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Effect of Phloroglucinol, Medium Type and Some Component on *In Vitro* Proliferation of Dwarf Rootstock of Apple (*Mallus domestica*)

MAHDI RUSTAEI, SONBOL NAZERI[1]†, MORTEZA GHADIMZADEH‡ AND SIYAVASH HEMMATY¶
Horticulture Department of Agriculture Faculty, Urmia University, Iran
†*Biotechnology Department of Agriculture Faculty, Bu-Ali Sina University of Hamedan, Iran*
‡*Agronomy and Plant Breeding Department, Agriculture Faculty, Urmia University, Iran*
¶*Academic Center for Education, Culture and Research (ACECR), Urmia Branch, Urmia University, Urmia, Iran*
[1]Corresponding author's e-mail: snblnazeri@yahoo.com; m.rusta@gmail.com

ABSTRACT

Proliferation of different plants is a need for *in vitro* studies of micropropogation. Maximizing shoot proliferation is a basic object of micropropagation and much attention has been focused on components that used in the culture medium and type of medium. Gami Almasi is a dwarf rootstock of apple (*Malus domestica* Borkh) suitable for different cultivars of apple that grow on North West of Iran. In this study two different basal media containing N6 and MS salts with different components involve phloroglucinol (PG), active charcoal (AC) and gibberellic acid (GA) were tested on proliferation of *in vitro* growing shoots. In our results MS medium was better than N6. Active charcoal did not affect *in vitro* proliferation. PG induced the transient proliferation and GA induced stable proliferation of shoots. The combination of PG and GA had the best results in proliferation of apple shoots. Proliferation of apple shoots showed lower occurrence of hyperhydric in the PG medium.

Key Words: Apple; Gami almasi; Phloroglucinol; Gibberellic acid; Proliferation

INTRODUCTION

Tree improvement programs, whether by conventional breeding or recombinant DNA technology, often rely on vegetative propagation to preserve superior genotypes, because of high inherent heterozygosity and selfincompatability mechanisms (Nicoll 1993; Tzfira, 1998). The micropropagation is very effective method for propagation in dwarf rootstock of apple. Two decade ago, micropropagation of apple was un-likely a useful technique for producing trees commercially due to several reasons (Webster *et al.*, 1986; Jones & Hadlow, 1989; Zimmerman & Miller, 1991) but by developing the high efficiency technique its commercial now. Shoot tips or lateral buds are used as explant materials with meristematic tissue to induce shoot growth. Axillary shoot proliferation is very similar to meristem tip culture in that the explant material is meristematic in nature. In axillary shoot proliferation, axillary buds or side shoots serve as the explant material rather than the apical meristem, as in meristem tip culture. Axillary shoot proliferation is used for multiplication of plant material and is commercially used to propagate foliage plants, African violets and other species (Gaspar *et al.*, 1996; Oliveira *et al.*, 2003). Newly formed shoots can either be propagated in new cultures to multiply exponentially, or transplanted in the field to produce a normal sized plant. Propagation of apple rootstocks by cuts are very difficult. The most likely commercial opportunity for micropropagation of apple is the use of micropropagated plants for conventional propagation either by cuttings or layering (Sriskandarajah & Mullins, 1981). Webster and Jones (1993) showed that apples rootstocks from micropropagated plants performed well as compared to rootstocks propagated by conventional methods. Selection of an appropriate culture medium and the use of correct growth regulators were critical for the optimum growth response of the explants. Phloroglucinol (PG) has been used previously for *in vitro* rooting of apple (Gaspar, 1991; Gaspar *et al.*, 1996; Modgil *et al.*, 1999) Demiralay showed that *Ficus carica* cultured on a medium with 89 mg L^{-1} PG or active charcoal, had the highest shoot formation ratio (50.1%) (Demiralay, 1998). Also another report showed that PG enhanced growth and rate of axillary shoot proliferation in potato shoot tip cultures (Debabrata & Prakash, 2000). In this study effect of Phloroglucinol, gibberllic acid, active charcoal and MS and N6 medium on axillary shoot proliferation and length of shoots were evaluated.

MATERIALS AND METHODS

Shoots from previously established cultures of apple rootstock Gami Almasi were grown on a basal medium of

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Murashige and Skoog (1962). In this study *in vitro* shoots grown in MS or N6 (Sriskandarajah and Mullins, 1981) medium contained 1 mg L^{-1} benzyl adenine (BA) and 0.1 mg L^{-1} naphthalene acetic acid (NAA) have been used. Different treatments contains 1 mg L^{-1}, active charcoal (AC), 80 mg L^{-1}, phloroglucinol (PG), 2 mg L^{-1}, gibberellic acid (GA) were used. These shoots divided into three buds each having shoots and then cultured horizontally in media. The cultures were placed at the conditions with 25±2°C and 16/8 h photoperiod provided by warm and cool white fluorescent tube lights with 4500 Lux. After 45 days on multiplication media, shoot multiplication was evaluated by counting the number and length of shoots that were more than 5 mm length. Significance was recorded at $p < 0.05$.

In all experiments 250 mL glass flasks each containing three shoots, with four replicates were used in random complete design. Each shoot have three buds. All data were analyzed by MSTAT-C software.

RESULTS

Results from *in vitro* experiments indicated enhanced proliferation of apple shoots in the medium with PG and GA. The occurrence of hyperhydric shoots was lower in PG medium. Explants cultured in PG was better, since leaves were wider and bright green and fresher than control treatments. These experiments showed that MS medium was better than N6 for shoot proliferation in this cultivar. Shoot number and lengths of shoots increased in MS medium (Fig. 1 & 2). This was due to the reason that MS medium had higher amount of nitrogen (nitrate & ammonium) than N6 medium. Active charcoal did not have any effect on shoot number and its lengths (Fig. 3 & 5).

Shoot production increased in both media in response to addition of PG and GA alone and in combinatin (Fig. 5 & 6). Shoot frequency in GA+PG medium was higher than other treatments although the difference was non-significant between GA and GA+PG treatments (Fig. 3). Addition of PG to MS medium increased growth and induced a higher number of internodal segments containing two leaves per node. Length of shoots in medium contain GA+PG was higher than other medium significantly ($P<0.05$). No significant effect was seen on length of shoots between PG and GA mediums (Fig. 4). Interaction (medium type × components) evaluation appeared that length and number of shoots increase significantly compared to control and AC medium, although no significant difference was seen in shoot number between GA, PG and PG+GA media (Fig. 5) Interaction results (medium type × PG, GA & PG+GA) appeared that in N6 medium; PG, GA and PG have significant effect on length of shoots ratio to AC and control treatments, but these treatments showed no significant difference (Fig. 6).

In MS medium effect of GA+PG on shoots lengths was significantly higher than PG treatment. Root formation occurred only in explants that cultured in the absence of GA

Fig. 1. Effect of medium on shoot number

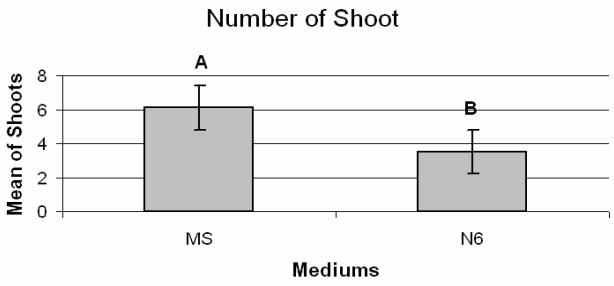


Fig. 2. Effect of medium on shoot length

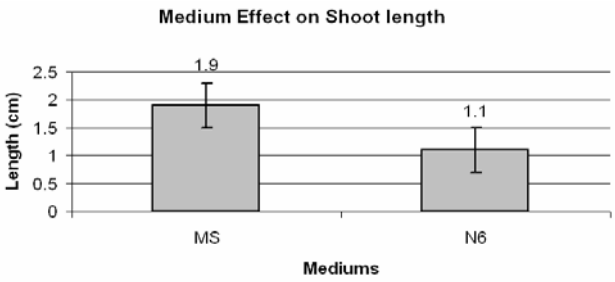


Fig. 3. Effect of different components on shoot number

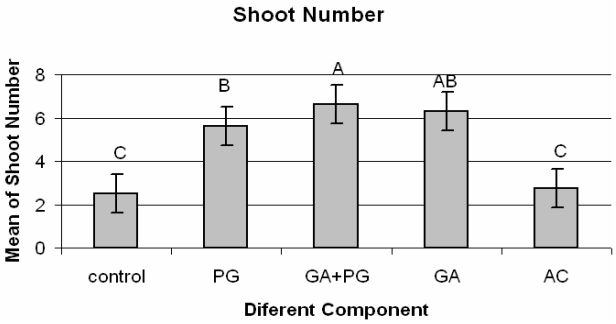
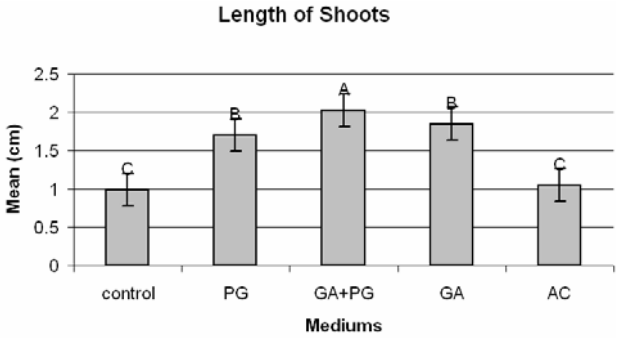


Fig. 4. Effect of medium on shoot lengths



or in MS medium supplemented with PG and 1000 mg L^{-1} active charcoal. In some cases both vitreous and non-vitreous clumps occurred in the same jar, indicating possible variability among explants. Results from *in vitro* experiments indicated enhanced proliferation of apple shoots and also the lower occurrence of hyperhydric shoots in the PG and PG+GA mediums.

Fig. 5. Interaction effect of medium type and component on shoot length

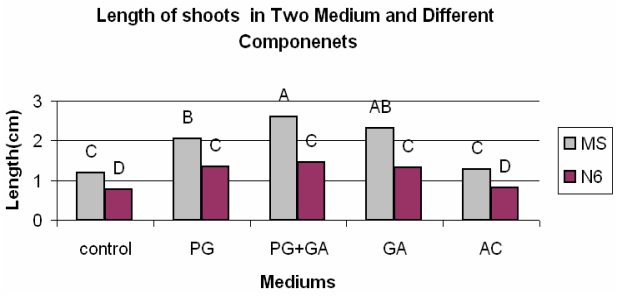


Fig. 6. Interaction effect of medium type and components on shoot numbers

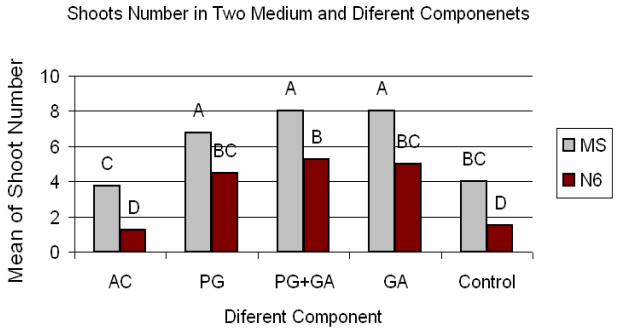
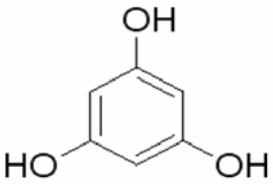


Fig. 7. Phloroglucinol (1,3,5-Trihydroxybenzene)



DISCUSSION

Improvemed performance of micropropagation by using of PG and GA has already been described in the literature (Gaspar *et al.*, 1996; Sharma *et al.*, 2000). PG is a phloridzin derivatives (Fig. 7) and its main use is in rooting (Bassuk *et al.*, 1981) but sometime it could be used as a component that reduce vitrification and increase proliferation (Gaspar, 1991; Aklan *et al.,* 1997). Positive effects of phloglucinol on the stimulation of growth and shoot proliferation have been reported for some cultivars and woody species (Jones, 1976).

A favorable effect of PG on apical and lateral buds from *Tabernaemontana fuchsiaefolia* has been reported. Hypocotyls achieved in medium containing no kinetin or in all kinetin concentrations (Oliveira *et al*., 2003). The addition of PG to MS medium induced rhizogenesis in the nodal segments transferred to MS medium in the absence of IBA (Oliveira *et al.*, 2003). Sharma *et al*. (2000) reported that 100 mg L^{-1} PG increased shoot proliferation in MM106 rootstock of apple.

Effects of PG on shoot proliferation caused by effect on reduce of vitrification of buds and shoots. Some factors that caused cell damage incur the vitrifications. Biosynthesis of acetyl phloroglucinols encoded by the *phlACBDE* gene cluster found in *Pseudomonas fluorescens* Pf-5. PG increased the catalase activity and its protein expression. In addition, catalase inhibitor abolished the protective effect of PG from H_2O_2, which induced cell damage (Kang *et al.*, 2006). Furthermore, PG increased phosphorylation of extracellular signal regulated kinase (ERK) (Kang *et al.,* 2006).

Dwarf plants appear better response to GA addition. In dwarf rootstocks that have deficiency in GA production, addition of GA enhanced shoots lengths more than other and non dwarf rootstock (Webster *et al.*, 1986). In our experiments MS medium enreached with 80 mg L^{-1} and 2 mg L^{-1} gibberellic acid is better than other mediums for apple (cv. Gami Almasi) shoot proliferation. High frequency multiple shoot formation in this medium will ensure a faster rate of shoot multiplication within a limited time.

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