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Screening for Cold Tolerance during Germination within Sweet and Fiber Sorghums [*Sorghum bicolor* (L.) Moench] for Energy Biomass

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Abstract: Within the project "BIOSEA" funded by the Italian Ministry of Agriculture and Forestry, a preliminary laboratory test was conducted to assess the variability for cold tolerance during germination in 30 cultivars of biomass sorghum, among fiber and sweet types. Seed germination (%) and mean germination time (MGT) were examined at seven constant temperatures (from 8 °C to 35 °C) and base temperature (Tb) and thermal time (θ_T) for 50% germination were calculated. A wide genetic diversity in the germination response of sorghum was ascertained at 8 °C (CV 45%) and 10 °C (CV 25.4%). At 8 °C, in cultivars of 'Padana 4', 'PR811F', 'PSE24213', 'PR849' and 'Zerberus', seed germination exceeded 80%. Seeds of 'Zerberus' were also the fastest, requiring less than 13 days for final germination at this low temperature. Great differences were found in Tb and θ_T among cultivars. Tb varied between 7.44 °C ('PR811F') and 13.48 °C ('Nectar'). Thermal time (θ_T) was, on average, 24.09 °Cd^{-1}, and ranged between 16.62 ('Nectar') and 33.42 °Cd^{-1} ('PSE24213'). The best combination of the two germination parameters (i.e., low Tb and θ_T) corresponded to 'Zerberus', 'Sucrosorgo 506', 'Jumbo' and 'PR811F'. Accordingly, these cultivars are more tolerant to cold stress during germination and, thus, more adapt to early spring sowings in Mediterranean areas (March-April). Cultivars 'PR811F' (fiber type) and 'Sucrosorgo 506' (sweet type) also combine high cold tolerance with good productivity in terms of final dry biomass, as assessed in open-field conditions (late spring sowing). The genetic variation in the germination response to a low temperature is useful for the identification of genotypes of sorghum suitable to early sowings in semi-arid areas. Selection within existing cultivars for cold tolerance during germination may also contribute to the expansion of biomass sorghum into cooler cultivation areas, such as those of Northern Europe, which are less suitable to this warm season crop.

Keywords: base temperature; germination; low temperature; *Sorghum bicolor*; thermal time

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1. Introduction

Sweet and fiber sorghums [*Sorghum bicolor* (L.) Moench], as fast growing and high-yielding species under a wide range of soil and environmental conditions [1,2], are considered promising industrial crops for the European Community, for the bioethanol (sweet types) and the combustion (fiber types) chains [3]. Moreover, both types of sorghums produce lignocellulose that could serve as feedstock for second generation biofuel [4].

Sorghum is a C4 plant, native to tropical areas, that can be adapted to most of the temperate and sub-tropical climates as annual crop, but it grows well on marginal and non-irrigated lands under Mediterranean-like climates [5]. Field experiments conducted in different areas of Europe confirmed the high yield potential of this crop under no water limitations [4,6].

As a drought resistant plant, sorghum well adapts to cultivation in arid and semi-arid regions. However, increasingly extensive drought or prolonged drought periods due to climate change are becoming major concerns even for sweet and fiber sorghums, in those areas where the irrigation water scarcity is the most important factor that limits summer crops' productivity [3,7].

A technical option for the cultivation of biomass sorghum in drylands of Southern Europe is the adoption of early sowings (e.g., in March), which offer a number of advantages to the crop, such as an escape from the dry period and a complete part of the growing season under more favorable weather conditions. In this way, the crop may benefit of soil water reserves accumulated during the rainy season and reduce irrigation requirements. Early sowings may also minimize the exposure to seed dehydration during germination.

Constraints for the adoption of early sowings in sorghum were found in its sensitivity to cold stress during germination [8]. Temperature plays a crucial role in the regulation of plant processes, including germination, and sorghum suffers chilling injuries when exposed to temperatures below 10 °C [9]. Cold stress at sowing may result in poor seedling establishment due to a delayed or reduced emergence [10]. To this purpose, the identification of cold tolerant cultivars during germination in sorghum is required.

The existence of genetic differences in cold resistance is useful in identifying genotypes of sorghum able to germinate at low temperatures, which are, therefore, suitable to early-spring sowings (March) in the Mediterranean environment [11]. The germination of sorghum under low temperatures has been widely studied in grain types [10,12,13], but little has been studied in sweet and fiber sorghums [14]. Furthermore, the changes in weather conditions over the years in the same experimental site, make the selection for cold tolerance difficult within a large number of genotypes [11]. A preliminary screening under controlled low temperature conditions may help to select biomass sorghums suitable to early sowings in semi-arid environments.

Within the project "BIOSEA" funded by the Italian Ministry of Agriculture and Forestry (MIPAF), a preliminary test was conducted in the laboratory to: (i) assess the existence and quantify the genetic variability, among sweet and fiber sorghum cultivars, for cold tolerance during germination, (ii) identify those genotypes with better performance during germination at low temperatures, (iii) define the limit to the early sowings in the semi-arid Mediterranean environments of Southern Italy through the identification of a minimum threshold of temperature allowing seed germination. The consistency of results from laboratory tests with those from field experiments reported in literature [11,15], suggests the reliability of using laboratory tests as a preliminary screening method for field evaluation.

2. Materials and Methods

2.1. Plant Material

The experiment was conducted in the laboratory on the seeds of 30 cultivars of biomass sorghum (*Sorghum bicolor* L. Moench) among fiber and sweet biomass types. The list of cultivars and their provenance are reported in Table 1. The cv. 'Keller' of sweet sorghum was adopted as a control, as it has been proven to be the most adaptable and best producing energy biomass in the Mediterranean environment [6,16]. When the experiment was conducted, the seeds were less than 12 months old, and kept at room temperature (10–20 °C) before being tested.

Table 1. List of the cultivars of sorghum assessed in the experiment.

Cultivar	Type	Seed Company	100-Seed Weight (g)
Jumbo	fiber	Padana Sementi (Italy)	3.19
Padana 1	fiber	Padana Sementi (Italy)	2.90
Padana 4	fiber	Padana Sementi (Italy)	3.36
Sugargraze	sweet	Padana Sementi (Italy)	3.41
PSE22043	fiber	Padana Sementi (Italy)	2.82
PSE22053	fiber	Padana Sementi (Italy)	3.66
PSE23431	fiber	Padana Sementi (Italy)	3.83
PSE24213	fiber	Padana Sementi (Italy)	3.85
PSE27677	fiber	Padana Sementi (Italy)	3.71
PSE98456	fiber	Padana Sementi (Italy)	2.61
HayDay	fiber	Padana Sementi (Italy)	3.36
Nectar	sweet	Padana Sementi (Italy)	1.51
Goliath	biomass	UNICATT (Italy)	3.99
Sucrosorgo 506	sweet	UNICATT (Italy)	3.38
Biomass 150	fiber	UNICATT (Italy)	2.85
Zerberus	fiber	UNICATT (Italy)	3.15
ABF26	fiber	UNICATT (Italy)	2.75
ABF306	fiber	UNICATT (Italy)	3.14
PR811F	fiber	Dupont Pioneer (USA)	3.28
PR849	fiber	Dupont Pioneer (USA)	3.03
PR895	fiber	Dupont Pioneer (USA)	2.65
Nicol	sweet	Dupont Pioneer (USA)	2.79
Topper	sweet	Mississippi State University (USA)	2.18
Dale	sweet	Mississippi State University (USA)	1.51
M81E	sweet	Mississippi State University (USA)	1.91
Keller	sweet	Mississippi State University (USA)	1.92
Bulldozer	sweet	KWS Italia (Italy)	1.88
Maya	sweet	KWS Italia (Italy)	2.92
Silage King	sweet	KWS Italia (Italy)	2.04
Biomass H133	fiber	Syngenta (Italy)	3.87

2.2. Germination Tests

The seeds of the 30 cultivars of sorghum were germinated at seven constant temperatures (T): 8, 10, 15, 20, 25 and 30 °C, with 25 °C considered as the optimum for seed germination of sorghum [17]. The germination tests were made in a thermostatically controlled (±1 °C) incubator. Samples of 150 seeds (three replicates of 50 seeds each) were placed in Petri dishes containing a single sheet of paper tissue, moistened with 7 mL of distilled water. Petri dishes were hermetically sealed with Parafilm to prevent evaporation. Thus, they were randomised within each temperature and incubated in the dark. Seeds germinated (those whose radicle reached at least 2 mm of length) were recorded and removed daily. The seed germination test was stopped when no additional germination occurred for 72 h. The germination tests were conducted in 2010, in a time span of approximately 6 months, starting in the spring.

2.3. Open Field Experiment

In the same year of germination tests in the laboratory (2010), a field experiment was conducted in a flat site of the Eastern coast of Sicily (South Italy, 10 m a.s.l., 37°25′ N Lat, 15°30′ E Long), on a Vertic Xerochrepts soil. The same 30 cultivars of sorghum tested in the laboratory assay were evaluated for biomass yield in the field, in a complete randomized blocks' experimental design with three replicates. Sowing was hand made on 3 June, in single plots of 6 m^2 (2 m long × 3 m wide). A 0.50 m row distance and a 12 plants m^{-2}

final density were adopted. Before sowing, 100 kg ha^{-1} of N (as ammonium sulfate) and 100 kg ha^{-1} of P_2O_5 (as mineral perphosphate) were distributed. Approximately a month after seedling emergence, a further 70 kg ha^{-1} N (as ammonium nitrate) were distributed as top dressing. During the growing season, the main meteorological variables (maximum and minimum air temperature, rainfall, reference evapotranspiration-ET_0) were recorded, using a weather station connected to a data logger (CR10, Campbell Scientific, Inc., Logan, UT, USA).

Irrigation was applied to meet crop requirements (100% evapotranspiration restoration) along the growing season. Total 3450 m^3 ha^{-1} were distributed to the crop. Final harvest was made on 11 November, on plants of central rows, after removing the border plants. At harvest, the total above-ground dry biomass was estimated. To this end, total biomass samples were oven-dried at 65 °C until constant weight for a dry matter measurement.

2.4. Calculations and Data Analysis

At the end of germination tests, final germination (FG, %) and Mean Germination Time (MGT, days) were calculated.

The time course of cumulative values of seed germination was described by a nonlinear iterative regression method (SIGMAPLOT® 9.0 software, Systat Software Inc., San Jose, CA, USA) using the following sigmoidal model with three parameters.

$$y = \frac{a}{1 + \left(\frac{x}{x_0}\right)^b} \tag{1}$$

where a is the maximal value of y (i.e., maximum germination), x is the time (days) after seed imbibition, x_0 is the time (days) to reach 50% of maximal germination, and b is a fitting parameter of the curve. The x value on the curve corresponding to 50% germination (y value of the curve) was assumed as theoretical time to 50% germination or t_{50} (days) [18,19].

Data set of germination rates of 50% germination fraction ($1/t_{50}$ or GR_{50}) of seed population, resulting from the germination time course (see Figure 1), was plotted against T, separately for the cultivars. The linear regression of GR_{50} vs. T allowed us to calculate the theoretical minimum or base temperature (Tb) at which seed germination of each cultivar is reduced to 50%. The slope b of the regression line is the germination rate with a decreasing temperature (the higher the b, the faster the germination with the temperature increase). The abscissa intercept is an estimate of the theoretical minimum temperature of germination (Tb) [19,20]. Thermal time (θ_T) to achieve 50% germination ($\theta_{T(50)}$) at each temperature was calculated for each cultivar using the equation below.

$$\theta_{T(50)} = (T - T\text{b}) \times t_{50} \tag{2}$$

where $\theta_{T(50)}$ = thermal time needed for 50% germination (°Cd), T = actual germination temperature (°C, constant in controlled environment), Tb = base germination temperature, t_{50} = the time to 50% germination (median response time) [19,20].

In order to compare the two methods of $\theta_{T(50)}$ calculation, thermal time was also calculated as the inverse of the slope b of the regression line used for Tb estimation.

Data of the final percentage germination, previously arcsine transformed, and those of MGT, were statistically analysed by a completely randomised two-way (*temperature* × *cultivar*) analysis of variance (ANOVA) using COSTAT version 6.003 (CoHort Software, Monterey, CA, USA). When 'F' ratios were significant, means were separated by the Least Significant Difference (LSD) test at $p \leq 0.05$. A one-way ANOVA was also performed on data of final dry biomass yield, using 'cultivar' as a source of variation, and means were separated by the LSD test at $p \leq 0.05$.

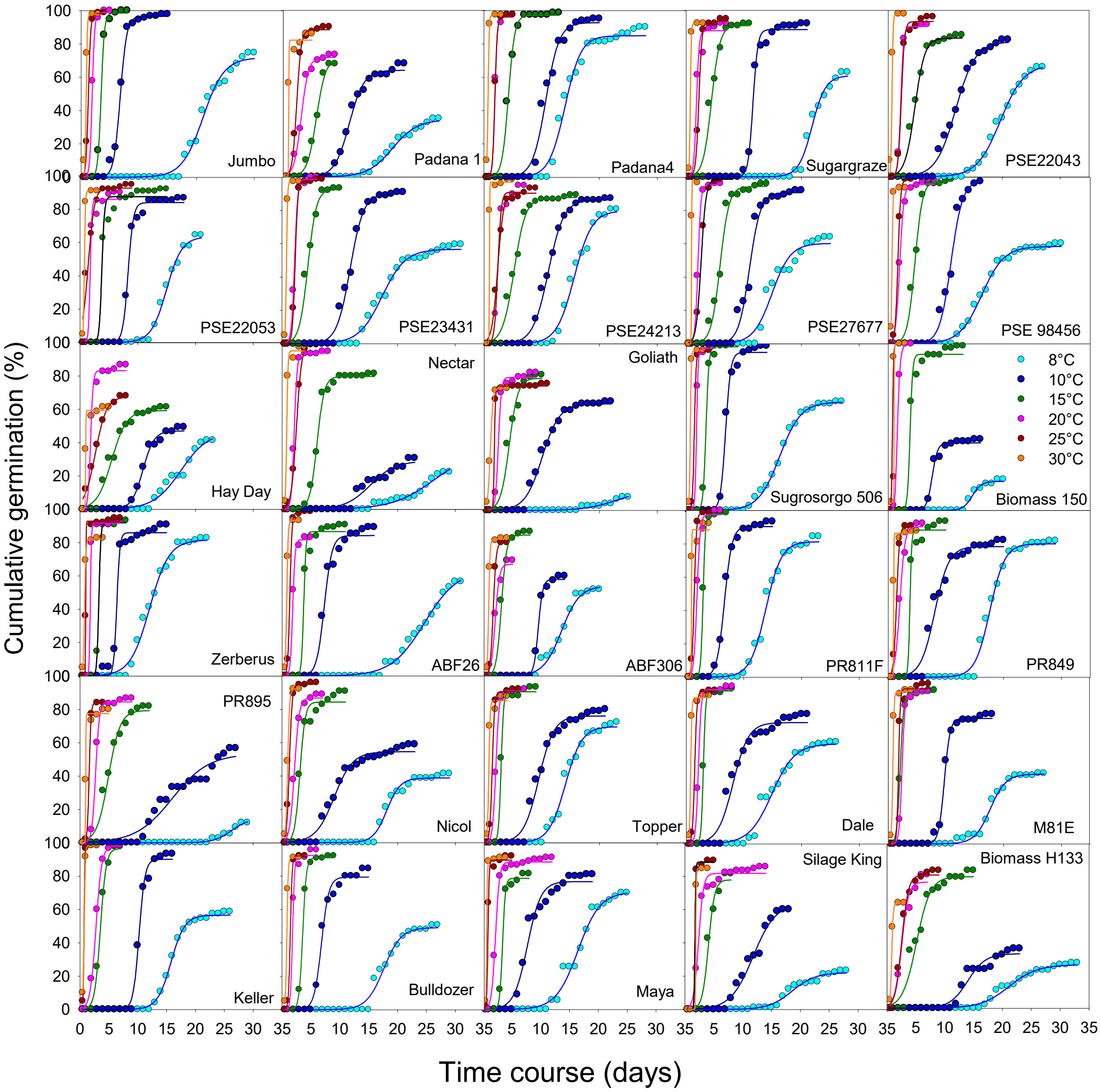

Figure 1. Cumulative germination time courses (solid curves) at different temperatures in 30 cultivars of biomass sorghum. Seeds were germinated at 8, 10, 15, 25 and 30 °C. Symbols represent the observed percentages with time.

2.5. Estimation of the Germination Date in an Open Field

In order to reproduce potential scenarios, the date of occurrence of 50% seed germination in the field was estimated for each cultivar of sorghum, considering three hypothetical early sowing dates: March 1, March 15, and April 1. For the prediction of the germination date in the field, individual thermal time and *T*b values of each cultivar, and mean soil temperatures from March to April, as measured over a 10-year period (2005–2014) by a weather station connected to a data logger (CR10, Campbell Scientific, Inc., Logan, UT, USA), located in the same site of the 2010 field experiment, were considered.

3. Results

3.1. Cumulative Germination Time Course

The cumulative seed germination time course of 30 sorghums during imbibition at different constant temperatures is illustrated in Figure 1. The course is well described

(R^2 > 0.90) by a three-parameter sigmoidal function whose trend reveals an initial phase of low germination, which is followed by a step rise up to a maximum. After that, germination stopped (at warmer temperatures) or slowly proceeded (at lower temperatures). The length of the initial phase of slow germination, minor or null at temperatures ≥ 25 °C, progressively increased as temperature decreases.

The lowering of the temperature from that optimal progressively inhibited and delayed germination to a different extent, depending on the cultivar. Some sorghums (e.g., 'Padana 4', 'PR811F', 'PSE24213', 'PR849', 'Zerberus') only postponed the start of the exponential increase of germination at lower temperatures, while exhibiting an overall similar cumulative germination trend and final germination percentage at all temperatures. In other cultivars ('Jumbo', 'PSE98456', 'Sugargraze', 'PSE22053', 'Sucrosorgo 506', 'ABF26', 'Keller'), the thermal lowering slowed down and depressed seed germination (i.e., final percentage of seeds germinated) only at the lowest temperature (8 °C). In some others (e.g., 'Padana 1', 'Hay Day', 'Nectar'), the depressive effects of thermal lowering on the germination time course were progressive, since a clear response was observed at 15 °C. Overall, the higher the temperature, the faster the germination process. However, at 30 °C, the final germination percentage in some cases was lower than that at 25 °C.

3.2. Final Germination under Controlled Temperatures

Final seed germination of sorghums was significantly affected by temperatures, decreasing with the lowering of temperatures, from 89.4% at 30 °C to 54.3% at 8 °C (Table 2). On average of cultivars, germination was the highest at 25 °C (92.7%), confirming this temperature as the optimum for the germination of sorghum.

Table 2. Final germination percentage in sorghums in response to temperature. Values for the main factors (temperature and cultivar) followed by the same letter do not statistically differ at $p \leq 0.05$ by LSD test.

	Germination (%)						
Cultivar	**8 °C**	**10 °C**	**15 °C**	**20 °C**	**25 °C**	**30 °C**	**Avg**
Jumbo	74.7	97.8	100.0	100.0	98.7	97.8	94.8 a
Padana 1	34.9	68.0	67.8	73.3	90.0	85.9	70.0 i–k
Padana 4	90.1	95.3	98.6	97.6	97.3	97.3	96.0 ab
Sugargraze	61.8	92.0	92.0	92.2	94.7	92.2	87.5 c–e
PSE22043	66.6	82.3	85.3	91.5	96.0	90.8	85.4 d–g
PSE22053	64.6	86.7	92.0	90.0	94.7	90.9	86.5 d–f
PSE23431	59.5	90.9	93.3	100.0	98.7	97.3	89.9 a–c
PSE24213	80.6	85.4	89.3	95.1	93.3	98.7	90.4 b–d
PSE27677	62.7	92.0	96.0	96.0	100.0	100.0	91.1 a–c
PSE98456	60.0	97.3	98.7	98.3	98.7	93.5	91.1 a–c
HayDay	41.3	49.3	61.3	86.7	68.0	61.3	61.3 k
Nectar	22.4	30.7	81.7	94.7	100.0	97.3	71.1 g–i
Goliath	8.0	65.3	81.3	82.7	76.0	73.3	64.4 k
Sucrosorgo 506	65.3	98.7	100.0	98.7	96.0	96.0	92.5 a–c
Biomass 150	18.7	42.7	98.7	100.0	100.0	100.0	76.7 d–e
Zerberus	82.7	90.7	93.3	93.3	94.7	82.7	89.6 c–e
ABF26	56.7	89.3	88.7	83.3	98.7	93.3	85.0 h–j
ABF306	52.0	60.0	86.7	69.3	80.0	82.7	71.8 h–j
PR811F	84.4	93.3	98.7	100.0	98.7	92.2	94.6 ab
PR849	82.0	82.2	93.4	92.2	90.7	87.8	88.1 d–f
PR895	12.0	56.7	81.9	86.7	84.3	80.0	66.9 jk
Nicol	41.3	58.9	90.8	88.9	96.0	92.2	78.0 f–h
Topper	72.2	80.0	93.3	92.1	92.0	87.8	86.2 d–g
Dale	61.1	77.8	93.3	94.4	92.0	88.9	84.6 d–g
M81E	42.2	77.8	92.2	92.2	96.0	92.2	82.1 e–g
Keller	58.7	93.3	97.8	98.3	98.3	98.3	90.8 a–c

Table 2. *Cont.*

	Germination (%)						
Cultivar	**8 °C**	**10 °C**	**15 °C**	**20 °C**	**25 °C**	**30 °C**	**Avg**
Bulldozer	50.7	84.4	92.0	95.6	92.1	91.1	84.3 d–g
Maya	70.0	81.1	81.3	91.3	92.0	91.1	84.5 e–g
Silage King	23.3	60.0	81.3	85.6	89.3	85.1	70.8 h–j
Biomass H133	27.4	34.2	82.7	80.1	82.7	63.1	61.7 k
Average ± σ	54.3 ± 22.6 d	76.5 ± 19.4 c	89.4 ± 9.2 b	91.3 ± 7.7 b	92.7 ± 7.6 a	89.4 ± 9.7 b	
CV (%)	41.70	25.36	10.23	8.46	8.25	10.83	
$LSD_{C \times T}$ (0.05)	10.37						

Cultivars exhibited a different behaviour at suboptimal temperatures ($C \times T$, significant at $p \leq 0.001$) (Table 3). The genetic variability (CV) increased (from 8.25% to 41.7%) with the lowering of germination temperature from an optimal temperature.

Table 3. Two-way analysis of variance for final germination of 30 biomass sorghums at a range of constant temperatures. Degree of freedom (df). Mean square (MS). Significant at $p \leq 0.001$ (***).

Source	df	MS	F	*p*
Cultivar (C)	29	1451.6	34.58	0.000 ***
Temperature (T)	5	12,592.6	300.00	0.000 ***
$C \times T$	145	208.5	4.99	0.000 ***
Error	360	41.7		
Total	539			

At 15 °C, germination exceeded 80% in all genotypes except 'Padana 1' and 'HayDay'. At this temperature, seed germination was full (100%) in 'Jumbo' and 'Sucrosorgo 506' and almost full (>98.5%) in 'PSE98456', 'Padana 4', 'PR811F' and 'Biomass 150', fiber sorghums all of them except 'Sucrosorgo 506'. At 10 °C, the variability for the germination percentage among sorghums became higher (CV = 25.36%). At this temperature, in four cultivars ('Jumbo', 'PSE98456', 'Padana 4', 'Sucrosorgo 506'), germination was still greater than 95%, while 'HayDay', 'Biomass H133', 'Nectar' and 'Biomass 150' did not achieve 50% of seeds germinated. At 8 °C, the variability was at a maximum (>40%) since seed germination dropped drastically in many cultivars. At this temperature, germination was ≥70% (significantly greater than a 58.7% in cv. Keller) in height cultivars with 'Padana 4', 'PR811F', 'PSE24213', 'PR849' and 'Zerberus', fiber types all, which were the best performing (germination > 80%). Among them, 'Padana 4' was the most tolerant to a low temperature (germination > 90%).

In ten cultivars, a very low temperature of 8 °C strongly depressed germination (<50%). Among them, 'PR 895', 'Biomass 150' and 'Goliath', had a final germination <20%, revealing a great sensitivity to cold stress during germination.

3.3. Mean Germination Time in Response to Temperatures

Mean Germination Time (MGT) varied with temperature and cultivar. Germination was faster at the highest temperatures and, at 30 °C, all cultivars were germinated in less than two days (Table 4).

Table 4. Mean germination time in sorghums in response to temperature. Values for the main factors (temperature and cultivar) followed by the same letter do not statistically differ at $p \leq 0.05$ by LSD test.

	Mean Germination Time (MGT, Days)						
Cultivar	**8 °C**	**10 °C**	**15 °C**	**20 °C**	**25 °C**	**30 °C**	**Avg**
Jumbo	22.28	6.34	4.05	2.47	1.83	1.24	6.37 f–i
Padana 1	19.43	13.71	6.07	3.82	2.29	1.52	7.81 c
Padana 4	15.53	11.58	4.96	2.44	2.01	1.05	6.26 f–j
Sugargraze	14.62	13.48	5.09	2.59	2.12	1.06	6.49 e–h
PSE22043	19.99	12.62	5.55	2.98	2.25	1.10	7.41 cd
PSE22053	15.70	10.19	4.76	2.44	1.69	1.07	5.98 h–j
PSE23431	18.65	12.50	5.13	2.78	2.08	1.11	7.04 d–f
PSE24213	16.43	11.87	5.97	3.13	2.50	1.26	6.86 d–g
PSE27677	16.26	11.83	6.53	2.63	2.40	1.23	6.81 d–g
PSE98456	16.83	11.40	5.28	2.75	2.00	1.03	6.55 e–h
HayDay	17.43	12.81	6.32	2.52	2.67	1.54	7.21 c–e
Nectar	23.07	17.45	6.83	2.71	2.28	1.24	8.93 a
Goliath	23.00	11.20	5.11	3.23	2.32	1.62	7.75 c
Sucrosorgo 506	16.71	7.70	4.08	2.28	2.03	1.05	5.64 i–k
Biomass 150	15.66	8.63	4.79	2.28	1.96	1.08	5.73 ij
Zerberus	12.87	7.26	4.26	2.23	1.73	1.18	4.92 k
ABF26	22.50	8.49	4.59	2.44	2.05	1.23	6.88 d–g
ABF306	13.38	10.09	3.74	2.60	2.11	1.23	5.52 jk
PR811F	14.74	5.45	3.50	2.54	1.94	1.34	4.92 k
PR849	18.34	8.33	4.58	2.71	1.98	1.42	6.23 g–j
PR895	25.41	16.20	5.76	3.38	2.08	1.65	9.08 a
Nicol	19.47	9.63	4.26	2.82	1.84	1.60	6.60 e–h
Topper	14.98	8.70	3.76	2.63	2.02	1.30	5.57 jk
Dale	15.80	7.69	3.59	2.79	2.02	1.18	5.52 jk
M81E	18.19	8.47	3.92	2.71	2.32	1.19	6.13 g–j
Keller	16.47	11.02	4.03	3.31	1.98	1.32	6.35 f–i
Bulldozer	18.51	6.87	4.16	2.44	1.97	1.21	5.86 h–j
Maya	16.95	7.04	4.10	3.03	1.41	1.23	5.63 i–k
Silage King	19.22	10.07	4.88	3.45	2.09	1.50	6.87 d–g
Biomass H133	21.47	15.29	6.21	3.39	2.47	1.21	8.34 b
Average ± σ	18.00 ± 3.10 a	10.46 ± 2.98 b	4.86 ± 0.94 c	2.78 ± 0.40 d	2.08 ± 0.26 e	1.26 ± 0.18 f	
CV (%)	17.22	28.49	19.34	14.39	12.50	14.29	
$LSD_{C \times T}$ (0.05)	1.18						

The lowering of temperature from that optimal level led to a progressive increase in germination time to a different extent depending on the cultivar ($C \times T$ significant, $p \leq 0.001$) (Table 5).

Table 5. Two-way analysis of variance for MGT of 30 biomass sorghums at a range of constant temperatures. Degree of freedom (df), Mean square (MS), and significant at $p \leq 0.001$ (***).

Source	**df**	**MS**	**F**	***p***
Cultivar (*C*)	29	19.93	36.8	0.000 ***
Temperature (*T*)	5	3802.92	7017.4	0.000 ***
$C \times T$	145	7.83	14.4	0.000 ***
Error	360	0.54		
Total	539			

At 15 °C, seeds took approximately 5 days to germinate, on average, with 'PR811F' being the fastest (MGT 3.50 days) and 'Nectar' being the slowest (MGT 6.83 days).

At 10 °C, the genetic variability for the germination rate was the greatest (28.49%), since cultivars responded quite differently from this cold temperature in terms of germination speed. 'Nectar' was the slowest (17.45 days MGT) under this temperature as well, while 'PR811F' was the fastest, reaching its final germination in a 5.45-day MGT.

At 8 °C, germination further slowed down and seeds took, on average, 18 days (MGT) to germinate. At this temperature, seeds of 'Zerberus' germinated the fastest, requiring less than 13 days to achieve final germination.

3.4. Final Germination vs. MGT

The relationship of final germination vs. mean germination time (MGT) at 8 °C was studied (Figure 2). An exponential decay model best fitted the data (R^2 = 0.50), whose trend reveals that cold-tolerant cultivars, i.e., those best performing as the total number of seeds germinated at this temperature, tend to germinate faster than those susceptible (i.e., little germinating) to a low temperature.

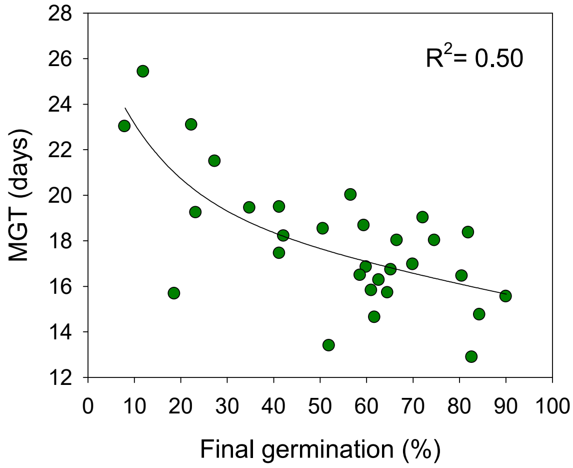

Figure 2. Relationship of final germination values vs. MGT values recorded at 8 °C.

3.5. Base Temperature and Thermal Time

A linear model was used to estimate the critical germination temperature, based on the germination rate GR_{50} (i.e., $1/t_{50}$). Minimum or base temperature allowing germination was 8.75 °C, on average of cultivars (Table 6). The base temperature varied with the cultivar (CV 18.7%), from 7.44 °C ('PR811F') to 13.48 °C ('Nectar'). It is interesting to notice that, among the thirty sorghums, more than half exhibited a low Tb (<8 °C), including 'Keller' (7.95 °C). Conversely, a high thermal threshold for germination (Tb > 10 °C) was calculated, beside 'Nectar', in 'PR895', 'HayDay', 'Biomass H133' and 'Biomass 150', which indicates a great sensitivity to suboptimal temperatures during germination.

Table 6. Values of base temperature (Tb), b coefficient of linear regression of GR_{50} vs. T, estimated (from the model, ±σ) and calculated (inverse of the slope b) thermal time θ_T, in thirty cultivars of sorghum. Confidence limits for Tb are reported.

	***T*b**	**Confidence**	**b**	**θ_T (°Cd)**	
Cultivar	**(°C)**	**Limits**	**(d^{-1} °C^{-1})**	**From Model**	**1/b**
Jumbo	7.79	5.66 to 9.51	0.047	21.09 ± 3.93	21.28
Padana 1	9.88	9.10 to 10.58	0.034	26.85 ± 4.92	29.41
Padana 4	7.71	6.32 to 8.95	0.039	27.11 ± 4.77	25.45
Sugargraze	8.36	6.88 to 9.51	0.040	26.64 ± 6.41	24.94

Table 6. *Cont.*

Cultivar	*Tb* (°C)	Confidence Limits	b (d^{-1} °C^{-1})	θ_T (°Cd) From Model	1/b
PSE22043	8.01	7.03 to 8.66	0.033	32.00 ± 4.14	30.30
PSE22053	7.94	6.47 to 9.14	0.046	22.49 ± 4.15	21.74
PSE23431	7.97	6.77 to 9.03	0.036	29.26 ± 5.56	27.78
PSE24213	7.81	5.29 to 9.77	0.032	33.42 ± 7.68	31.25
PSE27677	7.95	6.62 to 9.07	0.033	31.79 ± 7.61	30.03
PSE98456	7.98	6.81 to 9.07	0.039	26.49 ± 4.55	25.64
HayDay	12.03	9.77 to 13.85	0.045	26.13 ± 4.71	22.22
Nectar	13.48	12.81 to 14.07	0.061	16.62 ± 3.50	16.39
Goliath	8.52	8.03 to 8.92	0.035	27.31 ± 5.58	28.57
Sucrosorgo 506	7.92	6.15 to 9.40	0.049	20.84 ± 4.53	20.41
Biomass 150	11.37	9.59 to 12.80	0.070	14.24 ± 1.40	14.29
Zerberus	7.81	6.55 to 8.81	0.054	19.13 ± 3.92	18.52
ABF26	7.98	6.84 to 9.00	0.045	22.12 ± 4.43	22.22
ABF306	7.96	7.11 to 8.66	0.042	24.28 ± 4.49	23.81
PR811F	7.44	6.15 to 8.51	0.044	23.34 ± 6.69	22.73
PR849	7.74	6.36 to 8.88	0.042	24.47 ± 3.34	23.81
PR895	10.03	8.14 to 11.92	0.041	26.02 ± 2.14	24.39
Nicol	8.71	7.32 to 10.06	0.046	21.16 ± 3.24	21.74
Topper	7.90	6.15 to 9.32	0.046	22.50 ± 2.62	21.74
Dale	7.99	6.25 to 9.32	0.046	22.04 ± 3.98	21.74
M81E	8.21	6.81 to 9.40	0.046	21.86 ±5.97	21.74
Keller	7.95	7.15 to 8.73	0.047	23.93 ± 4.65	21.28
Bulldozer	7.98	6.15 to 9.37	0.047	21.39 ± 4.59	21.28
Maya	7.59	6.40 to 8.62	0.040	25.06 ± 3.48	25.00
Silage King	9.43	7.48 to 9.75	0.040	22.10 ± 8.07	25.00
Biomass H133	13.03	11.66 to 14.00	0.049	20.97 ± 3.20	20.41
Average (±σ)	8.75 ± 1.64		0.044 ± 0.0002	24.09 ± 4.27	23.50 ± 3.97
CV (%)	18.7		3.36	17.7	16.9

Cultivars with similar *T*b had different germination rates (i.e., b slope of linear regression of GR_{50} vs. T), thus, revealing a faster or slower response to increasing (or decreasing) temperature. In particular, while for most cultivars, a b ranging from 0.04 to 0.05 d^{-1} °C^{-1} was calculated, in 'Biomass 150', this value increased to 0.07 d^{-1} °C^{-1}, which indicates a great seed responsiveness to temperature shifting (above or below) from the optimum one. In this cultivar, seeds reached 100% germination at 25 °C, but at 8 °C, germination dropped to 18.7%. Differently, low b coefficients (0.032–0.033 d^{-1}°C^{-1}) calculated in 'PSE27677', 'PSE24213' and 'PSE22043', reveal a low sensitivity to increasing or decreasing temperature, little varying their final germination with a changing temperature.

Thermal time to 50% germination, as estimated from the model (Equation (2)), was, on average, 24.09 °Cd^{-1}. It significantly differed with cultivar (CV 17.7%), ranging between 16.62 ('Nectar') and 33.42 °Cd^{-1} ('PSE24213'). This last cultivar also had a low *T*b, revealing a great cold tolerance, but a slow germination speed. A similar behaviour was observed in 'PSE27677' with a low base temperature (7.95 °C) and high thermal time (31.79 °Cd^{-1}). High θ_T (>30 °Cd^{-1}) was also required in 'PSE22043'. Contrastingly, low θ_T (<20.00 °Cd^{-1}), beside 'Nectar', was estimated in 'Biomass 150' and 'Zerberus'.

Values of θ_T as estimated from the model (Equation (2)), closely matched those calculated (from the inverse of the slope b of x-axis intercept of GR_{50} vs. T). When calculated as 1/b, the average θ_T was 23.50 °Cd^{-1}, with a 16.9% CV.

The observations of the graph divided in four quadrants allows us to point out the best combination, i.e., low Tb and low θ_T (Figure 3, quadrant A). A good combination of the two parameters (Tb and θ_T) corresponded to 'Zerberus', 'Sucrosorgo 506', 'Jumbo' and 'PR811F' (all in quadrant A). Contrastingly, in the upper right side of the figure (quadrant C), the worst combination corresponded to cultivars 'HayDay' and 'PR895'.

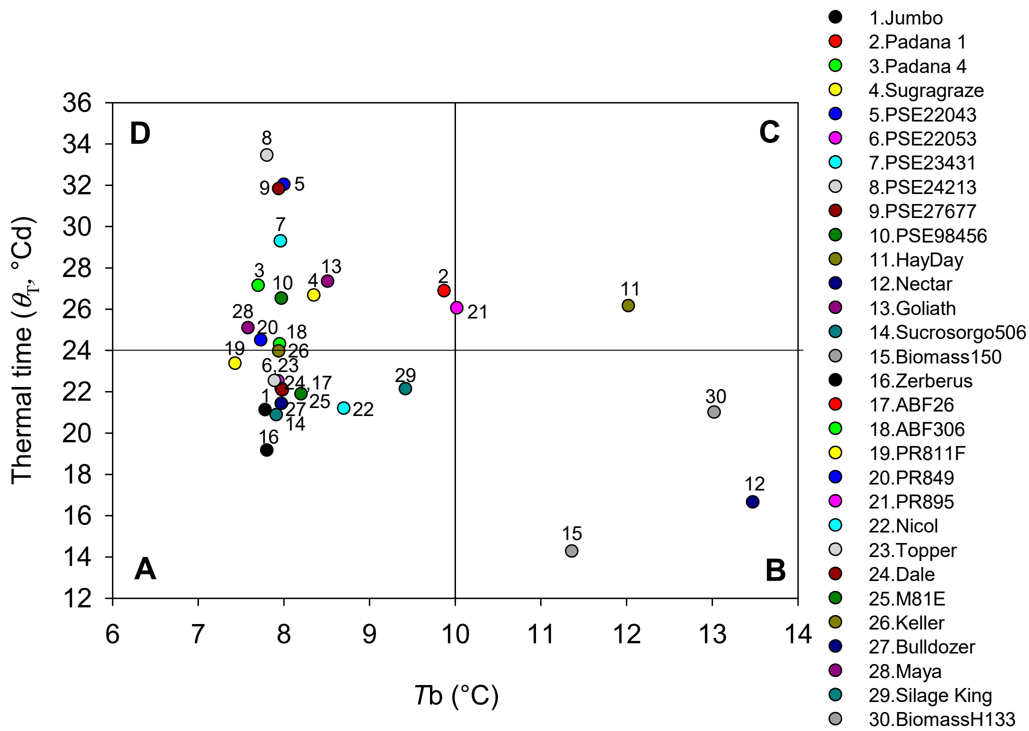

Figure 3. Thermal time (θ_T) vs. base temperature (*T*b) in the thirty cultivars of sorghum.

3.6. Biomass Yield in an Open Field

Final dry biomass measured in the thirty cultivars of sorghum under open field conditions was, on average, 17.51 ± 5.51 t ha^{-1} (Figure 4). A wide variability (CV 31.5%) among cultivars was calculated. Dry yield was maximized in 'PR811' (31.32 t ha^{-1}), which significantly differed from all cultivars of sorghum examined. Low biomasses (<11 t ha^{-1}) were harvested in 'Maya' and 'ABF26'.

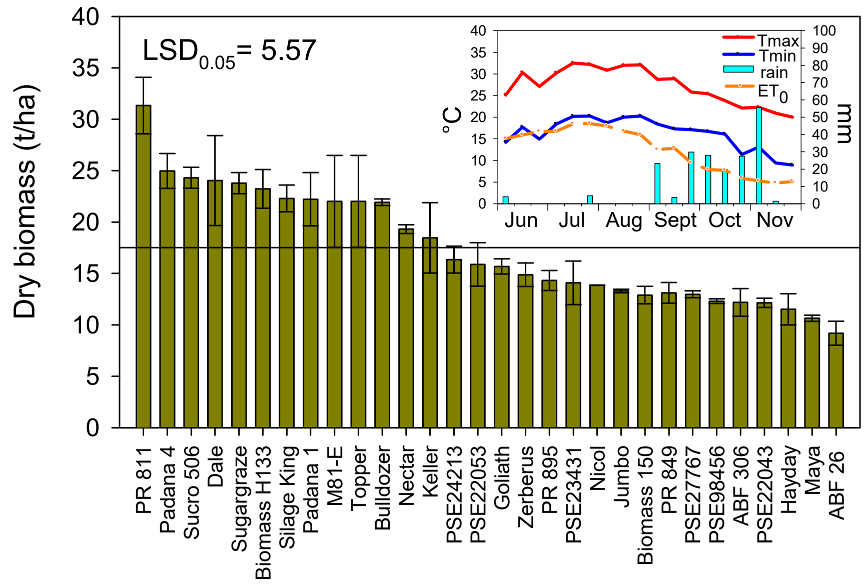

Figure 4. Dry biomass in thirty cultivars of sorghum measured in 2010 field experiment. Inset figure reports the meteorological trend (Tmax and Tmin as average values of 10-day period, rain and ET0 as cumulative values of 10-day period) recorded during the experiment (Catania, IT, 10 m a.s.l., 37°25′ N Lat, 15°30′ E Long).

3.7. Prediction of Germination Date in an Open Field

The dates of occurrence of 50% seed germination in the field in relation to three hypothetical times of sowing, as predicted for each cultivar of sorghum by θ_T and Tb values, are reported in Table 7. To this end, the mean soil temperatures recorded in the thirty-year period of 2005–2014 in the experimental site of the 2010 open field, were considered (Figure 5).

Table 7. Date of occurrence of 50% seed germination in the field in relation to three hypothetical early sowing dates, as predicted by thermal time.

	Sowing Date		
Cultivar	**March 1**	**March 15**	**April 1**
Jumbo	9/3	22/3	5/4
Padana 1	30/3	1/4	11/4
Padana 4	11/3	24/3	6/4
Sugargraze	16/3	26/3	7/4
PSE22043	16/3	26/3	7/4
PSE22053	10/3	23/3	5/4
PSE23431	14/3	25/3	7/4
PSE24213	15/3	26/3	7/4
PSE27677	15/3	26/3	7/4
PSE98456	13/3	24/3	6/4
HayDay	22/4	22/4	24/4
Nectar	4/5	4/5	4/5
Goliath	13/3	26/3	7/4
Sucrosorgo 506	10/3	23/3	5/4
Biomass 150	7/4	7/4	12/4
Zerberus	7/3	23/3	5/4
ABF26	10/3	24/3	5/4
ABF306	11/3	24/3	5/4
PR811F	9/3	22/3	5/4
PR849	10/3	23/3	5/4
PR895	31/3	2/4	11/4
Nicol	16/3	25/3	6/4
Topper	10/3	23/3	5/4
Dale	10/3	23/3	5/4
M81E	11/3	24/3	5/4
Keller	11/3	24/3	5/4
Bulldozer	10/3	23/3	5/4
Maya	10/3	23/3	5/4
Silage King	25/3	28/3	8/4
Biomass H133	19/4	19/4	19/4

With sowing in early March, predicted germination occurs over a window of almost two months (from March 9 to May 4). Cultivars that are better performing are 'Zerberus', 'Jumbo' and 'PR811F', that achieve 50% seed germination just after 6 ('Zerberus') and 8 days ('Jumbo' and 'PR811F'). Cultivars, as 'HayDay', 'Biomass H133', 'Biomass 150', take long to germinate (more than 1 month). 'Nectar' requires almost 2 months to 50% germination.

When the date of sowing is split to mid-March, the variability among cultivars decreases. Predicted germination occurs by the end of March in most cultivars, except 'Padana 1', 'HayDay', 'Biomass H133', 'Biomass 150', whose germination is delayed to April, and 'Nectar' that completes germination in early May. With a sowing in early April, 50% seeds germinate in 4–5 days in most cultivars. 'HayDay', 'Biomass H133' and 'Nectar' take much longer to germinate (20–30 days).

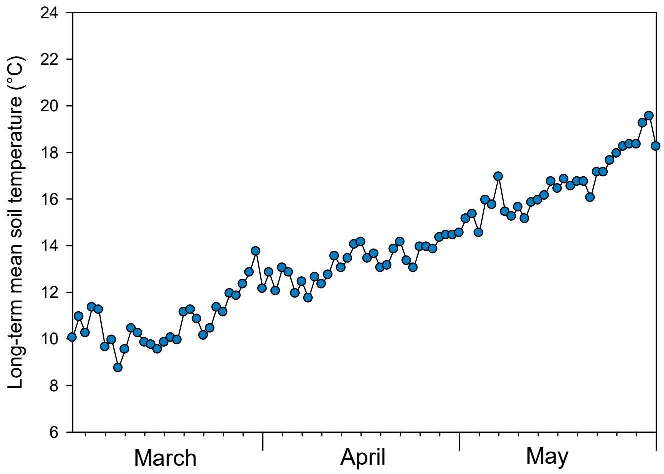

Figure 5. Mean soil temperatures recorded over the 10-year period of 2004–2015 in the 2010 field experimental site.

4. Discussion

Seeds have a critical temperature for germination, which means that those having a base temperature (*T*b) above actual *T*, fail to germinate. This occurs in all plants, including sorghum. Base temperature indicates the level of thermal stress the seeds may suffer, and the lower the *T*b, the greater the tolerance to stress due to a low temperature [14]. However, other factors, besides cold, which may affect the germination of seeds in soil, must be considered. In this regard, it has been observed that a delayed germination in cold soil, combined with a low seed tannin content, made the seeds of sorghum more susceptible to mould and other pathogens [21].

Results revealed that the seeds of sorghums studied responded differently when exposed to low temperature, both in terms of germination speed and extent. Significant variation of cold tolerance was also reported in literature in commercial sorghum hybrids under controlled low temperature in the laboratory [11]. In a previous research conducted on 242 accessions of sorghum at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) for seed germination and seedling vigor at 12 °C as a measure of cold tolerance, only one marker locus (Locus 7-2) was found to be significantly associated with low-temperature germination [22]. The use of this locus in molecular breeding of sorghum for low-temperature tolerance during germination has been suggested [22]. Harris et al. [23], working with nine genotypes of sorghum at a range of temperatures between 8 and 48 °C, found that, although final germination varied very little with temperature, *T*b widely differed among genotypes, between 8.5 and 11.5 °C. In our experiment, 18 of the 30 sorghums studied exhibited a *T*b lower than 8 °C.

The occurrence of a thermal dormancy in some cultivars could be suggested, at least in fractions of seed population, when seeds are incubated at low temperatures, which does not occur at optimal or supra-optimal temperatures. This form of dormancy may be considered as an adaptation strategy of seeds to survive in stressful conditions and guarantee favorable conditions for seedling growth [24].

However, cultivars with a final germination < 50% at 8 °C or even at 10 °C, have no agronomic value since they are not suitable to those regions, such as South Italy, when early sowings in sorghum are recommended. In other cultivars ('Padana 4', PR811F', 'Zerberus', 'PR849'), low temperatures only delayed the start of germination with minor effects on the final germination percentage (>90% at 8 °C). In these seeds, once the germination at low *T* started, it proceeded regularly. It has been observed how temperatures lower than optimal slow the rate of water absorption and the metabolic activation in seeds of sorghum [18,20].

In the thermal time, the model describes the pattern of seed germination in response to *T*, using only two parameters to predict germination: *T*b and θ_T. Once parameters are known, the germination time course at any *T* can be predicted by varying the value of *T* in

Equation (2). Since θ_T is constant, the larger the difference between actual T and Tb is, the faster the germination process (t) is and vice versa.

Low Tb allows seed germination at temperatures that, conversely, would inhibit germination in seeds of sorghums with higher Tb. However, a prolonged germination time, even in cultivars with low Tb, may result in altered seed performance in the field. Extended germination in the field longer exposes seeds to soil seedbed injuries and may result in poor crop uniformity and unsuccessful seedling establishment in the field [25].

Tiryaki and Andrews [8] found that the germination rate in cold conditions gives good separation among genotypes of sorghum. The authors also reported a highly significant correlation (R = 0.66) between cold germination measurements in the growth chamber and the rate of emergence in a field experiment. Similarly, Salas et al. [26] observed that twelve of the top fifteen accessions of sorghum exhibiting cold tolerance during germination in a 7-day test at 10 °C, were also ranked within the top 15 under field conditions. Accordingly, the authors suggested the breeders to perform a preliminary screening of sorghum germplasm for cold tolerant alleles adopting a 7-day cold test at 10 °C.

In our experiment, cultivars having similar Tb exhibited different θ_T requirements to germinate. In fact, no significant relationship was found between Tb and θ_T (R^2 = 0.14, data not shown). As an example, cultivars 'Bulldozer' and 'PSE23431' did not differ for Tb (7.98 and 7.97 °C, respectively), but the 'Bulldozer' needed to cumulate less θ_T (21.39 °Cd) than 'PSE23431' (29.26 °Cd) since this last one took longer to germinate.

Thermal time calculated from the inverse of the slope b of the x-axis intercept of GR_{50} vs. T, matched relatively well what was estimated by the model. In most cases (77% of cultivars), thermal time for germination was slightly overestimated by the model. These results highlight the validity of using both methods for thermal time estimation.

The wide genetic variability observed in thermal time requirements and Tb values was confirmed in the predicted time required by the different cultivars to reach 50% seed germination in the field, when three hypothetical dates of sowings (March 1, March 15, and April 1) are considered.

According to the results, it is possible to suggest the adoption of early sowings with seeds of 'Zerberus', 'Sucrosorgo 506', 'Jumbo' and 'PR811F', that may provide good plant stands at suboptimal soil temperature conditions (9–11 °C), as those occurring in late winter-early spring in a semi-arid environment.

Among all cultivars, only 'PR811F' (fiber type) and 'Sucrosorgo 506' (sweet type) combine good adaption to early sowings with high productivity in terms of final dry biomass. Cultivars such as 'Biomass H133' and 'Nectar' are high-yielding. However, they are not suitable to early sowings due to high cold sensitivity during germination.

5. Conclusions

The results of this study indicate that a decreasing temperature from the optimum level reduces seed germination of sorghum, to a greater extent, when the temperature is lower than 15 °C. However, wide differences for cold tolerance existed among sweet and fiber sorghums assessed under controlled temperatures in the laboratory. This genetic variation in germination response to a low temperature suggest the possibility of screening among cultivars for those with high thermal stress tolerance during germination, which are suitable to early sowings in semi-arid areas. In particular, criteria for selection are a low base temperature and low thermal time requirements (as observed in cultivars 'Jumbo', 'PR811F', 'Zerberus', all fiber types, and 'Sucrosorgo 506', sweet type) that, if coupled, ensure adequate seedling establishment standards when late winter-early spring sowings (March-April) are adopted in the Mediterranean environment. Among these cultivars, 'PR811F' and 'Sucrosorgo 506' combine high cold tolerance during germination with a good biomass yield potential.

The identification of cultivars cold tolerant during germination may also contribute to the expansion of biomass sorghum into cooler cultivation areas, such as those of Northern Europe, which are less suitable to this warm season crop.

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