Sustainable bioenergy for climate mitigation

Taylor, G.; Donnison, Iain; Murphy-Bokern, Donal; Morgante, Michele; Bogeat-Triboulot, M-B.; Bhalerao, R.; Hertzberg, M.; Polle, A.; Harfouche, A.; Alasia, F.; Petoussi, V.; Trebbi, D.; Schwarz, K.; Keurentjes, J. J. B.; Centritto, M.; Genty, B.; Flexas, J.; Grill, E.; Salvi, S.; Davies, W. J.

Published in:
Annals of Botany

DOI:
10.1093/aob/mcz146

Publication date:
2019

Citation for published version (APA):
Taylor, G., Donnison, I., Murphy-Bokern, D., Morgante, M., Bogeat-Triboulot, M-B., Bhalerao, R., ... Davies, W. J. (2019). Sustainable bioenergy for climate mitigation: developing drought-tolerant trees and grasses. Annals of Botany, 124(4), 513-520. [mcz146]. https://doi.org/10.1093/aob/mcz146

Annals of Botany **124**: 513–520, 2019
doi: 10.1093/aob/mcz146, available online at www.academic.oup.com/aob

ANNALS OF BOTANY
Founded 1887

VIEWPOINT: PART OF A SPECIAL ISSUE ON BIOENERGY CROPS FOR FUTURE CLIMATES

Sustainable bioenergy for climate mitigation: developing drought-tolerant trees and grasses

G. Taylor[1,2,*], I. S. Donnison[3], D. Murphy-Bokern[4], M. Morgante[5], M.-B. Bogeat-Triboulot[6], R. Bhalerao[7], M. Hertzberg[8], A. Polle[9], A. Harfouche[10], F. Alasia[11], V. Petoussi[12], D. Trebbi[13], K. Schwarz[14], J. J. B. Keurentjes[15], M. Centritto[16], B. Genty[17], J. Flexas[18], E. Grill[19], S. Salvi[20] and W. J. Davies[21]

[1]School of Biological Sciences, University of Southampton, Southampton, SO17 1BJ, UK, [2]Department of Plant Sciences, University of California at Davis, Davis, CA 95616, USA, [3]Institute of Biological, Environmental & Rural Sciences (IBERS), Aberystwyth University, Plas Gogerddan, Aberystwyth, Ceredigion, SY23 3EE, UK, [4]Lindenweg 12, Kroge-Ehrendorf, D-49393 Lohne, Germany, [5]Department of Agricultural and Environmental Sciences, University of Udine, Via delle Scienze, 206, 33100, Udine, Italy, [6]Université de Lorraine, INRA, AgroParisTech, UMR Silva, 54000 Nancy, 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, 37077 Göttingen, 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]Aix-Marseille University, CEA, CNRS, BIAM, UMR 7265, 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 and Food Sciences, University of Bologna, Viale Fanin 44, 40127 Bologna, Italy and [21]Lancaster Environment Centre, Lancaster University, Lancaster, UK
**For correspondence. E-mail gtaylor@ucdavis.edu*

Received: 9 July 2019 Returned for revision: 23 July 2019 Editorial decision: 3 September 2019 Accepted: 23 September 2019

• **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, 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. 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 genotyping by sequencing are particularly welcome. Genome editing 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.

Key words: *Miscanthus*, *Populus*, *Arundo*, molecular breeding, next-generation sequencing, marginal land, lignocellulosic crop.

INTRODUCTION

Biomass is required for a low-carbon future

The use of biomass for energy has a significant greenhouse gas mitigation potential and biomass resources are required now more than ever to ensure a sustainable low-carbon future for the planet. In the recent Intergovernmental Panel on Climate Change Fifth Assessment Report (IPCC, 2014), the majority of scenarios rely on bioenergy with carbon capture and storage (BECCS) to remove CO_2 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 carbon 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 and 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) that are 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 to be between 100 and 500 Mha by 2050 (Slade *et al.*, 2014). At the same time, it is recognized that competition for land is increasing. Land is required for future sustainable food production that optimizes human health (Willett *et al.*, 2019) and delivers afforestation and reforestation (Bastin *et al.*, 2019), and consequently there is potential for bioenergy crops to displace land for food and other ecosystem services, with negative impacts on the environment (Pretty and Bharucha, 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, are only just emerging. Most of the mitigation scenarios use a baseline bioenergy crop yield for modelling of 10 tonnes of biomass per hectare per year, 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 (Laurant *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 equivalent per year of net negative emissions up 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.

At current rates of crop performance, ~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 and Garnett, 2014), with the consequence that significant changes to the global food production system (Godfray *et al.*, 2010) are likely, and many of the challenges facing the food system are also relevant to land use for bioenergy crops (Godfray and Garnett, 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 overstated. 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

TABLE 1. *Ensuring future deployment of bioenergy for climate change mitigation*

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

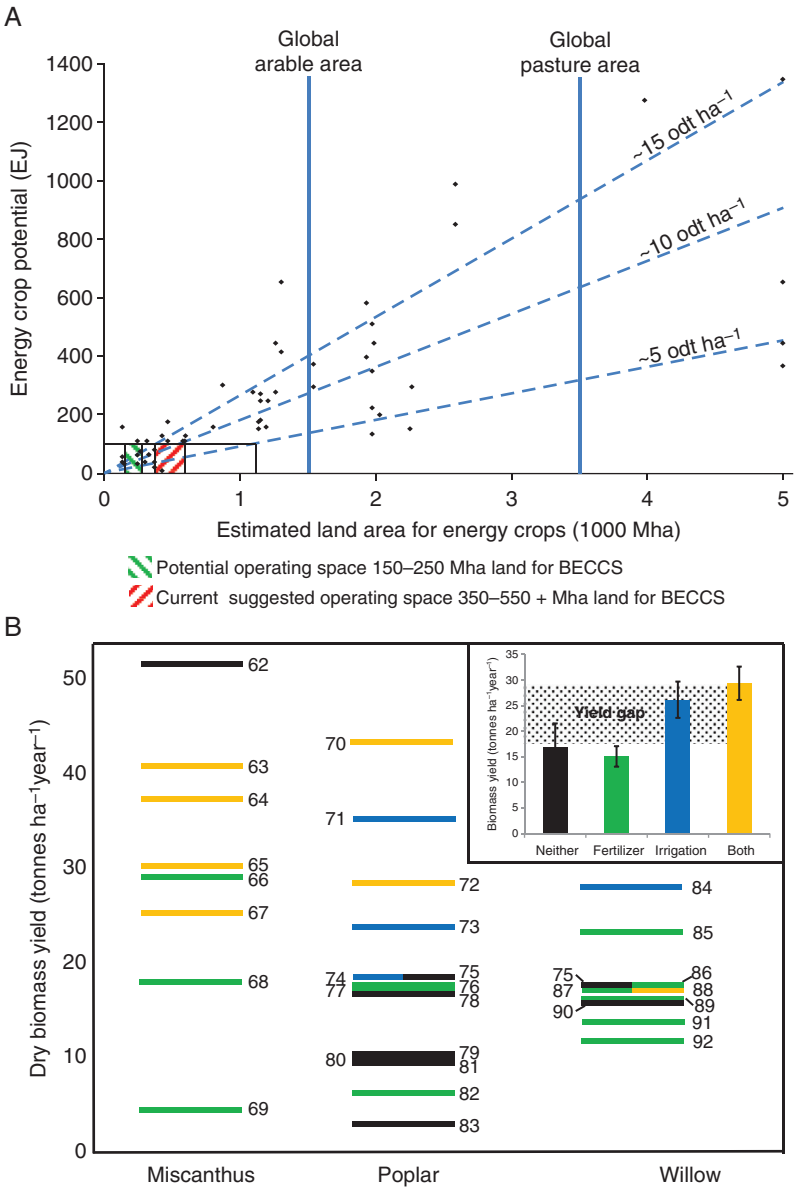

FIGURE 1. Land area and yield data for bioenergy crops. (A) Land area estimated to be required for global bioenergy crops to supply BECCS in relation to global arable and pasture area, with annual yield of 5, 10 or 15 oven-dried tons per hectare (ODT ha^{-1}). Given predictions for the bioenergy requirements for BECCS, the red hatched area represents the current land requirement to supply 100 exajoules of bioenergy, which could be significantly reduced by yield enhancements, shown as the green hatched area (redrawn from Slade *et al.*, 2014). (B) Synthesis of yield data from a survey of yield experiments with no water or fertilizer treatment (black bars), fertilization (green bars), irrigation (blue bars) or both (yellow bars) for three focal 2G lignocellulosic non-food crops, *Miscanthus*, poplar (*Populus* sp.) and willow (*Salix* sp.), where data were available. The inset illustrates a significant yield gap (grey area) that exists between yield with neither and both fertilizer and irrigation treatment. Each horizontal bar represents a single numbered study (modified from Allwright and Taylor, 2015).

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} $year^{-1}$ (Figure 1B), but this is rarely realized, because management regimes are often suboptimal, 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 5 years ago, including (1) genetic modification through gene editing, (2) plant breeding using molecular approaches and genomic selection, (3) precision agriculture and (4) agroecology as key to closing the yield gap. However, future sustainable intensification should also minimize fertilizer and water inputs, since they may add to environmental degradation and greenhouse gas emissions. In addition to greenhouse gas mitigation, a wide examination of ecosystem service 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 utilized 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 (Passioura, 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 (Oliver *et al.*, 2009; 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 and 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 in 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 vigour, 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 crops this means maximizing 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, 1992; Marron *et al.*, 2002; Tschaplinski *et al.*, 2006; Tardieu and Tuberosa, 2010; de Ollas and Dodd, 2016). Root traits are also important in maintaining biomass production during drought (Xie *et al.*, 2017; Parra-Londono *et al.*, 2018). 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.*, 2002; Sack and Holbrookman, 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 selected fast-growing genotypes originating from wetter areas of Europe (e.g. northern Italy) outperform 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 (where transpiration efficiency biomass gained per unti of water transpired*) to be best suited to drought environments across Europe. Moreover, although water use efficiency (WUE) and saccharification potential are less heritable breeding targets, genotypes that 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 overexpressed 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 hundreds 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 (genome-wide association study) traits]; and (2) measurements that are highly informative but too time-consuming for whole-population studies and may therefore be suited to genomic selection (GS) traits in training and test populations. One area that is significantly understudied in bioenergy crops with respect to drought tolerance is that of root traits (Parra-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).

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 the application of clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (CRISPR-Cas9) technology was twenty years in the making, the first reports of it appeared only a few years ago (Lander, 2016), enabling precise and efficient gene editing using an adaptive immune response of bacteria (Lander, 2016). It is one of a cadre of nuclease technologies able to make precise double-stranded DNA breaks, 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 backcross 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

TABLE 2. *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 hundreds 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)*

Traits of value for drought tolerance assessment in bioenergy crops	Relationship to drought tolerance	GWAS or GS trait	Reference*
Whole-plant biomass productivity	Non-destructive assessments of tree diameter and height as a measure of productivity, taken at 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, 6
Carbon isotope discrimination as a proxy for WUE (integrated)	Excellent proxy for integrated seasonal water use efficiency, using wood samples. Extensive evidence on heritability and usefulness in breeding.	GWAS	NA
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 thousands of measurements, daily.	GWAS	NA
Canopy and leaf infrared temperature	Hand-held IR probe and UAV-mounted camera as a proxy for stomatal opening, able to make thousands of measurements, daily.	GWAS	NA
Yield stress index	$YSI = \left(\frac{Geno_{drought}}{Pop_{drought}}\right) x \left(\frac{Geno_{control}}{Pop_{control}}\right) x \left(\frac{Pop_{drought}}{Pop_{control}}\right)$	GWAS	6
Drought resistance index	$DRI = \frac{(Geno_{drought}/Geno_{control})}{(Pop_{drought}/Pop_{control})}$ where Geno and Pheno represent genotypic and phenotypic means	GWAS	8

NA, not applicable; NDVI, normalized different vegetation index; Pop, population mean; SPAD, soil, plant analysis, development meter to measure leaf chlorophyll content D; UAV, unmanned aerial vehicle.

*References: 1, Larue *et al.*, 2019; 2, Rae *et al.*, 2004; 3, Allwright and Taylor, 2015; 4, Bertolino *et al.*, 2019; 5, Bartlett *et al.*, 2012; 6, Tschaplinski *et al.*, 2019; 7, Cochard *et al.*, 2007; 8, Fischer and Maurer, 1978. SPAD,

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 has 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 on bioenergy *Populus* appeared in 2015, spanning different species of the *Populus* genus (Fan *et al.*, 2015; Tsai and Xue, 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 (Begcy *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 so 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 (Porth *et al.*, 2013; Lopez-Alverez *et al.*, 2017) and staple food crops, such as wheat (Rasheed *et al.*, 2018), GWAS has been of limited value to breeding pipelines or marker-assisted selection (Jannink *et al.*, 2010). However, a paradigm shift has occurred in the last few years by the introduction of 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, 2012) to overcome some of the innate difficulties of undomesticated tree populations with rapid rates of linkage disequilibrium decay, halving the time of the breeding cycle. Using a sophisticated approach, Slavov *et al.* (2019) showed that it is possible to work with multiple breeding targets using GS in the absence of *a priori* knowledge, which 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.

Genomic selection relies on a high density of molecular markers, accurate phenotypic data and a training and validation population (Desta and Rodomiro, 2019). Molecular marker development in lignocellulosic crops has often been limited to relatively small panels of informative SNPs in 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 chromosomes 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 (Porth *et al.*, 2013; Evans *et al.*, 2014; Muchero *et al.*, 2019). Even for forest trees, where secondary cell wall biosynthesis is complex and different from that in many monocot crop plants (Meents *et al.*, 2018), progress is being made in wide-scale genotyping. Despite this progress, however, GBS remains costly and this may limit progress. In a novel 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, which 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 *Renewables 2018* report of the International Energy Agency 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 CO_2 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 underdeveloped, with limited breeding and selection. Their perenniality, long breeding cycles, wide and heterogeneous germplasm and untapped genomic resources have presented a bottleneck until recently. 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.

FUNDING

We thank WATBIO, www.watbio.eu (Development of improved perennial non-food biomass and bioproduct crops for water-stressed environments) which is a collaborative research project funded from the European Union's Seventh Programme for research, technological development and demonstration under grant Agreement No 311922.

ACKNOWLEDGEMENTS

We thank the reviewers of papers in this special issue, alongside those authors who contributed articles, who all gave their time and commitment to this collection of papers. Special thanks to Trude Schwarzacher, Pat Heslop-Harrison and Catherine Hyland for supporting us in all aspects of developing this special issue. Research in the laboratory of GT on defining sustainable bioenergy was supported by the MAGLUE and ADVENT projects (EPSRC EP/MO13200/1; NE/H010742/1))

LITERATURE CITED

Allwright MR, Taylor G. 2015. Molecular breeding for improved second generation bioenergy crops. *Trends in Plant Science* **21**: 43–54.

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. Overexpression of an evolutionarily conserved drought-responsive sugarcane gene enhances salinity and drought resilience. *Annals of Botany* **124**: 691–700.

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. Dynamic changes in ABA content in water-stressed Populus nigra: effects on carbon fixation and soluble carbohydrates. *Annals of Botany* **124**: 627–644.

Carlson CR, Gouker FE, Crowell CR, *et al.* 2019. Joint linkage and association mapping of complex traits in shrub willow (Salix purpurea L.). *Annals of Botany* **124**: 701–715.

Clark LV, Jin XP, Petersen KK, *et al.* 2019. Population structure of Miscanthus sacchariflorus reveals two major polyploidization events, tetraploid-mediated unidirectional introgression from diploid M. sinensis, and diversity centred around the Yellow Sea. *Annals of Botany* **124**: xxx–xxx, doi: 10.1093/aob/mcy161.

Cochard H, Casella E, 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 stopping global warming*. London: Committee on Climate Change.

da Costa RFM, Simister R, Roberts LA, *et al.* 2019. Nutrient and drought stress: implications for phenology and biomass quality in miscanthus. *Annals of Botany* **124**: 553–566.

Desta ZA, Rodomiro O. 2019. Genomic selection: genome-wide prediction in plant improvement. *Trends in Plant Science* **19**: 592–601.

Donald CM, **1968.** The breeding of crop ideotypes. *Euphytica* **17**: 385–403.

Evans LM, Slavov GT, Rodgers-Melnick E, *et al.* 2014. Population genomics of *Populus trichocarpa* identifies signatures of selection and adaptive trait associations. *Nature Genetics* **46**: 1089–1096.

Fan D, Liu T, Li C, Jiao B, Li S, Hou Y, Luo K. 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*. Shanhua: Asian Vegetable Research and Development Center, 257–270.

Foley JA, Ramankutty N, Brauman KA, *et al.* 2011. Solutions for a cultivated planet. *Nature* **478**: 337–342.

Fuss S, Canadell JP, Peter GP, *et al.* 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 HC, Garnett. T. 2014. Food security and sustainable intensification. *Philosophical Transactions of the Royal Society B: Biological Sciences.* **369**: 20120273.

Godfray HC, Beddington JR, Crute IR, *et al.* 2010. Food security: the challenge of feeding 9 billion people. *Science* **327**, 812–818.

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. The effect of summer drought on the yield of Arundo donax is reduced by the retention of photosynthetic capacity and leaf growth later in the growing season. *Annals of Botany* **124**: 567–579.

Huang LS, Flavell R, Donnison IS, *et al.* 2019. Collecting wild Miscanthus germplasm in Asia for crop improvement and conservation in Europe whilst adhering to the guidelines of the United Nations' Convention on Biological Diversity. *Annals of Botany* **124**: 591–604.

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*. Geneva: IPCC.

Jannink JL, Lorenz AJ, Iwata H. 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. Transcriptome characterization and differentially expressed genes under flooding and drought stress in the biomass grasses Phalaris arundinacea and Dactylis glomerata. *Annals of Botany* **124**: 717–730.

Lander ES, **2016.** The heroes of CRISPR. *Cell* **164**:18–28.

Larue F, Fumey D, Rouan L *et al.* 2019. Modelling tiller growth and mortality as a sink-driven process using Ecomeristem: implications for biomass sorghum ideotyping. *Annals of Botany* **124**: 675–690.

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.

Laurens LML, Chen-Glasser M, McMillan JD. 2017. A perspective on renewable bioenergy from photosynthetic algae as feedstock for biofuels and bioproducts. *Algal Research* **24**: 261–264.

Liu Z, 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, *et al.* 2002. Physiological traits of two *Populus* × *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, *et al.* 2016. Potential impacts on ecosystem services of land use transitions to second-generation bioenergy crops in GB. *Global Change Biology Bioenergy* **8**: 317–333.

Muchero W, Sondreli KL, Chen J-G, Breeanna R, *et al.* 2019. Association mapping, transcriptomics, and transient expression identify candidate genes mediating plant-pathogen interactions in a tree. *Proceedings of the National Academy of Sciences of the USA* **115**: 11573–11578.

Oliver, RJ, Finch, JW, 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, Grill E, 2019. Increased water use efficiency and water productivity of arabidopsis by abscisic acid receptors from Populus canescens. *Annals of Botany* **124**: 581–589.

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, *et al.* 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 the bioenergy water footprint by selecting SRC willow canopy phenotypes: regional scenario simulations. *Annals of Botany* **124**: 531–542.

Sack L, Holbrookman NM. 2006. Leaf hydraulics. *Annual Review of Plant Biology* **57**: 361–381.

Scaglione D, Pinosio S, Marroni F, *et al.* 2019. Single primer enrichment technology as a tool for massive genotyping: a benchmark on black poplar and maize. *Annals of Botany* **124**: 543–551.

Schaeffer SM, Nakata PA. 2016. The expanding footprint of CRISPR/Cas9 in the plant sciences. *Plant Cell Reports* **35**: 1451–1468.

Schnepf A, Leitner D, Landl M, *et al.* 2018. CRootBox: a structural-functional modelling framework for root systems. *Annals of Botany* **121**: 1033–1053.

Slade R, Bauen A, Gross R. 2014. Global bioenergy resources. *Nature Climate Change* **4**: 99–104.

Slavov GT, Davey CL, Bosch M, *et al.* 2019. Genomic index selection provides a pragmatic framework for setting and refining multi-objective breeding targets in Miscanthus. *Annals of Botany* **124**: 521–529.

Somerville C, Youngs H, Taylor C, Davis SC, Long SP. 2010. Feedstocks for lignocellulosic biofuels. *Science* **329**: 790–792.

Stavridou E. Webster RJ, Robson PRH *et al.* 2019. Novel Miscanthus genotypes selected for different drought tolerance phenotypes show enhanced tolerance across combinations of salinity and drought treatments. *Annals of Botany* **124**: 653–674.

Tardieu, F. 2012. Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. *Journal of Experimental Botany* **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–R783.

Taylor G. 2008. Bioenergy and the biorefinery concept. *Energy Policy* **36**: 4406–4409.

Tsai CJ, Xue LJ. 2015. CRISPRing into the woods. *GM Crops and 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, Abraham P, Jawdy S. 2019. The nature of the progression of drought stress drives differential metabolomic responses in Populus deltoides. *Annals of Botany* **124**: 617–626.

Valentine J, Clifton-Brown J, Hastings A, Robson P, Allison G, Smith P. 2011. Food vs. 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 K, Valdés-Rodríquez OA, Sánchez-Sánchez O, *et al.* 2019. High SNP diversity in the non-toxic indigenous Jatropha curcas germplasm widens the potential of this upcoming major biofuel crop species. *Annals of Botany* **124**: 645–652.

Viger M, Smith HK, Cohen D, *et al.* 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, *et al.* 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. *Lancet* **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 Botany* **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, Monti A. 2019. Deep root growth, ABA adjustments and root water uptake response to soil water deficit in giant reed, *Annals of Botany* **124**: 605–615.