**Sustainable bioenergy for climate mitigation – developing drought tolerant trees and grasses**

G Taylor1,2,4, IS Donnison3, D Murphy-Bokern4, M Morgante5, M-B Bogeat-Triboulot6, R Bhalerao7, M Hertzberg8, A Polle9, A Harfouche10, F Alasia11, V Petoussi12, D Trebbi13, K Schwarz14, JJB Keurentjes15, M Centritto16, B Genty17, J Flexas18, E Grill19, S Salvi20, WJ Davies21

1 School of Biological Sciences, University of Southampton, Southampton, SO17 1BJ, UK

2 Department of Plant Sciences, University of California at Davis, Davis, 95616 California, USA

3 Institute of Biological, Environmental & Rural Sciences (IBERS), Aberystwyth University, Plas Gogerddan, Aberystwyth, Ceredigion, SY23 3EE

4 Lindenweg 12, Kroge‐Ehrendorf, Lohne, D-49393 Germany

5 Department of Agricultural and Environmental Sciences, University of Udine, Via delle Scienze, 206, 33100, Udine, Italy

6 UMR EEF, INRA, Université de Lorraine, 54280 Champenoux, France

7 Department of Forest Genetics and Plant Physiology, Umea Plant Sciences Centre, Swedish University of Agricultural Sciences, 90183 Umea, Sweden

8 **SweTree Technologies AB,** P.O Box 4095, SE-904 03 Umeå, Sweden

9 Büsgen‐Institute, Department of Forest Botany and Tree Physiology, Georg‐August University, Göttingen, 37077 Germany

10 Department for Innovation in Biological, Agro-food and Forest Systems, University of Tuscia, Viterbo, Italy

11 Franco Alasia Vivai, Strada Solerette, 5/A, 12038 Savigliano, Italy

12 Department of Sociology,University of Crete,Gallos Campus,74100 Rethymno, Greece

13 Geneticlab, Via Roveredo, 20/B, 33170 Pordenone, Italy

14 Julius Kühn‐Institut (JKI) Bundesforschungsinstitut für Kulturpflanzen, Institute for Crop and Soil Science, Bundesallee 50, D‐38116 Braunschweig, Germany

15 Laboratory of Genetics, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands

16 Trees and Timber Institute, National Research Council of Italy, Sesto Fiorentino, Italy

17 Centre d'Etudes Atomiques, Centre National de la Recherche Scientifique, Université Aix-Marseille, Unité Mixte de Recherche 6191 Biologie Végétale et Microbiologie Environnementale, Laboratoire d'Ecophysiologie Moléculaire des Plantes, 13108 Saint Paul lez Durance, France

18 Research Group on Plant Biology under Mediterranean Conditions, Departament de Biologia, Universitat de les Illes Balears, Carretera de Valldemossa Km 7.5, 07122 Palma de Mallorca, Illes Balears, Spain

19  Lehrstuhl für Botanik, Technische Universität München, 85354 Freising, Germany

20 Department of Agricultural Sciences (DipSA), University of Bologna, Viale Fanin 44, 40127 Bologna, Italy

21 Lancaster Environment Centre, Lancaster University, Lancaster, UK

4  Corresponding author, gtaylor@ucdavis.edu

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Preface

**ABSTRACT**

**Background and Aims**

Bioenergy crops are central to climate mitigation strategies that utilize biogenic carbon, such as BECCS (Bioenergy with Carbon Capture and Storage), alongside the use of biomass for heat, power, liquid fuels and in the future, biorefining to chemicals. Several promising lignocellulosic crops are emerging that have no food role – fast growing trees and grasses – but are well suited as bioenergy feedstocks, including *Populus, Salix, Arundo, Miscanthus, Panicum,* and *Sorghum.*

**Scope**

These promising crops remain largely undomesticated and, until recently, have had limited germplasm resources. In order to avoid competition with food crops for land and nature conservation, it is likely that future bioenergy crops will be grown on marginal land that is not needed for food production and is of poor quality and subject to drought stress. Thus, here we define an *ideotype for drought tolerance* that will enable biomass production to be maintained in the face of moderate drought stress. This includes traits that can readily be measured in wide populations of several hundred unique genotypes for genome wide association studies (GWAS), alongside traits that are informative but can only easily be assessed in limited numbers or training populations that may be more suitable for Genomic Selection (GS). Phenotyping, not genotyping, is now the major bottleneck for progress since in all lignocellulosic crops studied, extensive use has been made of next generation sequencing such that several thousand markers are now available and populations are emerging that will enable rapid progress for drought tolerance breeding. The emergence of novel technologies for targeted genotype-by-sequencing are particularly welcome. Genome Editing (GE) has already been demonstrated for *Populus* and offers significant potential for rapid deployment of drought tolerant crops through manipulation of ABA receptors, as demonstrated in *Arabidopsis*, with other gene targets yet to be tested.

**Conclusions**

Bioenergy is predicted to be the fastest developing renewable energy over the coming decade and significant investment over the past decade has been made in developing genomic resources and in collecting wild germplasm from within the natural ranges of several tree and grass crops. Harnessing these resources for climate-resilient crops for the future remains a challenge but one that is likely to be successful.

**INTRODUCTION – Biomass is required for a low carbon future**

Biomass used for bioenergy has a significant greenhouse gas (GHG) mitigation potential and biomass resources are required now more than ever to ensure a sustainable low-carbon future for the planet. In the most recent Intergovernmental Panel on Climate Change 5th report (IPCC, 2014), the majority of scenarios rely on bioenergy with carbon capture and storage (BECCS) to remove CO2 from the atmosphere. More recently, in a consideration of pathways to limit global warming to 1.5 ◦C, there is a heavy emphasis on technologies to rapidly achieve net zero or even negative emissions by 2050 - so called Negative Emissions Technologies (NETs, Fuss et al., 2014). The most significant of these technologies include afforestation and reforestation, the use of biochar, Direct Air Capture and Sequestration (DACCS), enhanced weathering of minerals, soil carbon sequestration and BECCS. Of these, BECCS has more potential to deliver reduced global warming than afforestation and reforestation and biochar, although DACCS and soil carbon sequestration are considered to have an equivalent potential to BECCS (Fuss et al., 2018). For example, in the UK sustainable bioenergy is required to supply at least 10% of energy demand, even in a modest zero emissions scenario (Committee on Climate Change, 2019). Bioenergy and BECCS are significant elements of the technology options to limit global warming, where the favoured feedstocks are second generation (2G) dedicated cellulosic crops (e.g. *Miscanthus* and poplar). Although other innovative crops being considered include micro- and macro-algae, these are not included here since at present they remain uncompetitive due to the high costs of cultivation and harvest and, in the medium-term at least, may be more suited to high value chemical production (Laurens et al., 2017). Cellulosic crops have no food role, in contrast to many first generation bioenergy crops (e.g. oilseed rape and maize), and can be used for electricity, liquid fuel, biogas, hydrogen production and to supply feedstocks to complex biorefineries, producing energy alongside a range of high quality chemicals (Taylor, 2008). However, the merits of wide-scale bioenergy deployment to reduce greenhouse gas emissions are still questioned since the Integrated Assessment Models (IAMs), used to explore energy futures make assumptions about land availability, idealized management and also about the cost of carbon. The land that may be required, globally, for future BECCS deployment alone has been estimated between 100- 500 Mha, by 2050 (Slade et al., 2014). At the same time, it is recognised that competition for land is increasing.Land is required for future sustainable food production that optimizes human health (Willett et al., 2019), delivers afforestation and reforestation (Bastin et al., 2019) and as such there is potential for bioenergy crops to displace land for food and other ecosystem services, with negative impacts on the environment (Petty and Barucha, 2014). Where this large land use change may occur and what the implications are for a wide range of ecosystems services and natural capital, including food and nutrient security and the preservation of biodiversity and soil carbon stocks, is only just emerging. Most of the mitigation scenarios use a base-line bioenergy crop yield for modelling of 10 tonnes of biomass ha-1 y-1, but in a recent global analysis of lignocellulosic crop yields (Allwright and Taylor, 2015), it is apparent that yield may be double this baseline value (Laurent et al., 2015). The impact of this on fuel yield will vary, depending on species due to the varying energy densities of different bioenergy crops (Valentine et al., 2011). This gap between actual and potential yield is most often associated with drought stress and is reduced by using irrigation. Interestingly, this study also showed that the most productive lignocellulosic crop was *Arundo donax­*, a largely understudied and unimproved grass.

Despite its potential, significant unresolved challenges remain if BECCS is to be used at appropriate scale to deliver a 3 GtC eq. per year of net negative emissions out to 2100 as part of efforts to avoid a temperature rise in excess of 1.5◦C. Both the CCS and bioenergy components of BECCS require new insights, but significant challenges concerning the supply of sustainable biomass are highlighted in Table 1.

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| Table 1: Ensuring future deployment of bioenergy for climate change mitigation |
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| Significant challenges to ensure sustainable bioenergy  **Land availability** - finding marginal agricultural land for adequate bioenergy crop deployment |
| **Yield limitations** - addressing the gap between potential and actual yield |
| **Water use and climate resilience** of bioenergy crops– water-energy-food nexus |
| **Sustainability** with respect to ecosystem services, biodiversity and natural capital |

At current rates of crop performance, approximately 500 Mha of land dedicated to biomass supply is needed to fulfil the requirements of BECCS(Figure 1). Although this represents a fraction of that currently used for global arable crop production (1.5 billion ha) or pasture (3.5 billion ha), it is nevertheless significant, at approximately half of the land take estimated that might be available for improved global tree cover (Bastin et al., 2019). Growth in food consumption is predicted to continue for at least the next 40 years (Godfray et al., 2014), with the consequence that significant changes are likely to the global food production system (Godfray et al., 2010) and many of the challenges facing the food system are also relevant to land use for bioenergy crops (Godfray et al., 2014). Although (2G) non-food bioenergy crops – dedicated fast growing trees and grasses - have gained prominence as a potential source of sustainable lignocellulosic biomass that could feed the bioenergy, BECCS and bioeconomy technology requirements (Somerville et al., 2010), they must be delivered with minimal impacts on food crops, in a future where pressure to produce more food will remain. Thus, future bioenergy yield enhancement should be achieved alongside increasing environmental performance of production systems, with respect to fertilizer inputs, water use, carbon storage, food security and other management strategies to improve ecosystem services.

The importance of yield increase for BECCS cannot be over-stated. Foley et al., (2011), considered production intensification as imperative to enable the doubling of food delivery by 2050, thus avoiding further agricultural land expansion. Enhanced yield for BECCS will be particularly powerful, potentially enabling delivery of 100 EJ BECCS by 2100, *requiring less than 250 Mha*, rather than 300- 500 Mha (Figure 1a). As identified (Godfray et al., 2014) for food crops and in recent work for bioenergy crops as shown in Figure 1, significant gaps between potential and actual yield exist for both crop types. Current germplasm available has the technical potential to deliver >25 t ha-1 y-1 (Figure 1b), but this is rarely realised, because management regimes are often sub-optimal, and are likely to remain so on marginal land without targeting breeding for such specialist low-input conditions.

Significant new opportunities now exist to enhance yield, using technological approaches not available even five years ago, including i. genetic modification through gene editing, ii. plant breeding using molecular approaches and genomic selection, iii. precision agriculture and iv. agroecology as key to closing the yield gap. However, future sustainable intensification should also be minimize fertilizer and water inputs, since they may add to environmental degradation and GHG emissions. In addition to GHG mitigation, a wide consideration of ecosystem services impacts should also be considered for bioenergy cropping (Holland et al., 2015; Manning et al., 2015; Milner et al., 2016). There seems little doubt that land must be utilised effectively in a future resource-constrained world. In these circumstances, understanding the nature of bioenergy crop yield and how this may be impacted by future climate scenarios is particularly timely and in this context, drought-prone sites where drought tolerant crops can grow, may be particularly important. Alongside this, an assessment of new technologies and how they may be deployed for the perennial lignocellulosic crops is also warranted.

**DEVELOPING BIOENERGY CROPS FOR FUTURE DROUGHTED ENVIRONMENTS**

**Physiological and biochemical responses to water deficit**

Water limits crop growth more than any other environmental variable and it is perhaps not surprising that in the largely unimproved germplasm of lignocellulosic feedstock crops, water has a significant and variable impact on yield across a wide range of diverse genetic material (Richard et al., 2019). At the same time, marginal land is likely to be drought-prone land for future bioenergy crops for climate mitigation using BECCS. Thus, understanding how lignocellulose crops respond to limited water supply is critical for future crop breeding and selection. Observations and data acquired from model plants and food crops are likely to be, at best, only partially informative. Drought tolerance is a complex trait - difficult to quantify and highly variable (Passiora, 2012). For example, plant characteristics conferring positive drought tolerance in some circumstances may become detrimental in other circumstances, depending on the severity and duration of the drought (Tardieu, 2012) and thus it is essential to understand which drought scenarios are relevant for any particular cropping type. Non‐food bioenergy crops such as fast growing trees and grasses are thus required that are able to grow on marginal land where water supply is likely to be limited in future­ (Oilver et al., 2019; Viger et al., 2016). Understanding the genetic basis of adaptation to drought is therefore a pressing research priority in these crops (Allwright and Taylor, 2015), where water supply is of overriding importance in determining biomass yield. Genotypes adapted to low precipitation regimes have been identified in a wide range of arable crops and fragmented data are now emerging for bioenergy crops, including *Populus* and *Miscanthus* (Da Costa et al., 2019) and also *Arundo* (Howarth et al 2019), suggesting that enough diversity exists for targeted genetic improvement for drought tolerance. Wide variation has been reported in traits such as intrinsic water use efficiency, leaf carbon isotope discrimination, stomatal conductance, stomatal density, and differences in gene expression and metabolic changes in response to drought in *Populus* and several bioenergy grasses including *Phalaris, Dactylis*  (Klaas et al., 2019) and *Miscanthus* (Stavridou et al., 2019) have been identified. The ability of some bioenergy crops, for example *Populus* species to tolerate extremely droughted environments, including *Populus euphratica,* found in salty and arid environments such as the Negev desert (Bogeat-Triboulet et al., 2007), has also been noted. Some bioenergy crops, in contrast to those considered here, may be desert plants and highly tolerant of dry soils, however these extremes and severe droughts are outside the scope of this review and do not confirm to our ideotype for drought tolerance. Quantifying genetic diversity and understanding the physiological traits associated with genetic variation provides the first step to developing superior plants for drought tolerance that will underpin the release of trees and grasses resilient for future drought-prone climates. Here we define drought tolerance as the **‘*maintenance of plant biomass production in the face of moderate and persistent drought stress*’** since this type of drought scenario is likely to be more relevant to continued productivity in biomass bioenergy crops*.* Much of the research undertaken to date on drought tolerance in model plants and arable crops, such as that on early vigor, flowering time, grain fill, and partitioning, is of limited relevance to bioenergy crops, since these traits in annual crops tend to reflect end-of-life mechanisms to overcome drought stress and are highly dependent on plant developmental stage. Plants mitigate the effects of limited soil water availability through a range of mechanisms and improvements in drought tolerance should be specific to the targeted crop system and the type of drought that occurs most frequently (Tardieu, 2012) and for maintaining productivity in bioenergy cropsthis means maximising overall biomass accumulation. Thus, drought tolerant genotypes must maintain cell production and expansion, leaf growth, greenness and gas exchange rates under soil water deficits (traits identified as being drought responsive; Liu and Dickman, 1999; Tschaplinski et al., 2006; Marron et al., 2002; Tardieu and Tuberosa, 2010; Di Ollas and Dodd, 2016). Root traits are also important in maintaining biomass production during drought (Parra-Londono et al., 2018; Xie et al., 2017). In contrast, other traits important during terminal or very intense drought events like cell protection mechanisms and cavitation prevention may be less relevant for the drought ideotype being considered here (Marron et al.,2002b; Sack and Holbrook, 2006; Cochard et al., 2007).

**Developing an ideotype for drought tolerance – understanding complexity for future breeding**

An ideotype is defined as the idealized plant for a given environment (Donald, 1968) and here we are interested in the ideotype for drought tolerance in bioenergy crops. Strictly speaking, several bioenergy drought tolerance ideotypes may exist and are likely to be species specific. A drought tolerance ideotype for bioenergy poplar trees suggests that selection of fast growing genotypes originating from wetter areas of Europe (e.g. northern Italy) out-performs those from droughted environments (e.g. southern Spain), even during drought. In this way, we found genotypes with *high hydraulic capacity*, and *large leaves made up of many cells and high stomatal index* *and responsive stomata, with high transpiration efficiency (TE water loss per unit biomass gained)* to be best suited to drought environments across Europe. Moreover, although water use efficiency (WUE) and saccharification potential are less heritable breeding targets, genotypes which combine high yield, WUE and saccharification potential have been identified. Interestingly, saccharification potential increased under moderate drought (Wildhagen et al. 2018). At the same time, Papcek et al., (2019), have over-expressed poplar ABA receptors in *Arabidopsis* and observed a 26% increase in WUE. This suggests that ABA induced stomatal closure may be an important drought tolerance mechanism in *Populus*, a result supported by the findings of Brunetti et al., (2019), who demonstrated a tight link between ABA-related gene expression and altered photosynthesis and stomatal conductance in response to drought in this bioenergy tree. The overwhelming impact of ABA control on biomass production was also demonstrated with a range of transgenic poplars grown outdoors (Yu et al. 2019). From these and other findings, a list of key phenotypic traits that are tractable in very large populations of bioenergy crops and underpin the drought tolerance ideotype have been identified and are summarized in Table 2. Since phenotyping has become the costly, time-consuming and ‘bottle-necked’ activity in the study of links between phenotype and genotype (Tardieu et al., 2017), here we have identified two types of informative phenotyping measurement. 1): measurements that can be made in many 100s of samples, that are well-studied and conceptualized and representative of drought responses and used for association and other mapping to inform genetic loci (GWAS traits); 2): measurements that are highly informative but too time consuming for whole population studies and may, therefore, be suited to Genomic Selection traits in training and test populations (GS traits). One area that is significantly understudied in bioenergy crops with respect to drought tolerance is that of root traits (Para-London et al., 2018), for which there is a paucity of information, although a recent report suggests that deep roots may become more significant for water transport in droughted conditions, at least for *Arundo* (Zageda-Lizarazu and Monti, 2019). Whilst root architecture and function certainly remain an area where further research is required, new modelling approaches are emerging that are likely to be of value in the discovery of root ideotypes for drought tolerance (Schnepf et al., 2018).

**Table 2:** A summary of phenotyping measurements that provide insight into the ideotype for drought tolerance in bioenergy crops, where type of measurement is defined as either 1) made on many 100s of samples possible for use in GWAS analysis or 2) informative measurements but too costly or time consuming for GWAS and may be more suited to Genomic Selection (GS).

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| **Traits of value for drought tolerance assessment in bioenergy crops** | **Relationship to drought tolerance** | **GWAS or GS trait** | **Ref** |
| Whole plant  biomass  productivity | Non-destructive assessments of tree diameter and height as a measure of productivity, taken start of drought and end of season. For grasses, tillering has been shown to be linked to yield during drought | GWAS | 1 |
| Leaf size and shape | Strong evidence as a proxy for yield potential in *Populus* and a key trait for drought tolerance | GWAS | 2 |
| Leaf cell production and expansion | Leaf cell production is highly heritable and linked to leaf size. Cell size has low heritability but elasticity of cells is linked to drought tolerance | GWAS | 3 |
| Stomatal density and index | Evidence from several crops of links to water use | GWAS | 4 |
| Turgor loss point and osmotic adjustment | Literature has shown this to be a valuable trait and can be measured in many samples using an osmometer and metabolite analysis | GWAS | 5, 9 |
| Carbon isotope discrimination as a proxy for  WUE (integrated) | Excellent proxy for integrated seasonal water used efficiency, using wood samples. Extensive evidence on heritability and usefulness in breeding | GWAS | 6 |
| Hydraulic  conductivity | Found to be tightly linked to drought tolerance | GS | 7 |
| Stomatal closure | Rapid partial stomatal closure and maintenance of water potential linked to drought tolerance | GS | NA |
| Xylem water potential - - predawn | A useful indicator to assess plant strategy to avoid or tolerate reduced soil moisture, alongside stomatal conductance | GS | NA |
| Canopy greenness | Measured with proximal SPAD, an early indicator for drought tolerance for *P. trichocarpa* will now be improved using NDVI with a multi-spectral camera mounted on a UAV, with opportunity to quantify other spectra and make 1,000s of measurements, daily | GWAS | NA |
| Canopy and leaf  infra-red temperature | Hand held IR probe and UAV mounted camera as a proxy for stomatal opening, able to make 1000s, daily | GWAS | NA |
| Yield Stress Index |  | GWAS | 6 |
| Drought  Resistance Index | Where geno and pheno represent genotypic and phenotypic means | GWAS | 8 |

Where references are 1, Laurue et al., 2019; 2, Rae et al., 2004; 3, Allwright and Taylor, 2015; 4, Bertolino et al., 2019; 5, Bartlett et al., 2012; 6, Fernandez et al., 1992; 7, Cochard et al., 2007; 8, Fischer and Maurer, 1978; 9, Tschaplinski et al, 2019.

**ACCELERATING DISCOVERY - THE TECHNICAL POTENTIAL OF NEW GENOMIC TECHNOLOGIES FOR FUTURE BIOENERGY**

Game-changing molecular technologies made available in the last few years are now set to make possible the rapid development and deployment of lignocellulosic bioenergy trees and grasses. Although twenty years in the making, the first reports of the application of Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9 (CRISPR-Cas9) technology appeared only a few years ago (Lander et al., 2016), enabling precise and efficient gene editing using an adaptive immune response of bacteria (Lander et al., 2016). It is one of a cadre of nuclease technologies able to make precise double-stranded DNA breaks now, used for significant new discoveries in a range of crop plants, some of which have now been agreed for release by appropriate authorities (Schaeffer and Nakata, 2016). The advantage of CRISPR-Cas is the ability to back-cross and remove inserted DNA. This leads to fewer regulatory hurdles although the value of this for trees remains to be determined. The potential of these technologies cannot be overestimated – recent research comparing RNAi and CRISPR-Cas modified potatoes shows that although both technologies led to reduced vacuolar invertases, in CRISPR- Cas this was more effective and without the obligatory need to express the RNAi construct in commercial plants, in perpetuity. In the past the use of such new technologies have lagged behind in trees by years and often decades. However, with staggering speed, and only 2 years after the first plant experiment, two separate reports for bioenergy *Populus* were made in 2015, spanning different species of the *Populus* genus (Tsai and Xue, 2015; Fan et al., 2015). Many targets are already available that could potentially improve drought tolerance using a gene editing approach including genes coding for ABA receptor proteins and a drought response gene from sugar cane (Bagcy et al., 2019)

At the same time, yield and resilience to climatic stress are complex phenotypes, as described above, that may often be difficult to resolve at the level of a single gene and as such, other approaches are required to determine stacks of underpinning candidate genes for gene editing. Genome- wide association mapping provides the toolkit to develop such approaches and enables significant associations between complex traits and underlying genetic variation to be identified. Although useful in understanding the genetic basis of traits including in bioenergy crops (Lopez-Alverez et al., 2017; Porth et al., 2013) and staple food crops, such as wheat (Rasheed et al., 2018), GWAS has been of limited value to breeding pipelines or Marker Assisted Selection (MAS) (Janninck et al, 2010). However, a paradigm-shift has occured in the last few years by the introduction of Genomic Selection (GS). Rather than seeking to identify single markers associated with traits of interest, GS uses all marker data to predict the trait in a test population, providing a breeding value that can then be tested in a validation population, as described for *Miscanthus* by Slavov et al., (2019). This knowledge is now being applied to bioenergy trees and grasses (Resende et al., 2010) to overcome some of the innate difficulties of undomesticated tree populations with rapid rates of linkage disequilibrium (LD) decay, halving the time of the breeding cycle. Using a sophisticated, approach Slavov et al (2019) show that it is possible to work with multiple breeding targets using GS in the absence of *a priori* knowledge that may enable effective yield intensification in the future, where environmental constraints such as drought are considered. This approach is revolutionizing animal breeding and from initial results looks to be significantly more accurate for plant systems.

GS relies on a high density of molecular markers, accurate phenotypic data and a training and validation population (Desta et al., 2014). Molecular marker development in lignocellulosic crops has often been limited to relatively small panels of informative SNPs on small populations of individuals. However, rapid progress is now being made in these genetically diverse and unimproved crops. For example, going back to wild populations of  *Miscanthus sacchariflorus*, which is one of the parents of the commercial hybrid *Miscanthus*, Clark et al (2019) have identified over 34,000 SNPs in more than 700 individuals in the natural population range of this species across East Asia and have also resolved population structure. This has revealed enhanced genetic diversity relative to the other hybrid parent suggesting *M. sacchariflorus* as an important source of genetic diversity for future breeding efforts. Such wild collections also have a value in identifying early leads for breeding, since they are phenotypically diverse when assessed for yield and a recent report of such wild hybrids identified yield enhancement relative to the current commercial hybrid (Huang et al., 2019). Genotyping-by-sequencing (GBS) approaches, as described for *Miscanthus*  are rapidly providing outstanding resources for molecular breeding, including in the less well characterized bioenergy trees, such as shrub willow (Carlson et al 2019) and oil crops including Jatropha (Vandepitte et al, 2019). For shrub willow, GBS has provided the first insights into linkage analysis and linkage disequilibrium for this crop, and identified chromosome 5 and 10 as important hotspots for yield-related traits. For *Populus* extensive genotyping has been undertaken in a number of wild populations, but particularly in a collection of wild *P. trichocarpa* from the western USA that has been effectively used to unravel the genomic basis of stress tolerance and bioenergy wood chemistry traits (Muchero et al., 2018; Porth et al., 2013; Evans et al., 2014). Even for forest trees, where secondary cell wall biosynthesis is complex and different to many monocot crop plants (Meents et al., 2018), progress is being made for widescale genotyping. Despite this progress, however, GBS still remains costly and this may limit progress. In a novel genotyping-by -sequencing (GBS) approach that addresses the efficient and effective deployment of markers, in *P. nigra,* Single Primer Enrichment Technology (SPET) was used for the first time, to target SNPs within each gene model, thus reducing costs and providing a ‘smart’ enrichment technology. This provides a significant improvement over random sampling over genomic loci that may contain much redundancy and is inconsistent between laboratories (Scaglione et al., 2019).

**OUTLOOK - BIOENERGY FUTURES IN A RESOURCE-CONSTRAINED WORLD**

Bioenergy will lead the growth in renewables over the coming decades, according to the International Energy Agency renewables report, 2018 and will continue to make the largest contribution, globally to renewable energy, significantly through generation of heat and as a transport fuel and increasingly as part of the circular bioeconomy. Added to this, as BECCS technologies mature and are more widely deployed, bioenergy will also play a key role in the move towards net zero or negative CO2 emissions that will enable the global temperature rise to be held below 2.0 ◦C. Thus, bioenergy will be increasingly required for the emerging low carbon economy and society. At the same time, sustainable second generation, non-food, lignocellulosic crops that are key to these bioenergy developments remain largely under-developed with limited breeding and selection. Their perenniality, long breeding cycles, wide and heterogeneous germplasm and untapped genomic resources, until recently have presented a bottleneck. However, as this review reports, the utilization of emerging DNA technologies is providing a step-change in discovery and accelerating our understanding of complex drought tolerance traits and their links to underlying genes and suites of molecular markers that can be used to underpin an array of genomic approaches for breeding and improvement. At the same time, although phenotyping remains a bottleneck that is now limiting how these genomic technologies can be deployed (Tardieu et al., 2017), the research reported here provides a clear insight into the ideotypes for drought tolerance that should be targeted using molecular breeding approaches and modelling frameworks to ensure success over the coming decades. Alongside this, genome editing has already been demonstrated in bioenergy *Populus* trees and provides a significant opportunity to develop future bioenergy crops that can tolerate droughted environments without incurring a yield penalty.

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**LITERATURE CITED**

Allwright MR and Taylor G, 2015. Molecular breeding for improved second generation bioenergy crops. Trends in Plant Science, doi:10.1016/j.tplants.2015.10.002.

## Bartlett MK, Scoffoni C, Sack L. 2012. The determinants of leaf turgor loss point and prediction of a drought tolerance of species and biomes: global meta-analysis, Ecology Letters 15, 393-405.

## Bastin J-F, Finegold Y, Garcia C, et al., 2019. The global tree restoration potential. Science, 365, 76-79.

## Begcy K, Mariano ED, Lembke CG et al., 2019. ,Scdr2 improves tolerance to salinity and drought, *Annals of Botany* 124: xxx–xxx, doi: 10.1093/aob/mcz044.

Bertolino LT, Caine RS, Gray JE. 2019. Impact of stomatal density and morphology on water use efficiency in a changing world. Frontiers in Plant Science 10, 225.

Bogeat-Triboulot M-B, Brosché M, Renaut J., et al. 2007. Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in Populus euphratica, a poplar growing in arid regions. Plant Physiology 143, 876–892.

## Brunetti C, Gori A, Marino G, et al., 2019. Effects of ABA dynamics on poplar acclimation to drought, Annals of Botany 124: xxx–xxx, doi: 10.1093/aob/mcz005

Carlson CR, Gouker FE, Crowell CR, et al., 2019. Linkage and association mapping of complex traits in shrub willow, Annals of Botany, 124: xxx–xxx, doi: 10.1093/aob/mcz047

Campbell J. Elliot, Lobell DB, Genova RC, Field CB. 2008. The Global Potential of Bioenergy on Abandoned Agriculture Lands. Environ. Sci. Technol. 45, 5791-5794.

## ClarkLV, Jin, X Petersen KK et al., 2019. Population structure of *Miscanthus sacchariflorus,* Annals of Botany 124: xxx–xxx, doi: 10.1093/aob/mcy161.

Cochard H, Casella E and Mencuccini M. 2007. Xylem vulnerability to cavitation varies among poplar and willow clones and correlates with yield. Tree Physiology 27, 1761–1767.

Committee on Climate Change, 2019. Net zero. The UK’s contribution to stop global warming, CCC, May 2019.

Da Costa et al de Ollas C and Dodd IC. 2016. Physiological impacts of ABA–JA interactions under water-limitation. Plant Molecular Biology 91(6): 641–650.

## da Costa, RFM, Simister, R, Roberts, LA, Timms-Taravella, E, Cambler, AB, Corke, FM, Han, J Ward, RJ Buckeridge, MS, Gomez, LD, Bosch M. 2019. Environmental stress affects Miscanthus biomass accumulation and sugar release. Annals of Botany 124, xxx–xxx, 2019

doi: 10.1093/aob/mcy155.

Desta et al., 2014. Genomic selection: genomic-wide prediction in plant improvement. Trends in Plant Science, September, Vol 19, No. 9.

Donald CM, 1968. The breeding of crop ideotypes. Euphytica, 17, 385-403.

Evans LM, Slavov GT, Rodgers-Melnick E, Martin J, Ranjan P, Brunner AM et al., 2014. Population genomics of *Populus trichocarpa* identifies signatures of selection and adaptive trait associations. Nature Genetics, 46, 1089-96.

Fan [D](https://www.ncbi.nlm.nih.gov/pubmed/?term=Fan%20D%5BAuthor%5D&cauthor=true&cauthor_uid=26193631), [Liu T](https://www.ncbi.nlm.nih.gov/pubmed/?term=Liu%20T%5BAuthor%5D&cauthor=true&cauthor_uid=26193631), Li C, [Jiao B](https://www.ncbi.nlm.nih.gov/pubmed/?term=Jiao%20B%5BAuthor%5D&cauthor=true&cauthor_uid=26193631), [Li S](https://www.ncbi.nlm.nih.gov/pubmed/?term=Li%20S%5BAuthor%5D&cauthor=true&cauthor_uid=26193631), [Hou Y](https://www.ncbi.nlm.nih.gov/pubmed/?term=Hou%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=26193631), [Luo K](https://www.ncbi.nlm.nih.gov/pubmed/?term=Luo%20K%5BAuthor%5D&cauthor=true&cauthor_uid=26193631) 2015. Efficient CRISPR/Cas9-mediated Targeted Mutagenesis in *Populus* in the First Generation. Scientific Reports, 5, 12217.

Fernandez GGJ. 1992. Effective Selection Criteria for Assessing Plant Stress Tolerance. In: Proceedings of the International Symposium on Adaptation of Vegetables and Other Food Crops in Temperature and Water Stress. AVRDC, Shanhua, 257–70.

Foley JA et al.,2011. Solutions for a cultivated planet*.* Nature**,** 478(7369): p. 337-342.

Fuss S, Canadell JP, Peter GP, Tavoni M, Andrew RM, Andrew RM, Ciais P, Jackson RB, Jones CD, Kraxner F, Nakicenovic N, Le Quere C, Raupach MR, Sharifi A, Smith P, Yamagata Y 2014. Betting on negative emissions*.* Nature Climate Change, 4, 850-853.

Fuss S, Lamb WF, Callaghan MW, et al., 2018. Negative Emissions- Part 2: Costs, potential and side effects. Environmental Research Letters, 13, 063002.

Godfray HJC et al., 2010. Food Security: The Challenge of Feeding 9 Billion People. Science, 327, 812-818.

Godfray HJC et al., 2014. Food security and sustainable intensification*.* Phil. Trans. R. Soc. B, 369, 20120273.

Holland RA, Eigenbrod F, Muggaridge A, Brown G, Clarke D, Taylor G 2015. A synthesis of the ecosystem services impact of second generation bioenergy crop production. Renewable and Sustainable Energy Reviews, 46, 30-40.

## Howarth M, Marino G, Riggi, E et al., 2019. Morphological and physiological traits determine Arundo drought recovery, *Annals of Botany* 124: xxx–xxx, doi: 10.1093/aob/mcy223.

## Huang LS, Flavell R, Donnison IS, et al., 2019. *Miscanthus* germplasm collection for breeding, *Annals of Botany* 124: xxx–xxx, doi: 10.1093/aob/mcy231.

IPCC, 2014. Climate Change 2014, Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Jannink JL et al 2010. Genomic selection in plant breeding from theory to practice. Briefings in Functional Genomics, 9, 166-177.

## Klaas M, Haiminen N, Grant J et al., 2019. Gene expression under water stress in grasses, *Annals of Botany* 124: xxx–xxx, doi: 10.1093/aob/mcz.

Lander ES, 2016. The Heroes of CRISPR. Cell 164, January 14, 2016.

## Larue F, Fumey D, Rouan L et al., 2019. Modelling biomass sorghum ideotypes

Annals of Botany 124: xxx–xxx, doi: 10.1093/aob/mcz038.

Laurens LML, Chen-Glasser M, McMilla JD 2017. Letter to the Editor. Algal Research, 24, 261-264.

Laurant A, Pelzer E, Loyce C, Makawski D 2015. Ranking yields of energy crops: A meta-analysis using direct and indirect comparisons. 2015. Renewable and Sustainable Energy Reviews, 46, 41-50.

Liu Z and Dickman DI 1992. Abscisic acid accumulation in leaves of two contrasting hybrid poplar clones affected by nitrogen fertilization plus cyclic flooding and soil drying. Tree Physiology 11, 109–122.

Lopez-Alvarez D, Zubair H, Beckmann M, Draper J, Catalan P 2017. Diversity and association of phenotypic and metabolic traits in the close model grasses *Brachypodium distachyon*, *B. stacei,*  and *B. hybridum*. Annals of Botany, 119, 545-561.

Manning P, Taylor G, Hanley ME 2015. Bioenergy food production and biodiversity – an unlikely alliance? Global change Biology Bioenergy, 7, 570-576.

Marron N, Delay D, Petit JM, Dreyer E, Kahlem G, Delmotte FM, Brignolas F 2002. Physiological traits of two Populus x euramericana clones, Luisa Avanzo and Dorskamp, during a water stress and re-watering cycle. Tree Physiology 22, 849-858.

Marron N, Delay D, Petit JM, et al. 2002. Physiological traits of two Populus x euramericana clones, Luisa Avanzo and Dorskamp, during a water stress and re-watering cycle. Tree Physiology 22, 849–858.

Meents M, Watanbe Y, Samuels AL 2018. The cell biology of secondary cell wall biosynthesis. Annals of Botany, 121, 1107-1125.

Milner S, Holland RA, Lovett A, Sunnenberg G, Hastings A, Smith P. Wang S, Taylor G 2016.

[Potential impacts on ecosystem services of land use transitions to second‐generation bioenergy crops in GB](javascript:void(0)). Global Change Biology Bioenergy 8, 317-333.

Muchero W, Sondreli KL, Chen J-G, Breeanna R, et al., 2019. Association mapping , transcriptomics, and transiet expression identify candidate genes mediating plant-pathogen interactions in a tree. PNAS, 115, 11573-11578.

Oliver, RJ, Finch, JW, and Taylor G. 2009. Second generation bioenergy crops and climate change: a review of the effects of elevated atmospheric CO2 and drought on water use and the implications for yield. GCB Bioenergy 1, 97–114.

Passioura J 2012. Phenotyping for drought tolerance in grain crops: when is it useful to breeders? Functional Plant Biology, 39, 851-859.

## Papcek M, Christmann, A and Grill E, 2019. Poplar ABA receptors in Arabidopsis

Annals of Botany 124: xxx–xxx, 2019, doi: 10.1093/aob/mcy225.

# Parra-Londono S, Kavka M, Samans B, Sowdon R, Wieckhorst S, Uptmoor R 2018. Sorghum root-system classification in contrasting P environments reveals three main rooting types and root-architecture-related marker-trait associations. Annals of Botany, 121, 267-280.

Porth I, Klapste J, Skyba O, Lai BS, Geraldes A, Muchero W, Tuskan GA, Douglas CJ, El-Kassaby TA, Mansfield SD. 2013. Populus trichocarpa cell wall chemistry and ultrastructure trait variation, genetic control and genetic correlations. New Phytologist, 197, 777-790.

Pretty J, Bharucha ZP 2014. Sustainable intensification in agricultural systems. Annals of Botany 114, 1571-96.

Rae AM, Robinson KM, Street NR, Taylor G. 2004. Morphological and physiological traits influencing biomass productivity in short-rotation coppice poplar. Canadian Journal of Forest Research 34, 1488-1498.

Rasheed A, Mujeeb-Kazi A, Ogbonnaya FC, He Z, Rajara S 2018. Wheat genetic resources in the post-genomics era: promise and challenges. Annals of Botany, 121, 603-616.

Resende MFR 2012. Accelerating the domestication of trees using genomics selection: accuracy of prediction models across ages and environments. New Phytologist,193, 617-624.

## Richard B, Richter GM, Cerasuolo M 2019. Optimizing water footprint of SRC willow cultivars (Research in Context), Annals of Botany 124: xxx–xxx, doi: 10.1093/aob/mcz006.

Schaeffer SM and Nakata PA 2016. The expanding footprint of CRISPR/Cas9 in the plant sciences. Plant Cell Rep, 35,1451-1468.

Slade R, Bauen A, Gross R, 2014. Global bioenergy resources, Nature Climate Change 4, 99-105.

## Sack L, Holbrookman NM, 2006. Leaf Hydraulics. Annual Review of Plant Biology, 57, 361-381.

## Scaglione D, Pinosio S, Marroni F, et al., 2019, Focused genotyping by sequencing (GBS) made easy (Technical article), Annals of Botany124: xxx–xxx, doi: 10.1093/aob/mcz054.

Schnepf A, Leitner D, Landl M, Lobet G, Mai TH, Morandage S, Sheng C, Zorner M, Vanderborght J, Vereecken H 2018. CRootBox: a structural-functional modelling framework for root systems. Annals of Botany, 121, 1033-1053.

## Slavov GT, Davey CL, Bosch M et al., 2019. Genomic index selection provides multi-objective breeding targets in *Miscanthus* (Viewpoint), *Annals of Botany* 124: xxx–xxx, doi: 10.1093/aob/mcy187.

Somerville C, et al., 2010. Feedstocks for Lignocellulosic Biofuels. Science 329, 790.

Stavridou E. Webster RJ, and Robson PRH et al, 2019. *Miscanthus* response to combinations of salinity and drought, Annals of Botany124: xxx–xxx, 10.1093/aob/mcz009

Tardieu, F. 2012. Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. J. Exp. Bot. 63, 25–31.

Tardieu F., Tuberosa R. 2010. Dissection and modelling of abiotic stress tolerance in plants. Current opinion in plant biology 13, 206-212.

Tardieu F, Cabrera-Bosquet L, Pridmore T, Bennett M. 2017. Plant phenomics, From Sensors to Knowledge. Current Biology, 27, R770-783.

Taylor G, 2008. Bioenergy and the biorefinery concept. Energy Policy, 36, 4406-4409.

Tsai CJ, Xue LJ 2015. CRISPRing into the woods. GM Crops & Food, 6, 206-215.

Tschaplinski TJ, Tuskan GA, Sewell MM, Gebre GM, Donald ETI, Pendley C. 2006. Phenotypic variation and quantitative trait locus identification for osmotic potential in an interspecific hybrid inbred F-2 poplar pedigree grown in contrasting environments. Tree Physiology 26, 595-604.

## Tschaplinski TJ, Paul Abraham, Sara Jawdy 2019. Acute drought induces populosoides in *Populus deltoides.* Annals of Botany,124: xxx–xxx doi: 10.1093/aob/mcz002.

United Nations, 2015. Transforming Our World: the 2030 Agenda for Sustainable Development. Resolution adopted by the General Assembly on 25 September 2015. A/RES/70/1.

Valentine J, Clifton-Brown J, hastings A, Robson P, Allison G, Smith P 2011. Food versus fuel: the use of land for lignocellulosic ‘next generation’ energy crops that minimize competition with primary food production. Global change Biology Bioenergy, 4, 1-19.

## Vandepitte et al 2019. High genetic diversity in the non-toxic indigenous Jatropha curcas gene pool, Annals of Botany 124: xxx–xxx, doi: 10.1093/aob/mcz008.

Viger M, Smith HK, Cohen D, DeWoody J, Trewin H, Steenackers M, Bastien C, Taylor G 2016. Adaptive mechanisms and genomic plasticity for drought tolerance identified in European black poplar (*Populus nigra*). Tree Physiology, 36, 909-928.

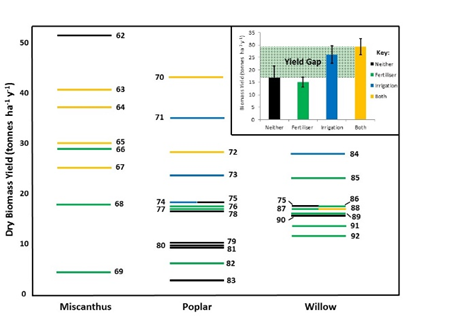
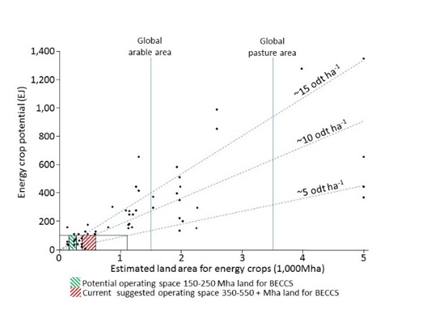
Wildhagen H, Paul S, Allwright M, Smith HK, Malinowska M, Schnabel S, Paulo J, Cattonaro F, Vendramin V, Scalabrin S, Janz D, Douthe C, Cohen D, Le Thiec D, Brendel O, van Eeuwijk F, Keurentjes JB, Flexas J, Morgante M, Robson P, Bogeat-Triboulot MB, Taylor G, Polle A, 2018. Genes and gene clusters related to genotype and drought-induced variation in saccharification potential, lignin content, and wood anatomical traits in *Populus nigra*. Tree Physiology, 38, 340-361.

Willett W, Rockstrom J, Loken B, et al., 2019. Food in the Anthropocene: the EAT – lancet Commission on healthy diets. The LancetCommissions, 393, 447-492.

Xie Q, Fernando KMC, mayes S, Sparkes DL, 2017. Identifying seedling root architecture traits associated with yield and yield components in wheat. Annals of Botnay, 119, 1115-1129.

Yu D, Wildhagen H, Tylewicz S, Miscolczi PC, Bhalerao RP, Polle A, 2019. ABA signaling mediates biomass trade-off and allocation in poplar. New Phytologist, 223, 1192-1203.

## Zageda-Lizarazu W and Monti A. 2019. Deep Rooting Characteristics and Water Uptake of Giant Reed, Annals of Botany 124: xxx–xxx, doi: 10.1093/aob/mcz001.

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**Figure 1:** A) land area required for global bioenergy crops [modified from Slade et al., 2014] and B) A synthesis of potential and actual yield data (inset) from a survey of yield experiments for three focal 2G lignocellulosic non-food crops, illustrating the existence of a significant yield gap [modified from Allwright and Taylor, 2015].