**stems age, nitrogen fertilizer and Salicylic acid application in Cutting induction of *Dendrobium nobile* Lindl.**

Jeferson João Soccol(1) \*Giorgini Augusto Venturieri(2) e Enio Luiz Pedrotti(3)

() Federal University of Santa Catarina UFSC/CCA-FIT. Agrarian Sciences Center, Admar Gonzaga Road, 1346, Itacorubi, 88040-900, Florianópolis SC, Brazil.

(2) Federal University of Santa Catarina UFSC/CCA-FIT. Agrarian Sciences Center, Admar Gonzaga Road, 1346, Itacorubi, 88040-900, Florianópolis SC, Brazil. \*E-mail [giorgini@cca.ufsc.br](mailto:giorgini@cca.ufsc.br)

(2) Federal University of Santa Catarina UFSC/CCA-FIT. Agrarian Sciences Center, Admar Gonzaga Road, 1346, Itacorubi, 88040-900, Florianópolis SC, Brazil.

**Abstract**

Cuttings induction on *Dendrobium nobile* Lindl. orchid was evaluated in two experiments experiment. In the first experiment were evaluated cuttings from already bloomed stems (mature) and from stems that had not bloomed yet (young), in function of the nitrogen (Nitrate and Ammonia forms) application under the following treatments: Calcium Nitrate at concentrations of 5.81gL-1; 11.61gL-1; 17.42gL-1; and 2.00gL-1; and Urea at concentrations of 4.00gL-1 and 6.00gL-1. In the second experiment the proportion of successful cuttings on mature stems was evaluated according to the application of Salicylic acid in concentrations of: 0.10mm, 0.50mm, 1.00mm and control (0.00mm). In experiment 1, analyzed variables were: proportion of successful cuttings, proportion of rotten cuttings stalks, vigor, number of roots/plant and root length/per plant. In experiment 1, the variable proportion of rotten cuttings stalks were negative correlated with the proportion of successful cuttings on young stems (*r* = - 0.34). Regarding the effect of fertilization there were no significant statistically increases in any of the variables. In experiment 2 variables evaluated were: proportion of successful cuttings, shoot height, length of the biggest roots and number of roots. The dosage of Salicylic acid 0.50mm had the highest result for proportion of successful cuttings with 43.3% against 23.3% in the control treatment.

**Key words:** cutting, propagation, scion, orchids

**INTRODUTION**

Flower cultivation in Brazil moves about $ 2 billion/year, representing 0.22% only of the world business of this sector however, in the last two decades, on the internal market, was observed a growth rate of 20%/year, that consumes almost all that is produced (Batalha and Buainain 2007). Exports of orchid scions totalized $152 thousands at 2010 and, in the following year US$103 mil, i.e., a decrease of 31.8%. However, for the same period, orchid plantlet imports increased 60.4%, passing form US$4.2 to US$6.7 million (Secex 2012). Considering that the item “plantlet” is a basic insume associated to the final orchid production can be said that expansion of such cultivation is occurring in the country and that it has been made with imported plantlets.

Flower cultivation is a profitable and attractive agricultural sector that involves significant manpower 3.8 persons/ha directly involved in the production (Reetze et al. 2007) but generates high incomes and does not require large planting areas. Therefore, flower cultivation is a business that is self-sustainable, able to generate many labor positions and is accessible to micro farm holders that use family members as a main work force. Noble Dendrobium is a fast-growing orchid that vegetates well under the majority of substrates indicated for orchids. It vegetates from plains to altitudes of 2000 m. The plant withstands temperatures up to 1 °C (Baker and Baker 1996) but requires temperatures above 22Co for good vegetative development; low temperatures induce flowering that occurs from late winter to early summer (Baker and Baker 1996). Indeed, Noble Dendrobium is one of the easiest orchids to grow (Campos 1998; Silva 1986).

The Noble Dendrobium orchid (*Dendrobium nobile* Lindl.) is one of the three species used in the formulation of "Shi-Hu" (with *D. tosaense* and *D. moniliforme*) (Lo et al. 2004; Ye and Zhao 2002), which is an antipyretic and tonic drink that is also described as an aphrodisiac in traditional Chinese medicine (Hanelt 2001). It also has antitumor and antimicrobial activities and inhibition of *in-vitro* lipid peroxidation (Devi et al. 2009) and anti HIV (Sánchez-Duffhues et al. 2008). If its potential for pharmaceutical industry been confirmed a high demand for plantlet production can increase vertiginously. Propagation Noble Dendrobium orchid, for hobbyists and for small producers, usually is made by clump division, cuttings and the shoots that appear in the axillary buds of leaves, called “Keikes”. For commercial purposes propagation by mericlones is preferred. Its propagation by seeds has only been adopted for purposes of genetic improvement because take the double the time to blossom compared to mericlones, clump division or cuttings.

Cloning by cuttings has the advantage to be made without laboratorial support as used to produce mericlones, however its potential to produce plantlets by propagule is higher than such clump division, cuttings and or Keikes. However, many stem cuttings do not emit leaves or roots and eventually rot. The highest proportion of successful cuttings obtained with stem cuttings layered on gravel sprinkled with Urea, a nitrogen fertilizer, at 2gL-1, was 40.7% (Venturieri and Pickscius 2012). According to Yen (2008) prolonged fertilizer application on *D. nobile* plants increases vegetative development and axillaries buds of leaves development. Salicylic acid is considered a growth regulator with many positive effects on plants as: flowering stimulant, disease resistance and Ethylene synthesis inhibition (Raskin 1992). Carvalho et al. (2007) observed that germination increased in *Calendula officinalis* L. and Campos et al. (2004) in beans an increase for anthracnose resistance.

Aiming to improve the propagation of *D. nobile* by cuttings, in the present study, the effects of stems’ age used for the stem cuttings, in interaction with nitrogen fertilizer and Salicylic acid application were evaluated on: proportion of successful cuttings and their quality.

**MATERIAL END METHODS**

Cuttings production was evaluated in *Dendrobium nobile* Lindl. in two different experiments. They are:

**Experiment 1:** *Evaluation of stems age and nitrogen fertilizer application on cutting production*. For this experiment were used stem cuttings, with 4 buds (approximately 20cm in length), from a bulk of *D. nobile* Yamamoto series cultivars (Yamamoto Dendrobiums s.d.), from stems of 2 ages: a) mature – from already bloomed stems on the year they were collected; and b) young – from stems that had not bloomed on the year they were collected. All stems cuttings were immersed in fungicide (Mancozeb[[1]](#footnote-1) 1.60g/L) and placed standing on gravel substrate at 44.2 x 28.0 x 7.5cm plastic trays. For drainage, trays were base perforated. Applied treatments were: aspersion of Calcium Nitrate[[2]](#footnote-2) at the concentration of: 5.81 gL-1 (T1), 11.61 gL-1 (T2) and 17.42 gL-1 (T3); and Urea[[3]](#footnote-3) at concentrations of: 2.00 gL-1 (T4), 4.00 gL-1 (T5) and 6.00 gL-1 (T6) the control treatment received water only at the same volume used for the other treatments. Aspersions were made up to dripping point. Treatments were applied fortnightly. Concentrations of Calcium Nitrate (mainly N at nitric form) and Urea (N at ammoniac form) were adjusted to make pairs of treatments with the same level of theoretical Nitrogen. Treatments were applied during 3 months. The adopted experimental design was entirely randomized with 4 stem cuttings by plot and 3 plots by treatment. The evaluated parameters were as follows: proportion of successful stem cuttings (considered when the stem cutting launched shoots and/or roots), proportion of rotten cuttings stalks; vigor (subjectively evaluated by assigning scores from 0 to 10, 0 attributed to rotten stem cuttings and/or without bud leaf or root emission, 5 to steams cuttings with 5cm in height of emitted leaf bud and 2 roots, and 10 for steams cuttings with more than 10cm of emitted leaf bud and more than 6 roots). Intermediate grades could be attributed). The proportion values were transformed to the square root of arcsine of *p*, where *p* = is the proportion (Sokal and Rohlf 1981). The data were subjected to two-factor analysis of variance (stems age x fertilizers) and the comparison between averages by the Tukey test for α=0.05. The association between variables was evaluated by Pearson correlation index and, due to the high variability of the vigor values, they were transformed to standard deviation units in relation to the average (the“Z” score) (Baldin 2005); the comparison of the means was performed using the Tukey test (Sokal and Rohlf 1981). The data were analyzed using Bioestat software package (Ayres et al., 2007).

**Experiment 2:** *Evaluation of Salicylic acid on cuttings production.* For this experiment was used stem cuttings with 1 bud (approximately 5cm in length), from mature stems, from the same genetic material of the previous experiment. The environmental condition; used substrate; period and system of application of the treatments all were as in the previous experiment. The adopted experimental design was entirely randomized with 5 replications (plots) each one with 6 stem cutting. Compared Salicylic acid concentrations were: T1) 0.10mM; T2) 0.50mM; T3) 1.00mM and Control) 0.0mM.

The evaluated parameters were as follows: proportion of successful cuttings (considered as in the experiment before), stem cutting leaves length (measured from the root insertion up to the top of the plant), length of the longest root and number of roots. Obtained values were subject to analysis of variance and regression analysis (Sokal and Rohlf 1981).

The two experiments were established in a greenhouse covered with transparent plastic film, superimposed by black plastic screen (Fitelamr - Engepol, Barueri - SP) able to block 60% of the incidental light, at the Center of Agrarian Science of the Federal University of Santa Catarina at Florianópolis, SC (27034`55``S; 48030`19`` W). Both experiments were irrigated 2 to 3 times a week. The data were analyzed using Bioestat software (Ayres et al. 2007).

**RESULTS AND DISCUSSION**

**Experiment 1:** *Evaluation of stems age and nitrogen fertilizer application on cutting production*. A summary of probabilities and their statistical significances for effects of all variables are presented in the table 1. There were differences for the variables proportion of successful cuttings and vigor only.

For the values of proportion of successful cuttings there was difference for stem age and use of fertilizer. The highest proportion of successful cuttings was achieved using mature stem cuttings from mature stems (59.5%), in other words, a value 1.6 times higher than obtained with stem cuttings from young stems (36.9%) (Table 2). According to Raven et al. (1996) auxins are produced at leaf primordium and young leafs that inhibit the growth of lateral buds. As mature stems lack leaves, is presumed that, stem cuttings formed from them, would have low concentration of auxins and consequently low inhibition of lateral buds, that justifies the high proportion of successful cuttings observed.

About the treatments with Nitrogen fertilizer, on proportion of successful cuttings, in the treatment with Calcium Nitrate, at the concentration of 11.61gL-1, was observed the lowest value (33.3%), the highest one (62.5%) was observed under the treatment of Calcium Nitrate at 17.42 g L-1. In other words, Nitrogen application, in 5 of the 6 applied treatments, showed similar effect to the Control treatment that had, in average, 50.0% (Table 2). Venturieri and Pickscius (2012) in a similar *D. nobile* experiment, where the same variables as in the present work were evaluated, but without stem cuttings differentiated by ages, was also observed that nitrogen fertilizer did not influenced or proportion of successful cuttings, nevertheless it influenced vigor positively, fact that was not observed in the present work. Apparently, lower proportion of successful cuttings observed for the treatment Calcium Nitrate at 11.61gL-1 would be an experimental artifact, because there was no interaction with the age of stems (Table 1).

About stems cuttings ages, the highest proportion of successful cuttings (59.5%) as the highest vigor, were obtained with stem cuttings from mature stems, a value superior to 40.7% obtained by Venturieri and Pickscius (2012) without differentiation of stem cutting age (Table 2).

Correlation between theoretical Nitrogen level and evaluated variables, in mature stem cuttings, significant *r* values (*p* = 0.05) was observed between proportion of successful stem cutting that was negatively correlated with proportion of rotten cutting stalks cuttings (*r =* - 0.58) and between proportion of successful cuttings and vigor (*r* = 0.55), been vigor also negatively correlated with proportion of rotten cutting stalks (*r =* -0.56). By the observed associations can be supposed that, successful stem cutting under mature cuttings stalks, could be improved avoiding stem cutting rot and vigor increasing. Venturieri and Pickscius (2012) observed, in a similar experiment, an association between application of Nitrogen and cuttings vigor suggesting that, application of a fungicide associated with Nitrogen fertilizer, vigor could be improved and decrease rotting on mature stem cuttings. A significant (*p* = 0.05)and negative correlation (*r =* -0.44) between theoretical Nitrogen level, on mature stem cuttings, with root length was observed (Table 3). A similar effect was also observed in *in vitro* cultivation of Phalaenopsis hybrids, where increases in NH+4 and NO−3 concentration provoked shoot growth and root reduction (Hinnen et al. 1989).

**Experiment 2:** *Evaluation of Salicylic acid on production of cutting*. A summary of probabilities and their statistical significance for effects of all variables are presented in the table 4. Among evaluated parameters, for proportion of successful cuttings only, was observed statistical difference (*p*<0.05) with the highest observed value (43.3%) in the treatment with 0.50mM of Salicylic acid, 1.86 times more than observed for the control treatment that that was 23.3% (Figure 1). Salicylic acid is involved in many physiological plants processes, among these stomata closure (Mori et al. 2001) and induction for abiotic stress resistance (Janda et al. 1999) that could contribute to proportion of successful cuttings increment. Nevertheless, the highest tested concentration, 1.0mM, showed a reduction of 7% in proportion of successful cuttings in relation to obtained with the dosage of 0.5mM (Figure 1). Çanakci and Munzuroglu (2009), in an experiment with application of Salicylic acid in barley and radish observed that concentrated doses provoke a reduction in fresh weigh and chlorophyll content. So, is supposed that the application of Salicylic acid, at the concentration of 1.0mM could have induced phytotoxic effect and diminished the proportion of successful cuttings.

From the regression analysis on the figure 1 is possible to visualize that the point of highest inflection of the curve is given with the x- axis of 0.61mM, point where the higher proportion of successful cuttings could reaches its maximum (44.8%), so, this would be the theoretical dosage for the maximum *D. nobile* cuttings production. This value is 3% superior to the best observed treatment by Venturieri and Pickscius (2012) where the best treatment was 2.5% superior to the control treatment only. At the present work, the best treatment was 20.5% superior to the control treatment, so there were environment restrictions that affected negatively the present experiment. In fact, during the execution of the second experiment significantly hot days were observed.

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**CONCLUSION**

With mature stems in achieved the highest vigor and proportion of successful cuttings.

Application of Nitrogen in the ammoniac or nitric forms did not benefit vigor or even cuttings production. Application of Salicylic acid at concentration of 0.50mM increased the proportion of successful cuttings of *D. nobile* been the best efficient dosage inferred as 0.61mM.

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**Table 1.** *Dendrobium nobile* cuttings in a selection of Nitrogen fertilizer and cuttings age. Summary of probabilities of significance by analysis of variance for all variables. Note: n.s = not significant statistic at a 5% level of probability. \* = significant statistic at a level inferior to 0.05% \*\* =significant statistic at a level inferior to 0.001%.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Factors** | **Proportion of successful cuttings** | **proportion of rotten cuttings stalks** | **Number of roots** | **Root length** | **Vigor** |
| Cuttings age (A) | 0.000\*\* | 0.437ns | 0.305ns | 0.087ns | 0,000\*\* |
| Fertilizer (B) | 0.040\* | 0.124ns | 0.783ns | 0.322ns | 0,262ns |
| A x B interaction | 0.206ns | 0.204ns | 0.840ns | 0.804ns | 0,127ns |

**Table 2:** Parameters averages of proportion of successful cuttings and vigor (assigned as statistically different by analysis of Variance), in function of used fertilizer and stem ages.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Parameter** | | | | | | |
|  | **Proportion of sucessfull cuttings (%)** | | | **Vigor (grade)** | | | |
| **Treatmen** | **Young** | **Mature** | **Average\*** | | **Young** | **Mature** | **Average** |
| Control | 41.7 | 58.3 | 50.0ab | | 3 | 5 | 4 |
| Calcium Nitrate 4.2 g L-1 | 41.7 | 75.0 | 58.3ab | | 3 | 5 | 4 |
| Calcium Nitrate 11.61 g L-1 | 16.7 | 50.0 | 33.3b | | 5 | 3 | 4 |
| Calcium Nitrate 17.42 g L-1 | 41.7 | 83.3 | 62.5a | | 3 | 5 | 4 |
| Urea 2g L-1 | 33.3 | 58.3 | 45.8ab | | 3 | 4 | 4 |
| Urea 4 g L-1 | 41.7 | 33.3 | 37.5ab | | 4 | 6 | 5 |
| Urea 6 g L-1 | 41.7 | 58.3 | 50.0ab | | 4 | 5 | 4 |
| Average by branch age \*\* | 36.9*b* | 59.5*a* |  | | 3.31*b* | 4.68*a* |  |
| CV% | 8.8 | | | | 62.3 | | |

\* Averages followed by the same letter by column do not differ statically by the Tukey test for (α=0.05). \*\* Idem by line for the parameter age of used branch.

**Table 3:** Correlation analysis between evaluated parameters in cuttings mature stems .

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Theoretic N level | Proportion of sucessfull cuttings | Proportion of rotten cutting stalks | Vigor | Root length |
| Theoretic N level | 1 | --- | --- | --- | --- |
| Proportion of sucessfull cuttings | 0.06ns | 1 | --- | --- | --- |
| Proportion of rotten cutting stalks | 0.14ns | -0.58\* | 1 | --- | --- |
| Vigor | 0.08ns | 0.55\* | -0.56\* | 1 | --- |
| Root length | -0.44\* | -0.22ns | -0.02ns | -0.13ns | 1 |

\*Values with statistical difference.

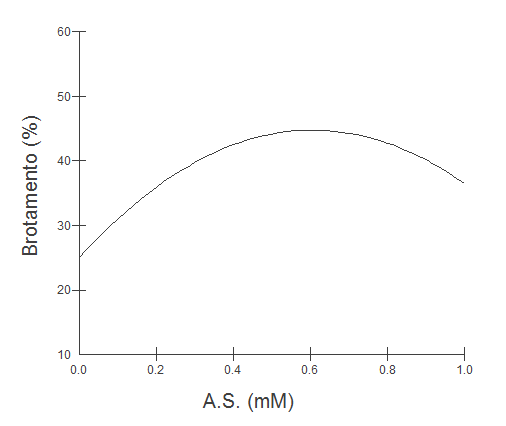
nsValues without statistical difference α=0.05.

**Table 4.** Analysis of variance for the parameters: shoot height, length of the biggest roots and number of roots and proportion of successful cuttings in function of the application of Salicylic acid.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parâmetro | GL | QM | F | *p* |
| Comprimento da parte aérea | 3 | 133.202 | 0.468 | 0.709ns |
| Comprimento de raízes | 3 | 76.376 | 0.406 | 0.750ns |
| Número de raízes | 3 | 1.801 | 0.973 | 0.429ns |
| Proporção de brotamento | 3 | 0.035 | 3.846 | 0.029\* |

\*Values with statistical difference.

nsValues without statistical difference α=0.05.



**Figure 1.** Frequencies of proportion of successful cuttings in stem stalks cutting of *D. nobile* in function of the concentration of Salicylic acid (*p<*0,5), \*Y = 25,0161 + 65.1709X -53.7073X2, R2 0.4008.

1. Manzate 800 is a product from DuPont, containg Mancozeb. [↑](#footnote-ref-1)
2. YaraLiva™Calcinit™ is a produt from Yara, containing 15.5% of Nitrogen (N) –been 14.4% N-Nitric and 1.1% N-Amoniacal forms and 19% of hidrossoluble Calcium (Ca). [↑](#footnote-ref-2)
3. Product from Buschle&Lepper S.A. containing 45% of Nitrogen. [↑](#footnote-ref-3)