

Soil water availability on biomass yield and water indicators of diverse warm-season perennial grasses in dryness conditions

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ARTICLE INFO

Keywords:

WUE
Water footprint
Perennial energy crops
Lignocellulosic
Marginal land
Mediterranean

ABSTRACT

Dryness triggers soil degradation processes and land abandonment in the south Mediterranean environment. Becoming unsuitable for food crops, it can be usable by low-iLUC risk biomass crops able to withstand prolonged dry periods due to drought-resilience traits and high biomass yield. The present experiment ascertained the biomass dry matter yield (DMY), water use efficiency (WUE) and water footprint (WF) by modulating soil water availability of six warm-season perennial grasses in the semiarid Mediterranean for two consecutive growing seasons. Two ecotypes of giant reed (*Arundo donax* L., ARCT and ARMO, respectively), one ecotype of African fodder cane (*Saccharum spontaneum* L. subsp. *aegyptiacum* (Willd.) Hack., SAC) and three hybrids of *Miscanthus* (the commercial *M. × giganteus* J.M. Greef, Deuter ex Hodk., Renvoize - M×G, and two new seed-based hybrids, GNT9 and GNT10, respectively) were subjected to 100%, 50% and 0% of maximum crop evapotranspiration (ET_m) restoration supplied during summer months. Crop water use (CWU) differed among crops, growing seasons and irrigation regimes, and significantly influenced DMY, WUE and WF. ARCT, ARMO and SAC had higher DMY and WUE, and lower WF than *Miscanthus* hybrids. Among *Miscanthus*, the new seed-based hybrids outperformed the M×G in all examined parameters. Genotypic response in DMY, WUE and WF by varying the CWU had different trends and rates of change. The relationship between WUE and WF combining data of all genotypes, growing seasons and irrigation regimes followed an exponential decay; it underlined the importance to select for crops with high resource use efficiencies to reduce the water required per ton of biomass.

1. Introduction

The European Union Directive EU (2018/2001) targets to reach 3.5% for advanced biofuels in the transport sector to progressively reducing the greenhouse gases emissions (GHG) at 50% by 2030 and set the EU free from net GHG emissions in 2050 (The European Green Deal, 2019), implying that significant agricultural areas have to be dedicated to the production of feedstock for a transition to a bio-based economy. However, the Directive EU (2015/1513) emphasized the need to promote low-iLUC risks feedstock by using lignocellulosic species on lower grade agricultural lands unsuitable for food production. In the Mediterranean, substantial agricultural areas are already becoming marginal and often abandoned by farmers since most food crops do not reach a marginal productivity due to increasing dryness (Panoutsou and Chiaramonti, 2020). In addition, more frequent extreme weather events due to climate change, like untypical long-dry and wildfires in summer, and heavy rainfall and flooding in autumn, are posing serious threats to

agricultural systems. In south Mediterranean, the long-term imbalance between available water resources and demands trigger negative effects on soil structure, soil physiological functions and soil moisture levels (BIO Intelligence Service, 2014). Under increasingly water limiting conditions, crops with drought-resilient mechanisms and high water use efficiency are necessary. Combined with drought-resilience strategies to conserve water in agricultural or industrial processes, suitable cultivation of industrial crops on dry areas can provide an economic, environmental and social benefits (Iglesias and Garrote, 2015). An indication of the sustainable freshwater resource used for anthropogenic purposes, such as the water footprint, can be used to quantify the virtual content of water, with larger volume per ton of product indicating lower efficiency (Lovarelli et al., 2016).

Among low-iLUC risk industrial crops, the warm-season, rhizomatous, perennial, lignocellulosic, bioenergy grasses are known to be affected by the pattern of rainfall in the Mediterranean area (Alexopoulou et al., 2015; Zanetti et al., 2019); however, these crops combine

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<https://doi.org/10.1016/j.indcrop.2022.114744>

Received 14 December 2021; Received in revised form 10 February 2022; Accepted 25 February 2022

Available online 8 March 2022

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high biomass yield level and water use efficiency, along with traits of resistance to multiple biotic and abiotic stresses (Scordia and Cosentino, 2019). *Miscanthus* (*Miscanthus* × *giganteus* J.M. Greef, Deuter ex Hodk., Renvoize) is the leading bioenergy crop, that has been investigated across almost all European climatic conditions due mainly to its wide geographical adaptability and the high biomass yield (Lewandowski et al., 2003). Although its C4 photosynthetic metabolism, it is too sensitive to drought for reliable yields in the south Mediterranean regions owing a minimal stomata regulation (Scordia et al., 2020a). Hence, large efforts have been placed to screen novel germplasm within miscanthus species to breed new competitive hybrids in the last decades (Clifton-Brown et al., 2019a). Recently, it has been reported a five-time higher yield of the new miscanthus hybrid GNT10 as compared with the commercial *M. × giganteus* under severe summer drought conditions in Poland (Clifton-Brown et al., 2019b).

Besides, wild plants originated or naturalized to dry prone areas possess a number of traits conferring adaptability to drought stress (Scordia et al., 2017). In such conditions, the undomesticated giant reed (*Arundo donax* L.) and African fodder cane [*Saccharum spontaneum* L. subsp. *aegyptiacum* (Willd.) Hack.], both naturalized in the Mediterranean basin, have demonstrated good performances under low soil moisture levels and other limiting growing conditions (Cosentino et al., 2014, 2015). African fodder cane is a C4 plant with high biomass yield and highly efficient in the use of natural resources (Scordia et al., 2015). Although a C3 photosynthetic pathway plant, giant reed is considered similar or superior to several C4 counterparts (Zegada-Lizarazu et al., 2010). The present experiment evaluated the response in biomass yield, water use efficiency and water footprint of six perennial grasses by modulating soil moisture levels for two subsequent growing seasons in a semiarid Mediterranean area.

2. Material and methods

2.1. Field trial set-up

The field trial was carried out at the Experimental Farm of the University of Catania (10 m a.s.l., 37°24' N, 15°03' E) in a soil with the following conditions: 11.8% silt, 55.9% sand, 32.3% clay, 1.4% organic matter, 1.4 g kg⁻¹ total N, 46.1 mg kg⁻¹ available P, 293.3 mg kg⁻¹ exchangeable K, and pH 7.6. The bulk density was 1.1 g cm⁻³. The soil moisture contents at field capacity (at -0.03 MPa) and nominal wilting point (at -1.5 MPa) were 27 and 11 g H₂O 100 g⁻¹ dry weight respectively.

In a split-plot design, two experimental factors were investigated: the irrigation levels and the perennial grass species. The former was the whole-plot factor and the latter the sub-plot experimental units, which were randomized and replicated three-times within the whole-plot. A single whole-plot measured 288 m² (24 × 12 m), while a single sub-plot 16 m² (4 × 4 m).

In the first growing season, the irrigation was the same in all plots to sustain rhizome establishment. Plantlets were kept well-watered through a drip irrigation system, from the establishment to the end of summertime, by restoring 100% of maximum crop evapotranspiration (ET_m). From the second growing season the drip irrigation was differentiated in three levels: 100% (I100), 50% (I50) and 0% (I0) of ET_m restoration. It was scheduled during the summer months (June-August), when the sum of daily ET_m, subtracting rainfall events, corresponded to the volume. The daily ET_m was calculated according to:

$$ET_m = E_0 \times K_p \times K_c$$

where ET_m is the maximum daily evapotranspiration (mm); E₀ is the evaporation of class-A pan (mm); K_p is the pan coefficient, equal to 0.80 in semi-arid environment. Crop coefficients (K_c) were those applied for *M. × giganteus*, *A. donax* and *S. spontaneum* subsp. *aegyptiacum* grown in the same environment (Cosentino et al., 2007, 2014, 2015). The

irrigation volume was determined as follow:

$$V = 0.66 \times (FC - WP) \times \phi \times D \times 10^3$$

where V = water amount (mm); 0.66 = readily available water not limiting for evapotranspiration; FC = soil water content at field capacity (27% of dry soil weight); WP = soil water content at wilting point (11% of dry soil weight); ϕ = bulk density (1.1 g cm⁻³); and D = rooting depth (0.6 m).

The experimental factor perennial grass species was differentiated in six levels: two *Arundo donax* L. ecotypes (ARCT – local clone Fondachello, and ARMO – clone Morocco), the commercial *Miscanthus* × *giganteus* J.M. Greef, Deuter ex Hodk., Renvoize (M×G), two seed-based *Miscanthus* hybrids (GNT9 and GNT10) obtained from the breeding program led by the Institute of Biological, Environmental and Rural Sciences of Aberystwyth University (UK) and Terravesta Ltd. (UK), and one ecotype of *Saccharum spontaneum* L. subsp. *aegyptiacum* (Willd.) Hack. (SAC – local clone).

Transplanting was carried out by hand in May 2018 at a density of one rhizome or one plantlet m⁻² in plots that were tilled in autumn to a depth of 25 cm and disk harrowed in spring before transplant. Rhizomes of ARCT, ARMO and SAC were collected from the in-situ germplasm collection located at the Experimental farm. Plantlets of M×G were provided by Energene sp. z o.o (Poland), while plantlets of the GNT9 and the GNT10 by Terravesta Ltd. (UK). Fertilization was neither applied as base nor as top dressing. Weeds were controlled by means of a grass trimmer during the year of establishment when necessary.

2.2. On-field measurements

Meteorological conditions and potential evapotranspiration (ET₀) were continuously measured through a weather station connected to a data logger (Delta-T, WS-GP1 Compact) and a Class A evaporation pan (mm d⁻¹), respectively. Both equipment were located about 50 m from the experimental field.

The first harvest after plant establishment was performed in January 2019, when aboveground biomass moisture content drops to the lowest levels (Monti et al., 2015). The whole aboveground fresh biomass was collected and weighted from a 4 m² after removing edge plants. Biomass was cut 5 cm above ground level and fresh sub-samples were randomly collected, immediately weighed and then dried to a constant weight at 65 °C. The percentage dry weight was used to calculate the dry matter biomass yield, which was referred to the unit land area (DMY, Mg ha⁻¹). The first year DMY is reported in Corinzia et al. (2020). Briefly, SAC had the significantly highest DMY (15.1 Mg DM ha⁻¹), followed by ARMO and ARCT (8.0 and 7.9 Mg DM ha⁻¹, respectively). Among miscanthus hybrids, GNT9 out yielded both GNT10 and M×G (6.0, 3.8 and 1.3 Mg DM ha⁻¹, respectively).

In the second (2019/20, hereinafter referred as 2019) and the third growing season (2020/21, hereinafter referred as 2020), the biomass was harvested in January 2020 and 2021, respectively, and DMY was determined according to the method described above. For each irrigation level the crop water use (CWU) was determined by means of water balance from plant re-growth up to the onset of senescence of genotypes:

$$CWU = I + P \pm \Delta C$$

where CWU = crop water use (mm); I = water supplied by means of irrigation (mm); P = precipitation (mm); ΔC = difference between soil water content at plant re-growth and soil water content at the onset of senescence. Soil samples were collected to a depth of 0.6 m in each irrigation treatment, growing season and species, at the beginning of each growing season and at the onset of senescence of each genotype. Fresh soil samples were immediately weighted, closed in plastic zip bags and then transferred in a ventilated oven to dry at 105 °C up to a constant weight. The CWU of genotypes and irrigation levels at the second and third growing season is shown in Table 1. The water use efficiency

Table 1

Crop water use in rainfed (I0), 50% of ET_m (I50) and 100% of ET_m (I100) of *Arundo donax* clones (ARCT and ARMO), *Saccharum spontaneum* spp. *aegyptiacum* (SAC) and *Miscanthus* hybrids (GNT9, GNT10 and M×G) in two subsequent growing seasons (2019 and 2020, respectively).

Irrigation	Genotype	2019 (mm)	2020 (mm)
I0	ARCT	296.81 ± 14.20	521.27 ± 18.62
I50	ARCT	553.48 ± 13.14	747.73 ± 15.93
I100	ARCT	786.60 ± 15.41	1061.80 ± 19.72
I0	ARMO	295.60 ± 16.91	517.05 ± 23.66
I50	ARMO	555.42 ± 38.71	747.54 ± 13.54
I100	ARMO	796.96 ± 15.84	1062.11 ± 21.10
I0	SAC	301.49 ± 12.85	518.15 ± 19.62
I50	SAC	558.26 ± 11.10	740.06 ± 29.73
I100	SAC	792.52 ± 15.43	1058.14 ± 18.37
I0	GNT9	263.13 ± 11.20	443.22 ± 14.84
I50	GNT9	519.07 ± 13.91	701.37 ± 15.54
I100	GNT9	733.14 ± 17.13	959.22 ± 16.19
I0	GNT10	264.34 ± 13.44	442.77 ± 12.58
I50	GNT10	516.54 ± 18.23	684.98 ± 13.65
I100	GNT10	729.11 ± 15.48	959.16 ± 18.43
I0	M×G	264.47 ± 22.12	441.78 ± 24.55
I50	M×G	501.95 ± 16.91	694.25 ± 21.43
I100	M×G	706.09 ± 19.70	958.61 ± 27.89

was calculated as DMY/CWU (g L⁻¹). The water footprint was calculated taking into account only the green and the blue water. Since the evapotranspiration was higher than the effective rainfall occurring during the crop growth, WF green is equal to the effective rainfall. The WF blue provided through irrigation was equal to the difference between evapotranspiration and rainfall. The grey water was set to zero for keeping a conservative approach (Lovarelli et al., 2016). The WF was thus calculated as CWU/DMY (m³ Mg⁻¹).

2.3. Statistical analysis

DMY, WUE and WF were analyzed by two-way ANOVA with repeated measure, where the growing season represents the within-factor, the irrigation and the genotype the between-factor (SPSS, PASW Statistics 18). When data failed Mauchly's sphericity test for sphericity, the univariate results were adjusted by using the Greenhouse-Geisser Epsilon and the Huynh-Feldt Epsilon correction factors. When univariate results satisfied sphericity for within-subject effects, the F-values and associated P-values for between-subject effects were tested.

Before conducting the ANOVA, the Bartlett's test was run to verify the assumption of homogeneity of variances according to the split-plot design. Differences between means were evaluated for significance using the Tukey's HSD test at 95% confidence level.

Relationships between dependent variables (DMY, WUE, WF) and independent variables (CWU) were modelled through linear and non-linear regressions. Coefficients were considered significant at $P \leq 0.05$. The Shapiro-Wilk test was developed to test residuals for normality, and the goodness of fit was assessed by calculating R² (SigmaPlot 11, Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Meteorological conditions

Air temperature was similar between the two growing seasons, averaging 18.3 °C in 2019 and 18.0 °C in 2020 (Fig. 1). The yearly reference evapotranspiration (ET₀) was also similar: 1291 mm in 2019 and 1257 mm in 2020. On the contrary, rainfall was lower in 2019 (473 mm) than the 2020 (779 mm) growing season. The average air temperature from March to October, namely the period of active CO₂ and water uptake of perennial grasses in this environment, was 21.3 °C and 21.0 °C in 2019 and 2020, respectively. In the same period, the cumulative rainfall was 213 and 470 mm, while the ET₀ was 1081 and 1063 mm, respectively.

3.2. Response to crop water use

The ANOVA showed a significant effect of growing season, genotype and irrigation on dry matter yield (DMY), water use efficiency (WUE) and water footprint (WF). The interaction of main effects was also significant for all examined parameters (Table 2).

Genotypic response of DMY to changing crop water use (CWU) given by different growing seasons and irrigation levels were fit by asymptotic regression models (Fig. 2). All regressions were significant per $P \leq 0.05$ and the models explained rather high percentages of the variance ($R^2 \geq 0.95$). Observed DMY were the highest when CWU was at the highest levels (I100) in all genotypes. The two giant reed genotypes (ARMO and ARCT) and SAC showed the significantly highest DMY overall (37.3, 34.1 and 34.0 Mg ha⁻¹ in ARMO, ARCT and SAC, respectively). At middle CWU levels (I50), DMY reduced by 16–20% in SAC and 11–21% in ARCT and ARMO. At the lowest CWU levels (I0),

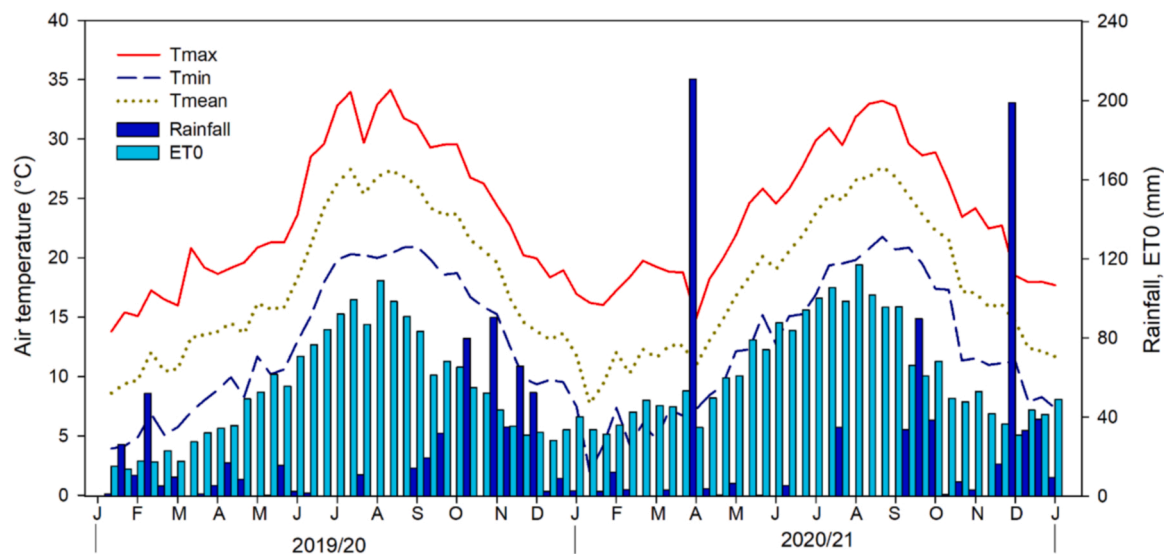


Fig. 1. Meteorological trend, maximum (Tmax), mean (Tmean) and minimum (Tmin) air temperatures (°C), rainfall, and reference evapotranspiration (ET₀) (mm), at the Experimental Farm of the University of Catania from 2019/20–2020/21 growing season.

Table 2

Repeated measure ANOVA for main effects and interaction on dry matter yield (DMY), water use efficiency (WUE) and water footprint (WF) of investigated perennial grasses. Growing season represents the within-factor, irrigation and genotypes the between-factor effects. Degree of freedom (df), adjusted mean square (Adj MS) and significance: $P \leq 0.001$ (***), Not significant (ns).

Source	df	DBY	WUE	WF
Growing season	1	325.24***	6.93***	98,113***
Irrigation (I)	2	1346.35***	2.64***	24,490***
Genotype (G)	5	1216.67***	25.91***	503,808***
I × G	10	34.16***	0.42***	1797***
Whole-plot Error	4	2.07	0.01	301
Error	83	2.59	0.05	1149

such DMY reduction was 31–48% in SAC, 34–50% in ARCT and 38–54% in ARMO. Among miscanthus, GNT10 out yielded both GNT9 and M×G in I100 (18.9, 16.1 and 12.9 Mg ha⁻¹, respectively). DMY in GNT10 and GNT9 at middle CWU levels (I50) was not different than the DMY of M×G in I100, and that of GNT10 in I0 was similar to the DMY of M×G in I50 (9.4 and 8.9 Mg ha⁻¹, respectively). The lowest DMY in I0 was in M×G (5.7 Mg ha⁻¹), which showed also the highest DMY reduction by varying the CWU (52–61% in I0 as compared with I100). Regression models predicted quite well the observed data, except in the GNT9 and particularly in the M×G, as evidenced by non-significant regression coefficients (Table 3). The *b* coefficient, which represents the rate of change of the DMY to one unit of CWU, was slightly higher in SAC than giant reed ecotypes (ARCT and ARMO), which showed similar values. Among miscanthus, the highest rate of change was in GNT10 and the

lowest in M×G.

Genotypic response of WUE to changing CWU was fit by polynomial linear regression models (Fig. 3). All regressions were significant per $P \leq 0.05$, except for GNT9 and M×G, which showed also the lowest goodness of fit ($R^2=0.56$ and $R^2=0.43$, respectively). Observed WUE data was the significantly highest in the drier growing season (2019) and at the lowest range of CWU in all genotypes, which corresponds to I0 irrigation level. ARMO, SAC and ARCT did not differ and the WUE ranged from 5.5, 5.4 and 5.2 g L⁻¹ at the lowest CWU (295.6–301.5 mm) to 3.4, 3.2 and 3.1 g L⁻¹ at CWU of 1058.1–1062.8 mm, respectively. The GNT10 was the highest among miscanthus hybrids (from 3.1 g L⁻¹ at CWU of 264.3 mm to 2.03 g L⁻¹ at CWU of 959.2 mm). The M×G showed the lowest WUE, ranging from 1.77 g L⁻¹ at CWU of 264.5 mm to 1.48 g L⁻¹ at CWU of 958.6 mm. The steepest rate of change was produced by SAC (−0.0029), while that of ARCT and ARMO was −0.0026 and −0.0024, respectively. The rate of change was −0.0014 in GNT10, −0.0009 in GNT9 and −0.0003 in M×G, the lowest overall (Table 4).

Genotypic response of WF to changing CWU was fit by polynomial linear regression models (Fig. 4). All regressions were significant per $P \leq 0.05$, except for the genotype GNT9 and M×G. In general, water provided through irrigation or wetter season significantly increased the WF in all investigated genotypes. The genotypes × irrigation interaction was not different in ARCT, ARMO and SAC up to middle CWU levels. However, when CWU was at the highest level, the WF in ARCT and SAC was larger than that in ARMO (319.6 and 311.1 vs 288.4 m³ Mg⁻¹). Among miscanthus hybrids, both GNT10 and GNT9 had similar WF, except in I0 in the wet season (2020), when GNT10 performed better

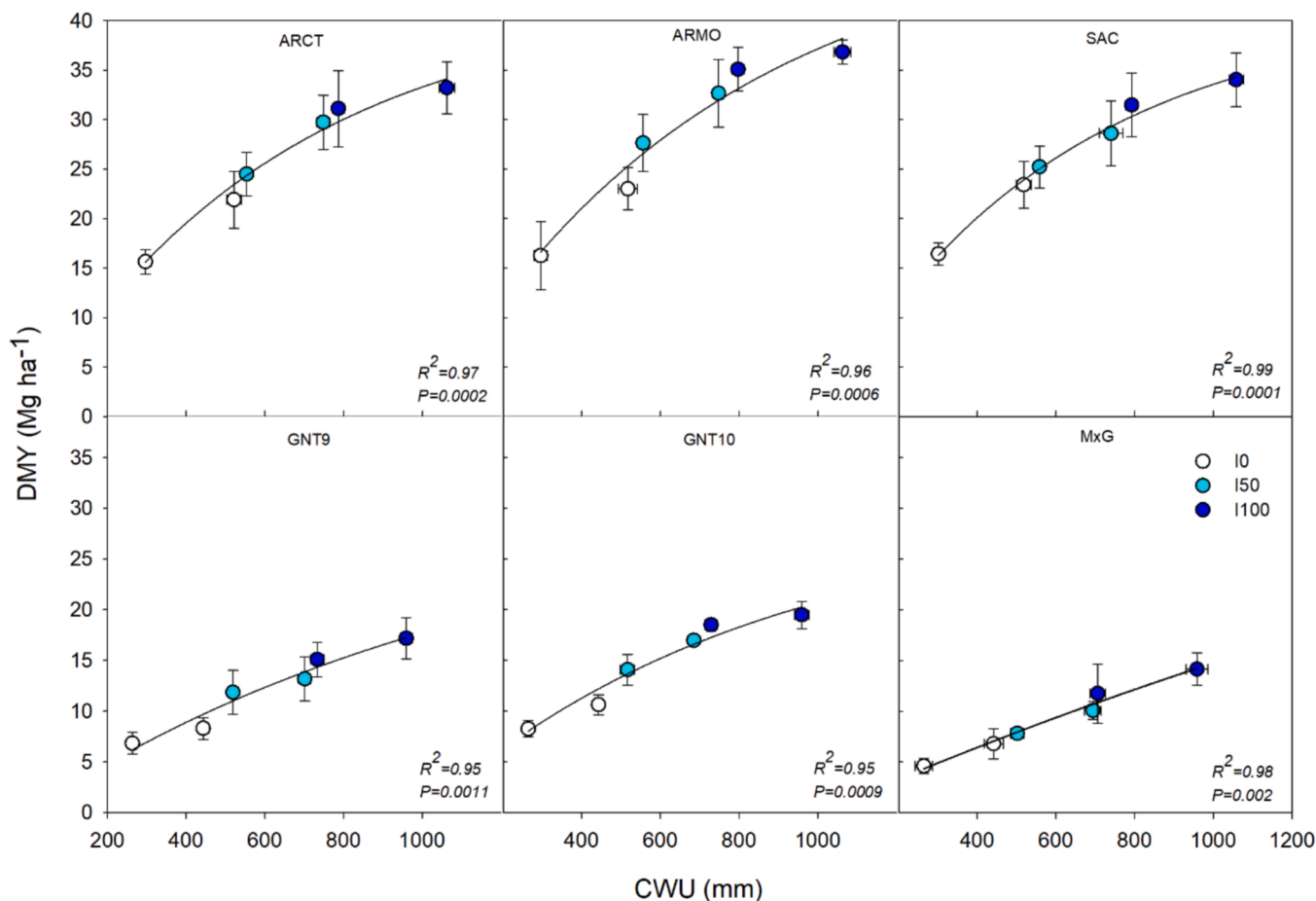


Fig. 2. Relationships between mean value \pm standard error of crop water use (CWU, mm) and dry matter yield (DMY, Mg ha⁻¹) of *Arundo donax* clones (ARCT and ARMO), *Saccharum spontaneum* subsp. *aegyptiacum* (SAC) and *Miscanthus* hybrids (GNT9, GNT10 and M×G). Least significant difference of genotype × irrigation interaction at $P \leq 0.05$ (2.18).

Table 3

Estimated coefficients, standard error (SE), t-value and P-value of the fitted equations between dry matter yield and crop water use of investigated genotypes.

Genotype	Equation	Coefficient	Value	SE	t-value	p-value
ARCT	$y = a(1 - e^{-bx})$	a	42.345	3.737	11.329	< 0.001
		b	0.0015	0.0002	6.180	0.003
ARMO	$y = a(1 - e^{-bx})$	a	49.750	6.9247	7.185	0.002
		b	0.0014	0.0003	4.166	0.014
SAC	$y = a(1 - e^{-bx})$	a	41.487	2.1242	19.531	< 0.001
		b	0.0017	0.0002	10.241	< 0.001
GNT9	$y = a(1 - e^{-bx})$	a	31.208	11.2074	2.784	0.049
		b	0.0008	0.0004	2.041	0.111
GNT10	$y = a(1 - e^{-bx})$	a	29.747	6.5098	4.569	0.010
		b	0.0012	0.0004	2.960	0.041
M×G	$y = a(1 - e^{-bx})$	a	47.587	45.949	1.253	0.278
		b	0.0003	0.0003	1.123	0.324

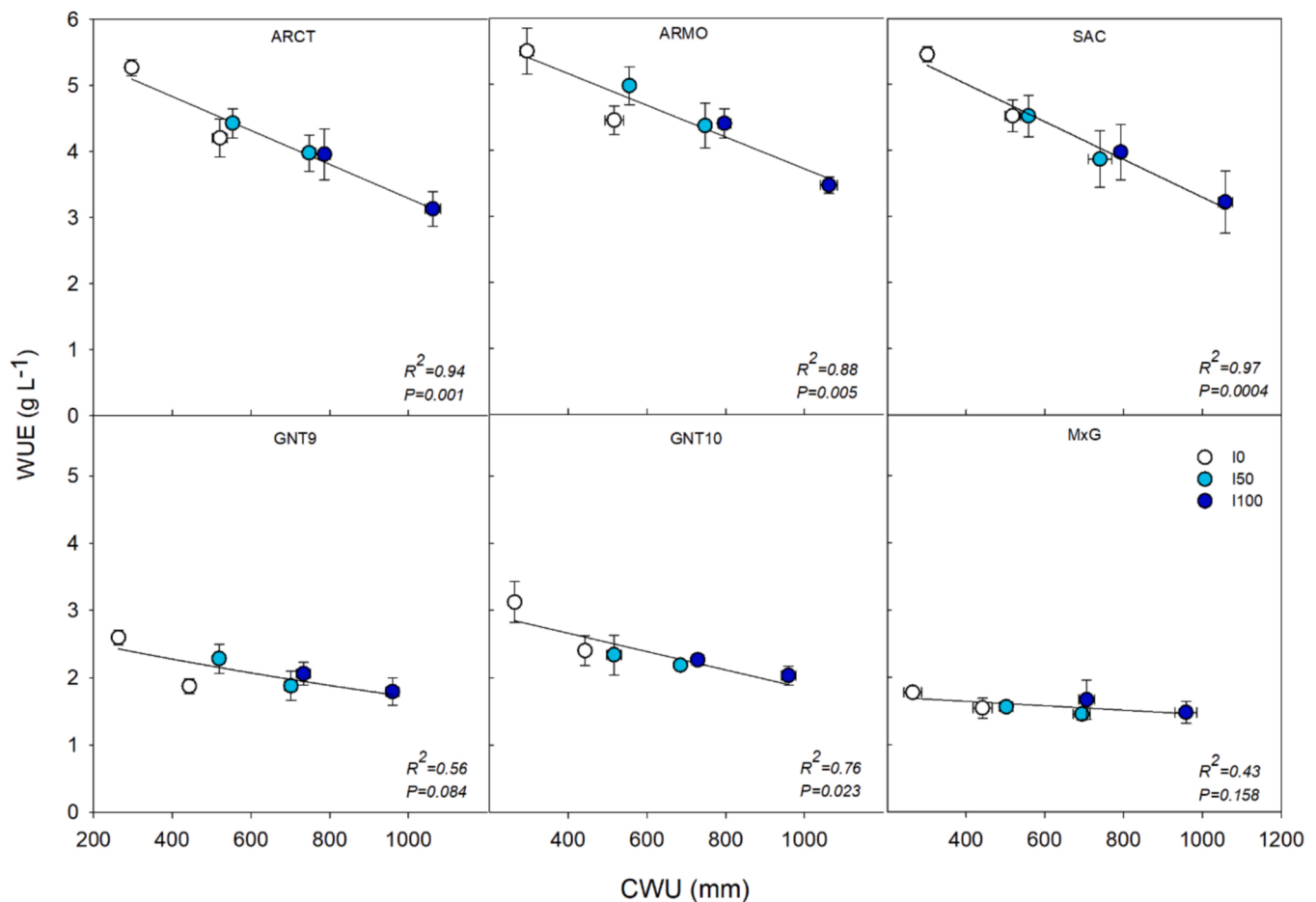


Fig. 3. Relationships between mean value \pm standard error of crop water use (CWU, mm) and water use efficiency (WUE, Mg ha^{-1}) of *Arundo donax* clones (ARCT and ARMO), *Saccharum spontaneum* subsp. *aegyptiacum* (SAC) and *Miscanthus* hybrids (GNT9, GNT10 and M×G). Least significant difference of genotype \times irrigation interaction at $P \leq 0.05$ (0.29).

(416.81 vs 534.13 $\text{m}^3 \text{Mg}^{-1}$). Generally, both genotypes had significantly larger WF from mid- as compared with the lowest CWU. M×G showed the largest WF overall (from 564.04 $\text{m}^3 \text{Mg}^{-1}$ at CWU of 264.4 mm to 689.5 $\text{m}^3 \text{Mg}^{-1}$ at CWU of 694.2 mm). The WF remained unchanged at higher CWU levels in M×G. The steepest rate of change of the WF to one unit of CWU (Table 5) was produced by the GNT10 (0.220), followed by GNT9 (0.203) and SAC (0.164). ARCT had a slightly higher rate of change than M×G (0.133 and 0.127, respectively), while ARMO had the lowest overall (0.107).

The relationship of pooled data between WUE and WF of investigated perennial, bioenergy grasses under changing growing seasons and irrigation levels was fitted by an exponential decay regression (Fig. 5).

The relative decrease of WF for a unit increase of WUE indicated a fast-exponential decay at WUE value from 1.4 to 3.1 g L^{-1} , which is the interval covered by the three miscanthus hybrids. Afterwards, the relative decrease of WF was rather slow from WUE higher than 3.2 g L^{-1} to a nearly flattened curve at WUE higher than 4.0 g L^{-1} . These WUE values were achieved by SAC, ARCT and ARMO genotype under reduced (I50) and rainfed (I0) water management.

4. Discussion

Unlike air temperature and evapotranspiration, the rainfall pattern greatly changed between the two growing seasons. This reflects the high

Table 4

Estimated coefficients, standard error (SE), t-value, P-value of the fitted equations between water use efficiency and crop water use of investigated genotypes.

Genotype	Equation	Coefficient	Value	SE	t-value	P-value
ARCT	$y = y_0 + ax$	y_0	5.894	0.227	25.78	< 0.001
		a	-0.0026	0.0003	-7.959	0.0013
ARMO	$y = y_0 + ax$	y_0	6.118	0.313	19.546	< 0.001
		a	-0.0024	0.0004	-5.413	0.005
SAC	$y = y_0 + ax$	y_0	6.142	0.184	33.338	< 0.001
		a	-0.0029	0.0003	-10.890	0.004
GNT9	$y = y_0 + ax$	y_0	2.651	0.266	9.957	0.006
		a	-0.0009	0.0004	-2.291	0.084
GNT10	$y = y_0 + ax$	y_0	3.208	0.2438	13.156	0.002
		a	-0.0014	0.0004	-3.581	0.023
M×G	$y = y_0 + ax$	y_0	1.771	0.1201	14.743	0.001
		a	-0.0003	0.0002	-1.728	0.159

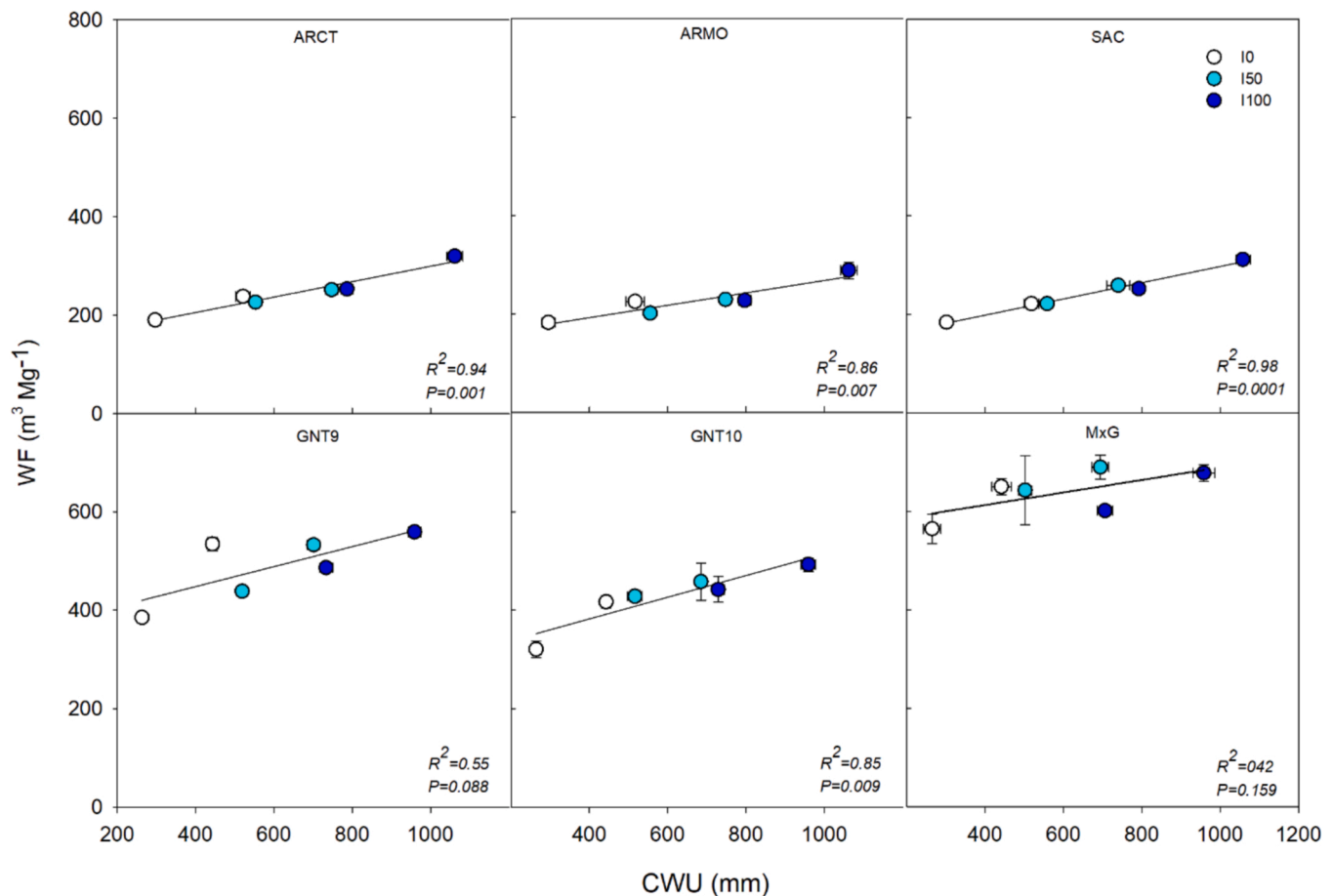


Fig. 4. Relationships between mean value \pm standard error of crop water use (CWU, mm) and water footprint (WF, $\text{m}^3 \text{Mg}^{-1}$) of *Arundo donax* clones (ARCT and ARMO), *Saccharum spontaneum* subsp. *aegyptiacum* (SAC) and *Miscanthus* hybrids (GNT9, GNT10 and M×G). Least significant difference of genotype \times irrigation interaction at $P \leq 0.05$ (45.94).

interannual variability and uneven rainfall distribution at lower latitudes of the European Mediterranean basin (Alexopoulou et al., 2015). The whole season ratio between precipitation and potential evapotranspiration, namely the dryness index, was 0.37 and 0.62 in 2019 and 2020, respectively, which is well below or just above the threshold set by the Joint Research Center for delineation of areas facing dryness (Confalonieri et al., 2014). If we narrow our focus in the period of active crop water uptake in this environment (March to October), the dryness index drops to 0.20 in 2019 and 0.44 in 2020. Therefore, dryness significantly impacts the growing cycle of warm-season perennial grasses in the present area.

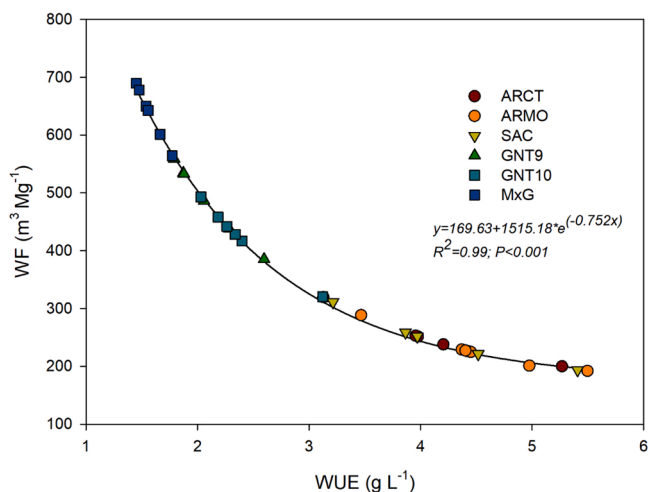
The crop water use (CWU), which included the water supplied by

irrigation, the precipitation, and the difference between soil water content at plant re-growth and soil water content at the onset of plant senescence, differed among crops, growing seasons and irrigation regimes. In rainfed conditions, the CWU was much higher in 2020 since it was wetter than 2019 growing season. In the present environmental conditions, the endemic giant reed and saccharum usually sprout earlier and reach canopy senescence later than miscanthus, building up more biomass due to extended water uptake and CO_2 assimilation (Scordia et al., 2014). Besides a stand age effect, since differences between the second and subsequent third growing season after the establishment might not be ruled out, all genotypes improved the biomass yield and linked water indicators by modulating the CWU. Giant reed likely

Table 5

Estimated coefficients, standard error (SE), t-value, P-value of the fitted equations of the water footprint and crop water use of investigated genotypes.

Genotype	Equation	Coefficient	Value	SE	t-value	P-value
ARCT	$y = y_0 + ax$	y_0	181.02	11.892	10.175	0.005
		a	0.133	0.0169	7.901	0.001
ARMO	$y = y_0 + ax$	y_0	190.614	14.993	8.044	0.001
		a	0.107	0.0213	5.0372	0.007
SAC	$y = y_0 + ax$	y_0	132.641	8.195	16.185	< 0.001
		a	0.164	0.0117	14.102	0.001
GNT9	$y = y_0 + ax$	y_0	366.830	58.026	6.322	0.003
		a	0.203	0.0902	2.2481	0.087
GNT10	$y = y_0 + ax$	y_0	294.242	29.669	9.917	0.006
		a	0.220	0.0464	4.745	0.009
M×G	$y = y_0 + ax$	y_0	561.734	46.755	12.014	0.003
		a	0.127	0.073	1.727	0.159

**Fig. 5.** Fitted regression of pooled data between water use efficiency (WUE, g L⁻¹) and water footprint (WF, m³ Mg⁻¹) of investigated perennial, bioenergy grasses under changing growing seasons and irrigation levels.

consumed more water than that actually measured since the calculations were made at 60 cm soil depth, where giant reed distributes approximately 40% of its root system (Monti and Zatta, 2009). Roots of giant reed can reach soil layer as deep as 180 cm, taking up 60% of soil available water in full irrigation; in rainfed conditions it can uptake nearly 20% of soil available water at soil depth of 150 cm (Cosentino et al., 2014). On the other hand, miscanthus root system distributes more on the top layers, with more than 90% of root in the top 35 cm (Monti and Zatta, 2009). The strategy to uptake water by exploring the available soil moisture at deeper soil layers, as in giant reed, allows to overcome prolonged moisture deficits near the soil surface that occur in the dry summer season. Erickson et al. (2012) showed that root biomass distribution was relatively uniform to a depth of 50 cm in giant reed, whereas root biomass declined more quickly with depth in other tall perennial grasses, such as *Saccharum* spp., and *Pennisetum purpureum* Schum. Hence, differences in crop phenology, root systems and water strategy uptake might in part explain the higher yields - and similar to that of the C4 saccharum - achieved by the C3 giant reed ecotypes.

Fitting models predicted most of the relationships analyzed quite well. The rate of growth of dry matter yield (DMY) was maximum from the lowest to mid CWU levels (up to around 600 mm) and the relative rate increase started to slowdown only at higher CWU. *Miscanthus* genotypes showed lower rate of change of one unit of biomass yield to one unit of water as compared with saccharum and giant reed ecotypes, and particularly M×G and marginally the GNT9 had a nearly linear trend and non-significant curvature coefficients. The asymptotic value showed that potential biomass yield would be 47 and 31 Mg ha⁻¹, respectively, well far from the actual yields of 14.1 and 17.2 Mg ha⁻¹ for M×G and

GNT9, respectively. This means that factors other than water availability might have contributed to differences between potential and actual yields. It is worth mentioning that crops were established unfertilized and in minimum tillage to cope with low-input practices; furthermore, top dressing fertilization was not provided, neither at the beginning of stem sprouting nor during stem elongation in any growing season. Although perennial grasses are low-nutrient requiring crops, it has been shown that *M. × giganteus* average annual productivity increased from 11.8 Mg ha⁻¹ for unfertilized crops to 22.0 Mg ha⁻¹ when nitrogen at 60 or 120 kg ha⁻¹ yr⁻¹ was applied in the USA, with no yield differences between plots fertilized with 60 and 120 kg N ha⁻¹ (Lee et al., 2017). Similar achievements were observed by Cosentino et al. (2007) in the present environmental conditions, with larger response to N fertilization when water was not limiting. Furthermore, N fertilization enhances root development which, in crops with a more superficial root structure like *Miscanthus*, may increase potential water uptake from the subsoil, allowing to withstand longer periods of low water availability in topsoil (Neukirchen et al., 1999). Hence, lack of fertilization and imbalanced nutrient requirements might have caused nutrient stress and exacerbated drought stress, which in turn limited the dry matter yield of *Miscanthus*.

The two giant reed ecotypes showed the second highest rate of DMY change (0.0014 and 0.0015 for ARMO and ARCT, respectively), which was very similar to that achieved in previous observation with the same local giant reed clone (ARCT) and environmental conditions but varying the N supply (from 0.0017 in unfertilized to 0.0018 in 120 kg N ha⁻¹). On the other hand, the rate of change in SAC, which was the overall highest (0.0017), was lower than that produced by a SAC plantation varying the CWU in either the 7–9 year (0.0023) or the 13–15 year (0.0024) of growth in the same experimental area (Cosentino et al., 2015; Scordia et al., 2020b). Nonetheless, the asymptotic coefficient was higher in the present observation (41.5 Mg ha⁻¹) than the previous ones (37.86 vs. 33.63 Mg ha⁻¹ for the plantation at 7–9 or 13–15 year, respectively), which agrees with the upward DMY trend of perennial grasses in the juvenile plantation phase (Alexopoulou et al., 2015).

Saccharum, owing a C4 photosynthetic pathway had the highest water use efficiency (WUE), but it was not different than the WUE of both giant reed ecotypes. Among miscanthus the GNT10 outperformed the GNT9 and this latter the M×G. The different rate of change associated with a one-unit shift in the WUE by increasing the CWU explains the response of investigated crops. The WUE improvement by reducing the CWU is typical for many crops that use a conservative growth strategy, such as giant reed, saccharum and the GNT10. Both the GNT9, and particularly the M×G, showed the lowest and non-significant rate of change of WUE to CWU, suggesting a non-conservative strategy for growth to cope with drought-stress conditions (Stavridou et al., 2019; Scordia et al., 2020a). WUE values of giant reed and saccharum were similar to previous observations (Cosentino et al., 2014, 2015; Scordia et al., 2015). Unlike GNT9 and GNT10, the WUE of M×G was rather low for a C4 crop (Cosentino et al., 2007; Triana et al., 2014); however, it was still within the range reported by Clifton-Brown and Lewandowski

(2000) and Scordia et al. (2020a).

The water footprint (WF) of saccharum was similar to that achieved previously (Scordia et al., 2020b), and matched the lowest range of the global average WF per ton of commodity food crops found by Mekonnen and Hoekstra (2011). The WF of giant reed ecotypes was similar that of SAC. ARMO produced a better WF than both SAC and ARCT only at the highest CWU. The WF of GNT9 and GNT10 grown in rainfed conditions in the dry year was similar to the WF of *M. × giganteus* grown in the North Europe (385 and 320 for GNT9 and GNT10 vs 334 m³ Mg⁻¹), and the average WF of the M×G was similar to the *M. × giganteus* grown in the United States, 637 vs 629 m³ Mg⁻¹ (Gerbens-Leenes et al., 2009). Generally, the linear increase of WF raising the CWU confirmed that rainfed crops used more efficiently the available water per ton of biomass produced in all investigated genotypes. The developed relationship between WUE and WF indicated a very fast improvement of WF at a narrow WUE range (i.e., the interval covered by the three miscanthus genotypes); hence, a sustainable intensification can be expected by combining genotypes with high WUE (like the locally adapted ones) and agronomic management strategies to reduce water in cropping systems (i.e., reduced irrigation or rainfed conditions).

5. Conclusions

Present findings demonstrated the importance to select for crops with high water use efficiency in areas affected by dryness. The endemic species, giant reed and saccharum, outperformed both the M×G and the improved miscanthus hybrids GNT9 and GNT10. These results are, however, not negative towards the new seed-based miscanthus hybrids. Both hybrids outperformed the commercial M×G under the present environmental conditions, and unlike the GNT9, the GNT10 seems to have a conservative growth strategy to cope with the drought-stress. Therefore, this study demonstrated that improvement of breeding more drought-resilient crops than the commercial M×G was achieved.

Irrigation water enhanced biomass yield in all crops, however, the water use efficiency and the water footprint were negatively impacted. Although a different response to drought-stress was observed, genotypes were able to withstand prolonged dry periods during the summers by improving the water use efficiency and consuming less water per ton of biomass produced.

The study demonstrated as can be improved the sustainability of the bioenergy cropping system by modulating the soil moisture so as to use more efficiently the available water, reducing the negative impact on freshwater resources. In view of increasingly dryness, it is strategically important to select for crops and breeding programs focusing on traits conferring high water use efficiency to improve drought-tolerance, which combined with sustainable agronomic management strategies might help to increase farming systems' resilience to climate change and land abandonment induced by dryness in the Mediterranean area.

CRedit authorship contribution statement

Danilo Scordia: Conceptualization, Methodology, Data curation, Formal analysis, Software, Validation, Project administration, Writing – review & editing. **Sebastiano Andrea Corinzia:** Investigation, Methodology, Data curation, Formal analysis, Software, Writing. **Salvatore L. Cosentino:** Project administration, Supervision, Funding acquisition, Validation, Review & editing. **Giorgio Testa:** Investigation, Methodology, Data curation, Formal analysis, Software, Writing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This paper is part of a project that has received funding from the "European Union's Horizon 2020 research and innovation programme under grant agreement No 727698" and by the "PIA.CE.RI. 2020–2022 Linea 2 - CROP2FUEL project (5A722192164), Italy". Authors gratefully acknowledge Matteo Maugeri, Dario Maugeri, Santo Virgillito and Giancarlo Patanè of the University of Catania for field trial set-up and maintenance.

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