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REVIEW: PART OF A HIGHLIGHT ON BREEDING STRATEGIES FOR FORAGE AND GRASS IMPROVEMENT

Advanced phenotyping offers opportunities for improved breeding of forage and turf species

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• *Background and Aims* Advanced phenotyping, i.e. the application of automated, high-throughput methods to characterize plant architecture and performance, has the potential to accelerate breeding progress but is far from being routinely used in current breeding approaches. In forage and turf improvement programmes, in particular, where breeding populations and cultivars are characterized by high genetic diversity and substantial genotype × environment interactions, precise and efficient phenotyping is essential to meet future challenges imposed by climate change, growing demand and declining resources.
• *Scope* This review highlights recent achievements in the establishment of phenotyping tools and platforms. Some of these tools have originally been established in remote sensing, some in precision agriculture, while others are laboratory-based imaging procedures. They quantify plant colour, spectral reflection, chlorophyll-fluorescence, temperature and other properties, from which traits such as biomass, architecture, photosynthetic efficiency, stomatal aperture or stress resistance can be derived. Applications of these methods in the context of forage and turf breeding are discussed.
• *Conclusions* Progress in cutting-edge molecular breeding tools is beginning to be matched by progress in automated non-destructive imaging methods. Joint application of precise phenotyping machinery and molecular tools in optimized breeding schemes will improve forage and turf breeding in the near future and will thereby contribute to amended performance of managed grassland agroecosystems.

Key words: Forage, turf, breeding, phenotyping, growth, biomass, imaging, marker-assisted selection, remote sensing.

DEFINITION AND CONCEPTUAL BACKGROUND OF PLANT PHENOTYPING

Plant phenotyping aims at a quantification of quality, photosynthesis, development, architecture, growth or biomass productivity of single plants or plant stands using a broad variety of analysis procedures. It presents an indispensible means to investigate physiological principles involved in the control of basic plant functions as well as for selecting superior genotypes in plant breeding programmes. Some of these procedures are well-known analysis tools of classical plant physiology based on visual observations, measurements or biochemical analyses. Others consist of target-specific and highly automated analysis procedures which have been established in recent years. Two lines of technological developments are currently converging towards each other, prospectively resulting in novel capabilities for improved phenotyping in the near future. On the one hand, devices originally designed for detecting total leaf area of small rosette model plants such as *Arabidopsis thaliana* (Leister *et al.*, 1999) have been improved, complemented by other techniques and brought to a stage at which a range of traits can be detected in laboratory-grown plants at high throughput (more than 1000 plants per day; Granier *et al.*, 2006; Walter *et al.*, 2007; Rajendran *et al.*, 2009). On the other hand, field monitoring and imaging methodologies used in remote sensing or precision agriculture have been improved and refined, providing relevant information on plant phenotypes in the field (Montes *et al.*, 2007).

Quantitative imaging in the field is more problematic than conceived intuitively. Variable illumination, dissected, reflecting plant canopies, altered spectral composition of the sunlight in different weather conditions, plant movements due to wind or rain, and many other factors complicate the retrieval of quantitative information from pictures in the field. Moreover, to provide meaningful information about the performance of plants in a certain environmental context, a set of environmental parameters needs to be recorded throughout a relevant time period to analyse genotype × environment interactions. Advanced phenotyping in the field also implies reconsideration of the experimental set-up regarding size and replication number of experimental plots in order to account for inhomogeneous soil and microclimate conditions. An experimental set-up specifically adapted to given environmental conditions will help to optimize statistical power of collected data and to reliably estimate phenotypes and interaction parameters.

Hence, automated phenotyping approaches are far more successful at the laboratory and greenhouse scale at present,

where they have already proved beneficial for certain applications, for example in rice research (Reuzeau *et al.*, 2005; De Wolf *et al.*, 2008). In these approaches, single plants are usually analysed in a static context, meaning that side-by-side comparisons of a range of plant genotypes are performed in a given set of environmental conditions. Yet, the dynamic response of plants is also analysed in some approaches (Walter *et al.*, 2007; Jansen *et al.*, 2009). In these, the reaction of growth towards an onset of drought stress, towards dynamical changes of light or temperature can be followed, requiring analysis of plant size at least at two consecutive points in time. In most cases, shoots or canopies are monitored, but root systems and root–soil interactions are beginning to be analysed non-destructively (Zhu *et al.*, 2011). Yet, even under more controlled glasshouse conditions, environmental factors such as light intensity or spectral composition of solar radiation vary to a certain degree, thereby complicating imaging-based phenotyping approaches.

The overall goal of phenotyping approaches with respect to plant breeding is to quantify or rank the success of a range of genotypes in certain environmental frameworks. Therefore, usually hundreds or thousands of genotypes have to be compared with each other. This requires rapid measurement procedures, a high throughput, a high degree of automation and access to appropriate, well-conceived databases (Kolukisaoglu and Thurow, 2010; Fabre *et al.*, 2011). Yet, methods that provide a high resolution at low throughput (fewer than 10 plants per day) can also be extremely helpful in depicting the performance of certain genotypes in a relevant environmental context (e.g. nuclear magnetic-resonance-based imaging of internal plant structure; Jahnke *et al.*, 2009).

Phenotyping of course can also include automated analyses of the plant transcriptome, proteome, metabolome or ionome (Kolukisaoglu and Thurow, 2010), but this review will focus on currently available techniques to monitor plant size, architecture, growth, photosynthesis and compound composition in a non-destructive and automated manner and will evaluate how phenotyping can contribute in the future to forage and turf breeding.

POSSIBILITIES AND PLATFORMS

Analysing plant morphology and biomass production

The most widely used concept in advanced phenotyping is to determine morphological parameters such as plant height, canopy width, total leaf area, leaf number or canopy shape of a plant from an ordinary colour picture or from a few pictures per plant taken from several angles. Although this concept has first been elaborated to a high degree of automation for rosette plants such as *Arabidopsis thaliana* (Leister *et al.*, 1999; Granier *et al.*, 2006; Walter *et al.*, 2007; Jansen *et al.*, 2009; Fig. 1), it is now also being applied for monitoring growth of major grain crops (Reuzeau *et al.*, 2005; Rajendran *et al.*, 2009; Hartmann *et al.*, 2011), or ornamentals (De Hert, 2011). In some of these approaches, plants are delivered to a camera system via conveyor belts, whereas in other approaches, individual plants are placed in the viewing field

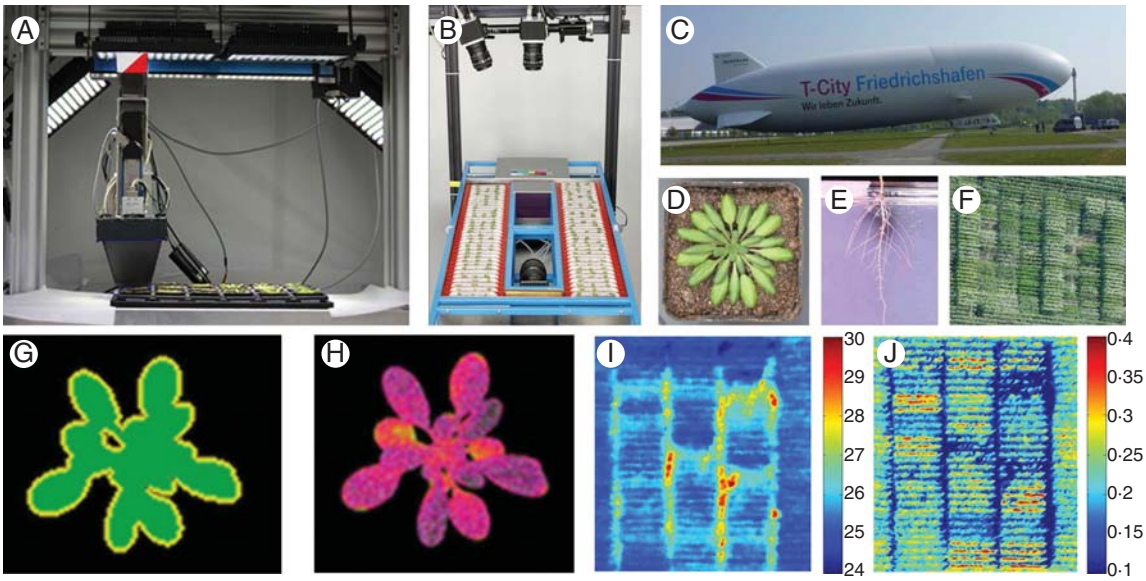

FIG. 1 Example phenotyping images. Top row: phenotyping set-ups and original images. Bottom row: result images. Phenotyping set-ups are: (A) GROWSCREEN FLUORO, a custom-made camera system that allows visualization of potted plants in the laboratory; (B) a set-up designed for automated imaging of root systems cultivated in translucent Petri dishes in the laboratory; (C) an airship used to visualize crop fields from a height of 300 m. Original images show (D) an *Arabidopsis thaliana* leaf rosette, (E) an oilseed rape root system and (F) a maize experimental field plot visualized with the above-mentioned techniques. Result images display (G) an automatically segmented leaf rosette with the outline indicated in yellow and with the green area detected as total leaf area (H) chlorophyll fluorescence image of the same plant with the colour coding for F_v/F_m – the potential efficiency of photosystem II, (I) a thermography image of a section of the maize field plot with colour coding for the surface temperature (°C) which is related to the canopy transpiration rate, and (J) NDVI as a typical spectral reflectance index, which is calculated from the canopy reflection at different wavelength bands and which refers to the greenness or healthiness of the canopy (high values indicating healthier, greener plants).

of the camera by manual or automatic positioning of the camera at a defined orientation towards the plant. Images are often acquired automatically, using a precisely defined source of illumination and are stored in a database. Proper image acquisition and image evaluation is crucial for successful extraction of the desired plant traits. The above-mentioned architectural or growth-related plant traits are extracted from images by exact calculation of the shoot outline and of enclosed pixel numbers. To achieve this, images have to be 'segmented', which means that plant and background have to be separated precisely, based on differences in colour or brightness. While this is a trivial process for an experienced human experimenter, numerous pitfalls lurk in the automated procedure: (1) overlap between canopies of neighbouring plants has to be prevented or the system needs to be provided with clear rules concerning plant separation, (2) brightness and colour values of non-target plant objects in the background must differ markedly from values on the plant, (3) shaded parts of the canopy need to be taken care of, (4) objects such as soil particles or insects situated on the plant need to be removed manually or the resulting 'holes' within the segmented image need to be filled automatically, (5) illumination conditions have to be equal for all plants to be compared and for all time points that are relevant to the experiment as coloration and segmentation can be affected enormously by varying light input, and (6) the colour information provided by most cameras (red, green, blue pixels) is often too imprecise for colour segmentation and hence needs to be transformed using specialized procedures. This list could be extended, which is the reason why automated plant phenotyping has not yet become a standard method, although high-quality imaging sensors are now available at low cost. Proper and standardized plant handling as well as exact definitions of the desired plant traits to be analysed are crucial for successful retrieval of phenotypic traits from colour images. Historically, the first approaches for such trait retrievals used custom-designed automation procedures that were adjusted to the imaging conditions in the lab (e.g. Leister *et al.*, 1999; Walter *et al.*, 2007). Nowadays, more flexible and interactive, freely available software solutions are available (e.g. Image J; http://rsbweb.nih.gov/ij/) and are being adapted for use by multiple experimenters (e.g. Hartmann *et al.*, 2011). Despite the aforementioned pitfalls, such methods hold great potential for the rapid phenotyping of complex traits. For example, digital analysis of total leaf area in the laboratory showed significant correlation of this trait with plant fresh and dry weight of *Arabidopsis* (Leister *et al.*, 1999), tobacco (Walter *et al.*, 2007) and cereals (Rajendran *et al.*, 2009). As plant height was also shown to be significantly correlated to dry matter yield of forage grasses grown in the field (Majidi *et al.*, 2009) a field-scale digital analysis of plant height may allow for a rapid prediction of dry matter yield in the field, which will be discussed in more detail later. Also, the benefit of such methods to detect genotypic differences to drought susceptibility or other environmental stresses has been shown in oilseed rape (Jansen *et al.*, 2009) and cereals (Rajendran *et al.*, 2009; Hartmann *et al.*, 2011). Therefore, an automated characterization of plant size in the field will also prove highly beneficial in forage and turf breeding in the near future.

Automated field-based extraction of morphological parameters in maize, which is mostly grown as a silage crop, has only recently been achieved (Montes *et al.*, 2011): morphological parameters such as shoot height and total leaf area were extracted for young maize plants using a so-called 'light-curtain system' (Fig. 2). This system consists of a tractor carrying a set of light barriers arranged on vertical poles. The light barriers are guided along rows of young maize plants, rendering integral values for leaf area, plant height and canopy density at defined height intervals. In addition, spectral reflectance of the canopy is analysed, which will be described in more detail later. This method would also be applicable to other plant systems such as forage crop swards and individual plants arranged in rows.

There are also non-optical approaches that have proved successful in direct determination of plant biomass without the detour of correlating leaf area to plant fresh or dry weight. One approach is the capacitive (electrical) determination of the water content of a plant that is covered by a hollow measurement device (Menzel *et al.*, 2009); other approaches determine even plant-internal structure, biomass and substance fluxes in shoots by utilizing positron emission tomography (Jahnke *et al.*, 2009) or portable nuclear magnetic resonance (NMR) imaging devices (Windt *et al.*, 2011). The latter approaches are far from being applied in agronomy on the field scale, but in the long term they will provide the opportunity to assess biomass non-destructively and in the field with a rapid measurement procedure. Moreover, these approaches can resolve plant architecture in three dimensions, which is not the case in all imaging solutions described above – even if multiple images are acquired from different perspectives. A three-dimensional representation of plant structures that helps to analyse shoot branching patterns, flower morphologies or traits related to flower development requires stereoscopic imaging approaches, in which not only are pixel numbers added up, but in which the positions of real landmarks on the shoot surface are determined from multiple views and in which these positions are then registered in three-dimensional image cubes. The first steps towards canopy reconstructions in the field have been undertaken for soybean (Biskup *et al.*, 2007), but due to the above pitfalls, their practical relevance for improving forage and turf grass breeding will not be high in the near future. Yet, other NMR-related analyses such as counting seeds are technically much easier to perform and are expected to reach applied sciences very soon, as discussed in more detail further below.

Analysing plant function

Chlorophyll fluorescence analysis (Fig. 1) is a widely used tool in plant physiology that allows determination of a number of parameters related to plant photosynthesis (Baker, 2008). It is also used in automated imaging platforms to derive the level of stress that is tolerable for plants (Woo *et al.*, 2008), to differentiate between genotypes with differing susceptibility to drought, salt or cold stress (Jansen *et al.*, 2009; Munns *et al.*, 2010; Lootens *et al.*, 2011) or as a tool to differentiate disease susceptibility (Bauriegel *et al.*, 2011). The measurement principle is based on a defined exposition of the plant with light of a low wavelength in the visible

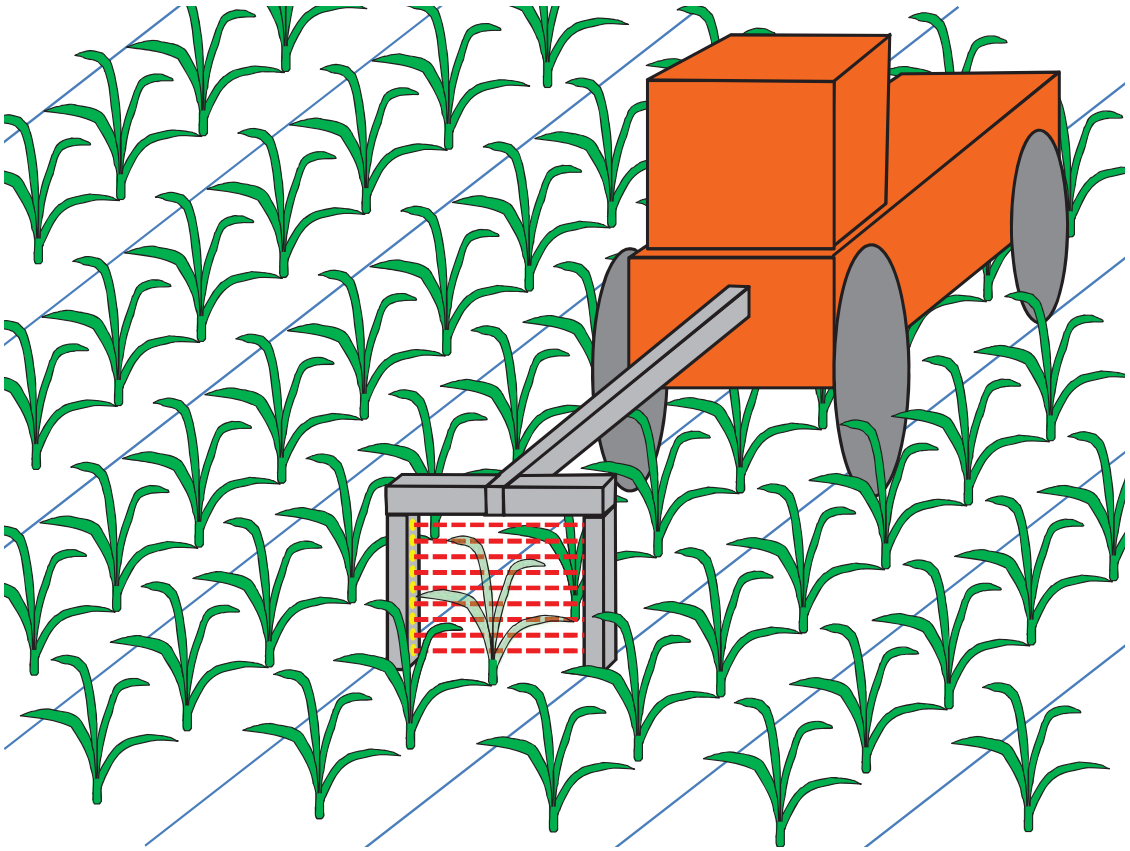

FIG. 2 Schematic drawing of the 'light-curtain' system developed by Montes *et al.* (2011) for analysis of maize in the field.

range and an exact registration of the re-emitted (fluorescent) light at a longer wavelength during a short time following the light pulse. Application of this technique can be powerful in the field, but either the plant has to be completely protected against incoming sunlight during the analysis by a shield or box (see, for example, Bauriegel *et al.*, 2011) or strong lasers have to be used to induce the fluorescence signal (Malenovsky *et al.*, 2009; Thoren *et al.*, 2010).

Thermal imaging (Fig. 1) in the infrared wavelength range (above 1000 nm) is becoming more and more widely used to monitor stomatal conductance of plant canopies in the lab and in the field (for a review see Munns *et al.*, 2010). Both in precision agriculture (Wang *et al.*, 2010) and in breeding (Sirault *et al.*, 2009; Jones *et al.*, 2009), thermographic analysis of canopy temperature, which can be related to leaf transpiration and canopy water use, has already proven beneficial. Therefore, a high impact of such methods on forage and turf breeding can also be expected in the near future. Care has to be taken, however, to perform precise calibrations of the temperature readings provided by the infrared camera with real canopy temperatures and real transpiration rates to avoid the following artefacts: (1) differing degrees of canopy closure can lead to superpositions of canopy and soil temperature, (2) differing developmental stages of genotypes to be compared with each other can be an underlying reason for differing transpiration, and (3) differing wind velocities or sunlight/shade conditions on different spots of the investigated canopy can lead to heterogeneous microclimates at different spots of an image.

Important information on compound composition and photosynthesis can be gained via analysis of the spectral composition of sunlight reflected by the shoot canopy (Fig. 1; spectral reflectance; Chen *et al.*, 2010; Winterhalter *et al.*, 2011). Spectral reflectance is analysed in the visual (or near-visual) wavelength range without the need to provide artificial illumination to plants in the field. Indices such as the normalized difference vegetation index (NDVI) make use of the fact that the chlorophyll and/or nitrogen content of the leaf as well as the relationship between water-filled, vigorous tissue and unhealthy, desiccated tissue leads to characteristic alterations in the colour of the leaves, which originates from differential reflection of sunlight at different wavelengths. These characteristic colours can be addressed more or less independent of the intensity of the incoming sunlight, either by calculating ratios of intensities at two wavelengths or by calculating the difference in intensity at two different wavelengths divided by the sum of intensities at these wavelengths (Haboudane *et al.*, 2004; Chen *et al.*, 2010; Winterhalter *et al.*, 2011). In contrast to most of the above-mentioned methods, which work best in the laboratory under controlled illumination, these spectral reflectance methods require natural sunlight as the illumination source as only then can the 'true colour' of the canopy be used to assess functional features of the monitored vegetation. These

methods even have the potential to obtain information on compound composition of the investigated genotypes. Such information can be used in near-infrared spectrometry approaches to deduct classes of compounds from plant surfaces or extracted plant material without wet chemistry analysis (Montes *et al.*, 2007; Lebot *et al.*, 2011). The advantage of such methods for improved breeding of the quality of forage and turf species is obvious; their application for this goal can hence be expected to be realized in the near future.

Analysing root phenotypes

The performance of any plant depends strongly on its root architecture and function (Lynch, 1995; De Dorlodot *et al.*, 2007; Zhu *et al.*, 2011). Currently, root biomass and architecture can be monitored in the laboratory either in pots using NMR (De Dorlodot *et al.*, 2007; Jahnke *et al.*, 2009) or X-ray-based computer tomography approaches (Tracy *et al.*, 2010) that allow segmenting the root from the surrounding substrate. Yet, most platforms set up for this purpose are based on a direct visualization of the root in aeroponic or hydroponic cultivation systems (Fig. 1; Hund *et al.*, 2009; Nagel *et al.*, 2009). In all of these systems, total root length, branching angles and other parameters can be determined. Approaches for the analysis of root phenotypes in the field comprise visualizations of excavated root systems (shovelomics; Trachsel *et al.*, 2011), analysis of root parameters via camera systems inserted into the soil in small plexiglas tubes (minirhizotrons; see review by Johnson *et al.*, 2001) or methods that are able to quantify root biomass indirectly via analysis of electrical properties of the soil that are altered by the intensity of water uptake via the roots (Srayeddin and Doussan, 2009).

Future automated phenotyping approaches for crop, forage and turf species need to be able to assess how efficiently the root system of individual plants or of plant communities can acquire below-ground resources. As practically all methods currently available are restricted to laboratory use, their impact on the field of forage and turf breeding is expected to increase in the mid-term only. To date, many of the above-mentioned technologies are primarily applied for answering specific questions related to plant physiology.

Overall, high-throughput automated phenotyping has tremendous potential, not only for reverse genetic approaches, where a large number of genotypes have to be screened for beneficial DNA sequence alterations, but mainly for improving complex traits in plant breeding programmes. Following up on the more general overview on phenotyping techniques given above, we will now discuss the specific requirements of breeding approaches related to phenotyping in forage and turf species in more detail.

FORAGE AND TURF GRASS BREEDING IN A CHANGING ENVIRONMENT

Grasslands represent one of the world's largest ecosystems and cover more than 40 % of the terrestrial area (Suttie *et al.*, 2005). They not only serve as a major source of nutrients for livestock and of biomass for energy production, they also provide a range of ecosystem services, such as the conservation of biodiversity, the storage and purification of water and the provision of attractive landscapes of high aesthetic value. Highly adapted and improved cultivars of forage crop species such as ryegrasses (*Lolium* spp.), fescues (*Festuca* spp.) or clovers (*Trifolium* spp.) form the basis of highly productive grassland agriculture in temperate regions, which provides for a major share of the world's production in beef and milk (Humphreys, 2005). To meet the growing global demand of food, feed and biomass and to mitigate challenges caused by changing conditions such as increased globalization and climate change, cultivars of forage crops have to be continually improved through efficient and targeted selection, thereby optimizing traits such as plant biomass, stress tolerance and metabolite composition.

Forage improvement programmes are faced with a considerable number of challenges. The number of species to be improved is large and the traits to select for are diverse. In addition, cultivars are often required to be able to adapt to a broad range of environments and management regimes. Consequently, the time required to produce novel cultivars is considerable and ranges between 15 and 20 years for species such as perennial ryegrass (Humphreys *et al.*, 2010). This, together with the rapidly changing requirements for well-adapted forage crop cultivars, calls for more efficient plant breeding schemes. Rapid developments in the area of molecular genetics and genomics offer a variety of possibilities for complementing conventional plant breeding with marker-assisted selection (MAS). However, while genetic improvement in breeding of crops such as maize and soybean has been substantially accelerated through MAS (Eathington *et al.*, 2007), there are only few reports on successful employment of MAS in forage crops (Roldán-Ruiz and Kölliker, 2010). This may be due to the initial lack of efficient genotyping platforms and sufficient gene-based markers together with the large and complex genome of many forage crop species, as well as population-based selection schemes. However, the enormous technical developments in the area of DNA sequencing and single nucleotide polymorphism genotyping have accelerated the development and deployment of molecular tools on a genome-wide scale, enabling molecular breeding concepts such as genomic selection (Meuwissen *et al.*, 2001). To be able to utilize these tools in forage crop breeding, conceptual models and adapted selection schemes as well as highly efficient and precise phenotyping pipelines are needed.

CHARACTERISTICS OF FORAGE AND TURF GRASS BREEDING AND ITS IMPLICATION FOR PHENOTYPING

Many of the most important forage species are allogamous with a high degree of self-incompatibility (reviewed in Yang *et al.*, 2008) and breeding is still largely based on open pollination. The resulting cultivars consist of highly heterozygous genotypes and represent panmictic populations (Posselt, 2010). Superior individuals are either selected directly based on their phenotype (phenotypic selection) or based on the performance of their progeny (genotypic selection). Phenotypic selection is mostly based on the evaluation of individual plants in spaced plant nurseries (mass selection) or the

evaluation of vegetative replicates (clones) planted in rows (clonal selection). Genotypic selection, on the other hand, is based on the evaluation of progenies (i.e. half-sib or full-sib families) in replicated plot trials, which allows us to estimate genetic variance (reviewed in Posselt, 2010). Evaluations are usually based on visual inspection (scoring) or measurement of the character of interest either on individual, spaced plants or in experimental swards. As spacing of plants in individual plant nurseries is markedly different from that in natural swards, observations for some complex traits such as biomass yield usually cannot be directly translated from spaced plants to swards (Casler *et al.*, 1996).

Forage crops are primarily grown for vegetative dry matter yield. Reproductive characteristics such as seed yield are of economic importance for novel cultivars to be successful in the market. As vegetative traits such as leafiness or persistency may be negatively correlated with seed yield, forage crop breeders are constantly challenged by trade-offs between vegetative and reproductive growth (Humphreys *et al.*, 2010). Therefore, efficient phenotyping of both reproductive as well as vegetative traits may allow for more sustainable breeding progress. Breeding objectives are defined by the trait limitations of the target species, the agricultural management targets as well as the target environments and include traits such as growth characteristics, biomass yield, nitrogen economy, forage quality, and resistance to biotic as well as abiotic stresses and seed yield (Casler and van Santen, 2010). Successful implementation of advanced phenotyping approaches for these traits implies (1) an initial evaluation regarding whether the target trait can be reliably described on individual, spaced plants or in field swards on family basis; (2) definition of an appropriate measurement reflecting the trait of interest; (3) transformation of the collected data into useful phenotypic information; and (4) a continuous validation if the collected data translate to the actual phenotype in the field.

BREEDING OBJECTIVES AND OPTIONS FOR ADVANCED PHENOTYPING IN FORAGE AND TURF GRASS SPECIES

Dry matter yield (DMY) is one of the most important traits as it is directly related to production costs. However, measurement of DMY in breeding programmes is not straightforward because there is often only poor agreement between yield measured on individual spaced plants and obtained yield in productive swards (Wilkins and Humphreys, 2003). Consequently, genetic gain in DMY over the past 60 years has been quite limited and ranged from 0 to 6 % per decade depending on the species investigated (van der Heijden and Roulund, 2010). Although intensive selection based on sward plots may lead to gains in DMY of up to 10 % per decade, such extensive family selection is very laborious and costly. Online measurements for DMY using field-portable near-infrared spectroscopy (NIRS) instruments have already facilitated direct selection in the field on family basis. In the future, portable NMR-scanners might become available to assess biomass non-destructively in the field. Indirect selection for leaf or stolon traits has been shown to positively influence DMY in white clover (Abberton and Marshall, 2005), but is less effective than direct selection for DMY. In forage grasses, imaging-based indirect selection for morphological traits such as leaf area, plant height, number of tillers or plant vigour that are related to DMY (Majidi *et al.*, 2009) constitute another tool for improving this central trait. More complex morphological characters such as tiller density, auxiliary formation, shoot branching and spike/spikelet morphology may be monitored by three-dimensional scanning as recently shown in maize (Montes *et al.*, 2011). In combination with spectral reflectance, this can be elaborated as a future tool for improving both DMY and morphological characteristics.

With regard to nitrogen economy, forage legumes are primarily selected for improved fixation of atmospheric nitrogen by screening plants for high tissue N concentrations. In forage grasses, nitrogen use efficiency is usually improved by selecting for increased DMY under uniform soil-N conditions (Casler and van Santen, 2010). Routine laboratory methods for the determination of N concentrations in grass samples such as Kjeldahl distillation (AOAC, 1990) or Dumas (Hansen, 1989) are widespread, even though they are time-consuming and expensive. It has been shown that NIRS can be implemented to more efficiently determine N concentrations in grass samples (Gislum *et al.*, 2004). A NIRS-based approach replacing wet chemistry methods with online field screening constitutes a more direct strategy to select for improved N uptake efficiency and total N concentration.

Improving forage quality mainly aims at improving dry matter digestibility, increasing the amount of compounds beneficial to livestock such as water-soluble carbohydrates (WSCs) and condensed tannins, and reducing the amount of unwanted substances such as toxins, oestrogenic compounds or alkaloids (Carbonero *et al.*, 2011). Due to the moderate to high heritability, genetic gain for forage quality has been substantial in recent decades (Casler and van Santen, 2010). Dry matter digestibility may be increased by breeding for decreased fibre and lignin concentration in the cell wall or by increasing the content of WSCs. These traits are traditionally determined using wet chemistry methods but can be streamlined by NIRS. In addition to DMY, N and WSC determination, NIRS has proven its value to predict ergovaline (Roberts *et al.*, 1997) and lignin concentrations in grasses (Andrés *et al.*, 2005). A NIRS-based lignin prediction would be useful to identify cultivars with beneficial properties for bioenergy production. However, the accuracy of calibration models developed to predict lignin and ergovaline is still limited and needs to be improved for online field applications (Gislum *et al.*, 2004). Of particular interest to sustain forage quality are fructans, fructose polymers deriving from sucrose and serving as reserve carbohydrates in many plant species (Ritsema and Smeekens, 2003). Fructans are key factors in crop plants to respond to abiotic stress in general, and drought, cold and freezing tolerance in particular (Livingston *et al.*, 2009). A NIRS-based approach to quantify fructan concentration in freeze-dried and ground grass samples has recently been reported (Shetty and Gislum, 2011). In contrast to WSCs, NIRS-based measurements for specific carbohydrates such as fructans are difficult to obtain online in the field. But given the fact that fructans constitute the main part of WSCs in grasses and the high correlation of fructans to

total WSCs (Sanada *et al.*, 2007), NIRS-based improvement of WSCs will not only increase digestibility and preference by ruminants, but might also provide an innovative approach to develop grasses with improved abiotic stress tolerance. This is in line with the improvement of persistence, a complex trait strongly affected by the environment and by management procedures applied. Although plants are routinely scored for persistence in many plant breeding programmes, greatest achievements for improved persistence arise when selection is focused on particular underlying traits such as tolerance to abiotic stress or disease resistance (Casler and van Santen, 2010). Chlorophyll fluorescence has been used in other grass species as a diagnostic tool for freezing and salt tolerance (Munns *et al.*, 2010; Rizza *et al.*, 2011). For drought, an important factor limiting forage production, high-throughput imaging has proven useful for the dissection of plant water stress response into several component traits (Berger *et al.*, 2010). Alternatively, water stress can be characterized by thermal imaging, as recently shown in maize (Romano *et al.*, 2011; Winterhalter *et al.*, 2011). Canopy temperatures of well-watered plants were lower when compared with water-stressed genotypes, indicating that genotypes that are better adapted to drought exhibit lower canopy temperatures. As drought might be influenced by root morphology and root physiology, current techniques used for root phenotyping such as imaging and X-ray microtomography play a crucial role here (Gregory *et al.*, 2009). A combination of the above-mentioned digital and functional phenotyping methods may allow for efficient, simultaneous selection on multiple traits related to persistence.

Durable resistance to major diseases and pests such as crown rust, snow mould, bacterial wilt, fusarium root rot or nematodes is a common objective in any forage and turf breeding programme. Resistance is usually improved through phenotypic recurrent selection using naturally occurring or artificial infection (Kimbeng, 1999; Boller and Lehmann, 1996). Although considerable genetic gain has been realized with regard to disease and pest resistance in many forage crop species (van der Heijden and Roulund, 2010), changing pathogen populations and newly emerging pathogens call for constant breeding efforts. As genotype × environment interactions often complicate efficient phenotypic selection, resistance assessments are often based on artificial inoculation in controlled environments to reliably mimic a specific host–pathogen interaction. Glasshouse assessments using artificial inoculation methods (Kauffman *et al.*, 1973; Birckensteadt, 1990) and *in vitro* leaf segment tests (Lellbach, 1994) have been applied to identify plants with increased resistance against crown rust (Schejbel *et al.*, 2007; Studer *et al.*, 2007) and bacterial wilt (Studer *et al.*, 2006; Wichmann *et al.*, 2010). However, all these procedures are based on visual observations which are laborious, often biased by the examiner and may not be sufficiently accurate for targeted improvement of disease resistance. Automated digital imaging of leaf area affected by the pathogen may enable more accurate quantification as well as the monitoring of dynamic changes of the pathogen attack on a large scale level. In addition, the detection and quantification of the pathogen in or on the host plant by means of quantitative real-time PCR may allow for efficient, accurate phenotyping of disease resistance (Zhu *et al.*, 2010; Qu *et al.*, 2011).

Seed yield is one of the most complex traits with a generally low heritability, highly affected by agricultural practices as well as environmental factors. Moreover, a highly efficient self-incompatibility system promotes cross-pollination and thus specific interactions between different genotypes to produce a viable seed. As a consequence, it is impossible to measure seed yield on single plants. The moderate to low correlation between seed yield evaluations on spaced plants compared with swards (Elgersma, 1990; Elgersma *et al.*, 1994) supports that only trials over several years in multiple environments will provide reliable values for seed yield. As seed set is of major importance for total seed yield (Elgersma, 1991), it has been suggested to breed for a more efficient realization of the seed yield potential rather than to increase the size of the reproductive system with possible negative effects on forage performance (Boelt and Studer, 2010). Automatic, X-ray imaging-based counts of seed numbers per spikelet could assist in selection towards a more efficient realization of the seed yield potential. Moreover, the percentage of seeds aborted post pollination, the abortion pattern within the spikelet and gradients in ovule dry weight within the spikelet are important seed yield components. Non-invasive three-dimensional imaging of caryopses from developing seeds by NMR as used on other grass species (Glidewell, 2006) may provide the opportunity to identify and select genotypes with a high and homogeneous seed weight at final harvest, which is beneficial for high seed yield (Warringa *et al.*, 1998).

CONCLUSIONS

Elaborating upon, adapting and using advanced phenotyping technologies is a promising way forward to efficiently and reliably improve agronomically important traits in the breeding process of forage and turf grass species. Practical realization includes a biological and a technical part: For the former, a thorough experimental design, the definition of a measurement reliably describing the target trait and a continuous validation regarding whether the collected phenotypic data translate to the actual phenotype in the field are key factors. The technological part consists of well-conceived selection, realization and application of the appropriate technology and of subsequent steps of data processing. In combination with optimized breeding schemes and cutting edge molecular tools, advanced phenotyping has the potential to substantially improve and fasten cultivar development, thereby contributing to a sustainable feed, food and biomass production on both the local and global level.

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