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Nondestructive Estimation Method of Water Status of Living Crops using Near Infrared Spectroscopy

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Mohammad Hanif Afzali

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ABSTRACT

For proper irrigation planning, a non-destructive and non-penetrating method of measuring water content of plants and soils is needed. Near-infrared (NIR) reflectance technology is designed to provide rapid, non-destructive, and accurate measurements of water inside materials.

As described in chapter 2, the NIRON-sensor technology (Spectral Engines NIRONE Sensor S2.0) was examined to calibrate by adjustment to achieve the best performance of certain model, and verification with ascertains that my assumption model implement correctly. A total of 40 reflectance spectra (1550–1950 nm) from different plant and soil samples were measured in the laboratory using an inexpensive hand-held sensor. The result of drying experiments showed that there were close relationships between spectrum parameters of the water percentage of leaves and soil. The result of log-linear model and linear regression analysis showed significant effects to spectrum parameters based on the water percentage of leaves and soil respectively. The result of my study confirmed that the absorption of NIR light by samples' water was highest around 1940 nm and lowest at around 1650 nm. It is suggested that the simple index using single wavelength (1940 nm) can be used for universal index to estimate the water content of leaves. The ANCOVA analysis showed significant differences of regression among soil types in Japan. This result suggests that different calibration models for the water percentage of the soil is needed for each soil type. These results reveal that an inexpensive hand-held NIR sensor can be applied to measure water status of living plants in the field.

Chapter 3 shows that, to validate my assumption by seeking and establishing the agreement between my prediction and observation a pot drying experiment was conducted. The objective of this study was to evaluate the ability of a hand-held near-infrared (NIR) sensor and water absorption index to estimate the water content in intact eggplant leaves. The NIR spectroscopy using a single wavelength (1940 nm) has already been used to estimate the water content of detached samples in a laboratory. However, it is not known whether this method can be used to estimate the water content of intact leaves of living plants. Therefore, I attempted to compare this method with destructive method by simulating drought stress and analyzing the relationship between leaf water content and soil matric potential. The water content of intact eggplant leaves was predicted with a calibration model using NIR reflectance. Both the measured and predicted water content of leaves decreased with decreasing soil matric potential. These results show that both destructive and NIR measurements can be used to estimate the water stress condition. Based on these findings, a hand-held NIR sensor is useful for estimating the water content of intact leaves of living plants.

Chapter 1

General introduction

One of the significant problem for the high quality and quantity of agricultural products in upland and dry regions such as Afghanistan is the irregular and inappropriate use of water for the plant. Water scarcity is one of the most important constraints for sustainable crop production, particularly in arid area such as Afghanistan. As noted by (Mancosu *et al.*, 2015; Parwani, 2018); the limitation of available water for agricultural production in most parts of Afghanistan is partly due to short seasonal precipitation and unimproved irrigation channels. Moreover, due to the country's dry climatic condition, most of its cultivable areas receives scarce rainfall when irrigation is needed (Rout, 2008).

To overcome the water scarcity and produce high quantity and quality of agricultural products, a sound irrigation planning is the most important element. Appropriate irrigation of plant cause reduction of water use and water waste, also prevent incidence of disease (Dumroese *et al.,* 2015). Sustainable and efficient use of natural resources particularly water can be ensuring high yield in improved agriculture methodology (Gadanakis *et al*. 2015; Çolak *et al*. 2015). Furthermore, wasteful use of underground and surface water severely affects the irrigation system and environment, although the efficient use of agricultural water can reduce water deficit and mitigate environmental problems (Deng *et al*., 2006).

For a sound irrigation planning, it is most important to estimate the available water amount in the plant and soil. One of the principal factors in plant-water related irrigation management is measuring the water used by the plant (Ferrara and Flore 2003). The water content of a plant is a key biochemical parameter, affecting photosynthesis efficiency and crop productivity (Zhang *et al*., 2012). Additionally, the water content estimation in living crops is a key element for proper irrigation planning and drought recognition, which can assist in determining the physiological status of vegetation (Peñuelas *et al*., 1993, 1994). In order to obtain high water use efficiency in crops, the accurate determination of plant water use is a significant requirement (Yunusa *et al*. 2000). Furthermore, irrigation management is usually based on evaluation of crops respond to the water content of soil, meteorological parameters and evaporative demand (Çolak *et al*. 2015; Yazar et al. 1999). Therefore, soil water management is a key material for high yield of crops. The estimation of accurate water content of soil is essential to make irrigation planning in the field of agriculture (Peñuelas *et al*. 1993).

For accurate measuring or estimation of water content in soil and in plant, a proper method is needed. There are different methods available to evaluate the water amount in a plant and the soil around its root. The oven drying method is used to measure the water content of materials (O'Kelly 2004). Gravimetric analysis is one such technique to measure plants' water content and soil water balance as well (Ferrara and Flore 2003). These methods can measure very accurate water content but these methods cannot be used for real time monitoring of water content of alive plant or soil in the field.

It is thought that, the Near Infrared (NIR) reflectance is expected to be used for real time monitoring of water contents not only soils but also leaves such as spectroscopy analysis in agriculture fields (Fan *et al*. 2010, Gillon *et al*. 2004). Water measurement is tested by NIR spectrum in many researches (Fernández *et al*. 2018; Suhandy *et al*. 2006: Jin *et al*. 2017; Neto *et al*. 2017), but previous NIR spectroscopy device was very large and/or expensive. Thus, it is not a field appropriate device. I think an inexpensive yet small NIR spectroscopy device is necessary to be used for real-time monitoring of water content in the field. Therefore, we selected a handheld, low-price NIR-sensor device called; "Spectral Engine's NIRONE sensor" whose wavelength range is 1550–1950 nm, giving us a high water absorption band (Bullock *et al*., 2004; Chen *et al*., 2005; Carter, 1991). Okamura *et al*., (2001) found that while water stress became more intense in leaf, the peaks of NIR reflectance formed around 1940 nm wavelengths. Then, it was anticipated that this system also can be used for living leaf.

Then, I thought that it is needed to examine the spectral absorption properties and features as indicators of water status in soils and plants within this narrow wavelength range (1550-1950nm). Thus, I firstly tested this nondestructive method successfully compare with oven-drying method as destructive measurement in the laboratory and found the highest and lowest light absorption range affected by water percentage, as well as the best calibration model for estimation of water percentages in leaf was derived. And, I also found that this method is useful to estimate the water content of small sample of soil (Afzali *et al*. 2021). However, it is unknown that this method is useful or not to estimate the water content of soil of around living root. Therefore, it is also unknown that this method is useful or not to analysis the relationship of water content between soil and leaf. It is very important information for proper irrigation planning and drought recognition in the field (Peñuelas *et al*., 1993).

In order to validate the NIR measurement, it is necessary to assess the precision and authenticity of this method compared to gravimetric as nondestructive method and oven-dry methods. The gravimetric method was used as very precise method for simulating drought stress in pot experiments (Earl, 2003). Then, for the validation of the ability of a hand- held NIR sensor and water absorption index to estimate the water-content in living leaf of plant and soil around its root-zoon, eggplant (*Solanum melongena*) was evaluated through a pot drying experiment using a fine-scale electronic balance in the greenhouse, because, eggplant is a hydrous, excellent product and economically a main vegetable crop in the world which is sensitive to water stress (Van and Snyder 2006; Kirnak *et al*. 2002; Chaves *et al*. 2003; Lovelli *et al*. 2007; Madramootoo & Rigby 1991).

The objectives of these studies were; 1) To investigate the feasibility of rapid and non-destructive measurement by NIR to examine spectral absorption properties and features as indicators of water status in soils and plants using inexpensive small hand- held NIR spectroscopy device "Spectral Engine's NIRONE sensor". 2) To evaluate the utility of a hand-held NIR sensor and a water absorption index for estimating the water content of intact leaves of eggplants grown in pots. 3) To simulate drought stress using a pot experiment, and analyze the relationship between leaf water content and soil matric potential.

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Chapter 2

Use of Hand-Held NIR Sensor to Estimate Water Status of Leaves and Soils

2.1 Introduction

An accurate water-status monitoring system is needed in the field to help develop effective irrigation plans (Peñuelas *et al.*, 1993). Water scarcity is the enemy of sustainable crop production, especially in arid areas such as Afghanistan. Moreover, water availability for agricultural production in most parts of Afghanistan is limited because of short seasonal precipitation and unimproved irrigation channels (Mancosu *et al.*, 2015; Parwani, 2018). Because the water supply is limited, efforts have been made to economize water consumption, especially in regions where the supply is critically low (Seckler *et al.*, 1998; Dinar and Yaron, 1992). For horticultural crop production in arid and semiarid regions, water shortages are major success blockers (Tesfaye *et al.*, 2011).

The land's evapotranspiration is affected by soil moisture, which is a focal subsystem of the climate and a chain of the water, carbon cycle, and energy (Falloon *et al.*, 2011; Garten *et al.*, 2009; Holsten *et al.*, 2009; Jung *et al.*, 2010; Wan *et al.*, 2007). The soil's moisture availability is a pre-requisite for nutrient movement inside plants (Dobriyal *et al.*, 2012). Thus, estimating accurate water content is essential for irrigation planning and agriculture (Peñuelas *et al.*, 1993). The water content of a plant is a key biochemical parameter, affecting photosynthesis efficiency and crop productivity (Zhang *et al.*, 2012).

Oven-drying methods are currently used to measure the water content of plant materials (O'Kelly, 2004). This process accurately measures water content, but it is destructive and cannot be performed in real time. For soils, a non-destructive probe insertion measurement is widely used to obtain real-time statuses (Noborio, 2001). However, this method cannot be used to measure the water content of plants. In search of a better solution, I looked at spectroscopic near-infrared (NIR) reflectance to gauge its use for real-time monitoring of both soil and plant water content (Fan *et al.*, 2010; Gillon *et al.*, 2004).

Water content can be estimated using regression models of relationships between the reflectance of NIR light when shined upon an object. However, the reflectance of NIR light is also affected by material components other than water (Gillon *et al.*, 2004). Therefore, adequate and accurate wavelengths should be selected, based on the types of plants. Water absorption is significantly stronger in the 1500–2500-nm region than in the 900–1300-nm region (Gao, 1996; Zhang *et al.*, 2012). The outstanding absorption features of liquid water near 970, 1200, 1450, and 1940 nm have been successfully used for these purposes (Curran, 1989; Gao and Goetz, 1994; Pu *et al.*, 2003). However, previous studies conducted similar studies with NIR spectroscopy devices that were very large and/or expensive, which prevents to apply the technique in operational conditions. Investigating similar study with an inexpensive hand-held NIR spectroscopy device, such as NIRONE sensor by Spectral Engine, contributes to make real-time monitoring of water content in fields. It is unknown the inexpensive hand-held NIR spectroscopy device can be used to estimate water content or not because the range of wavelength is very narrow compare to expensive NIR spectroscopy devices. It will be a disadvantage because of restricted number of available wavelength bands.

The aim of this study, therefore, is to investigate the feasibility of rapid and non-destructive measurement using NIR to examine spectral absorption properties and features as indicators of water status in soils and plants.

2.2 Materials and Methods

2.2.1 Study sites

The first experiment was conducted on an experimental farm laboratory at the University of Miyazaki, Japan, from 17–28 May, and from 2–11 August, 2018. The study site is located at 31°49'45" north latitude and 131°24'39" east longitude at an elevation of 35 m above sea level. The site receives a 2500-mm mean annual precipitation and is often humid (Weather Spark homepage).

The second experiment was conducted from 1–8 September, 2018, at Badam Bagh Agricultural Research Station, Kabul, Afghanistan. The site is located at 34°33'05" north latitude and 69°07’06" east longitude with an elevation of 1810 m above sea level. Kabul endures consistently dry climatic conditions (Weather Spark homepage).

2.2.2 Device for recording data

NIRONE sensors are designed with micro-electro-mechanical systems, including two integrated light sources (Spectral Engines homepage). The sensor can be adapted and controlled with a personal computer using a universal serial bus communication board (Figure 1) to measure the real-time water status of materials. This device is fully comparable to the laboratory instruments. It measures is light-weight (15 g), and is inexpensive (about $2000). (Spectral Engines homepage).

NIRONE sensors generally operate in the NIR wavelength range of 1100–2450 nm, but only at one selected wavelength at a time (Spectral Engines homepage). I selected the Spectral Engines' NIRONE Sensor S2.0, whose wavelength range is 1550–1950 nm, giving us a high water absorption band (Bullock *et al*., 2004; Chen *et al*., 2005; Carter, 1991) (Figure 2.1).

Figure 2.1: Spectral Engines' NIRONE sensor S2.0. on palm

2.2.3 Data collection

Leaves of Eggplant (*Solanum melongena*), Cherry blossom (*Prunus speciosa*), and Camellia (*Camellia japonica*) were collected from the experimental farm at the University of Miyazaki, Japan. Greenhouse soil (light black volcanic ash soil), bora (yellow volcanic ash soil), and andosol (dark-black volcanic ash soil) were also collected from same farm. Leaves of Tomato (*Solanum lycopersicum*), Apple (*Malus domestica*), Cherry (*Prunus avium*), and Apricot (*Prunus armeniaca*) were collected from open field and a greenhouse at Badam Bagh Agricultural Research Station in Kabul, Afghanistan. Compost soil, silt, and loam were also collected from same Station. All leaves were healthy and homogeneous in color without visible symptoms of damage. To minimize water loss during the transportation of samples, leaves and soils were immediately enclosed in a plastic bag after being picked. Then, all the leaves and soil samples were placed in opened plastic bags on a laboratory bench to slowly desiccate (Figure 2.2).

Figure 2.2: Drying experiment for leaves and soils.

Using my hand-held device, the reflectance spectra were obtained every day until the leaves and soils were dry (Figure 2.3). Each sample was weighed daily. Every day, prior to measuring the sample spectra, a baseline lamp spectrum was recorded using a standard white board. After the measurements were collected, the leaves and soils (according to Huisman *et al*., 2001) were oven-

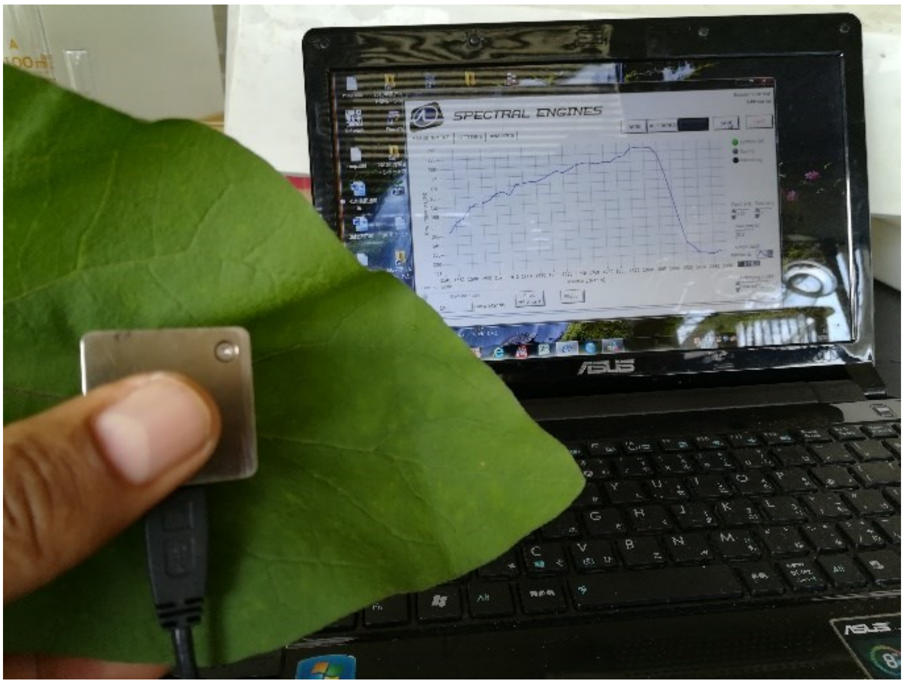

Figure 2.3: Using the hand-held NIRONE Sensor S2.0 to measure leaf reflectance spectra

dried at 70 ˚C for 48 h to obtain the final dry mass. The water content (percentage) was calculated using Eq. (1).

$$\textit{Water percentage} = (FW\text{-}DW)/(FW) \times 100 \qquad (1)$$

where FW is fresh weight, and DW is dry weight.

The spectral reflectance (R_x) were calculated from the reflectance values at each sample and standard (whiteboard) wavelength (sample/standard method).

$$R_\mathrm{x} = (NIR_\mathrm{x} \text{ of sample})/ (NIR_\mathrm{x} \text{ of standard}) \qquad (2)$$

where, NIR_x of sample and standard are reflectance values of x nm wavelength at sample leaf and at whiteboard, respectively.

The absorption index at 1940 nm was calculated using reflectance values of the whiteboard and sample wavelength (standard/sample method).

$$1/R_{1940} = (NIR_{1940} \text{ of standard})/ (NIR_{1940} \text{ of sample}) \qquad (3)$$

This type of index using 1940 nm was used by industrial moisture meter (NEAT homepage).

The normalized difference index (NDI) was calculated using Eq. (4).

$$NDI_{\mathrm{a:b}} =(R_\mathrm{a} - R_\mathrm{b})/ (R_\mathrm{a} + R_\mathrm{b}) \qquad (4)$$

The R_a and R_b are spectral reflectance at a nm and b nm wavelength, respectively.

This normalized difference type index was used by remote sensing studies (Wang *et al.*, 2009; Le Maire *et al.*, 2008; Cao *et al.*, 2015; Elsherif *et al.*, 2019).

2.2.4 Data analysis

Statistical analysis was accomplished via log-linear model and linear regression using ANCOVA, considering the soil types and/or plant species as categorical variables and using software R 3.5.1. The model for each variable's response was selected according the highest R^2 value.

2.3 Results and discussion

2.3.1 Spectral absorption features

As shown in Figures 2.4 and 2.5, both leaves and soils with low water percentages had relatively high reflectance in the region of 1550–1950 nm, compared to samples of high water percentage. Thus, there is a negative correlation between water percentage and R_x. This could be caused by the simulated increase of light absorption based on water percentage. Furthermore, it can be shown that light absorption, affected by water percentages, was highest at around 1940 nm and lowest at around 1650 nm. I expected that the normalized difference index (NDI) using these two wavelengths is effective to measure water content. The 1650 nm was used as water-insensitive wavelength of NDI (Elsherif *et al.*, 2019) and 1940 nm was used as water-sensitive wavelength (Wang *et al.*, 2009).

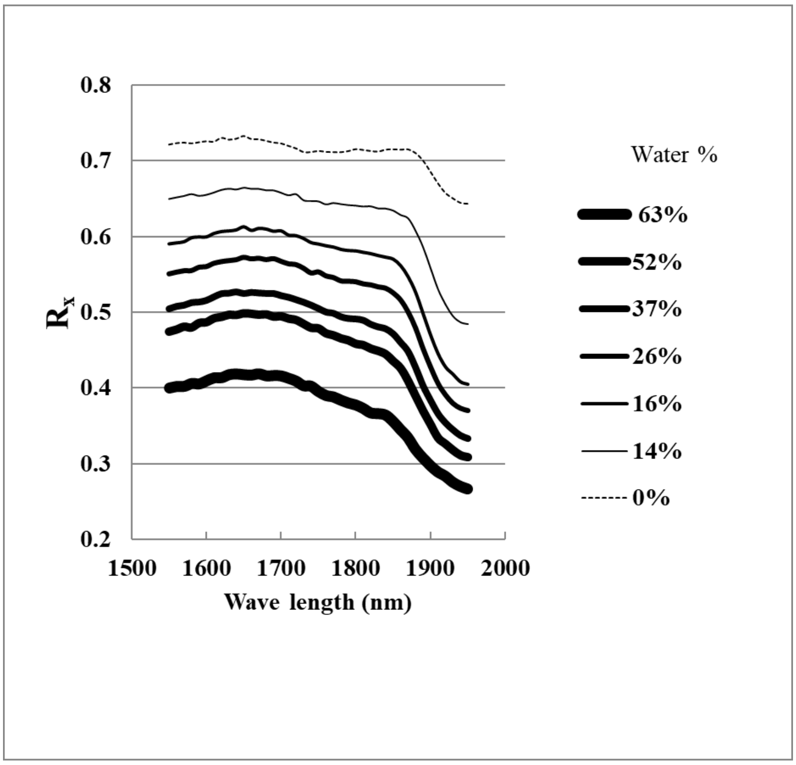

Figure 2.4: Spectral absorption features of cherry-blossom leaves

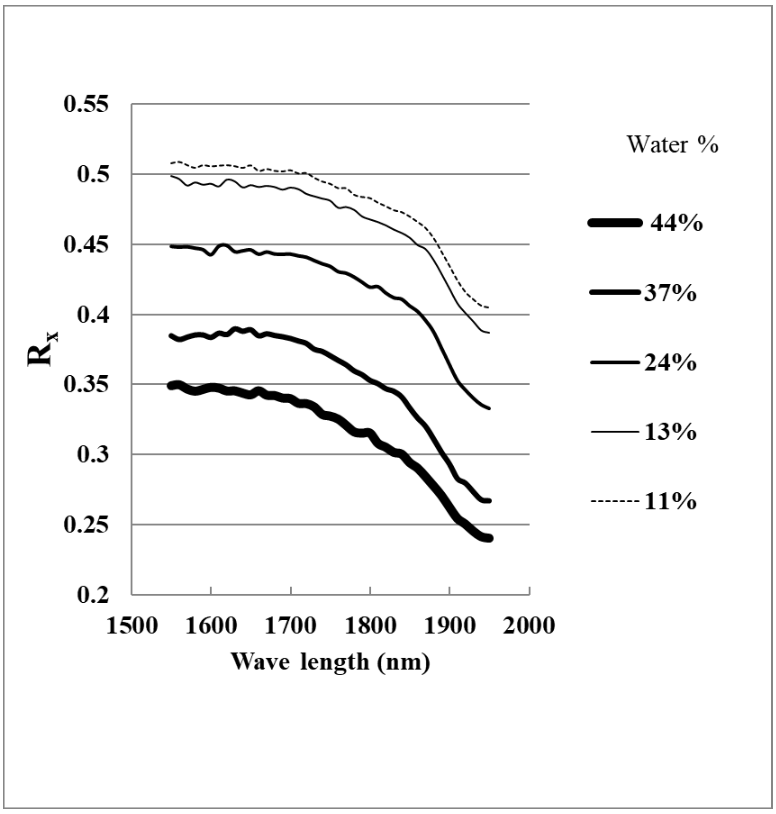

Figure 2.5: Spectral absorption features of Andosol

2.3.2 Relationships between spectrum parameters and water percentage of leaves

The result of log-linear model analysis showed significant effects on spectrum parameters based on the water percentage of leaves (Figure 2.6). However, in the case of the $NDI_{1650:1940}$, the ANCOVA analysis showed a significant difference of regression among plant species (Table 2.1), suggesting that different calibration models are needed for each species.

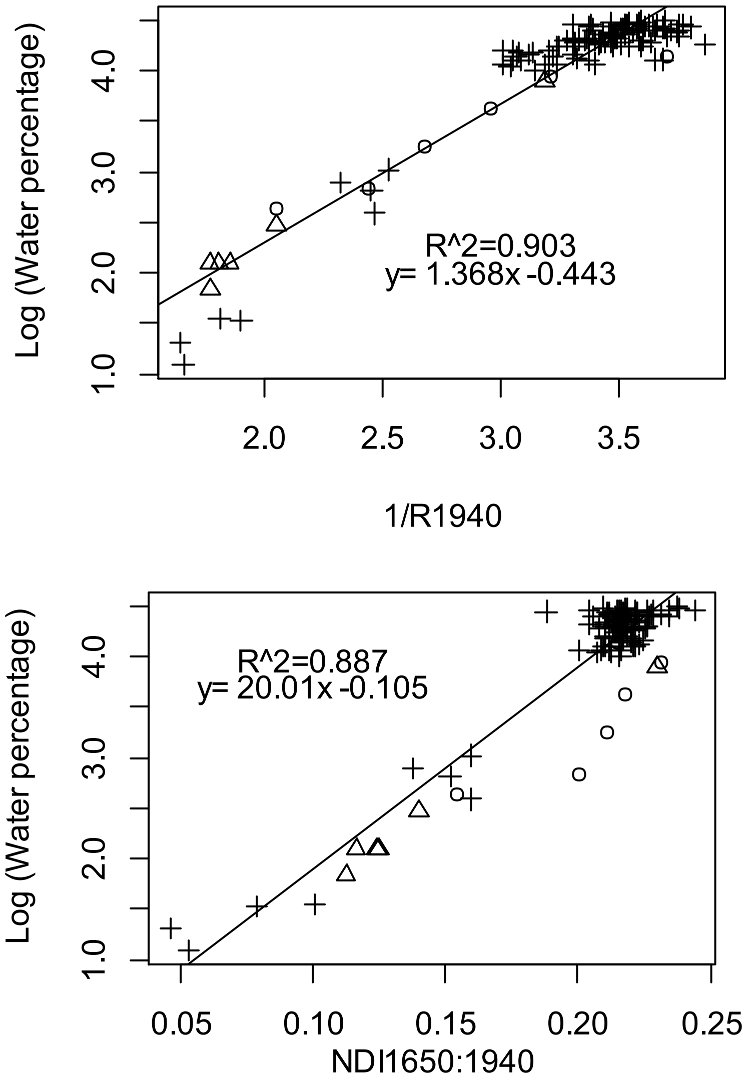

Figure 2.6: Relationships between spectrum parameters and water percentage of leaves. Eggplant (+), Camellia (○), Cherry blossom (△)

Compare to this index, in the case of the $1/R_{1940}$, the ANCOVA analysis showed a no-significant difference of regression among plant species (Table 2.1), suggesting that single calibration model is useful to estimate the water content of leaves for many plant species.

I think the difference of chemical component among plant species is a possible reason for different result between indexes. Absorption around 1650 nm is closely related to lignin content (Martin and Aber, 1997). The lignin content is lower in herbaceous plants, compared to woody plants (Kendall *et al.*, 2019). Therefore, it is predicted that absorption around 1650 nm is high in woody plants. This phenomenon should affect the value of $NDI_{1650:1940}$. Indeed, in my result (Figure 2.6), woody plants (Camellia, Cherry blossom) showed relatively high $NDI_{1650:1940}$ value compare to herbaceous plant (Eggplant).

I also think that absorption around 1940 nm is mainly related to water content compare to other chemical content, because in the case of the $1/R_{1940}$, the ANCOVA analysis showed a no-significant difference of regression among plant species. Therefore, I think that the $1/R_{1940}$ is useful to estimate the water content of leaves for many plant species.

2.3.3 Relationships between spectrum parameters and water percentage of soils

The result of linear regression analysis showed significant effects of spectrum parameters on the water percentage of soils (Figure 2.7). In both indexes, ANCOVA analysis showed no significant differences of regression among soil types in Afghanistan (Table 2.2). This result suggests that the same calibration model for water percentage of soil can be used for all soil types which I used in Afghanistan.

However, ANCOVA analysis showed significant differences of regression among soil types in Japan (Table 2.2). This result suggests that different calibration models for the water percentage of the soil is needed for each soil type.

It is known that soil structure and soil–water retention quality is depending on soil organic-carbon and soil texture, where soil–water retention characteristics effects on water's NIR spectra (Marakkala *et al.*, 2018). In japan, I used three types of volcanic ash soils (yellow, light-black and dark-black). It is known that these volcanic ash soils show quietly differences on organic-carbon and soil texture (Shoji and Takahashi, 2002).

The hygroscopic water, which is adsorbed to the soil surface especially on the surface areas of clay minerals and organic matter in thin layers and has absorption features near 1900 nm; and free water, which is present in soil pores (Ben-Dor, 2002; Stenberg *et al.*, 2010). Therefore, changes of clay and/or organic contents would be the possible reason for difference of NIR spectra among soil types. It is needed to get more information about difference of clay and/or organic contents of soil in the future.

Table 2.1: Results of log-linear regression analysis of spectrum parameters and water percentage of leaves

Spectrum parameters	Country	R^2	*P*	Intercept	Slope	ANCOVA test among plant species (*P*)
$1/R_{1940}$	Afghanistan	0.773	< 0.001	-4.2	2.2	0.093
	Japan	0.903	< 0.001	-0.4	1.4	0.723
$NDI_{1650:1940}$	Afghanistan	0.768	< 0.001	-1.4	24.2	0.224
	Japan	0.887	< 0.001	-0.1	20.0	< 0.001

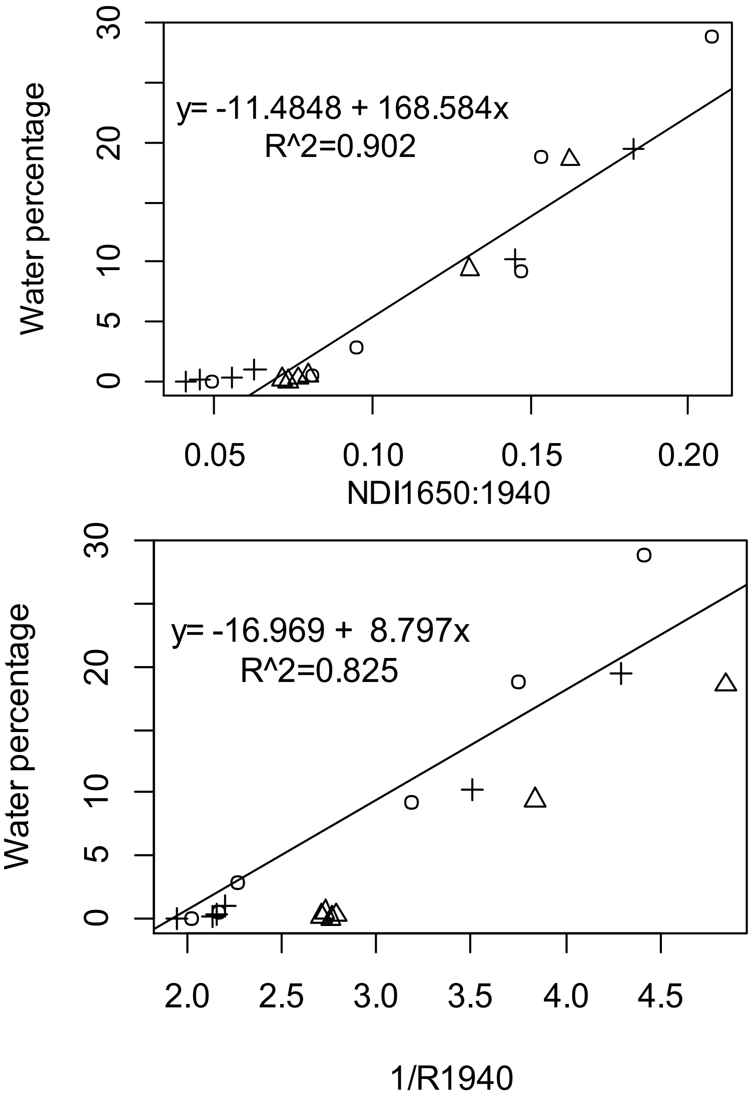

Figure 2.7: Relationships between spectrum parameters and water percentage of soils. Compost (○), Silt (△), Loam (+)

Table 2.2: Results of linear regression analysis between spectrum parameters and water percentage of soils

Spectrum parameters	Country	R^2	*P*	Intercept	Slope	ANCOVA test Among soil types (*P*)
$1/R_{1940}$	Afghanistan	0.825	< 0.001	-17.0	8.8	0.285
	Japan	0.831	< 0.001	-28.6	16.0	< 0.001
$NDI_{1650:1940}$	Afghanistan	0.902	< 0.001	-11.5	168.6	0.693
	Japan	0.826	< 0.001	-34.2	365.9	< 0.001

In addition, in this manuscript, I have done the adjustment of certain model and calibration for specific treatment, parameter and location. And for validation there is needed farther more experiment to appoint compromise among prediction and examination, that will be shown in the future.

2.4 Conclusions

The result of my study confirmed that the absorption of NIR light by samples' water was highest around 1940 nm and lowest at around 1650 nm. I expected that the normalized difference index using two wavelengths is effective to measure water content. However, different calibration models are needed for each plant species in this type of index.

It is thought that absorption around 1650 nm is affected by lignin content. It is also thought that absorption around 1940 nm is less affected by lignin content. Therefore, I think that the simple index using single wavelength (1940 nm) can be used for universal index to estimate the water content of leaves.

These results reveal that an inexpensive hand-held NIR sensor can be applied to measure water status of living plants in the field.

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Chapter 3

Non-Destructive Near-Infrared Sensor Method for Measuring Water Content of Intact Eggplant Leaves

3.1 Introduction

In upland and arid/semi arid areas such as those in Afghanistan, in particular, the unsystematic use of water for crops leads to suboptimal quality and quantity and is wasteful if used excessively (FAO, 2008). Afghanistan has a dry climate, and most of its cultivable area receives negligible rainfall during the irrigation season (Rout, 2008). The wasteful use of underground and surface water severely affects the irrigation system and environment (Deng et al., 2006). Estimation of the water content in living crops is a key element in effective irrigation planning and drought recognition, and can assist in determining the physiological status of vegetation (Peñuelas et al., 1993, 1994).

Non-destructive measurement of water in plant leaves by NIR spectroscopy has been conducted in many research projects (Suhandy et al., 2006, Jin et al., 2017; Steidle et al., 2017; Fernández-Novales et al., 2018). Okamura et al. (2001) found that peaks of NIR reflectance at approximately 1940 nm wavelengths indicated more intense water stress in leaves, and it was anticipated that this system could also be used for living leaves.

However, older NIR spectroscopy devices are very large and/or very expensive, and are very difficult to use in the field. I concluded that an inexpensive small NIR spectroscopy device is needed for real-time monitoring of water content in the field. Therefore, I wanted to know whether an inexpensive small NIR spectroscopy device was suitable for non-destructive measurement of the water content of leaves. I found that the water content in the detached leaves of some plant species could be estimated using a hand-held NIR sensor (Afzali et al., 2021). The procedure using a single wavelength (1940nm) was expected to be a suitable method for estimating the water content of living plants.

However, it is not known whether this method can be used to estimate the water content of living plants which is not only in detached leaves but also intact leaves. If this method could be used for intact leaves, then it could be used in the field. Moreover, it is not known whether this method can be used to analyze the relationship between soil and leaf water content. Since this information is very important for effective irrigation planning and drought recognition in the field (Peñuelas et al., 1993), it is necessary to compare the non-destructive water stress measurement by a small NIR spectroscopy with oven drying as a destructive method.

The objective of this study was to evaluate the utility of a hand-held NIR sensor and a water absorption index for estimating the water content of intact leaves of eggplants grown in pots. The relationship between the leaf water status of plants and soil water potential is important for understanding the water stress of plants (Millar et al., 1970; Araki, 1993). I then conducted a pot experiment to simulate drought stress and analyze the relationship between leaf water content and soil matric potential.

3.2 Materials and Methods

3.2.1 Study site

A pot experiment was conducted in a 60 m^2 greenhouse located at the experimental farm of the University of Miyazaki, Japan (31°49'45″ N, 131°24'39″ E, at 35 m elevation). The temperature and relative humidity in the greenhouse were monitored hourly using a data logger (Ondotori, TR-74Ui Illuminance UV Recorder; T&D Co., Matsumoto, Japan). The sensors were placed 2.2 m above the ground.

3.2.2 Experimental design and data collection

A full factorial (2 treatments×7 days) randomized complete block design experiment was carried out with 5 replicates on eggplant (*Solanum melongena*) cultivar ‘Semi-long’ (Sakata Seed Corporation, Japan) from 22-28 July 2019. Seventy plants (2 treatments×7 days×5 replicates) were prepared for this study to examine the effects of drought stress. Before starting the experiment, eggplant seeds were sown in a tray with NAFUCO cultured soil (bark compost, brown-ash soil, and chemical fertilizer) on May 20, 2019. After one month, at leaf stages 3-4, the seedlings were transplanted to 11 cm diameter plastic pots filled with 300 g (dry weight) NAFUCO cultured soil. All selected plants were healthy and homogeneous in color without visible symptoms of damage to reduce experimental error. The experiment was started at the 6-7 true leaf stage on July 22, 2019. During the experimental period, 100 ml of tap water was supplied to the wet-treatment pots after every 48 h at 07:00, while no water was supplied to the dry-treatment pots, to simulate drought stress. The gravimetric method has been used as a very precise method for simulating drought stress in pot experiments (Earl, 2003). Gravimetric measurements were performed for 10 pots for sampling every day by using a fine-scale electronic balance (SHIMADZU model TXB4201L, Japan) with a minimum read-ability of 0.1 g, as shown in Figure 3.1.

Figure 3.1: Pot gravimetric measurement using a fine-scale electronic balance

The NIR measurements were conducted every evening on the adaxial surface of the same leaf from the middle of the plant. The hand-held NIR sensor (NIRONE Sensor S2.0, Spectral Engines OY., Finland) was used in this study as in our previous study (Afzali *et al*., 2021), as shown in Figure 3.2. This sensor has a wavelength range of 1550-1950 nm, giving us a broad water absorption band.

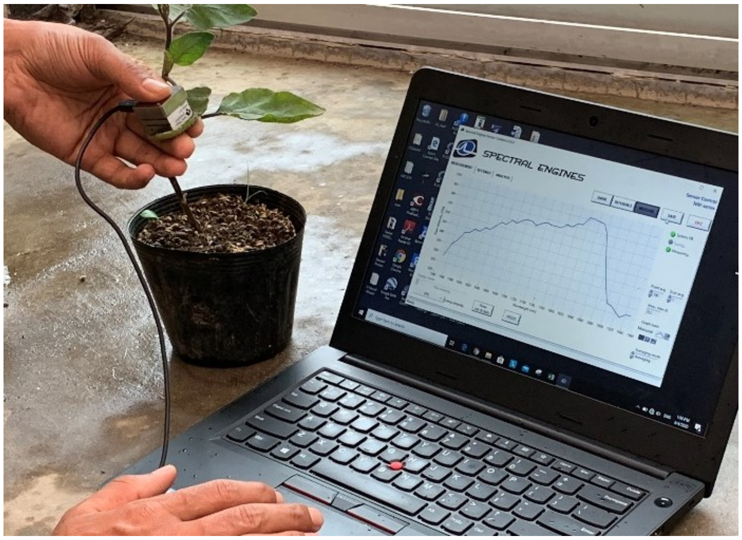

Figure 3.2: Data recording from an intact eggplant leaf using NIRONE Sensor S2.0 (Spectral Engines)

After pot gravimetric measurements and NIR measurements, all leaves and soils were harvested. To minimize water loss, the samples were placed in plastic bags immediately after being picked.

After the measurements were completed, the leaf and soil samples (100 cm^{-3}) were oven-dried at 105°C for 24 h to determine the dry mass and calculate the water content using Eq. (1) and (2).

$$\textit{Leaf water content} = (FW\text{-}DW)/(FW) \tag{1}$$

$$\textit{Soil water content} = Vw/Vt \tag{2}$$

where FW is the fresh weight, and DW is the dry weight. Weight measurements were performed using a fine scale electronic balance with a minimum readability of 0.001 g (SHIMADZU Moisture Balance Type MOC 120H, Japan). The volumetric soil water content is the ratio between the volume of water present and the total volume of the sample expressed by Eq. (2), where Vw is the volume of soil water and Vt is the total volume of soil.

The relative absorption index at a wavelength of 1940 nm was calculated by applying the reflectance values of a standard whiteboard and sample (standard/sample) using Eq. (3).

$$1/R_{1940} = (NIR_{1940}\ \text{of standard})/(NIR_{1940}\ \text{of sample}) \qquad (3)$$

The water potential of the soil samples was measured using a soil water matric potential sensor MPS-2 (Decagon Devices, Pullman, WA, USA) connected to an Em50 data logger (Decagon Devices, Pullman, WA, USA). MPS-2 is a dielectric water matric potential sensor (Decagon Devices, 2017). It measures the water content of porous ceramic discs using a capacitive reading and converts the measured water content to water potential using the moisture characteristic curve of the ceramic. The range of the measurement was -10 to -100,000 kPa (pF 2.01 to pF 6.01). The sensor was installed in a pot at depths of 10 cm below the soil surface. Data from the sensor were recorded at 30 min intervals. Measurements were conducted for over 24 hours per pot with 18 different soil water contents from March 9 to April 16, 2021. The soil was oven dried at 105°C for 24h to determine the dry mass for calculating the water content using Eq. (2).

3.2.3 Data analyses

To test the temporal change of measurements, the Tukey-Kramer multiple comparison test was conducted using software R 3.5.1 software. The regression models were also analyzed using software R 3.5.1.

The relationship between soil matric potential and water content was fitted using the model of Brooks and Corey (1964), which was selected based on the conspicuously higher degree of fitting and the unimodal behavior of my data. To obtain the soil water retention curve of Brooks and Corey model, the SWRC-Fit version 1.3 software (Seki, 2007) was used; it can also be executed directly from the web page (http://purl.org/net/swrc). The relationship between soil and leaf water content was also fitted using the model proposed by Brooks and Corey (1964).

3.3 Results and discussion

3.3.1 Temperature and Relative humidity

Temperature and relative humidity fluctuated in the ranges of 24–41°C, 39–100%, respectively (Figure 3.3). Fine day-time conditions with high temperatures continued during the entire experimental period.

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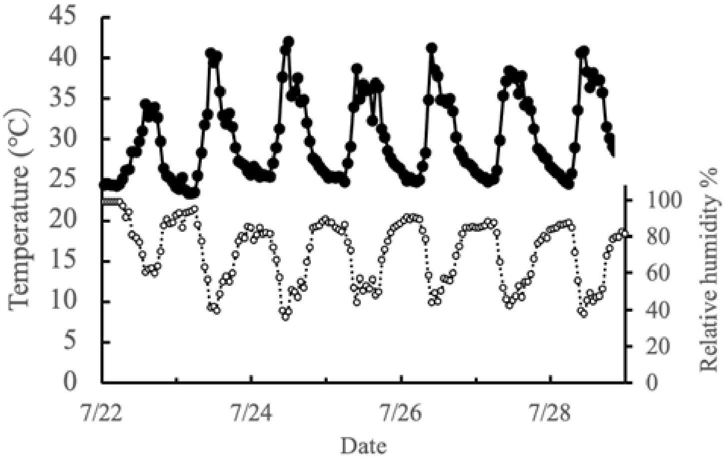

Figure 3.3: Temperature and relative humidity in the greenhouse

3.3.2 Temporal measurements

Figure 3.4 shows a boxplot of the measurements. Although the weight of the dry pots decreased over each of the seven days, the $1/R_{1940}$ or water content of the leaves decreased after four days of drying, and the leaf water content did not differ during the first four days due to water retention of the plant. In the wet treatment, the $1/R_{1940}$ of leaf and leaf water content did not change during the entire experimental period. The pot weight was increased after the second irrigation. Soil water content increased after the third irrigation. When soil is drying, plants are able to maintain a potential gradient for water uptake by means of low osmotic potential (Jensen et al., 1993; Jensen et al., 2000).

The results of the temporal pot experiments indicated close relationships between the NIR reflectance and water content of leaves and/or soil. NIR reflectance (at 1940 nm) was higher in stressed leaves than in non-stressed leaves, and changes can be observed in the curve of the time-course of the absorbance spectra (Okamura et al. 2001). These results show the strong drought effect, with sufficient variation to enable comparisons among these measurements.

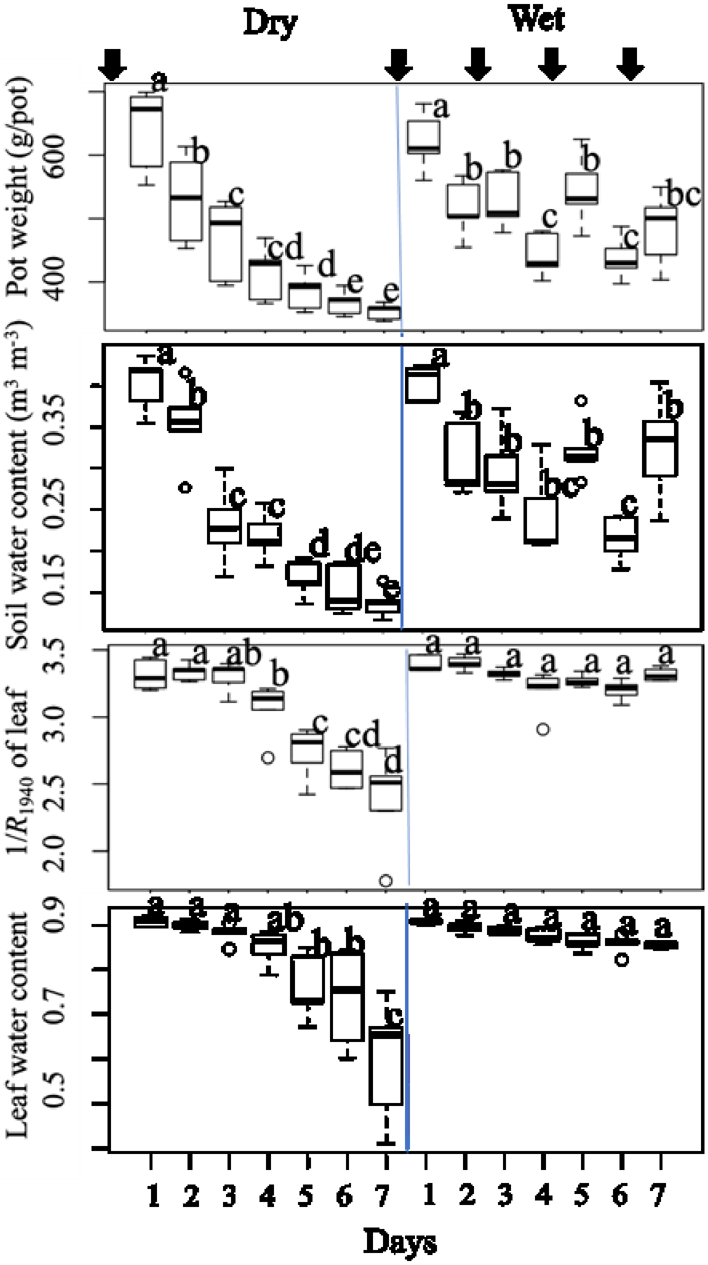

Figure 3.4: Temporal change in pot weight, soil water con-tent, $1/R_{1940}$ of leaves, and leaf water content over seven days of wet or dry treatments (arrows show irrigation timing). Means with different letters indicate significant differences by Tukey-Kramer multiple comparison test ($P < 0.05$, n=5 pots per day)

3.3.3 Relationship between $1/R_{1940}$ values and leaf water content

Log-linear regression analysis showed significant effects of $1/R_{1940}$ values on the leaf water content ($R^2=0.903$, $F=632$, $P<0.001$; Figure 3.5). The regression calibration model of $1/R_{1940}$ values, and the predicted leaf water content was derived from the sample pair as follows:

$$\textit{Predicted leaf water content} = 0.5411 \times \ln(1/R_{1940}) + 0.2545 \qquad (4)$$

There is a strong correlation between plant water and NIR reflectance, as the amount of water in the detached leaf can be determined from the absorbed and reflected near-infrared spectra (Bowman, 1989; Okamura *et al.*, 2001; Afzali *et al.*, 2021). My results showed that intact leaves can be used to estimate water content. This suggests the possibility of real-time monitoring of water stress status because this method can be used as a non-invasive measurement. Therefore, this non-destructive method could be used in field conditions.

Figure 3.5: Relationships between $1/R_{1940}$ and leaf water content

3.3.4 Relationships between soil water content and leaf water content

Figure 3.6 shows relationships between the soil water content and leaf water content (measured and predicted). The R^2 values indicated that the fitting of the Brooks and Corey model to the experimental data was highly appropriate. The leaf water content decreased with reductions in the soil water content, for both measured and predicted comparisons. However, the leaf water content showed no obvious changes when soil water content was 0.2 or higher. This might be because the water availability of the soil was sufficient for the plant. From the total amount of water available in the soil, a limited proportion is readily available for the plant to extract without being stressed (Milly, 1994). With soil water content lower than 0.17, there was a steep decline in leaf water content, probably because the water availability of soil was insufficient for the plant. A large number of plant responses to water deficits has been defined as a function of the fraction of transpirable soil water (King and Purcell 2017).

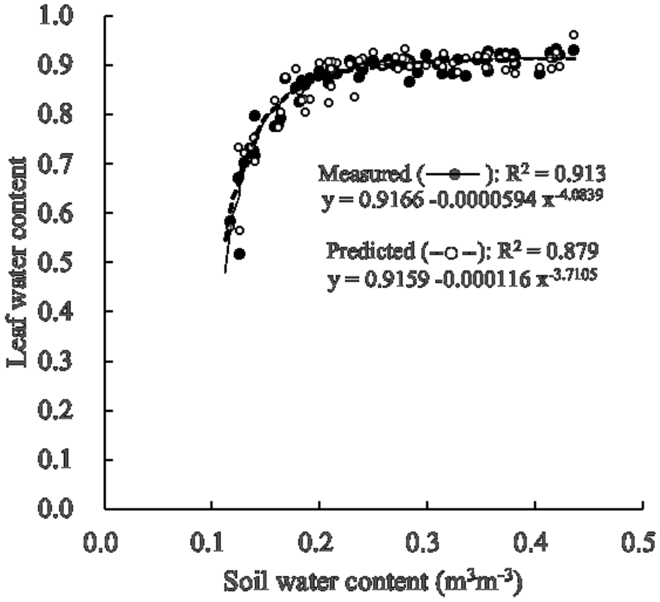

Figure 3.6: Relationships between the soil water content and leaf water content (measured or predicted)

3.3.5 Relationship between soil water potential and leaf water content

Figure 3.7 shows the soil water retention curve of NAFUCO cultured soil. The high R^2 value indicates that the fitting of the Brooks and Corey model to the experimental data was suitable. This demonstrates a relationship between the soil water content and soil matric potential. The nonlinear regression model correlated with water content and the matric potential was calculated as follows:

$$\text{Matric potential} = -8.2300 \times (3.5992 \times \text{soil water content} - 0.2247)^{-2.6444} \qquad (5)$$

Then, we converted the soil water content to matric potential using Eq. (5). The results of the linear model analysis showed significant effects of matric potential on leaf water content (Figure 3.8). The ANCOVA analysis showed no significant differences of regression models (t=0.001, P=0.999). A similar decrease in leaf water content with decreasing soil matric potential has been reported previously (Millar et al., 1970; Araki, 1993).

The soil water content at -1500 kPa matric potential is considered as the lower limit of available water to plants, and is called the "permanent wilting point" (Tolk, 2003). In this study, the soil water content at the permanent wilting point was calculated as 0.10 using a modified Eq. (5). The available water in the soil for plant growth is in the range between the field capacity and the permanent wilting point (Kramer, 1944). In this study, all pots had water available to plants because soil water content of pot was higher than 0.10. However, with soil water content lower than 0.17, corresponding to a soil matric potential of -100 kPa, there was a steep decline in leaf water content. The leaves of eggplants in soil with water content lower than 0.17 were under severe water stress. Millar et al. (1970) reported that barley leaves undergo severe water stress with decreasing leaf water content before ultimate wilt. Millar et al. (1970) also reported a soil matric potential of -1bar (-100kPa) was the first visible wilt.

Therefore, it is possible to evaluate the water stress status of eggplant by non-destructive measurement of leaf water content. I believe that the hand-held NIR sensor is very useful for determining optimal irrigation timing in the field.

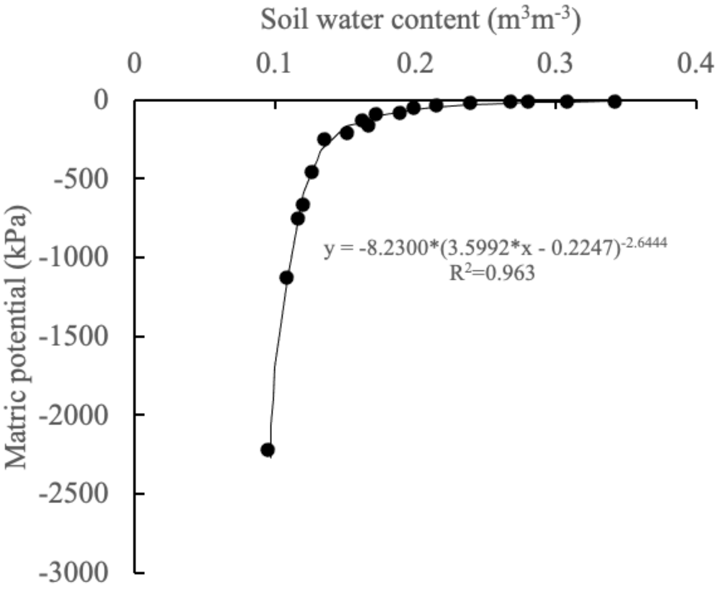

Figure 3.7: The soil-water retention curve of NAFUCO Culture Soil

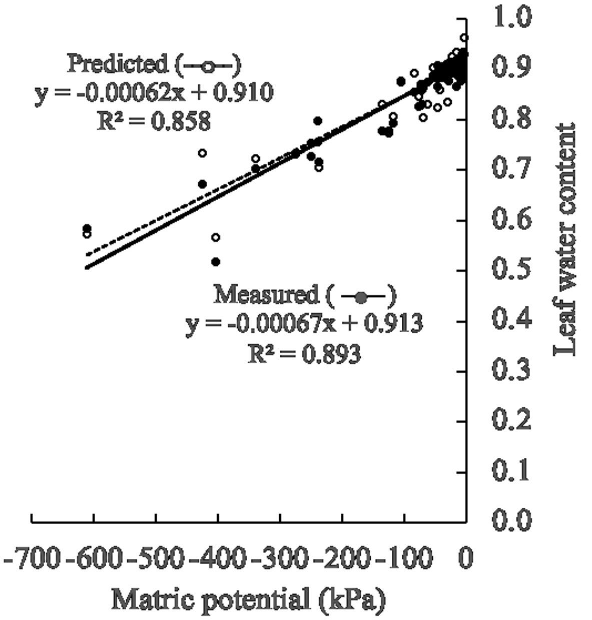

Figure 3.8: Relationships between the matric potential of soil and leaf water content (measured or predicted)

3.4 Conclusions

The objective of this study was to evaluate the ability of a hand-held NIR sensor and water absorption index to estimate the water content of intact living eggplant leaves using 1940 nm wavelength. I wanted to know whether this method could be used to analyze the relationship between soil and leaf water content. I conducted a pot experiment to simulate the drought stress.

The water content of intact eggplant leaves was predicted using a calibration model from NIR reflectance values. With soil water content lower than 0.17, there was a steep decline in leaf water content in response to decreasing soil matric potential. This result showed that both destructive methods and non-destructive NIR measurements can be used to estimate water stress conditions. A hand-held NIR sensor can be used to estimate the water content of intact leaves of living plants.

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Chapter 4

General conclusion

To overcome the water scarcity and produce high quantity and quality of agricultural products, a sound irrigation planning is the most important element, because the irregular and inappropriate use of water is the significant problem particularly in upland and dry regions. For this purpose; it is most important to estimate the available water amount in the plant and soil, which need a proper method. There are some destructive and nondestructive methods that measure the accurate water amount in soil and plant, which cannot be used for real-time monitoring of alive plant or soil in the field. As well as the Near Infrared (NIR) spectroscopy which were used previously for this purpose are very expensive and because of its big-size it is difficult to use it in the field. Therefore, I selected a handheld, low-price NIR-sensor device called; "Spectral Engine's NIRONE sensor" whose wavelength range is 1550–1950 nm, but it was unknown that this NIR spectroscopy device with restricted number of available wavelength bands can be used to estimate water content of plant and soil or not? Therefore, I examined the spectral absorption properties and features as indicators of water status in soils and plants within this narrow wavelength range (1550-1950nm) compare with oven-drying method as destructive measurement in the laboratory. Then, for the validation of the capability of a hand- held NIR sensor and water absorption index to estimate the water-content in living leaf of plant and soil around its root-zoon, eggplant was evaluated through a pot-drying experiment in the greenhouse as nondestructive module using a fine-scale electronic balance.

In the first experiment it was achieved that, both leaves and soils with low water percentages had relatively high reflectance in the region of 1550–1950 nm, compared to samples of high water percentage. The absorption of NIR light by soil and leaf samples' water was highest around 1940 nm and lowest at around 1650 nm. The normalized difference index using two wavelengths (1650 and 1940nm) was found effective to measure water content. However, different calibration models are needed for each plant species in this type of index. Therefore, I think that the simple index using single wavelength (1940 nm) can be used for universal index to estimate the water content of leaves. Finally, it can be suggesting that single calibration model ($1/R_{1940}$) is useful to estimate the water content of leaves for many plant species. Then, it can be concluded that the calibration model which derived in this study can be applied to measure water status of living plants in the field.

I also found that this method is useful to estimate the water content of small sample of soil. However, it was not known whether the NIR method could be used to estimate the water content of living plants which is not only in detached leaves but also intact leaves. If this method could be used for intact leaves, then it could be used in the field. Moreover, it is not known whether this method can be used to analyze the relationship between soil and leaf water content. Therefore, the objective of the second study was to evaluate the utility of a handheld NIR sensor and water absorption index for estimation of the water content of intact leaves of eggplants grown in pots. I want to know that this method is useful or not to analysis the relationship of water content between soil and leaf. I then conducted a pot experiment to simulate drought stress and analyze the relationship between leaf water content and soil matric potential.

The results of the temporal pot experiments indicated close relationships between the NIR reflectance and water content of leaves and/or soil. These results show the strong drought effect, with sufficient variation to enable comparisons among these measurements.

As well as, I found significant effects of $1/R_{1940}$ values on the leaf water content. Further, the water content of intact eggplant leaves was predicted using a calibration model from NIR reflectance values.

Also, the results of the linear model analysis showed significant effects of soil matric potential on leaf water content. Also, it was found that, with soil water content lower than 0.17, corresponding to a soil matric potential of -100 kPa, there was a steep decline in leaf water content, where leaves were found under severe water stress.

Finally, it can be concluded that, single calibration model ($1/R_{1940}$) is useful to estimate the water content of leaves for many plant species. The intact leaves can be used to estimate water content of plant using NIR measurement. It is possible to measure the water status of living plant in field condition by NIR non-destructively and in real-time monitoring.