

# Field-Grown Switchgrass (*Panicum virgatum* L.) as a Bioenergy Feedstock: A Review of Phenotype Data Collection and Analysis

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## Abstract

Switchgrass (*Panicum virgatum* L.) is noted for its high biomass yield and adaptability, making it a remarkable bioenergy source. Improving switchgrass for biofuel requires efficient collection and analysis of phenotype data. This review evaluates current methods and emphasizes the necessity of standardized, comprehensive data collection to enable reliable comparisons across studies. It examines the complex interplay between switchgrass genotypes and environmental conditions, advocating for extensive field tests and predictive models to create site-specific management strategies. The review emphasizes significant advancements in high-throughput phenotyping tools, including drones, hyperspectral imaging, and machine learning, which enhance data collection by increasing speed, accuracy, and detail. By integrating these cutting-edge technologies with a standardized methodology, this study establishes a framework for improving the efficiency and sustainability of biofuel production from switchgrass. It also offers practical recommendations for optimizing switchgrass as a bioenergy source.

**Keywords:** Switchgrass; Bioenergy feedstock; Phenotype data collection; High-throughput phenotyping; Genotype-by-environment interactions

## Introduction

Switchgrass is a lignocellulosic perennial warm-season grass native to North America. It has been considered one of the most promising perennial grasses for bioenergy production and is characterized by a higher biomass yield, adaptability, and potential placement to reduce greenhouse gas emissions by significant levels when compared with conventional fossil fuels [1]. Being native to North American prairies, switchgrass provides many ecological benefits, such as habitat provision, soil retention, and resistance to diseases and pests [2]. These features make switchgrass promising bioenergy feedstocks in the context of increasing global demands for sustainable and renewable energy sources. Recently, bioenergy from lignocellulosic biomass, an example of which is switchgrass, has gained much attention because it produces energy without competing with food crops; hence, it solves the debate about food vs. fuel [3]. The U.S. Department of Energy, among other agencies involved in the bioengineering of energy, has recognized the great potential of switchgrass to produce bioenergy, hence conducting extensive research to understand its productivity and suitability for biofuel production [4].

Despite its bioenergy potential, some challenges exist in optimizing switchgrass for biofuel production. One of the major difficulties is that accurate collection and analysis of phenotypic data must be carried out to support breeding and selection practices of genotypes that yield high, are stress-tolerant, and suitable for different environmental conditions [5]. The

relevant phenotypic traits are lignin content, leaf area, plant height, and biomass yield, which are essential factors influencing biofuel conversion efficiency. However, most data collection methods today suffer from a general lack of standardization, making comparing and integrating results from numerous studies and independent research groups challenging [6]. Lack of standardization in phenotyping protocols is one of the most significant challenges confronting the promotion of switchgrass as a bioenergy crop.

Besides that, switchgrass has enormous genetic variability, and its yield tends to depend so much on such elements as soil type, established climate conditions, and available water supply [7]. Understanding complex genotype-environment interactions underlies the development of site-specific management practices for optimizing biomass yield and quality, which are critical to biofuel production. This will be possible only through intensive field testing and using the newest and most potent data analysis techniques to select superior genotypes for given sets of conditions. The ability to predict and improve multiple switchgrass genotypes across diverse environments could, therefore, greatly enhance the overall efficiency of biofuel production.

These traditional phenotype data collection methods are labour- and time-consuming; hence, subjective variations and errors can occur frequently [8]. Therefore, standardization within these methods will be required to ensure that data collection is executed consistently and reliably, enabling more appropriate comparisons between studies and meta-analyses. A more integrated methodology for phenotyping needs to be developed that includes key traits such as biomass yield and those related to nutrient use efficiency, stress tolerance, and biofuel conversion efficiency. This would thus offer a more integrated approach to phenotyping across the wide range of traits that influence productivity and biofuel potential in switchgrass.

Recent breakthroughs in high-throughput phenotyping technologies offer promising solutions. HTP involves the tightening of advanced technologies in collecting large amounts of phenotypic data quickly and accurately [9]. Only a few tools, such as hyperspectral imaging, drones, and machine learning, can revolutionize phenotype data collection to quicker, more accurate, and more comprehensive services. Drones equipped with hyperspectral and/or multispectral cameras can scan huge plots of fields by field; the data from these aerial overpasses includes images that quantify canopy cover, plant height, and stress indicators. Then, machine learning algorithms can detect patterns in those data to determine phenotypic results. These technologies allow for comprehensive information on plant traits at a scale and resolution previously unreachable by using manual techniques, allowing researchers to detect subtle differences in plant performance that might go undetected otherwise [10].

Understanding the interaction between switchgrass genotypes and environmental factors is crucial for optimizing biomass yield and biofuel quality. Switchgrass growth and its potential conversion to biofuel are under the influence of climate, soil type, and water availability [11]. It has to be focused on the search for superior

genotypes in given environmental conditions. For this, a holistic and broad-based approach is required concerning field trials over various locations for data acquisition regarding environmental stressors' impacts related to various genotypes. Once this is done, predictive models can be generated which incorporate GxE interaction, and based on them, the selection of superior genotypes in regions of sub-continental levels would result in higher biomass production at a particular area with required biofuel yield accordingly brilliantly [12].

This review now discusses the present status of phenotype data acquisition and analysis in switchgrass. This involves genes in switchgrass, standardization, comprehensiveness, environmental interaction involved, and HTP. The critical aspects have been addressed in this study to present a holistic framework toward betterment in the phenotyping of switchgrass to offer more efficient and sustainable biofuel production. Advanced phenotyping technologies, understanding interactions between genotype and environment, and further prioritization in the standardization of data collection techniques are all essential steps toward switchgrass optimization for bioenergy production. This review presents practical recommendations for taking the field forward and significantly contributes to improving switchgrass as a bioenergy feedstock.

### **Switchgrass genes and their role in biofuel production**

Switchgrass has lately been under great scrutiny for its potential contribution to sustainable biofuel production. Some genetic research in switchgrass gets some of the essential controlling genes for critical traits regarding bioenergy yield and quality [13]. Full knowledge about these genes and their functions is vital for optimizing switchgrass as feedstock biomass. By manipulating these essential genes, switchgrass will be improved as a bioenergy feedstock. By targeting genes associated with sugar transport, photosynthesis, disease resistance, and lignin biosynthesis, new switchgrass varieties can be produced with higher biomass production, improved biofuel conversion efficiency, and better tolerance to various environmental stresses [1]. Such genetics-based improvement is highly imperative if biofuel production systems are to achieve sustainability and efficiency.

Table 1 summarizes some essential switchgrass genes and their respective traits, emphasizing biofuel production enhancement. For example, PvFPG1 affects biomass yield and ethanol production, a trade-off between growth and biofuel efficiency [1]. PvCOMT and PvMYB4 are responsible for lignin biosynthesis. The lower the amount of lignin present, the better the biofuel conversion [14,15]. PvSBPase enhances photosynthesis efficiency, which also increases biomass yield [16,17]. PvC4H and Pv4CL are related to disease resistance and connect lignin and flavonoid biosynthesis [18,19]. PvCESA and PvNST1 determine cell wall composition, decreasing biomass recalcitrance and increasing cellulose content [20-22]. PvSUT1 is responsible for sugar transport, essential in sustaining high biomass production [23]. These gene functions are critical to developing high-biomass-yielding, biofuel-efficient switchgrass genotype varieties.

**Table 1:** Comparison of switchgrass genes and traits.

Gene	Trait	Description	Impact on Biofuel Production	References
PvFPGS1	Biomass Yield	Folypolyglutamate synthetase gene in C1 metabolism, influencing plant growth and biomass production	Reduced expression may lower biomass yield but can boost ethanol yield	[1]
PvMYB4	Lignin Content	Transcription factor controlling lignin biosynthesis	Reduced lignin content increases biofuel conversion efficiency	[14]
PvCOMT	Syringyl/Guaiacyl Lignin Ratio	Caffeic acid O-methyltransferase gene in lignin biosynthesis	Modifies lignin composition, influencing digestibility and biofuel quality	[15]
PvSBPase	Photosynthesis Efficiency	Sedoheptulose-1,7-bisphosphatase gene in the Calvin cycle	Improved photosynthesis can improve biomass yield	[16,17]
PvC4H	Disease Resistance	Cinnamate-4-hydroxylase gene in phenylpropanoid pathway	Improves resistance to biotic stresses, enhancing plant health	[18]
Pv4CL	Lignin and Flavonoid Biosynthesis	4-coumarate ligase gene	Balances lignin and flavonoid production, impacting both biofuel and disease resistance	[19]
PvNST1	Secondary Cell Wall Formation	NAC transcription factor controlling secondary cell wall synthesis	Impacts cell wall composition and strength, affecting biomass recalcitrance	[20]
PvCESA	Cellulose Synthesis	Cellulose synthase gene responsible for cellulose production in cell walls	Higher cellulose content improves biofuel yield	[21,22]
PvSUT1	Sugar Transport	Sucrose transporter gene in phloem loading and sugar distribution	Efficient sugar transport facilitates higher biomass and biofuel yield	[23]
PvUbi1	General Growth	Ubiquitin gene employed as a reference for normalization in gene expression studies	Essential for accurate gene expression analysis	[24]

### Standardization and comprehensiveness

With its high biomass yield and ecological benefits due to its adaptability to different environmental conditions, switchgrass has been recognized as a suitable feedstock for bioenergy. However, this full deployment of switchgrass as a bioenergy crop requires rigorous and standardized phenotype data collection and analysis. Accordingly, phenotyping-any activity that assesses and analyzes plant traits-must be informed about the genetic and environmental factors that influence biomass production and biofuel conversion efficiency [25].

### Need for standardization

Among the notable deficiencies in switchgrass research is the absence of standardized methods of collecting data on phenotype. This is because different research groups commonly use different tools, protocols, and measurement standards that result in data discrepancies, which preclude valid comparisons and inclusions of results across studies [26]. Standardization ensures feasible reproducibility, consistency, and reliability during data gathering, forming the backbone for appropriate comparisons across studies and meta-analyses.

The lack of standardized protocols for phenotyping may lead to high variability in the measurements of the declared traits. Biomass yield, for instance-one of the critical traits of interest in bioenergy production-is susceptible to influences such as the timing of harvest, the method of measurement adopted, and even the definition applied for what constitutes biomass components

[27]. Since these protocols are not uniform, it can sometimes be challenging to determine whether differences in observed biomass yield result from methodological differences, environmental variation, or genetic effects.

### Full phenotyping

Full phenotyping encompasses a broad range of traits affecting growth, development, and the efficiency of switchgrass biofuel conversion. Conventionally, traditional phenotyping methods usually target key attributes such as leaf area, plant height, and biomass yield [28]. Although these characteristics are essential, they do not fully explain the factors affecting switchgrass productivity and, consequently, their suitability for bioenergy production.

To realize the full potential of switchgrass as a bioenergy crop, it is crucial to embrace a more holistic technique for phenotyping that includes both traditional and novel traits. This approach should assess disease resistance, nutrient use efficiency, stress tolerance, and biofuel conversion efficiency. For example, lignin content and composition are critical determinants of biofuel conversion efficiency, as high lignin content interferes with the enzymatic depolymerization of biomass into fermentable sugars [29]. Thus, assessing lignin content in addition to traditional traits can provide valuable information on the suitability of different switchgrass genotypes for biofuel production [30].

Table 2 summarizes some of the critical traits of switchgrass and their relative importance in biofuel production, clearly relating plant attributes and their impact on biofuel efficiency and yield.

**Table 2:** Major phenotypic traits of switchgrass for bioenergy production.

Trait	Description	Measurement Method	Importance for Bioenergy Production	References
Biomass Yield	Total dry mass-produced	Harvest and weigh, UAV-based	Determines the amount of feedstock available	[31]
Plant Height	Vertical growth of the plant	Ruler/tape measure, UAV-based	Indicator of overall growth and vigor	[32]
Lignin Content	Amount of lignin in cell walls	Chemical analysis, UAV-based	Influence ease of cell wall deconstruction	[33]
Nitrogen Content	Amount of Nitrogen in cell walls	Chemical analysis, UAV-based	Affects biofuel quality and conversion	[34]
Leaf Chlorophyll	Amount of Leaf Chlorophyll in cell walls	SPAD meter, UAV-based	Reflects photosynthetic capacity and health	[34]
Disease Resistance	Ability to withstand pathogens	Visual inspection, UAV-based	Affects overall plant health and yield stability	[35]

**Techniques for standardized and comprehensive phenotyping**

Implementing standardized and comprehensive phenotyping protocols entails combining conventional and advanced techniques. Traditional techniques like laboratory analyses and manual measurements have been the backbone of plant phenotyping

for decades [36]. These techniques are reliable but often time-consuming, labour intensive, and subject to human error. Table 3 compares different methods of phenotype data collection, highlighting their pros and cons. It gives a clear overview of various techniques and sets the stage for discussing the benefits of high-throughput technologies, such as UAV-based remote sensing.

**Table 3:** Comparison of phenotyping techniques for switchgrass.

Phenotyping Technique	Pros	Cons	References
Manual Measurements	High accuracy, direct observation	Labor-intensive, time-consuming	[34]
UAV-Based Remote Sensing	High-throughput covers large areas quickly	Initial setup cost, data processing complexity	[37]
Hyperspectral Imaging	Detailed spectral information	Expensive, requires advanced analysis tools	[38]
LiDAR	Accurate 3D structure data	High-cost, complex data integration	[34]
Machine Learning Approaches	Automated analysis can handle large datasets	Requires high-quality training data	[39]

Improvements in HTP technologies provide hopeful solutions to these challenges. HTP refers to applying various automated and semi-automated tools to rapidly and accurately acquire large quantities of phenotypic data in a high-throughput fashion [40]. As HTP technologies further improve, they promise much in enhancing efficiency and accuracy for phenotyping to allow the measurement of an increased number of traits at higher resolutions.

**HTP technologies**

Many HTP technologies have been developed and applied to switchgrass research. These are both reviewed as follows: hyperspectral imaging, drones, and machine learning-all with their own strengths in collecting phenotype data and its analysis.

**Drones**

HTP technologies that are especially noted in using the application of UAV have become transformative in plant phenotyping [41], offering broad advantages to various standards that could be reached regarding data collection and analysis. Recently, UAVs have become an essential tool in agricultural research studies because

they yield a fast acquisition of high-resolution imagery over large plots in the field [29]. The application epitomizes these advantages by UAV-based remote sensing for the automatic phenotyping of field-grown switchgrass using an intermediate-scale spatial and spectral data acquisition that improves accuracy and consistency compared to satellites and provides higher flexibility and throughput than ground-based methods.

Drones with hyperspectral/multispectral cameras acquire plant traits such as height, canopy cover, and stress indicators in high spatial and temporal resolution. The applications of drones in phenotyping enable the swift assessment of large numbers of plants and confirm fewer labour- and time-consuming manual phenotyping measurements.

The report prepared by Xu et al. [40] shows that UAV-based remote sensing adds more standardization and comprehensiveness to phenotype data for normally field-grown switchgrass by providing accurate, consistent, and scalable data on physiological and morphological traits. These technologies offer the possibility of high-resolution automation, enabling monitoring of several

traits that include lignin, nitrogen, chlorophyll content, and disease presence, among others, making the process uniform across different studies.

Including advanced imaging and statistical modelling, UAVs represent a whole suite of technology to date that standardizes the assessment of phenotypic traits and provides more excellent reliability to data for efficient breeding and management of switchgrass for bioenergy production. Li et al. [32] presented that UAV-based plant phenotyping in switchgrass was accomplished by applying LiDAR and multispectral imagery to validate high accuracy and consistency. Plant height and perimeter measurement strongly correlated with the manual approach:  $r=0.93, p<0.001$ . The high-performance biomass yield model integrating the CH, CP, and SI variables also correlated perfectly with the manual measurements with  $r=0.90$  and  $p<0.001$ .

Table 4 illustrates how to use UAV-based remote sensing, with key traits measured in switchgrass. It emphasizes the high correlation between UAV-based and manual measurements; hence, the accuracy and potential of the UAV technology in phenotyping [34].

**Table 4:** UAV-based remote sensing for switchgrass phenotyping.

Trait	Measurement Technique	UAV Sensor Type	Accuracy (Correlation with Manual Data)
Plant Height	LiDAR	LiDAR	$r = 0.93, p < 0.001$
Canopy Perimeter	Multispectral Imagery	Multispectral Camera	$r = 0.93, p < 0.001$
Biomass Yield	Biomass Yield Model	Multispectral Camera	$r = 0.90, p < 0.001$

**Hyperspectral imaging**

Hyperspectral imaging involves acquiring and processing a wide range of wavelengths in the electromagnetic spectrum to get reliable details about plant traits [41]. This technology will measure plant traits concerning health, nutrient contents, and stress responses. Hyperspectral imaging can detect slight changes in plant physiology that are not always visible to the naked eye. The process is an essential step toward describing the drivers that define productivity and efficiency in switchgrass biofuel conversion.

**Machine learning**

Machine learning algorithms can analyze massive datasets from HTP technologies to outline patterns and forecast the outcome of the pheno-typeable processing of complex, high-dimensional data [42]. Thus, they are well-suited for analyzing diverse traits collected via comprehensive phenotyping. Machine learning can help indicate the most important traits and their interactions affecting switchgrass performance to guide breeding and selection efforts. Hao et al. [37] have problems with alternative methods of forecasting P availability using leaf tissue chemical profiles rather than data on P content alone. A promising machine learning-based plant phenotyping for switchgrass has been presented here, which ensured high accuracy in model training, showing that plants

adapt to low P soils, resulting in actual P availability being more similar among contrasting sites than model predictions suggested. Although metabolically expensive, these adaptations influenced feedstock quality through changing cellulose-to-lignin ratios. Site-specific P allocation strategies were associated with successive biomass yields, again illustrating the strength of the model in its ability to capture detailed nutrient dynamics and their roles in switchgrass productivity.

**Case studies and applications**

Several authors reported the potential of HTP technologies for switchgrass research. Indeed, Xu et al. [40] employed UAV-based multispectral imaging to assess the growth and development of various switchgrass genotypes grown under contrasting environmental conditions; preliminary results indicate that drone-based phenotyping can offer reliable measurements of traits relevant to canopy cover, plant height, and biomass yield while delivering highly valued genotype assessment and selection data. Similarly, Decker et al. [43] measured the lignin switchgrass plants using hyperspectral imaging. According to the authors, hyperspectral data made accurate predictions of the lignin content, which shows the power of this technology in estimating biofuel conversion efficiency. Relating hyperspectral imaging technology with traditional methods of phenotyping, this study provided more information on factors that influenced the aptness of switchgrass for the production of bioenergy.

Machine learning algorithms have also been applied in the phenotyping of switchgrass. A study by Tong and Nikoloski [44] analyzed the data collected trials in switchgrass using machine learning algorithms. The algorithms identified critical traits and interactions influencing biomass yield and stress tolerance, providing the insights needed to substantiate breeding decisions. It unveiled the potentiality of machine learning for deciphering complex phenotypic data, improving the efficiency of switchgrass breeding programs.

**Standardization and comprehensiveness**

Standardization of phenotyping and its comprehensiveness is integral in optimizing switchgrass to a bioenergy feedstock. The major issues with switchgrass research include a lack of standardized methodologies and an increasing need for more diverse means of trait measurement techniques [45]. However, the development of HTP technologies has hailed a panacea for such challenges due to their enabling, and they have thereby increased the efficiency and accuracy of data collection and analysis each phenotype data elicits [46]. By applying standard protocols, traditional and novel approaches, and investment in HTPs, switchgrass research can take giant strides likely to act toward sustainable and efficient production of biofuels [47].

**Standardization of data collection**

The HTP technologies, such as UAVs equipped with multispectral cameras, are developing standardization in data collection among different research groups [48]. It standardizes data collection on the physiological and morphological characteristics of switchgrass,

reducing variability in the activity and increasing the comparability of results across several studies and locations. Since the mode of data collection with UAVs is automated, bias is minimized, and human error is reduced; therefore, such measurements are more reproducible and reliable [49]. Also, automated systems can be programmed to fly in similar environmental conditions on accurate flight paths, further strengthening data consistency.

High-resolution images can be taken regularly in UAVs for intensive and seamless data on switchgrass development and growth [50]. The capability allows researchers to accurately identify phenotypic traits and changes over time that accelerate the development of standardized growth models and phenotyping protocols.

**Comprehensiveness in phenotype data collection**

UAV-based multispectral imaging enables simultaneous acquisition of several phenotype traits, such as rust disease incidence, lignin, nitrogen, and chlorophyll content [51]. The comprehensiveness of the approach applied here means multiple relevant traits are covered, which helps in giving a wholesome overview of switchgrass phenotypic performance. The development of statistical models with vegetation indices, such as NDRE and NDVI, allows for predicting various traits [52]. These indices are then correlated with the ground-truth data to develop accurate models that will estimate superior traits. This ensures that all relevant phenotypic data are captured and analyzed in detail.

Ground-truthing UAV-based data against manually measured traits has shown accuracy and ensured the reliability of the collected phenotypic data [53]. This confirmation indicates that the UAV-based models are robust and can be trusted for large-scale phenotyping studies that improve the extensiveness of the data collected. The application of linear and nonlinear models for different traits further illustrates the flexibility of HTP technologies in handling such complex data. For example, such advanced analytic methodologies can capture these details for lignin and nitrogen contents where nonlinear relationships are more apparent. They can guarantee comprehensive data analyses of all phenotypic data [54].

**Integration with environmental data**

UAV-based HTP can enable researchers to phenotype under environmental conditions, enabling studies on genotype-environment interactions. Press and others pointed out that modelling switchgrass performance under different scenarios using environmental and phenotypic data was more accurate [55]. High-resolution data from UAVs could be used in a number of precision agriculture-based applications for site-specific management of pest control, fertilization, and irrigation activities [56,57]. This process

introduces multifunctionality of phenotypic data collection that is adequate and oriented toward the optimization of switchgrass quality and yield.

**Recommendations**

The operative recommendations go toward standardized and holistic phenotyping in switchgrass research: There should be collaboration between various research organizations and institutions in developing and adopting standardized protocols for phenotyping [58]. Standardized measurement criteria, methods, and tools must be defined to ensure the reproducibility and uniformity of the data being recorded for the traits under consideration. Phenotyping shall be holistic, considering conventional techniques and advanced high-throughput technologies [59]. Combining human measurement with automated tools will facilitate data collection, streamlining the process and increasing efficiency and accuracy. Investment in HTP technologies, such as hyperspectral imaging, drones, and machine learning, should be a priority for researchers and institutions [48,60]. These will significantly increase the speed, precision, and comprehensiveness of phenotype data collection. Training programs should be developed for researchers applying advanced phenotyping technologies [61]. This involves data collection, analysis, and interpretation training using high-throughput tools and machine learning algorithms. This will encourage collaborations and data sharing between research groups, fostering the development of standardized protocols for comprehensive phenotyping methods [62]. Shared databases and repositories will enable such researchers to compare and integrate results across studies, improving the understanding of switchgrass phenotyping.

**Environmental Interaction**

Understanding the interaction between switchgrass genotypes and environmental factors is crucial for optimizing biomass yield and biofuel quality. Switchgrass has considerable genetic variation and plasticity in response to environmental conditions [28]. For instance, ecological conditions IPHERS of native habitats, like water availability, climate, and soil type, are essential for determining its development, growth, and conversion efficiency in biofuel production [61]. This section deals with the complex GxE interactions in switchgrass, particularly considering comprehensive field trials, improved methods of analysis, and predictive models, which will be very useful for enhancing understanding and optimization in switchgrass cultivation for bioenergy production [62,63].

Table 5 presents essential environmental factors that affect switchgrass growth. Understanding these factors is of prime importance as it optimizes the field conditions to the best and enhances biomass yield for biofuels.

**Table 5:** Impact of environmental factors on switchgrass yield and biofuel quality.

Environmental Factor	Description	Impact on Yield and Biofuel Quality	References
Soil Type	Soil composition and fertility	Affects nutrient availability	[64]
Climate	temperature, precipitation, and sunlight	influences growth rate and biomass production	[61]

Water Availability	Irrigation and rainfall	Vital for photosynthesis and growth	[65]
Management Practices	Fertilization, pest control, and harvesting	Defines overall plant health and productivity	[61]
Genotype	Genetic variation among switchgrass cultivars	Specific traits for yield and resistance	[66]

## Soil type

Among the environmental factors affecting switchgrass growth and biomass production, soil type is among the most important. Switchgrass can grow on various diverse soil types, from sandy to clayey soils; however, performance greatly varies with diverse soil characteristics like organic matter content, fertility, pH, and texture [67]. According to reports done by several investigators, nutrient levels are one of the most important determinants of productivity in switchgrass. In this regard, Ameen et al. [62] observed that nitrogen fertilization significantly influenced the biomass yield of switchgrass genotypes evaluated across variable soil types. The genotype–soil types interaction in switchgrass is complex and might be another reason for selecting suitable genotypes for particular locations. Some genotypes are adapted well to high-fertility soils, and others do best in low-fertility or marginal soils. For instance, Alexopoulou et al. [65] & Peddy reddy [66] observed the general trend of the upland switchgrass ecotype to thrive on low-fertility, well-drained soil. In contrast, lowland ecotypes did well on land with high-fertility, poorly drained soils. The interactions described above are critical when developing management practices where biomass yield and sustainability are optimally balanced [42].

## Climate

Precipitation, temperature, and length of the growing season are some of the climatic parameters that highly influence switchgrass growth and biomass production. Indeed, switchgrass is highly adaptable to a range of climatic conditions, from temperate to subtropical. However, its productivity depends on several climatic factors. Temperature influences switchgrass's phenological development, though the best growth is witnessed within the temperature range of 23 °C to 30 °C [68]. Very low or high extreme temperatures can adversely influence yield and growth. Water availability and the amount of precipitation are other essential factors for the productivity of switchgrass. Sufficient water is necessary for sustaining the growth and accumulation of biomass, especially during the establishment phases. Drought conditions can significantly reduce biomass yield and impact biofuel conversion efficiency [69]. Several studies have indicated that there are different switchgrass genotypes with varying drought tolerance, hence the need for screening of tolerant genotypes for those regions susceptible to low water availability [70,71].

## Water availability and irrigation

Water availability is one of the key determinants of switchgrass productivity, especially in rainfall-limited or variable regions. Sufficient soil moisture is indispensable for germination, establishment, and continuous growth. Water stress, especially at critical growth stages, results in reduced biomass yield and

deteriorated physiological functions [72,73]. Proper irrigation management practices may mitigate the impact of water stress and optimize biomass production. The research on the effect of irrigation on switchgrass yield has given variable results. Whereas some report remarkable increases in yield with supplemental irrigation, others suggest limited benefits based on various conditions such as climate, soil type, and genotype, among other factors [74]. Some authors showed irrigation indeed increases switchgrass yield in arid regions. For instance, it has been reported that proper irrigation management can raise the yields of crops by up to even 30% under water-scarce conditions [75,76]. This is important in raising general agricultural productivity and ensuring food safety under unfavorable conditions.

Secondly, investigative studies agree on a consensus view that highly adapted switchgrass genotypes do not segregate much after minimum irrigation. For instance, studies have identified that, under appropriate conditions, such as limited water, some of the genotypes can perform excellently at low-input conditions [5,38]. This is because the characteristics related to efficient utilization and drought tolerance enable them to be used for bioenergy production under semi-arid and arid areas in a sustainable way [38]. Elucidation of the interaction between switchgrass genotypes and water availability is imperative to develop effective irrigation management and select the most water-efficient genotypes for productivity when water is limited.

## Nutrient management

Nutrient availability, particularly K, P, and N, is one of the critical factors in switchgrass development and biomass yield. Nitrogen is often the most limiting nutrient in many soils, and optimum N fertilization is crucial to achieving high biomass productivity [77]. However, excessive levels of N application result in environmental problems such as the emission of greenhouse gases and nitrate leaching. Optimization in rates and timing of N fertilization is thus of paramount importance for sustainable switchgrass production. While nitrogen needs are more often the focus, phosphorus and potassium nutritional needs are critical but generally lower than nitrogen. Soil K and P levels should be monitored, and adequate fertilization practices should be adopted to guarantee proper nutrient supply [78]. Some of these studies have also unravelled that switchgrass genotypes differ in nutrient use efficiencies under the same soil conditions. Thus, selecting an appropriate genotype for a particular soil condition is imperative for high nutrient use efficiency in the crop [79,80].

## Field trials and genotype evaluation

Determination of G×E interactions in switchgrass and superior performing crop genotypes suitable for specific regions, in-depth

field trials in diverse environmental conditions, will be explained. On the other hand, multilocation trials can provide worthwhile information on the response of different genotypes against varying climates, soil types, and management practices [67]. These trials should be designed to capture various environmental variables with multiple trait measurements including biomass yield, resistance to stress, and biofuel conversion efficiency. Field trials should consider performing well during their initiation stage and the long-term performance of switchgrass genotypes. Switchgrass is a perennial crop, and it grows through several seasons, depending on limiting factors such as the ageing of the plants, soil nutrient depletion, and changes in environmental conditions [61]. Long-term trials will be helpful in the study of phenotypic sustainability and flexibility of the various genotypes under different ecological conditions.

### Predictive modelling and decision support tools

Predictive modelling and decision support tools possess great potential to further our understanding of the G×E interactions in switchgrass and provide information to make effective decisions regarding the selection and management of genotypes. So far, crop growth models such as the ALMANAC (Agricultural Land Management Alternatives with Numerical Assessment Criteria) model have been applied to simulate switchgrass growth and yield across various environmental conditions [81]. These models can utilize data from soil type, climate, and management practices to better predict the performance of multiple genotypes and develop the best strategy for cultivation.

Decision support tools can also be used to aid farmers and researchers in choosing the most suitable switchgrass genotype for a particular location and management practice. The U.S. Department of Agriculture (USDA), for instance, developed the Switchgrass Selection Tool (SST), which makes recommendations regarding switchgrass varieties based on regional environmental conditions and intended uses such as conservation, forage, and bioenergy [82]. Such tools contribute to optimizing biomass yield with quality for biofuel by aligning genotype selection with environmental conditions and management objectives.

### Case studies on G×E interactions

G × E interactions have been investigated in various switchgrass studies; therefore, there is a valuable understanding of the factors affecting biomass yield and biofuel conversion efficiency. One of the pioneer works is by Alexopoulou et al. [65], where thirteen lowland and upland switchgrass ecotypes were evaluated for long-term productivity performances in the Mediterranean region. These authors reported significant G×E interactions, where the

upland ecotypes perform better under drier and cooler conditions, while the lowland ecotypes thrive well under warmer and wetter conditions. This means that selecting an appropriate ecotype based on specific environmental conditions can significantly improve switchgrass productivity in the Mediterranean region.

On the other hand, another study by Zhang et al. [83] focuses on the impact of precipitation and temperature on switchgrass productivity across a latitudinal gradient. Results present that climatic variables strongly influence biomass yield, with generally higher temperatures enhancing yield unless extreme heat reduces it. Adequate precipitation is essential for optimal biomass production, as excessive rainfall and drought conditions reduce yield. Additionally, it was observed that different switchgrass cultivars vary in their adaptation to local climatic conditions and thus perform differently in biomass production.

### High-Throughput Phenotyping

HTP is rapidly transforming plant breeding and other areas of agricultural research by providing a method for accurate, rapid, and high-throughput phenotyping of plant traits [40]. Regarding switchgrass as a bioenergy feedstock, HTP technologies are essential for identifying and selecting genotypes with higher stress tolerance, biomass yield, and biofuel conversion efficiency [84]. This section covers the current development of HTP technologies and their applications in switchgrass research and discusses the prospects of integrating these technologies with genomic and environmental data to accelerate breeding and optimize switchgrass for bioenergy production.

### Advances in HTP technologies

HTP encompasses high-throughput imaging, sensing, and data analysis techniques for assessing plant traits at a resolution and scale that is impossible with conventional methods [85]. The advanced HTP technologies, including multispectral imagery and UAV-based LiDAR, have allowed for the accurate and high-speed assessment of several plant traits, such as plant height, perimeter, and biomass yield in switchgrass [86]. These technologies provide highly correlated and consistent data from the physical measurements, thus ensuring the accuracy of bioenergy trait forecasts. By integrating perimeter and canopy height with spectral indices, robust biomass yield models can be developed that will significantly enhance efficiency in breeding and cultivar development of switchgrass for bioenergy production [34,87-89].

Table 6 provides an overview of crucial HTP technologies, their application, advantages, and limitations, giving a general view of how they can enhance switchgrass research and breeding programs.

**Table 6:** HTP technologies used in switchgrass research.

Technology	Application	Key Traits Measured	Benefits	Challenges	References
UAV-Based Imaging	Monitoring plant height, canopy, and health	Chlorophyll content, Biomass yield, Canopy cover, Disease symptoms and Plant height	Rapid, scalable, high-resolution data collection	Data management, Technical complexity	[90]



Multispectral Imaging	Vegetation indices calculation	Plant height, leaf chlorophyll	Improved data collection, Early disease detection, Nutrient status assessment	High initial investment, Weather dependence, Regulatory constraints, Complexity in data interpretation	[31]
LiDAR	3D structure and biomass estimation	Canopy structure, Plant height	Accurate height and structure measurements	Accurate height and structure measurements	[91]
Hyperspectral Imaging	Detailed spectral analysis	Disease resistance, nitrogen content	Detailed spectral information detects subtle changes	High cost, large data volumes	[92]
Thermal Imaging	Temperature mapping	Stress response, water use efficiency	Stress Detection, non-invasive, and spatial resolution	Sensitivity to wind and sunlight is not easy to interpret and requires frequent calibration	[93]
RGB Imaging	Visual inspection and monitoring	Plant health, disease symptoms	Relatively low-cost and easy to implement, Provides a wide range of visual information, Captures fine details of plant morphology	Changes in natural light can affect data quality. Limited Spectral Lacks the depth of spectral data provided by multispectral or hyperspectral imaging. Requires significant image processing and analysis.	[90]
Machine Learning	Analyzing complex phenotypic data		enhanced data interpretation and trait prediction	Requires large datasets, computational resources	[94]

Satellite, ground-based platforms, and UAVs with various sensors like thermal, multispectral, hyperspectral, and RGB cameras are major remotely operated-mounted equipment widely applied for HTP. These sensors capture high-resolution images and various data related to plant health, physiology, and morphology [95]. Hyperspectral imaging captures comprehensive spectral information across wavelengths, pinpointing physiological and biochemical traits of plants with great accuracy. This technology is helpful in detecting disease, assessing water content, and evaluating switchgrass’s nutritional status [96].

LiDAR technology produces 3D models of plant structures using laser pulses, allowing for the accurate assessment of plant height, canopy architecture, and biomass. LiDAR effectively records structural traits in dense stands of switchgrass. Thermal cameras utilize infrared radiation from plants to provide information about water stress and plant temperature [97]. Thermal imaging has been valued for measuring water use efficiency and drought tolerance in switchgrass [98]. Machine learning algorithms and AI methods have also become essential for analyzing large datasets from HTP platforms, supporting pattern recognition, automated trait extraction, and predictive modelling, which enhances efficiency and accuracy in phenotypic evaluation [99].

**Applications of HTP in switchgrass research**

HTP technologies make a rapid, transformative difference in phenotyping large populations and identifying valuable traits for switchgrass breeding programs. Biomass yield is one of the most critical factors in assessing switchgrass genotype potential as a bioenergy feedstock. UAV-based HTP platforms have been tested with multispectral and LiDAR sensors to assess canopy cover, plant height, and biomass across large field trials. These data provide important insights into genotype performance and environmental

interaction [97]. HTP technologies also enable sophisticated evaluation of switchgrass responses to abiotic stressors [100]. Hyperspectral sensing and thermal imaging are effective for detecting early stress signals and evaluating physiological reactions [101,102]. Such insights are crucial for selecting stress-resilient genotypes and developing management strategies that enhance resilience.

Nutrient use efficiency is a critical component of sustainable switchgrass production. UAV-based platforms and hyperspectral imaging can monitor nutrient status and uptake in switchgrass, including identifying superior genotypes for nutrient use efficiency. This information can be used to optimize nitrogen fertilization strategies and mitigate potential environmental impacts associated with intensive fertilization [42]. The early detection of diseases is critical for sustaining switchgrass health and productivity. Symptoms can be detected and disease progress monitored, enabling timely intervention and management using hyperspectral-thermal imaging. Some of these technologies also hasten the assessment of disease resistance in switchgrass breeding programs [103].

HTP data, when combined with genomic information, enhances the accuracy of genomic selection models. A combination of phenotypic data from an HTP platform with genotypic data enables researchers to ascertain genetic markers associated with desirable traits. It accelerates the process of developing superior cultivars of switchgrass [104]. Mazarei et al. [1] noted that genomic selection uses HTP to accelerate switchgrass improvement by integrating genome information with phenotypic traits. Other applications of HTP technologies, including UAV-based remote sensing, facilitate quicker acquisition with enhanced accuracy of bioenergy-related phenotypes like biofuel efficiency, biomass yield, and disease susceptibility [105]. Genetic markers correlated with these traits

enable the prediction of genotype performance under various conditions. This technique enhances the efficiency and speed in selecting optimal switchgrass varieties for biofuel production and sustainability.

### Integration of HTP with genomic and environmental data

The integration of HTP data with both genomic and environmental information holds promise for advancing switchgrass research and breeding. Such a comprehensive approach

can provide a detailed understanding of the genetic and ecological factors affecting phenotypic traits [106]. By associating genetic variations with phenotypic traits, research will ultimately develop enhanced switchgrass varieties with high adaptability to various environmental conditions and optimized biofuel yield and quality. Details in Table 7 describe the type of data presently being collected in switchgrass research and its respective integration purposes. A holistic understanding of switchgrass as a bioenergy feedstock requires data on phenotypic traits, genomics, biofuel conversion, environmental factors, and management practices.

**Table 7:** Genomic and phenotypic data integration in switchgrass research.

Data Type	Collection Method	Integration Purpose	References
Genomic Data	DNA sequencing, genotyping	Identifying genetic markers for traits	[70]
Phenotypic Data	Manual measurement, UAV-based remote sensing	Correlating traits with genetic markers	[107]
Environmental Data	Coil analysis, climate monitoring,	Understanding genotype-environment interactions	[70]
Biofuel Conversion Data	Chemical analysis, lab testing	Assessing biofuel quality and yield	[13]
Management Practices Data	Observational studies, field records	Optimizing cultivation techniques	[61]

### Integration strategies

A. Genotype-by-Environment Interactions: HTP technologies allow the gathering of detailed phenotypic data across multiple environmental conditions, providing insight into GxE interactions. Coupled with genomic information, this approach can support stable genotype performance across environments and strategies for location-specific breeding [107].

B. Precision Agriculture: Site-specific management practices are enabled by high-resolution data from HTP technologies on environmental conditions, plant health, and soil variability. This data aids in optimizing irrigation, fertilizer application, and pest control to enhance biomass yield and sustain switchgrass [108].

C. Predictive Modeling: Machine learning and AI algorithms can analyze data from HTP platforms to develop predictive models for switchgrass performance. The models provide genomic and environmental input data to predict phenotypic outcomes, guiding breeding decisions and management practices [109].

D. HTP Networks: Developing HTP networks for data and methodology sharing facilitates collaboration and data standardization in switchgrass research. These networks enable comparison across studies in different conditions, fostering robust and widely applicable results [45].

### Benefits of genomic and phenotypic data incorporation

The primary benefits of integrating genomic and phenotypic data include the identification of traits, precision breeding, in-depth phenotyping, superior prediction models, recognition of molecular mechanisms, optimized biofuel production, environmental adaptation, and data standardization and sharing. Genomic

data generated through genotyping and DNA sequencing allow researchers to track genetic markers essential for stress tolerance, disease resistance, and biomass yield [110]. These markers can be identified and validated more accurately when complemented by phenotypic data obtained through high-throughput phenotyping. Integrating phenotype data with genomic data expedites both GS and MAS [111], enabling breeders to select desirable traits more quickly, thereby speeding up the development of improved switchgrass varieties with high biofuel potential.

HTP technologies, including UAV-based remote sensing, provide comprehensive phenotypic data on traits like canopy structure, plant height, and biomass yield [31,112]. Combined with genomic data, these technologies offer deeper insights into GxE interactions influencing phenotypic expression, and they improve the accuracy of predictive models for trait performance [113]. For instance, machine learning algorithms can analyze these integrated datasets to predict genotype performance under various environmental conditions, aiding in selecting genotypes best suited for specific regions.

Integration also helps identify the molecular mechanisms governing complex traits. By correlating genetic variations with phenotypic observations, researchers can uncover candidate genes and pathways associated with traits like drought tolerance and lignin content, which are crucial for targeted genetic modifications [114]. Knowledge of the genetic basis of traits influencing biofuel yield and quality, such as lignin and cellulose content, supports the enhancement of switchgrass varieties for biofuel production [75]. An example includes manipulating genes in the C1 metabolic pathway to boost ethanol yield without compromising plant growth.

Mazarei et al. [1] discovered the novel PvFPGS1 gene in switchgrass and studied its role in cell wall composition and biofuel

production using an RNAi knockdown approach. They conducted field tests on PvFPGS1-downregulated plants over three seasons, finding that transgenic plants with a significant decline in PvFPGS1 grew slower and produced less biomass by the end of the season. Transgenic lines with moderate reductions in PvFPGS1 transcript levels accumulated biomass similar to control plants, with no significant differences in lignin content or syringyl/guaiacyl lignin monomer ratios compared to controls.

The sugar release efficiency also showed no difference between these transgenic and control lines. However, compared to non-transgenic controls, ethanol production was up to 18% higher in these transgenic plants without compromising plant growth and biomass. In field experiments, no differences in the severity of

rust disease were noted among the transgenic and control lines. Low-to-moderate PvFPGS1 down-regulated lines did not change their lignin content and composition. That may imply that the partial downregulation in PvFPGS1 expression did not change the negligible biosynthesis of lignin in switchgrass. Manipulating PvFPGS1 expression in bioenergy crops could be a valuable strategy for improving the biofuel potential with no growth penalty or increased vulnerability to rust in the feedstock. Table 8 Summary of the effect of the downregulation of the PvFPGS1 gene in switchgrass for various traits. This means that betterment in ethanol production could be done without much alteration in disease resistance and biomass yield, hence, the potentiality of genetic manipulation in bioenergy crops.

**Table 8:** Outcomes of PvFPGS1 gene knockdown in switchgrass.

Trait	High Reduction (PvFPGS1)	Low-to-Moderate Reduction (PvFPGS1)	Control (Non-Transgenic)
Biomass	Lower	Equivalent	Standard
Lignin Content	Unchanged	Unchanged	Standard
Ethanol Production	Increased by up to 18%	Increased by up to 18%	Standard
Disease Resistance (Rust)	Unchanged	Unchanged	Standard

Integrating environmental data with phenotypic and genomic information enables studying the adaptation of switchgrass genotypes to diverse ecological conditions [115]. This would further allow location-specific management practices to maximize yield and quality for biofuel production. This integration process assists in the normalization of data collection and analysis, making data comparable from different studies. Shared databases and collaborative platforms enhance data access and usage, fostering the collective advancement of switchgrass research [116].

### Challenges and future directions

While there are significant benefits from HTP technologies, several challenges persist in implementing and integrating such technologies. The vast amount of data generated through these platforms requires adequate data management and analysis pipelines. Standardization of data collection, storage, and sharing protocols is necessary to assure data quality and interoperability [117]. The HTP technologies are expensive and resource-intensive in terms of expert skills. For HTP to become more available for researchers and most breeding programs [40], efforts must be made toward cost reduction and developing user-friendly platforms. There is a need for strategic planning and coordination in integrating the HTP technologies into conventional breeding methodologies. High-throughput data allied with field testing and classic approaches to breeding will increase the efficiency and impact of improvement programs [118].

Environmental variation can lower the repeatability and accuracy of the HTP measurements. Establishing robust protocols that consider environmental factors and ensure reliable data collection for various conditions [119]. Of the many significant features, root architecture and microbial associations cannot yet be

pheno-typed with existing HTP technologies. Advancements in new sensors and image acquisition techniques will allow the capture of these complex phenotypes and further extend the applications of HTP in switchgrass studies [120]. Any effective incorporation requires close cooperation among bioinformaticians, agronomists, and geneticists. This indicates the interdisciplinary nature of research teams in such cases [120].

### Conclusion

With its innate potential as a feedstock for bioenergy, switchgrass is already on the frontline in renewable energy. However, realizing its full potential in biofuel production requires broad enhancements in phenotype data acquisition and analysis techniques. The present review summarizes the immediate need for standardization in research data collection methodologies at various research institutions and the adoption of high-dimensional phenotyping. Such steps are crucial in amalgamating research results and enabling swift comparisons for accelerating switchgrass's breeding and improvement cycle.

Besides, understanding the complex genotype-environment interactions of switchgrass is a major starting point. Developing better knowledge in this respect will lead to establishing site-specific management methods through optimizing biomass yield and biofuel quality. Integration of HTP technologies opens up a whole new dimension in keeping up with this demand by efficiently capturing diverse phenotypic traits of interest on a large scale. It could enable faster genotype selection processes using advanced tools such as hyperspectral imaging, remote sensing, and machine learning, accelerating innovation in switchgrass biofuel production.

Overcoming such challenges will be necessary for developing and fulfilling the sector's goals regarding efficiency and

sustainability in the production of switchgrass biofuel. Through this collaboration and ongoing advancement of phenotyping technologies, industry stakeholders will be able to enable a more prolific and environment-friendly future for bioenergy, ensuring that switchgrass remains at an advantageous position in renewable energy solutions across the globe.

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