**Original paper**

**IMPROVEMENT OF ROOTING IN SEMI-HARDWOOD *Nandina* *domestica* STEM CUTTING AS INFLUENCED BY EXOGENOUS IBA AND NAA AUXINS**

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**Abstract**

Here, we present a strategy for propagation of *Nandina domestica,* a semi-hardwood ornamental and medicinal plant, in presence of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA), which enables to stimulate the adventitious roots on stem cuttings. 1000 mg.L-1 IBA + 1000 mg.L-1 NAA significantly enhanced rooting of cuttings and subsequent growth.

**Key words:** Berberidaceae, *Nandina domestica* Thunb., ornamental plant, plant growth regulators, propagation

**Abbreviations:** IBA; indole-3-butyric acid, NAA; naphthalene acetic acid

**INTRODUCTION**

*Nandina domestica* (Berberidaceae) is a flat-topped shrub that can spread by root suckers (Dirr 1990, Whitcomb 1996). This species is an important ornamental and medicinal plant (Dirr 1990, Li et al. 2007). *Nandina* is propagated by seeds, rooted cuttings and plantlets from tissue culture. Seeds exhibit delayed germination due to a rudimentary embryo and slow rate of embryo development (Dirr and Heuser 1987), thus commercial propagation of *Nandina* is accomplished typically by vegetative means (Hartmann et al. 1997). Propagation through stem cuttings is most commonly used, that can be rooted any time of year except during the spring flush (Barr 1987, Hartmann et al. 1997). Successful rooting of *Nandina* cuttings can be achieved by the use of different auxins (Barr 1987, Dirr and Henser 1987). The rooting property of auxins has been widely recognized for long time (Thiman and Went 1934). The necessity of exogenous auxin application to stimulate root formation in cuttings has been reported in many species (Bashir et al. 2009, Berthon et al. 1990, Caboni et al. 1997, Edson et al. 1996, Fett-Neto et al. 2001, Greenwood and Weir 1994, Hashemabadi and Sedaghathoor 2006, Sharifi and Ebrahimzadeh 2010). Indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA), alone or in combination, are the most widely used auxins to rooting of stem cuttings (Griffin et al. 1999, Hartmann 1990, Hilaire 2003, Qaddoury and Amssa 2004, Weisman et al. 1988). The rooting ratio of hardwood and semi-hardwood cuttings was increased by IBA and NAA (Bashir et al. 2009, Cao and Gao 2003, Griffin and Schroeder 2004, Griffin et al. 1999, Qaddoury and Amssa 2004, Pair 1992, Singh et al. 2003, Zhou 2002). Rooting of *Nandina domestica* with semi-hardwood stem cuttings is not proper due to the deficit of root inductive materials. Therefore, the objective of current study was to investigate the influence of different concentrations of IBA and NAA on adventitious rooting of stem cuttings of *Nandina domestica*.

**MATERIALS AND METHODS**

Studies were carried out at a research greenhouse located in the suburbs of Amol city, Mazandaran province, in the northern part of Iran, during the year 2010-2011. Semi-hardwood cuttings of *Nandina domestica* containing at least 3-4 nodes was taken from high quality stock plants grown in a greenhouse in the month of March. The size of cuttings was 15 to 20 cm long and 5-8 mm in diameter. The experimental design was a randomized completely blocks design (RCBD) with a factorial arrangement of treatments containing of four IBA concentrations × four NAA concentrations × three replications × ten cuttings per treatment. Basal leaves were removed and the bottom of stem cuttings were inserted into a fungicide (1 mg/L) purchased from Moshkfam Co., Shiraz, Iran, for 10 min. Following fungicide treatment, bottom of stem cuttings were treated for 20 sec. with 0, 1000, 2000 and 4000 mg.L-1 of IBA in interaction with 0, 1000, 2000 and 4000 mg.L-1 of NAA. The treated stem cuttings were planted on sand and perlite (3:1 v/v) at a depth of 3-5 cm and kept in a greenhouse at 20°C. Stem cuttings were maintained under natural photoperiod and irrigated daily. After six weeks (on May, 2011), stem cuttings were harvested and data recorded. Data included rooting percentage, length of roots, diameter of roots, and number of roots, fresh weight and dry matter. Data in number were transformed by logarithmic transformation and arc sin (angular) transformation prior to statistical analysis. Data were subjected to analysis of variance (ANOVA) using MSTATC statistical software. Mean comparison were carried out by employing Duncan’s Multiple Range test at α = 5%.

**RESULTS**

The overall results of the effects of various concentrations of IBA and NAA on rooting percentage, length of roots, diameter of roots, and number of roots, fresh weight and dry matter are summarized in Table 1.

**Rooting percentage**

The results obtained from the effect of IBA, NAA and IBA × NAA on rooting percentage revealed the best concentration of these two plant growth regulators is 1000 mg.L-1 (Table 1). The stem cuttings treated with 1000 mg.L-1 IBA × 1000 mg.L-1 NAA led with maximum rooting (96.67%) (Table 1). However, rooting percentage in stem cuttings treated with 1000 mg.L-1 NAA without IBA (95.67%), 1000 mg.L-1 IBA (95.00%), and 1000 mg.L-1 NAA (91.67%) was suitable (Table 1). The lowest values of rooting percentage were recorded by the concentrations of 4000 mg.L-1 IBA × 4000 mg.L-1 NAA (63.33%) and control (70.00%) (Table 1). Analysis of variance indicated that differences among different concentrations of IBA, NAA and IBA × NAA on the percentage of root formation were significant (p<0.05, p<0.01, and p<0.05, respectively) (Table 2).

**Length of roots**

The results obtained show that maximum length of roots (11.66 cm) was noted in stem cuttings treated with 1000 mg.L-1 IBA × 1000 mg.L-1 NAA (Table 1). The stem cuttings treated with 4000 mg.L-1 IBA × 4000 mg.L-1 NAA showed the lowest length of roots (1.12 cm). However, length of roots in stem cuttings treated with 2000 mg.L-1 IBA × 4000 mg.L-1 NAA (1.50 cm) as well as control (1.96 cm) were low (Table 1). Length of root was significantly (p<0.05) affected by different concentrations of IBA, NAA and IBA × NAA (Table 2).

**Diameter of roots**

Maximum diameter of root (1.74 mm) was observed in stem cuttings treated with 1000 mg.L-1 IBA × 1000 mg.L-1 NAA (Table 1). However, diameter of root in stem cuttings treated with 1000 mg.L-1 NAA without IBA (1.59 mm) was proper (Table 1). Minimum diameter of roots (0.197 mm) was observed in stem cuttings treated with 4000 mg.L-1 IBA × 4000 mg.L-1 NAA (Table 1). Diameter of root was significantly (p<0.05) affected by different concentrations of IBA, NAA and IBA × NAA (Table 2).

**Number of roots**

As shown in Table 1, the largest (3.40) and the lowest (0.90) number of root was observed in stem cuttings treated with 1000 mg.L-1 IBA × 1000 mg.L-1 NAA and 4000 mg.L-1 IBA × 4000 mg.L-1 NAA, respectively (Table 1). Number of root in *Nandina domestica* was significantly improved by treatments of IBA, NAA and IBA × NAA (p<0.01) (Table 2).

**Fresh weight**

Stem cuttings treated with 2000 mg.L-1 IBA × 1000 mg.L-1 NAA and 1000 mg.L-1 IBA × 1000 mg.L-1 NAA had most fresh weight (0.77 and 0.71 g), respectively (Table 1). Stem cuttings treated with 4000 mg.L-1 IBA × 2000 mg.L-1 NAA and control had least fresh weight (0.06 and 0.08 g), respectively (Table 1). Data analysis showed that the effect of IBA, NAA and IBA × NAA was significant on the fresh weight (p≤0.01) (Table 2).

**Dry matter**

Stem cuttings treated with 2000 mg.L-1 IBA × 2000 mg.L-1 NAA and 2000 mg.L-1 IBA × 1000 mg.L-1 NAA had most dry matter (28.88 and 25.44%), respectively (Table 1). Stem cuttings treated with 4000 mg.L-1 IBA × 4000 mg.L-1 NAA had least dry matter (7.05%), respectively (Table 1). Data analysis showed that the effect of IBA, and IBA × NAA was significant on the dry matter (p≤0.05). No the effect of NAA on dry matter was significant (Table 2).

**DISCUSSION**

Rooting percentage of hardwood and semi-hardwood stem cuttings is poor and improves by auxins treatment (Griffin and Schroeder 2004). Auxin concentrations applied to the stem cuttings play an important role in formation of adventitious roots and subsequent growth. Our studies showed that the rooting percentage and subsequent growth of *Nandina domestica* stem cuttings affected by IBA, NAA and interaction of these two auxins. Data revealed that all measured traits were improved than that of control, when we applied proper concentrations of IBA, NAA and IBA × NAA. Many similar results were reported for other species (Bashir et al. 2009, Caboni et al. 1997, Fett-Nero et al. 2001, Griffin and Schroeder 2004, Hilaire 2003, Qaddouny and Amssa 2004). Current study demonstrated there were differences in rooting response between IBA and NAA auxins-treated and untreated stem cuttings of *Nandina domestica*. On semi-hardwood stem cuttings of *Ulmus parvifolia*, only 11% of non-treated cuttings rooted (Griffin and Schroeder 2004). Our studies revealed that the highest concentrations of IBA and NAA decreased rooting percentage and subsequent growth of *Nandina domestica* stem cuttings. Contrary to our results, Griffin and Schroeder (2004) showed the greatest rooting percentage of *Ulmus parvifolia* stem cuttings occurred with IBA concentrations ≥ 10,000 ppm. Mean number of roots increased in a linear fashion with increasing IBA concentration (Griffin and Schroeder 2004). Also, Bashir et al. (2009) and Lugman et al. (2004) demonstrated that the maximum values of root parameters of jojoba and guava cuttings were recorded at the highest concentration of IBA. These researchers proposed that higher concentration of auxins promoted the cell division which increased the number of roots. Similar to our findings, Hilaire (2003) showed root formation in terminal stem cuttings of catnip did not increase with higher levels of IBA, but IBA concentration had some influence on plant development. Bashir et al. (2009) showed that IBA significantly enhanced the root growth of jojoba semi-hardwood stem cuttings. Griffin et al. (1999) showed that rooting, root number and root length of semi-hardwood *Quercus* stem cuttings was unaffected by IBA concentration. Exogenous IBA had a significant positive effect on the rooting response of *Phoenix dactylifera* L. offshoot (Ahmed and Amssa 2004). Current study showed that the percent rooting and other traits for treatments of 1000 mg.L-1 IBA in combination with 1000 mg.L-1 NAA was better than those of IBA and NAA alone. Our findings are in contrast to reports of some other investigators. Studies of Griffin et al. (1999) on *Magnolia virginiana* revealed that the rooting was maximized for stem cuttings treated IBA alone. Increasing concentrations of NAA usually resulted in decreased rooting. Similar to our findings, studies of Rahman et al. (2004) on rooting of guava stem cuttings showed that the longest roots and maximum number of roots were observed in cuttings treated with NAA and IBA at 1000 ppm, while minimum length and number of roots were noticed in control.

**CONCLUSION**

In conclusion, proper concentration of IBA × NAA caused better response in rooting and subsequent growth of *Nandina domestica* stem cuttings than those of IBA and NAA alone. Thus, 1000 mg.L-1 IBA × 1000 mg.L-1 NAA could be applied to semi-hardwood *Nandina domestica* stem cuttings for rooting and subsequent growth in order to mass multiplication.

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**Table 1. Mean comparison of the effect of different concentrations of IBA and NAA on some traits of *Nandina domestica* stem cuttings.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | | Traits | |  | |  | |  | |
| Source of variations | Rooting percentage | Root No. | Root length (cm) | | Root diameter (mm) | | Fresh weight (g) | | Dry matter (%) | |
| I0 | 85.83ab | 1.94b | | 3.72b | | 0.98a | | 0.16c | | 21.84a | |
| I1 | 95.00a | 2.30a | | 6.34a | | 1.10a | | 0.49a | | 21.59a | |
| I2 | 83.33ab | 1.46c | | 3.83b | | 0.60b | | 0.37b | | 22.87a | |
| I3 | 79.17b | 1.41c | | 2.57c | | 0.49b | | 0.17c | | 12.65b | |
| N0 | 83.33bc | 1.60c | | 3.01c | | 0.70b | | 0.19b | | 20.51a | |
| N1 | 91.67a | 2.20a | | 6.82a | | 1.20a | | 0.48a | | 20.31a | |
| N2 | 85.83ab | 1.85b | | 4.23b | | 0.73b | | 0.27b | | 20.40a | |
| N3 | 77.50c | 1.50c | | 2.40c | | 0.53c | | 0.26b | | 17.70a | |
| I0N0 | 70.00cd | 1.13gh | | 1.96fg | | 0.55efg | | 0.08d | | 17.47cde | |
| I0N1 | 95.67a | 2.36b | | 5.12c | | 1.59a | | 0.29cd | | 24.20abc | |
| I0N2 | 90.00ab | 2.40b | | 4.86cd | | 0.97bc | | 0.12d | | 21.52abcd | |
| I0N3 | 86.67abc | 1.86cde | | 2.92ef | | 0.82cde | | 0.14d | | 24.17abc | |
| I1N0 | 90.00ab | 1.90cd | | 2.92ef | | 1.08b | | 0.14d | | 21.76abc | |
| I1N1 | 96.67a | 3.40a | | 11.66a | | 1.74a | | 0.71ab | | 24.75abc | |
| I1N2 | 86.67abc | 2.23bc | | 6.74b | | 0.88bcd | | 0.65ab | | 17.33cde | |
| I1N3 | 86.67abc | 2.03bcd | | 4.05cde | | 0.70cdef | | 0.48bc | | 22.50abc | |
| I2N0 | 86.67abc | 1.70def | | 3.91cde | | 0.59defg | | 0.33cd | | 25.44ab | |
| I2N1 | 83.33abc | 1.46efg | | 6.44b | | 0.72cde | | 0.77a | | 19.98bcde | |
| I2N2 | 90.00ab | 1.46efg | | 3.46de | | 0.67defg | | 0.25cd | | 28.88a | |
| I2N3 | 73.33bcd | 1.23gh | | 1.50g | | 0.42fgh | | 0.13d | | 17.18cde | |
| I3N0 | 86.67abc | 1.66def | | 3.24ef | | 0.60defg | | 0.20d | | 17.39cde | |
| I3N1 | 90.00ab | 1.80de | | 4.06cde | | 0.77cde | | 0.15d | | 12.30def | |
| I3N2 | 76.67bcd | 1.30fgh | | 1.88fg | | 0.41gh | | 0.06d | | 13.86def | |
| I3N3 | 63.33d | 0.90h | | 1.12g | | 0.20h | | 0.27cd | | 7.05f | |

In each column, means with the similar letters are not significantly different at 5% level of probability using Duncan’s Multiple Test.

**Table 2. Analysis of variance (ANOVA) for the effect of different concentrations of IBA and NAA on some traits of *Nandina domestica* stem cuttings.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | Mean squares |  |  |  |
| Source of variations | df | Rooting percentage | Root No. | Root length | Root diameter | Fresh weight | Dry matter |
| Replication | 2 | 214.58 | 0.13 | 0.63 | 0.048 | 0.072 | 17.72 |
| IBA | 3 | 247.22\* | 2.50\*\* | 30.26\* | 0.148\* | 0.316\*\* | 271.56\* |
| NAA | 3 | 413.88\*\* | 1.35\*\* | 45.94\* | 1.02\* | 0.194\*\* | 21.63ns |
| IBA × NAA | 9 | 208.33\* | 0.57\*\* | 7.99\* | 0.99\* | 0.095\*\* | 58.78\* |
| Error | 30 | 76.80 | 0.51 | 0.58 | 0.022 | 0.021 | 16.54 |
| c.v. (%) |  | 13.98 | 34.46 | 63.41 | 51.89 | 83.33 | 32.52 |

\*\*: Significant at α = 1%, \*: Significant at α = 5%, ns: Not significant