GENOTYPES SCREENING FOR COLD TOLERANCE DURING GERMINATION IN SORGHUM [*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 (MIPAF), a preliminary laboratory test was carried out with the aim of quantifying the variability for cold tolerance during germination in several cultivars of sweet and fiber sorghum, and defining the limit to the early sowings in the semi-arid Mediterranean environments of Southern Italy through the identification of minimum thermal threshold for seed germination. A genetic diversity in germination response of sorghum, greater at the lowest temperatures (8 and 10°C), was ascertained. Sweet types appeared more sensitive than fiber types to low temperature during germination. Base temperature varied with cultivar from 6.08°C to 10.68°C. The genetic variation in germination response to low temperature during germination is useful for the identification of genotypes of sorghum suitable to early sowings in semi-arid areas. In particular, criteria for selection are low base temperature and low thermal time requirements that if associated, ensure adequate seedling establishment standards when early sowings (March-April) are adopted in Mediterranean environment.
Keywords: genotype, germination, low temperature, sorghum bicolor L. Moench

1 INTRODUCTION

Sweet and fiber sorghum [*Sorghum bicolor* (L.) Moench], as high-yielding species, is considered a promising industrial crop for the European Community, for the bioethanol (sweet types) and the lignocellulosic (fiber types) chain [1]. Field experiments conducted in different areas of Europe confirmed the high yield potential of this crop, under no water limitations [2, 3, 4].

Constraints to the cultivation of sorghum, originating from tropical areas near the Equator, were found in the high temperature requirements for seed germination. Low temperature stress at planting may result in poor seedling establishment due to slow emergence rate or reduced emergence percentage [5]. However, germination at low temperatures may permit earlier sowings and allow the seminal root system to gain access to the water stored into the soil during winter, thus reducing irrigation requirements. In this view, the identification of cold tolerant cultivars during germination in sorghum is required.

Genetic differences in the response of germination at low temperatures are useful in identifying genotypes tolerant to low temperatures during germination and therefore suitable to early sowings in the Mediterranean environment [6]. The germination of sorghum under low temperatures has been widely studied in grain types [7, 8] but few researches have been carried out on sweet and fiber sorghums [9]. Furthermore, the weather changes which may occur from year to year in the same experimental site, make often difficult and unreliable the selection for cold evaluation within a broad number of genotypes under field conditions [6]. To this respect, a preliminary screening under controlled temperature conditions is required in the selection of 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 laboratory test was carried out with the aim of quantifying the variability for cold tolerance during germination in several cultivars of sweet and fiber sorghum, and defining the limit to the early sowings in the semi-arid Mediterranean environments of Southern Italy through the identification of minimum thermal threshold for seed germination.

2 MATERIALS AND METHODS

The germination tests were conducted in laboratory on the seeds of 23 cultivars of biomass sorghum among fiber and sweet types. When the experiments were conducted, the seeds were less than 12 months old, kept at room temperature (10-20°C) before being tested. The seeds were left to germinate at seven constant temperatures: 8, 10, 15, 20, 25, and 30°C, with 25°C considered as control, based on reports in literature as optimal for seed germination of sorghum [10]. The tests were conducted in a thermostatically controlled (± 1° C) incubator. Samples of 200 seeds (four 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 and then randomised within each temperature and incubated in the dark. Seed germination was scored daily on the seeds whose radicle reached at least 2 mm in length, until no additional germination occurred for 72 h. At the end of the test, final germination (%), Mean Germination Time (MGT, days) and the median response time (actual time to 50% of germination or t_{50}) were calculated. Data of the final percentage germination, previously arcsine transformed, and those of MGT, were statistically analysed by a completely randomised two-way (temperature x cultivar) analysis of variance (ANOVA). The theoretical minimum temperature of germination or base temperature (T_b) was calculated for each cultivar using a linear regression of the inverse values of t_{50} ($1/t_{50}$ or GR_{50}) vs. germination temperature, whose slope b is the germination rate with decreasing temperature (the higher the b, the faster the germination with temperature increase). The abscissa intercept is an estimate of the theoretical minimum temperature of germination (T_b) [11]. Thermal time (θ_T)

to achieve 50% germination at each temperature was also calculated for each cultivar using the equation:

$\theta_T(50) = (T\text{-}T_b)\, t_{50}$

where $\theta_T(50)$ = thermal time to 50% germination (°Cd^{-1}), T= germination temperature (°C, constant in controlled environment), T_b = base germination temperature, t_{50}= median response time [11].

3 RESULTS

3.1 Final germination under controlled temperatures

Seed germination of sorghum was significantly affected by temperatures, decreasing with the lowering of temperatures, from 92.9% at 25°C to 55.9% at 8°C (Table I). The results confirm 25°C as the optimum of temperature for the germination of sorghum.

Table I: Effects of temperature on final germination for 23 biomass sorghums (F= fiber sorghum: S= sweet sorghum).

Cultivar	Germination (%)					
	8°C	10°C	15°C	20°C	25°C	30°C
Jumbo (F)	74.7	97.8	100.0	100.0	98.7	97.8
PSE98456 (F)	60.0	97.3	98.7	98.3	98.7	93.5
Padana 4 (F)	90.1	95.3	98.6	97.6	97.3	97.3
PR811F (F)	84.4	93.3	98.7	100.0	98.7	92.2
Sugargraze (S)	61.8	92.0	92.0	92.2	94.7	92.2
PSE27677 (F)	62.7	92.0	96.0	96.0	100.0	100.0
PSE23431 (F)	59.5	90.9	93.3	100.0	98.7	97.3
Bulldozer (S)	50.7	86.7	92.0	95.6	92.1	91.1
PSE22053 (F)	64.6	86.7	92.0	90.0	94.7	90.9
PSE24213 (F)	80.6	85.4	89.3	95.1	93.3	98.7
PSE22043 (F)	66.6	82.3	85.3	92.6	96.0	90.8
PR849 (F)	82.0	82.2	93.4	92.2	90.7	87.8
Maya (S)	70.0	81.1	81.3	91.3	92.0	89.1
Topper (S)	72.2	80.0	93.3	92.1	92.0	87.8
Dale (S)	61.1	77.8	93.3	94.4	92.0	88.9
M81E (S)	42.2	77.8	92.2	92.2	96.0	92.2
Padana 1(F)	34.9	68.0	67.8	73.3	90.0	85.9
Silage King (S)	23.3	60.0	81.3	85.6	89.3	85.1
Nicol (S)	41.3	58.9	90.8	88.9	96.0	92.2
PR895 (F)	12.0	56.7	81.9	86.7	84.3	80.0
HayDay (F)	41.3	49.3	61.3	86.7	68.0	61.3
H133 (F)	27.4	34.2	82.7	80.1	82.7	63.1
Nectar (S)	23.6	30.7	81.7	94.7	100.0	97.3
Mean	55.9	76.4	88.6	92.0	92.9	89.2
σ	21.7	19.4	9.7	6.5	7.1	9.8
CV(%)	38.8	25.4	10.9	7.0	7.7	11.0

However, cultivars behaved differently at suboptimal temperatures (*cultivar* x *temperature* significant at p<0.001, Table II). Indeed, genetic variability increased with the lowering of germination temperature.

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 almost full (>98.5%) in 'PSE98456', 'Padana 4' and 'PR811F', fiber sorghums all of them.

At 10°C, variability for germination among sorghums became higher (CV= 25.4%). At this temperature, in seven cultivars, mostly fiber types, germination was greater than 90%, whilst cultivars 'HayDay', 'H133' and 'Nectar' did not achieve 50% of seeds germinated.

At 8°C variability was maximum (>38%) since seed germination dropped in many cultivars. At this temperature, height cultivars still germinated for more than 70%, with 'Padana 4' 'PR811F', 'PSE23431', 'PSE24213' and 'PR849', fiber types all of them, being the best performing (germination>80%). In particular, 'Padana 4' was the most tolerant to low temperature (germination >90%).

However, 8 cultivars did not achieve 50% of seeds germinated, thus reveling a great sensitivity to cold stress during germination. To this respect, these last cultivars are not suitable to early sowings. Generally, sweet types appeared more sensitive than fiber types to low temperature during germination.

Table II: Two-way analysis of variance for final germination of 23 biomass sorghums at a range of constant temperatures

Source of variation	df	SS	MS	F	P
Replicate	3	163.5	81.7	2.20	.11 ns
Cultivar (C)	22	31406.5	1427.6	38.3	.000***
Temperature (T)	5	41342.1	8268.4	222.1	.000***
C x T	110	14902.2	135.5	3.64	.000***
Error	411	10200.1	37.2		
Total	551	98014.4			

3.2 MGT under controlled temperatures

Mean Gemination Time (MGT) varied with temperature and cultivar. Germination was faster at the highest temperatures and at 30°C all cultivars germinated in less than 2 days (Table III).

Table III: Effects of temperature on Mean Germination Time for 23 biomass sorghums (F= fiber sorghum: S= sweet sorghum).

Cultivar	MGT (days)					
	8°C	10°C	15°C	20°C	25°C	30°C
Jumbo (F)	19.1	6.3	4.1	2.5	1.8	1.2
PSE98456 (F)	16.4	11.4	5.3	2.8	2.0	1.0
Padana 4 (F)	15.5	11.6	5.0	2.4	2.0	1.0
PR811F (F)	14.7	5.5	3.5	2.5	1.9	1.3
Sugargraze (S)	14.6	13.5	5.1	2.6	2.1	1.1
PSE27677 (F)	16.3	11.8	6.5	2.6	2.4	1.2
PSE23431 (F)	18.6	12.5	5.1	2.8	2.1	1.1
Bulldozer (S)	18.5	6.9	4.2	2.1	2.0	1.2
PSE22053 (F)	15.7	10.2	4.8	2.4	1.7	1.1
PSE24213 (F)	16.4	11.9	6.0	3.1	2.5	1.3
PSE22043 (F)	20.0	12.6	5.6	3.0	2.3	1.1
PR849 (F)	18.3	8.3	4.6	2.7	2.0	1.4
Maya (S)	17.0	7.0	4.1	3.0	1.4	1.2
Topper (S)	15.0	8.7	3.8	2.7	2.0	1.3
Dale (S)	15.8	7.7	3.6	2.8	2.0	1.2
M81E (S)	18.2	8.5	3.9	2.7	2.3	1.2
Padana 1(F)	19.4	13.7	6.1	3.8	2.3	1.5
Silage King (S)	19.2	10.1	4.9	3.5	2.1	1.5
Nicol (S)	19.5	9.6	4.3	2.8	1.8	1.6
PR895 (F)	25.4	16.2	5.8	3.4	2.1	1.7
HayDay (F)	17.4	12.8	6.3	2.7	2.5	1.5
H133 (F)	21.5	15.3	6.2	3.9	2.5	1.2
Nectar (S)	23.1	17.5	6.8	2.7	2.3	1.2
Mean	18.1	10.9	5.0	2.9	2.1	1.3
σ	2.7	3.2	1.0	0.4	0.3	0.2
CV(%)	15.0	29.8	19.7	15.4	12.9	15.0

The lowering of temperatures from the optimal one determined a progressive increase in germination time, although in a different extent depending on cultivar (*cultivar* x *temperature* significant at p<0.001, Table IV).

At 15°C seeds took 5 days to germinate, on average of cultivars, with 'PR811F' being the fastest (3.5 days MGT) and 'Nectar' the slowest (6.8 days MGT).

At 10°C the genetic variability for germination rate was the greatest (29.8%), since cultivars quite differently responded to this cold temperature in terms of germination speed. 'Nectar' was the slowest cultivar (17.5 days MGT) under this temperature as well, whilst 'PR811F' was the fastest, reaching its final germination in a 5.5 days MGT.

Table IV: Two-way analysis of variance for MGT of 23 biomass sorghums at a range of constant temperatures

Source of variation	df	SS	MS	F	P
Replicate	3	0.21	0.10	0.31	.74 ns
Cultivar (C)	22	3867.1	175.8	523.6	.000***
Temperature (T)	5	14999.7	2999.9	8935.9	.000***
C x T	110	19229.0	174.8	520.7	.000***
Error	411	92.0	0.34		
Total	551	38188.0			

At 8°C germination still slowed down, and seeds reached the final germination in more than 18 days (MGT), on average of cultivars. The longest MGT (>23 days) was recorded in 'Nectar' and 'PR895', the shortest MGT (<15 days) corresponded to cultivars 'Sugargraze' and 'PR811F'.

3.3 Final germination vs. MGT

The relationship of final germination values *vs*. mean germination time (MGT) values at 8°C was studied (Fig. 1). An exponential decay model best fitted the data (R^2 = 0.70), whose trend reveals how cold-tolerant cultivars tend to germinate faster than those susceptible to low temperature.

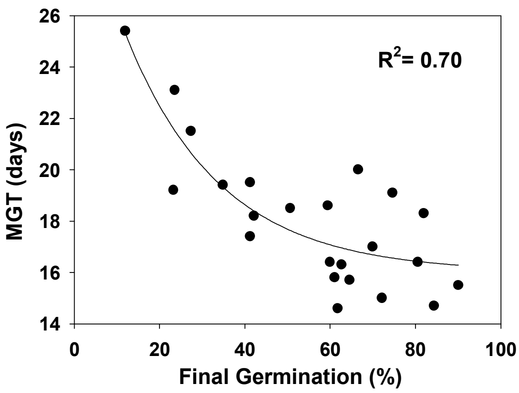

Figure 1: Relationship of final germination values vs. MGT values recorded at 8°C.

3.4 Base temperature and thermal time

Minimum or base temperature allowing germination was 7.87°C on average of cultivars (Table V).

Base temperature varied with cultivar from 6.08°C to 10.68°C. Low base temperatures (<7°C) were calculated for 'PSE24213', 'Padana 4', 'PR811F', 'Sugargraze', 'PSE27677', 'PR849' and 'Topper'. Conversely, a high thermal threshold for germination (>10°C) was calculated for 'Nectar' and 'H133'. No difference in terms of cold tolerance was observed among types (fiber or sweet).

Table V: Values of b coefficient, base temperature (T_b) and thermal time to germination (θ_T) calculated for 23 biomass sorghums (F= fiber sorghum: S= sweet sorghum).

Cultivar	b (d^{-1}°C^{-1})	T_b (°C)	$\theta_T \pm \sigma$ (°Cd^{-1})
Jumbo (F)	0.039	7.40	23.65
PSE98456 (F)	0.034	7.51	27.42
Padana 4 (F)	0.028	6.55	35.02
PR811F (F)	0.040	6.16	24.72
Sugargraze (S)	0.023	6.45	43.03
PSE27677 (F)	0.023	6.67	41.89
PSE23431 (F)	0.026	7.00	36.62
Bulldozer (S)	0.042	7.60	21.68
PSE22053 (F)	0.039	7.58	26.71
PSE24213 (F)	0.022	6.08	43.57
PSE22043 (F)	0.027	7.82	38.42
PR849 (F)	0.033	6.87	29.10
Maya (S)	0.038	7.64	23.75
Topper (S)	0.040	6.88	23.59
Dale (S)	0.034	7.20	27.74
M81E (S)	0.039	8.54	25.25
Padana 1(F)	0.027	9.17	40.42
Silage King (S)	0.040	9.23	24.68
Nicol (S)	0.041	9.22	23.73
PR895 (F)	0.039	9.35	25.30
HayDay (F)	0.030	9.26	36.81
H133 (F)	0.026	10.15	38.66
Nectar (S)	0.027	10.68	36.71
Mean	0.033	7.87	31.24
σ	0.007	1.32	7.47
CV(%)	20.61	16.80	23.91

Many cultivars with a similar T_b have different germination rates (b slope of the linear regression of GR_{50} vs. germination temperature), thus showing a faster or slower response to increasing temperature. However, beside cold tolerance, a fast germination rate with increasing temperatures is also important for sorghum, when a prompt emergence, in rapid drying soils as those of the semi-arid areas of Southern Europe, is required.

Thermal time to reach 50% germination differed with cultivar, ranging between 21.68 °Cd^{-1} ('Bulldozer') and 43.57 °Cd^{-1} ('PSE24213'). This last cultivar had the lowest base temperature, thus revealing a great cold tolerance but a slow germination speed. A similar behavior was observed in 'Sugargraze', with a low base temperature (6.45°C) but a high thermal time (43.03 °Cd^{-1}). Low thermal times (<24 °Cd^{-1}) was calculated for 'Jumbo', 'Maya', 'Topper', 'Nicol'; high thermal times (> 40°Cd^{-1}) corresponded to 'PSE27677' and 'Padana 1' as well.

4 CONCLUSIONS

Significant differences for cold tolerance existed among sweet and fiber sorghums assessed under controlled temperatures in laboratory.

Previous studies in sorghum [6] highlighted significant correlations among cold-tolerance traits, such as germination percentage and speed, measured in laboratory, and field assays for cold tolerance, indicating that the laboratory assay could be successfully used as a preliminary screening method for cold tolerance assessment in sorghum.

This genetic variation in germination response to low temperature during germination is useful for the identification of genotypes of sorghum 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 'PR811F' and 'PR849', both fiber types) that if associated, ensure adequate seedling establishment standards when early sowings (March-April) are adopted in Mediterranean environment.

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