







## Article

# Sustainable Cotton Production in Sicily: Yield Optimization Through Varietal Selection, Mycorrhizae, and Efficient Water Management

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## Abstract

This study explores the revival of cotton (*Gossypium* spp. L.) farming in Italy through sustainable practices, addressing economic and water-related challenges by integrating cultivar selection, arbuscular mycorrhizal fungi (AMF) inoculation, and deficit irrigation under organic farming. Field trials evaluated two widely grown Mediterranean cultivars (Armonia and ST-318) under three irrigation levels (I-100: 100% crop water requirement; I-70: 70%; I-30: 30%) across two Sicilian soil types (sandy loam vs. clay-rich). Under I-100, lint yields reached 0.99 t ha<sup>-1</sup>, while severe deficit (I-30) yielded only 0.40 t ha<sup>-1</sup>. However, moderate deficit (I-70) maintained 75–79% of full yields, proving a viable strategy. AMF inoculation significantly enhanced plant height (68.52 cm vs. 65.85 cm), boll number (+22.1%), and seed yield (+12.5%) ( $p < 0.001$ ). Cultivar responses differed: Armonia performed better under water stress, while ST-318 thrived with full irrigation. Site 1, with higher organic matter, required 31–38% less water and achieved superior irrigation water productivity (1.43 kg m<sup>-3</sup>). Water stress also shortened phenological stages, allowing earlier harvests—important for avoiding autumn rains. These results highlight the potential of combining adaptive irrigation, resilient cultivars, and AMF to restore sustainable cotton production in the Mediterranean, emphasizing the importance of soil-specific management.

**Keywords:** sustainable cotton cultivation; water management; mycorrhizal fungi; organic cotton; Mediterranean cotton



Academic Editor: John P. Thompson

Received: 9 July 2025

Revised: 3 August 2025

Accepted: 4 August 2025

Published: 6 August 2025

**Citation:** Vitale, G.S.; Iacuzzi, N.; Tortorici, N.; Indovino, G.; Franco, L.; Mosca, C.; Giovino, A.; Scavo, A.; Lombardo, S.; Tuttolomondo, T.; et al. Sustainable Cotton Production in Sicily: Yield Optimization Through Varietal Selection, Mycorrhizae, and Efficient Water Management.

*Agronomy* **2025**, *15*, 1892. <https://doi.org/10.3390/agronomy15081892>

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## 1. Introduction

Cotton (*Gossypium* spp. L.) is an annual open-field crop belonging to the *Malvaceae* family and represents one of the most important industrial crops in the world. With a cultivated area of 31.92 million ha<sup>-1</sup> across 80 countries and an estimated annual production of 25.51 million tons of fiber, cotton plays a crucial role in global agriculture [1]. Despite

its economic significance, cotton cultivation faces major sustainability challenges, primarily due to high water consumption, intensive pesticide use, and soil degradation. The increasing global demand for cotton, coupled with environmental concerns, underscores the need for efficient water management and innovative agricultural practices. Cotton cultivation is highly water-intensive, requiring between 700 and 1200 mm of water during the growing season, depending on the growing region [2,3]. Despite its drought tolerance, water remains critical for cotton growth, and seed cotton yield can be significantly enhanced through effective irrigation management [4]. It is estimated that global agricultural water consumption amounts to  $8053.6 \text{ km}^3 \text{ year}^{-1}$ , representing 87% of total global water consumption. Cotton production alone accounts for 2.6% of total freshwater use [5], and approximately 70% of the world's cotton-growing areas experience water stress [6].

In Mediterranean regions—including Sicily, Andalusia, Greece, and Turkey—cotton cultivation occurs under hot, and arid summers [7]. Among these regions, Sicily emerges as a critical case study due to its compounding hydroclimatic stressors. Climate models project a 10–17% increase in reference evapotranspiration ( $ET_0$ ) by 2091, with inland areas (e.g., Enna and Caltanissetta) facing the most severe intensification (+16.71%) [8]. These projections suggest reduced water availability coinciding with peak irrigation demand.

This issue is further exacerbated by the use of low-efficiency irrigation techniques. Currently, the most widely used irrigation systems in cotton cultivation are furrow and sprinkler irrigation, which have low efficiency and significantly increase water waste compared to more advanced systems such as drip irrigation [9–11].

The growing awareness of sustainability has led to an increase in the demand for organic cotton, driven by consumer requests for products made with sustainable production practices [12]. Although cotton accounts for only 2.4% of the world's arable land [13], it consumes 11% of total pesticide use, a figure that reaches 50% in developing countries [14]. According to the latest data provided by the United States Department of Agriculture [15], cotton ranks as the third-highest pesticide-consuming commodity crop in the United States, with about  $4 \text{ kg ha}^{-1}$  of pesticides applied annually. Herbicides are utilized in 96% of cotton-growing areas, with glyphosate being the predominant herbicide used [15]. Notably, the use of glyphosate in cotton production has doubled over the past decade [15]. This trend raises critical concerns due to several factors, particularly weed resistance [16], growth inhibition of some soil microbiome species [17,18], and potential risks to the environment and human health [19,20].

According to the latest USDA estimates, farmers applied an average of  $270 \text{ kg ha}^{-1}$  of synthetic fertilizer to cotton in the 2022 growing season. Nitrogen in particular was applied in 71% of the cultivated areas [15]. Globally, nitrogen consumption in cotton is estimated to range from 300 to  $450 \text{ kg ha}^{-1}$  [21,22]. This high level of fertilizer application poses a serious threat to crop sustainability and contributes to both direct and indirect greenhouse gas emissions [23]. Phosphorus (P), a nutrient classified as a non-renewable resource, is experiencing global depletion, leading to increased prices in recent years [24]. Moreover, most P fertilizers have low mobility in soils due to their strong adsorption to iron and aluminum cations in acidic soils and to calcium in alkaline conditions [25]. Sicilian soils (Typic Xerorthents/Calcixererts) differ significantly from those in other Mediterranean cotton-producing areas, being more alkaline (pH 7.5–8.2), poorer in organic matter (<1.5%), and richer in calcium carbonate (>25%) [26]. These characteristics reduce phosphorus availability compared to the alluvial soils of Çukurova (Turkey) or the Vertisols of Andalusia (Spain) [27,28].

A promising solution to this challenge lies in soil microorganisms, particularly arbuscular mycorrhizal fungi (AMF). Cotton is a mycorrhizal-responsive plant [29] and up to 90% of cotton root length can be colonized by AMF [30]. These fungi play a cru-

cial role in enhancing nutrient acquisition by effectively exploring larger volumes of soil, thereby significantly improving plant uptake of nutrients, especially P [31]. Among these, certain species such as *Funneliformis mosseae* are particularly effective in nutrient uptake, showing increases in P uptake of up to 110% [32–35]. Moreover, mycorrhizae exhibit a highly favorable response within the Sicilian production area, as extensively documented in the literature [11,36].

In Italy, the cultivation of cotton has gradually disappeared from the landscape. Today, despite the presence of only a few hundred hectares dedicated to this crop, there is no official data on its cultivation from Italian statistical sources [37]. The decline in cotton production can be attributed to several factors. First and foremost, agronomic problems, such as lower yields in non-irrigated conditions or with inefficient irrigation systems, together with high fertilizer and pesticide costs, have played a significant role. Economic challenges also contribute to this decline, including the low cash value of cotton production—around 1.86 EUR kg<sup>-1</sup> [13]—as well as labor shortages and rising labor costs [38].

Based on the above considerations, this study aims to examine specific aspects of agronomic techniques for the restoration of cotton cultivation in Sicily (Southern Italy). To achieve this objective, two upland cotton cultivars (*Gossypium hirsutum* L.), currently cultivated in the Mediterranean basin, were grown in two Sicilian environments. The study implemented organic farming principles and utilized mycorrhizal formulations containing *Funneliformis mosseae*, along with high-efficiency irrigation systems. Consequently, the purpose of this preliminary work was to understand the bio-agronomic behavior of cotton in Sicily, subjected to three different levels of crop water requirement, while ensuring the sustainability of water and soil resources, in accordance with organic farming principles.

## 2. Materials and Methods

### 2.1. Experimental Site

The experimental trial was conducted in 2023 at two sites in Sicily, Italy. The first site was the experimental farm “Campo Carboj” (Site 1) owned by the Agricultural Development Authority (Ente di Sviluppo Agricolo—ESA) of the Sicilian Region, located in Castelvetro (TP) in the Belice area (37°35′18.0″ N, 12°53′44.0″ E, 65 a.s.l.). The second site was the experimental farm of the University of Catania (Site 2), situated in Primosole (CT) (37°24′ N, 15°03′ E, 10 m a.s.l.). Chemical and physical characteristics of the two soils are shown in Table 1.

**Table 1.** Characteristics of the upper layer (0–50 cm) at Site 1 and Site 2.

Soil Characteristics	Unit	Value		Method
		Site 1	Site 2	
Sand	%	59	16.6	[39]
Loam	%	13	27.8	[39]
Clay	%	28	55.6	[39]
N total	g kg <sup>-1</sup>	1.3	1	Kjeldahl [40]
P	mg kg <sup>-1</sup>	9.16	2.18	Ferrari [41]
K	mg kg <sup>-1</sup>	112.9	203.3	Dirks and Scheffer [41]
Organic matter	%	1.46	1.1	Walkley and Black [41]
Electrical Conductivity	mS/cm	0.8643	0.15	[42]
Cation Exchange Capacity (CEC)	meq/%	27.05	14.8	[43]
pH		7.4	7.6	In water solution
Bulk Density	t m <sup>3</sup>	1.16	1.2	[44]
Field Capacity at −0.03 MPa	%	27.5	27	[45]
Wilting Point at −1.5 MPa	%	16.7	11	[46]

## 2.2. Experimental Design and Crop Management

The experimental trials at Sites 1 and 2 followed a split-plot design with three replications. The main plot factor consisted of three irrigation regimes: full irrigation (I-100), moderate deficit irrigation (I-70), and severe deficit irrigation (I-30). The subplot factor involved the application of a microbial biostimulant (Rizoplant, Biogard<sup>®</sup>, Grassobbio, Italy) during two phenological phases (BBCH-13 and BBCH-16) using a fertigation system. The sub-subplot factor included the evaluation of two *Gossypium hirsutum* L. varieties, ST-318 and Armonia. Each plot measured 16 m<sup>2</sup> (4 m × 4 m).

## 2.3. Irrigation Management

The irrigation system was designed with P5<sup>®</sup> dripline (Irritec S.p.A., Capo d'Orlando (ME), 98071, Italy) with emitters characterized by nominal flow rates of 2.1 L h<sup>-1</sup> at a pressure of 100 kPa. A self-compensating drip irrigation system was implemented along each row. The emitters were spaced 0.20 m apart and had a flow rate of 2 L h<sup>-1</sup>. Additionally, volumetric flow meters were used to monitor the volumes distributed for each irrigation scheduling method.

Differentiated irrigation began upon reaching the sixth true leaf (BBCH-16) to ensure that all factors successfully passed the emergence stage.

To determine the hydrological constants, each plot was sampled using a random sampling method at a depth ranging from 20 to 50 cm. The soil water content corresponding to field capacity (FC) was measured using pressure plate extractors [46] at a tension of −1 m. For each treatment, three samples were prepared by compacting the soil fraction through a 2 mm sieve into 5 × 1 cm samplers, based on the bulk density values measured from undisturbed soil [44]. Equilibrium with the applied tension was assumed when the samples ceased to drain for at least 24 h. The volumetric water content at equilibrium was determined using the thermogravimetric method after drying the samples in an oven at 105 °C for 24 h. All measurements were conducted under controlled conditions, with the temperature set to 22 ± 1 °C. After establishing the hydrological constants for the experimental sites indicated in Table 1, daily reference evapotranspiration (ET<sub>0</sub>) was calculated using the Penman–Monteith equation. Crop water consumption was estimated, for each phenological stage, by multiplying ET<sub>0</sub> by crop coefficients (K<sub>c</sub>) defined in FAO Irrigation and Drainage Paper No. 56 [47]. Additionally, the soil depth explored by the roots was considered in the calculation of available water quantity (Table 2).

**Table 2.** Phenological phases and K<sub>c</sub> values for cotton according to Allen et al. [47].

Phase	Description	K <sub>c</sub>	Depth of Soil Explored by Roots (cm)
Initial	Germination: from dry seed (00) to emergence of hypocotyl with cotyledons (09)	0.4–0.5	30
Development	Leaf development: from cotyledons completely unfolded (10) to canopy closure (39)	0.7–0.8	50
Mid-season	Inflorescence emergence: from first detectable bud (51) to about 90% of capsules having reached their final size (79)	1.05–1.25	50
End-season	Senescence: from about 10% of discolored or abscessed leaves (91) to above-ground parts of dead plants	0.65–0.70	50

Irrigation water productivity (IWP,  $\text{kg ha}^{-1} \text{mm}^{-1}$ ) was calculated following the methodology described by [48,49], using the following equations (Equation (1)):

$$IWP = \frac{\text{Yield}}{I} \quad (1)$$

where “Yield” represents the crop production, and “I” denotes the total amount of irrigation water applied.

#### 2.4. Microbial Biostimulants Management

The application of the microbial biostimulant was carried out at both sites using a fertigation system during two phenological phases. The first application occurred upon reaching the third true leaf stage (BBCH-13), while the second application was made at the sixth true leaf stage (BBCH-16).

The microbial biostimulant used was a commercial product (Rizoplant, Biogard<sup>®</sup>, CBC Europe S.p.A., Grassobbio, Bergamo, Italy), formulated on an inert carrier based on clay and peat. It contains  $1.35 \times 10^3$  propagules  $\text{g}^{-1}$  of arbuscular mycorrhizal fungi, specifically *Funneliformis mosseae*, *Funneliformis caledonium*, and *Rhizoglyphus irregulare*. The formulation also includes rhizosphere bacteria at a concentration of  $10^4$  CFU  $\text{g}^{-1}$ . The product was applied at a rate of  $0.25 \text{ kg ha}^{-1}$  per application.

#### 2.5. Variety Selection

Two varieties of *Gossypium hirsutum* L., ST-318 and Armonia, were evaluated at the two experimental sites. These cultivars were selected as non-GMO varieties widely cultivated in the Mediterranean region [50,51].

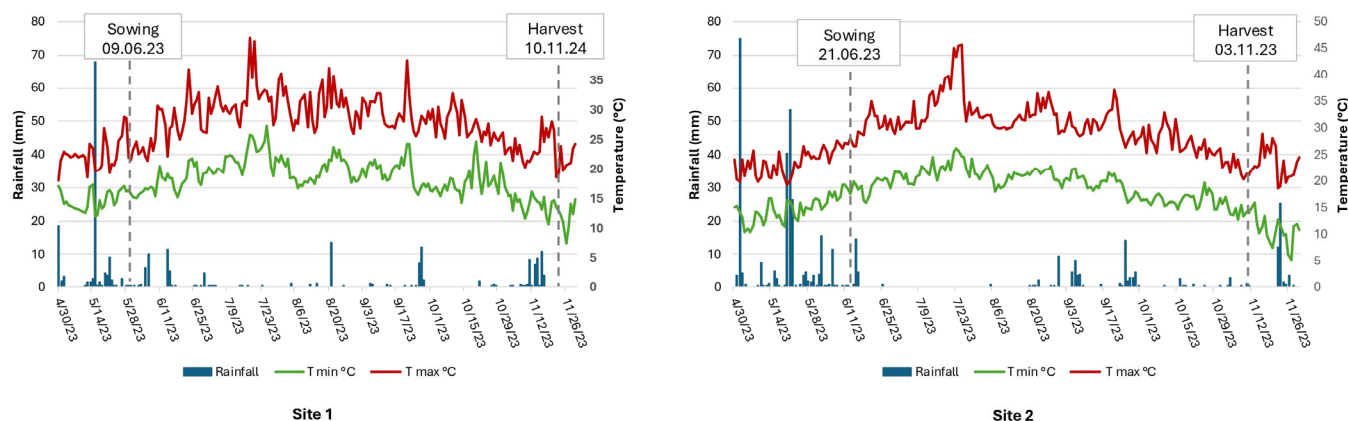
#### 2.6. Agronomic Management

The trial was conducted under organic management, and the soil at both experimental sites had undergone a fallow period in the previous year. Soil was plowed to a depth of 35 cm and tilled twice before sowing. In Site 2, seeding was performed on 29 May 2023, while in Site 1 it was performed on 9 June 2023. This shift in sowing was due to rainfall events that caused a delay in sowing at Site 1. The adopted plant density was  $12 \text{ plants m}^{-2}$  (in-row spacing: 0.08 m; inter-row spacing: 1.0 m). Weed control in the inter-row areas was carried out with a mower during the leaf development phase. Harvesting was conducted when approximately 90% of the bolls had opened (BBCH 89) and was performed manually. At Site 1, harvesting occurred on 10 November 2023, while at the Site 2, it took place on 3 November 2023. Fertilization was carried out before sowing and again just before the stem elongation phase, with a total application of  $110 \text{ kg ha}^{-1}$  of nitrogen (N),  $24 \text{ kg ha}^{-1}$  of phosphorus (P) and  $46 \text{ kg ha}^{-1}$  of potassium (K) as organic fertilizer. The base fertilization was applied in the form of pellets, containing 10% N, 2.2% P and 4.2% K (ORGA-KEM, Biolchim<sup>®</sup>). For surface fertilization, two applications of liquid organic nitrogen were administered during the phenological phases corresponding to the appearance of the first true leaves and flowering, using a liquid fertilizer with a concentration of 8.7% N (Tamarack, Growan Italia<sup>®</sup>, Faenza (RA), Italy). For both sites, no pest or disease control measures were applied during the trials.

#### 2.7. Weather Data

Rainfall and temperature data were collected from meteorological stations belonging to the Sicilian Agrometeorological Information Service 2024, located near the two experimental sites. The stations were equipped with an MTX datalogger (model WST1800) and various climatic sensors. Specifically, an MTX temperature sensor (TAM platinum PT100 heat-resistant model with radiation shield) and an MTX rainfall sensor (PPR model with

tipping bucket rain gauge) provided data on daily average maximum and minimum air temperatures (°C) and total daily rainfall frequency (mm). The thermo-pluviometric data are shown in Figure 1.



**Figure 1.** Temperature and precipitation trends at the two experimental sites of Site 1 (Campo Carboj) and Site 2 (Primosole) from April 2023 to November 2023.

### 2.8. Data Collection

#### 2.8.1. Growth Stage Data

The main growth stages of the two cultivars were determined according to the extended BBCH scale [52]: emergence (70% of plants with fully unfolded cotyledons), beginning of flowering (50% of plants with at least one open flower), and beginning of boll opening (50% of plants with at least one open boll). Growth degree days (GDDs) were also determined for each phenological stage of the two cultivars according to Equation (2):

$$GDD = \frac{(Tmin + Tmax)}{2} - Tb \tag{2}$$

where Tmin and Tmax are the daily minimum and maximum air temperatures, and Tb is the baseline temperature below which development ceases. The basal temperature for the identified cotton plant is 15.6 °C [53,54]. The accumulated GDDs for each phenological stage were calculated by summing the daily GDDs of each stage [55].

#### 2.8.2. Morphological Data

At harvest, within a delimited area of 2.0 m<sup>2</sup> within each subplot, these parameters were determined: height of the first fruiting branch (cm), plant height (cm), number of open bolls per plant and average weight of bolls after oven drying (g). These morphological measurements were determined at harvest (BBCH-89) at 154 DAS in Site 1 and 135 DAS in Site 2.

#### 2.8.3. Yield Data

Cotton lint was separated from the seeds of the raw material harvested from the parcel unit of each subplot using a laboratory ginning machine. Next, lint and seed yields (t ha<sup>-1</sup>) were determined.

### 2.9. Statistical Analysis

Statistical analyses were performed using the package MINITAB 19 (State College, PA, USA) for Windows. All collected data were subjected to the analysis of variance (ANOVA). The difference between means was analyzed using Tukey’s test ( $p \leq 0.05$ ). To ensure compliance with ANOVA assumptions, all data were tested for sphericity, normality and variance homogeneity through Mauchly’s test ( $\alpha = 0.05$ ), the Ryan–Joiner test ( $\alpha = 0.05$ )

and Levene's test ( $\alpha = 0.05$ ). The graphs were created using the Excel application Office 365 package for Windows.

### 3. Results

#### 3.1. Irrigation Data

Table 3 presents the water volumes applied for the different irrigation levels at the two sites. Rainfall data indicate that Site 1 received a total of 68.34 mm (equivalent to  $683.4 \text{ m}^3 \text{ ha}^{-1}$ ), whereas Site 2 received  $990 \text{ m}^3 \text{ ha}^{-1}$  over the entire vegetative period—a difference of approximately 31% less rainfall at Site 1.

**Table 3.** Water volume supplied (rainfall + irrigation) for irrigation level and phenological stage.

Irrigation Level	Phenological Phase	Site 1		Site 2	
		Rainfall ( $\text{m}^3 \text{ ha}^{-1}$ )	Irrigation ( $\text{m}^3 \text{ ha}^{-1}$ )	Rainfall ( $\text{m}^3 \text{ ha}^{-1}$ )	Irrigation ( $\text{m}^3 \text{ ha}^{-1}$ )
I-30	Initial (BBCH 00–09)	163.4	58.1	589	95
I-30	Development (BBCH 10–50)	92.3	0	17	0
I-30	Mid-season (BBCH 51–79)	365.3	0	335	0
I-30	End-season (BBCH 80–89)	62.4	0	49	0
Total water supplied ( $\text{m}^3 \text{ ha}^{-1}$ )		<b>741.5</b>		<b>1085</b>	
I-70	Initial (BBCH 00–09)	163.4	58.1	589	95
I-70	Development (BBCH 10–50)	92.3	92.2	17	108.9
I-70	Mid-season (BBCH 51–79)	365.3	90.7	335	115
I-70	End-season (BBCH 80–89)	62.4	0	49	0
Total water supplied ( $\text{m}^3 \text{ ha}^{-1}$ )		<b>924.4</b>		<b>1308.9</b>	
I-100	Initial (BBCH 00–09)	163.4	58.1	589	83.2
I-100	Development (BBCH 10–50)	92.3	127.3	17	154.6
I-100	Mid-season (BBCH 51–79)	365.3	141.7	335	250
I-100	End-season (BBCH 80–89)	62.4	0	49	0
Total water supplied ( $\text{m}^3 \text{ ha}^{-1}$ )		<b>1010.5</b>		<b>1477.8</b>	

I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc. Site 1 = Campo Carboj and Site 2 = Primosole.

Despite the lower rainfall, Site 1 required a reduced supplemental water input across all observed phenological phases. Under the I-30 treatment, for example, Site 1 received an additional  $58.1 \text{ m}^3 \text{ ha}^{-1}$  exclusively during the initial phenological phase (BBCH 00–09), compared to  $95 \text{ m}^3 \text{ ha}^{-1}$  at Site 2. This represents nearly 38.8% lower water input in Site 1 under the I-30 regime. Consequently, the total water resources applied (rainfall plus irrigation) amounted to  $741.5 \text{ m}^3 \text{ ha}^{-1}$  at Site 1 and  $1085 \text{ m}^3 \text{ ha}^{-1}$  at Site 2—about 31.7% less at Site 1. Under the I-70 treatment, Site 1 received  $241 \text{ m}^3 \text{ ha}^{-1}$  of supplemental water distributed from the initial phase up to mid-season (BBCH 79), while Site 2 was supplied with  $318.9 \text{ m}^3 \text{ ha}^{-1}$ —approximately 32.3% more water in Site 2 than in Site 1. Finally, under the I-100 treatment, supplemental irrigation was  $327.1 \text{ m}^3 \text{ ha}^{-1}$  for Site 1 and  $487.8 \text{ m}^3 \text{ ha}^{-1}$  for Site 2, indicating that Site 1 required roughly 33% less water than Site 2 under full water restoration conditions.

#### 3.2. Agronomic and Yield Data

Analysis of variance (ANOVA) revealed that the factors site (S), genotype (G), irrigation (I), mycorrhization (M), and their interactions showed significant effects in several cases, as observed in Table 4. In particular, ANOVA indicated a significant effect of G, M, I, and S on plant height, the height of the first fruiting branch, and the average number

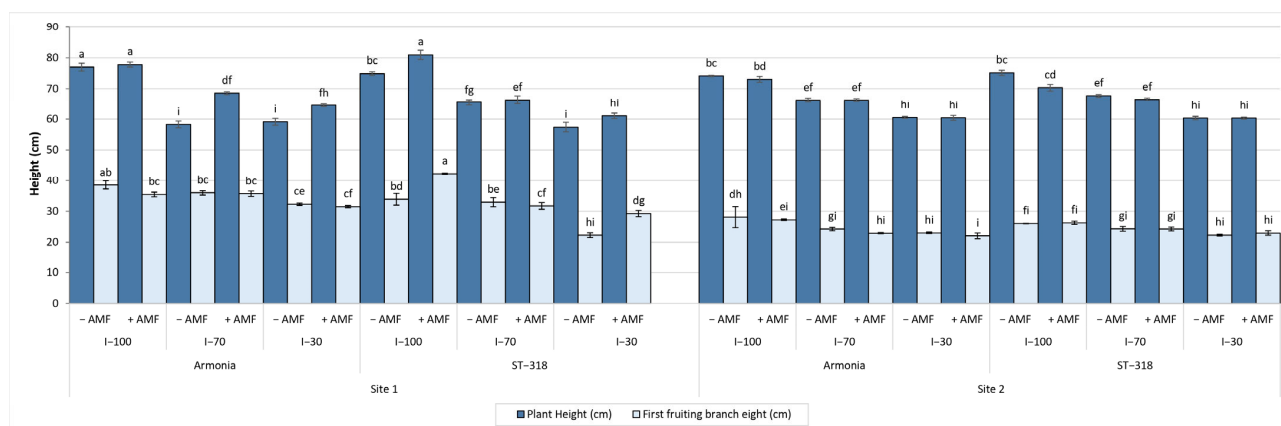
of open capsules per hectare. Additionally, the  $G \times I \times E$  interaction had a significant effect on the average capsule weight, raw yield, and lint yield. The  $M \times I$  interaction was significant only for seed yield.

**Table 4.** ANOVA for main effects and interactions on plant height, first fruiting branch eight, average number of open capsules per hectare, raw yield, lint yield and seed yield.

Source of Variation	df	Parameters						
		Plant Height	First Fruiting Branch Height	Number of Capsules ha <sup>-1</sup> × 10 <sup>-6</sup>	Average Capsule Weight	Raw Yield	Lint Yield	Seed Yield
G	1	0.01 ns	12.17 ***	1.16 ns	10.77 **	22.77 ***	41.18 ***	6.75 *
M	1	54.91 ***	2.3 ns	27.15 ***	18 ***	46.35 ***	42.9 ***	29.98 ***
I	2	591.55 ***	74.6 ***	52.4 ***	205.68 ***	612.07 ***	533.75 ***	418.65 ***
S	1	5.81 *	425.62 ***	5.44 *	6.34 *	10.53 **	3.25 ns	35.48 ***
G × M	1	0.06 ns	12.92 ***	2.05 ns	0.04 ns	0.04 ns	0.24 ns	0 ns
G × I	2	5.91 **	3.49 *	7.66 ***	1.63 ns	6.01 **	10.39 ***	1.95 ns
G × S	1	0.05 ns	8.78 **	2.56 ns	7.42 **	0.03 ns	1.91 ns	1.46 ns
M × I	2	0.42 ns	2.57 ns	1.24 ns	1.26 ns	3.94 *	2.21 ns	3.56 *
M × S	1	25.97 ***	5.42 *	13.57 ***	0.07 ns	0.02 ns	1.86 ns	1.22 ns
I × S	2	28.16 ***	10.24 ***	7.09 **	9.71 ***	1.8 ns	1.37 ns	2.64 ns
G × M × I	2	15.6 ***	3.88 *	3.06 ns	5.82 **	1.02 ns	2.34 ns	0.39 ns
G × M × S	1	8.93 **	12.04 ***	3.89 ns	0.07 ns	0.42 ns	0.48 ns	0.22 ns
G × I × S	2	3.29 *	9.45 ***	9.16 ***	4.92 *	6.27 **	11.86 ***	1.98 ns
M × I × S	2	2.15 ns	1.46 ns	9.3 ***	2.41 ns	1.8 ns	0.32 ns	3.09 ns
G × M × I × S	2	4.89 *	5.74 **	3.74 *	2.49 ns	0.16 ns	1 ns	0.34 ns

Values are given as F of Fisher. \*\*\*, \*\* and \* indicate significant at  $p < 0.001$ ,  $p < 0.01$  and  $p < 0.05$ , respectively. ns = not significant; df = Degrees of Freedom; I = irrigation; G = genotype; S = site; M = mycorrhizae.

The  $G \times M \times I \times S$  interaction had a significant effect ( $p < 0.05$ ) on plant height, the height of the first fruiting branch, and the average number of open capsules per hectare. As shown in Figure 2, the tallest plants were observed in Site 1, particularly for Armonia under the I-100 condition, reaching heights of 77.03 in +AMF and 77.83 cm in -AMF. Additionally, mycorrhized ST-318 under I-100 at Site 1 recorded an average height of 80.97 cm ( $p < 0.05$ ).

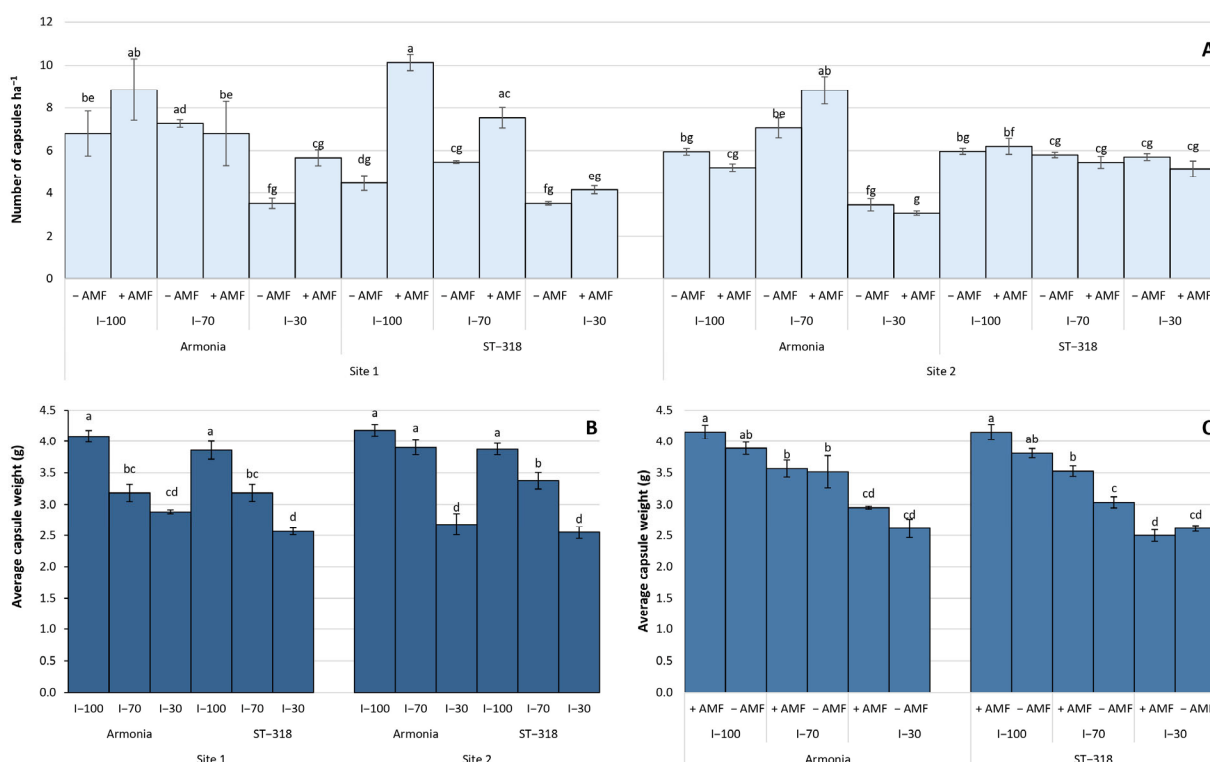


**Figure 2.** Effect of treatments on plant height and the first fruiting branch. For each data series, values with different letters are significantly different at  $p \leq 0.05$ , according to Tukey’s test. Error bars represent standard error. Site (Site 1 = Campo Carboj and Site 2 = Primosole), genotype (Armonia and ST-318), irrigation levels (I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc), and mycorrhization treatment (+AMF = mycorrhized; -AMF = non-mycorrhized). Each treatment was replicated three times. Different letters indicate statistically significant differences between means at  $p \leq 0.05$ .

The shortest plants were also found in Site 1, specifically in  $-AMF$  treatments. In particular, Armonia under I-70 and I-30 recorded heights of 58.3 cm and 59.1 cm, respectively, while ST-318 under I-30 reached 57.4 cm.

Regarding the first fruiting branch height, the  $G \times M \times I \times S$  interaction had a significant effect ( $p < 0.01$ ). The highest values were recorded in Site 1 for the ST-318 variety under I-100 +AMF, with an average of 42.33 cm, while the lowest values were observed in Site 2 for the Armonia variety under I-30 irrigation +AMF, showing a 47.77% reduction (Figure 2).

Figure 3A illustrates the statistically significant effect ( $p < 0.05$ ) of the  $G \times M \times I \times S$  interaction on the number of capsules per hectare. The highest values were recorded at Site 1 under I-100 +AMF, with a mean of  $10.13 \times 10^5$  capsules  $ha^{-1}$ , while the lowest values were observed at Site 2 under I-30 +AMF, with  $3.06 \times 10^5$  capsules  $ha^{-1}$ .



**Figure 3.** Effect of treatments on capsule production and weight. (A) Effect of M, I, G, S on capsule number per hectare. (B) Effect of I, G, S on average capsule weight. (C) Effect of M, I, G on average capsule weight. For each data series, values with different letters are significantly different at  $p \leq 0.05$ , according to Tukey’s test. Error bars represent standard error. Site (Site 1 = Campo Carboj and Site 2 = Primosole), genotype (Armonia and ST-318), irrigation levels (I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc), and mycorrhization treatment (+AMF = mycorrhized;  $-AMF$  = non-mycorrhized). Each treatment was replicated three times. Different letters indicate statistically significant differences between means at  $p \leq 0.05$ .

The  $G \times I \times S$  interaction had a significant effect on average capsule weight, raw yield, and lint yield.

Regarding average capsule weight, the highest statistically significant values ( $p < 0.05$ ) were observed in the treatments under I-100 in both Site 1 and Site 2, with a mean of 4.0 g per capsule (Figure 3B). Similarly, the Armonia variety under I-70 in Site 2 showed values (3.9 g) that were not statistically different from the I-100 treatments. In contrast, the treatments under I-30 in both sites exhibited significantly lower values ( $p < 0.01$ ) compared to the fully irrigated treatments, with an average of 2.67 g, representing a 33.1% reduction.

The  $M \times I \times G$  interaction also had a significant effect on average capsule weight ( $p < 0.01$ ). Both genotypes, Armonia and ST318, under the I100 +AMF treatment showed

the highest values, each with an average capsule weight of 4.15 g. The lowest value was recorded for genotype ST318 under the I30 +AMF treatment, with an average capsule weight of 2.50 g (Figure 3C).

As shown in Figure 4A, the highest raw yield values were recorded in treatments under I-100 irrigation ( $p < 0.01$ ). At Site 1, the ST-318 and Armonia varieties achieved average yields of 2.48 t ha<sup>-1</sup> and 2.33 t ha<sup>-1</sup>, respectively. At Site 2, the highest yields were observed for Armonia and ST-318, with average values of 2.33 t ha<sup>-1</sup> and 2.48 t ha<sup>-1</sup>, respectively. In contrast, the lowest yields were recorded under I-30 irrigation. At Site 1, the Armonia and ST-318 varieties had average yields of 2.51 t ha<sup>-1</sup> and 2.26 t ha<sup>-1</sup>, respectively. Regarding lint yield, as shown in Figure 4A, the highest values were recorded at Site 2 for the Armonia variety under I-100 irrigation, with an average yield of 1.12 t ha<sup>-1</sup> ( $p < 0.01$ ). The lowest values were observed in treatments subjected to severe water stress (I-30) at both sites, with an average yield reduction of 59.62% compared to I-100 treatments.

The  $M \times I$  interaction had a significant effect on seed yield ( $p < 0.05$ ), as shown in Figure 4B. The I-100 +AMF treatment resulted in the highest seed yield, averaging 1.50 t ha<sup>-1</sup>. In contrast, treatments under I-30 showed significantly lower yields, with values of 0.65 t ha<sup>-1</sup> for +AMF and 0.60 t ha<sup>-1</sup> for -AMF, representing a 55.46% reduction compared to the I-100 treatment. Moreover, the  $M \times I$  interaction had a significant effect on raw yield ( $p < 0.05$ ), as shown in Figure 4B. The I-100 +AMF treatment resulted in the highest raw yield, averaging 2.55 t ha<sup>-1</sup>. The lowest values were observed under the I-30 treatment, both with and without AMF, with mean yields of 1.07 and 0.98 t ha<sup>-1</sup>, respectively, representing a reduction of 58% and 61.6% compared to the I-100 +AMF treatment.

Seed yield was also influenced by the main factor S (Figure 4C) ( $p < 0.001$ ). In Site 1, seed yield was significantly higher, reaching 1.09 t ha<sup>-1</sup>, which represents an increase of 13.5% compared to Site 2 (0.96 t ha<sup>-1</sup>).

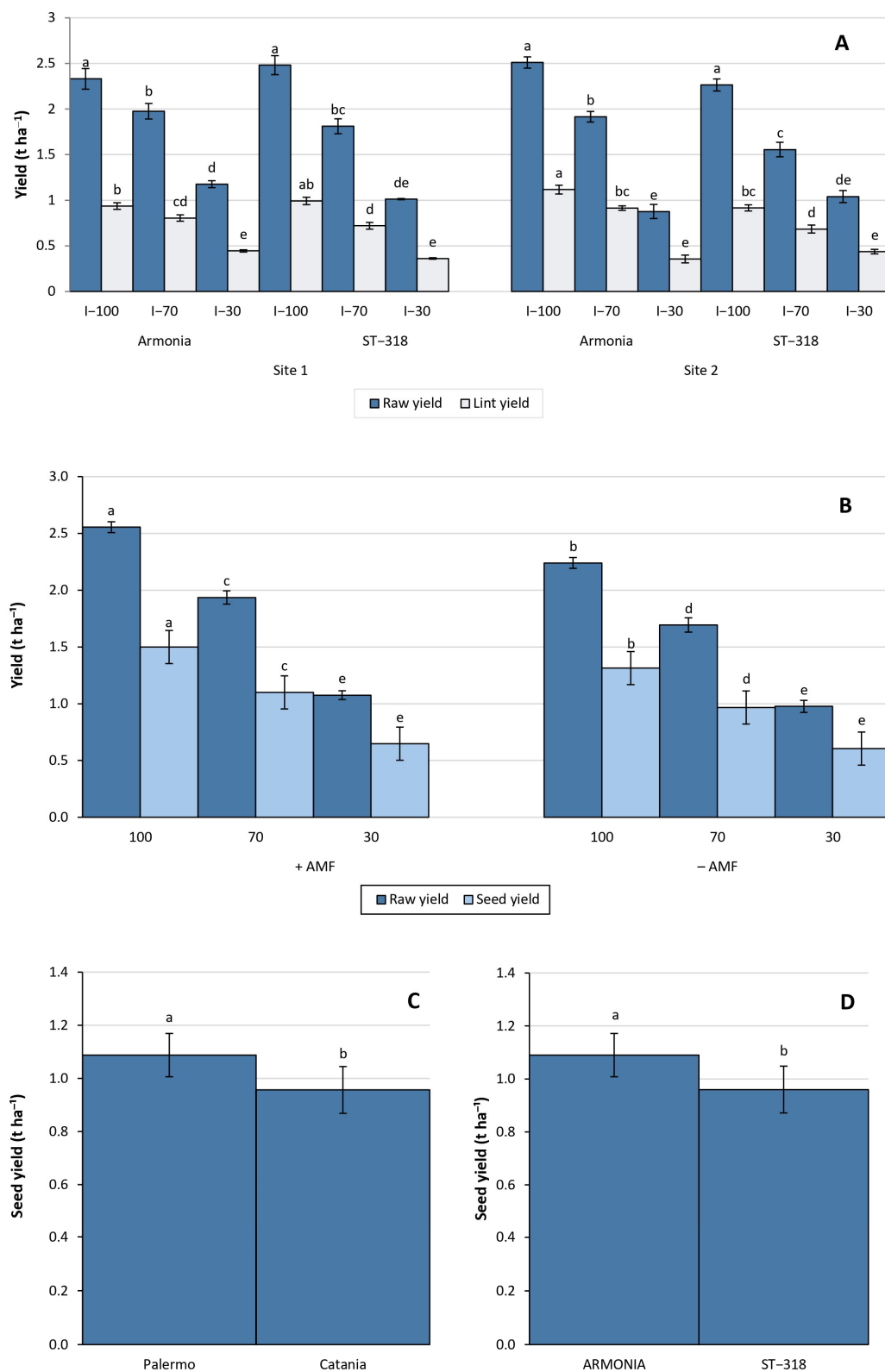
The main factor G also affected seed yield ( $p < 0.05$ ). Armonia showed the highest values, with an average of 1.05 t ha<sup>-1</sup>, which is 6.1% higher than ST-318 (0.99 t ha<sup>-1</sup>) (Figure 4D).

### 3.3. Phenological Data

Table 5 shows the output of the analysis of variance (ANOVA) regarding the duration in days of the phenological stages of cotton. Statistical differences, for the period of sowing–emergence (S–Em), were detected only by the I factor. For the duration of the phenological phases of emergence–flowering (Em–F), statistical differences were detected for the I factors, S, and by the interaction of the factors  $G \times I$ ,  $G \times S$ , and  $I \times S$ . For the duration of the phenological phases of flowering–capsule opening (F–Oc), statistically significant differences were found for the factors I and S and by the interaction  $G \times I$ ,  $G \times S$ ,  $I \times S$  and  $G \times I \times S$ . Finally, for the duration of the phenological phases of capsule opening–flowering (Oc–H), statistical differences were detected for the factors G, M, I, and S and in the interactions  $G \times I$ ,  $I \times S$ ,  $G \times M \times S$ , and  $G \times I \times S$ .

The main effect I was significant ( $p < 0.05$ ) for the S–Em phenological phase (Table 5). Significantly shorter durations were observed under the I-100 regime, with a mean of 18.75 days, while treatments subjected to I-30 and I-70 required on average 17.63 and 17.75 days, respectively, to complete this phenological phase (Figure 5).

During the Em–F phenological phase, the interaction between  $I \times S$  was significant ( $p < 0.05$ ) (Table 5). Under the I-30 regime, both sites showed a marked reduction in duration, averaging 38.58 days in Site 2 and 41.5 days in Site 1. Conversely, the I-100 treatment led to significantly longer phases, with means of 55.16 days in Site 1 (+32.9% vs. I-30) and 52.58 days in Site 2 (+26.6% vs. I-30) (Figure 6A).

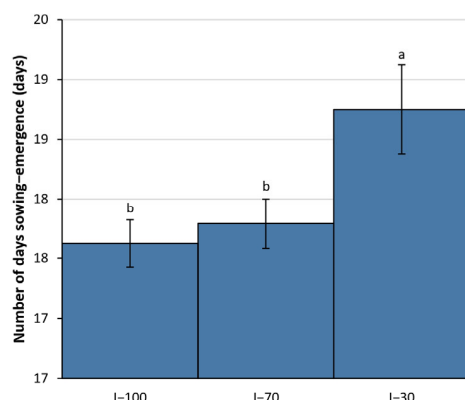


**Figure 4.** Effect of treatments on yield components. (A) Effect of I, G and S treatments on raw and lint yield. (B) Effect of I and M on raw yield and seed yield. (C) Effect of S on seed yield. (D) Effect of G on seed yield. For each data series, values with different letters are significantly different at  $p \leq 0.05$ , according to Tukey’s test. Error bars represent standard error. Site (Site 1 = Campo Carboj and Site 2 = Primosole), genotype (Armonia and ST-318), irrigation levels (I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc), and mycorrhization treatment (+AMF = mycorrhized; –AMF = non-mycorrhized). Each treatment was replicated three times. Different letters indicate statistically significant differences between means at  $p \leq 0.05$ .

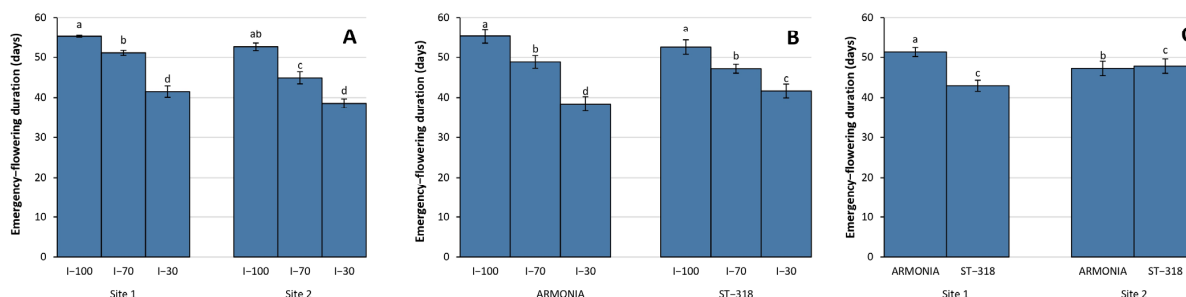
**Table 5.** ANOVA for main effects and interactions on the duration of cotton phenological stages.

Source of Variation	Parameters				
	df	S–Em	Em–F	F–Oc	Oc–H
G	1	0.27 n.s.	0.39 n.s.	3.88 n.s.	8.57 **
M	1	0.12 n.s.	0.96 n.s.	1.86 n.s.	14.56 ***
I	2	4.83 *	164.94 ***	54.57 ***	237.74 ***
S	1	0.76 n.s.	38.81 ***	28.12 ***	11.98 **
G × M	1	0.76 n.s.	0.07 n.s.	1.12 n.s.	0.07 n.s.
G × I	2	0.48 n.s.	8.75 **	8.92 **	31.25 ***
G × S	1	0 n.s.	51 ***	14.8 ***	1.77 n.s.
M × I	2	1.64 n.s.	0.49 n.s.	0.28 n.s.	1.28 n.s.
M × S	1	0.27 n.s.	1.14 n.s.	0.4 n.s.	0.95 n.s.
I × S	2	0.73 n.s.	3.35 *	130.18 ***	51.05 ***
G × M × I	2	0.33 n.s.	0.29 n.s.	0.71 n.s.	0.5 n.s.
G × M × S	1	3.03 n.s.	0.03 n.s.	0.452 n.s.	4.17 *
G × I × S	2	1.3 n.s.	1.31 n.s.	7.8 **	72.31 ***
M × I × S	2	2.07 n.s.	1.43 n.s.	0.02 n.s.	0.29 n.s.
G × M × I × S	2	0.14 n.s.	0.436 n.s.	0.29 n.s.	1.43 n.s.

Values are given as F of Fisher. \*\*\*, \*\*, and \* indicate significant at  $p < 0.001$ ,  $p < 0.01$ , and  $p < 0.05$ , respectively; Degrees of Freedom = df. ns = not significant. S–Em = sowing–emergence; Em–F = emergence–flowering; F–Oc = flowering–opening capsules; Oc–H = opening capsules–harvesting. I = Irrigation; G = genotype; S = site; M = mycorrhizae.



**Figure 5.** Effect of I on the length of phenological S–Em phase. For each data series, values with different letters are significantly different at  $p \leq 0.05$ , according to Tukey’s test. Each treatment was replicated three times. Error bars represent standard error. Irrigation levels (I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc).

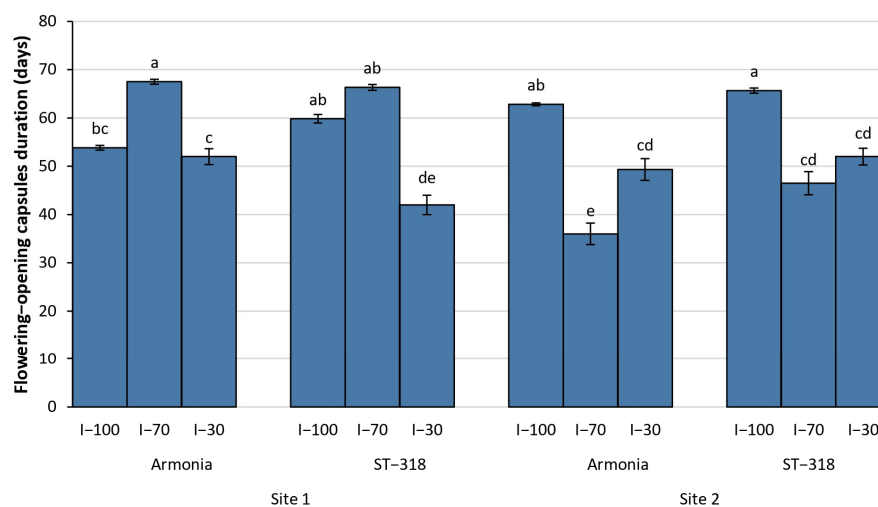


**Figure 6.** Effect of treatments on the duration of phenological Em–F phase. (A) Effect of I and S on the length of phenological Em–F. (B) Effect of I and G on the length of phenological Em–F. (C) Effect of G and S on the length of phenological Em–F. For each data series, values with different letters are significantly different at  $p \leq 0.05$ , according to Tukey’s test. Each treatment was replicated three times. Error bars represent standard error. Site (Site 1 = Campo Carboj and Site 2 = Primosole), genotype (Armonia and ST-318) and irrigation levels (I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc).

The  $G \times I$  interaction was also significant ( $p < 0.01$ ) for the Em–F phenological phase. Significantly shorter durations were observed for Armonia under the I-70 regime, with a mean of 38.4 days. On the other hand, significantly longer durations were recorded under the I-100 regime for both cultivars, with means of 55.2 days for Armonia and 52.5 days for ST-318 (Figure 6B).

The  $G \times S$  interaction was likewise significant ( $p < 0.001$ ) for the Em–F phenological phase. ST-318, in Site 1, showed the shortest duration, with 42.9 days, while Armonia in Site 2 recorded the longest duration to complete this phenological phase, with 51.3 days, representing a 19.6% increase compared to ST-318 within the same site (Figure 6C).

The  $G \times I \times S$  interaction was significant ( $p < 0.01$ ) for the F–Oc phenological phase (Table 5). Significantly shorter durations were observed for the Armonia in Site 2 under the I-70 regime, with a mean of 36 days. In contrast, longer durations were recorded for the ST-318 variety under I-100 in the same site (53.83 days) and for Armonia under I-70 in Site 1 (67.50 days) (Figure 7).

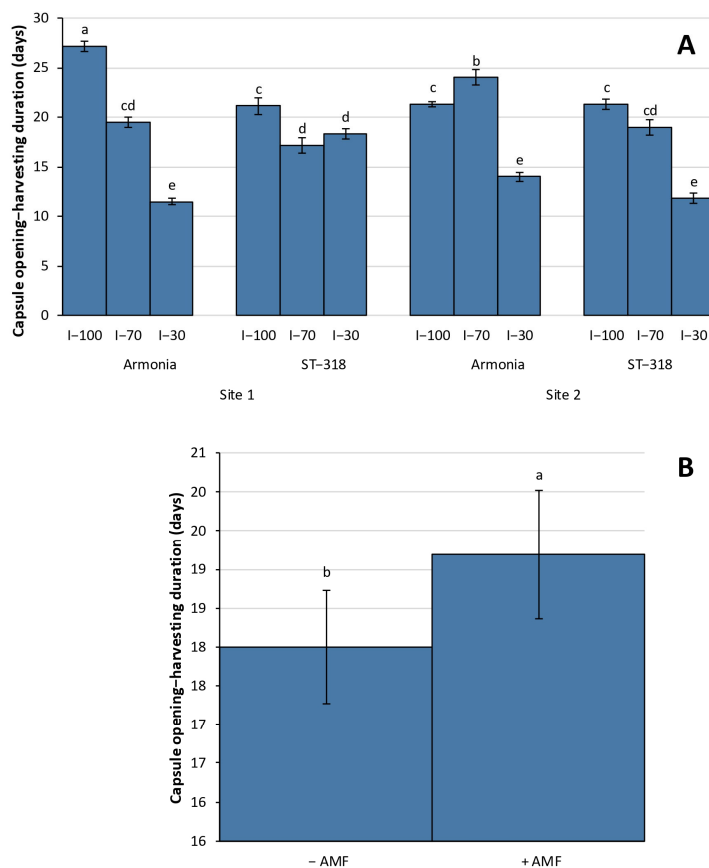


**Figure 7.** Effect of I, G and S on the duration of the Oc–H phenological phase. For each data series, values with different letters are significantly different at  $p \leq 0.05$ , according to Tukey’s test. Each treatment was replicated three times. Error bars represent standard error. Site (Site 1 = Campo Carboj and Site 2 = Primosole), genotype (Armonia and ST-318) and irrigation levels (I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc).

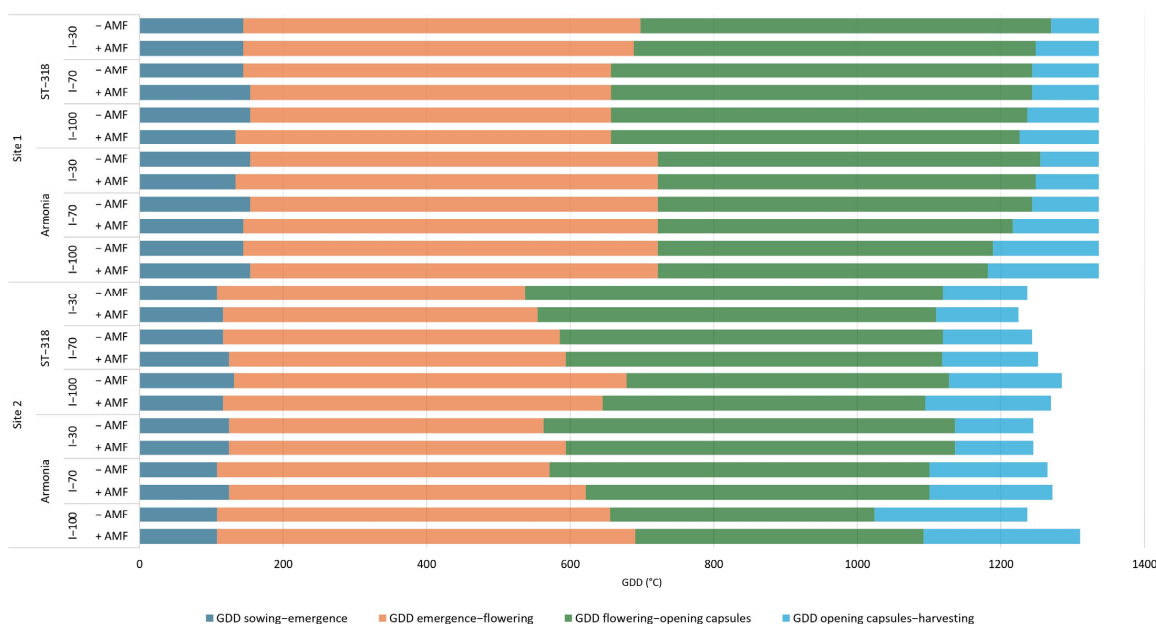
The  $S \times G \times I$  interaction had a significant effect ( $p < 0.001$ ) on the Oc–H phase (Table 5). Significantly shorter durations were observed in Site 2 under I-30 for ST-318 (11.8 days) and Armonia (14.0 days). Similarly, in Site 1, the Armonia variety under I-30 exhibited the shortest mean duration, averaging 11.5 days (Figure 8A).

The M factor also had a significant effect ( $p < 0.001$ ) on the Oc–H phenological phase (Table 5). Plots under +AMF showed significantly longer durations compared to –AMF, with an increase of 6.7% (19.2 days vs. 18 days) (Figure 8B).

Figure 9 shows the accumulation of GDDs across different phenological phases of cotton in the two experimental sites (Site 1 and 2) for the genotypes Armonia and ST-318, under different irrigation regimes (I-100, I-70, I-30). GDD accumulation was highest during the intermediate phases, such as Em–F and F–Oc. A reduction in accumulated GDD was observed under lower water availability (I-30 vs. I-100). Differences in thermal requirements were recorded between the two genotypes, with ST-318 generally requiring more GDD than Armonia in certain phases (e.g., Em–F phases). Variations in GDD accumulation were also detected between Site 1 and Site 2.



**Figure 8.** Effect of treatments on the duration of the F–Oc phenological phase. **(A)** Effect of S, G and I on the duration of the Oc–H phenological phase. **(B)** Effect of M on the duration of the Oc–H phenological phase. For each data series, values with different letters are significantly different at  $p \leq 0.05$ , according to Tukey’s test. Each treatment was replicated three times. Error bars represent standard error. Irrigation levels (I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc), genotype (Armonia and ST-318) and mycorrhization treatment (+AMF = Mycorrhized; –AMF = Non-mycorrhized).



**Figure 9.** Accumulation of growing degree days (GDDs) across different phenological phases of cotton. Site (Site 1 = Campo Carboj and Site 2 = Primosole), genotype (Armonia and ST-318), (I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc), and mycorrhization treatment (+AMF = mycorrhized; –AMF = non-mycorrhized).

### 3.4. Crop Water Productivity

Table 6 summarizes the IWP for seed yield and lint yield under different irrigation treatments at two experimental sites. For seed yield, the IWP was recorded under the I-100 treatment, with the highest value observed at Site 1 ( $1.43 \text{ kg m}^{-3}$ ). The lowest IWP value for seed yield was recorded at Site 2 under I-30, with a value of  $0.52 \text{ kg m}^{-3}$ . A similar trend was observed for IWP calculated based on lint yield, where Site 1 under I-100, recorded a higher value in IWP of 37.7% more than Site 1 under I-100. The lowest IWP value based on lint yield was recorded at Site 2 I-30, with a value of  $0.37 \text{ kg m}^{-3}$ .

**Table 6.** IWP of seed and lint yield under different irrigation treatments.

Site	Irrigation level	Water Supplied	IWP ( $\text{kg m}^{-3}$ )	
		( $\text{m}^3 \text{ ha}^{-1}$ )	Seed Yield	Lint Yield
Site 1	I-30	741.5	0.93	0.54
	I-70	924.4	1.22	0.83
	I-100	1010.5	1.43	0.95
Site 2	I-30	1203.9	0.52	0.37
	I-70	1308.9	0.72	0.61
	I-100	1477.8	0.93	0.69

I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc. Site 1 = Campo Carboj and Site 2 = Primosole.

## 4. Discussion

This study focused on reintroducing cotton cultivation in Sicily through sustainable practices to meet the growing demand for organic and “Made in Italy” cotton. For this reason, an integrated approach was developed, combining organic farming principles, efficient irrigation systems, and mycorrhizal formulations. The goal was to improve cotton yield while ensuring the sustainability of water and soil resources. The results show how the effects of E, I, M and G affect cotton growth, first fertile branch intersection, yield and phenological development, providing valuable insights for sustainable cotton production in Mediterranean environments.

### 4.1. Irrigation and Water Productivity

Despite receiving lower precipitation compared to Site 2, Site 1 required 38.4% less total water. This outcome is primarily attributable to the soil characteristics of Site 1. In fact, it has a higher organic matter content, which increases water retention [56], and a predominantly sandy texture that facilitates deeper root penetration, allowing plants to access water from greater depths [57,58].

In contrast, Site 2 is characterized by a heavy soil with a high clay content. Under drought conditions, such soils tend to form a hardpan, reducing infiltration and causing rapid water loss [59]; without sufficient irrigation, this condition exposes plants to water stress, thereby necessitating more frequent and abundant water applications.

During BBCH 10–50 supplemental irrigation was applied to I-70 and I-100 based on the ETc values. Notably, the total water supplied in this study was significantly lower than the literature values for similar pedoclimatic conditions ( $7000 \text{ m}^3 \text{ ha}^{-1}$ – $12,000 \text{ m}^3 \text{ ha}^{-1}$  per growing season; [11]). This reduction is largely due to the use of drip irrigation, which achieves efficiencies close to 90%, compared to common cotton irrigation systems such as furrow and sprinkler methods, whose efficiencies are typically around 65% and 75%, respectively [60,61]. Finally, both sites demonstrate good potential for the reintroduction of cotton in terms of water consumption, as their total water use remains well below that

reported for similar pedoclimatic conditions. However, among the two, Site 1 has proven to be the most suitable.

This study also showed that IWP is affected by both site and irrigation level. Specifically, as water supply increases, the IWP for both seed yield and lint yield increases as well. This happens because higher irrigation promotes plant growth and increases leaf area index (LAI) and dry matter accumulation by improving root water uptake and transport functions, as well as deep soil moisture and nutrient utilization [62]. The site also significantly influences IWP; Site 1 showed higher values, likely because sandy soils promote deep root development, reducing irrigation needs [63,64].

#### 4.2. Agronomic and Yield Performance

Water stress is well-documented to suppress cotton vegetative growth by limiting cell expansion and photosynthesis [56–58]. In Site 1, the tallest plants were observed under I-100 +AMF for both cultivars, as well as in Armonia I-100 –AMF, where irrigation alone promoted growth, suggesting a threshold effect under optimal water conditions. In contrast, I-30 led to a ~25–30% reduction in plant height.

Mycorrhization significantly increased plant height. As shown by Ortas & Iqbal [32], inoculation with *Funneliformis mosseae* enhances root exploration and nutrient uptake, boosting phosphorus acquisition in cotton by up to 110%. This effect is largely driven by AMF-induced modulation of phytohormones—particularly gibberellins—which stimulate stem cell division and elongation [65,66]. AMF also influence other hormones (ethylene, abscisic acid, cytokinins, salicylic acid, jasmonic acid, and auxins), improving water status under drought by enhancing hydraulic conductivity [66,67].

The mycorrhizal effect was observed across all treatments in Site 1, except for Armonia I-100 –AMF and Armonia I-100 +AMF, where plant height was primarily driven by irrigation. Conversely, in Site 2, soil characteristics may have compromised mycorrhizae colonization and nutrient uptake [25,31]; however, neither colonization rate nor nutrient uptake was directly measured in this experiment.

The height of the first fruiting branch is a key indicator of earliness in cotton, reflecting the plant's ability to transition from vegetative to reproductive growth [68]. Higher branch heights correlate with earlier phenological development, enabling earlier harvest—a critical trait in Mediterranean climates like Sicily, where autumn rains threaten cotton quality [69,70]. The tallest first fruiting branches were observed for ST318 under I-100 +AMF in Site 1, aligning with findings that AMF inoculation, particularly with *Rhizoglyphus irregularis*, accelerates reproductive development under optimal irrigation [71].

However, under I-30 and particularly in Site 2, AMF efficacy was limited by clay-rich soils and low fertility [25,31]. Cultivar choice also played a crucial role in determining first fruiting branch height. Some authors highlighted ST318's ability to thrive under optimal water availability [51], whereas Armonia, exhibited greater stability in water-limited environments [50].

The number of capsules  $\text{ha}^{-1}$  was highest under I-100, particularly for the ST-318 cultivar +AMF in Site 1. This is consistent with studies showing that it is a key indicator of reproductive efficiency and yield potential in cotton, reflecting proper carbohydrate translocation to developing bolls in response to water availability [72,73].

Moreover, the application of certain AMF species, may have contributed to improved boll retention and capsule development due to their ability to enhance P uptake and overall plant nutrient status [32,35].

Genotypic differences also influenced responses: ST-318 maintained higher capsule numbers even under suboptimal conditions, highlighting the importance of genotype-specific stress tolerance.

The average capsule weight reflects water status and the efficiency of resource allocation during boll development [74,75]. This parameter was higher in both sites under the I-100 treatment, as adequate water supply under full irrigation supports optimal boll filling and fiber development, leading to increased capsule weight [2]. In contrast, I-30 reduced capsule weight, likely due to limited water availability restricting nutrient translocation and boll maturation [76]. An interesting trend was observed with the Armonia cultivar, particularly under the I-70 treatment, where no significant differences were found compared to I-100. Therefore, genotypic variation was evident: Armonia not only showed the highest values under optimal conditions but also maintained stable weights under water stress, likely due to traits such as osmotic adjustment or improved resource partitioning, as highlighted by several authors [50,51].

Raw yield and lint yield responded positively to I, G, and S. Optimal water availability led to higher yields, in line with recent findings by Zhangjin et al. [48], which demonstrated that an adequate water supply enhances boll retention, carbohydrate partitioning, and fiber development, ultimately increasing yield. In contrast, water stress, particularly under severe deficit irrigation, reduces both raw and lint yield by limiting cell expansion, restricting carbohydrate translocation, and increasing boll abortion [76,77].

A particularly noteworthy result is the yield obtained under the I-70 regime, which, despite a physiological reduction in yield, remained economically viable. Specifically, raw yield decreased by 24.3% and lint yield by 21.1% compared to the I-100 treatment. However, a formal economic analysis comparing yield value and irrigation costs across regimes would be required to confirm this.

These findings highlight the strong adaptability of cotton, particularly the cultivars Armonia and ST-318, which maintained productivity and profitability even with a 30% reduction in water supply. This aspect is crucial, especially in regions severely affected by water scarcity, as also evidenced in Figure 1, where total rainfall during the entire vegetative period amounted to only 68.2 mm in Site 1 and 67.3 mm in Site 2.

Seed yield was significantly influenced by the  $M \times I$  interaction. This parameter was primarily affected by M, as evidenced by the I-100 and I-70 treatments, which showed increases in seed yield of 14.1% and 13.7%, respectively, compared to the –AMF treatments. AMF boosts seed yield by elevating phosphorus-dependent ATP synthesis, fueling energy-intensive seed development [78]. Additionally, they promote carbon allocation to reproductive sinks via upregulated sucrose transporter genes in mycorrhizal roots [79].

However, in the I-30 treatment such improvements were not observed. As documented by Madouh & Qureshi [80] and Pathan et al. [81], under strong water stress, AMF encounter considerable difficulties in both colonization and executing their symbiotic functions, thereby reducing or even nullifying their benefits [82,83].

#### 4.3. Phenological Performance

Deficit irrigation significantly reduced the S-Em stage at both experimental sites. This result is in line with studies indicating that water stress reduces germination and early establishment of cotton seedlings [82]. However, in Site 2, the I-70 treatment resulted in faster emergence compared to Site 1, likely due to differences in soil texture and thermal conditions. The higher clay content in Site 2 (55.6% vs. 28% in Site 1; Table 1) likely contributed to better soil warming, improved seed-soil contact, and greater water retention, accelerating germination [83,84]. It is important to highlight that a shorter emergence phase is critical for cotton establishment, as prolonged exposure to suboptimal soil moisture increases the number of failures [85]. The faster emergence observed at site 2 under I-70 suggests that clay soils in warmer environments may help mitigate some of the risks associated with deficit irrigation during the early growth stages.

The duration of the F–Oc phase is strongly influenced by the  $S \times G \times I$  interaction, reflecting complex trade-offs between boll development and yield outcomes. It is well documented that shorter F–Oc phases often correlate with an increased number of bolls but may compromise yield due to insufficient time for fiber accumulation and boll filling [86,87]. In this study, the I-100 treatments in both sites exhibited the longest F–Oc durations, maximizing boll development and yield potential. Notably, the I-70 treatment in Site 1 also extended this phase, likely due to its higher organic matter and nitrogen content [88]. Conversely, the nutrient-poor soil of Site 2 resulted in a significantly shorter F–Oc period.

The Oc–H phase was significantly influenced by the  $S \times G \times I$  interaction. A shorter duration of this phenological phase allows for earlier harvesting, reducing the risk of exposure to autumn rainfall events, which frequently affect southern Italy and can compromise cotton fiber quality [69,70]. In both sites, the Armonia variety under I-30 irrigation showed a marked reduction in the number of days to harvest, demonstrating its suitability for early maturation across different environmental conditions. This confirms its potential adaptability for cultivation in regions where early harvesting is advantageous. Meanwhile, the ST-318 variety exhibited a significant reduction in the Oc–H phase duration in Site 2, likely due to the sandy soil (Table 1) and thermopluviometric conditions (Figure 1) that better matched its growth requirements.

## 5. Conclusions

This study underscores the potential for revitalizing cotton cultivation in Sicily through integrated sustainable strategies. Optimized irrigation practices significantly enhanced yields, while moderate water reductions maintained viable productivity, achieving 75.7% and 78.9% of raw and lint yields, respectively, compared to full irrigation. The selected Mediterranean cultivars exhibited distinct adaptive responses: Armonia demonstrated stability under water-limited conditions, whereas ST-318 thrived under optimal irrigation. The inoculation of AMF contributed to many agronomic parameters analyzed, particularly in fertile soils (Site 1). However, its benefits were less pronounced under I-30 or on poor soils (Site 2). Soil properties and environmental factors played a crucial role in phenological development, with clay-rich soils (Site 1) prolonging boll maturation and sandy soils (Site 2) promoting earlier harvest readiness.

Future research should focus on refining adaptive management strategies, optimizing irrigation regimes, and developing stress-resilient genotypes to facilitate the reintroduction of cotton cultivation in these regions.

**Author Contributions:** Conceptualization, G.S.V. and N.I.; methodology, G.S.V. and N.I.; software, N.I., N.T. and C.M.; validation, N.I. and T.T.; formal analysis, N.I. and G.S.V.; investigation, G.S.V., N.I. and N.T.; resources, G.S.V. and N.I.; data curation, G.S.V., A.G., A.S. and N.I.; writing—original draft preparation, G.S.V. and N.I.; writing—review and editing, G.S.V., S.L., and N.I.; visualization, G.S.V., N.I., S.L., T.T., P.G., C.M., G.I., N.T., L.F. and A.G.; supervision, T.T., P.G. and S.L.; project administration, P.G.; funding acquisition, P.G. and L.F. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the OVS S.p.A. (Mestre, VE, Via Terraglio 17, 30174), which funded the PhD student enrolled in the PhD program in Agricultural, Food, and Environmental Sciences at the University of Catania; Irritec S.p.A. (d’Orlando, ME Via Gambitta Conforto, C.da S. Lucia, 98071 Capo) CN\_00000022 “National Research Centre for Agricultural Technologies (Agritech)”, D.R. n. 1032 on 17.06.2022, PNRR MUR–M4C2–1.4–“Centri Nazionali”–D.D. n. 3138 on 16/12/2021, CUP: B13D21011580004, which provided the drip irrigation system; and CBC (Europe) S.r.l. | Biogard Division (Via Zanica 25, 24050 Grassobbio, BG, Italy), which supplied “Rizoplant”, a mycorrhizal fungi inoculum.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Acknowledgments:** The authors would like to thank OVS S.p.A. for supporting the PhD student enrolled in the PhD program in Agricultural, Food, and Environmental Sciences at the University of Catania. Giuseppe Salvatore Vitale is currently conducting a research project titled “Agronomic and Qualitative Aspects of Cotton Cultivation under Organic Farming”. The authors would like to thank “Ente di Sviluppo Agricolo” (ESA) of the Sicilian Region for providing the experimental field.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

AMF	Arbuscular Mycorrhizal Fungi
FC	Field Capacity
ETc	reference crop evapotranspiration
GDDs	growth degree days
I-100	100% ETc
I-70	70% ETc
I-30	30% ETc
ET0	daily reference evapotranspiration
S	site
G	genotype
I	irrigation
M	mycorrhization
+AMF	mycorrhized plants
–AMF	non-mycorrhized plants
Kc	crop coefficients

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