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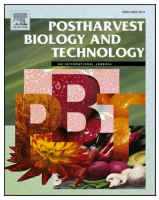

Prolonged vase life by salicylic acid treatment and prediction of vase life using petal color senescence of cut lisianthus

Hye Sook Kwon [a], Cody Leporini [b], Steven Kim [b], Seong Heo [a,*]

[a] Department of Horticulture, Kongju National University, Yesan 32439, South Korea
[b] Department of Mathematics and Statistics, California State University, Monterey Bay, Seaside, CA 93955, USA

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ABSTRACT

This study investigated the effects of exogenous salicylic acid (SA) concentrations (0, 0.1, 0.3, or 0.5 mM), treatment timing (vegetative or reproductive period), and cultivation methods (soil or hydroponic cultivation) on the vase life of four lisianthus cultivars after flower cutting. A 0.5 mM concentration of the SA application during the reproductive period resulted in the longest average vase life of 15.3 d for the Blue Picote cultivar after flower cutting. A colorimeter and a chlorophyll meter were used to observe and quantify petal color changes after cutting, and the color changes during the senescence stages are visualized using a three-dimensional color space. The three-dimensional visualization demonstrated the color senescence for each measurement period. Based on these data, we used automated machine learning algorithms to predict the vase longevity, and the predictive model showed a good accuracy. This model is expected to be widely used in the floricultural industry.

1. Introduction

Lisianthus (*Eustoma grandiflorum*) is one of the most important commercial cut flowers, and it is becoming increasingly valuable in flower markets worldwide (Bahrami et al., 2013; Darvish et al., 2021). Lisianthus originated in the United States and Mexico (López-Guerrero et al., 2020), and its consumer acceptance has grown rapidly in recent years owing to its varied colors and rose-like appearance. Like most cut flowers, lisianthus has a short vase life (Darvish et al., 2021; López-Guerrero et al., 2020), and this is highly variable depending on genetic (Bahrami et al., 2013; Shimizu and Ichimura, 2005) and environmental factors (El-Esawi et al., 2017; Idrees et al., 2010).

Cut flowers have a limited vase life because the senescence process occurs after cutting. Flower senescence is commonly accompanied by morphological, physiological, and biochemical deterioration (Ezhilmathi et al., 2007; Kazemi et al., 2018). It is induced by ethylene biosynthesis, which in turn, enhances oxidative stress related to the production of reactive oxygen species (ROS) and free radicals (Alaey et al., 2011). Additionally, ethylene activates the gene expression and enzyme activity of degrading enzymes such as lipoxygenases, lipases, pectinases, proteinases, polygalacturonases, and chlorophyllases (Darvish et al., 2021; Hurr et al., 2010; Iqbal et al., 2017; Liu et al., 2018). In addition, postharvest longevity of cut flowers is influenced by phenomena such as loss of water and nutrients due to increased respiration and pathogen infection. Bacteria and fungi cause vascular occlusion, hindering the transport of water and conservative solutions (Da Costa et al., 2021; Gómez-Merino et al., 2020; Yadeta and Thomma, 2013). Flowers are short-lived organs, and the most important quality of an ornamental plant is the longevity of its vase life while maintaining a fresh appearance.

Many researchers and growers have experimented with various plant growth regulators to extend the vase life of cut flowers. Salicylic acid (SA) has been widely applied in the industry to extend the vase life of roses, gladiolus, carnation, and other cut flowers (Ezhilmathi et al., 2007; Ghadimian and Danaei, 2020; Kazemi et al., 2011; Marandi et al., 2011). For instance, the treatment of roses with a 0.1 μM SA increased the expected vase life by six days compared to the control (Gerailoo and Ghasemnezhad. 2011), and the treatment of lisianthus with a 100 mg L^{-1} SA increased the expected vase life by four days and reduced wilting rates by 14% (Pourzarnegar et al., 2020).

The SA is an endogenous hormone, which plays an important role in systemic acquired resistance against pathogens and in acclimation to abiotic stresses (Bauters et al., 2021; Jandra et al., 2014). It is known to inhibit ethylene production by suppressing the activities of 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase (Kumar et al., 2023), thus it is known to delay the senescence process. The SA

* Corresponding author.
E-mail address: heoseong@kongju.ac.kr (S. Heo).

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treatment also increases the formation of ROS, reducing the number of PSII centers (Jandra et al., 2014) and inhibiting PSII functional activity (Uzunova and Popova, 2000). Moreover, several phenolic compounds, which are induced by SA, directly affect the photosynthetic electron transport chain or indirectly influence the photosynthetic machinery by controlling stomatal conductivity (Jandra et al., 2014). For instance, the application of SA to Arabidopsis leaves rapidly induced stomatal closure (Mateo et al., 2004). Depending on the concentration, duration, and method of SA application, as well as environmental conditions (e.g., temperature, light), the photosynthetic activity can be increased or decreased (Jandra et al., 2014). The SA treatment does not always increase chlorophyll with respect to its concentration. In sunflower cotyledons, the SA treatment at 0.001–10 μM increased chlorophyll and carotenoid contents; nonetheless, the treatment at 1 mM decreased them (Çağ et al. 2009; Jandra et al., 2014). As such, the effects of SA may be highly dependent on the concentration and other environmental factors.

Researchers have attempted to predict the postharvest longevity of cut flowers, but such predictive analyses are rare in literature. In et al. (2009) applied a neural network model to predict the vase life of cut roses, and it showed high predictive performance with an R-squared (R^2) value of 0.835. This model utilized the various morphological, physiological characteristics, and environmental parameters of cut roses at the harvest stage. Similarly, Choi and Lee (2020) recently developed a logistic regression model to predict the vase life of cut roses based on thermal images. However, this model solely relied on the thermal images and trained data of only one cultivar. Consequently, it is unknown whether the predictability can be extended to different cultivars. It is expected that the predictive models can be extended to other cut flowers and multiple cultivars, and in this study we attempted to predict the vase life of cut lisianthus with four cultivars.

Our research has three objectives. The first objective is to test the effects of SA treatment on the postharvest longevity of cut lisianthus with various conditions including cultivar (genotype), cultivation method, treatment time, and concentration (environment). We hypothesized that the expected vase life increases within our experimental concentrations (0–0.5 mM) and the effect of SA is different by the treatment time (during the reproductive period or the vegetative period) and method (hydroponics or soil cultivation). The second objective is to describe the degree of senescence over time after cutting of lisianthus based on the colorimetric and SPAD measurements. The third objective is to predict the vase life of cut lisianthus (classification of vase life stage) using automated machine learning (AutoML) for research purposes and practical uses in industry.

2. Materials and methods

2.1. Plant materials

Sixty-five-day-old seedlings of four lisianthus cultivars were planted on June 22, 2022 in the greenhouse of the Pocheon Agricultural Technology Center. The four cultivars were: Arena Green (AG), Blue Picote (BP), Corelli Pink (CP), and Kroma White (KW). These cultivars are currently popular in the market, and they attract consumers by various colors. The greenhouse was kept at an average temperature of 28 °C and an average duration of 13.5 h with natural daylight with shading from 11:00 AM to 2:00 PM when the light intensity is high or artificial light on cloudy days. The shipment of lisianthus was adjusted to coincide with holidays when there is a high demand for flower consumption. To induce rapid flowering, the cultivation of lisianthus was carried out at a temperature higher than the growth-optimal range of 24–26 °C (day) and 15–18 °C (night), specifically at 28 °C and 22 °C, respectively.

In addition, the greenhouse was maintained at a humidity of 65 ± 10% and a CO_2 concentration of 393.3 ppm. Separate beds for hydroponic (H) cultivation and soil (S) cultivation were set up in the greenhouse. One thousand lisianthus seedlings were randomly divided into the S cultivation and H cultivation groups. After cultivation, six samples were randomly selected according to the treatment. This process was repeated three times for each group. The soil for S cultivation was prepared by mixing peat moss, horticultural substrates, perlite, and oil-cake in the following proportions: 0.50: 0.38: 0.10: 0.02. The composite soil samples were analyzed: pH (1:5), 6.9; organic matter (OM), 54 g kg^{-1}; available phosphate (Av. P_2O_4), 790 mg kg^{-1}; exchangeable (Exch.) K, 14.8 $cmol_c$ kg^{-1}; Exch. Ca, 14.8 $cmol_c$ kg^{-1}; Exch. Mg, 4.8 $cmol_c$ kg^{-1}; and electrical conductivity (EC), 2.3 dS m^{-1}.

A 4-component compound fertilizer (N(5.1%), P(10), K(5), and micronutrients), BioNex Liquid Fertilizer (Bio Trading, Busan, Korea), was foliar-applied at 40 d after planting at a rate of 30 mL per application. In addition, the CalBungMa fertilizer (Ca (15.5%), N(5), B (0.1), and Mg (4.1), KoreaAgro, Chungbuk, Korea) were foliar-applied at 50 d after planting at a rate of 30 mL per application. Using a semi-automatic sprayer, the fertilizer standard solution was diluted in 12 L of water and uniformly foliar-applied.

The nutrient solution, based on a volume of 1000 L, was prepared for tank A by combining KNO_3 (303 g), $CaNO_3$ (944 g), and Fe-EDTA (22.62 mg) and for tank B by combining KNO_3 (303 g), $NH_4H_2PO_4$ (115 g), $MgSO_4{\cdot}7\ H_2O$ (492 g), $CuSO_4{\cdot}5\ H_2O$ (78.58 mg), H_3BO_3 (2858.5 mg), $MnSO_4{\cdot}H_2O$ (1538 mg), $ZnSO_4{\cdot}7\ H_2O$ (219.8 mg), and $NH_4Mo_7O_{24}{\cdot}4\ H_2O$ (121.3 mg). The prepared nutrient solution was applied using a drip irrigation system. The nutrient solution was analyzed as follows: pH (1:5), 6.7; OM, 54 g kg^{-1}; Av. P_2O_4, 674 mg kg^{-1}; Exch. K, 13.7 $cmol_c$ kg^{-1}; Exch. Ca, 13.7 $cmol_c$ kg^{-1}; Exch. Mg, 4.4 $cmol_c$ kg^{-1}; and EC, 2.7 dS m^{-1}. The media was composed of peat moss and perlite as 1:1.

The two treatment groups were both cultivated in the same greenhouse; however, there were differences in the moisture and nutrients supplied to the soil. In the S cultivation, lisianthus were planted three at a time in a container of 35 × 30 × 24 cm. For the first two weeks after planting, 2 L of water was supplied four times a week per container, and from the third week onward, the same amount of water was provided three times a week. Watering was consistently done between 9:00 AM and 10:00 AM.

On the other hand, in the H cultivation, lisianthus was replanted in a container measuring 30 × 40 × 2500 cm filled with media. During the first two weeks after planting, only tap water was provided. In the second week, a nutrient solution of 30 mL was supplied five times a day using a drip irrigation system, each lasting 10 s. From the fourth week onwards, a nutrient solution of 40 mL was supplied eight times a day, each lasting 10 s. As a result, lisianthus in the S cultivation occupied a smaller area compared to the H cultivation, and the two treatment groups had different supplied moisture and nutrients. The duration for the first flower to bloom was 56 d for S cultivation and 58 d for H cultivation, and the earlier flowering in the S cultivation might be due to the poor nutrition and drought stress (Takeno, 2016). Moreover, the time to harvest three flowers from each lisianthus was longer in H cultivation than in S cultivation.

2.2. Soil or hydroponic cultivation at vegetative or reproductive period

The hormone treatment was divided into two phases, the vegetative (V) stage and the reproductive (R) stage. The V stage is defined as three weeks after lisianthus seedlings develop roots in stages 1, 2, and 3 after planting. The R stage is defined as the period of SA treatment starting with development of the flower bud, when over 60–70% of flower budding has occurred in the average plant. The SA (Gooworl Co., Daegu, Korea) was dissolved in methyl alcohol as a 100 fold concentrate and then diluted with water for application. The SA was applied to six samples in each group. The experimental concentrations were 0, 0.1, 0.3, and 0.5 mM, and each concentration was applied three times at 3 d intervals, dispensing 10 mL each time between 10:00 AM and 12:00 PM. The control group was irrigated with 10 mL of tap water. For the S cultivation, SA was applied in Week 7 after lisianthus planting, and for the H cultivation, SA was applied in Week 8 after planting. Owing to differences in the timing of blooming in the S and H cultivation groups,

there was a 7 d difference in SA application.

There were four treatment groups based on the combinations of cultivation method and SA treatment timing as follows: SSV (soil cultivation, SA treatment, vegetative stage), SSR (soil cultivation, SA treatment, reproductive stage), HSV (hydroponics, SA treatment, vegetative stage), and HSR (hydroponics, SA treatment, reproductive stage).

2.3. Measuring the vase life of cut lisianthus

To measure the vase life after cutting, the flowers were cut on August 31, 2022 for the S cultivation groups (Day 68) and on September 16, 2022 for the H cultivation groups (Day 83). Considering factors such as flower size and flower opening (whether the flower was 60–70% open), we selected and cut flowers that had a similar blooming phase. The four lisianthus cultivars showed differences in their flowers, stems, and the appearance and location of their leaves. The flower stems were cut to 5 cm to match the stem length between the four cultivars, and all lower leaves were removed. The vase life was measured in a laboratory at a constant temperature of 20.4 °C, relative humidity of 60–76%, and illumination of 7.43–9.45 μmol m^2 s^{-1}. The water in the vase was sourced from tap water in the laboratory, and it was replenished with freshwater at intervals of 5 d. Three stems were placed in each vase for each lisianthus.

The vase life of each sample was defined as the time when an investigator observed a change of petal color and collapse of petal shape. CIELAB values were measured using a colorimeter (CR-300, Konica Minolta, Tokyo, Japan), and the color changes were observed from the initial flowering state of the cut flower until obvious wilting.

2.4. Additional measurement of colorimetric and SPAD data in the HSR group

To augment the training dataset for machine learning analysis, we targeted the HSR group and conducted additional investigations into colorimetric and SPAD data. From June 25 to July 9, 2023 (15 d), the same four cultivars (AG, BP, CP, and KW) were observed. For this additional data collection for machine learning analysis, the vase life was measured in the laboratory under the same conditions as aforementioned in Section 2.3. Color changes were investigated from the initial flowering state of the cut flower until the wilting. The flower stems were cut to a sufficient length of 30 cm, and the number of leaves and flowers was kept the same to reduce variations between the four cultivars. Nevertheless, the locations of leaves differed between the cultivars; therefore, SPAD values were measured repeatedly from the same locations. The vase life of HSR was about 15 d, and measurements were taken on Days 1, 6, 9, 11, and 14 after cutting, with three repeated measurements in 12 samples. As the vase life differed between each sample, measurements were taken under the same conditions for the longest surviving samples, even when flowers had fully opened, and the vase life was at an end (Day 14).

2.5. Statistical analysis

For the first objective, which was to test the effects of SA treatment on the postharvest longevity of cut lisianthus, we hypothesized that the expected vase life increases within the experimental concentrations (0.0, 0.1, 0.3, and 0.5 mM). Since we assumed that the effects are cultivar-specific and vary between treatment timing and cultivation methods (i.e., the four groups denoted by SSV, SSR, HSV, and HSR), we used the two-way interaction between concentration and group to explain the vase life of cut lisianthus. We used cultivar-specific regression models with the two-way interaction term to test whether the expected vase life increases with respect to the concentration in each group at a significance level of 0.05. See Section 3.1 for the results and discussion of this confirmatory analysis.

For the second objective, which was to describe the senescence over time after cutting of lisianthus, we visualized the color changes of flowers with respect to days. For the graphical demonstrations, the flowers treated by HSR were grouped by measurement day (Day 1, 6, 9, 11, and 14) and cultivar (AG, BP, CP, and KW). Each data point of (L*, a*, b*) was plotted in the 3-dimensional space where the x-axis, y-axis, and z-axis represent the value of b* , the value of L* , and value of a* , respectively. The values of L* , a* , and b* were converted to the standardized RGB values, and each data point was colored using the associated RGB values. The 3-dimensional mean vector and the covariance matrix was estimated for each cultivar and measurement day, and an ellipsoid was superimposed in the 3-dimensional space to represent a confidence region for the expected three color-parameters at a confidence level of 0.95. The plot3D and rgl packages were used in R version 4.3.0 for this graphic representation (Murdoch and Adler, 2023; R Core Team, 2023; Soetaert, 2021). See Section 3.2 for the results and discussion of this descriptive analysis.

For the third objective, which was to predict the vase life of cut lisianthus, we used machine learning algorithms to predict the vase life (days) and classify the five stages of the vase life using the cultivars, the colorimetric values, and the SPAD values. The h2o and lares packages were used to implement the machine learning algorithms in R (Fryda et al., 2023; Lares, 2023). For this predictive analysis, we used an additional dataset of 576 samples treated by HSR (observed five times for each stage per sample), and we divided the dataset into training set and testing set. For the regression-based prediction, the root mean square error (RMSE), the mean average error (MAE), and the R-square (R^2) were calculated for evaluating predictive performance. For the classification, the accuracy and the area under the ROC curve (AUC) were calculated. See Sections 3.3 and 3.4 for the results and discussion of the predictive analyses.

3. Results and discussion

3.1. Cultivar-specific effects of SA treatment concentration, timing, and cultivation method on vase life of cut lisianthus

The statistical analysis revealed that exogenous applications at various SA concentrations (0, 0.1, 0.3, and 0.5 mM) and treatment timings and cultivation methods (SSV, SSR, HSV, and HSR) affected the vase life of cut lisianthus differently between the cultivars (Fig. 1A), and the effect of SA concentration varied by treatment timing and cultivation method (Fig. 1B) and by the cultivar (Fig. 1C).

In this study, we established four treatment groups by combining the SA treatment timing (V or R period) and cultivation methods (S or H cultivation), namely SSV, SSR, HSV, and HSR. As depicted in Fig. 1A, the average vase life was shorter than 10 d for all cultivars treated by SSV, SSR, or HSV, whereas the average vase life was 15.3 d and 15.0 d for BP and KW, respectively, when they were treated by HSR. The AG cultivar had the shortest average vase life of 10.7 d in the HSR group, and it was longer than any cultivars treated by SSV, SSR, and HSV. It is evident from these results that all cultivars in the HSR group had significantly higher vase life compared to other groups. There was a trend of increased vase life in H cultivation compared to S cultivation, and similarly, treating SA during the R period had a more pronounced impact on vase life enhancement than when treated during the V period. Even in the comparison among S cultivation groups, the SSR group recorded a longer average vase life than the SSV group.

Fig. 2 presents the expected vase life with respect to the concentration for each treatment group (SSV, SSR, HSV, and HSR) and each cultivar (AG, BP, CP, and KW). The expected vase life of KW was the longest at a concentration of 0.5 mM with HSR among all cultivars, treatment times, and cultivation methods considered in this experiment. Table 1 presents the estimated slope (the change of the expected vase life with respect to the concentration), standard error, and p-value for each treatment group and cultivar. Under the cultivar-specific regression analysis, it was statistically evident that SSR is effective for BP

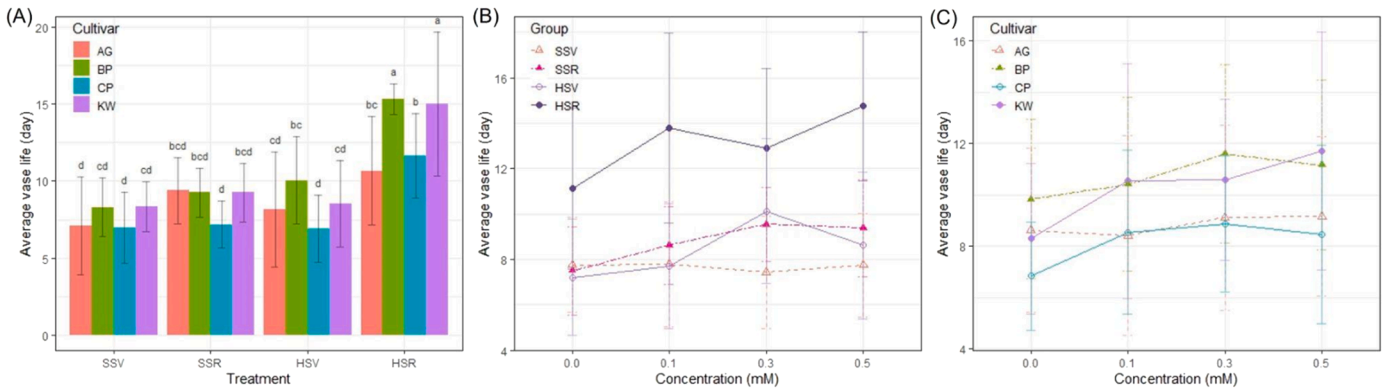

Fig. 1. The effect of treatment groups according to the cultivar on the vase life (A), the effect of salicylic acid (SA) concentration according to the treatment group (B), and the effect of SA concentration according to the cultivar (C).

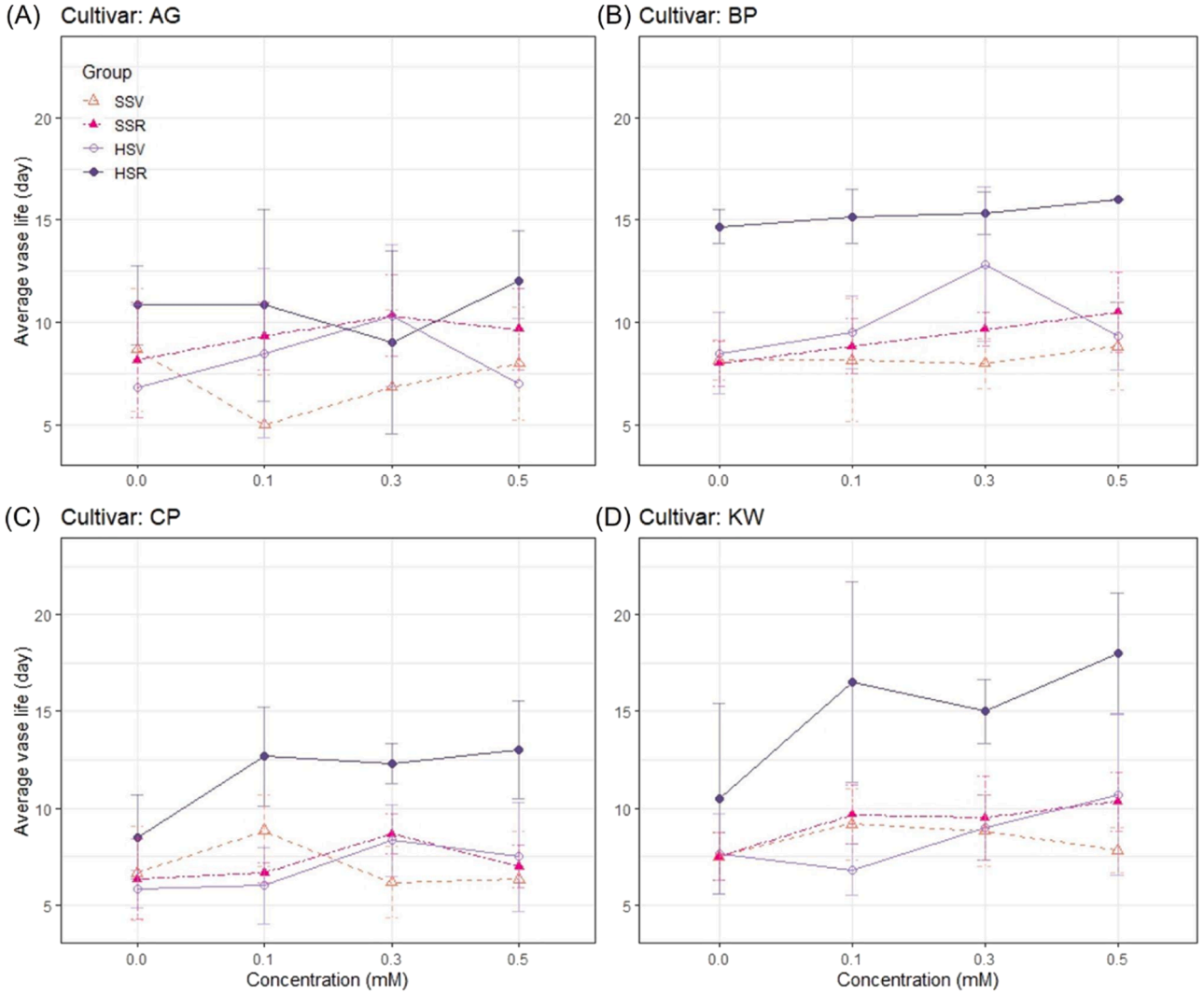

Fig. 2. Expected vase life (days) with respect to salicylic acid concentration (mM) for each treatment group (SSV, SSR, HSV, and HSR) and cultivar (AG, BP, CP, and KW).

($p = 0.008$); HSV for CP and KW ($p = 0.029$ and $p = 0.001$, respectively); and HSR for CP and KW as well ($p = 0.008$ and $p < 0.001$, respectively). For any results with lack of statistical significance due to large standard errors, we conclude the null hypothesis that the expected vase life does not change with respect to concentration. When the HSR treatment is applied, the expected vase life is the highest for the BP cultivar at the zero concentration (0 mM), and it is the highest for the KW cultivar at the highest concentration (0.5 mM). The results shown in Fig. 2 and Table 1 imply that, under the HSR treatment, the KW and CP cultivars benefit from an increased SA concentration, but the AG and BP

Table 1
Estimated slopes, standard errors, and p-values (for each treatment group under cultivar-specific regression).

Cultivar	Treatment Group	Estimate	Standard Error	Test Statistic (Z)	p-value
AG	SSV	0.932	3.465	0.269	0.394
	SSR	2.910	3.465	0.840	0.201
	HSV	0.678	3.465	0.196	0.422
	HSR	1.243	3.465	0.359	0.360
BP	SSV	1.158	2.005	0.578	0.282
	SSR	4.802	2.005	2.395	0.008
	HSV	2.910	2.005	1.451	0.073
	HSR	2.401	2.005	1.197	0.116
CP	SSV	-2.712	2.236	-1.213	0.887
	SSR	2.147	2.236	0.960	0.168
	HSV	4.237	2.236	1.895	0.029
	HSR	6.808	2.236	3.045	0.001
KW	SSV	-0.113	2.899	-0.039	0.516
	SSR	4.463	2.899	1.540	0.062
	HSV	6.977	2.899	2.407	0.008
	HSR	11.186	2.899	3.859	5.7e-05

do not.

The results showed the genetic advantages and disadvantages in the vase life after cutting. The AG and CP cultivars exhibited the shortest vase life within the HSR group, and there was a notable difference of 4 d approximately when compared to the BP and KW cultivars. Therefore, in addition to the most beneficial effect of HSR, the BP and KW cultivars showed additional genetic advantages, while the AG and CP cultivars showed a genetic limit in enhancing vase life. Irrespective of the cultivation method, the SA treatment should be applied during the R period to enhance vase life, and an additional benefit of the H cultivation depends on the genetic factor.

Though both BP and KW cultivars can benefit from HSR, the BP cultivar has a more predictable response to HSR when compared to the KW cultivar (Figs. 2B and 2D). The BP cultivar has a small standard variation (SD) of 0.99 d, while the SD of the KW cultivar is very large at 4.69 d. The BP cultivar is suitable for producing cut flowers with a uniform vase life in the HSR environment, and from an optimistic perspective, the KW cultivar has the potential to live up to 19 d under the HSR condition. In other words, the KW cultivar appears to have a genetic factor which triggers a longer vase life than the BP cultivar in a specific environment. However, this is thought to be influenced by a more delicate micro-environment than the HSR environment, and finding the cause will be a very challenging research topic.

As aforementioned, we hypothesized that the expected vase life increases with respect to SA concentration, and this monotonic assumption was used in the cultivar-specific regression analysis to test the hypothesis (Table 1). On the other hand, when data points were averaged without the monotonic assumption, the relationship between concentration and vase life may not be monotonic for all treatment groups and cultivars. More specifically, the average vase life was maximized at a concentration of 0.3 mM for AG, BP, and CP with HSV (Figs. 2A, 2B, and 2C). Since we did not specify a potential non-monotonic relationship prior to this study, finding an optimal concentration for each treatment group and cultivar is another important future study. If we have a larger sample size and obtain statistical evidence for a non-monotonic relationship for certain treatment timing, cultivation method, and cultivar, then we can provide a specific guideline to maximize the vase life for practitioners.

Table 2 informed us that the expected vase life depends on the concentration (A: 0, 0.1, 0.3 and 0.5 mM), treatment group (B: SSV, SSR, HSV and HSR), and cultivar (C: AG, BP, CP and KW). Furthermore, the A $\times$ B interaction (the effect of concentration depends on cultivation method and timing) is statistically significant ($p = 0.001$). As shown in Fig. 1B, the average vase life is longer at the highest concentration (0.5 mM) when compared to the control for SSR, HSV, and HSR. The

Table 2
Results of two-way ANOVA showing *p*-values and level of significance for the vase life of cut lisianthus.

	Concentration (A)	Group (B)	Cultivar (C)	A $\times$ B	A $\times$ C	B $\times$ C
Vase Life	3.7e-06	8.3e-45	3.5e-13	0.001	0.193	7.0e-05

average vase life for SSV is not only generally low, but it is also constant with respect to the concentration. The B $\times$ C interaction (the effect of cultivation method and timing depends on cultivar) is also statistically significant ($p < 0.001$). As shown in Fig. 1A, the effect of each treatment group is generally greater for the BP and KW cultivars, and it is generally lower for the CP cultivar. The A $\times$ C interaction (the effect of concentration depends on cultivar) is not statistically significant due to a lack of statistical power ($p = 0.193$). As shown in Fig. 1C and Fig. 2, the estimated cultivar-specific relationships between the expected vase life and concentration are mostly positive and vary depending on the cultivars, but the variation is not statistically significant given the current sample size.

In summary, (1) a higher concentration of SA treatment generally helps increasing vase life, especially it is statistically significant for BP with SSR, CP with HSV and HSR, and KW with HSV and HSR; (2) overall, HSR is the most helpful treatment timing and cultivation method; and (3) among all conditions experimented, the expected vase life is the longest under the following conditions: KW, HSR, and a concentration of 0.5 mM.

3.2. *Three-dimensional plot based on petal colorimetric data*

After the HSR cultivation, during the 14 d vase life, colorimetric data were collected additionally from petals of the four lisianthus cultivars. Day 1 is the day the flower was cut, and Day 6, 9, 11, and 14 indicate the measurement time (days). For instance, Day 6 is five days after Day 1. In the colorimetric data, the lightness value (L*) represents black at 0 and white at 100. The a* value represents the green-magenta opponent colors, with negative values toward green and positive values toward magenta, and the b* value measures the blue-yellow opponent, with negative values toward blue and positive values toward yellow. The 3-dimensional color space was arranged according to the cultivar and measurement time (Fig. 3). The data points were expressed in colors by converting the petal color of each cultivar into RGB. As senescence progresses after cutting, variations in the color space could be useful predictors for predicting the vase life or classifying the measurement time. Therefore, we have defined the changes in the space occupied by color points according to aging as color senescence.

3.2.1. *Arena Green (AG)*

Looking at Fig. S1, L* and a* showed no correlations from day 1 to day 14. However, the a* value gradually increased approaching Day 14, meaning that the color gradually exhibited less green and more red. On Day 1, the correlation between a* and b* was very strong, at -0.98. This trend continued until Day 9, but as senescence progressed, the correlation coefficient decreased, becoming a weak correlation by Day 14 ($R = -0.29$). Because the b* values increased irrespective of the a* values approaching Days 11 and 14, the long axis of the ellipse becomes parallel to the L* -b* plane, forming a wide ellipse with the L* -b* plane and a narrow ellipse with the a* -b* plane (Fig. 3). For the b* -L* relationship, the correlation coefficient is not high initially after cutting, but there is a moderate correlation on Day 14 ($R = -0.5$). As the b* value increases, the L* value decreases, resulting in lower brightness. In conclusion, the color changed from green on Day 1 to brown, which is red added to green, on Day 14. After initial cutting, as the a* value decreased (i.e., green color became more intense), the b* value increased, resulting in a stronger yellow color. However, the b* value

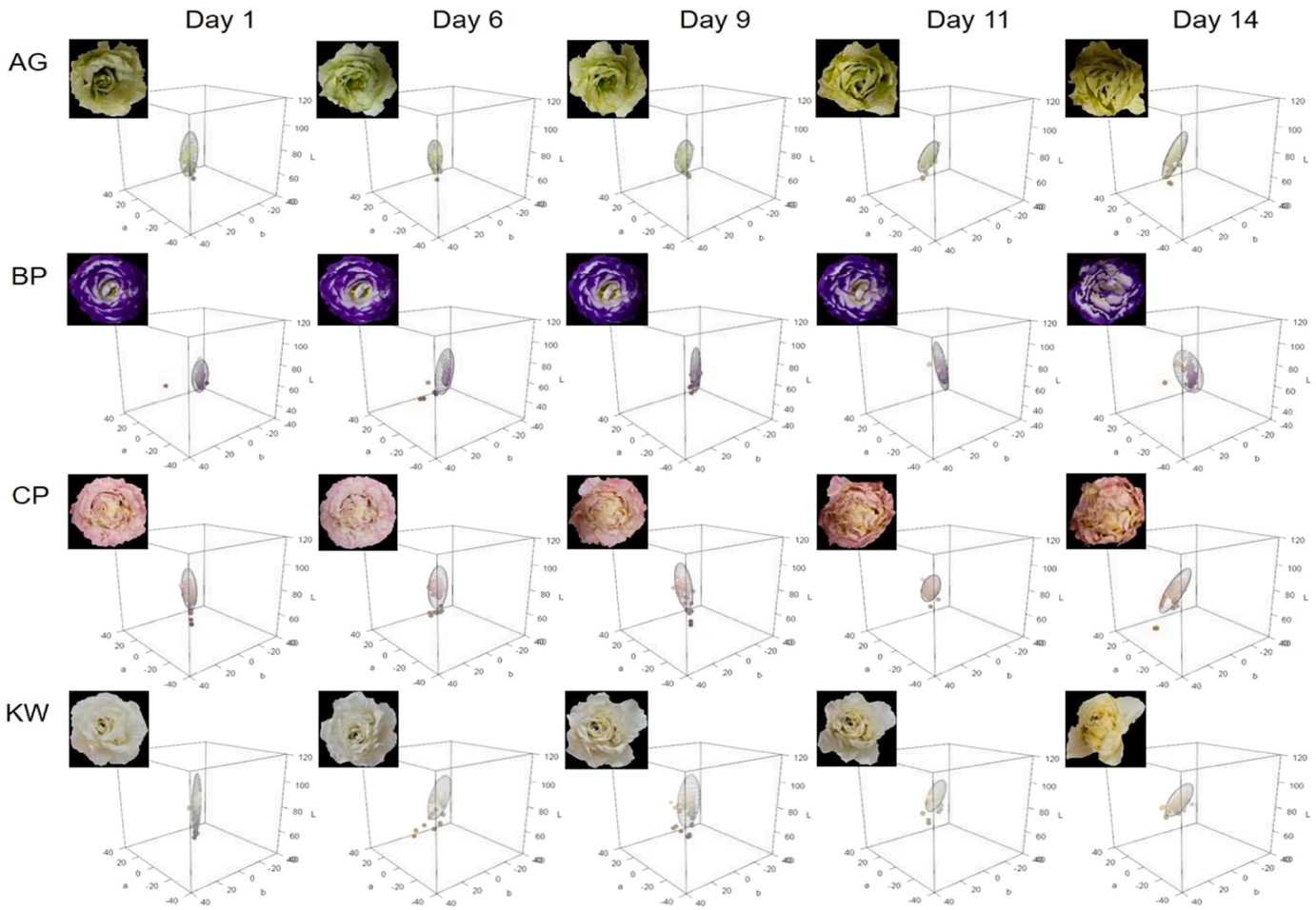

Fig. 3. Three-dimensional graphs indicating the color space (L*, a*, and b*) according to the measuring day (Day 1–14) and cultivar (AG, BP, CP, and KW) after flower cutting of lisianthus.

became larger irrespective of a* . In other words, as senescence progressed, the petals became increasingly yellow.

3.2.2. Blue Picote (BP)

The BP cultivar showed strong correlations among the L* , a* , and b* values (Fig. 3 and Fig. S2). Considering the relationship between L* -a* , on Days 1, 6, and 9, many samples showed a low L* value and an a* value close to 0 (i.e., the point color is close to dark white for many samples), but this trend declined as senescence progressed (i.e., the correlation coefficient increases), resulting in a clear color gradation from bright white to deep purple. In particular, there was a strong correlation between a* and b* , with the correlation coefficient close to − 1 at all time points (Fig. S2). As a result, there is a narrow ellipse in the a* -b* plane close to a straight line (Fig. 3). As the vase life increased, on Day 11, there were strong correlations between all components, forming narrow ellipses, but on Day 14, variation between the samples increased and the correlation coefficients gradually decreased, resulting in the largest, longest ellipses. Considering the relationship between a* and b* , most samples have large a* values and negative b* values (strong red and blue colors), increasing the expression of purple color. However, from Day 1 to Day 9, the b* values were below 0, but after Day 11, it began to increase above 0, resulting in an increase in yellow color. In the relationship between L* and b* , there was a trend of samples moving from the lower left corner on Day 1 (i.e., strong expression of dark, blue color) to the upper right corner on Day 14. Yellow color increased and red color was broadly distributed, forming an adjacent complementary relationship when mixing with blue, to express a deep purple color.

3.2.3. Corelli Pink (CP)

As seen in Fig. 3, the long axis of the ellipse on Day 1 was parallel to the L* axis, and there was little variation in the a* and b* values, resulting in a narrow ellipse. However, on Day 14, the correlation coefficient between L* and b* increased (Fig. S3), as did the variance of each component, resulting in a long, broad ellipse (Fig. 3). Considering the a* -b* relationship, initially after cutting, the b* value was close to 0 irrespective of the a* value, resulting in a distinct red color. As senescence progressed, the b* value increased above 0, leading to an increasingly yellow color (Fig. 3 and Fig. S3). Meanwhile, the red color became gradually weaker. In the b* -L* relationship, the b* values were between 0 and 10 on Day 1, but the b* values increased greatly over time. In other words, the expression of yellow color increased as senescence progressed.

3.2.4. Kroma White (KW)

Looking at the L* -a* relationship, the correlation coefficient was high on Day 1 (R = −0.76), decreased as senescence progressed, and then recovered again to R = −0.77 by Day 14. However, samples with a high L* value and negative a* value on Day 1 showed a sudden increase in the a* value from Day 6 to Day 14 approaching 0, and there was an increase in the number of samples with a value close to 4. In the relationship between b* and L* , b* values were low immediately after cutting, but as senescence progressed, the b* values gradually increased, and the L* value also showed an increasing trend. The KW cultivar showed a gradual increase in the expression of red color during the 14 d after cutting, changing to an orange color as shown in Fig. 3. In the a* -b* relationship, as senescence progressed, the a* and b* values both

increased; hence, the samples in the lower left (Day 1) moved towards the upper right (Day 14) (Fig. S4). Red and yellow colors both increased, resulting in the expression of an orange color.

3.3. Prediction of vase life of lisianthus cut flower based on colorimetric or SPAD data using AutoML

Based on the colorimetric and SPAD data from the four cultivars in the HSR group, we constructed a regression model to predict the vase life (Fig. 4). An additional experiment was conducted on 576 samples, which were divided into training and test datasets in an 8:2 ratio. Additional predictors, C* (chromas, brightness) and h (hue) were derived from the L* , a* , and b* values to predict vase life. The AutoML selected the gradient boosting machine (GBM) as the top model (RMSE = 0.4482; MAE = 0.2657, and R^2 = 0.9551). The b* value on Day 14 was the most important variable for predicting vase life, followed by cultivar and then the L* , h, a* values measured on Day 14.

Fig. 4A shows excellent predictive performance of the colorimetric data (R^2 = 0.9551), and Fig. 4B shows good, but inferior, predictive performance of the SPAD data (R^2 = 0.6966). Since the SPAD value is only an indicator of chlorophyll content in leaves of cut flowers, and the size and position of leaves vary by cultivar, using the SPAD value for predicting vase life can cause poor prediction performance. In other words, the unreliable measurement might be a main cause affecting the inferior model performance. These regression-based predictions are suitable for research purposes, but it is impractical to collect the colorimeter and SPAD data until Day 14. Therefore, a model that classifies the day measured after cutting would be more suitable for practical purpose and commercial use.

3.4. Classification of stage of vase life based on colorimetric data using AutoML

Note that the classification treats the day of measurement as a target (unlike treating the vase life as a numeric variable under the regression model). After running AutoML, a DRF-based stacked ensemble model showed the best accuracy, 0.7639. The AUC is not supported for the stacked ensemble model for multinomial classification, but the AUC and accuracy of the DRF model were 0.925 and 0.75, respectively. To this end, we expect that the AUC and accuracy of the stacked ensemble model would be at least 0.925 and 0.75, indicating that this ensemble model has good classification performance. If this technique is commercialized, it could be widely used in the logistics distribution stage of the floriculture industry, providing considerable commercial value and rapidly adapted to other floricultural crops.

We attempted various cultivation conditions that could affect vase life of cut lisianthus. First, we investigated the effects of SA concentration, timing of SA treatment, and cultivation method on vase life of four cultivars of lisianthus. We found that treating SA at a concentration of 0.5 mM in the R period under H cultivation enhanced vase life. Second, to develop a model that could be used more efficiently in industry, we investigated petal color and the chlorophyll content of leaves for the four cultivars using a colorimeter and SPAD meter. Based on these data, we showed that the color space of lisianthus cultivars varies depending on the time after cutting. Thus, we demonstrated that the vase life of each cultivar could be predicted based on the color senescence process of the petals. As shown in Fig. 3, each lisianthus cultivar has its own pattern of color senescence. All cultivars showed a trend for increasing b* value as senescence progressed, and the b* value increased greatly on Day 14, differentiating it from other days (Figs. S1–4). As senescence progresses, there is a greater increase in copigment expression (colorless or pale-yellow flavonol) compared to anthocyanin. Using a similar concept to the copigmentation index (total flavonol / anthocyanin contents) of Hashimoto et al. (2000), a senescence index that increases as senescence progresses could be defined. Rather than directly measuring anthocyanin and other flavonoids using HPLC, there is a more urgent need to develop phenomic approaches using equipment such as a spectrophotometer or hyperspectral camera, as we attempted in this study. Classification of vase life based on color senescence, as developed in this study, could also be combined with molecular studies of flower senescence. The correlation between phenomic data on senescence-related color changes and RNA-Seq data from flowers at different stages could provide a solution to solving the secrets of the aging process. Moreover, this phenomic approach could induce a synergistic effect when combined with genome wide association study (GWAS) research. Even within the same environment, vase life clearly differs depending on cultivar; hence, there will be distinct differences in the reaction norms of each cultivar. GWAS research based on breeding

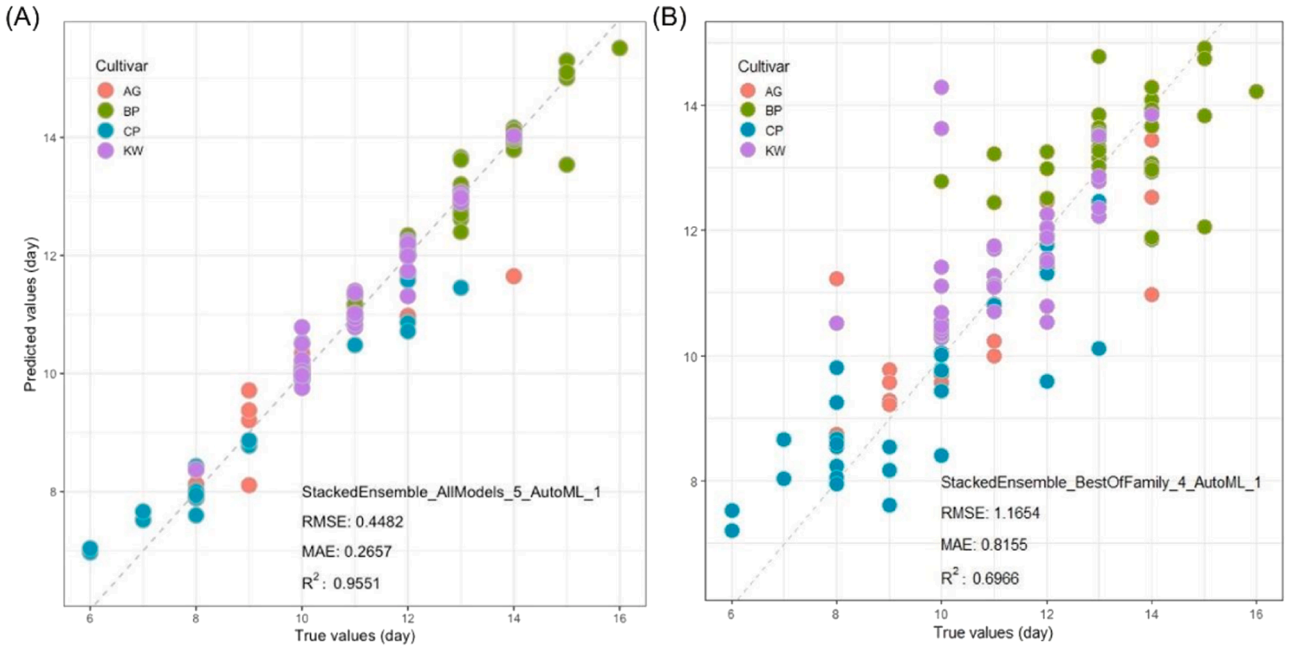

Fig. 4. Scatter plots of observed vs. predicted values for vase life of lisianthus cut flowers based on the test datasets from (A) colorimeter and (B) SPAD meter.

lines between these cultivars could provide clues to the major genes involved in vase life.

4. Conclusions

Flower longevity is a prerequisite for cut flower marketing. Previous studies have focused on improving the vase life of cut flowers through exogenous hormone treatment. This study aimed to advance the understanding of the effects of various cultivars (genotype) and cultivation conditions (environment), including SA treatment concentration, on the enhancement in vase life. It was confirmed that hydroponics and SA treatment at the reproductive period extends the expected vase life after cutting, and the longest vase life is expected at a 0.5 mM concentration for the Kroma White cultivar among all cultivars and treatment groups tested in this experiment. In addition, advanced machine learning models can improve the predictability of vase life using the colorimetric and SPAD data, and it will benefit the floriculture industry. We anticipate its applications to other popular flower crops such as roses.

CRediT authorship contribution statement

Kwon Hye Sook: Investigation, Writing – original draft. **Leporini Cody:** Formal analysis. **Kim Steven:** Formal analysis, Writing – review & editing. **Heo Seong:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request. All codes are available from Zenodo repository (https://doi.org/10.5281/zenodo.8299824) and GitHub (https://github.com/heoseong/Lisianthus).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.postharvbio.2023.112726.

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