

Germination and vigor in sorghum seeds under flood stress

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Abstract—*The objective of this study was to evaluate the physiological quality of sorghum seeds under water stress. For the experiment, five lots of sorghum seeds with the same genotype (CMX5156A) and different physiological qualities were used. Five lots were submitted to different combinations of hypoxia time (4, 8, 16, 24, 36, and 48 hours under water) and temperature (20, 30, and 40°C). The moisture content, percentage of germination and emergence, height, and dry matter of seedlings from soaked seeds, were determined. A completely randomized experimental design in a 5 x 6 x 3 factorial, with four replicates, was used. The effect of hypoxia stressed the sorghum seed, affecting the germination of the seeds and emergence, dry mass, and height of the seedlings. The greatest decreases in viability and vigor were observed at higher temperatures and flood times. It is concluded that the germination and vigor of sorghum seeds are adversely affected by flooding. The submersion of seed in water at 40°C for 24 hours allows the segregation of sorghum seed lots.*

I. INTRODUCTION

Modern agricultural markets aim for high-quality use of inputs, which encourages the development of new methodologies to determine the vigor and viability of seeds (AOSA, 2009). An efficient vigor test should be low-cost, fast, simple, objective, and reproducible, and the results must perform similar to the seedling emergence test in the field (Krzyzanowski et al., 2021). On the same hand, on-farm tests to estimate the physiological potential of seeds, which have low dependence on high-cost equipment and can provide fast, easy-to-interpret, and highly reproducible results, represent an additional advantage compared to traditional methods.

An alternative to determine seed vigor, is the flooding test, in which the seed to be evaluated is submerged in water and subject to stress by rapid imbibition and lack of oxygen. Under these conditions, there is an increase in the deterioration of physiological quality, compromising germination and seed vigor (Dantas et al., 2000; Zucareli et al., 2011). The limited amount of oxygen during the soaking period (hypoxia) is the mechanism and factor that underlies flooding as a test of vigor. This oxygen restriction induces a change from the aerobic airway to the anaerobic or fermentative one (Castro et al., 2004). This promotes the unbalanced formation of reactive oxygen species (ROS), which are highly deleterious, generating seed unviability or compromised seedling development and growth (Sharma et al., 2012).

Flooding stress on germination and seed vigor has been evaluated for several agricultural crops as a tool to evaluate seed lots, such as alfalfa (Bonacin et al., 2006), beans (Custodio et al., 2002; Bertolin, 2010), maize (Gazola et al., 2014), and soybean (Theodoro, 2013; Theodoro et al., 2018). There is a lack of studies evaluating the flooding methodology on sorghum seeds.

Hence, the objective of this study was to evaluate different periods of flooding and water temperatures on the vigor of sorghum seeds, in order to propose a new methodology of evaluating the physiological quality of sorghum seed lots.

II. MATERIAL AND METHODS

The research was carried out at the Seed Analysis Laboratory of Embrapa Milho e Sorgo. Seed lots with different levels of physiological quality were obtained by artificially aging the CMSX 5156 A genotype, at a temperature of 47°C for 0 (control), 24, 48, 72, and 96 hours (Lima et al., 2019). After the artificial deterioration of the lots, seeds were homogenized and stored in Kraft paper bags in a controlled environment (20°C and 60% relative humidity). The initial quality of each lot was characterized through evaluations of water content, carried out by the oven method at $105 \pm 3^\circ\text{C}$, for 24 hours (BRASIL, 2009); and germination percentage, using 4 replicates of 50 seeds per batch, with each batch sown on filter paper moistened with distilled water in the amount equivalent to 2.5 times the mass of the dry paper, and placed to germinate at 25°C in gerbox-type plastic boxes. The count was performed five days after the start of the test, according to the criteria established by ISTA (2011). The emergence of seedlings in sand was mensurated by counting the emerged and complete seedlings, with evaluations carried out daily until 14 days after sowing (Santana & Ranal, 2000). Results were expressed in percentage of seedlings.

Additionally, a cold test was carried out using a cold chamber at 10°C, in which seeds were held for seven days on a paper roll (Costa et al., 2011). After this period, the rollers were removed from the bags and placed in a germinator at 25°C for five days, after which point the percentages of normal seedlings were computed. Electrical conductivity was determined using four replicates of 50 physically pure seeds from each batch, previously weighed and immersed in 75 mL of distilled water, at 25°C, for 24

hours. Each solution had its electrical conductivity evaluated, with results expressed in $\mu\text{Scm}^{-1}\text{g}^{-1}$ of seeds.

To measure the effects of seed flooding on the five different lots obtained by accelerated aging, a combination of flooding times and temperatures was used: temperatures of 20, 30, and 40°C, for 4, 8, 16, 24, 36, and 48 hours of submersion. The experimental design was completely randomized, in a 3 x 5 x 6 factorial scheme of temperatures, lots, and flooding times respectively, with four replicates. For each repetition, 50 seeds were used, and submerged in 75 mL of distilled water in 250 mL plastic cups.

After each flooding period, the sorghum seed samples were removed from the water and washed with distilled water. The seeds were then evaluated daily until the germination and emergence values stabilized. The final percentage and mean germination time on paper, and seedling emergence in sand, were determined (Ranal; Santana, 2006). At the end of the emergency readings, 15 representative seedlings of the set were collected in each replicate to determine the height and dry matter mass of seedlings from seeds subject to flooding processes.

The collected data were analyzed with an analysis of variance (ANOVA), and, in cases of significant effects, the means were compared by the Tukey test at 5% significance. For the quantitative treatments, regression methodology was applied using surface responses. Correlations were performed between the means obtained in the test of emergence values, using the simple Spearman model; and the correlation coefficients ($p \leq 0.05$), using the test *t*.

III. RESULTS AND DISCUSSION

The water content of sorghum seeds remained similar between the lots. There was a variation in the initial water content of 1.7 percentage points between the highest observed value (lot 3 with 13.1%) and the lowest (lot 1 with 11.4%) (Table 1). The other quality attributes were affected by different periods of exposure to accelerated aging, which established the constitution of different sorghum seed lots.

Table 1- Water content (WC), germination (G), seedling emergence (SE), cold test (CT), seedling dry matter (DM), seedling height (SH) and electrical conductivity (EC) of five lots of sorghum seeds.

Lots	WC	G	SE (%)	CT	DM	SH	EC	Vigor
					mg	cm	$\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$	level
1	11.4	93.2	95.7 a	88 a	60 a	26.67 a	41.78 c	high
2	12.7	92.2	94.7 ab	86 a	61 a	26.40 a	44.80 b	high
3	13.1	91.5	91.2 ab	88 a	55 a	24.27ab	42.60 bc	medium
4	12.6	92.2	91.0 ab	88 a	53 a	23.37 b	47.83 a	medium
5	12.9	91.5	89.0 b	84 b	36 b	19.90 c	49.73 a	low
Average	12,5	92,12	92,2	86,8	53	24,12	46,54	
C.V. (%)	5,4	1,4	2,93	2,16	10,75	5,11	5,8	

Means followed by the same letter in the column do not differ from each other, according to the Tukey test ($p \leq 0.05$).

The lots showed high germination performance, with values ranging from 91.5 to 93.2%, with no difference between them. In terms of vigor presented by the emergency test and electrical conductivity readings, lot 1 was classified as superior. On the other hand, lot 5 was classified as inferior because it exhibited inferior performance in the other vigor tests (seedling emergence, cold test, seedling dry matter mass, seedling height, and electrical conductivity). The inferiority of lot 5 was

evident in majority of tests. Thus, the lots were segregated into three vigor categories for the analysis of the effect of seed flooding: high (lots 1 and 2), medium (lots 3 and 4), and low (lot 5).

It was observed that the viability and vigor of sorghum seeds were negatively affected by the increase in temperature and length of soaking, as seen in the variable germination, emergence, height, and dry matter mass of shoot (Table 2).

Table 2. Summary of analysis of variance for germination (G), seedling emergence in the field (E), seedling shoot height (SH), seedling dry matter (DM), mean germination time (MGT), and mean soil emergence time (MET) of five sorghum lots.

Source of variation	Mean squares					
	G	E	SH	DM	MGT	MET
Lot (L)	4	43.54**	5466**	103**	368.84**	0.17**
Temperature (T)	2	1636.42**	986**	256**	2276.74**	0.55**
Time (H)	5	562.61**	1273**	904**	6726.21**	0.23**
L x T	8	13.95 ns	206**	24.75**	25.81	0.03ns
L x H	20	21.65**	198*	15.28**	34.42**	0.06ns
T x H	10	416.24**	605**	14.41*	118.24**	0.10**
T x L x H	40	23.43**	113**	14.56**	334.02	0.04ns
Error	270	10.51	71	5.82	20.26	0.04
Average		89.18	70.7	19.18	24.65	3.87
CV (%)		3.64	12.04	3.64	18.26	4.09

*, **, 5 and 1% of significance, respectively, by the test F.

The germination percentage of the lots decreased as a function of the increase in period and temperature of flooding. The lots had greater decreases with exposure to

40°C water (Figure 1) over time. For all evaluated times, at the lowest temperature of 20°C, the viability losses showed the lowest range of germination values. Over time,

the decreases were intensified after 4 hours of flooding, except for lot 3 which showed greater initial resilience (Figure 1). Under stress conditions, seeds can increase the formation of reactive oxygen species (ROS), which are highly harmful to cells and tissues at elevated levels, and can promote the oxidation of ADN, proteins, cell membranes, and, in more serious cases, cellular death (Soares; Machado, 2007). Intensification in the production of ROS can reach toxic levels and thus promote membrane peroxidation, electrolyte leakage, cell death, and consequently reduce seed germination and seedling vigor (Tunes et al., 2011).

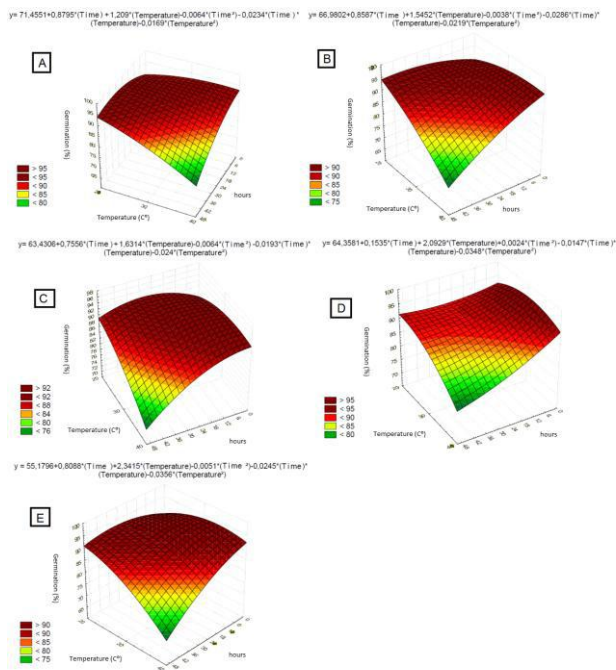


Fig.1. Surface responses to germination percentage of sorghum seed lots after flooding. (A) Lot 1 with high vigor, (B) Lot 2 with high vigor, (C) Lot 3 with medium vigor, (D) Lot 4 with medium vigor, (E) Lot 5 with low vigor.

In seedling emergence in the field, the sorghum lots showed different behavior at the temperatures studied, with the greatest reductions being observed at the highest temperature of 40°C. Decreases in this temperature ranged from 55.5% in lot 3 (medium vigor) to 67% in lot 5 (low vigor) (Figure 2). As the final temperature and the flooding time increased, the reduction in the percentage of emerged seeds for all lots were more significant (Figure 2). Therefore, it is important to highlight that under regular germination conditions, reactive oxygen species are controlled by antioxidant-metabolizing enzymes and used in the germination process as important signaling molecules (Bailly, 2004; Flores et al., 2014). However, when germination occurs in a hypoxic environment with high temperature, a redox imbalance occurs, observed in

the high levels of ROS, which compromise the integrity of cells and, consequently, seed germination. In this sense, the lot with greater initial vigor showed greater tolerance to oxidative stress by flooding, demonstrating the importance of using seeds of high physiological quality, especially with high vigor, for stress conditions (Moterle et al., 2006).

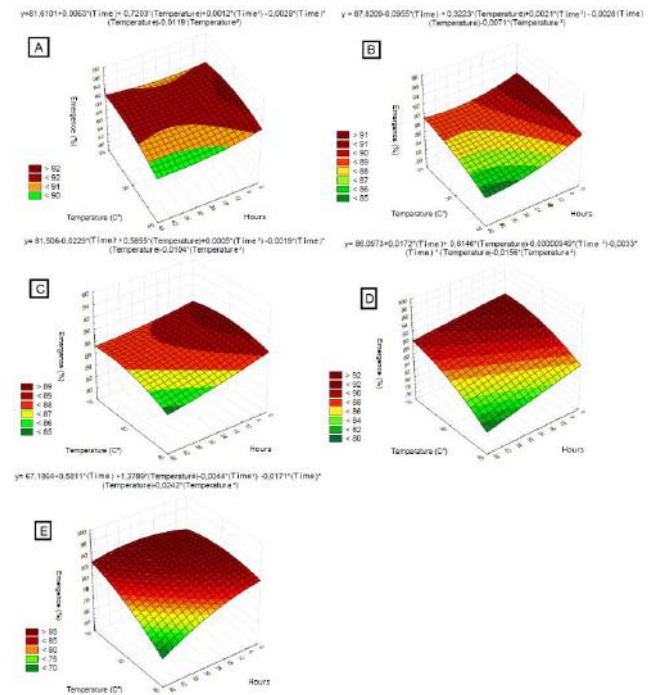


Fig.2. Surface responses of the percentage of emergence of sorghum seedlings after flooding. (A) Lot 1, (B) Lot 2, (C) Lot 3, (D) Lot 4, (E) Lot 5.

In terms of seedling dry matter mass, accentuated and linear decreases were observed in relation to the flooding periods in all lots (Figure 3). After 8 hours, the greatest reductions were observed, with the decrease in vigor being more accentuated when the seeds were exposed to temperatures of 40°C. The causes of reduced plantlet growth may be related to cellular turgor, which provides changes in metabolism according to the decrease in key protein synthesis during the germination process (Ge et al., 2014). These cellular turgor effects occur at the beginning of germination and can cause damage to the seeds (Oliveira et al., 2009).

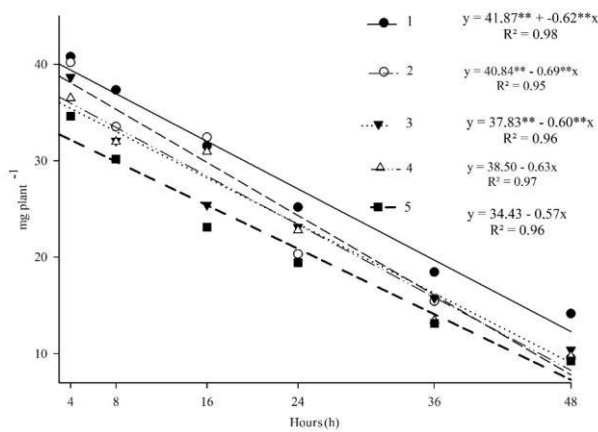


Fig.3. Dry matter mass of sorghum seedlings from submerged lots (1 and 2 with high vigor, 3 and 4 with medium vigor, and 5 with low vigor) at different times of hypoxia.

The hypoxia caused stress in sorghum seeds, particularly on seed vigor, influencing seedling development and growth (Table 3). There were lower rates of germination and emergence in the soil, with linear losses at the highest flooding temperatures.

Table 3. Regressions for mean emergence and germination time for sorghum seeds submerged in water at 20, 30, and 40°C, as a function of immersion time.

Temperature	Average emergence time		
20	$y = 5.38023725 - 0.14373980^{**}x + 0.0073051x^2 - 0.00009753x^3$	$R^2 = 0.9919$	< 0.0001
30	$y = 4.41550382 - 0.00538263^{**}x$	$R^2 = 0.7779$	0.0419
40	$y = 4.86044656 - 0.00904437x$	$R^2 = 0.7648$	0.0007
	Average germination time		
20	$y = 3.92979837 + 0.01057147x - 0.00127531x^2 + 0.00002251x^3$	$R^2 = 0.8547$	0.0398
30	$Y = 3.795$		0.1277
40	$y = 3.97978817 - 0.00236116x$	$R^2 = 0.6378$	0.0383

** and * significant at 0.05 and 0.01 probability by the test F, respectively

These phenomena are extremely important for crop plants, especially given that sorghum has small seeds, is difficult to plant and has a competitive advantage in terms of phytosanitary factors (especially weeds) and rapid establishment and development in the field. Greater exposure to a hypoxic environment during germination may represent the same damage in seeds as drought, salinity, and high temperatures (Visser et al., 2003), because there is an increase in cytosolic pH when activating the fermentative pathways, which compromises, among others, the effectiveness of water transport by aquaporins that are sensitive to low pHs (Reddy et al., 2015).

In turn, the average times of germination and emergence in the soil did not undergo substantial variations, with low decreases due the treatments in both variables. The germination values ranged from 3.72 to 4.03 days, as expected for lots of appropriate quality seeds of this species. The same applies to the emergence in soil;

it was found that the seedlings emerged at an average of 4.65 days. Treatments (time and temperature) with extreme values promote a low amplitude of only 0.91 days (Table 3).

The effect of flooding stress on sorghum seeds, as functions of the flooding periods and the reduction in vigor, partially corroborate the studies by Dantas et al. (2000) and Gazola et al. (2014) on maize seeds, and Custodio et al. (2002) on beans. These authors observed a significant effect on the seeds, and drastic reductions in vigor and viability of the seeds verified during the flooding test. Thus, it is believed that the high-water level, under conditions of high temperature during germination, caused seed metabolism to change from aerobic to anaerobic pathways. This change in metabolism can cause decreases in seedling yield attributes, according to species or genotype. Thus, when studying the effect of flooded soil on soybean plants, Ludwig et al. (2010) observed reduction in the physiological performance of seeds.

However, it is important to highlight that the speed of vigor-reduction during flooding can also be influenced by the seed size and permeability of the integument, which is variable among the species (Delouche, 2002).

Regarding the height of the aerial part of seedlings, it is observed that at a temperature of 20°C, the most vigorous lots (1, 2, and 3) showed greater resistance to flooding up to the 24-hour period of submersion in water (Figure 4). In general, lot 5, with the lowest vigor, showed greater sensitivity, as from 16 hours onwards there was a rapid reduction in this seedling attribute. At the highest temperatures, between 30 and 40°C, intolerance to flooding was noticed more quickly in the lower vigor lot, with an accelerated decrease in the height of the seedling shoots occurring after 8 hours of anoxia. The effect of temperature can be explained by the soaking process at the beginning of germination, when there is a high absorption of water by the seed, and oxygen becomes less available since its diffusion is up to 10,000 times slower in a liquid solution (Bewley et al., 2012). Absorption occurs faster at higher temperatures and in seeds with low vigor which present membrane disorder and decreases in vigor faster. Oxygen deficit compromises ATP synthesis, which contributes to the accumulation of harmful reducing power in tissues (Tommasi et al., 2001).

There was a significant correlation between flooding periods and temperatures, and seedling emergence (Table 3). Results correlating to emergence in field, indirectly represent the behavior of the lots in the field (Wendt et al., 2017). The use of flooding as a test for segregation of lots, is associated with stress that arises from the lack of oxygen and the release of toxic substances from the seeds' natural metabolic processes which initiate an energy expenditure higher than that of the seeds under regular conditions (Melo et al., 2012; Chen et al., 2013).

Table 3. Spearman's correlation coefficient (p) between the data obtained in the field emergence and flooding test of sorghum seed lots.

Flood period (h)	Flood temperatures (C°)		
	20	30	40
4	-0.10	0.36	0.79
8	0.20	0.10	0.46
16	0.36	0.30	-0.50
24	0.60	-0.30	0.97**
36	0.70	-0.10	0.70
48	0.50	0.95*	-0.60

* and **. Significant at 5% and 1%, respectively, by the test t.

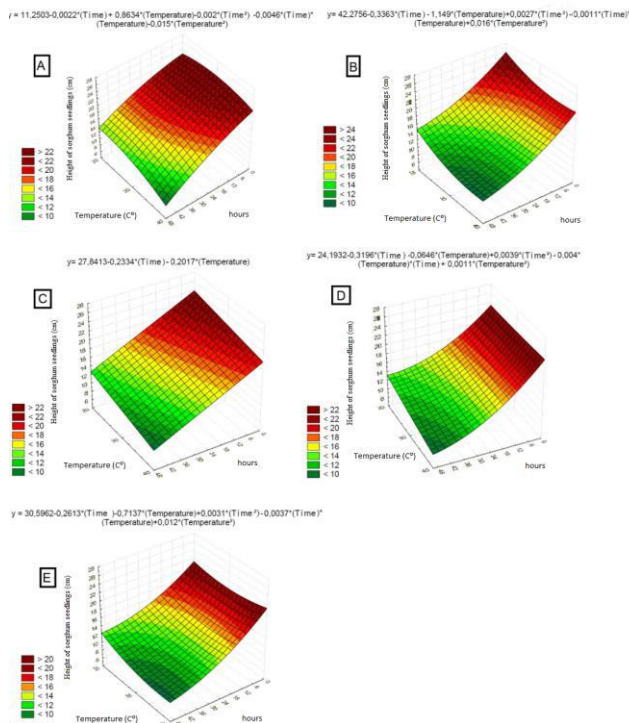


Fig.4. Surfaces response of height of sorghum seedlings after flooding. (A) Lot 1, (B) Lot 2, (C) Lot 3, (D) Lot 4, (E) Lot 5.

It was observed that, in a solution under 20°C, there was no significant correlation with the periods evaluated (Table 7). However, at temperatures of 30°C for 48 hours and 40°C for 24 hours, there is a significant and strong correlation. In maize seeds, it was possible to observe different behaviors between hybrids during flooding, with the best combination of time and temperature being 25°C for 24 hours for the 30F35R hybrid, and 24 and 48 hours at 25°C for the 30P70H hybrid (Grzybowski et al., 2015).

IV. CONCLUSION

The germination and vigor of sorghum seeds are negatively affected by water stress.

The increase in temperature and periods of immersion in water cause a reduction in germination, emergence, height, and dry matter mass shoot of sorghum seedlings.

Soaking at 30 °C for 48 hours or 40 °C for 24 hours promotes the vigor classification of sorghum seed lots.

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