheapest alternative among the studied gelling agents, and its use may reduce the costs of plant tissue culture. Nevertheless, starch is hydrolysed by plant amylolytic enzymes during the *in vitro* tissue culture (Lima et al., 2012).

Sucrose is a prime component of media for potato micropropagation (Khuri and Moorby, 1995). Media chemicals, account for less than 15% while the carbon sources such as grade sucrose contribute about 34% of the production cost. In potato, micropropagation using commercial grade sucrose and agar makes up approximately 80 % of the total medium cost (Naik and Sarkar, 2001). Identification of cheap or low-cost alternative gelling and carbon resource will greatly reduce the cost of production (90%) especially in a large-scale commercial potato micropropagation.

Guar gum is derived from *Cyamopsis tetragonoloba*, an annual herb, cultivated widely in India and Pakistan for its seeds and fodder (Babbar et al., 2005). Guar gum has a water-soluble (85%) non-toxic polysaccharide called guaran, composed of galactomannans (Windholz et al., 1983; Babbar et al., 2005). Guar gum is one of the best thickening additives, emulsifying additives and stabilizing additives. In the food industry, guar gum is used as gelling, thickening, viscosifying, clouding, and binding agent as well as for stabilization, preservation, emulsification, water retention and enhancement of water soluble fiber content. Therefore, in the present study, effects of using of guar gum as a gelling agent and sugar source agents on micropropagation and maintenance of potato plants *in vitro* were investigated.

Materials and Methods

The experiment were carried out during 4 Month (January to April 2013) in the plant tissue culture laboratory of Yuksel Seed, Antalya/Turkey. *In vitro* plants of *Solanum tuberosum* L. candidate cultivar PA99 (mid late) were multiplied routinely by sub-culturing single node cuttings every 3 weeks. Single node cuttings were propagated in Murashige and Skoog (1962)' MS basal medium with 30 gl^{-1} sucrose and 7 gl^{-1} agar (Sigma type A, A1296-1KG) in petri dishes (25x100 mm). Cultures were placed in a tissue culture growth room at 16 hour photoperiod and 25±1 °C (day and night) temperature regime for 3 weeks. Ten *in vitro* explants (0.5-0.8 cm long with single leaf) each having one node were placed into 10 petri dishes (25x100 mm) containing 15 ml of different growing medium (MS0: MS + 30 gl^{-1} sucrose + 7 gl^{-1} agar and other guar gum, white and brown sugar containing media) and were grown in a controlled environment room with light intensity (cool-white flourescent lamps, ca. 4000 lux). Totally 10 petri dishes and 100 plants were used each application. First part of the experiment, low guar gum concentrations (6 to 15 gl^{-1}) and different sugar sources were used. Second part of the experiment, 11 different media were used. The pH was adjusted to 5.7 prior to autoclaving for 20 min at 121°C. Cultures (10 plants) were incubated for 30 days and the following plant traits were measured: plant height (cm), number of nodes per plant, internode length (cm) and fresh weight (g). The data obtained represent repeated non-destructive measurements and the experiments were laid out in a completely randomised block design with five replications. Each replication 2 petri dishes and 20 plants was evaluated. Least Significant Differences were used to compare the means after Anova tests (Freed et al., 1989).

Table 1. Low level guar gum and sugar media

Medium No	Medium Content*	
1	MS0 (MS + 30 gl^{-1} Sucrose + 7 gl^{-1} Agar)	MSO
2	MS +30 gl^{-1} Sucrose+ 12 gl^{-1} Guar Gum	1.2%GG
3	MS +30 gl^{-1} Sucrose + 15 gl^{-1} Guar Gum	1.5%GG
4	MS + 30 gl^{-1} White sugar + 7 gl^{-1} Guar Gum + 3 gl^{-1} Agar	3%WS+0.7%GG+0.3%A
5	MS + 30 gl^{-1} White sugar + 6 gl^{-1} Guar Gum + 3 gl^{-1} Agar	3%WS+0.6%GG+0.3%A
6	MS + 30 gl^{-1} Brown sugar + 9 gl^{-1} Guar Gum + 3 gl^{-1} Agar	3%BS+0.9%GG+0.3%A

Results and Discussion

In this study we tested MS0 and different media combinations containing white and brown sugar, rice flour, potato starch, corn starch, low agar and guar gum as a preliminary study. Low cost effective five media was selected (Table 1). Medium selection was taken into consideration (solid, semi-solid, liquid), different sugar sources, low agar and alternatively guar gum content.

In the first part of research, plant height and number of nodes per plant varied in response to different sugar types and solidifiers. In the media such as 15 gl^{-1} guar gum medium, 30 gl^{-1} white sugar + 7 gl^{-1} guar gum + 3 gl^{-1} agar and 30 gl^{-1} brown sugar + 9 gl^{-1} guar gum + 3 gl^{-1} agar higher plant height and number of nodes values were obtained compare to the MS0 medium. In general, plant height and number of nodes per plant values were higher in 12 and 15 gl^{-1} guar gum media compare to other ½ agar containing media (Figure 1, 2). But these guar gum media are semi-solid. In semi-solid media, its highly viscous nature even at high temperatures, makes dispensing of the medium to petri dishes difficult. For this reason, potato plants were grown in medium containing between 1.5-2.5 % (w/v) guar gum containing solid media in the second part of the research.

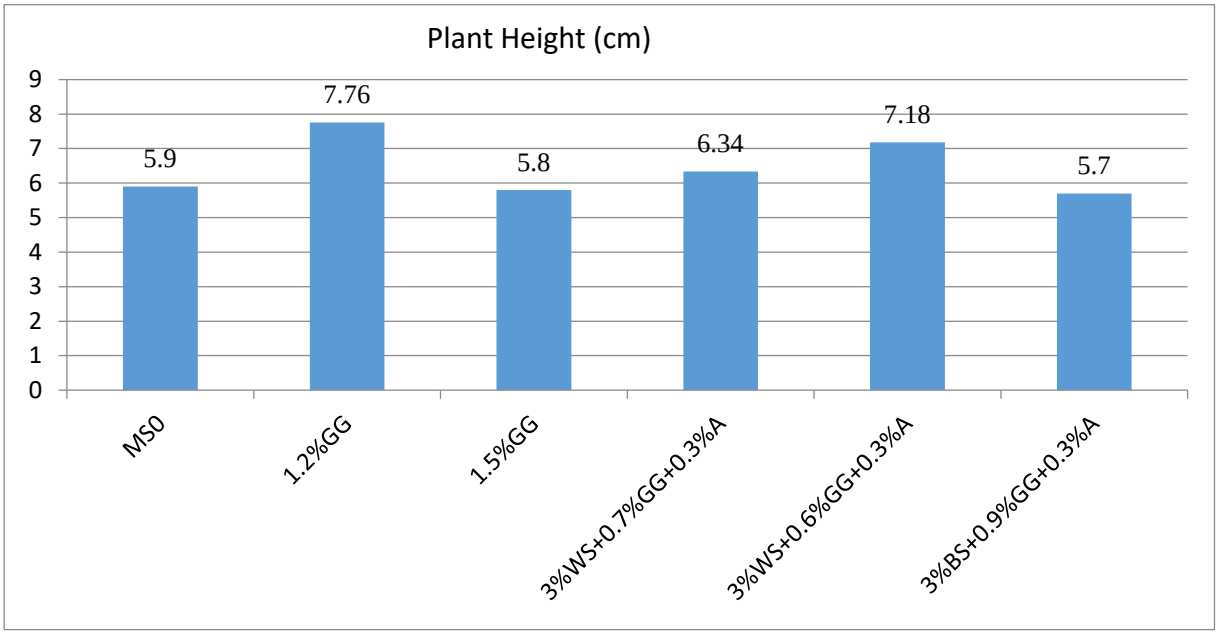

Figure 1. The effects of different media on plant height. LSD (0.05): 1.21.

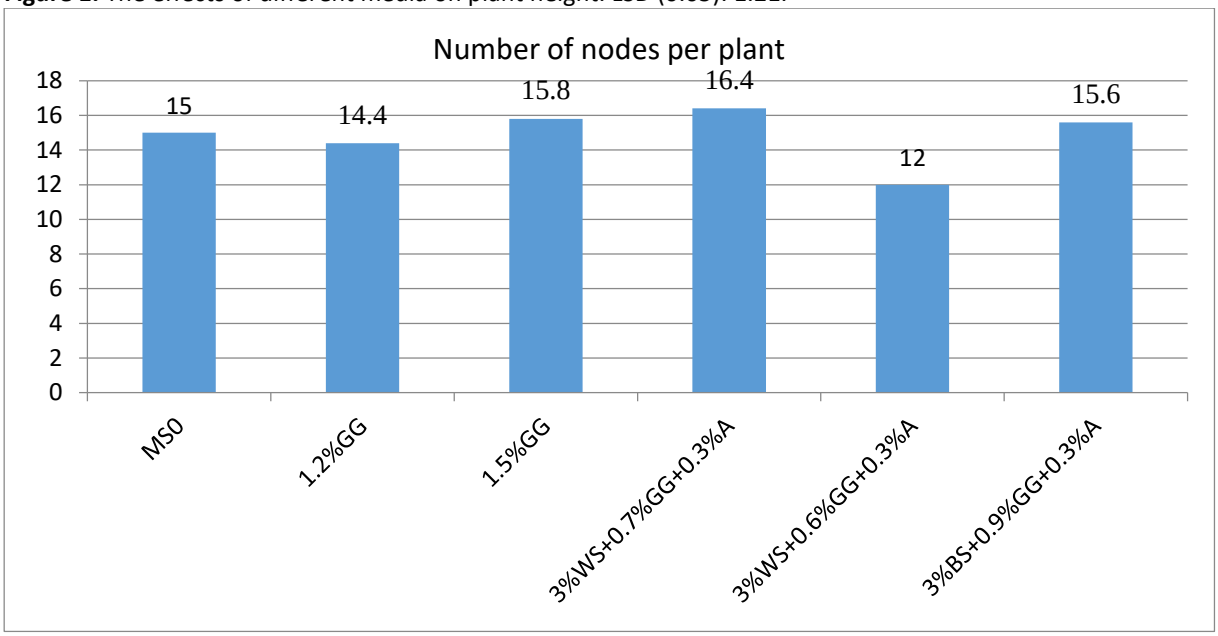

Figure 2. The effects of different media on number of nodes per plant. LSD (0.05): 1.33

Table 2. Means of various plant characteristics grown on different media

Medium	Medium Content*	Number of nodes per plant	Internode length (cm)	Fresh weight (g)	Plant height (cm)
MS0	MS + 30 gl^{-1} sucrose + 7 gl^{-1} agar	8.2e	0.42fg	0.71g	3.6h
MS1	MS + 30 gl^{-1} sucrose+ 18 gl^{-1} guar gum	14.8bc	0.58bcd	1.68d	5.5f
MS2	MS + 30 gl^{-1} sucrose + 20 gl^{-1} guar gum	11.2de	0.6bc	1.12f	6.62e
MS3	MS 30 gl^{-1} sucrose + 25 gl^{-1} guar gum	22.8a	0.34g	0.91g	4.8g
MS4	MS + 40 gl^{-1} white sugar + 18 gl^{-1} guar gum	12.8bcd	0.36g	1.65d	3.84h
MS5	MS + 40 gl^{-1} white sugar + 20 gl^{-1} guar gum	15.4b	0.62bc	2.9ab	7.48cd
MS6	MS + 40 gl^{-1}white sugar + 7 gl^{-1} corn starch + 18 gl^{-1} guar gum	13.4bcd	0.48ef	1.43e	5.42f
MS7	MS + 40 gl^{-1} white sugar+ 10 gl^{-1} rice flour + 12 gl^{-1} guar gum	12.4bcd	0.66b	2.74b	7.96c
MS8	MS + 40 gl^{-1} white sugar + 10 gl^{-1} corn flour + 15 gl^{-1} guar gum	14.7bc	0.8a	2.04c	9.2b
MS9	MS + 30 gl^{-1} brown sugar + 18 gl^{-1} guar gum	12cd	0.5def	0.9g	4.5g
MS10	MS + 30 gl^{-1} brown sugar + 20 gl^{-1} guar gum	14.2bcd	0.6bc	2.12c	9.98a
MS11	MS + 30 gl^{-1} brown sugar + 10 gl^{-1} rice flour + 18 gl^{-1} guar gum	14.2bcd	0.54cde	3.01a	6.96de
	Mean	13.84	0.54	1.77	6.32
	LSD (0.01):	3.28	0.09	0.21	0.53

*: Within columns, means followed by the same letter are not significantly different by ANOVA protected LSD test (P<0.01)

Second part of the research, 11 different media were investigated. Table 2 shows that all characters analysed growth *in vitro* conditions were statistically significantly different (P<0.01) among medium. In general, the highest values were obtained from MS3 for the number of nodes per plant, MS8 for internode length, MS11 for fresh weight and MS10 for plant height. Plant height over 4 weeks-time courses revealed significant differences among the media. The highest plant height was obtained in MS10 medium which ranged between 3.60 cm to 9.98 cm. Plants grown on media supplemented with 30 gl^{-1} brown sugar + 10 gl^{-1} ice flour + 18 gl^{-1} guar gum (M11), MS5, MS7, MS10 and MS8 had significantly higher fresh weights (3.01, 2.9, 2.74, 2.12 and 2.04 g $plant^{-1}$, respectively) compared to the MS0 (0.71 g $plant^{-1}$). Number of nodes per plant is a very crucial characteristic for *in vitro* potato micropropagation (Gopal et al., 2002; Özkaynak, 2015). The lowest and highest number of nodes per plant was determined between 8.2 and 22.8 for sugar and guar gum media. In the media MS1, MS5, MS8, MS10 and MS11 14 or higher number of nodes per plant were produced. Plant height and number of nodes per plant responded to different sugar types. Sugar is a prime carbon source of potato micropropagation and influence of developing vigour plants. Kubota et al., (2001) reported that supply of sugar to the culture medium promote plant development and compensate for the low net photosynthetic rate as a result of poor photosynthetic ability thus increasing the survival rates of tissue sections cultured in vitro. Thus, 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. In general, the optimum values were obtained from MS1, MS5, MS8, MS10 and MS11 for all plant characteristics.

In the last 15 years, a number of substances have been tried as alternative gelling agents with limited success. The *in vitro* cultivation of potato plants is generally carried out in a solid or semi-solid nutrient medium, using gelling agents. These are given in Table 3 along with their cost. The alternative gelling agents tested include starch, flour, different types of gums, alginates and agarose. These gelling agents are not expected to get universal acceptance due to various factors: starch metabolizes too readily, alginates gel only in the presence of specific ions, agarose is cost prohibitive (Jain and Babbar, 2002). Agar has been extensively used since it has suitable gelling characteristics and stability during potato micropropagation. In all mediums used for *in vitro* culture of plants, agar is the one of them source of unknown variations, besides it's a high costs (Lima et al., 2012).

Table 3. Comparative cost of different gelling agents and sugars used for potato tissue culture medium*

Gelling Agent	Cost (US$)	Concentration used (g/l)	Cost per liter (US$)
Agar (Sigma A4550)	683.30 per 1kg	7 gl^{-1}	4.78
Guar Gum	7.50 per 1kg	20 gl^{-1}	0.15
Rice Flour	4 per 1 kg	10 gl^{-1}	0.04
Corn Flour	4 per 1 kg	10 gl^{-1}	0.04
Sucrose (Sigma S5391)	62.04 per 1 kg	30 gl^{-1}	1.86
White sugar	2 per 1 kg	40 gl^{-1}	0.08
Brown sugar	5 per 1 kg	30 gl^{-1}	0.15

*: Guar gum (http://magaza.hammaddeler.com/Guar-Gam-1-kg,PR-7726.html)
Sucrose (http://www.sigmaaldrich.com/catalog/product/sigma/s5391?lang=en&region=TR)
Agar (http://www.sigmaaldrich.com/catalog/product/sigma/a4550?lang=en&region=TR)

Guar gum, is generally produced as a free-flowing, off-white powder that hydrates easily to generate solutions possessing high viscosity with pH ranging between 5.5 and 6. Guar gum, is a polysaccharide composed of sugars galactose and mannose, derived from the endosperms of *Cyamopsis tetragonoloba*, has been effectively used as a sole gelling agent for plant tissue culture medium (Babbar et al., 2005). The comparative efficacy of gelling agents like starches from various sources as barley, corn, potato, rice and wheat; synthetic polymers and gelrite 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 (Shah et al., 2003). But Lalitha et al., (2014) 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 paper describes the successful use of guar gum as a gelling agent for tissue culture medium used for *in vitro* potato micropropagation for which solid media are commonly used firstly. The advantages and drawbacks of guar gum as a gelling agent are discussed. Guar gum has been reported to be an inexpensive substitute of agar for microbial culture media (Gangotri et al., 2012). Guar gum, was used as gelling agents for *in vitro* propagation and regeneration as well for germination of Flax and *Brassica juncea*, for the multiplication of aerial parts of *Crateavea nurvala*, and their subsequent rooting, for the *in vitro* androgenesis of anthers on Tobacco, and for somatic embryogenesis of callus cultures on *Calliandra twedii*. The medium were gelled with 20, 30 or 40 gl^{-1} guar gum, as compared to agar (9 gl^{-1}), and for all species the morphogenetic responses were advanced in the medium gelled with guar gum (Babbar et al., 2005).

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 (George and Manuel, 2013). The design of cost efficient tissue culture protocols is a prerequisite in the adoption of the low cost tissue culture technology in developing countries (Lalitha et al., 2014). 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 (Demo et al., 2008). As introduced through the present study, being approximately 30 (cost per liter)–90 (total 1 kg cost) times cheaper than agar, guar gum would definitely be useful, particularly in the potato plant tissue culture commercial industry. Like guar gum, white sugar and brown sugar cost 23 and 12 (cost per liter)–31 and 12 (total 1 kg cost) times cheaper than sucrose. Guar gum source, *Cyamopsis tetragonoloba*, is an easily cultivated guar bean and, therefore, increased demands can be met without any fear of exploitation of the natural resource (Babbar et al., 2005). Guar gum, being a product of crop origin, is biodegradable and poses no threat to the environment on being disposed of after use.

Conclusions

The results of the present study offer new possibilities of using low cost materials as sugar and agar alternatives which will reduce materials costs considerably and will help popularizing potato micropropagation. The combination of 15-20 gl^{-1} concentration of guar gum and white and brown sugar containing 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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