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EFFECTS OF BA AND IBA CONCENTRATIONS AND SUBCULTURE FREQUENT ON MERISTEM CULTURE OF STRAWBERRY

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ABSTRACT

The meristems excised from runners of Camarosa and Redpearl strawberry cultivars were cultured in MS media enriched with 1H-indole-3-butanoic acid (IBA: 0.2, 0.3, 0.4, 0.5, 0.6 ppm) and BA (0.5, 0.6, 0.7, 0.8, 0.9 ppm) that were applied separately in three replications. BA generated plantlets were subcultured in the same media to produce S_i plantlets (i= 1st, 2nd, 3rd subculture) before root initiation. The result showed that all explants in IBA produced one plantlet and 0.2 ppm produced the best plantlet performance, while all of BA concentration induced multiple plantlets and 0.6 ppm produced 7-26 plantlets/explant. MS media without IBA was recommended for rooting induction. In both of hormones, Camarosa in vitro plantlet performance was better than Redpearl, but the performance of Redpearl plantlet was better than Camarosa after acclimatized. The number and length of Redpearl root was significantly higher so the stand ability was better than Camarosa.

Keywords: strawberry, meristem culture, IBA, BA, runner, daughter plant, variant.

INTRODUCTION

Strawberry is propagated by runners; therefore the health of daughter plant depends on their mother plants. Strawberry is affected by numerous viruses that greatly reduce the yield (Pisi, 2008). The viruses caused the smaller leaves, decreasing the photosynthesis rate and eventually reducing fresh and dry weight. In complex infections (more than one virus), the rate of photosynthesis per unit area also was profoundly reduced (Kang *et al*., 1981).

The method for avoiding virus infection used meristem culture of shoot tip of health mother stock, as propagation material. Many researchers have published the meristem culture of strawberry (Mohamed, 2007; Sim *et al*., 2007). Complete genetic fidelity of produced plantlet is important in micro-propagation technology, especially in commercial micro-propagation protocols giving rise to genetically uniform and true-to type plants (Rani and Raina, 2000). However, hormone concentration that stimulates the rapid differentiation could affect the magnitude of abnormality such as hyper-flowering, fruit malformation, small plant, lower yields, runner-less female fertile plants, as mentioned by Swart *et al*. (1981) and Anderson *et al*. (1982).

Anderson *et al*. (1982) showed that conditions favoring greater proliferation rates in culture may also favor multi-apexing. Multi-apexing could be avoided by using appropriate concentrations of growth regulators in culture media which will concomitantly maintain a good proliferation rate. James and Newton (1977) concluded that concentrations of the cytokinin ranging from 0.25-2.5 ppm, coupled with auxin concentrations ranging from 0.25-1.0 ppm, were most suitable. Borkowska (2001) applied lower concentrations (0.5 ppm BA and 0.1 ppm IBA) for growing plantlet. There was no information about effect of low concentration of BA and IBA for strawberry meristem culture, and comparison between IBA and BA concentration.

In this experiment, runner shoot tips of Camarosa and Redpearl cultivars were used to produce plantlets through meristem culture in the low BA and IBA concentration and also subculture frequent. Low concentration of BA or IBA was expected to produce true clone of intended strawberry cultivars. The experiment objective was to identify the effects of BA and IBA concentration on numbers and characteristics of plantlets.

MATERIAL AND METHODS

The mother plants such as Camarosa and Redpearl strawberry cultivars (Fragaria x ananassa) were grown in the opened field in high land research area (800 m asl, lat. 37.5°N., Korea). The maximum and minimum daily temperature was 7°C and 30°C respectively, and runner tips were taken when average temperature was about 20°C. The plant didn't prepare with special treatment as heating treatment chamber.

Runner tips were excised from mother plant, sterilized in 50% commercial bleach solution for 5 minute and rinsed three times with distilled water. The runner, in the clean bench, then sterilized with ethanol 70% for one minute and 0.1% sodium hypochlorite for ten minutes respectively, and after sterilization then rinsed three times with distilled water. The meristem consisted of dorm and one leaf (see Figure-1A) was excised aseptically, using scalpel and forceps under a stereoscope (8-100 x zoom). Scalpel was sterilized after each meristem excising finished to avoid contamination. Meristems are gently placed on MS (Murashige and Skoog salt) and vitamin, 3% sucrose, hormone according to the treatment, 0.6% agar and pH 5.8.

Randomized complete design with three replications was used in this experiment. The hormones with IBA (0.2, 0.3, 0.4, 0.5, 0.6 ppm) and BA (0.5, 0.6,

0.7, 0.8, 0.9 ppm) were applied separately. Each experiment unit consisted of 20 explants. Explants were stored in the dark room for one week, and then moved to light room with 16/8 hours light and dark, and temperature 23^{o}C.

After 8 weeks, the roots of the plantlets generated in IBA (Figure-1C) were cut and then transferred on the root initiation medium (MS medium without hormone). These sizes of plantlets were 0.8-1.2 cm and had 3-6 leaves. The meristem cultured in the MS medium + BA hormone produced multiple-apexing (Figure-1B) but had no root in rooting experiment and then subcultured to produce plantlets S1, S2, S3. S1, S2 and S3 plantlets were intended to detect effect of subculture frequency to somaclonal variation in the vegetative and generative stage. A half number of plantlets were subcultured in the similar medium and others was separated individually and plantlets transferred into rooting medium with IBA concentration: 0, 1, 2, and 3 ppm. It was applied to detect hormone requirement for root induction. The optimum concentration of IBA was used as root induction of plantlets S1 (first subculture), S2, and S3. All plantlets were splited as single plantlet before transferred to root initiation medium. The root number, root length, height, and leaves number of plants were recorded eight weeks after root induction. All plantlets then were acclimatized and planted in greenhouse.

Analysis of variance and means separation of quantitative data were performed with SAS 9.0, while the narrative used for explaining the qualitative data. Each sub culture in vitro data was analyzed separately.

RESULT AND DISCUSSIONS

Tissue culture is used for many purposes in biology research areas such as propagation, transformation tools, elimination of pathogen like especially virus, cryopreservation, and breeding. This study was focused on the propagation with low concentration of hormone for producing many plantlets through meristem culture.

The IBA and BA were applied independently in MS medium for growing meristem of two cultivars. The step of plantlet development from meristem was shown in Fig. 1. One month after meristem culture, the BA and IBA showed different effects in which BA induced multi-apexing (Figure-1B) plantlets while IBA induced single shoot with normal growth and leaf (1C). Subculture of plantlet induced BA increased the diameter size of multi-apexing that indicated the number of apex increased, otherwise no increasing of plantlet in subculture of IBA plantlets to new media, but plantlet size was bigger. The IBA enrichment of MS medium was appropriate for generating one plantlet per meristem.

In the medium + IBA, Camarosa shoot grew better than Redpearl with higher plantlet. Table 1 showed that plant height and leave number of Camarosa were significantly higher than Redpearl, otherwise Redpearl had longer root significantly than Camarosa. There was no significant difference on root number of both cultivars. With no difference of the root number, longer root and less leave number supported that Redpearl absorbed more water and nutrient, and reduced transpiration than Camarosa. As a result, Redpearl performance in acclimatization was better than Camarosa. Regardless of cultivars, between IBA concentrations, the lowest concentration (0.2 ppm) is the best concentration for producing single plantlet with longer root and more root number than higher concentration of IBA. The effect of IBA concentrations did not significantly differ to plant height; instead the higher concentration inhibited the root length development (Table-1).

Naturally, strawberry includes easy-rooted plants at the crown base and the daughter plant. The effect of IBA concentration in root induction of plant was shown in Table 2. Plant height and root number of Camarosa were significantly higher than Redpearl and the contrary was for root length. Between IBA concentrations, the plant height and root number of the control was significantly higher than others. It was linearly with Nankali and Azghandi (2009). Root length of Redpearl significantly was higher than Camarosa and the best concentration was achieved at 3 ppm, while 0 ppm IBA was recommended for Camarosa. Regardless of cultivar, 0 ppm IBA was recommended for applying in root initiation of S1, S2, S3.

Biswas *et al*. (2008) suggested that low concentration of BA is more effective for mass propagation of the studied three strawberry clones. In this experiment, BA 0.6 ppm was the best concentration for plantlet induction and produced 7-26 plantlets/explants. Nankali and Azghandi (2009) said that the greatest shoot proliferation was achieved in MS medium (full strength) containing 0.5 mg/L BAP. The different optimum concentration was allegedly caused by different cultivars. The BA concentrations and subculture (Si) effects to Camarosa and Redpearl performance were shown in Table 3. There was significant interaction between cultivar and BA concentrations. In all subculture (S1, S2, S3), the plant height and leaves number of Camarosa was higher than Redpearl, otherwise condition for length and number of root. Root and shoot characters of both cultivar were given characters determined by genetics.

Table 3 showed that generally for S1, S2, S3 plantlets, 0.6 ppm of BA produced best plantlets with more leaves, root and long root. But for inducing plantlets with best root in S2 and S3, 0.7 ppm was recommended to enrich MS medium. The concentration effect on Redpearl was all variable, but 0.6 and 0.7 ppm BA was the appropriate concentration on Camarosa. The cultivar and concentration effects had same trend in S1, S2 and S3 plantlet production.

Morphological and fruit characters were not reported in detail because plants looked uniform except for a variant. No variation was detected on Camarosa fruit while a variant that had multi crown (12 crowns while other plants produced 2-3 crown) and abnormal form of fruit in each inflorescence (Figure-1) was detected in S1 Redpearl cultivars. Mohammed *et al*. (2007) also indentified a white streaked variant from Camarosa plant derived from meristem culture. The results in both

experiment with Camarosa indicated that variant allegedly caused a genetic change. Camarosa cultivar has susceptible genotypes to methylation or change due to environmental conditions such as in vitro condition. Minor changes or genetic variation will be expressed if changing tissue grown in the suitable medium into intact plant.

Somaclonal variations occur in plants regenerated from cultures that have undergone a differentiation phase. Temporary variations may be due to methylation changes in the DNA. Biswas *et al*. (2009) showed a high concentration of BAP in culture medium and increasing number of subculture successfully induced somaclonal variation. In those studies, meristem culture was most effective for induction of somaclonal variation among the tissue culture techniques adopted. This result differed with Kumar *et al*. (1999) and Mohamed (2007) experiment, which showed the mass propagation via tissue cultures of meristem clones genetically similar to the mother plants based on the RAPD detection. The sub- and supra-optimal levels of plant growth substances, especially the synthetic ones, have been associated with somaclonal variation (Martin *et al*., 2006). Even at optimal levels, long-term multiplication often may lead to somaclonal or epigenetic variations in the micropropagated plants questioning the very fidelity of their clonal nature.

In conclusion, the plantlet production intended to produce mother plant in runner production was better using BA 0.2 ppm. Induction of plantlets in BA enriched media produced more plantlets but it cannot be guaranteed to produce genetically true type plantlets.

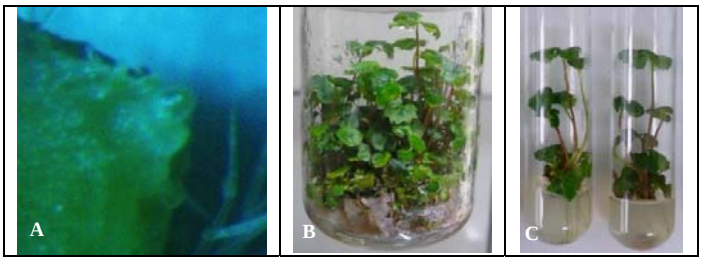

Figure-1. A. Meristem tissue with one leaf; Plantlets induced in BA (B) IBA (C) medium ± 4 months after cultured.

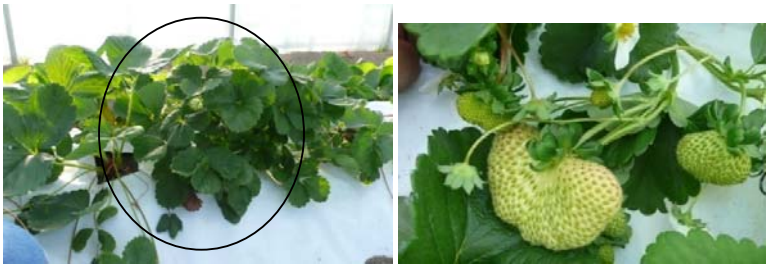

Figure-2. A variant of Redpearl with multicrown (left) and abnormal fruit form (right) plant produced in first subculture of 0.7 ppm BA.

Table-1. Plantlet characters of Camarosa and Redpearl cv. in different concentrations of IBA.

Cultivars	IBA Concentration	Leaves number	Plant height (cm)	Root length (cm)	Root number
Camarosa		14.2 x	5.9 x	3.4 x	14.7
Redpearl		9.3 y	2.9 z	4.4 z	14.6
	0.2	12.8 p	4.4	4.2 p	18.1 p
	0.3	12.0 pd	4.5	4.1 p	16.1 q
	0.4	11.5 pq	4.6	3.9 p	12.7 r
	0.5	12.0 pq	4.5	4.1 p	13.1 r
	0.6	11.1 q	4.6	3.1 q	13.3 r
Interaction	ns	*	*	*	*
	0.2	14.3 b	5.7 bc	3.6 bc	17.7 ab
Camarosa	0.3	16.5 a	6.3 a	3.1 c	17.7 ab
	0.4	13.5 b	5.5 c	3.8 bc	13.0cd
	0.5	13.8 b	5.9 abc	3.4 bc	11.5 d
	0.6	13.8 b	6.1 ab	3.1 c	15.2 bc
	0.2	11.2 c	3.1 d	4.9 a	18.4 a
Redpearl	0.3	9.0 d	3.2 d	4.8 a	14.5 c
	0.4	8.9 d	3.3 d	4.1 ab	12.4 cd
	0.5	10.1 dc	3.1 d	4.7 a	14.7 c
	0.6	5.5 e	1.4 e	3.0 c	5.5 e

Note: Means in column having different letters are significantly different according to LSD test (between cultivars) and Duncan's Multiple Range Test (Between concentration IBA or treatment combination) at α=5%.; * = significantly interaction

Table-2. Characters of BA induced plantlets (S0) of Camarosa and Redpealr cultivars growing in rooting medium with different concentrations of IBA.

Cultivars	IBA Concentration	Plant height (cm)	Root length (cm)	Leaves number (cm)	Root number
Camarosa		4.8 y	4.5 x	7.8	10.9 x
Redpearl		2.4 z	5.7 z	7.6	9.6 y
	0	4.1 p	5.0 q	7.5 qr	11.3 p
	1	3.7 q	5.1 q	7.8 pq	10.0 q
	2	3.5 q	4.9 q	8.2 p	9.9 q
	3	3.2 r	5.5 p	7.2 r	10.0 q
Cultivar*IBA		+	+	+	+
	0	5.4 a	5.2 dc	7.3 c	11.7 a
Camarosa	1	4.9 b	4.7 de	8.5 a	11.2 ab
	2	4.8 b	4.0 f	8.2ab	11.2 ab
	3	4.1 c	4.3 ef	7.1 c	9.6 c
	0	2.8 d	4.8 de	7.6 bc	10.8 b
Redpearl	1	2.6 ed	5.3 c	7.2 c	8.8 d
	2	2.1 f	5.9 b	8.2 ab	8.7 d
	3	2.2 ef	6.6 a	7.3 c	10.5 b

Note: Means in column having different letters are significantly different according to LSD test (between cultivar) and Duncan's Multiple range test (Between concentration BA or treatment combination) at α=5%.; +/- = significantly/not interaction

Table-3. S1-S3 plantlet characters of Camarosa and Redpearl cultivars produced in different BA concentration.

Cultivars	**Concentration**	**Plantlet height** (cm)			**Root length** (cm)			**Leaves number**			**Roots number**		
		S1	S2	S3	S1	S2	S3#	S1	S2	S3	S1	S2	S3#
Camarosa		4.9 x	6.1	5.0a	3.9 x	3.5 b	6.2	7.5	10.3 b	11.2	12.6 y	10.3 b	11.2
Redpearl		3.4 y	5.6	4.3q	5.7 y	5.5 a	5.5	7.4	8.6 b	5.1 b	16.7 x	16.7 a	13.0
	0.5	3.9 q	6.1 q	3.9	4.9 p	5.0 p	5.4 q	7.5 r	9.8 p	5.8 pq	13.7 q	14.0 pq	11.3 q
	0.6	5.1 p	7.3 p	5.4	5.0 p	5.0 p	6.8 p	7.8 q	10.0 p	6.6 p	15.6 p	15.0 pq	12.6 pq
	0.7	3.9 q	5.7 q	5.6	4.3 q	5.4 p	7.1 p	8.3 p	7.7 q	6.4 p	15.3 p	17.0 p	15.6 p
	0.8	4.0 q	5.0 q	3.2	5.1 p	4.2 q	5.0 q	7.1 s	9.1 p	5.0 q	14.4 pq	12.2 q	9.7 q
	0.9	4.1 q	5.1 q	5.2	4.6 q	4.1 q	5.1 q	6.6 t	9.5 p	5.3 q	13.7 q	12.8 q	11.1 q
Cultivars*concentrations		+	+	+	+	+	+	+	+	+	+	+	+
Camarosa	0.5	4.8 bc	7.5a	4.3bcde	3.4 f	3.9c	5.7bc	7.5 c	10.0bc	7.0ab	11.0 e	12.2bc	9.2bcd
	0.6	5.7 a	7.1ab	5.1bc	3.8 e	3.8c	5.6bc	7.1 cd	11.5a	6.7ab	12.9 de	12.2bc	10.2bcd
	0.7	5.1 b	-	7.0a	3.8 e	-	6.4ab	9.1 a	-	7.5a	14.6 cd	-	14.5abc
	0.8	4.1 d	4.9 c	3.4de	4.6 d	3.4c	4.3c	7.5 c	9.5bcd	5.7bcd	11.6 e	6.5d	8.5d
	0.9	4.7 bc	4.8 c	5.7abc	4.0 e	3.0c	5.7bc	6.5 e	10.5ab	6.0bcd	12.8 de	10.5c	13.5bcd
Redpearl	0.5	3.0 f	4.6 c	3.5de	6.4 a	6.0a	5.1bc	7.4 c	9.7bcd	4.7de	16.5 abc	15.7ab	13.5abcd
	0.6	4.6 c	7.5 a	5.7ab	6.3 a	6.1a	7.9a	8.5 b	8.5de	6.5abc	18.3 a	17.7a	15.0ab
	0.7	2.3 g	5.7bc	4.2cde	5.1 c	5.4ab	7.7a	7.3 c	7.7e	5.2cde	16.2 abc	17.0a	16.7a
	0.8	3.9 d	5.1 c	3.1e	5.6 b	5.3b	5.7bc	6.8 de	8.7cde	4.2e	17.2 ab	18.0a	11.0bcd
	0.9	3.4 e	5.2 c	4.8bcd	5.2 c	4.8b	4.6bc	6.7 e	8.5de	4.7de	15 bcd	15.2ab	8.7cd

Note: Means in column having different letters are significantly different according to LSD test (between cultivar) and Duncan's Multiple range test (Between concentration BA or treatment combination) at (=5%.; # = root did not be cut when sub culture for intact plantlet induction; + = significantly interaction

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