

Tolerance of cotton cultivars to water stress induced during seed germination

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ABSTRACT

There is a demand for cotton cultivars more tolerant of abiotic stress conditions, such as drought. This study assessed white and colored cotton genotypes for water stress induced during seed germination using polyethylene glycol 6000 (PEG). We evaluated three white cotton cultivars (UFUJP C, UFUJP T, and FM 966) and three colored cotton cultivars (UFUJP 5, UFUJP13, BRS Topázio), subjecting seeds to PEG-induced water stress at osmotic potentials -0.2, -0.4, and -0.6 MPa, during seed germination. The control plot (dose 0 of PEG) was performed for each genotype evaluated. We determined the dry matter of normal seedlings obtained in the germination test and performed the seed absorption curve in water and in PEG solution at -0.2 MPa. All cultivars evaluated showed a reduction in germination and imbibition speed when seeds were submitted to water stress. Cultivars FM 966 and BRS Topázio showed the highest tolerance. There was no difference between the groups of white and colored cotton genotypes for tolerance to water deficit. The better performance of the cultivars to tolerance did not reflect a higher imbibition speed of seeds.

Keywords: *Gossypium hirsutum* L.. Water deficit. Polyethylene glycol. physiological potential.

RESUMO

Existe uma demanda por cultivares de algodoeiro que sejam mais tolerantes a condição de estresses abióticos, tais como a seca. Nesse sentido, objetivou-se com o trabalho, a avaliação de genótipos de algodão branco e colorido quanto a tolerância ao estresse hídrico, submetido durante a germinação das sementes pelo uso do polietileno glicol 6000 (PEG). Foram avaliadas três cultivares de algodão do tipo branco (UFUJP C, UFUJP T e FM 966) e três de algodão colorido (UFUJP 5, UFUJP13, BRS Topázio) submetendo as sementes ao estresse hídrico com a utilização do PEG nos potenciais osmóticos de -0.2, -0.4 e -0.6 MPa, durante a germinação das sementes. Foi executado um tratamento controle (dose 0 de PEG) para cada genótipo avaliado. Foi determinada a matéria seca das plântulas normais obtidas no teste de germinação e realizada a curva de absorção das sementes em água e em solução de PEG a -0.2 MPa. Todos os materiais avaliados apresentaram redução da germinação e da velocidade de embebição quando as sementes foram submetidas à condição de estresse hídrico. As cultivares que demonstraram maior tolerância foram FM 966 e BRS Topázio. Não houve diferença entre os grupos dos genótipos de algodão branco e colorido na tolerância ao déficit hídrico. O melhor desempenho das cultivares não refletiu em maior velocidade de embebição das sementes.

Palavras-chave: *Gossypium hirsutum* L.. Déficit hídrico. Polietileno glicol. Potencial fisiológico.

1 Introduction

The cotton crop (*Gossypium hirsutum* L.) plays an important role in the Brazilian economy. Cotton production has increased in the last harvests in Brazil and output of the 2017/18 crop alone amounted to 2.7 million cotton tons, an increase of 32.8% in relation to the previous crop. This increase was attributed to the expansion of 35.4% of the planted area; nevertheless, the yield increase accounted to only 1.9% (CONAB, 2019). In the 2018/2019 crop, cotton production in Brazil reached 2.8 million tons with a record of exports (CONAB, 2020).

Increase in cotton crop yield, mainly in the context of global climate change, poses a great challenge due to higher average temperature and changes in precipitation patterns, affecting water availability for irrigation (DABBERT; GORE, 2014; ZONTA *et al.*, 2017; KHAN *et al.*, 2018). Furthermore, in Brazil, the conventional system for implanting the crop is still widely used (FERREIRA *et al.*, 2020). One way to increase crop yield is to keep residues after harvest to protect the soil and prevent water evaporation, a practice that has been widely used in the no-till system (FAROOQ *et al.*, 2011).

In recent years, many cotton growers, mainly in the states of Bahia and Mato Grosso, Brazil's major cotton producers, have implemented their crops soon after the soybean harvest, keeping residues (straw) on the soil surface (HOFFMANN *et al.*, 2020). This practice has improved the soil physical and chemical aspects, providing mild temperatures to cotton plants and greater water availability in the soil. However, even with the adoption of no-till in some regions, farmers who have not adopted an irrigation system are subject to irregular rains, compromising emergence and development of seedlings. Irregular rains are recurrent in the semi-arid region of northeastern Brazil, where yields of the cotton crop are the lowest in the country (CONAB, 2020).

Water deficit is one of the main abiotic stresses that affect cotton production, reducing crop yield (ABDELRAHEEM *et al.*, 2015). Although cotton species have an intrinsic tolerance to water deficit (BATISTA *et al.*, 2010), at least 400 to 500 mm of water are needed during the plant growth phase for good yields (CORDÃO SOBRINHO *et al.*, 2015). Dabbert and Gore (2014) observed that cotton production is highly vulnerable to changes in precipitation patterns.

Cotton plants under water stress show reduction of gas exchange, stomatal conductance, transpiration,

and photosynthesis with possible consequences to crop yield (ZONTA *et al.*, 2017; ARAÚJO *et al.*, 2019). Water deficit also influences aspects of fiber quality (CORDÃO SOBRINHO *et al.*, 2015; ZONTA *et al.*, 2015). Although studies have reported that water deficit at the initial growth phase of seedlings does not result in significant yield loss (ZONTA *et al.*, 2017; LIMA *et al.*, 2018), it is known that the seedling development phase is crucial for crop establishment. In this phase, plants are more sensitive to environmental stresses (RAJJOU *et al.*, 2012).

Tolerance to water stress is related to the plant genotype (MENESES *et al.*, 2006; ECHER *et al.*, 2010; CORDÃO SOBRINHO *et al.*, 2015; ZONTA *et al.*, 2015; ABDELRAHEEM *et al.*, 2015). Therefore, understanding the genetic behavior of cotton genotypes during water deficit could assist in strategies for the development of tolerant genetic materials (ABDELRAHEEM *et al.*, 2015). Some studies have evaluated tolerance of cotton genotypes to water stress during the initial developmental phase, using polyethylene glycol (PEG) to simulate water scarcity condition (MENESES *et al.*, 2006; MACHADO *et al.*, 2017). More tolerant cultivars could be used in regions where water availability is limited (MENESES *et al.*, 2006; OLIVEIRA *et al.*, 2017).

Drastic changes in global temperature have affected agricultural crops, requiring strategies to adapt genotypes to abiotic stresses. In this sense, this study assessed genotypes of white and colored cotton fibers for water stress tolerance, submitted to PEG-induced water stress during seed germination.

2 Materials and Methods

The experiment was carried out at the Seed Analysis Laboratory (LASEM) of the Federal University of Uberlândia (UFU), Uberlândia, Minas Gerais State, Brazil. Six cotton genotypes were used, three white cotton UFUJP C, UFUJP T, and Fiber Max (FM 966), and three colored cotton genotypes UFUJP 5, UFUJP13, and BRS Topázio. The Cotton Breeding Program (Promalg) at UFU developed the genotypes with the prefix "UFU". Genotypes FM 966 (Bayer®) and BRS Topázio (Embrapa Cotton) are commercial.

Cotton seeds were submitted to water deficit with polyethylene glycol 6000 (PEG) purchased from Synth®. Three levels of water deficit -0.2, -0.4, and -0.6 MPa were evaluated. For that purpose, the PEG solution was prepared to moisten germitest paper and perform the germination test. The dry germitest paper was weighed and its weight was multiplied by a factor

of 2.5, as prescribed in the Rules for Seed Analysis (RAS) (BRASIL, 2009), obtaining the amount of solution containing PEG to moisten the paper.

Solutions containing PEG at different osmotic potentials were prepared, as suggested by Michel and Kaufmann (1973) and cited by Villela; Doni Filho and Sequeira (1991). The PEG concentration per liter of deionized water to obtain the desired osmotic potential levels at 25 °C were: -0.2 MPa (119.571 g PEG L⁻¹ of water), -0.4 MPa (178.343 g PEG L⁻¹ of water), -0.6 MPa (223.739 g PEG L⁻¹ of water). A control plot (dose 0 MPa of PEG) was also prepared for each genotype evaluated, ensuring that the germitest paper was moistened only with deionized water without the addition of PEG.

The germination test was conducted to assess water deficit using 200 seeds arranged in four blocks. The procedure for assembling and evaluating the test followed Brasil (2009). At the end of test assembly, paper sheets were placed in two plastic bags to keep a favorable microclimate for experiment evaluations and avoid influence on the water deficit stipulated.

The germitest paper sheets protected with the plastic bags were kept in germinators regulated at a constant temperature of 25 °C (BRASIL, 2009). The first germination count was performed on the 4th day after sowing, evaluating normal seedlings and removing infected abnormal seedlings or infected dead seeds that could compromise the analysis.

Normal seedlings that were already counted and the other seedlings and seeds that had not yet developed into a normal seedling were kept on the paper roll for the last evaluation carried out on the 9th day after sowing. In this assessment, normal seedlings were counted and the germination result was obtained by adding the normal seedlings counted in the first and last evaluations (BRASIL, 2009).

The normal seedlings removed in the last evaluation were placed in paper bags properly identified, which were kept in a greenhouse with forced air circulation at 70 °C for 48 h. After this period, the dry matter was weighed on an electronic scale with accuracy of 0.001 g and the results obtained were expressed in grams (POSSE; SILVA; VIEIRA, 2004).

Finally, we performed the water absorption curves of seeds of the genotypes evaluated. For the evaluation, four sub-samples of 50 seeds of each cotton genotype were separated and weighed on a scale with precision to three decimal places. The seeds were placed on sheets of germitest paper and moistened with the equivalence in deionized water for

the germination test. Another test was performed using the PEG solution to moisten the germitest paper at the osmotic potential -0.2 Mpa.

The material was kept in a germinator regulated at 25°C. Absorption in water and in the PEG solution at -0.2 MPa of seeds (AbA) was determined in the soak times of 2, 4, 6, 8, 10, 12, 20, 28, and 36 h. At the end of each period, the seeds were removed, dried with paper towels, and weighed to obtain the wet weight. The AbA was calculated in percentage using the formula in accordance to as Oliveira and Gomes-Filho (2010)

$$\frac{(P_f - P_i)}{P_i} \times 100, \quad (1)$$

where: P_f represents the final weight of seeds at each time, and P_i denotes the initial weight of seeds before imbibition.

The experimental design adopted for the germination test was randomized blocks, with four blocks of 50 seeds, in a 6 x 4 factorial scheme, with treatments as a combination of the six cotton genotypes (three white types: UFUJP C, UFUJP T, and FM 966, and three colored cotton: UFUJP 5, UFUJP13, and BRS Topázio). The second factor included PEG doses of 0, -0.2, -0.4, and -0.6 MPa. The analysis of variance was performed using the F test and the means were compared by the Tukey test, both at 5% probability. The dosing behavior was assessed using the regression analysis. We also carried out a comparison of the white group and the colored group of genotypes. The statistical analysis was performed in the program Sisvar® (FERREIRA, 2011).

3 Results and Discussion

The analysis of variance for the first germination count, the germination test, and the normal seedlings dry matter have demonstrated that the factorial combinations between genotypes and PEG doses was significant for the three variables (Table 1).

Table 1 – Summary of the analysis of variance for the first germination count (FGC), germination test (GT), and the seedling dry matter (SDM) of cotton genotypes submitted to the PEG germination test.

Mean squares				
Variation source	DF ¹	FGC	GT	SDM
Genotype (G)	5	346.27**	1664.14**	2.53**
PEG dose (D)	3	29046.50**	12566.59**	3.46**
G * D	15	138.87**	118.76**	0.17**
Block	3	14.83**	44.15**	0.12**
Error	69	26.34	31.16	0.08
CV (%)		18.52	8.78	13.17

¹DF: Degrees of freedom. *Significant at 1% probability by the F test; **Significant at 5% probability by the F test.

The analysis of the genotypes genetic behavior, according to the osmotic potential to which seeds were submitted, demonstrated that when seeds were not under a stressful condition, colored genotype UFUJP 5 was superior to the others for the first germination count variable. As for germination, all three colored genotypes evaluated showed genetic behavior similar to the white cotton genotype FM 966 (Table 2).

Seeds that were submitted to the osmotic potential -0.2 MPa reduced the germination process in all genotypes evaluated. At -0.2 MPa, the genetic behavior of the genotypes evaluated was similar to the germination condition of seeds without exposure to water stress. The three colored genotypes have shown genetic behavior similar to the white cotton material FM 966 (Table 2).

Reduced concentrations of PEG, such as the osmotic potential -0.2 MPa, may have a priming effect on the seeds, stimulating seedling development. In this sense, Carneiro *et al.* (2011) submitted sunflower seeds to germination on paper moistened with osmotic solutions at potentials -0.2, -0.4, and -0.8 MPa, induced with PEG. The authors have found that the osmotic potential -0.2 MPa stimulated seed germination. However, the use of PEG at the potential -0.8 MPa, impaired germination, reducing speed and germination capacity of seeds. Queiroga *et al.* (2008) submitted cotton seeds to osmotic conditioning with PEG at the potential -0.25 MPa and observed an increase in seed vigor.

At osmotic potentials -0.4 and -0.6 MPa, the genotypes genetic behavior was similar for the first germination count. When seeds were submitted to the potential -0.6 MPa, no seed of the evaluated genotypes germinated on the 4th day of the germination test (Table 2).

Table 2 – First germination count, germination test, and dry mass of cotton seedlings submitted to PEG-induced water deficit.

First germination count (%)				
Genotypes ¹	0 MPa	-0.2 MPa	-0.4 MPa	-0.6 MPa
UFUJP C	57.0 c	25.0 b	0.0 a	0.0 a
UFUJP T	69.0 b	27.0 b	2.0 a	0.0 a
FM 966	69.5 b	40.5 a	1.5 a	0.0 a
UFUJP 5	90.5 a	42.5 a	1.5 a	0.0 a
UFUJP 13	77.5 b	35.5 ab	3.0 a	0.0 a
BRS Topázio	79.0 b	42.5 a	1.5 a	0.0 a
CV (%)	18.52			
SDM	10.6			
Germination (%)				
Genotypes ¹	0 MPa	-0.2 MPa	-0.4 MPa	-0.6 MPa
UFUJP C	64.5 b	56.5 b	49.0 c	17.5 d
UFUJP T	76.0 b	62.5 b	55.0 bc	31.5 bc
FM 966	93.5 a	81.0 a	70.5 a	47.5 a
UFUJP 5	94.5 a	84.0 a	65.0 ab	32.0 bc
UFUJP 13	89.5 a	86.0 a	60.0 abc	23.5 cd
BRS Topázio	92.5 a	82.0 a	69.5 a	43.0 ab
CV (%)	8.78			
SDM	11.6			
Seedling dry matter (g)				
Genotypes ¹	0 MPa	-0.2 MPa	-0.4 MPa	-0.6 MPa
UFUJP C	1.74 c	1.80 c	1.54 c	0.93 d
UFUJP T	2.21 bc	2.08 bc	1.82 abc	1.70 bc
FM 966	3.00 a	2.76 a	2.31 a	2.49 a
UFUJP 5	2.61 ab	2.42 ab	1.91 abc	1.54 bc
UFUJP 13	2.33 b	2.59 ab	1.59 bc	1.16 cd
BRS Topázio	2.70 ab	2.63 ab	2.16 ab	2.10 ab
CV (%)	13.17			
SDM	0.57			

* Means followed by different letters in the columns differ statically from each other by the Tukey test at 5% significance.

¹White cotton genotypes: UFUJP C, UFUJP T, and FM 966. Colored cotton genotypes: UFUJP 5, UFUJP 13, and BRS Topázio.

Regarding germination, genotypes FM 966 and BRS Topázio have shown considerable behavioral similarities, when their seeds were submitted to the osmotic potential -0.4 MPa. The other two colored cotton genotypes evaluated (UFUJP 5 and UFUJP 13) had a greater reduction in germination when the potential -0.4 MPa was used for seeds, exposing a condition of less tolerance to water deficit compared to commercial genotypes – FM 966 and BRS Topázio (Table 2).

At the potential -0.6 MPa, the analysis of two genotypes that performed better in the condition of

water deficit at -0.4 MPa, demonstrated that the BRS Topázio colored cotton genotype was also similar to UFUJP T and UFUJP 5, with lower performance in relation to germination and seedling dry matter, despite statistically similar to FM 966 (Table 2).

The regression analysis of cotton seeds germination, according to the osmotic potentials evaluated, showed a linear reduction behavior for all evaluated genotypes. The white cotton genotypes (UFUJP C and UFUJP T) showed a germination reduction of 73% and 59%, respectively, when their seeds were placed to germinate at the potential -0.6 MPa (Figure 1A).

However, the two white cotton genotypes already had low physiological potential of seed germination without water restriction. Genotype UFUJP C showed germination 64.5%, below the minimum standard for cotton seeds commercialization (BRASIL, 2013) and genotype UFUJP T had 76.0% of germination, very close to 75.0%, the minimum standard (Table 2).

Regarding the colored cotton genotypes evaluated, in the germination condition without water stress induction, the three genotypes showed similar genetic behavior for germination (Table 2). However, genotypes UFUJP 5 and UFUJP 13 were less tolerant to water restriction than the commercial genotype BRS Topázio. There was a reduction of 66% in germination of UFUJP 5 and 74% of UFUJP 13, when seeds were submitted to the potential -0.6 MPa. Conversely, BRS Topázio,

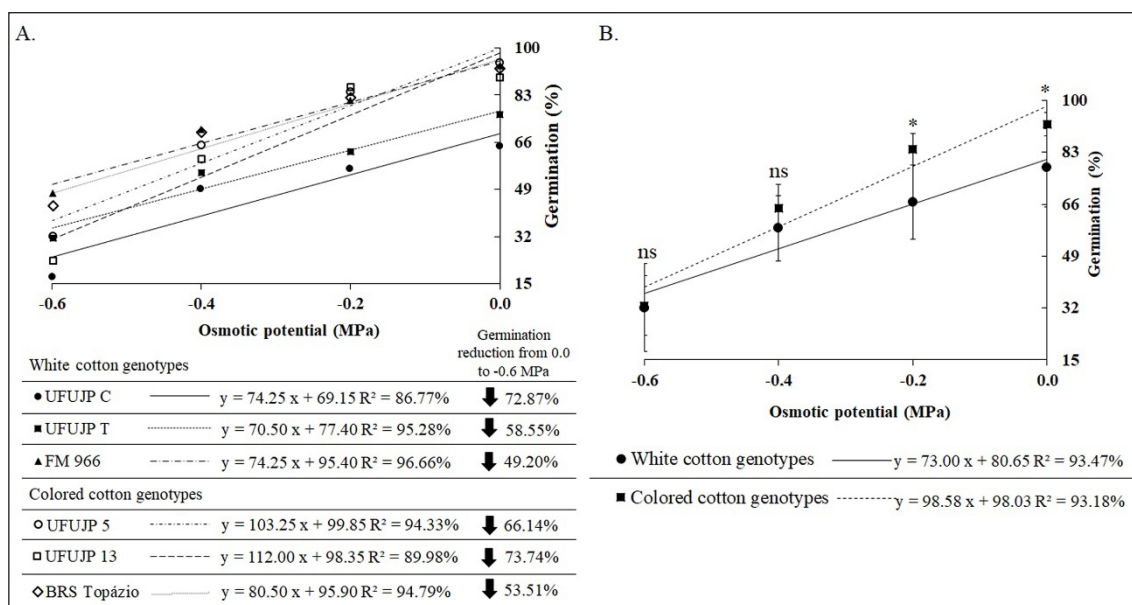
the most tolerant colored cotton material among the materials evaluated, showed a germination reduction of 54% in the condition at -0.6 MPa (Figure 1A).

Commercial genotypes FM 966 and BRS Topázio have displayed greater tolerance to water stress. Genotype FM 966 showed a lower germination reduction at the potential -0.6 MPa, with a value of 49%. In contrast, BRS Topázio had a greater germination reduction (54%) (Figure 1A) and dry mass of seedlings with 22% reduction, against 17% of FM 966 (Table 2).

The initial physiological quality of seeds affects water stress tolerance. Genotypes FM 966 and BRS Topázio have high initial physiological potential and showed greater tolerance to water stress (Table 2). Oliveira and Gomes-Filho (2010) have also found that sorghum seeds with superior physiological quality showed better tolerance to water stress. Moreover, genetics can contribute to tolerance to abiotic stresses. In this sense, Soares *et al.* (2018) found that cotton plants of genotype BRS Topázio, grown under salt stress, had a reduction of gas exchange; however, among the genotypes studied, BRS Topázio showed the best result. Oliveira *et al.* (2017) have also found tolerance of seeds of BRS Topázio to PEG-induced water stress at the potential -0.9 MPa.

Figure 1B shows a comparison between the groups of three white cotton and three colored cotton genotypes. Colored genotypes were superior in the condition without water stress and at the potential

Figure 1 – Germination of cotton seeds according to the osmotic potential, attributed by PEG solution (A) and comparison of white and colored cotton genotypes. (B) The error bar corresponds to an average of 12 germination values of the three genotypes. * Significant at 5%. ns denotes not significant at 5%.



-0.2 MPa. When genotypes were subjected to greater water restriction (-0.4 and -0.6 MPa), there was no difference between the groups of white and colored cotton genotypes.

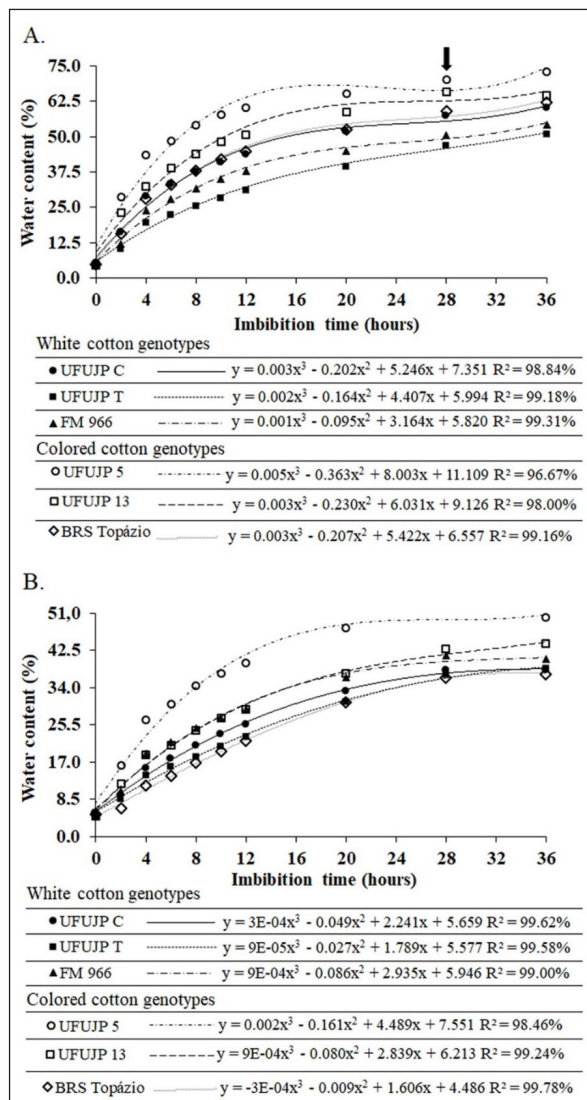
In addition to BRS Topázio, other cotton genotypes have shown relative tolerance to water stress. According to Meneses *et al.* (2006), the potential -0.4 MPa drastically reduced germination of cotton genotypes; however, genotype CNPA 187 8H was the least sensitive to stress conditions, while BRS 201 was the most sensitive with the greatest germination reduction and seed vigor. Echer *et al.* (2010) evaluated tolerance of four cotton genotypes to water deficit during the initial seedling development. Stress was induced by mannitol with the lowest rated potential -1.2 MPa. The authors concluded that the potential -0.9 MPa reduced the growth rate and the shoot dry matter of seedlings of all genotypes and LD CV 02 showed the best development under water deficiency.

In other cultures, studies have investigated water stress tolerant genotypes. Machado *et al.* (2017) have evaluated water stress effects on the physiological quality of seeds and on the performance of seedlings of two crambe genotypes. The authors simulated the stress condition using PEG at four osmotic potentials -0.25, -0.50, -1.0, and -1.50 MPa. The physiological quality of seeds and the performance of crambe seedlings were reduced under water stress from -0.25 MPa. Seeds of genotype FMSCR 1101 had greater tolerance to water stress, regardless of the osmotic potential used.

In addition to the distinct genetic behavior of genotypes for water stress tolerance, studies have also reported different behaviors of different species. Wu *et al.* (2011) submitted seeds of three perennial leguminous forage species to PEG-induced water stress. Seeds of *Medicago sativa* (L.) showed greater tolerance to hydric stress. According to the authors, this occurs because different species have different mechanisms to adapt to adverse conditions.

The seed absorption curve showed a reduction of the absorption rate in all genotypes subjected to water deficit (Figure 2). Factors, such as water stress or saline soils, can limit germination and development of different species in different regions and adaptation to stress conditions results in integrated events that occur at various levels, involving morphological, anatomical, cellular, biochemical, and molecular changes (CARNEIRO *et al.*, 2011).

Figure 2 – Imbibition curve of cotton seeds, kept in deionized water (A) and in a PEG solution at -0.2 MPa (B). Arrow in A represents the root protrusion of 50% of all seeds of cotton materials.



The gradient of water potential between substrate and seeds hinders the imbibition process and compromises germination (OLIVEIRA; GOMES-FILHO, 2010). When there was no water restriction, seeds of the colored cotton genotypes soaked water more quickly during germination. Regarding the bank fiber materials, UFUJP C had a water absorption behavior similar to BRS Topázio. FM 966 and UFUJP T showed lower efficiency in water absorption (Figure 2A).

Seeds of UFUJP 5 germinated more quickly, showing better results of the first germination count (Table 2). The water imbibition curve of UFUJP 5 seeds have shown greater speed in water absorption (Figure 2A). When the seeds of the genotypes evaluated were

submitted to the potential -0.2 MPa, BRS Topázio, which showed better tolerance to water stress together with FM 966, soaked water more slowly when compared to the others (Figure 2B). This result can be explained by the fact that rapid water absorption suggests a reduction in the physiological potential of the genotype, demonstrating loss of selective permeability of the seed membrane system.

During germination, water is necessary for digestion of reserves and translocation of metabolized products, and imbibition is the event that triggers the germination process. Water absorption by seeds follows a three-phase pattern with an initial period of rapid absorption, followed by a period of slow imbibition. The third phase begins with the event called root protrusion, as described by Bewley and Black (1994).

Still on the absorption curve without water restriction, the phase of greater absorption of deionized water occurred during the first 12 h of imbibition (imbibition phase 1). There is a reduction of water absorption between 20 and 28 h of the process (imbibition phase 2). Finally, imbibition phase 3 begins with root protrusion about 28 h of imbibition, marked by the arrow in Figure 2A.

After 36 h of imbibition, 50% or more of seeds of the six genotypes evaluated showed root protrusion (Figure 2A). On the other hand, after 36 h of imbibition, seeds in a solution containing PEG showed no evidence of the root protrusion level for the evaluated seeds and the test was terminated because the seeds started the process of mass loss (Figure 2B), possibly due to water loss to the environment with less osmotic potential. The reduction in movement and availability of water for soaking the seeds is related to the decrease in the osmotic potential of the solution; therefore, germination is delayed (MENESES *et al.*, 2006).

Other authors have reported germination reduction due to PEG-induced water stress. Oliveira *et al.* (2017) have noted that water stress induced by PEG concentrations reduced germination of cotton seeds at the potential -0.9 MPa and, when the seeds were submitted to the potential -1.2 MPa, there was no germination. Occurrence of pathogenic fungi is among the damages caused by the permanence of seeds in the soil for a longer period without germination. Barrocas *et al.* (2014) have used mannitol as an agent to promote water restriction (-0.8 MPa) exposing cotton seeds to the fungus *Colletotrichum gossypii* var. *cephalosporioides*. The authors observed an increase in the fungal incidence, as the water deficiency

period increased with delay and reduction of seed germination.

4 Conclusions

All the genotypes evaluated showed a reduction in germination and imbibition speed when seeds were submitted to PEG-induced water stress. Commercial cultivars FM 966 and BRS Topázio showed the highest tolerance and the former had less germination reduction at the potential -0.6 MPa.

There was no difference between the groups of white and colored cotton genotypes regarding tolerance to water deficit.

The better performance of BRS Topázio cultivar did not reflect a higher imbibition speed in a solution with an osmotic potential -0.2 MPa.

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