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Title: Image-based high-throughput phenotyping for the estimation of persistence of perennial ryegrass *(Lolium perenne L.)* - A review

A short running title: HTP for pasture persistence estimation

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Abstract: Perennial ryegrass (*Lolium perenne* L.) is considered the most important pasture species in temperate agriculture, with over six million hectares of sown area in Australia alone. However, perennial ryegrass has poor persistence in some environments because of low tolerance to a range of both abiotic and biotic stresses. To breed perennial ryegrass cultivars with greater persistence and productivity may require evaluation of genotypes over a number of years. Persistence assessment in pasture breeding depends on manual ground cover estimation or counting the number of surviving plants or tillers in a known area. These methods are subjective and labour intensive, which may limit data collection in large scale breeding programs. With the rapid development of sensors and image processing algorithms, image-based high-throughput phenotyping (HTP) is becoming commonplace in the breeding of major food crops. Image-based HTP approaches consist of the deployment of a wide range of sensors on ground-based or airborne platforms and data analyzed through image-processing pipelines. Image-based HTP show high potential for use in pasture phenotyping in breeding programs and may be able to reduce timeframes for releasing new cultivars. Moreover, existing image-based HTP approaches could be further developed to include precise tools for phenotyping pasture persistence traits such as pasture senescence, botanical composition, pathogen and pest resistance. In this paper, we reviewed existing image-based HTP approaches in precision agriculture and discussed their

feasibility for perennial ryegrass persistence estimation in pasture breeding. Although the paper focuses on application in perennial ryegrass, the principles equally apply to other perennial forage species.

Keywords: perennial ryegrass persistence; pasture breeding; high-throughput phenotyping; sensors; image analysis

1. Introduction

Perennial ryegrass (*Lolium perenne* L.) is the most important pasture species in temperate grazing systems, exceeding over six million hectares of sown area in Australia alone (Cunningham et al, 1994; Moate et al, 2012). Individual plants of perennial ryegrass may show low tolerance to both abiotic and biotic stresses, resulting in poor persistence. Poor pasture persistence creates a great challenge for livestock producers, who must utilise their agricultural lands productively and intensively within sustainable limits (Culvenor and Simpson, 2014). Perennial ryegrass is an outbreeding self-incompatible species (Wilkins and Humphreys, 2003), with a high degree of genetic diversity between individual plants in natural populations. Current limitations of pasture phenotyping methods coupled with its high degree of genetic diversity within breeding populations (Lin et al, 2017), results in new cultivar development in perennial ryegrass taking longer than most annual crops, taking 10-15 years from initial nursery establishment to the registration of a new cultivar (Hayes et al, 2013). Annual pasture dry matter production per unit area is an important consideration from the farmer's perspective since it directly influences the financial return of their capital investment (Wilkins, 1991). Long-term productivity of a sward depends on pasture persistence, which represents plant density or plant size (tiller number) in a known area (Waller and Sale, 2001). As such, the primary objectives of pasture breeding focus on increasing annual pasture productivity with an improved pattern of seasonal production and to extend the productive life of the pasture (persistence). In pasture breeding programs, individual plants with more desirable attributes are selected by phenotyping morphological, physiological and biochemical traits over multiple years. Trait assessment depends on traditional methods based on visual observations, manual measurements or biochemical analysis (Walter et al, 2012). These methods are time-consuming and sometimes subjective, which may limit data collection in large scale pasture breeding programs.

With rapid technology development, interest in the use of sensors for phenotyping plant traits has increased in agriculture research. The recent application of high-throughput phenotyping (HTP) across a range of crops includes a wide range of imaging and non-imaging sensors, deployed on ground-based or airborne platforms. However, image-based platforms have become the favoured approach in precision agriculture due to their low-cost, high resolution and high throughput. Image-based HTP platforms can detect reflectance values of plants, and reliably generate phenomic data representing plant development, architecture, growth or biomass productivity of single plants or populations. The existing HTP sensor-based tools utilised in other crops may have potential use in in pasture breeding programs (Walter et al, 2012) and could improve the efficiency of plant phenotyping for various aspects of pasture breeding. For example, image-based HTP pipeline showed robust estimation of herbage yield (Gebremedhin et al, 2019) and persistence (Jayasinghe et al, 2019; Jayasinghe et al, 2020) in perennial ryegrass breeding programs using both aerial-based and ground-based HTP platforms. However, the assessment of pasture persistence within breeding trials is not straightforward and still depends on conventional methods. Conventional plant phenotyping methods are often destructive that depend on expensive manual operations (Ubbens and Stavness, 2017). Therefore, conventional plant phenotyping methods have very limited throughput for comprehensive analysis of plant traits of an individual plant or across a population (Ubbens and Stavness, 2017). The objective of this review is to investigate existing image-based HTP approaches for field phenotyping and to evaluate their potential to replace conventional methods of persistence estimation in pasture breeding programs.

2. Conventional methods for pasture persistence estimation

Pasture persistence is usually evaluated through field observations in the second or third year after sowing using conventional methods (Wilkins, 1991). Ground cover of a pasture cultivar with low persistence decays rapidly over time and creates bare ground within the sward, that provides an opportunity for weed ingression (Fulkerson et al, 1993). Ingression of weeds changes sward composition (perennial ryegrass vs. weeds), generally lowers the nutritive value of the sward (Doyle et al, 1989) and can be considered as an indicator of persistence of a sward (Waller and Sale, 2001). Annual pasture dry matter production of a cultivar with poor persistence will decrease over time due to depletion of surviving plants. Therefore, analysing long term dry matter production data from a single species sward or breeding plots would be a way to assess the expression of persistence (Chapman et al, 2014). Pasture senescence may indicate cessation of plant growth, development and resistance to both abiotic and biotic stresses (Makanza et al, 2018a), and the amount of senescent pasture on the soil surface of breeding plots can be used as a key indicator of persistence in cultivar evaluation. Pasture ground cover, botanical composition, dry matter yield and pasture senescence can be used as measurements or processes to evaluate the expression of pasture persistence in breeding programs (Borra-Serrano et al, 2018).

2.1 Ground cover

Plant ground cover is the fractional area of soil covered by plants when viewed from the nadir position (Luscier et al, 2006). Plant ground cover may include pasture, weeds, other herbage and litter, and can be measured as a percentage or fractional unit (Calera et al, 2001). Techniques to estimate pasture ground cover include point quadrat (Cunningham et al, 1994), line intercept (Henry et al, 1995), visual estimation (Cayley and Bird, 1996), digital image analysis (Richardson et al, 2001), and gap counting methods. Point frame, ocular telescope, and line intercept methods are a variation of the point quadrat method, which have been used in many studies to estimate pasture ground cover (Freebairn and Boughton, 1981; Lang and McCaffrey, 1984; Eldridge and Rothon, 1992; Bari et al, 1995). A number of non-destructive techniques for plant ground cover estimation have been developed using digital red-green-blue (RGB) cameras

(McIvor et al, 1995; Zhou et al, 1998; Vanha-Majamaa et al, 2000; Borra-Serrano et al, 2018; Jayasinghe et al, 2019). However, the visual estimation method remains commonly used as it does not require any specialised equipment or training (Lodge and Murphy, 2006).

2.2 Species composition

Species composition of a sward can change over time (Minnee et al, 2017), and may provide a potential indicator of the persistence of a pasture population. Ingression of less desirable and invasive weed species can contribute to sward decline, reducing farm productivity and profitability (Brazendale et al, 2011). A simple technique for composition estimation is reviewing the presence or absence of species in randomly selected quadrats, and this method is subjective and has limited value for most agronomic research due to its lack of consistency throughout data collection (Lynch, 1966). The rod point technique is a straightforward alternative method to the point quadrat method (Little and Frensham, 1993) and offers a rapid estimation of sward botanical composition. This method requires a thin rod to be placed horizontally at random points of the sward, where species touching the rod are noted. The number of readings for each species can be compared over the total number of readings to acquire the botanical composition of a sward. This rod point technique shows less bias between different operators and does not require skilled labour to estimate botanical composition of a sward. (Mannetje and Haydock, 1963) introduced the dry matter ranking technique for pasture botanical composition estimation. The process of dry matter ranking requires a given area of pasture to be mechanically harvested and manually sorted into categories according to their species. Manual sorting depends on observer ability to assess the species. The DAFOR scale (D = dominant, A = abundant, F = frequent, O = occasional, and R = rare) is a method for visually assessing botanical composition in a sward, providing quantitative visual assessments of botanical composition within a known area of pasture mass (Martinson et al, 2017). . Dry matter ranking, point quadrat, rod point and the DAFOR scale methods rely on the same principles of taking multiple readings at points or in random patterns in a defined area (Rotz, 2006), these conventional techniques are highly dependent on observer knowledge about plant species. Manual methods have a higher possibility of under or over-estimating species, depending on the size of the species or the size of the investigation area, which will in turn lead to having poor accuracy of species composition estimation. However, image sensors and advanced image processing algorithms have a great potential to assess pasture composition under field conditions (Skovsen et al, 2017; Bateman et al, 2020).

2.3 Pasture dry matter

A study conducted by (Ludemann et al, 2015) measured pasture persistence as a change in the rate of decline in annual pasture dry matter production due to a decline in surviving plants in an area. Pasture dry matter of a known area can be determined by mechanically clipping the pasture, oven-drying the samples at 105 ^{0}C for 24 hours or until constant weight is achieved and weighing the dried sample (Cayley and Bird, 1996). This is the most straightforward dry matter yield measurement technique in pasture breeding (Martinson et al, 2017). However, it is a time-consuming and involves destructive sampling of the pasture area (Brummer et al, 1994). A pasture ruler is the first straightforward non-destructive tool to measure available pasture mass in sward or breeding plot (MLA, 2014). The single-probe capacitance meter, weighted-disc (Vickery and Nicol, 1982), rising-plate meter (Earle and McGowan, 1979), and "HFRO" sward stick (Hutchings, 1991) are all developments on the principle of a pasture ruler for point based assessment of pasture dry matter yield. The rising plate meter is widely used by researchers and farmers to estimate standing herbage mass (Martinson et al, 2017) due to its reasonably accurate estimation capability and ease of use. Due to the lack of efficiency of these conventional methods, gathering long-term dry matter data from a breeding trial is challenging for pasture persistence expressed as dry matter production changes over time (Ludemann et al, 2015). However, some existing phenomic approaches in precision agriculture, such as air-borne multispectral images and sensor-based plant height estimates may enable high-throughput phenotyping of yield in perennial ryegrass sward or breeding programs (Trotter et al, 2010; Alem et al, 2019; Gebremedhin et al, 2019; Karunaratne et al, 2020). Moreover, with varying climatic conditions each year, long term annual dry matter data may not be a precise element to attribute changes to pasture persistence (Tharmaraj et al, 2014; Ludemann et al, 2015).

2.4 Pasture senescence

Accumulated dried matter from leaves, stems and pseudostems in perennial ryegrass swards result from pasture senescence (Woodward, 1998), due to subsequent death of mature tissue. Pasture senescence is the process of remobilisation and transfer of soluble constituents from mature to immature plant tissues that occur with advancing age of plant parts, or through abiotic and biotic stresses (Allen et al, 2011). Pasture senescence rate may indicate a positive relationship between plant growth, development and resistance to abiotic and biotic stressors (Daily et al, 2013). For example, if pasture species are exposed to stress conditions such as water deficit and extreme temperature, strongly resistant cultivars may produce the smallest proportion of senescent tissue on the soil surface (Calviere and Duru, 1995). Therefore, estimation of the amount of senescent pasture in breeding plots can be used to monitor persistence of pasture species. The estimation of pasture senescence in breeding plots can be achieved using dry matter ranking methods or visual observations (Mannetje and Haydock, 1963). Advanced ML algorithms and some of the high-throughput phenotyping approaches in precision agriculture have a great potential to develop precise tools to estimate pasture senescence in breeding programs (Higgins et al, 2014; Ren et al, 2016).

Phenotypic data collected from conventional methods for pasture persistence estimation are recorded either visually or through destructive harvesting, however, data collection is time-consuming, sometimes subjective and labour intensive. The chance of errors in measurement can also be increased due to uneven ground, trampling of pasture, plant heterogeneity, and sward height differences (Murphy et al, 1995). The development of cost-effective efficient methods for pasture persistence evaluation in pasture breeding programs may be achieved by using high throughput phenotyping techniques (Borra-Serrano et al, 2018).

3. High-throughput plant phenotyping

In the past few decades, precision agriculture has emerged as a major discipline to optimise the use of natural resources in arable lands (Pratap et al, 2015). Plant phenotyping refers to a comprehensive assessment of plant morphological, physiological and biochemical traits such as plant growth, development, resistance against stresses, architecture and physiology (Walter et al, 2015). Consequently, traditional plant phenotyping methods have evolved towards HTP approaches. HTP consists of sensor technologies mounted on platforms to allow for data capture at scale and a data handling workflow to acquire targeted-specific plant traits for individual plants, plots or paddocks (Figure 1).

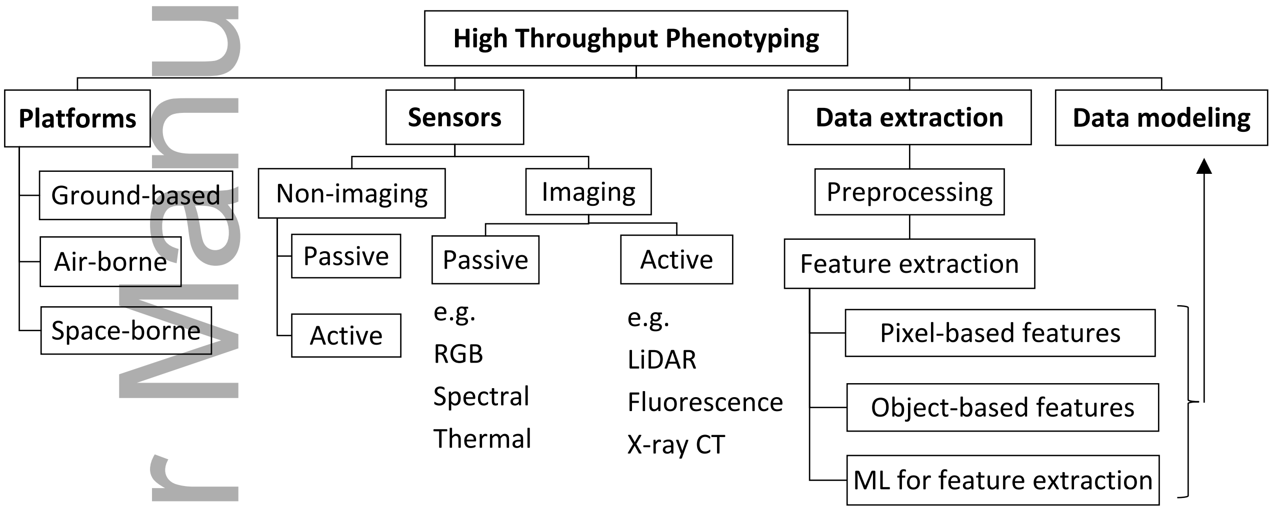

Figure 1. Elements of high throughput phenotyping technologies that could be used for estimating expression of pasture persistence under field conditions, where LiDAR is light detection and ranging, and ML is machine learning.

High-throughput phenotyping offers greater potential to enhance the efficiency and accuracy of phenotyping more complex traits in both indoor environment or under outdoor field conditions (Walter et al, 2012; Fiorani and Schurr, 2013). Due to the high genetic diversity of perennial ryegrass populations, phenotyping plant persistence may require examining many genotypes under field conditions (Wilkins, 1991). Therefore, in this review, we focused on image-based high throughput techniques for pasture phenotyping under field conditions.

4. High throughput phenotyping platforms

Field-based HTP platforms range from simple handheld devices to complex ground-based or space-borne systems (Figure 2). In order to facilitate high-throughput data capture, phenomic platforms are integrated with various sensors, that may allow the simultaneous measurement of multiple phenotypic traits under field conditions (Ruicheng et al, 2018).

4.1 Ground-based platforms

Ground-based platforms include handheld devices or modified vehicles. Handheld devices such as SPAD (soil plant analysis development) meters can be used to measure chlorophyll concentration, or GreenSeeker can be used to measure NDVI (Qiu et al, 2018). Poor accuracy and the time to measure multiple plant traits may be a key bottleneck of implementing handheld devices for pasture phenotyping under field conditions. Ground-based small motor vehicles like buggies, tractors and quad bikes have been modified and equipped with a wide range of sensors to overcome this bottleneck. These ground-based platforms can be driven in the field manually or automatically using computer technology (Lam et al, 2018). Ground-based modified vehicles could replace manual labour and provide more precise data for agricultural research (Gebremedhin et al, 2019). However, the application of ground-based vehicles is challenging under some field conditions due to uneven geometry (Bochtis and Sorensen, 2009), extreme weather conditions, and obstacles in the field (Bochtis et al, 2014).

4.2 Airborne platforms

Aerial-based platforms include unmanned aerial vehicles (UAVs), low altitude aircraft, hot air balloons and helicopters which can be used as a solution for some limitations associated with ground-based and spaceborne platforms in plant phenotyping (Pratap et al, 2015). However, the effectiveness of an airborne platform may depend on the capability for integration with multiple sensors, payload and weather conditions and associated acquisition time (Tsach et al, 2010). Due to the recent technology development, UAVs have been undergoing extraordinary development and are now considered a powerful sensor-bearing platform for many agricultural and environmental applications (Narayanan et al, 2015). Flying and maintenance costs of a UAV is relatively low, compared to the cost associated with other airborne and spaceborne platforms. UAVs can be integrated with ultra-high spatial resolution sensors for phenomics data acquisition and can be deployed as frequently as necessary (Hardin and Hardin, 2010). Flying time and battery-life of UAVs can be limited according to body type, payload, and operating conditions, and this may create a bottleneck for the use of UAVs for plant phenotyping.

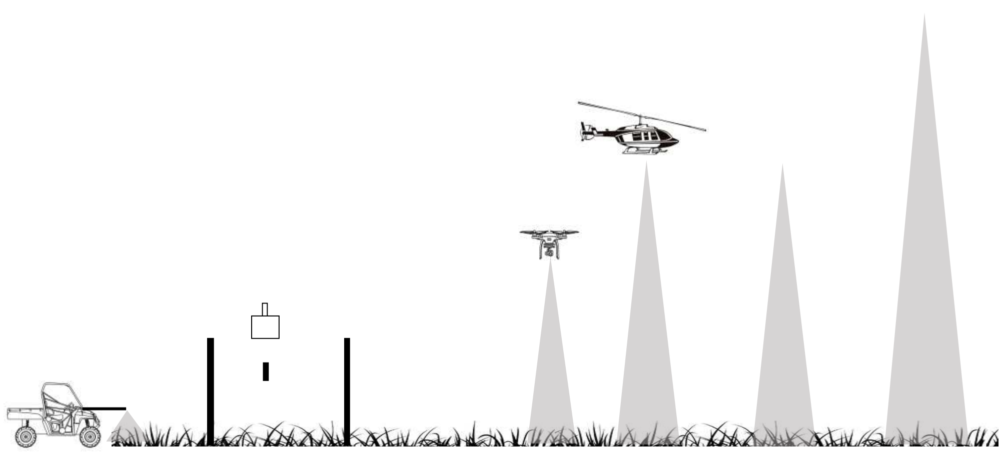

Figure 2. Scaling of high-throughput phenotyping platforms in precision agriculture, where (**a**) is field scanning using a handheld sensor, (**b**) ground-fixed environmental sensor, (**c**) a phenotyping ground vehicle, (**d**) a field scanning platform, (**e**) a phenotyping tower, (**f**) an unmanned aerial vehicle, (**g**) a low altitude phenotyping helicopter, (**h**) a phenotyping hot air balloon and (**i**) a satellite remote sensing platform.

4.3 Spaceborne platforms

The use of satellites for plant phenotyping began about three decades ago after satellites were equipped with high-resolution spectral sensors (Hoepffner and Zibordi, 2009), and presently, there are a total of 2,666 satellites orbiting earth (UCS, 2020). The majority of satellites provide data at 10-30 m spatial resolution with, on average, 16 days revisiting time (Chang and Clay, 2016). However, modern satellites offer high resolution spatial data with a shorter revisiting time. For instance, WorldView-2 has 2.4 m spatial resolution and a revisiting time of 1.1 - 3.7 days (Chang and Clay, 2016). Pasture persistence estimation may require data from plant traits over many years. Satellites are capable of providing long-term spectral data to the public for free, such as the moderate resolution spectral data from Sentinel 2, LANDSAT 7 ETM+ and LANDSAT 8 OLI. Satellite remote sensing platforms are considered to show great prospects for pasture persistence estimation of a sward (Phiri and Morgenroth, 2017). Buying high resolution data from satellites (e.g. GeoEye-1, WorldView-3 and QuickBird) is still a costly process (Chang and Clay, 2016; EOS, 2019), which makes use of high resolution satellite data for pasture phenotyping challenging in small scale breeding programs. Some of the sensors equipped with satellites such as spectral cameras and LiDAR (Light Detection and Ranging) sensors may have reduced quality depending on atmospheric conditions or may not penetrate through clouds or water vapour (Hoepffner and Zibordi, 2009). The spatial and temporal resolution of satellites combined with the dependency on good atmospheric conditions when passing over the target site may limit the regular application of satellite remote sensing for plant phenotyping.

5. Sensors for high-throughput phenotyping

The most important aspect of all remote sensing platforms is the sensors themselves. These, acquire energy in a specific region of the electromagnetic spectrum (EMS) that represents plant morphological, physiological and biochemical properties of the target object (Tsach et al, 2010; Qiu et al, 2018; Che Ya et al, 2019). Sensors can be classified into two different classes for precision agriculture in terms of imaging and non-imaging functionality (Zhu et al, 2018). Image sensors have become the dominant class of sensor used in precision agriculture for plant phenotyping due to the rapid progress in image capturing and processing technologies (Ruicheng et al, 2018).

5.1 Image sensors for phenotyping pasture persistence

Image sensors capture reflected energy of an object in the visible, near-infrared, or shortwave infrared region of the EMS and generate monochrome, RGB, multi- and hyperspectral, LiDAR or thermal imagery (Li et al, 2014; Perez et al, 2017; Ruicheng et al, 2018). Image sensors collect information about phenomenon through active or passive acquisition systems (Lillesand et al, 2015; Zhu et al, 2018). Active image sensors use their own energy source to generate reflected spectrum of a target object and capture reflected energy wavelengths from the target. In contrast, solar radiation is the primary energy source for passive image sensors. Existing image-based HTP approaches show that image sensors can be sensitive to discriminate morphophysiological characteristics of plants, and have higher throughput, reliability and repeatability at all scales of measurements than conventional plant screening techniques, such as, visual estimation and manual counting (Perez et al, 2017). The ground sampling distance (GSD) is the distance between two consecutive pixel centers measured on the ground, and it depends on the image acquisition height and the spatial resolution of the sensor (Popescu et al, 2016). GSD of modern imaging sensors is low compared to conventional analogue camera due to the high spatial resolution. This may allow quantification of complex plant traits related to growth, dry matter yield and stress resistance in a controlled environment or under field conditions (Walter et al, 2012). As such, image-based HTP approaches may have high applicability for persistence estimation in perennial ryegrass breeding programs.

5.1.1 RGB imaging

RGB imaging sensors capture reflected energy in the visible region of the EMS (400–700 nm) with the help of a charge-coupled device (CCD) or a complementary metal-oxide-semiconductor (CMOS) (Perez et al, 2017). Properties of spectral reflectance of pasture in the visible range of the EMS may depend on plant morphological and physiological traits such as plant size, leaf arrangement and chlorophyll concentration (Gates et al, 1965). RGB imaging sensors deployed on aerial or ground-based platforms may have feasibility for quantifying changes in plant growth, development and health status over time. These phenotypic traits may help to differentiate tolerant and sensitive cultivars in breeding programs (Rajendran et al, 2015). There are a number of RGB image-based phenomic tools available for perennial ryegrass persistence estimation. For example, recent studies have shown that proximal RGB images could be used to quantify and classify perennial ryegrass ground cover using automated phenomics pipelines (Figure 3), which could replace traditional visual estimations of perennial ryegrass persistence in pasture breeding programs (visual ground cover vs. RGB sensor-based ground cover, *r*- 0.75, *p* = 0.001; visual ground cover vs. multispectral sensor-based ground cover– *r* - 0.80, *p* = 0.001) (Jayasinghe et al, 2019). Moreover, vegetation indices extracted from airborne RGB were tested for use in perennial ryegrass persistence estimation (Borra-Serrano et al, 2018), pathogen detection in crops (Zhu et al, 2018), biomass estimation in wheat (Kipp et al, 2014) and biomass estimation in perennial ryegrass (Borra-Serrano et al, 2019). However, the overlapping of leaves in plants may reduce the accuracy of RGB image-based phenomics data (Golzarian et al, 2011). The background noise from soil brightness can also affect the quality of RGB image acquisition. Moreover, the visible region of reflected spectra provides only limited information about biochemical characters of the plants, which may limit the application of RGB sensors for phenotyping traits related to crop and pasture nutritive parameters (Fiorani and Schurr, 2013).

Figure 3. Application of proximal RGB imaging for perennial ryegrass ground cover estimation in pasture breeding plots; (**a**) A DSLR camera mounted on a tripod for RGB image acquisition (**b**) RGB image (12 megapixels) from nadir position and (**c**) classified RGB image using a pixel-based

image analysis techniques, where red colour represents perennial ryegrass green fraction, adapted from (Jayasinghe et al, 2019).

5.1.2 Spectral imaging

Spectral sensors acquire reflected radiation of an object in the visible region (400 - 700 nm) and the infrared (IR) region (700 - 2500 nm) of the EMS (Qiu et al, 2018). According to the spectral resolution, spectral cameras can be separated into two groups, namely multispectral and hyperspectral cameras (Ferrato and Forsythe, 2013). Multispectral cameras capture 3-25 discrete spectral bands (broadbands) across the EMS, including red, green, blue, and NIR (near infrared reflectance) with spectral resolution of >10 nm for each band (Perez et al, 2017). Hyperspectral cameras may capture continuous spectral bands (>25 bands) within a specific range of wavelengths and spectral resolution can be less than 10 nm per band (Kise et al, 2010). Pasture nutritive characteristics prediction (Pullanagari et al, 2015; Pullanagari et al, 2018; Shorten et al, 2019), mapping the spatial distribution of botanical composition and herbage mass in pasture (Kise et al, 2010), multi-temporal assessment of grassland dynamics (Gholizadeh et al, 2020), monitoring of grassland degradation (Wang et al, 2010), classification of grassland successional stages (Möckel et al, 2014), insect damage identification in crops (Jianrong et al, 2012), , identification of insect-damaged in wheat kernels (Singh et al, 2010) and early detection of rice blast at seedling stage (Yang, 2012) are some of the recent applications of hyperspectral sensors in precision agriculture. Airborne multispectral sensors have also been used in precision agriculture for mapping and monitoring of pasture biomass and grazing patterns (Michez et al, 2019), estimation of spatial and temporal variability of pasture growth and digestibility (Insua et al, 2019), prediction of biomass yield in perennial ryegrass breeding programs (Gebremedhin et al, 2020), estimation of pasture dry matter yield at paddock scale (Karunaratne et al, 2020). Sward botanical composition, herbage yield, pathogen and insect resistance and pasture ground cover can be used as a population trait to evaluate persistence of perennial ryegrass (described in more detail in section 2). (Jayasinghe et al, 2019) have recently developed a spectral camera-based tool for perennial ryegrass persistence estimation that allows for the estimation of perennial ryegrass ground cover in pasture breeding (Figure 4). However, further research is required to improve the accuracy and feasibility of the tool for paddock scale applications. Modification of these applications may be promising as a more precise sensor-based tool to assess persistence of perennial ryegrass in breeding plots or swards. Spectral sensors produce large datasets and may require advanced computational power for data handling and storing (Yang et al, 2020). The quality of image acquisition from spectral sensors may depend on ambient conditions and extensive calibration protocols may be required during data acquisition and data processing to improve the quality of phenomic outcomes.

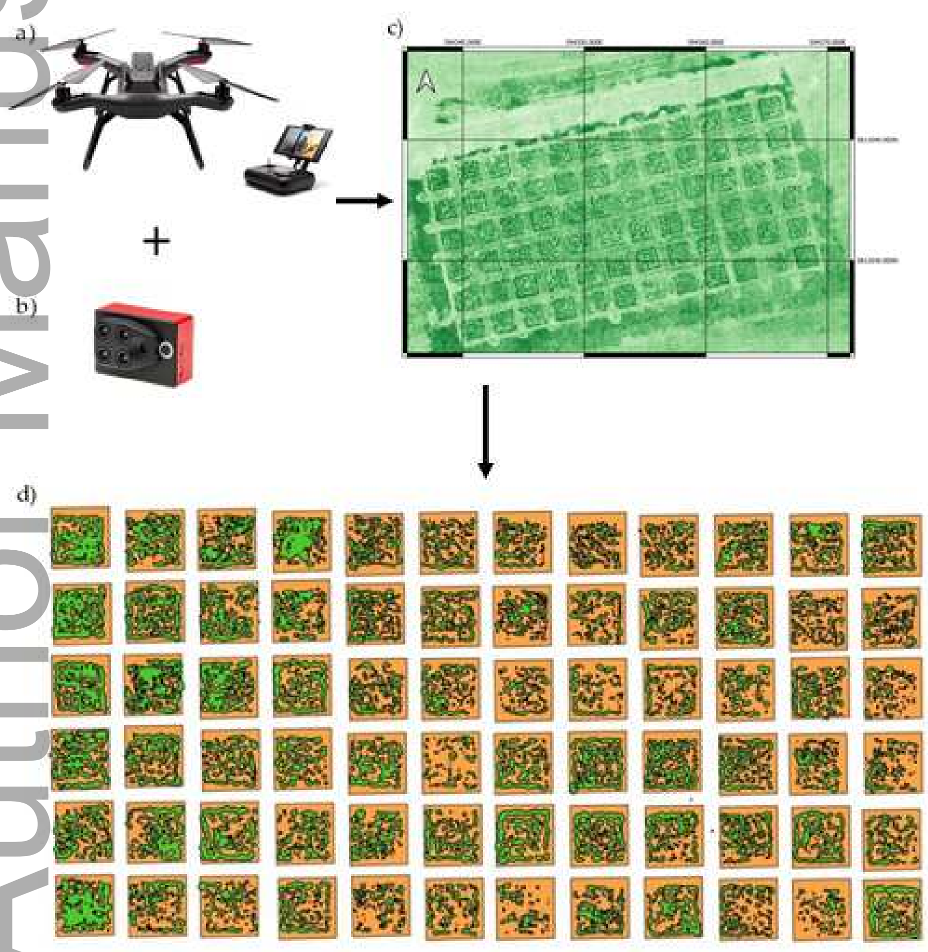

Figure 4. The multispectral spectral image-based high-throughput pipeline for perennial ryegrass ground cover estimation; (**a, b**) unmanned aerial vehicle and the ground control unit, (**c**) NDVI orthomosaic, where the intensity of pseudocolour green represents NDVI variation of the orthomosaic, (**d**) the ground cover classification map where green represents perennial ryegrass ground cover; orange represents soil and weed cover within the experimental plot boundaries, (adapted from Jayasinghe et al, 2019).

5.1.3 Thermography

Reflected radiation in the IR (infrared) region (wavelength range: 9-14 µm) shows a relationship with the overall heat of its surface, which is referred to as the "heat signature" (Gonzalez-Dugo et al, 2013). Thermography is an imaging technique of infrared radiation, which visualises an object using a heat signature (Walter et al, 2012). The transpiration rate can be reduced due to the stomata of leaves close, under water deficit conditions (Nanda et al, 2018). Due to lowering transpiration rate, plant canopy temperature of well-watered plants can be lower than plants that are water-stressed (Walter et al, 2012). Thermal imaging may have its greatest potential in identifying drought-tolerant genotypes in plant breeding programs (Gonzalez-Dugo et al, 2013; Zia et al, 2013). Drought resistance of perennial ryegrass may in turn support its persistence during drier summers, which can be phenotyped using thermal imaging in pasture breeding. Data acquisition time is very important in thermography as subtle changes in environmental conditions can affect acquired data (Still et al, 2019). A good knowledge of emissivity of the object and its surrounds is also required for more accurate image acquisition (Havens and Sharp, 2016). Thermal imaging is more suited to aerial vehicles with an altitude that may minimise the amount of time and images required to capture the whole trial site.

5.1.4 LiDAR

Light Detection and Ranging is laser technology, that uses a laser pulse to produce a point cloud by calculating the time difference between laser pulse emission and reflected light detection (Kumar et al, 2015). The point cloud is a constructed 3D structure (Lin, 2015), which may comprise physical dimensions of a target object. The LiDAR sensor has been a widely used technology in different agricultural studies since 1980 (Lee et al, 2010). Pasture biomass estimation (Wiering et al, 2019), dry matter yield and growth rate measurement in perennial ryegrass (Ghamkhar et al, 2019), mapping and monitoring of biomass and grazing in pasture (Michez et al, 2019), weed detection and discrimination from grass in agricultural lands (Escolà et al, 2012; Andújar et al, 2013), pest/disease monitoring in the field (Gebru et al, 2017; Pham et al, 2018; Song et al, 2020), determination of foliage yield and growth rate in perennial ryegrass (Ghamkhar et al, 2019) and estimation of herbage yield in tall fescue (Schaefer and Lamb, 2016)are recent applications of UAV-based LiDAR in precision agriculture. These approaches show prospects to use LiDAR sensors as an HTP tool for phenotyping pasture traits such as canopy cover, root architecture, herbage biomass, plant volume and plant structure, species composition and pest resistance. However, it may require complex data analysis pipelines for phenomics data extraction from LiDAR images as it generates “big data sets”(Qiu et al, 2019). LiDAR sensors are a relatively expensive technology compared to other imaging sensors and the quality of data acquisition may be impacted by environmental factors such as moisture and small particles in the air (Xharde et al, 2006).

5.1.5 Fluorescence imaging

Fluorescence imaging is an acquisition of the fluorescence signal in which particular chemical compounds, when hit with a specific wavelength emit a different specific wavelength (Wouterlood and Boekel, 2009). The fluorescing part of plant tissue is the chlorophyll complex. Plant pathogens are severe constraints to productivity and persistence of pasture in the temperate region, and early detection of pathogens is essential to minimise the spreading of infections. Investigation of pathogen resistance in pasture breeding is often based on artificial inoculation in controlled environments, and these procedures are biased and time-consuming for targeted improvement of disease resistance. In recent studies, fluorescence imaging was used as a precise tool to diagnose virus infection in crops and early response to biotic and abiotic stress in relation to changes of photosynthetic pigments in crops (Lohaus et al, 2000; Chaerle et al, 2007). Identification of perennial ryegrass genotypes with greater pathogen resistance could be achieved using fluorescence imaging. The fluorescence imaging technique could also be used in research for screening plant traits such as frost and salt tolerance (Buschmann and Lichtenthaler, 1998; Gorbea and Calatayud, 2013). An ability to survive in freezing temperatures is an important trait, which can be affected pasture survival rate in the temperate region (Wilkins, 1991). Most of the fluorescence imaging studies are limited at the level of a single leaf or an individual plant level, and the level of power requirement for generating fluorescence signal may limit the use of this technique in field-based applications (Li et al, 2014).

5.1.6 X-ray computed tomography

X-ray computed tomography (X-ray CT) is a non-destructive technique for visualising interior features of solid objects, which can generate a 3D image of an object from an extensive series of 2D radiographic images (Li et al, 2014). X-ray computed tomography has been applied for many crops, including barley, maize, wheat, and chickpea, to examine non-destructive root structure (Lontoc-Roy et al, 2006; Hargreaves et al, 2009). Perennial ryegrass plants with longer root systems can uptake available nutrition and water from deeper soil layers, which may support plants to survive under some abiotic stresses such as drought and nutrition deficiency. (Sokolovic et al, 2013) discovered that perennial ryegrass cultivars with better persistency showed higher proportions of deep roots which were 8 % heavier in total compared to poorly persisting cultivars. The implementation of X-ray CT for phenotyping pasture root morphology can be used to identify drought resistance genotypes in breeding programs. However, X-ray CT is a time-consuming technique and requires a high energy supply to generate an X-ray CT image (Li et al, 2014). Therefore, applications of X-ray CT for root phenotyping may limit for small scale under field conditions.

Field deployment of image sensors is promising as an effective and efficient source of reliable phenomics data for pasture phenotyping. However, data extraction from images, data interpretation and statistical analysis must also be considered when developing image-based tools for perennial ryegrass persistence estimation and these will be covered in the following sections.

6. Phenomic feature extraction from images

The process of quantitative feature extraction from images involves a pre-processing workflow and feature extraction pipeline. Prediction of some pasture traits such as dry matter yield can be achieved using vegetation indices (Gebremedhin et al, 2019). However, development of prediction tools for some measurements such as ground cover classification may require advanced machine learning algorithms for phenomic feature extraction. The complexity of the required pipeline for feature extraction may depend on the target trait. Estimation of perennial ryegrass persistence may require investigating changes in more complex processes such as botanical composition and plant senescence (described in more detail in section 2.). Therefore, we have reviewed a range of phenomic features such as VIs, object-based and pixels-based approaches and their feasibility for perennial ryegrass persistence estimation.

6.1. Image preprocessing

In preprocessing, images are imported into a computer-based script and run through a series of steps such as image cropping, contrast improvement, colour noise reduction, image smoothing, image reconstruction, geometric correction and radiometric calibration (Perez et al, 2017). Image preprocessing may improve the quality of data extraction in subsequent steps (Wang et al, 2017). Geometric error of acquired airborne images is a common defect of remote sensing data due to altitude differences between the camera and the target position on the ground (Gebremedhin et al, 2019). Geometric calibration (georectification) is the process to accurately link geotagged images with a known ground location. The most common way of geometric calibration is done by matching geo locations of ground control points (GCP) with geotagged airborne images (Hu et al, 2018).

Airborne and spaceborne images may have fluctuation in the radiometric resolution due to changes in environmental factors such as brightness, cloudiness or surface temperature during image acquisition (Wyatt, 1978b). Several approaches of radiometric calibration methods are available, depending on the data acquisition and extraction methods (Wyatt, 1978c). The use of known spectral values of ground-based panels is a widely implemented calibration method (Wyatt, 1978a; Guo et al, 2019). To estimate pasture persistence in breeding plots may require evaluating images from many years, and image data acquisitions could happen under a range of environmental conditions at different times of the year. Therefore, radiometric calibration in image preprocessing is an important element to maintain consistency of airborne images. After preprocessing, images analysis algorithms are executed through a computer vision workflow to extract target phenomic features such as VIs, ground cover and texture.

6.2 Feature extraction

Total incident solar radiation reflects, transmits, and absorbs on the surface of an object, and the ratio of the reflected portion to the entire incident solar radiation is called spectral reflectance (Zwiggelaar, 1998). The spectral reflectance of an object consists of a full spectrum, and the amplitudes of spectral reflectance within a specific region of the EMS may be subject to biophysical properties of a target object (Gates et al, 1965)(Figure 5). An image sensor captures particular regions of spectral reflectance, called "spectral bands", which stacks properties of spectral information in small regions, called "pixels" (Abdou et al, 1996). In plant phenomics, spectral properties of pixels may transform into a legible format such as vegetation indices, pixel-based and object-based features such as ground cover and plant volume using an automated or a semi-automated workflow.

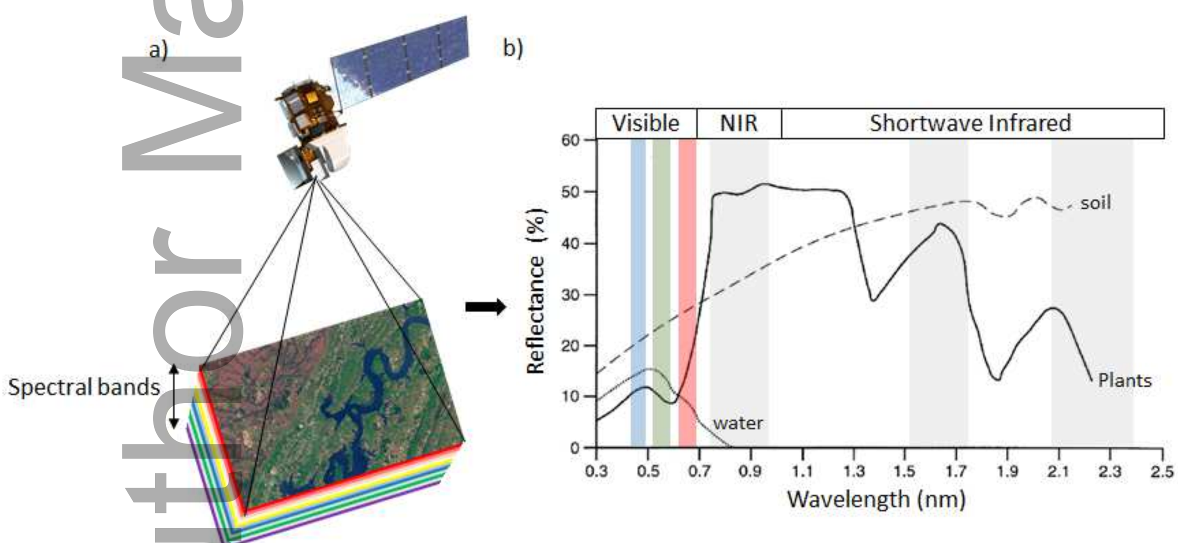

Figure 5. (**a**) The acquisition of reflectance bands from spaceborne hyperspectral sensor (e.g. Landsat-7 ETM; Enhanced Thematic Mapper), (**b**) typical spectral reflectance curves for soil, water, and plants where colour and grey area represents locations of acquired spectral bands of the hyperspectral sensor (e.g. Landsat-7 ETM+; data source for the graph from Roy et al., 2016).

6.2.1 Vegetation indices

Vegetative indices (VIs) are an algebraic combination of two or more spectral bands designed for quantitative and qualitative evaluations of plant physiological and biochemical properties in remote sensing studies (Abdou et al, 1996). The first two VIs; ratio index (RI) and vegetation index number (VIN) were developed by (Pearson and Miller, 1972) for mapping of standing crop biomass and estimation of the productivity of the shortgrass, combining NIR and red spectral bands (Abdou et al, 1996). With the recent technological progress, image sensors have been improved in acquisition speed, spatial resolution, and sensitivity range of spectral sensor, which may result in a wide range of VIs in precision agriculture. In this section, we discuss recent applications of VIs in precision agriculture and their feasibility for pasture persistence estimation.

Annual dry matter yield data across consecutive years is a useful means to assess persistence in pasture breeding. However, the weaknesses of conventional methods for pasture mass estimation may create a great challenge to breeders to use long-term dry matter yield data for assessment of persistence (Zwiggelaar, 1998). Normalized difference vegetation index (NDVI) is the normalised ratio between the red and near-infrared bands, proposed by Rouse (Rouse et al, 1974). Normalized difference vegetation index has a positive relationship with chlorophyll and nitrogen concentration in plant materials (Xue and Su, 2017). The mathematical equation of NDVI can be expressed as NDVI = $(R_n - R_r)/ (R_n + R_r)$, where R_n and R_r are the reflectance values of near-infrared and red bands. A recent study showed that combining NDVI and plant height offers a robust method to estimate herbage dry matter yield in perennial ryegrass breeding programs (Gebremedhin et al, 2019). Moreover, NDVI derived from seasonal time-series LANDSAT images showed a high throughput for forest aboveground biomass estimation (Zhu and Liu, 2015). However, the NDVI measure is known to saturate after a certain biomass density or ground cover is achieved (Lu, 2006). The green normalised difference vegetation index, GNDVI = $(R_n - R_g)/(R_n + R_g)$ is the normalised ratio between the green and near-infrared bands of EMS. Green normalised difference vegetation index is sensitive to variations in green vegetation (Xue and Su, 2017). However, the index can be saturated in dense vegetation conditions when the leaf area index becomes high. Green normalised difference vegetation index derived from satellite remote sensing was efficiently used for assessment of winter crop biomass (Raymond et al, 2013). Further development of these approaches for pasture breeding may open a great opportunity to assess persistence in large-scale breeding programs.

The estimation of fractional vegetation cover is feasible using image-based HTP (Balfourier et al, 1998), which may replace manual methods of ground cover estimation. UAV-based digital colour images were used to estimate the green fraction of pasture breeding plots using VIs (Borra-Serrano et al, 2018), and this approach has shown a strong relationship between manual ground cover and vegetation indices such as excess green (ExG2) = (2 x G - R – B)/(R + G + B), green leaf index (GLI) = (2 x G - R – B)/(2 x G + R + B) and normalised green intensity (NGI) = G/(R + G + B); where R, G, and B represent red, green and blue bands (visual ground cover vs. sensor-based ground cover - $r > 0.88$, $p = 0.001$).The senescent fraction of ground cover may be used as a parameter for pasture persistence estimation. However, current methods for pasture senescence estimation in pasture breeding methods depend on hand sorting clipped samples. A laboratory radiometric method has been developed for the rapid determination of green and senescent fractions in clipped perennial ryegrass samples using NDVI (Tucker, 1980). This method may permit the use of rapid green and senescent fraction determinations to replace hand sorting, and may also apply as ground-truth sampling where destructive dead and green biomass is necessary for validating remote sensing methods. With further development, this method could be implemented to estimate pasture senescence as an indicator of pasture persistence. However, separation of perennial ryegrass dead fraction from soil may be problematic using a multispectral camera as both soil, and dead fraction provide a relatively similar spectral signature in the visible region of EMS. However, spectral properties of the dead fraction may show different spectral properties in the shortwave NIR region of EMS due to the presence of cellulose and lignin (Nagler et al, 2003). The cellulose absorb index (CAI) and plant senescence reflectance index (PSRI) describe the average depth of the cellulose absorption feature at 2.1 nm wavelength in reflectance spectra and have been implemented to estimate plant litter in grassland (Ren et al, 2012). (Jayasinghe et al, 2020) have successfully used Vis, CAI and PSRI extracted from a proximal hyperspectral sensor for pasture senescence estimation.

$$\textbf{Cellulose Absorption Index (CAI)} = \mathbf{0.5}\ (\mathbf{R_{2.0}} + \mathbf{R_{2.2}}) - \mathbf{R_{2.1}} \text{ (Nagler et al, 2003)}$$

$$\textbf{Normalised Difference Lignin Index (NDLI)} = \frac{\log\left(\frac{1}{R_{1.7}}\right) - \log\left(\frac{1}{R_{1.6}}\right)}{\mathrm{Log}\left(\frac{1}{R_{1.7}}\right) + \log\left(\frac{1}{R_{1.6}}\right)} \quad \text{(Serrano et al, 2002)}$$

$R_{2.0}$, $R_{2.1}$, $R_{2.2}$, $R_{1.7}$ and $R_{1.6}$ are reflectance factors in bands at 2.00-2.05, 2.08-2.13, 2.19-2.24, 1.754, and 1.680 µm, respectively.

This study showed a positive relationship between these VIs and senescent pasture in perennial ryegrass breeding plots andthe application of these approaches may enable rapid estimation of plant senescence in pasture breeding to assess resistance to abiotic stress such as drought and nutrition deficiencies (Regression model for senescent fraction prediction, $y= 429x_1 + 21.83$, $R^2 = 0.57$, SE = 3.64; $y= 595.67x_2 + 17.335$, $R^2 = 0.43$, SE = 4.20; where y is senescent fraction, x_1 is CAI and x_2 is NDLI).

Estimation of sward botanical composition has received more attention from researchers in recent decades (Peng et al, 2018) because variations in sward botanical composition can effect pasture productivity (Waller and Sale, 2001; Reed et al, 2011). Spectral heterogeneity among perennial ryegrass and weeds may offer potential to develop a rapid non-destructive method to estimate weed ingression in breeding plots or a sward (Walter et al, 2012). Plant species diversity was precisely estimated at a fine-scale using hyperspectral indices such as ratio vegetation index (RVI) = R_{675}/R_{782}, NDVI = $(R_{782} - R_{675})/(R_{782} + R_{675})$, difference vegetation index (DVI) = $R_{810} - R_{680}$ and soil-adjusted vegetation index (SAVI) = $((R_{782}\text{-}R_{675})/(R_{782} + R_{675} + 0.2))$ (1.2), where the ratio of R represent reflectance at i^{th} band to the sum reflectance value (Peng et al, 2018). Moreover, a recent study has demonstrated the potential of hyperspectral-based NDVI for mapping the spatial distribution of botanical composition in pasture using linear discriminant analysis models (Suzuki et al, 2012). These studies have discovered the close connection between plant diversity and spectral indices, and it may enable the estimation of weed ingression to assess persistence in pasture breeding.

The automated estimation of plant diseases and insect attack at an early stage is vital for precision crop protection. Many studies have revealed that vegetation indices derived from remote sensing platforms have a high potential for discriminating healthy and diseased or pest stressed plants (Chew et al, 2014). Discrimination of plants that were non-inoculated or inoculated with *Uromyces betae* was achieved using the NDVI-based classification model at 71% accuracy (Rumpf et al, 2009). Moreover, VIs; chlorophyll absorption index (CAI) = R_n x R_r/R_gx2, where R_n, R_r and R_g are spectral reflectance of NIR, red and green bands; photochemical radiation index (PRI) = $(R_{531} - R_{570})/(R_{531} + R_{570})$ where the ratio of R represents reflectance at the ^{i}th band to the sum reflectance value. PRI has shown noticeable reduction of index value from the infected plants under light stress conditions (p = 0.001) (Chew et al, 2014). Analysing the relationship between manual disease rating and selected vegetation indices from pasture breeding plots could be used to develop a robust model for plant disease and pest susceptibility rating in terms of pasture persistence estimation.

Current remote sensing studies in precision agriculture show a robust empirical relationship between plant canopy characteristics and VIs. However, the relationships developed between ground truth sampling and remotely sensed data might not be accurate due to high-resolution saturations, soil brightness or other factors such as dust, cloud and shadowing. For example, in wheat, the mathematical relationships developed between ground truth observations of plant canopy characteristics, and corresponding values of vegetation indices for five different geographical locations were qualitatively similar but differed in the specific values of the coefficients in the relationships across the sites (Wiegand et al, 1992). Therefore, HTP approaches for pasture persistence estimation may need to move forward with more advanced image analysing procedures such as pixel-based or object image analysis scripts.

6.2.2 A pixel-based image analysis for feature extraction

The pixel-based phenomics pipeline analyses the spectral properties of every pixel within the area of interest using either a supervised or unsupervised classification or some combination (Weih and Riggan, 2010). It has been found that use of pixel-based methods for high-resolution images (at least 118 pixels per cm) results in low accuracy in image classification due to a "salt and pepper" effect (De Jong et al, 2001; Whiteside et al, 2011). Perennial ryegrass may have more heterogeneous individuals in large-scale breeding programs (Wilkins and Humphreys, 2003), and because perennial ryegrass is a densely tillered plant with leaves overlapping, this may cause shadows and scattering in reflectance spectrum. The increased spectral heterogeneity of neighboring pixels within ground cover classes often leads to an inconsistent classification (Whiteside et al, 2011). This can be overcome by averaging pixel number (e.g. 2x2-4 pixels) in image analysis pipeline, which is available with modern image analysis algorithms. Therefore, alternative learning algorithms such as an object-based image analysis (OBIA) may require extracting more accurate phenomic data for pasture phenotyping.

6.2.3 Object-based image analysis for feature extraction

An object-based image analysis was developed in the 1970s for remote sensing studies in the Alpine forest environment (De Kok et al, 1999). However, the initial application was limited by hardware, software, and poor resolution of images (Flanders et al, 2003). In the process of OBIA, image pixels cluster together into vector objects, based on their spectral, textural and contextual information (Figure 6) (Yan et al, 2006).

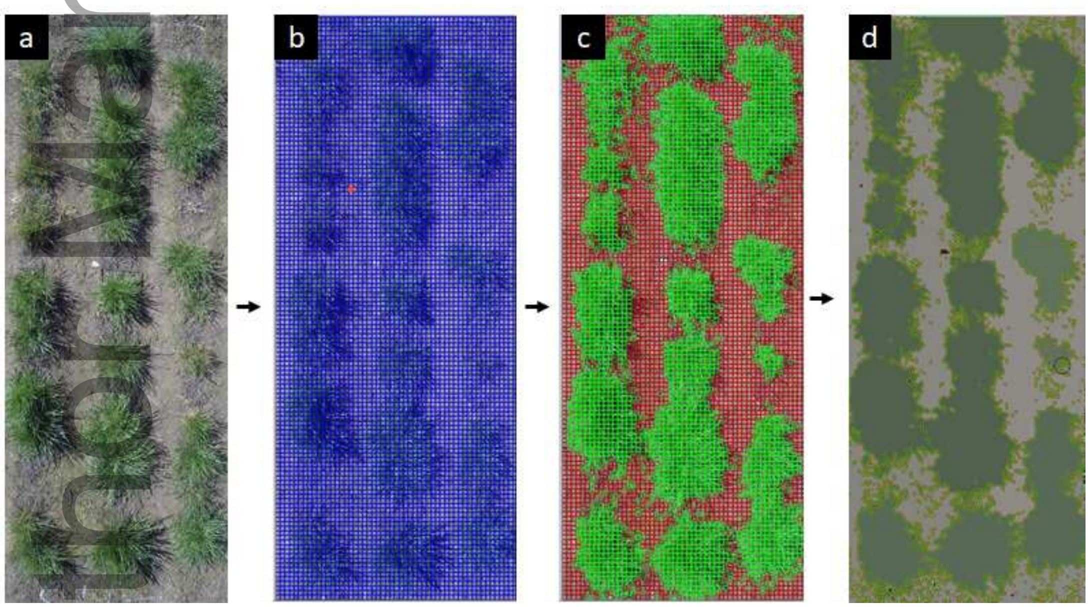

Figure 6. The process of image analysis in high throughput phenotyping pipeline for phenomics data extraction; a) image preprocessing, b) image segmentation, c) image classification, and d) classified objects of RGB image for feature extraction; where the image was classified in two types of objects to demonstrate an object-based image analysis pipeline using "k-nearest neighbor" classification algorithm in eCognition developer 9.3.2 software.

Image segmentation is a preliminary step in the OBIA. Image segmentation splits images into homogeneous logical partitions based on properties of pixels such as compactness, shape, and scale and knowledge about features of interest (Tan, 2016). After image segmentation, each logical particle is classified into classes on the basis of one or more statistical properties of the contained pixels (Perez et al, 2017). This means that all pixels within a segment are assigned to a class, eliminating problems associated with pixel-based approaches. Several studies have confirmed the superiority of OBIA over pixel-based classifications, especially in heterogeneous agricultural landscapes (Yan et al, 2006). As such, OBIA may have potential to extract phenomic features such as ground cover, plant number and senescent fraction to assess persistence of perennial ryegrass. Application of machine learning scripts such as k-NN (k-nearest neighbor), support vector machine (SVM) in OBIA optimise the accuracy of phenomics feature extraction (Perez et al, 2017).

6.2.4 Machine Learning approaches for phenomics feature extraction

Image pixels show a non-linear relationship with a range of plant traits such as leaf, fruit, plant organ, plant height, growth rate, and dry matter yield (Furbank and Tester, 2011). The standard image analysis pipelines depend on hand-engineered image processing parameters and have minimal throughput to extract these complex phenomic features from images causing a phenotyping bottleneck (Furbank and Tester, 2011; Walter et al, 2015). In the last two decades, machine learning techniques have become very popular in plant phenomics due to their robustness, accuracy and capability to handle more sophisticated "big data" (Liakos et al, 2018). Machine learning may be a supportive tool to combine phenomic, genomic and environment interactions to describe plant performance in a given environment (Dechter, 1986). Machine learning is expected to establish a prominent place in the future of image-based HTP under both controlled environment and field conditions. In terms of pasture persistence estimation, scientists cannot solely rely on linear function plant traits and may need to capture data on more complex traits such as root related traits or leaf morphology. Investigation of existing ML technology and their approaches in smart agriculture may enable the phenotyping of more complex plant attributes for pasture persistence estimation. For instance, the reflectance spectra of senescent pasture and bare ground lack the unique spectral signature in the visible region (400–700 nm wavelength) of the reflectance spectrum (Aase and Tanaka, 1991). This makes the discrimination between soil and senescent pasture in RGB images difficult or nearly impossible using a standard image analysis algorithm. However, a recent study used advanced ML algorithms; the k-nearest neighbor (k-NN) to discriminate senescent perennial ryegrass from the bare ground for persistence estimation (Regression model for senescent fraction prediction, y_1= 0.858x + 2.9845, R^2 = 0.65, SE = 3.08; y_2= 1.5207x + 2.73, R^2 = 0.71, SE = 4.07; where y_1 is visual senescent fraction, y_2 is dry matter percentage of senescent fraction and x is k-NN based senescent fraction) (Jayasinghe et al, 2020). In the k-NN analysis, RGB images are segmented into small equal partitions (1pixel x 1pixel) and classified according to the relationship of its k-NN with training samples (Figure 3.).

7. Phenomics data modelling

The current and future image-based platforms generate terabytes of information. Therefore, data modelling needs to become an essential framework for plant phenomics to develop hypotheses allowing multi-scale interpretations of features extracted from images (Tardieu et al, 2017). Due to the precision of image-based HTP, a phenomics pipeline may allow the extraction of multiple traits that contribute to pasture persistence to be measured at high temporal and spatial resolution. The data modelling can be used to identify the features most responsible for pasture persistence estimation in breeding trials. For instance, a recent study showed that an empirical model developed using features generated from airborne multispectral data and a machine learning modelling framework offers an excellent prospect for pasture dry matter yield prediction (manual dry matter vs. ML-based - Lin’s concordance values > 0.8, root mean squared error < 25%) (Karunaratne et al, 2020). Moreover, a machine learning technique, Cubist was used to analyse canopy spectra to predict perennial ryegrass nutritive characteristics, which may speed up the process of pasture nutritive value estimation under field conditions (Laboratory data vs. ML-based - R^2 = 0.49-0.82, Lin’s concordance values = 0.68-0.89) (Smith et al, 2020). Invasion of weeds and less productive species may reduce dry matter production in grazing systems and important pasture nutrient concentration of an animal intake such as crude protein (CP) acid detergent fibre (ADF) (Hume and Sewell, 2014). Therefore, the model developed in these recent studies could be adapted to monitor the expression of persistence of a sward. Perennial ryegrass adapts to manage biotic and abiotic stressors via changing morphological traits and adjusting their physiological behaviour (Waller and Sale, 2001). Perennial ryegrass may show complex interactions between genotypes, endophyte and environment at different scales to manage the plant development and mortality. The interactions between these three factors are still unclear. Statistical dynamic models such as artificial neural network and support vector machines have been proven to be an efficient phenomic approach to identify abiotic and biotic effects on plant phenotypes using machine learning algorithms (Safa et al, 2019; van Eeuwijk et al, 2019; Castro et al, 2020). Implementation of modern data modelling techniques may help to find answers to unsolved queries related to pasture persistence.

8. Challenges of image-based HTP for pasture breeding

Over the past few decades, plant phenomics has seen significant improvements through development of novel sensors and sensor-bearing platforms for phenotyping a wide range of traits, and biophysical processes. However, data handling and processing remain major challenges when translating sensor information into phenotyping knowledge (Yang et al, 2020). The primary aim of pasture phenotyping focusses on the quantification of biomass, nutritive characteristics and persistence at the single plant or population level under field conditions. Field conditions are heterogeneous and environmental factors make results difficult to interpret. However, indoor growth chambers or glasshouses are not the best substitutes for pasture phenotyping. Simulation and providing actual field conditions in a crossing room may create a real challenge for pasture breeders. Pasture breeding is driven by open pollination in field conditions, resulting in highly heterozygous populations. Therefore, understanding genotype x environment interaction using HTP is a challenging process due to the complexity of the perennial ryegrass genome (Araus and Cairns, 2013).

Application of sensor-based technologies for pasture persistence estimation will be a robust process. However, it may require long-term, sustained records of sensor-based data. Phenomics data, derived from airborne platforms are prone to have uncertainty due to changes in various environmental factors such as light conditions, solar radiation angle, atmospheric temperature, strong wind and air moisture (Ruicheng et al, 2018; Gebremedhin et al, 2019). Phenotyping technologies and protocols have no or inadequate capacity to assess some of the plant traits under field conditions (Araus and Cairns, 2013), and these traits may be vital factors for assessing pasture persistence. For instance, drought tolerance of forage grasses has a close relationship with both root morphology and physiology. However, available technology for root phenotyping are invariably destructive, time-consuming and their application for field phenotyping is limited or similar to ordinary traditional methods.

Current image analysis software offers a wide range of image processing algorithms. However, there is a lack of information available on the comparison of performances of these algorithms (Madabhushi and Lee, 2016). Therefore, validation of image processing algorithms requires comparing image data with ground truth data, which can be based on numerical real physical measurements. Some pasture breeding trials may occupy a large area (hectares in size) and collecting ground truth data from large breeding trials may be challenging and expensive (Gebremedhin et al, 2019; Gebremedhin et al, 2020).In an image, some of the pixels may potentially have a completely different colour profile from neighboring pixels due to leaf overlap and false colouring (Chianucci et al, 2018), and this will lead to overfitting or classification bias in image analysis. Therefore, It is important to have a quality inspection step in the image analysis pipeline using model cross/independent validation or appropriate algorithms, such as the watershed algorithm (Pahikkala et al, 2015; Wang et al, 2018).

Application of HTP platforms in precision agriculture may be costly due to high initial purchase and maintenance costs associated with HTP platforms, that may compensate with the cost related with manual phenotyping methods in large scale breeding programs (Yang et al, 2020). However, pasture farmers or small-scale pasture breeders may not be interested in using HTP platforms in their farm or breeding programs due to a low financial incentive. Moreover, applications of HTP platforms may require pre-training and knowledge, that may make use of HTP platforms problematic at the farm scale. Therefore, expenses associated with HTP platforms may create significant challenges in precision agriculture to develop low-cost, user-friendly solutions for small scale applications.

Conclusions

Persistence estimation in pasture breeding depends on manual ground cover estimation or counting number of plants in a given area. These conventional methods are time consuming and subjective and not suitable for persistence estimation in large scale field trials. The investigation of sensor-based technology for pasture persistence estimation has commenced in pasture breeding programs. Phenotyping data from a recently developed persistence estimation tool showed a strong relationship with manual ground-based observations. However, this tool was not sensitive enough to phenotype complex traits such as sward composition in breeding plots. Expression of persistence in perennial ryegrass populations can be estimated by investigating fractions of ground cover, long-term annual dry matter production, intensity of weed ingression, pest attack and disease infection. With the rapid development in sensor technologies and image processing software, image-based HTP has been widely implemented in precision agriculture to discover solutions for compelling issues in crop and pasture, including yield prediction, ground cover estimation, pathogen and disease severity estimation, weed discrimination and yield and nutritive characteristics prediction. Image-based HTP approaches have encountered various challenges due to a lack of knowledge in image processing and limitations of sensors such as poor temporal and spatial resolution. However, existing airborne and ground based HTP approaches in precision agriculture offers opportunities for pasture phenotyping, and further development of these approaches may enable a precise sensor-based tool to assess persistence of perennial ryegrass in pasture breeding. The use of image-based HTP for persistence estimation may reduce the required time for releasing new cultivars achieving industry targets in an acceptable time frame.

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Figure legend

Figure 5. Elements of high throughput phenotyping technologies that could be used for estimating expression of pasture persistence under field conditions, where LiDAR is light detection and ranging, and ML is machine learning.

Figure 6. Scaling of high-throughput phenotyping platforms in precision agriculture, where (**a**) is field scanning using a handheld sensor, (**b**) ground-fixed environmental sensor, (**c**) a phenotyping ground vehicle, (**d**) a field scanning platform, (**e**) a phenotyping tower, (**f**) an unmanned aerial vehicle, (**g**) a low altitude phenotyping helicopter, (**h**) a phenotyping hot air balloon and (**i**) a satellite remote sensing platform.

Figure 7. Application of proximal RGB imaging for perennial ryegrass ground cover estimation in pasture breeding plots; (**a**) A DSLR camera mounted on a tripod for RGB image acquisition (**b**) RGB image (12 megapixels) from nadir position and (**c**) classified RGB image using a pixel-based image analysis techniques, where red colour represents perennial ryegrass green fraction, adapted from (Jayasinghe et al, 2019).

Figure 8. The multispectral spectral image-based high-throughput pipeline for perennial ryegrass ground cover estimation; (**a, b**) unmanned aerial vehicle and the ground control unit, (**c**) NDVI orthomosaic, where the intensity of pseudocolour green represents NDVI variation of the orthomosaic, (**d**) the ground cover classification map where green represents perennial ryegrass ground cover; orange represents soil and weed cover within the experimental plot boundaries, (adapted from Jayasinghe et al, 2019).

Figure 5. (**a**) The acquisition of reflectance bands from spaceborne hyperspectral sensor (e.g. Landsat-7 ETM; Enhanced Thematic Mapper), (**b**) typical spectral reflectance curves for soil, water, and plants where colour and grey area represents locations of acquired spectral bands of the hyperspectral sensor (e.g. Landsat-7 ETM+; data source for the graph from Roy et al., 2016).

Figure 6. The process of image analysis in high throughput phenotyping pipeline for phenomics data extraction; a) image preprocessing, b) image segmentation, c) image classification, and d) classified objects of RGB image for feature extraction; where the image was classified in two types of objects to demonstrate an object-based image analysis pipeline using "k-nearest neighbor" classification algorithm in eCognition developer 9.3.2 software.