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Comparative Effects of Some PGRs Combination on Proliferation and Hyperhydricity of Sebri Pear Cultivar

S. Karimpour[1], G. H. Davarynejad[1], A. Tehranifar[1] and A. Bagheri[2]

1- Department of Horticultural Science, Ferdowsi University of Mashhad, Mashhad, Iran
2- Department of Biotechnology and plant breeding, Ferdowsi University of Mashhad, Mashhad, Iran

Corresponding author Email: *s.karimpour@yahoo.com*

ABSTRACT: In order to improve *in vitro* culture of Sebri pear cultivar several combinations of plant growth regulators of BAP, IBA and GA_3 were tested. In all PGRs combination, regenerated shoots increased with increasing BAP concentration In media containing of different combinations of BAP (3 mgL^{-1}) and BAP (2 mgL^{-1}) + GA_3 the highest of regenerated shoots were obtained. The higher regenerated shoot length showed in media containing IBA. The highest regenerated shoots length obtained in BAP (2 mgL^{-1}) + IBA and the lowest showed in BAP (3 mgL^{-1}). In BAP (3 mgL^{-1}) + GA_3 combination, internode length was the highest. Hyperhydricity rate was affected by BAP concentration and plant growth regulators combinations. A negative relation was obtained in internode length and hyperhydricity rate. The higher concentration of BAP induced more hyperhydricity rate and BAP + IBA + GA_3 produced the highest hyperhydric regenerated shoots, while BAP (2 mgL^{-1}) + GA_3 induced the lowest hyperhydricity rate.

***Keywords*:** BAP, GA_3, IBA, Micro propagation, Tissue culture
Abbreviations: BAP: 6-benzylaminopurine; IBA: Indole-3-butyric acid; GA_3: Gibberelic acid; MS: Murashige and Skoog medium (1962), PGRs: Plant growth regulators

INTRODUCTION

Pear is one of the most important fruit trees that widely have been grown in moderate regions such as Iran. It is reported Sebri cultivar is belonged to *P. communis* L. (Safarpour, 2008; Erfani et al., 2012) but some author are mentioned *P. pyrifolia* (Rahemi and Baghbani, 2002; Davarynejad and Davarynejad, 2007; Zafari nia et al., 2010). Micropropagation of Sebri was a strategy for its propagation, because of the difficulty of rooting and also budding or grafting of this cultivar on common rootstock have been incompatible (Amiri, 2002; Davarynejad and Davarynejad, 2007; Davarynejad el al., 2008).

Different concentration and combination of plant growth regulators may be have various effects on proliferation (Pierik, 1990; Grattapagali and Machado, 1998). Many workers have suggested combination of BAP and low concentration of auxins for proliferation of pear species (Yeo and Reed, 1995; Nadosy, 1997; Dwivedi and Bist, 1999; Nosrati et al., 2009). Hyperhydricity (previously known as vitrification) of regenerated shoots is an undesirable phenomena *in vitro* culture. In pear, hyperhydricity was affected by some factors such as cultivars, type and concentration of cytokinins (Rouzban et al., 2002; Kadota and Niimi, 2003) and medium type (Kadota et al., 2001). The aim of this study was to investigate the influence of BAP concentration and plant growth regulators combinations on regeneration and hyperhydricity rate in proliferation stage of Sebri pear cultivar.

MATERIALS AND METHODS

In vitro shoots of Sebri cultivar were used for this experiment. Shoots were cultured on MS (Murashige and Skoog, 1962) medium supplement with 30 gL^{-1} sucrose and different combinations of plant growth regulators whit two BAP concentrations (2 and 3 mgL^{-1}) and four combinations of PGRs (BAP, BAP + IBA, BAP + GA_3,

BAP + IBA + GA_3) with constant concentration of IBA (0.1 mgL^{-1}) and GA_3 (0.5 mgL^{-1}). Agar (0.8%) was added to medium after pH adjusted to 5.7. Medium containing BAP and IBA were autoclaved at 0.1 MPa pressure for 20 min at 121 ◦C, while GA_3 was filter-shrilled. The cultures were grown at 24±1 ◦C in a 16-h photoperiod at light intensity of 40 μmol $m^{-2}s^{-1}$ provided by white fluorescent tubes.

Regenerated shoots (shoot/explant), regenerated shoot length (mm), internode length (mm) and hyperhydricity rate (%) were evaluated after 4 weeks of culture (Singha et al., 1987). Experimental design of this study was complete randomized design with 5 replications containing 5 explants. Data were subject to ANOVA and treatment means were compared by the Duncan's multiple range test (DMRT) at 0.05 probability level using the SAS PROC GLM (SAS Institute Inc., 1989).

RESULTS AND DISCUSSION

The ANOVA showed that regenerated shoots and hyperhydricity rate were affected by BAP concentration, PGRs combinations and BAP concentration × PGRs combinations interaction, the regenerated shoot length by PGRs combinations and internode length by BAP concentration × PGRs combinations interaction (Table 1).

Proliferation

Regenerated shoots per explants in medium supplemented with 3 mgL^{-1} BAP was higher than medium containing 2 mgL^{-1} BAP except BAP (2 mgL^{-1}) + GA_3 (0.5 mgL^{-1}). BAP (3 mgL^{-1}), and BAP (2 mgL^{-1}) + GA_3 (0.5 mgL^{-1}) produced the most regenerated shoots (Table 2). It was reported that high concentrations of cytokinins led to producing more regenerated shoots in some cultivars of Asian pear (Rouzban et al., 2002). BAP (2 mgL^{-1}) + IBA (0.1 mgL^{-1}) were more effective than medium supplement with BAP that our results were in agreement with some workers. In *P.calleryana*, OPR 260 and OH×F 230, the highest proliferation rate was obtained in medium containing BAP (1.8 mgL^{-1}) + IBA (0.08 mgL^{-1}) combination (Yeo and Reed, 1995). It was reported BAP (0.5 mgL^{-1}) + IAA (0.05 mgL^{-1}) was more affective for proliferation in cultivars and hybrids of pear rootstocks (Nadosy, 1997). Successful proliferation reported for Japanese pear (Jinfeng and Zaosu cultivars) shoot tips with GA_3 pre-treatment that cultured in MS medium containing BAP (1 mgL^{-1}) + GA_3 (10 mgL^{-1}) (Zhao, 1982).

Regenerated shoots length decreased when BAP increased. More production in regenerated shoots led to less regenerated shoot elongation (Table 3). Similar results reported for Asian pears (Rouzban et al., 2002) and *P. syria* (Shibli et al., 1997) and *Pyrus* genus (Dolcet-Sanjuan, 1990). In *Pyrus*, Liaw et al. (1992) stated the highest proliferation obtained in higher BAP concentrations, while lower BAP concentrations led to longer shoot production. The higher regenerated shoot elongation was obtained in media containing IBA (BAP + IBA and BAP + IBA + GA_3) and the lowest elongation showed in BAP (Table 3). Singha (1980) reported addition of 0.1 mgL^{-1} GA_3 with IAA (0.5 mgL^{-1}) + BAP was effective for shoot elongation in *P.communis*, cv. Seckel. The effect of GA_3 in shoot elongation was reported on Lentil (Naeem et al., 2004).

In all combination, internode length decreased with increasing BAP concentration except for BAP + GA_3 that internode length increased and BAP combination this decreasing was not significant (Tabale 4). In *Pyrus calleryana* high concentrations of BAP led to reducing internode length of regenerated shoots (Berardi et al. 1998). The highest of internode length obtained in medium containing BAP (3 mgL^{-1}) + GA_3 (0.5 mgL^{-1}) and the lowest in medium supplement with BAP (3 mgL^{-1}) + IBA (0.1 mgL^{-1}) and BAP (3 mgL^{-1}) + IBA (0.1 mgL^{-1}) + GA_3 (0.5 mgL^{-1}). The positive effect of GA_3 on internode elongation was reported on *Pisum sativum* (Murfet and Barber, 1961).

Hyperhydricity

Hyperhydricity is a physiological disorder and malformation in cultured tissues that originated from hyper hydration and low lignifications. It is a commonly undesirable phenomenon *in vitro* culture of many plants such as pear. The Sebri hyperhydric regenerated shoots were more translucent, thick and brittle and leaves were small, compact with bright-green color. Hyperhydricity observed in all PGRs combinations but hyperhydricity rate was more affected by BAP concentration. Hyperhydricity rate increased with enhancing of BAP concentration (Table 5). It was reported hyperhydricity affected by BAP concentration, the higher concentration led to more hyperhydricity in pear (Rouzban et al., 2002; Rossi et al., 1991; Berardi et al., 1993). The lowest hyperhydricity rate (8%) obtained in BAP (2 mgL^{-1}) + GA_3 (0.5 mgL^{-1}) and it was the highest (67%) in medium containing BAP (3 mgL^{-1}) + IBA (0.1 mgL^{-1}) + $GA_{3\,(0.5}$ mgL^{-1}) (Table 5, Fig. 1). It seems in low concentration of BAP, GA_3 and IBA have synergic effect on hyperhydricity rate in Sebri regenerated shoots but in high concentration of BAP, different combination of PGRs did not control and reduce hyperhydricity rate.

A negative relation showed between hyperhydricity rate and internode length. In different combinations of plant growth regulators decreasing of internode length observed with increasing hyperhydricity rate (Table 5). In some Asian pear cultivars, it was observed that with increasing of BAP concentration, hyperhydricity rate increased and regenerated shoots length decreased (Rouzban et al., 2002). GA_3 (0.5 mgL^{-1}) with IBA (0.1 mgL^{-1}) induced more hyperhydricity rate rather than when one of them had been used.

Table 1. ANOVA of the influence of different combination of plant growth regulators on regenerated shoots, internode length and hyperhydricity of Sebri pear

Source	Means squares				
	Df	Regenerated shoots	Regenerated shoot length	Internode length	Hyperhydricity rate
BAp concentration	1	40.00**	3.35^{ns}	1.65^{ns}	20930.62**
PGRs combination	3	2.50*	50.69*	1.78^{ns}	2492.29**
BAp concentration× PGRs combination	3	3.46*	2.54^{ns}	5.74*	1672.29**
Error	32	0.97	18.13	1.91	117.18
Corrected total	39				

**: significant at 1%, *: significant at 5% and ^{ns}: not significant

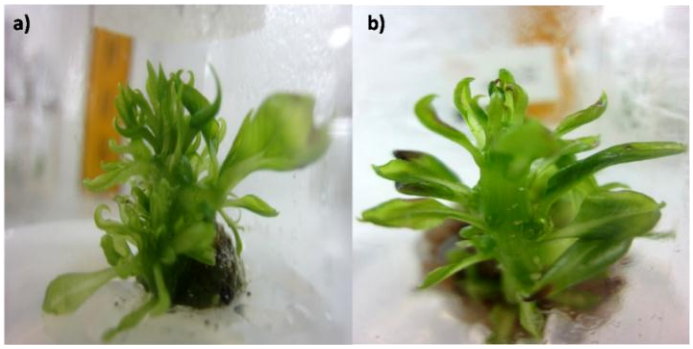

Figure 1. The effect of PGRs combination on regenerated shoot hyperhydricity a) in 2BAP + GA_3 and b) in 3BAP + IBA + GA_3

Table 2. The effect of BAP concentration, PGRs combinations and BAP concentration × PGRs combinations interaction on regenerated shoots (shoot/explants) in Sebri pear cultivar

		PGRs combination				Average
		BAP	BAP + IBA	BAP + GA_3	BAP + IBA + GA_3	
BAP concentration	2	0.40 b*	1.00 b	2.60 a	0.60 b	1.15 B
	3	3.60 a	3.40 a	3.00 a	2.60 a	3.15 A
Average		2.00 AB	2.20 AB	2.80 A	1.60 B	

* Means followed by the same letter (capital letter indicates simple and small letter indicates interaction) are not significantly different by Duncan's test at 5% probably level

Table 3. The effect of BAP concentration, PGRs combinations and BAP concentration × PGRs combinations interaction on regenerated shoot length (mm) in Sebri pear cultivar

		PGRs combination				Average
		BAP	BAP + IBA	BAP + GA_3	BAP + IBA + GA_3	
BAP concentration	2	3.400 a*	9.010 a	5.114 a	8.280 a	6.451 A
	3	4.224 a	8.182 a	4.382 a	6.700 a	5.872 A
Average		3.812 B	8.596 A	4.748 AB	7.490 AB	

* Means followed by the same letter (capital letter indicates simple and small letter indicates interaction) are not significantly different by Duncan's test at 5% probably level

Table 4. The effect of BAP concentration, PGRs combinations and BAP concentration × PGRs combinations interaction on internode length (mm) in Sebri pear cultivar

		PGRs combination				Average
		BAP	BAP + IBA	BAP + GA_3	BAP + IBA + GA_3	
BAP concentration	2	1.427 ab*	1.733 ab	1.283 ab	2.269 ab	1.678 A
	3	1.281 ab	0.490 b	2.890 a	0.424 b	1.271 A
Average		1.354 A	1.111 A	2.086 A	1.347 A	

* Means followed by the same letter (capital letter indicates simple and small letter indicates interaction) are not significantly different by Duncan's test at 5% probably level

Table 5. The effect of BAP concentration, PGRs combinations and BAP concentration × PGRs combinations interaction on hyperhydricity (%) in Sebri pear cultivar

		PGRs combination				Average
		BAP	BAP + IBA	BAP + GA_3	BAP + IBA + GA_3	
BAP concentration	2	28.00 b*	28.00 b	8.00 c	60.00 a	31.50 B
	3	63.00 a	62.00 a	59.00 a	67.00 a	62.75 A
Average		46.50 B	45.00 B	33.50 C	63.50 A	

* Means followed by the same letter (capital letter indicates simple and small letter indicates interaction) are not significantly different by Duncan's test at 5% probably level

CONCLUSION

The results of this experiment indicated that different combination of IBA and GA_3 had different results at various concentration of BAP. Higher concentrations of BAP produced higher regenerated shoots but induced higher hyperhydricity rate. Our results indicated BAP (2 mgL^{-1}) plus GA_3 (0.5 mgL^{-1}) is the most suitable PGRs combination for proliferation in Sebri pear cultivar, because of high regenerated shoot production and low hyperhydricity rate in regenerated shoots. The combination of BAP (2 mgL^{-1}) + IBA (0.1 mgL^{-1}) + GA_3 (0.5 mgL^{-1}) induced higher hyperhydricity rate rather than other PGRs combinations. Nevertheless, hyperhydricity was affected by high concentration of BAP concentration more than PGRs combination.

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