Rooting capacity of *Pelargonium* cuttings with special regard to carbohydrate availability and photosynthetic performance

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Mom and Dad – for you.

Abstract

Rapaka, V.K.

Rooting capacity of *Pelargonium* cuttings with special regard to carbohydrate availability and photosynthetic performance

This study investigated the relationship between carbohydrate availability and adventitious root formation of two cultivars ('Isabell' and 'Mitzou') of *Pelargonium x hortorum* cuttings, when affected by season of stock plant cultivation, stock plant age, cutting cold-storage (variable temperature regime for 4 days), as well as sustenance of current photosynthesis of those cuttings as additionally affected by rooting environment [greenhouse (GH); climate chamber (CC): PPFD 100 μmol m^{-2} s^{-1}, relatively low light]. Carbohydrate distribution, chlorophyll fluorescence parameters, chlorophyll quality and quantity, and net photosynthetic rates of the cuttings were analyzed during propagation.

Leaf carbohydrate levels of the cuttings were less influenced by season at harvest, but were significantly decreased after cold-storage. Starch, in particular, reduced to trace amounts as early as 1 day after cold-storage. Basal stem carbohydrate levels at harvest were higher in summer when compared to those in spring and winter, which was predominantly due to higher starch. Cold-storage significantly reduced the basal stem carbohydrates only in spring and winter. Pre-insertion non-photochemical quenching (qN) of chlorophyll fluorescence was less influenced by season but significantly decreased by cold-storage. However, during the subsequent course of propagation, there were no significant differences between unstored and stored cuttings for both qN (measured at day 1, 4, 7 & 14) and carbohydrate levels (at day 7) in any specific rooting environment, except in GH during winter for basal stem carbohydrates. Furthermore, the cuttings propagated in CC had significantly lower qN and carbohydrate levels when compared to those in GH during spring and summer. This appears to be a reflection of lower current photosynthetic performance, and consequently, lower production and basipetal transport of carbohydrates in the high light adapted cuttings propagated under relatively low light (CC). The carbohydrate distribution between the leaves and the basal stem during the rooting period indicates, that the newly assimilated carbon was predominantly partitioned between sucrose and starch in leaves, subsequently exported basipetally towards the basal stem, and there was mostly accumulated in sugar pools.

Highly significant positive correlations were found between both leaf sucrose levels and qN at insertion and root number when unstored and stored cuttings of 'Isabell' were propagated in CC (relatively low light) irrespective of the season. In GH, however, they were only correlated in winter (low light intensity). Thus, pre-insertion qN may be used as a rapid and non-destructive physiochemical index for assessing the rooting efficiency of the cuttings when propagated under defined relatively low light conditions. Mean leaf sucrose (day0 + day7) explained the whole variation in root number caused by three contrasting seasons, stock plant age, cold-storage, and rooting environment. Results indicate that even when basal stem carbohydrate levels are high at insertion, adventitious root formation of the cuttings is predominantly influenced by basipetal translocation of carbohydrates during propagation, derived from both pre-insertion leaf carbohydrate reserves and current photosynthesis, the latter of which is itself substantially affected by rooting environment.

Rapaka, V.K.

Bewurzelungsfähigkeit von *Pelargonium*-Stecklingen unter besonderer Betrachtung der Kohlenhydratverfügbarkeit und der Photosyntheseleistung

Diese Studie untersuchte die Beziehung zwischen Kohlenhydratverfügbarkeit und Adventivwurzelbildung zweier Genotypen (cv. „Isabell“ und cv. „Mitzou“) von *Pelargonium x hortorum* Stecklingen unter den Einflüssen der Jahreszeit der Mutterpflanzenkultur, dem Mutterpflanzenalter, einer Kühllagerung der Stecklinge (variable Temperaturführung für 4 Tage), als auch des Beitrages der aktuellen Photosynthese der Stecklinge in weiterer Abhängigkeit von den Bewurzelungsbedingungen (Gewächshaus, Klimakammer: 100 µmol m^{-2} s^{-1}). Untersucht wurden die Kohlenhydratkonzentrationen in den Blättern und in der Sprossbasis, Chlorophyllfluoreszenzparameter, Chlorophyllqualität und –quantität sowie die Nettophotosyntheserate der Stecklinge während der Bewurzelung.

Die Konzentrationen der Kohlenhydrate in den Stecklingsblättern zum Erntetermin wurden nur geringfügig durch die Jahreszeit beeinflusst, durch die anschließende Kühllagerung jedoch signifikant verringert. Stärke konnte bereits nach einem Tag Kältelagerung nur noch in Spuren nachgewiesen werden. Der Kohlenhydratgehalt der Sprossbasis zum Zeitpunkt der Ernte war im Sommer höher als im Frühling und Winter, was in erster Linie in einem höheren Stärkegehalt begründet war. Die Kühllagerung führte nur im Frühling und Winter zu einer signifikanten Reduzierung der Kohlenhydrate der Sprossbasis. Der nicht-photochemische Quenching-koeffizient (qN) der Chlorophyllfluoreszenz zum Zeitpunkt des Steckens wurde nur geringfügig durch die Jahreszeit beeinflusst, aber durch die Kühllagerung signifikant reduziert. Unabhängig von den Bewurzelungsbedingungen wurden diese Unterschiede jedoch bereits innerhalb eines Tages während der Bewurzelung ausgeglichen. Mit Ausnahme der Bewurzelung in Gewächshaus im Winter traf dies ebenfalls auf die am Tag 7 gemessenen Kohlenhydratkonzentrationen in den Stecklingen zu. Darüber hinaus waren während der Stecklingsbewurzelung im Frühling und Sommer qN und Kohlenhydratgehalte in der Klimakammer signifikant niedriger als unter den Gewächshausbedingungen. Dies deutet auf eine verminderte Photosyntheseleistung, eine dadurch verringerte Kohlenhydratsynthese und einen nachfolgend beeinträchtigten basipetalen Kohlenhydrattransport in den Starklicht-adaptierten Stecklinge unter den relativ niedrigen Lichtbedingungen der Klimakammer. Die Kohlenhydratverteilung in den Blättern und in der Sprossbasis während der Bewurzelung deutet darauf hin, dass der neu assimilierte Kohlenstoff in den Blättern vornehmlich zwischen Saccharose und Stärke verteilt, anschließend in Richtung Sprossbasis exportiert und dort bevorzugt in der Zuckerfraktion akkumuliert wurde.

Unabhängig von der Jahreszeit wurden für ungelagerte und gelagerte „Isabell“- Stecklinge hoch signifikante positive Korrelationen zwischen der Saccharosekonzentration in den Blätten bzw. dem qN zum Stecktermin und der Anzahl nachfolgend unter den Klimakammerbedingungen gebildeten Adventivwurzeln ermittelt. Im Gewächshaus konnten solche Korrelationen nur für den Winter gefunden werden (geringes Lichtangebot). Der nicht-photochemische Quenching-koeffizient qN kann möglicherweise als schneller und nicht destruktiver physico-chemischer Index zur Vorhersage der Bewurzelung verwendet werden, wenn diese unter definierten relativ schwachen Lichtbedingungen (praxisübliche Bedingung in Mitteleuropa) erfolgt. Mit Hilfe der mittleren Saccharosekonzentration der Blätter (Tag 1 + Tag 7) konnte die Variabilität der Wurzelanzahl, verursacht durch die unterschiedlichen Jahreszeiten, das Mutterpflanzenalter, die Kühllagerung und die Bewurzelungsbedingungen, gut erklärt werden. Die Ergebnisse zeigen, dass auch unter der Bedingung hoher Kohlenhydratgehalte der Sprossbasis zum Zeitpunkt des Steckens die Adventivwurzelbildung von *Pelargonium*-Stecklingen durch die basipetale Translokation der Kohlenhydrate limitiert wird, welche sowohl von den bereits vorhandenen Kohlenhydratreserven im Blatt als auch von der aktuellen Photosynthese abhängt, wobei Letztere im wesentlichen dem Einfluss des Lichtangebotes während der Bewurzelung unterliegt.

Key words

English: Carbohydrates, photosynthesis, adventitious root formation

Deutsch: Kohlenhydrate, Photosynthese, Bewurzelung

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ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
cm	centimeter
F-6-P	Fructose-6-phosphate
G-6-P	Glucose-6-phosphate
G-6-PPDH	Glucose-6-phosphate dehydrogenase
HK	Hexokinase
INT	Iodonitrotetrazolium chloride
NADP	Nicotinamide adenine dinucleotide phosphate
6-PG	6-phosphogluconate
PGI	Phosphoglucose isomerase
PMS	Phenazine methosulfate
PPFD	Photosynthetic photon flux density
qN	Non-photochemical quenching of chlorophyll fluorescence
qP	Photochemical quenching of chlorophyll fluorescence
RH	Relative humidity
RS	Reducing sugars (Glucose + Fructose)
TNC	Total non-structural carbohydrates (TS + Starch)
TS	Total sugars (RS + Sucrose)

1 INTRODUCTION

Pelargoniums have the ability to survive in arid conditions, they can be easily adapted, and last, but certainly not least, they have beautiful and captivating colored flowers, because of all these reasons probably there are few gardeners who have never grown them. Production of pelargoniums for bedding plants, potted plants and balcony plants is a worldwide industry because of their bewitching flowers. Their sales have proven to be the back bone of the floriculture industry for many decades, and the wholesale value of vegetatively and seed propagated pelargoniums increased almost by 60% from 1979 to 1990 (Berninger, 1993).

Vegetative propagation by means of leafy cuttings is widely used in ornamental plant production, especially when uniformity of the developing plants is required. The European production of pelargoniums principally relies on the import of vegetative cuttings that are produced worldwide. Germany requires 150 million cuttings per year, most from import. The production of cuttings takes place predominantly outside Central Europe, at so-called southern locations. Increasingly, these 'southern locations' are no longer confined to the Mediterranean regions, but include more distant locations like South America, North and Central Africa, Central America or the Middle East. These areas are climatically favorable for cutting production, moreover, have the added advantages of a ready supply of land and labor which are often less expensive than in Central Europe. During the winter season, the cuttings after harvest at southern locations are stored at relatively cool temperatures for a short time and transported to Central Europe. In Central Europe, the cuttings are then propagated in greenhouses during the winter months to meet the market requirements in the spring season (May/June). As a result of unsatisfactory cutting quality, some cuttings are unable to develop adventitious roots, and estimated losses are repeatedly around 20% in Central European markets. These problems are unpredictable at the very beginning by the visual assessment of the cuttings, but can be only perceived during the course of propagation in the form of leaf senescence, drop out of cuttings, or reduced adventitious rooting. The reason for the reduced adventitious root formation in pelargonium cuttings imported from Southern locations and propagated in Central Europe is the main theme of this investigation.

The successful shipment of pelargonium cuttings is the most difficult among all horticultural products (Sheely and Craig, 1993). The quality of cuttings is determined primarily by their capacity to initiate roots, the continued growth of the initiated roots, and also the occurrence of senescence symptoms in the leaves. Pelargonium cuttings are less tolerant to storage and transportation (Paton and Schwabe, 1987). They are especially

sensitive to elevated temperatures during dark storage (Krebs and Zimmer, 1977; Marousky and Harbaugh, 1979; Carow and Bahnemann, 1980). Pelargonium cuttings are best stored at 4°C (Paton and Schwabe, 1987), and the chlorophyll content and subsequently formed root weight are similar between short-term stored cuttings at 4°C and unstored cuttings (Arteca *et al*., 1996). However, the shipment of the cutting occurs under unfavorable conditions, such as low humidity, darkness, and extreme temperatures (Purer and Mayak, 1989) ranging from 3°C to 18°C during their four days transport before reaching Germany (Kadner *et al*., 2000). Leaf yellowing is an extremely important quality problem in the leafy cuttings. Wilting, chlorosis, and abscission of leaves typically follows after most shipping and storage procedures in pelargonium cuttings, but principally depends on the cultivars (Carow and Bahnemann, 1980). Pelargoniums are ethylene sensitive plants (Nell, 1993). At several occasions, ethylene was considered as the cause for quality deterioration of pelargonium cuttings by promoting leaf senescence (Purer and Mayak, 1989; Arteca *et al*., 1996). However, recently Kadner *et al*. (2000) demonstrated that ethylene accumulation in storage packages of pelargonium cuttings has no predominant role in subsequent leaf yellowing.

The adventitious root formation of imported cuttings is not only jeopardized by unfavorable conditions during storage (Carrow and Bahnemann, 1980; Kadner *et al*., 2000), but also by the problems in adaptation, as cuttings produced under high light conditions in southern locations are subsequently propagated under low light during winter in Central European greenhouses (Forschner and Reuther, 1984). Pre-storage loading of pelargonium cuttings with sucrose and low-intensity illumination during storage resulted in improved rooting of the cuttings when compared to those stored in the dark and without sucrose pretreatment (Paton and Schwabe, 1987). A recent study by Druege *et al*. (2003) on pelargonium cuttings produced under high light and subsequently propagated under low light, demonstrated that pre-rooting sugar levels in the basal stem of pelargonium cuttings positively correlated with cuttings survival and sugar levels in leaves with adventitious root formation. Druege *et al*. (2003) also confirmed those relations in a sample survey with 21 cultivars. These results indicate that, the adventitious root formation of pelargonium cuttings is predominantly influenced by the carbohydrate availability in the cuttings.

Carbohydrates are considered as the principal source of energy for adventitious root formation (Haissig, 1986; Veierskov, 1988) in the form of pre-rooting reserves and/or current photosynthesis. Kraus and Kraybill (1918), were the first to report a correlation between carbohydrate content and adventitious rooting ability in cuttings. Later, Went and Thimann

(1937) suggested that there is an interlocking system in which sucrose is the prime requirement for root formation, followed by auxin, biotin, and a factor X. However, high prerooting carbohydrate status of the cuttings has not always been associated with high rooting potential (Hansen *et al*., 1978; Veierskov *et al*., 1982a; Veierskov, 1988). Although there exists such conflicting data about the relationship between internal carbohydrate status and root formation, efficient utilization of carbohydrates has been repeatedly shown to be the most crucial factor in adventitious root formation (Okoro and Grace, 1976; Haissig, 1984; Tschaplinski and Blake, 1989). Inclusion of carbohydrates in the mechanistic model of adventitious root development in leafy cuttings further highlights their importance (Dick and Dewar, 1992; Piller *et al*., 2002). Friend *et al*. (1994) emphasized that carbon allocation should be a crucial process affecting the root initiation and development. However, the mechanisms that control the carbon allocation and partitioning during rooting may not be common, and may be more specific to species, genotypes and certain tissues (Friend *et al*., 1994).

Pre-rooting carbohydrate reserves are important during root initiation, while subsequent growth may be entirely dependent on current photosynthetic contribution (Friend *et al*., 1994). Although conflicting data exists regarding the importance of current photosynthesis on adventitious root formation of leafy cuttings (Van Overbeek *et al*., 1946; Breen and Muraoka, 1974; Gay and Loach, 1977), compelling evidence suggests that current photosynthesis can limit adventitious root formation (Davis and Potter, 1981; Davis, 1988) and also sustains a positive soluble carbohydrate balance that favors rooting of the cuttings (Hoad and Leaky, 1996). Light intensity during the rooting period has a strong influence on current photosynthesis and consequently on adventitious root formation (Davis, 1988). In a carbon based model of adventitious root formation, light intensity was considered as a pivotal factor which determines the photosynthetic rates in cuttings (Piller *et al*., 2002). However, photosynthetic rates of the cuttings are considerably lower than those for intact plants (Okoro and Grace, 1976; Eliasson and Brunes, 1980; Davis, 1988). Adventitious root formation should be more related to specific carbohydrate pools in certain tissues than total carbohydrate content in the whole cutting (Haissig, 1986; Veierskov, 1988). Attempts to correlate rooting response to changes in soluble and insoluble carbohydrate reserves have had varying and conflicting responses which depended mainly on the species studied and environmental conditions used (Hansen *et al*, 1978; Haissig, 1984; Tschaplinski and Blake, 1989; Druege *et al*., 2000; Druege *et al*., 2003). Thus, even after 85 years of intensive research since the first

reports by Kraus and Kraybill (1918) on carbohydrates in relation to adventitious root formation of leafy cuttings, this has remained a fertile field for continued research.

The quality of pelargonium cuttings after import from southern locations and/or during propagation is mostly judged by visual inspection. This will readily spot external abnormalities such as leaf wilting, necrosis, or senescence. However, the internal physiological status cannot be assessed and can be at a considerable variance with what can be perceived with the naked eye. Measurement of chlorophyll fluorescence on intact plant leaves serves as a unique non-destructive method for judging the physiological state of the plant (Schreiber and Bilger, 1987; Krause and Weis, 1991). In fact, the F_v/F_m ratio has become an intrinsic and easily measurable parameter of the physiological state of the photosynthetic apparatus of intact plant leaves (Krause and Weis, 1991), and it is the most common parameter used in stress studies. Björkman and Demmig (1987) found a positive linear correlation between F_v/F_m and optimum quantum yield of Photosystem II (PS II) in a variety of stressed plants. Analysis of photochemical (qP) and non-photochemical (qN) quenching coefficients of chlorophyll fluorescence provides the vital information on functional state of photosynthetic apparatus, and more specifically, about the efficiency of PS II (Krause and Weis, 1991). The introduction of the pulse amplitude modulation (PAM) fluorometer has permitted rapid and sensitive resolution and quantification of qP and qN under physiologically relevant conditions (Schreiber *et al*., 1986; Schreiber and Bilger, 1987; Peterson *et al*., 1988). Fluorescence measurements may provide a more sensitive indication of the photosynthetic activity of the cuttings than that of the traditional gas exchange measurements (Mesén *et al*., 1997). While, the gas exchange measurement provides absolute overall rates, the fluorescence measurement informs the relative rates and, in addition, informs the location of specific steps that are limiting the overall process (Schreiber and Bilger, 1987).

Although chlorophyll fluorescence parameters provide a more sensitive and rapid indication of photosynthetic activity, few reports are available on its usefulness in cutting propagation. Van Kooten and Peppelenbos (1993) used chlorophyll fluorescence (F_v/F_m, qP and Φ_{II}) to determine the rooting potential of chrysanthemum cuttings during storage. The authors found a curvilinear relationship between PS II photochemical yield (Φ_{II}, efficiency of photosynthetic electron transport) and the final quality of the rooted cuttings. Mesén *et al*. (1997) studied chlorophyll fluorescence (F_v/F_m) during the course of propagation of *Cordia alliodora* (Ruiz & Pavon) Oken cuttings to investigate the relationship between

photosynthetic activity and the rooting of leafy cuttings. They found positive correlations between final rooting percentage (week 6) and mean F_v/F_m at weeks 1, 2, and 3. In an other investigation, Bruce *et al*. (2001) studied the changes in chlorophyll fluorescence (F_v/F_m) over the course of propagation of *Taxus* stem cuttings. The authors attempted to correlate initial stock plant fluorescence parameters (F_v/F_m) with subsequent rooting of cuttings but they were in vain. To my knowledge, no studies were published using photochemical quenching and non-photochemical quenching of chlorophyll fluorescence to investigate the photosynthetic activity of the cuttings during propagation in any plant species.

The main aim of the present investigation was to provide detailed insight into the interaction between carbohydrate availability and adventitious root formation in pelargonium cuttings. The natural light condition in the greenhouse, changing substantially from season to season (spring, summer and winter), which is typical for Central Europe, was used as a tool for simulating the environments affecting stock plant cultivation at southern latitudes and northern latitudes. Three harvests were considered during stock plant growth in each season to test the influence of stock plant age and development. The cutting propagation in all the three seasons was studied concurrently in greenhouse and in climate chamber. The cuttings produced under high light conditions in green house during spring and summer, and/or additionally affected by cold-storage, were subsequently propagated under similar light conditions (greenhouse) on the one hand and at relatively lower light conditions (climate chamber) on the other hand. In contrast, cuttings produced under low light in greenhouse during winter, and/or additionally affected by cold-storage, were subsequently propagated under similar light conditions (greenhouse) on the one hand and at relatively higher light conditions (climate chamber) on the other hand. For the climate chamber propagation, a standard PPFD of 100 µmol m^{-2} s^{-1} was used irrespective of the season of propagation, which comes close to the light intensity (including additional assimilation light) provided by commercial propagators during rooting of pelargonium cuttings in Central European greenhouses during winter. Only a moderate cold-storage treatment was employed to reduce the side effects of leaf senescence on adventitious root formation of the cuttings. The whole study was conducted using a moderately cold-storage sensitive cultivar *viz*., ‘Isabell’, which is most renowned in the commercial sector. At a later stage of the experiment, an extremely cold-storage sensitive cultivar *viz*., ‘Mitzou’ was also included in the study to investigate only under certain standard rooting conditions.

The following objectives were formulated for the present study:

1) Elucidation of interplay between pre-rooting carbohydrate status, current photosynthetic performance, and carbohydrate availability during adventitious root formation of the cuttings.
2) Determination of relationship between carbohydrate availability and rooting success under the consideration of different factors during preharvest, storage, and rooting.
3) To evaluate the possibility of using chlorophyll fluorescence as non-destructive physiochemical index for predicting the rooting capacity of the cuttings.

2 MATERIALS AND METHODS

2.1 Plant material

The present study was conducted with two cultivars *viz.,* 'Isabell' and 'Mitzou' of *Pelargonium x hortorum* L. H. Bailey. The cultivar 'Isabell' is moderately sensitive to storage (moderate shipper) and 'Mitzou' is very sensitive to storage (poor shipper). The stock plants of these cultivars were cultivated in the greenhouse at the Institute of Vegetable and Ornamental Crops (IGZ), Erfurt e.V. Germany during the years 2002 & 2003.

2.2 Experimental design

2.2.1 Preliminary Experiments: Carbohydrate distribution

2.2.1.1 Within the leaf

Preliminary experiments were conducted on cv. 'Isabell' with an aim to study the carbohydrate distribution within the leaf from tip (proximal end) to base (distal end) and between the leaves of different ages. The main objective was to optimize the sampling procedure for carbohydrate analysis in the leaves. For the present study, typical cuttings having four leaves of different ages were taken. The experiment was commenced on 12th of April 2001 and comprised of 2 treatments: 1) freshly excised cuttings, one cutting per treatment and replicate (*n=10*); 2) stored cuttings (stored at 5°C for 7 days in perforated polyethylene bags under dark conditions), one cutting per treatment and replicate (*n=5*).

The cuttings were excised between 9 to 10 AM from the stock plants which were grown in the greenhouse. Four leaves of different ages *viz.,* oldest leaf (fully matured leaf, leaf 1), one/two maturing leaves (first fully expanded leaves, leaf 2 and leaf 3) and youngest leaf (developing leaf, leaf 4) were sampled for carbohydrate analysis in the following manner. Immediately after the excision and storage, the leaves of different ages were separated from the cuttings, the same were transversely cut into one cm sections from tip to base (Fig. 1) and the carbohydrate concentrations were analyzed separately in each individual section. This was done in all the leaf ages.

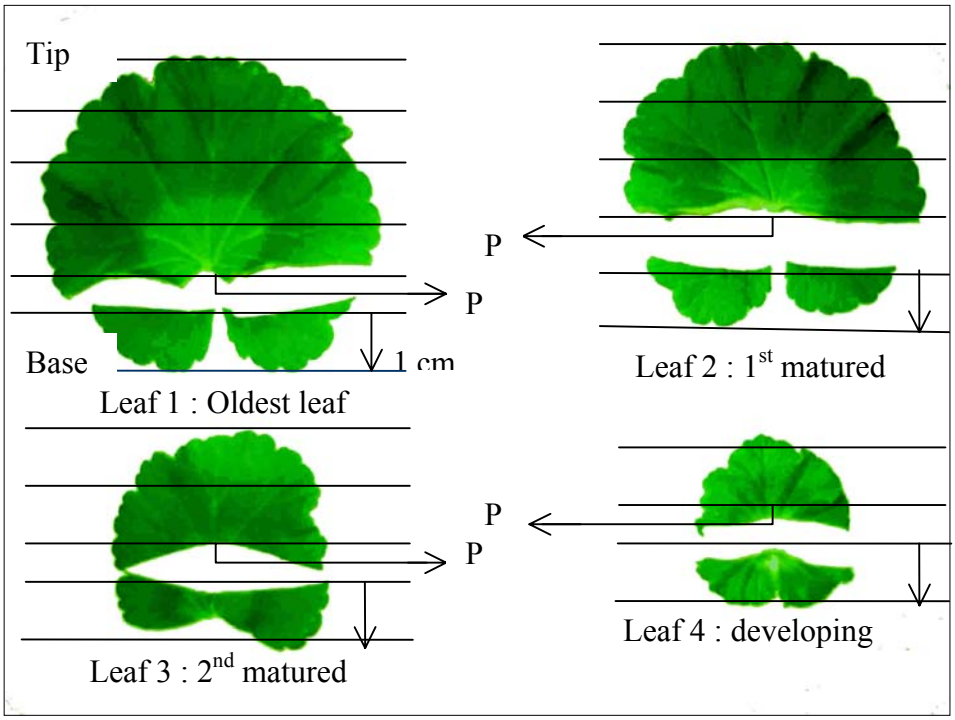


Fig 1:Diagrammatic presentation of sampling procedure undertaken for carbohydrate analysis in the leaves of different ages of a typical cutting. The letter 'P' indicates the position of petiole attachment with the individual leaf.

2.2.1.2 Validation of relation between basal 1 cm section of leaf and whole leaf

This experiment was conducted to check the validity of the relationships found in the experiment 2.2.1.1 for carbohydrate concentrations between basal 1 cm section and whole leaf, under additional consideration of various storage treatments. Typical cuttings with four leaves of different ages (as explained above in section 2.2.1.1) were collected from the stock plants which were grown in the greenhouse. This experiment was commenced on 19th of October 2001 and comprised of the following 5 treatments: 1) freshly excised cuttings; 2 & 3) cuttings stored at 5°C for 7 and 14 days, respectively; 4 & 5) cutting stored at 10°C for 7 and 14 days, respectively; in each case one cutting per treatment and replicate ($n=3$).

To check the validity of the relationships (as mentioned above), a different sampling procedure was carried out when compared with the sampling procedure in the earlier experiment 2.2.1.1. Immediately after excision and storage at different treatments, leaves of different ages were separated from the cuttings and the basal 1 cm section of each leaf was transversely cut down. Carbohydrate concentrations were analyzed separately in the basal 1 cm section and in the rest of the leaf. This was done in all the leaf ages.

2.2.2 Experiment 1: Effect of season, stock plant age, storage and rooting environment - cv. 'Isabell'

This complex experiment was designed with an aim to determine the influence of preharvest carbohydrate distribution in the cuttings, as affected by seasons, stock plant age, and cold-storage, as well as sustenance of current photosynthesis of those cuttings, as additionally affected by rooting environment, on subsequent adventitious root formation of the cuttings. The experimental design is schematically depicted in Fig. 2. As earlier mentioned in the introduction, season was used as a tool to obtain cuttings with different adaptation to light intensity and other associated parameters to cover the variability of those environmental conditions among different locations for cuttings production. Three harvests were considered in each season. The different climatic parameters prevailed during stock plant cultivation are presented in Table 1.

The stock plants cultivated in spring, summer, and winter seasons during the years 2002 and 2003 were used for the procurement of cuttings. Cuttings with three to four leaves of different ages were harvested at nine different occasions (three harvests in each season) as mentioned in Table 1. After harvest, the cuttings were chemically analyzed, non-destructively measured, and immediately rooted or stored. The cuttings were stored at a variable temperature regime (Fig. 3). Both unstored and stored cutting were rooted in greenhouse on the one hand and in climate chamber on the other hand. The chemical analysis and non-destructive measurements during the course of propagation of unstored cuttings as well as stored cuttings in both rooting environments include the following parameters:

1) Leaf and basal stem carbohydrates were analyzed on day 0 (at insertion) and on day 7 (during the course of propagation).
2) Chlorophyll fluorescence was measured on day 0, 1, 4, 7 and 14.
3) Chlorophyll content was analyzed on day 0 and 7.
4) Net photosynthetic rate was measured on day 1, 4, 7 and 14.
5) Number of senesced leaves were determined on day 21.
6) Number of subsequently formed adventitious roots were analyzed on day 21.

Replication was three fold for all the parameters (n=3).

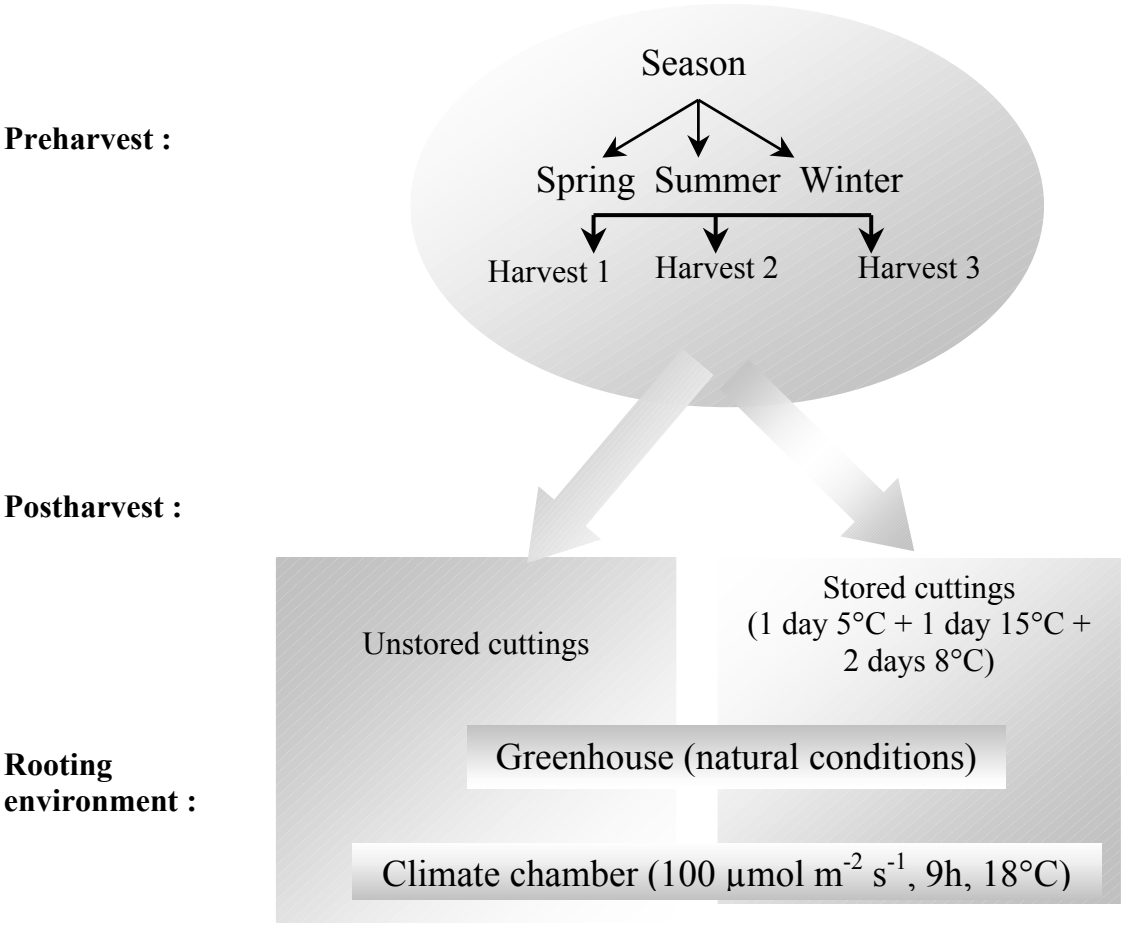


Fig 2 : Schematic presentation of experimental design depicting different factors *viz.,* preharvest, postharvest and rooting environment.

2.2.3 Experiment 2: Effect of storage temperature regimes – cv. 'Isabell'

The prime interest of this experiment was to provide detailed perception about the response of leaf and basal stem carbohydrates during the storage comparing two different temperature regimes. The influence of constant and variable storage temperature regimes on cutting carbohydrate dynamics during the storage, on functional status of photosynthetic apparatus after storage, and on subsequent adventitious root formation of the cuttings were investigated.

The stock plants cultivated during spring season in the year 2002 were used for the procurement of cuttings. Considering the stock plant age, the cuttings were harvested at three different occasions as mentioned in Table 1. After harvest, the cuttings were chemically analyzed, non-destructively measured, and immediately rooted or stored. In order to study the influence of temperature dynamics during storage on the response of carbohydrates in

different cuttings parts, the cuttings were stored at a variable temperature regime on the one hand and at a constant temperature regime on the other hand (both regimes amounting to the same mean storage temperature) (Fig. 3). Both unstored cuttings and stored cuttings were rooted in climate chamber. The chemical analysis and non-destructive measurements include following parameters:

1) Leaf and basal stem carbohydrates were analyzed after harvest and at every 24 hours interval during the 4 days of storage period.
2) Chlorophyll fluorescence was measured at harvest and after storage.
3) Number of subsequently formed adventitious roots were analyzed on day 21.

Replication was three fold for all the parameters (*n*=3).

2.2.4 Experiment 3 : Effect of stock plant age and storage – cv. 'Mitzou'

'Mitzou' is a very storage sensitive cultivar, so probably different responses (with regard to propagation) can be expected when compared to that of 'Isabell'. This experiment was designed to determine the influences of preharvest carbohydrate distribution in the cuttings, as affected by stock plant age and cold-storage, as well as sustenance of current photosynthesis of those cuttings, as affected by propagation under relatively low light, on subsequent adventitious root formation of the cuttings.

The stock plants cultivated during winter season in the year 2002 and 2003 were used for the procurement of the cuttings. Considering the stock plant age, cuttings were harvested at three different harvest dates as mentioned in Table 1. After harvest, the cuttings were chemically analyzed, non-destructively measured, and immediately rooted or stored. The cuttings were stored at a variable temperature regimes (Fig. 3). Both unstored and stored cutting were rooted in climate chamber. The chemical analysis and non-destructive measurements during the course of propagation of both unstored cuttings and stored cuttings include:

1) Leaf and basal stem carbohydrates were analyzed on day 0 and 7.
2) Chlorophyll fluorescence was measured on day 0 and 1.
3) Chlorophyll content was analyzed on day 0 and 7.
4) Number of senesced leaves were determined on day 25.
5) Number of subsequently formed adventitious roots were analyzed on day 25.

Replication was three fold for all the parameters (*n*=3).

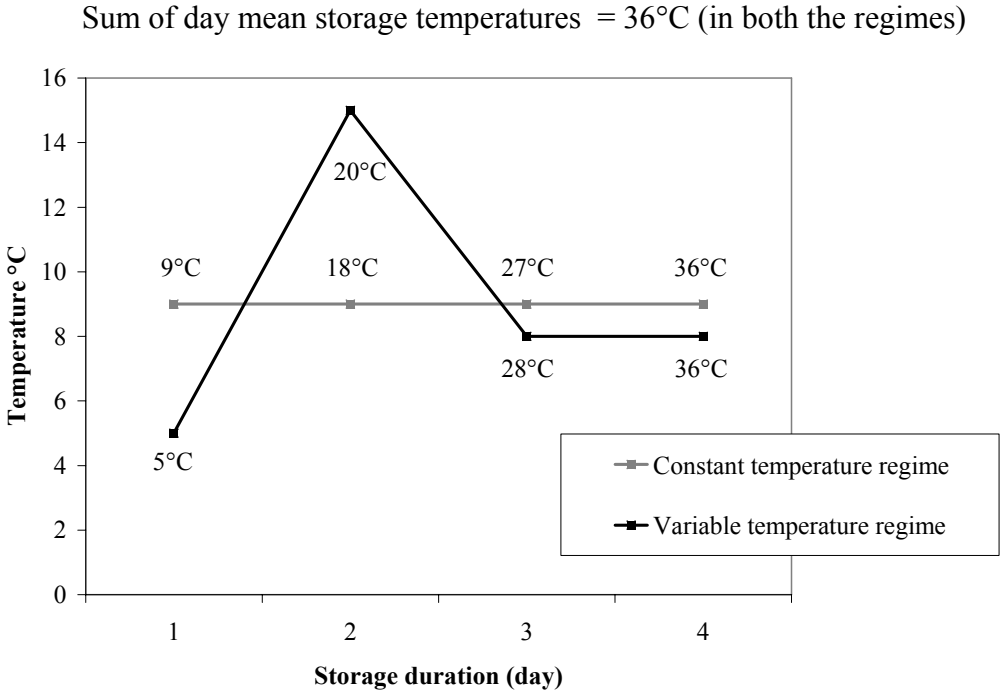


Fig. 3 : Diagrammatic presentation of two types of storage temperature regimes and resulting sum of day mean temperatures.

2.3 Experimental conditions

2.3.1 Stock plant cultivation and stock plant age

Zonal pelargonium cv. 'Isabell' stock plants were cultivated in spring, summer, and winter seasons whereas cv. 'Mitzou' in winter season during the years 2002 and 2003 in greenhouse. Rooted cuttings were planted in commercial peat (Einheitserde Typ ED 73, Patzer Company, Germany, containing 294 mg/L N, 135 mg/L P, 343 mg/L K, 90-130 mg/L Mg, 2.7 g/L KCl and 7.5 pH) at a density of 13 plants per square meter and maintained as stock plants over a period of 2 months in spring/summer and 3 months in winter season.

Fertilizers were applied once in a week, Hakaphos spezial (COMPO GmbH & Co, Germany) with liquid dispensers (Dosatron, France). The stock plants were irrigated manually and the fungicides were applied as per requirements. In greenhouse heating/ventilation setpoints for temperatures were 16/18°C during day and 15/16°C during night. The means of continuously measured photosynthetic photon flux densities (PPFD) were calculated over an

average day length of 9h, representing the mean natural day length during the winter season in Central Europe. Those PPFD values, relative humidity values (RH), and temperatures prevailed during the cultivation in each season are presented in Table 1. Cuttings with three to four leaves of different ages *viz*., oldest (leaf 1), one/two maturing (leaf 2 and leaf 3) and youngest (leaf 4) were always harvested between 9 to 11 AM.

Table 1. Details of stock plant cultivation and climatic conditions in the greenhouse at IGZ, Germany.

Cultivar	Season	Harvest date (DD/MM/YY)	PPFD * (μmol m^{-2} s^{-1})	RH* (%)	Temperature* (°C)
Isabell	Spring	H 1: 08/04/2002	233.9 (288.3)	73.9 (59.7)	23.1 (23.1)
		H 2: 13/05/2002	304.4 (334.0)	85.8 (85.8)	28.0 (28.0)
		H 3: 10/06/2002	361.8 (372.8)	88.7 (88.7)	31.4 (31.0)
Isabell	Summer	H 1: 25/07/2002	342.7 (318.5)	99.5 (93.9)	38.4 (24.3)
		H 2: 26//08/2002	386.9 (386.4)	100 (97.3)	35.4 (35.4)
		H 3: 19/09/2002	345.7 (315.5)	99.1 (82.2)	31.3 (24.0)
Isabell & Mitzou	Winter	H 1: 21/10/2002	75.8 (55.5)	79.0 (79.0)	19.8 (19.5)
		H 2: 08/01/2003	53.1 (64.2)	70.8 (70.8)	19.4 (19.4)
		H 3: 08/02/2003	117.6 (128.4)	71.4 (65.9)	19.8 (19.8)

*: Mean of values measured over three weeks and one week (values in parenthesis) before harvest .

2.3.2 Conditions of storage

Immediately after harvest, cuttings were kept in perforated polyethylene bags, packed in shipping boxes and stored under dark conditions for 4 days, unless designated otherwise. In this study, two types of storage temperature regimes were used: 1) variable temperature regime (5°C for 1 day + 15°C for 1 day + 8°C for 2 days, respectively) and 2) constant temperature regime (9°C through out the storage period, i.e. 4 days). So, the sum of day mean storage temperatures was 36°C under both the storage regimes (see also Fig. 3). Average relative humidity outside the storage boxes was maintained at 95% during storage.

2.3.3 Conditions of rooting

Both unstored and stored cutting were rooted in greenhouse on the one hand and in climate chamber on the other hand (Experiment 1) or only in climate chamber (Experiment 2 and 3). Cuttings were planted in commercial propagating cubes (Klasmann Steckmedium, Germany). These are widely used in the commercial sector, so to be close to the practice of the commercial propagators these cubes were chosen. The rooting substrate was a mixture of white sphagnum peat (75%) and perlite (25%) and surrounded by a fleece cloth. The medium contained 70 mg/L N, 80 mg/L P, 90 mg/L K, 85 mg/L Mg, 0.5 g/L KCl (equivalent for salt concentration) and 5.7 pH. Neither fertilizers nor plant hormones were applied.

2.3.3.1 Greenhouse

Rooting environment in greenhouse was similar to the natural climatic conditions prevailed during that specific period or, in other words, similar to the climatic conditions under which the stock plants were cultivated. When the global irradiation outside the greenhouse exceeded a PPFD of 450 µmol m^{-2} s^{-1} cuttings were shaded automatically (computer programmed) using overhead curtains.

In addition to the fog system, providing a high relative humidity (Table 2), the cuttings were periodically irrigated with intermittent mist-system using a fine sprinkler (5 times per day for 5 seconds). If required, they were irrigated manually once in a day during peak summer season. Heating/ventilation setpoints for temperatures were 19/21°C during day and 18/20°C during night. The means of continuously measured PPFD values were calculated over an average day length of 9 h. Those PPFD values, RH values and temperatures prevailed during the cutting propagation in each season are presented in Table 2.

Table 2. Details of cutting propagation and climatic conditions in the greenhouse at IGZ, Germany

Cultivar	Season	Propagation period (DD/MM/YY)	PPFD * (µmol $m^{-2}s^{-1}$)	RH* (%)	Temperature * (°C)
Isabell	Spring	08(12)/04 - 29(03)/04(05)/02	159.6 (158.9)	77.6 (77.6)	24.1 (24.1)
		13(17)/05 - 03(07)/06/02	206.5 (210.3)	81.4 (82.8)	31.4 (31.4)
		10(14)/06 - 01(05)/07/02	198.3 (196.6)	87.9 (87.9)	36.4 (36.4)
Isabell	Summer	25(29)/07 - 15(19)/08/02	185.2 (198.5)	88.2 (88.2)	34.3 (34.3)
		26(30)/08 - 16(20)/09/02	176.6 (179.4)	89.1 (88.9)	30.3 (30.3)
		19(23)/09 - 10(14)/10/02	99.2 (085.8)	73.3 (73.3)	25.7 (25.7)
Isabell	Winter	21(25)/11 - 12(16)/12/02	41.8 (041.3)	67.2 (67.2)	21.5 (21.5)
		09(13)/01 - 30(03)/01(02)/03	43.8 (046.7)	58.9 (58.9)	22.9 (22.9)
		08(12)/02 - 06(10)/03/03	89.7 (102.3)	63.3 (63.3)	24.0 (24.0)

*: Mean over 21 days of propagation period for unstored and stored cutting (values in the parenthesis).

2.3.3.2 Climate chamber

Rooting environment in climate chamber, simulated to climatic conditions accomplished by the commercial propagators under the supply of additional light for the propagation of cuttings during winter season in Central Europe. These were relatively low light conditions. The PPFD was 100 µmol m^{-2} s^{-1} per 9 h day length and was provided using Mercury lamps (Osram HQL – T 400 W) and Krypton lamps (Gluehlampen 100 W). The day and night temperatures, as well the air and soil temperatures were set at 18°C and relative humidity at 100%. The cuttings were irrigated manually twice in a day. All the above designated conditions were maintained constantly during the propagation period in all the seasons.

2.4 Chemical and Non-destructive measurements

2.4.1 Carbohydrate analysis

At insertion of unstored and stored cuttings (day 0), during storage, and during course of propagation (day 7) carbohydrates were analyzed in the basal 1 cm section of the leaves of different ages separately and in the basal 1 cm section of stem, three cuttings per treatment and replicate (*n*=3), unless designated otherwise (2.2.1.1, 2.2.1.2). The plant samples were always collected between 9 AM and 10 AM.

Soluble sugars were determined in a microplate assay by an enzyme-coupled colorimetric reaction (Hendrix, 1993). All chemicals used were at least pro analysis grade. Plant material was cut into small pieces (≤ 25 mm^3), immediately transferred into sealed polypropylene test tubes containing cold aqueous ethanol (80%, -20°C) and then stored below -20°C (Hendrix and Peelen, 1987) until they were analyzed. Tubes containing plant samples were incubated in water bath at 80°C for 30 min and the sugars were extracted in a five-fold excess of 80% ethanol (Druege *et al*., 1998). After the extraction period, ethanol in the tubes was decanted and replaced by a second wash of 80 % ethanol and residues were again incubated. This was repeated 4 times until all green color was removed. Aliquots (4 ml) of collected extracts were mixed with activated charcoal (2.5 g per g fresh mass of plant material, held for 1 h at room temperature) to remove ethanol-soluble materials in the plant extracts which interfere with subsequent enzyme coupled colorimetric reaction (Blunden and Wilson, 1985; Hendrix and Peelen, 1987). The extracts were cleaned up by filtration (Schleicher & Schuell No. 6, Germany) and centrifuged (20 min at 20 000 x *g*).

To analyze the sugars, 100 µl of alcohol extracts were pipetted into microplate wells (Greiner, Nürtingen, Germany) and dried in oven at 55°C overnight. Following the evaporation of ethanol, 20 µl of double distilled water was added to each sample well. A series of wells on the same plate containing 0.5 to 5 µg (each) of glucose, fructose and sucrose were also prepared as standards. The four corners of the microplate contained same amount of double distilled water for blanking. A glucose detection mixture (INT mixture) containing glucose-6-phosphate dehydrogenase (EC 1.1.1.49), hexokinase (EC 2.7.1.1) and iodonitrotetrazolium violet was purchased from Sigma Chemical Company, USA (Glucose kit 115 A) and reconstituted with double distilled water as indicated on the accompanying instructions. An aliquot (100 µl) of the INT mixture was added to each well on the microplate

under reduced illumination. Following the addition, plates were gently tapped to mix the contents well. The plates were covered with aluminum foil to exclude light and incubated in an oven at 37°C for 15 min. The INT mixture produces a color only with *D*-glucose-6-phosphate and so detects only ethanol extracted glucose in the sample. After incubation, absorption of each well was determined at 492 nm with a standard microplate reader (Spectro thermo, Tecan Deutschland GMBH, Germany).

In order to determine fructose after measuring absorbance value of glucose, 20 µl of a solution containing phosphoglucose isomerase (PGI) (EC 5.3.1.9, P-9544, Sigma Chemical Company, USA) was added to each well on the microplate, which converts fructose-6-phosphate created by the hexokinase. This PGI solution was prepared by adding 4 ml of 0.2 *M* HEPES [4-(2-hydroxyethyl)-1-pierazineethanesulfonic acid, pH 7.8] to a vial containing 1000 enzyme units (EU) of the enzyme. Following the PGI addition, the plates were again incubated in the dark at 37°C for 15 min and read at 492 nm. The absorbance values from the second readings are proportional to the sample hexose content (i.e. glucose + fructose). In the similar way adding 83 EU of invertase (Hendrix, 1993) (EC 3.2.1.26, I-4504, Sigma Chemical Company, USA) to each well, reincubating and reading at 492 nm produces an absorbance proportional to sucrose plus the hexose in each well. The invertase solution was prepared by adding 32 mg of powdered invertase (653 EU mg^{-1}) to 5 ml of 0.1 *M* citrate buffer (pH 6). The absorbance readings from the wells containing standards were used to construct a curve from which the sugar content of unknowns was determined.

Principle involved in enzyme coupled colorimetric assay.

Glucose (also for fructose and sucrose) :

$$\text{Glucose + ATP} \xrightarrow[Mg^{+2}]{HK} \text{G-6-P + ADP}$$

$$\text{G-6-P + NADP} \xrightarrow{\text{G-6-PPDH}} \text{6- PG + NADPH}$$

$$\text{NADPH + PMS} \longrightarrow \text{NADP + PMSH}$$

$$\text{PMSH + INT} \longrightarrow \text{PMS + INTH}$$

Fructose :

$$\text{F-6-P + ATP} \xrightarrow{PGI} \text{G-6-P}$$

Sucrose :

$$\text{Sucrose} \xrightarrow{\text{Invertase}} \text{Glucose + Fructose}$$

For the determination of starch, extraction residues from which sugars had been extracted were ground together with a pinch of quartz sand. To those, 15 ml of double distilled water per gram fresh mass were added and the suspensions were collected in polypropylene tubes and incubated in a water bath at 100°C for 3 h to gelatinize the starch (McRae, 1971). Following incubation, tubes were cooled to the room temperature, 15 ml of amyloglucosidase suspension (from *Aspergillus niger,* 1.5 U ml $^{-1}$ in 0.2 *M* acetate buffer, pH 4.8), (EC 3.2.1.3, A-3514, Sigma Chemical Company, USA) was added per gram fresh mass. Then the tubes were incubated at 60°C for 40 h in the dark to allow complete starch digestion. After filtration (Schleicher & Schuell No. 6, Germany), the starch concentration was determined via the glucose released (Druege *et al.,* 2000).

2.4.2 Chlorophyll fluorescence measurement

Chlorophyll fluorescence measurements were performed with a Photosynthetic yield analyzer MINI-PAM (Walz, Germany) (Fig. 1A in appendix) using a pulse-modulated light source. Two sets of measurements were made on the basal portion (upper surface) of leaf 1, one on the left and one on the right side. Mean of values measured on 8 cuttings (Experiment 1 and 3 in section 2.2.2 and 2.2.4, respectively) and on 5 cuttings (Experiment 2, section 2.2.3) per treatment and replicate (n=3) were used for the analysis. As per preliminary tests, no differences were found with the position of measurement on leaf 1, so basal portion was chosen, as carbohydrates were also measured in the basal section.

Cuttings were always temperature equilibrated and dark adapted at 20°C for 30 min before the measurement of fluorescence parameters, F_0 (minimum or initial fluorescence) and F_m (maximum fluorescence) (Schreiber, 1983; Krause and Weis, 1991). Similarly, cuttings after retrieval from cold-storage, which were packed in the shipping boxes, were taken to the greenhouse, unwrapped and equilibrated to 20°C for 30 min in darkness before the measurement of chlorophyll fluorescence. As dark adapted plant tissues are maximally primed to absorb photons or light energy by electron transport pathway because of Q_A, the primary electron acceptor of photosystem II (PS II) is in its fully oxidised state, and at the same time, chlorophyll fluorescence in response to a measuring light of low intensity is minimal (F_0). Upon exposure to a saturating light pulse, Q_A becomes fully reduced and chlorophyll fluorescence transiently achieves a maximum (F_m). A fluoremeter measuring light intensity of 0.15 µmol m^{-2} s^{-1} (<650 nm) and a saturating pulse of 4000 µmol m^{-2} s^{-1} for a duration of 1 second were used to measure F_0 and F_m, respectively. According to our preliminary tests, as per manufacturers instructions (Walz, Mess- und Regeltechnik 1999), this intensity was determined sufficient to saturate PS II. The difference between maximum and minimum fluorescence ($F_m - F_0$) is termed as variable fluorescence (F_v). In healthy plant tissues, the relative fluorescence ratio (F_v/ F_m) remarkably ranges around 0.83 (Krause and Weis 1991). Further, the samples were adapted with actinic light pulse of 300 µmol m^{-2} s^{-1} for 30 seconds and superimposed with a saturation light pulse for the second time at the end of the actinic illumination time. This allowed the determination of F_0' (minimum fluorescence) and F_m' (maximum fluorescence), respectively, for light adapted sample. From these data photochemical quenching qP and non-photochemical quenching qN were computed. According to Genty *et al*. (1989) photochemical quenching [$qP = (F_m' - F) / (F_m' - F_0)$], where

F defines the level of fluorescence at any time of the induction curve (see also Van Kooten and Snel, 1990), and non-photochemical quenching [qN = (F_m - F_m') / (F_m - F_0)], were calculated from the photosynthetic yield analyzer MINI-PAM (Walz, Germany).

The coefficients of ***photochemical quenching (qP)*** denotes the proportion of excitations captured by open traps and being converted to chemical energy in the PS II reaction center. The reoxidation of Q_A^- thus causes quenching. ***Non photochemical quenching*** (qN) is also called as energy-dependent quenching, which is the fastest phase ($t_{1/2}$ < 1 min). This is caused by the intrathylakoid acidification during light driven proton translocation across the thylakoid membrane (Krause and Weis, 1991).

2.4.3 Chlorophyll estimation

For chlorophyll analysis three leaves (leaf 1) per treatment and replicate (n = 3) were taken. After the determination of fresh mass (FM), they were sealed in aluminum foils, frozen in liquid nitrogen and stored in a freezer below - 20°C.

Chlorophyll estimation was performed according to modified Arnon (1949). The leaf material was taken in mortar and ground to make fine pulp. Then 20 ml of 80% acetone was added per 1 g FM. The suspension was stirred in darkness for 5 min and then it was filtered (Schleicher & Schuell No. 6, Germany). The leaf material was repeatedly washed for 4 times and the absorption was measured, using a U-VIS spectrophotometer, V-550 (Jasco, Germany) at 663 and 645 nm. Chlorophyll concentration was calculated using the following equations:

Chlorophyll a = 12.7 x (A_{663}) – 2.69 x (A_{645}) x (mg x l^{-1})
Chlorophyll b = 22.9 x (A_{645}) – 4.68 x (A_{663}) x (mg x l^{-1})

2.4.4 Stomatal gas exchange

Net photosynthetic rate was measured with a Portable Photosynthesis System, HCM-1000 (Heinz Walz GmbH, Germany) (Fig. 2A in appendix) using IRGA (Infrared gas analyzer). The measurements were taken on youngest developing leaf (leaf 3 or leaf 4). Mean of measurements on 2 leaves per treatment and replicate (n = 3) were used for the analysis. The system measures net photosynthesis or transpiration rates by calculating CO_2 and H_20 concentrations in the measuring cuvette containing leaf disc by differential mode of measurement.

2.5 Determination of adventitious root formation

Roots visible out side the propagator cube were determined after 21 and 25 days of propagation in case of 'Isabell' and 'Mitzou' cuttings, respectively. Visible number of roots were positively correlated (r=0.92 at p<0.001 level, n=1836) to total number of roots. Adventitious rooting of 17 cuttings per treatment and replicate (n=3) were studied in Experiment 1 and 3 (section 2.2.2 and 2.2.4, respectively). In Experiment 2 (section 2.2.3) adventitious rooting of 17 cuttings (unstored and variable temperature regime) and 5 cuttings (constant temperature regime) per treatment and replicate (n=3) were studied.

2.6 Statistical analysis

Data were analyzed with ANOVA/MANOVA and Regression modules of Statistica 6 software program (Statsoft, 2001). Influence of season, harvest date and cold-storage on pre-rooting carbohydrate concentrations in different cutting parts, chlorophyll fluorescence parameters, and chlorophyll concentrations were tested by analyses of variance. Similarly, influence of season, harvest date, cold-storage, and rooting environment on carbohydrate status, chlorophyll fluorescence parameters, chlorophyll concentrations and net photosynthetic rates during propagation, as well as on subsequent rooting performance, were tested by analysis of variance. Analysis of variance summaries (F-values) were presented to give an overview about the magnitude of influence of different factors as well the interactions between different factors in this complex design. At any occasion, maximum three fold interactions were considered. If significant effects were found between/at specified sampling dates during the course of propagation or during storage, mean values were compared using Newman-Keuls test with a significance level of at least $p \leq 0.05$. Linear regressions were calculated between carbohydrate concentrations in the basal 1 cm section of the leaves of different ages and concentrations in the corresponding whole leaves. Correlations and linear regressions were calculated between carbohydrate concentrations in different cuttings parts, chlorophyll fluorescence parameters and number of subsequently formed roots. Nonlinear regressions (exponential) were calculated between sum of day mean storage temperatures and carbohydrate concentrations during the storage period. At all the occasions, for calculating regressions, replicates were considered individually to cover the whole amount of variation.

3 RESULTS

3.1 Carbohydrate distribution within leaves of freshly excised and stored cuttings of 'Isabell'

Carbohydrate distribution within the leaves of different ages was investigated in the cuttings immediately after excision and after cold-storage at 5°C for 7 days.

3.1.1 Effect of leaf section – cv. 'Isabell'

Typical individual representatives showing carbohydrate distribution from tip to base within the leaves of different ages of freshly excised cuttings and stored cuttings are presented in Fig. 4. In freshly excised cuttings, there was no typical gradient from tip to base within leaves of different ages for glucose, fructose and sucrose, all the sections within a leaf of specific age were found to be having similar concentrations (Fig. 4a-d). Among different carbohydrate fractions, fructose concentrations followed by glucose were at lower levels within leaf 1 and leaf 2, but both of these monosaccharides were at relatively higher levels within leaf 3 and leaf 4. In contrast, sucrose concentrations were at higher levels within leaf 1 and leaf 2, but in leaf 3 and leaf 4 the concentrations were either equal or lower to the concentrations of monosaccharides (Fig. 4a-d). Starch concentrations displayed a typical gradient from tip to base within the leaves of different ages. Within leaf 1 and leaf 2, starch concentrations were higher at the tip of the leaf, but gradually declined towards the center, making a notable dip at the position of petiole attachment with the leaf, and again increased towards the basal section of the leaf (Fig. 4a, b). Similarly, at most of the occasions, starch concentrations within leaf 3 were higher at the tip and declined towards the petiole attachment position, but there was no further increase in the basal section (Fig. 4c). Starch concentrations were exceptionally low within leaf 4, which was the youngest leaf. (Fig. 4d).

Cold-storage of the cuttings at 5°C for 7 days, not only decreased the carbohydrate levels, but also completely eliminated the carbohydrate gradients within the leaves of different ages which were found at excision. Starch concentrations were undetectable within all the leaves of different ages after cold-storage (Fig. 4e-h). No typical gradient was observed for glucose within the leaves, almost all the sections within a leaf of specific age of a cutting were

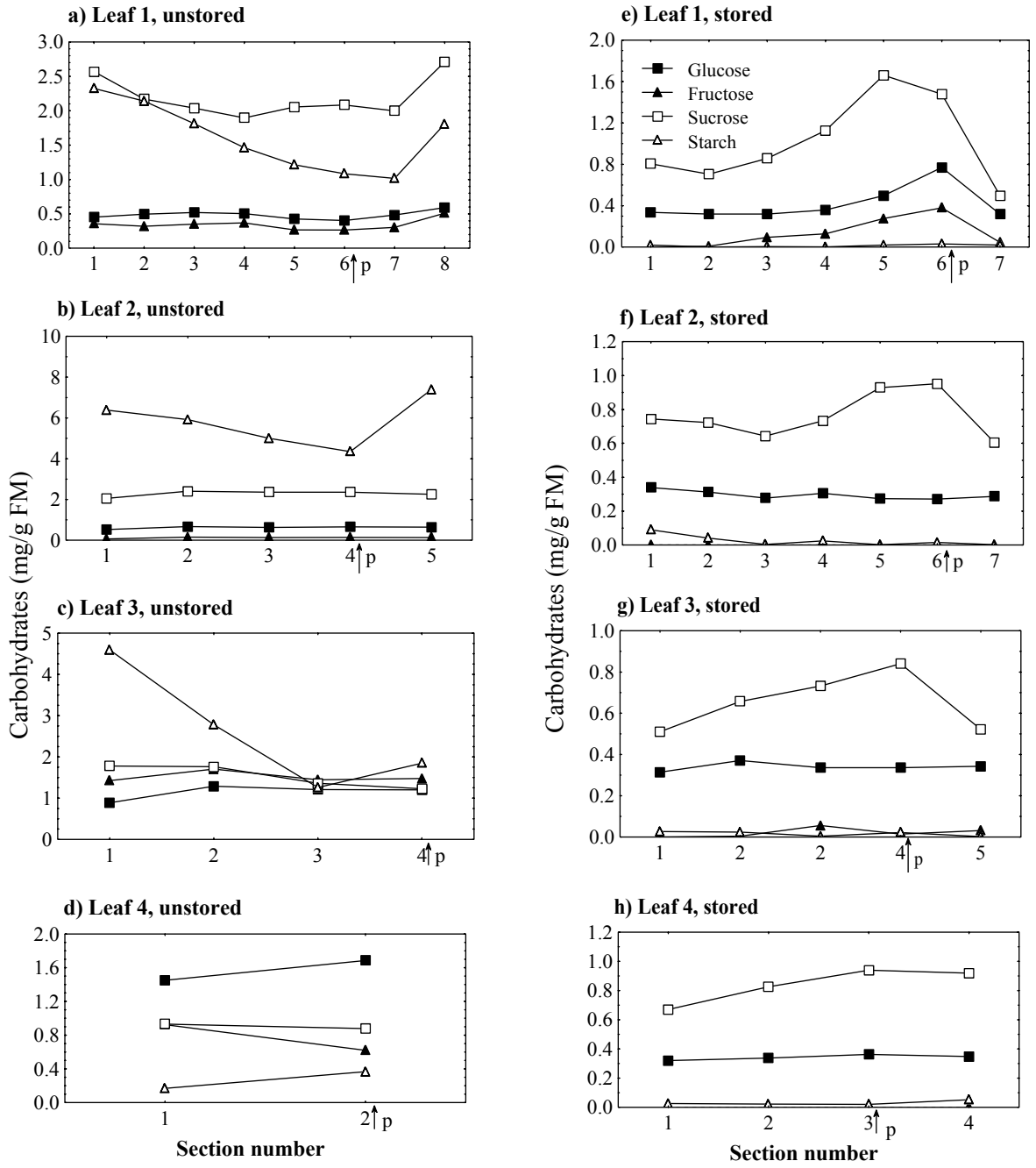


Fig. 4: Selected individual leaves of different ages (leaf 1: oldest leaf, leaf 4: youngest leaf) of unstored (a, b, c & d) and stored (e, f, g, & h) cuttings showing carbohydrate distribution (mg/g FM) from tip (section number 1) to base within the leaf, *n*=1. The letter 'p' on X – axis represent the position of petiole attachment with the individual leaf.

found to be having similar concentrations (Fig. 4e-h). Fructose was very low within all the leaves of different ages. Within all the leaves sucrose was found to be the major sugar after cold-storage. Interestingly, in leaf 1 and leaf 2, sucrose concentrations were significantly high at the position of petiole attachment (Fig. 4e, f), but this kind of gradient was not clear in leaf 3 (Fig. 4g).

3.1.2 Relationship between basal section and whole leaf – cv. 'Isabell'

Although the gradient for some carbohydrates varied from tip to base within the leaves of different ages for freshly excised as well as stored cuttings (5°C for 7 days), the individual and total carbohydrate concentrations measured in the basal 1 cm section of the leaf were equal to the mean concentrations in the corresponding whole leaf. Highly significant positive correlations were discovered between carbohydrate concentrations in the basal 1 cm section of the leaf and concentrations in the corresponding whole leaf, irrespective of the leaf age (data not shown). To check feasibility of those relationships, cuttings were stored at different temperatures for different durations (at 5°C and 10°C for 7 days and 14 days). Nevertheless, the relationship between the basal 1 cm section of the leaf and the corresponding whole leaf for different carbohydrate concentrations remained highly significant in all the leaves of different ages (Table 3), as presented for glucose in Fig. 5. As a result, basal 1 cm section of the leaf was found to be the representative sample for carbohydrate analysis. Consequently, instead of whole leaves, basal 1 cm sections of the leaves of different ages were used for the routine analysis, as it was faster and less laborious with the same precision.

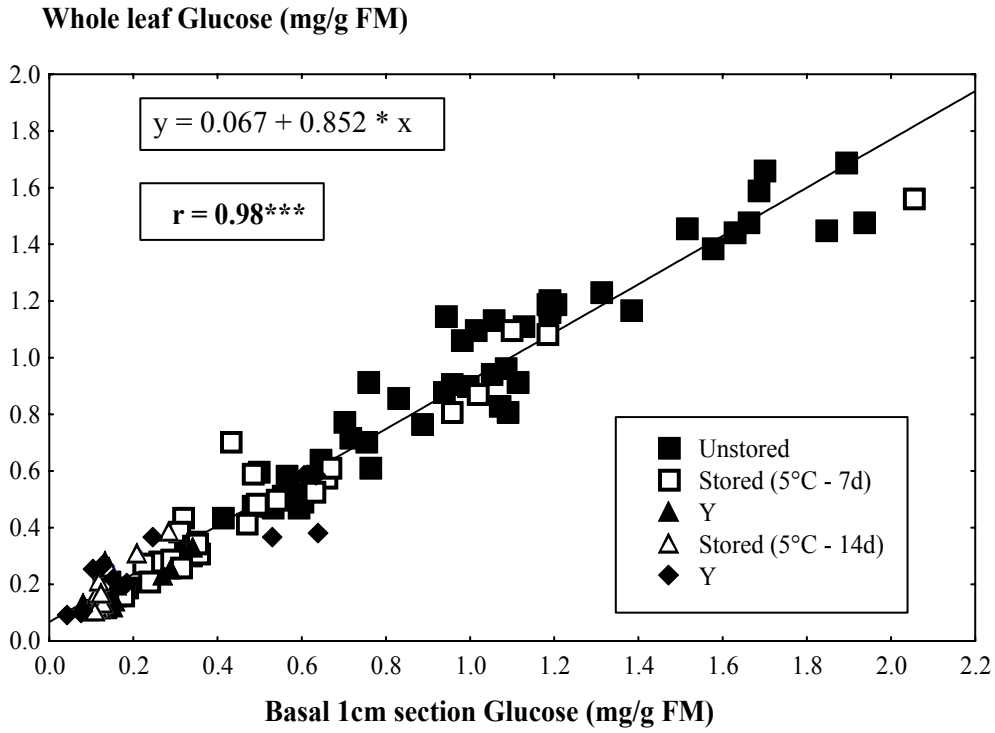


Fig. 5: Linear regression between glucose concentrations in basal 1 cm section of the leaves of four different ages and concentrations in the corresponding whole leaf at harvest and after storage at 5°C and 10°C for 7 and 14 days; ***: $p<0.001$; (Unstored: $n=52$; stored: 5° C for 7 days: $n=32$; 5° C for 14 days: $n=12$; 10° C for 7 days: $n=12$; 10° C for 14 days: $n=12$).

Table 3: Correlation coefficients, calculated between carbohydrate concentrations in the basal 1 cm section of leaves of different ages as independent variables and concentrations in the corresponding whole leaves as dependent variables. Includes unstored cuttings and cuttings stored at 5° C and 10°C for 7 and 14 days

Carbohydrate concentration	Tissue	Whole leaf (n = 120)
Glucose	Basal 1 cm section	0.98***
Fructose	Basal 1 cm section	0.97***
Reducing sugars (RS)	Basal 1 cm section	0.98***
Sucrose	Basal 1 cm section	0.96***
Total sugars (TS)	Basal 1 cm section	0.97***
Starch	Basal 1 cm section	0.97***
Total non-structural carbohydrates (TNC)	Basal 1 cm section	0.97***

RS: Glucose + Fructose; TS: RS + Sucrose; TNC: TS + Starch; ***: $p\leq0.001$.

3.2 Effect of season, stock plant age, storage and rooting environment on carbohydrate distribution, photosynthetic performance and root formation of 'Isabell' cuttings

The influence of pre-insertion carbohydrate distribution in different parts of the cuttings, when affected by season of stock plant cultivation, stock plant age and cold-storage, as well as current photosynthetic performance of those cuttings, when additionally affected by propagation environment, on subsequent root formation of the cuttings was investigated.

3.2.1 Pre-insertion condition

3.2.1.1 Carbohydrate distribution – cv. 'Isabell'

Significant interactions were found between the season of stock plant cultivation, stock plant age and subsequent cold-storage of cuttings with regard to carbohydrate composition in different parts of cuttings at insertion (Table 4). With regard to main factors, leaf carbohydrate levels were less affected by season of stock plant cultivation, but strongly influenced by cold-storage (Table 4). The influence of stock plant age (hereafter referred as harvest date for the sake of convenience) was either not significant or less significant, and inconsistent among seasons. The response of leaf carbohydrates to the season of stock plant cultivation, harvest date, and cold-storage are depicted in Fig. 6a-d.

At harvest, reducing sugar concentrations in leaves were higher in cuttings collected from stock plants cultivated in spring season when compared to those in summer and winter. Cold-storage of the cuttings resulted in the decrease of reducing sugars, and even minimized the differences found at harvest between different seasons and between harvest dates (Fig. 6a). Leaf glucose and fructose levels were almost similar at harvest in all the occasions. Cold-storage resulted in the significant decrease of both glucose and fructose, but this decrease was more pronounced in the case of fructose (data not shown). Leaf sucrose was less influenced by season of stock plant cultivation and harvest date (Table 4). Cold-storage resulted in a strong decrease of leaf sucrose concentrations in all the seasons (Table 4, Fig. 6b). At harvest, and also after cold-storage, sucrose constituted a major portion of total sugars. Leaf starch concentrations appeared to be higher when reducing sugar concentrations were higher (see Fig. 6a, c), other than that no significant differences were found between seasons.

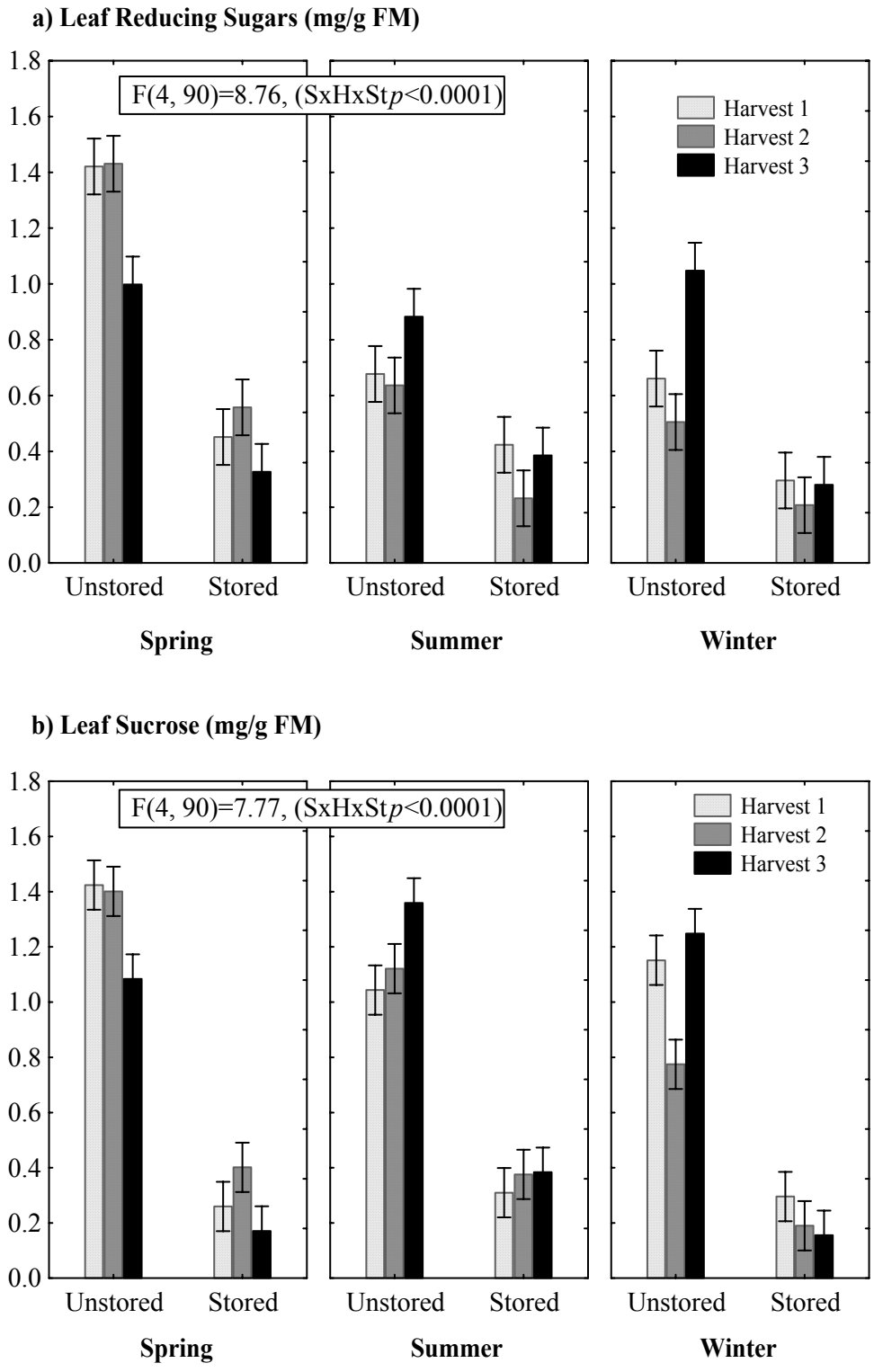


Fig. 6a & b: Effect of season of stock plant cultivation (S), harvest dates (H) and cold-storage (St) on pre-rooting concentrations of reducing sugars (a) and sucrose (b) in the leaves of 'Isabell' cuttings. *Vertical lines,* 0.95 confidence interval of least square mean values.

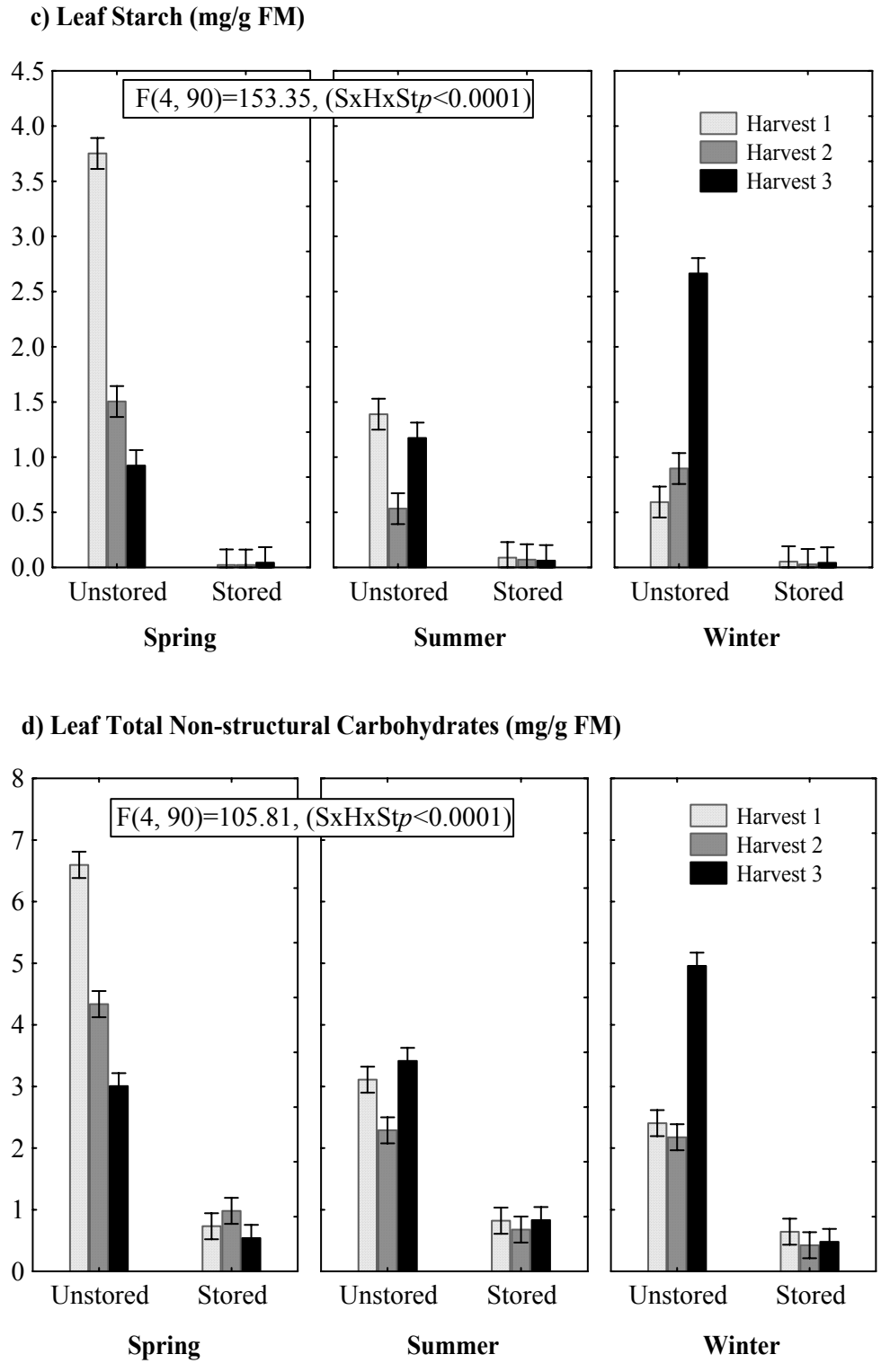


Fig. 6c & d: Effect of season of stock plant cultivation (S), harvest dates (H) and cold-storage (St) on pre-rooting concentrations of starch (c) and total non-structural carbohydrates (d) in the leaves of 'Isabell' cuttings. *Vertical lines,* 0.95 confidence interval of least square mean values.

A significant positive correlation was found between reducing sugar concentrations and starch concentrations at harvest (r = 0.60; $p<0.001$). However, cold-storage reduced starch to trace amounts at all the occasions (Fig. 6c). Total non-structural carbohydrates, which were strongly influenced by starch concentrations, varied strongly among harvest dates within a season and between the seasons, but these differences were nullified after storage, as starch totally disappeared (Fig. 6d).

In contrast to leaf carbohydrates, basal stem carbohydrates were strongly influenced by the season of stock plant cultivation, and relatively less influenced by cold-storage, but the influence varied from season to season (Table 4). In general, at harvest, basal stem carbohydrate levels were higher than leaf carbohydrate levels, especially glucose and starch, but varied strongly among seasons (Fig. 6a-d and Fig. 7a-d). The influence of harvest date on stem carbohydrates was minor and inconsistent among different seasons.

At harvest, basal stem glucose concentrations were significantly higher in the cuttings collected from stock plants cultivated in spring season when compared to those in summer and winter seasons. Basal stem glucose concentrations in winter season were very low, i.e. more than 4-fold lower than the concentrations in spring (Fig. 7a). Cold-storage reduced basal stem glucose concentrations by 47 %, 19 % and 67 % in spring, summer and winter, respectively (Fig. 7a). In all the seasons, at harvest, basal stem fructose concentrations were very low when compared to basal stem glucose, and more or less equal to leaf fructose concentrations (data not shown). Basal stem reducing sugar concentrations were declined after storage in spring and winter. In summer, in contrast, the concentrations remained unaffected, as glucose was only slightly decreased on the one hand (Fig. 7a) and fructose was slightly increased on the other hand (data not shown). Basal stem sucrose concentrations at harvest were higher in the cuttings produced in summer than those produced in spring and winter (Fig. 7b). The influence of cold-storage on basal stem sucrose concentrations was low (Table 4), and depended on season. In spring and winter (except the first harvest), sucrose concentrations were significantly decreased after storage, but in summer there was either a decrease as at first harvest, or alternatively an increase as at second (significant at $p<0.001$ level) and third harvests (n.s.) (Fig. 7b). Basal stem starch concentrations at harvest were strongly influenced by season (Table 4). The concentrations were higher in summer when compared to those in spring and winter (Fig. 7c). In summer, however, starch concentrations strongly varied among the harvest dates (Fig. 7c).

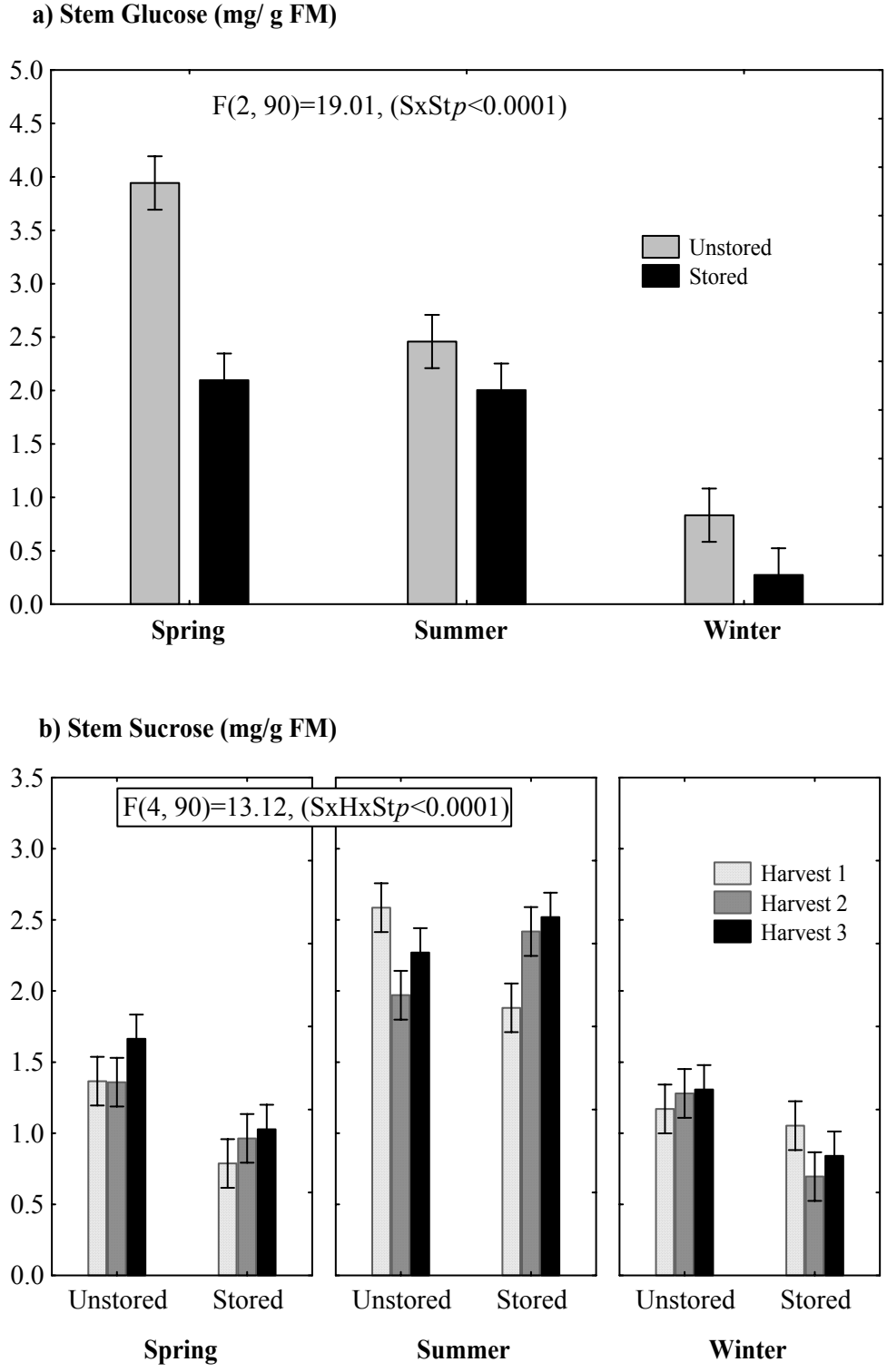


Fig. 7a & b : Effect of season of stock plant cultivation (S), harvest dates (H) and cold-storage (St) on pre-rooting concentrations of glucose (a) and sucrose (b) in the basal stems of 'Isabell' cuttings. *Vertical lines,* 0.95 confidence interval of least square mean values.

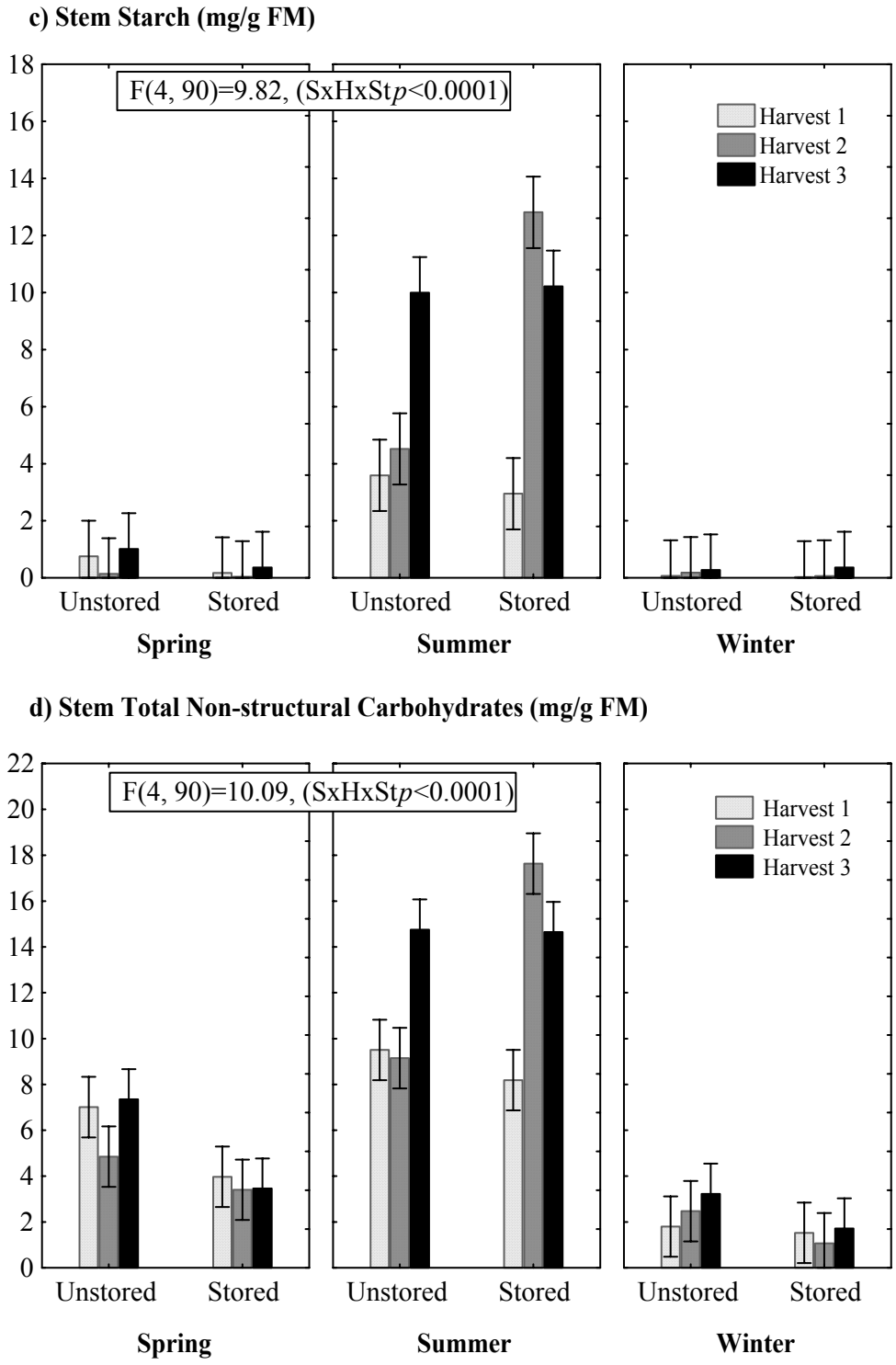


Fig. 7c & d: Effect of season of stock plant cultivation (S), harvest dates (H) and cold-storage (St) on pre-rooting concentrations of starch (c) and total non-structural carbohydrates (d) in the basal stems of 'Isabell' cuttings. *Vertical lines,* 0.95 confidence interval of least square mean values.

Table 4: Analysis of variance summaries (F-values) of data for pre-rooting carbohydrates (day 0) in leaves and basal stems of 'Isabell' cuttings as affected by season of stock plant cultivation (S), harvest dates (H) and cold-storage (St)

Factors	DF (n-1)	Leaf							Basal stem						
		Glucose	Fructose	RS	Sucrose	TS	Starch	TNC	Glucose	Fructose	RS	Sucrose	TS	Starch	TNC
S	2	128***	81***	95***	20***	63***	76***	126***	201***	79***	228***	359***	351***	252***	385***
H	2	4*	n.s.	n.s.	n.s.	n.s.	70***	45**	11***	n.s.	10***	6**	11***	24***	16***
St	1	670***	464***	571***	1784***	1439***	1889***	3342***	86***	6*	75***	58***	131***	6*	n.s.
SxH	4	28***	25***	24***	23***	30***	149***	117***	12***	50***	9***	n.s.	7***	21***	15***
SxSt	2	32***	37***	34***	9***	28***	89***	105***	19***	21***	20***	15***	33***	10***	23***
HxSt	2	n.s.	5**	n.s.	9***	6**	66***	45***	6**	3*	6**	4*	6**	11***	15***
SxHxSt	4	13***	6***	9***	8***	11***	153***	106***	n.s.	4**	n.s.	13***	5**	10***	10***

RS (Reducing sugars): Glucose + Fructose; TS (Total sugars): RS + Sucrose; TNC (Total non-structural carbohydrates): TS + Starch; n.s.: non significant; ***: $p \leq 0.001$; **: $p \leq 0.01$; *: $p \leq 0.05$.

The influence of cold-storage on basal stem starch was quite contrasting when compared to its influence on leaf starch (Table 4). In summer, basal stem starch levels were not changed at first and third harvests after cold-storage, but at second harvest the concentration was increased by more than 2-fold when compared to the corresponding value at harvest (Fig. 7c). It becomes clear that, basal stem sugar concentrations were only substantially decreased by storage (Fig. 7a-c) in those cases where prestorage starch levels in the same tissues were low (Fig. 7d). Basal stem non-structural carbohydrate levels, which were strongly influenced by starch levels both at harvest and after cold-storage, were high in cuttings procured from the stock plants cultivated in summer season, followed by spring and winter. After cold-storage, the concentrations were reduced in all seasons excepting summer, where there was either an increase as detected at second harvest or no change (Fig. 7d).

Correlations were calculated between PPFD prevailed in the greenhouse during stock plant cultivation (mean over 3 weeks and 1 week before the harvest of the cuttings) as independent variables and carbohydrate concentrations in different cutting parts as dependent variables. No significant correlations were calculated between PPFD and leaf carbohydrates, neither at harvest nor after cold-storage (data not shown), as leaf carbohydrates were less influenced by season of stock plant cultivation (Table 4). In contrast, highly significant positive correlations were calculated between PPFD and all individual basal stem carbohydrate fractions at harvest (except fructose and starch) (Table 5), as presented for total sugars in Fig. 8. Lower PPFD during stock plant cultivation in winter season (Table 1) resulted in substantially lower concentrations of carbohydrates in the basal stems and higher PPFD during spring and summer seasons resulted in higher carbohydrate concentrations. Even after the subsequent cold-storage of the cuttings, positive correlations calculated at harvest remained highly significant (Table 5, Fig. 8). These relationships even explained the variation in carbohydrate distribution between harvest dates within a season. Thus, the differences found in basal stem carbohydrate concentrations among seasons as well as among harvest dates were merely an out come of the PPFD perceived by the stock plants.

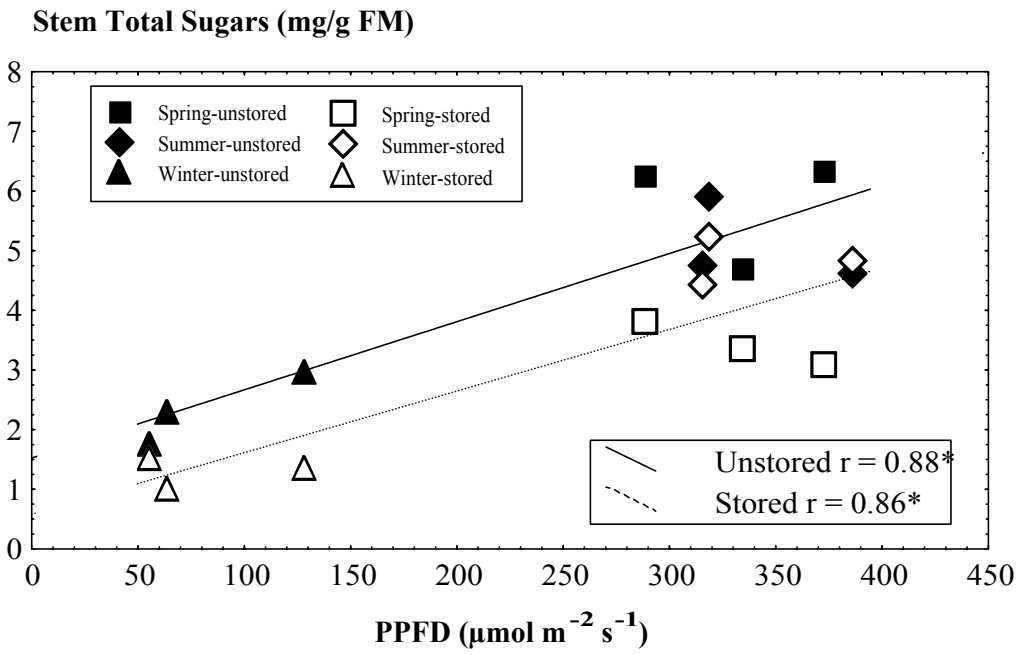


Fig. 8: Linear regression between PPFD prevailed (mean of values measured in greenhouse over one week before cutting harvest) during stock plant cultivation in three seasons (three harvest dates in each season) and pre-rooting total sugar concentrations in basal stems of 'Isabell' cuttings. *n*=9, each for unstored and stored cuttings. Each value for total sugars is a mean per three replications.

Table 5: Correlation coefficients, calculated between photosynthetic photon flux density (mean of values measured in greenhouse over 3 weeks and 1 week before cutting harvest) as independent variables and pre-rooting carbohydrate concentrations in basal stems of 'Isabell' cuttings as dependent variables. Includes three seasons (spring, summer and winter) and three harvest dates in each season (*n*=9)

Carbohydrate concentrations	At harvest		After storage	
	3 Weeks	1 Week	3 Weeks	1 Week
Glucose	0.69*	0.79*	0.79*	0.83**
Fructose	n.s.	n.s.	n.s.	n.s.
Reducing sugars (RS)	0.69*	0.80**	0.82**	0.85**
Sucrose	0.72*	n.s.	0.67*	n.s.
Total sugars (TS)	0.82**	0.88**	0.90**	0.86**
Starch	n.s.	n.s.	n.s.	n.s.
Total non-structural carbohydrates (TNC)	0.80**	0.73*	0.71*	0.63*

RS: Glucose + Fructose; TS: RS + Sucrose; TNC: TS + Starch; n.s.: non significant; **: $p \leq 0.01$; *: $p \leq 0.05$.

3.2.1.2 Chlorophyll fluorescence values – cv. 'Isabell'

Chlorophyll fluorescence values of the cuttings were measured at harvest and after retrieval from cold-storage. Initial F_v/F_m at harvest were 0.719, 0.724 & 0.669, and after cold-storage the values were 0.702, 0.685 & 0.704 in spring, summer and winter, respectively. At harvest, photochemical quenching (qP) of chlorophyll fluorescence values were high for the cuttings collected from stock plants cultivated in spring and summer when compared to those cultivated in winter. Cold-storage significantly reduced qP in all seasons. The diminution was strong in spring, followed by summer, alternatively in winter the values were only slightly (statistically significant) decreased (Fig. 9a). Non-photochemical quenching (qN) of chlorophyll fluorescence was found to be the main varying component to cold-storage. At harvest, qN values of the cuttings collected from the stock plants cultivated in different seasons were found to be more or less equal. After cold-storage, qN values of the cuttings were strongly decreased in all the seasons (Fig. 9b).

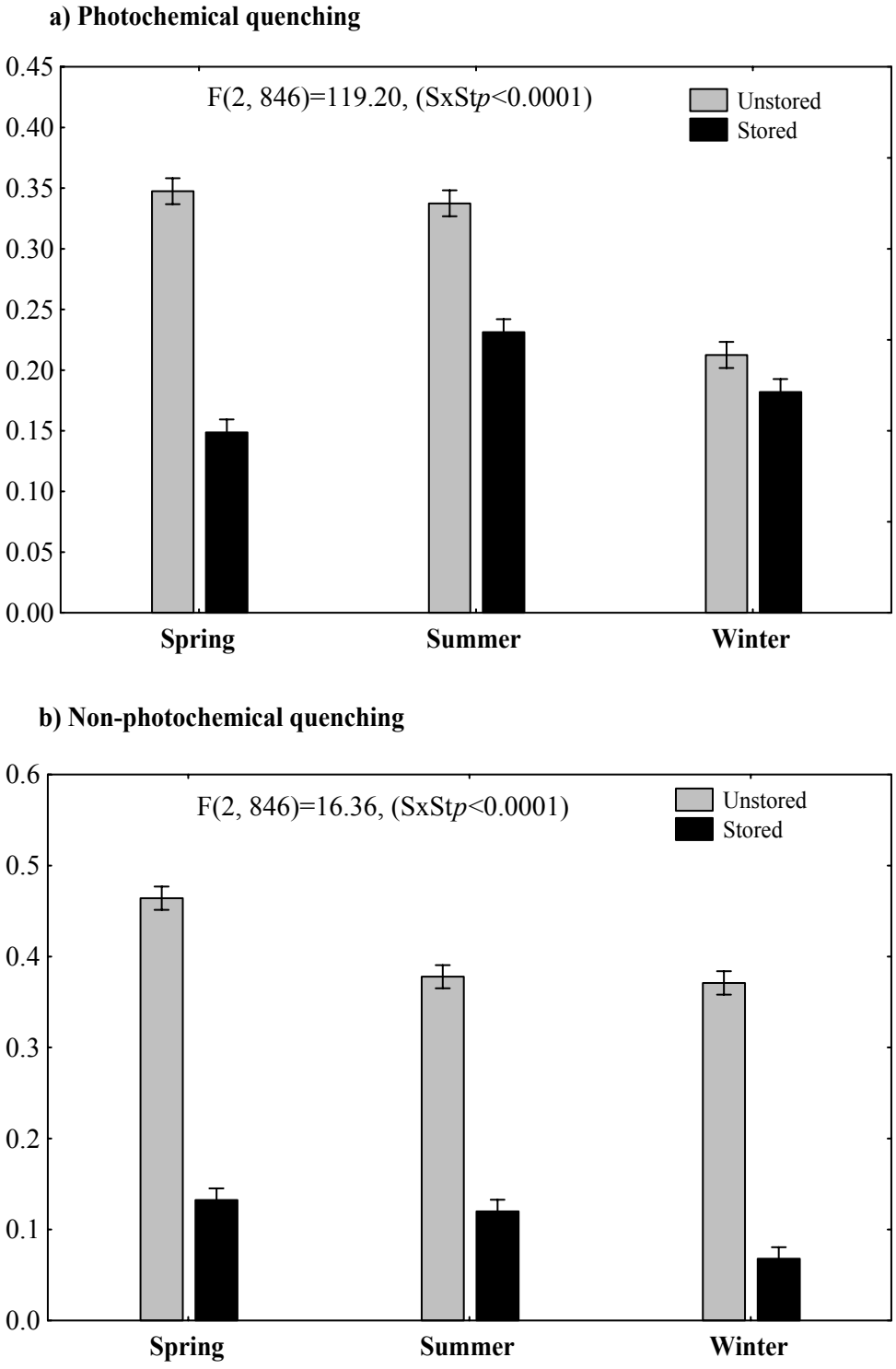


Fig. 9: Photochemical quenching (a) and Non-photochemical quenching (b) of chlorophyll fluorescence of 'Isabell' cuttings at insertion (day 0) as affected by season of stock plant cultivation (S) and cold-storage (St). Mean of three harvest dates. *Vertical lines,* 0.95 confidence interval of least square mean values.

3.2.1.3 Chlorophyll content – cv. 'Isabell'

Chlorophyll content of the oldest leaf (leaf 1) was analyzed immediately after harvest and after the cold-storage of the cuttings. There was no decrease in the chlorophyll content of the leaves after cold-storage, regardless of the season of stock plant cultivation (Table 6). In summer and winter, chlorophyll content (a + b) values were slightly higher in stored cuttings when compared to unstored cuttings.

Table 6: Influence of season of stock plant cultivation and cold-storage on chlorophyll content at insertion (day 0) in 'Isabell' cuttings

Season	Chlorophyll (a + b) (mg/g FM)	
	Unstored	Stored
Spring	1.1 b	1.1 b
Summer	1.1 b	1.3 a
Winter	1.1 b	1.4 a

Mean values of three harvest dates. Different subscripts indicate significant differences ($p \leq 0.05$).

3.2.2 During course of propagation

3.2.2.1 Chlorophyll fluorescence values – cv. 'Isabell'

With regard to a specific rooting environment, i.e. either in greenhouse or in climate chamber, during the course of propagation principally there were no significant differences between unstored cuttings and stored cuttings for different chlorophyll fluorescence parameters, although the quenching coefficients at insertion were significantly lower for stored cuttings. In all the seasons, F_v/F_m of both unstored and stored cuttings significantly raised to 0.801 ± 0.01 during 1 day of propagation in both unstored and stored cuttings. Thereafter, F_v/F_m was maintained at the same level during the rest of the propagation period at all the occasions (data not shown). With regard to photochemical quenching (qP) values, although there were strong differences between unstored cuttings and stored cuttings at the time of insertion in spring and summer, alternatively less difference in winter, during the course of propagation, i.e. from day 4 to day 14, there were no significant differences between

unstored cuttings and stored cuttings in any of the seasons in any specific rooting environment, i.e. either in greenhouse or in climate chamber (see Fig. 10a, 11a). In all the seasons, non-photochemical quenching (qN) values of stored cuttings which were very low at day 0, turned equal to the values of unstored cuttings during 1 day of propagation. Thereafter, no differences were detected between unstored cuttings and stored cuttings for qN values in any specific rooting environment (see Fig. 10b, 11b).

Nevertheless, when either unstored cuttings or stored cuttings rooted in greenhouse and climate chamber were separately regarded, highly significant interactions were found with regard to different chlorophyll fluorescence parameters. Although cold-storage strongly influenced different parameters at day 0, the after effect of storage principally relied on the rooting environment in which the cuttings were propagated as well as the season of propagation.

With regard to unstored cuttings, in both rooting environments F_v/F_m values were raised to 0.801 ± 0.01, and remained at same levels during the whole course of propagation in all the seasons (data not shown). Rooting environment had no major influence on qP values. Irrespective of rooting environment, qP values of the unstored cuttings decreased shortly after insertion during spring and summer propagation (Fig. 10a). However, the values were slightly increased by day 7 during spring and strongly increased by day 4 during summer propagation (Fig. 10a). Alternatively, in winter, qP values of the cuttings which were relatively low at the time of insertion when compared to other two seasons, were found to be increasing above the day 0 values during the course of propagation (Fig. 10a). Irrespective of the rooting environment, qN values of the cuttings were strongly reduced until day 1 during the course of propagation in spring and summer and until day 4 in winter (Fig. 10b). However, qN values of the cuttings propagated in greenhouse were recovered almost equal to the day 0 values by day 7 and day 4 during propagation in spring and summer, respectively, but in climate chamber no further recovery was detected and remained at the same low levels during the whole course of propagation in both the seasons. Alternatively, in winter, qN values of the cuttings propagated in both the rooting environments were equally increased by day 7 and reached the levels equal to day 0 values by day 14 (Fig. 10b).

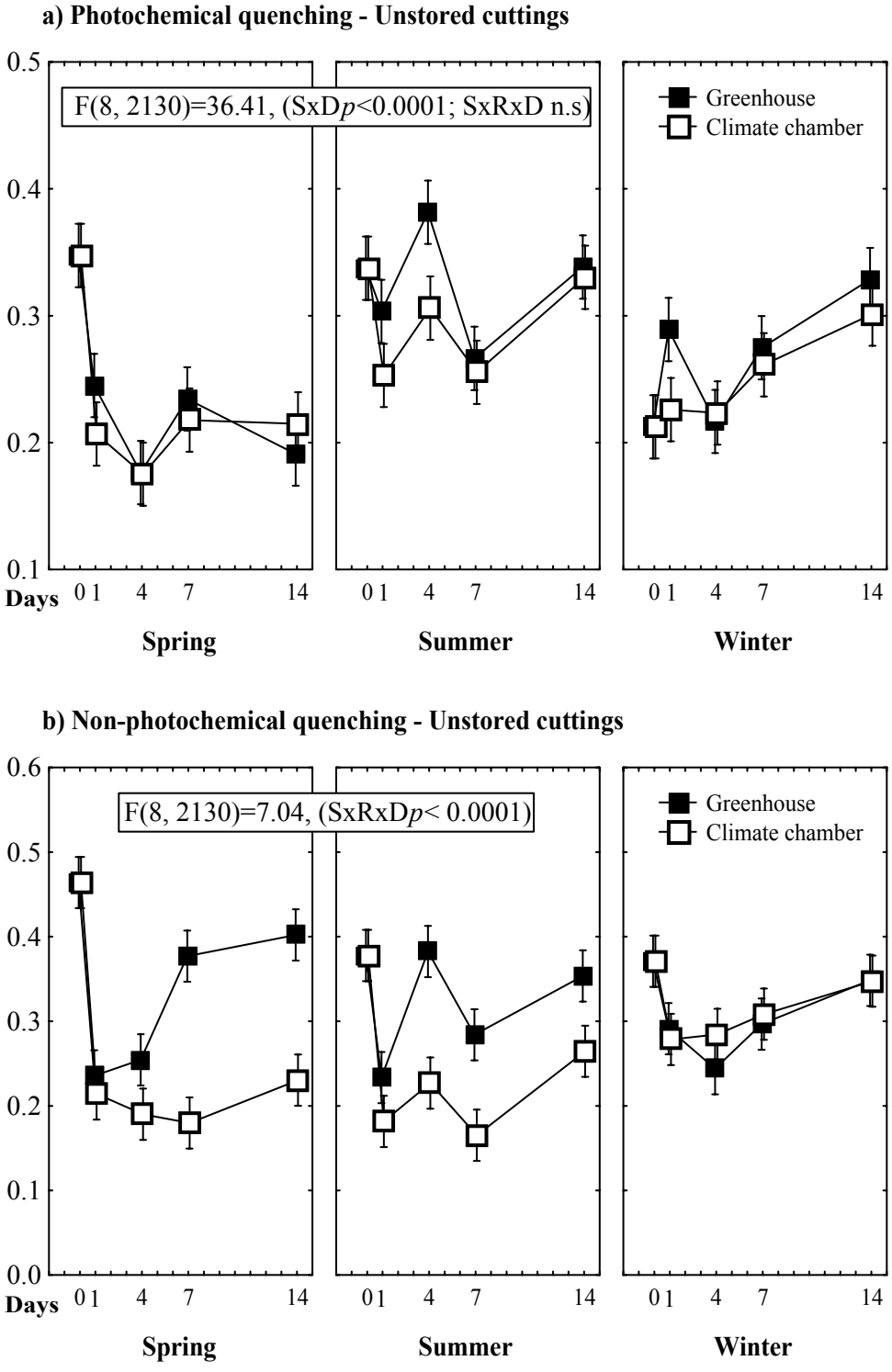


Fig. 10: Effect of season of propagation (S) and rooting environment (R) on photochemical quenching (a) and non-photochemical quenching (b) of chlorophyll fluorescence of unstored 'Isabell' cuttings during the course of propagation (D). Mean of three harvest dates. *Vertical lines,* 0.95 confidence interval of least square mean values.

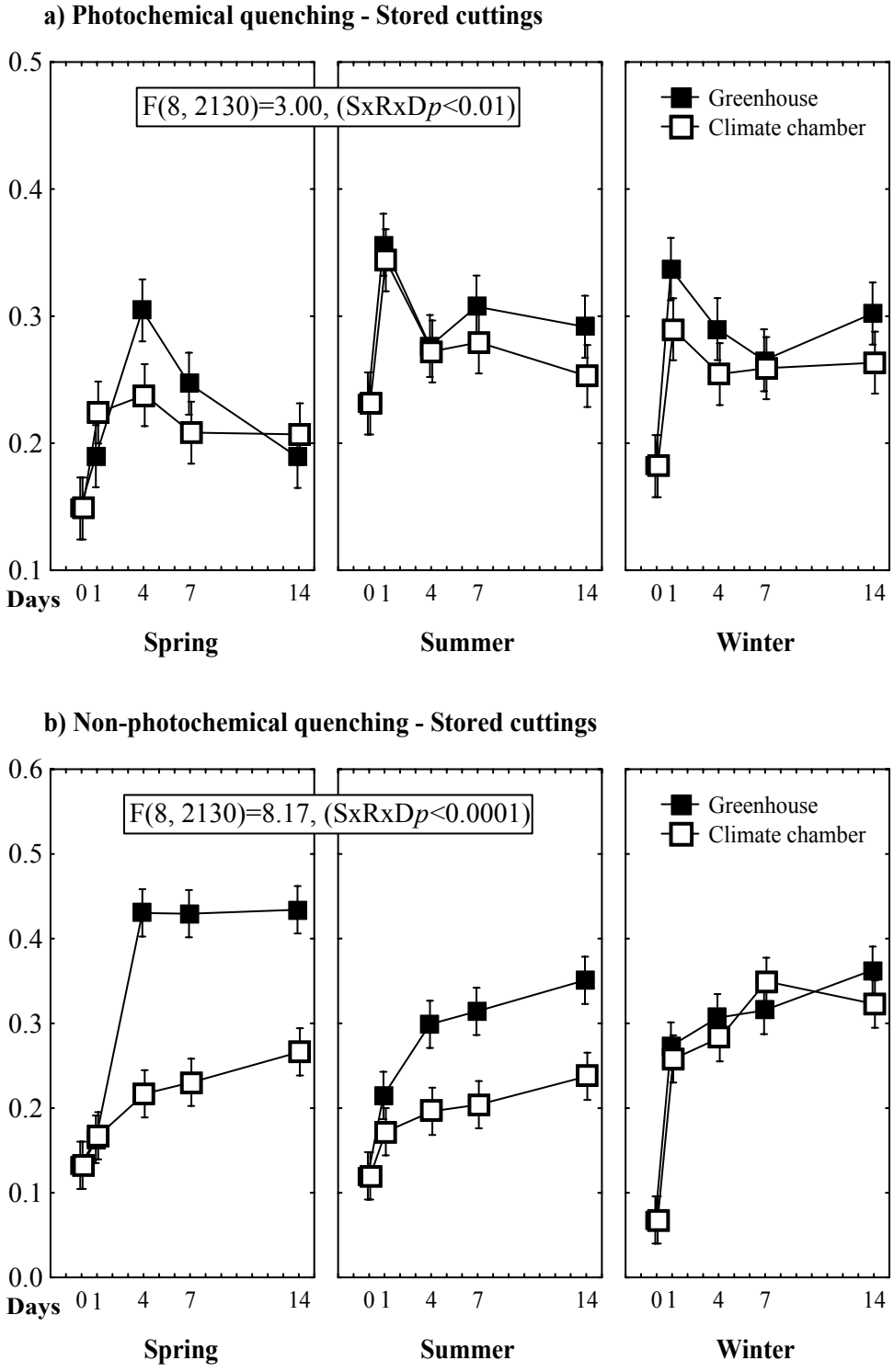


Fig. 11: Effect of season of propagation (S) and rooting environment (R) on photochemical quenching (a) and non-photochemical quenching (b) of chlorophyll fluorescence of stored 'Isabell' cuttings during the course of propagation (D). Mean of three harvest dates. *Vertical lines,* 0.95 confidence interval of least square mean values.

When stored cuttings were rooted in greenhouse and climate chamber, rooting environment had absolutely no influence on F_v/F_m of the cuttings. During 1 day of propagation in spring, summer and winter, F_v/F_m values were raised to 0.801 ± 0.01 and remained at similar levels for the rest of the propagation period (data not shown). In all the seasons of propagation, qP values of stored cuttings were significantly and equally increased in both the rooting environments during 1 day of propagation above the values measured at day 0 (Fig. 11a). Moreover, during the rest of the propagation period, no major significant differences were found between the two rooting environments (Fig. 11a). Pre-insertion qN values of the cuttings, which were strongly influenced by the cold-storage, were once again greatly influenced by the rooting environment during the course of propagation in all the seasons. During spring propagation, qN values of the cuttings were remained almost at the same low levels until day 1 in both the rooting environments, but by day 4 the values were drastically raised in greenhouse and remained higher during the rest of the propagation period, whereas in climate chamber there was only a smaller increase above the day 0 values (Fig. 11b). A similar phenomenon was also observed in summer propagation. Alternatively in winter, both in greenhouse as well as in climate chamber, qN values were equally and greatly increased by day 1 and continued to increase slightly until the last measurement day. (Fig. 11b).

3.2.2.2 Chlorophyll content – cv. 'Isabell'

With regard to chlorophyll content at day 7, cuttings propagated in greenhouse (1.00 mg/g FM) were found to have slightly lower content when compared to the cuttings propagated in climate chamber (1.08 mg/g FM), (significant at $p \leq 0.001$). Alternatively, stored cuttings (1.01 mg/g FM) were found to have slightly lower content when compared to unstored cuttings (1.07 mg/g FM), (significant at $p \leq 0.01$).

3.2.2.3 Net photosynthetic rate – cv. 'Isabell'

Cuttings, during different seasons, maintained measurable net photosynthetic rates at most of the measuring days during the course of propagation when exposed to a PPFD of 100 µmol m^{-2} s^{-1}. However, increasing the PPFD to 500 µmol m^{-2} s^{-1} did not result in any further

increase in the photosynthetic rates. In both the rooting environments and in all the seasons, net photosynthetic rates of the cuttings varied strongly during the course of propagation, and no clear trend emerged. Nevertheless, analysis of variance revealed that during spring and summer propagation, net photosynthetic rates of the cuttings were higher when propagated in greenhouse than in climate chamber irrespective of storage treatment. For those seasons, mean of net photosynthetic rates measured over the whole course of propagation were 0.43 and 0.26 µmol CO_2 m^{-2} s^{-1} in greenhouse and climate chamber, respectively (significant at $p \leq 0.001$ level). In winter, however, there were no clear differences between unstored cuttings and stored cuttings or between the rooting environments, average net photosynthetic rate during the course of propagation was 0.41 µmol CO_2 m^{-2} s^{-1}.

3.2.2.4 Carbohydrate status – cv. 'Isabell'

Leaf carbohydrates which were less influenced by season at day 0, were also only slightly affected by the same at day 7 (Table 7). Alternatively, basal stem carbohydrates which were predominantly influenced by season at day 0, were either less influenced or not influenced by the same at day 7 (Table 7). Cold-storage which had a strong influence on leaf carbohydrates at day 0, had no influence at day 7 (Table 7), whereas stem carbohydrates which were less influenced by cold-storage at day 0, were also either not influenced or less influenced at day 7 (Table 7). Either in unstored cuttings or in stored cuttings, carbohydrate levels in different cutting parts at day 7 were principally influenced by rooting environment (Table 7), and also interacted with season.

At insertion, basal stem carbohydrate levels were significantly influenced by season. However, the persistence or elimination of the differences among different seasons during propagation primarily depended upon rooting environment irrespective of storage treatment. With reference to the propagation in greenhouse, reducing sugars in basal stems significantly increased during 6 days of propagation in all the seasons. However, at day 7 the concentrations were highest in spring and lowest in winter (data not shown). Basal stem sucrose concentrations were also significantly increased during 6 days in all the seasons. The concentrations at day 7 were relatively lower in winter when compared to those in spring and summer (Fig. 12a).

Table 7: Analysis of variance summaries (F-values) of data for carbohydrates at day 7 during the course of propagation in leaves and basal stemsof ‘Isabell’ cuttings as affected by season of propagation (S), cold-storage (St) and rooting environment (R)

Factors	DF (n-1)	Leaf							Basal stem						
		Glucose	Fructose	RS	Sucrose	TS	Starch	TNC	Glucose	Fructose	RS	Sucrose	TS	Starch	TNC
S	2	38***	10***	21***	6**	11***	10***	13***	30***	7***	19***	n.s.	14***	24***	18***
St	1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	6**	12***	9**	n.s.	6**	n.s.	n.s.
R	1	14***	12***	14***	n.s.	7**	n.s.	n.s.	22***	23***	24***	6*	25***	n.s.	15***
SxSt	2	n.s.	n.s.	n.s.	n.s.	n.s.	9***	4**	n.s.	n.s.	n.s.	6**	n.s.	n.s.	n.s.
SxR	2	10***	4*	7***	21***	17***	14***	17***	10***	8***	9***	7**	11***	n.s.	6**
StxR	1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	4*	n.s.	n.s.	n.s.	n.s.
SxStxR	2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

RS (Reducing sugars): Glucose + Fructose; TS (Total sugars): RS + Sucrose and TNC (Total non-structural carbohydrates): TS + Starch;
n.s.: non significant; ***: $p \leq 0.001$; **: $p \leq 0.01$; *: $p \leq 0.05$.

During 6 days of propagation in spring, summer and winter, total sugar concentrations were increased by 147 % (6.7 mg/g FM) , 111 % (5.5 mg/g FM) & 225 % (4.1 mg/g FM), respectively (Fig. 12b). As a consequence, the concentrations remained significantly lower in winter season when compared to those in spring and summer (Fig. 12b). Starch concentrations were significantly reduced from day 0 to day 7 during summer propagation, but in spring and winter they remained at the same lower levels (Fig. 12c). Total non-structural carbohydrates were significantly increased in spring and winter, but in summer remained at the same level (data not shown) because starch was significantly decreased (Fig. 12c) and total sugars were increased (Fig. 12b).

With reference to the propagation in climate chamber, reducing sugar concentrations in the basal stems were only increased in winter during 6 days of propagation, but in spring and summer the concentrations remained at the same levels. Basal stem sucrose concentrations were significantly increased during 6 days in all the seasons, and at day 7 there were no significant differences among different seasons (Fig. 13a). It was surprising that under the same conditions of propagation in three seasons, basal stem total sugars were increased by only 60 % (2.8 mg/g FM) and 29 % (1.4 mg/g FM) in spring and summer, respectively, but in winter they were increased by 271 % (4.9 mg/g FM) (Fig. 13b). As a consequence, at day 7, total sugar concentrations were found to be equal in all the seasons, although there existed outstanding differences at day 0 (Fig. 13b). Basal stem starch decreased during propagation in summer, while in spring and winter there was no change and remained at the same lower level (Fig. 13c). Total non-structural carbohydrates increased during spring and winter propagation [as total sugars increased during those seasons (Fig. 13b)] and decreased during summer propagation [as starch decreased during summer (Fig. 13c)]. As a result, at day 7, the differences among different seasons were minimized (data not shown). Thus, when the cuttings were propagated in climate chamber the differences found at insertion among different seasons for basal stem carbohydrates were minimized during the course of propagation, whereas in greenhouse the differences persisted.

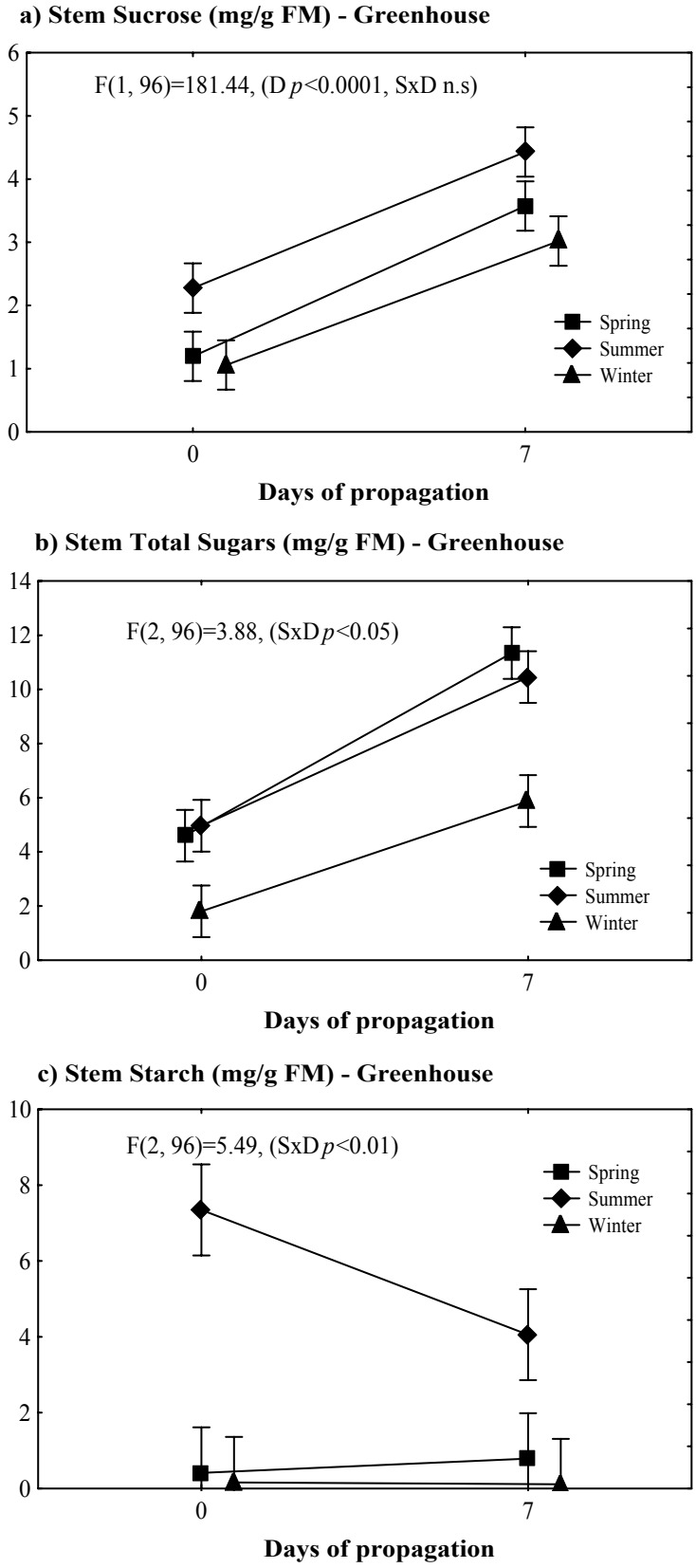


Fig. 12: Effect of season of propagation (S) on concentrations of sucrose (a), total sugars (b) and starch (c) in basal stems of 'Isabell' cuttings during the course of propagation (D) in greenhouse. Mean of unstored and stored cuttings and three harvest dates. *Vertical lines,* 0.95 confidence interval of least square mean values.

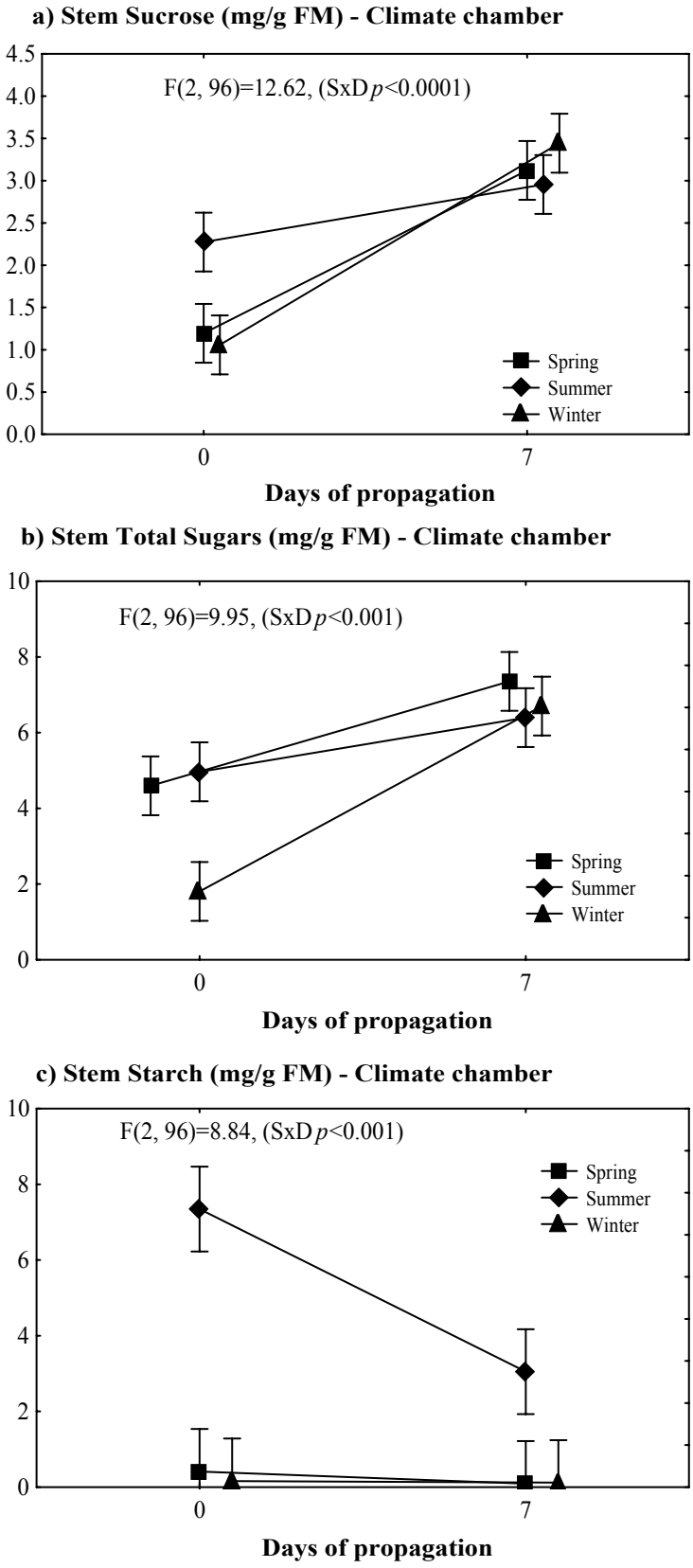


Fig. 13: Effect of season of propagation (S) on concentrations of sucrose (a), total sugars (b) and starch (c) in basal stems of 'Isabell' cuttings during the course of propagation (D) in climate chamber. Mean of unstored and stored cuttings and three harvest dates. *Vertical lines,* 0.95 confidence interval of least square mean values.

Carbohydrate levels in different parts of cuttings at day 7 (during propagation) were predominantly influenced by season, rooting environment and interaction of both (Table 7). To elucidate the influence of rooting environment on sustenance of current photosynthesis, greenhouse propagation and climate chamber propagation were compared separately for unstored cuttings and for stored cuttings. Furthermore, to explicate the impact of cold-storage on carbohydrate status during propagation, unstored and stored cuttings propagated in a specific rooting environment (i.e. either in greenhouse or in climate chamber) were compared.

Strong interactions were found with reference to the carbohydrate shift in different cutting parts during the course of propagation when either unstored cuttings or stored cuttings rooted in greenhouse and climate chamber were separately regarded. With regard to unstored cuttings, irrespective of the season and rooting environment, leaf glucose and fructose concentrations were significantly reduced during the first 6 days of propagation (data not shown). During spring propagation, leaf sucrose levels were decreased in both greenhouse and climate chamber, whereas during summer, sucrose levels were only reduced in climate chamber (Fig. 14a). At day 7, however, leaf sucrose levels were significantly lower in climate chamber when compared to the levels in greenhouse during both spring and summer (Fig. 14a). In contrast, during winter, leaf sucrose levels were only significantly decreased in greenhouse (Fig. 14a). Leaf total sugars were significantly reduced by day 7 in both the rooting environments during spring and winter propagation, but the decrease was significantly higher in climate chamber during spring and *vice versa* in winter (n.s.), (Fig. 14b). In summer propagation, however, the concentrations were only decreased in climate chamber, reaching significantly lower levels compared to those in greenhouse (Fig. 14b). Leaf starch was not principally influenced by rooting environment. Irrespective of the season, leaf starch was significantly reduced by day 7 (from 1.49 mg/g FM to 0.97 mg/g FM; $p<0.01$). During 6 days of propagation in spring, leaf total non-structural carbohydrates were significantly decreased in both rooting environments, but during summer the levels in climate chamber and during winter the levels in greenhouse only declined (data not shown).

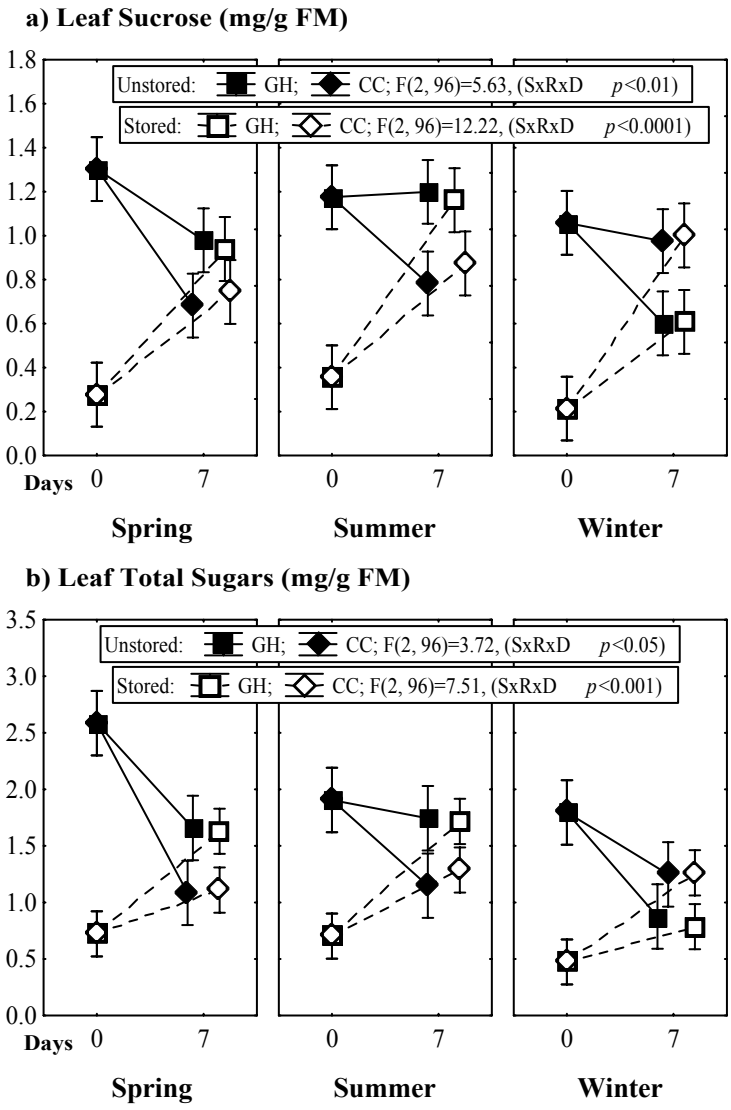


Fig. 14: Effect of season of propagation (S) and rooting environment (R) on concentrations of sucrose (a) and total sugars (b) in leaves of unstored cuttings and stored 'Isabell' cuttings during the course of propagation (D). Mean of three harvest dates. *Vertical lines,* 0.95 confidence interval of least square mean values. GH: Greenhouse; CC: Climate chamber.

Stored cuttings which had substantially low leaf carbohydrate concentrations at day 0 either increased or remained at the same levels during 6 days of propagation. Furthermore, in any specific rooting environment, i.e. either in greenhouse or in climate chamber, there were absolutely no differences between unstored cuttings and stored cuttings for any of the individual leaf carbohydrate fractions (Table 7, Fig. 14a, b). Leaf glucose slightly (statistically significant, $p<0.05$) increased in greenhouse, but remained at the same lower level in climate chamber during 6 days of propagation in spring and summer, whereas in winter there was no change in both of the rooting environments (data not shown). Neither rooting environment nor

day of propagation influenced leaf fructose levels (data not shown). Irrespective of the season and rooting environment, leaf sucrose increased significantly during 6 days of propagation. However, the increase was significantly higher in greenhouse than in climate chamber during spring and summer, and *vice versa* during winter season (Fig. 14a). Leaf total sugars at day 7, influenced by leaf sucrose, were significantly higher in greenhouse during spring and summer, and in climate chamber during the winter season (Fig. 14b). Leaf starch, which was absent at day 0, significantly and equally increased during 6 days of propagation in both the rooting environments in summer. Alternatively, during spring the levels were only increased in greenhouse and during winter the levels were only increased in climate chamber (data not shown). Total non-structural carbohydrates increased significantly in all the seasons and in both rooting environments, except in greenhouse during winter (data not shown). Thus in essence, leaf carbohydrate concentrations at day 7 were significantly higher in the cuttings propagated in greenhouse than in climate chamber during spring and summer seasons, and in climate chamber than in greenhouse during winter season, and storage had no influence.

With regard to the basal stem carbohydrates of unstored cuttings, in spring there was a significant increase of glucose in greenhouse and decrease in climate chamber, but in summer and winter there were no significant changes during 6 days of propagation. However, at day 7 the levels in greenhouse were significantly higher than the levels in climate chamber in both spring and summer, but in winter there was no difference between rooting environments (Fig. 15a). Basal stem fructose levels which were very low at day 0, significantly increased during 6 days of propagation in both rooting environments and in all seasons. However, the increase was significantly higher in greenhouse during spring ($p<0.001$) and summer ($p<0.001$), but in winter there were no differences between rooting environments (data not shown). Rooting environment had no influence on basal stem sucrose and starch levels at day 7. Irrespective of the season, sucrose significantly increased during 6 days of propagation (Fig. 15b). Starch decreased (6.03 mg/g FM to 3.16 mg/g FM; $p<0.001$) during summer propagation, but in spring and winter there was no change and remained at the same lower levels. During spring and summer propagation, basal stem total sugars increased significantly in greenhouse, but in climate chamber the increase was not significant, and at day 7 the levels were significantly higher in greenhouse when compared to the levels in climate chamber (Fig. 15c). During winter propagation, in contrast, total sugars were significantly and equally increased in both of the rooting environments (Fig. 15c).

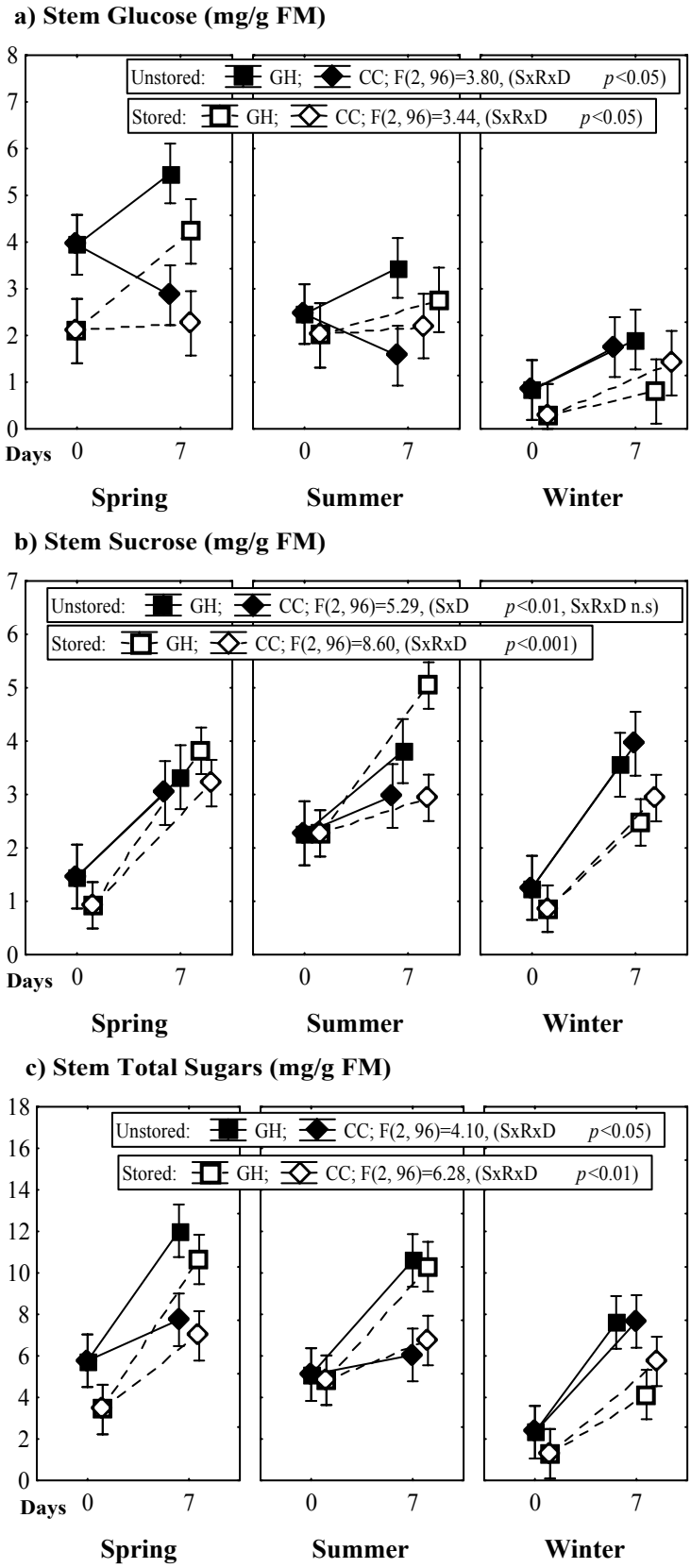


Fig. 15: Effect of season of propagation (S) and rooting environment (R) on concentrations of glucose (a), sucrose (b) and total sugars (c) in basal stems of unstored cuttings and stored 'Isabell' cuttings during the course of propagation (D). Mean of three harvest dates. *Vertical lines*, 0.95 confidence interval of least square mean values. GH: Greenhouse; CC: Climate chamber.

In stored cuttings, basal stem carbohydrates were either significantly increased during 6 days of propagation or remained at the same levels. When propagated in climate chamber, in all the three seasons there were no significant differences between unstored cuttings and stored cuttings for all the basal stem carbohydrate fractions at day 7 (Fig. 15a-c). Alternatively, when propagated in greenhouse, there were some minor differences between unstored cuttings and stored cuttings, which principally depended on season (Fig. 15a-c). With reference to basal stem glucose concentrations in stored cuttings, excepting a significant increase in greenhouse during 6 days of spring propagation, there were no significant changes in any of the seasons in both the rooting environments (Fig. 15a). Basal stem fructose exhibited a pattern similar to that evident in unstored cuttings (data not shown). Basal stem sucrose increased significantly in all the seasons and in both rooting environments, except in climate chamber during summer propagation (Fig. 15b). Moreover, at day 7, there were no significant differences between rooting environments for sucrose levels in spring and winter, but in summer the levels in greenhouse were significantly higher than the levels in climate chamber (Fig. 15b). Basal stem total sugars exhibited a pattern similar to sucrose. At day 7, however, the levels were significantly higher in greenhouse during spring and summer, but in winter there were no differences between rooting environments (Fig. 15c). Rooting environment had no influence on basal stem starch and total non-structural carbohydrate levels. During 6 days of propagation, starch was reduced in summer (8.66 mg/g FM to 3.95 mg/g FM; $p<0.0001$), but remained at the same lower levels in spring and winter. Total non-structural carbohydrates were increased during spring and winter, but there was no change during summer (data not shown). With regard to the influence of cold-storage on basal stem carbohydrates at day 7 when propagated in greenhouse, there were no significant differences between unstored cuttings and stored cuttings during spring season, as shown for glucose (Fig. 15a), sucrose (Fig. 15b) and total sugars (Fig. 15c). Similarly, in summer there were no significant differences between unstored cuttings and stored cuttings expect for sucrose (Fig. 15b), where stored cuttings had significantly ($p<0.01$) higher sucrose concentrations when compared to those of unstored cuttings. In winter, however, glucose ($p<0.01$, Fig. 15a), fructose ($p<0.01$, data not shown), total sugars ($p<0.05$, Fig. 15c) and total non-structural carbohydrates ($p<0.05$, data not shown) were lower in stored cuttings compared to unstored cuttings. Although there existed some minor differences, the majority of the results reveal that both unstored and stored cuttings propagated in greenhouse had higher basal stem

carbohydrate levels when compared to the cuttings propagated in climate chamber during spring and summer seasons, but in winter there were no differences between the rooting environments.

3.2.2.5 Visual observations – cv. 'Isabell'

Almost no leaf senescence was observed during the propagation of 'Isabell' cuttings. Irrespective of the season of propagation, influence of cold-storage on leaf senescence was less and rooting environment had no influence (data not shown). The number of senesced leaves determined per 17 cuttings were 0.12, 0.29 & 0.26 in spring, summer and winter seasons, respectively.

3.2.3 Rooting efficiency – cv. 'Isabell'

Rooting efficiency of the cuttings as affected by season of propagation, cold-storage, and rooting environment was investigated. Root number was determined after 21 days of propagation. Rooting efficiency of the cuttings was strongly influenced by storage treatment (Table 8), but the influence depended on the rooting environment under which the cuttings were rooted and the season of propagation. Root number varied among the harvest dates within season, but the variation among the harvests in different seasons was not consistent (Fig. 16).

When cuttings were rooted in greenhouse, during spring and summer propagation there were no major differences between unstored cutting and stored cuttings for number of roots formed, except at second harvest in summer propagation, where unstored cuttings formed significantly higher number of roots than stored cuttings. (Fig. 16a). In winter propagation, however, root number was significantly lower for stored cuttings when compared to unstored cuttings, except at second harvest (Fig. 16a). The survival rate of those stored cuttings in winter remained high, but the root number was extremely reduced as most of the cuttings either had small roots which were still inside the propagator cube or remained at callus stage. In contrast, when cuttings were propagated in climate chamber, in all the seasons, root number was higher for unstored cuttings when compared to stored cuttings (statistically not significant at some harvests in spring and summer) (Fig. 16b).

Table 8: Analysis of variance summaries (F-values) of data for adventitious root formation of 'Isabell' cuttings as affected by season of propagation (S), cold-storage (St) and rooting environment (R)

Factors	DF (n-1)	Visible roots[1]	Total roots[2]
S	2	13 ***	19 ***
St	1	165 ***	191 ***
R	1	n.s.	n.s.
S x St	2	39 ***	47 ***
S x R	2	28 ***	31 ***
St x R	1	14 ***	5 ***
S x St x R	2	n.s.	n.s.

[1]: Roots visible outside the propagator cube; [2] Total roots: Visible roots + small roots which were still inside the propagator cube; n.s.: not-significant; ***: $p \leq 0.001$.

With regard to unstored cuttings, during spring propagation, there was no difference between greenhouse and climate chamber for number of subsequently formed roots (Fig. 17a). In summer propagation, however, root number was slightly but significantly higher in greenhouse when compared to that in climate chamber. During winter propagation, by contrast, the cuttings rooted in climate chamber formed significantly higher number of roots when compared to the cuttings rooted in greenhouse. Moreover, it was the best when compared to propagation in any other seasons, either in greenhouse or climate chamber (Fig. 17a). With regard to stored cuttings, during spring and summer propagation, the cuttings rooted in greenhouse formed significantly higher number of roots when compared to the cuttings rooted in climate chamber. In contrast, cuttings rooted in climate chamber formed significantly higher number of roots during winter propagation (Fig. 17b). It is noteworthy that stored cuttings when rooted in climate chamber, produced similar number of roots with minor discrepancies, irrespective of the season of propagation.

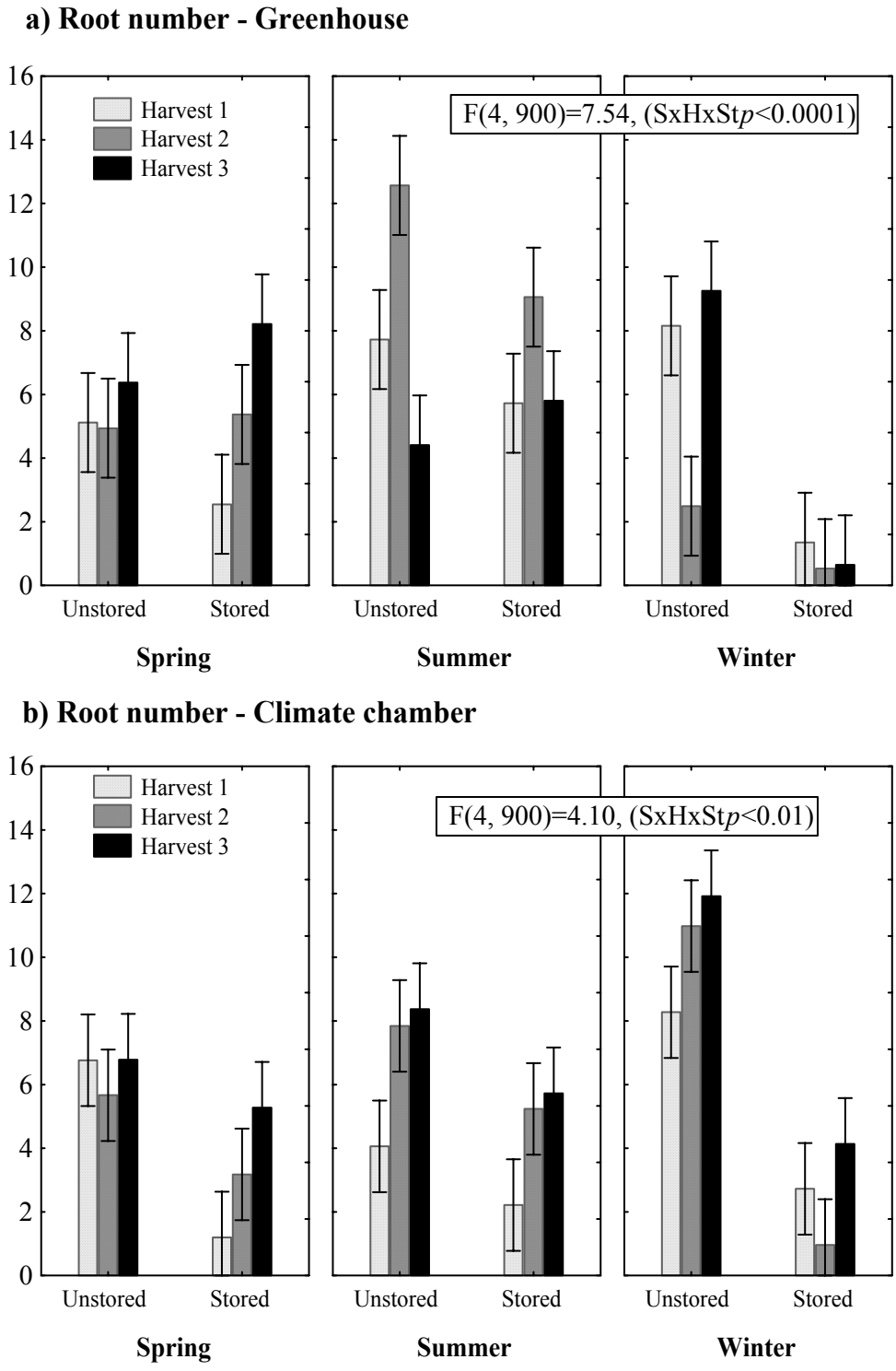


Fig. 16: Adventitious root formation of 'Isabell' cuttings in greenhouse (a) and in climate chamber (b) as affected by season of propagation (S), harvest dates within the season (H) and cold-storage (St). Root number: Visible roots outside the propagator cube. *Vertical lines,* 0.95 confidence interval of least square mean values.

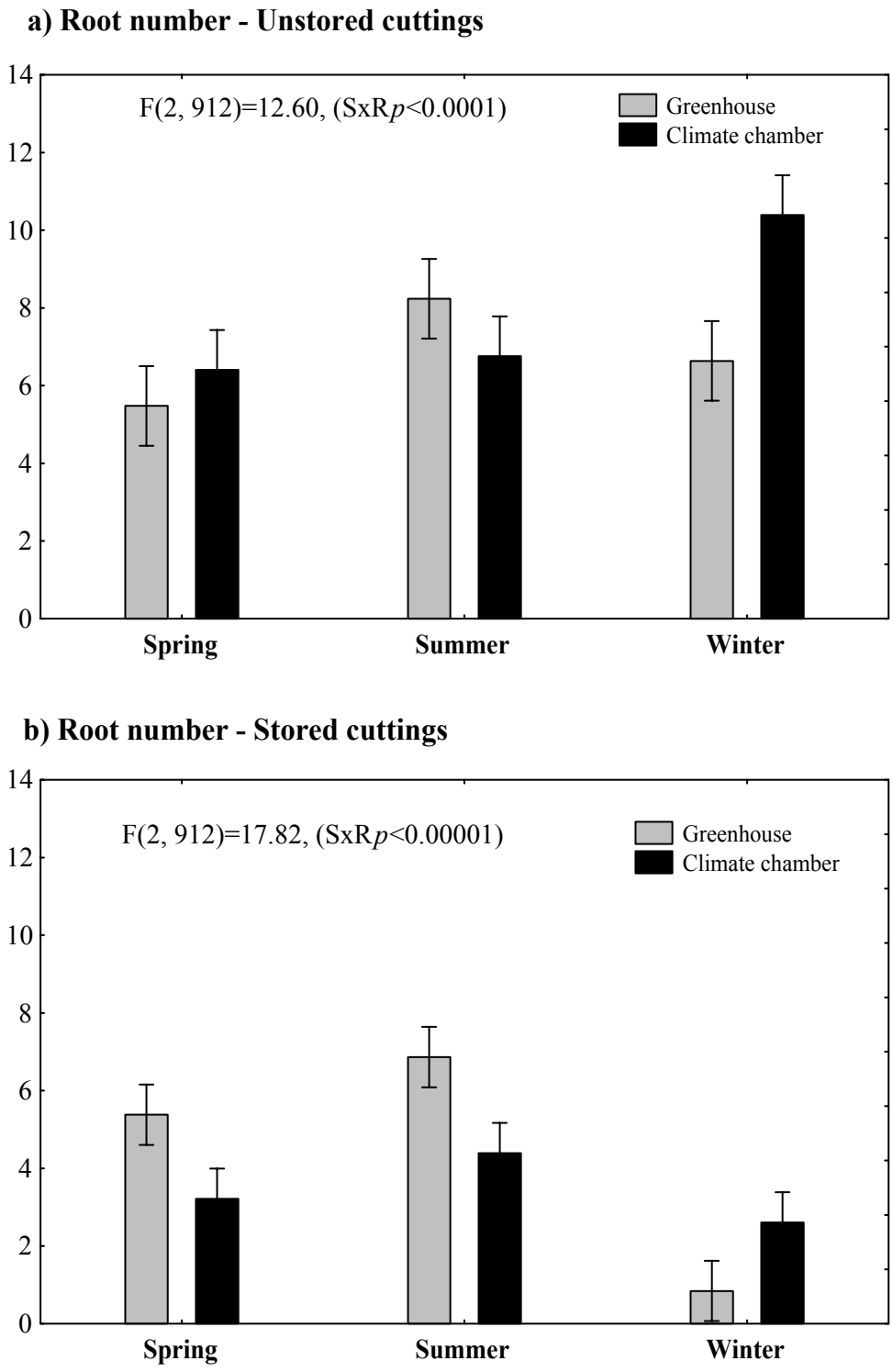


Fig. 17: Adventitious root formation of unstored (a) and stored (b) cuttings of 'Isabell' as affected by season of propagation (S), and rooting environment (R). Mean of three harvest dates. Root number: Visible roots outside the propagator cube. *Vertical lines,* 0.95 confidence interval of least square mean values.

3.2.4 Relationship between pre-insertion condition and subsequent root formation

3.2.4.1 Correlation between pre-insertion carbohydrates and root number – cv. 'Isabell'

Correlations, calculated between pre-rooting carbohydrate concentrations in different parts of the cutting as independent variable and the number of subsequently formed roots as dependent variables, are presented in Tables 9 and 10. When unstored and stored cuttings were rooted in climate chamber, where similar environmental conditions were maintained during all the seasons of propagation (relatively low light), significant positive correlations were found between pre-rooting carbohydrates in different cutting parts and the rooting response (Table 9). All the pre-rooting leaf carbohydrates in spring, summer and winter propagation, except leaf glucose and starch in summer, positively correlated with the number of roots formed, but highly significant correlations were found with leaf sucrose concentrations irrespective of the season of propagation (Table 9). Similar significant correlations were also found when pre-rooting carbohydrates in leaves of different ages (leaf 1 and leaf 2) were attempted to correlate with root number (Table 1A in Appendix). During all the seasons of propagation, the higher carbohydrate presence in unstored cuttings compared to stored cuttings, resulted in positive linear regression with the number of subsequently formed roots, as presented for leaf sucrose (Fig. 18a). The regression slopes over the same broad range of sucrose concentrations for unstored and stored cuttings were different for the three seasons of propagation, which depended principally on rooting response of unstored cuttings. The steepness of the regression line was higher for winter propagation, followed by summer and spring, with regard to number of roots formed (slopes of 8, 3.4 and 2.7, respectively). This demonstrates the higher rooting capability of unstored cuttings during winter propagation (Fig. 18a). Similar significant correlations were found between all pre-rooting basal stem carbohydrates in spring and winter (except fructose, reducing sugars and starch in spring, and starch in winter) and number of subsequently formed roots. Like leaf sucrose, basal stem sucrose was also best fitted among different carbohydrates in the linear regression with root number (Table 9). The steepness of the regression line which was mainly determined by unstored cuttings was significantly higher for winter propagation when compared to spring (slopes of 13.4 and 5.5, respectively), (Fig. 18b). This once again mirrored the higher rooting capacity of unstored cuttings in winter. In summer, in contrast, no regressions were found

Table 9: Correlation coefficients, calculated between pre-rooting (day 0) carbohydrate concentrations in different parts of 'Isabell' cuttings propagated during spring, summer and winter seasons as independent variables and number of subsequently formed adventitious roots as dependent variables. Pooled data of unstored and stored cuttings propagated in greenhouse or climate chamber

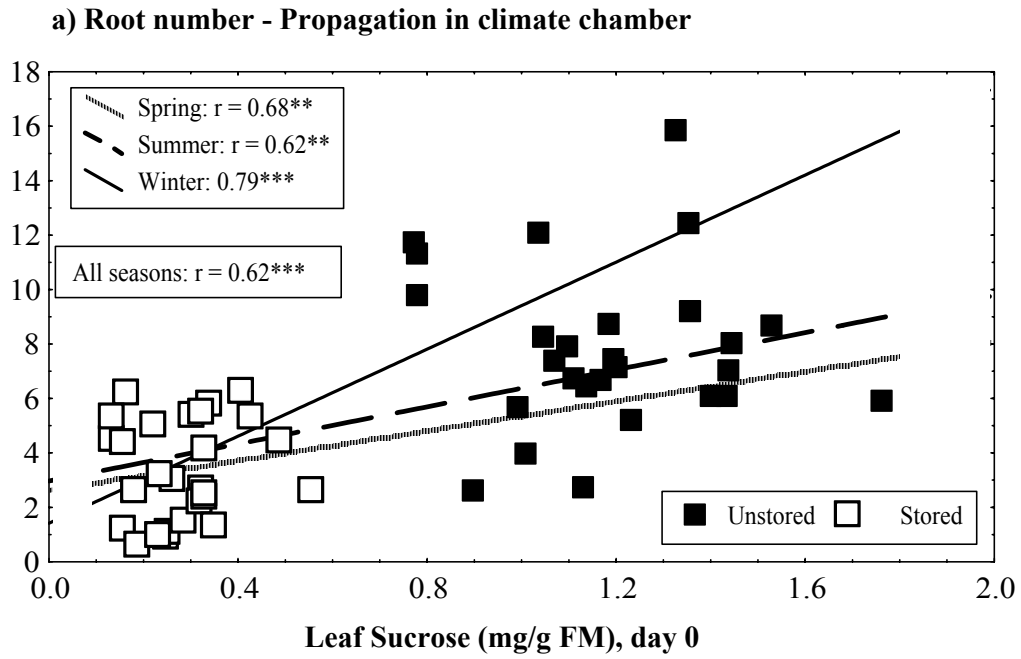
Carbohydrate concentrations	Tissue	Greenhouse (day 0)				Climate chamber (day 0)			
		Spring (*n*=18)	Summer (*n*=18)	Winter (*n*=18)	All seasons (*n*=54)	Spring (*n*=18)	Summer (*n*=18)	Winter (*n*=18)	All seasons (*n*=54)
Glucose	Leaf	n.s.	n.s.	0.90***	0.27*	0.58*	n.s.	0.72***	0.34*
	BS	n.s.	n.s.	0.72***	0.32*	0.53*	n.s.	0.73***	n.s.
Fructose	Leaf	n.s.	n.s.	0.90***	n.s.	0.49*	0.52*	0.73***	0.41**
	BS	-0.61**	n.s.	n.s	n.s.	n.s.	n.s.	0.68**	n.s.
RS	Leaf	n.s.	n.s.	0.91***	n.s.	0.54*	n.s	0.73***	0.39**
	BS	n.s.	n.s.	0.66**	0.31	n.s.	n.s.	0.76***	n.s.
Sucrose	Leaf	n.s.	n.s.	0.92***	0.38**	0.68**	0.62**	0.79***	0.62***
	BS	n.s.	n.s.	0.67**	0.48***	0.80***	n.s.	0.73***	n.s.
TS	Leaf	n.s.	n.s.	0.94***	0.31*	0.62**	0.57*	0.79***	0.53***
	BS	n.s.	n.s.	0.70**	0.43**	0.56*	n.s.	0.80***	n.s.
Starch	Leaf	n.s.	n.s.	0.77***	n.s.	0.60**	n.s.	0.78***	0.52***
	BS	n.s.	n.s.	n.s.	0.33*	n.s.	n.s.	n.s	n.s.
TNC	Leaf	n.s.	n.s.	0.87***	n.s	0.64**	0.47*	0.81***	0.55***
	BS	n.s.	n.s.	0.67**	0.42**	0.60**	n.s.	0.78***	n.s.

RS (Reducing sugars): Glucose + Fructose; TS (Total sugars): RS + Sucrose, TNC (Total non-structural carbohydrates): TS + Starch; BS: Basal stem; n.s.: non significant; ***: $p \leq 0.001$; **: $p \leq 0.01$; *: $p \leq 0.05$.

Table 10: Correlation coefficients, calculated between carbohydrate concentrations in different parts of 'Isabell' cuttings propagated during spring, summer and winter seasons as independent variables and number of subsequently formed adventitious roots as dependent variables. Pooled data of unstored and stored cuttings propagated in greenhouse and climate chamber

Carbohydrates concentration	Tissue	Spring (*n*=36)		Summer (*n*=36)		Winter (*n*=36)		All seasons (*n*=108)	
		Day 0[1]	Mean[2]	Day 0[1]	Mean[2]	Day 0[1]	Mean[2]	Day 0[1]	Mean[2]
Glucose	Leaf	n.s.	n.s.	n.s.	n.s.	0.76***	0.77***	0.30**	0.29**
	BS	n.s.	n.s.	n.s.	n.s.	0.68***	0.63***	n.s.	n.s.
Fructose	Leaf	n.s.	n.s.	n.s.	0.39*	0.76***	0.77***	0.26**	0.24*
	BS	-0.50**	n.s.	n.s.	0.39*	0.44**	0.48**	n.s.	0.26**
RS	Leaf	n.s.	n.s.	n.s.	0.38*	0.76***	0.78***	0.29**	0.28**
	BS		n.s.	n.s.	0.40*	0.67***	0.57***	n.s.	0.21*
Sucrose	Leaf	n.s.	n.s.	n.s.	0.68***	0.80***	0.82***	0.50***	0.61***
	BS	0.53***	0.42*	n.s.	n.s.	0.66***	0.52**	0.34***	0.42***
TS	Leaf	n.s.	n.s.	n.s.	0.61***	0.80***	0.84***	0.42***	0.49***
	BS	n.s.	n.s.	n.s.	0.35*	0.71***	0.57***	0.26**	0.32***
Starch	Leaf	n.s.	n.s.	n.s.	0.34*	0.73***	0.72***	0.36***	0.46***
	BS	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.20*	n.s.
TNC	Leaf	n.s.	n.s.	n.s.	0.58***	0.79***	0.81***	0.41***	0.51***
	BS	n.s.	n.s.	n.s.	n.s.	0.69***	0.56***	0.25**	0.29**

RS (Reducing sugars): Glucose + Fructose; TS (Total sugars): RS + Sucrose, TNC (Total non-structural carbohydrates): TS + Starch; BS: Basal stem; [1] and [2] are carbohydrate concentrations at insertion and mean of day 0 + day 7, respectively; n.s.: non significant; ***: $p \leq 0.001$; **: $p \leq 0.01$; *: $p \leq 0.05$.



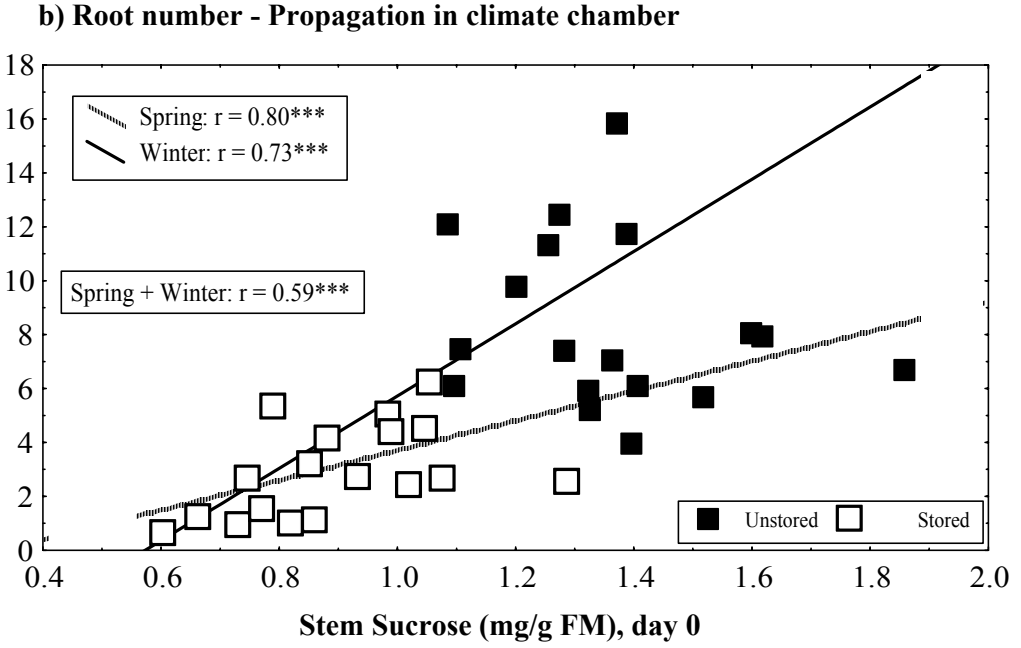


Fig. 18: Linear regressions between sucrose concentrations at day 0 in leaves (a) or basal stems (b) of the cuttings and number of subsequently formed roots by unstored and stored cuttings of 'Isabell' propagated in climate chamber during spring, summer and winter (a) or spring and winter (b) seasons. *n*=9 per season and storage treatment.

between basal stem carbohydrates and number of roots of unstored and stored cuttings, as there was no change in the carbohydrate levels after cold-storage of the cuttings (Fig. 7a-d). Although there was no decrease in basal stem carbohydrates after storage, the decrease in root number for stored cuttings was elucidated by a decrease in leaf carbohydrates. In essence, the regressions found above reveal the dependency of root formation on the pre-rooting carbohydrate situation in different cutting parts (particularly in leaves) when propagated under relatively low light conditions (climate chamber) irrespective of the season of propagation. However, the contrariety in the steepness of the slope over same broad range of sucrose concentrations with regard to root formation (Fig. 18a, b) among different seasons, mainly caused by unstored cuttings in winter, discloses that the dependency was only partial, and acknowledges further influences beyond pre-rooting carbohydrates.

In contrast, when propagated in greenhouse where complex environmental conditions were involved, no correlations were found between pre-rooting carbohydrates and subsequent root formation, except in winter (Table 9). In winter propagation, all pre-rooting carbohydrate concentrations in different cutting parts (except basal stem fructose and starch) were positively correlated with the root number of unstored and stored cuttings.

When all the treatments were put together, i.e. unstored and stored cuttings propagated in greenhouse and climate chamber, no significant regressions were calculated in spring and summer, except two low regression coefficients with basal stem sucrose and starch in spring (Table 10). Alternatively, in winter, except with basal stem starch, highly significant correlations were calculated between all the pre-rooting carbohydrates in different cutting parts and root number, as presented for leaf sucrose (Fig. 19) (Table 10). However, the higher level of the regression line for climate chamber propagation (as indicated by a higher ‘a’ value of the regression equation, $y = 1.4 + 8.0*x$) when compared to that for greenhouse propagation ($y = -1.0 + 7.5*x$), represents the higher rooting efficiency in climate chamber, although the initial carbohydrate levels were similar for both of the rooting environments at day 0.

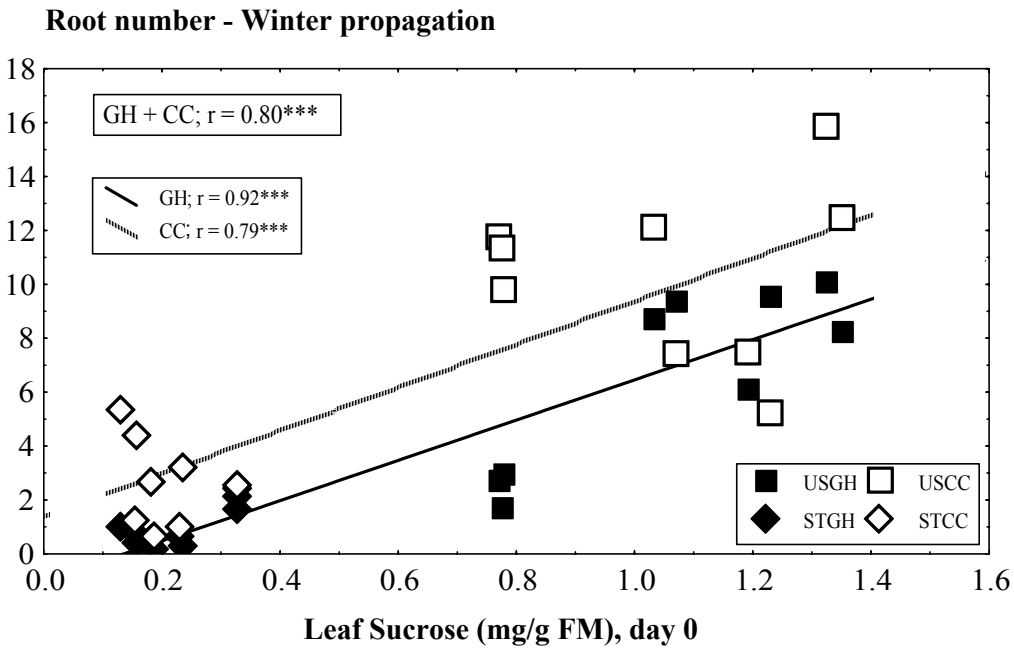


Fig. 19: Linear regression between leaf sucrose concentrations of the cuttings at day 0 and number of subsequently formed roots by unstored (US) and stored (ST) cuttings of 'Isabell' propagated in greenhouse (GH) and climate chamber (CC) during winter season. *n*=9 per storage treatment and rooting environment.

3.2.4.2 Correlation between pre-insertion quenching values and root number – cv. 'Isabell'

Correlations were calculated between quenching coefficients (qP and qN) of unstored and stored cuttings measured at day 0 as independent variables and number of subsequently formed adventitious roots as dependent variables (Table 11). When unstored and stored cuttings rooted in climate chamber (relatively low light) were regarded, root number positively correlated with both qP and qN in spring, summer, and winter seasons. When all the seasons were combined, the correlation between qP and root number became insignificant because of the strong differences in qP values among different seasons for unstored cuttings (Fig. 9a). In contrast, the correlation between qN and root number remained highly significant (Table 11). In all the seasons of propagation, unstored cuttings which had higher qN values when compared to the stored cuttings resulted in a highly significant positive correlation with the number of subsequently formed roots (Fig. 20a). However, regressions slopes over the same broad range of qN values varied strongly among the three seasons of propagation, which depended mainly on rooting response of unstored cuttings. As demonstrated earlier for leaf sucrose concentrations, the steepness of the regression line was higher for winter propagation,

followed by summer and spring, with regard to the number of roots formed (slopes of 24.2, 9.1 and 7.3, respectively). This once again demonstrated the higher rooting capability of unstored cuttings during winter propagation (Fig. 20a). Although the degree of relationship between qN and root number changed among the different seasons of propagation, the principal relationship was found to be the same for all the seasons of propagation, indicating the dependency of root formation on pre-rooting qN. However, the hierarchy in the steepness of the slopes among different seasons, caused mainly by unstored cuttings in winter season, reveals the involvement of factors over pre-rooting status.

In contrast, when propagated under nature defined environmental conditions, i.e. greenhouse, no such relationships were found in spring and summer, but in winter qN was linearly positively correlated with the root number (Table 11). When all the treatments were put together, i.e. unstored and stored cuttings propagated in greenhouse and climate chamber, no significant correlations were found in spring and summer, except a low level correlation in summer propagation with qP (Table 11). In winter, however, both qN and qP (day 0) values of the cuttings were positively correlated with number of subsequently formed roots, as illustrated for qN in Fig. 20b. As demonstrated earlier with pre-rooting leaf sucrose, the regression line was at a higher level for climate chamber propagation (as indicated by higher 'a' value of the regression equation, $y = 1.1 + 24.2 *x$) when compared to that for greenhouse propagation ($y = 0.07 + 16.6 * x$), although the initial qN values of cuttings were similar in both of the rooting environments at day 0. This reveals that both unstored cuttings and stored cuttings propagated in climate chamber exhibited higher rooting capability than those in greenhouse during winter season.

Intercorrelations were calculated between pre-rooting quenching coefficients (qP and qN) of unstored and stored cuttings as independent variables and leaf carbohydrate concentrations of corresponding cuttings as dependent variables (Table 2A in Appendix). Both qP and qN positively correlated with leaf carbohydrate levels. Among those, the best correlations were found between qN and leaf sucrose, starch, total sugars and total non-structural carbohydrates (Table 2A in Appendix).

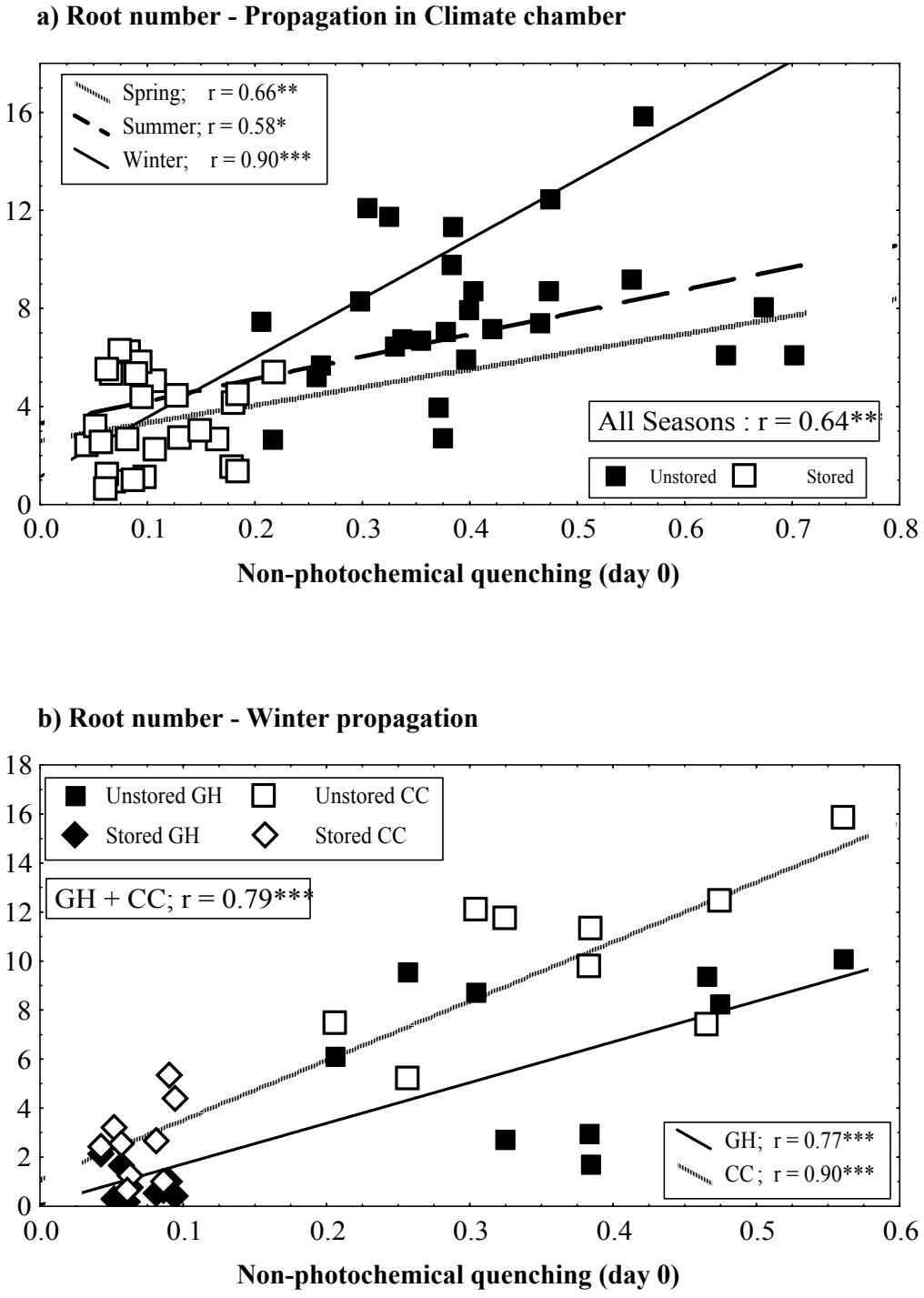


Fig. 20: Linear regressions between non-photochemical quenching of chlorophyll fluorescence of the cuttings at day 0 and number of subsequently formed roots by unstored and stored cuttings of 'Isabell' propagated in climate chamber during spring, summer and winter seasons (a) or in both greenhouse (GH) and climate chamber (CC) during winter season (b). *n*=9 per season, storage treatment and rooting environment.

Table 11: Correlation coefficients, calculated between chlorophyll fluorescence values of unstored and/or stored cuttings of 'Isabell' propagated in greenhouse and/or climate chamber during spring, summer and winter seasons as independent variables and number of subsequently formed adventitious roots as dependent variables

Season	C.F. parameters	Green-house[a]	Climate chamber[a]	Unstored cuttings[b]	Stored cuttings[b]	All treatments[c]	
		Day 0[1]	Day 0[1]	Mean[2]	Mean[2]	Day 0[1]	Mean[2]
Spring (*n*=18)	qP	n.s.	0.53*	n.s.	0.74***	n.s.	0.63***
	qN	n.s.	0.66**	n.s.	0.63**	n.s.	0.49**
Summer (*n*=18)	qP	n.s.	0.67***	0.64**	n.s.	0.35*	0.55***
	qN	n.s.	0.58*	0.79***	0.64**	n.s.	0.76***
Winter (*n*=18)	qP	n.s.	0.59*	n.s.	n.s.	0.44**	n.s.
	qN	0.77***	0.90***	n.s.	n.s.	0.79***	n.s.
Seasons (*n*=54)	qP	n.s.	n.s.	0.32*	n.s.	0.27**	0.28**
	qN	0.29*	0.64***	0.34*	n.s.	0.46***	0.37***

qP: Photochemical quenching; qN: Non-photochemical quenching; [1]: measured at day 0; [2]: mean of values measured during the course of propagation (day 0, 1, 4, 7 and 14); [a]: includes unstored and stored cuttings; [b]: includes propagation in greenhouse and climate chamber; [c]: includes unstored and stored cuttings propagated in greenhouse and climate chamber; n.s.: non significant; ***: $p \leq 0.001$; **: $p \leq 0.01$; *: $p \leq 0.05$.

3.2.5 Relationship between pre-insertion condition, performance during propagation and subsequent root formation

3.2.5.1 Correlation between quenching values and root number – cv. 'Isabell'

Correlations were calculated between mean quenching coefficients [mean calculated over whole propagation period (mean qP and mean qN)] as independent variables and the number of subsequently formed adventitious roots as dependent variables (Table 11). With reference to unstored cuttings rooted in greenhouse and climate chamber, only in summer the root number was positively correlated to mean qP and mean qN, where the number of roots formed was significantly higher in greenhouse when compared to climate chamber. Alternatively, in spring and winter, no correlations were found (Table 11). This missing relationship can be attributed to the lack of difference between the two rooting environments for the number of roots formed in spring (Fig. 17a) and for the chlorophyll fluorescence parameters in winter (Fig. 10a, b). In case of stored cuttings propagated in both rooting environments, root number positively correlated with mean qP in spring, and mean qN in spring and summer, but in winter no correlations were found (Table 11). When all the treatments were combined, i.e. unstored and stored cuttings propagated in both rooting environments, mean qP and qN in spring and summer were significantly positively correlated with number of subsequently formed roots. In winter propagation, however, the root number which was correlated with day 0 quenching values did not show any further correlation with mean values (Table 11). The highly significant positive correlation between root number and mean qP and especially with mean qN (Fig. 21), clearly reflects the dependency of root formation on cuttings performance during the whole course of propagation. Thus, the large variation in root formation between unstored and stored cuttings (that depended on rooting environment) during spring and summer, which could not be correlated to pre-insertion qP and qN (functional status of photosynthetic apparatus) or pre-insertion carbohydrate reserves of the cutting, could be partially related to mean qP and mean qN (Fig. 21) values. In addition, the homogenous distribution of root number formed by stored cuttings in greenhouse along the regression line documents that the reduced activity of PS II of stored cuttings at insertion or rooting-inhibitory low initial leaf carbohydrate levels in those cuttings did not impede the subsequent rooting when higher qN values were maintained during propagation (Fig. 21).

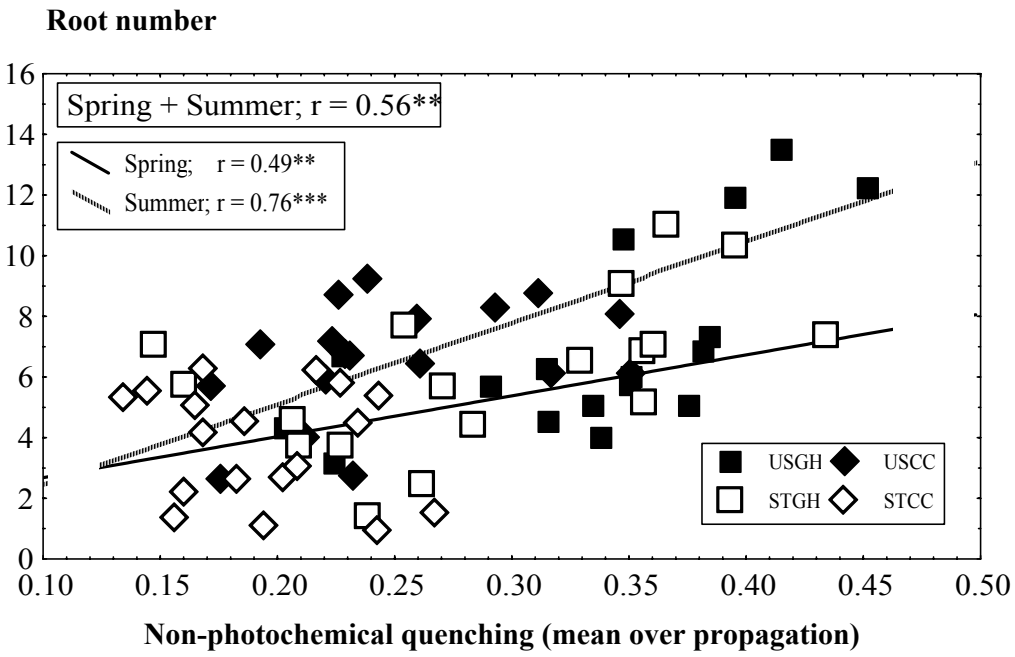


Fig. 21: Linear regression between non-photochemical quenching of chlorophyll fluorescence (mean over propagation period) of the cuttings and number of subsequently formed roots by unstored (US) and stored (ST) cuttings of 'Isabell' propagated in greenhouse (GH) and climate chamber (CC) during spring and summer seasons. *n*=9 per season, storage treatment and rooting environment.

3.2.5.2 Correlation between carbohydrates and root number – cv. 'Isabell'

Correlation coefficients were calculated between the mean of pre-rooting carbohydrate situation and the performance during propagation (mean of day 0 + day 7) as independent variables and the number of subsequently formed adventitious roots as dependent variables (Table 10 and 12).

With regard to unstored cuttings propagated in greenhouse and climate chamber, root number was positively correlated with leaf mean (day 0 + 7) sucrose, mean total sugars, and mean total non-structural carbohydrates in summer and winter (Table 12). The positive correlations in summer were a function of the better performance of the cuttings in greenhouse when compared to that in climate chamber, whereas in winter the correlations were a function of the better performance of the cuttings in climate chamber when compared to that in greenhouse. Correlations were not calculated for spring propagation, as there were no significant differences between rooting environments for the number of roots formed (Fig.

17a). For unstored cuttings, irrespective of season of propagation, no correlations were found between basal stem mean carbohydrate concentrations and root number (Table 12).

With regard to stored cuttings, highly significant positive correlations were found between all mean carbohydrate concentrations in different cutting parts and root number in summer and winter propagation, except with mean reducing sugars in basal stems in summer, and mean sucrose and mean starch in basal stems in winter (Table 12). In spring, the correlations were not significant, except for a significant positive correlation with basal stem mean sucrose and a negative correlation with leaf mean fructose (Table 12). When regarded over all the seasons of propagation for stored cuttings, all mean carbohydrates in different cutting parts were positively correlated with adventitious root number, except leaf mean fructose. Among all the carbohydrates in different cutting parts, basal stem mean sucrose concentrations were best correlated with the number of roots formed by the stored cuttings (Table 12, Fig. 22a). This emphasizes the importance of pre-insertion reserves, as well as the accumulation of carbohydrates in the basal stems during propagation, especially for adventitious root formation of stored cuttings.

When all the treatments were combined, no significant regressions were calculated in spring propagation, excepting a positive correlation between basal stem mean sucrose concentrations and root number. Alternatively, in summer and winter, significant positive correlations were found between almost all of the mean carbohydrates in different cutting parts and root number (Table 10). However, when all of the treatments in all three seasons were considered, relatively low but significant, positive correlations were calculated between all mean carbohydrates in different cutting parts and root number (Table 10). The whole range of variation in the adventitious root formation (from zero – 16 roots), caused by three seasons of stock plant cultivation, three harvest dates under each season, cold-storage under variable temperature regime, rooting under two contrasting environments, and three replicates, could be explained using a characteristic – ‘leaf mean sucrose concentrations’ (Fig. 22b). This reflects the importance of both pre-rooting carbohydrate status as well as photosynthetic performance during the course of propagation.

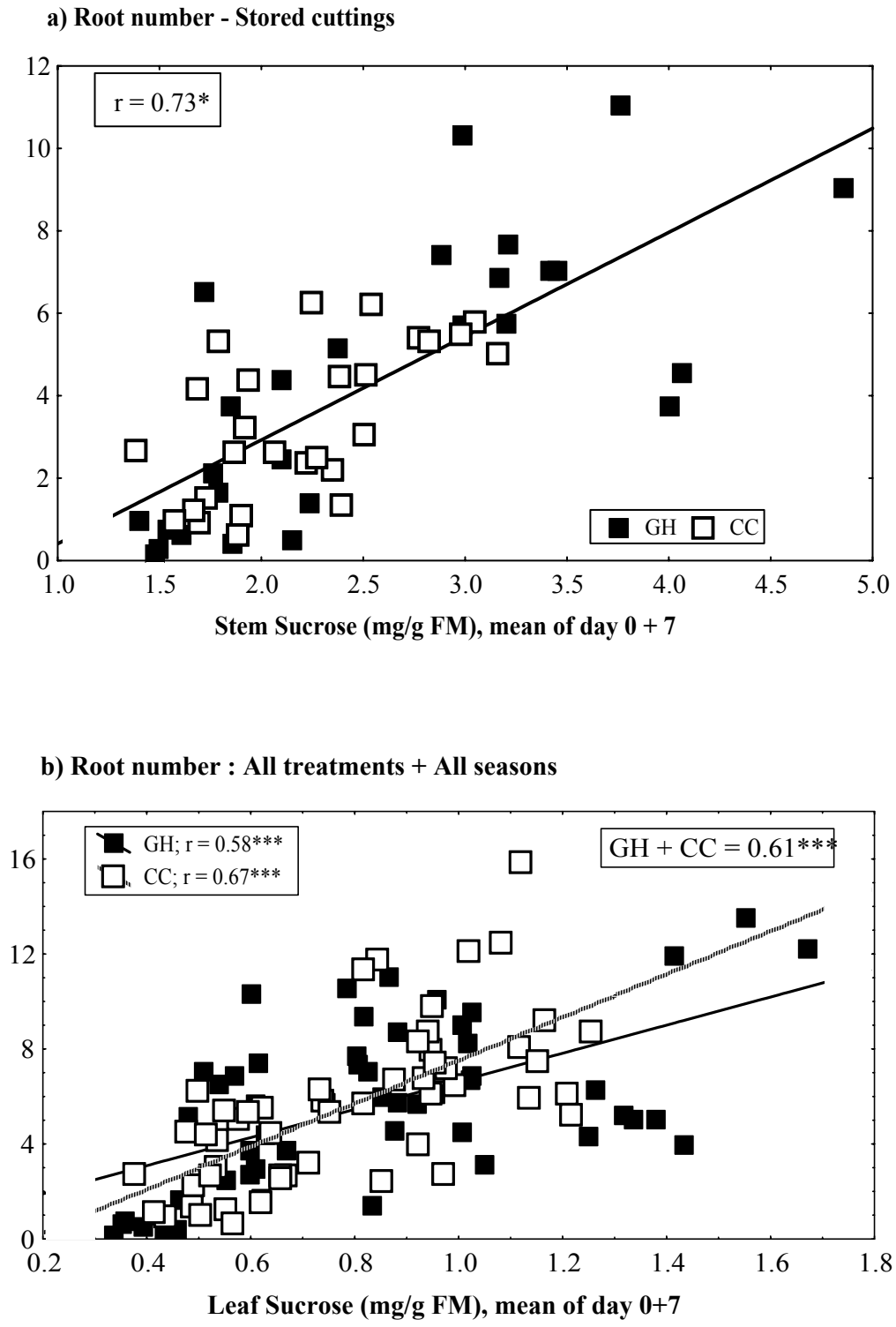


Fig. 22: Linear regressions between sucrose concentrations (mean of day 0 + 7) in basal stems (a) or leaves (b) of the cuttings and number of subsequently formed roots by stored cuttings (a, *n*=54) or unstored and stored cuttings (b, *n*=108) of 'Isabell' propagated in greenhouse (GH) and climate chamber (CC) during spring, summer and winter seasons.

Table 12: Correlation coefficients, calculated between mean carbohydrate concentrations (mean of day 0 + day 7) in different parts of 'Isabell' cuttings propagated during spring, summer and winter seasons as independent variables and number of subsequently formed adventitious roots as dependent variables. Pooled data of unstored or stored cuttings propagated in greenhouse and climate chamber

Carbohydrate concentrations	Tissue	Unstored cuttings (Mean of day 0 + day 7)				Stored cuttings (Mean of day 0 + day 7)			
		Spring (*n*=18)	Summer (*n*=18)	Winter (*n*=18)	All seasons (*n*=54)	Spring (*n*=18)	Summer (*n*=18)	Winter (*n*=18)	All seasons (*n*=54)
Glucose	Leaf	-	n.s.	n.s	n.s	n.s	n.s	0.69**	0.33*
	BS	-	n.s.	n.s	n.s	n.s	n.s	0.76***	0.35**
Fructose	Leaf	-	n.s.	n.s	n.s	-0.58*	0.66**	0.55*	n.s
	BS	-	n.s.	n.s	n.s	n.s	n.s	0.50*	0.34*
RS	Leaf	-	n.s.	n.s	n.s	-0.49*	0.51*	0.67**	n.s
	BS	-	n.s.	n.s	n.s	n.s	n.s	0.65**	0.37**
Sucrose	Leaf	-	0.64**	0.54*	0.29*	n.s	0.71***	0.56*	0.58***
	BS	-	n.s.	n.s	n.s	0.67**	0.56*	n.s	0.73***
TS	Leaf	-	0.51*	0.55*	n.s	n.s	0.69**	0.61**	0.44***
	BS	-	n.s.	n.s	n.s	n.s	0.48*	0.61**	0.55***
Starch	Leaf	-	n.s.	0.49*	n.s	n.s	n.s	0.50*	0.48***
	BS	-	n.s.	n.s	n.s	n.s	0.52*	n.s	0.52***
TNC	Leaf	-	0.49*	0.55*	n.s	n.s	0.55*	0.61**	0.49***
	BS	-	n.s.	n.s	n.s	n.s	0.59**	0.58*	0.61***

RS (Reducing sugars): Glucose + Fructose; TS (Total sugars): RS + Sucrose, TNC (Total non-structural carbohydrates): TS + Starch; BS: Basal stem; n.s.: non significant; ***: $p \leq 0.001$; **: $p \leq 0.01$; *: $p \leq 0.05$.

3.3 Effect of storage temperature regimes on rooting of 'Isabell' cuttings under low light conditions (climate chamber)

Because the shipment of cuttings and rooting under low light conditions are inevitable for pelargonium propagation in Central Europe, and pre-insertion sugar levels were revealed to be important for the subsequent adventitious root formation of the cuttings under those relatively low light conditions, the influence of two temperature regimes on carbohydrate depletion during simulated storage transport were investigated.

3.3.1 Carbohydrates during storage – cv. 'Isabell'

Carbohydrate levels in different cutting parts as influenced by constant and variable storage temperature regimes were determined at every 24 hours during storage. Both of the storage temperature regimes resulted in a decrease in all the carbohydrates in the leaves as well as in the basal stems (Table 13).

Table 13: Effect of storage temperature regimes on leaf and basal stem carbohydrate concentrations of 'Isabell' cuttings. Mean of three harvest dates

Leaf carbohydrates	Tissue	Concentration (mg/g FM)		
		Unstored [1]	Constant [2] regime	Variable [2] regime
Glucose	Leaf	0.688 a	0.325 b	0.301 b
	BS	3.943 a	2.646 b	2.097 b
Fructose	Leaf	0.595 a	0.166 b	0.144 b
	BS	0.364 a	0.404 a	0.397 a
Reducing sugars (RS)	Leaf	1.284 a	0.492 b	0.445 b
	BS	4.307 a	3.050 b	2.494 b
Sucrose	Leaf	1.303 a	0.323 b	0.277 b
	BS	1.463 a	0.982 b	0.926 b
Total sugars (TS)	Leaf	2.586 a	0.815 b	0.723 c
	BS	5.770 a	4.031 b	3.420 b
Starch	Leaf	2.060 a	0.024 b	0.030 b
	BS	0.635 a	0.187 a	0.190 a
Total non-structural carbohydrates (TNC)	Leaf	4.647 a	0.839 b	0.752 b
	BS	6.404 a	4.218 b	3.610 b

RS: Glucose + Fructose; TS: RS + Sucrose; TNC: TS + Starch; Different subscripts indicate significant differences ($p \leq 0.05$) between different storage treatments, for specific carbohydrate in a specified tissue; BS: Basal stem; [1]: at harvest; [2]: after 4 days of storage period.

Interestingly, the concentrations of different individual carbohydrate fractions in the leaves and basal stems found at the final sampling date were similar under both storage temperature regimes (Table 13). Leaf carbohydrates were strongly decreased when compared to basal stem carbohydrates under both the storage temperature regimes.

With regard to leaf carbohydrates, during the whole storage period, glucose and fructose levels were similarly influenced and there were no significant differences between the two storage regimes in the pattern of decline at different sampling dates, as presented for glucose (Fig. 23a). During 1 day of storage, leaf sucrose was found to be relatively less decreased under variable temperature regime when compared to constant temperature regime, but by the next sampling date there were no differences (Fig. 23b). This effect, evident on day 1, was due to the fact that initial temperatures under the variable temperature regime were low (5°C) when compared to those in constant temperature regime (9°C). Initial leaf sucrose concentrations were higher than reducing sugar (glucose + fructose) concentrations, but at the final sampling date the concentrations were even lower than reducing sugars (Table 13). Leaf total sugars exhibited a pattern similar to that of leaf sucrose, but the concentrations at the final sampling date were found to be slightly higher in the constant storage temperature regime (Table 13). Leaf starch concentrations varied strongly between the harvest dates, but irrespective of the initial level, starch was reduced to trace amounts by day 1 during storage under both the temperature regimes (Fig. 23c). Leaf total non-structural carbohydrates which were strongly influenced by starch levels, exhibited a strong decrease by day 1 during storage under both the temperature regimes.

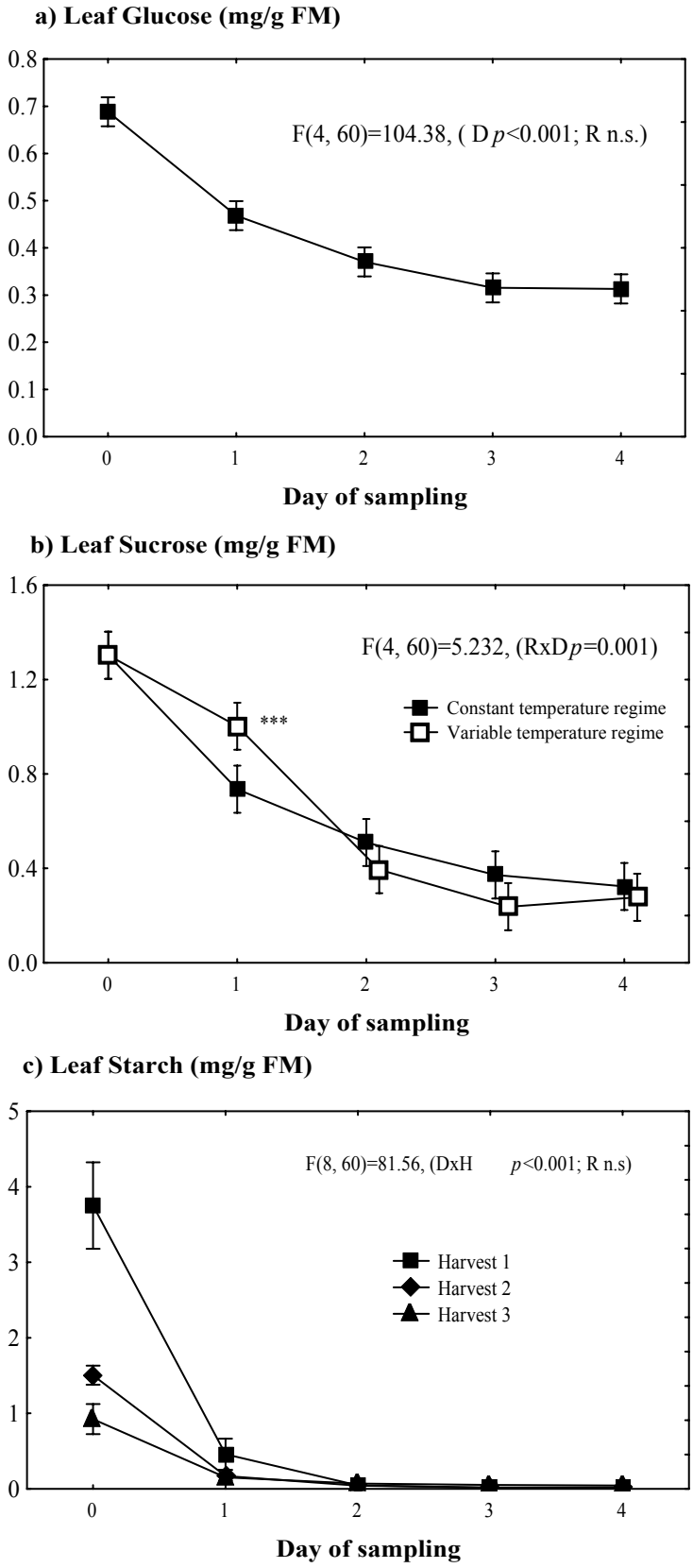


Fig. 23: Effect of storage temperature regimes (R) and day of sampling during storage (D) on concentrations of glucose (a), sucrose (b) and starch (c) in leaves of 'Isabell' cuttings. Mean over three harvest dates or per each harvest date in case of significant interactions. Asterisks indicate significant differences between storage temperature regimes at specified day during storage at (***) $p<0.001$ level. *Vertical lines*, 0.95 confidence interval of least square mean values.

At harvest, basal stems of the cuttings were found to have higher glucose levels, but lower levels of fructose and starch when compared to those of leaves. Alternatively, sucrose concentrations were similar in both tissues (Table 13). Storage temperature regimes had no influence on basal stem glucose concentrations. The pattern of decrease of basal stem glucose during storage was generally similar to that of the leaf glucose but the magnitude of decrease was less (Fig. 24a). Unlike the influence experienced by leaf fructose and starch concentrations, basal stem fructose and starch concentrations, which were low at the time of harvest, were not influenced during the storage period and remained at the same levels or slightly decreased (Table 13). Similar to the pattern exhibited by leaf sucrose, stem sucrose was also relatively less decreased by day 1 during storage under variable temperature regime, but this difference was not persistent until the next sampling date (Fig. 24b). Basal stem sucrose concentrations were less decreased when compared to leaf sucrose concentrations and were found to be at a higher level at the final sampling date.

In essence, stem carbohydrates exhibited a slower and less pronounced decrease when compared to leaf carbohydrates under both storage regimes.

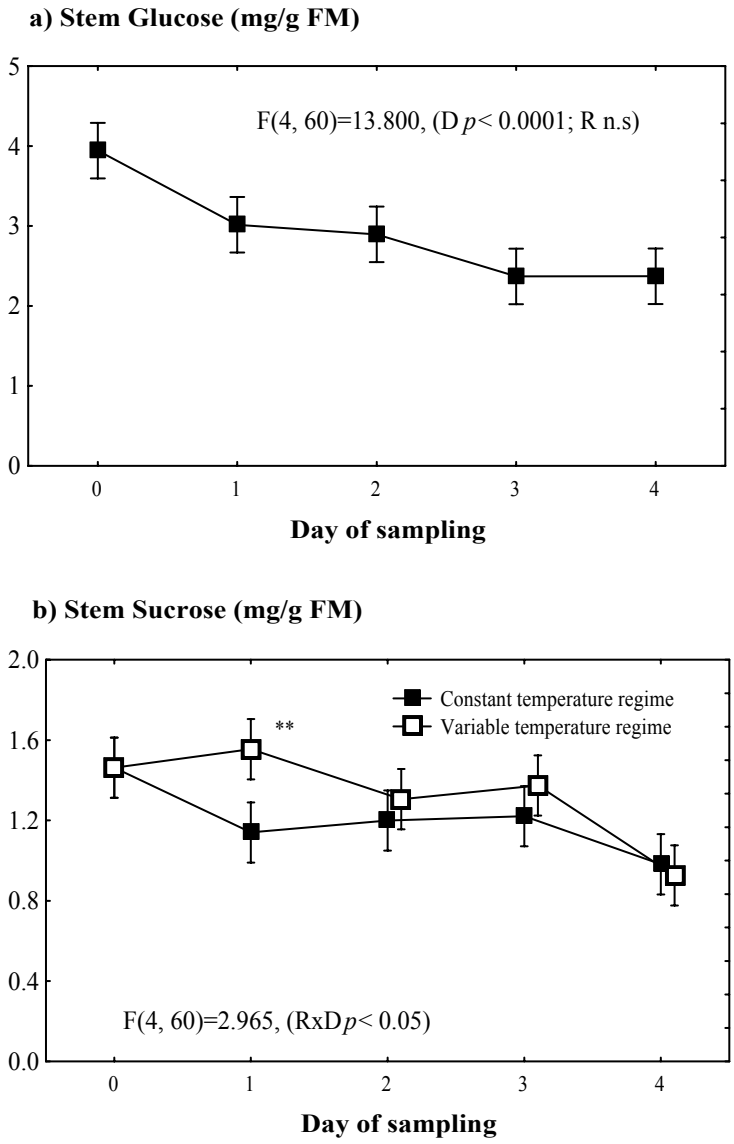


Fig. 24: Effect of storage temperature regimes (R) and day of sampling during storage (D) on concentrations of glucose (a) and sucrose (b) in basal stems of 'Isabell' cuttings. Mean of three harvest dates. Asterisks indicate significant differences between storage temperature regimes at specified day during storage at (**) p<0.01 level. *Vertical lines*, 0.95 confidence interval of least square mean values.

3.3.2 Regression between storage temperatures and carbohydrate depletion – cv. 'Isabell'

Regressions were calculated with sum of day mean storage temperatures (°C) during storage as independent variables and carbohydrate concentrations in different parts as dependent variables (Table 14). Leaf carbohydrates were decreased in a generally exponential fashion, as presented for total sugars and total non-structural carbohydrates in Fig. 25a, b. Leaf total non-structural carbohydrates, which were strongly influenced by leaf starch levels (Fig. 23c), experienced a substantial decrease initially (Fig. 25b). Similar, but relatively weak regressions were calculated with sum of day means storage temperatures (°C) during storage and stem carbohydrates concentrations (Table 14). The relatively weak regressions were an outcome of a slower and less pronounced decrease of basal stem carbohydrates.

Table 14: Regressions (y=a*exp(-b*x)+c), calculated between sum of day mean storage temperatures as independent variables and carbohydrate concentration in leaf and basal stem of 'Isabell' cuttings as dependent variables

Carbohydrate concentration	Tissue	Sum of day mean storage temperatures (n= 30)
Glucose	Leaf	0.93*
	BS	0.62*
Fructose	Leaf	0.90*
	BS	n.s.
Reducing sugars (RS)	Leaf	0.92*
	BS	0.62*
Sucrose	Leaf	0.92*
	BS	0.58*
Total sugars (TS)	Leaf	0.93*
	BS	0.65*
Starch	Leaf	0.82*
	BS	0.64*
Total non-structural carbohydrates (TNC)	Leaf	0.90*
	BS	0.64*

RS: Glucose + Fructose; TS: RS + Sucrose; TNC: TS + Starch; n.s.: not significant; *: significant; BS: Basal stem.

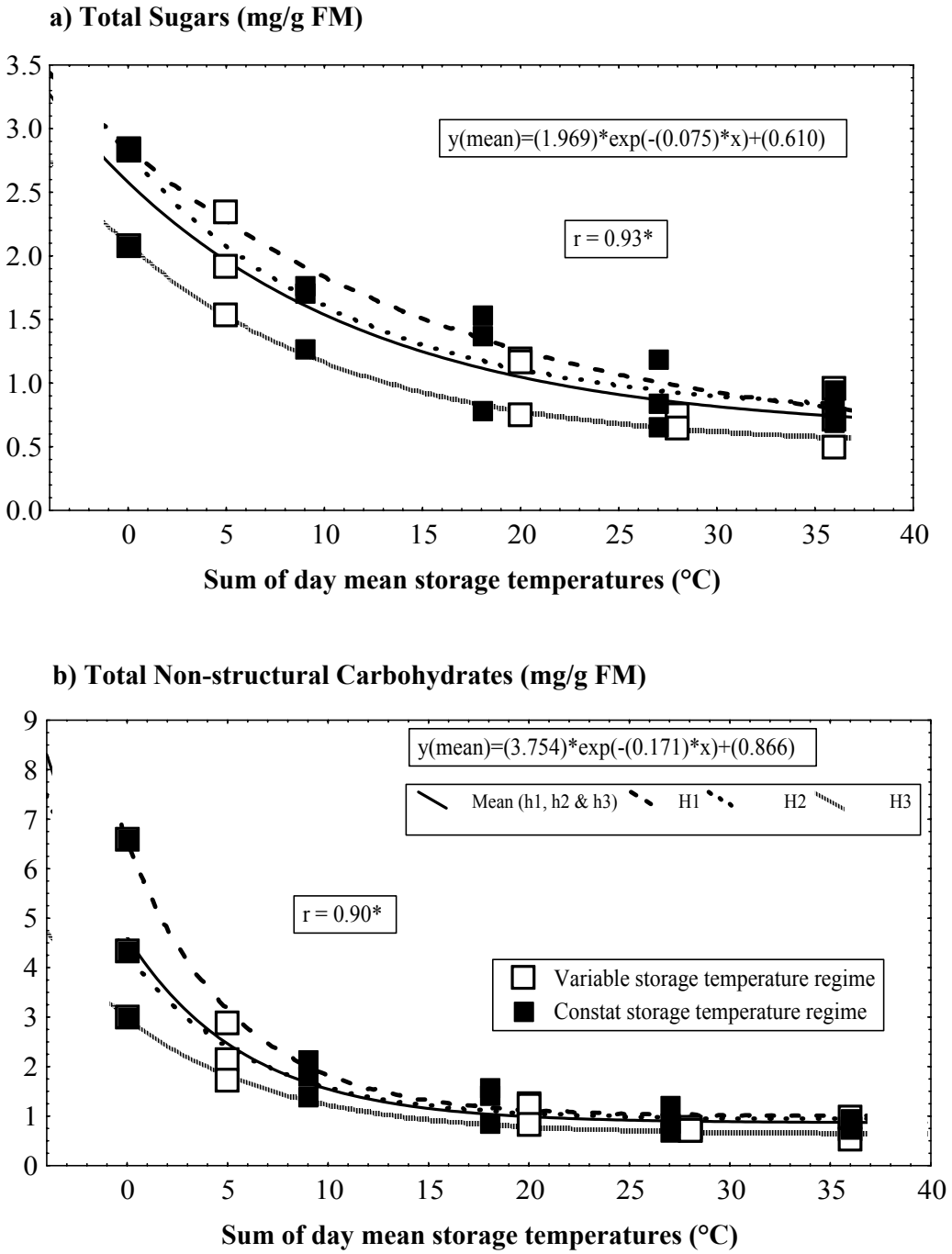


Fig. 25: Depletion of total sugars, TS (a) and total non-structural carbohydrates, TNC (b) in leaves of 'Isabell' cuttings as a function of sum of day mean storage temperatures during the storage. Each value for TS or TNC is a mean per harvest date. *n*=15 per storage temperature regime; Values on X - axis at 0 represent carbohydrate concentrations at harvest.

3.3.3 Chlorophyll fluorescence values – cv. 'Isabell'

Photochemical quenching (qP) and non-photochemical quenching (qN) of chlorophyll fluorescence of the cuttings were measured at harvest and after retrieval from cold-storage under constant and variable temperature regimes. qP and qN were significantly reduced after storage under both temperature regimes, and there were no significant differences between the two temperature regimes (Fig. 26).

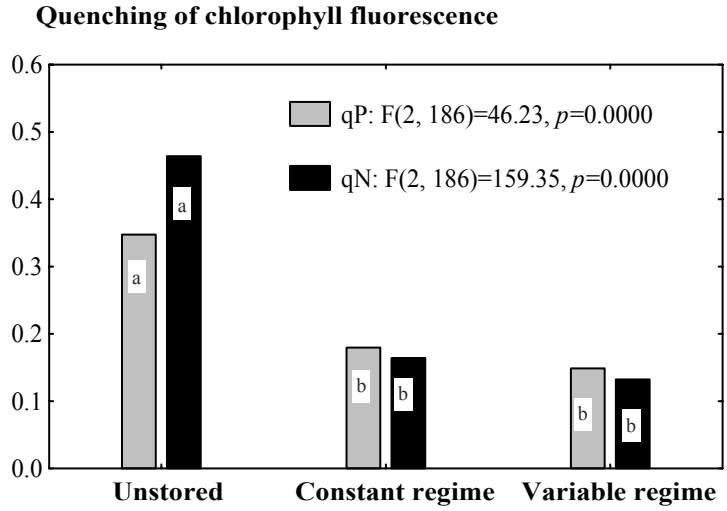


Fig. 26: Effect of constant and variable storage temperature regimes on photochemical quenching (qP) and non-photochemical quenching (qN) of chlorophyll fluorescence of 'Isabell' cuttings at insertion (day 0). Mean of three harvest dates. Different subscripts indicate significant differences.

3.3.4 Rooting efficiency – cv. 'Isabell'

After 21 days of propagation under relatively low light conditions (climate chamber), unstored cuttings formed higher number of adventitious roots when compared to cuttings stored under both the temperature regimes. There were no significant differences between cuttings stored under constant temperature regime and those stored under variable temperature regime for the number of subsequently formed roots (Fig. 27).

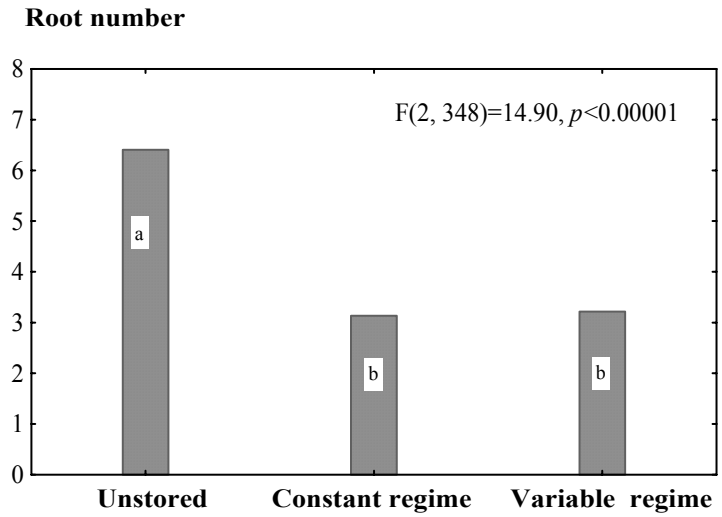


Fig. 27: Adventitious root formation of 'Isabell' cuttings as affected by constant and variable storage temperature regimes, propagated under low light conditions during spring season. Mean of three harvest dates. Root number: Visible roots outside the propagator cube. Different subscripts indicate significant differences.

3.4 Effect of stock plant age and storage on carbohydrate distribution, photosynthetic performance and root formation of 'Mitzou' cuttings under climate chamber conditions

During the later part of the study, 'Mitzou' was included to determine how the pre-insertion carbohydrate reserves and current photosynthesis affect root formation in this postharvest sensitive cultivar. The influence of pre-rooting carbohydrate composition in different parts of the cuttings, when affected by stock plant age and cold-storage, as well as current photosynthetic performance of those cuttings, when affected by propagation under relatively low light conditions, on the rooting efficiency of the cuttings was investigated.

3.4.1 Carbohydrate status – cv. 'Mitzou'

Analysis of variance summary for pre-rooting carbohydrates in different cutting parts as affected by stock plant age and cold-storage in 'Mitzou' cuttings are presented in Table 3A in Appendix. Pre-insertion leaf carbohydrates were less influenced by harvest date, but strongly influenced by cold-storage, whereas stem carbohydrates were either not affected or less affected by harvest date and cold-storage (Table 3A in Appendix).

Leaf glucose and fructose concentrations were similar at harvest, except at third harvest, where fructose concentrations were relatively higher. Leaf glucose moderately and fructose substantially decreased after cold-storage (data not shown). Reducing sugars in leaves were high at third harvest, but cold-storage eliminated the differences among harvest dates (Fig. 28a). Leaf sucrose levels were higher at third harvest when compared to the first two harvests, however, storage strongly reduced the concentrations and eliminated the differences between different harvest dates (Fig. 28b). Starch levels exhibited the strongest variation among harvest dates, with the lowest at first and the highest at third harvest. Cold-storage reduced starch to trace amounts at all the occasions (Fig. 28c). With regard to carbohydrates in basal stem, reducing sugar concentrations were influenced by cold-storage and not dependent on harvest date, whereas sucrose and starch concentrations were more influenced by harvest date and less or not influenced by storage (Table 3A in Appendix). Cold-storage slightly reduced basal stem glucose and fructose levels (Fig. 29a). Basal stem sucrose concentrations, which were relatively more influenced by harvest date than by cold-storage (decreased from 3.11 mg/g FM to 2.49 mg/g FM; $p<0.05$), were significantly high at second harvest (Fig. 29b). Basal stem starch, which was not influenced by cold-storage and strongly influenced by harvest date, was very low at first and second harvest dates and relatively high at third harvest (Fig. 29b).

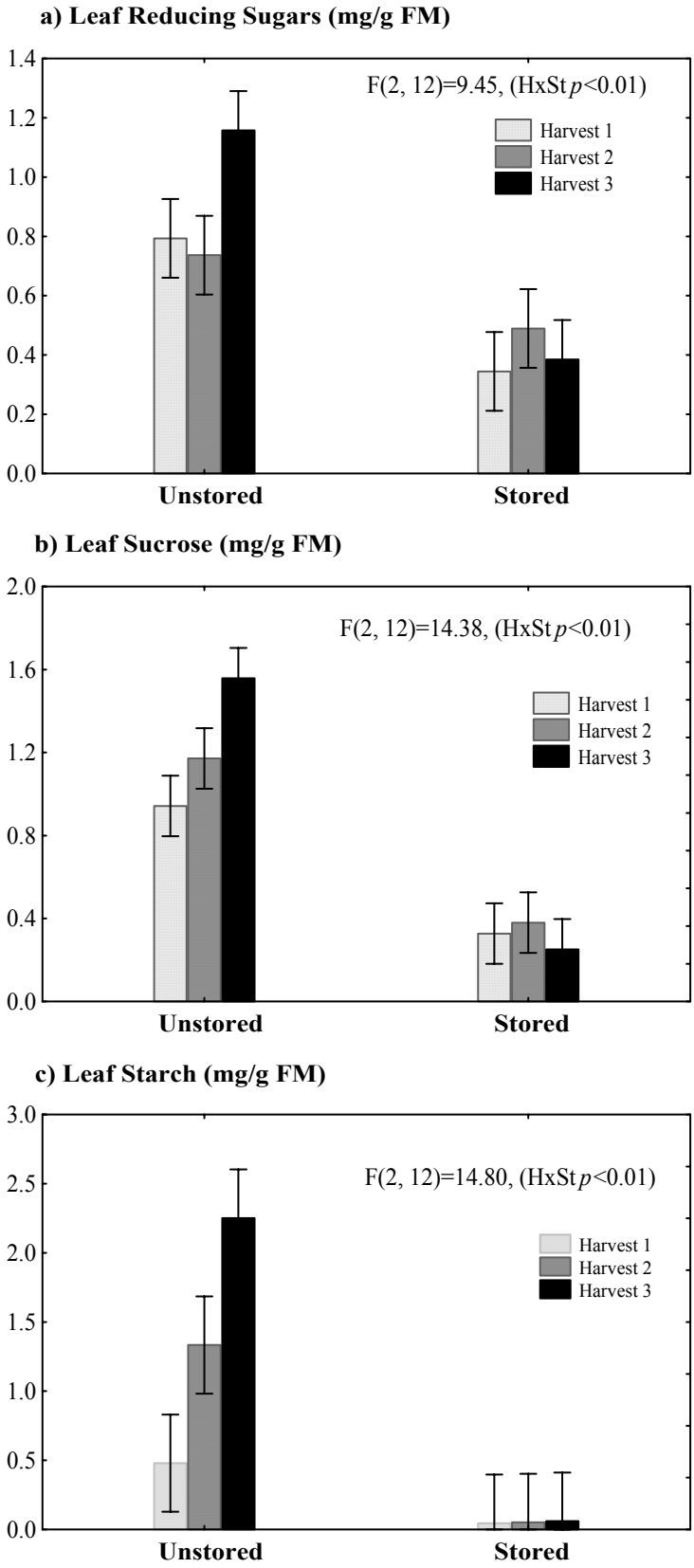


Fig. 28: Effect of harvest date (H) and cold-storage (St) on pre-rooting concentrations of reducing sugars, RS (a), sucrose (b) and starch (c) in the leaves of 'Mitzou' cuttings procured from stock plants cultivated in winter season. *Vertical lines,* 0.95 confidence interval of least square mean values.

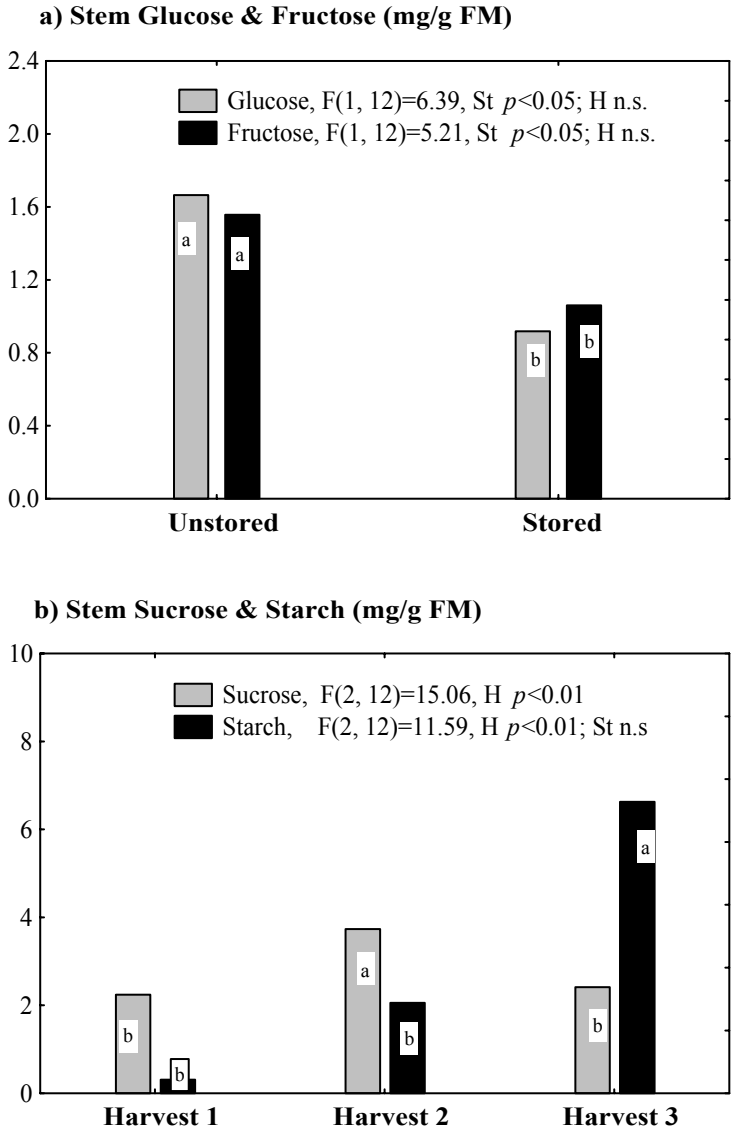


Fig. 29: Effect of cold-storage (a) and harvest date (b) on pre-rooting concentrations of glucose & fructose (a) and sucrose & starch (b) in the basal stems of 'Mitzou' cuttings procured from stock plants cultivated in winter season. Different subscripts indicate significant differences.

With reference to carbohydrate status during the course of propagation of cuttings in climate chamber, at day 7, leaf carbohydrates were influenced by neither cold-storage nor by harvest date, except fructose, where stored cuttings had higher fructose levels when compared to unstored cuttings (Table 4A in Appendix). Stem carbohydrates were influenced by cold-storage and not dependent on harvest date (Table 4A in Appendix). In case of unstored cuttings, reducing sugar concentrations in leaves were strongly decreased during 6 days of

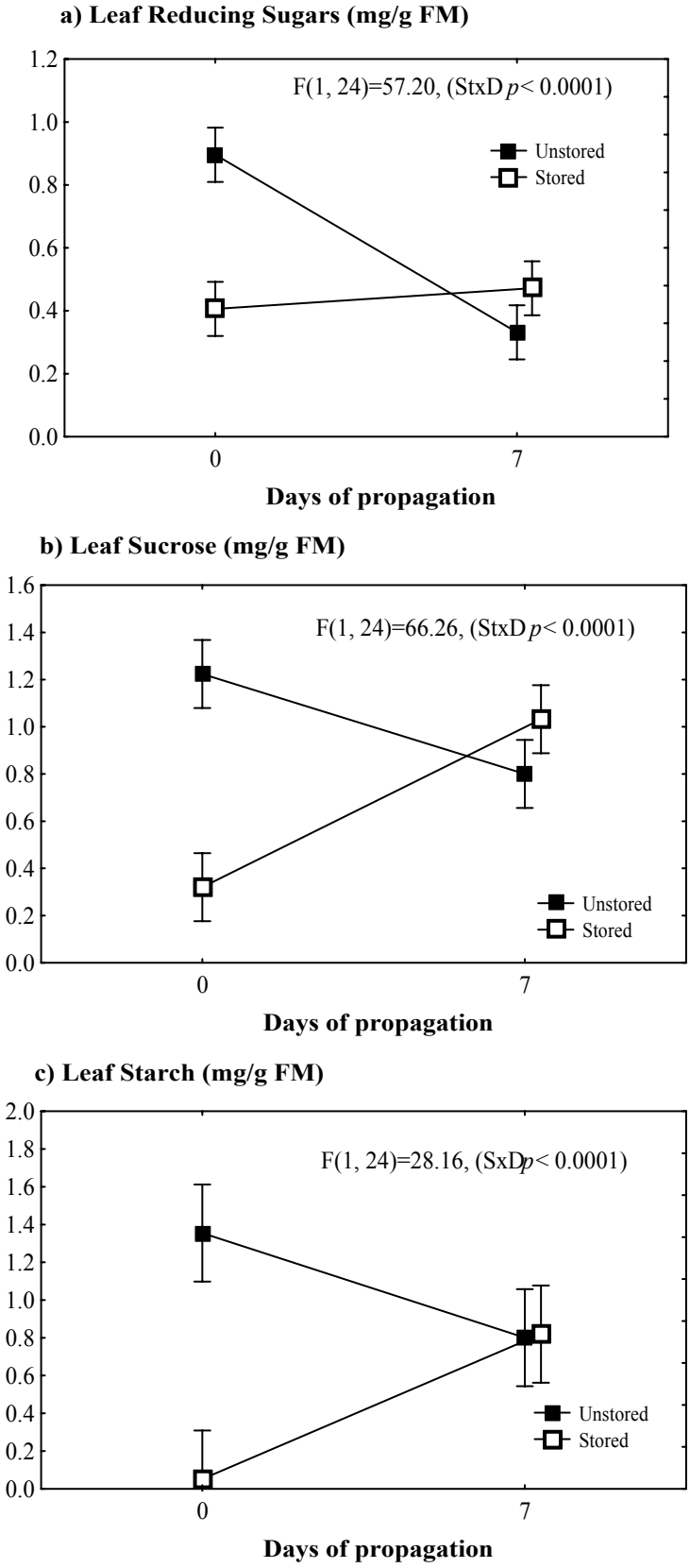


Fig. 30: Effect of cold-storage (St) on concentrations of reducing sugars (a), sucrose (b) and starch (c) in the leaves of 'Mitzou' cuttings during the course of propagation (D) in climate chamber. Mean of three harvest dates. *Vertical lines*, 0.95 confidence interval of least square mean values.

propagation, but in stored cuttings they remained at the same lower levels (Fig. 30a). Leaf sucrose concentrations were slightly reduced (statistically significant) in unstored cuttings but strongly increased in stored cuttings, and at day 7 the levels in stored cuttings were equal to the levels in unstored cuttings (Fig. 30b). Leaf starch was slightly deceased in unstored cuttings, but in stored cuttings, starting from trace amounts the concentrations were significantly increased during 6 days of propagation (Fig. 30c). At day 7 during the course of propagation, all basal stem carbohydrate levels were significantly lower in stored cuttings when compared to those in unstored cuttings, except starch, which was similar in both unstored and stored cuttings (data not shown). Irrespective of storage treatment, all basal stem sugars remained at the same pre-rooting levels during 6 days of propagation (data not shown). Basal stem starch, in contrast, decreased to trace amounts, as at second and third harvests, or remained at substantially lower levels, as at first harvest (Fig. 31). No interactions were found between storage treatment and day of propagation with regard to any of the basal stem carbohydrates (data not shown).

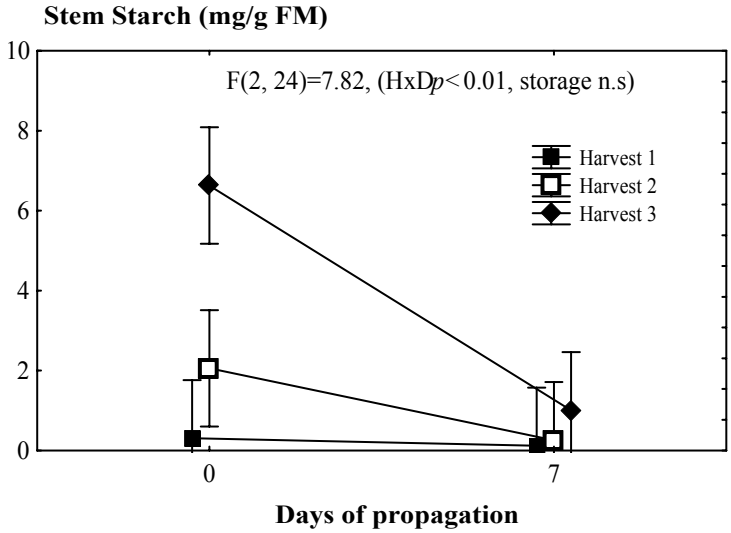


Fig. 31: Interaction between harvest date (h) and days of propagation for starch concentrations in basal stems of ‘Mitzou’ cuttings. Mean of unstored and stored cuttings. *Vertical lines*, 0.95 confidence interval of least square mean values.

3.4.2 Chlorophyll fluorescence values – cv. 'Mitzou'

F_v/F_m at harvest and after cold-storage were 0.676 & 0.690, respectively. Cold-storage of the cuttings resulted in significant diminution photochemical quenching (see Fig. 32a at day 0) and non-photochemical quenching (see Fig. 32b at day 0) of chlorophyll fluorescence. Among different chlorophyll fluorescence parameters, qN was most responsive to storage.

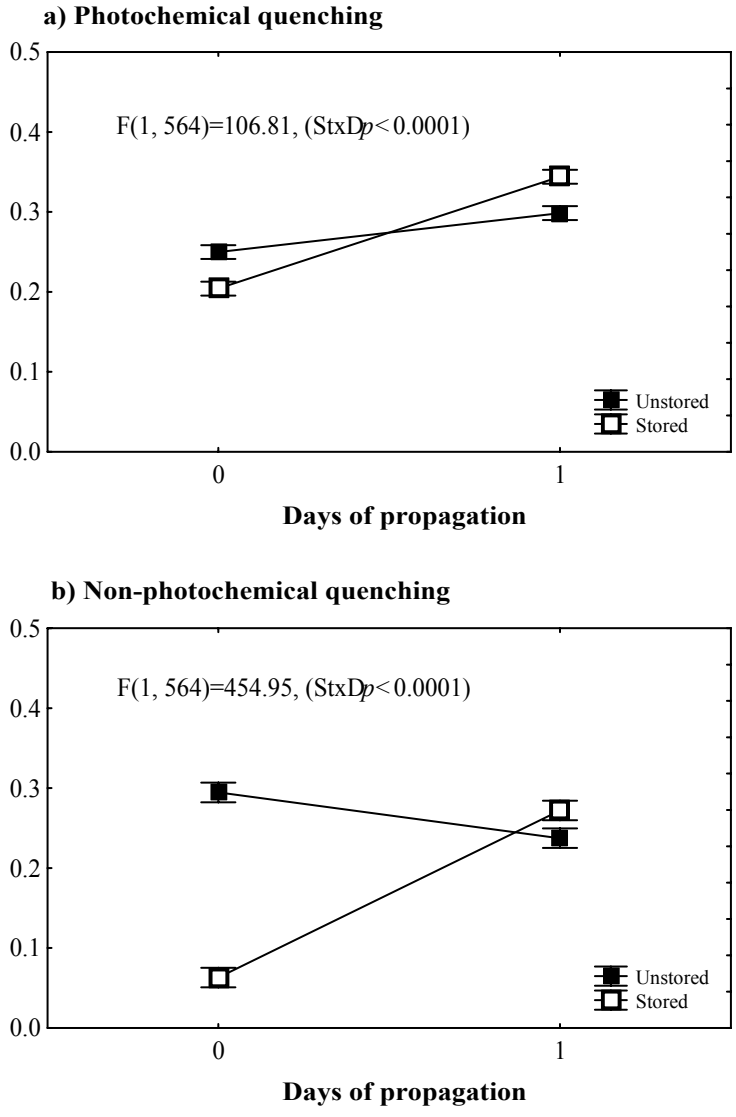


Fig. 32: Effect of cold-storage (St) on photochemical quenching (a) and non-photochemical quenching (b) of chlorophyll fluorescence of 'Mitzou' cuttings during the course of propagation (D) in climate chamber. Mean of three harvest dates. *Vertical lines*, 0.95 confidence interval of least square mean values.

During propagation in climate chamber, F_v/F_m was increased from 0.676 to 0.808 (p<0.001) by day 1 in unstored cuttings, and from 0.690 to 0.789 (p<0.001) in stored cuttings. In unstored cuttings, qP slightly increased (Fig. 32a) and qN slightly decreased (Fig. 32b) by day 1 during the course of propagation. In stored cuttings, both qP (Fig. 32a) and qN (Fig. 32b) strongly increased by day 1 during propagation. Chlorophyll fluorescence was not measured beyond day 1 during the course of propagation, as leaf senescence was visible by day 4.

3.4.3 Chlorophyll content – cv. 'Mitzou'

Cold-storage of the cuttings had no influence on initial (day 0) leaf total chlorophyll content and chlorophyll a : b ratio. Both unstored and stored cuttings were found to have similar chlorophyll content (Table 15). By day 7 during the course of propagation, total chlorophyll content was significantly reduced in stored cuttings when compared to unstored cuttings. Chlorophyll a : b ratio was reduced by day 7 irrespective of the storage treatment (Table 15). Leaf senescence was relatively high at third harvest when compared to other harvest dates, and only in that case did storage significantly increase the number of senesced leaves (Table 16).

Table 15: Chlorophyll content and chlorophyll a : b ratio of leaves during propagation of 'Mitzou' cuttings as affected by storage

Days of Propagation	Chlorophyll content (a+b) (mg/g FM)		Ratio * Chl a : Chl b
	Unstored	Stored	Unstored & stored
0	1.2 ab	1.3 a	3.0 a
7	1.1 b	0.8 c	2.7 b

Mean of three harvest dates; *: storage not significant; Different subscripts indicate significant differences at p=0.001 level

Table 16: Number of senesced leaves after 25 days of propagation of 'Mitzou' cuttings as affected by harvest date and cold-storage

Harvest number	Senesced leaves (No)[1]	
	Unstored	Stored
1	0.8 c	0.9 c
2	1.1 c	1.1 c
3	1.5 b	2.0 a

[1]: Per 17 cuttings; Different subscripts indicate significant differences at $p<0.05$ level.

3.4.4 Rooting efficiency – cv. 'Mitzou'

Root number was determined after 25 days of propagation. Analysis of variance revealed that harvest date had a stronger influence on the rooting efficiency of the cuttings than cold-storage treatment. Regardless of storage treatment, root number was significantly lower at second harvest when compared to first and third harvests (Fig. 33). The influence of cold-storage on rooting efficiency was not consistent at all the harvest dates. Except at first harvest, where unstored cuttings produced higher number of roots than stored cuttings, there were no significant differences between unstored cuttings and stored cuttings (Fig. 33).

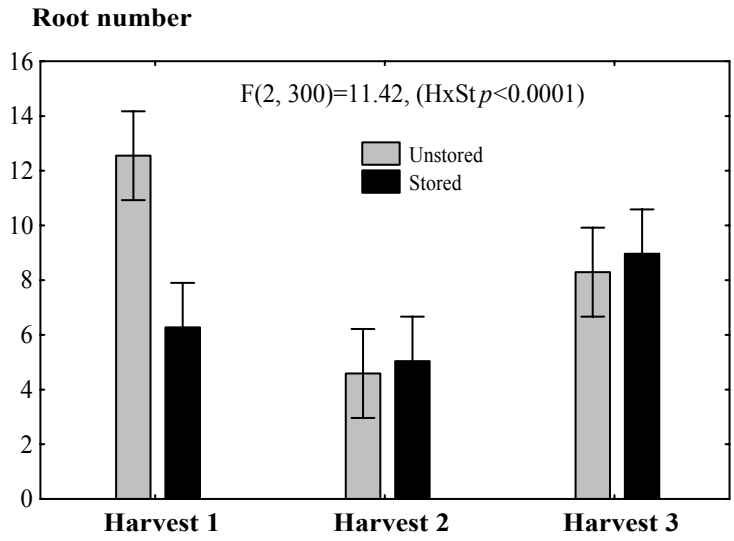


Fig. 33: Adventitious root formation of 'Mitzou' cuttings as affected by harvest date (H) and cold-storage (St), propagated in climate chamber during winter season. Root number: Visible roots outside the propagator cube. *Vertical lines,* 0.95 confidence interval of least square mean values.

3.4.5 Relationship between pre-insertion condition, performance during propagation and subsequent root formation – cv. 'Mitzou'

No significant correlations were found between pre-rooting carbohydrate concentrations (day 0) or mean carbohydrate concentrations (mean of day 0 + 7) in different cutting parts and number of subsequently formed adventitious roots. In addition, no correlations were found between chlorophyll fluorescence parameters and root number.

4 DISCUSSION

4.1 Carbohydrate distribution within the leaves

In unstored cuttings, the relatively higher sucrose and starch levels in leaf 1 (Fig. 4a) and leaf 2 (Fig. 4b) reveal the source status of those leaves, alternatively, the higher monosaccharides in leaf 3 (Fig. 4c), and especially leaf 4 (Fig. 4d), reveal their sink status. Young leaves, which are still importing assimilates (sinks), also synthesize sucrose (Turgeon and Webb, 1972). Nevertheless, low sucrose concentrations are maintained in those leaves, either by respiration or by conversion into the compounds needed for the growth (Taiz and Zeiger, 1998). In dicots, source to sink relationship varies from tip to base within a developing leaf, while tip is the source and base is the sink (Turgeon and Webb, 1972 and 1973). This is possibly the reason behind higher starch levels in the tip region of the leaves (Fig. 4a-c), however, storage eliminated this trend as starch totally disappeared (Fig. 4e-g). In stored cuttings, sucrose levels were higher at the position of petiole attachment in leaf 1 (Fig. 4e) and leaf 2 (Fig. 4f), which could be due to the redistribution of carbohydrates within cuttings during storage.

4.2 Pre-insertion condition of the cuttings as affected by season, harvest and storage

4.2.1 Carbohydrate distribution

The most obvious way through which light affects the internal quality of the cuttings is via direct effects of photosynthesis on assimilate production and consequently on the carbohydrate distribution and content in the cuttings. It is apparent that stock plants grown at higher light intensities do accumulate higher content of carbohydrates (Veierskov, 1988). In the present study with ‘Isabell’, basal stems of the cuttings had significantly higher level of carbohydrates when cuttings were procured from the stock plants cultivated at higher PPFD, but in leaves this trend was not evident. Especially leaf sucrose (Fig. 6b), the main transportable form of sugar that is exported from source-leaves to sinks in different organs, was similar among the cuttings from different seasons. Similarly, leaf starch (Fig. 6c), which is the transitory carbon reservoir (Geiger *et al*., 2000), also did not show any trend in accordance with the PPFD prevailed during stock plant cultivation. The fact that this trend

was not evident for leaf carbohydrates in the current study, is in contradiction with the finding of Forschner and Reuther (1984) on pelargonium stock plants. In that study, leaf sucrose and especially leaf starch levels were significantly higher in the plants grown at higher light intensities when compared to that grown at lower light intensities. The possible explanation for this apparent contradiction can be the time of sampling. In the present study, samples for carbohydrate analysis were collected during the early part of the day, i.e. between 9 AM and 10 AM, but in that study the authors did not mention the time of sampling. Generally, starch accumulation in the leaves reaches maximum during afternoon hours, as shown by Huber (1983) in the leaves of soybean plants. However, this contrariety between the present study and the study by Forschner and Reuther (1984) could be also due to the cultivar effect. For example, in the present study with cv. 'Mitzou', leaf sucrose (Fig. 28b) and starch levels (Fig. 28c) in cuttings increased from first harvest to third harvest when global irradiation gradually increased during stock plant cultivation in winter season. In the current study, leaf reducing sugar levels (Fig. 6a) in 'Isabell' cuttings were higher in spring when compared to those in summer. Perhaps in summer most of the newly fixed carbon could have been actively transported to various sinks. Leaf starch, which has the capability to accumulate and degrade, may enable plants to counteract the high transitory increases of leaf soluble sugar levels that may adversely effect the regulation of photosynthetic capacity and other processes (Sun *et al*., 1999). This could be the reason behind higher leaf starch (Fig. 6c) concentrations at the occasions where leaf reducing sugar concentrations (Fig. 6a) were higher.

Basal stem carbohydrates of 'Isabell' cuttings were strongly influenced by the season of stock plant cultivation. The stock plants cultivated in summer season, which had received higher PPFD, apparently accumulated higher levels of carbohydrates when compared to the stock plant cultivated in spring and winter seasons (Fig. 7d). These results are in agreement with the observations made on pea cuttings, obtained from the stock plant grown at 16 W m^{-2} and 38 W m^{-2} (Veierskov *et al*., 1982a), and stock plants grown at 350 µE m^{-2} s^{-1} and 600 µE m^{-2} s^{-1} (Davis and Potter, 1981). The highly significant positive correlations between PPFD prevailed during the stock plant cultivation and glucose, sucrose and total sugar concentrations (Fig. 8) in basal stems, explain the whole variation among different seasons and even between the harvest dates. This makes clear that in the present study the prevailing light intensity during stock plant cultivation had a predominant influence on basal stem carbohydrate levels, whereas the effect of stock plant development was of minor importance. Basal stem starch concentrations (Fig. 7c) were significantly higher only in the cuttings that

were procured from the stock plants cultivated during summer season, but varied among the harvest dates. These results are in agreement with the observations made by Leakey and Storeton-West (1992), who have reported that *Triplochiton scleroxylon* hard wood cuttings possessed higher starch levels when stock plants were grown at 650 µmol m^{-2} s^{-1} compared to those grown at 250 µmol m^{-2} s^{-1}. Starch can be an important quantitative component of carbon balance in sources and sinks of plant, but it is not essentially necessary that it is involved in the control of any plant process (Farrar, 1991). Although stock plants cultivated during spring received relatively higher light intensities when compared to those cultivated in winter, starch concentrations in basal stems of the cuttings were substantially lower, but the basal stems had significantly higher glucose levels (Fig. 7a). Significantly higher carbohydrate levels in the basal stems of the cuttings procured from stock plants cultivated under higher PPFD when compared to those cultivated under lower PPFD, explains the missing trend in leaf carbohydrates among different seasons. Therefore, it can be concluded that, most of the photosynthetically assimilated carbon during stock plant growth was transported to long term storage locations such as stem and to other active sinks. In 'Mitzou' cuttings, harvest date had no influence on basal stem reducing sugars, but starch levels (Fig. 29b) were significantly higher at the third harvest. These higher levels can be attributed to the gradual increase in global radiation during stock plant cultivation. However, 'Isabell' cuttings procured from the stock plants cultivated during the same period had absolutely no starch in the basal stems (Fig. 7c). This reveals that the partitioning of assimilated carbon within the plant is strongly influenced by cultivar. From the entire pelargonium stock plant cultivation in the present study, it can be concluded that the differences in the light intensity during stock plant cultivation (between different seasons simulated to different locations of stock plant cultivation) can tremendously influence the carbohydrate distribution in the cuttings. However, other effects could also occur. For example, in the present study, the cuttings procured in different seasons were also morphologically different, as the cuttings produced in winter season had higher shoot length, internode length and larger leaf area when compared to the cuttings produced in summer season.

In the current study, the main effect of cold-storage at variable temperature regime was a significant reduction of all the leaf carbohydrate levels in both cultivars without exception (Fig. 6a-d & 28a-c). Principally, leaf starch was reduced to trace amounts, and among the soluble sugars, fructose was strongly reduced. This decrease in carbohydrates agrees with the observations made on cuttings and young plants of other species (Davies and

Potter, 1985; Behrens, 1988; Kubota *et al*., 1997; Druege *et al*., 2000) and also on pelargonium cuttings (Purer and Mayak, 1989; Arteca *et al*., 1996; Druege *et al*., 2003). This kind of decrease is very obvious, because although low temperatures slow down metabolic processes (Behrens, 1988), there is certainly a need for energy to carry out the respiration and other vital metabolic processes, which occurs at a low pace (Kubota *et al*., 1997; Wilson *et al*., 1998). The storage instigated decline of basal stem carbohydrates was moderate in both cultivars. In 'Isabell' cuttings procured during spring and winter, extremely low levels or the absence of starch in the basal stems at harvest (Fig. 7c) may have resulted in the excessive reduction of glucose (Fig. 7a), and also sucrose (Fig. 7b) to a certain extent, by cold-storage in the same tissue in those seasons. In 'Mitzou' cuttings procured during winter, all basal stem carbohydrate fractions, except starch (Fig. 29b), were reduced by cold-storage. In contrast, in 'Isabell' cuttings procured during summer, only glucose levels in the basal stems (Fig. 7a) were slightly decreased after cold-storage, and all other carbohydrates, especially starch (Fig. 7c), either remained at the same level or increased. The lack of any stringent influence of cold-storage on basal stem carbohydrates in summer, as was seen in other two seasons, could be due to the presence of significantly higher carbohydrate levels in summer at harvest and probably their redistribution within the cuttings during cold-storage. The altered pattern of carbohydrate composition in certain plant tissues in response to cold-storage may depend either on the inter connections between the plant tissues or integration within a whole plant (Druege *et al*., 2000). Moreover, low temperature effects on source and sink relationships and on the differential sensitivity of related enzymes are little known to date (Hällgren and Öquist, 1990).

4.2.2 Chlorophyll fluorescence

The optimal quantum yield of PS II (F_v/F_m) for non-stressed healthy leaves is always close to 0.83, independently of the plant species and ecotypes studied (Björkman and Demmig, 1987). In the present study, shortly after harvest F_v/F_m of both 'Isabell' and 'Mitzou' cuttings were lower than the values of non-stressed stock plants (data not shown). These results clearly indicate that leafy cuttings undergo physiological shock immediately after severance. In cold-stored cuttings also F_v/F_m values were lower for both cultivars. A reduction in F_v/F_m after cold-storage was also reported by Bruce *et al*. (2001) on *Taxus* cuttings. In addition to the decline of optimal quantum yield of PS II, the photochemical

quenching (qP) of chlorophyll fluorescence of the cuttings was strongly decreased after dark-cold-storage [Fig. 9a & 32a (see at day 0)]. The decline in qP reveals the reduced reoxidation rate of Q_A^-, which depends on electron transport via PS I, and on ultimate consumption of reducing equivalents in carbon fixation (Krause and Weis, 1991). For example, stomatal closure results in the decrease of internal CO_2 concentration and thus in an inhibition of carbon fixation. This repression in carbon fixation down-regulates the photosynthetic electron transport system (Ott *et al*., 1999), which in turn results in reduced reoxidation of Q_A^-. This could also be the reason for the reduction in non-photochemical quenching (qN) of chlorophyll fluorescence after dark-cold-storage of the cuttings [Fig. 9b & 32b (see at day 0)]. The down-regulation of electron transport may have resulted in reduced generation of proton gradient across the thylakoid membrane leading to the decrease in qN. While, proton gradient causes the conformational changes in a thylakoid membrane constituent, resulting in the formation of a quencher (Horton *et al*., 1996). To date, there is no paper published about the influence of cold storage of cuttings on photochemical and non-photochemical quenching of chlorophyll fluorescence. In a recent study on intact spinach plants by Toledo *et al*. (2003), during the postharvest dark-storage of the plants, both photochemical quenching and non-photochemical quenching values were maintained high until the onset of leaf yellowing, which occurred 14 days after the commencement of storage. The difference between the present study and that by Toledo *et al*. (2003) could be explained by the effect of stomatal closure on carbon fixation. In contrast to the present investigation, in the study of Toledo *et al*. (2003) the leaves were given enough atomized water during the storage of intact spinach plants. Probably that kept the stomates open and carbon fixation to take place immediately on adaptation to actinic light during chlorophyll fluorescence measurement after the retrieval from cold-storage.

4.2.3 Chlorophyll quality and quantity

Chlorophyll content (a + b) was not influenced by the season of stock plant cultivation. Furthermore, cold-storage of the cuttings did not result in the decrease of chlorophyll content in both cultivars (Table 6 & 15). The slightly higher chlorophyll content (a + b) values for stored cuttings when compared to that for unstored cuttings can be explained by probable loss in water content of leaves during simulated storage treatment and determination of chlorophyll content on fresh weight basis. Thus, the moderate storage temperatures in the

present study did not allow the breakdown of chlorophyll pigment. The observations by Arteca *et al*. (1996) on pelargonium cuttings (of other cultivars), supports these findings. In that study, the authors reported that short term storage at moderate temperatures (4 °C and 10°C) did not cause any change in chlorophyll content. However, at higher temperatures (20°C and 25°C) the content was significantly reduced, which was also observed by Serek *et al*. (1998) in 'Isabell' cuttings.

4.3 Post-insertion performance

The potential to perform photosynthesis and the rate at which it proceeds influences the plant's ability to maintain its vigor. Current photosynthesis can contribute significantly to the actual carbon budget of leafy cuttings (Davis, 1988; Veierskov, 1988). The cuttings should be rooted in an environment that is conducive for photosynthesis (Davis, 1988), with optimal light to provide appropriate energy (Eliasson and Brunes, 1980), and without causing any water deficit stress (Loach, 1988). To cover the whole gamut of variation from southern latitudes to northern latitudes with regard to the production and propagation of pelargonium cuttings, the cuttings procured from stock plants cultivated at high light intensity were propagated under the same light intensity on one hand and under relatively low light intensity on the other hand, and *vice versa*. To elucidate the photosynthetic efficiency and performance of the cuttings under different rooting environments, which were previously influenced by season, harvest and cold-storage, chlorophyll quantity and quality, chlorophyll fluorescence, net photosynthetic rate and carbohydrate status were measured during the course of propagation.

4.3.1 Current photosynthetic efficiency

With regard to chlorophyll content in 'Isabell' cuttings, at day 7 during propagation, there were no major differences between unstored cuttings and stored cuttings. These results are similar to the observations of Arteca *et al*. (1996). However, in the present study cuttings propagated in greenhouse suffered a very slight reduction in chlorophyll content when compared to the cuttings propagated in climate chamber. This response was mainly due to spring and summer propagation. The possible reason could be the higher leaf surface temperature (Smart, 1994) under relatively high light conditions in greenhouse when

compared to that in climate chamber (see Table 2). Leaf senescence is a genetically programmed process with an ordered series of cytological and biochemical events (Smart, 1994). Substantial evidence do suggest that cytokinins delay leaf senescence by inhibiting degradation of chlorophyll, proteins and nucleic acids (Smart, 1994; Wingler *et al*., 1998; Druege, 2000). It is likely that the cuttings, which are devoid of roots [the actual site of cytokinin production (Feldman, 1979)], are more sensitive to the slight external or internal abnormalities that cause leaf senescence. With regard to 'Mitzou' cuttings in winter season, at day 7 stored cuttings had lower chlorophyll content when compared to that of unstored cuttings, and irrespective of the storage treatment, chlorophyll a : b ratio was reduced during 6 days of cutting propagation (Table 15). Moreover, in 'Mitzou' cuttings, visual leaf senescence was prominent regardless of the storage treatment (Table 16), whereas, in 'Isabell' almost no senescence was observed irrespective of season of propagation and storage treatment. In this case, it apparently highlights the sensitivity of this specific cultivar.

With regard to chlorophyll fluorescence measurements, in unstored 'Isabell' cuttings, F_v/F_m (optimal quantum yield of PS II) increased to the normal levels within 1 day after insertion and maintained at the same high levels during the whole course of propagation in all the seasons. These results are in agreement with the findings of Mesén *et al*. (1997) on *Cordia alliodora* (Ruiz & Pavon) Oken cuttings, who demonstrated that F_v/F_m was maintained high during the whole course of propagation under moderate light conditions. Similar to that in unstored cuttings, in stored cuttings also F_v/F_m increased to the normal levels within 1 day after insertion in all the seasons. Bruce *et al*. (2001) reported a similar phenomenon during the propagation of stored *Taxus* cuttings. In that study, however, F_v/F_m reached the peak at around 35 days after insertion, which coincided with the root initiation. The immediate increase of F_v/F_m in the present study was probably due to the fast recovery of leafy cuttings compared to the woody cuttings in the experiment of Bruce *et al*. (2001). Thus, irrespective of storage treatment and rooting environment, F_v/F_m was maintained at normal levels during the whole course of propagation in all the seasons. This reveals that the efficiency of energy transfer from antenna pigments to the PS II reaction center remained high and similar under all the circumstances. The absence of any significant chlorophyll (total chlorophyll content) degradation during 6 days propagation in the present study (as discussed above) supports these observations.

Although net photosynthetic rates (gas exchange) of the cuttings did not show a clear trend during propagation in the present study, it is obvious that photosynthetic rates of the

cuttings drop swiftly after severing, especially during first 24 - 48 h of the rooting period, and this drop is presumably due to the stomatal closure (Gay and Loach, 1977; Eliasson and Brunes, 1980) which consequently reduces internal CO_2 concentration. As mentioned earlier, reduction in carbon fixation down regulates the electron transport system (Ott *et al*., 1999), as assimilatory powers are not consumed. This down regulation might be a reason for the decrease of qP (Fig. 10a) and qN (Fig. 10b) in unstored cuttings immediately after insertion, as already discussed above in relation with cold-storage. However, during the subsequent rooting period, qP of unstored cuttings increased in all the seasons (Fig. 10a). In contrast, in stored cuttings, qP which was already decreased by cold-storage, significantly increased after 1 day of insertion (Fig. 11a), and after that they remained equal to the values of unstored cuttings in both the rooting environments.

Non-photochemical quenching of chlorophyll fluorescence was found to be the most responsive component in relation to different rooting environments (differences in light intensity) during propagation in all the seasons. In unstored cuttings, during spring and summer, qN recovered fully and reached pre-insertion levels when propagated under relatively high light conditions (greenhouse), but when propagated under relatively low light conditions (climate chamber) qN was not fully recovered and remained at lower levels during the whole propagation period (Fig. 10b). In stored cuttings, qN which was substantially reduced by cold-storage, significantly increased when propagated in greenhouse during spring and summer, but in climate chamber qN remained at lower levels (Fig. 11b). Moreover, either in greenhouse or in climate chamber qN of unstored and stored cuttings were similar during propagation. Chlorophyll fluorescence quenching has to be viewed in close context with regulation of photosynthesis and adjustment to external environment (Krause and Weis, 1991). It is known that light driven electron flow connecting PS II reaction center and PS I reaction center results in building up of proton gradient across the thylakoid membrane, and it is the principal component of proton motive force under steady state (Taiz and Zeiger, 1998), which powers the synthesis of ATP. The energy dependent quenching component of qN is linearly related to intrathylakoid H^+ concentration (Laasch, 1987; Krause and Weis, 1991), and this part of qN is very sensitive to change in pH gradient (Ruban and Horton, 1995). Thus, a possible explanation for low qN of cuttings propagated in climate chamber during spring and summer could be a low proton gradient across the thylakoid membrane. The relatively low light intensity in climate chamber, may not have been sufficient enough to drive optimal electron flow for building a strong proton gradient across the thylakoid membrane.

An assumed low proton gradient in the cuttings propagated in climate chamber may have resulted in lower ATP production in those cuttings when compared to that in greenhouse during spring and summer (higher qN values). This response in the present study was likely due to the shift in light intensity, because the cuttings adapted to high light intensity during spring and summer cultivation were subsequently propagated under relatively low light intensity in climate chamber. In contrast, during winter season, when low light adapted cuttings were propagated under similar light (greenhouse) on the one hand and under relatively higher light (climate chamber) on the other hand, there were no significant differences between rooting environments for qN values (Fig. 10b & 11b). Furthermore, when even propagated under the same light intensity in climate chamber during different seasons, irrespective of storage treatment, the cuttings in winter season had higher qN during propagation when compared to that in spring and summer seasons, which again reveals the shift from high light to low light intensity in spring and summer and *vive versa* in winter. These results support the conclusion that high light adapted cuttings when subsequently rooted under relatively low light conditions obtained a deficiency for light intensity, which was reflected as low qN of chlorophyll fluorescence in the present study. From the varied responses of qN in different rooting environments, it can be concluded that current photosynthetic efficiency was likely higher in the cuttings propagated in greenhouse when compared to their counterparts in climate chamber during spring and summer. Alternatively, in winter, there may not have existed significant differences between the two rooting environments. Within the climate chamber propagation, current photosynthetic efficiency appeared to be higher in winter when compared to that in spring and summer. Net photosynthetic rates (gas exchange) further supports the above conclusions, as in spring and summer the rates were higher in greenhouse compared to that in climate chamber, whereas in winter there were no significant differences between the rooting environments.

4.3.2 Current photosynthetic performance

In the present study considerable amounts of carbohydrates were accumulated in ‘Isabell’ cuttings during the initial 6 days of propagation in all the seasons and in both the rooting environments. However, the magnitude of accumulation principally relied on the type of carbohydrate fraction, part of the cutting, rooting environment, and season of propagation. Net photosynthetic rates in leafy cuttings are reported to be much lower than in corresponding

stock plants (Eliasson and Brunes, 1980; Davis and Potter, 1981; Forschner and Reuther 1984). However, carbohydrates can accumulate under such diminished photosynthesis (Haissig, 1984), because they are devoid of root system, which can reduce plant respiration by 40 % (Veen, 1980).

With reference to leaf carbohydrates of 'Isabell' cuttings, at day 7 during the course of propagation, there were no significant differences between unstored cuttings and stored cuttings in any specific rooting environment, i.e. either in greenhouse or in climate chamber (Fig. 14a, b). Similarly, in 'Mitzou' cuttings propagated in climate chamber during winter season, there were no differences between unstored cuttings and stored cuttings at day 7 (Fig. 30a-c). During 6 days of propagation, leaf glucose and fructose levels were significantly reduced in unstored cuttings, but in stored cuttings both the reducing sugars remained at the same lower level. In contrast, leaf sucrose (Fig. 14a & 30b) and starch were only slightly decreased in unstored cuttings, but both these carbohydrate fractions strongly increased in stored cuttings. This reveals that, both in unstored cuttings as well as in stored cuttings, newly assimilated carbon did not enter reducing sugar pools in the leaves, but was predominantly partitioned among sucrose and starch. Jiao *et al*. (1999) showed that sucrose and starch are the primary pools of newly fixed carbon in the source leaves of pelargonium plants. Starch mobilization supplements sucrose synthesis from photosynthesis during dark, and supplies carbon skeletons for sucrose formation (Geiger and Batey, 1967; Ho, 1978; Gordon *et al*., 1980). Growth is accelerated when transitory starch turnover and newly assimilated carbon provide complementary sources for sucrose synthesis and export to sink throughout the day and night cycle (Fondy *et al*., 1989; Schulze *et al*., 1991). However, in unstored cuttings the decrease in leaf sucrose levels was more pronounced in climate chamber when compared to those in greenhouse during spring and summer, and *vice versa* in winter (Fig. 14a). In stored cuttings the increase in leaf sucrose levels was significantly higher in greenhouse when compared to those in climate chamber during spring and summer, and *vice versa* in winter (Fig. 14a). At all the occasions, either in unstored or in stored cuttings, leaf total sugars displayed a similar trend like that of leaf sucrose except in unstored cuttings in winter season, where total sugar levels of the cuttings were similarly decreased in both the rooting environments (Fig. 14b). This multi-faceted phenomenon exhibited by unstored cuttings, as well as by stored cuttings in different rooting environments among different seasons, can be attributed to the available light intensity during the course of propagation. It is evident that, light intensity during propagation strongly influences the current photosynthesis of the

cuttings (Foster, 1963; Howard, 1965; Eliasson, 1978; Davis and Potter, 1981). In the present study, leaf carbohydrate levels, combined with qN and net photosynthetic rates (gas exchange), reveal that current photosynthetic performance of the cuttings was significantly higher under high light conditions, i.e. in greenhouse compared to climate chamber during spring and summer, and also in greenhouse during spring and summer compared to the same rooting environment during winter. Furthermore, under the same relatively low light (climate chamber) current photosynthetic performance of the cuttings was higher in winter when compared to that in spring and summer, which can be attributed to the higher photosynthetic efficiency as discussed above.

4.3.3 Contribution towards basal stem

With regard to basal stem carbohydrates of 'Isabell' cuttings, all most all carbohydrate fractions except starch were significantly increased during 6 days of propagation in both unstored cuttings and stored cuttings, and in all the seasons (Fig. 15a-c). Current photosynthesis or mobilization of carbohydrates reserves during the cutting propagation results in basipetal transport of carbohydrates, and their accumulation in the basal stem of the cutting (Altman and Wareing, 1975; Hansen *et al*., 1978; Davis and Potter, 1981; Haissig, 1982 and 1984, Veierskov *et al*., 1982b). In the current study, at day 7 during propagation, there were no significant differences between unstored cuttings and stored cuttings in any specific rooting environment, excepting greenhouse propagation during winter season (Fig. 15a-c). Alternatively, irrespective of storage treatment, basal stem carbohydrate levels were significantly higher in the cuttings propagated under higher PPFD when compared to those under lower PPFD, i.e. higher in greenhouse compared to climate chamber during spring and summer, but in winter no significant differences were found between the two rooting environments (Fig. 15a-c). These observations are in accordance with Davis and Potter (1981), who demonstrated that pea cuttings propagated at 280 μE m^{-2} s^{-1} had higher basal stem carbohydrates than did the cuttings at 50 μE m^{-2} s^{-1}. At insertion, stored cuttings when compared to unstored cuttings had relatively low basal stem carbohydrate levels in all seasons except in summer, and substantially low leaf carbohydrates in all the seasons. Nevertheless, the differences found at insertion between unstored cuttings and stored cuttings for different carbohydrate fractions were eliminated in all the seasons during 6 days of propagation in any specific rooting environment, i.e. either in climate chamber or in greenhouse, except during

winter propagation in greenhouse. Thus, the deficiency in carbohydrates acquired during cold-storage was rectified within the first 6 days of propagation at most of the occasions. The high performance of stored cuttings could be due to upregulation of genes for photosynthesis, remobilization, and carbohydrate export, by low initial leaf carbohydrate levels (Koch, 1996). During winter season, when propagated in greenhouse, at day 7 all the basal stem carbohydrate concentrations, except sucrose, were significantly lower in stored cuttings when compared to those in unstored cuttings. In this whole complex investigation, this was the only exceptional phenomenon, but not a strange phenomenon. While the PPFD during the propagation in winter season was very low, probably, the low PPFD did not allow for current photosynthesis in stored cuttings to cover up the differences with unstored cuttings. Thus, it becomes apparent that the differences between unstored cuttings and stored cuttings for basal stem carbohydrate levels can be eliminated only when propagated above certain critical light intensities (for example, in the present study it was 100 μmol m^{-2} s^{-1}).

In the current study, under any occasion, the basipetally translocated carbohydrates did not enter into the starch pools in the basal stem of the cutting during 6 days of propagation (Fig. 12c & 13c). As a consequence, in spring and winter starch levels remained low during propagation. Furthermore, in summer both unstored and stored cuttings, which were initially rich in starch levels, displayed decreased levels during 6 days of cutting propagation. However, in an investigation by Costa *et al*. (2001) on rose cuttings, it was shown that basal stem starch concentrations were substantially higher at day 21 during propagation when compared to those of day 0. It is probable that during the initial phase of propagation, basipetal translocation of carbohydrates may result in accumulation of soluble sugars in the basal stem region, and at a later phase they may be driven to storage pools (starch accumulation). The observations of Druege *et al*. (2003) on pelargonium cuttings cv. 'Isabell', supports this reasoning. In that study, basal stem starch concentrations were either reduced or maintained at the same level during first 6 days of propagation, but by day 13 there was an increase in the levels. However, starch accumulation may also depend on the sink strength and sink activity of the cuttings of a specific cultivar. The accumulation of basal stem fructose in both unstored and stored cuttings was higher in greenhouse when compared to that in climate chamber during spring and summer. Furthermore, in those seasons basal stem glucose levels were higher in greenhouse compared to those in climate chamber (Fig. 15a). Probably, the translocated sucrose was readily converted to reducing sugars in the region of root generation (basal stem), as discussed by Haissig (1984). This could be the reason for the lack

of influence of rooting environment on basal stem sucrose levels at day 7 during propagation (Fig. 15b). Basal stem total sugars in both unstored and stored cuttings, substantially influenced by the fructose and sucrose levels, increased enormously during 6 days of propagation in both rooting environments and in all seasons, except in climate chamber during summer season (Fig. 15c). However, irrespective of storage treatment, the magnitude of increase was significantly higher in greenhouse when compared to that in climate chamber during spring and summer seasons, but in winter there were no differences between rooting environment either in unstored or stored cuttings. Thus it can be concluded that in spring and summer, the cuttings which had higher leaf carbohydrate levels, concurrently those cuttings also had higher basal stem carbohydrate levels. The simultaneously higher leaf carbohydrate levels and basal stem carbohydrate levels in greenhouse-rooted cuttings during spring and summer indicate, that a higher current photosynthesis contributed to a higher carbohydrate synthesis and subsequent basipetal transport. In winter, leaf carbohydrates of the cuttings were higher in climate chamber when compared to those in greenhouse only in the case of stored cuttings but not in unstored cuttings (Fig. 14b). However, basal stem carbohydrate levels of the cuttings did not differ among rooting environments, neither in stored cuttings nor in unstored cuttings (Fig. 15a-c). It is possible that the current photosynthetic contribution towards basipetal transport of carbohydrates may not have been substantially higher in climate chamber when compared to that in greenhouse in order to manifest consequent higher carbohydrate levels in the basal stems of the cuttings in climate chamber.

When the environmental conditions of cutting production and cutting propagation were similar (greenhouse propagation) in different seasons, irrespective of storage treatment, basal stem total sugar concentrations of the cuttings were increased by 6.7 mg/g FM (147 %), 5.5 mg/g FM (111 %), and 4.1 mg/g FM (225 %) in spring, summer, and winter seasons, respectively (Fig. 12b). As a consequence, the differences for basal stem carbohydrate levels which existed among different seasons at the time of insertion were maintained at the same magnitude during 6 days of propagation. In contrast, when the cuttings produced under high light intensity during spring and summer, and under very low light intensity during winter, were propagated under similar relatively low light conditions during different seasons (climate chamber propagation), the total sugar levels were increased by 2.8 mg/g FM (60 %), 1.4 mg/g FM (29 %), and 4.9 mg/g FM (271 %) in spring, summer, and winter seasons, respectively (Fig. 13b). As a result, the differences for total sugar levels found at day 0 among different seasons were eliminated during propagation. It can be concluded that, although there

existed outstanding differences in basal stem carbohydrate levels among three seasons, which resulted because of the adaptation to different grades of light intensities and associated parameters, they were eliminated during propagation under a controlled (relatively low light) environment. These findings emphasizes the pivotal role of rooting environment (especially light intensity) in the propagation of leafy cuttings. These results are in accordance with the observations made on pea cuttings (Veierskov *et al*., 1982a). In that study, cuttings obtained from stock plant grown at 38 W m^{-2} had higher extractable carbohydrates than did those grown at 16 W m^{-2}. When both of those cutting types were propagated at 16 W m^{-2}, in spite of the initial difference, carbohydrate content in both types increased to similar levels during initial 6 days of propagation.

The trivial increase in basal stem total sugar levels in climate chamber (under relatively low light) during spring and summer (2.8 mg/g FM and 1.4 mg/g FM, respectively) when compared with their counterparts in greenhouse (6.7 mg/g FM and 5.5 mg/g FM, respectively), discloses the reduced current photosynthetic contribution towards the basipetal transport of carbohydrates in those cuttings propagated in climate chamber, which could be due to lower current photosynthetic performance of those cuttings (the lower photosynthetic performance of the cuttings propagated in climate chamber during spring and summer was already discussed above). This reduced current photosynthetic performance in the cuttings propagated in climate chamber during spring and principally during summer cannot be simply attributed to the absolute lower PPFD itself, because under the same environmental conditions in winter, basal stems of the cuttings accumulated 4.9 mg/g FM of total sugar. Furthermore, even at substantially lower PPFD in greenhouse during winter propagation, basal stems of the cuttings accumulated considerable amounts of carbohydrates (4.1 mg/g FM increase in total sugars). Light saturation of unrooted leafy cuttings occurs generally at a much lower light intensities when compared to intact plants (Davis, 1988). Perhaps the reduced current photosynthetic performance in climate chamber during spring and summer could be due to the steep decline in PPFD from greenhouse (stock plant cultivation) to climate chamber (cuttings propagation), which may have caused severe adaptation problems. The stock plants grown at high light intensities have higher light compensation point when compared to the plants grown at low light intensities (Mortensen and Olsen, 1987). When the cuttings taken from stock plants cultivated at high light intensity and subsequently rooted at low light intensity, they face adaptation problems (Moe, 1988) and the net photosynthetic rate reduces (Forschner and Reuther, 1984), as the current study demonstrated. In the present investigation, the

reduced current photosynthetic efficiency resulted to lower carbohydrate synthesis (lower leaf carbohydrate levels) in the cuttings during spring and summer in climate chamber (under relatively low light), which could be due to the limitation of assimilatory force (ATP) for photosynthetic carbon fixation (as shown from low non-photochemical quenching of chlorophyll fluorescence during propagation in climate chamber). Therefore, it can be concluded that high light adapted cuttings when propagated under relatively low light, they encountered adaptation problems and as a consequence photosynthetic performance was reduced, which was reflected by lower leaf carbohydrate synthesis and basal stem carbohydrate accumulation in the cuttings during spring and summer propagation in climate chamber. The present results highlight the reasons behind the practical problems frequently observed in pelargonium cuttings imported from southern locations and subsequently propagated under low light during winter season in Central European greenhouses.

In ‘Mitzou’ cuttings, during 6 days of propagation, leaf sucrose and starch almost maintained at the same pre-rooting levels in unstored cuttings, whereas in stored cuttings both of those carbohydrate fractions were significantly increased (Fig. 30a-c). However, irrespective of storage treatment, all basal stem carbohydrate fractions (sugars) remained at the same level during 6 days of propagation. In addition, basal stem starch levels exhausted or remained at substantially lower levels during 6 days (Fig. 31). Thus, irrespective of storage treatment, the high levels of carbohydrates in leaves and concurrent depletion in basal stems indicate that there was substantially low or probably no basipetal transport of carbohydrates from the source leaves to the regions of root regeneration during propagation. Because, soluble carbohydrate levels or total non-structural carbohydrate levels in basal stem regions of the cuttings decline under the occasion where basipetal translocation does not occur or photosynthetic carbon fixation is impeded (Haissig, 1986). A recent study by Druege e*t al.* (2003) on zonal pelargonium cuttings cv. ‘Isabell’ demonstrated that sugars were accumulated in leaf lamina and concurrently depleted in the basal stems of the cuttings, after those cuttings had been stored under very unfavorable conditions causing leaf senescence. Thus, also in the present study the pronounced leaf senescence of ‘Mitzou’ cuttings irrespective of storage treatment may have upset the basipetal transport of carbohydrates. Moreover, the contribution of current photosynthesis could have been also very low in ‘Mitzou’ cuttings because of the early and pronounced leaf senescence.

4.4 Rooting response

Season itself had no predominant influence on adventitious root formation of the cuttings. However, season in combination with cold-storage treatment or rooting environment showed significant interactions for number of roots formed. Internal quality of cold-stored pelargonium cuttings is inferior to unstored cuttings (Kaltaler, 1966; Eisenberg et al., 1978; Carow and Bahnemann 1980; Paton and Schwabe 1987; Steinitz *et al*., 1987; Purer and Mayak, 1989; Arteca *et al*., 1996, Serek *et al*., 1998; Kadner et al., 2000; Druege *et al*., 2003), even short term cold-storage results in reduced rooting capacity of the cuttings. In the current investigation also, irrespective of the season of propagation, number of adventitious roots formed by 'Isabell' cuttings was reduced by storage (variable temperature regime) when propagated under relatively low light conditions (in climate chamber) (Fig. 16b). Alternatively, when propagated under relatively high light conditions (in greenhouse during spring and summer), there were no differences between unstored cuttings and stored cuttings with regard to the number of roots formed (Fig. 16a). In 'Mitzou' cuttings, storage had no major influence on adventitious root formation, when propagated in climate chamber during winter season (Fig. 33). The potential influences which have resulted in the varied rooting behavior of 'Isabell' cuttings in climate chamber and in greenhouse will be discussed in the following section.

4.4.1 Rooting response as related to pre-insertion condition

Like any other developmental process, neo-root formation is also an endergonic process (Haissig, 1986). Substantial evidence offers an indication that cuttings root best under conditions that yield optimum internal total non-structural carbohydrate levels either before rooting or during course of propagation (Reuveni and Adato, 1974; Veierskov *et al*, 1976; Reuveni and Raviv, 1980; Leakey, 1983). The unique importance of carbohydrates does not simply reside in providing energy and carbon skeleton, but also affects sugar sensing systems that modulate the gene expression (Koch, 1996) and hormonal responses (Leon and Sheen, 2003).

In the present study, irrespective of the season of propagation, highly significant positive correlations were found between pre-rooting leaf carbohydrates, especially sucrose (Fig. 18a), and root number of unstored and stored cuttings of 'Isabell' propagated in climate chamber

(relatively low light). These results are in agreement with Druege *et al*. (2003) on the same cultivar. This strongly supports the conclusion that low initial leaf carbohydrate reserves in stored cuttings are causally related to their reduced rooting performance, when rooted under relatively low light conditions where there could be less scope for current photosynthetic contribution. Similar positive correlations were found with pre-rooting basal stem carbohydrates and root number only in spring and winter (Fig. 18b), but not in summer season, as there was no decrease in carbohydrates after cold-storage. Despite the lack of decrease in basal stem carbohydrate levels after cold-storage during summer, the number of roots formed by stored cuttings was lower when compared to that of unstored cuttings. Furthermore, those stored cuttings during summer season rooted no better than corresponding stored cuttings during spring and winter in climate chamber. This reduction in root number during summer was addressed by the decrease in leaf carbohydrates, or to be more specific, decrease in leaf sucrose levels. These findings presented a new insight, as they uncoupled certain relations and elevated the pivotal relationships. This reveals that pre-rooting steady state (basal stem) carbohydrate abundance is not the main basic criteria, but rather the availability of transportable form of carbohydrates (leaf carbohydrates) is more important for adventitious root formation in 'Isabell' cuttings. This implies that cuttings which had higher potential to carry out basipetal transport of carbohydrates at the time of insertion resulted in the formation of higher number of roots and *vice versa*. Consequently, unstored cuttings which had higher initial leaf sucrose levels principally produced higher number of roots when compared to stored cuttings. Sucrose, which is non-reducing and the main transportable form of sugar, supplies carbohydrates to the region of root regeneration, where they can promote root initiation and development by diverse mechanisms (Haissig, 1986; Veierskov, 1988; Koch, 1996). In the present study, despite the differences found at day 0 between unstored and stored cuttings with regard to leaf and basal stem carbohydrate levels were abolished during propagation in climate chamber, and also net photosynthetic rate and qN during propagation were similar for both unstored and stored cuttings, the number of subsequently formed roots was lower in stored cuttings when compared to unstored cuttings. These observations further ascertain that the relationship between pre-rooting carbohydrate reserves and number of subsequently formed roots was causal. This does not categorically mean that there was no contribution of current photosynthesis towards the subsequent root formation in the present study. The increase in the leaf and basal stem carbohydrates during 6 days of propagation in different seasons provides promising evidence about the involvement of

current photosynthesis. Nevertheless, pre-rooting cutting carbohydrate levels overrode the predominance of current photosynthesis, as the performance of current photosynthesis and contribution towards basal stem were substantially low under relatively low light conditions in spring [2.8 mg/g FM (60 %) increase] and summer [1.4 mg/g FM (29 %) increase], which could be attributed to adaptation problems (as discussed earlier). Thus, the positive correlations between initial leaf sucrose concentrations and root number unequivocally indicate that pre-rooting leaf carbohydrate status is the most crucial factor for subsequent root formation, when the contribution of current photosynthesis is curtailed, which may result from the dearth in light intensity because of adaptation problems during the propagation or other factors (Druege *et al*., 2003). Stored cuttings in spring and summer propagated in climate chamber in the current study should be similar to pelargonium cuttings that are imported from southern latitudes and propagated in Central European greenhouses during winter season under low light with additional assimilation light. One can imagine that the cuttings imported from southern latitudes also depend on pre-insertion cutting status when propagated under those relatively low light conditions (Druege *et al*., 2003).

Similar to that of leaf sucrose, irrespective of the season, highly significant positive correlation was found between pre-rooting qN (day 0) and root number of unstored and stored cuttings (Fig. 20a). It appears that the cuttings which had higher pre-rooting ATP production efficiency predominantly resulted in the formation of higher number of roots and *vice versa*. Consequently, unstored cuttings which had higher carbon fixation capability at insertion principally produced higher number of roots when compared to that of stored cuttings. However, it should be noted that qN values of unstored cuttings decreased to the levels of stored cuttings as early as 1 day after insertion, and remained equal to the values of stored cuttings for the rest of the propagation period. Therefore, a causal relationship behind this highly significant positive correlation between qN and root number has to be questioned. Nevertheless, pre-rooting ‘qN’ of unstored and stored ‘Isabel’ cuttings may be used as a non-destructive physiochemical index for assessing rooting efficiency of the cuttings when propagated under defined environmental conditions (relatively low light), because of the fact that pre-rooting qN of unstored and stored ‘Isabel’ cuttings positively correlated with both leaf sucrose ($r=0.88$, $p<0.001$) and rooting performance ($r=0.64$, $p<0.001$) of those corresponding cuttings.

In contrast to climate chamber, when propagated in greenhouse no significant correlations were found between pre-rooting leaf or basal stem carbohydrate levels and root

number of unstored and stored cuttings, as well as between pre-rooting quenching coefficient (qP and qN) and root number of unstored and stored cuttings, except in winter season. The reason behind the missing bond was due to lack of significant differences between unstored cuttings and stored cuttings for number of subsequently formed roots. Thus, neither low pre-rooting carbohydrate levels in different cutting parts of stored cuttings, especially in leaves, nor their reduced pre-rooting PS II activity (low qP and qN) impeded the rooting performance of those cuttings in greenhouse during spring and summer. The importance of current photosynthesis to actual carbohydrate status and to subsequent root formation is repeatedly emphasised (Haissig, 1986; Davis, 1988; Veierskov, 1988; Druege *et al*., 1998 and 2000). It can be concluded that high light during propagation compensated the initial lower carbohydrate availability in stored cuttings to such an extent that their adventitious root formation was not inhibited. In winter, however, when the light intensity was very low during propagation in greenhouse, both pre-rooting carbohydrate levels [in leaves (Fig. 19) and basal stems] and pre-rooting quenching coefficient [qN (Fig. 20b)] were positively correlated with root number of unstored and stored cuttings. This reveals that adventitious root formation of those cuttings relied on the pre-rooting carbohydrate status of the cuttings as in climate chamber.

4.4.2 Rooting response as related to pre-insertion condition and performance during propagation

In the present study, it was interesting that even unstored cuttings differed in their rooting performance when they were propagated in different rooting environments (Fig. 17a). Especially, unstored cuttings propagated in climate chamber during winter season, in spite of having similar pre-rooting leaf total sugar concentrations when compared to corresponding cuttings in spring and summer on one hand, and lower basal stem total sugar levels on the other hand, formed the highest number of roots. This higher rooting performance was probably due to enhanced current photosynthetic contribution allowing for a stupendous increase in basal stem total sugar levels [4.9 mg/g FM (271 %) increase] during 6 days. This increased rooting performance also explains the hierarchy in the steepness of the slope in climate chamber propagation among different seasons [with regard to the correlations between root number of unstored and stored cuttings and both pre-rooting sucrose levels in different cutting parts (Fig. 18a) and pre-rooting qN (Fig. 20a)]. The explicit role of

carbohydrates from pre-rooting and current photosynthesis is obscure (Veierskov and Andersen, 1982; Veierskov *et al*., 1982a,b). However, the regulating effect of current photosynthesis even in the propagation of unstored leafy cuttings has been demonstrated frequently in different species (Davis and Potter, 1981; Leakey and Coutts, 1989; Newton *et al*., 1992; Hoad and Leakey, 1996; Costa *et al*., 2001). Thus, it can be concluded that current photosynthesis does influence the rooting performance of unstored cuttings, even when no deficiencies in pre-rooting carbohydrate (leaf) levels exists. With regard to stored cuttings, adventitious root formation was promoted with increasing mean (day 0 + day 7) leaf and basal stem carbohydrate levels. Principally, a highly significant positive correlation between mean basal stem sucrose concentrations and root number of stored cuttings (Fig. 22a), when investigated over three contrasting seasons and two rooting environments, emphasizes the importance of both pre-rooting reserves as well as the accumulation occurred by basipetal transport during propagation. Therefore, even in stored cuttings best rooting can be obtained when soluble carbohydrate pools are well maintained by current photosynthesis, as current photosynthesis contributes significantly to the actual carbon balance in leafy cuttings (Davis, 1988; Veierskov, 1988). The above positive correlation also explains the reduction in number of subsequently formed roots by stored cuttings in climate chamber during winter season [although basal stem total sugars increased by 4.9 mg/g FM (271%) during propagation], and especially, the greatest setback in root formation by stored cuttings in green house during the same season [total sugars increased by 4.1 mg/g FM (225 %) during propagation]. Thus, even when the current photosynthetic contribution towards the basal stems of the cuttings was considerably high in winter season, the cuttings which had substantially low pre-rooting basal stem carbohydrate reserves principally resulted in the reduction of number of subsequently formed adventitious roots. This explicitly supports the conclusion that both steady state carbohydrates (pre-rooting carbohydrate levels in basal stems) and carbohydrates accumulated during propagation were causally related in the adventitious root formation of stored ‘Isabell’ cuttings.

The highly significant positive correlations between mean quenching coefficients [qP and qN (Fig. 21)] and root number of unstored and stored cuttings in spring and summer reveal the importance of current photosynthetic efficiency (consequently photosynthetic performance) over the whole rooting period of the cuttings for successful adventitious root formation. The whole set of variation in this complex investigation was explained using the ‘mean’ (day 0 + day 7) sucrose (Fig. 22b) and total sugar concentrations in leaves, which once

again repeats the above underlining importance of pre-rooting carbohydrate status as well as current photosynthetic performance (consequently photosynthetic contribution). The increase in root number with the increase in mean leaf sucrose levels implies that the cuttings which had a higher capacity for carbohydrate export from leaves during rooting, resulting from both initial status and current photosynthesis, were able to form higher number of roots. However, apart from this translocating ability, the function of sucrose as an osmoticum and regulator of gene expression (Koch, 1996; Wobus and Weber, 1999) cannot be excluded. From this complex investigation, structured with a whole gamut of variations in production and in propagation of pelargonium cuttings, it can be concluded that basipetal carbohydrate fluxes are utmost important for determining the rooting success of pelargonium cuttings. These observations are in accordance with the views of Druege *et al*. (2003) on the same cultivar, and Druege *et al*. (2000) on chrysanthemum cuttings, who emphasized that basipetal carbohydrate fluxes during propagation are more important than carbohydrate reserves *per se* for determining the rooting success. Effective utilization of sugars depends principally on the activity of sucrose metabolizing enzymes (Claussen, 1983; Miller and Chourey, 1992; Morris and Arthur, 1984; Pfeiffer and Kutschera, 1995; Sung *et al*., 1988). It was reported that during the ex vitro rooting of micropropagated *Spathiphyllum* 'Petite', all sucrose metabolizing enzymes, i.e. sucrose-*P*-synthase, acid invertase and sucrose synthase activities were elevated (Van Huylenbroeck and Riek, 1995). This evidence further supports the present results that active sucrose translocation takes place during the process of adventitious root formation. Thus, the capability or success of adventitious root formation of a cutting depends on active contribution of the source-leaves towards basipetal influx of carbohydrates during the course of propagation.

It can be hypothesised that carbohydrate reserves (when adequate, as in spring and summer) present in the basal stem (steady state) meet the energy requirements, whereas basipetal translocation resulting from pre-insertion leaf carbohydrate reserves and/or current photosynthesis may aid in supplying other essentials (as detailed below) for adventitious root formation of cuttings. The basipetal transport of sucrose is known to be instrumental in carrying out multiple functions. Compelling evidence does suggest that endogenous indole-3-acetic acid (IAA) levels elevate transiently in the rooting zone and this is causally associated to adventitious root initiation (Blakesley, 1994 and Gaspar, 1997). Absolute auxin levels are important in stimulating primary events of root initiation (Blakesley and Chaldecott, 1993), and they are basipetally translocated in the phloem along with photoassimilates (Lomax *et al*.,

1995; Baker, 2000). It is likely that enhanced basipetal transport of photoassimilates aid in increasing the supply of auxins for root initiation as well as subsequent root growth, where auxins control the growth by modulating cellular responses to gibberellin (GA) (Fu and Harberd, 2003). Substantial evidence admit that polyamines (Jarvis *et al*., 1983; Haissig, 1986; Gaspar *et al*., 1997) during the initial stage, and amino acids (Suzuki, 1982; Haissig, 1986) during the course of root formation, accumulate in the basal portion of the cuttings, which are causally related to root development. Amino acid pools which change in response to daily photosynthetic activities (Ito *et al*., 1996) are translocated from the source leaf (cytosol) via the sieve tubes along with the mass flow of sucrose driven by active sucrose transport (Riens *et al*., 1991; Winter *et al*., 1992). From this it is apparent that enhanced basipetal transport of sucrose in the cuttings would facilitate increased co-transport of amino acids in the phloem.

In the present study, irrespective of the season, harvest date, and cold-storage of the cuttings, rooting environment (light intensity) played a pivotal role in adventitious root formation of ‘Isabell’ cuttings. The present study also clearly demonstrates that the current photosynthetic performance and the shift in carbohydrate status in the basal stems of ‘Isabell’ cuttings were substantially affected by the rooting environment. All of these observations highlight the underlining importance of rooting environment in the propagation of leafy cuttings. Rooting period is not quite short in the case of pelargonium cuttings: generally, first visible roots emerge 6 days after the cutting insertion as was observed by Steinitz *et al*. (1987) and also in the present study. Thus, it is probable that the deficiencies in pre-rooting carbohydrate levels in the cuttings resulting from shipment can be rectified, and also the adaptation problem causing by the shift from southern latitudes to northern latitudes can be minimised, during the course of propagation by controlling the rooting environment (light intensity) in Central Europe. As a result, the rooting performance of those cuttings can be improved. However, the light intensity that should be provided during cutting propagation in Central Europe depends on the environmental conditions at which stock plants are being cultivated.

In ‘Mitzou’ cuttings, root number of unstored and stored cuttings was not correlated with either pre-insertion or post-insertion carbohydrates and chlorophyll fluorescence parameters, which was mainly due to the lack of differences between unstored cuttings and stored cuttings for number of subsequently formed roots. Probably, because of the disturbance in basipetal transport of carbohydrates in both unstored and stored cuttings (as discussed

earlier), the pre-rooting leaf carbohydrate levels may not have exerted influence on the subsequent adventitious root formation of the cuttings. It was likely that both unstored and stored 'Mitzou' cuttings depended on pre-rooting basal stem carbohydrate reserves. From the present study, it appears that in 'Mitzou' cuttings adventitious root formation of the cuttings is not a primary problem, but leaf senescence, irrespective of the storage treatment, appears to be the main problem. However, further experimentation is needed to understand the relationships between carbohydrates and adventitious root formation in this highly post-harvest senescence sensitive and abandoned (from commercial usage) cultivar.

4.5 Internal quality and rooting response of the cuttings as affected by storage temperature regimes

In the present study, under both constant and variable storage temperature regimes, all the leaf and basal stem carbohydrates were decreased (Table 13, Fig. 23a-c & 24a b). Leaf starch concentrations varied strongly among the harvest dates. Nevertheless, the concentrations were reduced to trace amounts after the very first day of dark-cold-storage irrespective of the temperature employed (9° C in constant temperature regime and 5°C in variable temperature regime) (Fig. 23c). This early exhaustion of leaf starch in the present study reveals its transitory nature, which is a normal diurnal phenomenon. Leaf starch, a transitory carbon reservoir, mobilizes during the night or even at low light during the day (Geiger *et al*., 2000) and exits in the form of glucose (Trethewey and ap Rees, 1994) by chloroplast glucose translocator. This mobilization appears to involve an endogenous regulated circadian component (Geiger *et al*., 2000). Pelargonium cuttings are extremely sensitive to elevated temperatures during storage (Krebs and Zimmer 1977; Carow and Bahnemann 1980; Steinitz *et al*., 1987). However, in the present study two different storage temperature regimes (variable and constant regime) did not show any significant differences in the pattern of decrease of individual or total sugars either in leaves or in basal stems, except sucrose at the initial stage. Although the depletion of carbohydrates did not show a very fine regulation accordingly to the temperatures employed, the concentrations at the final sampling date were equal in both the temperature regimes (Table 13). Possibly, it is not the fluctuation in temperatures, but rather the sum of day mean storage temperatures that are responsible for carbohydrate depletion. Similarly, pre-insertion functional status of photosynthetic apparatus [qP and qN (Fig. 26)] and rooting performance (Fig. 27) of the cuttings stored under two

different temperature regimes were similar. From these results it can be concluded that a variable temperature regime had an influence similar to that of a constant temperature regime on carbohydrate depletion, functional status of photosynthetic apparatus, and rooting performance of the cuttings.

Gradual and less pronounced decrease of basal stem carbohydrates during storage yielded lower significant regressions with sum of day mean storage temperatures. The interconnections of basal stem with the rest of the cutting or basipetal transfer of carbohydrates during storage likely resulted in a reduced decrease. Leaf total non-structural carbohydrates, strongly influenced by early starch exhaustion, showed a rapid and drastic decrease at the beginning of the storage (Fig. 25b). Leaf total sugars also decreased at a relatively higher pace at the beginning of cold-storage, but with the increasing sum of day mean storage temperatures the magnitude of decrease was reduced (Fig. 25a). It is likely that respiration rates decrease with duration in darkness because of the decline in carbohydrate levels that can be used as respiratory substrates, as discussed by Kubota *et al*. (1997). Nevertheless, relatively little is known about low temperature effects on respiration and mitochondrial activity (Hällgren and Öquist, 1990), and respiratory changes related to sugar-modulated gene expression (Koch, 1996). However, the pattern of decrease of leaf total sugars was well explained by the sum of day mean storage temperatures. Since temperature determines the pace of all metabolic processes in the plant, it is perhaps not surprising if such a fine relationship exists between the carbohydrate depletion during dark storage and the sum of temperature.

4.6 Predicting the rooting capacity of cuttings

Pelargonium production losses in Central Europe are often around 20%. Currently, there is no reliable method to assess the quality of the cuttings after shipment from southern locations, but if a quick method of determining the rooting capacity of the cuttings based on internal quality of those cuttings were available for commercial propagators, then success of rooting could be predicted prior to an investment in resources, labor, and time.

In the present study it was shown that: 1) cuttings produced under high light intensity (example: spring & summer) had higher basal stem carbohydrate levels at harvest, 2) basal stem carbohydrate levels of the cuttings were less influenced by cold-storage, especially when their levels were higher at harvest (example: summer), 3) irrespective of the light regime

(season) under which the cuttings were produced, leaf carbohydrate levels (especially sucrose) were similar at harvest, 4) leaf carbohydrates were significantly reduced by cold-storage at all occasions, and variable temperature regime had no additional influence when compared to constant temperature regime, 5) pre-insertion leaf sugar levels, especially sucrose levels, were found to be crucial for subsequent adventitious root formation of the cuttings, when current photosynthetic performance and consequent contribution towards basipetal transport was curtailed by relatively low light conditions. So, one can imagine that: 1) cuttings produced at southern location (southern cuttings) also may have higher basal stem carbohydrate levels at harvest, 2) during the shipment of southern cuttings, basal stem carbohydrate levels may not decline drastically, 3) leaf carbohydrate levels (especially sucrose levels) of southern cuttings at harvest may be similar to those in the present study, 4) leaf carbohydrate levels of southern cuttings may decline significantly during shipment, 5) pre-insertion leaf sugar levels could be crucial when southern cuttings propagated under winter climatic conditions in Central Europe. As a result, the rooting capacity of the cuttings may revolve around the magnitude of decrease in leaf sugar concentrations during shipment.

Irrespective of the season, the number of subsequently formed roots of unstored and stored cuttings propagated in climate chamber, which demonstrated highly significant positive correlation with pre-insertion leaf sucrose concentrations, also exhibited a similar correlation with pre-insertion leaf 1 sucrose concentrations (Table 1A in Appendix). The present study demonstrated that there exists a highly significant positive correlation between carbohydrate concentrations in the basal 1 cm section of the leaf and carbohydrate concentrations in the corresponding whole leaf (Fig. 5). Therefore, rooting capacity of the cuttings can be predicted by measuring the sugar concentrations in the basal 1 cm section of leaf 1. This information could provide an opportunity in decision making, i.e. whether the cuttings received from southern locations are to be propagated or not.

Sugar analysis by enzyme-coupled colorimetric reaction (Hendrix, 1993) is very accurate and specific, but the analysis requires sample preparation and single determination of each compound, which is time-consuming and expensive analysis. Sugars can be also principally measured accurately, rapidly, and non-destructively using FT-NIR spectroscopy as demonstrated by Rodriguez-Saona *et al*. (2001) in fruit juices. However, the possibility of measuring the same in intact leaves has to be investigated. Thus, with these two methods leaf sugar concentrations can be estimated and thereby rooting capacity can be predicted.

However, in the present study it was shown that, without measuring sugars, rooting capacity can be predicted non-destructively using two approaches. Firstly, with qN values at insertion [because highly significant positive inter-correlation was found between qN of unstored and stored cuttings and leaf sugars of corresponding cuttings (Table 2A in Appendix)]. However, for using qN as a non-destructive physiochemical index in predicting the rooting capacity, further investigation is indispensable to establish whether or not a quantitative relationship exists between qN and leaf carbohydrates. Secondly, with sum of day mean storage temperatures [because highly significant regressions were found between sum of day mean temperatures and leaf sugars depletion during storage (Fig. 25a)]. Therefore, if the sum of day mean temperatures during the shipment of pelargonium cuttings can be measured using some sensitive temperature recorders (like Tiny Talks or temperature tags), it may provide an opportunity to assess the pre-insertion leaf total sugar concentrations in those cuttings. However, in developing this method as a predicting tool, the fine relationship between sum of day mean temperatures and leaf sugar depletion during storage which was found in the present study has to be evaluated under the consideration of various storage temperature regimes. Furthermore, it is also necessary to know the maximum sum of day mean storage temperatures a cutting can experience, and consequently the minimum leaf total sugar levels (especially sucrose) a cutting may possess, for subsequent root formation of a cutting without potentially affecting its survival rate.

Outlook:

1) Using qN as a non-destructive physiochemical index in predicting the rooting capacity requires further investigation, to establish whether or not a quantitative relationship exists between qN and leaf carbohydrates.
2) Developing sum of day mean storage temperatures as a predicting tool also needs further investigation.
3) To generalize the dependencies found in the present study (between carbohydrates and rooting performance of the cuttings) for all pelargoniums needs further investigation using different cultivars.
4) To define the critical light intensity for improved rooting performance of pelargonium cuttings that are imported from southern locations and subsequently propagated in Central European greenhouses during winter season, needs further investigation.

5 SUMMARY

The aim of the present investigation was to provide detailed insight into the interaction between carbohydrate availability and adventitious root formation in pelargonium cuttings, and to establish stabilized relations at least for certain defined basic rooting conditions. In addition, the current study seeks to evaluate the possibility of using chlorophyll fluorescence as a non-destructive index for predicting the rooting capacity of the cuttings. Carbohydrate levels in leaves and basal stems, chlorophyll fluorescence parameters, chlorophyll quality and quantity, and net photosynthetic rates of the cuttings were analyzed during propagation.

The salient findings of the present study are as follows:

Pre-insertion condition of cuttings:

1) Leaf carbohydrate levels in 'Isabell' cuttings were less influenced by season of stock plant cultivation. Cold-storage significantly reduced all the carbohydrate fractions, especially starch, which was reduced to trace amounts as early as 1 day after storage.
2) Basal stem carbohydrate levels in 'Isabell' cuttings were higher in summer when compared to those in spring and winter, which was predominantly due to higher starch in summer and its absence in the other two seasons. In 'Isabell' cuttings, cold-storage significantly reduced basal stem sugar levels in spring and winter but not in summer. In contrast, starch remained at the same level or increased (as at second harvest in summer). In 'Mitzou' cuttings, cold-storage resulted in the decrease of sugars, but starch remained unaffected.
3) F_v/F_m at harvest and after cold-storage was lower than the optimal F_v/F_m of stock plants. Both photochemical quenching (qP) and non-photochemical quenching (qN) of chlorophyll fluorescence were reduced by cold-storage at all occasions.

Post-insertion performance of cuttings:

4) Chlorophyll content of 'Isabell' cuttings was not principally influenced by both cold-storage and rooting environment until day 7 during propagation. In 'Mitzou' cuttings, chlorophyll content was lower in stored cuttings when compared to unstored cuttings at day 7.

5) Net photosynthetic rates (mean over propagation) in ‘Isabell’ cuttings were higher for the cuttings propagated in greenhouse compared to those in climate chamber during spring and summer, but in winter there were no significant differences between the two rooting environments.
6) Non-photochemical quenching of chlorophyll fluorescence did not differ between unstored cuttings and stored cuttings during the subsequent course of propagation in any specific rooting environment irrespective of the season. Regardless of storage treatment, qN was higher for the cuttings propagated in greenhouse when compared to that in climate chamber during spring and summer. In contrast, in winter there was no significant difference between the two rooting environments.
7) Leaf carbohydrate levels in stored cuttings were significantly increased during 6 days of propagation and at day 7 there were no differences with the levels in unstored cuttings in any specific rooting environment irrespective of the season. However, irrespective of storage treatment, cuttings propagated in greenhouse had higher levels when compared to those in climate chamber during spring and summer. In contrast, during winter, cuttings propagated in climate chamber had higher levels in stored cuttings, but in unstored cuttings there were no differences between the rooting environments.
8) In ‘Isabell’ cuttings, basal stem carbohydrate levels in stored cuttings did not exhibit differences with the levels in unstored cuttings at day 7 in any specific rooting environment irrespective of the season, except in greenhouse during winter, where stored cuttings had lower levels. When cuttings were rooted in greenhouse, total sugars were increased by 6.7 mg/g FM, 5.5 mg/g FM, and 4.1 mg/g FM in spring, summer, and winter, respectively. As a consequence, the differences which existed at harvest for total sugar levels among different seasons persisted at the same magnitude during 6 days. When rooted in climate chamber, however, total sugars were increased by 2.8 mg/g FM, 1.4 mg/g FM, and 4.9 mg/g FM in spring, summer, and winter, respectively. As a result, the differences which existed at harvest were minimized during 6 days of propagation. The carbohydrate levels at day 7 were significantly higher in the cuttings propagated in greenhouse when compared to those in climate chamber during spring and summer, but in winter there were no significant differences between the two rooting environments. In ‘Mitzou’ cuttings, either in unstored or in stored cuttings, basal stem carbohydrates remained at the same level during 6 days of propagation. At day 7, stored cuttings had lower carbohydrate levels when compared to

unstored cuttings. Starch either remained at the same level or decreased during 6 days of propagation in both cultivars.

Rooting performance and dependencies

9) In ‘Isabell’ cuttings, irrespective of the season, the number of subsequently formed roots was significantly lower in stored cuttings than in unstored cuttings when rooted in climate chamber. In greenhouse, by contrast, there was no significant difference between unstored and stored cuttings, except in winter, where stored cuttings formed lower number of roots. In ‘Mitzou’ cuttings, principally there were no significant differences in root number between unstored and stored cuttings.
10) In ‘Isabell’ cuttings, highly significant positive correlations were found between both leaf sucrose levels and qN at insertion and number of subsequently formed roots when unstored and stored cuttings were rooted in climate chamber irrespective of season. In greenhouse, however, they were correlated only in winter. Regardless of season, a highly significant positive correlation was obtained between mean basal stem sucrose (day 0 + day 7) and root number when stored cuttings were rooted in greenhouse and climate chamber. The significant positive linear correlation between mean leaf sucrose (day 0 + day 7) and the number of subsequently formed roots explained the whole range of variation in root number caused by season of stock plant cultivation, stock plant age, cold-storage treatment, and rooting environment. In ‘Mitzou’ cuttings, in contrast, no correlations were found between root number and both carbohydrates and quenching coefficients.
11) Results indicate that even when basal stem carbohydrate levels are high at insertion, adventitious root formation of pelargonium cuttings is predominantly influenced by basipetal translocation of carbohydrates during propagation, derived from both pre-insertion leaf carbohydrate reserves and current photosynthesis, the latter of which is itself substantially affected by rooting environment.

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7 APPENDIX

Fig. 1A. Picture showing the device for measurement of chlorophyll fluorescence (Photosynthetic yield analyzer MINI-PAM).

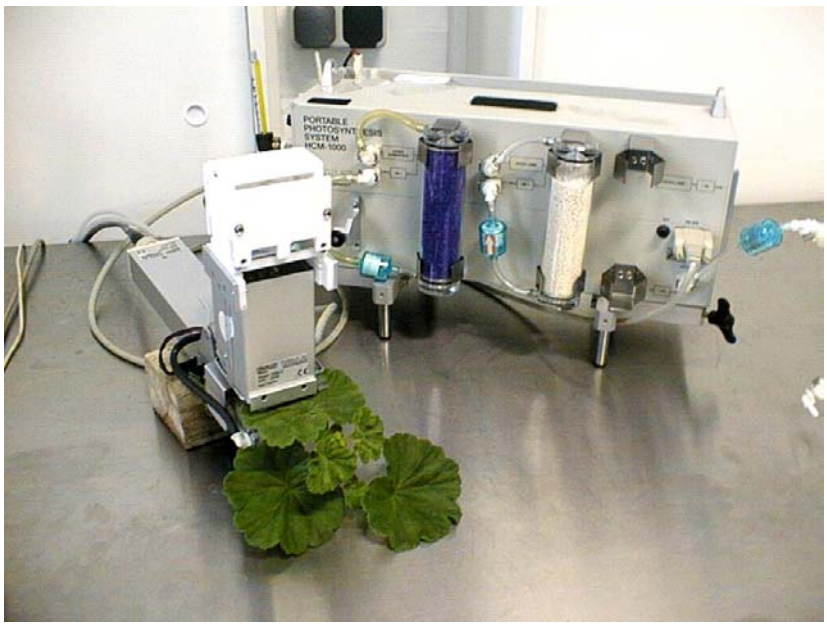

Fig. 2A. Picture showing the device for measurement of photosynthetic rate (Portable Photosynthesis System, HCM-1000

Table 1A: Correlation coefficients, calculated between pre-rooting (day 0) carbohydrate concentrations in leaves of different ages (leaf 1 and leaf 2) of 'Isabell' cuttings propagated during spring, summer and winter seasons as independent variables and number of subsequently formed adventitious roots as dependent variables. Pooled data of unstored and stored cuttings propagated in greenhouse or climate chamber

Carbohydrate concentrations	Leaf age	Green house (day 0)				Climate chamber (day 0)			
		Spring (n=18)	Summer (n=18)	Winter (n=18)	All seasons (n=54)	Spring (n=18)	Summer (n=18)	Winter (n=18)	All seasons (n=54)
Glucose	L1	n.s.	n.s.	0.83***	n.s.	0.56*	n.s.	0.69**	0.43**
	L2	n.s.	n.s.	0.88***	n.s.	n.s.	n.s.	0.75***	n.s.
Fructose	L1	n.s.	n.s.	0.79***	n.s.	0.50*	0.56*	0.77***	0.49***
	L2	n.s.	n.s.	0.87***	n.s.	n.s.	n.s.	0.63**	n.s.
RS	L1	n.s.	n.s.	0.82***	n.s.	0.53*	n.s.	0.74***	0.47***
	L2	n.s.	n.s.	0.88***	n.s.	n.s.	n.s.	0.68**	n.s.
Sucrose	L1	n.s.	n.s.	0.91***	0.37**	0.62**	0.57*	0.79***	0.61***
	L2	n.s.	n.s.	0.90***	0.38**	0.68**	0.53*	0.80***	0.58***
TS	L1	n.s.	n.s.	0.89***	0.30*	0.59**	0.55*	0.79***	0.57***
	L2	n.s.	n.s.	0.93***	0.28*	0.55*	n.s.	0.79***	0.45***
Starch	L1	n.s.	n.s.	0.77***	n.s.	0.58*	n.s.	0.74***	0.56***
	L2	n.s.	n.s.	0.70**	n.s.	0.57*	n.s.	0.82***	0.49***
TNC	L1	n.s.	n.s.	0.84***	n.s.	0.61**	0.48*	0.77***	0.59***
	L2	n.s.	n.s.	0.84***	n.s.	0.61**	n.s.	0.85***	0.51***

RS (Reducing sugars): Glucose + Fructose; TS (Total sugars): RS + Sucrose, TNC (Total non-structural carbohydrates): TS + Starch;
n.s.: non significant; ***: $p \leq 0.001$; **: $p \leq 0.01$; *: $p \leq 0.05$.

Table 2A: Correlation coefficients, calculated between photochemical (qP), and non-photochemical (qN) quenching of chlorophyll fluorescence of unstored and stored cuttings at insertion (day 0) as independent variables and pre-rooting leaf carbohydrate concentrations of corresponding cuttings as dependent variables. Data includes three seasons and three harvest dates in each season

Carbohydrates	qP (*n*=54)	qN (*n*=54)
Glucose	0.50***	0.80***
Fructose	0.46***	0.79***
Reducing sugars (RS)	0.49***	0.81***
Sucrose	0.55***	0.88***
Total sugars (TS)	0.54***	0.88***
Starch	0.65***	0.89***
Total non-structural carbohydrates (TNC)	0.63***	0.93***

RS: Glucose + Fructose; TS: RS + Sucrose; TNC: TS + Starch; ***: $p \leq 0.001$.

Table 3A: Analysis of variance summaries (F-values) of data for pre-rooting carbohydrates (day 0) in leaves and basal stems of 'Mitzou' cuttings as affected by harvest date (H) and cold-storage (St)

Factors	DF (n-1)	Leaf							Basal stem						
		Glucose	Fructose	RS	Sucrose	TS	Starch	TNC	Glucose	Fructose	RS	Sucrose	TS	Starch	TNC
Harvest (H)	2	8**	4*	6*	8**	16***	15***	22***	n.s	n.s	n.s	15***	n.s	12**	5*
Storage (St)	1	53***	106***	97***	273***	419***	98***	255***	6*	5*	6*	7*	9*	n.s	6*
HxSt	2	n.s	12**	9**	14***	25***	15***	24***	n.s	n.s	n.s	n.s	n.s	n.s	n.s

RS (Reducing sugars): Glucose + Fructose; TS (Total sugars): RS + Sucrose and TNC (Total non-structural carbohydrates): TS + Starch; n.s.: non significant; ***: $p \leq 0.001$; **: $p \leq 0.01$; *: $p \leq 0.05$.

Table 4A: Analysis of variance summaries (F-values) of data for carbohydrates at day 7 during the course of propagation in leaves and basal stems of 'Mitzou' cuttings as affected by harvest date (H) and cold-storage (St)

Factors	DF (n-1)	Leaf							Basal stem						
		Glucose	Fructose	RS	Sucrose	TS	Starch	TNC	Glucose	Fructose	RS	Sucrose	TS	Starch	TNC
Harvest (H)	2	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Storage (St)	1	n.s	6*	n.s	n.s	n.s	n.s	n.s	12**	14**	13**	13**	14**	n.s	14**
HxSt	2	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

RS (Reducing sugars): Glucose + Fructose; TS (Total sugars): RS + Sucrose and TNC (Total non-structural carbohydrates): TS + Starch; n.s.: non significant; **: $p \leq 0.01$; *: $p \leq 0.05$.

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