The Micropropagation *Begonia boliviensis* Crackling Fire© Series©

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In this research we used TDZ (0.1, 1.0, 10 μM), 4CPPU (0.1, 1.0, 10 μM), BAP (0.1, 1.0, 10 μM) with NAA (0.0, 0.1 μM) to test the formation ability of adventitious buds, and we found that NAA (0.1 μM) is essential for the formation of adventitious buds. For the explants, stem segment, petiole section, leaf lamina and pedicel section were chosen, and we found that stem segment had the highest formation ability of adventitious buds. Comparing different cytokinins and those concentrations with NAA, we found the index of adventitious bud formation in ½N MS medium containing TDZ (10 μM) and NAA (0.1 μM) was the highest, so we considered that TDZ (10 μM) and NAA (0.1 μM) is optimal for the formation of adventitious buds for *Begonia* Crackling Fire® series.

INTRODUCTION

The *Begonia* genus which contains about 1400 different species is a perennial herb with soft succulent stems. *Begonia* 'Crackling Fire®' series (*Begonia boliviensis* var. *sunjiraore*) bred by Suntory Flowers Ltd., contains short internodes, showy and naturally compact flowers, with a much higher flower count. However, most axillary buds differentiate into flower buds in this series. Vegetative propagation, such as cuttings, cannot provide high propagation efficiency and seed propagation cannot maintain phenotypic stability. In this study, we tried to develop an efficient micropropagation system for *B. boliviensis* Crackling Fire® series.

MATERIALS AND METHODS

Plant Materials and Sterilization

Plants of *B. boliviensis* Crackling Fire® Orange, Creamy Yellow, Pink, and White growing in pots were collected in May 2013 from Suntory Flowers's greenhouse.

Stem segment (2 mm), petiole section (2 mm), leaf lamina (2×2 mm), and pedicel section (2 mm) were used as explants. After washing with neutral detergent and rinsing, the explants were surface sterilized with ethanol (70%, 60 s) and then sterilized by 10 min immersion in 1% sodium hypochlorite solution (NaClO) with a drop of Tween 20 in 100 ml NaClO solution. They were rinsed four times with sterile water then transferred to the culture media.

Media Preparation

Murashige and Skoog (MS) medium was chosen as the inorganic formulation with half strength nitrogen content of the original recipe (½N MS). The media were supplemented with sucrose (30 $g \cdot L^{-1}$) and gelling agent (agar, 7 $g \cdot L^{-1}$), and pH was adjusted to 5.8 by NaOH and HCl. Growth regulators were added and then the media were autoclaved (120°C, 15 min).

Experiment 1: Formation of Adventitious Buds by Different Explants on Various Propagation Media

Explants were placed in the ½N MS medium with different concentrations and combination of thidiazuron (TDZ; 0.1, 1.0, 10.0 μM), N-(2-chloro-4-pyridyl)-N'-phenylurea (4CPPU; 0.1, 1.0, 10.0 μM), and 6-benzylaminopurine (BAP; 0.1, 1.0, 10.0 μM) with

α-naphthaleneacetic acid (NAA; 0.0, 0.1 μM). Each treatment involved 10 replications. There are 20 treatments in all (Table 1). Observation was conducted 6 weeks later after culturing.

Table 1. The growth regulators and concentrations for treatments.

Treatment	TDZ (μM)	4CPPU (μM)	BAP (μM)	NAA (μM)
A	–	–	–	–
B	0.1	–	–	–
C	1.0	–	–	–
D	10.0	–	–	–
E	–	0.1	–	–
F	–	1.0	–	–
G	–	10.0	–	–
H	–	–	0.1	–
I	–	–	1.0	–
J	–	–	10.0	–
K	–	–	–	0.1
L	0.1	–	–	0.1
M	1.0	–	–	0.1
N	10.0	–	–	0.1
O	–	0.1	–	0.1
P	–	1.0	–	0.1
Q	–	10.0	–	0.1
R	–	–	0.1	0.1
S	–	–	1.0	0.1
T	–	–	10.0	0.1

Experiment 2: Formation of Adventitious Buds, Leaf Number, and Maximum Leaf Length on Various Propagation Media

Multiple shoots obtained in Expt. 1 were cut into blocks (4 × 4 mm). All explants were placed in the ½N MS medium with TDZ (10 μM) or 4CPPU (0.01, 0.1 μM) with NAA (0.1 μM), and we set the number of treatments as follows: (A) TDZ (10 μM) with NAA (0.1 μM), (B) 4CPPU (0.1 μM) with NAA (0.1 μM), (C) 4CPPU (0.01 μM) with NAA (0.1 μM). Each treatment involved 15 repetitions. After 6 weeks observation was conducted.

RESULTS AND DISCUSSION

Experiment 1: Formation of Adventitious Buds by Different Explants on Various Propagation Media

For observation we set the indexes for counting adventitious buds, "0" means no buds, "1" means few, "2" means some, "3" means many, "4" means a large number of buds.

Almost no adventitious buds were observed on media without NAA (Fig. 1). The effectiveness of TDZ (10 μM) with NAA (0.1 μM) was remarkable, and index of adventitious bud formation reached 0.79. Reducing the TDZ concentration reduced adventitious buds formation. High cytokinin concentration with NAA was more effective than cytokinin used alone. In vitro regeneration of four *Begonia* genotypes, *B.*

Semperflorens Cultorum Group, *B. rex*, *B. elatior*, and a hybrid of *Begonia* with unknown parents 'Tiger' was carried out from leaf and petiole segments as explants (Espino et al. 2004). It was mentioned that shoot regeneration was preferentially induced on media containing BAP; quantitative differences being observed among explants and genotypes. However in our research, BAP was not effective for the formation of adventitious buds (Fig. 1). We feel that the reason may be related to genetic differences. Mendi et al. (2009) mentioned that cytokinins are often used to stimulate growth and development. Cytokinins promote cell division when it added together with auxins. With the decreasing concentration of 4CPPU, the formation of adventitious buds was increasing, so we think lower concentration of 4CPPU can induce better formation of adventitious buds.

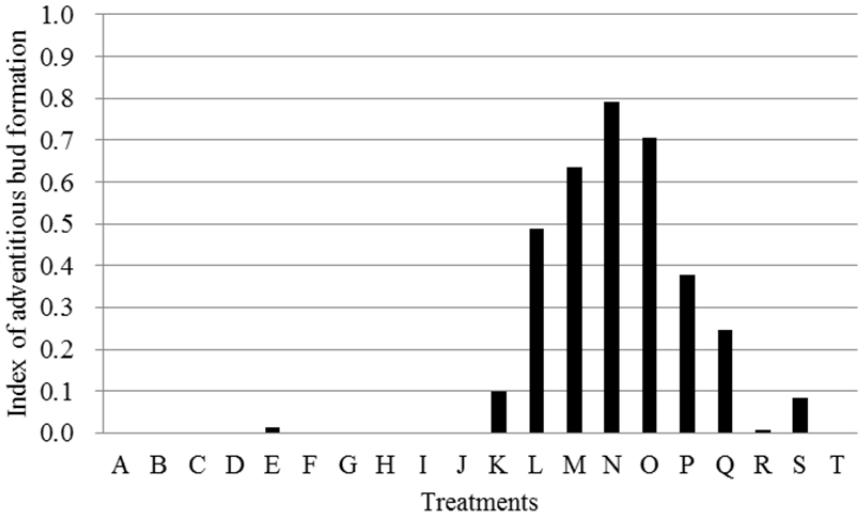

Fig. 1. Formation of adventitious buds on various treatments.

It is clear that only stem segments and petiole sections could produce adventitious buds (Fig. 2). Stem segments especially showed a higher index than petiole sections and the index reached 0.48, when we compared only stem segments and petiole sections in different concentration of cytokinins. Stem segments on medium containing with TDZ (10 μM) and NAA (0.1 μM) showed highest index of adventitious bud formation (Fig. 3). Therefore, we considered stem segment to be the optimal explant for the formation of adventitious buds in the *B. boliviensis* Crackling Fire® series.

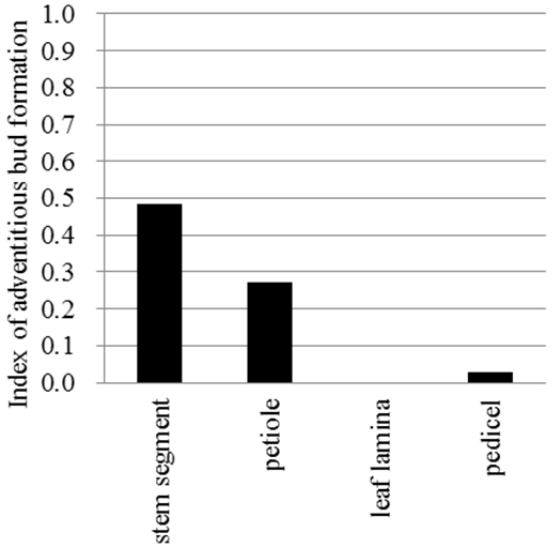

Fig. 2. Formation of adventitious buds from different explants.

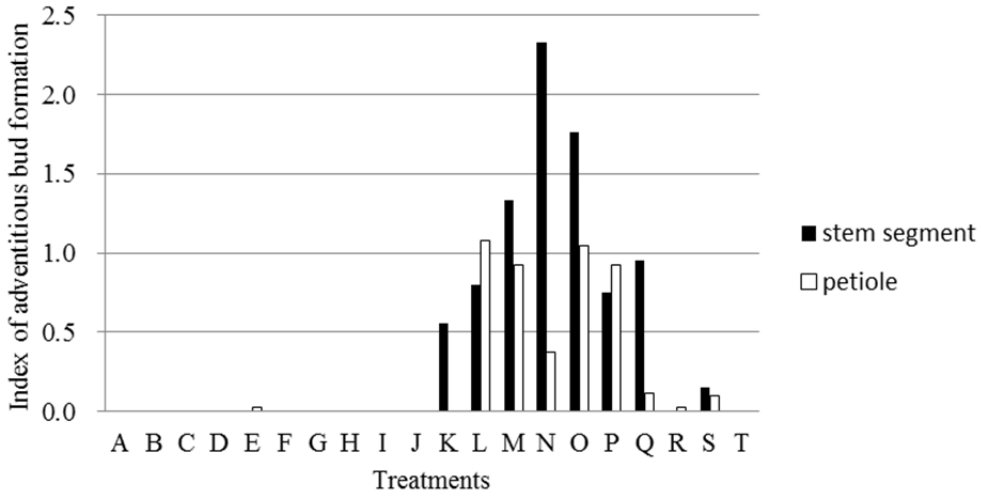

Fig. 3. Formation of adventitious buds from stem segment and petiole section on various treatments.

Experiment 2: Formation of Adventitious Buds, Leaf Number and Maximum Leaf Length on Various Propagation Media. Considering the results from Expt. 1, in Expt. 2 we chose three media to confirm the hypothesis of Expt. 1 that lower concentration of 4CPPU may promote adventitious bud formation. In comparison with Treatment C, the index of adventitious bud formation in Treatment B was higher (Fig. 4), so lower concentration of 4CPPU (0.01 μM) could not induce higher formation of adventitious buds. In the medium containing TDZ (10 μM) and NAA (0.1 μM), explants differentiated many adventitious buds with small leaves (Figs. 5, 6, and 7), and it was clear that that medium was the most effective for the formation of adventitious buds and was a suitable medium for micropropagation of *B. boliviensis* Crackling Fire® series.

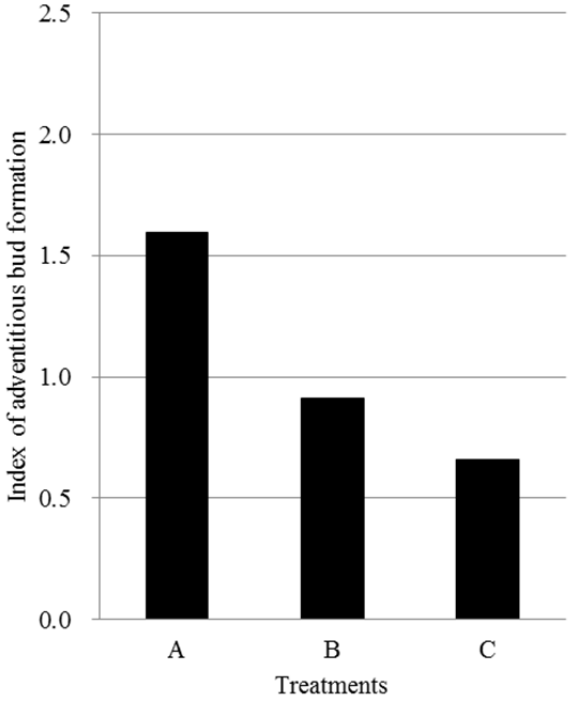

Fig. 4. Formation of adventitious buds on different treatments: (A) TDZ (10 μM) with NAA (0.1 μM), (B) 4CPPU (0.1 μM) with NAA (0.1 μM), and (C) 4CPPU (0.01 μM) with NAA (0.1 μM).

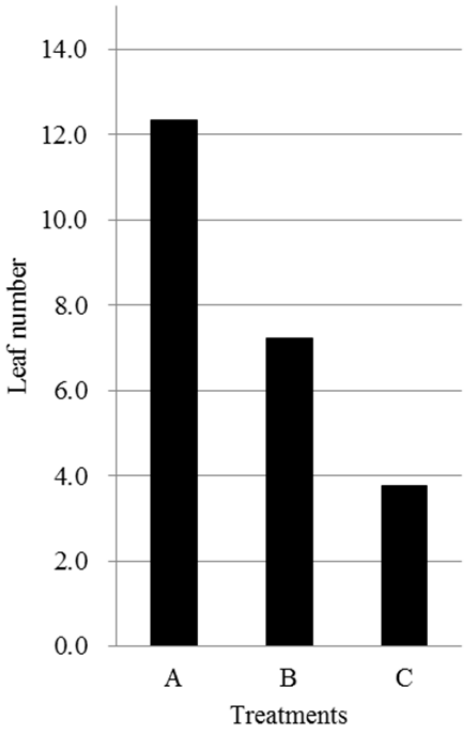

Fig. 5. Leaf number in different treatments (A) TDZ (10 μM) with NAA (0.1 μM), (B) 4CPPU (0.1 μM) with NAA (0.1 μM), (C) 4CPPU (0.01 μM) with NAA (0.1 μM).

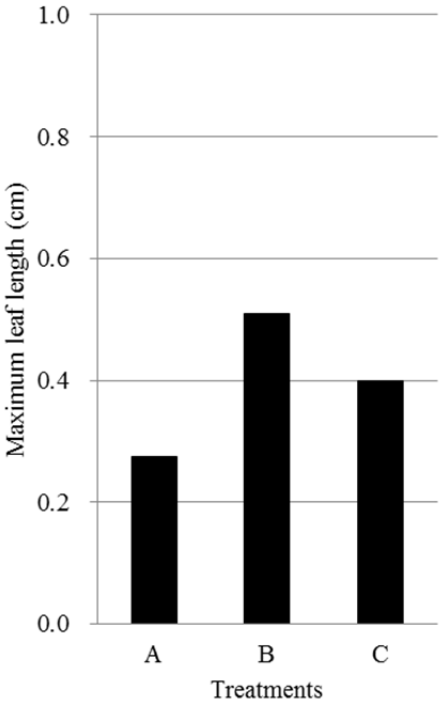

Fig. 6. Maximum leaf length in different treatments: (A) TDZ (10 μM) with NAA (0.1 μM), (B) 4CPPU (0.1 μM) with NAA (0.1 μM), (C) 4CPPU (0.01 μM) with NAA (0.1 μM).

Fig. 7. Growth situation of Experiment 2 after 6 weeks: (A) TDZ (10 μM) with NAA (0.1 μM), (B) 4CPPU (0.1 μM) with NAA (0.1 μM), and (C) 4CPPU (0.01 μM) with NAA (0.1 μM).

Although explants on media containing 4CPPU (0.1, 0.01 μM) and NAA (0.1 μM) developed a few adventitious shoots, leaves were larger than those on Treatment A (TDZ (10 μM) with NAA (0.1 μM)). Especially the maximum leaf length was the longest in medium contained 4CPPU (0.1 μM) (Fig. 6), and there was a large number of roots in media containing 4CPPU (Fig. 7). So, micropropagated bud on medium containing with TDZ (10 μM) and NAA (0.1 μM) were able to make elongate shoots, leaves, and roots by transplanting to medium containing 4CPPU (0.1 μM) and NAA (0.1 μM), and it was able to make plantlets for acclimation.

Literature Cited

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