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Morphological and Physiological Traits that Explain Yield Response to Drought Stress in Miscanthus

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Abstract: Miscanthus is a high yielding perennial grass capable of high biomass yields with low inputs. Traits associated with yield have been identified in miscanthus, but less is known about the traits associated with sustaining biomass production under drought stress. The commercial hybrid *M. × giganteus* and high yielding examples from the parental species *M. sinensis* and *M. sacchariflorus* were grown under well-watered and moderate drought conditions. Growth, morphology, physiology and phenotypic plasticity were analyzed. Functional data were parameterized and a matrix of traits examined for associations with yield, genotype and drought treatment. Phenotypic plasticity was determined, indexes were then calculated to determine the plasticity of trait responses. All genotypes assessed responded to moderate drought stress, and genotypic differences in yield decreased under drought. Genotypes with low tolerance exhibited greater plasticity than highly drought tolerant *M. sinensis*. In well-watered plants variance in yield was explained by a relatively simple empirical model including stem length and stem number, whereas under drought a more complex model was needed including the addition of leaf area and stomatal conductance data. This knowledge can help us to define ideotypes for drought tolerance and develop miscanthus varieties that sustain high yields across a range of environmental conditions.

Keywords: bioenergy; energy crops; miscanthus; drought; water use efficiency; phenotypic plasticity; modelling; resilience

1. Introduction

To produce sustainable biomass for the bioeconomy, crops are required, which can displace carbon-intensive feedstocks while also limiting any potential negative impacts [1]. Important characteristics of energy crops include high energy output to input ratios, a low requirement for agronomic interventions such as irrigation [2], an ability to grow on underutilized marginal land to minimize pressure on food production [3,4] and increasing opportunities for rural development and diversification [5]. It is, therefore, necessary to develop energy crops which exhibit high yields and resilience to environmental stresses. Drought, in particular, may be a factor in making land marginal and more generally is one of the most limiting factors affecting crop yield worldwide [6]. For example, the production of energy crops, under future climate change scenarios, will be limited in most regions of southern Europe [7]. Thus, for bioenergy crops, there is a need to improve abiotic stress tolerance and improve understanding of drought tolerance mechanisms [8,9]. The ability of plants to not only withstand drought but also efficiently produce biomass under moderate drought is important [10].

Miscanthus is a genus of C4 perennial grasses capable of high biomass production even in temperate regions and has a number of characteristics that make it an ideal crop for the renewable supply of energy and chemicals [2,11–13]. However, the yield is strongly linked to water availability,

and many environments have limited water supply where otherwise irradiation and temperature are favourable for biomass production. Miscanthus though is largely undomesticated with considerable natural variation available for crop improvement [14–16]. Miscanthus is perennial and develops yield over a longer period than conventional annual grain crops, and as such, the importance of some trait-to-yield associations may be expected to be different. To test this, a large number of phenotypic traits associated with yield accumulation [17–20], and complex traits such as seasonal duration and harvestable yield [21–23] have been measured in diverse miscanthus populations. A number of studies have examined responses to, and genotypic variation in traits associated with, drought [24–29]. However, drought resistance in undomesticated miscanthus is the result of natural evolution and may not necessarily favour growth under stress but rather survival. Few studies have attempted to examine yield associations across a broad range of traits to help define ideotypes that sustain yield or improve yield resilience under drought. In this study we examined five high yielding and diverse miscanthus genotypes to determine how associations between yield and morphological and physiological traits changed between well-watered and drought conditions. The aim was to identify the traits that most contributed to the accumulation of higher biomass in optimal and in droughted conditions.

2. Materials and Methods

2.1. Plant Material

Five miscanthus genotypes were selected (Table S1) which were in the top 10% for biomass accumulation from a population of 47 diverse genotypes investigated in a drought stress study using phenomics [30]. The designations for genotypes have the same numerical suffix as reported in Kalinina et al. [13]: *M. × giganteus* a commercial triploid hybrid between *M. sacchariflorus* and *M. sinensis* [31] (WAT09); a triploid *M. sinensis* hybrid (Goliath) (WAT11), a diploid *M. sacchariflorus × M. sinensis* hybrid (WAT10); and two wild accessions of *M. sacchariflorus* (WAT03 and WAT04). The genotypes chosen were representative of two morphotypes, *M. sacchariflorus* types which morphologically tend to have large rhizomes and thick stems [14,32] and *M. sinensis* types which tend to be tussock forming with smaller rhizomes and are reported to have superior abiotic stress tolerance [14,25]. The wild accessions of *M. sacchariflorus* are high yielding but were reported as less resilient [13].

2.2. Experimental Setup

Plants were grown in a glasshouse (Venlo), in a 14 h photoperiod using supplemental lighting in Aberystwyth, U.K. (52.4336° N, 4.0173° W). The glasshouse was fitted with automatic vents and an extractor fan to help reduce variance attributable to environmental factors. Plants were grown in 5 L pots, with John Innes No. 2 compost, from approximately 20–30 g of rhizome cuttings.

Field or soil water-holding capacity defined as 'the amount of water held in the soil after the excess of gravitational water had drained' [33] was estimated from a pilot experiment. Pots were weighed daily for the 35 days of the experiment, to calculate water loss due to evapotranspiration, and the required amount of water added to maintain the desired water holding capacity of the soil. Target weight for each genotype was evaluated individually and was adjusted during the experiment to account for the biomass accumulation. Plants were grown under well-watered (85% field capacity) and moderate drought stress (15% field capacity). All genotypes reached target weight set for the soil moisture deficit after 8 ± 1 days after the last irrigation.

2.3. Determination of Traits

On the last day of the experiment (75 days after potting), plants were harvested to determine fresh and dry biomass. Fresh material was dried at 60 °C until constant weight was achieved at which point dry biomass was recorded.

2.3.1. Measurements of the Morphological Traits

Growth measurements were made every 2–3 days over the course of 5 weeks. The length of the primary stem was measured, using a graduated ruler, from the soil surface to the ligule of the youngest fully expanded leaf. Leaf area was calculated from measurements of leaf length (from the ligule to the leaf tip) and width (midpoint between the leaf tip and the ligule) using the equation described by Clifton-Brown & Lewandowski [34]: Area (mm^2) = 0.74 × length (mm) × width (mm).

Due to plant growth and new leaf development, leaf area was measured on a different but standard leaf throughout the experiment, the youngest leaf with a differentiated ligule, this method was previously shown to successfully detect the quantitative impact of drought stress on leaf area in *M.* × *giganteus* [26]. Additionally, the number of leaves (on the main stem) and stems were counted bi-weekly.

2.3.2. Physiological Measurements

All physiological measurements were taken between 8:00 h and 14:00 h to avoid significant potential diurnal variation in stomatal conductance (g_s) [35,36]. Conductance of leaf surfaces was measured once a week using AP4 Leaf Porometer (Delta-T Devices) from the middle, avoiding the midrib, of the youngest fully expanded leaf with the ligule.

Net CO_2 assimilation rate (A_N), stomatal conductance, and net transpiration rate (E) were measured using a calibrated portable Infrared Gas Analyzer (IRGA) equipped with an integrated fluorometer (Walz GFS-3000, Heinz Walz, Germany). Measurements were recorded, three times throughout the experiment, on five plants per genotype per treatment at approximately 2-week intervals: before, and two and four weeks after treatment began.

2.4. Data Analysis

2.4.1. Drought and Productivity-Related Indexes

A selection of indexes was calculated to describe the plant performance and responses to stress. Water use efficiency defined as g of dry biomass produced per kg of water [37,38], was calculated by measuring total water applied during the experiment and above-ground dry biomass at the end of the experiment. Gravimetric data for pots without plants were used for all treatments to adjust for evaporative water loss.

Additionally, three indexes of phenotypic plasticity (*IP*) [39], plant productivity and resilience to water deficit [40] were calculated. Index of plasticity was calculated according to Equation (1) where *trait$_{max}$* is the trait average with highest values (control) and *trait$_{min}$* the trait average with lowest values (drought condition).

$$IP = (trait_{max} - trait_{min})\ trait_{max}^{-1} \tag{1}$$

Yield stress score index (*YSSI*) was calculated according to Equation (2) and combined the stress susceptibility index (*SSI*) [41], and stress tolerance index (*STI*) defined by Fernandez [42] (Table S2).

$$YSSI = 0.5\ (STI + SSI) \tag{2}$$

Yield potential score index (*YPSI*) summarised mean productivity index (*MP*) [43], stress tolerance index (*STI*), stress susceptibility index (*SSI*), and tolerance index (*TOL*) [44] (Table S2), and is shown in Equation (3).

$$YPSI = 0.5\ (MP + STI) - 0.5\ (SSI + TOL) \tag{3}$$

2.4.2. Growth Curves Analysis

Growth and elongation data were summarised using non-parametric characteristics of curves. The main stem length and leaf area for each plant were interpolated using a univariate penalized cubic

regression spline using the R package (mgcv) [45] based on the method by Hurtado et al. [46] and adjusted to study plant growth as described by Malinowska et al. [30]. The period of analysis was six weeks, starting 32 days after the potting, one week before the drought stress was applied. The fitting process resulted in smooth curves for leaf and stem growth for every plant analyzed. The features of the fitted curves were obtained using the first derivative calculated from the smoothed curves. Mean progression rate (*mprate*), as defined by Hurtado et al. [46], indicated the average rate of change of the growth curve throughout the experiment and was the mean of the first derivative calculated for each 2–3 days interval. The mprate reflected the speed of growth or the change in size across the experiment.

2.4.3. Statistical and Graphical Analyses

Data were analyzed using Statistical Software in R version 3.2.1 [47]. Statistical significance (when appropriate) was indicated as follows:·$p \leq 0.1$, * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. Regression models were built to test which phenotypic and physiological traits contributed to variation in the dry biomass of the plants at the end of the experiment. The analysis was performed separately for the subset of plants under control and drought treatments to eliminate the substantial treatment effect at the population level. Traits that were tested included: main stem length, number of stems, number of leaves on the main stem, leaf length, leaf width, leaf area, maximal and average growth rate of stems and accumulation of leaf area, and area under the curve for stem growth, leaf area, and stomatal conductance. General linear models were obtained using automated model selection and dredge function from the MuMIn package [48] and the best model selected based on the Aikake information criterion value and delta <6 [49]. All time course, longitudinal studies were analyzed with linear mixed-effects models using the lme4 [50] package. The overall time course of various measurements was modelled with an orthogonal polynomial and fixed effect of treatment (control vs. drought; within genotypes) on all time terms. The models also included the individual plant random effect on all time terms. *p*-values and the best models were obtained by likelihood-ratio test [51] for the general effect and interactions against the null model (without the effect). Outliers, which exceeded 2.5 standard deviations, were removed. For the single time point analyses (e.g., biomass, WUE, and other end-of-experiment measurements) single stratum analysis of variance was performed using built-in R functions.

3. Results

3.1. Biomass Accumulation

Dry biomass varied 45–84 g for summed leaves and stems and 38–61 g for roots and rhizomes. WAT03 and WAT09 accumulated the highest dry biomass above- and below-ground under both treatments (Figure 1). Under well-watered conditions, there was significant variation in above-ground biomass accumulation between genotypes, whilst under moderate drought biomass accumulations were relatively similar. Drought had the most severe effect on WAT03 with a decrease of leaf and stem biomass of up to 35% in comparison to control plants. When compared to control plants leaf biomass of WAT04 was significantly lower under drought stress (Table 1). Drought stress significantly lowered leaf and stem yield (30–35%) of *M. × giganteus* but not dry biomass of roots and rhizome. Biomass of WAT10 and WAT11 genotypes was not significantly affected by drought stress.
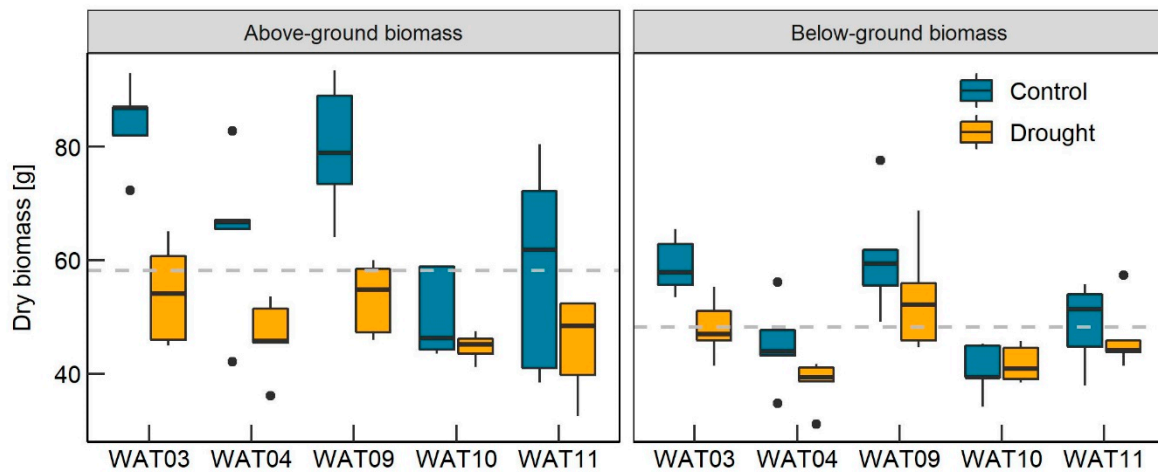
Figure 1. Boxplot of above and below-ground dry matter under control and drought stress treatments (*n* = 5). Horizontal, dashed, line is showing the populations average; outliers (which exceeded 2.5 standard deviations) are plotted as individual points.

Table 1. Effect of drought stress treatment on biomass accumulation, above and below-ground, separated into leaves, stems, rhizomes, and roots, compared to control; *p*-value indicates the statistical significance for a given term within the model. Significance of differences are shown as * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns = not significant.

	Rhizome (g)	Root (g)	Stem (g)	Leaf (g)	Total Biomass (g)
WAT03	**	*	***	***	***
WAT04	ns	ns	ns	*	ns
WAT09	ns	ns	**	**	*
WAT10	ns	ns	ns	ns	ns
WAT11	ns	ns	ns	ns	ns

3.2. Miscanthus Morphology

3.2.1. Length of the Main Stem

Stem elongation curves were highly variable (*p* < 0.001) between genotypes. The main stem of WAT09 was significantly longer than the other four genotypes. In WAT04 stem elongation declined rapidly, in comparison to control plants, after seven days of drought treatment (*p* < 0.01). After 22 days a moderate response to drought treatment resulted in stem length under drought being significantly lower in WAT09 than in control plants. WAT11 plants showed no significant impact on the main stem length under drought stress (Figure 2A).
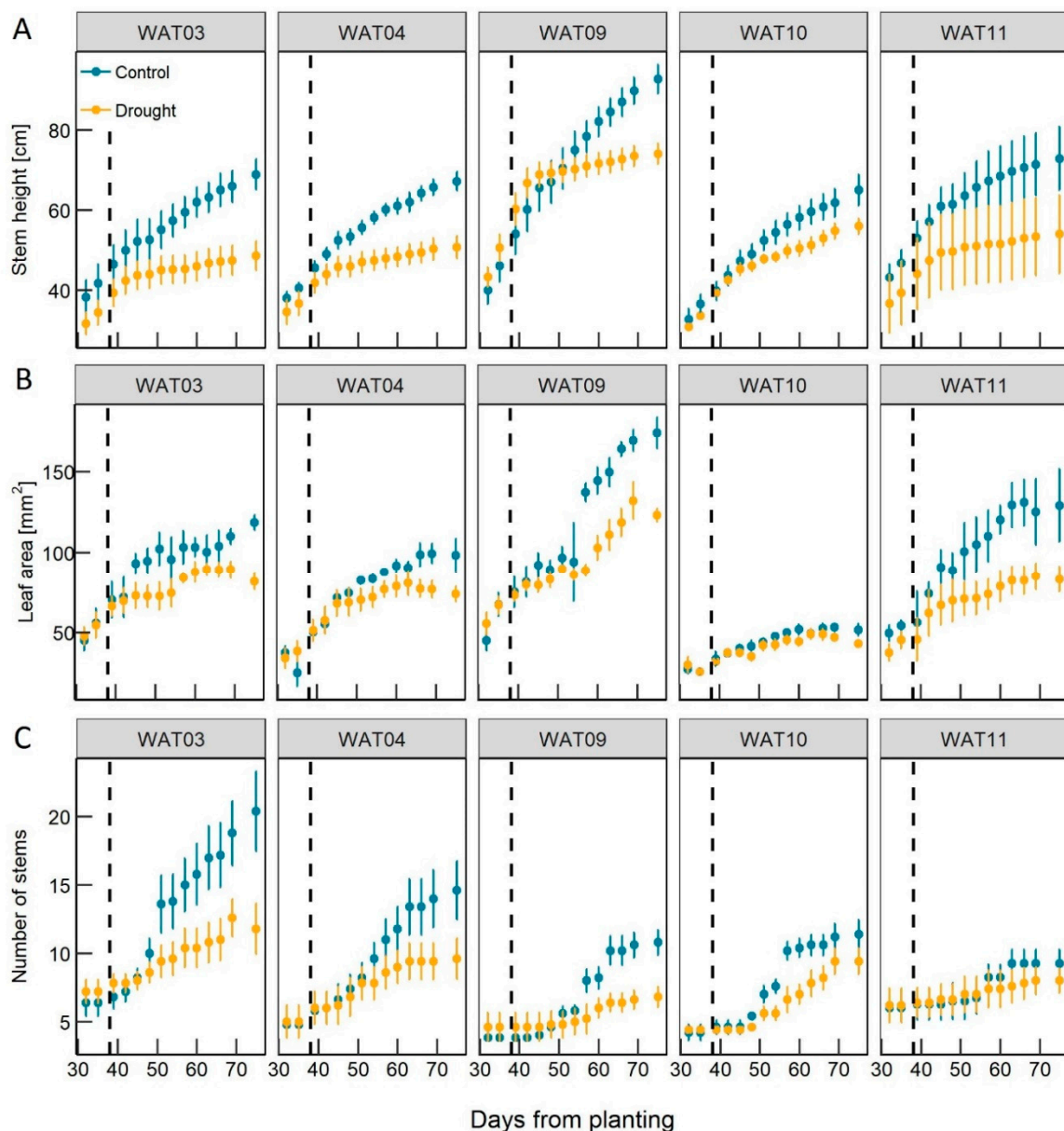
Figure 2. Above-ground morphology characteristics. The main stem elongation (**A**), youngest fully expanded leaf area development (**B**), and increase in stem number (**C**) curves for control and drought treatments of five genotypes. The dashed vertical line indicates the onset of drought stress. Values represent means ± SEM ($n = 5$); SEM—standard error of the mean.

3.2.2. Leaf Area

At the population level, drought stress negatively affected the area of newly formed leaves ($p < 0.05$). The area of the youngest fully expanded leaves in WAT03 plants was significantly lower in drought-stressed than control treated plants after only seven days ($p < 0.1$). Leaf area was small and very similar between treatments in WAT10, but differences were observed 37 days after the start of drought stress. The leaf areas of the other three genotypes diverged between the treatments between 22 and 28 days after the drought was applied (Figure 2B). By the end of the experiment leaf area of WAT09 was significantly larger ($p < 0.001$) than the other four genotypes under both treatments (Figure 2B).

3.2.3. Number of Stems

WAT3 and WAT4 produced the most stems, whilst, in other genotypes stem numbers were comparable. Under moderate drought stress, stem number was lower in all genotypes except for WAT11 which was not significantly affected by treatment. A significant reduction in stem number was observed for WAT10 at 16 days of the treatment ($p < 0.01$). Stem number in WAT03, WAT09 and WAT04 was reduced in response to drought after between 25 and 31 days (Figure 2C).

3.2.4. Growth Curves Analysis

The *mprate* identified differences between slow and fast growing plants. Variation in *mprate* could be explained by both treatment and genotype. Changes in leaf and stem growth and final sizes differed among genotypes in response to drought. Drought stress resulted in a lower stem *mprate* for all genotypes. WAT09 plants showed a considerably higher *mprate* for stem elongation and leaf expansion when compared to plants of other genotypes (Figure 3). Leaf area and leaf *mprate* were the lowest in WAT10 plants, with drought having the smallest effect on leaf expansion in this genotype (Table 2).
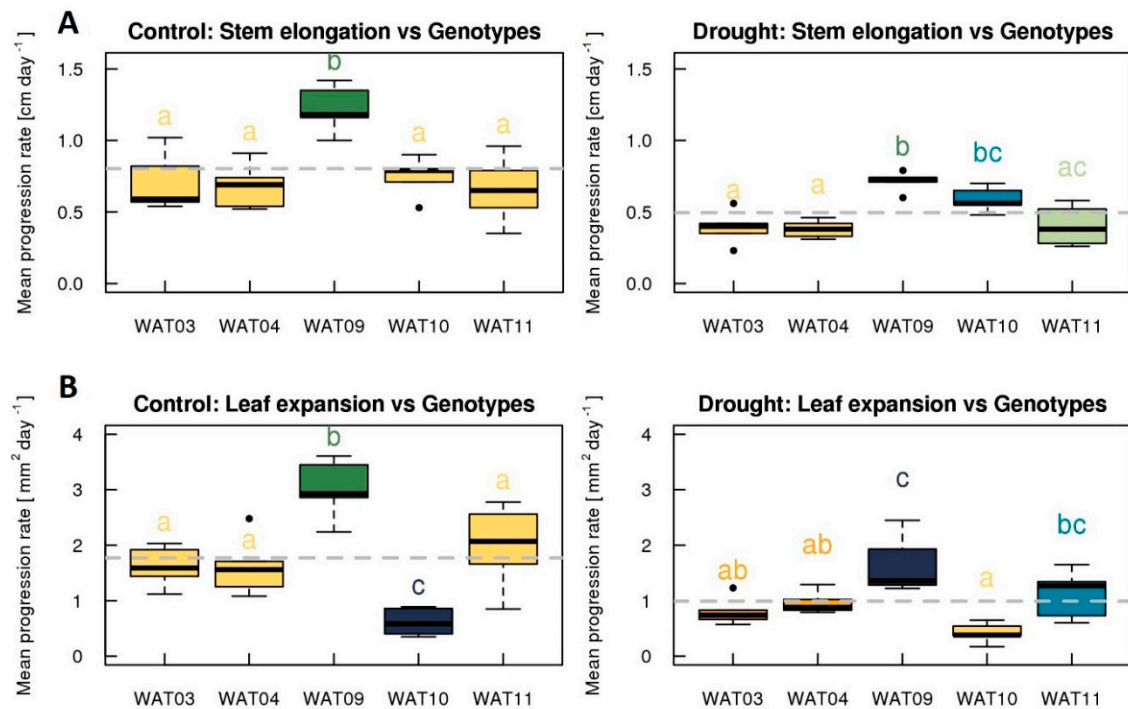
Figure 3. Mean stem elongation rate (**A**) and leaf expansion rate (**B**) of five miscanthus genotypes with varying dry biomass under control (left) and drought stress (right) treatments; significant differences between genotypes are based on Tukey multiple comparison test and denoted by lettering above the boxes (conf. level = 0.95); each statistical group is drawn with the same colour; grey horizontal, dashed, line is showing the populations average; outliers (which exceeded 2.5 standard deviations) are plotted as individual points.

The final morphology and curve characteristics of morphological traits were assessed by PCA. Under each treatment, WAT10 was grouping with two *M. sinensis* types (WAT03 and WAT04) while WAT09 and WAT11 were clustering together (Figure S1).

Table 2. Analysis of variance on effects of applied treatment on growth curve characteristics and phenotypic traits at harvest of drought-stressed miscanthus genotypes compared to controls; mprate—the mean progression rate of the growth curve; SE—measure of the uncertainty in the coefficient; *p*-value indicates the statistical significance for a given term within the model. Significance of differences are shown as $p \leq 0.1$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns = not significant.

| | The length of the Main Stem (cm) | | | |
| | *Mprate* (cm d^{-1}) | | Stem Length at Harvest (cm) | |
	Coefficient ± SE	*p*	Coefficient ± SE	*p*
WAT03	−0.32 ± 0.11	*	−20.40 ± 5.29	**
WAT04	−0.30 ± 0.08	**	−16.80 ± 3.81	**
WAT09	−0.51 ± 0.08	***	−18.60 ± 4.32	**
WAT10	−0.16 ± 0.07	*	−9.60 ± 4.51	.
WAT11	−0.26 ± 0.12	.	−12.80 ± 13.24	ns
	Leaf Area (mm^2)			
	Mprate (mm d^{-1})		Leaf Area at Harvest (mm^2)	
	Coefficient ± SE	*p*	Coefficient ± SE	*p*
WAT03	−0.82 ± 0.20	**	−36.52 ± 6.81	***
WAT04	−0.66 ± 0.26	*	−24.36 ± 11.12	.
WAT09	−1.37 ± 0.34	**	−50.80 ± 10.26	**
WAT10	−0.20 ± 0.14	ns	−8.52 ± 4.39	.
WAT11	−0.86 ± 0.39	.	−36.34 ± 21.28	ns

3.3. Physiological Traits

3.3.1. Stomatal Conductance

At the whole population level, there was a significant effect of drought stress on g_s ($p < 0.001$). WAT09 plants exhibited the highest values of g_s and stomata responded most rapidly to drought in this genotype (Figure 4) with a significant decline in g_s after ten days ($p < 0.001$). Stomatal conductance significantly decreased in the two *M. sacchariflorus* genotypes after 13 days of drought ($p < 0.01$). In WAT10 and WAT11 plants, a significant reduction in stomatal conductance occurred a week later ($p < 0.05$).
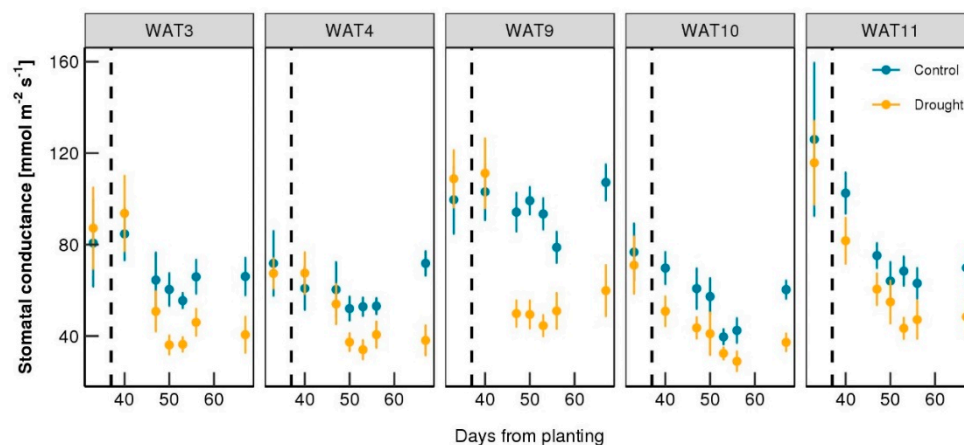
Figure 4. Stomatal conductance (g_s). The change in stomatal conductance of 5 miscanthus genotypes growing for 75 days, drought stress was applied to half the plants at day 37 indicated by the vertical dashed line. Values represent means ± SEM ($n = 5$); SEM—standard error of the mean.

3.3.2. Photosynthetic Gas Exchange

Net photosynthesis (A_N) of WAT09 was significantly higher than for plants of other genotypes ($p < 0.001$) except for WAT04, which exhibited an almost identical pattern of net CO_2

assimilation rates. The values of E, as well as A_N in well-watered plants, were lowest in WAT11 (1.2 ± 0.06 mmol H_2O m^{-1} s^{-1} and 12.54 ± 1.24 µmol m^{-2} s^{-1} respectively) and highest in WAT09 (2.1 ± 0.1 mmol H_2O m^{-1} s^{-1} and 24.04 ± 0.68 µmol m^{-2} s^{-1}). Drought stress significantly affected A_N and E of three genotypes WAT04, WAT09, and WAT11 (Table S3). The applied drought stress was insufficient to affect A_N or E of WAT03 and WAT10 at any time point (Table S3).

3.4. Water Use Efficiency

The average shoot WUE in well-watered plants varied from as low as 6.7–10.1 g kg^{-1} in WAT10 and WAT04 plants, and under drought stress varied from as high as 12.6–17.3 g kg^{-1} in WAT03 and WAT11, respectively (Table S4). The drought treatment resulted in a statistically significant increase of WUE for all five genotypes (Table S4). WUE more than doubled in *M. sinensis* plants grown under drought stress compared to controls. WAT03 and WAT09 plants exhibited the smallest changes in WUE between treatments, whilst WAT04 plants exhibited an intermediate response.

3.5. Drought and Productivity-Related Indexes

Phenotypic plasticity describes the dynamic change in morphology or physiology of a single genotype (or at the population level) in response to the environment [52,53]. The index of phenotypic plasticity (Figure 5) revealed that below-ground dry biomass was the least responsive (plasticity) trait. Response to drought stress in WAT10 and WAT11 could be most readily observed through the changes in stomatal conductance and area of the youngest fully expanded leaf. For WAT09 and the *M. sacchariflorus* genotypes above-ground dry biomass was the most plastic trait, followed by the length of the main stem. WAT09 and WAT03, exhibited the greatest summed plasticity, whilst WAT10 with the most fixed traits, was at the opposite end of the gradient (Figure 5).
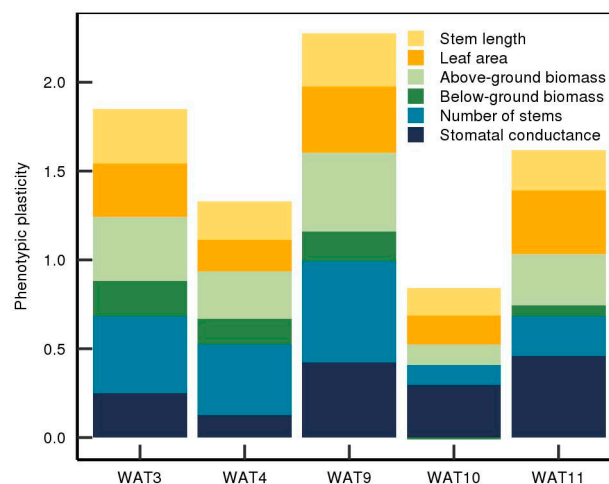
Figure 5. Index of phenotypic plasticity for six phenotypic traits for five genotypes: the length of the main stem, youngest fully expanded leaf area, above-ground biomass, and below-ground dry biomass at the end of the experiment, number of stems, and stomatal conductance.

Yield, stress and potential, score indexes that summarised drought tolerance, susceptibility, and productivity were plotted against above-ground dry biomass under control (Figure 6A) and drought (Figure 6B) conditions. Plants of WAT03 and WAT09, which exhibited the highest YSSI also exhibited the highest biomass accumulation under both drought and control treatments. Plants of WAT10, which exhibited lower yield and YSSI, also exhibited the highest YPSI of all genotypes. YSSI correlated well with the yield under drought, while YPSI favoured genotypes with the lowest yield penalty but not necessarily the highest biomass accumulation.
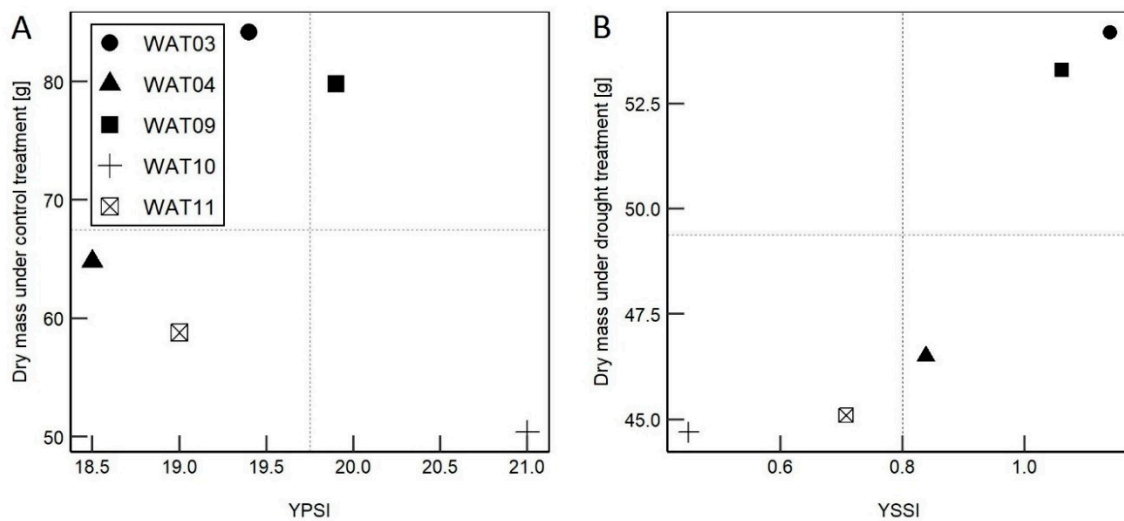
Figure 6. The values of the yield potential score index (YPSI) versus above-ground biomass under control conditions (**A**), and the yield score stress index (YSSI) versus above-ground dry biomass under drought stress (**B**) for each genotype.

3.6. Linear Regression Models

The variation in the above-ground dry biomass of the control plants was explained by the length of the main stem and the number of stems with ~85% variation explained by the model (Table 3). The dry biomass of the aerial parts of the plants under drought was explained by the length of the main stem, the number of stems, as well as the youngest leaf area ($p < 0.01$) and area under the stomatal conductance curve, with ~70% of variation explained by the model. In comparison to the control plants, the dry biomass of the plants under drought was explained by a higher number of variables, and less variation was described (Table 3). Additionally, the effect of stem length on the dry biomass was less significant under drought than under control conditions, while the number of stems as a measure was highly significant under both treatments. The variation in dry biomass within the population under moderate drought decreased compared to variation under control conditions.

Table 3. The variables explaining the above-ground biomass accumulation under well-watered and drought conditions in the tested population; SE—measure of the uncertainty in the Coefficient; t value is derived from the t-statistic and p-value indicates the statistical significance for a given term within the model. Significance of differences are shown as $p \leq 0.1$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

	Coefficient ± SE	*t*-Val	*p*
	Control		
Intercept	−36.19 ± 10.36	−3.49	**
Stem length	1.06 ± 0.12	8.68	***
Number of stems	2.06 ± 0.32	6.52	***
	Drought Treatment		
Intercept	12.67 ± 5.78	2.19	*
Stem length	0.20 ± 0.09	2.17	*
Number of stems	0.47 ± 0.09	4.96	***
Leaf area	0.65 ± 0.09	1.83	.
Stomatal conductance curve	0.17 ± 0.08	2.28	*

4. Discussion

A broad range of traits associated with biomass accumulation were compared across five miscanthus genotypes under well-watered and drought treatments. The comparison included the industry-standard *M. × giganteus* (WAT09), so results would be transferable to commercial

production [13,15,22]. It was demonstrated that despite a rapid decline in yield under drought, the superior ideotype was high yielding under well-watered conditions and plastic under drought. We conclude, based on the genotypes studied, that resilience *per se* is unlikely to produce a superior yield in an environment with changing water availability because of the associated yield penalty in the absence of stress.

The genotypes studied included two broadly different morphotypes so that the impact of contrasting traits could be assessed. All genotypes grown in our study reached the target weight at approximately the same time (±1 day), so uniform water limitation within the population could be assumed. A moderate soil moisture deficit was chosen (15% FC) to generate physiologically relevant responses to biomass accumulation under stress, i.e., we were focused on traits involved in sustaining growth not escape from terminal drought. In three genotypes (WAT03, WAT04 and WAT09), the applied moderate drought treatment resulted in a reduction in dry matter, compared with cloned plants growing in control treatments. Growth inhibition by drought may result from turgor loss, stomatal closure and molecular rearrangements which lead to disturbance in photosynthesis, nutrient and carbohydrate metabolism, or ion uptake [54–56]. A broad range of these traits was assessed, and it was demonstrated that the five genotypes responded differently to the application of moderate drought treatment.

4.1. High Yielding Miscanthus Genotypes Demonstrated a Diversity of Responses to Drought

Plants of WAT09 produced the highest above-ground biomass and were characterized as having long stems, large standard leaf area and high stem and leaf growth rates. WAT03 and WAT04 accumulated intermediate levels of above-ground biomass in the well-watered treatment. WAT10 accumulated lower amounts of above-ground biomass, showed the smallest area of the uppermost leaf with a ligule and the lowest leaf growth rate when compared with other genotypes. WAT11 accumulated lower amounts of above-ground biomass and produced a low number of stems, but was characterised by leaf area and growth rates.

Not all genotypes showed gross differences in morphological traits between the two treatments. However, for all plants trajectories of stem growth, leaf expansion, and tillering (stem number) curves were significantly affected by the applied treatment. Several studies, both in greenhouse and field conditions, reported that stem elongation and leaf area were significantly affected in *M. × giganteus* and *M. sacchariflorus* but not in *M. sinensis* [17,25–27,29,34,57]. In the present study, the morphological trait changes under applied drought conditions were in line with previous findings for pots experiments [25,28,29,34,58].

Yield reduction is the most important effect of limited water availability on biomass crops. Multiple studies, conducted in the field, and glasshouse, are showing the adverse effect of the poor water supply on the miscanthus biomass accumulation [11,26,28,29,34,58]. In the present study, applied treatment resulted in a reduction in dry matter only in *M. × giganteus* and *M. sacchariflorus*. The decrease in dry matter was mostly due to lowered leaf weight, with very little to no change in below-ground biomass.

The correlation between above and below-ground biomass declined under drought from an adjusted R^2 of 0.80 for well-watered plants to 0.44 (data not shown) for drought treated plants, indicating the simple relationship between above- and below-ground biomass in control plants was more complex under drought stress. In our study, except for WAT11, plants responded to drought by increasing the flux of biomass underground. Increased below-ground biomass may reflect increased scavenging for water or an escape from drought to vegetative rhizome production. However, such strategy may not necessarily be beneficial for the final yield, as according to Poorter et al. [59] under mild to moderate drought stress changes in biomass allocation may results in suboptimal growth after re-watering.

In contrast to some previous studies [25] and in agreement with others [26] we showed the commercial type *M. × giganteus* (WAT09) had high water use, low water use efficiency and high stomatal conductance in control treatments but that stomata were responsive to drought. Considering stomatal responses and growth kinetics, we would classify WAT09 as an optimistic plant that grows as

if water will be forever plentiful. This is similar to the accessions from North Taiwan discussed by Weng [24]; "optimistic" accessions from the north, where summer rainfall was plentiful, had higher photosynthetic rates even under drought compared to accessions from the south where seasonal drought was common.

4.2. Changes in Photophysiology Occurred Late and Did Not Have a Significant Impact on Biomass Accumulation

The predicted consequence of lowered stomatal conductance, and therefore limited CO_2 diffusion and decreased transpiration, would be impaired photosynthesis [60]. In our study, even though all genotypes exhibited a reduction in stomatal conductance, a significant decrease in A_N was restricted to three genotypes. In maize and miscanthus under moderate drought stress, only minor fluctuations in net carbon assimilation have previously been reported [25,29,61]. Changes to A_N in drought-stressed plants depend not only on stomatal closure but also on metabolic limitation [62–64].

Even though stomatal conductance was lower under drought stress in all genotypes, and net carbon assimilation rate was lower in three genotypes, only *M. sacchariflorus* and *M. × giganteus* exhibited significant morphological responses to drought. The assimilation rate of *M. sacchariflorus* genotype, WAT03, was not affected, even though biomass accumulation and growth rate were significantly decreased under drought. For all genotypes, the reduction of stomatal aperture did not change linearly and nor were stomata completely closed. Despite fluctuations in the g_s values, once conductance was significantly decreased, it maintained a similar level, which may indicate the acclimation to new homeostasis under lower water availability. Thus, growth alteration under moderate stress is not the consequence of reduced photosynthetic rate and resource limitation but an adaptive response [55,65–67].

4.3. High Tolerance to Drought Was Associated with Low Plasticity in Phenotypic Traits and Low Overall Biomass Accumulation

Independent of final yield, drought stress resulted in a decrease in main stem length, leaf area, and stem number to differing extents in all genotypes. However, within the time frame of the experiment, all plants exposed to moderate drought were able to maintain some level of growth. *M. × giganteus* was most sensitive to drought and exhibited the highest values of phenotypic plasticity for most of the analyzed morphological and physiological traits. With the most fixed traits, WAT10 was the most drought-tolerant genotype. Morphological traits in WAT10 were moderately impacted by drought stress, yield penalty was insignificant, and it was considerably smaller than the other genotypes under control conditions. This was in agreement with the study of Clifton-Brown and Lewandowski [34] in which moderate drought reduced yield, relative to controls, of *M. sacchariflorus* and *M. × giganteus* but not *M. sinensis*. However, even with low yield accumulation and low phenotypic plasticity WAT10 exhibited relatively high mean stem elongation rate (under both treatments), and a rapid decrease of stomatal conductance under drought stress. The plasticity rankings indicated that the two genotypes (WAT03 and WAT09) most responsive to drought also had the highest yield under optimal conditions, while the least plastic and most tolerant genotype produced the smallest yield. In analyzed genotypes, drought tolerance was associated with reduced phenotypic plasticity and a water-conserving strategy. While conservative use of water may promote drought-resilience, under favourable conditions when resources are abundant, the benefit of plant survival may be out-weighed by the inferior growth performance and meagre biomass gain [68–70]. Therefore if a low level of plasticity is associated with a high level of drought tolerance but low biomass accumulation, it is unlikely that selecting for fixed traits will maximize biomass accumulation throughout a long growing season.

Biomass values were plotted against yield score stress index and yield potential score index, proposed by Thiry et al. [40] and captured two major characteristics. The results confirmed the conclusions above. Under a given stress level, WAT10 was most tolerant but accumulated the lowest quantities of biomass while WAT03 and WAT09 were high performing genotypes despite the high yield penalty. This suggests that high resilience will not necessarily result in the best performance integrated

across a long season, including periods of varying water availability. Plants, with conserved water use, could be the right choice for arid regions but would not produce suitable amounts of biomass under temperate climates [71,72].

Drought indexes indicated a significant drought-related decline in biomass accumulation. However, absolute differences in biomass between genotypes decreased under soil moisture deficit, suggesting there was no intrinsic penalty associated with high biomass in well-watered genotypes giving rise to low biomass under drought. *M. sacchariflorus* species, such as WAT03, were identified as good candidates for maintaining biomass accumulation under drought similar to that of *M. × giganteus*. The production of higher amounts of biomass; however, required more water. Genotypes WAT03 and WAT09, in particular, used more water under both treatments, which under field conditions may deplete soil water and exacerbate drought stress or have an adverse effect on local soil hydrology [73].

4.4. A More Complex Linear Model Was Required to Explain the Yield from Drought-Stressed Miscanthus

Stem and leaf measurements over time were used to produce a functional description of any changes occurring. We used these functional traits along with accumulated biomass, morphological and photophysiological traits that had been associated with responses to drought stress in plants [25,55,74–76] to identify the most parsimonious model for biomass accumulation under both conditions. Yield under well-watered treatment was best explained by stem traits, following the previous studies which identified these traits as major correlates with biomass accumulation in miscanthus in the field and controlled environment conditions [22,77]. However, a more complex model was required to explain variation in the biomass accumulation under drought stress, and this model explained less of the variation, i.e., we did not add more variables to explain more variance. Stem length and stem number remained in the model but explained less of the variation and the most parsimonious model included leaf area and stomatal conductance, suggesting that selection for biomass accumulation resilience, i.e., superior yield across a range of water availabilities in miscanthus should include these additional traits.

5. Conclusions

The effect of drought stress at the physiological level was evident for all five genotypes and different morphotypes and allowed us to examine different combinations of responses to drought. Stomatal conductance was reduced in response to water deficit. However, assimilation capacity was not significantly affected; plants did not senesce, reduced physiological activity and wilting were limited. The lower final yield of *M. sinensis* morphotypes under both conditions was mainly due to the low mean leaf development rate, small plant leaf area (WAT10) and very few stems (WAT11). Genotypic diversity in accumulated biomass yield declined under drought stress, but sufficient variation was present across a broad range of traits studied to test associations with yield. The traits that most contributed to the accumulation of higher biomass in optimal and in droughted conditions were stem number and canopy height. Selection for superior performance *per se* under more optimal conditions appears to be a viable strategy and perhaps a most practical one given the incredible diversity of severity and duration of drought stress possible in the natural environment. Therefore given the importance of yield and the small number of additional traits identified, a selection strategy such as Genotype by Yield × Trait would seem most appropriate, focusing on improving stem and leaf growth and stomatal traits in combination with yield.

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