

Relation between Nitrogen Status, Carbohydrate Distribution, and Subsequent Rooting of *Chrysanthemum* Cuttings[©]

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INTRODUCTION

Adventitious root formation (ARF) of cuttings is substantially affected by the initial nitrogen and carbohydrate status (Haissig, 1986; Blazich, 1988; Veierskov, 1988). High nitrogen supply to stock plants, meeting or even surpassing the level necessary for maximum growth, has often been observed to decrease subsequent rooting of cuttings (Roerber and Reuther, 1982; Haissig, 1986; Henry et al., 1992). Such effects have been discussed repeatedly in relation to decreased carbohydrate levels and decreased C : N ratio, which was first suggested by Kraus and Kraybill (1918) to be a crucial characteristic for ARF. However, there exists a large amount of conflicting data (Hansen et al., 1978; Leakey, 1983; Haissig, 1986; Veierskov, 1988; Leakey and Storeton-West, 1992). In a recent study, ARF of chrysanthemum cuttings at natural radiation in a greenhouse during spring and summer was found to be positively correlated with initial nitrogen concentrations, and not impeded by simultaneously lower sugar concentrations in cuttings (Droege et al., 1998).

Nitrogen is needed for synthesis of diverse nitrogenous compounds, but the promotive influence of nitrogen in root formation may also be manifested by the manner in which it relates to carbohydrate content and metabolism (Blazich, 1988). ARF should be related to individual carbohydrate pools in certain tissues (Haissig, 1986; Veierskov, 1988) and to the efficiency of carbohydrate utilization (Okoro and Grace, 1976; Haissig, 1984; Tschaplinski and Blake, 1989) rather than to total content in cuttings. Nitrogen supply strongly influences carbon allocation and partitioning in plants (Rufty et al. 1988; Kaiser, 1997). Also cold storage, which is a standard procedure in the propagation of chrysanthemums, affects the carbohydrate status of cuttings (Behrens, 1988).

With these relationships in mind, and regarding the results obtained in a previous study (Droege et al., 1998), the present investigation was carried out to provide a more detailed insight into the interactions between nitrogen status and the distribution of nonstructural carbohydrates and their role in subsequent ARF of two chrysanthemum cultivars differing in their rooting response.

MATERIALS AND METHODS

Details of all materials and methods are also described by Droege et al. (2000).

Plant Material, Experimental Design, and Determination of ARF. *Chrysanthemum* (syn. *Dendranthema ×grandiflorum* Ramat. Kitamura) 'Puma' *Chrysanthemum* 'Puma' and 'Cassa' were planted in a greenhouse in commercial peat (Einheitserde Typ P- Sinta, Patzer Company, Germany) at a density of 100 plants m⁻². Plants were managed as stock plants with one harvest of cuttings per

week. Apart from nitrogen, all plants received the same adequate nutrient supply. The two cultivars were combined with three nitrogen treatments as preharvest factors using three replicates. The nitrogen dosages (N-low, N-medium, N-high: 0.6, 1.5, 4.0 g N m⁻² week⁻¹, respectively) were adjusted by combining a constant dose of calcium nitrate with different doses of ammonium nitrate. Cuttings were harvested on three occasions (21 April, 2 June, 14 July 1997) and either chemically analysed and rooted immediately, or first stored in nonperforated polyethylene bags in the dark at two temperatures (0.5, 5°C) for three different periods (2, 3, 4 weeks). ARF of 20 cuttings per treatment and replicate (n = 3) was studied in a greenhouse under intermittent misting using perlite as rooting substrate. No fertilizers or plant hormones were applied. After 12 days the number of roots per cutting and mean root length were determined.

Chemical Analysis. Total nitrogen concentration in the dry matter (Nt) of 50 harvested cuttings per treatment and replicate (n=3) was analysed with a CHN-Rapid analyser by using the Dumas method (Ehrenberger, 1991). As Nt in cuttings during 4 weeks of cold storage remained unchanged (Druege et al., 1998), this data was used for all storage combinations. At harvest, and after the different storage intervals, carbohydrate concentrations in the dry matter of leaves, upper stems and basal stems (1 cm) of six cuttings per treatment and replicate (n = 3) were analysed. Sugars were extracted in 80% ethanol and quantified in a microplate assay by an enzyme-coupled colorimetric reaction (Hendrix, 1993). Starch and fructan concentrations in the extraction residues were determined via the glucose released after digestion with amyloglucosidase and via fructosyl residues released after acid hydrolysis, respectively.

Statistics. Effects of treatments were tested by analyses of variance using harvest date as a potential interacting factor. Because results were not substantially affected by storage temperature, the two temperatures were not differentiated. Comparison of mean values was carried out using the Newman-Keuls test (Statsoft, 1995) with a significance level of at least P 0.05. Linear regressions and correlations were calculated between concentrations of nitrogen and carbohydrate characteristics for the different cutting parts and number and length of subsequently formed adventitious roots.

RESULTS

Nitrogen Status of Cuttings. Fresh weight and dry matter concentrations of cutting samples were similar for the different nitrogen treatments and cultivars (Druege et al., 2000). As expected, increasing nitrogen supply resulted in higher Nt in the cuttings, which were on the same level for both cultivars (mean values for N-low, N-medium and N-high: 3.9%, 4.9%, and 5.6% Nt, respectively).

Carbohydrate Distribution as Influenced by Nitrogen Supply, Cold Storage, and Cultivar. With regard to the carbohydrate composition of different cutting parts, strong interactions were found between nitrogen supply of stock plants and duration of cold storage. Responses of leaf carbohydrates are presented in Fig. 1. One primary effect of increasing nitrogen was a decrease in starch levels at harvest (Fig. 1A). Also, concentrations of fructans at harvest, which were lower than 5% of the concentrations of starch, decreased when more nitrogen was supplied (Druege et al., 2000). The

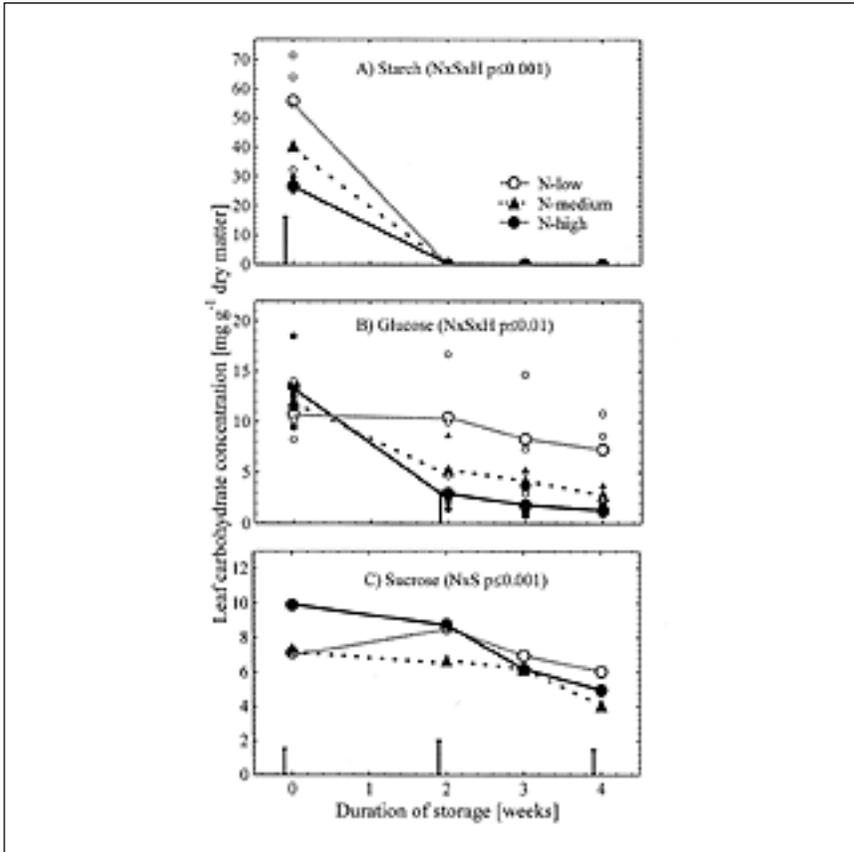


Figure 1. Effects of nitrogen supply (N) and cold storage (S) on starch (A), glucose (B) and sucrose (C) concentrations in leaves of chrysanthemum cuttings. Average of two cultivars. Vertical bars represent LSD ($P = 0.05$) between nitrogen treatments at specified storage durations. Small symbols represent mean values per harvest date (H) in cases of interactions with this factor.

influence of nitrogen supply on sugar concentrations was less pronounced as can be seen from glucose in leaves (Fig. 1B). But significantly higher sucrose levels were measured in the leaves when cuttings were collected from high nitrogen-supplied stock plants (Fig. 1C). Starch completely disappeared from leaves within 2 weeks of storage regardless of the nitrogen treatment (Fig. 1A). Cold storage also resulted in a decrease of sugar concentrations. For all sugars, the decrease was lowest in the low-nitrogen cuttings and highest in the high-nitrogen cuttings. As a consequence, the ranking of sugar concentrations in leaves amongst the nitrogen treatments found at harvest was reversed by storage, with low-nitrogen cuttings having highest sugar levels after 3 weeks of storage (Fig. 1).

Interactions between nitrogen and cold storage were similar for the different cutting parts. They were also observed in the basal stems of cuttings, where adventitious roots were subsequently regenerated (Fig. 2A). In addition to these

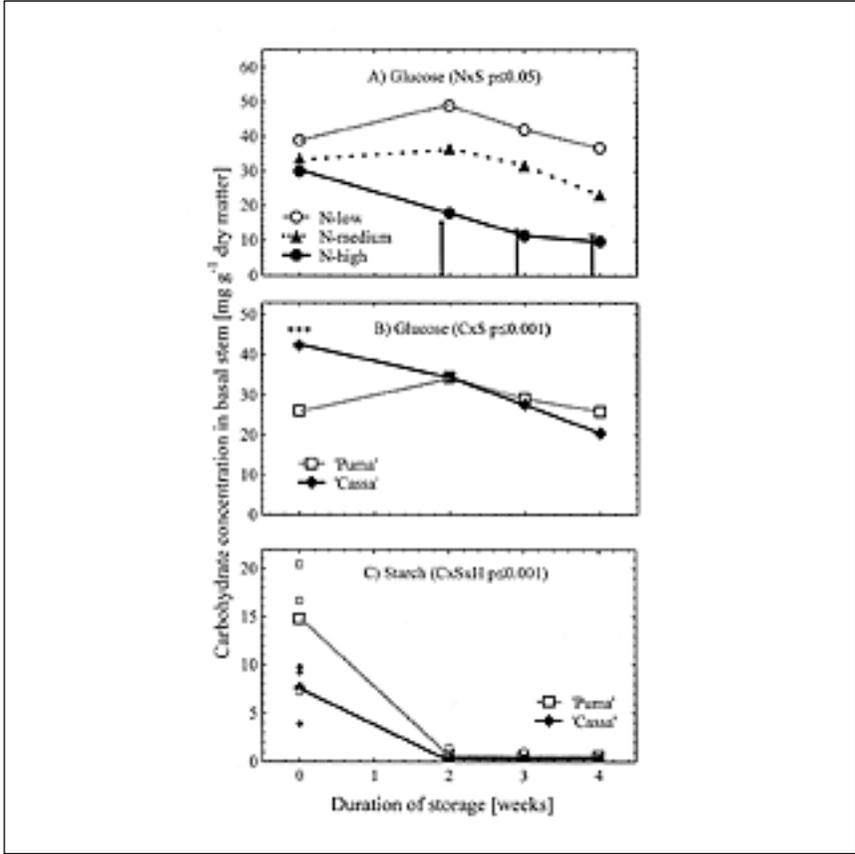


Figure 2. Interactions between nitrogen supply (N) and cold storage (S) on glucose (A) and between cultivar (C) and cold-storage (S) on glucose (B) and starch (C) concentrations in basal stems (1 cm) of chrysanthemum cuttings. Vertical bars represent LSD (P 0.05) between nitrogen treatments (average of two cultivars) at specified storage durations. Asterisks indicate significant differences (P 0.001) between the two cultivars (average of three nitrogen treatments) at specified storage durations. For explanation of small symbols see Fig. 1.

interactions, the two cultivars showed different responses to cold storage. At harvest, sugar concentrations in all tissues were higher for 'Cassa' than for 'Puma', as presented for glucose in basal stems (Fig. 2B). By contrast, 'Cassa' had lower starch levels (Fig. 2C). Cold storage, which caused a dramatic loss of starch in cuttings of both cultivars (Fig. 2C), resulted in a much slower decrease of sugar levels in tissues of 'Puma' than in 'Cassa' (Fig. 2B). As a result, after 4 weeks of storage 'Puma' had similar (Fig. 2B) or even higher sugar concentrations in all cutting parts when compared to 'Cassa'.

Relationship Between Internal Nitrogen Status and Carbohydrate Distribution. Correlations, calculated with Nt as independent variable and carbohydrate characteristics of the different cutting parts as dependent variables,

generally highlighted the effects noted above. Since correlations were similar for both cultivars, only data of 'Cassa' is presented (Table 1). Strong negative correlations were calculated between Nt and both starch and fructan concentrations in leaves of nonstored cuttings. By contrast, sucrose levels in same organs were positively correlated with Nt. Thus, the partitioning between sucrose and starch in leaves was particularly sensitive to the nitrogen level. This became also apparent from strong positive correlations calculated between Nt and the sucrose : starch ratio in leaves at harvest (Fig. 3). The linear regression lines document a stronger nitrogen response of this characteristic for 'Cassa' (slope: 0.15) than for 'Puma' (slope: 0.09). Cold storage had a pronounced effect on the nitrogen carbohydrate relationships (Table 1). The positive correlation between nitrogen and sucrose in harvested cuttings was reversed into a negative relationship. In most cases, negative correlations were found between nitrogen concentration and carbohydrate concentrations in the cutting parts of stored cuttings.

Subsequent Rooting of Cuttings as Related to Nitrogen and Carbohydrate Status. As shown for number of roots for 'Cassa' (Table 2), irrespective of cultivar and cold storage, the number and length of subsequently formed adventitious roots were positively correlated with pre-rooting Nt in cuttings. The steepness of the regression line was higher for 'Cassa' than for 'Puma' in regards to number of roots formed by the nonstored cuttings (Fig. 4A, slopes of 5.3 and 2.7, respectively). This indicated a stronger nitrogen response for 'Cassa'. Whereas the regression of 'Puma' was not significantly affected by cold storage (slope: 2.6), the steepness of the regression of 'Cassa' was substantially decreased (slope: 3.1, Fig. 4B). Thus, the stronger nitrogen response of rooting of 'Cassa' was reduced by storage.

Table 1. Correlation coefficients between nitrogen (Nt) in cuttings as independent and carbohydrate concentrations in leaves and basal stems of cuttings as dependent variables for 'Cassa'.

Carbohydrate concentration	Organ	At harvest (n=27)	After storage (n=162)
Glucose	leaves	n.s.	-0.71 ***
	basal stems	n.s.	-0.70 ***
Fructose	leaves	n.s.	-0.62 ***
	basal stems	n.s.	-0.46 ***
Sucrose	leaves	+0.54 **	-0.28 ***
	basal stems	n.s.	-0.40 ***
Starch	leaves	- 0.82 ***	-0.44 ***
	basal stems	n.s.	-0.49 ***
Fructans	leaves	- 0.61 ***	n.s.
	basal stems	- 0.57 **	-0.21 **

n.s., nonsignificant; ** P < 0.01; *** P < 0.001.

The correlations between pre-rooting carbohydrate concentrations in the different cutting parts of nonstored cuttings and the number of adventitious roots were, in most cases, either insignificant or else negative, as presented for 'Cassa' (Table 2). By way of contrast, significant positive correlations were calculated between sucrose concentration in leaves and number of roots. However, a causal relationship was not evident from these data because the same sugar concentrations also revealed positive correlations to nitrogen level (Table 1). The positive relationship between sucrose concentrations and number of roots mainly reflects intercorrelations with nitrogen, and becomes evident from the absence of such a correlation for stored cuttings (Table 2). These relationships provide evidence that decreased pre-rooting carbohydrate concentrations in the parts of nitrogen-rich cuttings did not substantially impede their improved rooting.

Whereas pre-rooting carbohydrate concentrations in the different cutting parts did not affect rooting, the more roots formed by nitrogen rich cuttings, as well as the stronger nitrogen response of 'Cassa' were associated with increased sucrose : starch ratios in leaves at harvest (Fig. 3). Consequently, strong correlations were calculated between the sucrose : starch ratio in leaves at harvest and numbers of subsequently formed roots (Fig. 5). Even after combining the data obtained from nonstored cuttings of both cultivars, a highly significant regression covered both the principal positive nitrogen effect as well as the stronger nitrogen response of 'Cassa' (Fig. 5A). While 23% of the variability of root numbers formed by the nonstored cuttings of both cultivars could be predicted by Nt, 57% of the same variability could be explained by using the sucrose : starch ratio. In addition, the rooting response of stored cuttings increased with increasing sucrose : starch ratio at harvest. The less steep regression line of stored cuttings (Fig. 5B) was mainly attributed to the higher storage sensitivity of nitrogen-rich cuttings from 'Cassa' (Fig. 4B). However, 40% of the total variability in number of roots affected by the two cultivars, three nitrogen treatments, three harvest dates, seven storage combinations, and three replicates,

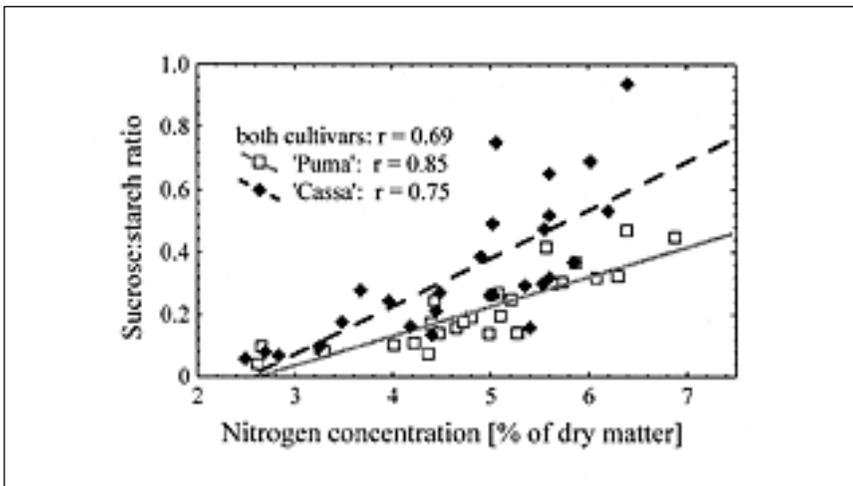


Figure 3. Linear regressions between total nitrogen concentrations (Nt) in cuttings and sucrose:starch ratios in leaves of cuttings at harvest.

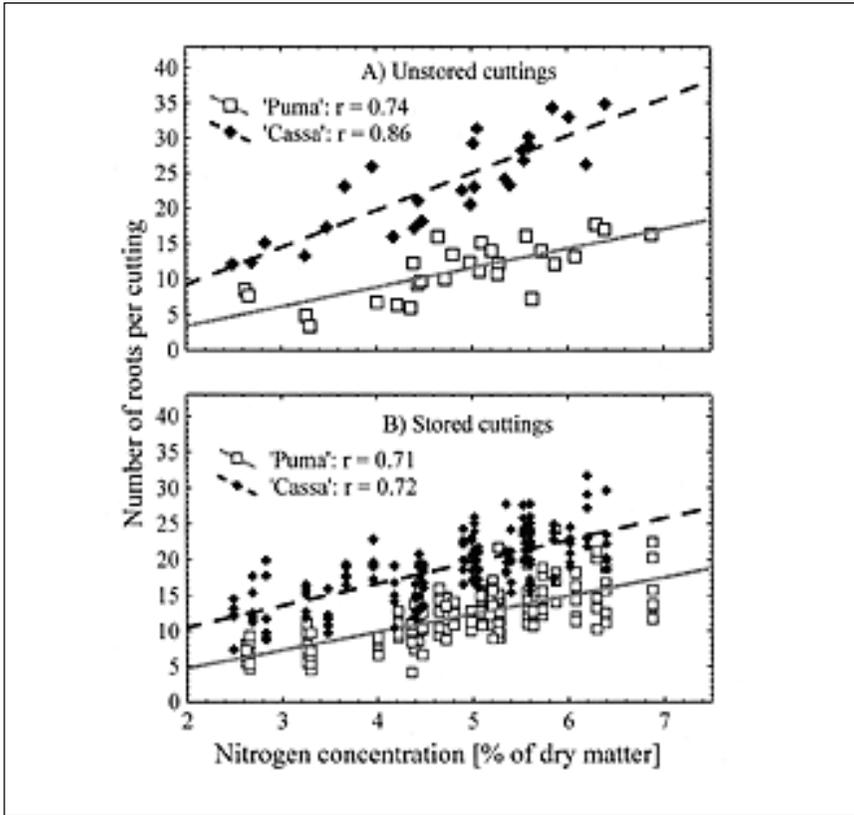


Figure 4. Linear regressions between pre-rooting nitrogen concentrations in cuttings and number of roots subsequently formed by unstored (A) and stored (B) cuttings.

ranging from 3 to 35 roots per cutting, could be explained by using this characteristic ($r = 0.63$), whereas only 25% could be predicted from Nt.

DISCUSSION

With regard to carbohydrate distribution, the most pronounced effect of increasing nitrogen supply and correspondingly increasing Nt was a decrease in starch concentrations in leaves at harvest, whereas sucrose concentrations in the same organs slightly increased. Similar results were found for spinach (Reddy et al., 1996) and for peach (Nii et al., 1997). The resulting increase in sucrose : starch ratio in the leaves indicates an increased partitioning of carbohydrates towards assimilate export (Galtier et al., 1993). The main effect of cold storage observed in the present study was a decrease of carbohydrate concentrations in the cutting parts, which agrees with observations on cuttings of other species (Davies and Potter, 1985; Behrens, 1988). Low temperatures during dark storage slow down metabolic processes including respiration (Behrens, 1988), but there is still a need for energy and organic compounds, and respiration is not completely prevented (Kubota et al., 1997). Starch turned out to be much more sensitive to cold storage than the sugars.

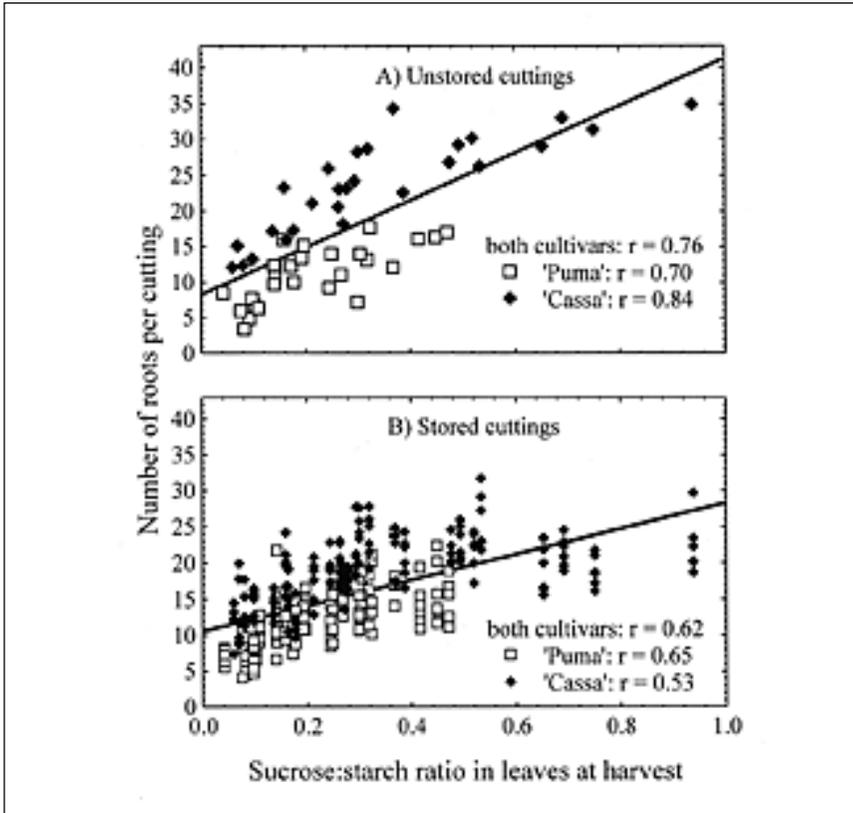


Figure 5. Linear regressions between sucrose:starch ratios in leaves at harvest and number of roots subsequently formed by unstored (A) and stored (B) cuttings.

The stronger decrease in sugar concentrations for the cultivar 'Cassa' and the more pronounced decrease after increased nitrogen supply, both associated with lower starch levels at harvest, indicate that initial starch levels affected the sugar responses to storage.

Confirming the results obtained in a previous study (Druege et al., 1998), ARF of chrysanthemum cuttings was principally promoted by an increasing nitrogen supply and a corresponding increase in internal nitrogen concentrations. This was regardless of the cultivar and whether or not cuttings received cold storage. The correlations found are evidence that the initial (pre-rooting) concentrations of individual and total carbohydrates in the different cutting parts did not impede ARF to such an extent that the positive effect of increased nitrogen supply was significantly inhibited. Taking into account that chrysanthemum cuttings have a high photosynthetic capacity within a day after excision (Kadner and Druege, 1999), the findings strongly support the conclusion that under adequate light conditions (present study: average PPFD per 16-h day outside the greenhouse $>300 \mu\text{mol m}^{-2} \text{s}^{-1}$, 400 to 700 nm) carbohydrate reserves per se do not limit root formation, because the carbohydrate demand is covered by current photosynthesis.

Table 2. Correlation coefficients between pre-rooting concentrations of nitrogen (Nt) in cuttings and of carbohydrates in leaves and basal stems as independent variables and number of subsequently-formed adventitious roots as dependent variables for 'Cassa'.

Carbohydrate concentration	Organ	At harvest (n = 27)	After storage (n = 162)
Nt	whole cutting	+0.86 ***	+0.72 ***
Glucose	leaves	n.s.	-0.54 ***
	basal stems	n.s.	-0.55 ***
Fructose	leaves	n.s.	-0.48 ***
	basal stems	n.s.	-0.41 ***
Sucrose	leaves	+0.62 ***	n.s.
	basal stems	n.s.	-0.23 **
Starch	leaves	-0.82 ***	-0.24 **
	basal stems	n.s.	-0.33 ***
Fructans	leaves	-0.69 ***	n.s.
	basal stems	-0.47 *	-0.21 **

n.s., nonsignificant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Increased number of roots produced by nitrogen-rich cuttings and the stronger nitrogen response of the cultivar 'Cassa' were associated with higher sucrose : starch ratios in leaves, reflecting increased carbohydrate partitioning towards assimilate export (Galtier et al., 1993). The correlations found support the conclusion that enhanced carbohydrate export in cuttings manifested at the time of excision was causally involved in the changed rooting response observed. Increased export activity may lead to an accelerated subsequent export, transport, and supply of carbohydrates to the region of root regeneration, where they can promote root initiation and development by diverse mechanisms (Haissig, 1986; Veierskov, 1988). However, also other mechanisms like increased export and basipetal transport of auxins (Cambridge and Morris, 1996; Goldsmith et al., 1974; Lomax et al., 1995) and/or accelerated co-transport of amino acids (Riens et al., 1991; Winter et al., 1992) may be causally involved in the associations found. The stronger nitrogen response of rooting of 'Cassa', associated with lower starch levels and higher sucrose : starch ratios in leaves at harvest, was partially lost during cold storage, and coincided with a stronger decrease of initially higher sugar concentrations. These results indicate that increased partitioning of carbohydrates towards carbohydrate export can only favour subsequent rooting unless carbohydrate depletion of the supplying tissue becomes the crucial factor.

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LITERATURE CITED

- Behrens, V.** 1988. Storage of unrooted cuttings, pp. 235-247. In: Davis, T.D., B.E. Haissig, and N. Sankhla (eds.), Adventitious root formation in cuttings. Dioscorides Press, Portland, Oregon.
- Blazich, F.A.** 1988. Mineral nutrition and adventitious rooting, pp. 61-69. In: Davis, T.D., B.E. Haissig and N. Sankhla (eds.), Adventitious root formation in Cuttings. Dioscorides Press, Portland, Oregon.
- Cambridge, A.M. and D.A. Morris.** 1996. Transfer of exogenous auxin from their phloem to the polar auxin transport pathway in pea (*Pisum sativum* L.). *Planta* 199:583-588.
- Davis, T.D. and J.R. Potter.** 1985. Carbohydrates, water potential, and rooting of stored *Rhododendron* cuttings. *HortScience* 20:292-293.
- Druege, U., S. Zerche, and R. Kadner.** 1998. Relation between nitrogen and soluble carbohydrate concentrations and subsequent rooting of *Chrysanthemum* cuttings. *Advan. Hort. Sci.* 12:78-84.
- Druege, U., S. Zerche, R. Kadner, and M. Ernst.** 2000. Relation between nitrogen status, carbohydrate distribution and subsequent rooting of chrysanthemum cuttings as affected by pre-harvest nitrogen supply and cold-storage. *Ann. Bot.* 85:687-701.
- Ehrenberger, F.** 1991. Quantitative organische Elementaranalyse. p. 863. VCH, Weinheim.
- Galtier, N., C.H. Foyer, J. Huber, T.A. Voelker, and S.C. Huber.** 1993. Effects of elevated sucrose-phosphate synthase activity on photosynthesis, assimilate partitioning, and growth in tomato (*Lycopersicon esculentum* var UC82B). *Plant Physiol.* 101:535-543.
- Goldsmith, M.H.M., D.A. Cataldo, J. Karn, T. Brenneman, and P. Trip.** 1974. The rapid non-polar transport of auxin in the phloem of intact *Coleus* plants. *Planta* 116:301-317.
- Hansen, J., L.-H. Stroemquist, and A. Ericsson.** 1978. Influence of the irradiance on carbohydrate content and rooting of cuttings of pine seedlings (*Pinus sylvestris* L.). *Plant Physiol.* 61:975-979.
- Haissig, B.E.** 1984. Carbohydrate accumulation and partitioning in *Pinus banksiana* seedlings and seedling cuttings. *Physiol. Plant.* 61:13-19.
- Haissig, B.E.** 1986. Metabolic processes in adventitious rooting of cuttings, pp. 141-189. In: Jackson, M.B. (ed.), New root formation in plants and cuttings. Martinus Nijhoff Publishers, Dordrecht, Boston, Lancaster.
- Hendrix, D.L.** 1993. Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. *Crop Sci.* 33:1306-1311.
- Henry, P.H., F.A. Blazich, and L.E. Hinesley.** 1992. Nitrogen nutrition of containerized eastern redcedar. II. Influence of stock plant fertility on adventitious rooting of stem cuttings. *J. Amer. Soc. Hort. Sci.* 117:568-570.
- Kadner, R. and U. Druege.** 1999. Unbewurzelte Stecklinge von Chrysanthemen brauchen viel Licht. *Taspo Gartenbaumagazin* 8(2):24-25.
- Kaiser, W.M.** 1997. Regulatory interaction of carbon- and nitrogen metabolism, pp. 150-163. In: Behnke, H.D., U. Luettge, K. Esser, J.W. Kadereit, and M. Runge (eds.), *Progress in Botany*. Vol. 58, Springer-Verlag, Berlin, Heidelberg.
- Kraus, E.J. and H.R. Kraybill.** 1918. Vegetation and reproduction with special reference to Tomato. Oregon Agricultural Experimental Station Bulletin 149.
- Kubota, C., N.C. Rajapakse, and R.E. Young.** 1997. Carbohydrate status and transplant quality of micropropagated broccoli plantlets stored under different light environments. *Postharvest Biol. Technol.* 12:165-173.
- Leakey, R.R.B.** 1983. Stockplant factors affecting root initiation in cuttings of *Triplochiton scleroxylon* K. Schum., an indigenous hardwood of West Africa. *J. Hort. Sci.* 58:277-290.

- Leakey, R.R.B. and R. Storeton-West.** 1992. The rooting ability of *Triplochiton scleroxylon* cuttings: The interactions between stock plant irradiance, light quality and nutrients. *Forest Ecol. Mgt.* 49:133-150.
- Lomax, T.L., G.K. Muday, and P.H. Rubery.** 1995. Auxin transport, pp. 509-530. In: Davies, P.J. (ed.), *Plant hormones*. Kluwer Academic Publishers, Dordrecht, Boston, London.
- Nii, N., K. Yamaguchi and M. Nishimura.** 1997. Changes in carbohydrate and ribulose biphosphate carboxylase-oxygenase contents in peach leaves after applications of different amounts of nitrogen fertilizer. *J. Japan. Soc. Hort. Sci.* 66:505-511.
- Okoro, O.O. and J. Grace.** 1976. The physiology of rooting *Populus* cuttings.I. Carbohydrates and photosynthesis. *Physiol. Plant.* 36:133-138.
- Reddy, A.R., K.R. Reddy, R. Padjung, and H.F. Hodges.** 1996. Nitrogen nutrition and photosynthesis in leaves of *Pima cotton*. *J. Plant Nutrit.* 19:755-770.
- Riens, B., G. Lohaus, D. Heinecke, and H.W. Heldt.** 1991. Amino acid and sucrose content determined in the cytosolic, chloroplastic, and vacuolar compartments and in the phloem sap of *Spinach* leaves. *Plant Physiol.* 97:227-233.
- Roeber, R. and G. Reuther.** 1982. Der Einfluss unterschiedlicher N-Formen und-Konzentrationen auf den Ertrag und die Qualitaet von Chrysanthemen-Stecklingen. *Gartenbauwissenschaft* 47:182-188.
- Rufty, T.W., Jr., S.C. Huber, and R.J. Volk.** 1988. Alterations in leaf carbohydrate metabolism in response to nitrogen stress. *Plant Physiol.* 88:725-730.
- Statsoft, Inc.** 1995. *Statistica for Windows* [Computer program manual]. Statsoft Inc., 2325 East 13th street, Tulsa, Oklahoma.
- Tschaplinski, T.J. and T.J. Blake.** 1989. Correlation between early root production, carbohydrate metabolism, and subsequent biomass production in hybrid poplar. *Can. J. Bot.* 67:2168-2174.
- Veierskov, B.** 1988. Relations between carbohydrates and adventitious root formation. pp. 70-78. In: Davis, T.D., B.E. Haissig and N. Sankhla (eds.), *Adventitious root formation in cuttings*. Dioscorides Press, Portland, Oregon.
- Winter, H., G. Lohaus, and H.W. Heldt.** 1992. Phloem transport of amino acids in relation to their cytosolic levels in barley leaves. *Plant Physiol.* 99:996-1004.