Effects of Substrate and Exogenous Auxin on the Adventitious Rooting of *Dianthus caryophyllus* L.

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Abstract. In this study, *Dianthus caryophyllus* L. was used as the experimental plant to investigate the effects of rooting substrate and exogenous auxin concentration on the adventitious rooting of the stem cuttings. Our results showed that the formulated substrates with different physicochemical properties significantly affected the root formation. The substrate with a ratio of cocopeat to perlite at 1:1 (v:v) resulted in the optimum rooting of *D. caryophyllus* cuttings. Different Indole-3-butyric acid (IBA) and 1-naphthalene acetic acid (NAA) concentrations affected the rooting percentage and seedling rate of *D. caryophyllus*. Application of NAA at 1000 mg·kg⁻¹ with IBA at 100 mg·kg⁻¹ resulted in the greatest rooting percentage and improved breeding speed. The rooting percentage and seedling rate did not increase with the increase in auxin concentration. Based on these results, we concluded that an appropriate rooting substrate is required to fulfill proper rooting of *D. caryophyllus* cuttings, whereas an exogenous application of IBA and NAA at 1000 mg·kg⁻¹ and 100 mg·kg⁻¹ promoted the rooting and a higher auxin concentration inhibited rooting.

Dianthus caryophyllus L., commonly known as carnation, is one of the most popular cut flowers; it ranks third after chrysanthemum and rose in the worldwide market (Liu et al., 2018). Vegetative propagation by cutting is one of the main methods used for *D. caryophyllus* seedling production, which is economically important for high-scale breeding of elite commercial genotypes from stem cuttings of the mother plants (Garrido et al., 1998).

Adventitious rooting is an essential step in the vegetative propagation, it is a complex physiological, biochemical, and metabolic process affected by multiple factors (da Costa et al., 2013). Endogenous factors such as genetic characteristics, hormone synthesis, and transport unavoidably influence the rooting capacity (Pacurar et al., 2014). Exogenous environmental conditions including temperature, water and oxygen supply, and auxin application are also critical for promoting the quality of rooting and improving the survival rate of cuttings (Geiss et al., 2009).

Several previous studies have been performed to investigate the factors that influence the root formation in cutting propagation, including the effects of rooting auxin, storage

temperature, light, and oxygen requirements (Garrido et al., 1998; Kreen et al., 2002; Van de Pol and Vogelesang, 1983). Auxin has a crucial role in the induction of the meristems of root primordia, redistribution of nutrients, and biosynthesis of endogenous hormones in the cuttings, thereby stimulating adventitious root regeneration (Garrido et al., 2002). Indole-3-butyric acid (IBA) and 1-naphthalene acetic acid (NAA) feature different mechanisms in the induction of adventitious roots. For example, IBA was more effective than NAA for the rooting of stem cuttings (Husen and Pal, 2007). IBA has a strong, long-term promotional effect on the initiation, emergence, and development of root primordia. NAA promotes the hydrolysis of starch into sugar, thereby increasing the sugar availability for the formation of roots; it also promotes rooting and induces short, thick roots (Altman and Wareing, 1975). In practice, IBA is commercially accepted as an exogenous rooting hormone for many plant species because of its high root-inducing capacity and light stability (Pacurar et al., 2014). The optimal auxin dose for rooting varies between species and genotypes (Exadaktylou et al., 2009; Luwig-Müller et al., 2005; Rosier et al., 2004). IBA can be used alone or in combination with other auxins such as NAA, depending on the specific condition, to achieve better rooting performance (Cano et al., 2014).

Plant root formation is affected by the physical and chemical properties (bulk density, porosity, water/air-holding capacity, pH, EC) of the rooting substrates (Altman and Freudenberg, 1983). The ideal substrate is able to provide a proper water and oxygen balance, thus promoting oxygen availability,

transpiration, nutrient uptake, growth, and aeration during root initiation of stem cuttings (Mabizela et al., 2017). The water content is a key factor in the survival of cuttings, especially during the initial rooting phase when the transpiration and physiological activities rely on the stem insertion in the substrate (Grange and Loach, 1983). For example, the survival rates of *Juniperus horizontalis*, *Rhododendron*, and *Ilex crenata* stem cuttings were the highest at the highest studied medium moisture level (625%) (Rein et al., 1991). A sufficient oxygen supply is indispensable for root initiation, growth, and development; furthermore, maintaining suitable pH and electrical conductivity (EC) is important during the rooting period (Holt et al., 1998). Generally, a mixture of two or more substrate types is used to obtain the ideal rooting substrate with the expected characteristics, such as organic components (peat), which provide the water-holding capacity, and inorganic components (perlite), which increase aeration, in this study.

The objectives of the current study were to improve carnation breeding quality and provide a theoretical basis for factory breeding. We investigated the effects of the auxin concentration and the ratio of the substrate on carnation rootings by evaluating the rooting percentage and survival rate of the experiments.

Materials and Methods

Experiments were conducted in the solar greenhouse of Xingqing Ornamental Breeding Center in Yinchuan, China (lat. 38.48° N, long. 106.23° E). The temperature of the experimental greenhouse was set to 25 °C/18 °C during the day and night, respectively. The experimental plant was *Dianthus caryophyllus* L. cv. Master, and the unrooted cuttings were obtained from a local nursery. Stem cuttings (15 cm in length) with four leaf-pairs and three developed internodes were subjected to the rooting experiment in 72 cell trays that were filled with the studied substrates and placed on breeding beds in the greenhouse.

Experimental design

Expt. 1: Effect of the rooting substrate on the rooting of carnation cuttings. The substrate was a mixture of cocopeat (Galuku International Pty Ltd., Vaucluse, Australia)

Table 1. Substrate formulation of the studied treatments.

	Substrate formulation (v:v)
B ₁	cocopeat:perlite = 1:1
B ₂	cocopeat:perlite = 1:2
B ₃	cocopeat:perlite = 1:3

Table 2. Auxin concentrations of the experimental treatments.

Treatment	Auxin	Concn (mg·kg ⁻¹)
A ₁	NAA + IBA	500 + 50
A ₂	NAA + IBA	1000 + 100
A ₃	NAA + IBA	1500 + 150

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Table 3. Physical and chemical properties of the different rooting substrates.

Substrate	Bulk density (g·cm ⁻³)	Total porosity (%)	Air-filled porosity (%)	Water-holding capacity (%)	Electrical conductivity (μS·cm ⁻¹)	pH
Cocopeat	0.171 ± 0.002	85.9 ± 1.0	43.3 ± 2.4	42.6 ± 1.4	0.28 ± 0.05	6.87 ± 0.16
Perlite	0.074 ± 0.001	69.5 ± 1.2	48.8 ± 2.4	20.7 ± 1.3	0.02 ± 0.001	6.90 ± 0.00
B ₁	0.130 ± 0.004 a	82.8 ± 0.5 a	34.1 ± 2.1 a	48.6 ± 1.8 a	0.15 ± 0.02 a	6.85 ± 0.04 a
B ₂	0.117 ± 0.002 b	76.8 ± 1.0 b	34.5 ± 3.3 a	42.2 ± 3.9 a	0.11 ± 0.02 b	6.88 ± 0.01 a
B ₃	0.104 ± 0.002 c	74.8 ± 0.7 b	22.5 ± 4.5 a	52.4 ± 3.8 a	0.09 ± 0.004 c	6.89 ± 0.00 a

Different letters within the column indicate significant differences at $P < 0.05$ according to Tukey's test.

Table 4. Effects of rooting substrate on the rooting percentage and seedling rate of carnation.

	Rooting percentage (%)	Seedling rate (%)
B ₁	98.8 a	98.2 a
B ₂	98.0 a	97.2 ab
B ₃	97.4 a	95.5 b

Different letters within the column indicate significant differences at $P < 0.05$ according to Tukey's test.

and perlite (obtained from a local supplier). The substrate formulations were set as follows: cocopeat:perlite = 1:1 (v:v; B₁); 1:2 (v:v; B₂); and 1:3 (v:v; B₃) (Table 1). Fresh cuttings were dipped with rooting powder (1000 mg·kg⁻¹ NAA and 100 mg·kg⁻¹ IBA) and planted in the trays of each treatment; then, they were subjected to the rooting experiment for 3 weeks.

Expt. 2: Effect of auxin concentration on the rooting of carnation cuttings. The experiment was conducted based on the results of Expt. 1. The rooting substrate used was cocopeat:perlite (1:1), and three treatments were set with differences in the auxin concentration (Table 2). Then, 400 cuttings of each treatment were set, and each treatment was replicated three times. Before planting in the substrates, the basal cuttings were dipped in the rooting powder that was prepared with the different auxin concentrations (IBA and NAA dissolved in ethanol) and talcum powder in the proper proportions (Table 2).

Management during the rooting period

Substrates were sufficiently watered before planting; after the cuttings were planted, they were covered with perforated transparent plastic. During the first 2 weeks, water spraying was applied to ensure moisture of the leaf surface and prevent wilting. Beginning from the third week, water spraying was reduced to only maintain leaf evaporation to promote root development. The general substrate temperature was kept at 20 to 23 °C.

Physiochemical property analysis of the substrates

Physical properties of the substrates were determined according to the work of Atiyeh et al. (2001). Oven-dried substrate was placed in a container with a known volume (V) and weight (W₁); nonwoven material covered the base. The container filled with substrate was weighed (W₂) and then subjected to water saturation for 48 h to obtain the weight of the substrate with saturated water (W₃). Then, the container was inverted for 8 h to drain water freely. Next, it

was weighed to obtain W₄. The bulk density (g·cm⁻³) was calculated as follows: $(W_2 - W_1)/V$; total porosity (% volume) = $(W_3 - W_2)/V \times 100$, air-filled porosity (% volume) = $(W_3 - W_4)/V \times 100$; water-holding capacity (% volume) = total porosity - air-filled porosity.

The pH was determined with a pH meter (PHS-3C; INESA Instrument Co., Ltd., Shanghai, China) using a deionized water suspension of the substrates (1:5, W:W) that was agitated mechanically for 1 h and filtered with filter paper. The same solution was used to measure EC with a conductance meter (INESA Instrument Co., Ltd.).

Rooting assessment

The planted cuttings were allowed to root for 21 d; then, the rooting percentage and seedling rate were calculated. The length of the longest adventitious root (cm) was measured with a ruler and the root fresh weight (g) was determined. Then, it was oven-dried until a constant weight was reached to obtain the root dry weight (g).

Statistical analysis

Data presented were analyzed using SPSS Statistics 22 (IBM Software, Chicago, IL) for the one-way analysis of variance after verifying homoscedasticity using Levene's test. Tukey's honestly significant difference test was used to compare means at $P = 0.05$.

Results and Discussion

Physiochemical properties of the studied substrates

The rooting substrate was used to fix plants and to supply nutrients, water, and oxygen to the plant root system (Lemaire, 1995). Cocopeat is widely used as a component of nursery substrates (Salvador et al., 2005) because of its relatively high water-holding capacity and good cation exchange capacity. Perlite has very low bulk density and high porosity to supply aeration (Bunt, 1988; Pascual et al., 2018). A mixture of these two compositions is commonly used in soilless culture.

As shown in Table 3, the physiochemical properties of the studied substrates were affected by their formulation. With the increase in perlite proportion, the bulk density, total porosity, and EC decreased. The air- and water-filled porosity and pH resulted in no significant differences. The bulk density was highest for B₁ (0.130 g·cm⁻³) and lowest for B₃ (0.104 g·cm⁻³). The distributions of air and water in the substrate depended on its

physical properties (Lemaire, 1995). Porosity is an important criterion used to evaluate the substrate. The greatest total porosity was recorded for B₁ (82.8%); however, it was significantly decreased for B₂ (76.8%) and B₃ (74.8%). The air-filled porosity of the three formulated substrates was highest for B₁ (34.1%) and lowest for B₃ (22.5%); there was no statistical difference between treatments. The water-holding capacity was greatest for B₃ (52.4%), followed by B₁ (48.6%) and B₂ (42.2%).

Chemical properties of the substrates could also affect plant growth and the nutritional response. The pH of the studied substrates was ≈6.8, which is suitable for carnation growth (James and Topper, 1993). EC reflects the concentration of soluble salt in the substrates, which has a certain relationship with its fertilizer-supplying capacity. B₁ resulted in the greatest EC, and decreased in B₂ and B₃, although they were all relatively low for supporting sufficient nutrients for plant growth and development (Abad et al., 2001). Therefore, the nutrient solution was applied for a sufficient nutrient supply in this study.

Effects of the studied substrates on adventitious rooting of carnation

As shown in Table 4, the substrate composition unavoidably influenced the rooting percentage and seedling rate of carnation cuttings. The rooting percentage and seedling rate of B₁ were 98.8% and 98.2%, respectively. There was no significant difference between treatments for the rooting percentage. The seedling rate was highest for B₁, followed by B₂; it significantly decreased with B₃.

The root length, root fresh weight, and root dry weight were significantly affected by the substrate formulation (Fig. 1). The greatest root length was found with B₁, whereas it was significantly decreased with B₂ and B₃. The fresh root weight and dry root weight of B₁ were significantly greater than those of B₂ and B₃.

Substrate selection mainly depends on the biological characteristics of the target plant and is closely related to the root zone environmental conditions (Lemaire, 1995). Substrate quality is an important factor affecting the rooting percentage and root growth quality in vegetative cutting reproduction (King et al., 2011). Physicochemical properties differ with different substrate ratios. The bulk density and total porosity of B₁ were higher than those of B₂ and B₃. The seedling rate, longest root length, root fresh weight, and

Fig. 1. Effects of rooting substrate on the longest root length, root fresh weight, and root dry weight of carnation. Different letters within the column indicate significant differences at $P < 0.05$ according to Tukey's test.

Table 5. Effects of auxin concentrations on rooting percentage and seedling rate of carnation.

	Rooting percentage (%)	Seedling rate (%)
A ₁	99.3 a	98.5 b
A ₂	99.8 a	99.7 a
A ₃	99.8 a	98.9 b

Different letters within the column indicate significant differences at $P < 0.05$ according to Tukey's test.

Fig. 2. Effects of auxin concentration on the root length, root fresh weight, and root dry weight of carnation. Different letters within the column indicate significant differences at $P < 0.05$ according to Tukey's test.

root dry weight of B₁ were all higher than those of B₂ and B₃. This was likely caused by the greater porosity of B₁. During a study of baldcypress stem rooting, a tradeoff was suggested between greater rooting percentage/root quality in a substrate with greater aeration or water-holding capacity (King et al., 2011). Therefore, the studied substrate with cocopeat:perlite (volume) at 1:1 resulted in positive effects on carnation root formation and development, thus suggesting rooting substrate for carnation root regeneration. However, further studies should be performed to investigate if it could be better if the perlite proportion in the substrate was further reduced.

Effects of the auxin concentration on adventitious rooting of carnation

According to Table 5, the rooting percentage (99.8%) and seedling rate (99.7%) were both greatest for A₂. A₁ resulted in the lowest rooting percentage and seedling rate. The increase in the auxin concentration resulted in a decreased seedling rate for A₃, which indicated the inhibition of adventitious root formation caused by an excessive auxin concentration (Pacurar et al., 2014). This was in agreement with the results of King et al. (2011), who performed a study of baldcypress. Differences in the auxin concentration resulted in significant differences in the root fresh weight and dry weight of

carnation seedlings (Fig. 2). No significant difference between treatments was found for the longest root length. The root fresh weight and dry weight were the greatest with A₂ and significantly decreased with A₁ and A₃.

The results showed that the auxins accelerated the rooting of carnation, which was consistent with the results of previous studies of smokebush (Pacholczak et al., 2013) and rose (Al-Saqri and Alderson, 1996). The combined auxin (NAA and IBA) application achieved good rooting induction and development. In this study, the combination of NAA (1000 mg·kg⁻¹) and IBA (100 mg·kg⁻¹) effectively improved the propagation speed of carnation. Root formation and development with the auxin combination treatment were attributed to the synergistic and complementary effects that effectively promoted the metabolism of the stem cuttings (Ragonezi et al., 2010). Therefore, for the reproduction of carnation seedlings, proper application of the combined auxin treatment reduces the dose usage of auxin and achieves ideal root formation and development.

Conclusion

The ideal rooting substrate requires a good balance between the water-holding capacity and air/water permeability. The mixture of cocopeat and perlite with a volumetric ratio of 1:1 improved the substrate structure and yielded the root formation capacity in this study. The combined application of IBA and NAA significantly improved the rooting percentage and seedling rate. In practice, the proper concentrations of plant growth regulators are important because excessive NAA or IBA concentrations may inhibit root initiation.

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