University of Southern Queensland

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Can Machine Vision Assess the Eating Quality of a Growing Leafy Plant?

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ABSTRACT

Key Words: Lettuce, Machine Vision, RGB, Features, Machine Learning, Support Vector Machine

Maximising the nutritional yield from lettuce grown on space stations for consumption by astronauts is critical. A well-rounded, low-cost tool which uses a combination of machine vision and machine learning components, was conceptualised to autonomously assess the eating quality of lettuce plants based on the plant's external features. Romaine lettuce (*Lactuca sativa* L. var. *longifolia*) was chosen as the model plant. TPCA (Top-Projected Canopy Area), colour and texture (contrast, correlation, energy and homogeneity) were selected as morphological features for investigation. These morphological features were extracted from RGB images taken of the lettuce plants. An SVM binary classification algorithm was trained to detect water stress in lettuce plants and classify whether the plant is healthy or not healthy. From the RGB images, there were 9 parameters selected for training, including TPCA, energy, homogeneity and six colour indices (GCC, MGRVI, HUE, BI, ExB and BGI). The SVM model had 81.2% accuracy for training data, and 75% accuracy for testing data, with an F1 score of 0.8. PCA was used to reduce the dimensionality of the parameters, which explained at least 95% variance of the data. Additionally, leaf crispness is an indicator of leaf water content. Analysis was performed on audio features peak level, amplitude range and waveform length to determine whether audio could be used as an additional quality metric for assessment. These audio features were compared to NGRDI for biomass estimation. Amplitude range had positive correlation to NGRDI, where $r = 0.5196$ for the watered group, and $r = 0.2908$ for the non-watered group. These results indicate that amplitude range can be used to estimate leaf water content and may be used as an additional quality metric to assess the eating quality of a lettuce plant.

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7 November 2024

Date

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GLOSSARY

The following abbreviations have been used throughout the text:

TPCA	=	Top-Projected Canopy Area
GLCM	=	Gray Level Co-Occurrence Matrix
NGRDI	=	Normalised Green Red Difference Index
GCC	=	Green Percentage Index
RGBVI	=	Red Green Blue Vegetation Index
MGRVI	=	Modified Green Red Vegetation Index
GLI	=	Green Leaf Index
VEG	=	Vegetative Index
vNDVI	=	Visible Normalised Difference Vegetation Index
HUE	=	Overall Hue Index
GR	=	Simple Red-Green Ratio
ExB	=	Excess Blue
BI	=	Brightness Index
BGI	=	Simple Blue-Green Ratio
PCA	=	Principal Component Analysis
SVM	=	Support Vector Machine
LDA	=	Linear Discriminant Analysis
ELM	=	Extreme Learning Model
CNN	=	Central Neural Network
ANN	=	Artificial Neural Network

CHAPTER 1 INTRODUCTION AND BACKGROUND

Autonomous and non-autonomous methods to support the optimal growth of healthy plants are crucial to the sustainability of life on Earth and in space. Machine vision tools are becoming more accessible and are rapidly gaining popularity in medicine, agricultural, manufacturing, automation, and IT sectors. In this research project, an automatic detection tool using machine vision is proposed, aiming to identify stress qualities in a growing leafy plant; qualities that reduce the overall nutritional and health benefits of the plant. Leafy plants, for example, lettuce, are abundant in vital vitamins, minerals, and antioxidants, and improve and physical health (Kim et al. 2016).

Optimizing the nutritional value of a plant is desirable on Earth and in space. In space, there is a need for growing high quality food crops as vegetables are a necessary supplement to the dried foods eaten by astronauts (Khodadad et al. 2020). High quality food crops also enable the possibility of manned, deep space exploration missions. Khodadad et al. (2020) investigated the growth of lettuce in space inside an enclosed payload, dubbed Veggie, and determined that the lettuce was safe for human consumption (Figure 1.1). The need to automate the production and inspection of plants grown in space is driven more by astronauts being limited in time and lacking the skill to grow the crops to a high-quality standard. On Earth, current global and climate conditions contribute to increased crop stress, with predictions of societal and economic impacts. An exponential increase in population implies an increase in food demand and resulting food scarcity (Mishra et al 2023, Shin et al. 2022). This highlights the importance of minimizing food waste, improving crop yields and thus, optimizing plant nutritional quality.

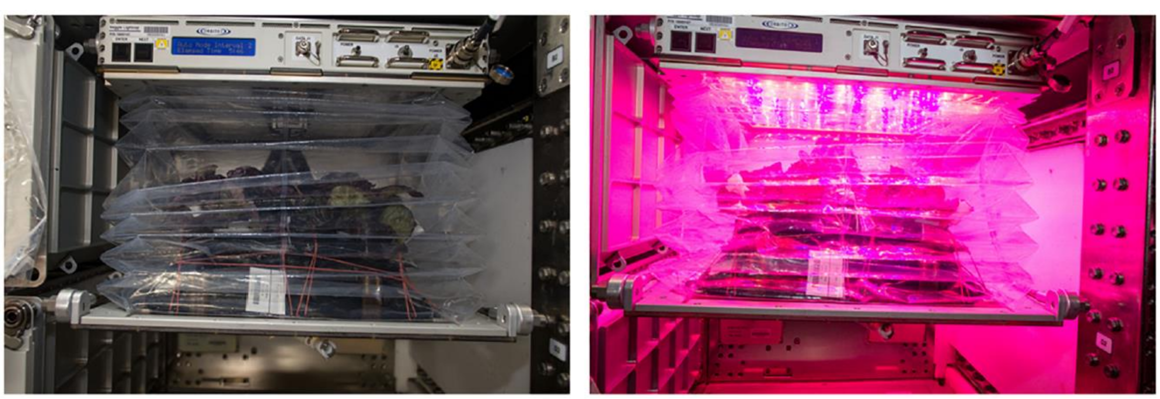


Figure 1.1. *VEGGIE payload aboard the ISS, growing red romaine lettuce (Khodadad et al. 2020).*

Firstly, it is to be noted that there may be a difference between cosmetic defects that do not affect the eating quality of a plant, and qualities that do. Current research indicates that biotic and abiotic stresses on plants directly affect its eating quality, and in most cases, there are visual indicators of the plant stress during the growing cycle. Abiotic stress is caused by numerous factors, including but not limited to water, temperature, light, micronutrient and macronutrient soil stress, and biotic stress is caused by pests and diseases (Kumari et al. 2022). These stresses cause various physiological, metabolic, and developmental inconsistencies in a plant and impact plant tissue cells, break protein bonds, and impede photosynthesis (Mishra et al. 2023).

Many of the current methods of detecting stresses in plants are non-autonomous, quite laborious and in most cases, require some form of human intervention. Detecting plant stress with machine vision is a relatively new concept, but emerging literature is auspicious and supports its usefulness as a tool in the detection and prevention of plant stress. In this project, the proposed solution aims to assist in addressing the shortcomings of the performance of current methods in terms of efficiency, reliability, and cost. The proposed solution is an electronic, machine vision-based system built on a machine learning model, that has the capability to autonomously detect visual stresses in a growing leafy plant if present and classify whether the plant is healthy or not. To fit the scope of the research project due to limitations in time and resources, this project will investigate one type of leafy plant (lettuce), one type of stress (drought) and will consider training one machine vision model to draw conclusions on whether machine vision as a tool can appropriately assess the eating quality of a plant. A ground truth quality metric will be chosen to assist in discerning which qualities of a plant are “good” vs “bad” when concerned with nutritional benefits. Additionally, crispness is an audible feature that was manually extracted, to evaluate whether this feature could be used as an additional metric to assess the eating quality of a lettuce plant.

The aim of the research is to enhance methods already being used, to explore more suitable methods, and make recommendations for a more ideal system. The implications of this research can be applied to growing crops in space to optimize nutrient gain for astronaut consumption, and on Earth, where crops are susceptible to pests and disease. Furthermore, there are other environmental and socio-economic benefits of this research, including the reduction of food waste, optimization of nutrient gain and an increase in crop yields.

1.1 PROJECT OBJECTIVES & OUTLINE

This section lists specific objectives that this research aims to address and summarises an outline of the project. The specific objectives are aligned with the primary objective of this project. This primary objective, or problem statement, is to determine whether machine vision can be used as a tool to autonomously assess the eating quality of a lettuce plant based on the plant's external features.

The first specific objective was to conduct a literature review of current machine vision systems in this area of research, as well as eating quality metrics of food crops. The second specific objective was to identify an appropriate quality metric and conceptualise a machine vision system that can autonomously extract plant features for eating quality assessment. Then, the third specific objective was to develop a system prototype including software and hardware. This prototype uses a combination of machine vision and machine learning tools. The fourth specific objective was to collect data of the plants using the system prototype. The fifth specific objective was to evaluate the performance of the system in classifying lettuce based on stress indicators. The findings were compared with other methods discussed in the literature review. Lastly, recommendations for improvements and further research were provided.

To outline the project, first, an extensive literature review is conducted, and a knowledge gap is identified. Then, the machine vision system is conceptualised, where an initial investigation is performed to develop a methodology and to make initial observations in the data. Following this, a main trial is performed, to gather a larger data set. Changes to the methodology are mentioned, then the results of the main trial are presented. These results were used to guide parameter selection and thresholding for class labels, to train and test the model. Lastly, practical design considerations are made to provide suggestions on developing the system beyond conceptualisation. Then, the project is concluded, and further research is recommended.

CHAPTER 2 LITERATURE REVIEW

Water is vital for plant morphological development and physiological health and is a necessary component in many plant cellular processes. Transpiration is a cellular process, in which the water molecules inside the plant's cells change from liquid to vapor and diffuse through the leaf stomata into the surrounding atmosphere, concomitantly reducing the plant temperature (Kacira et al. 2002). The rate of this transpiration process is related to the aperture of leaf stoma; a pore in the epidermis layer of leaves and stems of a plant (Cheong 2022). This is regulated by water availability and evapotranspiration demand. In a healthy plant, its turgidity is maintained by sufficient leaf moisture content. Plant stresses caused by water occur when the evapotranspiration demand exceeds water supply, resulting in deleterious effects on the plant's health. In response to this imbalance, turgor pressure falls, the stoma closes, photosynthetic activity decreases, discoloration appears, wilting occurs and plant temperature increases (Shin et al. 2022, Zuber & Yoon 2020, Lak et al. 2021). Consequently, the nutritional content of the plant deteriorates since the absorption and translocation of important ions, micronutrients and macronutrients from the root zone is affected when a plant is under stress (Kumari et al. 2022, Ling et al. 1996). Therefore, it can be inferred that the eating quality of a plant is directly related to its health.

Stress induced plant senescence is accompanied by both visual and non-visual symptoms. These visual and non-visual symptoms can be detected using machine-vision techniques. Machine vision is a non-destructive, smart technology that has multi-dimensional sensing capabilities (Li et al. 2019). It has been researched extensively in literature to extract spectral, morphological, and temporal plant features pertaining to abiotic and biotic stresses, to distinguish between and classify healthy and unhealthy plants. Morphological features include plant shape, size and texture. Spectral features include colour, temperature, and reflectance characteristics. Temporal features include growth rate and plant movement (Ling et al. 1996). Leaf wilting and discoloration are clear visual indicators that something is wrong with the plant. However, by the time that stress symptoms of a plant can be visually detected by the human eye, irreparable damage to the plant's health may have already occurred. Therefore, non-contact, early detection of plant stresses is desirable in many applications to prevent crop yield loss and maximize nutritional quality.

Romaine lettuce was chosen as the model leafy plant. This was due to local availability and high nutritional value compared to other lettuce cultivars (Kim et al. 2016a). Quality metric specifications for commercially sold Romaine lettuce consider the plant's size, colour, texture and crispness (BG Brisbane n.d.). These criteria, presented in Table 2.1, were selected for comparison when determining the eating quality of the lettuce plants. Features to be extracted using machine vision include TPCA (top-projected canopy area) which relates the plant's size, vegetation indices to assess the plant's colour, and GLCM (grey level co-occurrence matrix) to assess the structural complexity and texture of the plant. On the other hand, crispness is an audible quality that cannot be extracted using machine vision. Lettuce is 95% water, and it can be assumed that leaf crispness is an indicator of leaf water content (Kim et al. 2016a). The midrib of the leaf has a high moisture content, averaging 91.71% of leaf's total water (Wang et al. 2020). Furthermore, outer leaves of a lettuce plant are more mature compared to inner leaves, and wilt first when subjected to water stress conditions (Nyakwende et al. 1996).

Table 2.1. *Quality metric specifications as per supermarket criteria for commercially sold Romaine (Baby Cos) lettuce (BG Brisbane n.d.).*

Criteria	Specifications
Colour	“Mid-green leaves grading to pale green at the base...”
Visual Appearance	“Fresh, bright loosely overlapping outer and inner leaves...” “Crinkly to very crinkly and undulating leaves...” “Firm, white mid ribs...”
Sensory	“Firm, compact heads; crisp leaves with fleshy mid ribs...”
Size	“... span of main leaves > 140 mm diameter...”
Maturity	“... crisp leaves easily snapped away from core...”

2.1 EXISTING APPLICATIONS OF MACHINE VISION AS A DECTECTION TOOL

Machine vision-based detection tools have been developed in a myriad of applications to prevent or ameliorate plant stress and have shown promising results. This section of the literature review discusses some existing applications and approaches that relate to the research question of this project.

Kacira et al. (2002) developed a non-invasive sensing technique using machine-vision extracted plant features at the canopy level to monitor water stress in varieties of New Guinea Impatiens, under low and high humidity conditions. Its effectiveness was evaluated against the time of stress detection by an operator, and the research suggested that early plant water stress detection using top-projected canopy area (TPCA) based images was feasible. Lak et al. (2021) captured thermal measurements and red-green-blue (RGB) images of canopies to detect water stress in tomato plants grown in greenhouse conditions. Story et al. (2010) used plant morphological features such as TPCA, colour features (RGB and hue-saturation-luminosity (HSL)), and textural features (entropy, energy, contrast, and homogeneity) to detect calcium deficiency in lettuce plants. Li & Ling (1996) used spectral (reflectance and average grey levels in images) and morphological (TPCA and profile) features to detect temperature stress in tomato plants. Ling et al. (1996) determined from spectroscopic studies that stresses could be detected from visual and non-visual symptoms. The research used spectral and morphological features to study the effects of water and nutrient stresses on lettuce plants. Li et al. (2019) used spectral features in the visible and infrared wavelengths to measure leaf and canopy temperature of strawberry plants under water stress. Nguyen et al. (2020) used night-based hyperspectral imaging to capture leaf reflectance in multiple spectral regions to assess nutrient status in Bok Choy and Spinach under different fertilization regimes. Mao et al. (2015) used spectral, morphological feature analysis to measure total nitrogen of lettuce plants. These existing applications highlight important plant features that reflect water status and nutrient level in plants.

2.2 MACHINE VISION EXTRACTED FEATURES

2.2.1 MORPHOLOGICAL FEATURES

Top-projected canopy area (TPCA) provides information about plant movement and canopy expansion or growth. TPCA and plant movement were selected as features in the studies conducted by Kacira et al. (2002), and the findings concluded that plant water stress detection using TPCA was feasible. This is possible because when plants are under water stress, the leaves of a plant transition from a turgid state to a wilting state and will generally show symptoms like wilting leaves. Growth is also inhibited and is reflected as a decrease in plant TPCA. These symptoms make it possible to use TPCA of plants as an indicator of water stress. To reduce the effects of environmental and electronic noise, multiple images should be taken consecutively and the TPCA of the images should be averaged (Story et al. 2010). TPCA is best calculated when the image is transformed to a grey level binarized (black and white) image to distinguish the plant from the background. The area of interest (plant canopy) corresponds to the number of white pixels and the background corresponds to the number of black pixels (Mao et al. 2015). Plant TPCA is determined by the summation of the number of plant element pixels in the image. Story et al. (2010) found that TPCA was a promising marker for detecting nitrogen deficiency in lettuce crops. Therefore, TPCA is a good indicator of water stress and nutrient deficiencies in plants. However, TPCA data alone does not provide specificity. Literature has combined TPCA with other sensor data to draw more confident conclusions. An approach that uses multiple stress detection markers is more reliable (Story et al. 2010). For example, using crop or leaf reflectance data from spectroscopic studies provides information about levels of nutrients in the plant.

Colour is an external visual indication of a plant’s internal health. Colour features of a plant can be examined using RGB vegetation indices. Biró et al. (2024), compared 37 commonly used RGB vegetation indices, and summarised these into groups of similar indices. Out of the 16 individual vegetation indices determined by Biró et al. (2024), 11 were selected for this research. Table 2.2 contains information about the selected vegetation indices. Additionally, Jannoura et al. (2014) and Hunt et al. (2005), observed significant correlation between NGRDI (Normalised Green Red Difference Index) derived from RGB images, and biomass of soybean, pea, corn, oat, and alfalfa crops, with a correlation coefficient of $r = 0.57 – 0.78$, and $r = 0.63 – 0.94$, respectively. Therefore, NGRDI as a vegetation index can be used as a biomass estimator.

Table 2.2. *Selected vegetation indices and associated formula (Biró et al. 2024), where R is average red channel value, G is average green channel value, and B is average blue channel value of the RGB image.*

Index	Description	Formula
GCC	Green Percentage Index	$G/(R+G+B)$
RGBVI	Red Green Blue Vegetation Index	$(G^2-(B*R))/(G^2+(B*R))$
MGRVI	Modified Green Red Vegetation Index	$(G^2-R^2)/(G^2+R^2)$
GLI	Green Leaf Index	$(2G-R-B)/(2G+R+B)$
VEG	Vegetative Index	$G/(R^{0.667}*B^{0.334})$
vNDVI	Visible NDVI	$0.5268(R^{-0.1294}*G^{0.3389}*B^{-0.3118})$
HUE	Overall Hue Index	$\mathrm{atan}\,(2*(B-G-R)/30.5*(G-R))$
GR	Simple Red-Green Ratio	G/R
ExB	Excess Blue	$1.4*B-G$
BI	Brightness Index	$((R^2+B^2+G^2)/3)^2$
BGI	Simple Blue-Green Ratio	B/G
NGRDI	Normalised Green Red Difference Index	$(G-R)/(G+R)$

2.2.2 SPECTRAL FEATURES

Spectroscopic studies have been used extensively to estimate nutrient levels and deficiencies in plants, by measuring the reflectance of a leaf or canopy. Reflectance is measured as the ''ratio between energy reflected from the sample and the energy reflected from a reference sample' (Li & Ling 1996). The surface area and coloration of the plant sample alters the wavelength reflected back to the sensor. Reflectance studies are also subject to noise such as lighting fluctuation and surface geometry. Li & Ling (1996) used spectroscopic studies to monitor water and nutrient deficiency stress in hydroponically grown lettuce plants and found strong correlation between leaf water content and the reflectance wavelengths centred at 1394 nm and 1870 nm. These wavelengths are beyond the visual spectrum and require expensive resources such a NIRSystems Model 6500 spectrophotometer system and Li-Cor 1800 Spectroradiometer system (Li & Ling 1996), making it difficult to use spectroscopic data to detect water stress on lettuce plants with financial limitations. On the other hand, there were strong correlations between nutrient content and the reflectance wavelengths between 425nm and 720nm. These wavelengths are within the visual spectrum. Thus, it would be viable to use spectroscopic studies to estimate nutritional content of lettuce plant.

However, chemical composition analysis as a ground truth measure to compare the spectroscopic data against was not possible due to financial and time limitations of the project.

2.2.3 TEXTURAL FEATURES

Texture can be described as the underlying patterns of the plant's external morphological appearance, status of the plants internal physiological state (Story et al. 2010). The four textural features, homogeneity, entropy (correlation), energy, and contrast are the most significant and useful in identifying plant health (Mao et al. 2015, Story et al. 2010). 'Changes in canopy texture and surface structure are external symptoms of the plant's internal physiological status' (Story et al. 2010, pp. 241-42). Healthy plants usually appear colourful and darker due to higher levels of chlorophyll and have complex leaf textures. Plants under stress, appear lighter and more uniform in colour, and have reduced complexity in leaf textures (Story et al. 2010). From these visual indicators, and using textural analysis, information can be gathered about the plant's health.

Entropy (correlation) is measured as a level of randomness in grey level distribution of a plant. Healthy plants (colourful and complex) have a higher level of entropy and stressed plants have (dull and uniform) a lower level of entropy (Story et al. 2010). Contrast is calculated from the local variations in an image. Healthy plants (colourful) have higher contrast levels and plants that are stressed (dull) have lower contrast values (Story et al. 2010). Energy is a numerical representation of the grayscale brightness of a plant. The darker the leaf or canopy (healthy), the lower the energy value. The brighter the leaf or canopy (stressed), the higher the energy value (Story et al. 2010). Homogeneity refers to the grey level distribution of the pixels in an image. Healthy plants (colourful and complex) have a decreased grey level pixel distribution. Stressed plants (dull and uniform) have an increased grey level pixel distribution (Story et al. 2010).

GLCM (Grey Level Co-Occurrence Matrix) is a conventional method for texture analysis, first coined by Haralick et al. (1973). GLCM examines the spatial relationship of pixels in a grayscale image, by monitoring the frequency of patterns contributing to the perception of texture (Gomede 2024, MathWorks n.d.b, Story et al. 2010). One thing to note is that the perceived texture in an image is orientation dependent. Based on the studies conducted by Mao et al. (2015) and Story et al. (2010), images should be analysed at different angles relative to the pixel of interest (0°, 45°, 90° and 135°). See Figure 2.1 for a visual representation of performing GLCM on an image. For GLCM analysis, the grey-scale image must be quantized. Quantizing an image reduces complexity and increases efficiency and performance (Abbood & Al-Assadi 2021). Medina and Chen (2010) found that 16 to 32 grey levels are suitable for texture representation in images. Therefore, grey-scale images taken of the leaves will be quantized to 16 grey levels.

In the study conducted by Story et al. (2010), nutrient deficiencies were detected one day earlier by machine vision compared to human vision. However, doubt was cast on using contrast as a textural parameter in early detection of plant stress due to the wide confidence interval observed by Story et al. (2010). Therefore, contrast will not be considered as a textural feature in this project.

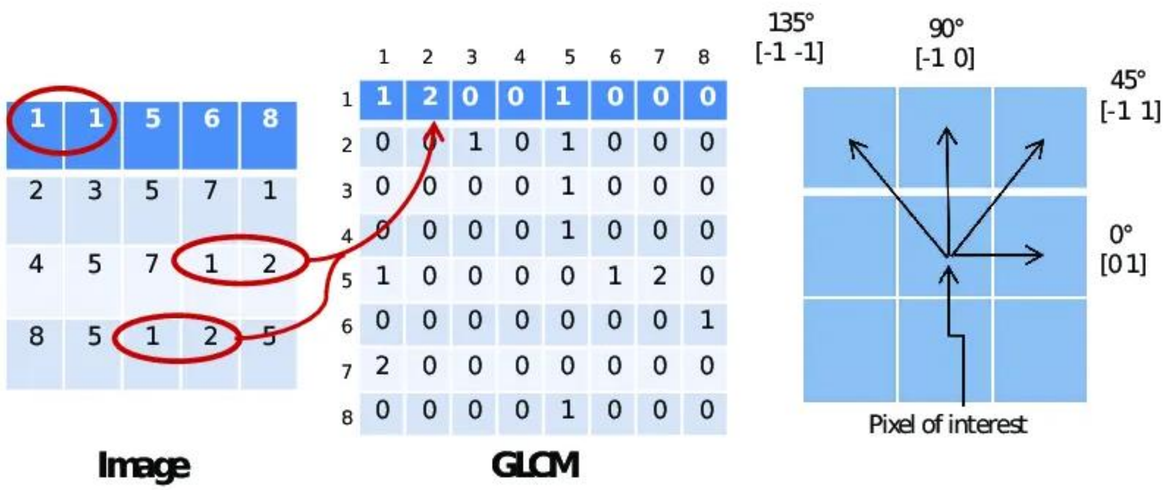


Figure 2.1. *Visual representation of performing GLCM on an image, at different relative angles surrounding the pixel of interest (Gomede 2024).*

2.3 IMAGE ANALYSIS

RGB images taken of the plant's should be segmented to separate vegetation pixels from background pixels, so that extracted features pertaining to the plant only are isolated. An automatic plant masking algorithm using the Hue channel of an image was developed. Vegetation segmentation using the isolated hue channel has been extensively researched for precision agriculture applications with high accuracy (Hassanein et al. 2018, Yang et al. 2015, Kumar & Miklavcic 2018). The hue channel contains information about the colour of an object, decoupled from lighting variables (Hassanein et al. 2018). The hue channel values range from 0° to 360°, as can be seen in

Figure 2.2. Values that represent vegetation range from 60° (yellow hue) to 180° (cyan hue) (Hassanein et a. 2018). In MATLAB, hue values range from 0 to 1 (MathWorks n.d.h). When it comes to image thresholding for binarization, Otsu's method of thresholding is conventionally used. Otsu's thresholding method minimises the intra-class variance binarized pixels (Otsu 1979, MathWorks n.d.c).

Figure 2.2. *An illustration of the HSV colour space wheel with degree values, uploaded to Pinterest by Aaron Lewis (n.d.).*

2.4 MACHINE LEARNING MODELS

Machine vision and image processing tools have been widely used in the detection of biotic and abiotic plant stress, but there are limitations in the tools' ability to detect or classify multiple stresses. Machine learning tools used in conjunction with machine vision data is known to improve classification accuracy (Mao et al. 2015). This is a comprehensive strategy to assess the quality of a growing plant or crop, as it can provide real time feedback and control on a closed loop system, and trend data to predict stress onset. Another advantageous feature of machine learning is the ability to provide information pertaining to the cause of stress, and recommendations of treatments to alleviate it. One drawback, however, is their tendency to overfit data ‘caused by the Hughes phenomenon or violation of the principle or parsimony’ (Nguyen et al. 2020, p. 4), leading to incorrect classifications. To avoid data overfitting, and to improve the accuracy of a model, data should be pre-processed to introduce more variation, and training data should represent all classes equally. As explained by Zubler & Yoon (2020), this can be achieved by image cropping or flipping, background removal, contrast enhancement, colour correction and noise removal or injection. For these reasons, a system that fuses machine vision imaging processing and machine learning modelling to predict, detect, assess, and classify stresses is ideal.

Machine learning is a sub-set of Artificial Intelligence (AI), in which a machine replicates human intelligence, problem solving and pattern recognition, without explicitly being programmed to do so (Zubler & Yoon 2020, Njazi et al. 2023). Its accuracy is improved with large data sets and training. Quality of data is also important. Machine learning is shallow compared to deep learning. Deep learning is based on the human brain’s network of neurons. Machine learning can extract features of images efficiently when trained with labelled or semi-labelled sets of data, but deep learning can extract features on its own from unlabelled data when passed through a series of layers and nodes (Shin et al. 2022, Zubler & Yoon 2020). Nonetheless, these models can be used to find relationships and patterns with the data, and form predictions based on what it has learnt (Zubler & Yoon 2020). It is useful for automating tasks previously performed by humans.

Literature investigating machine learning models is finite when it comes to assessing the nutritional quality or detecting water stress in lettuce plants. However, some of the machine learning and deep learning models used to detect plant stresses that have shown high accuracy in the literature include support vector machine (SVM), linear discriminant analysis (LDA), extreme learning machine (ELM) and deep convoluted neural network (CNN).

2.4.1 SUPPORT VECTOR MACHINE (SVM)

Support Vector Machines (SVM) are primarily used for binary classification and fall under the category of supervised learning algorithms (IBM 2023). SVM models make use of features extracted from segmented images for training and can achieve high classification accuracy ranging from 82% to 99.6% (Bhange & Hingoliwala 2015, Karthickmanoj et al. 2021). SVM utilizes principal component analysis (PCA) to reduce the dimensionality of image and spectral data, down to principal components (PCs) which are responsible for a large portion of the variation in the data (Lak et al. 2021). These PCs are plotted on a 2D coordinate plane, and a decision boundary (line) separates the data into two (or more) classes (i.e. healthy vs unhealthy). Data to be classified is processed through the trained model, and the decision boundary determines the class (Zubler & Yoon 2020). This process is displayed in Figure 2.3.

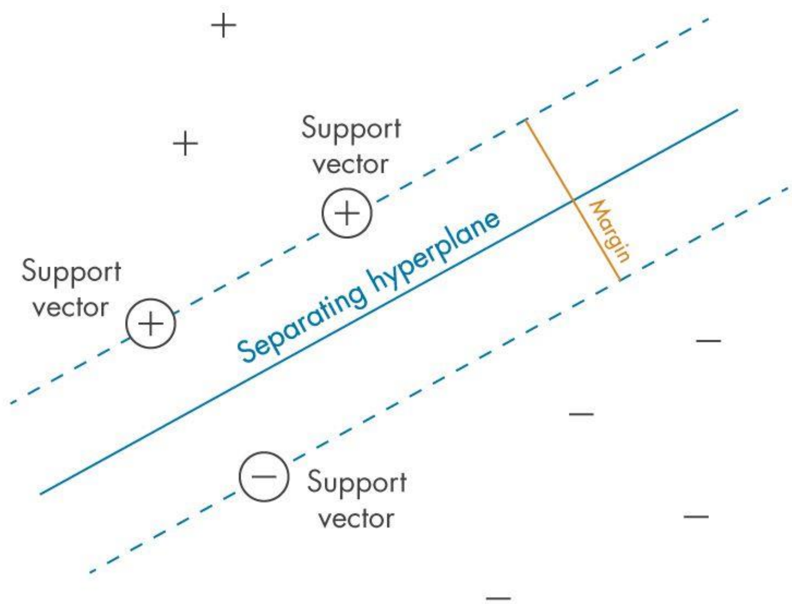


Figure 2.3. *SVM optimisation to separate the data into classes, where the separating hyperplane is the decision boundary (MathWorks n.d.i).*

A recent review article written by Zubler and Yoon (2020), summarises that SVM models are mainly developed for disease detection and classification in plants. For example, Karthickmanoj et al. (2021) developed a framework for classifying diseased leaves of Brinjal plants by extracting textural features using GLCM, including energy, homogeneity, contrast, and entropy of healthy and unhealthy leaves. SVM was used as the classifier model. The collected images were pre-processed to enhance contrast (Karthickmanoj et al. 2021). Then, the images were segmented to magnify affected areas of the Brinjal plants, using a segmentation threshold. This pre-processing step reduced the time it took to train the model, and the classification accuracy of the framework proposed by Karthickmanoj et al. (2021) was 99.6%.

2.4.2 LINEAR DISCRIMINANT ANALYSIS (LDA)

Like SVM, Linear Discriminant Analysis (LDA) is a supervised learning model, in which the data points are plotted on a 2D coordinate plane and separated by a decision boundary into classes. In SVM, the decision boundary is drawn so that the distance from the boundary to the nearest point in each class is optimally minimized. In LDA, however, it is assumed that the data points are more densely clustered around the mean of the class, and the decision boundary separates the means of the data (Pennsylvania State University 2018a, Pennsylvania State University 2018b). Thus, they are not dissimilar, and the model with greatest accuracy is determined by the nature and spread of the data.

Nguyen et al. (2020) conducted a comprehensive study on using leaf reflectance data in combination with LDA to classify spectral ranges that are associated with macronutrient contents (N,K, Mg and Ca) in Bok Choy and Spinach. Additionally, these studies conducted a cross-validation sensitivity analysis which determined that classification accuracy was very similar when 50% to 80% of the data was used for training, implying that less data is required for training an LDA model compared to other studies and models (Table 2.3). The classification accuracies were 78.4% and 86.4% for Bok Choy and Spinach respectively. Similar to the literature reviewed for SVM applications, the LDA classification model used in the study conducted by Nguyen et al. (2020) was visualized on a 2D plane, where the PCs (LDA1 and LDA2) were the axes, where LDA1 explained most of the variation in the data. LDA used in conjunction with hyperspectral remote sensors has been investigated as a tool in studies concerned with assessing the macronutrient content of a plant, as there are 'significant correlations between spectral bands and the levels of macronutrients' (Nguyen et al. 2020, p. 2).

Table 2.3. *Ratio of training and testing data used in literature.*

Source	Ratio of Data used for Training/Testing
Mao et al. (2015)	66% / 33%
Nguyen et al. (2020)	50-80% / 20-50%
Njazi et al. (2023)	80 / 20%
Mishra et al. (2020)	70% / 30%
Sibiya & Sumbwanyambe (2019)	70% / 30%

2.4.3 EXTREME LEARNING MACHINE (ELM)

‘Extreme Learning Machines are a type of artificial neural network with one or more hidden layers that are trained under supervised, unsupervised, or semi-supervised learning approaches’ (Huérfano-Maldonado et al. 2020, p. 1). ELM was conceptualized by Huang et al. (2006). ELM is primarily a supervised, multi-layer model, which is fast to learn, easy to implement and performs more efficiently than other feed-forward neural network models (Chen et al. 2017, Huang et al. 2006).

In the research conducted by Mao et al. (2015), three ELM models were trained based on sensor data collected from spectroscopic studies and morphological, colour and textural features of lettuce plants, which makes this study relevant to the proposed research question. The morphological feature was TPCA, the colour features included RGB and hue-saturation-intensity (HSI), and the textural features included contrast, homogeneity, energy, and entropy. The models used three layers: an input layer, a hidden layer with multiple nodes, and an output layer. The results gathered concluded that a model based on spectral data performs better than the model based of image data, but a model that uses combinatory sensor data from both spectroscopy and image data is superior to a single inspection technique (Mao et al. 2015). To estimate the nitrogen (N) levels of lettuce, this model achieved a correlation coefficient of 0.8864 and a root-mean square error of 0.3231%.

Again, PCA was used to reduce the dimensionality of the data from 73 spectral variables and 11 image features, to three spectral and two image PCs. The input layers of the model consisted of the three spectral and two image PCs, and the output layer was the nitrogen content of the lettuce plant. It was determined that the optimal ELM model to assess nitrogen content in lettuce had 30 hidden nodes in the hidden layer. A total of 90 samples were randomly selected from the treatment groups and divided into two sets. One set was used to calibrate or train the model and consisted of 60 samples or 66% of the data. The other set was used for testing the model and consisted of 30 samples or 33% of the data (Mao et al. 2015) (Table 2.3).

2.4.4 CENTRAL NEURAL NETWORKS (CNN)

Central neural networks (CNNs) utilize artificial neural network (ANN) as a framework but are typically more advanced and contain more layers. The key difference between ANNs and deep CNNs, is that deep CNNs have shorter connections between layers, increasing efficiency and accuracy even further (Njazi et al. 2023). CNNs work well to learn significant features on their own to classify raw, unlabelled data. The major drawback of using CNNs is the large data sets required to produce accurate results, potentially in the thousands (Njazi et al. 2023). When there is insufficient data, image manipulation is useful to increase the size of the dataset without requiring mode images. The benefit of CNN models is that they require little to no preprocessing steps for feature extraction (Njazi et al. 2023). Often, pretrained models such as GoogleLeNet, ResNet, VCG and DenseNet make use of images from databases such as Kaggle or ImageNet, which reduces the time it takes to train the model (Zubler & Yoon 2020). In CNN models, features are extracted from inputs (images) as they are passed through each layer. The model eventually learns how to discern the features and will classify an input appropriately.

Njazi et al. (2023) used a pre-trained, deep CNN to detect plant disease in Maize crops, such as Common Rust, Gray Leaf Spots, and Blight. The model used was a modified version of ResNet50, pretrained on images from the ImageNet database. A total of 2000 images were taken from an open-source database, Kaggle, to train and test the model. To train the model, 80% of the data set was used, and 20% was used for testing (Table 2.3). Using an equal number of each ensures that the model does not overfit the data by getting too good at classifying one type. For example, if 1000 images of Common Rust were included instead of 500, the model would get better at classifying Common Rust and may incorrectly classify Gray Leaf Spot or Blight. The accuracy of the model was 92.63% after 15 epochs. Mishra et al. (2020) proposed a CNN system that was 11 layers deep, which classified Northern Corn leaf Blight at an accuracy of 99.9% after 24 epochs, and had high accuracy in classifying Gray Leaf Spot, Common Rust, and Healthy images too. Sibiya & Sumbwanyambe (2019) proposed a CNN system with 50 hidden layers, utilizing Neuroph Studio Framework, to detect and classify Maize Common Rust and Blight diseases at an average accuracy of 92.85% after 150 epochs. Zhang et al. (2021) conducted a study using the Pytorch framework in different types of CNN models including the VCG series, ResNet series and DenseNet series to detect diseases of Maize. The experiment consisted of determining how many epochs were necessary to receive the highest classification accuracy. It was concluded that ResNet50, VCG19, and DenseNet161 achieved an accuracy of 0.96%, 0.98% and 0.96% respectively after 30 epochs. These results suggest that the VCG19 model is the best performer to detect diseases in Maize plants.

2.4.5 MODEL PEFORMANCE ANALYSIS

Conventionally, model performance can be measured in terms of accuracy using a confusion matrix, or by precision, recall and F1 score metrics. A confusion matrix measures the performance of a classification model by comparing correct and incorrect predictions (Sokolova et al. 2005). In order to use a confusion matrix, the actual class labels for the data must be known (Kulkarni et al. 2020). For binary classification, a confusion matrix has four outcomes, true positive (TP), true negative (TN), false positive (FP) and false negative (FN). An example is given in Table 2.4. Accuracy, precision, recall and F1 score are calculated using these four outcomes from the confusion matrix, in equations 2.1, 2.2, 2.3 and 2.4, respectively (Sokolova et al. 2005, Karthickmanoj et al. 2021). The F1 score is a number between 0 and 1. The higher the F1 Score, the better the performance of the model (Karthickmanoj et al. 2021). It is an overall metric, that combines precision and recall values (Kundu 2022).

Table 2.4. *An example of a confusion matrix for binary classification models.*

		Predicted Class	
		Positive	Negative
True Class	Positive	TP	FP
	Negative	FN	TN

$$Accuracy = \frac{TP + TN}{TP + FP + FN + TN} \tag{2.1}$$

$$Precision = \frac{TP}{TP + FP} \tag{2.2}$$

$$Recall = \frac{TP}{TP + FN} \tag{2.3}$$

$$F1\ Score = \frac{2 * TP}{2 * TP + FP + FN} \tag{2.4}$$

2.5 CURRENT LIMITATIONS AND KNOWLEDGE GAP

Existing machine vision tools have successfully detected nutrient-deficiency and water stress in lettuce plants, but this research focuses mainly on spectral data in non-visible wavelengths, using high-cost spectroscopy equipment. This project aimed to develop a well-rounded, low cost, small-scale system, using visible RGB images, which can be applicable in space, but also translates well to applications on earth. Research concerned with detecting water stress in lettuce plants is lacking, and to the best of the author's knowledge, there is no literature to date that explores using a combination of image data and machine learning classification specifically in this application.

Support vector machine models in literature are mainly used for disease classification, indication and quantification using spectral data, so it is unknown how they would perform when used to detect water stress in lettuce plants. Due to the financial and time restrictions of the project, an open-source machine learning algorithm that does not require a lot of time for training or computational resources would be ideal. For this reason, the chosen model that will be trained from images taken during trials will be SVM. Data will be split so that 60% of the images will be used for training and the remaining 40% will be used for testing. This model has shown high accuracy in literature (Zubler & Yoon 2020) and is accessible through MATLAB's classification learner app.

Additionally, there are many ground truth techniques to measure water stress on crops, including but not limited to moisture content analysis (Li & Ling 1996), crop water stress index (Li et al. 2019), and biomass (Mao et al. 2015). However, in this area of research, there is no literature to date that explores using audio analysis to measure water content in lettuce leaves. Audio analysis will be compared against NGRDI to evaluate how accurately audio features can estimate leaf water content. NGRDI is a vegetation index commonly used to estimate biomass (Jannoura et al. 2014).

CHAPTER 3 INITIAL INVESTIGATION

An initial investigation was conducted, followed by a main trial. The aim of the initial investigation was to gauge which data was required to satisfy the objectives of the project, to understand the processes involved in data collection and feature extraction, and to identify ways in which the robustness of the machine vision system can be improved. A specific objective of the initial investigation was to confirm whether suitable audio snippets could be captured, and to determine the usefulness of this feature when used as an indication of eating quality, by comparing it to the NGRDI vegetation index.

First, the methodology and materials used in the initial investigation are listed, and then the results are presented and discussed. Data was collected during the initial investigation pertaining to morphological and audio features of the watered (control) and not-watered (treatment) lettuce plants. The morphological features include TPCA, colour and texture. Data was collected in the morning (AM data) and in the evening (PM data). Conclusions are drawn about which features best reflect the plants' health status, and whether AM or PM data should be used to train the classification model. These conclusions will assist in refining parameter selection for the classification model.

3.1 MATERIALS AND METHODOLOGY

The core of the methodology for the initial investigation consists of data acquisition, and feature extraction through image processing software. The model plant in the initial investigation was the lettuce cultivar, Romaine (*Lactuca sativa* L. var. *longifolia),* purchased as established plants with a root ball (Woolworths, Clifford Gardens, Toowoomba). This species of lettuce was selected due to availability, maturity and its high nutritional value compared to other lettuce cultivars (Kim et al. 2016a). The initial investigation was conducted inside, in an uncontrolled environment in Toowoomba, Queensland during May. The watered and non-watered groups consisted of two lettuce plants each. Each group requires two plants because the nature of collecting audio data is destructive, and morphological data, such as TPCA, requires a whole, intact plant for measurement.

Data was collected on four days, twice a day at 6:00 am and 6:00 pm, over the course of a week, beginning on 16th May and ending on 22nd May. At the beginning of the data collection period, the roots of both groups were submerged in a hydroponic media, dosed with a ratio of two drops of a liquid fertiliser with a NPK ratio of 3:1:4 (We The Wild™, Grow Concentrate Plant Food) to one litre of local tap water. This was to reduce nutrient deficiency stress, and to provide a common baseline for data collection from both groups. Data was collected on the first day (16th May) to gather information from both groups while the plants remained healthy. On the fourth day, the watered group remained in the hydroponic media until the end of the data collection period, and the not-watered group was placed on a bench to induce water stress (drought). Data collection continued on the fifth day, one day after the not-watered group was placed on the bench. The collection period continued until the end of the seventh day (22nd May).

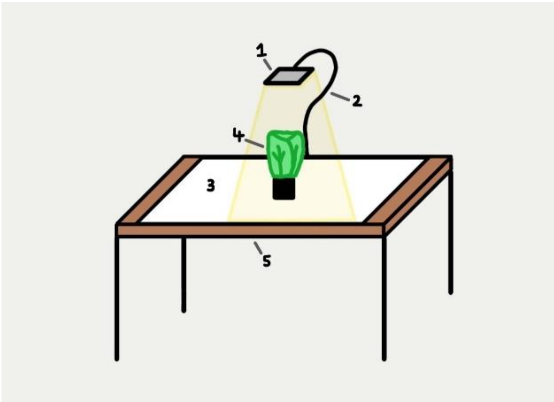
3.1.1 DATA ACQUISTION

A mobile phone was used to capture the images and record the audio files. See Table 3.1 for device specifications. Plant and leaf images of both groups were captured to extract morphological features. The morphological features extracted include the plants' TPCA, colour (using RGB vegetation indices) and texture (using GLCM analysis). Specific colour, textural and audio features extracted are discussed later in the report.

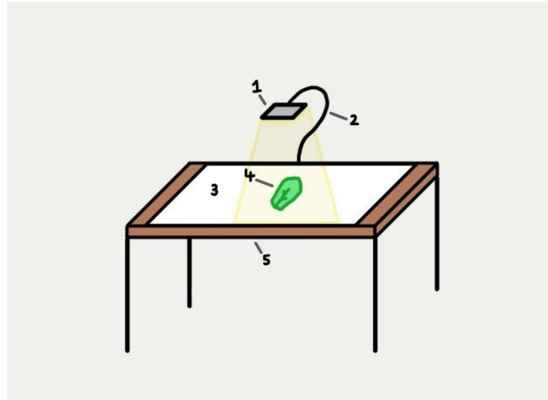
The process of collecting TPCA data involved taking a series of four images from a top-down view of the whole lettuce plants against a white background. Four images taken in series was required to remove electronic noise from the images and improve segmentation accuracy. The TPCA count of each of the four images was calculated, then averaged together to represent the plant's overall TPCA count. Colour and textural features were also extracted from the whole plant image. The images were taken at a constant distance from the table (30 cm) at 1x zoom. The process of collecting leaf data to extract colour information involved removing one of the outer most leaves off of the partial lettuce plants and taking a photo of the leaf profile against a white background. The images of the leaves were taken at a constant distance from the table (30 cm), at 1.5x zoom. The experimental setup for the initial investigation can be seen in Figure 3.1. A mobile phone was used to collect data (RGB images and audio recordings) throughout the duration of the data acquisition period. See Table 3.1 for specifications of the mobile phone. A gooseneck phone holder was used to ensure that the distance between the camera and the plant remained constant while collecting data. A 510 mm x 635 mm piece of white card was used as a background when taking images. This white card made image segmentation (background vs. foreground) simpler and more accurate. A flat table was used in the experiment setup as seen in Figure 3.1. An artificial light source (ceiling light) improved light uniformity between images. The use of the light source reflects conditions on a space station.

Table 3.1. *Technical specifications of the mobile phone apps being used to record audio files and capture images. The mobile phone model is a Samsung S10 5G.*

Type of Data	Characteristics	Specifications
Visible Images	Colour Space	RGB
	Resolution	12 MP, 4032 x 3024 pixels
	Lens Size	26 mm
	ISO	400-500
	Aperture	F2.4
	Exposure	0.0ev
	Shutter Speed	1/50s
	File Extension	.jpg
	Distributor	Samsung, Camera App
	Version	12.0.01.87
Audio	Resolution	128 kbps
	Quality	Medium
	Frequency	44.1 kHz
	Channel	Mono
	File Extension	.m4a
	Distributor	Samsung Electronics Co., Voice Recorder App
	Version	21.4.16.041



(a)



(b)

Figure 3.1. *Diagram of the experimental setup for the initial investigation [1. Mobile phone, 2. Gooseneck phone holder, 3. White card, 4. Lettuce plant/leaf, 5. Table].*

After the profile image of the leaf was taken, audio data of the leaf was collected. The hypothesis was that a lettuce leaf with a higher moisture content would produce a louder, more crisp sound compared to a leaf that has low water content, indicative of plant under water (drought) stress. The process of recording audio data in the initial concept trials was sensitive to human error. Thus, a protocol was developed to consistently snap the lettuce leaves as follows:

1. Start audio recording.
2. Hold leaf at two points of the stem (towards the base the leaf) between thumb and index fingers.
3. Hold leaf approximately 3 cm away from the microphone of the audio recording device.
4. Snap stem upwards, away from the audio recording device, in a rapid motion.
5. Stop audio recording.

3.1.2 SOFTWARE

<u>*Data Storage*</u>

At the end of each day during the data acquisition period, image and audio files were transferred from the mobile phone to a Microsoft OneDrive folder. Microsoft OneDrive is a cloud based storage service. Image and audio files were also saved on a removable USB for backup.

Data Processing

The software package, MATLAB (version R2023b) was used to develop the code to process the image and audio data. At the time this research was completed, MATLAB version R2023b was required for all Image Processing and Audio Toolbox functions to work. The images collected were processed using image processing functions from the Image Processing toolbox, to extract the morphological features. The audio files collected were processed using functions from the Audio Toolbox, to extract audio features. Extracted data was manually logged into a Microsoft Excel (version 2408) spreadsheet, for analysis.

3.1.3 COMPUTER SPECIFICATIONS

Operating system (OS) and hardware specifications of the PC in which the MATLAB code was executed on is listed in Table 3.2. This hardware impacts execution time of the code.

Table 3.2. *Specifications of hardware and operating system used to run the MATLAB code.*

Item	Specification
OS	Microsoft Windows 11
Processor	AMD Ryzen 9 5900HS with Radeon Graphics 3301 Mhz 8 Cores 16 Logical Processors
RAM	16GB
Graphics Card	NVIDIA GeForce RTX 3080 Laptop GPU

3.2 IMAGE AND DATA ANALYSIS

Feature specific methodology is discussed in the following sub-sections.

3.2.1 SEGMENTATION FOR TOP PROJECTED CANOPY AREA (TPCA)

Feature Extraction Process

A segmentation algorithm was implemented using image data collected in the RGB colour space. These images were then converted to the HSV colour space, and hue channel analysed to perform plant segmentation. The hue channel in combination with Otsu's thresholding method (Otsu 1979) were used to segment the whole plant images for TPCA calculation. Since Otsu's thresholding method minimises the intra-class variance binarized pixels (Otsu 1979), an additional step was implemented to remove pixels in the hue image that are not representative of vegetation before binarization. In this investigation, pixel values less than 60° (0.1667 in MATLAB) and more than 120° (0.3334 in MATLAB) were removed from the hue image. After pixel removal, the hue image is binarized, where white pixels represent vegetation, and black pixels represent the background. The result is an image mask for the lettuce plant. This mask was used to isolate the plant pixels and extract morphological features. See Figure 3.2 for the binarization process, and Figure 3.3 for the segmentation algorithm flowchart.

TPCA counts of the watered and non-watered whole plants were estimated automatically by the developed segmentation algorithm. The sum of the white pixels is the TPCA count of the whole plant, using the equations defined by Kacira et al. (2002):

$$b(x,y) = \begin{cases} 1 \; if \; \mathrm{h}(x,y) < \gamma \\ 0 \; if \; \mathrm{h}(x,y) \geq \gamma \end{cases} \tag{3.1}$$

$$TPCA = \sum b(x,y) \tag{3.2}$$

Where $\mathrm{h}(x,y)$ is hue value at pixel coordinate (x,y), γ is the thresholding value determined by Otsu's method, and $b(x,y)$ is the corresponding binarized pixel value (1 or 0).

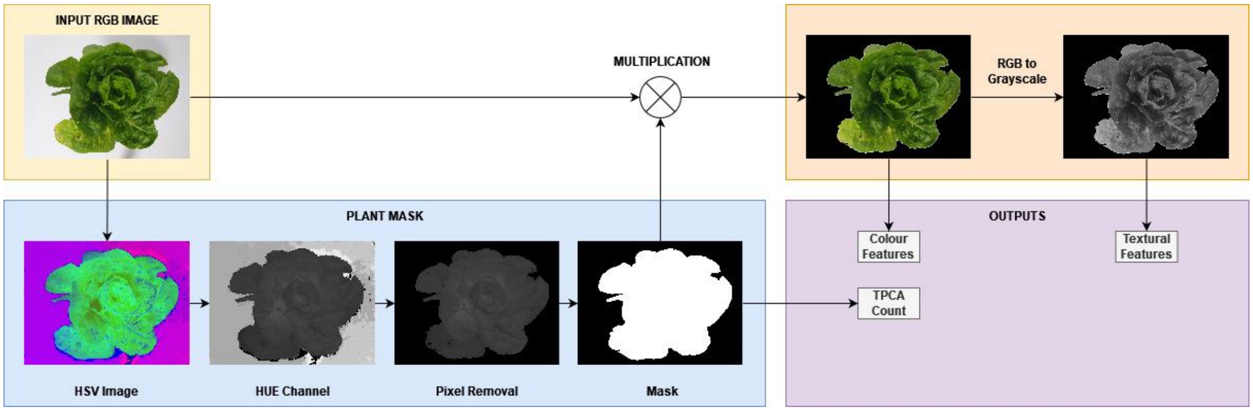


Figure 3.2. *A diagram of the segmentation algorithm to automatically create a plant mask.*

The MATLAB Image Processing Toolbox and the included generic functions were used to construct the segmentation algorithm. See Appendix C for the code of the segmentation algorithm, as well as the version of MATLAB required for each function to work. The functions used were, imread, imresize, rgb2hsv, imbinarize, imfill, bwareopen, imshow and imshowpair. Using the mask created from the machine vision system, means that morphological features can be extracted automatically.

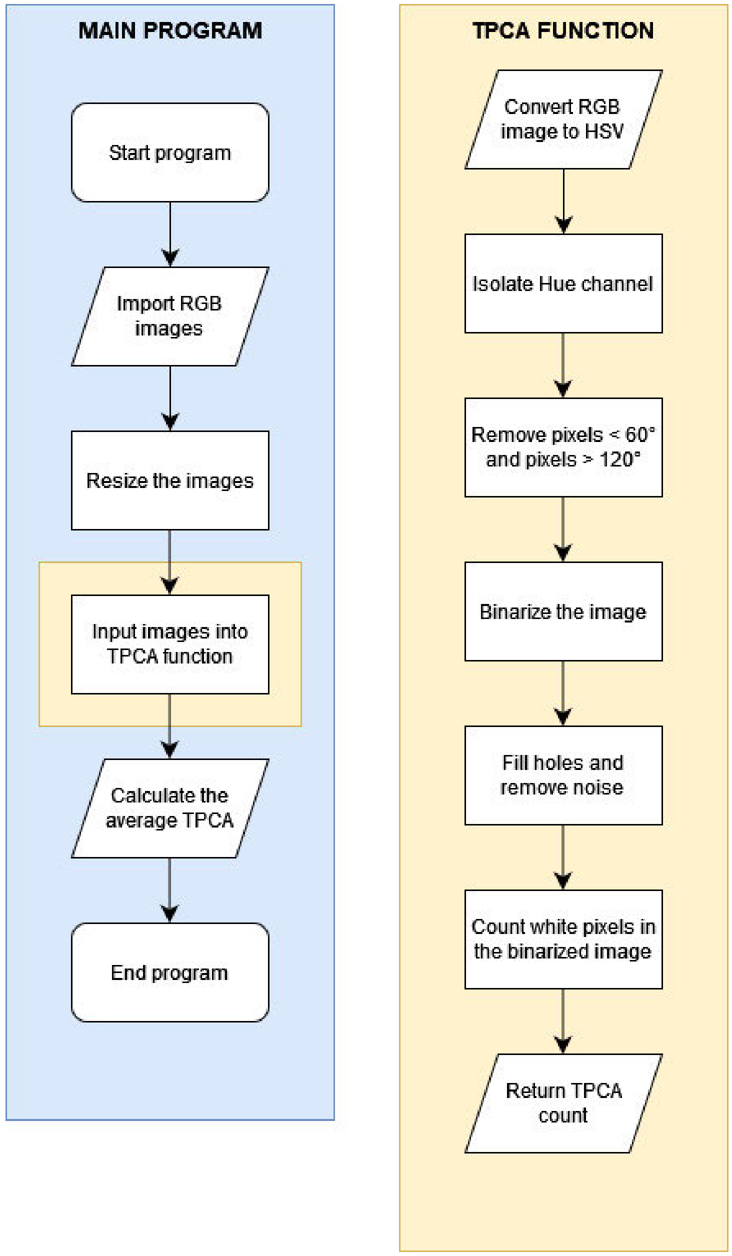


Figure 3.3. *From left to right: RGB image, HUE image, Binarized image using the developed segmentation algorithm.*

<u>*Segmentation Accuracy Method*</u>

The accuracy of the image segmentation algorithm was evaluated. The TPCA count calculated by the segmentation algorithm was compared to a ground truth TPCA count. The ground truth TPCA count was collected by importing an RGB image to the Color Thresholder app in MATLAB (introduced in version R2014a), from the Image Processing toolbox. This app is interactive and allows a polygon to be drawn around the desired pixel colours. This tool was used to remove white and grey pixels from the image and was found to accurately segment the vegetation from the image. The resulting binarized image from this app gave an accurate TPCA count to compare the algorithm results against, as see in Figure 3.4. Accuracy was calculated using equation 3.3.

$$Accuracy\ (\%) \quad = \quad 100 - \left(\left(\frac{TPCA_{true} - TPCA_{calculated}}{TPCA_{true}} \right) * 100 \right) \tag{3.3}$$

(a) (b)

Figure 3.4. *(a) RGB image (b) resulting binarized image using the developed segmentation algorithm.*

3.2.2 COLOUR FEATURES

Feature Extraction Process

Using the plant mask output from the proposed segmentation algorithm, the area of interest (lettuce plant) was separated from unwanted background pixels, resulting in a masked RGB image. RGB images in MATLAB are in the form of a matrix, where each pixel contains a ratio of R, G and B values. Therefore, to calculate the R, G and B values of the plant to be used in the vegetation index calculations, the pixels values were summated, then divided by the number of pixels in the area of interest to get the average R, G and B values. Then, the average R, G and B values are substituted into each of the colour index equations presented in Table 2.2.

3.2.3 TEXTURAL FEATURES

Feature Extraction Process

Using the plant mask output from the proposed segmentation algorithm, the area of interest (lettuce plant) was separated from unwanted background pixels, resulting in a masked grey-scale image. To perform GLCM, a grey-scale image is quantized to a number of grey levels. Images were quantized to 16 grey levels. The size of the GLCM matrix depends on the number of grey levels. The functions graycomatrix and graycoprops from the MATLAB Image Processing Toolbox were used to perform GLCM analysis. The function graycomatrix were used to create a GLCM of the grey level image at relative angles of 0°, 45°, 90°, and 135°, with grey level limits enabled. Then the function graycoprops was used to return the features: contrast, energy, correlation and homogeneity. These functions were developed based off research done by Haralick et al. (1973), and Haralick and Shapiro (1992). Please see supporting documentation and in-depth instructions on how to use these functions online (MathWorks n.d.b, MathWorks n.d.f). To simplify, GLCM analysis can be broken down into the following steps (Gomede 2024):

1. Image quantization to a number of gray-levels.
2. Define distance and angles of adjacent pixels around pixel of interest.
3. Matrix calculation.
4. Normalize matrix.
5. Texture feature extraction from GLCM.

3.2.4 AUDIO FEATURES

Feature Extraction Process

Audio analysis is a novel approach in determining the eating quality of a growing leafy plant. Audio data samples were collected as an additional measure of quality to be used in conjunction with the machine vision extracted information. In this investigation, audio samples were collected by removing an outer leaf from the lettuce plant and snapping it at the stem. This was a destructive, manual process.

The audioLevelMeter object from the MATLAB Signal Processing Toolbox was used to analyse the audio data (requires version R2023a). This tool was developed based off the standard IEC TR 60268-18:1995 (International Electrotechnical Commission 1995) and algorithm recommendations guideline ITU-R BS.1770-4 (International Telecommunication Union 2015). This tool takes the audio file as input, digitizes the signal and displays it in an interactive window. Screen cursors can be manipulated to measure aspects of the waveform. Features were extracted from the audio data including the digital peak level in dBFS, amplitude range and length of waveform. Peak level was output to the MATLAB workspace as a decimal number. However, amplitude range and length of waveform were measured manually using the screen cursors; therefore, the measurements were subject to human error. Methods were developed to measure these features to try and remain consistent across measurements. The method for measuring the amplitude range was done by finding the greatest peak of the waveform and placing the vertical cursors at either of the peak. The greatest peak is defined as the peak with the largest amplitude range. Amplitude range is unitless, but measured values are between 0 and 2. See ΔValue in Figure 3.5. The method for measuring the waveform length was done by placing the horizontal cursors at the first and last peak of the waveform that was caused by the snapping of the leaf (exclude external noise at the beginning and end of the recording if present). Length of waveform is measured in seconds. See Δsecs in Figure 3.5. Please see supporting documentation and in-depth instructions on how to use these functions and objects online (MathWorks n.d.e).

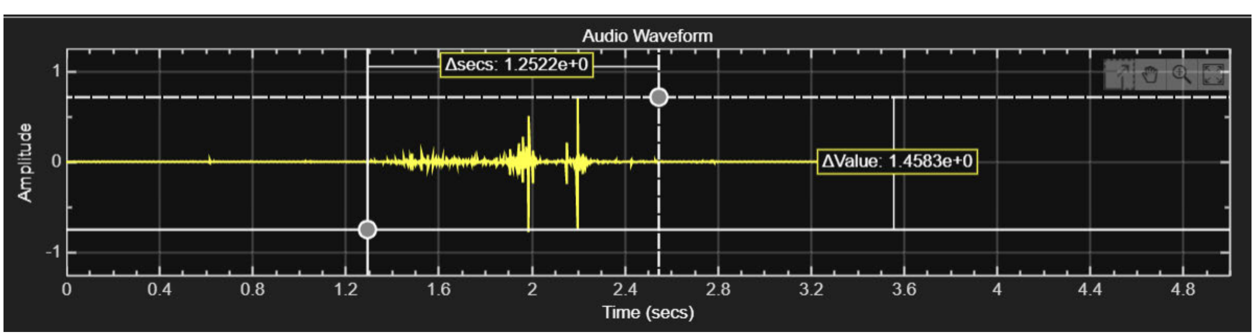


Figure 3.5. *Method of recording waveform length (Δsecs) and amplitude range (ΔValue). Amplitude ranges from -1 to 1.*

Audio data will not be selected as a parameter for the classification model, but rather the goal was to evaluate whether this feature could be used as an additional metric for assessing the eating quality of a growing, lettuce plant. Audio data was compared against the vegetation index, NGRDI as a ground truth measure, to estimate biomass and therefore, the relative water content of the lettuce leaf. Using NGRDI estimation of biomass instead of a manual experimental method such as biomass calculated using fresh and dry weights, aligns with the objectives of the research by using a computer vision system to gather information from the growing leafy plants.

Ground Truth Methods

Each audio features were compared against NGRDI of the leaf, measured form the RGB image taken before the leaf was snapped. Correlation coefficient was calculated to determine which features best estimated leaf water content. Leaf images were masked manually using the Color Thresholding App in MATLAB. Processing the leaves through the same plant segmentation algorithm did not result in accurate masking of the leaves.

3.3 RESULTS AND DISCUSSION

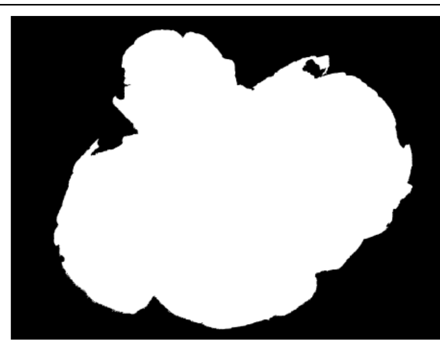
AM and PM data were evaluated separately due to plant diurnal and nocturnal behaviour. Data pertaining to TPCA, colour and textural features of the watered and non-watered plants were plotted on scatter graphs. Audio data and NGRDI were plotted on the same scatter graphs, to observe similarities in the trends. A correlation coefficient was also calculated to determine how accurately the extracted audio features estimate leaf water content. NGRDI is a vegetation index commonly used to estimate biomass.

3.3.1 TOP PROJECTED CANOPY AREA

Table 3.3 shows the accuracy of plant segmentation over 98%, indicating that the features could be automatically extracted by the machine vision system with high accuracy. Four images were picked from the data set to display the output of the two segmentation methods.

Table 3.3. *Comparison of ground truth method vs segmentation algorithm method. Accuracy for the segmentation algorithm was rounded to four significant figures.*

** suffix _AM_A*

IMG	Ground Truth Method	Segmentation Algorithm Method	Accuracy (%)
20240516*			99.12
20240520*			99.28
20240521*			99.26
20240522*			98.04

For the segmentation algorithm, when values between 120° and 180° were included, there was a decrease in accuracy of the segmentation algorithm. This was because cyan colours were being picked up in the background. This may have been due to the lettuce plant being reflected on the white card background. Removing these values showed a dramatic improvement in segmentation accuracy.

For a healthy plant, during the day, when the temperature rises and light increases, the plant perks up which can result in a decreased TPCA count. During the night, when temperature and light decreases, the plant rests, which can result in an increase in TPCA count. Wilting is a visual stress indicator that affects the TPCA count of the plant. For the AM data of the watered plant, it was expected that the TPCA count would decrease at a slower rate than the non-watered plant. The results in Figure 3.6 meet expectations. There was an overall decrease of 10.39% for the watered plant, and a decrease of 20.11% for the non-watered plant from the beginning of the data collection period to the end. The results of the PM data, shown in Figure 3.7, follow similar trends as the AM data. There was an overall decrease of 19.8% for the watered plant, and a decrease of 32.32% for the non-watered plant. There is a clear separation between the two groups, implying that this feature can be used as a parameter for classification. For the AM and PM data of the watered and non-watered plants, there was no significant difference in the TPCA count or linear trends as seen in Figure 3.8 and Figure 3.9, respectively.

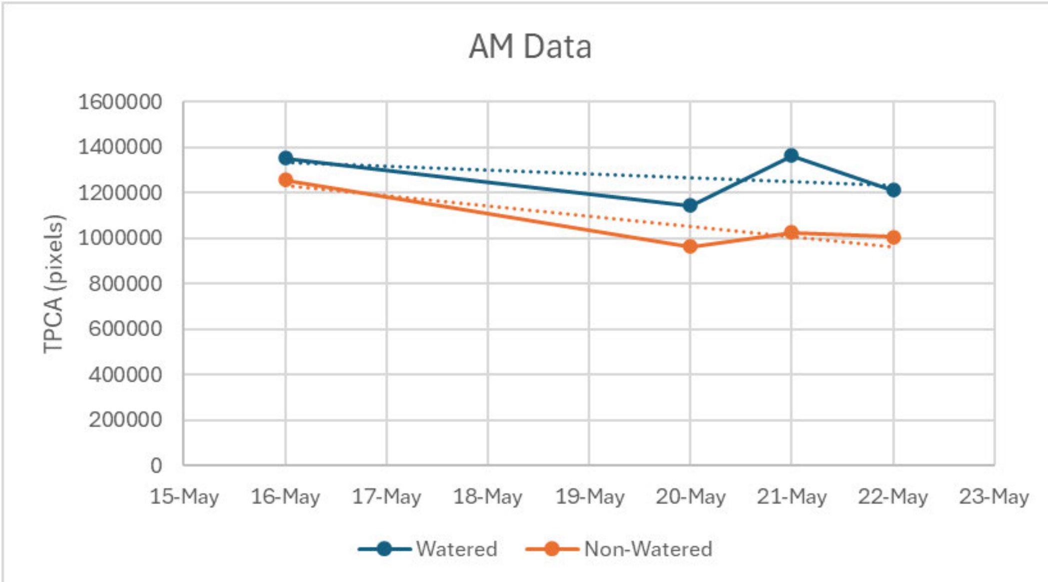


Figure 3.6. *TPCA (Top-Projected Canopy Area) of watered and non-watered plants, calculated from AM data. TPCA is the count of white pixels in a 1414 x 1883 image.*

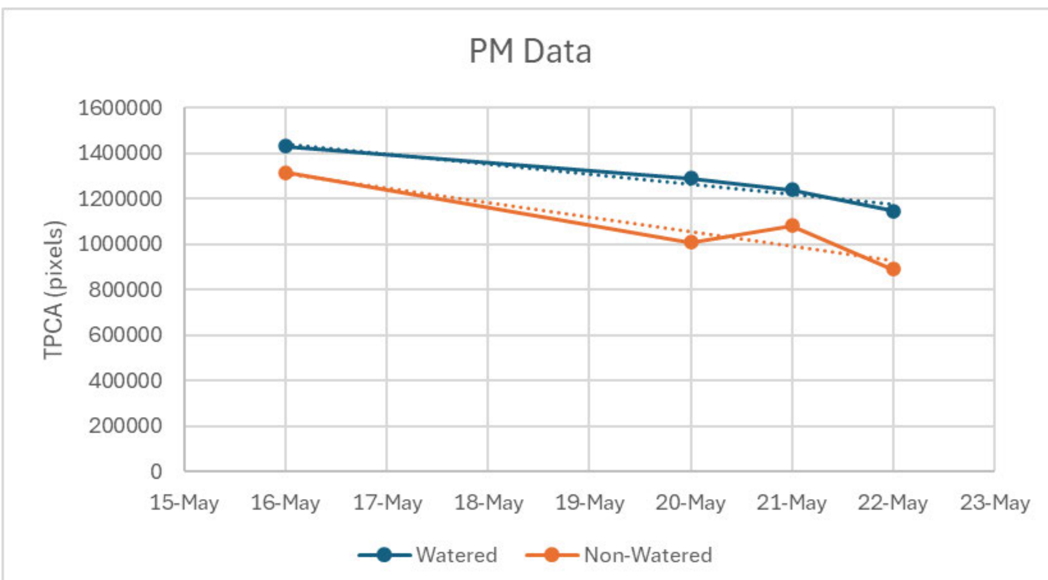


Figure 3.7. *TPCA (Top-Projected Canopy Area) of watered and non-watered plants, calculated from PM data. TPCA is the count of white pixels in a 1414 x 1883 image.*

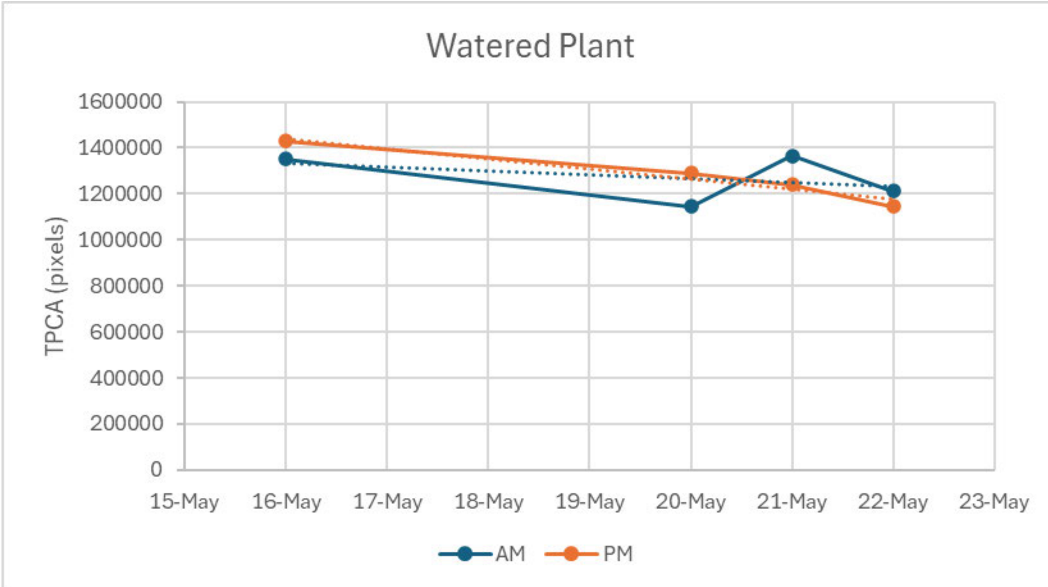


Figure 3.8. *TPCA (Top-Projected Canopy Area) of the watered plant, calculated from AM and PM data for comparison. TPCA is the count of white pixels in a 1414 x 1883 image.*

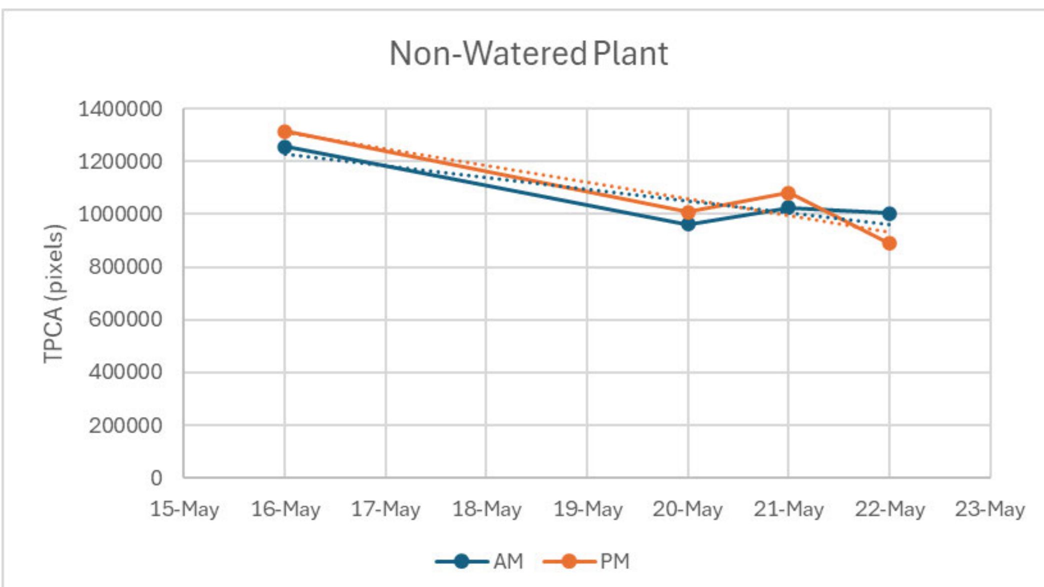


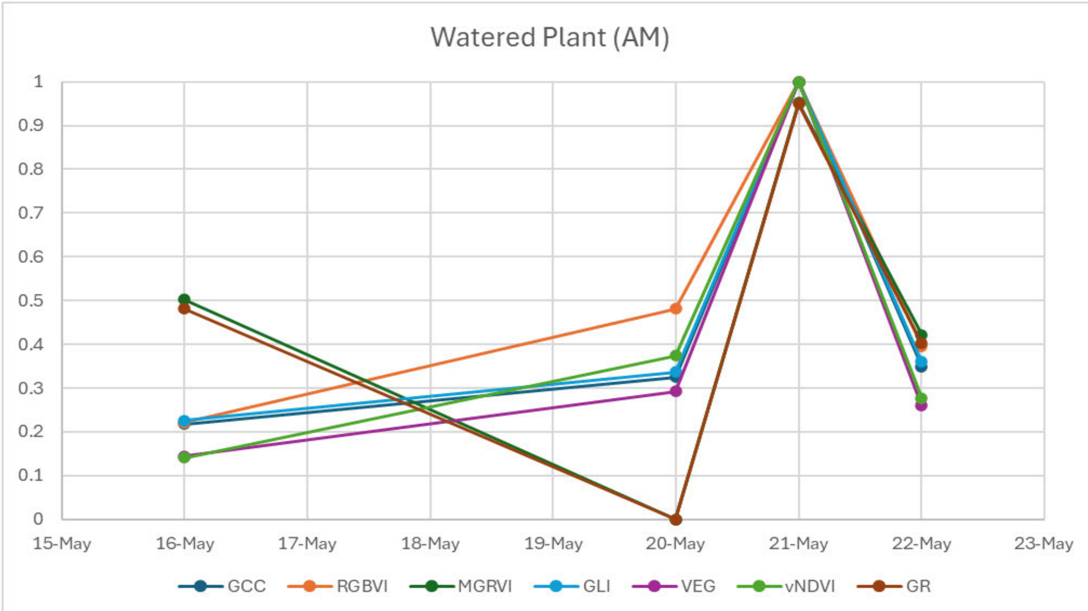
Figure 3.9. *TPCA (Top-Projected Canopy Area) of the non-watered plant, calculated from AM and PM data for comparison. TPCA is the count of white pixels in a 1414 x 1883 image.*

3.3.2 COLOUR FEATURES

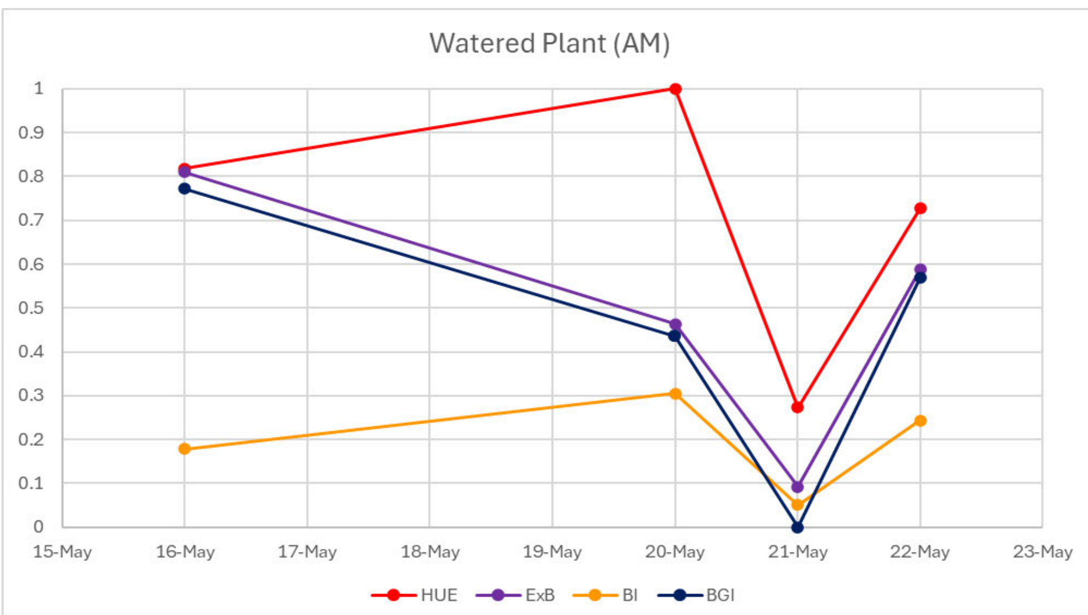
Colour indices were graphed together based on similar trends, to simplify the results for interpretation. The results presented in Figure 3.10 for the AM data of the watered plant, and the results presented in Figure 3.11 for the AM data of the non-watered plant, shows similarities between:

- GCC, RGBVI, GLI, VEG and vNDVI
- MGRVI and GR
- BGI and ExB

For the AM data, other vegetation indices that appeared significantly different include BI and HUE. Similar results were seen in Figure 3.12 and Figure 3.13 for PM data of the watered and non-watered plant, with some differences in BGI, ExB, BI and HUE colour indices. These results suggest that either AM or PM data can be used. Also, the eleven individual colour features can be reduced to six: GCC, MGRVI, HUE, ExB, BI and BGI. Overall, the watered plant had higher values than the non-watered plant, and significant differences in trends between groups was observed on the last day of data collection (22nd May). This suggests that there may be larger differences observed in the trends if the data collection period was extended beyond seven days.

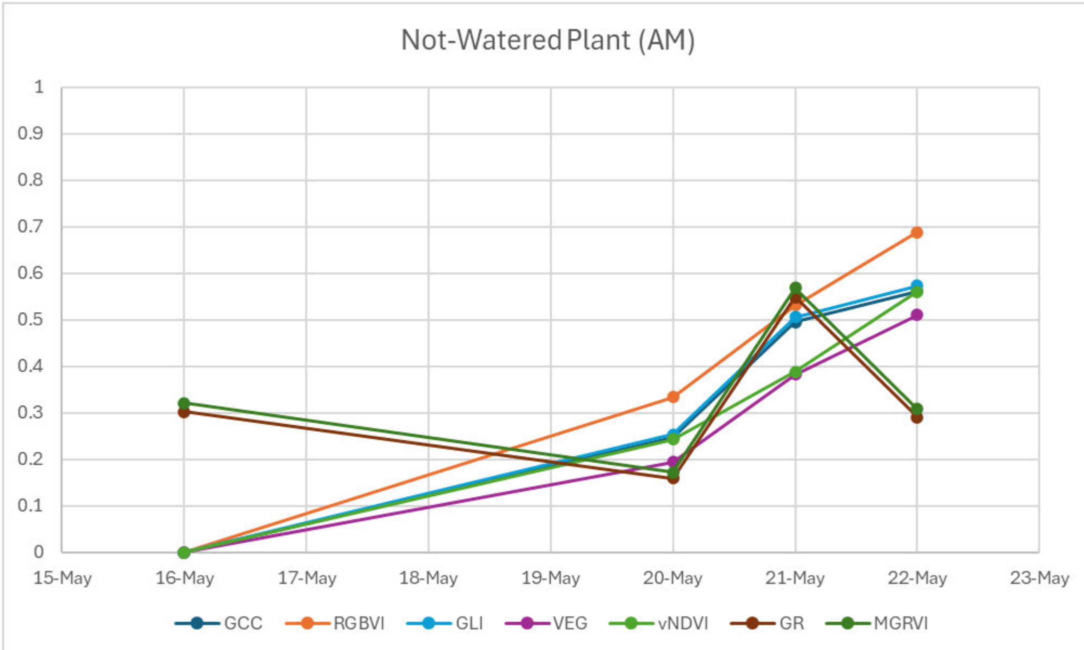


(a)

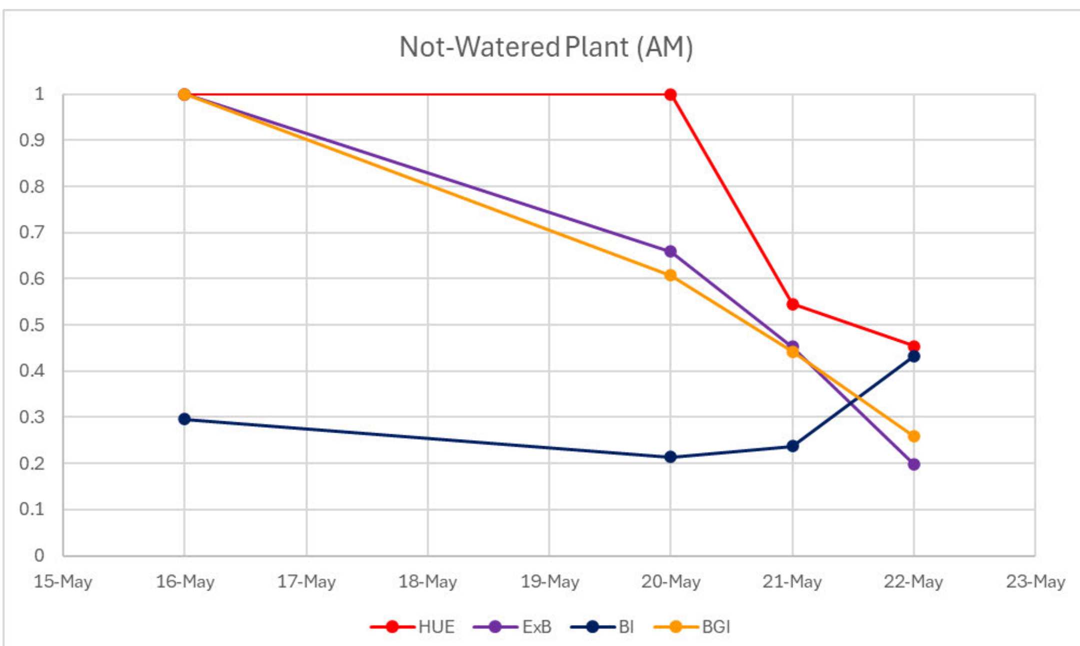


(b)

Figure 3.10. *Vegetation indices of the watered plant (AM data, normalised between 0 and 1). Vegetation indices are separated into two charts (a) and (b) to show similarities and differences between trends.*

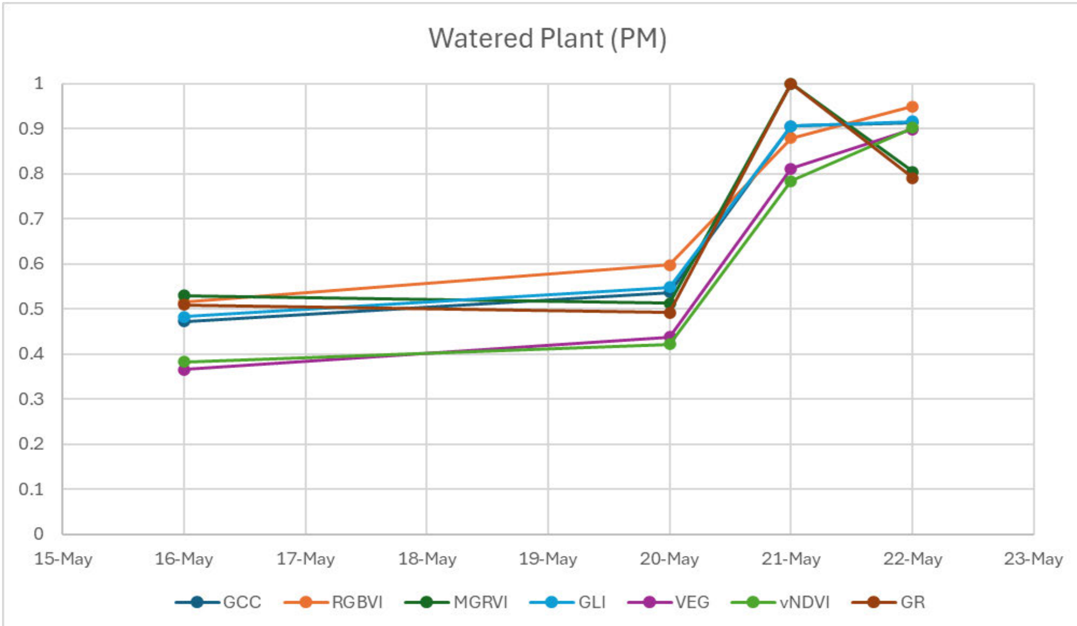


(a)

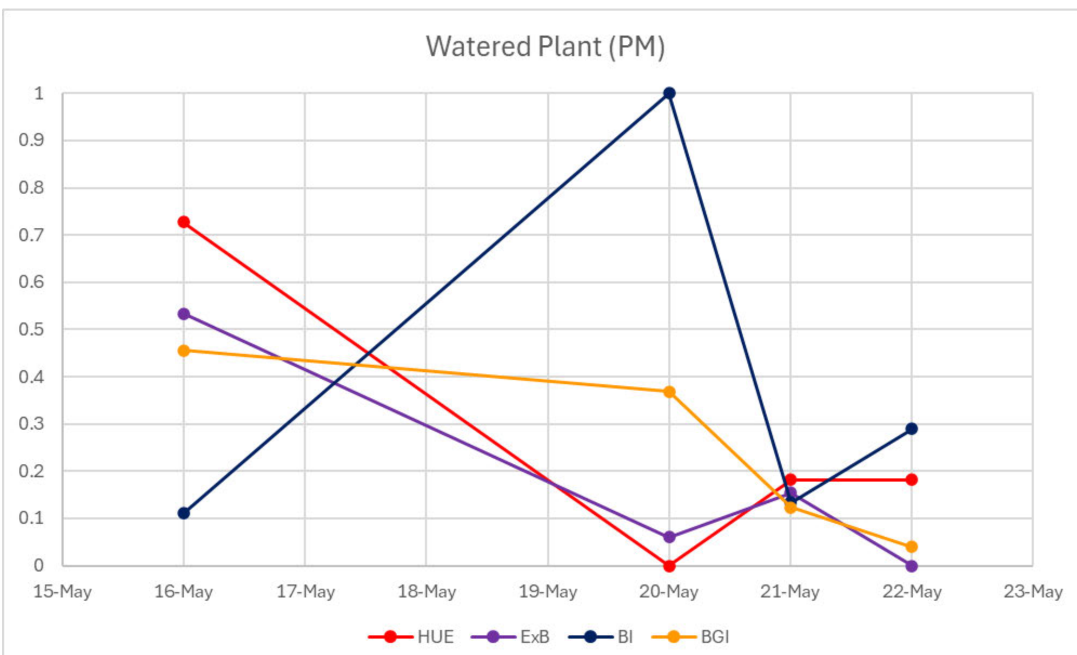


(b)

Figure 3.11. *Vegetation indices of the non-watered plant (AM data, normalised between 0 and 1). Vegetation indices are separated into two charts (a) and (b) to show similarities and differences between trends.*

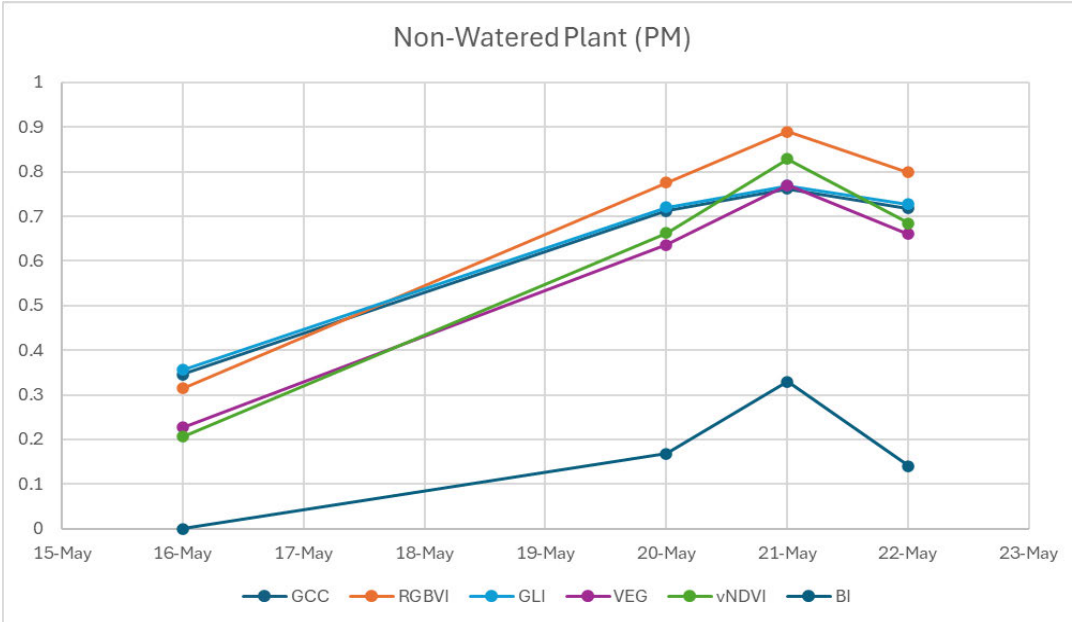


(a)

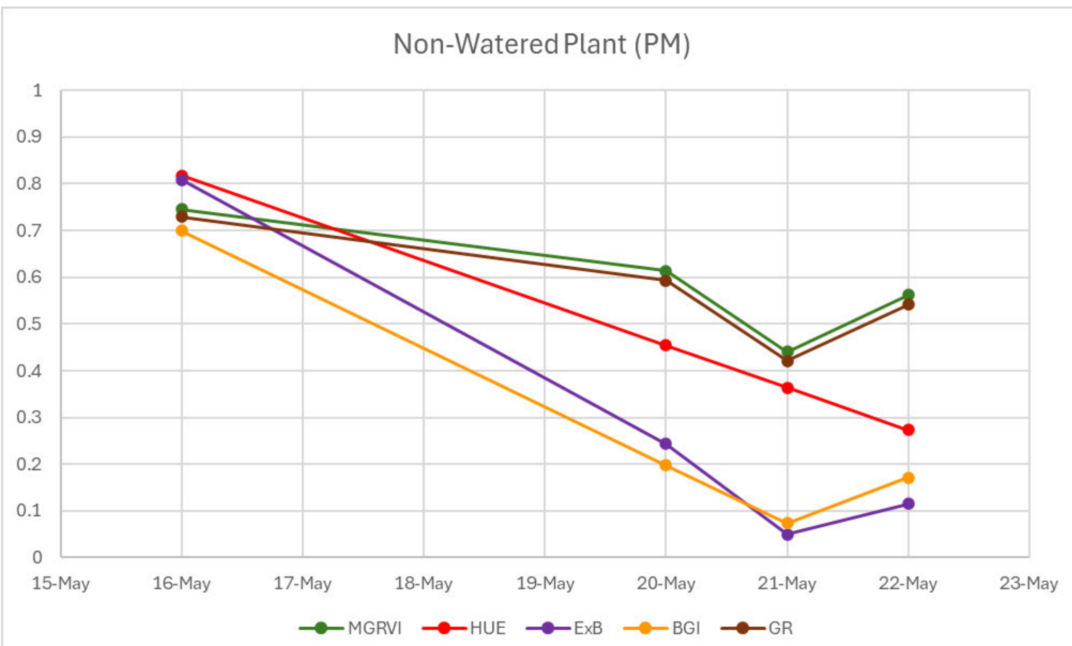


(b)

Figure 3.12. *Vegetation indices of the watered plant (PM data, normalised between 0 and 1). Vegetation indices are separated into two charts (a) and (b) to show similarities and differences between trends.*



(a)



(b)

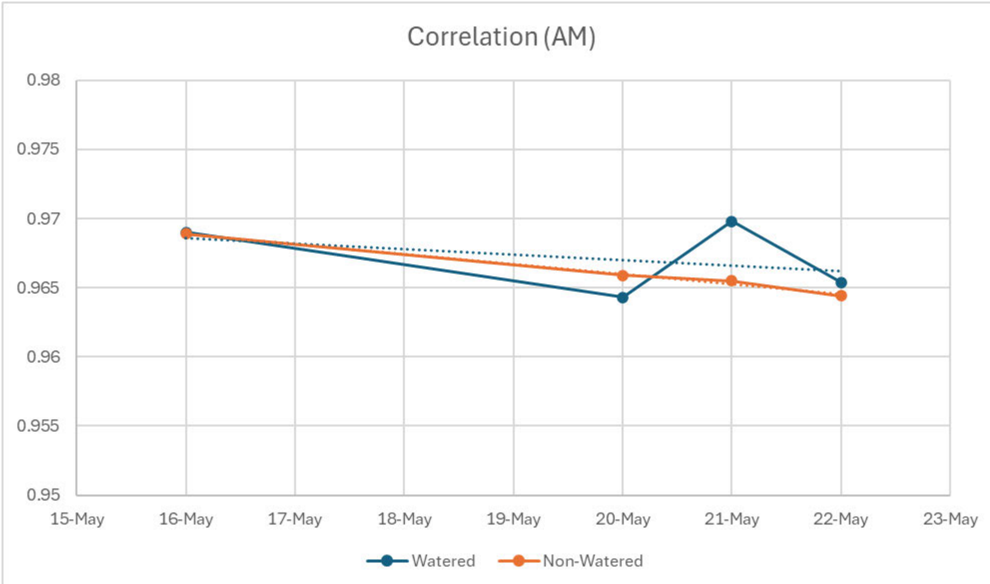
Figure 3.13. *Vegetation indices of the non-watered plant (PM data, normalised between 0 and 1). Vegetation indices are separated into two charts (a) and (b) to show similarities and differences between trends.*

3.3.3 TEXTURAL FEATURES

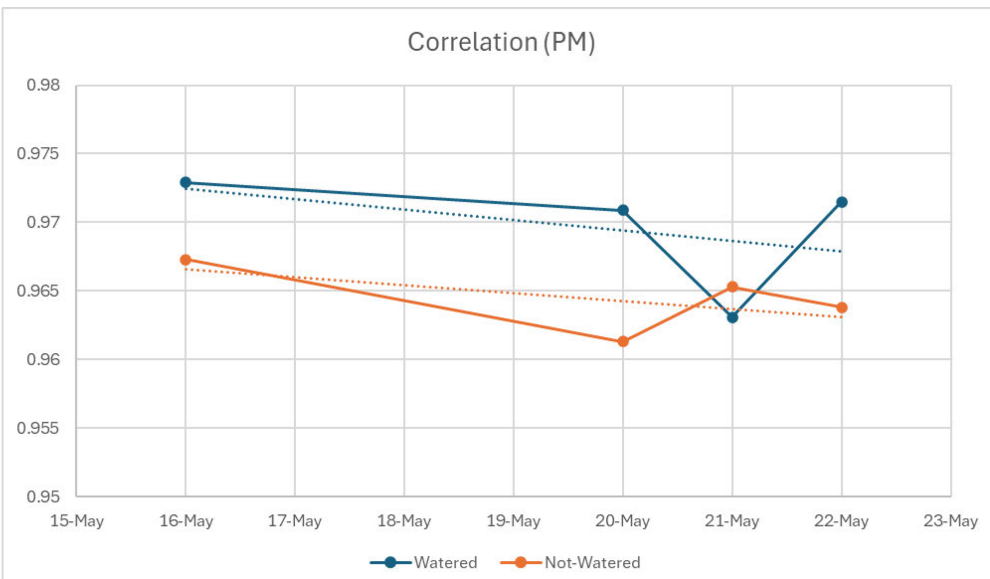
Correlation of AM data decreased for both the watered plant and the not-watered plant, as seen in Figure 3.14 (a). There was a decrease of 0.37% and 0.46% from the beginning of the data collection period to the end for the watered plant and non-watered plant, respectively. These results are supported by those observed by Story et al. (2010), who reported high levels of entropy values for the control group, and lower levels of entropy values for the treatment group as structural complexity reduced.

As seen in Figure 3.15 (a), energy of AM data increased for both the watered plant and the non-watered plant. There was an increase of 24.05% and 42.47% from the beginning of the data collection period to the end for the watered plant and non-watered plant, respectively. An increase in energy is supported by the results observed by Story et al. (2010), who reported increased energy levels due to the yellowing leaves of the treatment group. Dissimilarly, Story et al. (2010) observed decreasing energy levels in the control group as the plants became darker green in colour. This means that the watered plant was showing signs of stress, but at a much slower rate when compared to the non-watered plant. A factor that may have contributed to the increasing energy values of the watered plant, is that the lettuces were bought as established, mature plants. This means that there was little room for further plant growth.

From Figure 3.16 (a), an increase in homogeneity of the AM data was observed for the non-watered plant, but a decrease was observed for the watered plant. However, the differences were minimal between the beginning of the data collection period to the end. Homogeneity values increased by 1.66% for the non-watered plant and decreased by 0.26% for the watered plan. Again, these results are supported by the findings of Story et al. (2010), which saw decreased values of homogeneity for the control group over time, as it remained colourful and healthy, and increased values of homogeneity for the treatment group as the colour became dull and uniform.

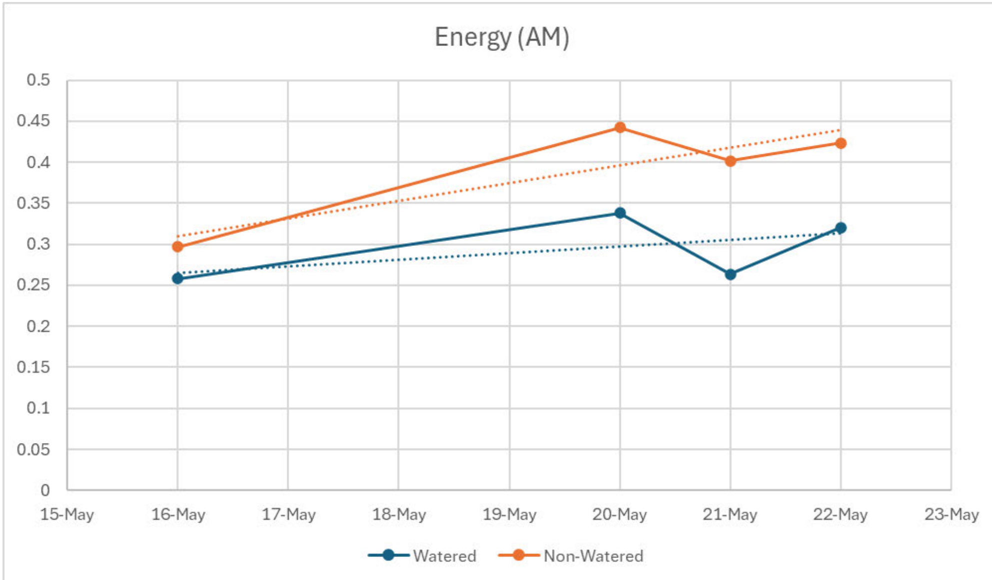


(a)

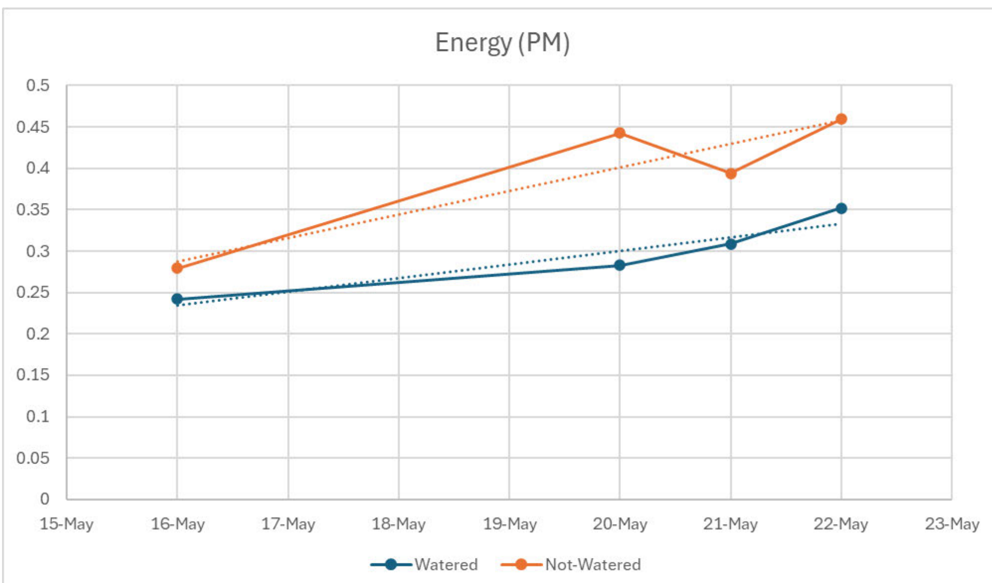


(b)

Figure 3.14. *(a) Correlation of watered and non-watered plants (AM data). (b) Correlation of watered and non-watered plants (PM data). Correlation is unitless but measured between 0 and 1.*

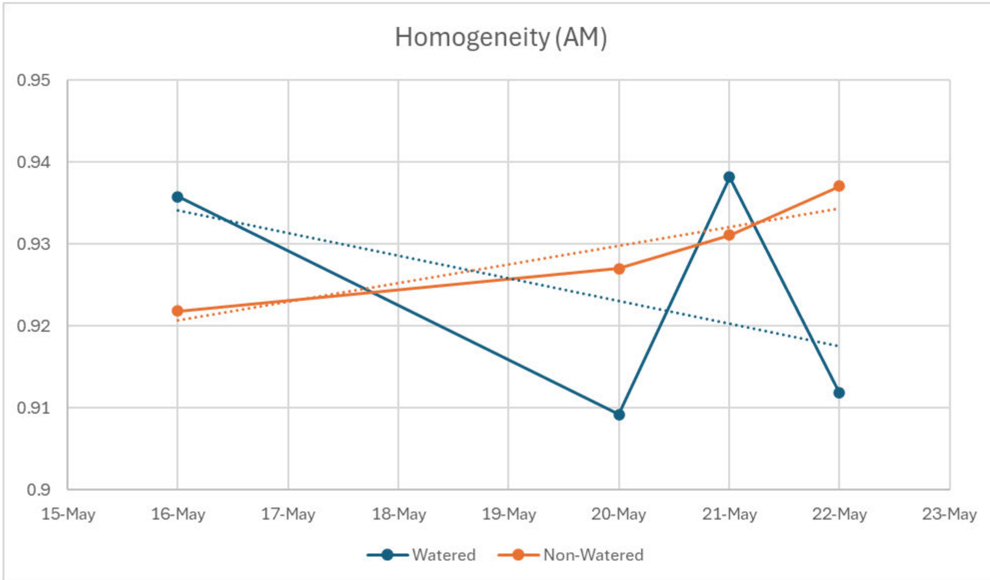


(a)

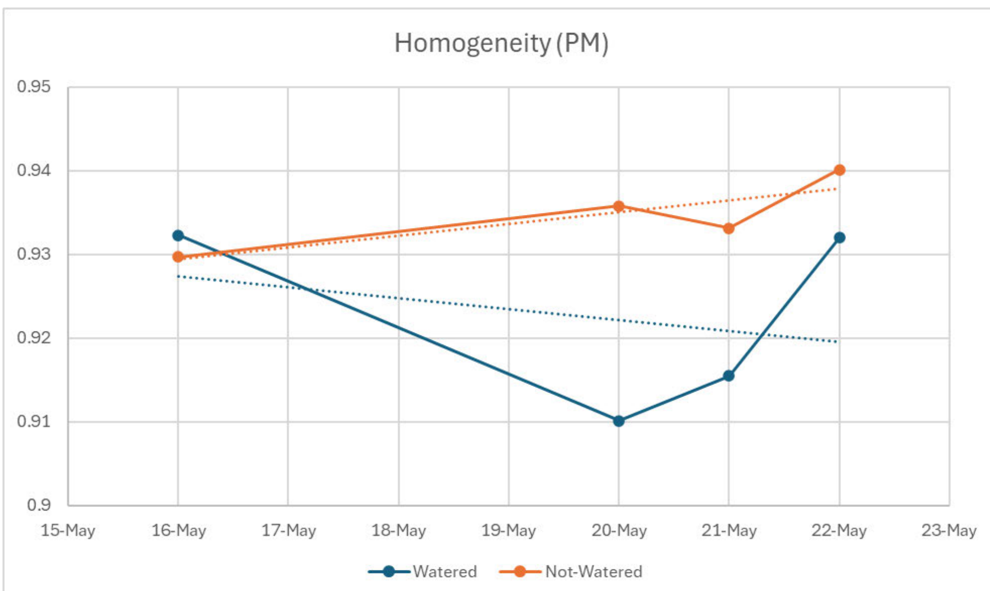


(b)

Figure 3.15. *(a) Energy of watered and non-watered plants (AM data). (b) Energy of watered and non-watered plants (PM data). Energy is unitless but measured between 0 and 1.*



(a)



(b)

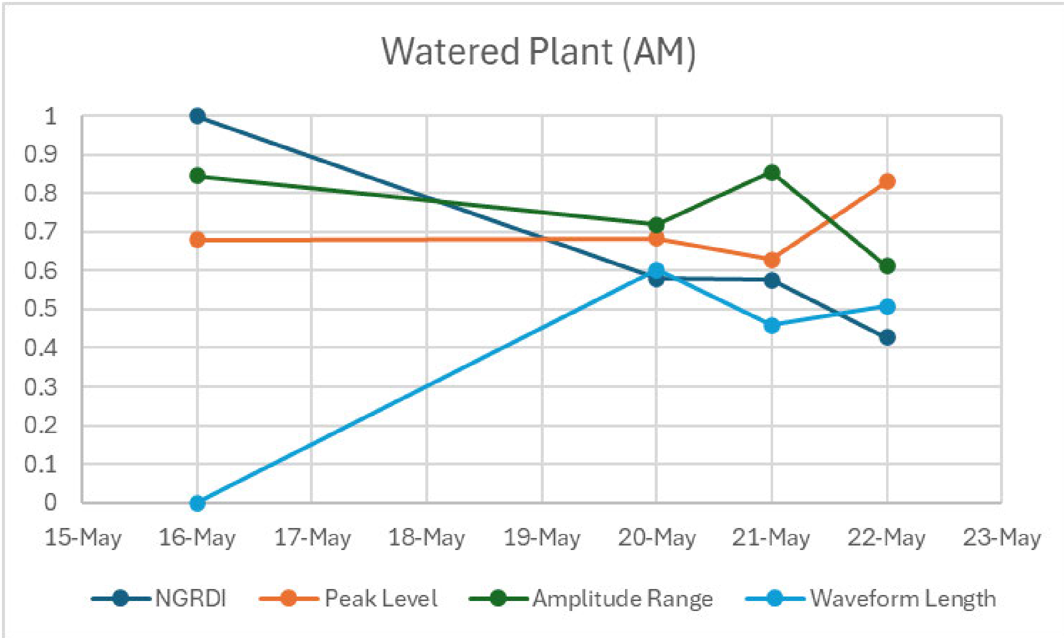
Figure 3.16. *(a) Homogeneity of watered and non-watered plants (AM data). (b) Homogeneity of watered and non-watered plants (PM data). Homogeneity is unitless but measured between 0 and 1.*

Similar linear trending was observed between the AM and PM data of correlation values (Figure 3.14), energy values (Figure 3.15) and homogeneity values (Figure 3.16) of the watered and non-watered plants. These results suggest that either AM or PM data can be used to gather information about the health status of the plants. For textural data, the biggest difference was observed in the energy values between the watered and non-watered groups, making it the most meaningful textural feature. In contrast, there was minimal difference in correlation and homogeneity features. These features may not be able to differentiate between healthy and non-healthy lettuce plants.

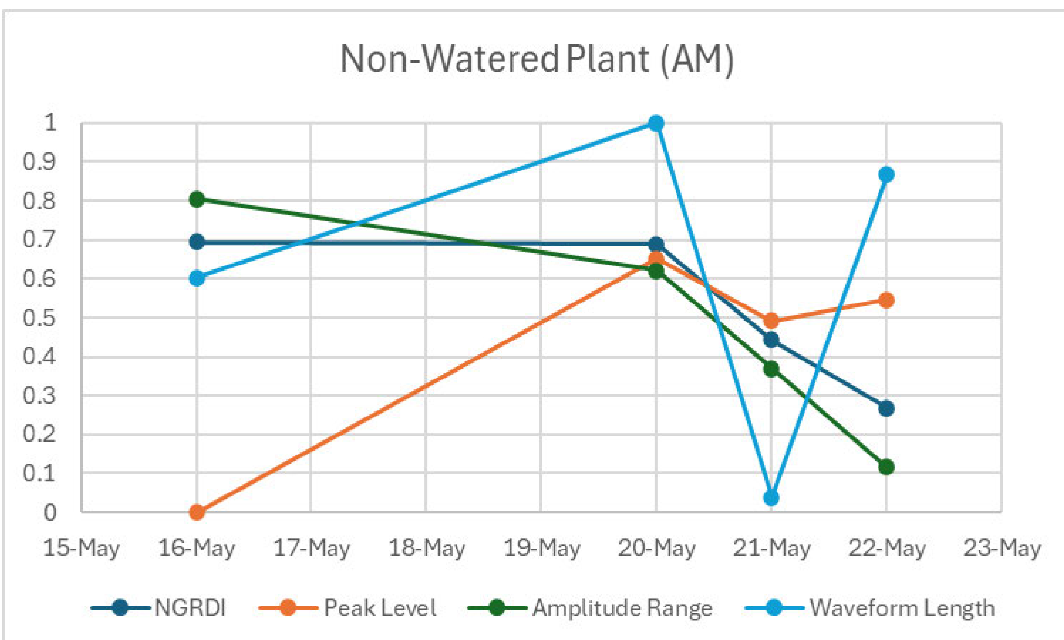
3.3.4 AUDIO FEATURES

For digital waveforms, dBFS is a measure of amplitude relative to full scale, where 0 dBFS is the maximum level. A signal that does not reach the maximum level, is measured in negative values (FADGI n.d.). Some qualitative observations were made during the analysis of the audio data, relating to the ‘crispness’ of the sound, and the resulting waveform. A leaf that produces a crisp sound, has a shorter wavelength with defined peaks and minimal noise. A leaf that produces a less crisp sound which is indicative of a lack of moisture content in the leaf, has a longer wavelength with less defined peaks and appears noisy. It was expected that the control group would have higher peak levels (lower negative values), a wider amplitude range and a shorter waveform length than the treatment groups. Audio features, peak level, amplitude range and waveform length were plotted on the same scatter graph as NGRDI to determine which features confidently reflects moisture content of the lettuce leaves.

From the correlation coefficient table (Table 3.4) and the graphs for the AM data (Figure 3.17), amplitude range had high positive correlation to NGRDI values for both the watered and non-watered groups. Peak level had negative correlation to NGRDI for both the watered and non-watered group. Waveform length had high negative correlation for the watered plant but had low positive correlation for the non-watered group. Thus, from the AM data it can be concluded that amplitude range can confidently estimate the moisture content and biomass of the leaves. More investigations should be performed to assess whether peak level and amplitude range can be considered as estimators of moisture content.



(a)



(b)

Figure 3.17. *Scatter graph of AM data for the (a) watered and (b) non-watered plants, to compare NGRDI to audio features: peak level, amplitude range and waveform length. Data was normalised between 0 and 1.*

Table 3.4. *Correlation coefficients of audio features against NGRDI, rounded to four decimal places, for AM data. Correlation coefficient values are between -1 and 1, where 1 is a high positive correlation, and -1 is a high negative correlation.*

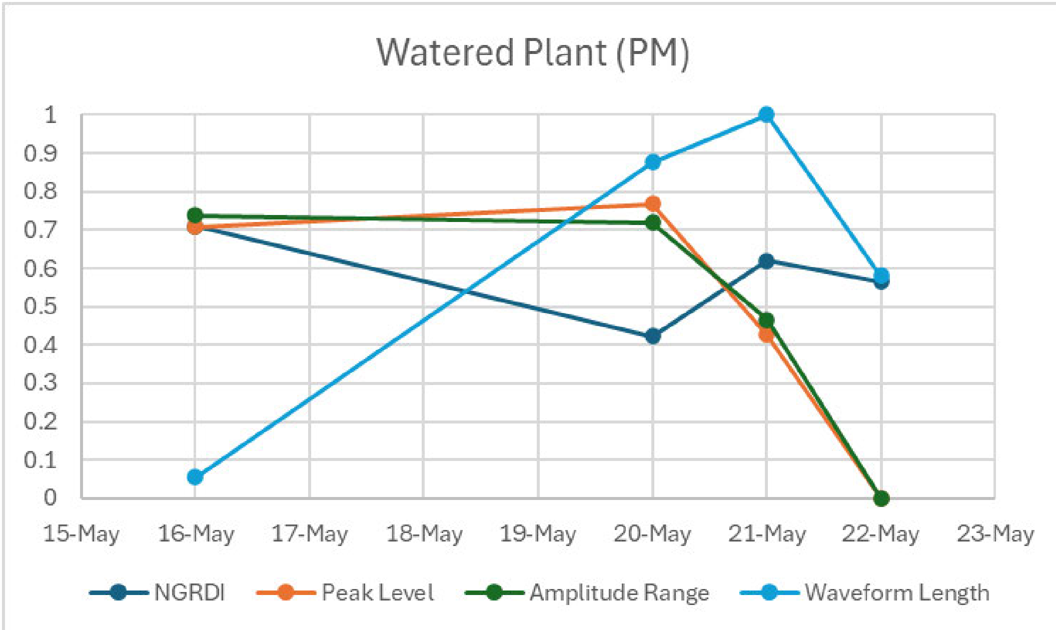
Group	Peak Level	Amplitude Range	Waveform Length
Watered Plant	-0.4621	0.6886	-0.9214
Non-Watered Plant	-0.3963	0.9700	0.1664

From the correlation coefficient table (Table 3.5) and the graphs for the PM data (Figure 3.18), amplitude range for the watered group had low positive correlation to NGRDI, whereas it had high negative correlation for the non-watered group. Peak level had low negative correlation to NGRDI for both the watered and non-watered groups. Waveform length had high negative correlation to NGRDI for both the watered and non-watered groups. Thus, the PM data did not reflect the results of the AM data. While the peak level correlation to NGRDI was roughly 0 (neither strong negative nor positive correlation), the PM did not draw any confident conclusions about whether these audio features could estimate moisture content.

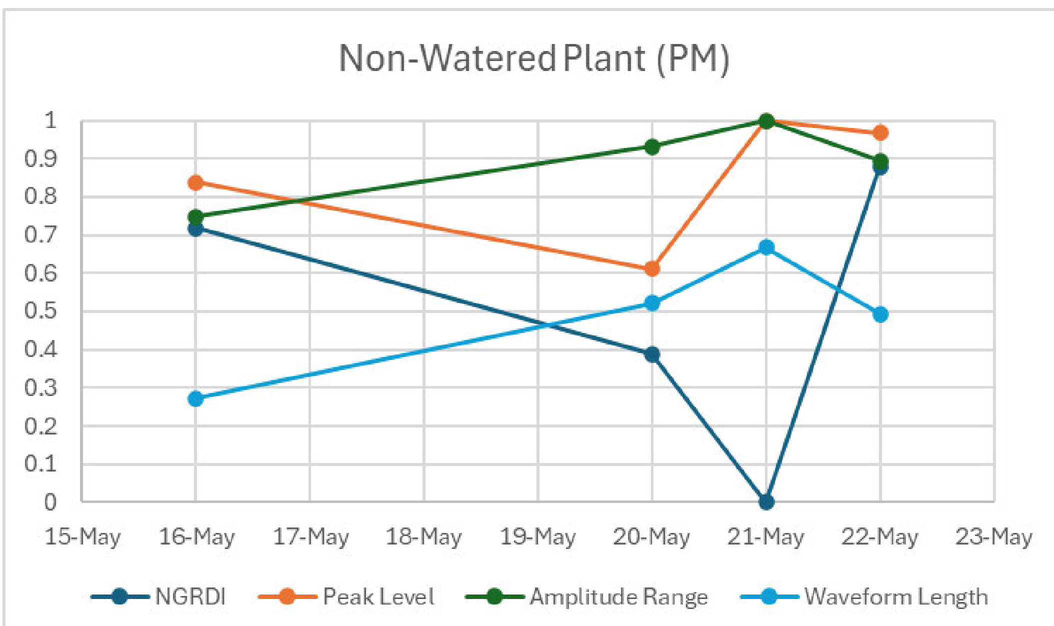
An observation was made between AM and PM data, suggesting that the non-watered lettuce plant at night held onto more water, resulting in higher peak levels, larger amplitude range and longer waveform lengths compared to the watered plant. Little to no difference between AM and PM data was observed for the watered plant. This is interesting because as temperature drops, and light disappears, transpiration of water through the leaf is not needed, so the plants would retain more water. However, a plant under drought stress should not be able to retain its moisture content. This unexpected behaviour may explain the difference between AM and PM data correlation to NGRDI.

Table 3.5. *Correlation coefficients of audio features against NGRDI, rounded to four decimal places, for PM data. Correlation coefficient values are between -1 and 1, where 1 is a high positive correlation, and -1 is a high negative correlation.*

Group	Peak Level	Amplitude Range	Waveform Length
Watered Plant	-0.0837	0.0217	-0.6470
Non-Watered Plant	-0.0290	-0.7185	-0.7242



(a)



(b)

Figure 3.18. *Scatter graph of PM data for the (a) watered and (b) non-watered plants, to compare NGRDI to audio features: peak level, amplitude range and waveform length. Data was normalised between 0 and 1.*

3.4 CONCLUSIONS AND IMPROVEMENTS

One limitation in the data collection process which was discovered during the analysis of the TPCA data was that the plant orientations were rotated. TPCA count depends on the orientation of the plant, and rotating the plant will produce different TPCA counts. Additionally, the environment that the plants were kept in during data collection was not controlled and the plants were subjected to varying levels of light, temperature and humidity, which may affect the results. Therefore, improvements to be made for the main trials when collecting image data includes maintaining plant orientation. Environmental factors cannot be controlled due to that nature of this project. Additionally, for the collection of audio data, the process of removing the outer most leaves must remain consistent. Initially, rotted or rotting leaves were omitted during the initial investigation, which may skew the results. Other data that will be collected during the main trials, is the fresh and dry weights of the lettuce plants at the end of the data collection period, for moisture content analysis to compare the treatment methods.

A conclusion that can be made from the initial investigation, is that either AM or PM data can be used to train the classification model. Besides the audio data, there wasn't a significant difference in the results between the different data sets, implying that both AM and PM data reflect the health of the plant equally. For the sake of simplifying the mail trial, and because of the positive correlation of audio features to NGRDI, AM data only will be collected during the main trials. Interestingly, there seemed to be some sort of anomaly in the data on day six (21st May), across all graphs. The cause of this is not certain but it was worth noting.

The results of the initial investigation confirm that the conceptualised machine vision system can successfully extract plant features automatically, with high segmentation accuracy. Currently, the biggest indicators of plant health include TPCA and energy values. Additionally, from the results of the initial investigation for the AM data, amplitude range can confidently reflect the plants moisture and could be used as an additional quality metric for assessment. Lastly, features began to show significant difference between the two groups on the last day. A larger data set was collected during the main trials, and all morphological features were re-assessed during the main trials.

CHAPTER 4 MAIN TRIAL

Now that the processes involved in data collection and processing are better understood, the main objective of the main trial was to collect a larger data set of healthy and non-healthy features of lettuce plants. This data set will be used to train and test the classification model. The main trial will aid in supporting observations seen in the initial investigation. Furthermore, since the data collection period of the main trials is longer than the initial investigation, the aim is to see clearer separation in the trends between the features of the watered and non-watered groups. This will assist in drawing conclusions about which features can be selected as parameters for training the classification model, as well as defining thresholding values for each class (healthy or not healthy) when labelling the training data set. Lastly, audio data collected during the larger, main trial will be re-evaluated to determine which features can confidently estimate the water content and comparably, the eating quality of the lettuce leaves.

The core of the methodology remained the same as the initial investigation in terms of data acquisition, hardware and software. Specific changes to the methodology will be discussed, followed by the results and discussion of the main trial.

4.1 UPDATES TO METHODOLOGY

The lettuce cultivar, Romaine, remained as the model plant in the main trial. For the main trial, lettuce plants were kept and grown during August, under similar environmental conditions to the initial investigation. Again, the watered and non-watered groups consisted of two plants each, of relative size and maturity (Figure 4.1).

Data was collected once a day at 6:00 am, over a period of fourteen days, starting on 16th August, and ending on 29th August. Dissimilarly to the initial investigation, the non-watered plants were placed on a bench to induce water stress (drought) from the beginning of the trial. The roots of the watered plants were submerged in a hydroponic media from the begging of the trial, dosed with a ratio of two drops of liquid fertilizer with a NPK ratio of 3:1:4 (We The Wild™, Grow Concentrate Plant Food) to one litre of local tap water. Every fourth day, the hydroponic media of the watered plants was refreshed. Data collection continued until the fourteenth day (29th August).

Figure 4.1. *Image of the four lettuce plants at the beginning of the main trials, showing similar size, maturity and health.*

Methods for data acquisition in the main trials reflected the methods used in the initial investigation. However, fresh and dry weights of the whole watered and non-watered plants were collected at the end of the data collection period for the main trial. This data was collected to compare the whole lettuce plants at the end of the trial to verify the overall differences in the treatments, rather than being used as metric throughout the trial. Each leaf of the intact was removed from the core of the lettuce plant, and the fresh weights were collected. After the fresh weights were collected, the leaves of the plants were placed on a tray and dried in a conventional fan-forced oven at a temperature of 80°C for 5 hours. The fresh and dry weight were used to calculate the moisture content (MC) of the plants at the end of the main trial, using equation 4.1 (Ling et al. 1996).

$$MC\ (\%) \quad = \quad 100 * \left(\frac{FW - DW}{FW}\right) \tag{4.1}$$

Where MC is leaf moisture content measured as a percentage, FW is the fresh weight in grams and DW is the dry weight in grams.

As discussed in the conclusion section of the initial investigation, a change was made to improve the quality of the data collected to calculate the TPCA count of the plants. As TPCA is orientation dependent, the orientation of the plant was maintained throughout the data collection period. This removed some errors in the data caused by rotation, and resulted in a TPCA count that better represents the health of the plant. This adjustment would replicate the target application of this tool, as lettuce plants being grown in space would maintain their orientation as they growing and being monitored. Otherwise, data collection methods remained the same for colour, texture and audio features, and data processing methods remained the same for all features.

Data pertaining to TPCA, colour and textural features of the watered and non-watered plants were plotted on scatter graphs. Data analysis was performed to determine whether there was a clear separation in trends between the two groups and determine whether the features could be used as parameters of the classification model. Audio data was re-assessed, and plotted against NGRDI on scatter graphs, to determine a correlation coefficient and measure how accurately the extracted audio features measure and estimate leaf water content.

4.2 RESULTS AND DISCUSSION

To preface, by the end of the main trials, the healthy plants were also showing signs of significant stress, which is reflected in the trends. See (Figure 4.2).

4.2.1 TOP PROJECTED CANOPY AREA

Both the watered and non-watered plants followed a downward trend in Figure 4.3, which was also observed in the initial investigation. The watered plant decreased by roughly 60.39% and the non-watered plant decreased by 90.11% by the end of the data collection period. The watered plant decreased rapidly around day 10 of data collection (25th August), which aligns with the visual indication of severe stress in Figure 4.2. Between the first day of data collection (16th August), to this turning point (25th August), there was a decrease in TPCA for watered group of only 22.91%, compared to 60.49%. Regardless, the difference in TPCA between the two groups is significant enough to consider it as a parameter for the classification model, to distinguish between healthy and not healthy Romaine lettuce plants.

Figure 4.2. *A visual comparison showing health status of the watered plant (left) and non-watered plant (right) on day 10 of data collection (25th August).*

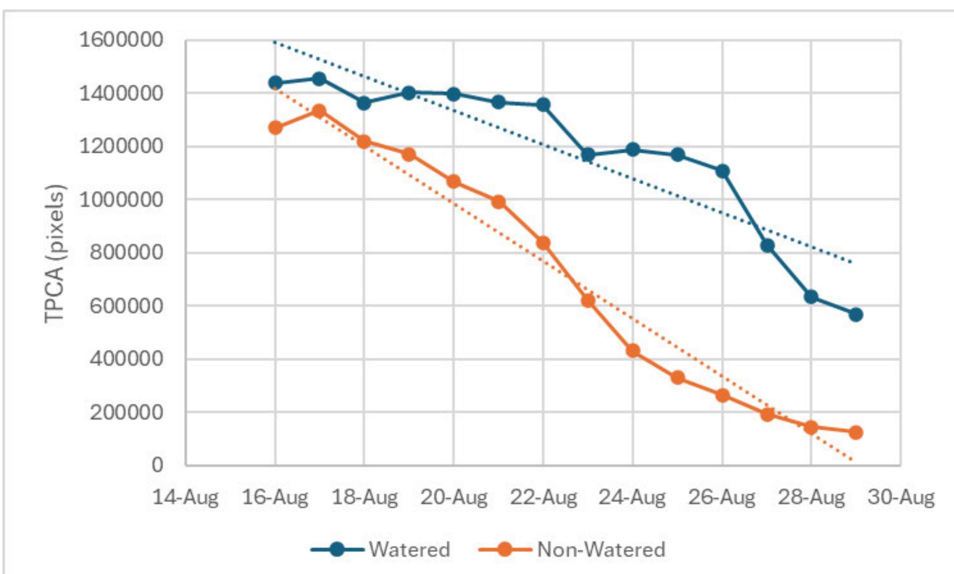
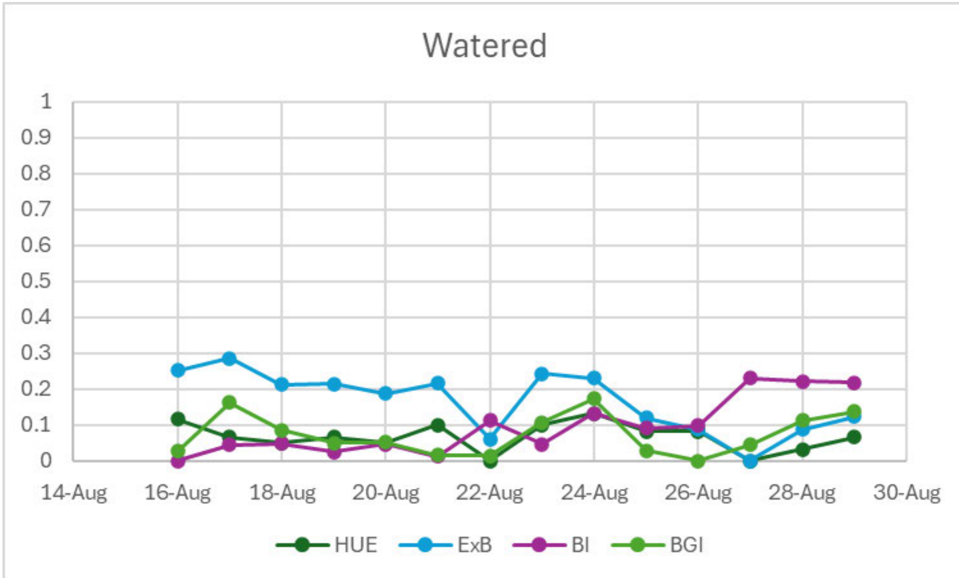


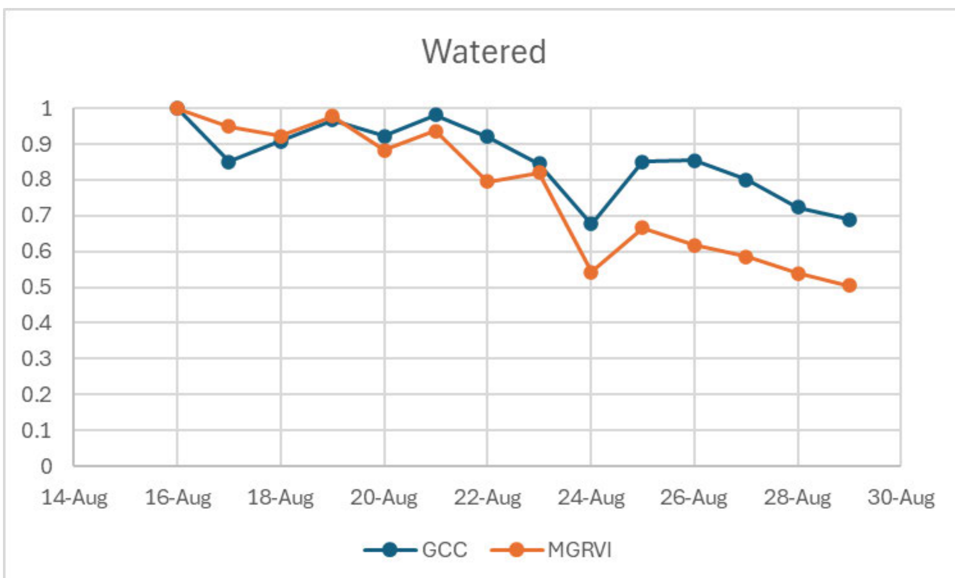
Figure 4.3. *TPCA (Top-Projected Canopy Area) of the watered and non-watered plants. TPCA is the count of white pixels in a 1414 x 1883 image.*

4.2.2 COLOUR FEATURES

Data collected for each of the vegetation indices was normalised between 0 and 1 for comparison and analysis. For vegetation indices HUE, ExB, BI and BGI of the watered plant in Figure 4.4 (a), values remained below 0.3 over the course of the data collection period. Comparatively, these vegetation indices of the non-watered plant in Figure 4.5 (a) rose above 0.3, especially towards the end of the data collection period. For vegetation indices GCC and MGRVI of the watered plant in Figure 4.4 (b), values remained above 0.5 over the course of the data collection period. Comparatively, these vegetation indices of the non-watered plant in Figure 4.5 (b) dropped below 0.5, especially towards the end of the data collection period. Notably, there is a clear downward trend in GCC and MGRVI values for both groups on the last four days of data collection (26th August to 29th August). Again, this aligns with visual indication of severe stress in both groups, shown in Figure 4.2. The difference in these six colour features between the two groups is significant enough to consider them as individual parameters for the classification model, to distinguish between healthy and not-healthy Romaine lettuce plants.

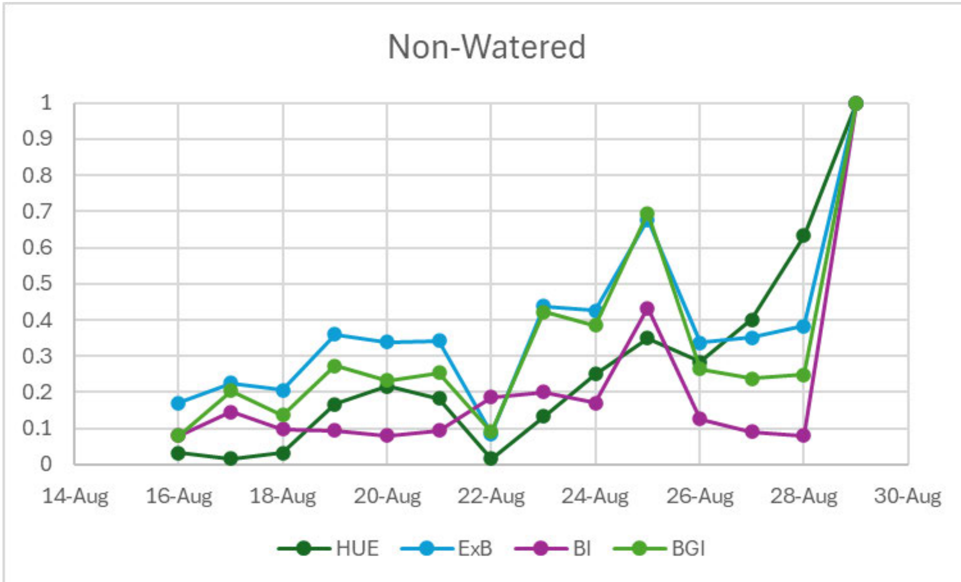


(a)

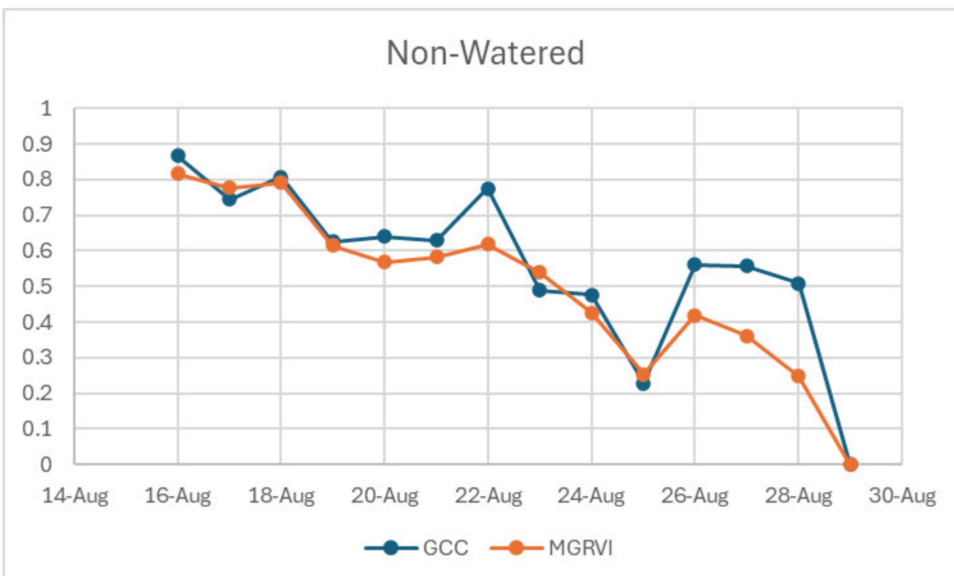


(b)

Figure 4.4. *Colour features (vegetation indices) of the watered plant, where (a) HUE is Overall Hue Index, ExB is Excess Blue, BI is Brightness Index, BGI is Simple Blue-Green Ratio and (b) GCC is Green Percentage Index and MGRVI is Modified Green Red Vegetation Index. Values were normalised between 0 and 1.*



(a)



(b)

Figure 4.5. *Colour features (vegetation indices) of the non-watered plant, where (a) HUE is Overall Hue Index, ExB is Excess Blue, BI is Brightness Index, BGI is Simple Blue-Green Ratio and (b) GCC is Green Percentage Index and MGRVI is Modified Green Red Vegetation Index. Values were normalised between 0 and 1.*

4.2.3 TEXTURAL FEATURES

As seen in Figure 4.6, correlation increased for both the watered and non-watered plants. There was an increase of 2.26% for the watered group, and an increase of 0.75% for the non-watered group between the beginning and end of the trial. These results did not reflect the observations and results in the initial investigation, which showed a decrease in correlation for both groups.

Story et al. (2010) reported higher levels of entropy (correlation) value for the control group, and lower levels of entropy for the treatment group. Thus, the results in Figure 4.6 did not meet expectations as the non-watered plant had higher correlation values than the watered plant, and the watered plant increased in correlation more rapidly than the non-watered plant. Furthermore, the separation between correlation values for the watered and not watered plants is not significant enough to justify using this textural feature as a parameter for the classification model. In other words, this feature does not seem to represent the healthy and not healthy lettuce plants based on expectations.

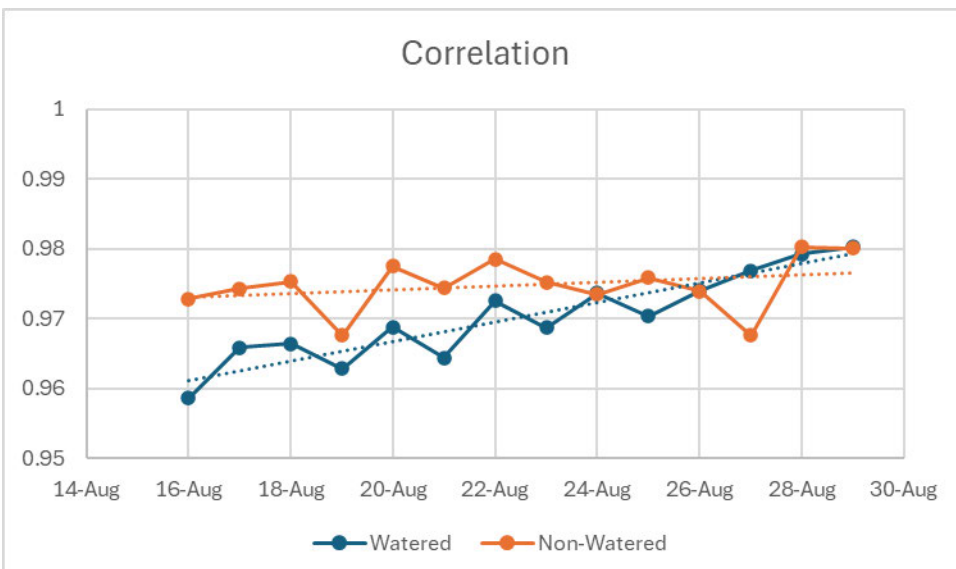


Figure 4.6. *Correlation of watered and non-watered plants. Correlation is unitless but measured between 0 and 1.*

Energy values increased for both the watered and non-watered plants (Figure 4.7). There was an increase of 62.2% for the watered plant and an increase of 92.1% for the non-watered plant between the beginning of and end of the main trial. These results reflect those observed in the initial investigation, and the trending is very similar. While the watered group increased in energy, it was at a much slower rate compared to the non-watered group. It is worth nothing that a majority of the increase in energy values for the watered group can be described by the last four days in the data collection period (26th to 29th August), which aligns with the visual indication of severe stress in both groups, shown in Figure 4.2. If these days were omitted, energy would have only increase for the watered plant by 38.19%.

In the initial investigation, energy was the most meaningful textural feature, and this statement also applies to the results of the main trial. There is a large difference between the two groups, meaning that energy could be used as a parameter for the classification model, to confidently distinguish between healthy and not healthy lettuce plants.

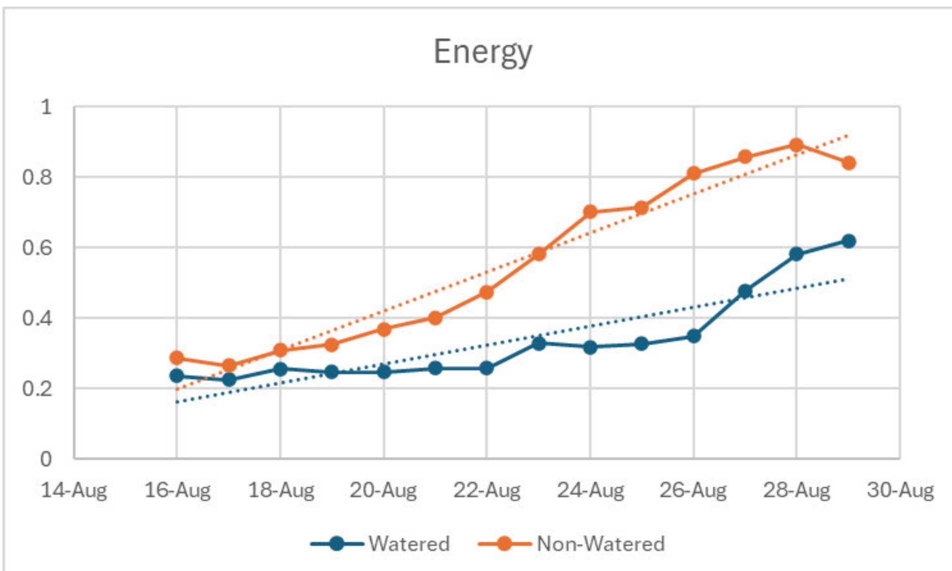


Figure 4.7. *Energy of watered and non-watered plants. Energy is unitless but measured between 0 and 1.*

As seen in Figure 4.8, homogeneity for both the watered and non-watered plants increased. There was an increase of 6.33% for the watered plant, and an increase of 7.75% for the non-watered plant between the beginning and end of the trial. These results do not quite resemble those observed in the initial investigation, but the data of the initial investigation is rather limited, so it was difficult to draw conclusions about the usefulness of this feature. Now that a larger data set has been collected, there is a clear trend. Similar to the energy feature, while the watered group increased in homogeneity, it was at a much slower rate compared to the non-watered group. It is worth noting that a majority of the increase in homogeneity for the watered group can be described by the last four days of data collection (26th to 29th August), which aligns with visual indication of severe stress in both groups, shown in Figure 4.2. If these days were omitted, energy would have only increase for the watered plant by 2.82%.

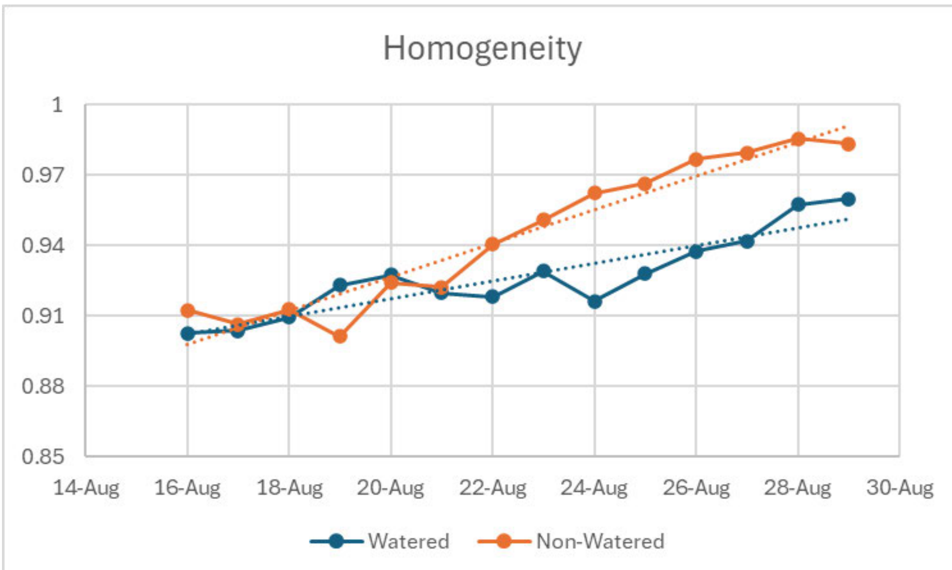


Figure 4.8. *Homogeneity of watered and non-watered plants. Homogeneity is unitless but measured between 0 and 1.*

4.2.4 AUDIO FEATURES

In the main trials, all three audio features were re-assessed as the results in the initial investigation for peak level and waveform length were not clear. In Figure 4.9 and Table 4.1, there is a negative correlation between peak level and NGRDI for both the watered and non-watered groups. Between the initial investigation and the main trial, it can be determined that peak level cannot confidently estimate the water content of a lettuce leaf, when compared to NGRDI as a biomass estimator. When comparing amplitude range and NGRDI, there is a positive correlation for both the watered and non-watered groups, as can be seen in Figure 4.10 and Table 4.1. This confirms the results of the initial investigation and suggests that amplitude range can confidently estimate leaf water content. Lastly, from the results presented in Figure 4.11 and Table 4.1, there is a low positive correlation between waveform length and NGRDI for both the watered and non-watered groups. In the initial investigation, there was high negative correlation and low positive correlation between waveform length and NGRDI for the watered and non-watered group, respectively. Therefore, waveform length cannot confidently estimate leaf water content.

Table 4.1. *Correlation coefficient of audio features (peak level, amplitude range and waveform length) against NGRDI (Normalised Green Red Difference Index), rounded to four decimal places. Correlation coefficient values are between -1 and 1, where 1 is a high positive correlation, and -1 is a high negative correlation.*

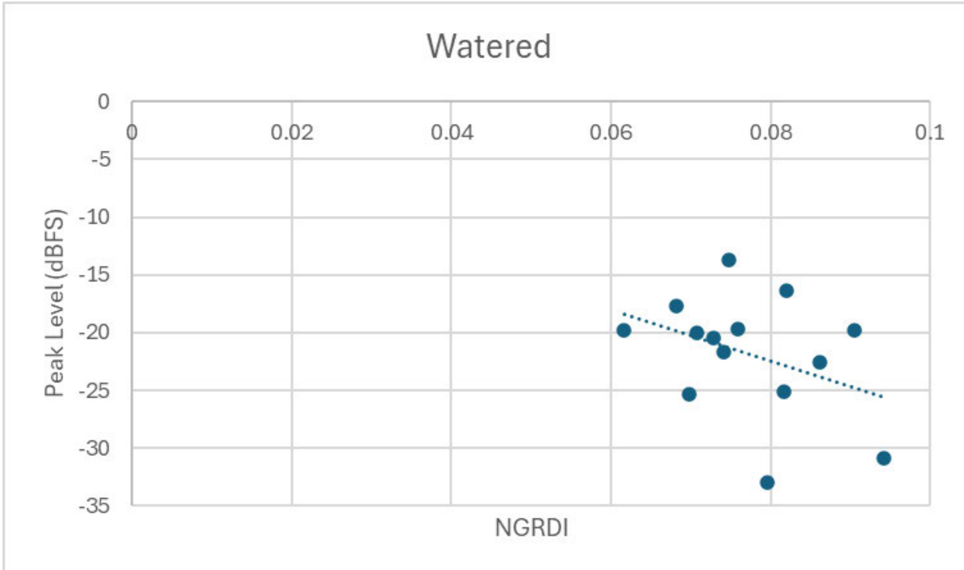
Group	**Peak Level**	**Amplitude Range**	**Waveform Length**
Watered Plant	-0.3782	0.5196	0.1181
Non-Watered Plant	-0.0117	0.2908	0.1523

4.2.5 MOISTURE CONTENT ANALYSIS

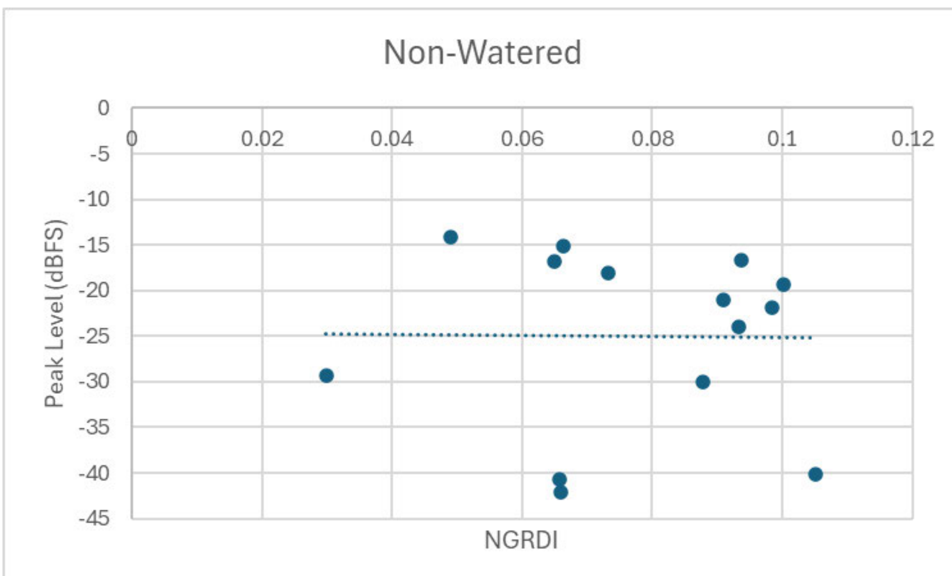
The results presented in Table 4.2, reflect the different plant treatments. The non-watered group had a moisture content of 82.76%, whereas the watered plant had a moisture content of 90.67%, implying that the non-watered plant had less moisture content at the end of the trial compared to the watered plant. This implies that the different treatments did affect the plant's health.

Table 4.2. *Moisture content analysis of plants at the end of the main trial, using equation 4.1.*

Group	**Fresh Weight (g)**	**Dry Weight (g)**	**Moisture Content**
Watered Plant	75	7	90.67
Not-Watered Plant	29	5	82.76

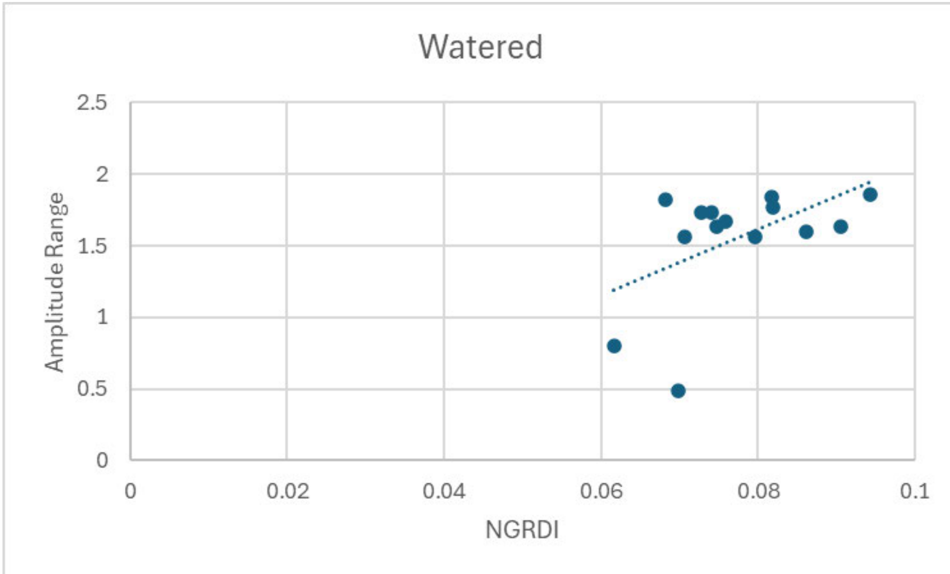


(a)

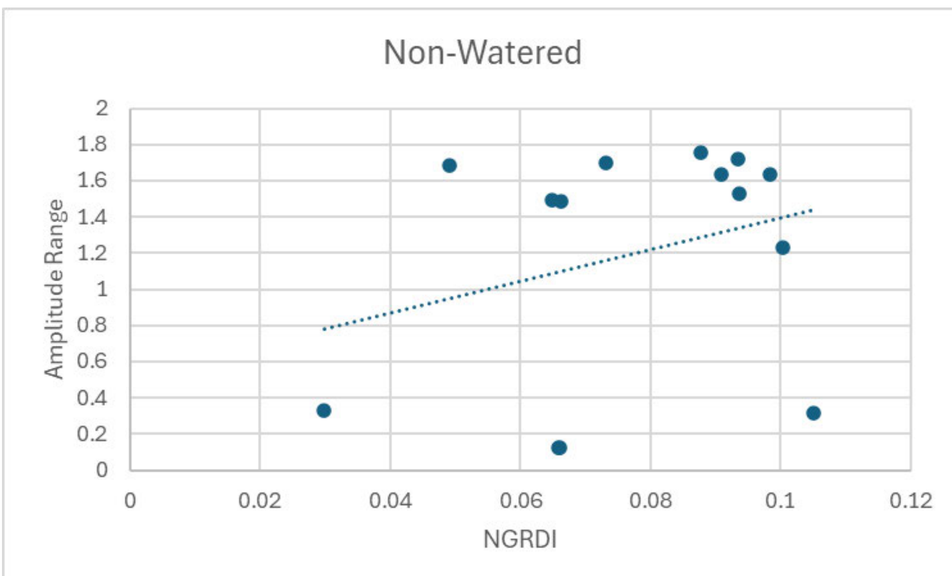


(b)

Figure 4.9. *Correlation of NGRDI vs peak level of (a) watered and (b) non-watered plants. Peak level is measured in dBFS. NGRDI (Normalised Green Red Difference Index) is unitless, but is calculated using the average red, green and blue channel values in the leaf image.*

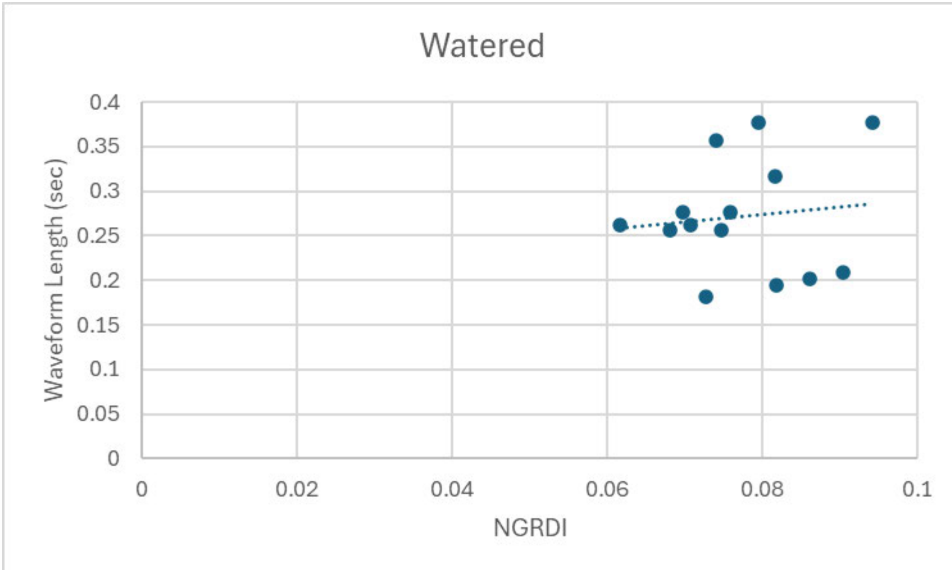


(a)

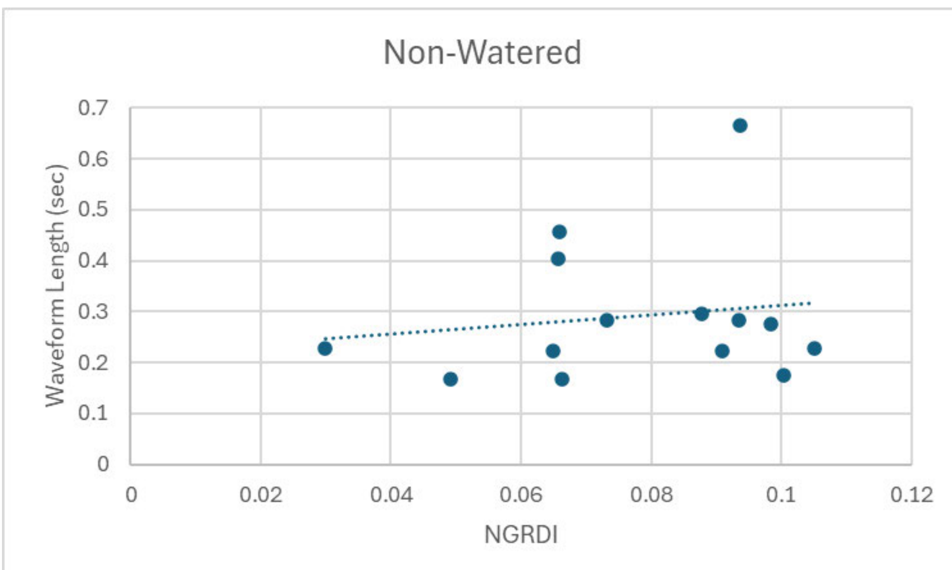


(b)

Figure 4.10. *Correlation of NGRDI vs amplitude range of (a) watered and (b) non-watered plants. Amplitude range is unitless, but values range from 0 to 2. NGRDI (Normalised Green Red Difference Index) is unitless, but is calculated using the average red, green and blue channel values in the leaf image.*



(a)



(b)

Figure 4.11. *Correlation of NGRDI vs waveform length of (a) watered and (b) non-watered plants. Waveform length is measured in seconds. NGRDI (Normalised Green Red Difference Index) is unitless, but is calculated using the average red, green and blue channel values in the leaf image.*

4.3 CONCLUSIONS

Compared to the initial trial, which had a limited data set (a total of 8 data points), there was a total of 28 data points collected during the data collection period of the main trial. Ultimately, there weren't many discrepancies in the results between the initial investigation and the main trial. However, the results of the textural feature, correlation, were unexpected and did not match the results in the initial investigation or the results found by Story et al. (2010). Additionally, adjusting the method of collecting TPCA data seemed to have a positive impact on quality and accuracy. Re-assessment of the audio features came to the same conclusion as the initial investigation, and amplitude range could be used as an additional quality metric of lettuce. Moisture content analysis of the leaves after image and audio data is collected could support these results. The results of the main trials will help determine which features should be selected to train the classification model.

CHAPTER 5 CLASSIFICATION MODEL

This chapter discusses which features were selected as parameters to train the classification model, determines thresholding values to label the data, discusses software required to train and test the model, and evaluates the performance of the trained model.

5.1 PARAMETER SELECTION

Parameters were selected based on the results of the main trial. Chosen parameters (features) were determined based on whether the collected data could be used to distinguish between healthy and unhealthy lettuce plants. Parameters selected include TPCA, the six colour features (MGRVI, GCC, HUE, ExB, BI and BGI), and two textural features, energy and homogeneity.

5.2 METHODOLOGY

The MATLAB classification learner app was used to train all SVM algorithms (MathWorks n.d.a), and the SVM with the highest training and testing accuracy was be selected as the ideal SVM model. The model classified testing data as either healthy or not-healthy based on the patterns it learnt during training.

Since SVM is a supervised learning algorithm, it requires a labelled data set. To determine which label (healthy or not healthy) to allocate to the data, thresholding is required. Thresholding values will determine the class (label) of the data. The first step to determine the thresholds was to observe and record at what point during data collection, that the plants began to show visible change from healthy to not healthy in the collected images. This change point separates the data into the two classes. This visible changed was determined by an amount of yellowness and drooping compared to the start of the trial. For healthy data, visual change was recorded on day 10 of data collection (25^{th} August). For not healthy data, visual change was recorded on day 4 of data collection (19^{th} August). See Figure 5.1 for a visual comparison of the lettuce plants on these days (change points).

(a) (b)

Figure 5.1. *Images taken that coincide with visual change where (a) is an image taken on the 10th day of data collection (25th August) and (b) is an image taken on the 4th day of data collection (19th August).*

Thresholding values were calculated by taking the values of each feature on the 10th day (25th August) for the healthy group, and values of each feature on the 4th day (19th August) for the unhealthy group. These values are averaged to give the thresholding value of each feature, collated in Table 5.1. Data will be re-evaluated and compared to the thresholding values, and each individual feature will be labelled as healthy or unhealthy. The overall class of the data will be labelled based on the majority of the feature labels.

After labelling the data, it was split into 60% for training, and 40% for validation. For the training data, half of it reflected the healthy class, and the other half reflected the unhealthy class, so that the model did not overfit the data or favour one class. Out of the 28 samples collected, 16 samples were used for training and 12 samples were used for testing. For the 16 samples used for training, 8 samples were manually selected to represent the strongest of the healthy plant, and 8 samples were manually selected to represent the strongest of the unhealthy plant. In this project, "strongest" refers to which images had the most individually classed features for each respective group. Usually random data is selected, but the data set was small enough to manually select an equal amount of healthy and not-healthy plants for the training data.

Table 5.1. *Thresholding values determined from data based on change points. Thresholds of features are in raw values (not normalised). Features are classed as healthy or not healthy based on whether it is greater than or less than the threshold.*

Feature	**Threshold (λ)**	**Healthy (> λ or < λ)**	**Unhealthy (> λ or < λ)**
TPCA	1169918	> λ	< λ
Energy	0.3267375	< λ	> λ
Homogeneity	0.914675	< λ	> λ
GCC	0.5085	> λ	< λ
MGRVI	0.28045	> λ	< λ
HUE	-1.56775	> λ	< λ
ExB	-79.4318	< λ	> λ
BI	49926000	< λ	> λ
BGI	0.21985	< λ	> λ

5.3 MODEL PERFORMANCE

All SVM types were trained to find which kernel gave the highest accuracy. Different types of SVM's include Linear, Quadratic, Cubic, Fine Gaussian, Medium Gaussian and Coarse Gaussian. Training and testing accuracies of each SVM are presented in Table 5.2. Results for precision, recall and F1 score calculated using equations 2.2, 2.3 and 2.4 of each SVM are presented in Table 5.3.

Automatic PCA was enabled, to reduce parameter dimensionality to two features, that explained 95% variance in the data. The app determined that TPCA and energy were the two features that explained most of the variance in the data, which was not unexpected, as the results of these features in the initial investigation and the main trials have the biggest difference between the two groups. Out of the six SVM kernels trained and tested, the cubic SVM performed the best. It had the highest training accuracy, testing accuracy and an F1 score of 0.8. Additionally, it only took 6.97 seconds to train the Cubic SVM model. The confusion matrix of the Cubic SVM is shown in Figure 5.2, and the resulting scatter graph showing correct and incorrect classifications is shown in Figure 5.3.

Table 5.2. *Training and testing accuracies of the different SVM kernel's, using the MATLAB Classification Learner app. Results are rounded to one decimal place, and measured as a percentage.*

Kernel	Training Accuracy (%)	Testing Accuracy (%)
Linear	68.8	75.0
Quadratic	75.0	66.7
Cubic	81.2	75.0
Fine Gaussian	75.0	66.7
Medium Gaussian	50.0	33.3
Coarse Gaussian	37.5	33.3

Table 5.3. *Precision, recall and F1 score for each SVM kernel, where values are measured between 0 and 1.*

Kernel	Precision	Recall	F1 Score
Linear	0.875	0.6364	0.7368
Quadratic	0.875	0.7	0.7778
Cubic	0.75	0.8571	0.8
Fine Gaussian	1	0.6667	0.8
Medium Gaussian	0.625	0.5	0.5556
Coarse Gaussian	0.5	0.4	0.4444

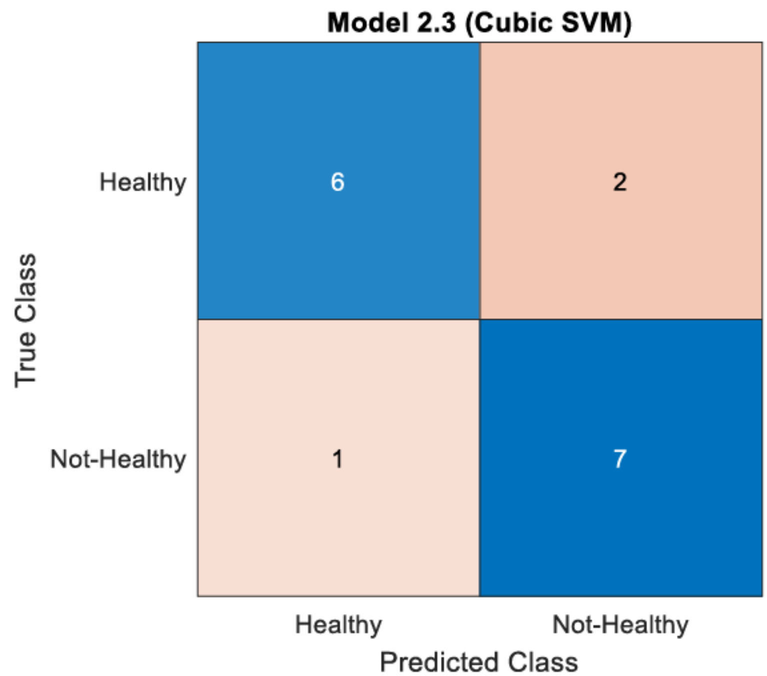


Figure 5.2. *Confusion matrix of training data for the Cubic SVM model.*

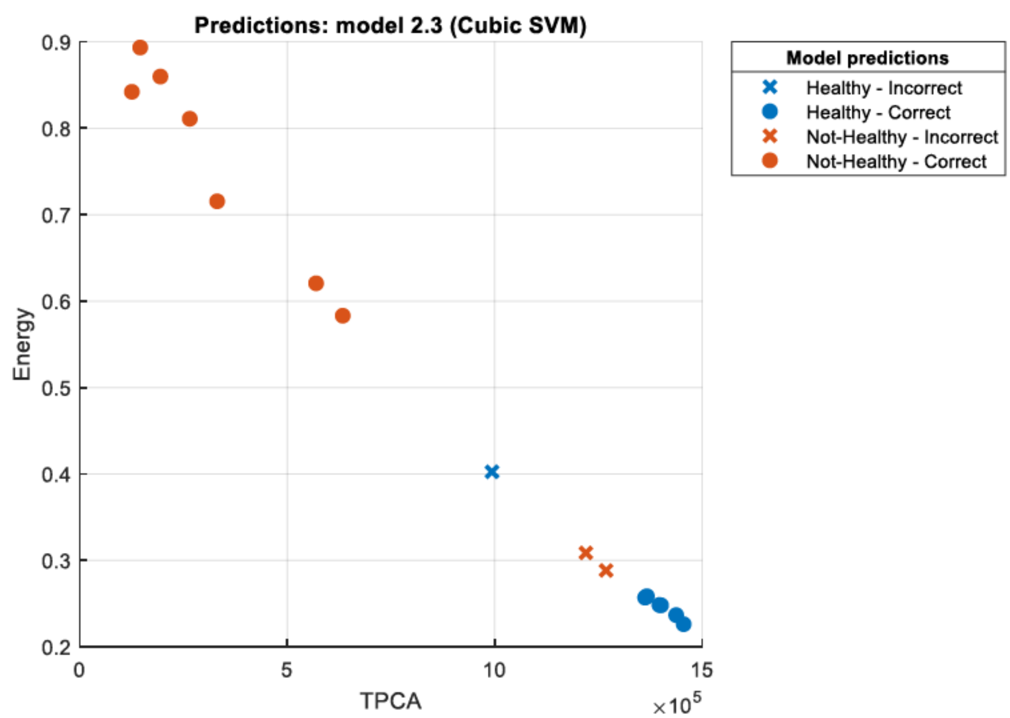


Figure 5.3. *Scatter graph results of Cubic SVM model for testing data. Where energy and TPCA were determined as predictors by PCA.*

5.4 CONCLUSIONS

There were six SVM algorithms trained to detect water stress and classify lettuce plants as healthy or not-healthy. Out of the six SVM kernels trained and tested, the cubic SVM performed the best. It had the highest training accuracy, testing accuracy and an F1 score of 0.8. These results are promising, and it didn't require much data to achieve this accuracy. Additionally, training the SVM model took less than 10 seconds. It is important to compare the performance of this model with methods discussed in the literature to detect water stress in plants.

Literature investigating detection of water stress in lettuce plants is limited, but the review of literature examined successful methods of measuring water stress in other types of plants such as tomato plants (Lak et al. 2021), strawberry plants (Li et al. 2019), and New Guinea Impatiens (Kacira et al. 2002). Li et al. (2019) used infrared and visible light images captured using a machine vision system to measure average temperature of the plant. However, the results of this study that successfully correlated area crop water stress index (CWSI) (r = 0.8805) to stomatal conductance of the strawberry plants were measured using temperature data that was manually collected. So, it is unclear how the automatic temperature measurements would perform. This method also uses spectral data in the infrared wavelengths, which was outside the scope of this project. Kacira et al. (2002) developed a system for the early detection of water stress of New Guinea Impatiens under low humidity and high-water demand conditions. Coefficient of relative variation of TPCA (CRV_{TPCA}) data had a high success rate in detecting water stress 5 to 29 hours prior to visual detection. There was one paper that induced water and nutrient stress on lettuce plants to measure plant development (Ling et al. 1996). This research used machine vision extracted spectroscopic data and concluded that a ratio of two wave bands centered at 1450 nm and 780 nm could be used to determine moisture content of the lettuce plants. However, these methods do not provide automatic classification of water stressed lettuce plants and require some form of human intervention for data analysis. This is where a combination of machine vision and machine learning methods is superior.

To the best of the author's knowledge at the time this research was conducted, there is no model developed to detect water stress in lettuce plants. However, Mao et al. (2015) developed an extreme learning machine (ELM) to measure total nitrogen in lettuce plants using a fusion of spectroscopy and computer vision extracted features. This model had optimal performance when 30 hidden nodes were used, with a correlation coefficient of prediction of 0.8864 and root-mean-square error of prediction of 0.3231%. ELM is a fast-learning algorithm, but it is a multi-layer network model. Training the ELM model did not require a large data set (90 samples in total), but it was undeclared how long it took to train and test the model. Community developed toolboxes are available in MATLAB to train and test ELM's. One thing to consider is that when spectroscopic data was removed from the calibration data set, the model performed worse, with a correlation coefficient of prediction of 0.6537, and a root-mean-square error of prediction of 0.6015. Lak et al. (2021) developed a multilayer perceptron neural network (MLPNN) to classify water stressed tomato plants using features extracted from visible and thermal images. This model had optimal performance when 10 hidden nodes were used, with a correlation coefficient of 0.9905 and mean-square error of 0.0061 for the testing data. The overall algorithm accuracy was 83.3%. The error of the algorithm to classify normal plants was 26.7% and the error to classify water-stressed lettuce plants was 6.7%. Again, this is a multi-layer network model. It was undeclared how much data was required, and how long it took to train and test the model (processing time), so it is difficult to compare results. Community developed toolboxes are available in MATLAB to train and test MLPNN's.

The model developed in this project was simple SVM classifier, that was quick to train, didn't require a large data set, and used two features, TPCA and energy, collected from visible RGB images, with relatively high accuracy and F1 score, to classify healthy and water stressed lettuce plants. These results are promising, and more consideration should be given to improve the robustness of the system.

CHAPTER 6 PRACTICAL DESIGN CONSIDERATIONS

This chapter contains practical designs considerations, including hardware, software and cost, to develop the system beyond conceptualisation.

6.1 HARDWARE

Hardware requirements for the physical system based on the conceptualised prototype includes a camera capable of taking RGB images, and a microcontroller to collect and transmit, or process the data. Other auxiliary hardware requirements include a way to mount the system, and an optional serial cable.

For the camera, since this project did not consider the effect of resolution on the accuracy of the system, it is unclear what kind of resolution is required. However, a Raspberry Pi Camera has a resolution similar to the mobile phone used in this project and is capable of taking RGB images (Raspberry Pi n.d.). A Raspberry Pi V3 Camera costs around $50 on the Pi Australia website (Pi Australia n.d.). This camera is a part of the Raspberry Pi suite and is designed to work with a Raspberry Pi microcontroller. A microcontroller, like a Raspberry Pi 5 board, could handle all data collection, processing and transferring, and is relatively cheap. A Raspberry Pi 5 (4GB) board costs roughly $101 AUD on the Core Electronics website (Core Electronics n.d.). Processing power or RAM requirements to collect and process data is unclear. However, data can be processed externally after data has been transferred, thus the microcontroller would not need much computing power. This Raspberry Pi board has Bluetooth and WiFi capabilities, which could be used to transmit data wirelessly. However, on a space station, this may not be desirable or possible. Instead, a serial data cable would be needed. A USB cable could be used and would cost roughly $10 from Officeworks (Officeworks n.d.).

6.2 SOFTWARE

Data can be processed onboard the microcontroller and would require to MATLAB code to be rewritten in a language that is compatible with Raspberry Pi boards, like Python. Otherwise, data can be transferred from the microcontroller board to a remote PC. PuTTY can be used to remotely access the SSH port of the Raspberry Pi to transfer the data to an external device. PuTTY is an open source remote access tool that uses a File Transfer Protocol (FTP) (On Logic 2023). Similarly, it is possible to download MATLAB support software on a Raspberry P microcontrolleri, to access the data via an online MATLAB account. Using this method means the data can be acquired from imaging devices and sensors connected to the Raspberry Pi (MathWorks n.d.g), but it would require a device with internet connection to access MATLAB online, or the MATLAB app can be downloaded on the external device. MATLAB cannot be directly downloaded on the Raspberry Pi board. This would require a MATLAB licence, which would cost roughly $209 for a Home license according the MathWorks Pricing and Licensing website (MathWorks n.d.d).

6.3 IMPLEMENTATION

It would be feasible to develop a physical system that could be integrated into existing systems relatively easily. It is small and cheap, and the estimated cost of a system like this is roughly $160 to $370. Further analysis would need to be done to determine hardware specification requirements to run this code, and it's possible that this estimated cost could be even cheaper as the developed code and SVM are not computationally intensive.

CHAPTER 7 PROJECT CONCLUSIONS

To conclude, this project successfully addressed a gap in the existing knowledge. The proposed system is affordable and can successfully classify water stressed lettuce plants as unhealthy. A cubic SVM model was developed to classify water-stressed lettuce based on features extracted from RGB images, with high accuracy. Audio, a novel approach in assessing eating quality, was successfully correlated to leaf water content. Specifically, amplitude range. This was a positive outcome for this project, but further research could be conducted.

At the beginning of the project, specific objectives were specified. To conclude this project, it is pertinent to reflect whether the objectives were met. The first objective detailed conducting a comprehensive literature review. Chapter 2 of this dissertation gave a detailed literature review of eating and nutritional quality metrics of lettuce, existing machine vision systems, machine learning models, and associated plant features related to stress. The second objective was to identify an appropriate quality metric and conceptualise a machine vision system to autonomously assess the eating and nutritional quality of a lettuce plant. In chapter 2, a quality metric was identified, that specified eating quality characteristics of commercially sold Romain lettuce plants. In chapter's 3, 4 and 5, an autonomous machine vision system was conceptualised successfully to detect water stress and classify whether a lettuce plant was healthy or unhealthy, relating to the plant's eating and nutritional quality. The conceptualised system automatically extracts plant features from RGB images, processes the data, then associates a class using the developed Cubic SVM model. The second objective ties into the third project objective, which develops a prototype of the machine vision system, including hardware and software for data collection and model training. Throughout the dissertation, hardware and software used to satisfy this objective was highlighted. Hardware considerations included a camera that could take RGB images, and MATLAB software to process the data. Furthermore, in chapter 6 of this dissertation, practical design considerations were made to develop the system beyond the prototype stage. The system prototype and a ground truth measure of quality was used to collect data of the lettuce plant under stress, which addresses the fourth project objective. The ground truth measures of quality include NGRDI for biomass estimation, audio data to measure the crispness, and the supermarket quality metrics for commercially sold Romaine lettuce. The fifth objective considers validation and evaluation of the performance of the system in identifying plant stress indicators under different conditions. To further explain this objective, two groups of lettuce plants were given different conditions (watered vs non-watered), and the system was able to classify the plants into healthy or not-healthy based off the plant stress indicators. Chapter 5 gives an in-depth analysis into the overall performance of the trained classification model, and the results were considered to quite high given the context, with an accuracy of 75%, and an F1 score of 0.8. This is not a critical application and could be used in conjunction with visual interpretation of plant health, to identify whether the plant is okay to eat, or not. The findings of the developed model were compared to other method of detecting water stress in lettuce plants, in the conclusion of Chapter 5. The last objective, which was to provide recommendations for improvements and further research, is discussed next.

7.1 RECOMMENDATIONS FOR FURTHER RESEARCH

This section lists some recommendations for further research which could be investigated. The first recommendation is an adjustment to the methodology, which would improve the quality of the data. White-balance calibration should be performed before the images of the plants are taken. This would ensure the brightness index is consistent across all images and would remove the effect of lighting variation in the data. Another recommendation, which is an extension of the first one, is collecting data in a controlled environment. This would remove humidity, temperature and light variance and reduce the effect of these variables on the health of the plant, and better isolate water stress plant responses. Robustness of the model could be improved by increasing the number of plants per group (watered and non-watered) to get more variation in the data. The SVM model should be retrained and compared to the results presented in this project. Similarly, the SVM model is specific to Romaine lettuce. Different types of lettuce cultivars (green and red cultivars) could be investigated by following the same methodology. This project focused on drought stress, but flood stress could also be investigated. This will aid in drawing more confident conclusions about whether machine vision can be used as a tool to assess the eating quality of a growing leafy plant. More specifically, the tool may perform better or worse at detecting water stress symptoms when considering different types of lettuce plants. Human error was introduced during collection of audio data. This could be removed by developing a mechanism to snap the leaf without human intervention. The quality of the audio data could also be improved by recording audio files in an acoustic chamber. Audio features and their correlation to NGRDI should be re-evaluated using this tool. Another recommendation is using chemical analysis or spectral equipment to obtain nutritional composition as a ground truth to compare water stress status of the lettuce plants to, but this may not be possible in an undergrad project as it would be expensive. Lastly, building the system prototype beyond conceptualization, into a physical device, to take images of the plant periodically and process them automatically, before feeding them through the SVM classification model in real time. This would solidify the possibility of using such a device in space and on earth.

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APPENDIX A – PROJECT SPECIFICATION

Title : Can Machine Vision Assess the Eating Quality of a Growing Leafy Plant?

Name : Sarah Mabee

Student ID : [redacted]

Supervisors : Dr Alison McCarthy

1.2 Objectives and Aims :

The primary objective of this research is to determine if machine vision can be used as a tool to assess the eating quality of a growing leafy plant, and consequently, assess the nutritional quality, with potential applications in Space and on Earth.

1.3 Specific Objectives :

This section lists some more specific objectives that this research project aims to address. These specific objectives are aligned with the phases of the project work plan. These include:

1. Conduct literature review of current machine visions systems, as well eating and nutritional quality metrics of food crops.
2. Identify an appropriate quality metric and conceptualize a machine vision system that can autonomously assess the eating and nutritional quality of a plant.
3. Develop a machine vision system prototype including software and hardware for data collection and model training.
4. Collect data on the plant under stress using the system prototype and a ground truth measure of quality.
5. Validate and evaluate the performance of the machine vision model in identifying plant stress indicators under different conditions. Compare findings with other conventional methods.
6. Provide recommendations for improvements and further research.

1.4 Expected Outcomes :

The expected outcomes for this research project are as follows:

- Draw conclusions about whether machine vision is or is not an effective and superior tool in identifying plants under stress, by evaluating the performance and accuracy of the detections.
- Gain a better understanding of which qualities of a growing plant have a direct correlation to the eating and nutritional quality of a leafy plant.
- Identify limitations and provide recommendations for further improvements. Recommend which applications this tool could be used in and the benefits of optimizing yield and minimizing food waste.

1.5 Materials :

Resources were acquired by the University of Southern Queensland (UniSQ) and the student, with a financial limit of $150 provided by UniSQ to assist in securing resources. The materials required for the methodology of the initial investigation are itemised below, and a summary of how each item was used is provided.

- The model plant in the initial investigation is Romaine lettuce (*Lactuca sativa* L. var. *longifolia*).
- Liquid fertiliser was used to reduce nutrient deficiency stresses in the control group.
- A mobile phone was used to collect data (RGB images and audio recordings) throughout the duration of the data acquisition period.
- A gooseneck phone holder was used to ensure that the distance between the camera and the plant remained constant while collecting data.
- A 510 mm x 635 mm piece of white card was used as a background when taking images. This white card made image segmentation (background vs. foreground) simpler and more accurate.
- A flat table was used in the experiment setup.
- An artificial light source (ceiling light) improved light uniformity between images. The use of the light source reflects conditions on a space station.

- A conventional oven was used to dehydrate the control and treatment lettuce plants at the end of the data acquisition period. Dry mass was used to determine water content.
- MATLAB (Version R2023b) and associated toolboxes were used for data processing.
- Microsoft Excel (Version 2408) was used to data logging and data analysis.
- Microsoft Word (Version 2408), a laptop, a mouse, a keyboard, a monitor, and a USB are auxiliary resources used for the preparation of this research.

APPENDIX B - RISK ASSESSMENT

4672	RISK DESCRIPTION	STATUS	TREND	CURRENT	RESIDUAL
	Project: Analysis of Lettuce Quality using Machine Vision	Live		Low	Very Low

RISK OWNER	RISK IDENTIFIED ON	LAST REVIEWED ON	NEXT SCHEDULED REVIEW
Sarah Mabee	11/05/2024	11/05/2024	11/05/2025

RISK FACTOR(S)	EXISTING CONTROL(S)	CURRENT	PROPOSED CONTROL(S)	TREATMENT OWNER	DUE DATE	RESIDUAL
Microbial colonies and pathogens can grow on the lettuce plants. There is a potential for coming into contact with harmful bacteria such as e.coli.	Control:	Low	Wear gloves when handling the plant.		11/05/2024	Very Low
Writing the dissertation requires sitting for long periods of time. Typing may cause repetitive strain injuries.	Control: Take regular breaks. Stretch.	Low	Use a standing desk.		11/05/2024	Very Low
There is a risk of delaying the progress of the project if power is lost for an extended period of time.	Control: Prepare to make arrangements if notified of upcoming power loss. Take back ups of project work on an external storage device/cloud service. Use university computers and printers.	Very Low	No Control:			Very Low

APPENDIX C – TPCA CODE

```
% ---------------------------------------------------------------------- %
%   Author: Sarah Mabee
%   Date: 24/07/2024
%   Description: This code uses the function from the Image Processing and
%   Computer Vision MATLAB toolbox to segment images of lettuce.
%
%   Input: This code takes image files as inputs.
%
%   Outputs: TPCA count.
% ---------------------------------------------------------------------- %

%   clear the workspace and command window
clear;
clc;

%% IMAGE IMPORT
rgb_1 = imread("20240516_AM_A(1).jpg");
rgb_2 = imread("20240516_AM_A(2).jpg");
rgb_3 = imread("20240516_AM_A(3).jpg");
rgb_4 = imread("20240516_AM_A(4).jpg");

%% IMAGE RESIZE

size = size(rgb_1);

% Resize images to 1414 px by 1885 px
rgb_1 = imresize(rgb_1,[1414 1885]);
rgb_2 = imresize(rgb_2,[1414 1885]);
rgb_3 = imresize(rgb_3,[1414 1885]);
rgb_4 = imresize(rgb_4,[1414 1885]);

% Dynamic resizing for accuracy test
% rgb_1 = imresize(rgb_1,[size(1) size(2)]);
% rgb_2 = imresize(rgb_2,[size(1) size(2)]);
% rgb_3 = imresize(rgb_3,[size(1) size(2)]);
% rgb_4 = imresize(rgb_4,[size(1) size(2)]);

%% FUNC CALL
[tpca_1,hue_1,hue_bin1] = tpca_func(rgb_1);
[tpca_2,hue_2,hue_bin2] = tpca_func(rgb_2);
[tpca_3,hue_3,hue_bin3] = tpca_func(rgb_3);
[tpca_4,hue_4,hue_bin4] = tpca_func(rgb_4);

%% TPCA AVG
% find the average tpca of the four images
tpca_avg = (tpca_1+tpca_2+tpca_3+tpca_4)/4;

%% ACCURACY TEST
% ground truth TPCA
BW_test = createMask4(rgb_1);
BW_test = imfill(BW_test,'holes');

tpca_true = sum(BW_test(:));

accuracy = abs(((tpca_true-tpca_avg)/tpca_true)*100);
accuracy_percent = 100 - accuracy

%% DISPLAY

% imshow(rgb_1);
% imshow(BW_test);
imshow(hue_bin1);

% imshowpair(rgb_1,BW_test,'montage');
```

```matlab
% ---------------------------------------------------------------------- %
%   Author: Sarah Mabee
%   Date: 24/07/2024
%   Description: This function code converts the RGB image to the HSV colour
%   space, then isolates the hue channel. Pixel values less than 60 degrees
%   and more than 120 degrees are removed. The hue image is then binarized
%   and TPCA is calculated.
%
%   Input: This code takes an image file as the input parameter.
%
%   Outputs: TPCA count.
% ---------------------------------------------------------------------- %

function [TPCA,hue_thresh,hue_bin] = tpca_func(rgb)

% convert rgb image to hsv colour space
hsv = rgb2hsv(rgb);

% isolate hue channel
hue = hsv(:,:,1);

% remove pixel values less than 60 degrees and more than 120 degrees.
hue_thresh = hue.*(hue<0.3334);
hue_thresh = hue_thresh.*(hue_thresh>0.1667);

% binarize the hue image
hue_bin = imbinarize(hue_thresh);

% fill holes in image
hue_bin = imfill(hue_bin,8,'holes');
% removes all blobs of pixels less than 150000
hue_bin = bwareaopen(hue_bin, 150000);

% count white pixels in image
TPCA = sum(hue_bin(:));

end
```

Below is a list of the functions used, and the version of MATLAB required to use them. These functions are included in the MATLAB Image Processing Toolbox:

- imread (R2006a)
- imresize (R2006a)
- rgb2hsv (R2006a)
- imbinarize (R2016a)
- imfill (R2006a)
- bwareaopen (R2006a)
- imshow & imshowpair (R2006a and R2012a respectively).

APPENDIX D – COLOUR CODE

```
% ---------------------------------------------------------------------- %
%   Author: Sarah Mabee
%   Date: 31/8/2024
%   Description: This code uses the functions from the Image Processing
%   toolbox to extract colour features from the RGB image.
%
%   Input: This code takes image files as inputs.
%
%   Outputs: Colour features
%
%   Functions:
%       1. tpca_func.m
% ---------------------------------------------------------------------- %

%   clear the workspace and command windowclear;
clear;
clc;

%% IMPORT IMAGE
rgb_plant = imread("20240816_AM_A(1).jpg");

% leaf_masked = imread("20240827_AM_B_leaf_masked.jpg");
% leaf_mask = imread("20240827_AM_B_leaf_mask.jpg");

%% MASK IMAGES
% Plant Mask
[tpca_plant,hue_plant,hue_bin_plant] = tpca_func(rgb_plant);
% Overlay
masked_plant = bsxfun(@times, rgb_plant, cast(hue_bin_plant,'like',rgb_plant));


%% COLOUR FEATURES
% isolate colour channels
% PLANT
red = masked_plant(:,:,1);
grn = masked_plant(:,:,2);
blu = masked_plant(:,:,3);
% LEAF
% red = leaf_masked(:,:,1);
% grn = leaf_masked(:,:,2);
% blu = leaf_masked(:,:,3);


% calculate average values
num_pixels = tpca_plant;
% num_pixels = sum(leaf_mask(:));

red_avg = sum(red,"all")/num_pixels;
grn_avg = sum(grn,"all")/num_pixels;
blu_avg = sum(blu,"all")/num_pixels;


%% VEGETATION INDICES
%
GCC = grn_avg/(red_avg+grn_avg+blu_avg)
RGBVI = (grn_avg^2-(blu_avg*red_avg))/(grn_avg^2+(blu_avg*red_avg))
MGRVI = (grn_avg^2-red_avg^2)/(grn_avg^2+red_avg^2)
GLI = (2*grn_avg-red_avg-blu_avg)/(2*grn_avg+red_avg+blu_avg)
VEG = grn_avg/(red_avg^(0.667)*blu_avg^(0.334))
vNDVI = 0.5268*(red_avg^(-0.1294)*grn_avg^(0.3389)*blu_avg^(-0.3118))
HUE = atan(2*(blu_avg-grn_avg-red_avg)/30.5*(grn_avg-red_avg))
GR = grn_avg/red_avg
ExB = 1.4*blu_avg-grn_avg
BI = ((red_avg^2+blu_avg^2+grn_avg^2)/3)^2
BGI = blu_avg/grn_avg
NGRDI = (grn_avg-red_avg)/(grn_avg+red_avg)
```

APPENDIX E – TEXTURAL CODE

```
% -------------------------------------------------------------------- %
%   Author: Sarah Mabee
%   Date: 31/8/2024
%   Description: This code uses the functions from the Image Processing and
%   Computer Vision MATLAB toolbox to determine GLCM features of lettuce
%   plants.
%
%   Input: This code takes image files as inputs.
%
%   Outputs: GLCM features (entropy, correlation, energy, homogeneity)
%
%   Functions:
%       1. tpca_func.m
% -------------------------------------------------------------------- %

%   clear the workspace and command windowclear;
clear;
clc;

%% IMPORT IMAGE
rgb_plant = imread("20240816_AM_A(1).jpg");


% convert images from rgb to grayscale
gray_plant = rgb2gray(rgb_plant);


%% MASK IMAGES

% Plant Mask
[tpca_plant,hue_plant,hue_bin_plant] = tpca_func(rgb_plant);

% Overlay
masked_plant = bsxfun(@times, gray_plant, cast(hue_bin_plant,'like',gray_plant));
% imshow(masked_plant);


%% GLCM FUNCTION

offsets = [0 1; -1 1;-1 0;-1 -1];

% Perform GLCM with 16 gray-levels and graylimits
[glcm_plant, SI_plant] = graycomatrix(masked_plant,'NumLevels',16,'GrayLimits',[],'Offset',offsets);

% Return textural features
stats_plant = graycoprops(glcm_plant)
```

APPENDIX F – AUDIO CODE

```
% ------------------------------------------------------------------- %
%   Author: Sarah Mabee
%   Date: 24/07/2024
%   Description: This code utilisez the audioLevelMeter object from the
%   Signal Processing and Communications MATLAB toolbox.
%
%   Input: This code requires a valid audio file as input.
%
%   Output: This code will play back the audio file and display it in an
%   interactive tool for analysis. The audioLevelMeter also finds the peak
%   level in dBFS.
% ------------------------------------------------------------------- %

%   Clear the workspace and command window
clc;
clear;

%% Create audioLevelMeter object

% input audio file to be analysed to reader
reader = dsp.AudioFileReader("20240516AM_A.m4a");
player = audioDeviceWriter(SampleRate=reader.SampleRate);
lvlMeter = audioLevelMeter(SampleRate=reader.SampleRate,WindowLength=reader.SamplesPerFrame);

% create a timescope object

scope =
timescope(SampleRate=reader.SampleRate,LayoutDimensions=[2,1],TimeSpanSource="property",TimeSpan=5);
scope.Title = "Audio Waveform";
scope.YLabel = "Amplitude";
scope.YLimits = [-1.25, 1.25];

scope.ActiveDisplay = 2;
scope.Title = "Peak Level";
scope.YLabel = lvlMeter.Units;
scope.YLimits = [-40, 6];
%scope.ChannelNames = ["Channel 1", "Channel 2", "Channel 1", "Channel 2"];

% play back the audio file
while ~isDone(reader)
    audioIn = reader();
    peakLevel = lvlMeter(audioIn);
    peakLevelHold = peakLevel.*ones(size(audioIn));
    scope(audioIn,peakLevelHold);
    player(audioIn);
end
```