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Multivariate Analysis of Relations between Preharvest Environmental Factors, Postharvest Morphological and Physiological Factors, and Vase Life of Cut 'Asami Red' Roses

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Rose (*Rosa hybrida* L.) 'Asami Red' plants were grown in a glasshouse for a year and the preharvest environmental parameters, and morphological and physiological parameters of individual cut flowers at harvest and during the postharvest period were recorded. Principal component analysis showed interrelations between the parameters: rose plants grown under "dry" conditions, i.e., high temperature, low relative humidity, and consequent high vapor pressure deficit, produced cut flowers having delayed wilting symptoms, resulting in a long vase life; cut roses with a high transpiration rate in the dark at harvest could not maintain their water relations properly, resulting in a shorter vase life; roses grown under "dry" conditions had small stomata and a low transpiration rate in the dark at harvest. These results indicate that humidity conditions are key preharvest environmental factor affecting the vase life of cut roses, and roses grown under "dry" conditions develop more functional stomata, regulate their water relations properly after harvest, and have a longer vase life. Multiple regression analysis to predict the vase life from preharvest environmental parameters and morphological and physiological parameters at harvest generated a significant equation ($Y = -0.0971 \cdot X_1 + 0.0242 \cdot X_2 - 0.3275 \cdot X_3 - 2.84792 \cdot X_4 - 0.4859 \cdot X_5 + 15.397$, where Y is the number of days of vase life; X_1–X_5 are the daily minimum relative humidity, ratio of the stem diameter of the neck and cut end, stomatal width, water potential in the light, and transpiration rate in the dark, respectively; $R^2 = 0.618$; $P < 0.001$).

Key Words: multivariate analysis, preharvest environment, stomata, transpiration, vase life of cut rose.

Introduction

The flower market has attached the greatest importance to the appearance of cut flowers, such as stem length, and flower color, and shape. Recently, longevity has been considered of great commercial value as the need to guarantee a longer vase life increases.

The vase life of cut roses varies not only between cultivars but also between seasons. Cut roses show many kinds of senescence symptoms that reduce the ornamental value; bent neck and petal wilting are the most common disorders. Preharvest environmental factors and consequent morphological and physiological characteristics of cut flowers influence the vase life, but their relations are complicated. The longevity, i.e., potential vase life, of cut roses primarily depends on the genetic characteristics of the varieties (Ichimura et al., 2002; Mayak et al., 1974; Mortensen and Gislerød, 1999). A constantly high humidity before harvest reduces the vase life of cut roses (Mortensen and Fjeld, 1995; Mortensen and Gislerød, 2000; Torre and Fjeld, 2001). Among postharvest factors, vapor pressure deficit (VPD) and atmospheric temperature certainly affect the vase life of cut roses (Doi et al., 2000a, 2000b).

Techniques to predict the potential vase life of cut roses are effective means to grade cut flowers and guarantee the vase life of flowers bought by consumers. First, clarifying the factors that mainly influence the fluctuation of vase life is necessary. Most experiments with preharvest factors affecting the vase life of cut roses used controlled, constant environmental conditions (Mortensen and Fjeld, 1995; Mortensen and Gilslerød, 1999, 2000; Torre and Fjeld, 2001; Torre et al., 2003), except for a study by Slootweg et al. (2001) who observed a seasonal

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change in rose vase life.

Factors affecting rose vase life can be separated into three stages: production (preharvest), marketing (at harvest), and retailing and consumption (postharvest). In this study, we cultivated roses in a glasshouse for a year and recorded various preharvest environmental parameters, and morphological and physiological parameters of individual cut flowers at harvest and during the postharvest period in the vase. We analyzed the interrelations between the parameters obtained and the vase life using multivariate analysis and then established a multiple regression model to predict the vase life at harvest.

Materials and Methods

The parameters prepared for the analysis and their abbreviations in this study are shown in Table 1.

Cultivation and preharvest environmental data

Young plants of *Rosa hybrida* L. 'Asami Red' (syn. 'Rote Rose') were planted on rockwool slabs in a glasshouse in October 2003. The plants were trained using an "arching" method and drip-irrigated hourly with half strength Enshi formula nutrient solution. The glasshouse was heated to 16°C and ventilation was provided

Table 1. Preharvest environmental parameters and morphological and physiological parameters of cut roses at harvest and postharvest used for analysis.

Parameter	Abbreviation	Unit	Note
Preharvest			
Daily integrated PPF	PPF	$mmol \cdot m^{-2}$	total of 15 days before harvest
Daily maximum temperature	Tem-Max	°C	average of 15 days before harvest
Daily minimum temperature	Tem-Min	°C	average of 15 days before harvest
Daily average temperature	Tem-Ave	°C	average of 15 days before harvest
Daily maximum relative humidity	RH-Max	%	average of 15 days before harvest
Daily minimum relative humidity	RH-Min	%	average of 15 days before harvest
Daily average relative humidity	RH-Ave	%	average of 15 days before harvest
Daily maximum vapor deficit	VPD-Max	kPa	average of 15 days before harvest
Daily minimum vapor deficit	VPD-Min	kPa	average of 15 days before harvest
Daily average vapor deficit	VPD-Ave	kPa	average of 15 days before harvest
At harvest			
Fresh weight	FW	g	
Stem length	Len-Stem	cm	
Stem diameter of neck	Dia-Stem-Ne	cm	
Stem diameter of cut end	Dia-Stem-En	cm	
Ratio of stem diameter	Rat-Dia-Stem	%	="Dia-Stem-Ne"/"Dia-Stem-En"
Leaf Area	Area-Leaf	cm^2	
Stomatal length	Len-Stom	mm	at Dark 0; including guard cell
Stomatal width	Wid-Stom	mm	at Dark 0; including guard cell
Stomatal density	Den-Stom	mm^{-2}	
Ratio of stomatal size	Rat-Stom	%	="Wid-Stom"/"Len-Stom"
Transpiration rate in the dark	Tran-Dar	$mg \cdot cm^2 \cdot s^{-1}$	at Dark 0
Transpiration rate in the light	Tran-Lig	$mg \cdot cm^2 \cdot s^{-1}$	at Light 90
Ratio of transpiration rate	Rat-Trans	%	="Trans-Dar"/"Trans-Lig"
Water potential in the dark	WatPot-Dar	MPa	at Dark 0
Water poteintial in the light	WatPot-Lig	Mpa	at Light 90
Ratio of water potential	Rat-WatPot	%	="WatPot-Dar"/"WatPot-Lig"
Brix of leaf	Brix	%	at Dark 0
Postharvest			
Maximum relative fresh weight	RFW-Max	%	
Daily transpiration on the intial day	DTran-Init	$mg \cdot cm^{-2} \cdot day^{-1}$	
Number of days to maximum fresh weight	Days-FW-Max	day	
Number of days keeping intial fresh weight	Days-FW-Init	day	Calculated from a formula (see Fig. 1)
Number of days keeping intial water uptake	Days-WatUpt-Init	day	
Number of days keeping water balance	Days-WatBar	day	water balance=water uptake - transpiration
Rate of decrease in relative fresh weight	Rat-Dec-RFW	$\%\cdot day^{-1}$	Calculated from a formula (see Fig. 1)
Number of days of vase life	VL	day	

Preharvest parameters are averages of daily values for 15 days before harvest.

automatically when the air temperature in the glasshouse exceeded 25°C. From May 28 to September 20, 2004, the plants were shaded by a layer of translucent plastic film (permeability: 60%) to avoid severe irradiation.

We set thermo recorders (RS-11, Espec Mic, Aichi, Japan) at a distance of 2 m along the slabs and logged the local temperature and relative humidity (RH) at intervals of 10 min. The local photosynthetic photon flux (PPF) was measured using an OPTLEAF sensor system (R-2D film and THS-470 T-METER, Taisei E&L, Tokyo, Japan), and the data were standardized according to the value of a PPF radiation sensor (IKS-27, Koito, Yokohama, Japan) connected to a data logger (HR-1300, Yokogawa, Tokyo, Japan). The VPD was calculated from the corresponding temperature and RH.

We used 104 cut roses harvested from March 2004 to February 2005. We conducted a preliminary investigation, from which the decision was made that the environmental parameters assigned for the individual cut flowers were to be decided on for 15 days before their harvest with the nearest thermo recorders (Table 1).

Harvesting and morphological and physiological data at harvest

This stage corresponds to the time from harvest to auction at market by transportation. Cut flowers at normal maturity (the top of buds dehiscing) were harvested at 17:00. The cut flowers were immediately placed in a bucket containing tap water and carried to our laboratory. The stems were trimmed to 50, 60, or 70 cm long and to the five uppermost foliage leaves with three or five leaflets. The base of each cut stem was placed in a sterilized glass jar containing 500 mL distilled water through a hole (1 cm diameter) in the center of a plastic cap. The cut flowers were kept at 25°C and 50% RH in the dark for more than 12 h. At 09:00 on the next day after harvest, the size (length and width) and density of stomatal apparatus, transpiration rate, brix, and leaf water potential were measured for the dark condition (Dark 0). Then, the cut flowers were irradiated using three-band fluorescent lamps at 80 $\mu mol \cdot m^{-1} \cdot s^{-1}$ for 90 min up to 10:30 (Light 90), and the transpiration rate and leaf water potential were measured again. The room temperature and RH were kept at 25°C and 50%, respectively, throughout these measurements.

The transpiration rate was measured using a steady state porometer (LI-1600, LI-COR, Lincoln, NE, USA) fitted on a terminal leaflet of the uppermost three-leaflet leaf. For the measurement of water potential, a fresh leaf disk of 5 mm diameter sampled from the second uppermost three-leaflet leaf, was placed in a sample chamber (C-52, Wescor, Logan, UT, USA) connected to a microvolt meter (HR-33T, Wescor), and was allowed to stand for 2 h to equilibrate. The dew point of the headspace was measured according to the instrument's instruction manual.

The stomatal size and density of the adaxial side of the lowermost five-leaflet leaf were measured using a "sump" method. Images of the leaf surface impressions were taken using a digital camera (Coolpix 4500, Nikon, Tokyo, Japan) connected to an optical microscope (model B202, Olympus, Tokyo, Japan). The stomatal size and density were calculated using Scion Image ver. 4.02 (Scion, Frederick, MD, USA.) software on a personal computer.

A tissue sample (0.1 g) of the lowermost leaf was ground in a mortar with 0.9 mL distilled water and the resulting liquid was used to find the brix (%) using a digital refractometer (PR-101, Atago, Tokyo, Japan).

When the vase life ended, all leaves were sampled and scanned using an image scanner (FB1210U, Canon, Tokyo, Japan) to find the leaf area.

Postharvest morphological and physiological data

Postharvest parameters were collected during the vase life evaluation. After the measurements at harvest, the cut roses were transferred to a reference room at 25°C, 50% RH, and a photoperiod of 12 h supplied by fluorescent tubes at 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity. Cut flowers and vase water were weighed every day and the daily water uptake and transpiration rates were calculated. The vase life was regarded to end when a bent neck or petal wilting, i.e., loss of petal turgor, occurred; all cut flowers showed these symptoms before petal abscission in this study.

We approximated the changes in fresh weigh of individual cut flowers after harvest using a quadratic formula against the number of days of evaluation (Fig. 1), and then "the number of days keeping initial fresh weight" and "the rate of decrease in relative fresh weight" were read from the formula.

Principal component analysis

To clarify the relations between the parameters and vase life, three steps of principal component analysis (PCA) were performed using SPSS 13.0 software (SPSS Japan, Tokyo, Japan): 1) preharvest parameters vs vase life, 2) parameters at harvest and postharvest parameters

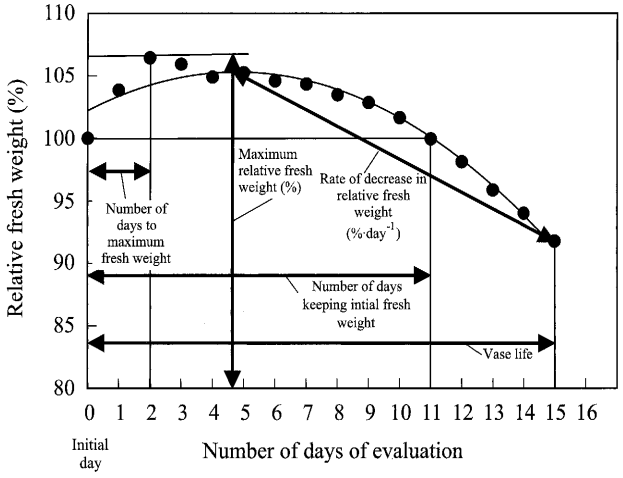

Fig. 1. Change in cut flower fresh weight during the postharvest period and related parameters. The values are represented as the percentage of the fresh weight on the initial day.

vs vase life, and 3) preharvest parameters vs parameters at harvest. Table 1 lists the parameters used and their abbreviations. In the PCA, the data were standardized using a correlation matrix.

Multiple regression analysis

Independent from the PCA, we performed a multiple regression analysis (MRA) of vase life against preharvest environmental parameters and morphological and physiological parameters at harvest using SPSS 13.0 software. An all-possible subset selection method was used to select parameters.

Results

Relations between preharvest environmental parameters and vase life

Fig. 2A–D shows the changes in PPF, temperature, RH, and VPD in the glasshouse. The actual temperature in winter fell to about 10°C occasionally despite the heating. VPD-Max was high in summer and low in winter. The vase life (VL) was longer in spring and summer and gradually decreased from August to January (Fig. 2E).

Fig. 3 shows the PCA results for preharvest parameters and VL. The contributions of the first and second components were 51.5% and 27.7%, respectively. In the first component (Fig. 3A), VPD-Max, VPD-Ave, Tem-Ave, Tem-Max, Tem-Min, PPF, and VL showed large positive eigenvectors; RH-Min and RH-Ave showed large negative eigenvectors and VPD-Min and RH-Max showed small eigenvectors. In the second component (Fig. 3B), the eigenvector of VL was very small.

Relations between morphological and physiological parameters and vase life

PCA was performed for parameters at harvest and of the postharvest period from cut flowers (Fig. 4). The proportions of the first and second components were 20.7% and 15.4%, respectively. VL showed the greatest negative eigenvector in the first component. Tran-Dar, Rat-Stom, Rat-Trans, and Wid-Stom at harvest and DTran-Init and Rat-Dec-RFW of the postharvest period showed large positive eigenvectors; Rat-Dia-Stem at harvest and Days-WatUpt-Init, Days-WatBar, RFW-Max, and Days-FW-Init of the postharvest period showed

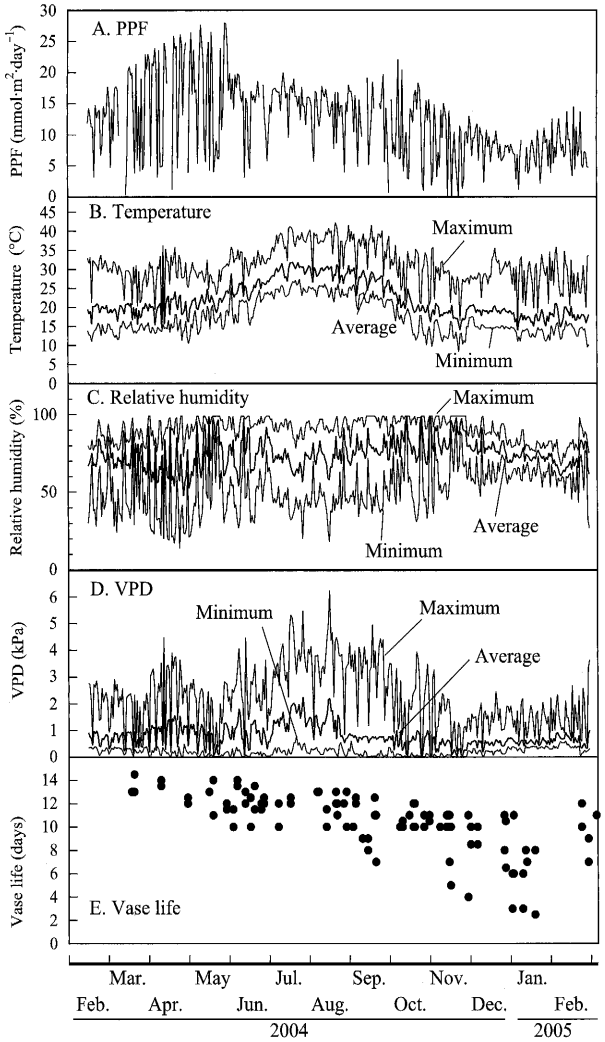

Fig. 2. Changes in PPF, temperature, relative humidity, and vapor pressure deficit in the glasshouse, and vase life of cut flowers.

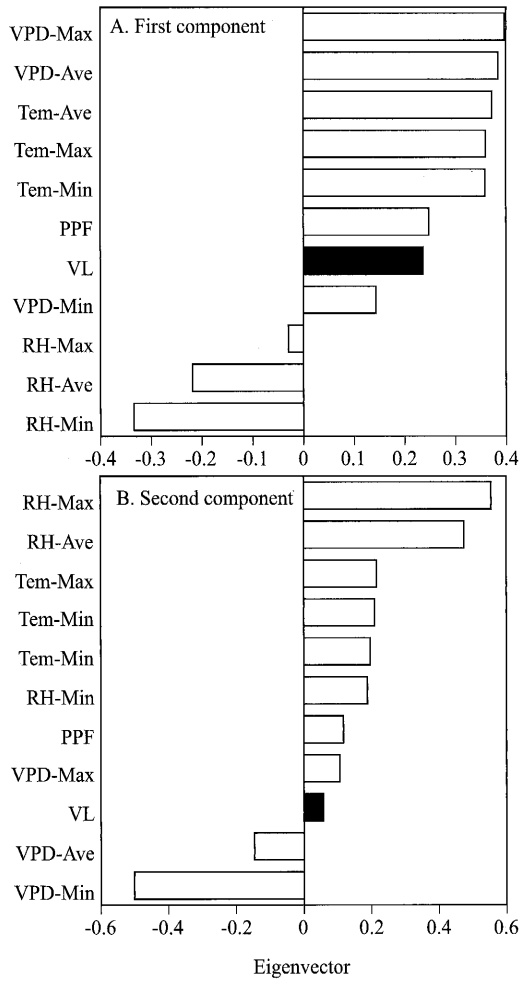

Fig. 3. Eigenvectors of the first and second components in the principal component analysis of preharvest parameters and VL. The proportions were 51.5% and 27.7% for the first and the second components, respectively.

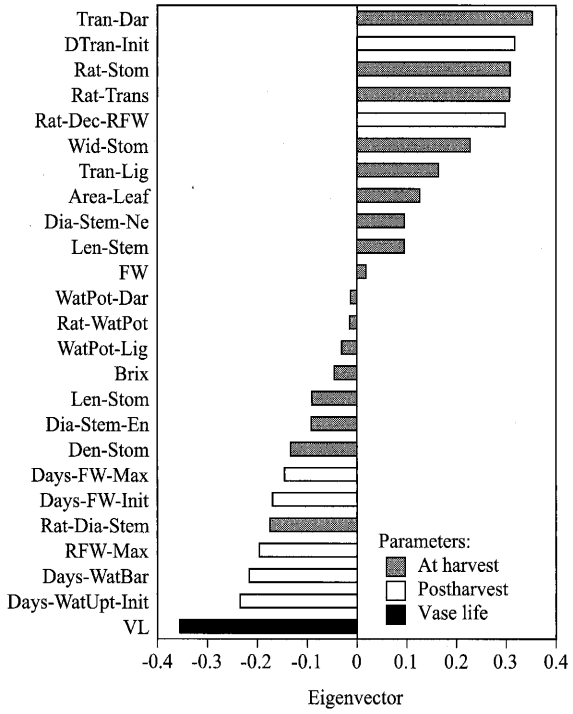

Fig. 4. Eigenvectors of the first component in the principal component analysis of morphological and physiological parameters at harvest and of the postharvest period, and vase life. The proportion was 20.7%.

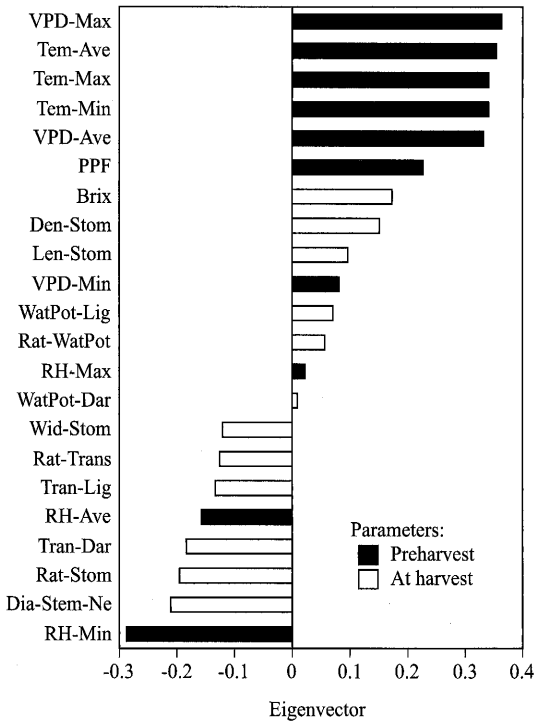

Fig. 5. Eigenvectors of the first component in the principal component of preharvest parameters and morphological and physiological parameters at harvest. The proportion was 30.2%.

large negative eigenvectors. In the second component, the eigenvector of VL was too small to assess (data not shown).

Relations between preharvest parameters and morphological and physiological parameters at harvest

For this PCA, FW, Len-Stem, Dia-Stem-En, Rat-Dia-Stem, and Are-Leaf were excluded because they were modified artificially before the vase life evaluation. The proportions of the first and second components were 30.2% and 15.4%, respectively. In the first component, preharvest parameters such as VPD-Max, Tem-Ave, Tem-Max, Tem-Min, VPD-Ave, and PPF showed large positive eigenvectors and RH-Min showed a large negative eigenvector (Fig. 5). Parameters at harvest such as Dia-Stem-Ne, Rat-Stom, and Tran-Dar were large negative eigenvectors and Brix was a large positive eigenvector. In the second component, the eigenvectors did not show any meaningful trend (data not shown).

Multiple regression analysis

As a result of MRA, five parameters were selected and a regression formula was generated as follows (Fig. 6):

$Y = -0.0971 \cdot X_1 + 0.0242 \cdot X_2 - 0.3275 \cdot X_3 - 2.84792 \cdot X_4 - 0.4859 \cdot X_5 + 15.397$ ($R^2 = 0.618$, $P < 0.001$), where Y is, VL; X_1–X_5 are RH-Min, Rat-Dia-Stem, Wid-Stom, WatPot-Lig, and Tran-Dar, respectively. The 95% confidence interval was ±3 days.

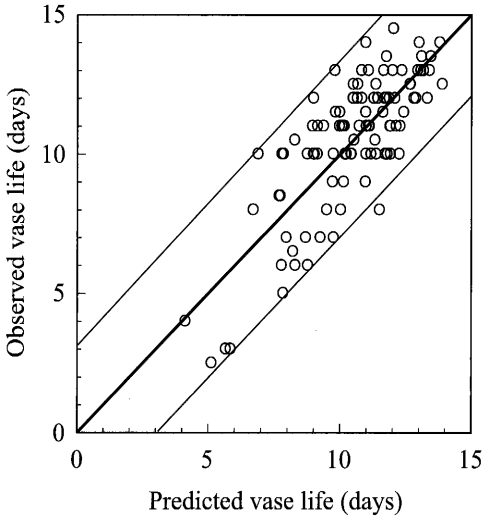

Fig. 6. Relation between the number of days of vase life predicted by multiple regression and observed ones. $Y = -0.0971 \cdot X_1 + 0.0242 \cdot X_2 - 0.3275 \cdot X_3 - 2.84792 \cdot X_4 - 0.4859 \cdot X_5 + 15.397$ ($R^2 = 0.618$, $P < 0.001$), where Y is VL; X_1–X_5 are RH-Min, Rat-Dia-Stem, Wid-Stom, WatPot-Lig, and Tran-Dar, respectively. The thin lines are 95% confidence intervals.

Discussion

We evaluated seasonal changes in the vase life of 'Asami Red' roses under fixed postharvest conditions (25°C, 50% RH) for a year. Many cut flowers had a short (10 days or less) vase life from November 2004 to January 2005 (Fig. 2), which is consistent with the observation by Slootweg et al. (2001). The growing conditions in this period were characterized by low irradiance, low

temperatures, and a high daily minimum RH (Fig. 2A–C). Low temperature and high humidity resulted in a low VPD (Fig. 2D).

We examined relations between the preharvest environmental parameters and VL using PCA (Fig. 3). The contribution of the first principal factor was rather high at 51.5%. In this principal factor, it is axiomatic that close interrelations existed among temperature, RH, and VPD. High temperature and high irradiance were accompanied by low RH and high VPD; such conditions should be referred to as "dry". VL also showed a large eigenvector in this principal factor. In the second principal factor, of which the contribution was 27.7%, the eigenvector of VL was so small that parameters closely related with vase life could not be detected. In the first principal factor, the directions of the vectors (positive or negative) of the environmental parameters suggest that rose plants grown under "dry" conditions produce cut flowers with a long vase life. Doi et al. (2000a) reported that the prevailing postharvest vapor pressure is proportional to the transpiration rate of leaves of cut stems. Our study shows that the preharvest vapor pressure may also affect physiological characteristics of cut flowers. Before this study, we expected that a high preharvest temperature reduces vase life, but we detected no negative effects of temperature on vase life in any of the principal factors (data not shown). Motomura et al. (2002) reported that seasonal changes in vase life were due to postharvest environmental conditions, and that a low humidity and high temperature during the postharvest period are major causes of a short vase life in summer; therefore, postharvest handling (storage and transportation) of cut roses in summer is to be crucial.

A PCA was performed for the morphological and physiological parameters that were obtained at harvest and during the postharvest period, and vase life (Fig. 4). The contribution of the first principal factor was considerably low (20.7%), reflecting the complicated and unclear interrelations between the parameters. However, favorably, VL showed the largest negative eigenvector in the first principal factor (Fig. 4), suggesting that this component indicates parameters closely related to vase life. Cut flowers having a high dark transpiration rate (Tran-Dar) and large stomata (Wid-Stom and Rat-Stom) could not regulate their water relations properly, typically shown by small Days-WatUpt-Init and Days-WatBar; this trend clearly coincided with a shorter vase life. Leaves that draw water from petals as well as from vase water (Hu et al., 1998; Mayak et al., 1974) contribute to most of the transpiration of cut roses. Generally, roses open their leaf stomata in light, close them in dark, and maintain diurnal stomatal transpiration rhythms in a daily light-dark cycle (Doi et al., 1999; Mayak et al., 1974). Excessive transpiration at night results in failure in the recovery of water balance that worsens during the daytime and causes bent neck and petal wilting (Blom-Zandstra et al., 1995; De Stigter, 1980; Doi et al., 2000a, 2000b; Torre and Fjeld, 2001). Previous studies attributed a deterioration of water relations to the plugging or cavitation of xylem vessels caused by microorganisms (Burdett, 1970; Mensink and Van Doorn, 2001; Van Doorn, 1995; Van Doorn and De Witte, 1991; Van Doorn and Suiro, 1996). However, the seasonal fluctuations in vase life detected in our study were unlikely to be caused by xylem plugging, because we used sterilized vases, clean distilled water, and identical reference room conditions; they may have been caused by congenital transpiration characteristics (Van Doorn and De Witte, 1997).

Preharvest environmental parameters and the morphological and physiological parameters at harvest were relevant to a considerable extent (Fig. 5). The contribution of the first principal factor in PCA was 30.2%. The low transpiration rate in the dark just after harvest was attributed to a "dry" condition (Fig. 5). Plants grown under high humidity experience no water stress and may lose the function of stomatal closure (Torre and Fjeld, 2001). Roses grown in winter fail to close stomata and show remarkable water loss, resulting in a short vase life (Slootweg et al., 2001). A high, constant, preharvest humidity results in large stomata of rose leaves (Torre et al., 2003), and we observed large Rat-Stom, i.e., round stomatal apparatuses in roses grown under low humidity.

It is interesting that RH-Max and VPD-Min were scarcely related to VL, whereas RH-Min and VPD-Max were related to VL markedly (Fig. 3). Therefore, a "constantly" low preharvest humidity may not be required to maintain the stomatal function, and this point is worth further clarification. The stem diameter of the neck (Dia-Stem-Ne) tends to be short at a high VPD or high temperature, or both (Fig. 5), but vase life (VL) was hardly correlated with stem diameter in our study (Fig. 4).

Leaves are a source of nutrient materials, such as sugars for the corolla in intact and cut roses (Nichols and Ho, 1979). The sugar content can explain varietal differences in vase life (Ichimura et al., 2002). As we expected, brix, that reflects the sugar content, was affected by preharvest PPF (Fig. 5). However, unexpectedly, a relation between brix and VL was not detected in our experiment. Moreover, seasonal changes in brix were relatively slight within the cultivar 'Asami Red'. Exogenous application of sugars to cut roses markedly improves vase life (Ichimura et al., 2003), while fluctuations in the congenital sugar content of intact stems may only slightly affect vase life.

We concluded from the PCAs that humidity is the key preharvest environmental factor affecting the vase life of cut 'Asami Red' roses. Roses grown under a "dry" condition can have functional stomata, regulate their water relations properly after harvest, and have a long vase life. Hence, we tried to predict the vase life from preharvest environmental parameters and morphological and physiological parameters at harvest using multiple regression analysis; we could obtain a significant ($R^2 = 0.618$, $P < 0.001$) equation (Fig. 6). However, a 95% confidence interval for ±3 days is not enough to guarantee

the vase life practically. Moreover, we used an all possible subset selection method to avoid multicollinearity, and parameters such as Rat-Dia-Stem and WatPot-Lig that were not significant in the results of PCAs were chosen. Now, we realize the limitations in applying statistical methods to the complicated system of the vase life of cut roses for estimation and prediction. For an accurate and practical vase life prediction, we have to examine more competent data processing methods, such as non-parametric methods and mechanistic modeling, in addition to rapid and nondestructive detection techniques at harvest to measure transpiration that is strongly related to rose vase life.

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