African Journal of Biotechnology Vol. 9 (1), pp. 012-016, 4 January, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Corn and potato starch as an agar alternative for *Solanum tuberosum* micropropagation

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Accepted 29 June, 2009

Potato single nodes were subcultured onto fresh MS medium gelled with 0, 1 and 2 g/l of agar + 40, 50 or 60 g/l of commercial corn and potato starch (CS or PS, respectively). After 4 weeks of culture, the pH of medium supplemented with 50 or 60 g/l of CS or 60 g/l of PS was significantly decreased to 3.91 - 4.00. This reduction coincided with a significant increment in electric conductivity (EC) which was 172 - 214 µmhos/cm^{-1} for media containing 50 or 60 g/l of CS, or 60 g/l of PS. Corn and potato starch had no significant effect in plantlet height nevertheless, they significantly increased the number of shoots/explant over the control treatment (2.5) which had 7 g/l of agar. The highest number of shoots/explant (6.8) was achieved in medium with 50 or 60 g/l of PS + 1 g/l of agar. Plantlets developed on media with 40 g/l of CS or PS had higher shoot fresh and dry weight ($p < 0.5$) compared to those in the control one. Media with 50, 60 g/l of PS or 60 g/l of CS and 50 g/l of CS + agar at 1 g/l significantly enhanced the percentage of dry weight. Moreover, 92 - 98% of plantlets were acclimatized to the greenhouse conditions regardless the type of gelling agent. The results suggest that the combination of agar and PS or CS could offer a firm support for plant tissues and could be successfully used for potato micropropagation.

Key words: Cheap gelling agent, starch, potato, micropropagation.

INTRODUCTION

The main advantage of potato micropropagation technology is the production of high quality and uniform plantlets. However, production of low cost high value plantlets is an ultimate objective which could be achieved by appropriate choice of media components. Prakash (1993) stated that gelling agent such as agar which is usually added to increase media viscosity contributes 70% of the media costs. Various brands and grades of agar, agarose, phytagel and gelrite were used for in vitro micropropagation (Debergh, 1983). However, plantlets growth is strongly influenced by the physical consistency of the culture media.

Agar, the conventional gelling agent, has a number of drawbacks that negatively affect culture growth and differentiation in many cases (Scholten and Pierik, 1998). Cheaper agar alternatives include various types of starch and gums have been investigated in commercial micropropagation (Pierik, 1989; Nagamori and Kobayashi, 2001). For example gelrite can be replaced with starch-gelrite mixture (Kodym and Zapata-Arias, 2001). Other options include white 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% (Prakash, 1993).

Corn starch has been used with a low concentration of gelrite (5 + 0.5%) for the propagation of different plant species (Zimmerman, 1995). Smykalova et al. (2001) found that the growth of proliferated shoot of *Humulus lupulus* on CS-medium was better than that on agar one. They added that the cost of CS was $1.8/kg compared to $200/kg of agar. Naik and Sarkar (2001) substituted agar on potato micropropagated medium with 13% of sago and found that the number of shoots and leaves and root length were significantly higher compared to the agar

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Abbreviations: CS, Corn starch; **PS,** potato starch.

medium. Isubgol which is derived from the seeds of *Plantago ovata* had a good gelling activity for the propagation of chrysanthemum (Babbar and Jain, 1998; Bhattacharya et al., 1994). Lucyszyn et al. (2007) found that shoot proliferation and plantlet growth of *Nicotiana tabacum* on medium solidified with mixtures of agar/galactomannan were better compared to agar-solidified one. The aim of this study is to investigate the effect of different combination commercial potato starch (PS) or corn starch (CS) with agar on potato micropropagation.

MATERIAL AND METHODS

The 2nd and 3rd single nodes (from the base) of micropropagated potato (cv. Sandy) were subcultured onto fresh Murashige and Skoog (1962) medium supplemented 30 g/l of sucrose and 1 mg/l of 6-benzylaminopurine. Medium was solidified with combinations of agar at 0, 1 or 2 g/l (BDH Laboratory Supplies, England) and 40, 50 or 60 g/l of CS and PS. Medium with 7 g/l of agar was used as a control. The pH was adjusted to 5.7 prior to autoclaving for 20 min at 121°C. After sterilization 5 random samples form each type of media were individually homogenized then pH and EC were measured with Beckman 3500 digital pH-meter (Arregui et al., 2003). Cultures were incubated 4 weeks under 14/10 h light/dark provided by white fluorescent tube with 40 µmol m^{-2} s^{-1} light intensity and room temperature 24 ± 1ºC.

After the growth period, 12 randomly selected plantlets represent the 6 replicates were washed under running water and gently dried on filter papers to remove excess water. Plantlet height, number of shoots/explant and fresh weight were measured; thereafter, plantlets were dried at 70°C to estimate their dry weights. The other plantlets were acclimatized to the greenhouse conditions as descried by Mohamed et al. (1999). Media were homogenized after removing the plantlets then, the pH and EC were measured as described before.

There were 30 explants for each treatment; 5 in each baby jar containing 21 ml of the growth medium. The experiment was laid in complete randomized design and the obtained results were submitted to an analysis of variance and means were compared using LSD test ($p < 0.05$) (Clewer and Scarisbrick, 2001).

RESULTS AND DISCUSSION

No physiological disorders were observed on developing plantlets regardless the type of solidified agents during the 2 periods of cultures. The type of gelling agents had no significant effect on plantlet height which varied between 5 and 6.4 cm (Figure 1a). Explants cultured on control medium had the minimum number of shoots/ explant (2.5). However, this value was significantly ($p < 0.50$) increased to 6.8 when media solidified with 50 or 60 g/l of PS + 1 g/l of agar. There were no significant differences in that trait when media supplemented with any combinations of PS and agar. Plantlets developed on media with CS and agar had no significant difference in their shoot number compared to the control medium (Figure 1b).

Plantlets grown on media supplemented with 40 g/l of CS or PS had significantly higher fresh weights (1037 and 1320 mg/plantlet, respectively) compared the control ones (462 mg/plantlet). But, when combinations of agar and PS or CS were used, plantlets on media with PS had higher fresh weights than those on media with the same level of CS. The lowest fresh weight (228 mg/plantlet) being on media supplemented with agar at 2 g/l + 60 g/l of CS (Figure 2a). Moreover, plantlets grown on media with 50 g/l of PS had fresh weight 251% higher than control ones.

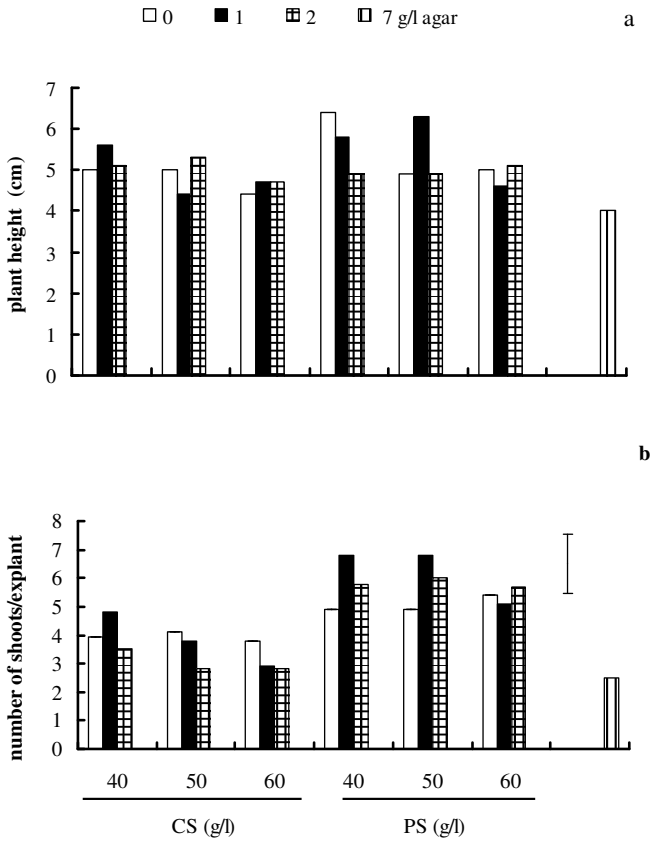

Figure 1. Effect of agar and different combination of corn starch (CS) and potato starch (PS) in potato micropropagated plantlets height (a) and number of shoots/explant (b) after 4 weeks of culture. The bar on graph shows LSD for all pairs comparisons at p = 0.05.

Media with 2 g/l of agar and CS significantly developed lower plantlets dry weight compared to the control treatment whereas, all plantlets developed on medium with 5 or 6 g/l of PS (except 1 g/l agar + 6 g/l PS) had significantly higher dry weight than those on media contains 7 g/l of agar (Figure 2b). It was interestingly to find that plantlets grown on media gelled with 50 or 60 g/l of PS had significantly higher percentage of dry weight (12.6 and 10.9%, respectively) than the control ones (7.3%). Using media with 40 g/l of PS or in with a mixture of 1 g/l of agar significantly decreased the percentage of dry weight to 3 and 4.8% respectively. However, there were no significant differences among the other PS treatments and the control one. When CS was used as a

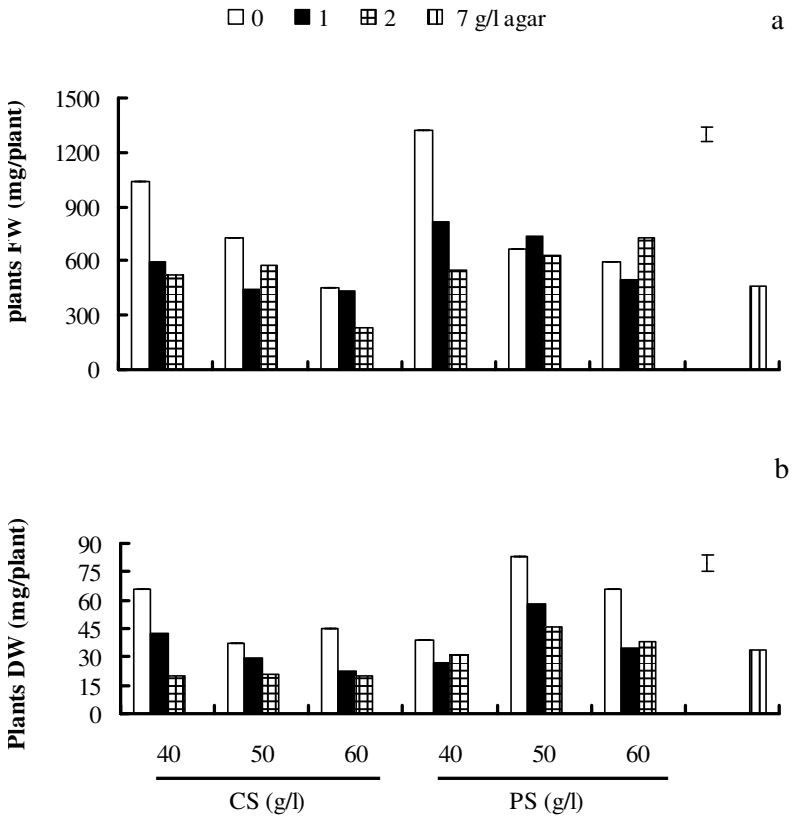

Figure 2. Effect of agar and different combination of corn starch (CS) and potato starch (PS) in potato micropropagated fresh weights and (a) dry weights (b) after 4 weeks of culture. The bars on graphs show LSD for all pairs comparisons at p = 0.05.

gelling agent, only media with 60 g/l of CS increased the percentage of plantlets dry weights over the control plants ($p < 0.5$) whereas, the plantlets grown on media with 40 or 50 g/l of CS in combination with 2 g/l of agar had lower percentage of dry weight compared to the control.

Results showed that PS, CS or a mixture of them with 1 g/l of agar had good results for potato micropropagation than medium with 7 g/l of agar. For example fresh and dry weight of plantlets on media with the lowest concentration of PS or CS were significantly higher than those on control treatment. These differences in fresh and dry weights were coincided with distinct differences in the percentage of dry weights. Similar results were achieved by Lucyszyn et al. (2006 and 2007) who observed that tobacco and strawberry plantlets developed on media with 6 g/l of agar were shorter, had less number of shoots and biomass compared to medium with the agar-alternative galactomannans.

Scholten and Pierik (1998) inferred that agar could has a number of drawbacks that negatively affect morphogenesis and culture growth on some plants. In addition to that, toxic exudates from the cultured explants may take longer time to diffuse (Powell and Uhrig, 1987). Percentage of dry weight of plantlets grown on medium with 50 g/l of PS was about 2 times that of plantlets on control one. Obtained results were similar to those achieved by Owens and Wozniak (1991) on sugar beet and Henderson and Kinnersley (1988) on tobacco and wild carrot. The reduction on plantlet growth which achieved on media contained 2 g/l of agar and 60 g/l of PS or CS could be due to the reduction on plant water potential below threshold value for cell expansion which is a prerequisite for shoot formation and growth (Owens and Wozniak, 1991).

The media pH was slightly reduced to 5.31 ± 0.02 following autoclaving. After 4 weeks of culture the pH of media with no agar + (50 or 60 g/l of CS), or 60 g/ l of PS was significantly reduced ($P < 0.5$) to 3.91 - 4.00 compared to the control treatment (4.93). Whereas, medium with 2 g/l of agar + 60 g/l of CS had the highest pH value (5.49) with no significant difference with the control one (Figure 3a). The reduction in pH coincided with a significant increment in EC. Media with no agar + (50 or 60 g /l of CS) or 60 g/l of PS (Figure 3B) had significantly higher EC (172 - 214 µ mhos/cm) compared to the control medium (129 µ mhos/cm). Nevertheless, the reduction on pH and increment on EC did not affect the

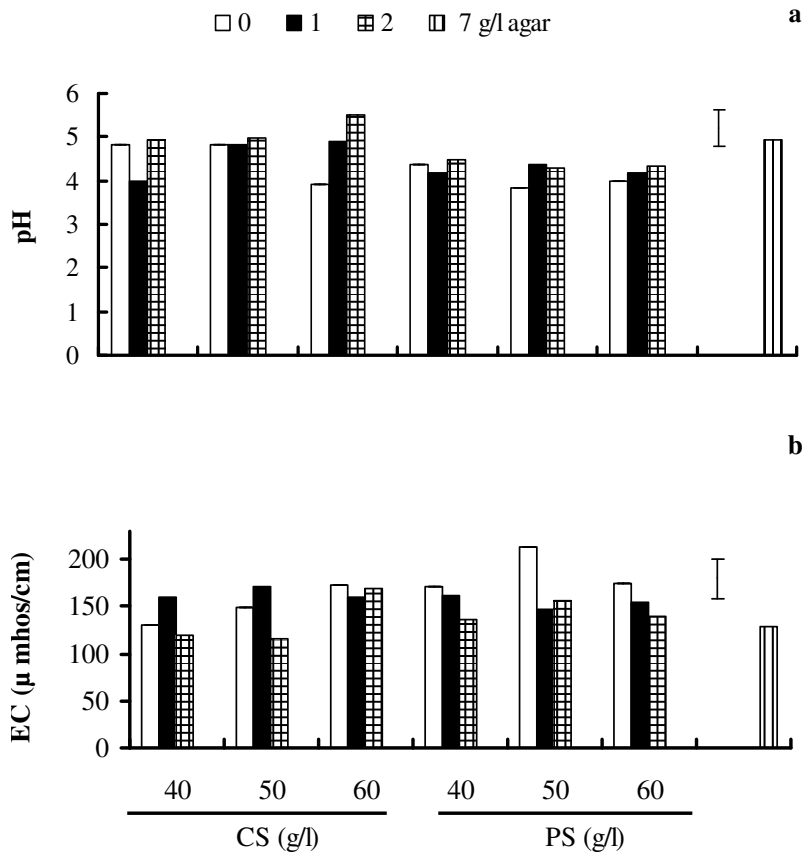

Figure 3. Effect of agar and different combination of corn starch (CS) and potato starch (PS) in pH of the growth medium (a) and EC (b) after 4 weeks of culture single nodes. The bars on graphs show LSD for all pairs comparisons at p = 0.05

media consistency.

The reduction in pH after sterilization is a common phenomenon during media preparation. 4 weeks in culture increased the acidification of the media containing CS and PS depending on their concentration. Sarma et al. (1990) observed that autoclaving alters the medium pH depending on its concentration of agar. Prknov (2007) found that the pH of growth medium of *Sorbus sudetica* was reduced to 4.5 due to hydrolysis. The changes in the pH during culture growth could be explained by the nutrients uptake and the possible formation of complex molecule such as organic acids. The initial decrease in pH is known to be caused by the preferential ammonia uptake, leaving the more acidic nitrate in the media (George, 1993). Lucyszyn et al. (2007) stated that starch can be easily metabolized by the α-amylases present in the medium, resulting in a gradual decrease in the consistency of the medium during the culture period which is the main reason for the limited use of starch as a gelling agent in tissue culture (Naik and Sarkar, 2001). However, in our study potato plantlets with better growth traits were developed on media gelled with PS which had the lowest pH and highest EC. Interestingly these did not affect media consistency which indicated that plantlets had higher ability for ions uptake form the media. Gelling agents had no significant effect on plantlets acclimatization which was successfully adapted to the greenhouse conditions with a survival rate varying between 92 and 98%.

The results of this study suggest that using cheap commercial PS or CS instead of agar as solidifying agent are efficient for potato micropropagation by single node. Potato plantlets grown on medium with 50 g/l of PS as a gelling agent had better growth characters compared to using medium with 7 g/l of agar. The combination of low concentration of agar (1 g/l) and PS or CS in the semisolid 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.

ACKNOWLEDGEMENTS

This study was supported by the College of Food and Agricultural Research Center, King Saud University.

Critical review by Prof. Abdulazia M. Alssaeed is highly appreciated.

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