Biomass for the Future: Miscanthus and Sorghum for New End-Uses in France

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The present special issue of *BioEnergy Research* entitled "Biomass for the Future: Miscanthus and sorghum for new end-uses in France" covers studies carried out in the framework of the French national project "Biomass for the Future (BFF)". This project was financed by the "Investissement d'Avenir" initiative managed by the French National Research Agency, covering an unusually long period from 2012 to 2020.

BFF addressed the typical "catch 22" problem hampering the development of a bioeconomy: "The lack of biomass production for lack of biomass users and the lack of biomass users for lack of biomass production", by treating both sides of the equation. To this end, BFF brought together not only researchers in agronomy, ecophysiology, plant genomics, and private plant breeders and farmers but also experts in material science, anaerobic digestion and life cycle analysis, downstream users of biomass (i.e., companies producing concrete or plastic fiber composites), and even local governments, interested in promoting biomass-based value chains on their territories.

BFF focused on two species: sorghum, which is a high-yielding annual drought-tolerant grass adapted to southern Europe, and miscanthus, which is a productive perennial grass adapted to more temperate climates, with a longer growing season and a lower demand for chemical inputs than annual species. BFF has worked on the development of new miscanthus and sorghum biomass end-uses for anaerobic digestion, materials such as polymer composites for automotive parts and construction materials. In addition, maize was used as a model crop for comparative genomics between these three plants. This special issue gathers some of the articles focused on miscanthus. In France, some 8000 ha were cultivated with this crop in 2021, and the area has been growing with 10% per year since 2015. At the moment, only a single clone of the interspecific sterile hybrid *Miscanthus × giganteus* is being grown. To diversify the varietal offer, BFF focused on the species *M. sinensis* for its high genetic diversity and its adaptability to diverse environments. The nine articles cover topics relevant for the production and utilization of miscanthus biomass, related to material science, plant genomics, cell-wall chemistry, ecophysiology, agronomy, bio-process engineering, and life cycle analysis. Given its close taxonomic proximity with maize and sorghum, miscanthus was compared to maize and/or sorghum in half of the papers. Along with these results, additional studies on sorghum and maize in the framework of the BFF project have been reported elsewhere (https://anr.fr/ProjetIA-11-BTBR-0006).

An important obstacle for biomass production is the yield instability of *M. × giganteus*. The article of Outtara et al. [1] investigated through an agronomic diagnosis the causes of this instability by analyzing the main factors limiting *M. sinensis* and *M. × giganteus* yield across several pedo-climatic conditions in France. For both species, they found that water stress and crop age were the main critical factors. *M. sinensis* yields were also affected by the sum of degree-days of the previous year of growth. This species achieved higher and more stable yields than *M. × giganteus* in shallow sandy soils or at locations with low rainfall.

Nitrogen is a major source of agricultural pollution and greenhouse gas emissions. Miscanthus is an interesting feedstock due to its low nitrogen fertilizer requirement. Leroy et al. [2] studied nitrogen recycling as a key underlying mechanism. As little is known on the subject in miscanthus species other than commonly cultivated *M. × giganteus*, they compared *M. × giganteus* and *M. sinensis* for plant biomass and nitrogen stock dynamics during 2 years. Their study

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provides the first experimental comparison between these species of nitrogen pools and endogenous and exogenous nitrogen fluxes. The nitrogen remobilized during spring and autumn differed for the two species, with *M. sinensis* showing smaller nitrogen fluxes. However, nitrogen recycling efficiency, defined as the ratio between nitrogen remobilization fluxes and the maximum above-ground nitrogen content, did not differ significantly between the two species. These new results show that *M. sinensis* is as efficient as *M.* × *giganteus* in nitrogen recycling and nitrogen use efficiency as well as other perennial species. Therefore, *M. sinensis* appeared as a relevant alternative to *M.* × *giganteus* for producing lignocellulosic biomass, provided that these results are confirmed under situations of lower soil resources.

Understanding what causes yield instability is not only of great interest for the agronomy of miscanthus but also for its breeding. Flowering-time is an important trait as it affects both adaptation and yield potential. In the first of their two companion papers, Hou et al. [3] estimated the genetic parameters — genetic variance and genetic heritability — and genetic correlations for flowering-time related traits in a biparental *M. sinensis* diploid population evaluated during its third and fourth growing year in a French location. The originality of the approach is the use of a staggered-start design, which allowed the authors to distinguish between the effects of age and of climatic conditions within the year effect. Interestingly, the genotype × climatic condition interaction turned out to be much greater than the genotype × age interaction for flowering-time-related traits. The climatic condition effect reduced the genetic parameters for all flowering-time related traits. The study also explained the large climatic condition effects by climatic indicators determined for different plant growth periods, with the most pronounced effects preceding the floral transition stage, which had not yet been studied in miscanthus.

In their second paper, Hou et al. [4] studied the same population to detect QTL for several flowering-time related traits. They detected several QTL corresponding to effects of genotype as well as genotype × age and genotype × climatic condition interactions. A subset of both types of QTL formed seven clusters. For anther appearance, the most obvious trait to represent flowering-time, the allelic effects varied with climatic conditions, which provided interesting genetic evidence explaining the instability over the growing seasons studied. Moreover, they showed that QTL effects for the genotype × climatic condition interaction were larger than for genotype × age interaction at the mature stage of the crop. By decomposing the year effect into age and climatic condition effects, the staggered-start design improved QTL detection, which helped decipher, for the first time in miscanthus, the genetic determinism that corresponds to the interaction effects of genotype with age and climatic conditions. Finally, Hou et al. [4] took advantage of the genetic

proximity of miscanthus with maize and sorghum to identify, within the QTL intervals, candidate flowering-time genes based on their sequence similarity to known flowering-time genes in maize and sorghum. They identified two candidate genes, *D8* and *HD6*, which enriched the genetic information on *M. sinensis* flowering-time and may provide a reference for future breeding programs.

In two other companion papers, Raverdy et al. [5, 6] focused on traits related to biomass production and composition, which are rarely studied in combination. Understanding the genetic correlations between biomass composition- and yield-related traits is essential for the selection of productive genotypes adapted for different end-uses. The objective of the first paper of Raverdy et al. [5] was to estimate the genetic parameters of these traits for the same population studied by Hou et al. [3, 4], grown for 4 years at two French locations. In order to comprehensively name each trait, they developed a miscanthus ontology using the GnpIS multispecies integrative information system (https://urgi.versailles.inra.fr/ephesis/ephesis/ontologyportal.do) Thirteen datasets related to phenotyping resources, based on two staggered-start designs at each location are available at https://doi.org/10.15454/1NVRNJ. Raverdy et al. [5] showed that the heritability increased with time for most of the biomass production and composition traits. They also observed that the low heritability at young plant ages reflected a low genetic variance along with large variances in genotype × age and genotype × climate interactions. Most of the production traits showed large interaction variances for age and climate at both locations, in contrast to biomass composition traits, which showed large interaction variances at one location only. The genetic and phenotypic correlations between biomass production and composition traits were moderate and positive, while hemicelluloses were negatively correlated with all the traits. The results underscore that efficient genetic gain in miscanthus breeding requires the evaluation of older plants. This significantly slows down phenotype-based selection and emphasizes the need for new approaches involving genomics and genomic selection in the future.

In the second paper of Raverdy et al. [6], aimed at understanding the genetic basis of biomass production and composition traits in *M. sinensis*, in relation to plant age and environmental conditions, by using the thirteen datasets mentioned in the previous paper [5]. They built an integrated genetic map (https://doi.org/10.15454/ET8MJE) that was aligned with the *M. sinensis* reference genome. This alignment is a novelty compared to previous genetic maps in the literature. Raverdy et al. [6] detected QTL related to biomass production and composition traits and observed that a subset of QTL was stable across different climatic conditions and ages. Based on this stability, they detected twelve QTL clusters that were established based on either biomass production or composition traits and validated by the high

genetic correlations between the traits. Raverdy et al. [6] also identified orthologous *M. sinensis* genes based on cell-wall-related gene lists developed for sorghum and maize in the framework of the BFF project (Virlouvet, Pot and Reymond, personal communication). Within the twelve QTL clusters of biomass composition traits, Raverdy et al. [6] identified 62 cell wall-related candidate genes with orthologs in sorghum and maize. Twelve of them were differentially expressed and belonged to gene families related to cell-wall biosynthesis identified in other miscanthus studies. These stable QTL can be used for marker-assisted breeding for the simultaneous improvement of biomass production and composition traits.

Regarding the end-uses, the article of Brancourt-Hulmel et al. [7] deals with a comparative study in miscanthus and maize of cell-wall composition and stem anatomy in relation to the production of bioethanol and polymer composites. The results highlight distinct features of each crop regarding stem anatomy and biochemistry and shows that maize and miscanthus stems are good models to study digestibility and mechanical properties, respectively. This study paves the way for further comparisons at the genomic level between the two crops, but also with sorghum.

In the context of the development of biorefineries, sorghum and miscanthus are relevant candidates for energy production, in particular methane production by anaerobic digestion. As for other lignocellulosic crops, pretreatments can be used to improve the accessibility of carbohydrates to microorganisms during the anaerobic digestion. In this context, the paper of Thomas et al. [8] studied the impacts of alkaline pretreatments for anaerobic digestion at cell-wall and tissue scales in sorghum and miscanthus. They used histochemical approaches to decipher the effect of pretreatments on the cellular and macromolecular architecture of biomass and on its digestibility by anaerobic microbial consortia. This in situ approach provides complementary information to the in vitro biochemical analysis. At the environmental level, Jury et al. [9] carried out a life cycle assessment to estimate the environmental impacts of two pretreatment methods, lime and soda, for sorghum and miscanthus in co-digestion with cow manure, in comparison to natural gas. For both species, they showed that the biggest environmental impacts are related to the crop production and the purification-injection step that corresponds to the production of pure methane and its grid injection. Heat produced from biogas derived from sorghum straw had a better environmental performance than heat based on miscanthus for all the impact categories. However, natural gas outperformed biogas produced from either feedstock for all the impact categories except for the impact on greenhouse gas emissions. They concluded that competition with food production should be avoided to limit the environmental impact of anaerobic digestion.

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