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The Effect of Improvised Media and Gelling Agents on *In Vitro* Germination of Cotton (*Gossypium hirsutum.* L.)

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Abstract

The significance of cotton can hardly be over emphasized in Turkish economy as it contributes momentously to agricultural value added products. The success of propagating cotton in *in vitro* conditions or to modify it genetically largely depends on an efficient, reproducible and cost effective protocol for *in vitro* germination of seeds, seedlings development and different explant sources. The present study was conducted to investigate the effect of different media (various sucrose concentrations) and gelling agents (Agar and Phytagel) on *in vitro* germination and seedling growth of different cotton cultivars. Results showed that MS medium devoid of sucrose contents induced maximum germination rates. The decreased sucrose concentration resulted in increased germination rates. Furthermore, experiments with different concentrations of gelling agents exhibited that MS media supplemented with 0.4 (w/v) % Phytagel results in induction of maximum *in vitro* germination rates; leading to robust seedling development suitable for further *in vitro* propagation experiments. According to our knowledge, this is the first report of increased germination rates of cotton cultivars incubated on MS media without the presence of sucrose; hence MS medium devoid of sucrose and solidified with 0.4% of Phytagel is most suitable for *in vitro* seed germination of cotton cultivars to obtained healthy seedlings in a shorter period.

Keywords: Decreased sucrose; Enhanced; Germination; Gelling agent; Economical

Farklı Besin Ortamlarının ve Katılaştırıcıların Pamuk Tohumunun *In Vitro* Koşullarda Çimlenme ve Fide Gelişimine Etkisi

Öz

Pamuğun Türk ekonomisindeki yeri oldukça önemlidir. Pamuğun *in vitro* koşullardaki çoğaltım başarısı veya genetiğinin değiştirilmesi tohumların, bitkiciklerin ve farklı eksplantların ucuz, etkili ve tekrarlanabilir bir şekilde in vitro koşullara aktarılabilmesi için geliştirilecek protokollere bağlıdır. Bu çalışmayla farklı büyüme ortamlarının (değişen sukroz konsantrasyonu) ve çeşitli katılaştırıcıların (agar ve fitojel) yedi farklı pamuk çeşidinde *in vitro* çimlenme ve bitki gelişimi üzerindeki etkisine bakılmıştır. Sukroz içermeyen MS ortamındaki çimlenme oranının en yüksek olduğu gözlenmiştir. Ayrıca sukroz konsantrasyonundaki azalma ile çimlenme yüzdesindeki artış arasında doğru bir orantı vardır. Dahası, farklı konsantrasyonlarda katılaştırıcı içeren ortamlarla yapılan *in vitro* çimlenme deneylerinde en yüksek oran %0,4 fitojel içeren MS ortamında gerçekleşmiştir. Ayrıca bu oranın güçlü *in vitro* bitki gelişimini de sağladığı *in vitro* çoğaltım deneylerinde gözlenmiştir. Bu çalışmayla ilk defa sukroz içermeyen MS ortamının farklı pamuk çeşitlerindeki çimlenmeyi arttırdığı gösterilmiştir. Böylece, kısa sürede sağlıklı pamuk bitkileri elde edebilmek için en uygun ortamın % 0,4 (w/v) fitojel içeren sukrozsun MS ortamı olduğu söylenebilir.

Anahtar kelimeler: Azaltılmış sukroz; Geliştirilmiş; Çimlenme; Katılaştırıcı; Ekonomik

Introduction

Agriculture serves as a jugular vein in the economy of Turkey and a good chunk of the population in the country depends directly and in directly on agriculture. The importance of cotton can hardly be over emphasized in the economy of Turkey as Turkey is one of 10 largest cotton producers in the world. It is an important crop that is grown as a source of fiber, food and feed. The most important product lint provides a source of high quality fibre for the textile industry. The cotton seed is an important source of oil and a high protein product used as livestock feed (Bakhsh et al., 2009). Because of its high economic importance, considerable attention has been paid for improving cotton plants by conventional plant breeding methods (Agrawal et al., 1997).

The plant tissue culture and genetic modification technologies have enabled the researchers to produce biotic and abiotic resistant crops. The different insect and herbicide resistant traits in cotton have been incorporated since the commercialization of first GM crop in 1996 (Rahman et al., 2012). For *in vitro* culture and as well as genetic modifications, the seed, embryonic axes, hypocotyl and cotyledon explants have been used in numerous studies (Gould et al., 1998; Bakhsh et al., 2012). However, establishing aseptic conditions as a result of cotton surface sterilization and less germination rates during *in vitro* propagation experiments have been of concern for the researchers.

The *in vitro* propagation of cotton is difficult and challenging process (Gupta et. al., 1997). The improvement of an effective *in vitro* cotton regeneration protocol is crucial for gene transformation and other genetic studies (Firoozabady and Deboer, 1993). A healthy *in vitro* germination without contamination is very important for plant regeneration (Huda et. al., 2009). Genotype (Seabrook and Douglass 2001), explant type (Lu et al., 1984), growth regulators (Trolinder and Goudin, 1988; Sun et al., 2006), sugar (Swankar, 1986), and medium (Popelka and Altpeter, 2001) is very important for plant regeneration especially for plants like cotton. Therefore, the present study was conducted to investigate the effect of different manipulated media and gelling agents on *in vitro* propagation of cotton cultivars.

Materials and Methods

The present research work was conducted at Tarbiotek laboratories; Department of Field Crops, Faculty of Agriculture, University of Ankara. The seeds of cotton cultivars used in this study were freshly collected from open field in Cotton growing area (Kahramanmaraş) city of Turkey and sent to our laboratory. Approximately 100 sinker seeds of each cultivar were placed in a beaker. The seeds were delinted with commercial H_2SO_4 at a rate of 100 ml/kg of seed. While delinting, seeds were stirred slowly with spatula for 60 seconds. After removing the fuzz, seeds were again washed with tap water four times to remove the remaining acid from the surface of seeds and further subjected to surface sterilization. Here on, all steps were performed in laminar flow cabinet to maintain aseptic conditions. Surface sterilization of seeds was performed in small bottles (500 ml) and 16 seeds of each replication were cultured in magenta box (Duchefa, Cat No. 1682). For surface sterilization of cotton seeds, we have already a protocol using H_2O_2 in combination with n-Hexane (Bakhsh et al., 2016). The cultures of

disinfected seeds of cultivars were placed in growth chamber at 26° C and 45 µMol photons m^{-2} s^{-1} light intensity in three replications.

In an initial experiment, the effect of different sucrose concentration (0%, 1%, 2% and 3% (w/v)) was observed on seed germination of Coker-312. On achieving interesting results, the same experiment was applied to seven locally cultivated cotton cultivars i.e. STN-468, BA-119, GSN-12, Özbek-100, Ayhan-107, Furkan-1 and SG-125. The experiment was designed with three repeats and each magenta had 16 seeds. The seeds were incubated for germination on MS medium (Murashige and Skoog, 1962) at 24°C with 16/8 h photoperiod for a week. A cultivar with highest germination rate was selected further for second round of experiments with different concentration of gelling agents i.e. Phytagel and Agar (as provided in Table 1). The comparison of concentrations of gelling agent was based on available literature. Every magenta box had 16 seeds for this experiment as well and was incubated for germination at same photoperiodic conditions for a week.

Table 1. Different media with different gelling agents
Çizelge 1. Farklı katılaştırıcılar içeren farklı besi ortamları

Medium Number *Ortam numarası*	**Medium contents** *Ortam içeriği*
1	0.6% agar + MS salt with vitamins
2	0.8% agar + MS salt with vitamins
3	0.6% Phytagel + MS salt with vitamins
4	0.4% Phytagel + MS salt with vitamins

All chemicals used in this study were purchased from Duchefa Biochemie B.V. (Haarlem, the Netherlands) and Sigma-Aldrich Co. (St. Louis, MO, USA). The pH was adjusted between 5.6-5.8 prior to autoclave. Media were autoclaved at 104 kPa atmospheric pressure and 120 °C for 20 min. All experiments were replicated thrice and data was recorded accordingly.

Results and Discussion

As we have already optimized an efficient protocol for cotton seed surface sterilization (Bakhsh et al., 2016), all seed cultures were free of microbial contamination. Our results of seed germination of Coker-312 with different concentration of sucrose (0%, 1%, 2% and 3% sucrose) were promising in achieving maximum germination rates (85%) without any sucrose contents in MS medium (Data not shown here). The same experiment was applied to seven locally cultivated cotton cultivars i.e. STN-468, BA-119, GSN-12, Özbek-100, Ayhan-107, Furkan-1 and SG-125. The germination rates of cultivars with sucrose contents (1%, 2% and 3% sucrose) and without sucrose (0%) were observed as 72.7%, 69.4% and 60.6% and 80.5% respectively (Figure 1). The very less or zero sucrose concentration resulted in maximum germination. Sucrose is widely used at concentration of 3% as a standard in *in vitro* seed germination and propagation of different crops (Day et al. 2016), however there are studies by Han et al. (2009), Özyiğit and Gözükırmızı (2008) and Mushke et al. (2012) who have reported the use of 2% and 1.5% (w/v) of sucrose concentration respectively in their MS media for successful

growth. We found that MS medium devoid of any sucrose contents resulted in maximum *in vitro* germination of cotton cultivars (Figure 1).

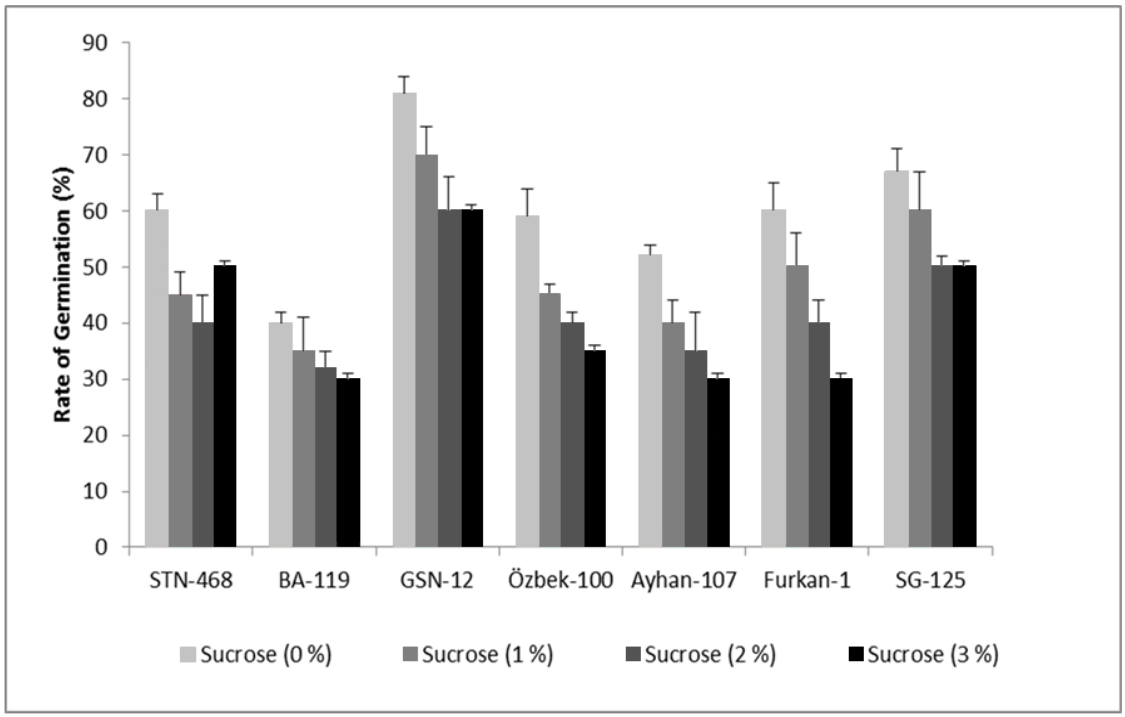

Figure 1. *In vitro* germination rates (%) of different cotton cultivars in MS0 medium without sucrose. GSN-12 cultivar showed maximum germination percentage

Şekil 1. Farklı pamuk çeşitlerinin sükroz içermeyen MS0 besi ortamlarındaki in vitro çimlenme oranları (%). GSN-12 çeşidi en yüksek çimlenme oranına sahiptir

A perusal of the graph will show that GSN-12 showed more than 80% germination on MSO medium devoid of sucrose. Others cotton cultivars also exhibited more germination percentage when compared media containing to 1, 2 or 3% (w/v) of sucrose contents. However, the germination percentage varied among cotton cultivar; GSN-12 being the notable one. Doman et al., (1982) described that composition of cotton seeds constituting of oil, protein, starch, oligosaccharide and sugars. Good germination in absence of sucrose can be attributed to the presence of sugar in cotton seed.

The experiments were also conducted to compare the various concentrations of gelling agents based on reports available in scientific literature. Plant agar (0.6 and 0.8 %) and Phytagel (0.6 and 0.4%) were used as gelling agents for this purpose. MSO media was devoid of any sucrose contents. Based on our earlier data, GSN-12 cultivar was selected. The results showed that MS medium solidified with 0.4 % (w/v) of Phytagel induces 96 % germination of GSN-12 cultivar (Figure 3). Phytagel and agar are mostly used gelling agents for invitro propagation experiments of cotton (Bakhsh et al., 2012; Khan et al., 2011). The less germination (25-30%) of cotton was

reported by Agrawal et al. (1997) and Afolabi-Balogun et al. (2011) on MS0 medium containing 3% (w/v) sucrose and 0.8% (w/v) agar. They also reported the delayed elongation of seedling. In our experiments, the robust seedling growth was recorded in a period of one week (Figure 2 &3)

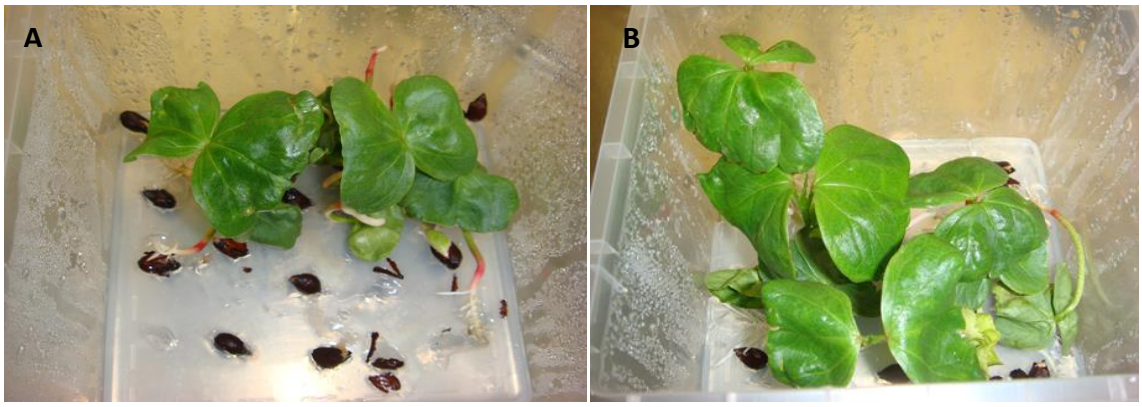

Figure 2. In vitro germination of GSN-12 cultivar on MS medium containing 0.6% (w/v) (A) and 0.8 % (w/v) agar (B). The more germination rate was recorded on MS containing 0.8% (w/v) Agar

Şekil 2. GSN-12 çeşidinin %0.6 (A) ve %0.8 (w/v) (B) agar içeren MS ortamında in vitro çimlenmesi. %0.8 agar içeren MS ortamında en yüksek çimlenmeyi göstermiştir

Figure 3. *In vitro* germination of GSN-12 cultivar on MS medium containing 0.6% (w/v) (A) and 0.4 % (w/v) plant agar (B). The more germination rate was recorded on MS containing 0.4% Phytagel

Şekil 3. GSN-12 çeşidinin %0.6 (A) ve %0.4 (w/v) (B) bitki agarı içeren MS ortamında in vitro çimlenmesi. %0.4 fitajel içeren MS ortamında en yüksek çimlenmeyi göstermiştir

The germination rate of GSN-12 cultivar on MS medium devoid of sucrose contents and solidified with 0.4% Phytagel was increased from 80.5% to 96.6% when Phytagel replaced with agar as gelling agent (Table 2). The results exhibit the suitability of Phytagel as solidifying agent for *in vitro* propagation of cotton. All seedlings elongated were healthfully and without contamination. The healthy seedling development is very important for vitro experiments to obtain various explants.

Table 2. Effect of different gelling agents and vitamins on GNS-12 cultivar's *in vitro* seed germination

Çizelge 2. GSN-12 çeşidinin in vitro çimlenmesine faklı katılaştırıcıların ve vitaminlerin etkileri

Medium *Ortam*	**Gelling Agent** *Katılaştırıcı*	**Germination Ratio (%)** *Çimlenme oranı (%)*
MS salts and vitamins	%0.6 (w/v) Agar	48.1±3.20
MS salts and vitamins	%0.8 (w/v) Agar	72.9±4.4
MS salts and vitamins	%0.6 (w/v) Phytagel	73.5±2.22
MS salts and vitamins	%0.4 (w/v) Phytagel	96.6±3.21

Conclusion

We conclude that MS medium devoid of sucrose and solidified with 0.4% (w/v) of Phytagel is most suitable for *in vitro* seed germination of cotton cultivars to obtained healthy and contaminated free explants in a shorter period for *in vitro* propagation experiments.

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