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*Full Length Research Paper*

# Agar alternatives for micropropagation of African violet (*Saintpaulia ionantha*)

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**Agar is one of the most popular solidifying agents in plant tissue culture. High price of pure grade agar and fear of over exploitation of its resources caused searching for low cost alternatives. In this study, liquid medium with cotton substratum and different combinations of starch, semolina, potato powder and agar in two steps of micropropagation (shoot induction and proliferation) were investigated. The highest frequency of regeneration was found in media containing agar (0.8%), combination of starch: semolina: potato powder (2:1:1) in 9 and 12% and combination of starch (6%) plus agar (0.4%), but maximum shoot numbers were produced in media containing agar (0.8%), combination of starch (6%) plus agar (0.4%) and liquid medium with cotton substratum. The best shoot proliferation take place in liquid medium with cotton substratum. The results show that the combination of starch: semolina: potato powder (2:1:1) in 9% and starch (6%) plus agar (0.4 %) can be suitable alternatives for agar in regeneration stage but the shoot number is lower than agar alone. These options are very cheaper than agar. The best shoot proliferation can be done in bioreactors or liquid medium with suitable substratum like cotton.**

**Key words:** Gelling agent, low cost, micropropagation, liquid culture.

## INTRODUCTION

In plant tissue culture, the composition of medium like mineral salts, organic supplements, growth regulators and gelling agents affect the culture responses (Gamborg et al., 1968; Gamborg and Phillips, 1995). There are special media compositions for some plants (Nitsch and Nitsch, 1969; Pierik, 1989). Based on gelling agents, media are classified as solid, semi solid, semi liquid and liquid. Agar, as solidifying agent, due to its stability, high clarity, nontoxic nature and resistance to its metabolism, is commonly used in the plant tissue culture (McLachlan, 1985).

Recently, researchers try to find a suitable substrate instead of agar, because of high price of pure grade agar and there are some doubts about its nontoxic nature. Moreover, the exclusive use of agar may result in over exploitation of its resources (Jain and Babbar, 2005; Deb and Pongener, 2010). Different materials such as, starch from various sources (Sorvari and Schieder, 1987; Zimmerman, 1995; Dabai and Muhammad, 2005), starch with low concentration of gelrite (Zimmerman, 1995), combination of starch: semolina: potato powder (2:1:1) (Prakash et al., 2002), Isubgo (Babbar and Jain, 1998; Jain et al., 1997), sago (Bhattacharya et al., 1994), Xanthan gum (Babbar and Jain, 2006) and guar gum (Babbar et al., 2005; Jain et al., 2005) were used as alternative gelling agents. Nowadays, commercial micropropagation labs are using low cost agar alternative in routine protocols. Even liquid medium and micropropagation in bioreactors which eliminate agar contributes 70% of the costs (Prakash et al., 2002). Other researchers evaluated poly-urethane foam, coconut coir and betel nut coir in liquid medium (Deb and Pongener, 2010). This study was carried

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**Abbreviations: MS,** Murashige and Skoog; **BA,** N6-benzyladenine; **NAA,** naphthalene acetic acid.

**Table 1**. Different media with combination of starch, semolina, potato powder and agar as gelling agent.

| Medium | Composition | Solidified degree |
|---|---|---|
| A | 12% (Starch 2: Semolina 1: Potato powder 1) | Solid |
| B | 9% (Starch 2: Semolina 1: Potato powder 1) | Solid |
| C | 9% (Starch 1: Semolina 1: Potato powder 1) | Semi liquid |
| D | 6% (Starch 2: Semolina 1: Potato powder 1) | Semi liquid |
| E | 6% (Starch 1: Semolina 1: Potato powder 1) | Semi liquid |
| F | 6% (Starch 2: Semolina 1: Potato powder 1) + 2 g/l agar | Semi liquid |
| G | 6% (Starch 1: Semolina 1: Potato powder 1) + 2 g/l agar | Semi liquid |
| H | 6% (Starch 2: Semolina 1: Potato powder 1) + 4 g/l agar | Semi solid |
| I | 3% (Starch 2: Semolina 1: Potato powder 1) + 4 g/l agar | Semi solid |
| J | 3% (Starch 1: Semolina 1: Potato powder 1) + 4 g/l agar | Semi liquid |
| K | 9% Starch | Semi solid |
| L | 6% Starch + 4 g/l agar | Solid |
| M | 3% Starch + 4 g/l agar | Semi solid |
| N | 9 g/l agar | Solid |
| O | Liquid medium with cotton substratum | Liquid |

out to find a low cost option for micopropagation of African violet.

## MATERIALS AND METHODS

In the first step of this experiment, the effect of different gelling combinations (Table 1) in Murashige and Skoog ($MS^1$) medium supplemented with 1 mg/l N6-benzyladenine ($BA^1$), 0.2 mg/l Naphthalene acetic acid ($NAA^1$), 30 g/l sucrose and pH= 5.7 were investigated on shoot induction, regenerated shoot number, height and dry weight of plantlets in the first subculture. Young leaves were cut in to pieces of $1\times1$ $cm^2$ as explants. In the second step, regenerated explants were cut in to 4 pieces and transferred to the media containing agar, combination of starch: semolina: potato powder (2:1:1) in 9%, combination of starch (6%) plus agar (0.4%) and cotton substratum in liquid MS medium containing 1 mg/l BA, 0.2 mg/l NAA, 30 g/l sucrose and pH= 5.7 for shoot proliferation. Then the shoot number, plantlet height and dry weight were determined. Cultures were incubated at 25°C in 16 h, light photoperiod with cool white fluorescent at 5000 lux.

## RESULTS AND DISCUSSION

There were differences among media based on solidified degree. Media with composition of A, B, L and N were solid, C, D, E, F, G and J media were semi liquid and H, I, K and M media were semi solid (Table 1).

Two weeks after culture, some of explants got brown and died. After 3 weeks, regeneration signs were observed in green and inflamed explants. Regenerated shoots in all treatment were normal. Media containing A, B and N had maximum frequency of regeneration (100%, Figure 1). Explants in media containing M, N and O compositions produced higher shoot number (12, 12.7 and 11.3 shoots, respectively) followed by B, I, J, K and L media (Figure 2). Media containing compositions of starch, semolina and potato powder except B medium produced a few shoot numbers. Although, A and B media were solid and had maximum regeneration frequency but the shoot number in A medium was low (5 shoots per explants) and in B medium was 10 shoots per explants. Conversely, liquid medium (O) had low shoot induction (36.7%) but shoot production was much more (11.3 Shoot per explant). Plantlets in O medium had maximum dry weight and height (95.8 mg and 14.3 mm, respectively), while these parameters were the same in A, B, I, J, K, L, M and N media (Figures 3 and 4).

Shoot induction and their growth of them are strongly influenced by the physical consistency of the medium. Apparently, the viscosity of medium and its component play an important role in African violet regeneration. Medium containing high (12%) and low (6 and 3%) percentage of starch, semolina and potato powder combination in both ratio (2:1:1 and 1:1:1) were not suitable but by adding agar to these combination, the regeneration frequency was increased, whereas, growth of regenerated shoot was better in liquid medium. Maybe the low shoot production in starch combination was due to the inhibitory effect of this compound especially in low concentration. Powell and Uhrig (1987) reported that some solidifying agents have inhibitory substances that affect morphogenesis. However, Prakash (1993) used combination of laundry starch, semolina and potato powder in a ratio of 2:1:1 as an alternative for agar in ginger and turmeric micropropagation. Zimmerman (1995), Stanley (1995) and Kodym and Zapata (2001) used starch with low concentration of gelrite for propagation of apple, pear, raspberry, banana and sugarcane. They reported that shoot proliferation in this medium was better than agar. Dabai and Muhammad (2005) introduced cassava starch as a potential solidifying agent in microbiological nutrient media.

These results show that, agar is the best gelling agent

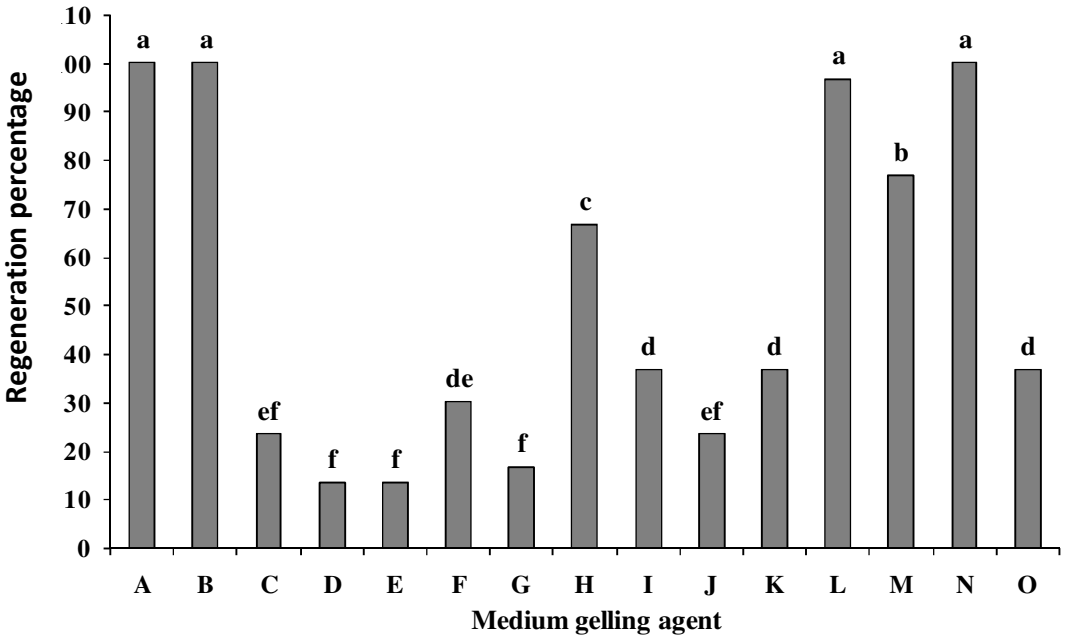


**Figure 1.** The effect of medium composition on regeneration frequency in first subculture.

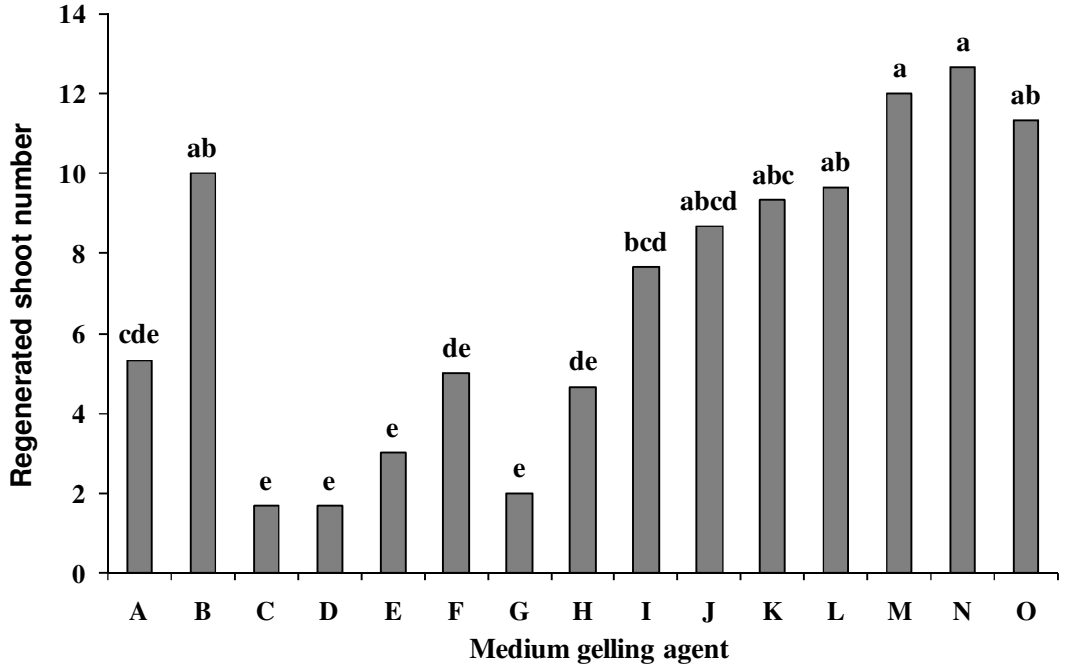


**Figure 2.** The effect of medium composition on regenerated shoot number in first subculture.

for shoot induction in African violet but due to the high price of this compound, the combination of starch, semolina and potato powder or combination of starch and agar can be low cost options.

In proliferation step, liquid medium with cotton substratum was the best and plantlets grew normally and fast. Media containing agar proliferated the same number of shoots like liquid medium but the plantlets were smaller. Other two media (combination of starch, semolina, potato powder and combination of starch and agar) were not suitable for African violet proliferation (Table 2). These results suggest that we can proliferate African violet in

**Table 2.** Effect of medium composition on shoot number, height and dry weight of plantlets in proliferation step.

| Medium | Shoot number | Height (mm) | Dry weight (mg) |
|---|---|---|---|
| B | 10 c | 1.5 b | 120.7 b |
| M | 16 b | 1.5 b | 143.9 ab |
| N | 22 a | 1.6 b | 164.9 ab |
| O | 22 a | 2.0 a | 201.9 a |

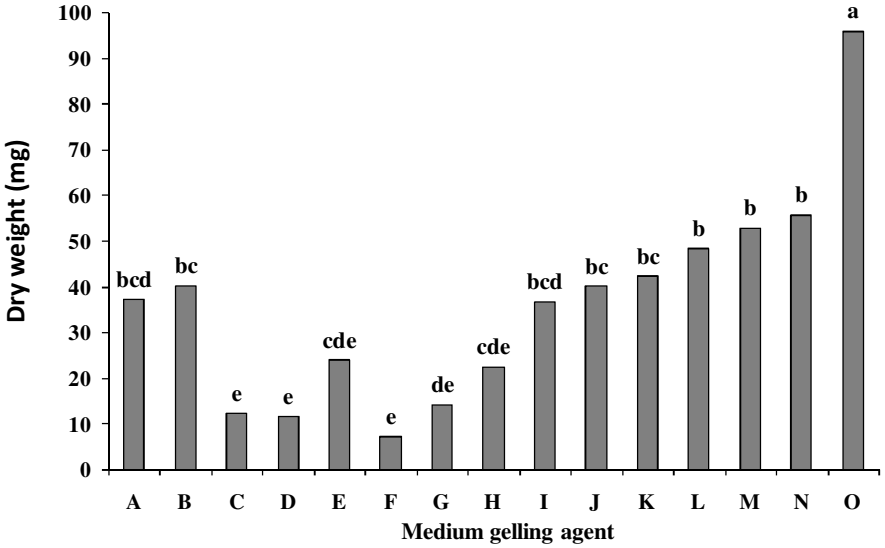


**Figure 3.** The effect of medium composition on dry weight of regenerated shoots in first subculture.

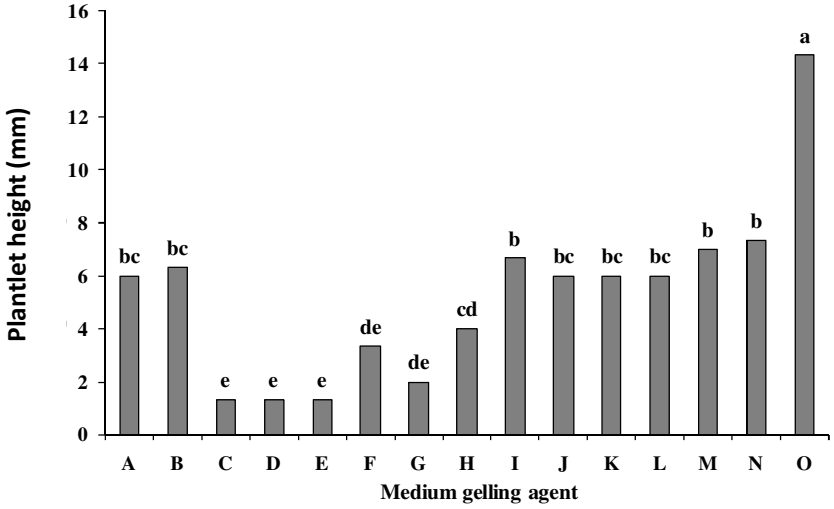


**Figure 4.** The effect of medium composition on plant height of regenerated shoots in first subculture.

bioreactors easily. Bioreactors are used for propagation of apple, chrysanthemum, coffee, garlic, ginseng, grape, lilium, phalaenopsis, potato, strawberry and sugarcane (Etienne and Berthouly, 2002; Paek et al., 2005).

Regenerated shoots produced roots spontaneously in first subculture and it is not depending on gelling agent. Researchers successfully used different substratum like forest titter, moss, polyurethane foam, coconut coir and betel nut coir in liquid medium as alternative to the agar (Temjensangba and Deb, 2005; Deb and Temjensangba, 2006; Deb and Pongener, 2010).

During the last decade, efforts for finding a suitable alternative for agar has been increased and different substances were investigated. Starch as the cheapest gelling agent, has inferior gelling ability, poor clarity and metabolizable nature, which leads to softening of the media. Combination of starch, semolina and potato powder, and starch plus agar in accurate ratio will improve the starch characteristics as gelling agent. In addition, use of bioreactors in whole or part of micropropagation process in African violet will decrease the production cost by 70%. Moreover in this way, explants are in the same vessel and only the fresh medium is added to the system at regular interval and proliferated propagules are exit from vessels.

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