BIOLOGIA PLANTARUM 47 (4): 481-485, 2003/4

# Polar transport of indole-3-acetic acid in relation to rooting in carnation cuttings: influence of cold storage duration and cultivar

G. GARRIDO*, M. B. ARNAO**, M. ACOSTA** and J. SÁNCHEZ-BRAVO**[1]

*Departamento de Investigación y Selección, Barberet and Blanc, E-30890 Puerto Lumbreras, Murcia, Spain**
*Departamento de Biología Vegetal (Fisiología Vegetal), Facultad de Biología, Universidad de Murcia, E-30100 Espinardo, Murcia, Spain***

## Abstract

The influence of cold storage of cuttings on the transport and metabolism of indole-3-acetic acid (IAA) and the rooting were studied in two carnation (*Dianthus caryophyllus* L.) cultivars (Oriana and Elsy), which are known to exhibit very distinct rooting characteristics. The percentage of rooting at 11 d after planting increased with the storage period particularly in Oriana, but the values in Elsy were higher than in Oriana. Auxin transport was measured by applying $^3$H-IAA to stem sections. Irrespective of the section localization, the oldest node (node) or the basal internode (base), the transport increased as the storage period increased from 2 to 12 weeks in Oriana and from 2 to 8 weeks in Elsy cuttings. The auxin transport rate was higher in bases than in nodes and also in Elsy than in Oriana at a given storage period. IAA oxidation and hydrolyzation of IAA conjugates (determined by extracting the sections with acetonitrile and NaOH once the basipetal IAA movement ceased after a 24 h transport period) showed a negative, highly significant correlation with the amount of IAA transported. Although the rooting percentage and IAA transport were higher in Elsy than in Oriana, the differences in rooting between the cultivars could not be explained solely by differences in IAA transport.

*Additional key words*: auxin metabolism, *Dianthus caryophyllus*.

## Introduction

The involvement of auxin in the formation of adventitious roots has been reported in many studies. Auxin treatments are a common practice to induce rooting in reluctant species and promote rooting in spontaneous rooting species such as carnation (Eliasson and Areblad 1984, Boggetti *et al.* 2001). Studies on these species have shown that endogenous IAA from the upper part of cuttings (apex, buds, leaves) was necessary for the formation of adventitious roots in the base of cuttings (Gatineau *et al.* 1997). Thus, rooting was inhibited by removal of the presumed auxin sources by decapitation, debudding or defoliation (Haissig 1970, Eliasson and Areblad 1984, Garrido *et al.* 2002). Conversely, application of exogenous auxin induced rooting in cuttings with the auxin source removed (Liu and Reid 1992). Specific inhibitors of polar auxin transport (PAT) such as 1-N-naphthylphthalamic acid (NPA) also inhibited rooting (Katsumi *et al.* 1969, Liu and Reid 1992, Garrido *et al.* 2002). All the above suggest that PAT might play a decisive role in providing the IAA needed for spontaneous rooting to the rooting zone.

In commercial production of many ornamental rooted plants, cold storage of cuttings before their planting is a common practice used to match production and demand. Previous studies have shown that cold storage of carnation cuttings could produce significant variations in the subsequent rooting (Van de Pol and Vogelezang 1983, Garrido *et al.* 1996, 1998). These changes depended on the storage period and the carnation cultivar. Thus, long storage periods (8 - 10 weeks) could be as effective as an auxin treatment in rooting promotion (Garrido *et al.* 1996). These results suggested that changes in auxin concentration at the rooting zone, occurring during and/or after storage, might be responsible for the changes observed in the rooting. Taking into account that the origin of the IAA responsible for the rooting in carnation cuttings is the leaves and that exogenous IAA applied to mature leaves was polarly

*Received* 11 November 2002, *accepted* 21 March 2003.
*Abbreviations*: IAA - indolyl-3-acetic acid; $I_T$ - (polar) transport rate; ORP - optimal rooting period; PAT - polar auxin transport.
*Acknowledgements*: This work was supported by grant (AGL2001-0465) from MCYT/FEDER, Spain.
[1] Corresponding author; fax: (+34) 968364936; e-mail: jsbravo@um.es

transported through the stem to the rooting zone (Garrido *et al.* 2002), it could be suspected that the final IAA concentration in the rooting zone should be regulated by the transport and metabolism of IAA along the cutting stem. The aim of the present study is to investigate the influence of cold storage on the transport of IAA in an attempt to explain the changes in rooting produced by cold storage on different carnation cultivars. In addition, a tentative evaluation of the IAA metabolism during PAT was carried out. A recent study showed a parallel variation in PAT and rooting during cold storage of carnation cultivar Virginie (Guerrero *et al.* 1999). Here data are presented which suggest a possible relationship between the variations in PAT and rooting produced by cold storage of carnation cuttings. Among the several carnation cultivars previously studied, Oriana and Elsy cultivars showed notable differences in rooting after cold storage (Garrido *et al.* 1996) and for this reason were used in the present study.

## Materials and methods

**Plants:** Carnation (*Dianthus caryophyllus* L. cvs Oriana and Elsy) cuttings (*Barberet and Blanc*, Puerto Lumbreras, Murcia, Spain) were pinched from mother plants and stored in a cold chamber as described by Garrido *et al.* (1996). After different storage periods (2, 8 and 12 weeks) cuttings were planted for rooting. Before planting, the transport and metabolism of IAA in sections from cutting stem were studied.

**Rooting of cuttings:** Cold stored cuttings were planted in a peat-perlite substrate (70:30, v/v) in the greenhouse as described by Garrido *et al.* (2002). The influence of cold storage on the subsequent rooting was evaluated according to Garrido *et al.* (1998) by using the following indicators: *1*) percentage of plants that were rooted at day 11 after planting and *2*) the optimal rooting period (ORP) defined as the minimum time required for 95 % of plant to show commercial rooting quality, characterized by the presence of 30 - 40 roots and an average root length of about 45 mm.

**Transport and metabolism of IAA:** The transport and metabolism of exogenous IAA were measured in sections with different localization along the cuttings before their planting for rooting. To evaluate as quickly as possible the effects of cold storage, and for practical reasons, the study was done before planting rather than during the rooting period. Nevertheless, the transport assays were carried out at optimal rooting temperature (24 ± 1 °C) and in darkness since the stem region used would have been submerged in the substrate during rooting. IAA transport was studied in sections of stem cutting as described previously (Guerrero *et al.* 1999). Briefly, a 5 $mm^3$ drop of a water solution containing 335 Bq of [5-$^3$H]-IAA (*Amersham*, Buckinghamshire, UK) was applied to the upper cut surface of excised sections placed on a receiver agar block. To measure radioactivity transported at different time periods, the agar block was replaced at hourly intervals for up to 6 h, the transport from 6 to 8 h and from 8 to 24 h also being measured. The radioactivity accumulated in the replaced agar blocks was plotted against time to obtain the corresponding transport curves. The linear parts of these curves were fitted using the least-squares method. The slope of the regression equation represents the transport rate (intensity), $I_T$, [Bq $h^{-1}$]. This parameter is commonly used to measure PAT since a minor part of the basipetal IAA transport during the first hours is not polar (Botía *et al.* 1992). Transport was studied in 5 mm sections from two different localization in the cuttings, the region from 3 mm below to 2 mm above the oldest node (node) and the region immediately below (base). A negligible amount of mobile IAA was present in the transporting sections at the end of the transport period (24 h) (see Results). At this time, individual sections were successively extracted with acetonitrile and 1 M NaOH as described by Sánchez-Bravo *et al.* (1990). This previous study showed that acetonitrile efficiently extracted the IAA oxidation products while the radioactivity extracted with NaOH included the IAA from hydrolyzed IAA conjugates. As discussed in Results, in the conditions of the present study the total radioactivity extracted with acetonitrile and NaOH could be used for a tentative evaluation of the IAA metabolism. It must be noted that the radioactivity recovered (transported + metabolized) in the different assays represented 60 ± 5 % of that applied. Therefore a part of the IAA applied remained in the tissue sections either compartmentalized and/or metabolized as insoluble compounds.

**Radioactivity measurements:** The activity of $^3$H in the receiver agar blocks and in the different extracts (acetonitrile and NaOH) was measured in a *Rack Beta, model 1211*, liquid scintillation counter (*LKB*, Turku, Finland). Using a standard, the efficiency of $^3$H counting was calculated to be 52 ± 1 %.

**Statistical analysis:** Experiments were repeated at least twice. Fisher's least significant difference (LSD) multiple range test was used to analyze the variance. Data showing significant differences at the 95 % or greater confidence level were taken into account in the interpretation of the results. The correlation coefficients and their significance (* - $P \leq 0.05$, ** - $P \leq 0.01$; $^{NS}$ - not significant) were calculated in the linear regression analysis carried out. The number of XY pairs used in this calculation was 15 for nodes and bases and 30 for nodes + bases in each cultivar (twice when the data of both cultivars were used) in the linear regression between total metabolism

(acetonitrile + NaOH) and transport (total in 24 h) of IAA, and 9 for nodes and bases in each cultivar (18 when the data of both cultivars were used) in the linear regression between rooting percentage and auxin transport rate.

## Results and discussion

**Influence of cold storage on the rooting of cuttings:** A previous study showed that in general, cold storage favored rooting compared to unstored (*i.e.* fresh) cuttings in different carnation cultivars (Garrido *et al.* 1998). In addition, cuttings that have been stored for a given period rather than fresh cutting are generally used in commercial production of rooted plants. For these reasons the study was carried out using cuttings stored for 2, 8 and 12 weeks. The rooting percentage and the optimal rooting period (ORP) used in evaluating the rooting showed notable variations after cold storage of cuttings (Fig. 1). As a rule, an increase in rooting percentage and a shortening in ORP were observed as the storage period increased although differences between the two cultivars were evident. Thus, rooting percentage was lower and ORP higher in Oriana than Elsy after a given storage period. It must be noted that rooting percentage was increased more than three fold in 12 week- compared to 2 week-stored Oriana cuttings (Fig. 1). In Elsy cuttings,

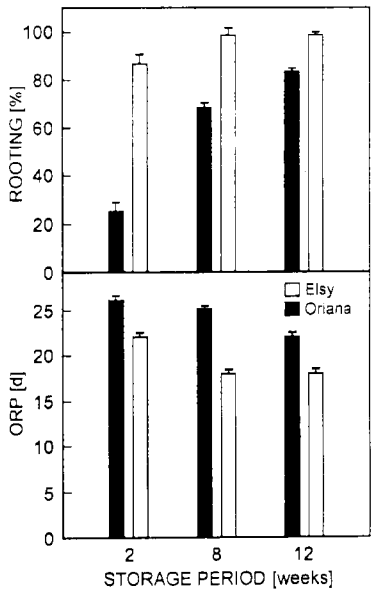


Fig. 1. Rooting percentage and optimal rooting period (ORP) of Oriana and Elsy cuttings stored for different periods in a cold chamber. Means ± SE, $n = 3$.

an increase in the storage period from 2 to 8 weeks resulted in a small increase in rooting percentage, no further variations being noted at longer storage periods. These effects of cold storage on rooting confirm the results obtained in previous studies, which suggested that cold storage might produce changes in the level of IAA (Garrido *et al.* 1996, 1998). These changes could be caused by alterations in the biosynthesis, transport and catabolism of IAA.

**Transport of IAA in cutting sections:** The curves in Fig. 2 show almost no IAA transport after 8 h since the $^3$H-IAA from 8 to 24 h was negligible irrespective of the localization of the sections (nodes or bases), the cultivar or the storage period of the cuttings. Transport was dependent on the localization since $I_T$ and the total amount of transported $^3$H-IAA at 24 h were higher in bases than nodes (Table 1, Fig. 2), which indicates that transport in nodes could be decisive in providing IAA to the rooting zone as discussed by Garrido *et al.* (2002). Transport also varied with the storage period irrespective of the section localization and cultivar. Thus, the lowest values in nodes and bases were observed in 2 week-stored cuttings, the transport being clearly increased when the storage period lengthened to 8 weeks (Fig. 2, Table 1). An increase in the storage period from 8 to 12 weeks produced a significant increase in transport in Oriana while no further variation was observed in Elsy (Fig. 2, Table 1). Although the ultimate reason for this effect of cold storage on IAA transport is unknown, it should be noted that the loss in PAT produced by removal of the endogenous auxin source was prevented by application of exogenous IAA or by exposure to low temperature (Morris and Johnson 1990). The presence of the endogenous auxin source (the leaves) during cold storage of carnation cuttings might favor PAT by some unknown mechanism.

Table 1. Influence of cold storage on $^3$H-IAA transport rate, $I_T$ [Bq h$^{-1}$] in sections from different parts (nodes and bases) of stem cuttings from two carnation cultivars. Means ± SE of five sections. Different superscripts denote significant differences ($P < 0.05$) between the means.

| Cultivar | Storage [week] | Nodes | Bases |
|---|---|---|---|
| Oriana | 2 | $6.9 \pm 0.2^{a}$ | $20.1 \pm 0.6^{f}$ |
| | 8 | $27.8 \pm 0.8^{b}$ | $35.9 \pm 0.7^{g}$ |
| | 12 | $30.9 \pm 0.6^{c}$ | $43.1 \pm 1.0^{h}$ |
| Elsy | 2 | $12.4 \pm 0.2^{d}$ | $23.5 \pm 0.7^{i}$ |
| | 8 | $16.7 \pm 0.8^{e}$ | $30.1 \pm 1.0^{c,j}$ |
| | 12 | $18.5 \pm 0.9^{e,f}$ | $28.0 \pm 1.2^{b,j}$ |

**IAA metabolism during PAT in cutting sections:** In an attempt to evaluate the IAA metabolized during PAT, sections were successively extracted with acetonitrile and NaOH at the end of the transport period (24 h). At this time no mobile IAA was present in the sections (Fig. 2) and no significant amount of IAA was detected in the acetonitrile fraction (data not shown). Therefore, in the conditions of the present study and bearing in mind

previous results (Sánchez-Bravo *et al.* 1990, 1991) most of the radioactivity extracted with acetonitrile corresponds to decarboxylation products while the NaOH fraction corresponds to conjugated IAA and conjugated products. The radioactivity was detected in acetonitrile and NaOH fractions obtained from all the transporting sections (Fig. 3). This indicates that part of the applied auxin was metabolized during its transport, a phenomenon that has already been described (Goldsmith and Thimann 1962, Sánchez-Bravo *et al.* 1991). This phenomenon might be explained taking into account that during PAT, there was a lateral diffusion of IAA from the PAT pathway to the adjacent tissues where IAA was metabolized (Sánchez-Bravo *et al.* 1991) since no significant IAA metabolism was detected in the transporting cells (Botía *et al.* 1992). The total IAA metabolized (acetonitrile + NaOH) was higher in Elsy than in Oriana and decreased as the storage period

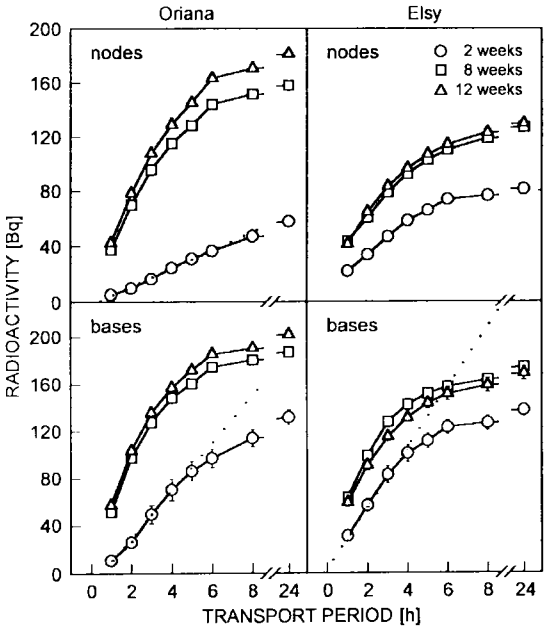


Fig. 2. Transport of radioactive IAA in nodes and bases of Oriana and Elsy cuttings stored for 2, 8 and 12 weeks in a cold chamber. The radioactivity recovered in the receiver agar blocks after different transport periods is presented. Means ± SE of five sections. The linear parts of the transport curves were fitted by the least squares method (dotted lines) to obtain $I_T$.

increased from 2 to 8 weeks, small or no significant differences being observed when the storage period increased from 8 to 12 weeks (Fig. 3). Negative linear regression between the total IAA metabolism and the total IAA transport in sections from cuttings stored for different periods, irrespective of the section localization and the cultivar was measured (the correlation coefficients for nodes, bases and nodes + bases, respectively, were: -0.94**, -0.96** and -0.90** in Oriana; -0.94**, -0.86** and -0.93** in Elsy; -0.66**, -0.80** and -0.74** in Oriana and Elsy). Negative correlation between transport and metabolism has been also observed in other plant materials (Kaldewey 1984, Sánchez-Bravo *et al.* 1988). These results clearly show that stem tissues in carnation cuttings have a capacity to metabolize IAA.

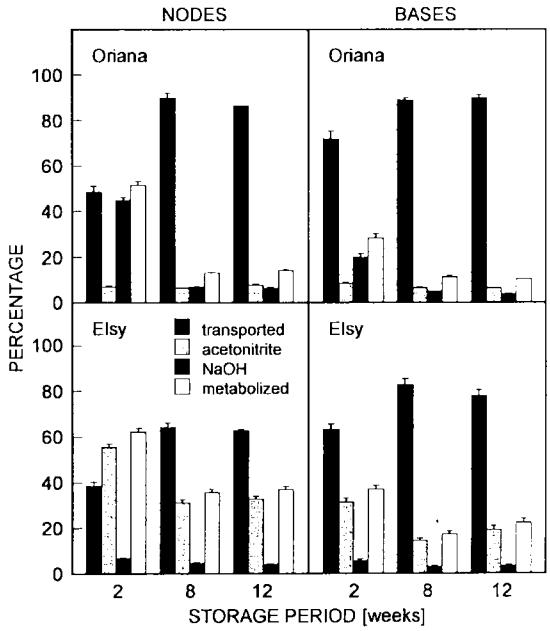


Fig. 3. Metabolism of IAA in nodes and bases of Oriana and Elsy cuttings stored for different periods in a cold chamber. After a 24 h transport period, the sections were successively extracted with acetonitrile and NaOH. The total IAA metabolized (acetonitrile + NaOH) and transported are also presented. Data are expressed as a percentage of the total radioactivity recovered (transported + metabolized) and correspond to the mean ± SE of five sections.

**Correlation between rooting and IAA transport:** Taking into account that the IAA responsible for spontaneous rooting in carnation cuttings was transported from the leaves through the stem (Garrido *et al.* 2002), the question is whether the rooting variations produced by cold storage (Fig. 1) is associated with variations in IAA transport in the stem (Fig. 2, Table 1). The linear regression analysis carried out by fitting precocity as a function of $I_T$ at the different storage periods showed positive and significant correlation coefficients in the two cultivars, irrespective of the section localization (nodes or bases) (the values for nodes and bases, respectively, were: 0.98** and 0.97** in Oriana; 0.74* and 0.82** in Elsy). This means that in a given cultivar the onset of rooting appears to be dependent on the $I_T$. However, no significant correlation was obtained when the values of both cultivars were used in the same regression plot ($0.39^{NS}$ for nodes and $0.38^{NS}$ for bases), which suggest that the auxin content required for the onset of rooting was different in each cultivar. Differences between species and cultivars in their auxin requirements for rooting have been described (James 1983, Berthon

*et al.* 1991).

The present results suggest that the changes in $I_T$ along the stem observed during cold storage and the capacity of the stem tissues to metabolize IAA could alter the provision of auxin to the rooting zone of carnation cuttings.

## References


Berthon, J.Y., Boyer, N., Gaspar, T.: Uptake, distribution and metabolism of 2,4-dichlorophenoxy-acetic acid in shoots of juvenile and mature clones of *Sequoiadendrom giganteum* in relation to rooting *in vitro*. - Plant Physiol. Biochem. **29**: 355-362, 1991.

Boggetti, B., Jásik, J., Mantell, S.H.: *In vitro* root formation in *Anacardium occidentale* microshoots. - Biol. Plant. **44**: 175-179, 2001.

Botía, J.M., Ortuño, A., Acosta, M., Sabater, F., Sánchez-Bravo, J.: Influence of 2,3,5-triiodobenzoic acid on the transport and metabolism of IAA in lupin hypocotyls. - J. Plant Growth Regul. **11**: 135-141, 1992.

Eliasson, L., Areblad, K.: Auxin effects on rooting in pea cuttings. - Physiol. Plant. **61**: 293-297, 1984.

Garrido, G., Cano, E.A., Arnao, M.B., Acosta, M., Sánchez-Bravo, J.: Influence of cold storage period and auxin treatment on the subsequent rooting of carnation cuttings. - Sci. Hort. **65**: 73-84, 1996.

Garrido, G., Cano, E.A., Acosta, M., Sánchez-Bravo, J.: Formation and growth of roots in carnation cuttings: influence of cold storage period and auxin treatment. - Sci. Hort. **74**: 219-231, 1998.

Garrido, G., Guerrero, J.R., Cano, E.A., Acosta, M., Sánchez-Bravo, J.: Origin and basipetal transport of the IAA responsible for rooting of carnation cuttings. - Physiol. Plant. **114**: 303-312, 2002.

Gatineau, F., Fouché, J.G., Kevers, C., Hausman, J.F., Gaspar, T.: Quantitative variations of indolyl compounds including IAA, IAA-aspartate and serotonin in walnut microcuttings during root induction. - Biol. Plant. **39**: 131-137, 1997.

Goldsmith, M.H.M., Thimann, K.V.: Some characteristics of movement of indoleacetic acid in coleoptiles of *Avena*. I. Uptake, destruction, immobilization, and distribution of IAA during basipetal translocation. - Plant Physiol. **37**: 492-505, 1962.

Guerrero, J.R., Garrido, G., Acosta, M., Sánchez-Bravo, J.: Influence of 2,3,5-triiodobenzoic acid and 1-N-naphthylphthalamic acid on indoleacetic acid transport in carnation cuttings. Relationship with rooting. - J. Plant Growth Regul. **18**: 183-190, 1999.

Haissig, B.E.: Influence of indole-3-acetic acid on adventitious root primordia of brittle willow. - Planta **95**: 27-35, 1970.

James, D.J.: Adventitious root formation *in vitro* in apple rootstocks (*Malus pumila*) II. Uptake and distribution of indole-3-acetic acid during the auxin sensitive phase in M.9 and M.26. - Physiol. Plant. **57**: 154-158, 1983.

Kaldewey, H.: Transport and other modes of movements of hormones (mainly auxins). - In: Scott, T.K. (ed.): Hormonal Regulation of Development II. Encyclopedia of Plant Physiology New Series. Vol. 10. Pp. 80-148. Springer-Verlag, Berlin 1984.

Katsumi, M., Chiba, Y., Fukuyama, M.: The roles of the cotyledons and auxin in the adventitious root formation of hypocotyl cuttings of light-grown cucumber seedlings. - Physiol. Plant. **22**: 993-1000, 1969.

Liu, J.H., Reid, D.M.: Adventitious rooting in hypocotyls of sunflower (*Helianthus annuus*) seedlings. IV. The role of changes in endogenous free and conjugated indole-3-acetic acid. - Physiol. Plant. **86**: 285-292, 1992.

Morris, D.A., Johnson, C.F.: The role of auxin efflux carriers in the reversible loss of polar auxin transport in the pea (*Pisum sativum* L.) stem. - Planta **181**: 117-124, 1990.

Sánchez-Bravo, J., Ortuño, A., Acosta, M., Sabater, F.: *In vivo* metabolism of labeled indole-3-acetic acid during polar transport in etiolated hypocotyls of *Lupinus albus*: Relationship with growth. - Plant Growth Regul. **7**: 271-288, 1988.

Sánchez-Bravo, J., Ortuño, A., Botía, J.M., Acosta, M., Sabater, F.: Lateral diffusion of polarly transported indoleacetic acid and its role in the growth of *Lupinus albus* L. hypocotyls. - Planta **185**: 391-396, 1991.

Sánchez-Bravo, J., Ortuño, A., Botía, J.M., del Rio, J.A., Caballero, M., Acosta, M., Sabater, F.: Identification of the metabolites of indole-3-acetic acid in growing hypocotyls of *Lupinus albus*. - Plant Growth Regul. **9**: 315-327, 1990.

Van de Pol, P.A., Vogelezang, J.V.M.: Accelerated rooting of carnation "Red Baron" by temperature pretreatment. - Acta Hort. **141**: 181-188, 1983.