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Application of sensor based high-throughput phenotyping methods to study drought stress in soybean

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TABLE OF CONTENTS

LIST OF TABLES

1. Chapter I

2. Chapter II

LIST OF FIGURES

1. Chapter I

2. Chapter II

I. MAIN RESEARCH GOALS

1. High throughput phenotyping for breeding drought resistance cultivars

Erratic climate change due to global warming is deriving frequent and severe drought. Drought especially affects as a crucial threat to the agricultural industry, therefore, drought solutions such as improving irrigation systems, rainwater harvesting, damming, cloud seeding, changing cultivation methods, and breeding resistance cultivars are developed and applied. Among all the current drought solutions, breeding resistance cultivar is the most efficient, effective method. However, conventional plant breeding requires an amount of time and resources during all processes. This led to the high throughput phenotyping (HTP), which is rapid, massive, accurate, non-invasive, automated, and reliable breakthrough in the plant breeding cycle researches by the combination of latest computing and sensor technologies. Applying HTP methods in developing drought resistance cultivar must choose the sensors and platforms that are appropriate to evaluate drought related traits. Therefore, the current chapter will introduce various methods of HTP in drought stress detection and possibilities to provide helpful guidelines for breeders and researchers under their circumstances.

2. Sensor based drought evaluation method in soybean (*Glycine max* L.)

Soybean (*Glycine max* L.) is one of the major food crops worldwide. Affected by the drought impact, existing soybean cultivars might resist some degrees of drought stresses. However, increased drought frequency and severity under global warming are now forcing rapidly responded solutions through accelerated breeding cycles for more powerful resistance cultivars. Conventional methods for drought evaluation in soybean are non-repeatable or not fast enough to shorten the selection process. Manageable and reliable drought-related traits are required in HTP methods for the acceleration. Recent researchers found out that biomass and yield correlate positively with the number of nodes and green area of canopy. Thus, we applied the RGB sensor for fast and reliable screening green area of drought-stressed soybeans and compared with other drought related traits. The goal of the current chapter is to develop a fast and cost-effective method of screening drought tolerance by means of image analysis in the early vegetative stages. This thesis will focus to provide a useful tool for drought evaluations and the basis for selection criteria in further drought tolerance experiments and breeding processes.

II. Chapter I: Literature review: Comparisons of High-Throughput Phenotyping Methods for Detecting Drought Tolerance

Abstract

Drought is crucial threat worldwide for crop production, especially present rapid climate changing situation. Current drought solutions: improving irrigation system, rainwater harvesting, damming, cloud seeding, and some changes of cultivation methods, although they are effective each has their economic, environmental, and temporal drawbacks. Among all solutions, the most effective, inexpensive and manageable method is the use of drought resistance cultivars, via plant breeding. However, conventional plant breeding is a time-consuming and laborious task especially for the phenotypic data acquisition of the targeting traits of numerous progenies. The recently emerged method, high-throughput phenotyping (HTP), has potential to overcome the foresaid issues. Its massive, accurate, rapid, and automatic data acquisition in breeding procedure can be the breakthrough for developing drought resistant/tolerant cultivar to solve current drought problems. Thus, the current article will introduce various methods of HTP to detect drought stress, which can accelerate the drought resistance cultivar breeding processes in order to provide helpful guidelines for to choose the appropriate methods for breeders and researchers under their circumstances.

1. Introduction

Expanding global population demands the doubled crop production by 2050, which will be a significant challenge to achieve the goal (Araus and Cairns, 2014). However, recently variation of drought frequency and location are increasing tremendously due to the global warming and climate change causing severe yield loss (Spinoni *et al*., 2014). Severe and frequent drought would decrease yield significantly causing global food security.

Developing drought tolerant cultivar is an effective method to deal with current situation by providing farmers to relatively inexpensive and manageable plantation (Cattivelli *et al*., 2008). However, only few drought tolerance cultivars of crops have been developed so far. Moreover, conventional breeding takes many years even with the modern breeding such as marker-assisted selection (Collard and Mackill, 2008; Tester and Langridge, 2010). To enable shorter breeding cycles, great rates of genetic gain with the sufficient number of samples and reliable data set are required. This led the advent of new field, high throughput phenotyping (HTP) (Rutkoski *et al*., 2016). HTP is based on various kinds of sensors and computing technology in order to accelerate phenotypic data acquisition process in accurate, fast, non-invasive, automated, and reliable manners. Therefore, it would be worth to reviewing various methods of HTP to evaluate drought stress in crop plants so that researchers who want to phenotype drought tolerance level could compare them to utilize in their own purposes under their circumstances.

In order to monitor plant performance and identify traits under drought condition, defining phenotypes of drought stresses are crucial.

Dehydration under drought condition results in critical damage to plant, by changing leaf and canopy temperature, transpiration rate and biomass distribution decreasing growth rate and production (Khodarahmpour, 2011; Passioura, 1983). Thus, there should be various ways to screen drought stress level with various kinds of sensors to screen each of those components mentioned above. This article will focus on reviewing high throughput phenotyping methods and platforms.

2. High throughput phenotyping methods for drought stresses in plants

1) Red, green, and blue (RGB) image

Multispectral sensors generally comprise several bands including RGB channels and near infrared (NIR) channels (Kelcey and Lucieer, 2012). Relatively insensitive accessibility of spectral imagery made various forms of its usage. RGB (Red, green, blue) band sensor is the most affordable and accessible instrument because it takes images of most all of the morphological features of plants, such as whole image or partial image of plant, plant structure, shoot biomass, leaf density, leaf area, height, and color. Due to its rapid measurement and affordable access, it has various applications. For example, plant density of wheat was estimated with light platform fixed RGB camera (Liu *et al.*, 2017), time series of plant penology was monitored with an automated time-lapse photography (Crimmins and Crimmins, 2008), and leaf segmentation of sorghum was estimated by RGB camera on UAVs (Chen *et al.*, 2018). RGB images also can be applied to acquire sophisticated information of water stress responses based on its shape, compactness, solidity, and other visible parameters (Deery *et al.*, 2014).

2) Infrared imaging

In the 700 nm to 1300 nm near infrared (NIR) wavelengths, its reflectance on plant green area shows highest rates (Broge and Mortensen, 2002). It also shows relatively low reflection at the wavelength beyond 1300 nm. The former causes the scattering wave within the leaf mesophyll, and later is absorbed by water strongly (Knipling, 1970). Consequently, these characteristics verifies compatibility on meaningful parameters against drought stresses. Bei *et al.* (2011) measured grapevine water potentials using custom-made spectrophotometer and handheld spectrometer to have significant correlation with the results of pressure chamber in fields and glasshouses. In addition, vegetation indices requires NIR channel shows significant correlation on vegetation statuses. Bendig *et al.* (2015) and Yang *et al.* (2017) estimated normalized difference vegetation index (NDVI) in order to monitor biomasses in projected area expeditiously easily with combination of RGB and NIR imagery on Unmanned Aerial Vehicles (UAVs). These sensors can be adapted on not only UAVs but also on other platforms such as ground vehicles and chambers to produce images of wide range and continuous images at each platform (Chapman *et al.*, 2014; Deery *et al.*, 2014; Gago *et al.*, 2015).

3) Hyperspectral imaging

Hyperspectral sensors consist of hundreds and thousands of bands per one pixel compared to the multispectral sensors (Thenkabail *et al.*, 2002). By its narrow and numerous bands, band selection is relatively complicated for imaging than the multispectral sensors. Nevertheless, it can differentiate various stress responses by its feasibility in acquiring images in high resolution and narrow spatial range. Thereby, generally it is used on indoor imaging and high altitude aerial platforms based on the high level of details in hyperspectral imagery. Due to its narrow ranges vegetation and water indices, soil coverage status, photosynthesis rates, and levels of phytochemicals such as nitrogen, cellulose, lignin, and pigments can be derived (Hamada *et al.*, 2007; Stagakis *et al.*, 2010; Zhao *et al.*, 2013). However, the illumination issues on close range and the inconstant imaging by environmental changes could be problematic for high-throughput phenotyping (Mishra *et al.*, 2017). Nonetheless, physiological and phytochemical parameters with hyperspectral imaging to detect drought stress responses in crop plants is highly effective (Behmann *et al.*, 2014).

4) Thermal imaging

Thermography, also known as infrared thermography, produces images using the emitted radiation of object that increases with the object temperature above absolute zero (Shekhawat, 2016). Thermal sensor can detect temperature changes cause by transpiration occurrence due to the stomatal closure with visualized image data (Peñuelas *et al.*, 1992). Thereby, temperature related traits such as water content, transpiration rate, and stomatal conductance could be measured through the thermal imaging (Prashar *et al.*, 2013; Tattaris *et al.*, 2016). For examples, stomatal conductance in grapevine (*Vitis vinifera*) was estimated with a handheld thermometer camera (Leinonen *et al.*, 2006), and water stress in olives was evaluated through correlation between soil and tree water status and thermal imagery (Ben-Gal *et al.*, 2009). HTP methods with thermal imagery are often applied with other sensors to have comprehensive data. For instance, applying thermal and multispectral sensors on UAVs for vegetation monitoring (Berni *et al.*, 2009) and water status was assessed in vineyard (Baluja *et al.*, 2012; Gago *et al.*, 2015). Since thermal images have significant correlation with water stress indicators, it may be one of the most useful sensors to phenotype drought tolerance. However, various environmental factors such as solar radiation, air temperature, wind speed, and background temperature can easily influence the field measurement, which requires technical expertise to overcome this limitation (Sugiura *et al.*, 2007)

5) Fluorescence imaging

Fluorescence is luminescence of longer wavelength photons of fluorescence lifetime after photon absorption by certain susceptible atom or molecule. These longer wavelength and lower energy photon decays slow, so that it can be measured by the sensors for 10E-12 seconds (picoseconds) (Berezin and Achilefu, 2010). Thereby, plant fluorescence can be obtained through those response of fluorescence by irradiating chloroplasts with blue or actinic light. As fluorescence and chlorophyll contents are strong indicators of drought tolerance to determine the metabolic status of plants, fluorescence imaging can be effective for dissecting drought related traits such as photosynthetic rate changes and pigment proportion changes (Li *et al.*, 2006; Ögren and Öquist, 1985; Zlatev and Yordanov, 2004). However, impropriety for the early water stress detection, inadequacy on broad range imagery, inconsistent illumination, environmental disruptions under field conditions for remote sensing, and high-power requirements is its limitation (Jansen *et al.*, 2009; Shakoor *et al.*, 2017). Despite fluorescence sensor applicable platforms are limited, efficiency of fluorescence imaging is proved under drought conditions by the combination with other sensors or automated facilities to screen photosynthetic rates (Chaerle *et al.*, 2006).

6) Light Detection and Ranging (LiDAR)

Lidar is newly emerged remoted sensing technology that measures distance of target objects by analyzing the reflected light (Lefsky *et al.*, 2002). Various parameters of canopy and leaves, such as vegetation cover, height, canopy structure, leaf area index, and nitrogen status are acquirable (Eitel *et al.*, 2014; Lin, 2015; Madec *et al.*, 2017; Omasa *et al.*, 2006; Zhang and Grift, 2012). Furthermore, LiDAR measuring via 3D structuring can be done in only several minutes. It is generally applied in aerial platforms, ground vehicles, and ground fixed & stationary platforms. Among them, UAVs shows a highest potential and efficiency than the other platforms for 3D LiDAR mapping. However, this method has limited application for drought stress study. One such application could be biomass, which results from slow growth and wilting due to drought stress based on 3D images of canopy. In summary, aerial platforms with LiDAR are effective for measuring canopy area, while rough images might be unsuitable for accurate data for drought tolerance. To overcome this, ground based platforms are suggested with current image resolution of LiDAR.

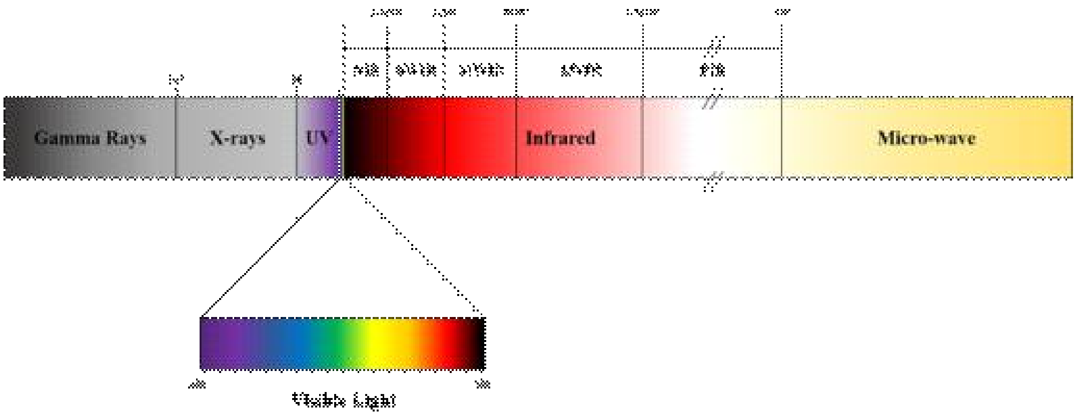

Fig 1. Electromagnetic spectrum scheme (nm).

Table 1. Sensors for high throughput imaging and obtainable traits.

Sensors	Range	Features	Traits	Reference
RGB sensor	400~700nm	Images of visible wavelengths are obtained. Most easily accessible sensor.	Vegetation indices, plant height, plant structure, growth rates, and morphological traits.	Crimmins and Crimmins (2008); Deery *et al.* (2014); Liu *et al.* (2017)
NIR sensor	700~1400nm	Shows highest reflectance of plant green area in 700~1300nm, while beyond 1300nm shows more absorbance by water than the visible spectrum.	Chlorophyll conductance, water status, and vegetation indices.	Bei *et al.* (2011); Bendig *et al.* (2015); Yang *et al.* (2017)
Hyperspectral sensor	-	Consists thousands of bands per one pixel. More detailed images can be obtained than the multispectral imaging if the requirements are set.	vegetation and water indices, soil coverage status, photosynthesis rates, and levels of phytochemicals.	Hamada *et al.* (2007); Stagakis *et al.* (2010); Zhao *et al.* (2013)
Thermal sensor	$700 \sim 10^6$ nm	Imaging sensor using the emitted radiation of object that increases with the object temperature above absolute zero. Suitable to image temperature changes.	Canopy temperature, transpiration rates, and water stress responses.	Baluja *et al.* (2012); Berni *et al.* (2009); Gago *et al.* (2015); Leinonen *et al.* (2006)
Fluorescence sensor	180~800 nm	Capable to measure fluorescence emitted by short wave light absorption of susceptible molecule.	Chlorophyll conductance, photosynthetic rates, and pigment composition.	Chaerle *et al.* (2006)
LiDAR	250 ~ 2,000nm	Distance measuring and surface scanning of target objects by analyzing the reflected light.	Canopy and leaves, vegetation cover, plant height, and nitrogen status.	Lin (2015); Eitel *et al.* (2014); Madec *et al.* (2017); Omasa *et al.* (2006); Zhang and Grift (2012);

3. Platforms for sensors to evaluate the drought tolerance

As mentioned above, various sensors can derive parameters for high-throughput phenotyping. They are powerful imaging instruments that allow accurate and massive phenotyping data at a glance off. However, it is important to have appropriate platform such as aircrafts, vehicles, ground fixed, and automated facilities to mount sensors in order to obtain visualized parameters of plant response under drought conditions.

Traits from canopy and leaf area such as leaf area, transpiration rates, canopy temperature, phytochemicals, and photosynthetic rates are highly related with drought encounter. Among various platforms, aerial detection is the most effective and most efficient way in terms of phenotyping speed. Its rapid and accurate remote sensing allows to image massive amount of plant in wide area within very short time. Visible traits of above canopy area including plant height can be easily measured by aerial imaging with RGB sensor (Bendig *et al.*, 2014; Jin *et al.*, 2017). Chlorophyll contents that can be estimated by NIR and Red range by aerial imaging (Bendig *et al.*, 2015; Yang *et al.*, 2017). Thermal sensor mounted on aerial vehicles is capable of aerial water status detections (Baluja *et al.*, 2012; Berni *et al.*, 2009; Gago *et al.*, 2015). Also, aerial platform with high payload can apply hyperspectral sensor for phytochemical and photosynthetic traits. However, application of thermal and fluorescence sensors might be more appropriate to be mounted on ground vehicles, ground fixed & stationary platforms, and indoor facilities for higher resolution images due to formally mentioned issues (Busemeyer *et al.*, 2013; Deery *et al.*, 2014; Shafiekhani *et al.*, 2017; Tisné *et al.*, 2013).

Although aerial platform has benefits for high throughput phenotyping, its detectable area is limited on canopy area only. Therefore, drought influenced phenotypes below canopy area such as stem structure, biomass and branching are remotely sensed by ground vehicles (Salas Fernandez *et al.*, 2017), ground fixed & stationary (Busemeyer *et al.*, 2013; Shafiekhani *et al.*, 2017), and indoor (Hartmann *et al.*, 2011) platforms. Ground vehicles are be relatively less expensive than other two kinds of platforms, while its images need analyzing process same as the aerial platform (Deery *et al.*, 2014). They are also very advantageous in term of much higher capacity for loading many sensors with higher weights than aerial platform does. However, the phenotyping speed is much slower than aerial platform. Indoor platforms have benefits that can control the objective environment due to the inhibition of other uncontrollable disturbances. By restricting interference of extrinsic factors, almost all the sensors are available on this platform. Proper posture rectified for each imaging sensor can make the measurement more accurate and rapid with easier operation. Ground fixed & stationary platforms has advantage that can produce time-lapsed image easily due to their fixed imaging angle and constant imaging time although this must be durable under the outdoor condition. Indoor facilities are also capable to phenotype root whose formation under drought condition also provides important hints to be tolerance to drought (Wasaya *et al.*, 2018). However, restricted individual numbers, high cost, and environmental settings are its limits. Cylinder growth systems, hydroponic growth systems, aeroponic growth systems, X-rays, nuclear magnetic resonance microscopy, magnetic resonance imaging, and laser scanning are currently available for indoor root phenotyping (Clark *et al.*, 2013; Iyer-Pascuzzi *et al.*, 2010; Marié *et al.*, 2014; Taras *et al.*, 2012).

Table 2. Platforms for High Throughput Phenotyping.

Platforms	Categories	Features	Limits	Reference
Aerial	Satellites	Rapid imaging of broad area is available. Payload limits. Screening process is possible irrespective of plant height. Only orthoimages can be obtained.	Relatively low resolution images than platforms on lower altitude.	Hamada *et al.* (2007); Stagakis *et al.* (2010)
	Aircrafts		Manual control requires expertise.	Chapman *et al.* (2014)
			Easily influenced by environmental factors. Relatively low payloads.	Baluja *et al.* (2012); Bendig *et al.* (2014); Bendig *et al.* (2015); Berni *et al.* (2009); Gago *et al.* (2015); Jin *et al.* (2017); Yang *et al.* (2017)
Ground	Tractors & Buggies	Manually or remotely controlled. High resolution images obtainable. Sensor payload is irrespective.	Inappropriate to screen very tall crops.	Deery *et al.* (2014); Salas Fernandez *et al.* (2017)
	Bicycles			Liu *et al.* (2017)
	Ground-Fixed & Stationary	Suitable to time-lapsed images. More sensors are mountable than the aerial platforms.	Requires durability against outdoor conditions.	Busemeyer *et al.* (2013); Shafiekhani *et al.* (2017)
	Indoor Facilities	Environmental factors can be controlled. Uncontrollable disturbances are inhibited. Almost all sensors can be applied. Capable to root phenotyping.	Limited individuals.	Clark *et al.* (2013); Iyer-Pascuzzi *et al.* (2010); Hartmann *et al.* (2011); Marié *et al.* (2014); Taras *et al.* (2012); Tisné *et al.* (2013); Wasaya *et al.* (2018)

4. Conclusion

Present drought problems are one of the main casual factors of incoming world food crisis, which can be overcome by developing drought tolerance cultivars via plant breeding. Since drought occurs more often in severe forms, the breeding cycle should be significantly shortened. To achieve this, massive and accurate phenotypic data is crucial. Considering drought stress responses are related with various morphological and physiological traits, numerous methods could be applied using sensors such multispectral, hyperspectral, thermal, fluorescence sensors, laser sensors on various platforms. In the current article, currently developed methods were reviewed to help researchers who need to do high throughput phenotyping for drought responses. We sincerely hope that this article could help those who consider to study drought response or to breed drought tolerance cultivars.

III. Chapter II: Application of image analysis method to study drought stress in soybean

Abstract

The steep increase of drought frequency under global warming has resulted in massive losses to world crop production. Consequently, drought-tolerant cultivars are required to overcome this crisis under the given circumstances. In order to develop new drought-tolerant cultivars efficiently, it is crucial to phenotype massive numbers of individuals in a fast, reliable, and precise manner, which has led to the advent of high throughput phenotyping. In this report, we demonstrate fast and reliable phenotyping methods to screen drought tolerance in soybeans (*Glycine max* L.). Recent studies have revealed that biomass and yield are positively correlated with the number of nodes and canopy/green area. The results showed that green pixel percentage has a significant correlation with the number of main nodes. This case study demonstrates that the green pixel percentages would be useful for drought evaluations in further experiments.

1. Introduction

Climate change impacts on crop productivity and field water balance. In particular, water scarcity during the early growing season and reproductive stage poses a severe threat to crop yields (Spinoni *et al.,* 2014). One of the most sustainable methods to overcome the unpredictable occurrence of drought is to introduce drought-tolerant cultivars within a short time frame (Cattivelli *et al.,* 2008). Consequently, quick and reliable phenotyping of the correct traits is essential to achieve this.

Soybean (*Glycine max* L.) is one of the most important field crops and requires sufficient water and temperature levels during its life cycle (Wang *et al.,* 2006). Nevertheless, studies of drought tolerance are heavily focused on the reproductive stage. Furthermore, not many target traits were developed for high throughput phenotyping, although Bai and Purcell (2019) reported that the greenness intensity using vertical images demonstrated the possibility for screening yields and responses under drought. While this method is reliable and repeatable it is not fast enough to make breeding cycles shorter. Most importantly, it requires expensive equipment.

There are several conventional yield components, such as root formation, node formation, flowering, and pod formation (Dornbos *et al.,* 1989; Desclaux and Roumet, 1996; Fenta *et al.,* 2014). Among those, biomass and yield were found to be related to the number of nodes and canopy/green area (Thomas and Raper, 1977; Cui and Yu, 2005). In addition, Kobraei *et al.* (2011) reported that the number of nodes is associated with the number of pods. This is possibly because the formation of main stem nodes results in the formation of branches, which also increases the number of branch nodes

(Nakano *et al.,* 2019), having a positive effect on biomass and yield (Board, 1987; Cui and Yu, 2005). Therefore, it is possible to estimate biomass and yield through the number of nodes by following the correlations.

Thus, we developed a fast and cost-effective method of screening drought tolerance by means of image analysis in the early vegetative stages using a commercial digital camera. To achieve this, the vertical images of the green area of canopy were examined, which demonstrated a correlation between the number of nodes and the number of pods.

2. Materials and methods

1) Plant materials and experiments

The experiment was conducted between May 21, 2019 and September 20, 2019, at the greenhouse of Jeju National University, Korea (33°27′19.1″N, 126°33′41.8″E, DMS). The average temperature of the greenhouse was maintained at 29 ℃ during the day and 22 ℃ at night.

Parents of 28 nested association mapping (NAM) populations of soybean (Glycine max L.) were provided by the Rural Development Administration (RDA), Korea (Table 3). Each parent was planted using three replications of individuals in each of the four pots, sized 38.5 × 28 cm. All pots were randomly placed in 7 rows × 4 columns × 4 plots (total 112 pots) at the greenhouse. Watering was evenly applied until June 9, 2019, 20 days after planting (DAP), when 90% of the soybeans were in the 4th vegetative stage (V4) and from June 24, 2019 to September 20, 2019 (35 DAP to 123 DAP). Drought stress with irrigation control was carried out from June 10 to June 23, 2019 (21 DAP to 34 DAP). One pot of each parental line was fully irrigated as a control sample, while the remaining three pots were irrigated with 75ml once every three days.

At the end of drought treatment (June 23, 2019, 34 DAP) and after 14 days of recovery (July 6, 2019, 47 DAP), the number of main nodes was counted and vertical RGB images of the whole plant were taken 120 cm above ground with a Nikon COOLPIX A100 (Nikon, Japan). On September 20, 2019 (123 DAP), main stem nodes were measured, and the number of pods were counted for the final yield evaluation. The treatment schedule is summarized in Fig 2.

Table 3. Twenty-eight nested association mapping (NAM) population of soybean.

NAM Number	Varieties	NAM Number	Varieties
Common	Daepung	NAM14	Willians82
NAM1	Bangsa	NAM15	Saedanbek
NAM2	Pungwon	NAM16	Daewon
NAM3	Hannam	NAM17	Hwanggeum
NAM4	Sowon	NAM18	Chungja
NAM5	Galche	NAM19	Chungja 3ho
NAM6	Somyeong	NAM20	Sochung 2ho
NAM7	Sinhwa	NAM21	Ilpumgeomjung
NAM8	Pureun	NAM22	Daeheuk
NAM9	Taegwang	NAM23	Josangseori
NAM10	Wuram	NAM24	Yeunpung
NAM11	Danbek	NAM25	Chunal
NAM12	PI96983	NAM26	Heukchung
NAM13	Haman	NAM27	Seoritae

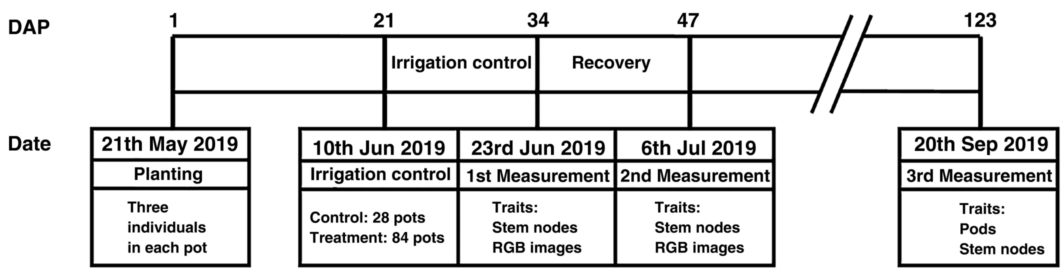

Fig 2. Treatment schedule of current experiment. (Control) well-watered; (Treat) drought stressed; (Traits) measured traits; RGB image: red, green, and blue image.

2) Image process

Vertical images of the whole plant acquired 34 DAP and 47 DAP were analyzed by MATLAB (R2019a update 3 9.6.0.1135713, MathWorks) application Canopeo (v1.1, canopeoapp.com) using a noise reduction value of 1000 and color thresholds with a 1.0 Red to Green (R/G) ratio and 1.0 Blue to Green (B/G) ratio (Patrignani and Tyson, 2015). The process of estimating GPP through Canopeo is shown in Fig 3.

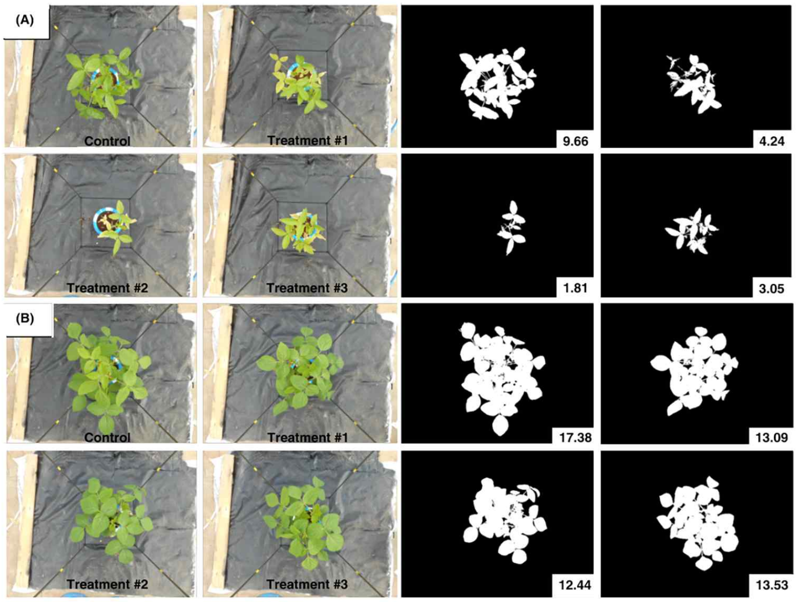

Fig 3. Images of before/after (left/right) images and Canopeo process of NAM number 20. Numbers in the white box indicate the percentage of green area. (A) Images of after drought treatment, June 23th, 2019; (B) Images of after recovery, July 6th, 2019.

3) Statistical analysis

Multivariate analysis of variance (MANOVA) was carried out to investigate the null hypothesis of no treatment and varieties effects or interactions on the main nodes and GPP (Johnson and Wichern, 2007, p. 301-307). The data were gathered at the end of the drought treatment and after 14 days of recovery.

The statistical model is as follows:

$$Y_{rlk} = \mu + \beta_r + \tau_l + \gamma_k + (\tau\gamma)_{lk} + e_{lkr}$$

Where $\boldsymbol{Y}_{rlk}$ is the r-th replication of the vector of the characters (main node and GPP) measured at l-th variety and k-th treatment; μ is the intercept; β_r is the vector of the block effects; τ_l is the vector of the variety effects; γ_k is the vector of the treatment effects; $(\tau\gamma)_{lk}$ is the vector of the interaction effects and $\boldsymbol{e}_{lkr}$ is the random error effects.

Pillai's trace was used as a test statistic in MANOVA, i.e.

$$V^{(s)} = tr\left((E+H)^{-1}H\right) = \sum_{i=1}^{s} \frac{\lambda_i}{1+\lambda_i}$$

Where $V^{(s)}$ is the Pillai's statistics; $\boldsymbol{H}$ is the hypotheses matrix; $\boldsymbol{E}$ is the error matrix and λ_i is the eigenvalue of the $\boldsymbol{E}^{-1}\boldsymbol{H}$. Orthogonal Contrast was defined in an incidence matrix of a multivariate linear model to test the null hypothesis of no difference between control and treatment.

A simple linear regression analysis was performed to verify the relationships between main node and GPP and main node and number of pods gathered, as measured at the final yield evaluation. A hypothesis test for the slope of the regression line was applied to verify the null hypothesis of no relationship between the variables. All computations were conducted using R statistical software (R Core Team, 2020).

3. Results

Results from MANOVA revealed significant outcomes for treatments and varieties without interaction (Table 4). Each variety also had significant differences from one another. Considering there is no difference among blocks, this experiment in the greenhouse seems to be reliable. Furthermore, the orthogonal contrasts for control versus treatments for GPP and main node showed that GPP is a promising parameter considering both GPP and the number of main nodes could discriminate the difference from control (Table 5). This becomes more evident with high correlation (0.71) between both parameters (Fig 4). However, the correlation between the number of main nodes and the number of pods was moderate (0.40). One of the possible explanations could be that this is due to the outlier, as shown (b) in Fig 4. Another reason, which may be more important, could be the different recovery rate of each variety from drought stress based on the significant differences between them, as stated above. This can also be supported by Figure 2. This specific variety (NAM20) was treated with drought stress (a) and recovered (b) (Fig 3). During the treatment, those treated individuals were severely damaged; however, they recovered almost as much as the control. Thus, the number of main nodes taken after the recovery stage was not correlated with the number of pods figure as the GPP and the number of main nodes was, given those two parameters are highly associated (Board and Tan, 1995; Ball *et al.*, 2001; Kahlon *et al.*, 2011).

	Df	Pillai	F statistics	P value
Blocks	2	0.00703	0.9579	0.4297
Treatments	3	0.25977	27.0183	< 0.001
Varieties	27	0.3569	4.3677	< 0.001
Interaction	81	0.2577	0.9917	0.561
Residuals	543			

Table 4. Multivariate analysis for data from percentage of number of main nodes and green pixels ($P < 0.05$).

Features	Estimate	t statistics	P value
Green pixel percentage	1.81545	11.954	< 0.001
Main node	0.79651	13.413	< 0.001

Table 5. T test for orthogonal contrasts: Control versus treatments for GPP and number of main nodes ($P < 0.05$).

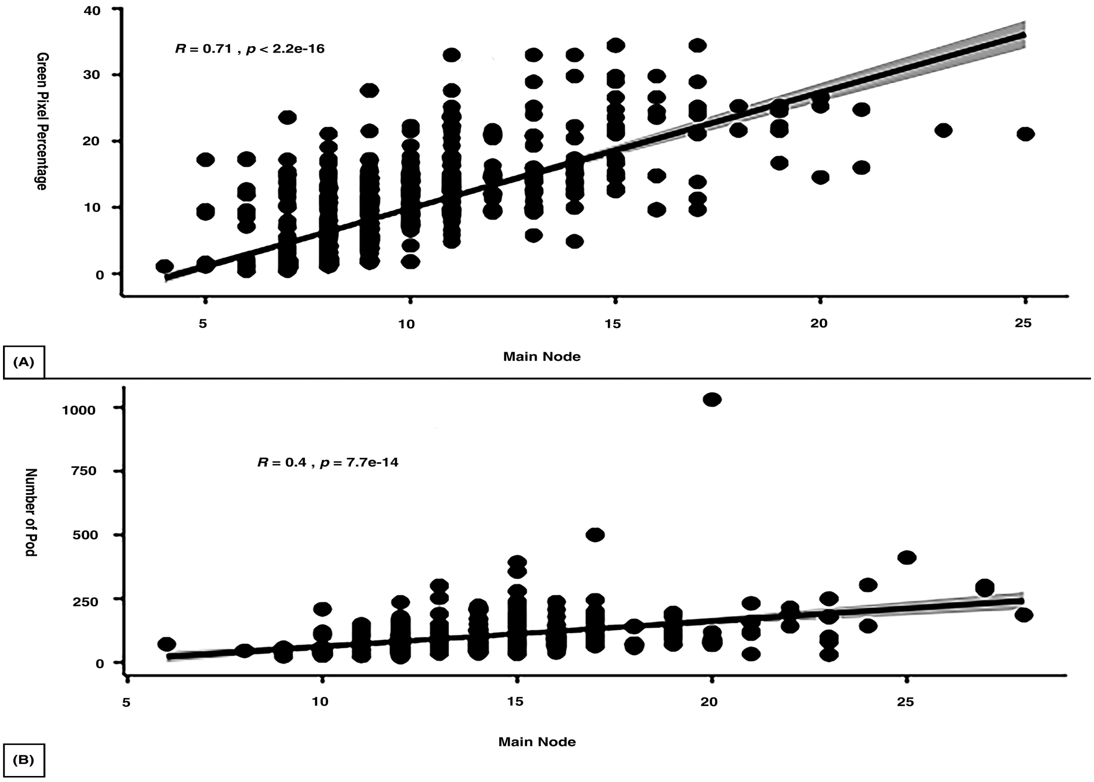

Fig 4. Pearson correlation and simple regression analysis. (A) Pearson correlation between the number of main nodes and green pixel percentage; (B) simple regression analysis for the number of main nodes and number of pods.

4. Discussion

From this preliminary study, we conclude that GPP is useful in measuring biomass as an indicator for drought stress in the early stage of soybean cultivation by estimating the number of pods for those drought susceptible varieties. Furthermore, this method provides the exact digital data, enabling the quantitative measure, unlike the conventional methods that provide only categorical data. In addition, GPP correlates estimate the number of pods for those drought susceptible varieties and detect varieties that recover from drought stress quickly. We expect that this method would be helpful to researchers and breeders who are not familiar with complex technologies and who have tight budgets for studying and pre-screening drought-tolerant lines of soybean populations in a fast and cost-effective manner.

IV. Conclusions of this thesis

The main goal is to introduce the various high throughput phenotyping methods with an appropriate guide to the drought researches and to develop an efficient basis for the drought resistance cultivar breeding processes and researches. Present extreme drought due to global warming is menacing the agricultural industry. Immediate development of drought resistance cultivar against frequent drought cycles will be the most effective solution. Acceleration of the breeding cycle requires drought indicator evaluation for selection criteria. Applying HTP method, which is feasible to a rapid, massive, and accurate data analysis, is the consequent process to achieve present demands.

Chapter I suggests the sensors such as RGB, NIR, multispectral, hyperspectral, thermal, and fluorescence will be appropriate for image data analysis and drought evaluation in HTP manners. We applied RGB sensor, which is the most accessible and the most efficient among the spectral sensors, to assess drought related traits in the early vegetative stages of soybean. Concluding GPP has the possibility to evaluate biomass as an indicator of drought stress of soybean in the early cultivation period by estimating the number of pods for those drought susceptible varieties. Results also demonstrated that while the conventional methods provide only categorical data, digital data from sensors makes feasible of quantitative measurement.

Although it has some difficult issues of technological, financial, and other issues to apply HTP in drought researches, it is obvious that the HTP method which has enormous benefits of acquiring and analyzing phenotyping data is a powerful tool for screening drought responses. Such challenges for proper application of sensors and platforms from researchers worldwide will circumspectly make the HTP technology more solid. Therefore, we hope this paper to be a helpful guideline for researchers and breeders who considers sensor based HTP drought screening methods under their circumstances and objectives.

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