

**PREDICTION OF LEAF MECHANICAL  
PROPERTIES BASED ON GEOMETRY  
FEATURES WITH DATA MINING**

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**2019**

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PROPERTIES BASED ON GEOMETRY  
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**By**

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**Thesis submitted in fulfilment of the  
requirements for the degree of  
Master of Science**

**October 2019**

## **ACKNOWLEDGEMENT**

I wish to express my deepest gratitude to all kind-hearted individuals who have contributed to this valuable thesis in many ways throughout my adventure in the whole study. First and foremost, I would like to acknowledge my supervisor, Dr. Loh Wei Ping, for her patience, tireless effort and prompt assistance. I must appreciate the wisdom of her supervision which had broaden my perspective and led me to be relatively independent. Without her suggestions, encouragement and guidance, this thesis would not have been materialized.

Secondly, I would like to thank Universiti Sains Malaysia for funding my studies, which have relieved my financial burden and provided all the support I needed to complete this work.

I would also like to express my sincere gratitude to lecturers, technicians and fellow friends for their encouragements, supports and suggestions. I am grateful for their constant support and motivations. Last but not least, my gratitude is extended to my family members for their continuous moral support throughout this journey in the pursuit of my goals.

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## LIST OF SYMBOLS

$A$	Attribute
$c_i$	Equation constant
$F_T$	Tearing force or load applied on leaf specimen in UTM
$j_i$	Attribute constant
$k_i$	Constant for species attribute
$K_i$	Constant for other attributes
$l_a$	Apical extension length
$l_b$	Basal extension length
$r$	Pearson correlation
$S$	Species
$S_T$	Tear strength
$W_T$	Work-to-tear
$SW_T$	Specific work-to-tear

## LIST OF ABBREVIATIONS

ANN	Artificial Neural Networks
ANOVA	Analysis of Variance
LM	Linear Model
LMA	Leaf Mass per Area
MLP	MultilayerPerceptron
RRSE	Root Relative Square Error
SPSS	Statistical Package for the Social Science
SVM	Support Vector Machines
USM	Universiti Sains Malaysia
UTM	Universal Testing Machine
WEKA	Waikato Environment for Knowledge Analysis

# RAMALAN SIFAT MEKANIK DAUN BERDASARKAN CIRI-CIRI GEOMETRI DENGAN PERLOMBONGAN DATA

## ABSTRAK

Sifat mekanikal daun biasanya ditentukan dengan pendekatan ujian mekanikal untuk mengkaji jangka hayat daun, daya ketahanan anti herbivoranya dan fungsi-fungsi ekologi. Pengaruh habitat dan sumber alam sekitar seperti nutrien, cahaya matahari, dan air, serta kepelbagaian spesies dari segi perbezaan anatomi daun dan komposisi kimianya telah dipertimbangkan dalam kajian lampau. Walau bagaimanapun, sifat mekanik daun dari segi ciri-ciri geometri dan morfologi masih lagi kabur. Tujuan utama kajian ini adalah untuk mengkaji pengaruh ciri-ciri atribut geometri untuk meramal sifat mekanik daun berdasarkan empat penunjuk yang berbeza dengan menggunakan pendekatan perlombongan data. Kajian eksperimen yang membabitkan 20 spesies tumbuh-tumbuhan terestrial telah dijalankan. Sejumlah 600 x 23 ciri-ciri atribut yang terdiri daripada ciri-ciri geometri daun, ciri-ciri pembeza layan dan kuantiti terbitannya telah diperolehi melalui pengukuran, pemerhatian lapangan dan ujian koyakan dengan menggunakan Mesin Uji Serbaguna (UTM). Data rekod ditapis dengan normalisasi data sementara data terpercil dibuang sebelum analisis regresi dilakukan dengan bantuan perisian Waikato Environment for Knowledge Analysis (WEKA). Penunjuk sifat mekanikal daun: daya koyakan ( $F_T$ ), kekuatan koyakan ( $S_T$ ), kerja untuk koyak ( $W_T$ ), dan kerja untuk koyak tertentu ( $SW_T$ ) ditakrifkan sebagai atribut kelas. Sifat mekanik daun diramal dengan menggunakan algoritma-algoritma GaussianProcess, LinearRegression, MultilayerPerceptron (MLP), SMOreg, M5P dan REPTree oleh perisian WEKA, disahkan dengan indeks ralat punca kuasa dua relatif (RRSE). Penemuan menunjukkan bahawa ramalan

berangka bagi  $F_T$  dan  $S_T$  (RRSE  $\sim 25\%$ ) adalah dua kali ganda lebih baik daripada  $W_T$  dan  $SW_T$  (RRSE  $\sim 50\%$ ) dalam keenam-enam algoritma yang diuji. Prestasi ramalan terbaik diperolehi bagi penunjuk  $F_T$  dengan algoritma M5P (RRSE = 22.44%). Model linear dan peraturan yang dibangunkan oleh algoritma M5P telah digunakan untuk pemodelan ramalan penunjuk  $F_T$  yang terdiri daripada 14 atribut. Atribut 'Spesies' memberi sumbangan terbanyak dalam model regresi M5P. Penemuan juga menunjukkan bahawa ciri-ciri geometri daun sahaja tidak mencukupi untuk mewakili sifat mekanik daun. Model regresi M5P telah dipermudah lagi kepada 9 atribut yang menunjukkan tidak perbezaan signifikan antara model regresi M5P dan model mudah (RRSE = 21.37%).

# PREDICTION OF LEAF MECHANICAL PROPERTIES BASED ON GEOMETRY FEATURES WITH DATA MINING

## ABSTRACT

The leaf mechanical properties are typically determined by mechanical tests to study the leaf's lifespan, its anti-herbivore defences and the ecological functions. The influences of habitats, environmental resources such as nutrient, light, and water, and species diversity on the leaf anatomies and their chemical compositions were previously considered. However, the mechanical properties of the leaves from the geometry and morphology aspects are still vague. The main goal of this study is to examine the effect of various geometrical attributes to predict the leaf mechanical properties based on four different indicators using data mining approach. An experimental study involving 20 different species of the terrestrial plants were conducted. A total of 600 x 23 features attributes comprising of leaf geometrical features, discriminant features and its derived quantities were collected by measurements, field observations and the tearing test performed using the Universal Testing Machine (UTM). The recorded data were screened on data normalization while the outliers were discarded prior to regression analysis aided by the Waikato Environment for Knowledge Analysis (WEKA) tool. The leaf mechanical property indicators: Tearing Force ( $F_T$ ), Tearing Strength ( $S_T$ ), Work-to tear ( $W_T$ ), and Specific Work-to-tear ( $SW_T$ ) identified were predefined as the numeric class attribute. The leaf mechanical properties indicators were predicted using the GaussianProcess, LinearRegression, MultilayerPerceptron (MLP), SMOreg, M5P and REPTree algorithms of WEKA tool, verified on Root Relative Squared Error (RRSE) evaluation index. Findings showed that the numerical predictions on  $F_T$  and

$S_T$  (RRSE ~ 25%) were about two folds better than the  $W_T$  and  $SW_T$  (RRSE ~50%) in the six algorithms tested. The best prediction performance was gained on  $F_T$  indicator using the M5P algorithm (RRSE = 22.44%). The linear models and rules developed from the M5P algorithm were adopted for the  $F_T$  indicator prediction modelling of 14 attributes. The ‘Species’ attribute contributes the most for the M5P regression model. Findings also indicate that leaf mechanical properties were insufficient to be represented by its geometry features alone. The M5P regression model was further simplified into 9 attributes showing insignificant difference determined on the paired T-test between the RRSE achieved by M5P regression and the simplified model (RRSE = 21.37%).



## **CHAPTER ONE**

### **INTRODUCTION**

#### **1.0 Overview**

The leaf mechanical properties were commonly quantified by various mechanical testings such as punching test, shearing test and tearing test adopted from material engineering concepts. This chapter presents the background of the leaf mechanical properties' predictions. This is followed by the research challenges encountered in mechanical testings such as large amounts of data, tiresome process and failure to measure successively leading to the alternative approach; developing a predictive model based on the concept of data mining. This research aims to construct a predictive model to determine the mechanical properties of the leaf with the relevant qualitative and quantitative attributes being modelled.

## 1.1 Study background

Leaf mechanical properties are associated with its physiological processes and functional bases including the light interception, photosynthesis rate, water transpiration, metabolism level, and energy balance (Jing et al., 2016; Onoda et al., 2008, 2011; Read & Sanson, 2003; Read et al., 2006). Through the evolutionary process of environments adaptation, the unique structures of the leaves with ‘brilliant’ mechanical characteristics were shaped (Wang et al., 2010). The leaf structures and geometry vary considerably from species to species, which are intensely subjected to climate, light intensity, nutrients availability, maturity, ecological competition with neighbourhood plants and other factors such as herbivores (Chitwood & Sinha, 2016; Givnish, 1979; Nicotra et al., 2011; Tsukaya, 2005).

The leaf geometry features referred to its structures constructed by a set of geometric elements like points, lines, curves or surfaces. Most of the time, lamina thickness, lamina area, lamina width, lamina length, lamina density and the vein density are denoted as leaf geometry in the studies related to leaf mechanical properties (Anten & Schieving, 2010; Kitajima & Poorter, 2010; Lucas et al., 2012; Onoda et al., 2011; Pierantoni et al., 2019; Sack & Scoffoni, 2013). Meanwhile, other factors such as leaf perimeter, leaf diameter, leaf margins, leaf convexity, leaf ratios and shapes are also considered in the study of plants identification in relation to features (Cobo-Quinche et al., 2019; Ellis et al., 2009; Lee et al., 2017; Musić & Gagula-Palalić, 2016; S. G. Wu et al., 2007).

The physical properties inherent in the leaf are assessed via mechanical testing. Three typical leaf mechanical tests commonly adopted are the punching,

tearing and shearing tests (Onoda et al., 2008; Sanson et al., 2001). Comprehensive guidelines on the procedures and techniques for leaf mechanical testing approaches have been outlined previously (Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013; Vincent, 1990). The measurement techniques, machinery, test morphology and fracture modes were specified in the context of Vincent (1990). The apparatus, leaf specimen selection and preparation, and limitations of the approaches were highlighted in the context of Cornelissen et al. (2003) and Pérez-Harguindeguy et al. (2013). In addition, previous studies (Enrico et al., 2016; Onoda et al., 2011) proved that the leaf mechanical property indicators strongly correlated with each other. Therefore, the choice of testing approach was dependent on the research interests and any of the mechanical tests can be used as the general indicator. Previous studies on the mechanical properties of the leaves (Anten et al., 2010; Enrico et al., 2016; Onoda et al., 2015) were lengthy and tedious in the sense that it took few hours for every single testing including the sampling, measuring, setting up and testing steps. Moreover, typical mechanical testing is destructive methods and successive measurements on the same leaf are impossible.

## 1.2 Problem statement

Leaf mechanical properties were usually examined by experimental approaches such as punching, shearing and tearing tests. In order to perform the testing, previous works either use simple and inexpensive customized experimental setups such as weight loads with a scale for tearing test protocols and penetrometer for punching test (Balsamo & Orkwiszewski, 2008; Balsamo et al., 2005; Niklas, 1993), or some expensive and sophisticated instruments like Chatillon Universal Tension and Compression Tester or Instron Universal Testing Machine (UTM) for greater accuracy (Anten et al., 2010; Balsamo et al., 2003; Edwards et al., 2000; Hernández, 2010; Liu et al., 2007; Onoda et al., 2015; Read & Sanson, 2003; Read et al., 2006; Sanson, et al., 2006; Witztum & Wayne, 2014). However, these destructive methods are time-consuming and labour-intensive in terms of setups and leave specimen preparation are necessary for each testing. These approaches also caused inconvenience and difficulties for repetitions, in particular, to repeat the measurement on the same leaf specimen. There might be a bias in results obtained without repetitions due to the composite, anisotropic and heterogeneous characteristics of the leaf.

Past studies usually compare the results with statistical analysis methods to investigate the bivariate relationship between leaf geometry and leaf mechanical properties. There has been an abundance of recorded data from various leaf mechanical tests conducted. The information stored in voluminous recorded data could be fruitful to return the multivariate classification analysis as well as to identify unknown patterns. Thus, data mining approach could support the study of leaf mechanical properties mainly to shorten the computational or analytical time as compared to the conventional statistical analyses. Besides, data mining adoption in

plant science area is currently limited to plant recognition, diseases detection and yields prediction. Data mining implementation from the perspectives of predicting the leaf mechanical properties based on the plant geometry features is relatively a new study.

### **1.3 Objectives**

The general objective of this research is to develop a predictive model to determine the leaf mechanical properties based on the leaf geometry. The specific objectives include:

- a) To correlate between the leaf geometry features and its mechanical properties.
- b) To identify the best leaf mechanical properties indicator that can be represented by leaf geometry features using the WEKA software.
- c) To identify the appropriate algorithm and optimal modelled attributes for the predictive classification model.

#### **1.4 Scope of the study**

This research is an experimental case-study based involving 20 species of terrestrial plants grown at the nursery of the Development Department in Universiti Sains Malaysia (USM) Engineering Campus. The project involves different stages of data collection, data pre-processing and data analysis. In the data collection stage, 600 instances of 23 featured attributes were acquired through field observation, measurements and tearing test. The geometrical attributes of the leaf were measured quantitatively by using measuring instruments and ImageJ software. The mechanical properties of the leaf are investigated using the tearing test conducted on Instron UTM, model 3367 equipped with a 500N static load cell. The Statistical Package for the Social Sciences (SPSS) and WEKA tools for data pre-processing and data analyses stages. The study attributes consists of quantitative and qualitative data. At the data analysis level, the statistical data analysis that includes descriptive statistics, Pearson correlation test and Welch statistical test were used prior to the data mining approach. The data mining levels involve regression studies, result validation with paired T-test on various algorithms or indicators for comparative breakdown, and model interpretation and improvement efforts (residuals analysis and attribute relative importance analysis). Six WEKA algorithms: GaussianProcess, LinearRegression, SMOreg, MultilayerPerceptron (MLP), M5P and REPTree were used to predict the mechanical properties of the leaf. This study shall benefit the botany, paper and textile industries which commonly performed the mechanical testing in order to understand the impact of habitats or relationships between leaf biomechanical properties with the associated insect herbivores densities (plant-insect studies) and findings of alternate natural materials used as reinforcement materials or replacement for existing fibre composites.

## **1.5 Organization of thesis**

This thesis consists of five chapters:

Chapter 1 presents an introduction of the research, the background of leaf mechanical properties, leaf geometry features and a brief summary of the application of data mining in developing the predictive model. The problem statement is presented in Section 1.2, the objectives and study scope are discussed in Sections 1.3 and 1.4.

Chapter 2 explores the literature on the existing leaf mechanical properties studies, leaf related modelling and an overview of the elements in data mining applications. The first section describes plant biomechanics and discusses the mechanical property analysis focusing on the leaf organ. Meanwhile, the second section presents the leaf modelling and classification studies in plant science. The related works done by previous studies were reviewed on existing approaches and the study attributes are considered. The various numerical classification algorithms, data mining software tools and the performance measures for the algorithm are reviewed in the following sections.

In chapter 3, the methodology of the study is explained. First, the leaves sampling, data collection including the methods and tools used, and the data attributes are presented. This is followed by the description of data mining implementations aided by WEKA including the data pre-processing, regression analysis and the performance measures. The interpretation and simplification efforts of the prediction model are discussed subsequently and defined by residuals analysis and relative importance of modelled attributes.



Chapter 4 presents the results of numeric classification predictions on qualitative and quantitative data perspectives. The prediction on four different indicators and the corresponding algorithm's performance are compared and discussed. In the latter part, a mathematical model for prediction is developed and the model simplification efforts based on residuals analysis and relative importance of modelled attributes evaluations are presented. Findings are also supported by previous studies.

Chapter 5 is the concluding section which summarizes the overall research. The chapter details how the objectives set are met and provides further suggestions for the future extended study.

## **CHAPTER TWO**

### **LITERATURE REVIEW**

#### **2.0 Overview**

This chapter provides a literature review on the past studies concerning the leaf mechanical property analysis, the application of data mining and modelling in plant science. The chapter begins with the review on plants biomechanics and different application of data mining in the plant science areas. The subsequential sections discussion of leaf mechanical property analysis which provides a solid basis for the present study to conduct the experimental data collection and the existing modelling works on leaf studies. The chapter also assesses the leaf observable and measurable attributes that were employed in previous works for leaf mechanical properties investigations. Subsequently, the leaf modelling and classification studies and numerous algorithms are assessed. The following sections present the background study on the software tools utilised for data mining and the performance measures approaches. The chapter summarizes the gap from the past studies that motivate the conceptual framework of the present study.

## 2.1 Plant biomechanics

The plant biomechanics discipline emerged to provide valuable insights to address the current plant science issues. The plant biomechanics (Hatze, 1974; Niklas, 1992) or sometimes referred to as the mechanobiology (Mouliya, 2013) discipline investigate different plant forms and functions from the physics and engineering perspectives. At a fundamental basis, the plants' ability to survive adverse environmental conditions such as extreme temperature, deficient sunlight, water scarcity (drought), and mechanical stimuli like strong wind and snow is important. Therefore, the primary research interests from biomechanics perspectives were on the internal structures and mechanical properties of cell-wall extracts (Burgert & Keplinger, 2013), seed (Steinbrecher & Leubner-Metzger, 2017), fruit skin (Domínguez et al., 2011; Li & Thomas, 2016), woody stem (Fournier et al., 2013), plant organs (Guzmán & Cordero, 2016; Loades et al., 2015) and plant architectures (de Langre, 2008; Gardiner et al., 2016; James et al., 2014; Onoda & Anten, 2011) on the basis of plant morphology under various dynamic conditions (Figure 2.1).

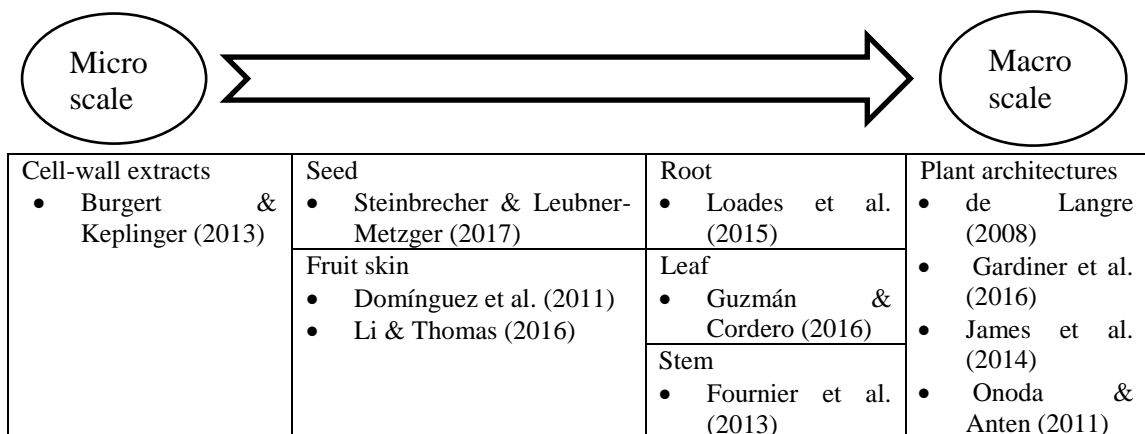


Figure 2.1 Example of plant biomechanics research interests covering from microscales to macroscales

In a microscopic view, Burgert & Keplinger (2013) overviewed the micro- and nanomechanical methodological aspects for the study of the structure and mechanical

design of plant cell walls. The study focused on the new developments and advancements of the mechanical characterization techniques in the field. Meanwhile, Steinbrecher & Leubner-Metzger (2017) presented the biological materials of seed tissues, the biomechanics of embryo cell growth during seed germination and the balancing of two opposing forces (growth potential of the embryonic axis and restraint of the seed-covering layers) for successful seed germination from a biomechanical perspective. Domínguez et al. (2011) reviewed the biophysical impact of the lipid polymer cutin particularly on its structural, thermal, biomechanical, and hydric properties and relationships perspectives which enable the plants to survive in vary hydration and temperature environmental conditions. Li & Thomas (2016) studied the cuticles characteristics of the tomato fruits from mechanics aspect and pointed out that the bruising and other mechanical damage to the fruit would result of the failure of cells at the microscale.

From a macroscopic view, Fournier et al. (2013) demonstrated the biomechanical traits of tree involved in sustaining an upright position, tropic motion velocity and posture control against winds and self-buckling. The combination of tree size, shape and wood properties were considered in the study, in which the variations throughout the environments and functional groups greatly influenced the tree shape and wood properties were observed. Meanwhile, Guzmán & Cordero (2016) discovered that neighbouring size and distance-dependence interference are associated with changes in biomechanics, allometry and branching of plants. The neighbouring size and distance-dependence interference should be considered as significant factors that contribute to plant adaptation and coexistence in the highly diverse forest environment, although the layout of the plant also varies depending on plant density and light accessibility. Loades et al. (2015) suggested that the variation of the

biomechanical characteristics (Young's modulus and tensile strength to diameter relationship) was due to the age effect. Whereas, de Langre (2008), Gardiner et al. (2016), James et al. (2014) studied the architectures of plants as a whole subject to survive various wind conditions. De Langre (2008) emphasized that the motion of plants or parts of plants was caused by flow-induced vibration mechanisms and strong coupling between plants and wind exists (plant motion modifies the wind dynamics). Gardiner et al. (2016), whereas, looked into the changes of chemical composition, physical structure and morphology at from the cell to the whole plant in order to survive in varying wind conditions. These included the plant's re-orientation, canopies reconfiguration, needle leaves to reduce drag, and even the mechanism of root and stem failure. James et al. (2014) explored the biomechanics of open-grown trees found in urban areas based on their form with simple tree models and multimodal approach and the finding indicated that form and morphology of the tree and branches play an important role in tree dynamics. Onoda & Anten (2011) demonstrated that the plant responses to wind vary between different parts of a plant and between plant species due to the extent of water stress. Hence, plant size, plant architecture and the signal sensing and transduction of both the mechanical and drought signals associated with wind should be taken into consideration.

The growth of terrestrial plant structured by root and shoot systems consisting mainly of meristematic tissue and permanent tissue for support, anchorage, and protection to ensure its growth and reproductive performance (Karam, 2005; Read et al., 2006; Stokes et al., 2006) is another concern. The genomics, biochemistry or ecology domain knowledge are sometimes integrated into the biomechanics or mechanobiology studies to support informative discovery. Data mining techniques

have been implemented for microarray classification (bioinformatics), yield prediction, plant identification, and disease detection as summarized in Table 2.1.

Table 2.1 Summary of data mining applications in plant science studies

Application	Microarray classification	Yield prediction	Disease detection	Plant identification
Paper	LaBonte et al. (2018)	Dey et al. (2017)	Predic et al. (2018)	Dyrmann et al. (2018)
	Torres-Avilés et al. (2014)	Medar & Rajpurohit (2014)	Ilic et al. (2018)	Lee et al. (2017)
	Kantety et al. (2002)	Johann et al. (2013)	Kim & Sharma (2016)	Musić & Gagula-Palalić (2016)
		Le Ber et al. (2006)	Hill et al. (2014)	Padao & Maravillas (2015)
			Tripathy et al. (2014)	Zhao et al. (2015)
			Phadikar et al. (2013)	Yamamoto et al. (2014)
Description	Simple sequence repeat markers for comparative mapping. (Kantety et al., 2002) A bioinformatics pipeline to identify suspected fungal coding sequences. (LaBonte et al., 2018) Identify potential new resistance genes in tomatoes vaccinated with <i>Phytophthora infestans</i> from microarray data. (Torres-Avilés et al., 2014)	Rainfall, area of sawing and fertilizers used for rice yield prediction. (Dey et al., 2017; Medar & Rajpurohit, 2014) Harvesting patterns, water management for plantation area prediction. (Johann et al., 2013; Le Ber et al., 2006)	Weather and pathogen data for fruit pathogen diseases. (Hill et al., 2014; Ilic et al., 2018; Kim & Sharma, 2016; Predic et al., 2018) Rice plant images for disease detection. (Phadikar et al., 2013) Wireless sensory and field level surveillance data for leafspot disease detection in groundnut crop. (Tripathy et al., 2014)	Geometrical and morphological features for plant identification. (Dyrmann et al., 2018; Lee et al., 2017; Musić & Gagula-Palalić, 2016; Padao & Maravillas, 2015; Zhao et al., 2015) Geometrical and colour features for tomato fruit detection. (Yamamoto et al., 2014)

Data mining helps to identify unknown patterns from the vast database for knowledge discovery and in better decision-making through classification, association rule mining, prediction and regression (Fayyad et al., 1996; I. H. Witten et al., 2016).

Researchers realized the importance to understand the mechanical properties of leaves to withstand different stresses for the plants' sustainable performance and survival (Huber et al., 2014; Niklas, 1992; Wang et al., 2009). Huber et al. (2014) demonstrated that leaves responded through cellular adjustments when undergoing different stress factors such as being overcrowded by neighbours and succumbing to sudden mechanical stress. Niklas (1992) discussed the trade-offs of the design of the leaves in order to meet their daily requirements such as light interception, mechanical stability, hydraulics exchange, gas exchange, and reproduction. Wang et al. (2009)

proved that the interactive effects of water availability and mechanical stress have contributed to the growth of plants in windy environments. Yet, trade-offs in the responsiveness of cellular characteristics to different environmental stresses could be a restriction on the plant's ability to respond adequately to different scenarios. The diversity of the leaf as a result of the plant evolution (Chitwood & Sinha, 2016; Dkhar & Pareek, 2014), influences of environmental stresses such as temperature and light (Chabot & Chabot, 1977; Hernández, 2010; Onoda et al., 2008), and as a response against herbivory (Alves-Silva & Del-Claro, 2015; Campitelli et al., 2008; Clissold, 2007) as shown in Figure 2.2.

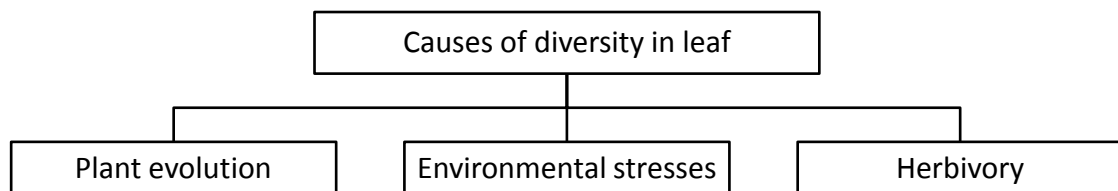


Figure 2.2 Causes of diversity in leaf

The unique geometry of leaves allows for light interception and exchange of gases simultaneously between the plants and the surroundings (Dkhar & Pareek, 2014; Givnish, 1979; Sack & Scoffoni, 2013). The form of the leaf is revealing in relation to the function of the leaf, especially owing to its intimate connection and interaction with the surrounding environment. Chitwood & Sinda (2016) demonstrated that distinct molecular pathways that regulated leaf dissection were modulated by specific environmental inputs through historical patterns and conserved plastic responses in existing plants. Besides, the evolution of simple leafless vascular plants into branched veins and planate forms as a countermeasure to atmospheric carbon dioxide decline (Beerling, 2005). The leaves developed more but smaller stomata to avoid water loss and improve the capacity of gas exchange capacities to adapt to the decline in carbon dioxide (de Boer et al., 2012). Moreover, the evergreen, elliptical leaves with entire

margins and deciduous, shifted to more rounded leaves with toothed or lobed margins in response to climate and temperature changes (Schmerler et al., 2012). Chabot & Chabot (1977) proved that light and temperature environmental factors have a different effect on the leaf structure. Hernández (2010) also demonstrated that inclination angles became steeper in the upper canopy of the sunflower plant with increased light availability for plant photosynthetic balance. On the other hand, Campitelli et al. (2008) showed that visual aspects of leaf morphology (leaf colouration, leaf shape, leaf size) can reduce levels of herbivory and act as physical defences for leaf, especially in the early stage. Alves-Silva & Del-Claro (2015) demonstrated a significant impact of herbivory as a major source of plant stress and it can decrease plant fitness, cause developmental instability in plant and influence the normal pattern of growth and expansion of leaf blades.

Leaf primarily respond through avoidance or tolerance mechanisms towards the environmental stresses (Hanley et al., 2007; Kozłowski & Pallardy, 2002; Verslues et al., 2006). The examples of stress avoidance mechanism are plant rapidly dominating gaps in a canopy to maximize sunlight interception (Ruberti et al., 2012) and decrease the leaf conductance to minimize water loss through transpiration and prevent leaf turgor loss (Rodríguez et al., 2012). Meanwhile, the active osmotic adjustment was triggered at maximum stress levels to control the leaf turgor (Rodríguez et al., 2012) and leaf adaptations during photosynthesis to function optimally under low-light conditions (Ruberti et al., 2012) are some examples of stress tolerance mechanism. Some leaves have impenetrable barriers wax, thorns or trichomes as protections against herbivory (War et al., 2012). There could be an abnormal pattern of plant growth and the expansion of leaf blades such as thicker cell



wall, more lignification and reduced leaf digestibility as the physical defences mechanism (Alves-Silva & Del-Claro, 2015; War et al., 2012).

There has been plenty of data mining application in the field of plant science but limited to bioinformatics, plant recognition, diseases detection and yields prediction. The leaf images were used in the plant's disease detection and plant identification studies where the morphological features of the leaf were usually only explored when plant identification or species recognition.

## **2.2 Leaf mechanical property analysis**

The mechanical properties of the leaf usually examined in terms of maximum force or stress that the leaf structure able to handle prior to failure. The carbon allocation or so-called 'investment in leaf physical defence' via thickening tissues against abiotic (wind) and biotic (herbivore) mechanical damages contribute to a longer leaf lifespan (Kitajima & Poorter, 2010). In ecological studies, the internal structures of the leaf are often express as biomechanical properties or fracture toughness (Vincent, 1990). Fracture toughness is a fundamental material property, defined as the work done to produce a unit area by a crack propagating at a constant velocity (Atkins & Mai, 1985).

From the context of plants, leaves have different physical properties depending on the direction of force applied. Therefore, it is possible to define and measure the mechanical properties of the leaves in different ways (Wright & Vincent, 1996). The mechanical properties of leaf were commonly assessed by punching, shearing and testing tests as described in Table 2.2.

Table 2.2 Classification of mechanical testing utilized in leaf mechanical properties studies

Mechanical test	Reference	Description	Calculation
Punching (punch and die)	Anten et al. (2010) Onoda et al. (2008) Read & Sanson (2003) Read et al. (2006) Read et al. (2000)	The measurement of force to break the bonding between leaf tissues through penetration. The specimen is located between the punch and die unit. The force needed to break through the particular thickness of the specimen is determined.	$Punch\ strength = F/A$ $Specific\ punch\ strength = (F/A)/T$ $Work\ to\ punch = (F/A) \times D$ $Specific\ work\ to\ punch = ((F/A) \times D)/T$  <i>F = maximum force detect;</i> <i>A = area of punch;</i> <i>T = thickness of leaf specimen;</i> <i>D = displacement of moving head of test machine</i>
Shearing	Enrico et al. (2016) Read & Sanson (2003) Read et al. (2000)	Involved cutting off the leaf specimen between two blades in order to measure the toughness of intercostal lamina. The force needed to cut through specimen along the transverse planes by the blade is determined.	$Work\ to\ shear = (F \times D)/W$ $Specific\ work\ to\ shear = ((F \times D)/W)/T$  <i>F = maximum force detect;</i> <i>D = displacement of moving head of test machine;</i> <i>W = width of leaf in plane of shear;</i> <i>T = thickness of leaf specimen</i>
Tearing (tensile)	Anten et al. (2010) Balsamo et al. (2003) Balsamo & Orkiszewski (2008) Balsamo et al. (2005) Enrico et al. (2016) Onoda et al. (2011) Read & Sanson (2003) Read et al. (2000) Witztum & Wayne (2014) Witztum & Wayne 2016)	The property of leaf structure under the pulling load is examined. The specimen is usually elongated in the direction of the constantly applied forces until break. The point of fracture or notch was introduced into the test specimen probably to characterize the leaf resistance to fracture in a neutral environment with the presence of a sharp edge crack under severe tensile constraint such as chewing of insect.	$Tear\ strength = F/C$ $Work\ to\ tear = F \times D$ $Specific\ Work\ to\ tear = (F \times D)/C$  <i>F = maximum force detect;</i> <i>C = cross section of leaf specimen;</i> <i>D = displacement of moving head of test machine</i>

The three testing approaches are associated with the feeding mechanisms with different modes of fracture in herbivores. The tearing test is used to investigate the herbivory by mammalian grazers and other tearing herbivores (snail) in which crack propagation caused by tension or crack-opening. The shearing test is used to study the herbivory (small vertebrates and chewing insects (grasshopper)). Meanwhile, the punching test is to reflect the condition of chewing or sucking of the insects (aphids). In addition, these three mechanical tests are generally used for the decomposition and identify plant resource-allocation strategies studies (Clissold, 2007; Pérez-Harguindeguy et al., 2013; Sanson, 2006; Wright & Vincent, 1996).

The mechanical properties of leaf were measured from the perspectives of ‘structural’ properties as a response to a particular action and ‘material’ properties inherent in the material regardless of its geometry. The ‘structural’ properties such as strength and toughness are usually divided by width of the specimen (work to fracture) while the ‘material’ properties divided by both width and thickness and labelled with ‘specific’ such as specific strength and specific work to fracture (Kitajima & Poorter, 2010; Read & Sanson, 2003). Strength is the maximum stress that causes the leaf specimen breaks while the work to fracture is used as a measure of the leaf specimen resistance against the crack propagation.

In recent studies, the punching test was used for leaf mechanical properties in relation to sclerophylly (hard, tough texture of leaf) investigation (Read & Sanson, 2003; Read et al., 2000), and to understand the environmental impacts such as light, nutrient, rainfall and wind on the phenotypic variation of the leaf (Anten et al., 2010; Onoda et al., 2008; Read et al., 2006). Majority studies showed that hard and tough leaves (higher sclerophylly index) basically had high punch strength and work to punch (Edwards et al., 2000). The punch strength and work to punch of the leaf

increased with the light condition but did not change on the nutrient factor (Onoda et al., 2008). On average, the leaves of maquis plants (high rainfall) were structurally tougher and stronger than those in the dry forest (Read et al., 2006). The wind factor, whereas, had an insignificant impact on leaf strength (Anten et al., 2010).

Furthermore, the shearing test has also been used to characterize sclerophylls (Edwards et al., 2000; Read & Sanson, 2003) and comparing the results with tearing test (Enrico et al., 2016). The outcomes showed that hard and tough leaves had high strength and toughness fracture (work to shear) (Edwards et al., 2000; Read & Sanson, 2003). A positive and significant correlation was found between work to shear and tearing force of leaves (Enrico et al., 2016). In addition, the tearing test was used to compare the mechanical properties of mesophytic leaves and sclerophyllous leaves (Balsamo et al., 2003), compare the mechanical properties of leaf at different dehydration states (full dehydrated, partial dehydrated, naturally air-dried, flash-dried) (Balsamo et al., 2005), investigate the mechanical properties of *Zea May* leaves during vegetative phase change (Balsamo & Orkwiszewski, 2008), compare the properties of leaf epidermis and mesophyll layers (Onoda et al., 2015) and study the properties of fibre in *Typha* leaves (Witztum & Wayne, 2014, 2016). The sclerophyllous leaves have higher tearing strength than the mesophytic leaves likely due to the complex internal structure and the physical/chemical differences in their respective cell walls (Balsamo et al., 2003). The tensile strength increased with tissue dehydration but there were no significant differences in naturally air-dried and flash-dried (Balsamo et al., 2005). The tensile strength of *Zea May* leaves increased with phase change from juvenility to adulthood and it might due to the lignification of tissues (Balsamo & Orkwiszewski, 2008). The studies aforementioned which utilizing

punching, shearing and tearing tests to measure leaf mechanical properties were illustrated as in Figure 2.3.

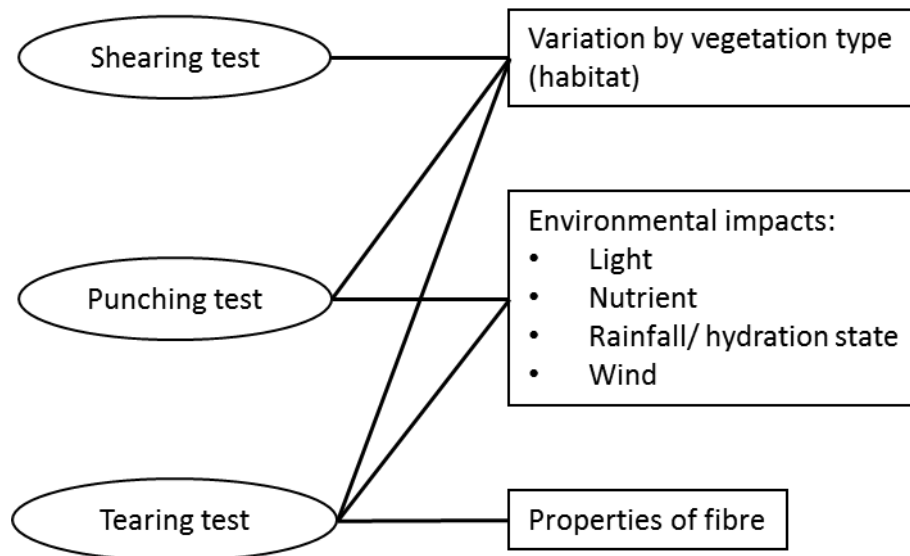


Figure 2.3 Existing works on punching, shearing and tearing tests to measure leaf mechanical properties

Besides, the leaf mechanical properties were strongly influenced by the combination, arrangement and characteristic of various cells made up the leaf structures. Previous studies aforementioned exposed that the mechanical properties of a leaf affected by the leaf hardness, thickness, relative water content and maturity state; while the leaf morphological characteristics affected by the environmental factors.

### 2.2.1 Mechanical test setups

Most of the studies utilized sophisticated instrument (Chatillon Universal Tension and Compression Tester, Instron Universal Testing Machine) for all mechanical testing except for those reported in Balsamo et al. (2005) and Balsamo & Orkwiszewski (2008) that used customized setup which consists of a portable

tensometer constructed with Pesola scale, clamp and a mounting bucket for tearing test.

In the punching test, an averaging of multiple measurements at random positions on the leaf specimen is recorded (Vincent, 1990). The punch and die design directly influence the punch strength. A chamfering in the flat end of the punch enhances the smoothness during punching (Aranwela et al., 1999; Pérez-Harguindeguy et al., 2013). Unfortunately, there is no standard design for the punch and die have been used in the past due to differences in the leaf size and structure been studied. The punching test was found inappropriate for monocots (Pérez-Harguindeguy et al., 2013) due to the arrangement of venations and lignification fibres. The great advantage of a shearing test is that the properties of the whole structure of a leaf can be measured for better control on the crack propagation (Lucas & Pereira, 1990; Sanson et al., 2001). However, the shearing test is highly sensitive to external noise such as vibration and wind (Pérez-Harguindeguy et al., 2013). Besides, the protocol setup in the shearing test is very critical on the parameters like blade clearance, blade angle and blade sharpness (Aranwela et al., 1999; Pérez-Harguindeguy et al., 2013). Tearing test involved a tedious setup and specimen preparation as well. There is a risk that direct clamping the leaf specimen in the testing will damage the leaf specimen (Sanson et al., 2001). The limitations of punching, shearing and tearing test were summarized as in Table 2.3.

Table 2.3 Limitation of punching, shearing and tearing tests

Mechanical test	Limitation
Punching	<ul style="list-style-type: none"> <li>• No standard design for the punch and die</li> <li>• Inappropriate for monocots</li> </ul>
Shearing	<ul style="list-style-type: none"> <li>• Highly sensitive to external noise (eg. vibration)</li> <li>• Critical on the parameters and protocol setups</li> </ul>
Testing	<ul style="list-style-type: none"> <li>• Direct clamping will damage the leaf specimen</li> </ul>

The mechanical testing (punching, shearing, tearing) based on material engineering concepts cannot be precisely quantified as the actual leaf properties. This is due to the plant leaves are complex composite structures with anisotropic properties and heterogeneous growth (Choong et al., 1992; Clissold, 2007; Sanson, 2006). Each of the mechanical test approaches has its own limitations and therefore require careful considerations prior to conducting the experimental leaf property measurements (Aranwela et al., 1999; Lucas & Pereira, 1990; Read & Sanson, 2003; Sanson et al., 2001). Besides, the choice of a test should be considered with reference to previous experience, the type of leaf structures, and the availability of experimental and computational resources (Srikar & Spearing, 2003). Nevertheless, the previous studies disclosed that the mechanical tests were significantly correlated and any of the mechanical tests can be used as the general indicator (Enrico et al., 2016; Onoda et al., 2011). The results in Enrico et al. (2016) showed positive and significant correlations ( $r$  ranged from 0.47 – 0.75) between force-to-tear and specific work-to-shear. In addition, Onoda et al. (2011) findings showed that the ranges of variation in the three tests (punching, shearing and tearing) and their associations to leaf thickness and tissue density were generally similar. Overall studies showed on average 55–59% of the variation in leaf mechanical properties was due to variation in leaf traits rather than individual studies setup.