ENRAIZAMENTO *IN VITRO* DE *Lavandula angustifolia*

IN VITRO ROOTING OF Lavandula angustifolia

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RESUMO

O enraizamento das brotações produzidas *in vitro* representa uma das mais importantes etapas da micropropagação de plantas, uma vez que favorece o balanço hídrico das plântulas, auxiliando na fase de mau funcionamento dos estômatos durante a aclimatização, podendo melhorar o processo de transplante. A micropropagação se apresenta como uma maneira rápida e eficiente de propagar e multiplicar genótipos superiores como os do gênero *Lavandula,* cujas espécies apresentam dificuldades de enraizamento. O objetivo deste trabalho foi avaliar o enraizamento de brotações de *Lavandula angustifolia* propagadas *in vitro* utilizando ácido naftaleno acético (ANA). Brotações *in vitro* de *L. angustifolia* contendo duas folhas foram transferidas de meio MS (6 g L^{-1} de agar e pH 5,8) contendo 30 g L^{-1} de sacarose + 1 mg L^{-1} 6-benzilaminopurina (BAP) + 0,5 mg L^{-1} ácido giberélico (GA_3) para meio MS básico mais 0 mg L^{-1} (controle), 2 mg L^{-1}, 5 mg L^{-1} ou 10 mg L^{-1} de ANA. Porcentagem de raízes, número de raízes e comprimento da maior raiz por plântula foram avaliados após 60 dias. O número de raízes não diferiu estatisticamente entre os tratamentos. A utilização de ANA favoreceu o enraizamento, aumentando a porcentagem de enraizamento com maiores doses desta auxina. Observou-se maior comprimento de raízes quando se utilizou até 5 mg L^{-1} de ANA.

Palavras-chave: auxina, micropropagação, alfazema.

ABSTRACT

Rooting of *in vitro* produced shoots constitutes an important step to plant micropropagation as it favors the plantlets' water balance, compensates the phase of stomata malfunctioning during acclimation and may improve the transplanting process. Micropropagation has presented itself as a fast and efficient mean to propagate and multiply superior genotypes of the genus *Lavandula* since its species root poorly. The objective of this work was inducing rooting on *Lavandula angustifolia in vitro* shoots by using naphthalene acetic acid (NAA). Tissue culture habituated *L. angustifolia* elongated shoots containing two leaves each were transferred from MS medium (6 g L^{-1} of agar and pH 5.8) containing 30 g L^{-1} of sucrose + 1 mg L^{-1} 6-benzylaminopurine (BAP) + 0.5 mg L^{-1} gibberelic acid (GA_3) to basal MS medium plus 0 mg L^{-1} (control), 2 mg L^{-1}, 5 mg L^{-1} or 10 mg L^{-1} of NAA. Percentage of rooting, number of roots and length of the longest root per plantlet were evaluated after 60 days. Root number did not differ statistically among treatments. NAA favored rooting as percentage of roots increased along with NAA concentration. Root length increased until 5 mg L^{-1} of NAA.

Key words: auxin, micropropagation, lavender.

Lavender (*Lavandula* spp), is among the most economically important aromatic plants in the world (FAHLÉN et al., 1997; TSURO et al., 2001; NOGUEIRA & ROMANO, 2002) and many *Lavandula* species have been micropropagated such as *L. dentata* (JORDAN et al.,1998; SUDRIÁ, 2001; ECHEVERRIGARAY et al., 2005), *L. angustifolia* (ANDRADE et al.,1999; QUAZI, 1980) and *L. stoechas* (NOBRE, 1996). Micropropagation has presented itself as a fast and efficient mean to propagate and multiply some superior genotypes of the genus *Lavandula* since its species, especially *L. angustifolia*, root poorly when asexually propagated by conventional techniques such as cutting propagation (PANIZZA et al., 1993). Information about lavender *in vitro* rooting is still contradictory. Some authors such as NOBRE (1996), who obtained 100% rooting of *L. stoechas* (Spanish lavender) shoots with 1,2 mg L^{-1} naphthalene acetic acid (NAA), recommended the use of auxin in the culture media as a mean to incentive rooting while others authors considered their use unnecessary (JORDAN et al. 1998; ECHEVERRIGARAY et al., 2005).

Rooting of *in vitro* produced shoots constitutes an important step to plant micropropagation and presents a differentiated behavior. It may occur spontaneously or may be induced by growth regulators; it may be easily induced, as normally happens in

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herbaceous species, or be more difficult, as for many hardwood species (HARTMANN et al., 2002; KAJIKI & SHEPHERD, 2006). Additionally, *in vitro* root development may improve the transplanting process because the presence of roots favor the plantlets' water balance (DIAZ-PEREZ et al., 1995). Not only that but, accordingly to SEELYE et al. (2003), *in vitro* developed roots are believed to compensate the phase of stomata malfunctioning during acclimation and that the improved performance and increase in dry weight observed on *in vitro* rooted plantlets may be due to an extra nutrient uptake by the roots.

The objective of this work was to find out if presence of external auxin is necessary for rooting induction of *L. angustifolia in vitro* cultivated shoots and to observe the response of such shoots to different NAA concentrations.

Elongated shoots of *L. angustifolia* containing two leaves each were transferred from MS medium (MURASHIGE & SKOOG, 1962) containing 30 g L^{-1} of sucrose (6 g L^{-1} of agar and pH 5.8) + 1 mg L^{-1} 6-benzylaminopurine (BAP) + 0.5 mg L^{-1} gibberelic acid (GA_3) to basal MS medium containing four different naphthalene acetic acid (NAA) concentrations to induce rooting. The following NAA concentrations were tested: 0 mg L^{-1} (control); 2 mg L^{-1}; 5 mg L^{-1}; and 10 mg L^{-1}. Parcels consisted of 10 flasks, containing one shoot each, per treatment. Four replications were performed. Cultures were maintained at 25±2 ºC under a 16-h photoperiod provided by cool-white fluorescent tubes. Percentage of rooting, root number and the length of longest root per plantlet were evaluated 60 days after experiment installation. Analysis of variance (ANOVA) followed by Tukey Test at 5% of significance or regression analysis was used to statistically analyze the obtained data.

Percentage of rooting of *L. angustifolia* increased with NAA concentration until the maximum point estimated by the regression equation of 9,2 mg L^{-1} (Figure 1), showing a benefic effect of auxin. External application of NAA is known to benefit rooting because it may increase the internal-free auxin, or may synergistically modify the actions or the endogenous synthesis of plant tissue's internal naturally occurring auxin (IAA, indole-3-acetic acid), or even enhance the sensitivity of the plantlet to IAA, increasing rooting (HARTMANN et al., 2002). Almost 90% mortality was observed in the control while 0% mortality occurred in NAA containing treatments, showing that auxin was important to survival after shoots were transferred to basal MS medium. The rate of mortality in control could have been avoided by presence of auxin in the media since it presence, as well as increase in agar or sucrose, are also supposed to help to trigger rooting (HARTMANN et al., 2002).

Not only the presence and dose of auxin, but its type, and concentration of sucrose seems to be important, since DIAS et al. (2002) observed that *Lavandula viridis* plantlets rooted on GD medium supplemented with 2,4 mg L^{-1} NAA, but not with IAA or IBA (indole-3-butyric acid), however, double of sucrose had to be used in the media for rooting induction. IAA, however, was important for *in vitro* rooting of *Lawsonia inermis*, as ROUT et al. (2001) observed that elongated shoots were rooted on MS basal medium supplemented with various concentrations of either IAA or IBA but the rooting in the microshoots was inhibited if shoots were placed on medium devoid of growth regulator, similar to what was observed herein.

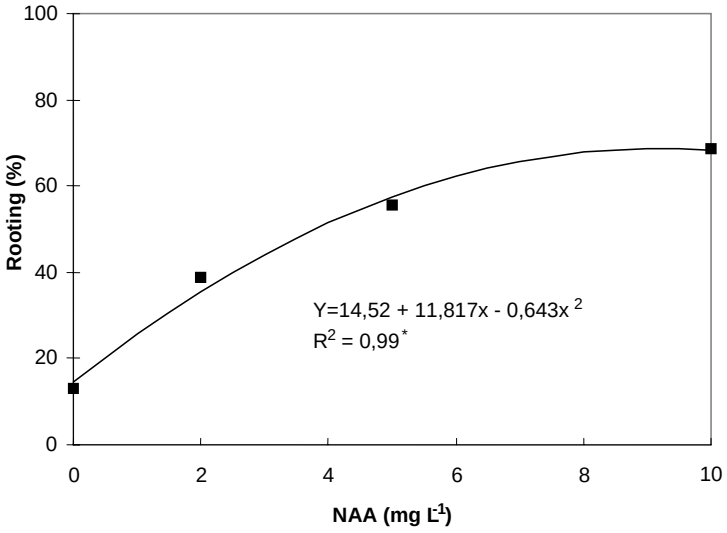

Figure 1. Effect of naphthalene acetic acid (NAA) on rooting percentage of *in vitro L. angustifolia* shoots.

TYUB et al. (2007), contrarily, observed that rooting on *Lavandula officinalis* and microshoot

elongation were favored by MS (x1/2) basal medium but rooting in isolated shoots was also recorded with root inducing hormone NAA, with similar results reported by CHISHTI et al. (2006), and in *Mentha arvensis*, another member of the Lamiaceae family, rooting occurred without growth regulators in the media (BHAT et al., 2002).

Root number did not differ statistically among treatments, however, the lower number was observed in the control (Table 1). A good distribution of roots among the plantlets' stand is desirable to increase the chance of survival and homogenize the whole stand. In spite of not being statistically significant, the extra root per plant (differences from 1.2 to 1.8 extra roots per plantlet were obtained with NAA) may cause a substantial difference in the plantlet's fixation to the substrate as well as to nutrient and water uptakes. As observed herein, rooting of *Lawsonia inermis* microshoots was also inhibited in media devoid of growth regulator and rooting was readily achieved upon transferring the microshoots onto MS basal semi-solid medium supplemented with 0.25 mg L^{-1} indole-3-butyric acid (IBA) after ten days of culture (ROUT et al., 2001).

Even tough the NAA concentrations were not statistically superior to the control, an increase of 40 to 60% roots more than the control is important since it may be beneficial to the survival of the explants during acclimatization (KALININA & BROWN, 2007).

Table 1. Effect of naphthalene acetic acid (NAA) on the average root number by plantlet of *L. angustifolia*.

NAA (mg L^{-1})	Root number
0	3,0 a
2	4,8 a
5	4,5 a
10	4,2 a
Coefficient of variation (%)	22,5

Means marked by the same letter did not differ at the 5% significance level (ANOVA, Tukey's test)

Length of roots increased until 5,1 mg L^{-1} NAA but started to decrease with the higher doses (Figure 2), showing a possible change on the rooting response to auxin towards percentage of roots, in detriment of length, or a possible toxicity induced by the higher auxin doses. Accordingly to BLYTHE & SIBLEY (2007), the use of auxin at a moderate rate can sometimes result in larger root systems by the end of the rooting period; however, a high concentration of auxin can adversely affect rooting. Percentage, rather than length is important for plantlet establishment because a greater amount of roots allow better fixation of plantlets in the substrate and a more efficient exploration of water and nutrients. For roots, more, rather than longer, is better, since longer roots may easily be broken during handling and planting.

NAA (10 mg L^{-1}) was considered benefic for rooting of *in vitro L. angustifolia* shoots because it increased percentage of rooting.

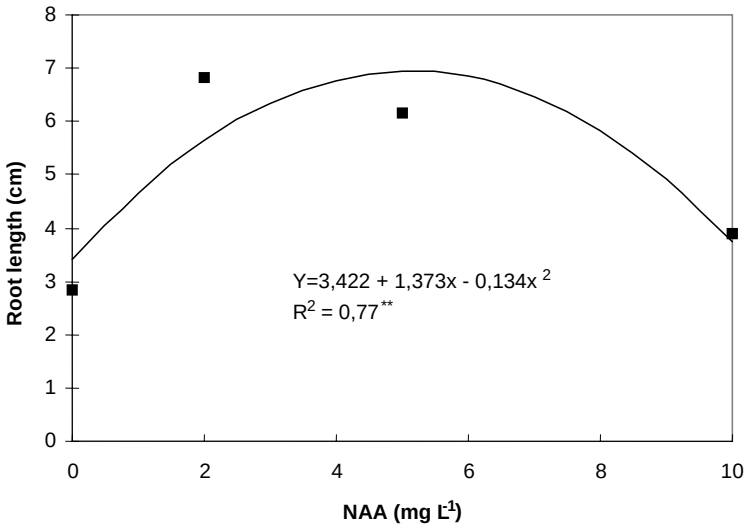

Figure 2. Effect of naphthalene acetic acid (NAA) on root length of *in vitro L. angustifolia* shoots.

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