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Rescue of Peach Embryo in Culture Media with Additional of 6-benzylademine and Gibberellic Acid

Nonglak Jeengool[1] and Unaroj Boonprakob[2]

ABSTRACT

Culture media and plant growth regulators (PGRs) are important factors for successful embryo rescue. Four medium formulas: Brooks and Hough (BH), Woody Plant (WP), Gilmore, and Monet, were compared for their effects on rescuing immature embryo of peach and later development into seedlings. The results indicated that all media allowed embryos to continue their development and generated high percent germination (>80%). Seedlings germinated in WP and BH were significantly larger in size than those in either Monet or Gilmore. Survival rate of transplanted seedlings was highest in WP (54.4%). Overall observation pointed to WP as the best culture medium in rescuing immature peach embryos. In the following experiment, the response of immature embryos to PGRs: BA and GA_3, in WP was evaluated for germination and seedlings formation. Most embryos germinated well (>85%) with or without PGRs; however, resulting seedlings showed much difference. Numbers of seedling with dead shoot tips were much lower when either or both PGRs were added in the medium. The results showed that WP with 0.1 mg/l GA_3 and either 0.5 or 1.0 mg/l BA yielded 100% germination and no rosetting seedlings. Significant linear responses ($P = 0.04$) of seedling height and quadratic responses ($P = 0.03$) of seedling dry weight were observed with GA_3. Seedlings showed significant linear response ($P < 0.01$) with BA for their leaf number, but total leaf area remained comparable. Positive correlations between seedling dry weight with root length ($r = 0.23$), root number ($r = 0.49$), leaf number ($r = 0.24$) and leaf area ($r = 0.3$) were significant, while that between dry weight and seedling height was not significant.

Key words: *in vitro*, embryo culture, plant growth regulator, breeding, *Prunus persica* (L.) Batsch

INTRODUCTION

Annual production of dessert and processing type peach in Thailand was approximately 20-30 and 400-500 metric ton, respectively. Ripening season of dessert type peach extends from late March to late April in which most fruits come to market in mid-April. Peach fruit ripening in late March gets higher price because there is no competition of tropical fruits such as durian and rambutan during the same period. Therefore, any early ripening peach variety gets advantages of marketing window and high profit.

The early ripening trait in peach is genetically controlled and is an important objective in many stonefruit breeding programs (Ramming, 1993; Anderson and Byrne, 2001). Crosses between both early ripening parents could generate

1 Department of Agriculture, Ministry of Agriculture and Cooperatives, Muang District, Sakon Nakhon 47000, Thailand.

2 Department of Horticulture, Faculty of Agriculture Kamphaengsaen, Kasetsart University, Kamphaengsaen Campus, Nakhon Pathom 73140, Thailand. e-mail: unaroj.b@ku.ac.th

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greater proportion of progeny with this trait resulting in better chance of making new selections. However, hybrid seeds from these parents germinated poorly due to their immature embryos or embryo abortion when fruits are ripe (Turkey, 1933). Embryo rescue is necessary to recover viable hybrid seeds and allows embryos to continue growing to seedling stage. Embryo culture was successfully performed by Turkey (1933) in sweet cherry and since then much research work has been conducted to optimize embryo culture technique in several crops.

Successful embryo rescue depends on a culture medium, developmental stage of embryo and culture conditions (Chaparro and Sherman, 1994; Kuden *et al*., 1999). Embryos were successfully cultured in several media such as Gilmore and Monet media (George *et al*., 1987), Brooks and Hough medium (Scorza and Sherman, 1996) and Woody Plant medium (Emershad and Ramming, 1994). In addition, plant growth regulators enhance embryo growth. Kuden *et al*. (1999) reported the beneficial effect of BA and GA_3 on the development of embryo and seedling germination. Koukhartehik and Semenas (2000) found that media with BA and GA_3 produced good shoots and those with only GA_3 stimulated good rooting. For a particular variety, developmental stage of embryos when fruits are ripe depends on climatic conditions where the trees are grown, therefore, successful reports of embryo culture in one place may not confirm similar results in another place (Scorza and Sherman, 1996). In Thailand, embryo culture in peach has not been accomplished and there is a need for this technique in developing early ripening peach variety. The objectives of this research were to compare the effects of culture media on rescuing immature embryos and to evaluate the response of immature embryos to BA and GA_3 added in culture medium.

MATERIALS AND METHODS

Comparison of culture media

Four culture medium formulas, Brooks and Hough (1958), Woody Plant (Lloyd and McCown, 1981), Gilmore (1950), and Monet (1968), were used to compare the growth and development of 'EarliGrande' peach embryos. The media were prepared with 2% sucrose, 0.65% agar and adjusted pH to 5.7. Approximately 15-20 ml of media was added into 100 ml wide-mount food jars and autoclaved at 121°C for 15 min.

Firm ripe fruits were collected in mid-April in 2000 from Angkhang Royal Agricultural Station, Chiang Mai. Fruits were surfaced sterilized in 1.2% sodium hypochloride for 15-20 min. and seeds were aseptically removed from endocarp. Seedcoat was peeled off and an embryo (hylum side) was placed into culture media. One hundred and forty-two replications (one embryo per jar) were set for each medium.

Jars containing embryo cultures were stored at 2-4°C in the dark for 10 weeks for stratification, then they were transferred to a culture room (16 h photoperiod with white fluorescent at 23°C) for 4 weeks and germination number was recorded. Ten jars were randomly selected from each medium to determine their seedling properties, i.e., plant height, leaf number, lateral root number, lateral root length, fresh weight and dry weight. All seedlings were transplanted into a sterile growing medium (1 sand : 1 peat moss, v:v) and kept in the culture room for 7 weeks. Survival rate was determined, then 10 vigorous seedlings were selected from each medium and recorded for plant height, leaf number and leaf area. The Statistical Analysis System (SAS) general linear model procedure was used to analyze the data and the Duncan's New Multiple Range Test (DMRT) was performed to compare the effect of each medium.

Response of embryos to 6-benzylademine (BA) and gibberellic acid (GA_3)

After the best medium was selected, additional of BA at three concentrations: 0, 0.5 and 1.0 mg/l and GA_3 at three concentrations: 0, 0.1 and 0.2 mg/l were included in this selected medium. The medium with BA was sterilized in an autoclave, then GA_3 stock filtered through 0.2 μm cellulose nitrate membrane was later added. The 3×3 factorial experiment in CRD was arranged in 2001. Fifty embryos (50 replications) were done for each treatment combination. Embryos were stratified and raised in similar conditions as previously described. Data was collected in the same manner except no survival rate of seedling after transplanting into growing medium was recorded. The SAS general linear procedure was used to analyze the data. Contrast analysis was used to test for significant response of embryo development to plant growth regulators. Seedling parameters were analyzed using correlation analysis.

RESULTS AND DISCUSSION

Comparison of culture media

In this experiment, the seeds had approximately 13% dry weight. All four medium formulas gave more than 80% germination rate and embryos cultured in WP germinated better than those in BH, Gilmore or Monet media (Table 1). The better performance of WP was similar to other reports on peaches and nectarines having approximately 90-100% germination rate (Emershad and Ramming, 1994; Rizzo *et al*., 1998). The resulting seedlings in WP and BH media were significantly taller and had more fresh weight than those cultured in Monet or Gilmore media (Table 1 and Figure 1). No significant difference was detected in the other growth parameters such as leaf number and root growth. Seven weeks after transplanting, seedlings from WP had the highest survival rate and other growth parameters were significantly greater (Table 2). The vitamins and amino acids included in WP seemed to enhance the seedling growth. The beneficial effects of vitamins and amino acids on development of immature embryo were observed in several plant species (Raghavan, 1994). In addition, its lower salt concentration than that in BH permitted greater root development in peaches and nectarines (Emershad and Ramming, 1994).

Response of embryos to 6-benzylademine (BA) and gibberellic acid (GA_3)

In this experiment, the seeds had approximately 15% dry weight. Embryo germination rate was greater than 85% with or without both PGRs. One hundred percent

Table 1 Germination rates and seedling growth parameters in culture media after 4 weeks in culture room.

Medium	Germination rate (%)	Weight[1/] (g)		Height[1/] (cm)	Leaf number	Lateral root	
		Fresh	Dry			Number	Length (cm)
BH	84.5	0.55 a	0.073	4.1 a	9.2	5.4	12.6
WP	88.7	0.53 a	0.074	4.4 a	6.3	6.2	13.8
Monet	80.3	0.35 b	0.062	2.7 b	6.9	4.3	9.8
Gilmore	80.3	0.44 ab	0.064	3.2 ab	6.7	3.4	10.5
P < F-test		0.01	0.47	0.02	0.38	0.25	0.78

[1/] Values within a column followed by a different letter are significantly different at the 95% confident level using DMRT

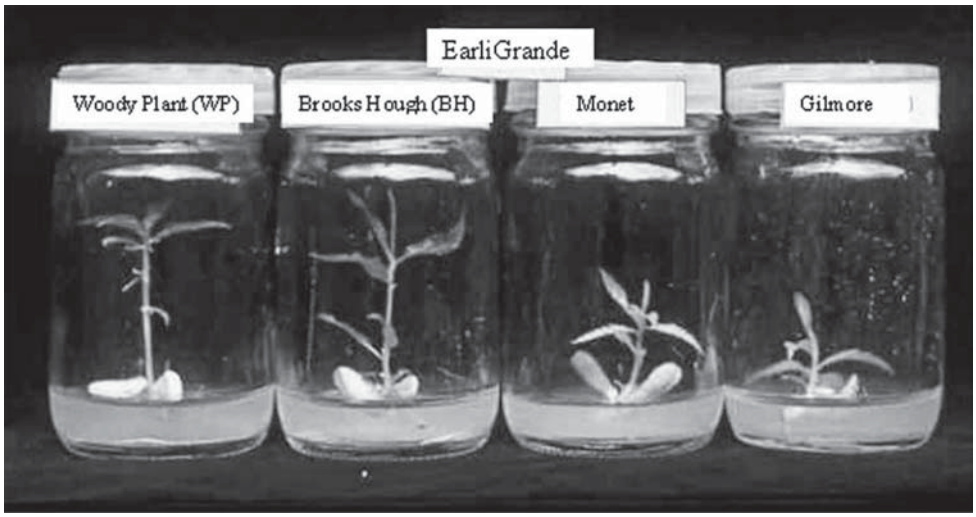

Figure 1 Seedlings of 'EarliGrande' peach resulted from embryo rescue on four media: Woody Plant (WP), Brooks and Hough (BH), Monet, and Gilmore.

Table 2 Survival rate and growth parameters of seedlings seven weeks after transplanting.

Medium	Survival rate (%)	Height[1/] (cm)	Leaf number[1/]	Leaf area[1/] (cm^2)
BH	33.8	4.3 b	11.6 ab	4.7 a
WP	54.4	5.8 a	14.1 a	5.2 a
Monet	35.2	4.0 b	10.0 b	2.1 b
Gilmore	33.8	3.9 b	11.0 ab	3.2 b

[1/] Values within a column followed by a different letter are significantly different at the 95% confident level using DMRT

germination rate was observed with embryos in 0.1 mg/l GA_3 plus either 0.5 or 1.0 mg/l BA (Table 3). Seedlings grown with neither BA nor GA_3 developed higher incident of dead shoot indicating that these seedlings might be weaker and became more sensitive to desiccation. Increasing BA concentration was associated with increasing number of seedlings with small leaf. No obvious effect of PGRs on the number of normal and rosetting seedlings was detected during four weeks in culture.

Interaction effect of the PGRs was significant on leaf number (Table 4) in which increasing GA_3 concentrations at 0.5 mg/l BA had positive effect but increasing GA_3 concentrations at 1 mg/l BA had negative effect (Figure 2). Leaf number was also increased linearly with the amount of BA used, however, these leaves were smaller in size and total leaf area per seedling was not increased. Significant effect of GA_3 on seedling dry weights and heights was observed. Dry weight had quadratic response to the amount of GA_3 and the highest value was obtained at 0.1 mg/l. Taiji *et al*. (2002) suggested that total plant dry weight was an appropriate measurement for plant growth *in vitro*. In this experiment seedling height responded linearly to the amount of GA_3, known to enhance cell elongation and hence made the distinctive difference in height of those treated with high concentrations of GA_3. Nonsignificant

Table 3 Effects of BA and GA_3 in WP on embryo germination and seedling condition.

Treatment combination		Germination	Seedling condition (%)			
BA (mg/l)	GA_3 (mg/l)	rate (%)	Normal	Rosetting	Small leaf	Dead shoot
0	0	91	69	3	9	16
0	0.1	86	90	7	0	3
0	0.2	97	73	6	3	18
0.5	0	94	82	0	6	12
0.5	0.1	100	71	0	20	9
0.5	0.2	91	87	0	13	0
1.0	0	94	65	0	27	3
1.0	0.1	100	88	0	9	3
1.0	0.2	97	70	3	27	0

Table 4 Seedling growth parameters on WP with BA and GA_3.

	Height	Weight (g)		Leaf		Lateral root	
Factor	(cm)	Fresh	Dry	Number	Area (cm^2)	Number	Length (cm)
BA (mg/l)							
0	2.7	0.806	0.087	8.4	0.63	10.4	6.5
0.5	3.3	0.881	0.094	14.2	0.60	8.7	7.3
1.0	3.3	0.892	0.093	18.1	0.58	8.4	6.2
			Probability of contrast analysis				
Linear	0.06	0.22	0.36	<0.01	0.91	0.11	0.83
Quadratic	0.27	0.60	0.59	0.80	0.96	0.54	0.32
GA_3 (mg/l)							
0	2.7	0.766	0.081	12.7	0.60	9.0	5.9
0.1	3.2	0.932	0.100	15.1	0.70	9.0	7.3
0.2	3.4	0.880	0.092	13.1	0.50	9.5	6.8
			Probability of contrast analysis				
Linear	0.04	0.11	0.14	0.59	0.31	0.67	0.38
Quadratic	0.59	0.08	0.03	0.08	0.11	0.78	0.29
			Probability of interaction effect				
BA and GA_3	0.05	0.86	0.79	<0.01	0.55	0.92	0.58

effect of PGRs was observed on other growth parameters.

Because dry weight was the best indication of *in vitro* growth, statistical correlation was analyzed between dry weight with other growth parameters: height, average lateral root length, lateral root number, leaf number and leaf area. Significant correlation coefficient (r) was detected

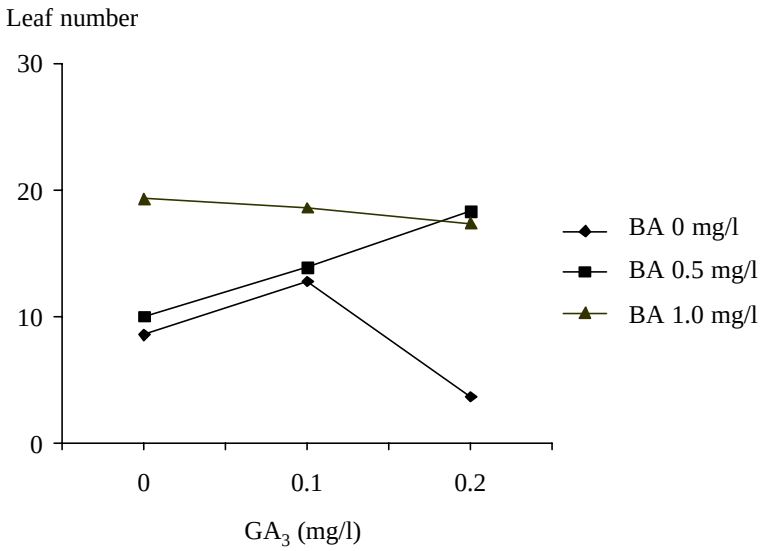

Figure 2 Interaction effect of BA and GA_3 on leaf number.

between dry weight with average lateral root length ($r = 0.23$; $P = 0.02$), lateral root number ($r = 0.49$; $P < 0.01$), leaf number ($r = 0.24$; $P = 0.02$) and leaf area ($r = 0.3$; $P < 0.01$). This indicated that an increase in dry weight was a result of an increase of lateral root number, root length, leaf number and leaf area but seedling's height. These growth parameters significantly correlated with dry weight were vital for seedling survival.

CONCLUSION

Immature embryos of peach from seeds having about 13-15% dry weight could be successfully rescued and stimulated to develop *in vitro* to seedlings using different culture media. The most suitable medium for best germination and survival rate was WP medium. The PGRs had beneficial effects on embryo growth and development. Adding BA (1.0 mg/l) and GA_3 (0.1 mg/l) resulted in 100% germination, prevented rosetting and improved seedling growth. Increasing in seedling dry weight was positively associated with lateral root number, lateral root length, leaf number and leaf area.

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