ISSN 1023-1072

Pak. J. Agri., Agril. Engg., Vet. Sci., 2015, 31 (1): 42-53

EFFECT OF BASAL APPLICATIONS OF NAPHTHALENE ACETIC ACID (NAA) ON SPROUTING AND ROOTING POTENTIAL OF STEM CUTTINGS OF BOUGAINVILLEA (*BOUGAINVILLEA GLABRA)*

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ABSTRACT

Bougainvillea is a beautiful ornamental climbing plant of tropical and subtropical regions of the world. An experiment was conducted to assess the effect of basal methods of Naphthalene acetic acid (NAA) application on sprouting and rooting potential of stem cuttings of bougainvillea. The stem cuttings of about 6 inches in length were obtained from two year old plants. The basal ends of the cuttings were treated with Naphthalene acetic acid (NAA) using basal methods of auxin application including basal quick dip method for 10 seconds and basal long soak method for 12 hours in NAA solutions viz. 2000 and 4000 mg L^{-1} plus control treatment with no NAA. Two groups were set, one group was covered with polyethylene and another left opened. The better response for mean number of sprouts (5.22) per stem cutting, sprouting percentage (67.8), rooting percentage (67.28), number of roots (20.83), fresh weight of the roots (44.47 mg) and number of leaves (27.33) was observed in response to the treatment where basal long soak method of NAA application was followed at 4000 mg L^{-1}. Chlorophyll content of leaf and electrolyte leakage of leaf was not significantly influenced by any basal method of NAA application under both uncovered and covered conditions. The covered stem cuttings exhibited more pronouncing and significant response for most of the plant traits in comparison to untreated and uncovered cuttings. The maximum sprouts per stem cutting (5.61), sprouting percentage (78.62), rooting percentage (73.33), number of roots (21.87), fresh weight of the roots (47.43 mg) and number of leaves (28.81) were recorded from covered stem cuttings.The minimum mortality percentage (14.66) was observed from covered stem cuttings.

Keywords: NAA**,** *Bougainvillea glabra,* stem cutting, rooting potential.

INTRODUCTION

Bougainvillea is an evergreen ornamental vine of tropical and subtropical areas of the world. Typically multi-trunked or with clumping stems, it has a spreading, round plant habit with a height and spread of up to 20 feet (Kobayashi *et al*.,

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2007). It is used in mass plantings, as shrubs or bushes, and as ground cover (Singh *et al*., 2011). The plant is also used as hedges, barriers, and slope coverings. For large, difficult to maintain areas, bougainvillea is an excellent ground cover. The general practice of multiplication for most of the perennial ornamental plants is by the use of vegetative plant parts including stem, leaves, terminal buds and roots due to its simplicity and practicability in developing countries (Elgimabi, 2009). Vegetative propagation of ornamental plants through stem cutting is one of the cheapest and sometimes, the only method available for multiplication. However, under normal conditions, wide variability is noticed in different cultivars of the same species; while some cultivars root easily, others are either difficult or fail to root. In bougainvillea the success of propagation by stem cutting is very limited. Under normal conditions mostly growers observed poor rooting percentage. However, the rooting responses vary from variety to variety. It is well known that the success of rooting of the woody stem cuttings, in the majority of ornamental plants and fruit trees depends mainly on the physiological stage of the mother plant (Day and Loveys, 1998), the time of planting of the cutting (Hartman and Loreti, 1965; Darwesh, 2000) and the type of growth regulators used (Rowezak, 2001).

Treatment of cuttings for ease in rooting is an old horticultural practice. In the early historic days, people used to treat cuttings with manganese, boron and phosphorus but with the discovery of auxins in 1934 for their stimulative effects on root initiation was a major milestone in the history of plant propagation. Various auxins such as Indole Acetic Acid (IAA), Indole Butyric Acid (IBA), Naphthalene Acetic Acid (NAA) and 2,4-Dichloropheoxy Acetic Acid (2,4-D) have been reported to promote rooting in cuttings of the most of the plant species (Griffith, 1998). Cuttings of some species root readily without an auxin treatment, while cuttings of other species benefit from auxin treatment through enhanced promotion of rooting; benefits may be dependent upon the species and cultivar, condition of the cutting wood, time of year, and other factors (Griffith, 1998). These auxins are generally applied to the plants using basal and foliar methods of applications. The basal methods include basal quick dip method, basal long soak method and basal dry dip method by dipping the basal end of the cuttings for few seconds, few hours in hormone solutions and sticking of the powder at basal ends of cuttings, respectively (Kroin, 2012). Commercial root promoting chemicals are normally applied to the basal portion of cuttings using a liquid or talc formulation of auxin. The quick dip method is often preferred by commercial propagators for application of liquid auxin formulations for reasons of economy, speed, ease, and uniformity of application and results. An extended basal soak method may be utilized for some difficult to root species (Hartman *et al.,* 2002). The prevailing environmental conditions at the site i.e. light, temperature and humidity play also an important role in rooting and succeeding growth of the cuttings. At present use of polyethylene for the success of rooting of the cuttings is also well reported due to increase in temperature inside the plastic that promotes callus formation of the buds and roots as reported by Pipattanawong *et al*. (2008) and Elgimabi (2009). The present study was therefore conducted to explore and compare basal methods of auxin applications with and without plastic for the success of rooting and sprouting of the cuttings of bougainvillea.

MATERIALS AND METHODS

The experiment was conducted to assess the effect of basal methods of Naphthalene acetic acid (NAA) application on sprouting and rooting potential of stem cuttings of bougainvillea. The stem cuttings of cultivar Torch glory (pink flower variety) about 6 inches in length were obtained from two year old plants and planted at Horticulture garden, Sindh Agriculture University, Tandojam during the year 2013. The basal ends of the cuttings were treated with Naphthalene acetic acid (NAA) using basal methods of rooting including basal quick dip method for 10 seconds and basal long soak method for 12 hours in NAA solutions viz. 2000 and 4000 mg L^{-1} plus control treatment with no NAA. The solutions were prepared by dissolving the NAA compound in 95% ethanol and adding distilled water. Two groups were set, one group was covered with polythelene and another left opened.The growing medium was prepared by mixing equal ratio of sand, farm manure and garden soil which was analyzed for soil texture by Bouyoucos Hydrometer method (Jackson, 1962), pH of 1:5 soil-water extract by pH meter, EC of 1:5 soil-water extract by EC meter, organic matter by Walkley-Black method. The soil was sandy loam, non-saline in nature with EC 0.93 dS m^{-1} and slightly alkaline in reaction with pH 7.8. The soil was also low in organic matter (0.71%). The mixture of the growing medium was filled in earthen pots leaving one inch space at the top. The experiment was conducted in Completely Randomized Design (CRD) replicated thrice with 10 cuttings in each replication and total 30 cuttings in each treatment. The data was recorded for number of sprouts $cutting^{-1}$,sprouting percentage $treatment^{-1}$, mortality percentage of the cuttings $treatment^{-1}$, rooting percentage $treatment^{-1}$, number of roots $cutting^{-1}$, fresh weight of the roots (mg), number of leaves $cuttings^{-1}$, chlorophyll content of leaf (mg ml^{-1}) and electrolyte leakage of leaf (%). Sprouting, mortality and rooting percentage was calculated by using following equation:

$$GP = \Sigma n/ N \times 100$$

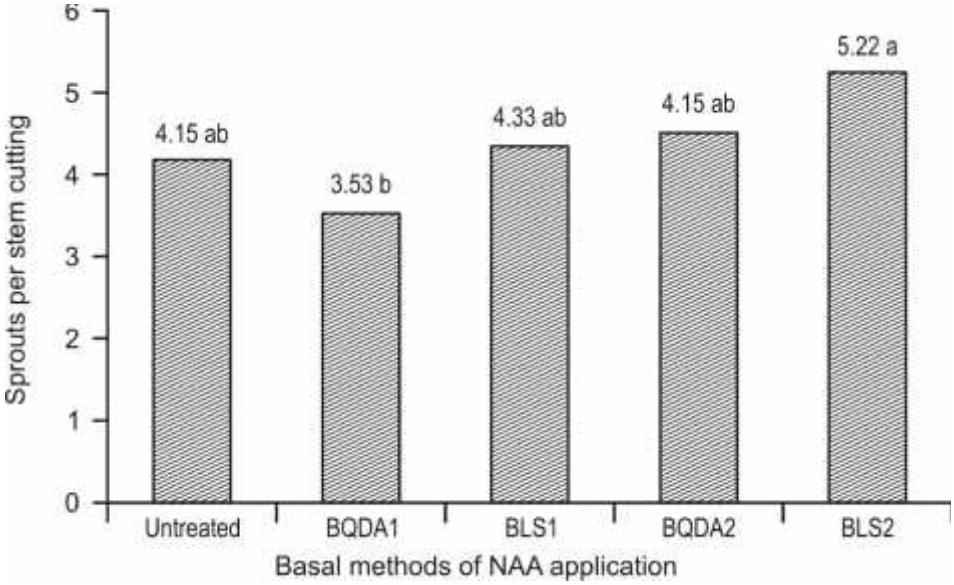
Where; n is number of sprouted cuttings at each counting and N is total cuttings sprouted in each treatment. For chlorophyll content, leaf sample of 0.5 g was grinded in 10 mL of 80% acetone and filtered with Whatman No.1 filter paper by adding more 10 ml of acetone. Samples were poured in cuvettes at 663 and 645 nm using UV-Vis spectrophotometer (Bruinsma, 1963). Electrolyte leakage of leaf was measured by taking leaf discs of size 1 cm^2 and weight 0.5 g from random samples of leaf. The leaf discs were rinsed well with deionized water prior to incubation in 25 ml of deionized water for 3 h at room temperature. After incubation, the conductivity (value A) of the bathing solution was measured with the conductivity meter. The petal discs were boiled with bathing solution for 15 min to lyse all cells. After cooling at room temperature, the conductivity (value B) of the bathing solution was again measured. The electrolyte leakage was expressed as percent value according to the formula given below:

Electrolyte leakage of leaf % = (Value A/Value B) 100

RESULTS

The Figure 1 reveals that number of sprouts per stem cutting was significantly influenced by basal methods of NAA applications. Maximum mean number of sprouts (5.22) per stem cutting was recorded from the treatment where basal long soak method of NAA application was applied at 4000 mg L^{-1}. These results are statistically similar with the results obtained from the cuttings where basal long soak method was followed for NAA application @ 2000 mg L^{-1} (4.33) and basal quick @ 4000 mg L^{-1} (4.15). Reduced response (3.53) was observed from the cuttings which were treated by quick dip method @ 2000 mg L^{-1} NAA. On the basis of un-covered and covered stem cuttings presented in Figure 2, more pronouncing results were observed from the cuttings covered with polyethylene which produced mean 5.61 sprouts per stem cutting as compared to un-covered stem cuttings (1.31). Sprouting, mortality and rooting of the stem cuttings was also significantly influenced by basal methods of NAA application (Figure 3). The maximum sprouting (67.80%) and rooting (67.28%) was observed from the cuttings where basal long soak method of NAA was applied at the concentration of 4000 mg L^{-1}. These results are at par with the results obtained from the cuttings where quick dip method of NAA was applied at the same concentration of 4000 mg L^{-1}. There was no significant effect of basal methods on the mortality of the plants.

Sprouting, mortality and rooting exhibited statistically similar response when cuttings were planted and left as un-covered. However, minimum mortality of 14.66% was observed when cuttings were covered with polyethylene sheet as compared to the results obtained from un-covered stem cuttings (45.31%). The sprouting (78.62%) and rooting (73.33%) response was also observed much better when cuttings were placed under polyethylene. The data in Table 1 showed that number of roots per cutting and fresh weight of the roots was significantly influenced by basal methods of NAA applications under un-covered and covered conditions. The covered stem cuttings exhibited maximum response for mean number of roots per cutting producing 21.87 and fresh weight of the roots (47.43 mg). However, on the basis of basal methods, basal long soak method was observed better @ 4000 mg L^{-1} NAA for potential number of roots (20.83) and fresh weight of the roots (44.47 mg), and covered stem cuttings (Figure 5). The data in Table 2 indicated that number of leaves per cutting of bougainvillea was significantly influenced by basal methods of NAA applications under un-covered and covered conditions. The stem cuttings exhibited statistically better response for number of leaves (27.33) in response to the treatment where basal long soak method of NAA was applied at 4000 mg L^{-1}. These findings are at par with the results (26.83) obtained from the cuttings where quick dip method of NAA was applied at 4000 mg L^{-1}. The minimum mean number of leaves (17.0) was obtained from untreated cuttings. The covered cuttings exhibited better response for mean number of leaves (28.81) as compared to un-covered stem cuttings (17.0).



BQD_1 = Basal quick dip with naphthalene acetic acid @ 2000 mg L^{-1}
BLS_1 = Basal long soak method naphthalene acetic acid @ 2000 mg L^{-1}
BQD_2 = Basal quick dip with naphthalene acetic acid @ 4000 mg L^{-1}
BLS_2 = Basal long soak method naphthalene acetic acid @ 4000 mg L^{-1}

Figure 1. Sprouts per stem cutting of bougainvillea as influenced by basal methods of naphthalene acetic acid application.

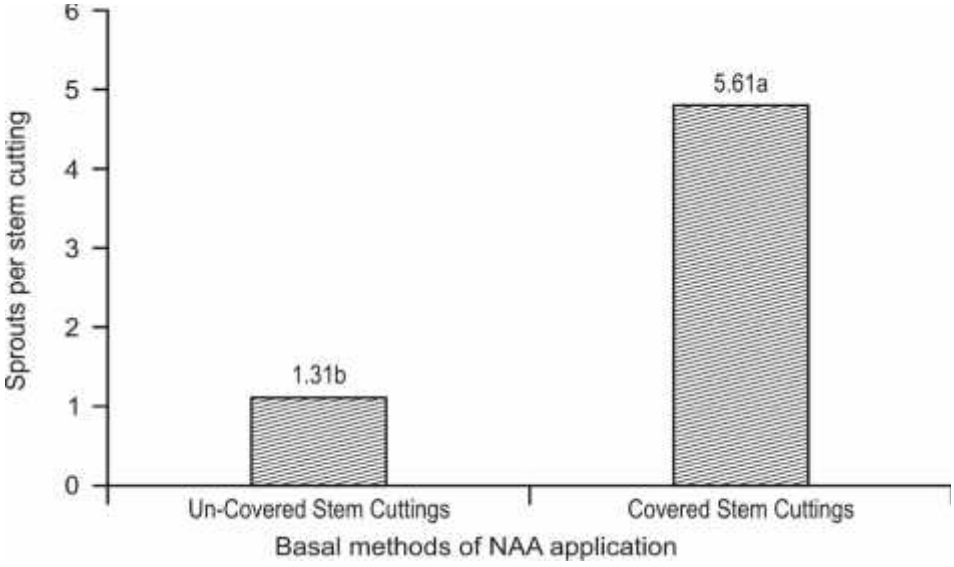
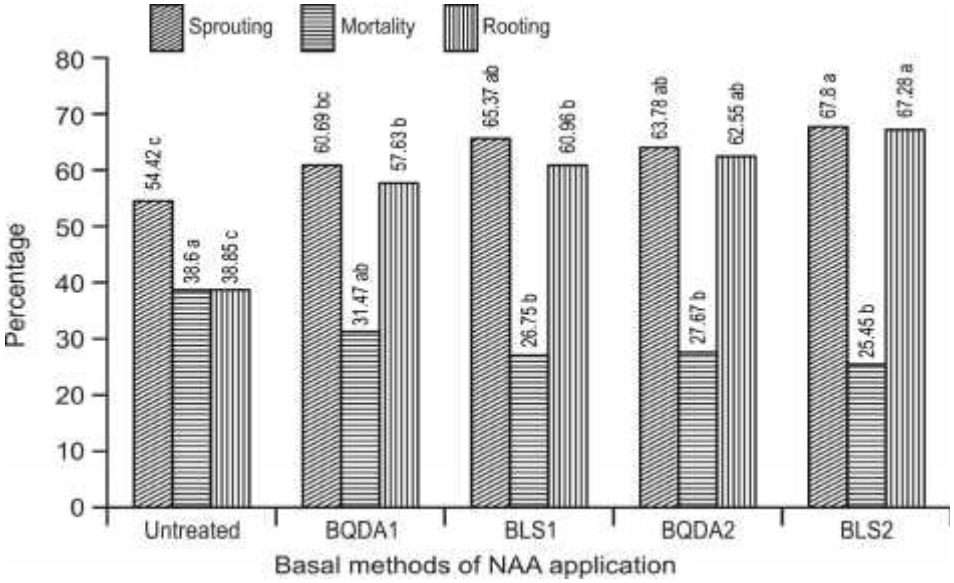


Figure 2. Comparison of sprout obtained from uncovered and covered stem cuttings of bougainvillea.



BQD_1 = Basal quick dip with naphthalene acetic acid @ 2000 mg L^{-1}
BLS_1 = Basal long soak method naphthalene acetic acid @ 2000 mg L^{-1}
BQD_2 = Basal quick dip with naphthalene acetic acid @ 4000 mg L^{-1}
BLS_2 = Basal long soak method naphthalene acetic acid @ 4000 mg L^{-1}

Figure 3. Sprouting, mortality and rooting (percentage) from bougainvillea stem cuttings as influenced by basal methods of naphthalene acetic acid application.

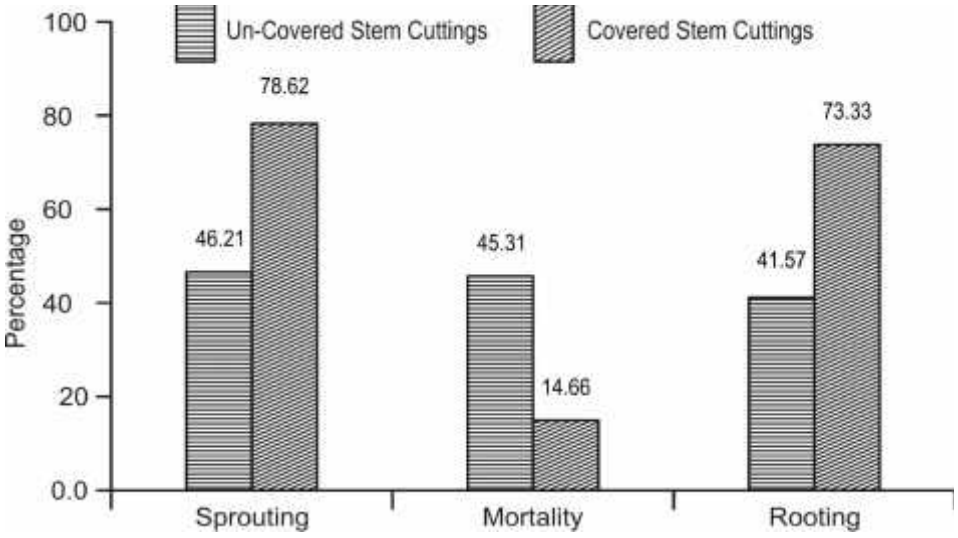
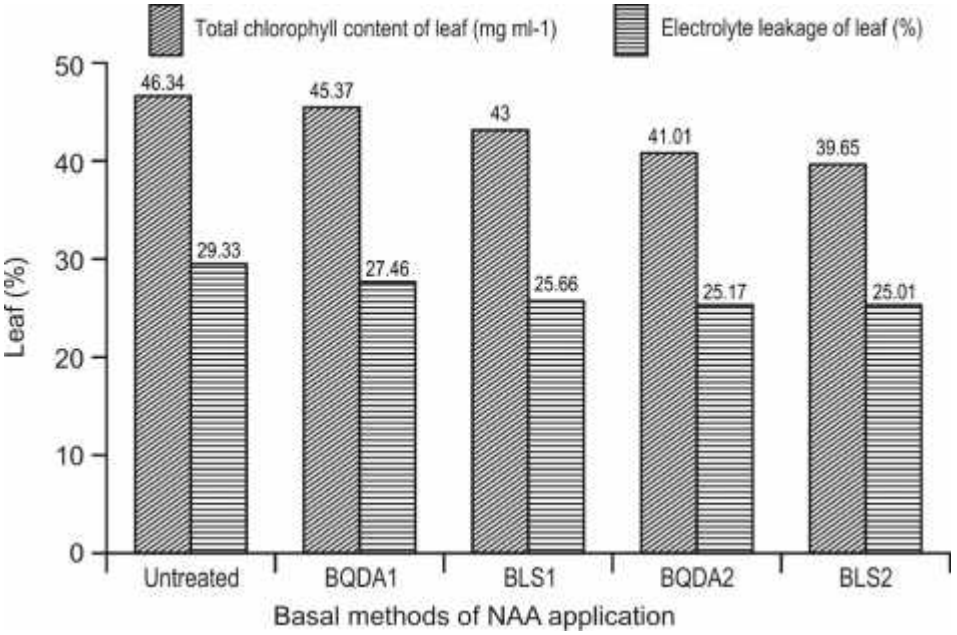


Figure 4. Comparison of sprouting, mortality and rooting percentage obtained from uncovered and covered stem cuttings of bougainvillea.



BQD_1 = Basal quick dip with naphthalene acetic acid @ 2000 mg L^{-1}
BLS_1 = Basal long soak method naphthalene acetic acid @ 2000 mg L^{-1}
BQD_2 = Basal quick dip with naphthalene acetic acid @ 4000 mg L^{-1}
BLS_2 = Basal long soak method naphthalene acetic acid @ 4000 mg L^{-1}

Figure 5. Total chlorophyll content of leaf (mg ml^{-1}) and electrolyte leakage of leaf (%) as influenced by basal methods of naphthalene acetic acid application.

DISCUSSION

The vegetative propagation of the plants by cutting have many benefits such as greater stock stand uniformity, true to type plants, faster growth rate and disease free varieties of economically and commercially important plants (Ooyamma and Toyoshima, 1965; Fielding, 1969; Kochhar *et al*., 2008). Rooting of stem cuttings is one of the critical steps in plant propagation of woody plants. It varies from species to species. However, propagation through seeds cause genetic variability and makes the crops prone to different diseases. The covered stem cuttings treated with basal long soak method of NAA @ 4000 mg L^{-1} exhibited more pronouncing response for number of sprouts per cutting as compared to rest of the treatments. In contrast Singh *et al*. (2011) obtained maximum number of sprouts (30.22) under mist chamber in response to the treatments where 50 cm stem cuttings of bougainvillea were treated with IBA @ 3000 ppm by quick dip method and planted in February. Singh *et al*. (2011) also reported somewhat similar results for number of sprouts per cutting. They reported more number of sprouts from November planted stem cuttings of bougainvillea variety Torch glory

treated by quick dip method with auxin (IBA) at the concentration of 3000 mg L^{-1}. However, they also observed the lowest number of sprouts with the increasing levels of IBA upto 4500 mg L^{-1} in the same bougainvillea variety Torch glory. Awad *et al*. (1988) reported higher percentage of bud sprouts from the bougainvillea stem cuttings of 12 mm thickness treated with auxin at the concentration of 6000 ppm of Indole butyric acid (IBA). Kochhar *et al*. (2008) also observed maximum number of sprouts from stem cuttings of *Jatropha curcas* when treated with auxin i.e. NAA @ 100 ppm as compared to 10 ppm. This concentration of NAA is much lower than the concentration we applied in our study. This may be the cause due to different types of plants, season variability, and thickness of the stem cutting. Wahab *et al*. (2001) observed better sprouting from cuttings of guava when treated with NAA @ 2000 ppm. Jadhav (2007) obtained maximum number of sprouts and length of sprout from stem cuttings of phalsa treated with NAA @ 200 ppm.

In present study, maximum percentage of sprouting was also observed from covered stem cuttings treated with basal long soak method of NAA @ 4000 mg L^{-1}. Singh *et al*. (2011) obtained 90.0% sprouting percentage from the treatment where 50 cm long cuttings were treated with IBA @ 3000 ppm by quick dip method and planted under mist chamber in Feburary. Singh *et al*. (2011) obtained 100% sprouting from January planted stem cuttings of bougainvillea treated with various concentrations of IBA including 2000, 2500 and 3000 mg L^{-1}. However, Tiwari and Das (2010) observed better response for sprouting on much reduced levels of NAA (1000 ppm).

This may be the cause due to different type of plants and may be due to diameter of the stem cutting. They propagated two shrub medicinal species viz *Caesalpinia bonduc* and *Embelia tsjeriam* through stem cuttings by applying exogenous plants growth regulators including Indole Acetic Acid (IAA), α- Naphthalene Acetic Acid (NAA) and Indole 3 Butyric Acid (IBA). Higher effects of plant growth regulators including NAA, IAA and IBA on sprouting of rose buds was also reported by Sun and Chen (1998). Majeed *et al.* (2009) reported 75% sprouting from the stem cuttings of *Aesculus indica* treated with IBA (4000 ppm). Khan *et al*. (2006) obtained maximum percentage of bud sprouts from rose cuttings treated with NAA @ 50 mg L^{-1}. They used two auxins viz indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA) at various concentrations and reported NAA superior over IAA in the range of 50 to 75 mg L^{-1}. Jadhav (2007) reported the maximum sprouting of 85.00% from stem cuttings of phalsa treated with NAA @ 200 ppm which was at par with IBA, 200 ppm (81.67%).

Auxin (NAA) treated cuttings had more potential in increasing rooting percentage. Long soak method of covered stem cuttings @ 2000 and 4000 mg L^{-1} of NAA exhibited better response for rooting percentage. Shahba and Alshmmary (2013) treated hard wood cuttings of *Bougainvillea peruviana* cv. Shurba with naphthalene acetic acid (NAA) and indole butyric acid (IBA). They reported better rooting percentage 55.6%, 59.4% after 12 and 24 hours using IBA at 500 ppm, respectively. This concentration of IBA is much lower than the concentration we applied in our study. This may be the cause due to different species of the

bougainvillea and size of the stem cuttings. However, in the present study more than 80% root induction was achieved. This may be due to more concentration of auxins or due to covered stem cuttings. However, Seyedi *et al*. (2013) observed better rooting response from the cuttings treated with IBA @ 4000 mg L^{-1}.

Table 1. Roots and fresh weight of the roots (mg) as affected by various basal methods of Naphthalene acetic acid (NAA) application.

Basal methods of NAA application	Roots per stem cutting		Mean	Fresh wt. of the roots		Mean
	Un-covered stem cuttings	Covered stem cuttings		Un-covered stem cuttings	Covered stem cuttings	
Un-treated cuttings	4.33	16.00	10.16 d	16.67	36.67	26.67 b
Basal Quick dip with NAA @ 2000 mg L^{-1}	5.67	19.00	12.33 cd	20.53	47.45	34.60 ab
Basal Long Soak with NAA @ 2000 mg L^{-1}	7.67	21.67	14.67 c	25.67	47.77	36.50 ab
Basal Quick dip with NAA @ 4000 mg L^{-1}	10.67	24.67	17.67 b	26.83	48.67	37.00 ab
Basal Long Soak with NAA @4000 mg L^{-1}	13.67	28.67	20.83 a	32.33	56.61	44.47 a
	8.40 b	21.87 a	-	24.41 b	47.43 a	-

Table 2. Number of leaves per stem cutting as affected by various basal methods of Naphthalene acetic acid (NAA) application.

Basal methods of NAA application	Leaves per stem cutting		Mean
	Un-covered stem cuttings	Covered stem cuttings	
Un-treated cuttings	11.33	22.67	17.00 d
Basal Quick dip with NAA @ 2000 mg L^{-1}	15.33	25.67	20.50 cd
Basal Long Soak with NAA @ 2000 mg L^{-1}	18.33	27.40	22.87 bc
Basal Quick dip with NAA @ 4000 mg L^{-1}	19.33	34.33	26.83 ab
Basal Long Soak with NAA @4000 mg L^{-1}	20.67	34.00	27.33 a
	17.00 b	28.81 a	-

Asl *et al*. (2012) reported no effect of auxins (IBA) on rooting percentage of bougainvillea stem cuttings. However, they observed maximum number of roots from the cuttings treated with IBA @ 2000 mg L^{-1}. In the present study higher number of roots was observed from the covered stem cuttings in response to the treatment where basal long soak method of NAA was applied at the concentration of 4000 mg L^{-1}. Shahba and Alshmmary (2013) treated hard wood cuttings of *Bougainvillea peruviana* cv. Shurba with naphthalene acetic acid (NAA) and indole butyric acid (IBA). They also obtained higher number of roots per cutting after 12 (20.5) and 24 (19.8) hours using IBA at 500 ppm. Gupta *et al*. (2002) reported that treatment of bougainvillea cuttings with 1000 ppm IBA gave maximum rooting (100%) with higher number of roots in soaking method. Panwar *et al*. (1994) observed the best rooting in hard wood cuttings of bougainvillea var. *Alok* treated with IBA 2000 ppm. Jadhav (2007) observed more rooting percentage and number of roots from the stem cuttings of phalsa when treated with IBA @ 200 ppm. Shahba and Alshmmary (2013) observed maximum number of leaves per cutting soaked for 12 (49) and 24 hours (40) with IBA at 500 ppm. Singh *et al*. (2013) observed maximum number of leaves on new growth (7.48) from 50 cm long cuttings of bougainvillea in response to the treatment where IBA was used at 5000 ppm. In present study maximum number of leaves was observed from covered cuttings soaked with quick or long soak method of NAA @ 4000 mg L^{-1}. Shahba and Alshmmary (2013) observed also better response for fresh weight of roots from the cuttings soaked for 12 hours and 24 hours with IBA at 500 ppm.

CONCLUSION

It was concluded that sprouting and rooting performance of bougainvillea cuttings was remarkably better from covered stem cuttings in response to basal long soak method of NAA @ 2000-4000 mg L^{-1}. However, most of the parameters responded well to the quick dip method of NAA.

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(Accepted: November 11, 2014)