# Effects of Cytokinins on Multiplication and Rooting of Micropropagated Shoots of *Spathiphyllum*

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**Using micropropagated shoots of *Spathiphyllum*, the effects of cytokinins, including new types which were recently introduced into micropropagation, on shoot multiplication and rooting of multiplicated shoots were examined. Phenylurea-type cytokinins (CPPU and thidiazuron) markedly promoted shoot multiplication and inhibited rooting of multiplied shoots at more than 0.1 mg liter$^{-1}$. A water soluble BA (TG-19) showed a promotive effect on shoot multiplication similar to BA. The results showed that BA was the most effective cytokinin for micropropagation of *Spathiphyllum*.**

## INTRODUCTION

Recently, new types of cytokinins, such as, N-phenyl -N$^1$- (1,2,3 - thiadiazol-5- yl) urea (thidiazuron) and N-(2-chloro-4-pyridyl)-N$^1$-phenylurea (CPPU or forchlorofenuron) (Mok et al., 1987), and one of water soluble-BA (6-benzylaminopurine), N$^6$-[2-(N-methoxy-N-methylamino) ethyl] adenine (TG-19) (Sasaki et al., 1993), were introduced to plant tissue culture. *Spathiphyllum* is one of the typical ornamental plants propagated through tissue culture. In the micropropagation system for *Spathiphyllum*, BA is the most popular cytokinin to promote shoot multiplication (Hikosaka, 1988).

In this report, the effects of these new cytokinins on the growth and rooting of *Spathiphyllum* micropropagated shoots were examined and compared to those of BA.

## MATERIALS AND METHODS

In the following experiments, micropropagated shoots of *S. wallisii* 'Merry' (syn. *S. clevelandii* 'Merry') were used. The shoots were obtained through shoot-tip culture and multiplied through subculture on Murashige and Skoog (1962) medium (MS) supplemented with 1 mg liter$^{-1}$ BA (Hikosaka, 1988). All cultures were incubated in test tubes ($\phi$26 × 120 mm) at 24C under 16-h light period (40 μmols$^{-1}$m$^{-2}$ PPFD).

**Experiment 1. Effects of Cytokinins on Shoot Multiplication.** Shoots with two unfolded leaves (3 cm long, cat 50 mg FW) and without roots were prepared from the multiplied shoots. The shoots were cultured on MS supplemented with BA, kinetin (Kin), TG-19, CPPU, or thidiazuron (TDZ) at the concentrations of 0.1, 0.5, or 1.0 mg liter$^{-1}$. After culture for 2 months, the number and fresh weight (FW) of shoots (more than 6 mm long) and roots (more than 5 mm long) of respective test tubes were recorded.

**Experiment 2. Carryover Effects of Cytokinins on Rooting and Shooting of Cultured Shoots.** Shoots with two unfolded leaves and without roots were

prepared from each of the media supplemented with different cytokinins. All were subcultured to the cytokinin-free MS medium. At 4 weeks after subculture, percentages of rooting were recorded. After culture for 6 weeks on cytokinin-free medium, the cultured shoots were potted into plastic pots containing Metromix 360. At 4 weeks after potting, the number of shoots longer than 10 mm were recorded.

## RESULTS

**Experiment 1. Effects of Cytokinins on Shoot Multiplication.** Total FW increased with the increased concentration of cytokinins (Fig. 1). The largest value of total FW, FW of shoots, and the number of multiplied shoots was obtained on the

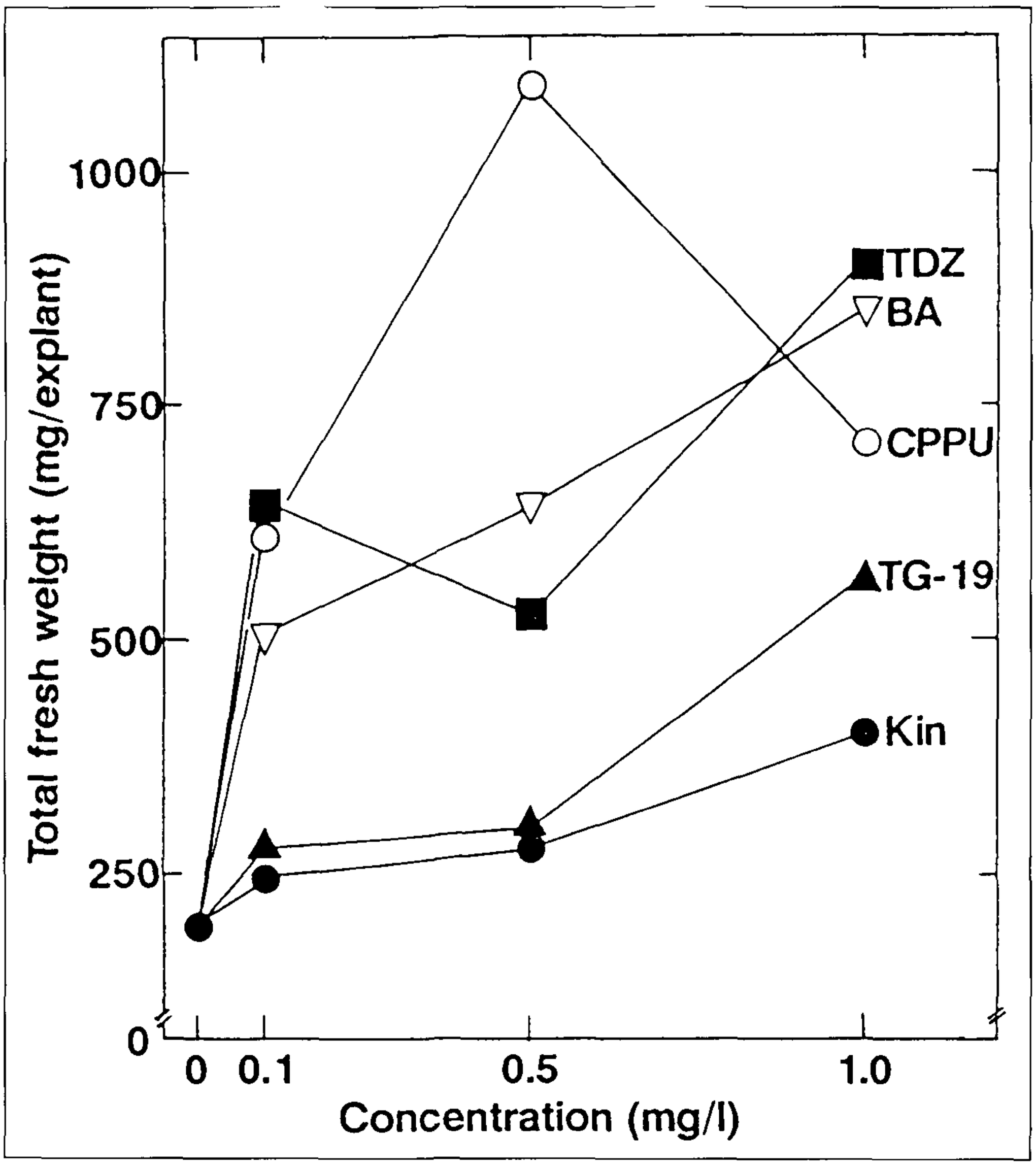


**Figure 1.** Total fresh weights of *Spathiphyllum* shoots after culture for 2 months on media supplemented with different cytokinins. Shoots with two unfolded leaves and without roots were used as explants. The average fresh weight of explants (initial shoot) was 50 mg.

medium supplemented with 0.5 mg liter$^{-1}$ CPPU. On the other hand, shoots grew without shoot multiplication on media supplemented with 0.1 or 0.5 mg liter$^{-1}$ KIN, and 0.1 mg liter$^{-1}$ TDZ as in the control (cytokinin-free medium). The average FW per shoot, multiplied on the medium supplemented with 0.5 mg liter$^{-1}$ BA, was 51.7 mg, the largest result on the media examined. The effects of TG-19 on shoot multiplication were almost the same as those of BA (Table 1).

**Table 1.** Effects of cytokinins on growth and multiplication of shoots during the multiplication stage in *Spathiphyllum wallisii* 'Merry'.

| Cytokinin | Conc. (mg liter$^{-1}$) | Total FW of shoots per explant (mg per explant) | Multiplied shoots per explant: Number | Multiplied shoots per explant: Average FW (mg per shoot) |
|---|---|---|---|---|
| Control | 0 | 148.0±15.7 | 0 | 0 |
| BA | 0.1 | 288.8±29.4 | 1.5±0.6 | 11.4±3.31 |
| | 0.5 | 322.8±85.5 | 2.8±1.2 | 51.7±6.18 |
| | 1.0 | 350.0±56.4 | 7.5±1.7 | 23.6±3.42 |
| TG-19 | 0.1 | 183.3±25.1 | 1.3±0.4 | 14.6±4.75 |
| | 0.5 | 197.3±25.5 | 2.3±1.1 | 26.7±10.5 |
| | 1.0 | 344.0±55.1 | 6.3±1.6 | 26.7±3.74 |
| Kinetin | 0.1 | 173.3±7.54 | 0 | 0 |
| | 0.5 | 171.8±13.0 | 0 | 0 |
| | 1.0 | 230.0±33.6 | 1.0±0 | 28.3±6.53 |
| CPPU | 0.1 | 253.5±20.4 | 3.8±1.6 | 23.3±10.7 |
| | 0.5 | 522.8±166 | 18.3±5.9 | 18.5±3.41 |
| | 1.0 | 179.3±53.3 | 3.3±1.7 | 22.2±7.30 |
| TDZ | 0.1 | 122.0±14.0 | 0 | 0 |
| | 0.5 | 168.8±25.8 | 1.0±0.6 | 22.8±17.2 |
| | 1.0 | 274.5±65.9 | 9.5±3.1 | 19.7±2.25 |

**Experiment 2. Carryover Effects of Cytokinins on Rooting and Shooting of Cultured Shoots.** BA and KIN showed a promotive effect on root formation, at 0.1-1.0 mg liter$^{-1}$ and at 1.0 mg liter$^{-1}$, respectively. However, TDZ completely inhibited root formation (Fig. 2 and 3). CPPU also inhibited root formation at 1 mg liter$^{-1}$, but promoted it at 0.1 mg liter$^{-1}$ (Fig. 2).

As shown in Table 2, the rooting of shoots multiplied on media supplemented with CPPU and TDZ at 0.5 and 1.0 mg liter$^{-1}$ was obviously delayed. The number of shoots derived from media supplemented with CPPU and TDZ at 1 month after potting was more than those from other cytokinins. Shoots from media supplemented with 1.0 mg liter$^{-1}$ of CPPU and TDZ showed strong after-effects on shoot multiplication even after potting (they produced 6.8 and 3.4 shoots, respectively).

**Table 2.** Effects of applied cytokinins during the multiplication stage on rooting after transplanting to a cytokinin-free medium and shooting after potting in *Spathiphyllum wallisii* 'Merry'.

| Cytokinin | Conc. (mg liter$^{-1}$) | Rooting (%) of multiplied shoot on cytokinin-free medium (after 1 month) | Number of shoots after 1 month from potting |
|---|---|---|---|
| Control | 0 | 100 | 1.0±0 |
| BA | 0.1 | 66.7 | 1.0±0 |
| | 0.5 | 80.0 | 1.0±0 |
| | 1.0 | 100 | 1.1±0.1 |
| TG-19 | 0.1 | 71.4 | 1.1±0.1 |
| | 0.5 | 100 | 1.1±0.1 |
| | 1.0 | 100 | 1.3±0.2 |
| Kinetin | 0.1 | 66.7 | 1.0±0 |
| | 0.5 | 75.0 | 1.0±0 |
| | 1.0 | 100 | 1.1±0.1 |
| CPPU | 0.1 | 100 | 1.4±0.2 |
| | 0.5 | 40.0 | 2.5±0.5 |
| | 1.0 | 30.0 | 3.4±0.4 |
| TDZ | 0.1 | 70.0 | 1.8±0.4 |
| | 0.5 | 0 | 3.3±0.6 |
| | 1.0 | 0 | 6.8±0.6 |

## DISCUSSION

Since Fonnesbech and Fonnesbech (1979) showed the promotive effect of cytokinins on the micropropagation of *Spathipyllum* using PBA (a tetrahydropyranyl derivative of BA), commercial tissue-culture laboratories have generally utilized cytokinins to multiply *Spathiphyllum*. Chu and Kurtz (1990) described micropropagated *Spathiphyllum* as characterized by a greater degree of basal branching when the culture medium was supplemented with cytokinins, and they called the effect the "carryover effect". However, BA also showed an inhibitory effect on rooting at more than 2.5 mg liter$^{-1}$ BA (Werbrouck and Debergh, 1995). Hikosaka (1988) pointed out that the optimal concentration of BA to multiply shoots and not to inhibit rooting in *Spathiphyllum* shoot-tip culture was 1.0 mg liter$^{-1}$.

TG-19 is characterized by its high solubility in water, while it has a comparable cytokinin activity to that of BA (Maruyama et al., 1993). In the present experiments, 1 mg liter$^{-1}$ TG-19 also showed similar effects on shoot multiplication to that of BA and uninhibited rooting of plantlets after potting.

By contrast, TDZ, one of phenylurea type, showed a strong inhibitory effect on rooting during in vitro culture and even after transplanting to pots. CPPU also had an inhibitory effect on rooting, but to a lesser degree than TDZ. Henny (1995) reported that *Spathiphyllum* plants given a soil drench of TDZ at 4 to 10 mg liter$^{-1}$ had more than double the number of shoots without inhibition of root growth in a glasshouse. At present, there is no known reason for the difference in response to TDZ between in vitro plantlets and intact plants. However, it is known that

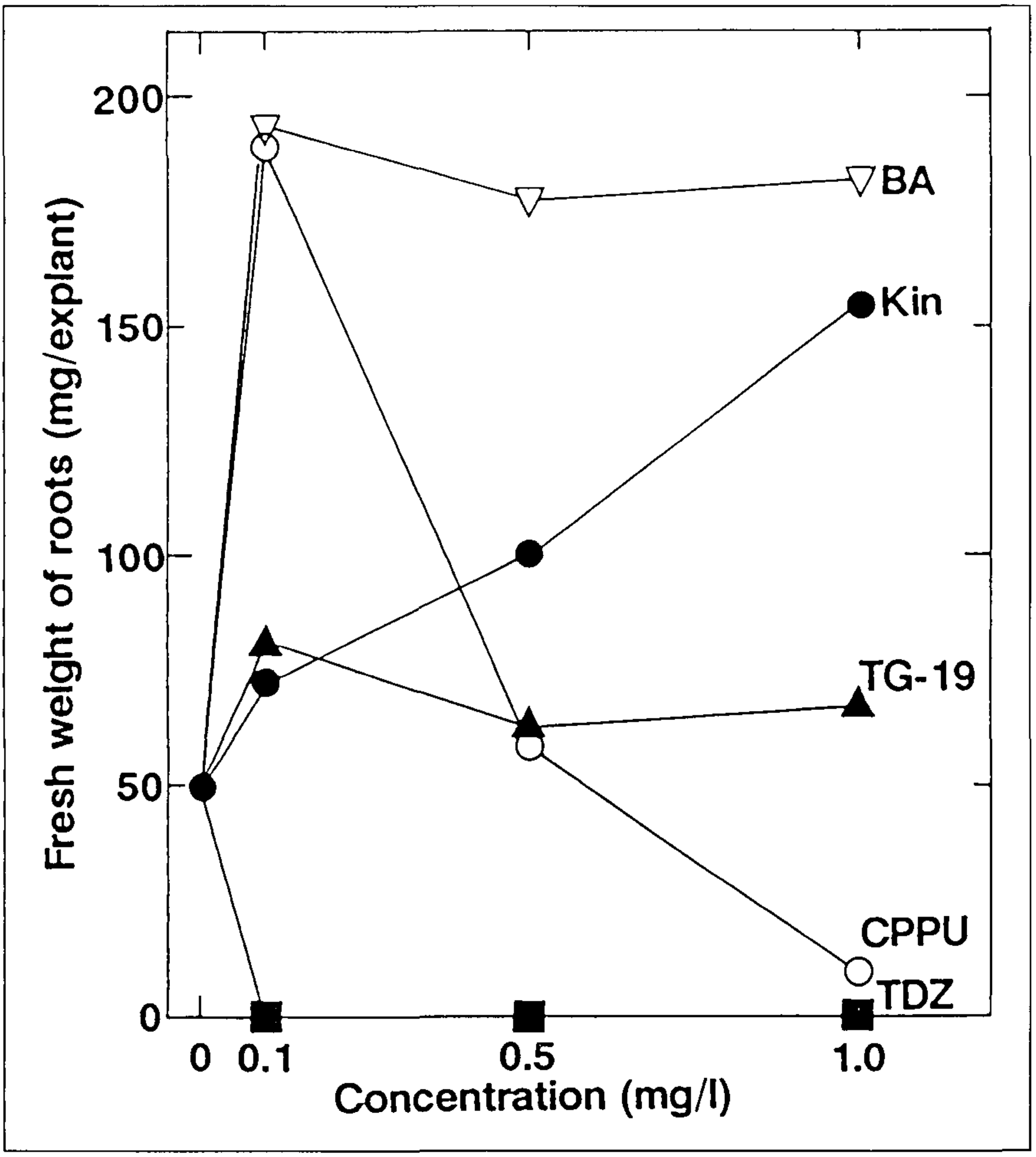


**Figure 2.** Root fresh weights of *Spathiphyllum* shoots after culture for 2 months on media supplemented with different cytokinins. Shoots with two unfolded leaves and without roots were used as explants.

*Spathiphyllum* plantlets take BA rapidly from the culture medium and accumulate large amounts of BA in the stem tissue when they are cultured on a medium containing 5 mg liter$^{-1}$ BA (Werbrouck et al., 1995). It seems that TDZ is also accumulated in the *Spathiphyllum* plantlets at multiplication stage, and the accumulation could cause the carryover effect, that is, the strong inhibition of rooting after potting.

In conclusion, the effects of TG-19 on multiplication and rooting of multiplied shoots were similar to those of BA, and the carryover effect of the urea-type cytokinins inhibited rooting after potting. Such a carryover effect is undesirable for the practical production of potted *Spathiphyllum* plants.

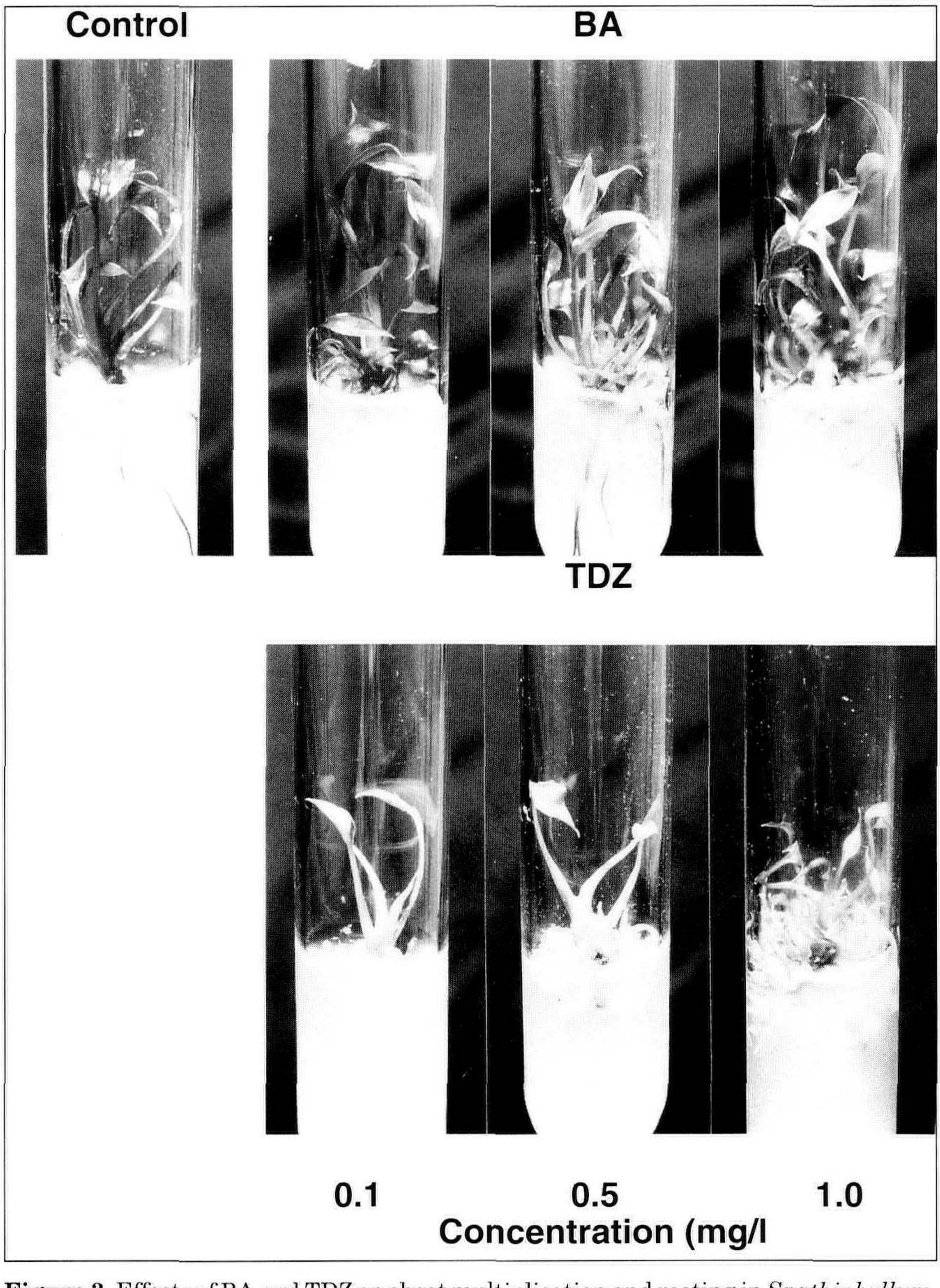


**Figure 3.** Effects of BA and TDZ on shoot multiplication and rooting in *Spathiphyllum*. Photographs were taken after 2 months of culture.

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