A DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Non-destructive Measurement of Plant Internal Electrical Conductivity of Hydroponically Grown Paprika

수경재배 파프리카의 비파괴적 식물 내부 전기전도도 측정

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NON-DESTRUCTIVE MEASUREMENT OF PLANT INTERNAL ELECTRICAL CONDUCTIVITY OF HYDROPONICALLY GROWN PAPRIKA

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ABSTRACT

The electrical properties of plant stems represent physiological activities including water and ion transport. Plant responds to changes in environmental condition, which can be reflected in internal electrical conductivity of plant stems (EC_{ps}). Therefore, monitoring of EC_{ps} may help understand the plant physiological changes related to environmental stress. Because direct and stable measurement of EC_{ps} was very difficult, complicated, and expensive, the EC_{ps} could not be easily adapted to monitor plant responses to stressed environmental conditions. The objectives of this study were to develop a stable and simple measuring method of internal EC_{ps} and investigate the relationship between the EC_{ps} and environmental factors in greenhouse. Two electrodes with three needles were inserted into both sides of paprika stem to monitor paprika EC_{ps} and stable EC_{ps} was acquired. Environmental factors such as temperature, irradiance, and relative humidity were recorded and compared with the EC_{ps}. The EC_{ps} was positively correlated with light intensity and temperature (R^2=0.642 and 0.815, respectively), while negatively correlated with relative humidity (R^2=-0.416). The EC_{ps} was predicted using a regressed equation describing environmental data, and the predicted EC_{ps} corresponded well to measured ones. The EC_{ps} was higher during the day than at night, which was attributed to higher daytime water content in the stems. The EC_{ps} was better correlated with water content than ion concentrations in the stem. To use EC_{ps} for monitoring of paprika responses to environmental stress, relationship of EC_{ps} with plant physiological responses was established. The relationships between EC_{ps} and photosynthetic responses of paprika as well as sap flow were evaluated. Monitoring of paprika EC_{ps} relative to various environmental conditions such as low irradiance and water shortage showed that plant responses to environmental stress could be explained by changes in EC_{ps}. High EC_{ps} was related to high photosynthetic rate, stomatal conductance, and transpiration rate. Sap flow of the plant was also associated with EC_{ps}, with a correlation coefficient of 0.606. However, the sap flow reflected only water flux, while EC_{ps} was determined by both water and ion contents in stem of paprika. Comparison of measured and predicted EC_{ps}s could be used to detect unusual cultivation conditions of paprika. Plant responses to water shortage could be reflected on lower EC_{ps} compared with predicted value. Therefore, continuous monitoring of EC_{ps} can be used to detect plant responses to water stress. In order to use this method in the field, it will be necessary to test and develop field application techniques through further experiments such as comparison with sap flow.

Keywords: electrical conductivity; ion concentration; moisture content; paprika; water stress

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D, diameter of paprika stem (mm)

d, diameter of needle (mm)

EC_{ps}, internal electrical conductivity of plant stem ($\mathrm{mS \cdot m^{-1}}$)

EC_{ps20}, calibrated EC_{ps} at 20°C

$EC_{ps\ predicted}$, predicted EC_{ps} using Eq. I-3

IR, irradiance ($\mathrm{W \cdot m^{-2}}$)

L, length (mm) of needle inserted into bundle sheaths of paprika

$R_{20°C}$ resistance of paprika at 20°C

R_A, resistance (kΩ) of A section

R_B, resistance (kΩ) of B section

R_T, resistance (kΩ) of total paprika stem

$R_{T°C}$, resistance of paprika at T°C

RH, relative humidity (%)

T, temperature (°C)

INTRODUCTION

Various techniques to diagnose plant physiological status have been developed to assess plant biomass and potential for plant stress. The techniques include measurement of spectral reflectance, chlorophyll fluorescence, and plant hydraulic properties (Čermák et al., 2004; Naumann et al., 2007; Peñuelas and Filella, 1998; Stiller et al., 2003). The techniques used to measure plant physiological responses could be selected based on the experimental objectives. Spectral reflectance and chlorophyll fluorescence can be used to assess photosynthesis rate or plant biomass and plant hydraulic properties can be applied to monitor plant water status. However, none of these techniques provide an integrated view of plant growth and water status.

The electrical properties of plants have been used to estimate plant growth and physiological characteristics including water and ion transportation. The electrical properties of plants occur at the cellular level and are caused by differences in the flow of electrical current (Gora and Yanoviak, 2015). The electrical properties that can be measured include electrical potential, resistance, conductance, and impedance. In this study, electrical resistance of paprika was measured, which was converted to electrical conductivity to evaluate plant physiological responses to environmental conditions. Although some studies have been tried to assess plant physiological responses by measuring electrical properties, stable electrical signals could not be acquired and relationship between electrical signal and plant physiological properties was not established. Therefore, it is necessary to acquire stable and accurate electrical signals that reflect plant responses to environmental conditions, if electrical properties are to be used as plant physiological indicators.

Electrical properties are frequently related to rapid responses to plant stress to environmental conditions (Fromm and Lautner, 2007). Plant stress can be grouped into biotic and abiotic stresses. Abiotic stress occur by non-living factors including temperature, salt, drought and flood while biotic stress is induced by other living organisms such as bacteria, viruses, and fungi as well as weed (Jan et al., 2017). Individual plant responds differently to stress conditions and physiological aspect observed in response to stress is different. The responses of plants are sometimes not apparent or constant regarding visual indicators such as wilting and changes in the color of leaves. Therefore, evaluation techniques that are non-destructive, easily practical in the field, and cost effective need to be developed to effectively assess plant response to stress or environmental conditions. In addition, the developed evaluation technique was validated by comparing plant response data acquired by the developed method with plant physiological response obtained by other conventional techniques.

The measurement of electrical properties was proposed as a method to assess plant response to stress (Gora and Yanoviak, 2015). The objectives of this study are to develop methods that provide more stable and accurate plant internal electrical conductivity (EC_{ps}) and to understand the relationships of the EC_{ps} with environmental factors such as temperature, irradiance and relative humidity. In addition, effect of water content of a plant and the pH, electrical conductivity (EC), and ion content of plant sap on EC_{ps} was evaluated. To evaluate the relationships between EC_{ps} and plant physiological responses, the EC_{ps} was compared with the photosynthetic responses of paprika and sap flow.

LITERATURE REVIEW

Measurement of plant internal electrical conductivity

The electrical potential of plants was first measured by Fensom (1963) and more recently, Gibert et al. (2006) measured the electrical potential of poplar trees (*Populus nigra* L.) (Fig. 1). Electrodes were inserted in both of the soil and plants, and the electrical potential was measured using a voltmeter. Gibert et al. (2006) indicated that the flow of sap in the poplar is associated with electrical variation caused by electrochemical effects including membrane diffusion potentials and active transport of ions. Love et al. (2008) used a Pt electrode instead of stainless steel and a 1 M KCl agar salt bridge to complete the circuit through the soil/root interface. Differences in electrical potential can be used to indicate the daily variation of sap flow (Morat et al., 1994). This method was beneficial because it can measure inherent electrical potential differences. However, the data was influenced by environmental factors such as atmospheric electricity, point discharge, and the geomagnetic and electric fields of the Earth (Koppán, 1999; Koppán, 2000).

Electrical resistance was first quantified for trees using a Wheatstone bridge in the early 1900s (Stone and Chapman, 1912). In the 1970s, a device called a “Shigometer” was used to measure the resistance of trees (Fig. 2). The measurement was non-destructive, simple, and rapid (Shigo and Shigo, 1974). It was known that electrical resistance in the cambial zone of plants was inversely proportional to their growth rate (Shortle et al., 1977). However, the differences in resistance was observed only when there were significant differences in plant growth (Piene et al., 1984). In addition, resistance was not highly correlated with growth in individual plant (Smith and Ostrofsky, 1993).

Recently, it was reported that the electrical resistivity of English oak was inversely correlated with sap pH, and with the potassium and magnesium content of the measured cross section rather than with moisture content (Bieker and Rust, 2010). Resistance data can be converted to resistivity using the equation $\rho = RA/L$, where R = resistance in ohms, A = cross-sectional area of the plant stem or wood block, and L = distance between the electrodes (Gora et al., 2017). However, the resistivity was not measured using the entire cross-sectional area of the plant stem, but by some part of the cross-sectional area of the plant stem (Fig. 3), so the accurate electrical resistivity of the plant could not be measured.

Recently, Jeon et al. (2017) tried to measure real-time EC_{ps} of tomato stem using microsensor to evaluate tomato growth model and physiological properties. They concluded that there are still lots of technological defects that hamper measuring stable EC_{ps} that reflects the physiological conditions of plants. Ono et al. (2018) proposed a pure photosynthates extraction sensor device which can separately measure electrical conductivity of phloem and xylem. The position of phloem and xylem could be identified by measuring different electrical conductivity.

Plant responses to stress

Plant stress is a common aspect in plant life cycle, which often leads to crop loss. Approximately 75% of plant yield can be reduced by environmental stresses (Mousavi et al., 2016). Environmental conditions cause plant stress and plant stress induced by exposing to unfavorable conditions results in changes in physiology (Jansen and Potters, 2017). Environmental stress to plant induces production of stress proteins, up-regulates antioxidant machinery and accumulates compatible solutes. The physiological characteristics of plants under the drought and heat stresses are high respiration, low photosynthesis, stomata closure and increased leaf temperature. During the drought and heat stresses, starch decomposition was coupled with energy production in mitochondria (Mittler, 2006). Analysis of plant stress responses include genomics, proteomics, metabolomics and transgenic-based techniques (Pérez-Clemente et al., 2013). Plant stress responses also can be detected at the whole-plant level.

Genetic level studies are in relation to plant stress responses and helpful to understand plant stress adaptation and to produce engineered plant species with greater stress tolerance (Cushman and Bohnert, 2000). Metabolites and hormones involved in plant responses to drought, salinity and cold stress includes abscisic acid, jasmonic acid, salicylic acid, polyamines, proline, glycine-betaine, unsaturated fatty acids and reactive oxygen species (ROS). Metabolites related to metal detoxification are phytochelatins and metallothioneins (Pérez-Clemente et al., 2013). ROS is involved in the plant signaling pathways including membranes, redox homeostasis, plant hormones and photosynthesis (Sewelam et al., 2016). Drought and salt stress affected photosynthesis by regulation of photosynthetic genes and salt stress intensely affected genes because of the combined effects of dehydration and osmotic stress (Chaves et al., 2009).

Since photosynthetic processes were affected by plant stress conditions, chlorophyll fluorescence techniques can be used to evaluate photochemical and non-photochemical processes within thylakoid membranes, chloroplasts, and plant tissues (Roháček et al., 2008). Remote sensing has been used to assess crop vigor and health by motoring changes in the angle of leaves under water stress and color of plant leaves. The color of leaves provide information about nutrient limitations and imbalances. Light reflectance by a leaf depends on leaf surface properties, structure, and components in the leaf such as nitrogen, lignin and cellulose. Variation in reflectance is also related to absorption of water and other compounds (Barton, 2011). Hyperspectral imaging was used to assess drought stress in barley and the method could capture the distribution of leaf senescence and detect drought stress before visible symptom was developed (Behmann et al., 2014). Thermal imaging was employed to detect plant stress for shaded leaves. The infrared thermography could determine the onset of plant stress by detecting changes in stomatal aperture (Stoll and Jones, 2007).

Plant stress include biotic and abiotic stresses. Biotic stress is caused by living organisms such as weed and plant pathogens, and abiotic stress is caused by environmental conditions. Unfavorable environmental conditions which affect plant growth are drought, flooding, salt and metal stresses, and temperature. Siddiqui et al. (2008) evaluated combined drought and salt stress to *Brassica napus*. Combined drought and salt stress resulted in significant reduction in fresh and dry weights and decreased osmotic potential, and stomatal conductance was retarded. High temperature also affected plant growth by reducing leaf chlorophyll content, photosynthetic rate, and Rubisco activity (Almeselmani et al., 2012).

Drought stress reduces leaf size, stem extension, and proliferation of root, resulting in reduced water use efficiency (Farooq et al., 2009). Therefore, whole plant hydraulic conductance can be affected by the drought stress. Sap flow reflect the hydraulic conductance of plants. When soil water is enough for plants, sap flow rate depends on changes in solar radiation and vapor pressure deficit (Stöhr and Lösch, 2004). Drought in summer decreased leaf gas exchange and water potentials, and increased hydraulic conductance in olive tree, which caused modifications in hydraulic properties between soil and root (Tognetti et al., 2004). Patakas et al. (2005) evaluated grapevine inter-water status under drought conditions and showed that sap flow rates decreased in water stressed grapevine compared to irrigated plants, and stomatal conductance and photosynthetic rate were also significantly lower in stressed plants than irrigated plants.

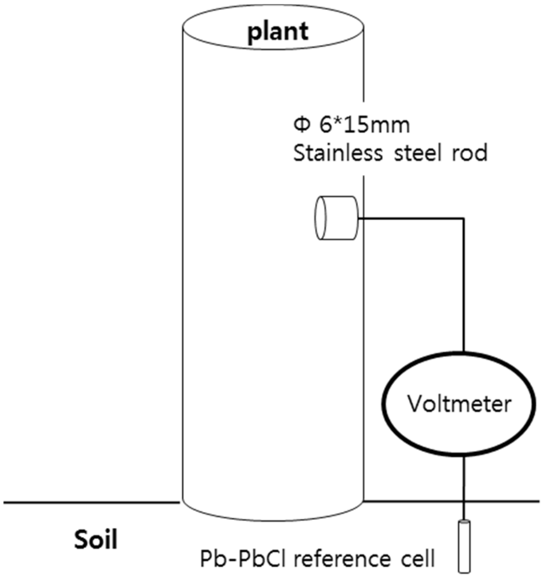

Fig. 1. Schematic diagrams of measuring plant electrical potential

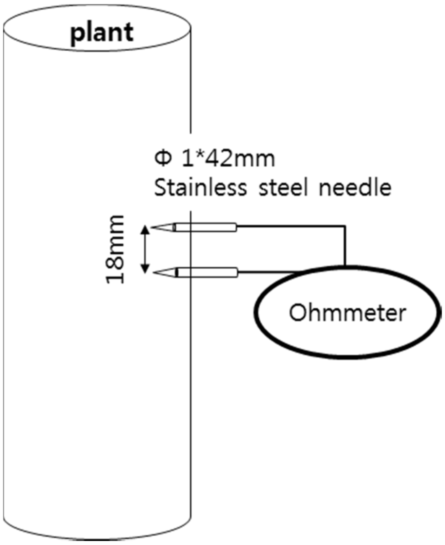

Fig. 2. Schematic diagrams of measuring plant electrical resistance

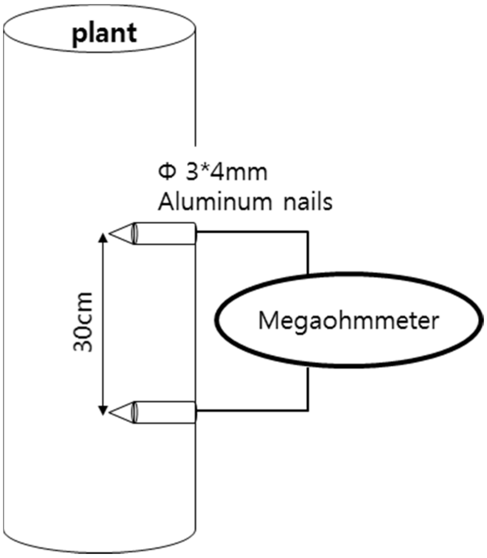

Fig. 3. Schematic diagrams of measuring plant electrical resistivity

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CHAPTER I

MEASUREMENT OF NON-DESTRUCTIVE PLANT INTERNAL ELECTRICAL CONDUCTIVITY OF PAPRIKA AND ITS RELATION TO ENVIRONMENTAL FACTORS

ABSTRACT

The electrical properties of plants represent physiological activities including water and ion transport in their stems. Therefore, monitoring of electrical conductivity of plants (EC_{ps}) can be used to detect plant responses to changes in environmental conditions. Stable measurement of internal EC_{ps} was developed and the relationship between the EC_{ps} and environmental factors was evaluated. Two electrodes with three needles were inserted into each side of paprika stem to monitor paprika EC_{ps} and environmental factors in terms of temperature, irradiance, and relative humidity were recorded. The EC_{ps} was positively correlated with irradiance and temperature

(R^2=0.642 and 0.815, respectively), while negatively correlated with relative humidity (R^2=-0.416). The EC_{ps} was higher in May than in February due to increment in the size of vascular bundle sheaths as well as higher irradiance and temperature. The EC_{ps} was predicted using a regressed equation describing environmental data, and the prediction results corresponded well to measured ones. The EC_{ps} was higher during the day than at night, which was attributed to higher daytime water content in the stems. Measurement of EC_{ps} is a non-destructive method to monitor plant responses to environmental conditions.

Additional keywords: electrical conductivity, ion concentration, moisture content, paprika, resistance

INTRODUCTION

The electrical properties of plants have been used to estimate plant growth and physiological characteristics. The electrical properties of plants occur at the cellular level and are caused by differences in the flow of electrical current involving cell membrane functions and extracellular and intracellular fluids. Among the electrical properties, the electrical potential, resistance, and impedance are the most commonly used in trees (Gora and Yanoviak, 2015). The electrical potential of plants was first measured by Fensom (1963) and recently Gibert et al. (2006) measured the electrical potential of poplar trees (*Populus nigra* L.). Electrodes were inserted in both of the soil and plants, and the electrical potential was measured using a voltmeter. Gibert et al. (2006) indicated that the flow of sap in the poplar is associated with electrical variation caused by electrochemical effects including membrane diffusion potentials and active transport of ions. Love et al. (2008) used a Pt electrode instead of stainless steel and a 1 M KCl agar salt bridge to complete the circuit through the soil/root interface. Differences in electrical potential can be used to indicate the daily variation of sap flow (Morat et al., 1994). This method was beneficial because it can measure inherent electrical potential differences. However, the data was influenced by environmental factors such as atmospheric electricity, point discharge, and the geomagnetic and electric fields of the Earth (Koppán et al., 1999; Koppán et al., 2000).

Electrical resistance was first quantified for trees using a Wheatstone bridge in the early 1900s (Stone and Chapman, 1912). In the 1970s, a device called a “Shigometer” was used to measure the resistance of trees. The measurement was nondestructive, simple, and rapid (Shigo and Shigo, 1974). It was known that electrical resistance in the cambial zone of plants was inversely proportional to their growth rate (Shortle et al., 1977). However, the differences in resistance was observed only when there were significant differences in plant growth (Piene et al., 1984). In addition, resistance was not highly correlated with growth in individual plants (Smith and Ostrofsky, 1993).

Recently, it was reported that the electrical resistivity of English oak was inversely correlated with sap pH, and with the potassium and magnesium content of the measured cross section rather than with moisture content (Bieker and Rust, 2010). Resistance data can be converted to resistivity using the equation $\rho = RA/L$, where R is resistance in ohms, A is cross-sectional area of the plant stem or wood block, and L is distance between electrodes (Gora et al., 2017). However, the resistivity was not measured using the entire cross-sectional area of the plant stem, but by some part of the cross-sectional area of the plant stem, so the accurate electrical resistivity of the plant could not be measured. Therefore, it is necessary to acquire stable and accurate electrical signals that reflect plant responses to environmental conditions, if electrical properties are to be used as plant physiological indicators.

Although, there have been many efforts to understand the relationships between physiological properties and electrical properties, it is still unclear whether environmental conditions are correlated with electrical conductivity of plants (EC_{ps}). The EC_{ps} is not easily acquired because of the instability of the data. The measurement of EC_{ps} to evaluate plant physiological properties was tried by Jeon et al. (2017). They concluded that there are still lots of technological defects that hamper measuring stable EC_{ps} that reflects the physiological conditions of plants.

Electrical properties are frequently related to rapid responses to stress (Fromm and Lautner, 2007). The responses of plants are sometimes not apparent or constant regarding visual indicators such as wilting and changes in the color of leaves. Therefore, evaluation techniques that are non-destructive, easily practical in the field, and cost effective need to be developed to effectively assess plant response to stress or environmental conditions. Sap flow measurement was proposed to evaluate transpiration rates of plants, which can be measured through heat balance by moving sap stream (Smith and Allen, 1996). However, sap flow measurement only provide information about water movement within plants. The measurement of electrical potential was proposed as a method to assess plant response to stress, but this is greatly affected by soil water and weather conditions (Dobbertin, 2005). Therefore, the relationships between environmental conditions and EC_{ps} should be clarified. The objectives of this study are to develop methods that provide more stable and accurate EC_{ps} and to understand the relationships of the EC_{ps} with environmental factors such as temperature, irradiance and relative humidity. In addition, effect of water content of a plant and the pH, electrical conductivity (EC), and ion content of plant sap on EC_{ps} was evaluated.

MATERIALS AND METHODS

Growth conditions of paprika

Paprika (*Capsicum annuum* L. ‘Cupra’) seedlings sown on 25 August, 2016 were transplanted into a venlo-type greenhouse at Protected Horticulture Research Institute, National Institute of Horticultural and Herbal Science, located at Haman, Korea on 22 September, 2016. Nutrient solutions of Dutch PBG were used for the paprika plants and contained N, P, K, Ca, and Mg at concentrations of 0.91, 0.12, 0.15, 0.19, and 0.10 mM, respectively, and the solution was supplied 10 times every day by the timer control for 4 minutes every hour to the growing media using pump. The EC of the nutrient solutions was maintained at 2.5–3.0 $dS \cdot m^{-1}$ and the pH at 5.8–6.0. Ventilation windows were opened when the room temperature rose above 25°C during the day, and heating was activated when the temperature fell below 18°C at night. Shading screens on the roof were closed when the temperature rose above 33°C. Environmental factors in terms of temperature, irradiance, and relative humidity of the greenhouse were monitored every hour during the experimental period inside the greenhouse.

Measuring EC_{ps} in paprika

A test plant was selected 90 days after transplanting when the plant had developed its cambium zone and its outer layer was lignified. It was possible to measure the EC_{ps} of paprika continuously with little influence from the needle insertions. To test the relationships between the EC_{ps} and environmental conditions, two electrodes with three stainless steel needles were inserted into each side of the lower stem of a paprika plant as shown in Fig. I-1. Insertion of three pins provided more stable data than did insertion of one or two pins (determined in preliminary experiments). The resistance was measured by the bridge circuit method after pulsing direct current on both sides of the two electrodes. Electrical resistance and environmental data were received every minute 3–5 February and 18–20 May, 2017 using a data logger (CR1000, Campbell Scientific, Logan, UT, USA) and the received data were averaged every hour. The resistance was converted to EC_{ps} following Eq. I-1.

Electrical conductivity measured between opposite faces of needles was calculated per cross-sectional area of the stem contacted by needles and proportional to the length of the stem between the needles. The area of the stem contacted by three needles is $L \times \pi d \times 3$ and the length of the stem between the needles is half of circumference and the average length of the stem between the needles was calculated at the middle of the needles inserted in the stem, which was calculated as $\pi\ (D\text{-}L)$.

$$EC_{ps} = \frac{1}{R_A} \times \frac{\pi(D-L)}{L \times \pi d \times 3} = k\,\frac{1}{R_T} \times \frac{(D-L)}{L \times d} \qquad \text{(Eq. I-1)}$$

where EC_{ps} ($\mathrm{S \cdot m^{-1}}$) is electrical conductivity of paprika; R_A and R_T are resistance (kΩ) of A section and total paprika stem, respectively, in Fig. I-1; D is the diameter (mm) of the paprika stem; L is the length (mm) of needle inserted into bundle sheaths of paprika; d is the diameter (mm) of the needle; and k is constant.

After R_T was measured, vascular bundle sheath of the paprika was removed and R_B was consequently measured. In this experiment, R_T occupied 79% of R_A. R_A could be calculated using R_T and R_B as described in Fig I-1B. k was obtained as 0.316 by calculating with the ratio of R_A and R_T, and it was also related to the conductivity ratio measured by the sensor comprised of actual surface (5 mm × 10 mm) and the three needles to the surface area.

According to the above equation, the EC_{ps} increased as the diameter of the paprika stem at the needle insertion position increased (i.e., as D of paprika stem at the lower end increased as the paprika grew). As the resistance of the paprika decreased, the EC_{ps} increased.

Temperature calibration

For temperature calibration, the paprika was cut 30 cm above the bottom and inserted into a rockwool cubic bed (10 cm × 10 cm × 10 cm) in a water container. The rockwool bed was filled with water and evaporation from the top of the paprika stem was prevented by a film covering. In these ways, the water content in the paprika stem was kept constant. A temperature sensor was inserted into the paprika stem at a depth of 5 mm. The rock wool cubic bed with the paprika stem was kept in a refrigerator at 4°C for 8 h; then placed in an incubator at 45°C. The temperature of the paprika was monitored and its resistance was measured every 10 min.

EC_{ps} ***modelling and its validation***

All resistance data were converted to EC_{ps} and calibrated for 20°C. The EC_{ps} and the temperature-calibrated EC_{ps20} were correlated with temperature, relative humidity, and irradiance. A correlation matrix (Pearson's correlation coefficient) of each type of data was created to assess the relationship between environmental conditions and the EC_{ps}. Regression was employed to find a model to predict the EC_{ps} using different environmental conditions (temperature, irradiance, and relative humidity) 3–5 February and 18–20

May, 2017. The goodness-of-fit of the regression model was evaluated using the coefficient of determination (R^2). A model was developed using the data from February and May to predict the EC_{ps} on 25 May, 2017 in relation to monitored environmental data such as temperature, relative humidity, and irradiance The predicted EC_{ps} was validated using the EC_{ps} measured on 25 May, 2017 to evaluate whether EC_{ps} model made from environmental data measured at a certain date is generally applicable to other day's environmental conditions. Statistical analysis of the data was carried out using SPSS version 24 (IBM, New York, NY, USA).

Water contents and sap extract properties in paprika stem

To measure the water content, EC, pH, and ion content of paprika stems, two individual paprika plants were cut at 15:00 (day) and 22:00 (night) on 6 February, 2017. Paprika stems were cut into five pieces, severed every 5 cm from their bottom and were weighed. Distilled water was added to five of the paprika stem pieces at a ratio of 10:1 (wt / wt). After 24 h of shaking, EC, pH, and ions (NO_3, PO_4, SO_4, Cl, Ca, Mg, K, and Na) in the extracted water were measured using an EC and pH meter (Star A215, Thermo Scientific, Waltham, MA, USA) after calibration, and ion chromatography (DX-500, Dionex, Thermo Scientific, Waltham, MA, USA), inductively coupled plasma optical emission spectroscopy (ICP-OES, ICAP7400, Thermo Scientific, Waltham, MA, USA), respectively. The remaining five pieces of paprika were dried at 60°C for 24 h; then, the water content of paprika was determined by measuring the dry weight. Comparison of the mean values of EC_{ps}, pH, EC, water, and ion content of the paprika between day and night was made using the t-test (SPSS) to verify which factors affected the EC_{ps}.

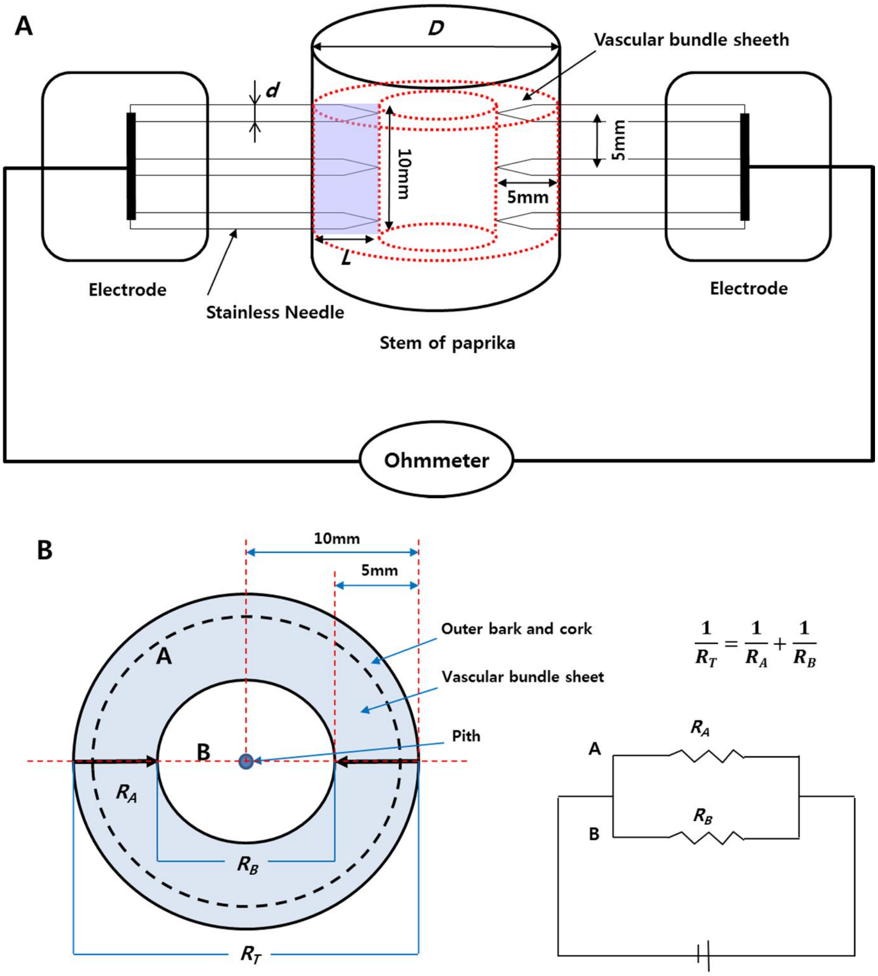

Fig. I-1. Measurement of electrical conductivity (EC_{ps}) of vascular tissue in a paprika stem with radius *r*. Two electrode with three needles each were inserted 5 mm deep into the stem on both sides (A). Calculation of resistance (R_A) of vascular tissue in a paprika stem. R_A is resistance of outer bark and cork, and vascular bundle sheath. R_T is resistance of whole stem and R_B is resistance of B section in a paprika stem (B).

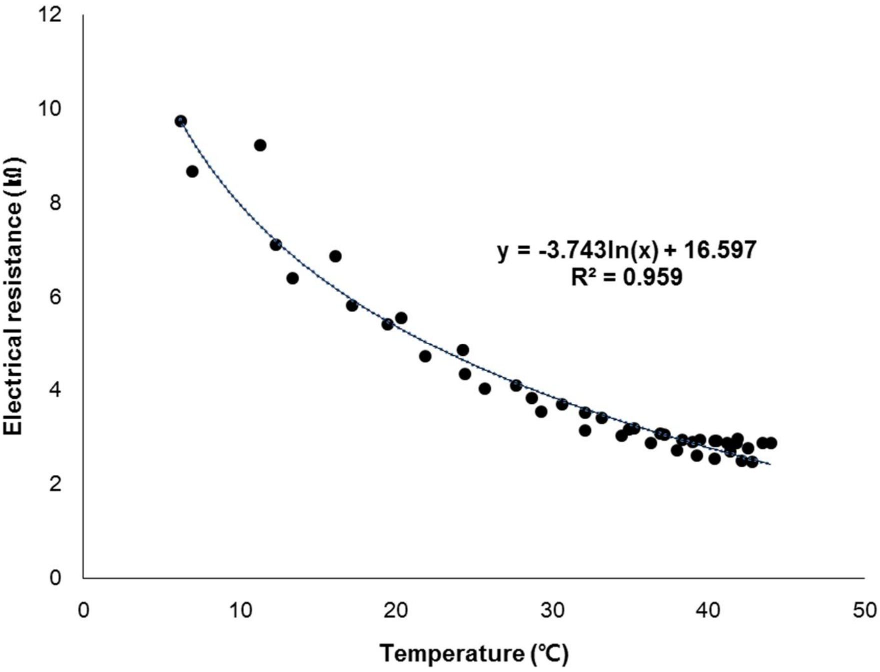

Fig. I-2. Changes in electrical resistance of a paprika stem with temperature.

RESULTS AND DISCUSSION

Resistance calibration with temperature

The resistance of paprika decreased with increasing temperature as indicated in Fig. I-2. The temperature-dependent values of paprika resistance were best represented by a logarithmic fit. Therefore, the resistance of paprika measured at a given temperature was calibrated to resistance at 20°C using Eq. I-2.

$$R_{20°C} = R_{T°C} + 3.743\,[\ln(T) - \ln(20)] \qquad \text{(Eq. I-2)}$$

where $R_{20°C}$ is the resistance of paprika at 20°C and $R_{T°C}$ is the resistance at T°C.

Correlation between EC_{ps} and environmental conditions

When the irradiance inside the greenhouse increased, the air temperature and EC_{ps} increased while the relative humidity decreased (Fig. I-3). Increment in EC_{ps} with increasing irradiance and temperature can be attributed to the high transpiration rate of paprika and to increase in water transport in the paprika stem during daylight hours. As irradiance increased, the stem electrical capacitance of spruce and fir trees was strongly influenced by sap water (Lekas et al., 1990). Temperature and irradiance were the highest around 13:00–14:00 and the EC_{ps} was the highest at 13:00 on 3 and 4 February, 2017. The relative humidity was the lowest at 13:00–15:00 (Fig. I-3A). However, the EC_{ps}, temperature, and irradiance of the paprika were all the highest at 11:00 on 5 February, 2017 due to cloudy weather in the afternoon, as shown by irradiance (Table I-1).

In May, irradiance was the highest at 09:00–11:00. This was 2–3 hours faster than in February, because in May, the shading screens of the roof were closed at temperatures above 33°C (Table I-2). The screens were operated to maintain optimum temperature for plant growth when irradiance was too high in summer. Temperature was highest at 12:00–14:00 and the EC_{ps} was the highest at 14:00–15:00 (Fig. I-3B). The time for paprika to reach maximum EC_{ps} was delayed by 1–2 hours in February, compared with the data in May.

The mean value of the EC_{ps} during daytime was calculated when irradiance had a positive value. The maximum and the means of EC_{ps} were higher during the day than night, and higher on clear days than cloudy days. The mean values of EC_p in May increased by 7–10 $mS \cdot m^{-1}$ compared with the data in February, which could be probably attributable to the increased intensity of irradiance followed by increased temperature. The mean values of temperature and irradiance in February were lower than those in May, which corresponds with the study by Mancuso (2000). Mancuso (2000) reported that electrical resistance, which is inversely proportional to EC in olive trees, increased as the temperature declined.

In this study, some differences in EC_{ps} could also be attributed to the growth stages of paprika. The same paprika plants were used for monitoring EC_{ps} in February and May, 2017. In the February measurements, the paprika plants were three and half months younger than in May, therefore there should be differences in biomass and physiological activities of paprika depending on the degree and stage of plant growth. Lekas et al. (1990) also reported seasonal trends in stem capacitance. The stem capacitance of trees increased following bud break until early June, although it slowly declined after that. Electrical resistivity varies with stem diameter, growth form, and plant species (Gora and Yanoviak, 2015). Increased EC_{ps} can be related to increased physiological activity represented by increase in ion and water transport (Fromm and Lautner, 2007).

The EC_{ps} was positively correlated with temperature and irradiance, with correlation coefficients of 0.815 and 0.642, respectively. The EC_{ps} and relative humidity were negatively correlated with a correlation coefficient of -0.416. Inverse correlation of EC_{ps} with relative humidity can be explained by the high transpiration rate of plants under conditions of low relative humidity and high water flow in stems. The EC_{ps20} was calibrated for 20°C, which was less correlated with environmental conditions than the non-calibrated EC_{ps}. Temperature calibration decreased the correlation coefficient because temperature greatly affected the EC_{ps} (Table I-3). Regression analysis between EC_{ps} and environmental conditions of temperature (T), irradiance (IR), and relative humidity (RH) was as follows.

$$EC_{ps} = 0.765 \cdot T + 0.002 \cdot IR + 0.105 \cdot RH + 0.012$$

$$(R^2 = 0.715, p < 0.01) \qquad \text{(Eq. I-3)}$$

The model was only applicable to paprika plant and basic EC_{ps} information should be provided when it is used for other plants. Eq. I-3 was used to predict the EC_{ps} using environmental conditions, and the predicted values were validated by comparing with measured EC_{ps}. The values predicted using Eq. I-3 corresponded well with the EC_{ps} measured on 25 May, 2017 (correlation coefficient 0.931; $p < 0.01$) (Fig. I-4). The high correlation coefficient indicated that the growth status of paprika is in accordance with environmental conditions. If the EC_{ps} predicted by environmental conditions is higher than the measured EC_{ps}, there might be some physiological problems within the plant. The lowered EC_{ps} can be related to water and salt stress conditions within the plant. Sap flow and hydraulic conductance of paprika was affected by salt stress (Carvajal et al., 1999). Under salt stress conditions caused by NaCl and Na_2SO_4 in hydroponic conditions, *Capsicum annuum* L. showed reduced stomatal conductance, root hydraulic conductance, and increased solutes flow into xylem (Navarro et al., 2003). Changes in solute flow and hydraulic conductance under salt and water stress condition will result in the changes in measured EC_{ps}. Therefore, differences in predicted and measured $EC_{ps}s$ might indicate that the growth of paprika is affected by other factors such as salt and water stress.

Relationship between EC_{ps}, and water content and ion concentration of paprika

As discussed in previous section, EC_{ps} can be influenced by changes in the water content and the ion concentration in the paprika stem. Therefore, the relationships between EC_{ps} and the water content and ion concentration of paprika were investigated. The water content and the EC_{ps} of the paprika stem were significantly higher at 15:00 (day) than at 22:00 (night). The EC of 1:10 paprika stem extract was slightly higher at 15:00 compared to 22:00, and the sum of anions and cations was also slightly higher at 15:00 than at 22:00 (Table I-2). Because the EC was high and pH was low at 15:00 compared to those at 22:00, organic acids in the sap might contribute to the higher EC at 15:00. Organic acids (e.g., malic acid and glutamic acid) decreased the pH and increased the EC of water (data not shown).

Most ion concentrations were similar between 15:00 and 22:00. However, the ion concentrations of potassium, sodium, phosphate, and nitrate were significantly higher at 15:00 than at 22:00. This could be related to the higher EC at 15:00. Peuke et al. (2001) reported that solute concentrations were constant during light and dark cycles (except for major solutes such as potassium and nitrate) in the phloem and xylem. When the aerial parts of olive plants were exposed to moderately high temperature, potassium accumulated in the stems (Benlloch-González et al., 2016). Potassium is known to regulate stomatal opening to avoid dehydration of the aerial parts when the air temperature increases and evaporation also increases (Fischer and Hsiao, 1968; Benlloch-González et al., 2016). Bieker and Rust (2010) reported that increasing potassium and magnesium was correlated with decreasing electrical resistivity in the heartwood of English oak. Lower phosphate concentrations at 15:00 than at 22:00 can be attributed to ATP production through photophosphorylation and oxidative phosphorylation during the day (Gardeström and Igamberdiev, 2016).

The electric field of a paprika stem is generally consistent because the structure of the vascular bundle sheaths of paprika does not significantly change in a day. The current density of a paprika stem will increase with increased current flow as the water and ion contents in the vascular bundle sheaths of paprika increase. Therefore, the EC_{ps} of paprika is positively correlated with the water and ion content in the vascular bundle sheath of paprika (Tattar et al., 1972).

The increment of the EC_{ps} of paprika might be influenced by increasing in the water content of the paprika stem and the ion concentration in the paprika vascular bundle sheaths with incline in transpiration rate, when the irradiance and temperature increased during the day. The EC_{ps} of paprika was correlated primarily with the moisture and mineral contents of the vascular bundle sheaths of paprika (Table I-2). Below the cambium saturation point, the degree of resistance was correlated primarily with the amount of moisture, while above the cambium saturation point; resistance was correlated primarily with the concentration of mobile ions (Koppán et al., 2002). Nutrient absorption by the roots is constant during the day and is not different between day and night, but the evaporation rate is lower at night, thereby resulting in higher ion concentration in the sap of paprika at night. Therefore, the difference in EC_{ps} of paprika at 15:00 (day) and 22:00 (night) seems to be more affected by water than by the ion content of the sap in the vascular bundle sheaths of paprika. Al Hagrey (2006) showed that electrical resistivity was inversely related to the moisture content in tree trunks. Lekas et al.

(1990) reported that stem electrical capacitance of spruce and fir trees was strongly influenced by water when irradiance increased.

Table I-1. EC_{ps} (mS·m^{-1}) in paprika plants vs environmental factors of temperature (°C), irradiance (W·m^{-2}), and relative humidity (RH, %) measured in February, 2017.

Measured time and environment factor		Mean			Standard deviation	Max		Min	
		Day	Night	Sum		Value	Time	value	time
3 Feb	EC_{ps}	21.28	17.73	19.29	2.27	23.43	13:00	15.75	23:00
	Temperature	24.18	17.89	20.66	4.04	27.82	14:00	16.64	23:00
	Irradiance	77.23	0.00	33.45	54.02	193.20	13:00	0.00	-
	RH	30.27	34.00	32.36	7.45	45.68	19:00	21.88	14:00
4 Feb	EC_{ps}	20.91	17.61	20.86	2.18	24.66	13:00	16.40	0:00
	Temperature	24.88	18.60	20.69	3.98	28.46	13:00	16.76	22:00
	Irradiance	92.99	0.00	33.09	55.91	192.50	14:00	0.00	-
	RH	35.01	48.31	43.52	13.86	62.78	22:00	17.04	15:00
5 Feb	EC_{ps}	19.18	15.92	17.35	1.98	21.32	11:00	14.03	0:00
	Temperature	22.11	18.35	20.00	2.96	26.33	11:00	16.70	3:00
	Irradiance	52.45	0.00	22.63	36.46	120.80	11:00	0.00	-
	RH	55.84	50.12	52.63	7.42	63.81	17:00	33.16	12:00

Table I-2. EC_{ps} (mS·m^{-1}) in paprika plants vs environmental factors of temperature (°C), irradiance (W·m^{-2}), and relative humidity (RH, %) measured in May, 2017.

Measured time and environment factor		Mean			Standard deviation	Max		Min	
		Day	Night	Sum		Value	Time	Value	Time
18 May	EC_{ps}	27.53	25.13	26.28	2.15	29.15	15:00	22.35	7:00
	Temperature	29.69	21.03	25.19	6.56	34.43	14:00	15.90	6:00
	Irradiance	177.76	0.00	125.50	103.28	312.50	9:00	-1.02	0:00
	RH	33.74	53.13	43.83	16.63	66.87	8:00	20.32	15:00
19 May	EC_{ps}	29.32	25.17	27.16	2.93	31.55	14:00	22.37	6:00
	Temperature	32.95	22.44	27.49	7.64	38.85	12:00	17.15	6:00
	Irradiance	279.57	0.00	194.00	161.05	623.70	11:00	17.59	5:00
	RH	28.71	51.64	40.63	19.00	65.37	7:00	15.08	12:00
20 May	EC_{ps}	28.74	24.73	26.65	2.87	32.63	14:00	22.56	6:00
	Temperature	32.34	22.44	27.19	6.94	38.47	13:00	17.82	6:00
	Irradiance	320.26	0.00	228.95	128.73	532.60	10:00	42.03	5:00
	RH	29.68	49.41	39.94	17.08	64.79	7:00	17.06	11:00

Table I-3. Correlation coefficient matrix for electrical conductivity (EC_{ps}, mS·m^{-1}) of paprika plants, calibrated EC_{ps20} at 20°C, and inside environmental factors of temperature (°C), irradiance (W·m^{-2}), and relative humidity (RH, %) measured from 3–5 February and 18–20 May, 2017.

Measured parameter	EC_{ps}	EC_{ps20}	Temperature	Irradiance	RH
EC_{ps}	1				
EC_{ps20}	0.974**	1			
Temperature	0.815**	0.674**	1		
Irradiance	0.642**	0.569**	0.764**	1	
RH	-0.416**	-0.264**	-0.713**	-0.538	1

Table I-4. Differences between moisture content and sap properties in paprika plants measured at 15:00 (day) and 22:00 (night) on 6 February, 2017.

Measured parameter	Day (15:00)	Night (22:00)	Difference (Day-Night)
EC_{ps} ($mS \cdot m^{-1}$, on 3–5 February)	19.40	15.91	3.49*
Moisture content (%)	70.61	67.52	3.09*
EC (1:10, $mS \cdot m^{-1}$)	11.67	11.40	0.27
pH (1:10)	6.46	6.58	-0.12
Ion concentration ($\mu mol \cdot g^{-1}$)			
NO_3^-	11.78	8.46	3.31*
PO_4^-	1.19	2.80	-1.61*
SO_4^-	0.96	1.00	-0.04
Cl^-	1.85	1.96	-0.11
Sum of anions	15.77	14.23	1.55
Ca^{2+}	1.89	2.29	-0.39
Mg^{2+}	1.84	2.38	-0.54
K^+	28.81	22.55	6.25*
Na^+	1.13	2.72	-1.59*
Sum of cations	33.67	29.93	3.73
Sum of anions and cations	49.44	44.16	5.28

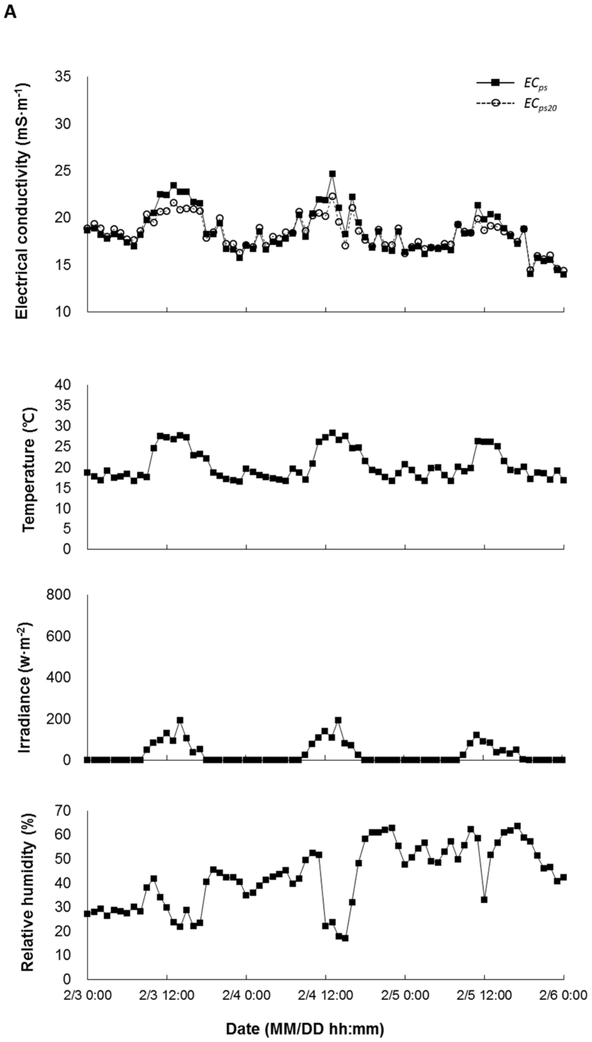

Fig. I-3. Electrical conductivity (EC_{ps}) in paprika plants in relation to inside temperature, irradiance, and relative humidity in the greenhouse from 3 to 5 February (A) and from 18 to 20 May (B), 2017. EC_{ps20} is the calibrated EC_{ps} at 20°C (continued).

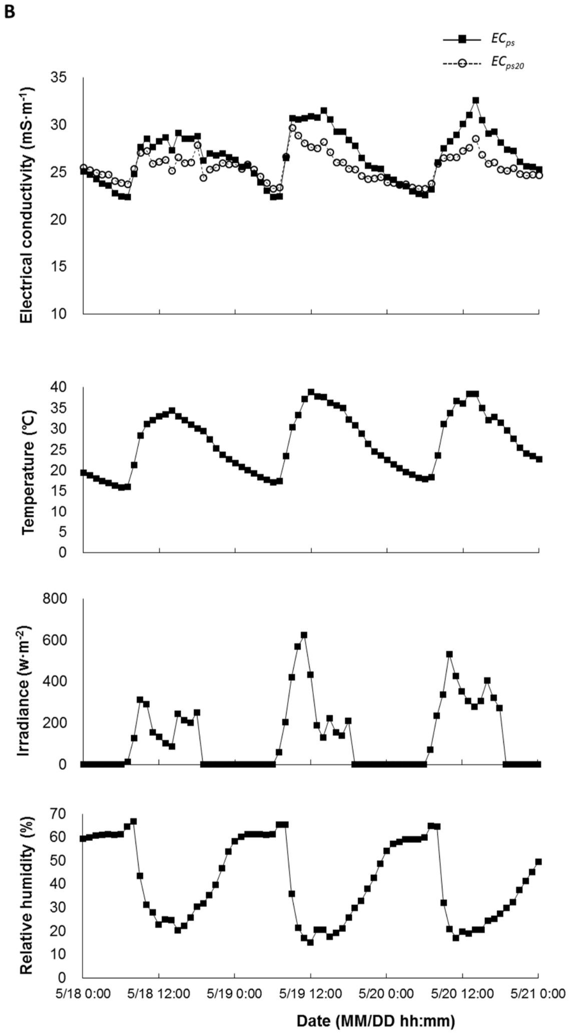

Fig. I-3. Electrical conductivity (*EC_{ps}*) in paprika plants in relation to inside temperature, irradiance, and relative humidity in the greenhouse from 3 to 5 February (A) and from 18 to 20 May (B), 2017. *EC_{ps20}* is the calibrated *EC_{ps}* at 20℃.

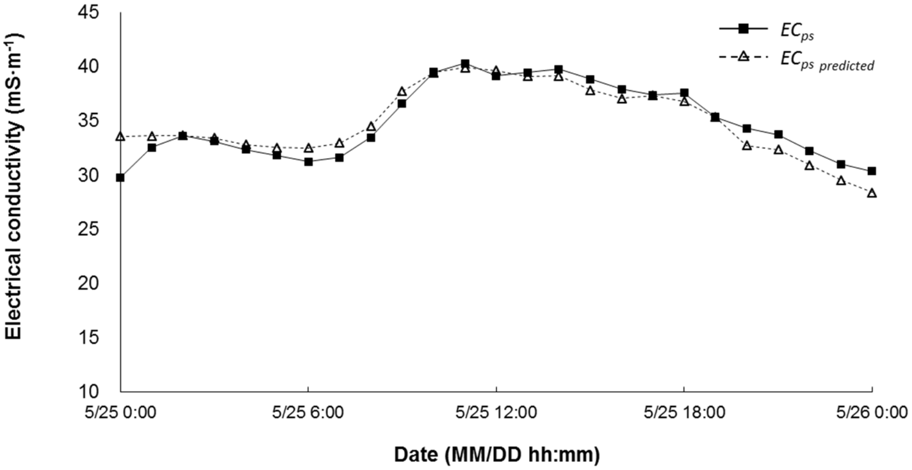

Fig. I-4. Comparison of the electrical conductivities (EC_{ps}) in paprika plants measured by the sensor and predicted by Eq. I-3 ($EC_{ps\ predicted}$) on 25 May, 2017.

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CHAPTER II

EVALUATION OF PLANT STRESS CONDITIONS IN PAPRIKA BY COMPARING INTERNAL ELECTRICAL CONDUCTIVITY, PHOTOSYNTHETIC RESPONSE, AND SAP FLOW

ABSTRACT

A non-destructive analytical method to measure plant internal electrical conductivity (EC_p) was developed to monitor plant responses to changes in environmental conditions. However, the relationship between EC_p and plant physiological responses has not yet been established. The objective of the study was to evaluate the relationships among EC_p, photosynthetic responses, and sap flow in paprika to monitor EC_p in relation to changes in environmental conditions. High EC_p levels were related to high photosynthetic rate, stomatal conductance, and transpiration rate. Sap flow in the plant was also associated with EC_p with a correlation coefficient of 0.606. However, the sap flow reflected only water flux, while EC_p was determined by both water and ion content in stems of paprika. The EC_p was predicted using environmental factors including temperature, irradiation, and relative humidity. A comparison of measured and predicted EC_ps could be used to detect unusual cultivation conditions for paprika such as drought and high temperature. Plant responses to water shortage were reflected by lower EC_p compared to the predicted value.

Additional key words: plant response, stomatal conductance, transpiration rate, water stress

INTRODUCTION

Changes in environmental conditions result in the crop yield losses because plant growth is affected by environmental stresses (Alcázar et al., 2006). The external symptoms of plant growth inhibition caused by changes in environmental conditions are not immediately expressed and can be slow to develop. When symptoms are expressed, this means that plant growth and yield have already been affected by the environmental stresses. Therefore, various analytical methods that reflect plant physiological status are used to monitor plants responses to environmental stresses. Water stress causes decrease in water potential of plant leaf and stomatal opening, thus leading to the reduced photosynthesis (Osakabe et al., 2014). Therefore, photosynthetic rate, stomatal conductance can be used to monitor plant responses. Photosynthesis is closely related to crop productivity and can be measured by chlorophyll fluorescence (Bresson et al., 2015; Kalaji et al., 2016). Water availability from the soil is an essential factor for determining plant productivity and water availability for transpiration in plants can be measured by sap flow (Ježík et al., 2015); high transpiration drives sap flow, which is influenced by atmospheric conditions such as irradiation, vapor pressure deficit, temperature, and CO_2 concentration (Cruiziat et al., 2002). Innovation in the measurement of physiological responses of plants has been performed by omics-driven research, including phenotyping bioinformatics and metabolomics with aid of mass spectrometry based analytical techniques (Humplík et al., 2015; Jorge et al., 2015; Negrão et al., 2017).

The electrical properties of plant cells can be used to evaluate plant physiological conditions affected by growth stage, cell structure, and nutrient, water, and temperature conditions (Repo, 1988). A method for non-destructive measurement of plant internal electrical conductivity (EC_{ps}) was developed. Electrical resistance of plant stem was measured by inserting two electrodes with three neeldles into each side of plant stem and converted to electrical conductivity. The EC_{ps} is correlated with environmental factors such as temperature, irradiance, and relative humidity (Park et al., 2018).

Accordingly, EC_{ps} can be used to monitor plant responses to changes in their environmental conditions. Compared to other techniques including omics-driven methods and chlorophyll fluorescence, in this method EC_{ps} can be relatively easily and cost-effectively acquired; however, no relationship between EC_{ps} and physiological responses of plants has been established. The objectives of this study were to evaluate the relationships among EC_{ps}, the photosynthetic responses, and sap flow in paprika, and to monitor EC_{ps} in relation to changes in environmental conditions.

MATERIALS AND METHODS

Monitoring EC_{ps} in paprika plants

Paprika (*Capsicum annuum* L. ‘Cupra’) seedlings were transplanted into rockwool based growing media and grown in a Venlo-type greenhouse in Haman, Korea. Dutch PBG nutrient solution was comprised of N, P, K, Ca, and Mg at concentrations of 0.91, 0.12, 0.15, 0.19, and 0.10 mM, respectively, and the solution was supplied 10 times every day by the timer control for 4 minutes every hour to the growing media using pump. Electrical conductivity (EC) and pH of the nutrient solutions were maintained at 2.5–3.0 $dS{\cdot}m^{-1}$ and 5.8–6.0, respectively. The inside temperature of the greenhouse was maintained at 18 to 33°C by closing shading screens on the roof when temperatures were above 33°C. Environmental conditions such as air temperature, irradiance, and relative humidity in the greenhouse were recorded every hour during the experimental period.

Internal electrical conductivity (EC_{ps}) of paprika plants were measured 100 days after transplanting by using the bridge circuit method after pulsing direct current on both sides of the two electrodes with three stainless steel needles and converting electrical resistance to electrical conductivity (Jun's meter, Prumbio, Suwon, Korea) as previously described (Park et al., 2018). Electrical resistance and environmental data were recorded every minute 23–24 March, 11–14 May, and 31 May to 2 June, 2017, using a data logger (CR1000, Campbell Scientific, Logan, UT, USA); the received data were averaged every hour. Stable EC_{ps} data was acquired for more than 4 days and EC_{ps} was monitored for 1–4 days, which was enough to evaluate changes in plant electrical responses. Measurements of the EC_{ps} from 23 to 24 March was used to compare EC_p with paprika photosynthetic performance, which included photosynthetic rate, stomatal conductance, and transpiration rate. EC_{ps} measured from 31 May to 2 June was used to compare EC_{ps} with paprika sap flow. Monitoring of EC_{ps} from 11 to 14 May was conducted to evaluate plant electrical responses under various environmental conditions. Three individual paprika plants (paprika 1, 2, and 3) in identical growing media were used to monitor the EC_{ps} of each paprika.

Measurement of photosynthetic rate, stomatal conductance, transpiration rate, and sap flow

Net CO_2 assimilation rate, stomatal conductance of water vapor and transpiration rate were measured for 20 minutes at 10 s intervals using a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) equipped with an infrared gas analyzer. A fully developed leaf was placed across the 6 cm^2 leaf chamber. The temperature of the leaf chamber was maintained at 25ºC and relative humidity at 60–70%. Light was beamed through a transparent window at 400 $\mu mol \cdot mol^{-1}$ of CO_2 concentration. Photosynthetic rate was determined by the net CO_2 assimilation rate as a function of light curves and stomatal conductance of water vapor was calculated using the Ball, Woodrow and Berry (BWB) model (Ball et al., 1987).

The sap flow in the stem of paprika plants was measured using the heat-balance technique (Steinberg et al., 1989) with a handgrip sap flow meter with a 19-mm diameter (SGB19, Dynamax Inc., Houston, TX, USA). The sap flow meter had a flexible heater, a layer of foam insulation, and an aluminum coated PVC weather shield. Sap flow was measured every 10 s and recorded every 10 min using the data logger, and then averaged over 1 h.

RESULTS AND DISCUSSION

Relationship between EC_{ps} and photosynthetic responses in paprika plants

The EC_{ps} in paprika was positively correlated with temperature and negatively correlated with relative humidity (Fig. II-1), which were consistent with a previous study (Park et al., 2018). Gora and Yanoviak (2014) also explained that electrical properties of trees were related to temperature and relative humidity. The higher irradiation on 23 March compared to 24 March resulted in a higher photosynthetic rate (Table II-1, Fig. II-1). Photosynthetic rate is related to environmental conditions such as CO_2 concentration, light, and temperature (Farquhar et al., 1980); high light intensity leads to high photosynthesis and an increased light saturated oxygen evolution rate (Bailey et al., 2001). Photosynthetic rate is also related to the hydraulic conductivity of leaf vascular system, which transports water to the photosynthesizing mesophyll cells (Brodribb et al., 2007). Because water content in paprika plants was found to be related to EC_{ps} of paprika in our previous study, it was expected that high EC_{ps} would be related to a high photosynthetic rate.

Stomatal conductance and transpiration rate were also higher on 23 March than on 24 March due to higher temperature and irradiance on 23 March than on 24 March (Table II-1, Fig. II-1). Stomatal conductance governs water use and carbon uptake in plants and is used to predict plant functions such as stem hydraulic conductivity and photosysnthesis (Medlyn et al., 2011). Stomatal conductance was higher during the day than at night because of the higher transpiration rate during the day. Stomatal conductance was proportional to the water potential difference between the soil, and leaf and reversely proportional to the vapor pressure deficit (Hubbard et al., 2001).

Photosynthetic rate is related to stomatal conductance because intercellular CO_2 concentration is affected by photosynthesis and influx of CO_2 regulated by stomatal conductance. Therefore, stomatal conductance is coupled with photosynthesis, intercellular CO_2 concentration, the flux of water from the soil to the plant, and stem hydraulic resistance (Tuzet et al., 2003). The relatively low photosynthetic rate in the paprika plants can be attributed to plant age (kim et al., 2003). When the photosynthetic rate was measured, paprika reached its maximum growth and photosynthetic rate was not fully active. It was reported in various experimental studies that photosynthesis and stomatal conductance generally decreased with increasing age of shrubs and trees (Bond, 2000). Stomatal conductance was relatively low in taller and older plants, which resulted in lower intercellular CO_2 concentrations and a decline in the foliar photosynthesis rate (Niinemets, 2002).

Transpiration rate was related to stomatal opening and water condition. Generally, transpiration rates in plants at night are known to be 5 to 15% of daytime transpiration rates, although nighttime transpiration rates sometimes reaches 30% that of daytime rates (Caird et al., 2007). Transpiration rate at night on 24 March was 75% of the daytime transpiration rate, which might be attributed to low transpiration rate during daytime caused by the low irradiance on that day (Schulze et al., 1985; Lanoue et al., 2018). In addition, incomplete stomatal closure at night can result in relatively high transpiration rates at night (Caird et al., 2007). Accordingly, the EC_{ps} did not decline much in the nighttime compared to the daytime, and this effect was observed on cloudy days (Table II-1). Overall, when the photosynthetic rate, stomatal conductance, and transpiration rate were high, the EC_{ps} was also high and photosynthetic responses were well reflected in EC_{ps} values. These results show that monitoring of EC_{ps} can be used to predict the photosynthetic responses and productivity in paprika plants.

Relationship between EC_{ps} and sap flow in Paprika

Sap flow was correlated with temperature, irradiance, and relative humidity with correlation coefficients of 0.915, 0.915, and -0.802, respectively (Table II-2). In particular, irradiance and temperature had significantly high correlations with sap flow. It was reported that sap flow was highly correlated with solar radiation and vapor pressure deficit measured by single-probe heat pulse method (López-Bernal et al., 2017). Baek et al. (2018) also showed that sap flow measured by micro-needle sensor was correlated with solar intensity and house temperature. In field experiments by Nardini et al. (2001), the hydraulic conductivity of *Laurus nobilis* branches exposed to sun was 60% higher than those for shaded branches. Higher irradiance and temperature increased the water demand of plants as well as sap flow and transpiration. When irradiance and temperature are high, plant demand for water increases along with sap flow, which leads to an increase in transpiration (Wilson et al., 2001). Wullschleger et al. (2001) reported that sap flow could be used to estimate transpiration, which can be described by the vapor pressure deficit, radiation, and leaf area index. Transpiration was the highest prior to canopy closure. Therefore, high sap flow indicates high transpiration and sap flow data reflect water demand of plants.

The EC_{ps} was also correlated with sap flow; we calculated a correlation coefficient of 0.606. However, the correlation coefficient between $\mathrm{EC_p}$ and environmental factors was lower compared to the correlation coefficient between sap flow and environmental factors, which might be attributed to an indirect reflection of EC_{ps} under different environmental conditions. The value of EC_{ps} reflects internal water and salt contents in plants in response to changes in environmental conditions. Sap flow shows the water flux rate in the plant stem, while EC_{ps} represents both water and ion contents. Because $EC_{ps,}$ was concomitantly affected by water and ion contents, it fluctuated less than the sap flow over the course of one day. Peuke et al. (2001) reported that xylem sap flow rates were significantly higher during the day than at night, which was compensated for by solute concentration changes between the day and night. Koppán et al. (2002) also reported that there was no linear relationship could be observed between sap flow and electric potential difference because of daily fluctuations in ion concentration in the plant stem. Since water flown and ion transport are not independent each other, the two factors should be considered together for monitoring of plant physiological responses. Therefore, $\mathrm{EC_p}$ is useful to monitor plant physiological responses under different environmental conditions.

Sap flow was the highest on 31 May, followed by 2 June and 1 June, over which period the highest EC_{ps} value gradually decreased with time (Fig. II-2). The sap flow was higher on 2 June than on 1 June, irrespective of the lower EC_{ps}, which can be attributed to the lowest measured relative humidity that occurred on 2 June. Low relative humidity might facilitate transpiration and result in relatively high sap flow. High evaporation demand resulted in high sap flow (Nadezhdina et al., 2002). Therefore, sap flow was more closely related to water demand and flow than ion transportation within the stem.

Monitoring EC_{ps} in relation to environmental conditions

EC_{ps} was monitored for 4 days 11–14 May, during which various environmental conditions occurred (Fig. II-3). EC_{ps} can be predicted using environmental factors including temperature, irradiance, and relative humidity, which are used as an indicator of abnormal growth conditions and plant responses (Park et al., 2018). Generally, EC_{ps} was the highest in the hours between 12:00 and 15:00 and the lowest during the night. However, during the day on 12 May EC_{ps} did not reach a peak because of the overcast conditions, as shown by irradiance values.

Measured EC_{ps} values in paprika plants 1 and 3 on a cloudy day (12 May) were higher than the predicted EC_{ps}. Predicted EC_{ps} was calculated from the environmental factors including temperature, irradiance, and relative humidity, which are related to the expected growth performance of paprika under the given environmental conditions. Thus, the higher measured EC_{ps} compared to the predicted value for a cloudy day indicates either a better growing state or higher ion flux in paprika was expected. The measured EC_{ps} 2 of paprika plant 2 was lower than the predicted value over the monitoring period, indicating that the growth responses of paprika plant 2 were worse than expected and also worse than those of paprika plants 1 and 3. Although the growth parameters for paprika plants were not measured in this study, apparently the external growth of paprika 2 was lower than those of paprika plants 1 and 3. As plant growth proceeded, water and ion transportation increased, resulting in high EC_{ps} values. Electrical signals in living organisms reflect plant physiological responses to environmental conditions and continuous growth (Gurovich and Hermosilla, 2009). Electrical potential values were related to not only soil water availability and environmental conditions, but also plant species characteristic (gurovich and Hermosilla, 2009). Therefore, the absolute $\mathrm{EC_p}$ will be different for individual plant, however, the pattern change along with time according to environmental conditions will be same within same plant species.

The measured EC_{ps} values decreased and were lower than those predicted after 14 May, which indicated abnormal conditions from this date. Irradiance on 14 May was higher than on 13 May, and the relative humidity on 14 May was significantly lower than 13 May, indicating that plant transpiration was relatively high on 14 May. Because the water supply was constant over the experimental period, the demand of paprika plants for water could have been high on 14 May compared to water demand on 13 May, which could result in water stress in the plants. Hence, the EC_{ps} was lower than the values predicted using these environmental conditions. Water stress in plants leads to retarded development (Ortuño et al., 2006). Water stress caused by disturbed irrigation resulted in stomatal closure, and affected plant hydraulics and transpiration rate (Cochard et al., 2002). Water shortage conditions reduced sap flux density (Masmoudi et al, 2011). Decreased sap flow might lead to low water contents in the stems of paprika plants and to a decline in measured EC_{ps}. Consequently, a comparison of measured and predicted values of EC_{ps} can be used to identify abnormal conditions or stress in plants.

Table II-1. Relationship among internal electrical conductivity (EC_{ps}), photosynthetic rate, stomatal conductance, and transpiration rate in paprika plants during the day and night from 23 to 24 March, 2017.

Parameters	23 March		24 March	
	13:00	19:00	12:00	19:00
EC_{ps} ($mS \cdot m^{-1}$)	27.898	25.584	25.402	21.745
Photosynthetic rate ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)	5.758	-1.514	2.982	-1.723
Stomatal conductance ($mol\ H_2O \cdot m^{-2} \cdot s^{-1}$)	0.106	0.067	0.084	0.061
Transpiration rate ($mol \cdot m^{-2} \cdot s^{-1}$)	1.074	0.669	0.866	0.653

Table II-2. Correlation coefficient matrix for sap flow, internal electrical conductivity (EC_{ps}) in paprika plants, and environmental factors such as temperature, irradiance and relative humidity (RH) measured from 31 May to 1 June, 2017.

Parameter	Sap flow	EC_{ps}	Temperature	Irradiance	RH
Sap flow	1				
EC_{ps}	0.606**	1			
Temperature	0.915**	0.822**	1		
Irradiance	0.915**	0.472**	0.766**	1	
RH	-0.802**	-0.464**	-0.807**	-0.657	1

**$p < 0.01$ (Pearson's correlation coefficient).

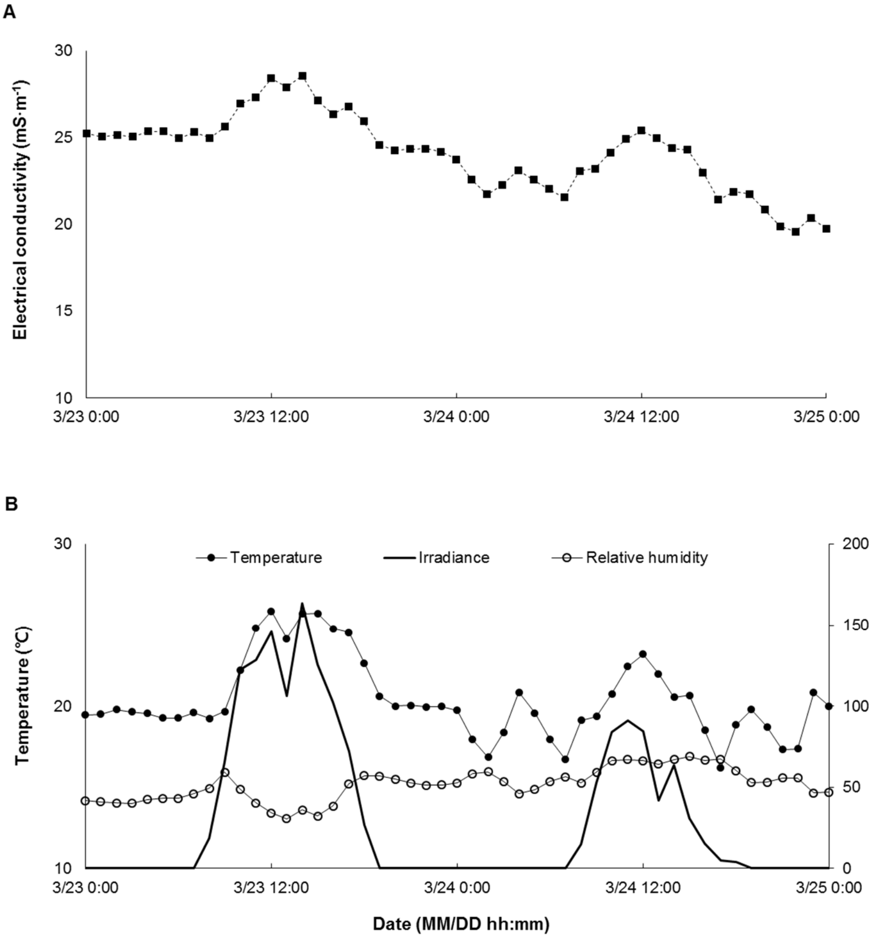

Fig. II-1. Internal electrical conductivity in paprika plants (A) and monitored meteorological data such as temperature, irradiance, and relative humidity (B) from 23 to 25 March, 2017.

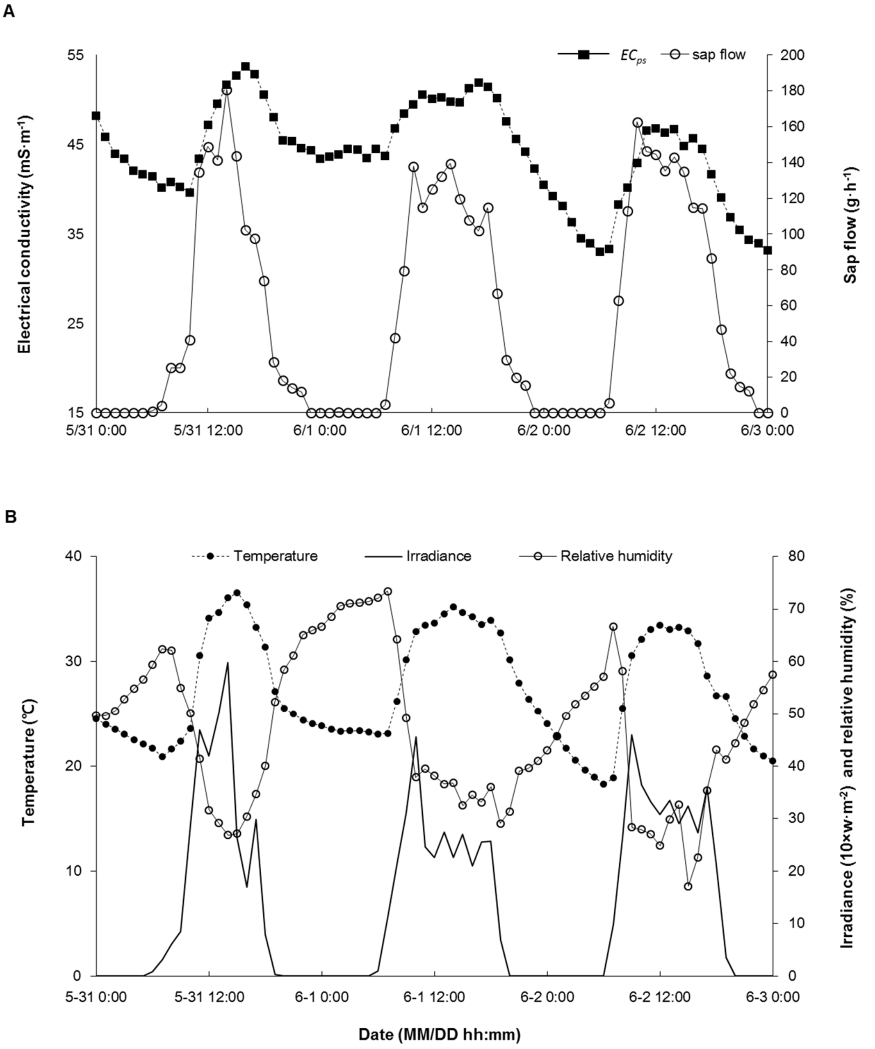

Fig. II-2. Internal electrical conductivity and sap flow in paprika plants (A) and monitored meteorological data such as temperature, irradiance, and relative humidity (B) from 3 May to 3 June, 2017.

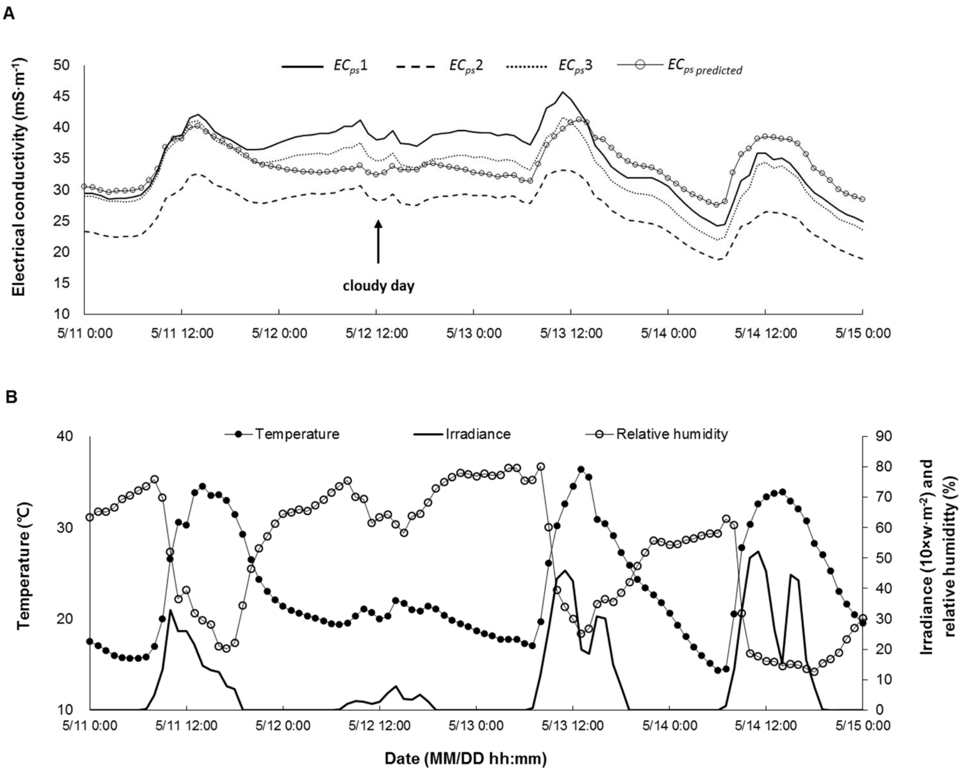

Fig. II-3. Monitoring of internal electrical conductivity ($EC_{ps}1$, $EC_{ps}2$, and $EC_{ps}3$) in paprika plants 1, 2, and 3 and their comparison with predicted $EC_{ps\ predicted}$ (A) calculated from environmental conditions in relation to various environmental conditions (B).

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CONCLUSION

Measurement of EC_{ps} is a non-destructive method to monitor plant responses to environmental conditions. The EC_{ps} in paprika plants could be stably monitored and well represented the water and ion contents of paprika stem. The EC_{ps} could be precisely predicted using environmental factors such as temperature, irradiance, and relative humidity. Therefore, unusual cultivation conditions for paprika such as drought and high temperature can be predicted by comparing the monitored and predicted EC_{ps}.

EC_{ps} is positively related to photosynthetic responses and sap flow, which was influenced mainly by water flux in the paprika stem. In fact, sap flow represents only water flux, while EC_{ps} is related with both ion and water contents. Consequently, EC_{ps} can describe physiological plant responses to changes in their environments. Dynamic environmental conditions such as cloudy days and water stress were reflected in EC_{ps} of paprika. However, more research is needed to identify species-specific EC_{ps} patterns and the relationships between plant growth stage and EC_{ps} change.

Further studies on electrical conductivity variance by plant stress conditions such as drought and temperature stress are expected to use EC_{ps} as an indicator for evaluating plant stress responses.

ABSTRACT IN KOREAN

식물 줄기의 전기적 특성은 줄기에서 수분과 이온 수송을 비롯한 여러 가지 생리적 활동을 나타낸다. 식물은 환경조건에 따라 반응하며 이는 식물의 내부 전기 전도도(EC_{ps})에 반영될 수 있다. 따라서 EC_{ps} 를 측정·관찰하는 것은 환경 스트레스와 관련된 식물 생리학적 변화를 이해하는 데 도움이 될 수 있다. 그러나 EC_{ps} 를 측정하는 방법은 복잡하고 어려워 환경 스트레스에 따른 식물 반응을 관찰하는 데 적합하지 않았다.

따라서 본 연구에서는 3 개의 바늘을 가진 2 개의 전극을 파프리카 하부 줄기의 양쪽에 삽입하여 내부 전기전도도($\mathrm{EC_{ps}}$)를 간단하고 안정적으로 측정할 수 있는 방법을 개발하였으며, 기온, 상대습도, 광량 등 온실 내부 환경요인과 EC_{ps}를 비교하였다. EC_{ps}는 기온 및 광량과 양의 상관 관계(각각, $R^2=0.82$ 및 0.64)를 보였으나 상대습도와는 음의 상관 관계($R^2=-0.42$)를 보였다. 기온, 광량, 상대습도 등의 환경요인을 이용한 회귀식($R^2=0.72$, $p<0.01$)을 구하였으며, 이를 이용하여 EC_{ps} 를 예측

하였다. 예측된 EC_{ps}는 측정된 EC_{ps} 값과 잘 일치하였다. EC_{ps}는 야간보다 주간에 높았으며, 파프리카 줄기의 수분함량은 주간에 높았으나, 이온 농도는 주야간 큰 차이를 보이지 않았다.

EC_{ps}를 이용하여 환경 스트레스에 대한 파프리카 반응을 관측하기 위해 생리반응과 EC_{ps}의 관계를 관찰하였으며, 여러 가지 생리반응 중 파프리카의 광합성 및 sap flow 와 EC_{ps} 관계를 평가하였다. 낮은 광량 및 수분 부족과 같은 환경 스트레스에 대한 파프리카 EC_{ps}의 관측은 환경 스트레스에 대한 식물 반응이 EC_{ps}의 변화에 의해 설명될 수 있음을 보여 주었다. EC_{ps}는 광합성 속도, 기공 전도도 및 증산 속도와 양의 관계가 있었다. 또한 증산류(sap flow)는 상관 계수 0.61 으로 양의 상관관계가 있었다. 하지만 증산류가 파프리카 줄기 내 수분 흐름만을 반영한 반면, EC_{ps}는 도관에서 수분과 이온 함량을 동시에 반영하였다. EC_{ps}의 측정값과 예측값을 비교하면 파프리카의 비정상적인 재배 조건을 감지할 수 있었으며, 실제로 수분 부족의 경우에는 EC_{ps}의 측정값이 예측값보다 낮게 나타났다. 따라서 지속적으로 EC_{ps}를 측정함과 동시에 예측값과 비교하면 파프리카의 수분스트레스에 대한 반응을

감지할 수 있을 것으로 판단되며, 추후 수분 스트레스에 대한 증산류와의 비교 등 실증 실험을 통하여 현장 적용이 가능할 것으로 판단된다.

주요어: 수분 스트레스, 수분 함량, 이온 함량, 전기전도도, 파프리카

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