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APPLICATION OF OJIP CHLOROPHYLL FLUORESCENCE TRANSIENT ANALYSIS TO STUDY THE POSTHARVEST CHANGES IN PHOTOSYNTHETIC APPARATUS IN CUT FOLIAGE SPECIES

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ABSTRACT

Chlorophyll fluorescence (ChlF) is a non-invasive technique that can be potentially used in postharvest research to gain useful information on early responses to postharvest stresses. This study was conducted to validate the application of ChlF transient analysis in determining the postharvest changes in photosynthetic apparatus in three ornamental foliage species, *i.e.*, *Cordyline fruticosa* 'Willy's Gold' and 'Rubra', *Dracaena sanderiana* 'White', and *Nephrolepis exaltata*. Salicylic acid (100 and 300 mg·L^{-1}), glucose (10 g·L^{-1}), and their combinations were used as holding solutions with control treatment (distilled water) at room temperature (25±2°C). Vase life was evaluated using OJIP analysis. OJIP parameters, *i.e.*, specific energy fluxes per reaction center (ABS/RC, TR/RC, ET/RC, and DI/RC), flux ratios (maximum quantum yield of primary photochemistry-φPo), electron transport efficiency (ψo), and quantum yield of electron transport (φEo), and performance index (PI) were recorded every other day, using a fluorometer (FluorPen 100). Leaf chlorophyll contents of all species and anthocyanin contents of two cordyline cultivars were determined. Data were subjected to ANOVA in a completely randomized design. Mean separation was done by DMRT ($p \leq 0.05$). Clear variations in ChlF were observed in every foliage species with the time. OJIP analysis showed species-depended variations. The higher ABS/RC and DI/RC were recorded for *D. sanderiana* and *N. exaltata* compared to the PI of those species. At the end of the experiment, the chlorophyll contents were decreased, while anthocyanin contents were increased. Consequently, chlorophyll fluorescence changes in photosynthetic apparatus can be used for the prediction of the postharvest stresses and longevity of cut foliage.

1. INTRODUCTION

In Sri Lanka, floriculture is a lucrative agribusiness that includes both export and domestic sectors. The industry creates significant foreign exchange and jobs, strengthening rural people. Various channels are used to provide a variety of tropical and temperate cut flowers and foliage species to retail locations. Customer satisfaction depends on a consistent supply of high-quality cut flowers (Idirisinghe et al., 2013). Hence, the postharvest quality of cut flowers and cut foliage has been attracting the attention of growers and researchers for many years. The major concern of cut flower/foliage postharvest quality is the longevity of the vase life. Researchers have determined the postharvest longevity (vase life) in cut flowers/foliage by the number of days from harvest until senescence.

There are different methods to extend the postharvest life of cut foliage and cut flowers, including control temperature, optimizing postharvest handling, preharvest and postharvest chemical treatments, introducing packaging materials, and application of different harvesting methods. When monitoring the postharvest quality by using these methods some problems such as delayed and subjective observations, some compounds that use for preharvest and postharvest treatments can be hazardous for the human body, and they can be under or overdosing of treatments and most of these trials are difficult and time-consuming. Hence to overcome these limitations, there are different newly-introduced improved techniques such as OJIP chlorophyll fluorescence transient analysis, RGB image analysis, UV/VIS spectroscopy, NIR spectroscopy, and fluorescence spectroscopy (Abbott, 1999; Mota et al., 2005).

The chlorophyll fluorescence (ChlF) technique also known as the OJIP technique has been rapidly developed and applied to plant physiology studies, which shows great potential to gain insights into the postharvest physiology of ornamental cut flowers/foliage. This technique is useful to detect non-destructive measurements that enable for early detection of stress conditions, during and quickly after storage or transportation. Moreover, OJIP technique has a high rate of sample analysis approximately about 100–300 samples per working hour, and this is an easy technique that can be applied for intact plants as well as any plant part (Abbott, 1999).

The OJIP technique can be used to explain the sequential flow of energy through the photosystem (PS II) at the reaction center or a cross-section of a region that has been exposed to exciting (Force et al., 2003). Subsequently, the OJIP technique is an information-rich source about the photosynthetic apparatus that can be used to identify the postharvest changes of cut flowers/foliage. This study was conducted to validate the application of ChlF transient analysis in determining the postharvest changes in photosynthetic apparatus in three ornamental foliage species.

2. METHODS

2.1 Experimental Site

The experiment was conducted at the Research Laboratory, Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka. Experiment on vase life evaluation was carried out in the laboratory under a relative humidity of 90-95 ± 2 % and room temperature of 25±2°C.

2.2 Plant Materials and Postharvest Treatments

Three species of foliage plants, including *Cordyline fruticosa* 'Willy's Gold' and 'Rubra', *Dracaena sanderiana* 'White', and *Nephrolepis exaltata* were selected. From each foliage species, the healthy leaves with the same maturity level were collected.

Six different holding treatments (vase solutions) were used, including control (distilled water: (T1), 100 mg·L^{-1} salicylic acid (SA, T2), 300 mg·L^{-1} salicylic acid (T3), 10 g·L^{-1} glucose (T4), 100 mg·L^{-1} salicylic acid + 10 g·L^{-1} glucose (T5), and 300 mg·L^{-1} salicylic acid + 10 g·L^{-1} glucose (T6).

The holding solutions (150 mL) were filled into cleaned glass bottles. The leaves were recut while holding in water using a disinfected sharp secateur. The uniform cut leaves were dipped in 150 mL of holding solutions (treatments) separately. And organized in a Completely Randomized Design (CRD) with 02 replicates per treatment. Each replicate consisted of 02 glass bottles (technical replicates) with 03 leaves per glass bottle (biological replicates).

2.3 Measurements

2.3.1. *Measurements of Chlorophyll fluorescence*

Chlorophyll fluorescence (ChlF) data were recorded using a portable fluorometer (FluorPen, FP 100, Photon Systems Instruments Inc). The data were collected every other day commencing from the first day of treatment application throughout the vase life. Three ChlF data were collected from three different places of a single leaf of a treatment. During taking measurements, the cells were exposed to a 3000 μmol$m^{-2}s^{-1}$ saturating light pulse. ChlF were captured at 10 μs intervals across a period of 10 μs to 1 s (Beneragama et al., 2014). Each transient was analyzed using raw data according to the OJIP-test (Strasser et al., 2005). The major OJIP derived parameters were taken; specific energy fluxes (ABS/RC, TR/RC, ET/RC, and DI/RC), flux ratios (maximum quantum yield of primary photochemistry-φPo), electron transport efficiency, and quantum yield of electron transport (φEo), and performance index (PI) (Beneragama et al., 2014) were recorded every other day.

2.3.2. *Leaf chlorophyll contents*

Leaf chlorophyll contents (milligrams of chlorophyll per gram of leaf tissue extracted) were measured on the first day and the last day of the experiment using the 80% acetone extraction method. Absorbance was determined by spectrophotometry at 645 nm and 663 nm (Witham, 1971).

2.3.3. *Leaf anthocyanin contents*

Leaf anthocyanin content was assessed using the UV/VIS spectroscopy method on the first day and the last day of the vase life. Here we considered that the day which leaves could not be used for floral arrangements as the last day of the vase life according to visual appearance. Collected leaf samples were ground and extracted with potassium chloride (pH 1) and sodium acetate (pH 4.5) solvents. Absorbance was determined by spectrophotometry at 700 nm. The monomeric anthocyanin content of the samples was calculated according to (Giusti & Wrolstad, 2001). The present study was carried out as a single experiment.

3. RESULTS AND DISCUSSION

In this study, we discussed only the 3rd and last day OJIP data of the selected foliage species. The longevity of vase life for *Cordyline fruticose* 'Rubra' and *Nephrolepis exaltata* was nine days, while it was fifteen days for *Cordyline fruticose* 'Willy's Gold' and *Dracaena sanderiana* 'White'.

3.1. Results

3.1.1. *Chlorophyll Fluorescence (OJIP) Measurements*

On the 3rd day of the experiment the significantly highest ($p \leq 0.05$) performance index was showed by 100 mg·L^{-1} salicylic acid + 10 g·L^{-1} glucose treatment for the *Cordyline fruticosa* 'Willy's Gold' (Fig.1A). The significantly lowest ($p \leq 0.05$) performance index was reported for the 300 mg·L^{-1} salicylic acid treatment (Fig.1A). On the last day of the experiment, the performance index of 'Willy's Gold' was significantly higher ($p \leq 0.05$) in 100 mg·L^{-1} salicylic acid + 10 g·L^{-1} glucose treatment (Fig.1A). Similarly, the highest performance index was recorded for the 300 mg·L^{-1} salicylic acid-treated leaves even on the last day (Fig.1A). The lowest performance index of 'Willy's Gold' was shown by 100 mg·L^{-1} salicylic acid on the last day (Fig.1A). From 3rd day to the last day (15th day) the maximum performance index of 'Willy's Gold' was reduced. The performance index value was higher than other OJIP parameters of 'Willy's Gold'. The flux ratios were less in values even on the last day of the experiment. But there were significant differences between treatments.

Cordyline fruticosa 'Rubra' showed a significantly higher ($p \leq 0.05$) performance index in 100 mg·L^{-1} salicylic acid treatment both on 3rd day and last day (9th day) (Fig.1B). The lowest performance indexes of 'Rubra' were recorded on 3rd day and 9th day for 10 g·L^{-1} glucose treatment and the combination treatment of 100 mg·L^{-1} salicylic acid + 10 g·L^{-1} glucose (Fig.1B). 'Rubra' showed the highest performance index at the beginning and it was gradually reduced at the end of the vase life and the lowest performance index at the beginning was increased to some extent in 'Rubra' (Fig.1B). The other parameters which are affected for the performance index; $\phi o, \psi o,$ and ABS/RC were in lower values than the performance index of 'Rubra' (Fig.1B).

In *Draceana sanderiana* 'White' (Fig.1C), the specific energy flux for absorption, heat dissipation, and trapping per reaction center (ABS/RC, DI/RC, and TR/RC) were higher than the performance index. Highly deviated ABS/RC was clearly observed (Fig.1C). Further, *D. sanderiana* 'White' held in 100 mg·L^{-1} salicylic acid treatment and 10 g·L^{-1} glucose treatment recorded the significantly highest ($p \leq 0.05$) values of the above parameters on the 3rd day and last day (15th day) (Fig.1C). However, those values were slightly decreased on the last day. The performance index of *D. sanderiana* 'White' held in 300 mg·L^{-1} salicylic acid + 10 g·L^{-1} glucose was significantly higher ($p \leq 0.05$) on the 3rd day (Fig.1C). However, the *D. sanderiana* 'White' held in the distilled water (control) showed the significantly highest ($p \leq 0.05$) performance index on the last day (15th day) of the experiment (Fig.1C).

According to figure 1D for *Nephrolepis exaltata*, all the treatments maintained the same antenna size at the end of the experiment (9th day). The performance index (PI) was lower than the ABS/RC and TR/RC parameter values (Fig.1D). On the 3rd day, the combination treatment of 300 mg·L^{-1} SA + 10 g·L^{-1} glucose reported a significantly lower ($p \leq 0.05$) performance index in *Nephrolepis exaltata*, and the highest PI was reported from the control treatment (Fig.1D). On the last day (9th day), the combination of 300 mg·L^{-1} SA+ 10 g·L^{-1} t glucose showed the highest performance index. The other parameters for quantum efficiencies on the 3rd and 9th day showed significantly lower ($p \leq 0.05$) values for all treatments.

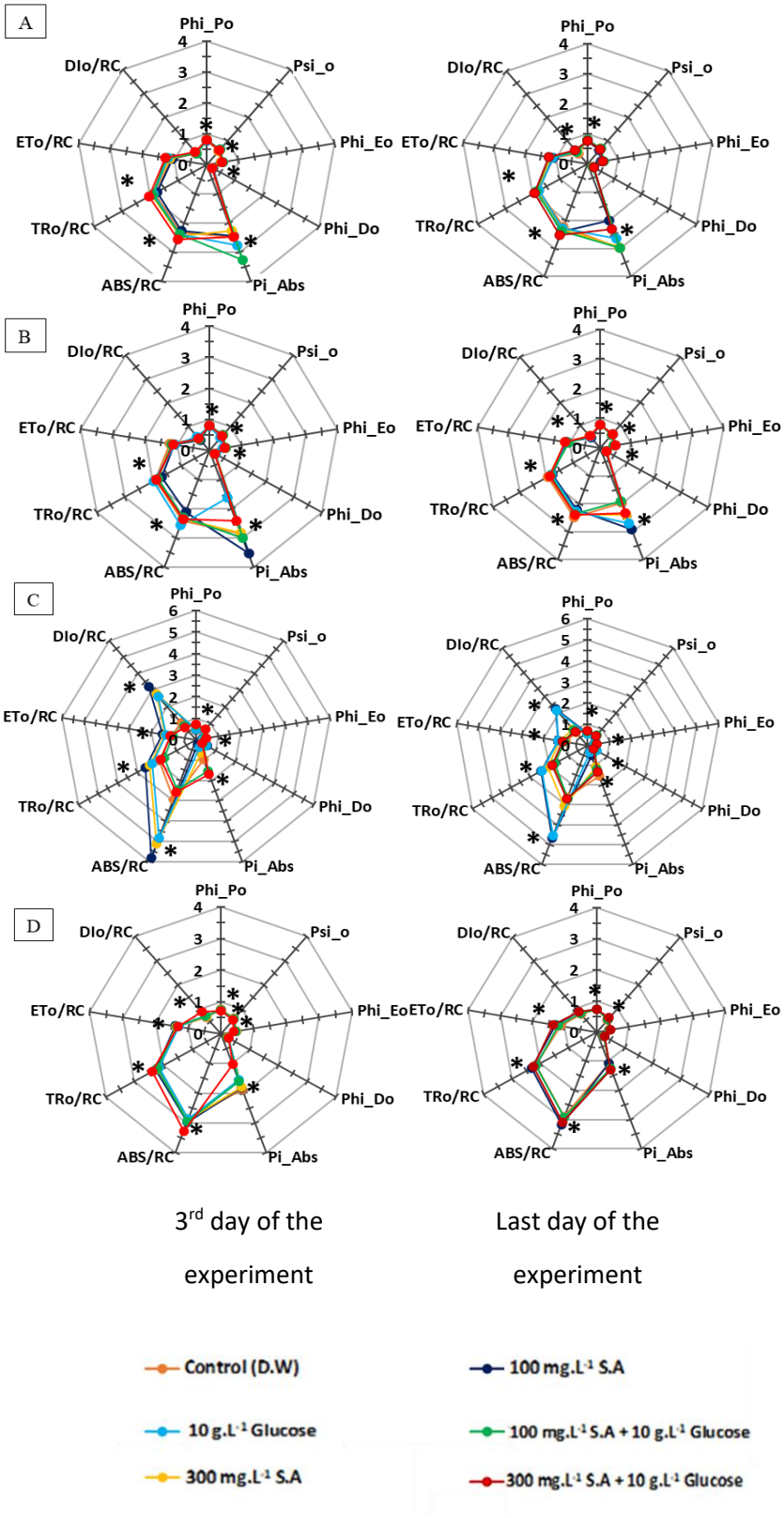

Figure 1 Specific fluxes, flux ratios, and performance indexes on the 3rd and last day of the experiment for the selected foliage species; (A) *Cordyline fruticosa* 'Willy's Gold'" (B) *Cordyline fruticosa* ''Rubra' (C) *Draceana sanderiana* 'White', and (D) *Nephrolepis exaltata* held in six different holding solutions, including distilled water (DW, control), 100 mg·L^{-1} SA, 300 mg·L^{-1} SA, 10 g·L^{-1} Glucose, 100 mg·L^{-1} SA + 10 g·L^{-1} Glucose, and 300 mg·L^{-1} SA + 10 g·L^{-1} Glucose). The asterisk marks show the significant difference ($p \leq 0.05$).

3.1.2. *Total Chlorophyll Content*

As shown in figure 2A, there was a significant reduction ($p \leq 0.05$) in total chlorophyll content of *Cordyline fruticosa* 'Willy's Gold' at the end of the experiment (15th day). However, the greatest reduction was observed in 100 mg·L^{-1}salicylic acid + 10 g·L^{-1} glucose treatment, while the least reduction was recorded from the 10 g·L^{-1} glucose treatment.

Cordyline fruticosa 'Rubra' also showed a significant reduction ($p \leq 0.05$) in total chlorophyll content (Fig. 2B) at the end of the experiment (9th day). The significantly lower chlorophyll content was recorded in leaves of 'Rubra' held in distilled water (control) and the highest value was recorded for 100 mg·L^{-1}salicylic acid+ 10 g·L^{-1} glucose treatment.

There was a significant reduction ($p \leq 0.05$) in total chlorophyll content of *Dracaena sanderiana* 'White' held in 100 mg·L^{-1} salicylic acid treatment and control treatment (Fig. 2C). However, the leaves treated with 300 mg·L^{-1}salicylic acid and the combination of 300 mg·L^{-1}salicylic acid and 10 g·L^{-1} glucose showed the significantly highest ($p \leq 0.05$) total chlorophyll content that was similar to the chlorophyll content on the 1st day of the experiment.

A significant reduction ($p \leq 0.05$) in the total chlorophyll content was observed in leaves of *Nephrolepis exaltata* held in distilled water (control), 10 g·L^{-1}glucose, and the combination treatment of 100 mg·L^{-1}salicylic acid+ g·L^{-1} glucose (Fig. 2D). Further, the total chlorophyll content was significantly higher ($p \leq 0.05$) in *Nephrolepis exaltata* leaves held in 100 mg·L^{-1}salicylic acid that was similar to the chlorophyll content on the 1st day of the experiment.

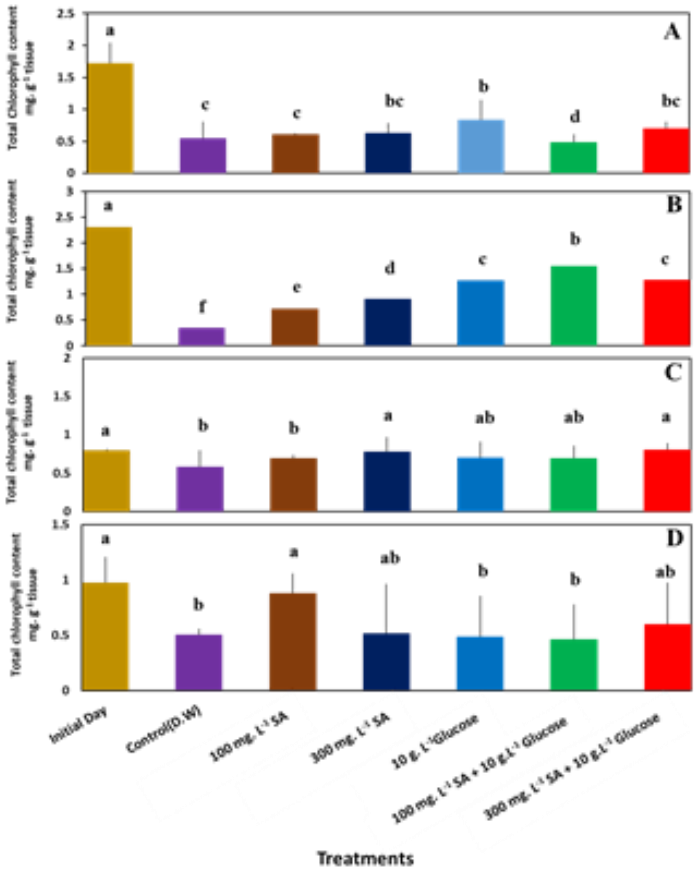

Figure 2 Leaf total chlorophyll content (mg·g^{-1} tissue) on the first and the last day of the experiment for the selected foliage species; (A) *Cordyline fruticosa* 'Willy's Gold' (B) *Cordyline fruticosa* 'Rubra' (C) *Draceana sanderiana* 'White', and (D) *Nephrolepis exaltata* held in six different holding solutions, including distilled water (DW, control), 100 mg·L^{-1} SA, 300 mg·L^{-1} SA, 10 g·L^{-1} Glucose, 100 mg·L^{-1} SA + 10 g·L^{-1} Glucose, and 300 mg·L^{-1} SA + 10 g·L^{-1} Glucose. The means with the same letters are not significantly different ($p \geq 0.05$) according to DMRT.

3.1.3. *Anthocyanin content*

According to figure 3A, the significantly highest ($p \leq 0.05$) anthocyanin contents of were observed in *Cordyline fruticosa* 'Willy's Gold' leaves held in both 100 and 300 mg·L^{-1} salicylic acid treatments, respectively. In contrast, *Cordyline fruticosa* 'Rubra' leaves showed the highest ($p \leq 0.05$) anthocyanin contents at the last day of experiment compared to the first day, regardless of the type of holing solution (Fig. 3B).

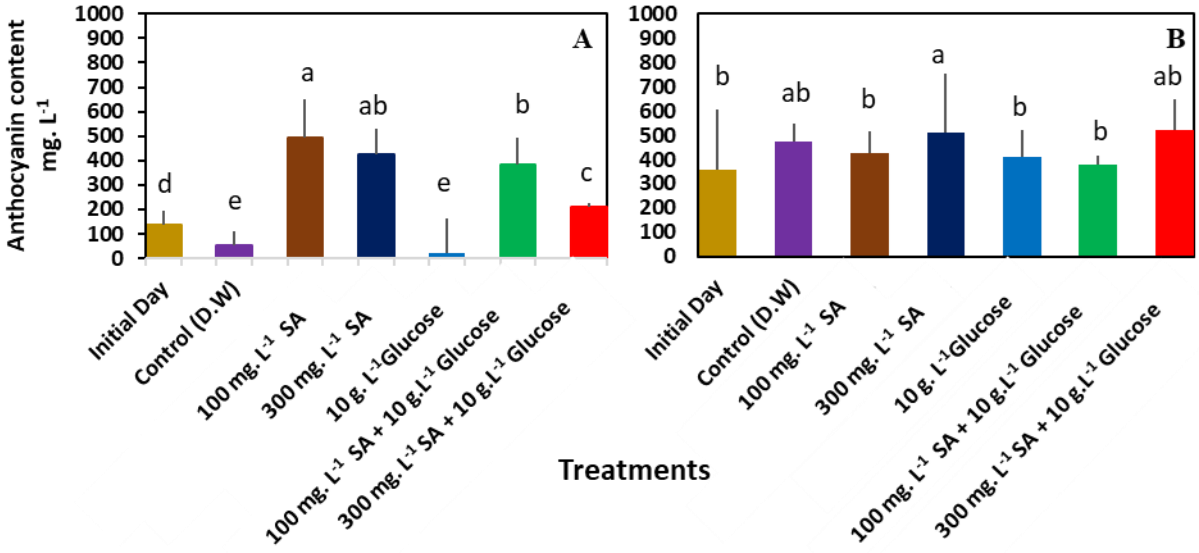

Figure 3 Leaf anthocyanin content ($mg \cdot L^{-1}$) on the first and the last day of the experiment for (A) *Cordyline fruticosa* 'Willy's Gold', and (B) *Cordyline fruticosa 'Rubra',* held in six different holding solutions, including distilled water (DW, control), 100 mg·L^{-1} SA, 300 mg·L^{-1} SA, 10 g·L^{-1} Glucose, 100 mg·L^{-1} SA + 10 g·L^{-1} Glucose, and 300 mg·L^{-1} SA + 10 g·L^{-1} Glucose. The means with the same letters are not significantly different ($p \geq 0.05$) according to DMRT.

3.2 Discussion

The overall performance of the *Cordyline fruticosa* 'Willy's Gold' was gradually decreased by the end of the experiment. Similarly, Cocetta & Ferrante (2018) reported that stems of cut flowers of *Rosa hybrida* L. treated with salicylic acid showed a loss in the leaf PSII functionality after 7 days of vase life, evidenced by a significant decline in the performance index (PI) value. Further, the significant changes were not observed in Fv/Fm, RC/CS and in DIo/RC even though these indexes showed the similar trend with the PI changes in cut flowers of *Rosa hybrida* L. Salicylic acid is a plant hormone that has a role in a variety of plant processes, including stress responses, development, and signaling (Alaey et al., 2011). Moreover, SA has been shown to increase the activity of the ROS-scavenging enzyme catalase and improve the water balance in cut rose flowers thus, extending the vase life (Cocetta & Ferrante, 2018).

According to T. Janda et al., (2014) the exogenous SA may also create the protection against the negative effects of various stressors and this protection revealing by the increased photosynthetic capacity. The endogenous SA levels of the plants have been demonstrated to rise in response to unfavorable environmental conditions. Controlled SA levels in plants are critical for good photosynthetic performance and adaptation to changing environmental stressors.

The reduction of the performance index in *Cordyline fruticosa* 'Willy's Gold' can be attributed to the overall status of the photosynthetic apparatus of this species.

The OJIP results observed in 100 $mg \cdot L^{-1}$ salicylic acid treated leaves of *Cordyline fruticose* 'Rubra' were contradicted with the visual observations of those cut foliage. This can be probably owing to the masking effect of chlorophyll by anthocyanin.

Anthocyanin forms a pigment layer in the palisade mesophyll layer of red-osier dogwood (*Cornus stolonifera*) that reduces light capture by chloroplasts (Feild et al., 2001). They observed that maximum photosystem II (PSII) photon yield of red-senescing leaves recovered from a high-light stress treatment using chlorophyll 'a' fluorescence measurement. The differences in light response curves of effective PSII photon yield for red- and yellow-senescing leaves were not observed thus, differences between red- and yellow-senescing leaves cannot be explained by differences in the capacities for photochemical and non-photochemical energy dissipation (Feild et al., 2001). Consequently, the chlorophyll fluorescence technique is not much applicable with the colorful foliage plant species, including *Cordyline fruticose* 'Rubra'.

A linear relationship was obtained between the quantum yields of photochemical and non-photochemical quenching, irrespective of the CAM phase and prevailing irradiance, based on an integrative analysis of diurnal changes in gas exchange, chlorophyll fluorescence parameters, and organic acid decarboxylation during the different CAM phases in *Clusia hilariana* Schlecht (Franco et al., 1999). These results can be used to interpret the findings of the present study on *Draceana sanderiana* 'White'.

The non-photochemical quenching parameter is thought to be proportional to both the effective rate constant for energy dissipation in the antennae and the concentration of quenching agents that cause thermal energy dissipation.(Franco et al., 1999) Even though the stomata were closed during phase III of CAM, decarboxylation of malate and citrate provided high internal CO_2 concentrations, allowing optimal use of light energy, as evidenced by non-saturating rates of electron transport rate (ETR) in light response curves and the attainment of maximum rates of ETR in diurnal curves. CO_2 loss due to CO_2 evolution would account for around 3% of the possible CO_2 fixation rates calculated by organic acid decarboxylation rates.(Franco et al., 1999).

As the effect of glucose on chlorophyll fluorescence parameters of CAM palnts, the thylakoid energization was enhanced (Krapp et al., 1991) The following findings suggest that reduced demand in the Calvin cycle has an indirect effect on light harvesting and electron transport. At first, the light-response curve's initial slope is not reduced. Second, leaves given glucose have a very high ATP/ADP ratio, a higher stromal NADPH/NADP ratio, a higher decrease of QA (PSII's principal electron acceptor), and a higher non-photochemical quenching of chlorophyll fluorescence. This indicates that the thylakoids' reflexes are being hindered by a lack of 3-PGA. (Krapp et al., 1991).

The cut foliage of *Nephrolepis exaltata* held with different salicylic acid and glucose treatments did not show distinctive changes in their chlorophyll florescence parameters. It may be due to the vascular nature of these plants and their poor evolutionary development. Hence, they may be unable to change their inner systems according to the effects of treatments.

The specific fluxes of light absorption per reaction center of *Nephrolepis exaltata* was similar to the *Draceana sanderiana* 'White'. However, they absorb more light energy by antenna pigment molecules and also the consistency of the trapping this energy is not changed. The portion from absorb energy for trapping is significantly high other than the dissipated portion. Because of this trapping rate it can be damaged their reaction centers, and this may be cause for the quick yellowing of leaflets of *Nephrolepis exaltata* foliage.

Even if there are variances among plant species, the maximum quantum efficiency of PSII (FV/FM) steadily diminishes and is hardly visible at the onsets of senescence. When the plastoquinone electron acceptor pool (Qa) is entirely oxidized, the Fo, or minimal fluorescence, measures the fluorescence intensity at 20 seconds. The increase in Fo is likely

due to PSII damage, as it is unable to discharge all electrons. Specifically, the OJIP test is used to determine the leaf senescence and quality (Ivlev, 2014).

The literature on the effect of SA on ornamental foliage is still scanty. However, the effects of exogenously applied SA on plant physiological processes under optimal environmental conditions are controversial. The effects of exogenously applied SA on plant physiological processes under optimal environmental conditions, on the other hand, remain debatable. The Calvin cycle is hindered by the indirect influence of salicylic acid (SA) on stomata closure, and the slower rate of CO_2 absorption affects ATP and NADPH accumulation, resulting in a reduced quantum yield of PSII (ϕPo) than PSI. (K. Janda et al., 2012; T. Janda et al., 2014).

The positive effects on chlorophyll contents in gladiolus cut spikes treated with salicylic acid was reported by Hassan & Ali, (2014). Moreover, the salicylic acid enhanced the chlorophyll content Yusuf et al., (2013) and significantly extend the vase life of cut roses (Zamani et al., 2011). Additionally, the chlorophyll degradation process was hindered by the effect of glucose treatment.

Exogenous SA has been shown to stimulate the biosynthesis of anthocyanin pigments (Ram et al., 2013) and it is possible that SA treatment could delay the degradation of pigments in petals of rose flowers. As seen in model plants, increasing the concentration of phenolic compounds and anthocyanins may help to counteract senescence and prolong lifespan of cut rose flowers. Despite the low dose used, SA helped cut rose petals retain higher quantities of anthocyanins after four days in the vase. Also, the petal coloration was affected by the salicylic acid after 7 days (Cocetta et al., 2018). These findings convinced the present results of the experiment.

Sugars in vase solutions help cut flowers like Eustoma last longer in the vase (Islam et al., 2003). Carbohydrates are a major structural material used in cell growth and enlargement, as well as a soluble component in leaves and petal tissues, making them an important osmotic regulator of water potential. Sugars have also been found to delay climacteric ethylene production and reduce ethylene sensitivity, though the mechanisms of action remain unknown (Pun & Ichimura, 2003).

4. CONCLUSION

Based on this study it can be concluded that there is no significant effect of different postharvest holding solutions for the vase life of *Neprolepis exaltata* cut foliage. Moreover, specifically salicylic acid increases the color pigments throughout their vase life. Thus, it is possible to maintain the pigments by using different concentrations and a combination of salicylic and glucose treatments.

Also, there were species-dependent changes in the OJIP chlorophyll fluorescence parameters. For *Cordyline fruticosa* 'Willy's Gold' and 'Rubra' performance index was the species-dependent chlorophyll fluorescence parameter while ABS/RC and DI/RC were specific to *Draceana sanderiana* 'White'. For *Nephrolepis exaltata* ABS/RC and TR/RC were specific chlorophyll fluorescence parameters. Therefore, these species-dependent parameters are suitable in determining the postharvest life of different ornamental and horticultural crops.

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