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Review article

Wild biomass perennial grasses show tolerance to dry conditions in a Mediterranean climate

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ABSTRACT

The productivity of biomass perennial grasses (BPGs) in the marginal areas of the Mediterranean is mainly limited by water scarcity. To address this constraint, this study investigated the impact of different irrigation levels on the morphological and productive traits of seven BPG genotypes belonging to *Arundo donax* L., *Saccharum spontaneum*, and *Miscanthus* hybrids, adopting a split-plot experimental design under the Mediterranean climatic conditions of Sicily (Italy) during a three-year experiment. Results showed that water availability significantly affects biomass yield, particularly during early crop development, with all genotypes benefiting from irrigation through increased stem and leaf biomass. However, notable differences emerged among species: while *Arundo donax* and *Saccharum spontaneum* exhibited strong drought tolerance, maintaining stable aboveground biomass and stem density without irrigation, *Miscanthus* × *giganteus* was more susceptible to water stress, showing marked biomass declines under rainfed conditions. Conversely, *Miscanthus* hybrids GNT3 and GNT43, bred for drought tolerance, outperformed *S. spontaneum* under rainfed environments and achieved biomass yields comparable to *A. donax* when irrigated. Notably, despite biomass reduction under drought conditions, the chemical composition of the biomass (lignin, cellulose, hemicellulose, and ash) remained stable, indicating that biomass quality was not adversely affected by water stress. These findings confirm the role of effective water management and genotype selection for optimizing biomass yield and resource efficiency, providing valuable insights for enhancing crop productivity in Mediterranean and other semi-arid regions. The evaluated species represent promising candidates for cultivation under water scarcity due to their high water use efficiency and tolerance to water stress.

1. Introduction

The study addresses the need to identify and develop sustainable bioenergy cropping systems suitable for Mediterranean and semi-arid regions. Climate projections for these regions suggest an increase in summer drought duration, air temperature, and evapotranspiration, together with a decline in the frequency of precipitation. These changes are expected to cause land degradation (Panoutsou and Chiaramonti, 2020) and a reduction in biomass crops yields (Cosentino et al., 2012). Under these circumstances, conventional irrigation practices are becoming less viable due to competition with food crops and increasing pressure on water resources (Jewitt and Kunz, 2011; Mathioudakis et al., 2017).

The erratic rainfall pattern and the frequent occurrence of summer drought and heatwaves typical of the climate in the Mediterranean area significantly influence the productivity of biomass crops (Alexopoulou et al., 2015; Ciardini et al., 2016; Hatfield and Prueger, 2015). In this context, biomass perennial grasses (BPGs) are particularly promising because they are water-efficient and can support low-energy cultivation systems. However, their productivity is often limited by low soil moisture availability and high vapour pressure deficit during the summer months, which reduce CO_2 assimilation and limit biomass production in spring-summer crops (Panoutsou and Chiaramonti, 2020).

Biomass perennial grasses are generally more productive than annual crops in terms of biomass yield (Scordia et al., 2022; Scordia and Cosentino, 2019) and exhibit high resource-use efficiency, particularly with respect to radiation (RUE) and water use efficiency (WUE) (Corinzia et al., 2023; Scordia et al., 2022).The relationship between

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irrigation and biomass yield is complex and depends on multiple environmental and management factors. Studies have shown that BGPs can achieve higher biomass yields than conventional food crops while using similar or lower amounts of water owing to their higher water use efficiency (WUE) (Scordia et al., 2022). This efficiency is crucial for achieving sustainable biomass production under water-limited conditions. Consequently, it is essential to identify BPG species and genotypes capable of maintaining high productivity on marginal lands under low water availability, thereby supporting both renewable energy generation and environmental sustainability.

Among BPGs, giant reed (*Arundo donax L.*), *Miscanthus* (*Miscanthus x giganteus* (Greef & Deuterand)) and African fodder cane (*Saccharum spontaneum* L. *ssp*. *aegyptiacum* Wild (Hack.)) have been suggested as the most suitable for Mediterranean environments (Scordia, et al., 2022). Both *A. donax* and *S. spontaneum* are now naturalized in the Mediterranean area (Cosentino et al., 2007). Their high suitability can be attributed to the C4 metabolism of *Miscanthus* and *S. spontaneum* and the capacity of *A. donax* to withstand low soil water content (Cosentino et al., 2014, 2015). Previous studies have shown that *Miscanthus* productivity is constrained by its sensitivity to drought, prompting the development of new hybrids adapted to the low water availability typical of southern Europe (Clifton-Brown et al., 2019). In contrast to these C_4 species, *A. donax* is a C_3 plant that nonetheless exhibits high intrinsic water use efficiency (WUE) (Scordia et al., 2022) and photosynthesis rates comparable to those of C4 species, which typically dominate in warm climates (Sánchez et al., 2021; Zegada-Lizarazu et al., 2010a). *Arundo donax* maintains high photosynthesis rates even under water stress, a trait attributed to its efficient leaf structure, xylem morphology, and efficient physiology (Cosentino et al., 2016; Haworth et al., 2017a, 2017b, 2017c, 2018, Pompeiano et al., 2019; Webster et al., 2016). In addition, its high biomass productivity under water stress results from delayed leaf senescence in comparison to well water plants (Haworth et al., 2019) and the development of an extensive root system, which extends uniformly to a depth of 50 cm (Erickson et al., 2012), allowing access to deeper soil moisture and enables performance comparable to C_4 plants.

The present study focuses on the response of BPGs to agronomic marginality caused by climatic constraints, specifically the ratio between precipitation and potential evapotranspiration (P/PET). Areas with a P/PET ratio below 0.6 are classified as drought-affected according to the criteria set by the Joint Research Centre (JRC) of the European Commission for defining marginal lands in terms of biophysical restrictions (Terres et al., 2014). This field trial evaluated a heterogeneous group of seven genotypes comprising *Miscanthus* hybrids selected for drought tolerance (Clifton-Brown et al., 2017), clones of *A. donax*, and a clone of *S. spontaneum* over three years, under two irrigation regimes reflecting summer drought conditions typical of the southern Mediterranean environment.

The study aims to assess how water availability influences key growth parameters such as stem density, height, and weight, with the goal of identifying the genotypes most suitable for biomass production on marginal land affected by prolonged drought. By addressing this objective, the research supports sustainable use of agricultural resources in arid regions, facilitates the transition toward bio-based energy systems, and helps reduce competition with food crops through the productive use of underutilized marginal lands.

2. Materials and methods

2.1. Experimental site description

The field trial was carried out during three years from 2020 to 2023 at the Experimental Farm of the University of Catania (10 m a.s.l., 37°24′ N, 15°03′ E) in a typical Xerofluvents soil (USDA, 1999).

2.2. Experimental factors and design

The following factors were studied:

- Genotype (seven levels: three *Arundo donax* L. clones, three *Miscanthus* hybrids and one clone of *Saccharum spontaneum*);
- Irrigation (two levels, 100 % and 0 % restoration of ETm).

The experimental design was a randomized complete block with three blocks and a split-plot randomization restriction with irrigation levels as the whole-plot factor and genotypes as the sub-plot factor. Each subplot measured 100 m^2 (10 m × 10 m).

2.2.1. Genotype factor

Seven genotypes were evaluated: three *Arundo donax* L. clones, named ARCT (local clone from Catania province Italy); ARMO (from Marrakesh, Morocco), provided by Tree and Timber Institute, National Research Council of Italy; and ARPI (from Piacenza province, Italy) provided by the Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Piacenza, Italy.

The commercial *Miscanthus x giganteus* (Greef et Deuter) named MxG was provided by Energene sp. z.o.o (Wroclaw, Poland); and two commercial *Miscanthus* hybrids (*M. sinensis x M. sacchariflorus*), named GNT3 and GNT43 and selected for drought tolerance within the breeding program led by the Institute of Biological, Environmental and Rural Sciences of Aberystwyth University (UK), were provided by Terravesta Ltd. (Lincoln, UK). The clone of *Saccharum spontaneum*, named SAC, was collected from wild plants in eastern Sicily.

2.2.2. Irrigation factor

The irrigation factor had two levels: irrigated and rainfed. During the first year, irrigation was applied for both irrigation levels to ensure the plant establishment: the irrigated plot received 250 mm and the rainfed plot 150 mm, representing 100 % and 60 % of the crop's maximum evapotranspiration (ETm). In the following two years, the irrigated plot was maintained at 100 % of the ETm during the summer months (June-August), corresponding to approximately 540 mm of irrigation, while the rainfed plot received 0 % of ETm. Irrigation was provided by a drip irrigation system.

Daily ETm was calculated according to:

$$ETm = ETo \times Kc$$

where ETo is the evapotranspiration rate from the reference surface (mm) calculated trough the FAO Penman-Monteith method (Allen et al., 1998) and Kc is the highest crop coefficients (Kc) among those applied for *Miscanthus* × *giganteus*, *Arundo donax* and *Saccharum spontaneum* grown in the same environment (Cosentino et al., 2014; Panoutsou and Chiaramonti, 2020).

The irrigation volume was calculated using the formula:

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

where V is the water volume (mm), 0.66 is the amount of readily available water not limiting evapotranspiration, FC is the soil water content at field capacity (27 % of soil dry weight), WP is the soil water content at wilting point (11 % of soil dry weight), ϕ is the soil bulk density (1.1 g cm^{-3}), and D is the depth of the soil explored by the roots (0.6 m).

At the end of summer season, when the available soil moisture was adequate to the crops, irrigation was suspended.

2.3. Agronomic management

Soil preparation was performed in autumn 2019 through harrowing to a depth of 25 cm and subsequently by disc harrowing in spring before transplant. No fertilization was applied, and the preceding crop was hemp.

Rhizomes of *Miscanthus*, sourced from Terravesta Ltd. (Lincoln, UK), and *S. spontaneum*, already available at the farm, were first potted to produce plantlets for later transplanting. *Arundo donax* clones were directly transplanted as rhizomes. Manual transplanting of the three crops took place in April 2020 at a density of 1 plant m^{-2} using plantlets (*Miscanthus* and *S. spontaneum*) or rhizomes bearing two or three buds (*A. donax*). Weed control was performed only during the first year of establishment through manual weeding.

2.4. Measurements

Weather conditions and potential evapotranspiration (ETo) were continuously measured using a weather station connected to a data logger (Delta-T, WS-GP1 Compact) and a Class A evaporation pan (mm d^{-1}). The first harvest was carried out in February 2021, the second in February 2022 and the third in February 2023. Fresh biomass was collected in an area of 4 m^2, excluding plot edges, with three replicates per plot. All stems within this area were counted and then harvested by making a cut 5 cm above the ground. Fresh weight (FW) of stems and leaves and stem length were measured from samples of five representative stems collected adjacent to the harvested area. Samples were dried in an oven at 65 °C until constant weight, after which dry weight (DW) was calculated.

2.5. Qualitative determinations

Dried stem and leaf samples were ground separately and the composition of the biomass was analysed by near-infrared spectroscopy (NIR) using the SpectraStar™ XL model 2500XL-R (Unity Scientific Milford, MA, USA). Analyses were performed using ring cells in diffuse reflectance between 680 and 2500 nm at 1 nm intervals. From the absorption spectra, concentrations of hemicellulose, cellulose, acid detergent lignin (ADL), ash, and neutral detergent soluble fraction (NDS) were determined using a calibration developed for perennial lignocellulosic grasses with the Ucal InfoStar version 3.11.0 software, as reported by Scordia et al. (2017).

2.6. Climatic conditions

The climate at the experimental site is classified as sub-tropical Mediterranean. Throughout the experiment, temperature patterns, rainfall, and reference evapotranspiration followed the typical pattern of the Mediterranean climate (Fig. 1). Average air temperatures during the growing seasons (March to November) were slightly higher in 2022 compared to 2021 and in 2023 compared to 2022, with monthly mean temperatures of 21.5 °C in 2023, 21.2 °C in 2022 and 21.0 in 2021. Monthly averages of daily minimum temperatures during the growing seasons were 15.9 °C in 2021, 15.8 °C in 2022 and 10.1 °C in 2023. Monthly averages of daily maximum temperatures were 26.1 °C in 2021, 26.7 °C in 2022 and 26.8 °C in 2023. Reference evapotranspiration (ETo) was higher during the 2021 growing season compared to the 2022 and 2023, with seasonal totals of 1285, 1188 and 1222, respectively. Precipitation was greatest from November 2020 to November 2021 (1016 mm), followed by November 2019 to November 2020 (641 mm), and lowest from November 2021 to November 2022 (397.6 mm) (Fig. 1).

2.7. Statistical analysis

The measured data were processed using R CRAN software (R Core Team, 2021). A mixed-model analysis of variance was performed with year as a fixed factor, irrigation as the whole-plot fixed factor, genotype as the sub-plot fixed factor, blocks as random effect, and the block x irrigation interaction as the whole-plot error term. This analysis was applied to biomass yield, stem density, plant height, stem diameter, ash, hemicellulose, cellulose, lignin, and NDS. Genotypes were analysed individually, not grouped by species. Residuals were tested for normality using the Shapiro–Wilk test. The Bartlett test was used to test homoscedasticity. Tukey's Honestly Significant Difference (HSD) test at a 95 % confidence level was used for pairwise genotype comparisons within each year × irrigation combination. Correlation among variables were assessed using the Pearson's product-moment correlation test.

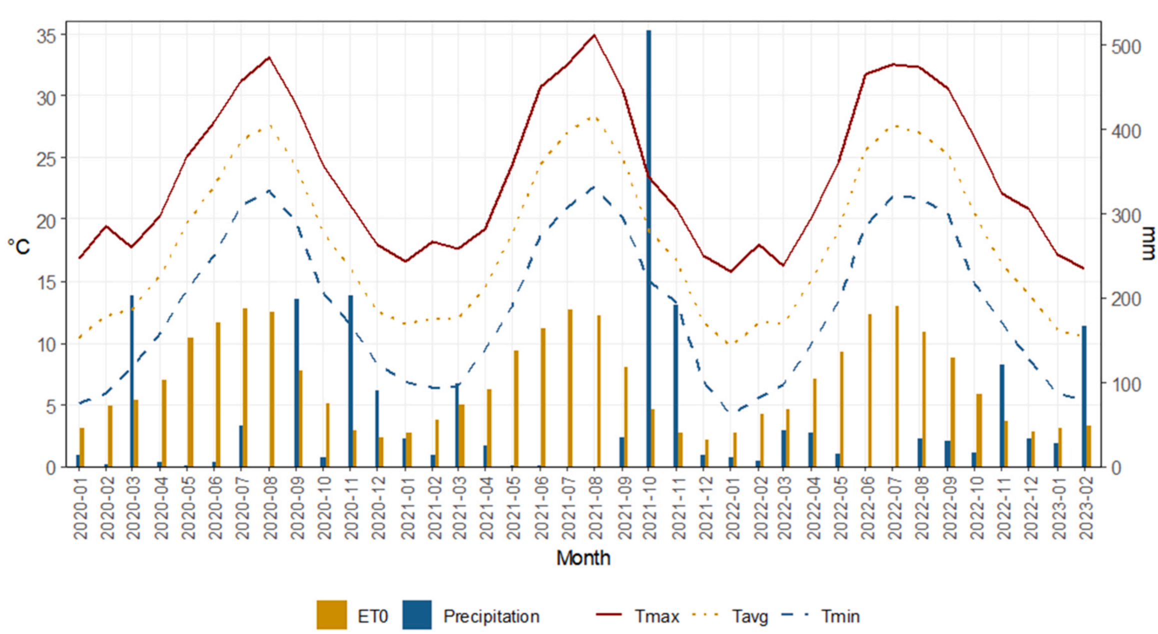

Fig. 1. Meteorological variables (Tmax = monthly average of daily maximum temperature, Tavg = monthly average of daily mean temperature, Tmin = monthly average of daily minimum temperature, ETo = monthly sum of daily reference evapotranspiration according to Allen et al. (1998), Precipitation = monthly precipitation from January 2020 to February 2023 at the experimental site (Catania, 37°24′ N., 15°03′ E., 10 m a.s.l.).

3. Results

3.1. Stem height

Stem height was significantly affected by year, irrigation, genotype, and their interactions (Table 1).

Miscanthus hybrid MxG showed the most pronounced difference between irrigated and rainfed conditions, with the exception of the first year, when the greatest height occurred under non-irrigated conditions (Fig. 2).

Miscanthus hybrids GNT3 and GNT43 showed a moderate response to irrigation. Height increase under irrigation was greater in 2023, whereas the difference was less conspicuous in the earlier years. *Saccharum spontaneum* genotype SAC achieved its greatest stem height in 2022 under irrigated condition, reaching 340 cm.

Arundo donax clones ARCT and ARMO reached the greatest heights, exceeding 300 cm under both rainfed and irrigated conditions (Fig. 2, Table 2). Significant effects were observed for the interactions Genotype × Irrigation, Genotype × Year, and Irrigation × Year on stem height (Table 1), highlighting the complex interplay among these factors.

3.2. 3.2 Stem density

The results of the ANOVA (Table 1) showed that irrigation, genotype, and year had highly significant effects on stem density. The interactions Irrigation × Genotype, Irrigation × Year, Genotype × Year, and the three-way interaction (Genotype × Irrigation × Year) were also significant, indicating that stem density was influenced in a complex, multifactorial manner. Stem density was generally higher under irrigated than under rainfed conditions, with mean values of 36.4 ± 3.2 stems m^{-2} for irrigated and 26.5 ± 2.6 stems m^{-2} for rainfed conditions. *Miscanthus* genotypes showed particularly high stem densities in 2023, with values reaching almost 80 stems m^{-2}. The hybrid GNT3 had the highest stem density, with a value of 61.3 ± 3.0 stems m^{-2}, while MxG had a relatively low density of 37.1 ± 5.8 stems m^{-2}. The *S. spontaneum* SAC genotype had a similar density to MxG, with 32.6 ± 3.9 stems m^{-2}. The increase in stem density under irrigated conditions compared to rainfed conditions was stronger in 2023, particularly for *A. donax and S. spontaneum* genotypes (Fig. 3).

3.3. Stem weight

Stem weight was significantly affected by genotype, year, the irrigation × genotype interaction, and the genotype x year interaction, whereas irrigation alone had a non-significant effect. *Arundo donax* clones exhibited high stem weights, whereas *Miscanthus* hybrids had significantly lower values (Fig. 4). The effect of irrigation on *A. donax* stem weight was non-consistent and the highest values in 2023 were achieved under rainfed conditions. In contrast, the other genotypes consistently exhibited higher values under irrigated conditions than under rainfed conditions across all years (Fig. 4).

Table 1
Three-way ANOVA for main effects (Irrigation, Genotype, Year) and interactions (Irrigation x Genotype, Irrigation × Year, Genotype × Year, Genotype × Irrigation × Year) on Above-Ground Biomass (AGB) yield, Leaf-to-Biomass (LB) ratio, stem density, stem height, and stem weight. The p-values are reported; values lower than 0.05 indicate statistical significance.

Source of Variation	AGB yield	LB ratio	Stem density	Stem height	Stem weight
Irrigation	< 0.01	< 0.01	< 0.01	< 0.01	0.50
Genotype	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Year	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Irrigation × Genotype	< 0.01	0.02	< 0.01	< 0.01	0.025
Irrigation × Year	< 0.01	0.33	< 0.01	< 0.01	0.05
Genotype × Year	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Irrigation × Genotype × Year	0.20	0.55	0.01	< 0.01	0.26

3.4. Biomass yield

Biomass yield was significantly affected by year, irrigation, genotype, and their interactions, with the exception of the three-way interaction (Genotype × Irrigation × Year) (Table 1). The results confirmed that soil water availability was a limiting factor for biomass yield of the seven genotypes across the three years of production (Table 2; Fig. 5). Irrigation generally resulted in higher biomass yields for both leaves and stems. In some genotypes, such as ARCT and MxG, the yield gap between irrigated and non-irrigated treatments was less evident in the initial years (2021–2022) but became more pronounced in 2023. Across genotypes, ARCT, ARMO, and ARPI showed the highest biomass yields under both irrigation regimes. In contrast, MxG produced 70 % less biomass than ARMO under rainfed conditions and 50 % less than ARPI under irrigated conditions (Fig. 5). Biomass yield increased progressively from 2021 to 2023 for all genotypes. This increase was mainly driven by greater stem production, which reached its highest portion of total biomass in 2023. The leaf to stem ratio tended to increase over time from 2021 to 2023 in most genotypes, especially under irrigated conditions (Fig. 5). *Miscanthus* hybrids GNT3 and GNT43 showed a higher leaf to stem ratio than the others genotypes, particularly in the rainfed treatment. Conversely, ARCT, ARMO, ARPI and SAC had a higher proportion of stems, particularly in the irrigated treatment. The highest yields were obtained in the third year under irrigated condition by the clones ARMO, ARPI, and the hybrid GNT3, with no statistically significant differences among them. The genotypes GNT3, GNT43, and SAC maintained low AGB yields under rainfed conditions across all years, with no notable increase between the second and third year after plant establishment. Variability among replicates was minimal in 2021, but increased in the subsequent years. The genotypes ARCT and GNT43 showed lower overall variability, suggesting more uniform responses.

3.5. Correlation between variables

Pearson correlation values ≥ 0.197 or ≤ - 0.197 were significant at the 95 % confidence level in a two-tailed test (Fig. 6). Stem height was significantly correlated with AGB yield (r = 0.74). Stem weight was also positively correlated with AGB yield (r = 0.73). However, a negative correlation was observed between LBR and AGB yield (r = -0.73). The LBR was negatively correlated with stem height (r = -0.62) and stem weight (r = -0.61). Stem weight and stem height were positively correlated (r = 0.81), whereas stem weight and stem density showed a negative correlation (r = -0.54). Stem height and stem density were also negatively correlated (r = 0.29).

3.6. Biomass composition

Biomass composition was similar among all genotypes and across both irrigation conditions in 2022 and 2023 (Figs. 7 and 8). Genotypes ARCT and SAC maintained similar levels of lignin and cellulose under both irrigated and rainfed conditions, whereas the other genotypes exhibited more pronounced variation.

4. Discussion

The study examined the impact of the water input on the biomass yield and quality of several biomass perennial grass (BPG) genotypes during the first years following crop establishment, with the objective of identifying the most suitable genotypes for biomass production on marginal land affected by low water availability during the summer months.

Meteorological data confirmed the climatic pattern typical of the

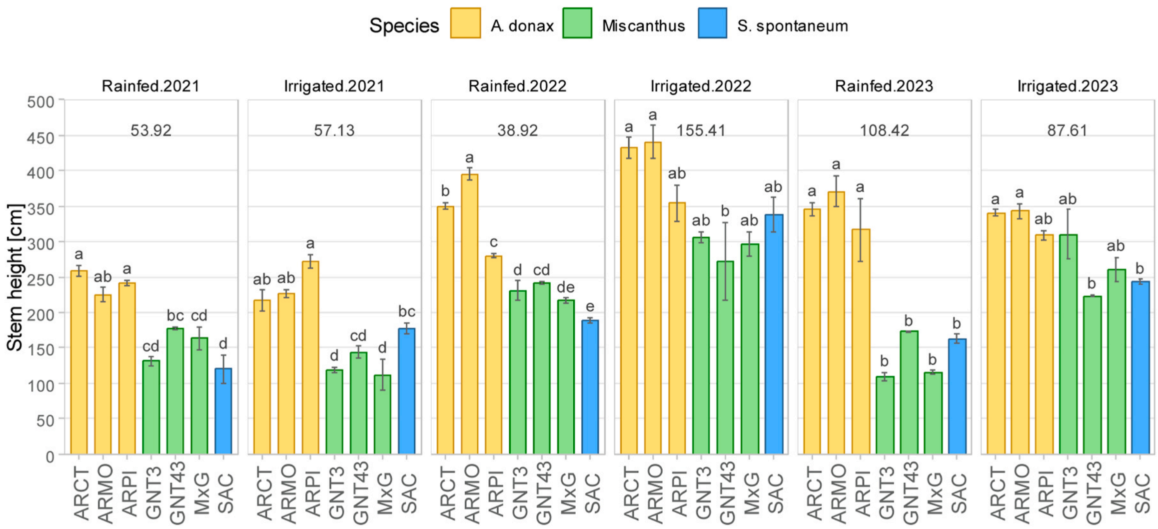

Fig. 2. Stem height of the seven genotypes examined in this study (ARCT, ARMO, ARPI, GNT3, GNT43, MxG, SAC), over the years 2021, 2022, and 2023 under two irrigation conditions. Tukey's Honestly Significant Difference (HSD) values are reported for pairwise genotype comparisons at each level of the year × irrigation interaction.

Table 2
Mean values of Aboveground Biomass (AGB) yield, Leaf to Biomass (LB) ratio, stem density, stem height and stem weight for the irrigation and the genotypes factors. Tukey's Honestly Significant Difference (HSD) values are reported to compare pairwise means across irrigation levels and genotypes.

Trait		AGB yield	LB ratio	Stem density	Stem height	Stem weight
Irrigation	Rainfed	11.0	24.0 %	26.5	219.3	65.2
	Irrigated	16.9	19.0 %	36.4	257.3	67.4
	HSD	1.2	2.4 %	2.7	9.0	4.7
Genotypes	ARCT	16.2	11.2 %	13.6	310.3	115.3
	ARMO	17.6	16.9 %	14.5	319.8	118.4
	ARPI	18.6	18.8 %	14.7	284	120.8
	GNT3	14.2	30.4 %	61.3	194.2	22.2
	GNT43	11.8	31.6 %	46.2	202.2	26.4
	MxG	7.3	24.0 %	37.1	181.9	17.0
	SAC	11.9	17.6 %	32.6	188.4	44.0
	HSD	2.2	4.40 %	5.0	16.8	8.7

Mediterranean area, characterised by hot, dry summers with high evaporative demand, followed by cooler, rainy winters (Cos et al., 2022). The precipitation pattern and amount were more favourable during the first and the second year after the establishment of the crop, whereas the third year was affected by lower-than-average precipitation.

4.1. Morphological and productive traits

Results from this study confirmed that all the examined genotypes responded positively to irrigation, showing significantly higher leaf and stem biomass. During the early years after establishment, BPG biomass yields were further constrained by a shallow root system and limited rhizome reserves, which reduced canopy expansion (Curt et al., 2017; Kantola et al., 2022; Zegada-Lizarazu et al., 2010b). After the establishment phase, AGB yield reached its maximum and usually stabilised within three to four years (Alexopoulou et al., 2015; Kantola et al., 2022; Kering et al., 2012). These findings support the hypothesis that reduced

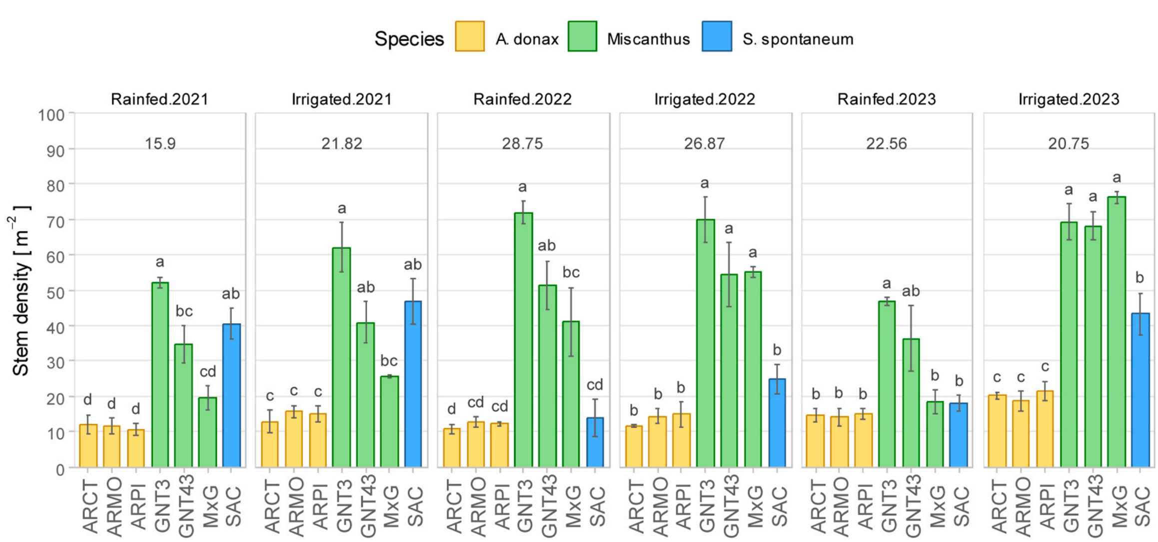

Fig. 3. Stem density of the seven genotypes examined in this study (ARCT, ARMO, ARPI, GNT3, GNT43, MxG, SAC), over the years 2021, 2022, and 2023 under two irrigation conditions. Tukey's Honestly Significant Difference (HSD) values are reported for pairwise genotype comparisons at each level of the year × irrigation interaction.

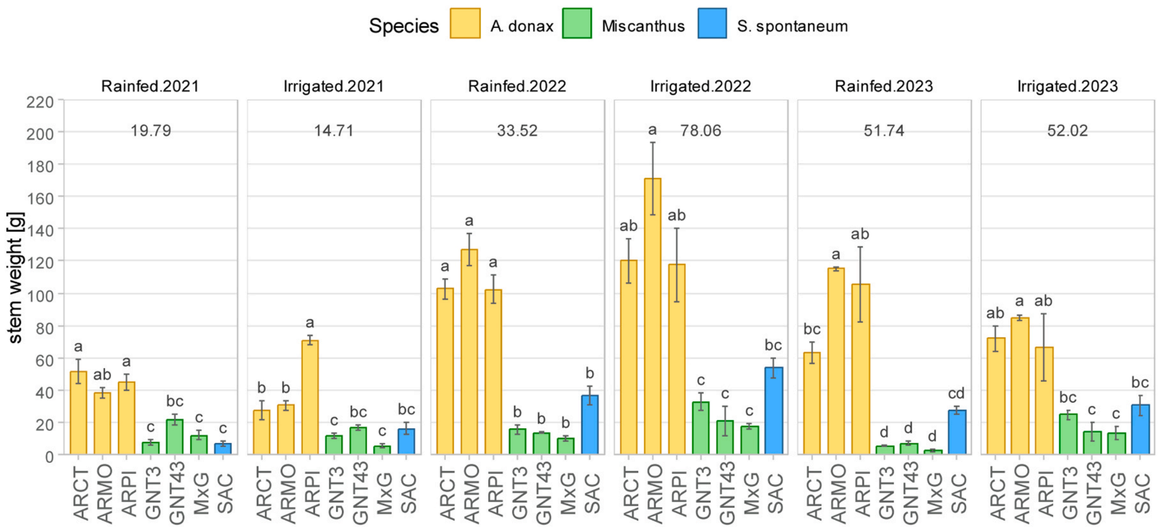

Fig. 4. Stem weight of the seven genotypes examined in this study (ARCT, ARMO, ARPI, GNT3, GNT43, MxG, SAC), over the years 2021, 2022, and 2023 under two irrigation conditions. Tukey's Honestly Significant Difference (HSD) values are reported for pairwise genotype comparisons at each level of the year × irrigation interaction.

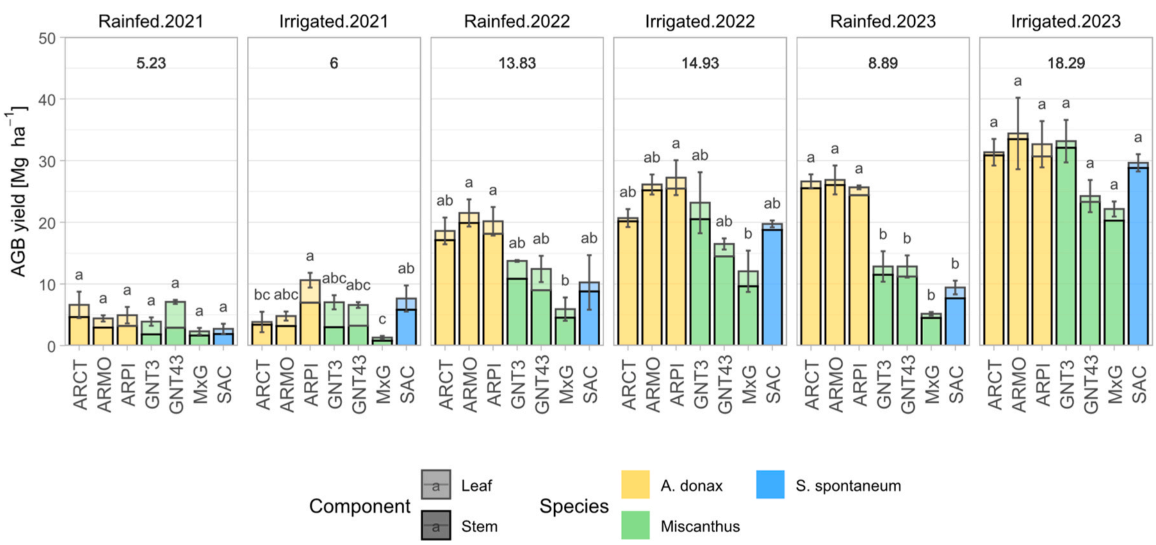

Fig. 5. AGB yield and composition (stems and leaves) of the seven genotypes tested in this study (ARCT, ARMO, ARPI, GNT3, GNT43, MxG, SAC), over the years 2021, 2022, and 2023 under rainfed and full irrigated conditions. Tukey's Honestly Significant Difference (HSD) values are reported for pairwise genotype comparisons at each level of the year × irrigation interaction.

soil moisture under rainfed conditions limits plant growth, particularly in environments with prolonged summer droughts (Scordia et al., 2022). Consequently, optimizing water use and adopting drought-tolerant genotypes remain essential to mitigate water source depletion and competition with food crops (Cai K. et al. 2020). Among the tested species, *Arundo donax* clones, originating from the Mediterranean area, showed smaller yield differences between irrigated and rainfed conditions than *Miscanthus* hybrids, consistent with previous findings (Haworth et al., 2019). In contrast, *M.* × *giganteus* was more sensitive to low soil moisture, exhibiting a pronounced reduction in AGB yield under rainfed conditions. This reduction was expected, as *M. x giganteus* (MxG) is native to humid East Asian regions and poorly adapted to the dry Mediterranean conditions (Ben Fradj et al., 2020). A similar reduction in AGB under rainfed conditions was also observed in *S. spontaneum* during the third year after establishment, likely due to below-average precipitation, contrasting with previous studies (Corinzia et al., 2023; Scordia et al., 2022). The *Miscanthus* hybrids selected for drought tolerance (GNT3 and GNT43) (Krzyżak et al., 2023) outperformed *S. spontaneum* under rainfed conditions and achieved AGB yields similar to *A. donax* under irrigated conditions.

Biomass yield differences among the tested PBG were mainly driven by the efficiency of the photosynthetic apparatus and Leaf Area Duration (LAD) under non-limiting water conditions (Jones et al., 2015), while Water Use Efficiency (WUE) became dominant under limiting water conditions (Scordia et al., 2022). Local clones of *A. donax* and *S. spontaneum* have been shown in previous studies to maintain high Radiation Use Efficiency (RUE) and WUE in the Mediterranean climatic zone under both limiting and non-limiting water and nitrogen inputs (Corinzia et al., 2023; Scordia et al., 2022; Scordia, Papazoglou, et al., 2022). The high RUE and WUE of *A. donax* are linked to its superior photosynthetic performance under both irrigated and rainfed conditions, supported by its capacity for both RuBP-limited and RuBP-saturated photosynthesis, comparable to that of C4 plants, its effective stomatal control, and its extensive root system that accesses deep soil moisture (Haworth et al., 2017a, 2017b, 2018, Sánchez et al., 2021). In contrast, *M.* x *giganteus* maintains high photosynthetic rates

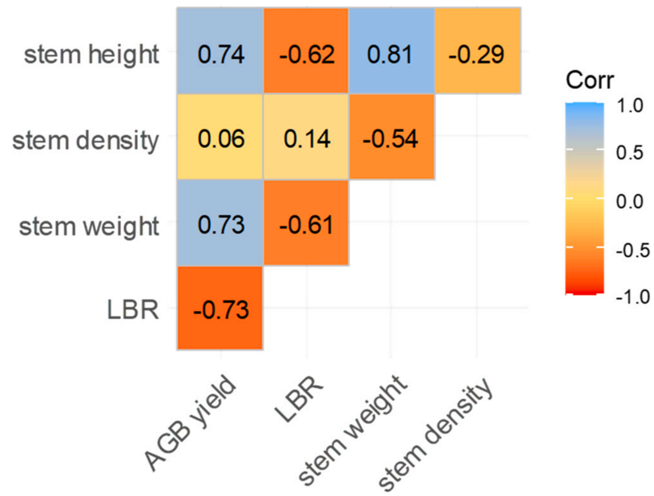

Fig. 6. Pearson correlation matrix for the measured variables (stem height, stem density, stem weight, LBR = Leaf to Biomass Ratio and AGB yield = Above Ground Biomass). Correlation values ≥ 0.197 or ≤ - 0.197 are significant at the 95 % confidence level in a two-tailed test.

under irrigation but is more sensitive to drought, partly due to poor stomatal control (Malinowska et al., 2020), which accelerates leaf senescence.

Both *A. donax* and *S. spontaneum* exhibit an extended growing season and high LAD (Haworth et al., 2019, Corinzia et al., 2023), allowing them to absorb more water and nutrients and assimilate CO_2 for a longer period. This resulted in higher biomass accumulation compared to *M.* x *giganteus*, which has a shorter growing season. Earlier sprouting and prolonged canopy maintenance are key factors in maximizing growth and productivity, particularly in environments with limited water and nutrients (Scordia et al., 2014). Leaf biomass represented a smaller fraction of total biomass, regardless of year or irrigation conditions. The fraction of leaf biomass relative to stem biomass was higher during the first year and tended to decrease over time. This pattern suggests that plants initially allocated most of the photosynthate to leaf development when the amount of nutrient from rhizome reserves and photosynthesis was limited, while allocated the extra photosynthate to stem growth when the nutrient availability exceeded the demand for the leaf area expansion. This finding confirmed the patterns reported by Corinzia et al. (2023). Furthermore, crops may adopt strategies that prioritise resilience over rapid growth by investing in deeper, more extensive root systems, which keep the plant well anchored and promote optimal water uptake (Sofi et al., 2017). Consequently, the leaf to stem ratio was higher under rainfed conditions across most genotypes, especially in *Miscanthus* hybrids, reflecting limited photosynthate availability and reduced stem growth. Under severe water stress, BPGs may further reduce leaf production and allocate resources in larger root system to access deeper soil water reserves deeper (Yan et al., 2023).

High stem biomass yield in BPGs can result either from the emission of a high number of stems per plant (i.e. stem density), as observed in *Miscanthus*, or from the production of fewer, larger stems, as observed in *A. donax*. Stem density was promoted by irrigation, although the response to water availability varied significantly among genotypes. The difference between stem density under rainfed and irrigated conditions was higher for *Miscanthus* hybrids in 2023, whereas *A. donax* and *S. spontaneum* maintained stable stem density under drought conditions. This trend confirms what had previously been observed regarding the drought resistance of these plants (Li et al., 2021; Sánchez et al., 2021).

Variation in stem weight between rainfed and irrigated conditions was generally low, particularly for *A. donax* clones. Stem weight peaked during the second year for *A. donax* and *S. spontaneum*, suggesting that the increase in AGB in the third year was mainly driven by higher stem density. In contrast, the stem weight of *Miscanthus* hybrids increased during the third year.

4.2. Biomass composition

Biomass composition showed low variability between different irrigation conditions, suggesting stability of chemical composition even under water stress. Consistent with previous studies, lignin and cellulose contents remained stable despite water stress (Hussain et al., 2021), and hemicellulose levels were similarly unaffected, preserving the structural integrity of cell walls (Allahdadi and Bahreininejad, 2019). The neutral detergent soluble fraction (NDS) and ash content were unaffected by water stress in prior studies (Hussain et al., 2021; Piccitto, 2022); however, in the present experiment they represented the most variable components across irrigation treatments. Specifically, *S. spontaneum* and *Miscanthus* GNT43 exhibited higher soluble fraction (NDS and ash) under irrigated conditions, whereas other genotypes showed the opposite trend. Overall, biomass quality for energy conversion remained stable across irrigation regimes and between years, suggesting that

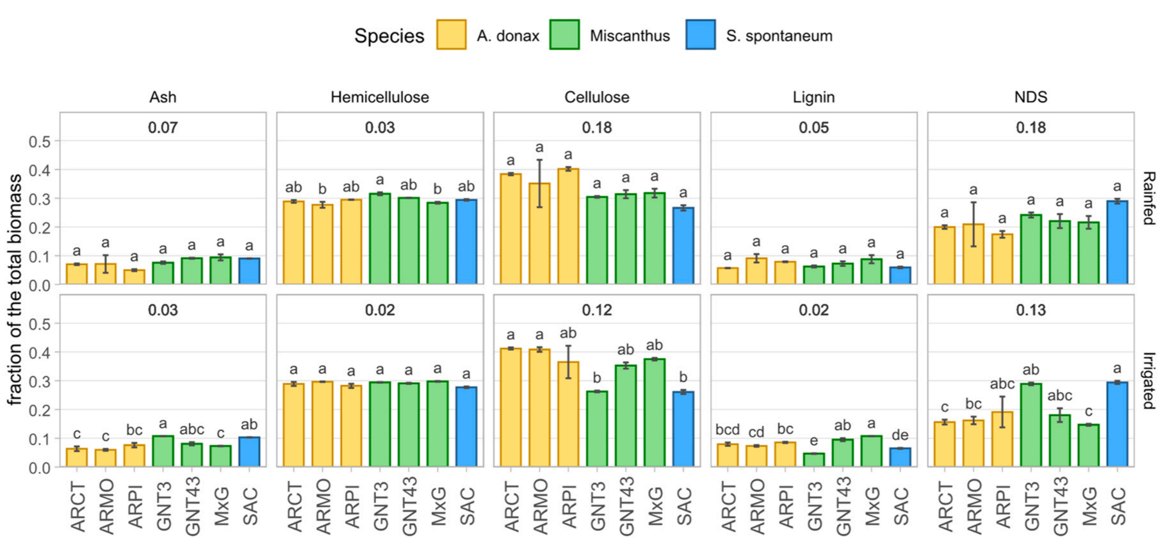

Fig. 7. Biomass fractions for the seven genotypes examined in this study (ARCT, ARMO, ARPI, GNT3, GNT43, MxG, SAC), in the year 2022 under two irrigation conditions in 2022. NDS = Neutral Detergent Soluble fraction. Tukey's Honestly Significant Difference (HSD) values are reported for pairwise genotype comparisons at each level of the irrigation factor.

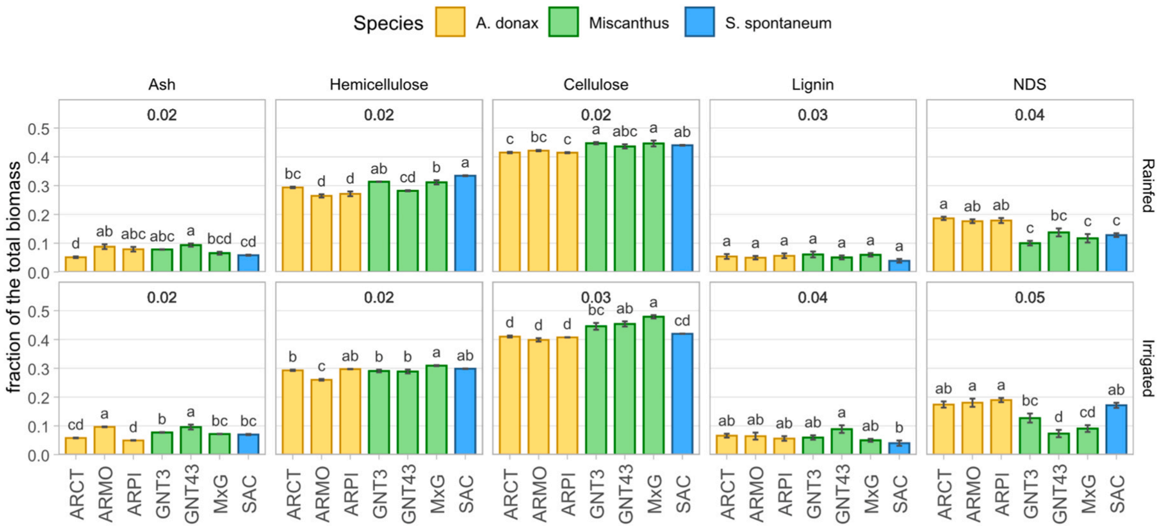

Fig. 8. Biomass of the biomass for the seven genotypes examined in this study (ARCT, ARMO, ARPI, GNT3, GNT43, MxG, SAC), in the year 2023 under two irrigation conditions in 2023. NDS = Neutral Detergent Soluble fraction. Tukey's Honestly Significant Difference (HSD) values are reported for pairwise genotype comparisons at each level of the irrigation factor.

water limitation did not compromise feedstock suitability.

5. Conclusions

This study investigated the performance of perennial bioenergy grasses under contrasting water availability during the early years after establishment, aiming to identify drought tolerant and water-efficient genotypes capable of maintaining biomass production under reduced water supply. The results of the present study confirmed that water availability strongly influences biomass yield, particularly in the early stages of crop development. All genotypes responded positively to irrigation, showing significant increases in both stem and leaf biomass. However, notable interspecific differences emerged. *Arundo donax* and *Saccharum spontaneum* demonstrated greater drought tolerance, maintaining relatively stable aboveground biomass (AGB) and stem density under rainfed conditions. In contrast, *Miscanthus* × *giganteus* was more sensitive to water stress, exhibiting a pronounced reduction in AGB under rainfed conditions. The *Miscanthus* hybrids (GNT3 and GNT43) selected for drought tolerance outperformed *S. spontaneum* under rainfed conditions and achieved biomass production similar to *A. donax* under irrigation, suggesting their potential as suitable alternatives. Beyond yield, biomass composition remained relatively stable across irrigation regimes. This suggests that drought does not significantly compromise biomass quality for energy conversion, particularly regarding lignin, cellulose, and hemicellulose content. These findings highlight the relevance of genotype selection in designing low-input, water-efficient biomass production systems suited to Mediterranean environments. While acknowledging the fundamental role of irrigation in biomass production within the Mediterranean basin, future strategies should prioritize the prudent management of water resources. This includes adopting agricultural practices that minimise water consumption, such as cultivating drought tolerant crops, optimising irrigation techniques, and selecting species better adapted to water deficit conditions. Further research is required to deepen understanding of water use efficiency, drought resistance and biomass composition among different genotypes and across varying environmental conditions. Understanding how these crops respond to fluctuations in water availability over extended periods and under varying climate scenarios will be essential for developing sustainable agricultural systems in water scarce regions.

CRediT authorship contribution statement

Elena Crapio: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Sebastiano Andrea Corinzia:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Danilo Scordia:** Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Simona Giannoccaro:** Writing – original draft, Investigation. **Salvatore Luciano Cosentino:** Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis. **Giorgio Testa:** Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

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