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CROP PRODUCTION

Primary leaf growth and coleoptile length in maize seedlings

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ABSTRACT. Evaluation of the physiological potential of maize seeds during the certification process is performed by a germination test. However, some tests show a delay in the growth of aerial seedling organs that does not reflect seed lot behavior in the field. This study aims to investigate the effects of genotype, water, light and temperature on differential primary leaf growth compared to coleoptile length in maize seedlings. Seeds from two simple hybrids and their reciprocal crosses that showed slow primary leaf growth were evaluated according to physiological quality, morphology, histochemistry, and enzymatic expression. There were differences among maize genotypes regarding initial primary leaf growth when compared to the coleoptile, especially when germination was tested without light. Water availability and optimal temperature range did not affect the differential growth of the primary leaf in the hybrids. Pericarp thickness, lignin content and differences in enzyme expression in different genotypes were also not related to changes in the relationship of primary leaf growth with coleoptile length during the maize seed germination test.

Keywords: *Zea mays* L.; germination test; lignin; enzymes.

Crescimento diferencial da plúmula e do comprimento do coleóptilo de plântulas de milho

RESUMO. A avaliação do potencial fisiológico de lotes de sementes de milho no processo de certificação é realizada pelo teste de germinação. No entanto, em alguns testes, são observados atrasos no crescimento das estruturas da parte aérea das plântulas, o que leva a considerá-las plântulas anormais, mas não reflete o comportamento do lote no campo. Objetivou-se, neste trabalho, investigar o efeito do genótipo, água, luz e temperatura sobre o crescimento diferencial da plúmula em relação ao comprimento do coleóptilo de plântulas de milho. Sementes de dois híbridos simples e dos seus recíprocos com característica de crescimento lento da plúmula, foram avaliadas em sua qualidade fisiológica, morfológica, histoquímica e expressão enzimática. Existem diferenças entre genótipos de milho no que se refere ao crescimento inicial da plúmula em relação ao coleóptilo, principalmente na ausência de luz no teste de germinação. Disponibilidade hídrica e temperatura não afetam a resposta dos híbridos em relação ao crescimento diferencial da plúmula. Espessura do pericarpo, presença de lignina ou diferenças na expressão das enzimas dos genótipos não estão relacionadas às variações de comprimento plúmula/coleóptilo, durante o teste de germinação.

Palavras-chave: *Zea mays* L.; teste de germinação; lignina; enzimas.

Introduction

The germination test is the typical way to evaluate the physiological potential of maize seeds during the certification process (minimum 85% germination according to the commercialization standards established in normative instruction 45 and law 10 711). The germination percentage is determined by the number of normal seedlings at the end of the test, which is carried out under optimal conditions for germination. At the time of the evaluation, seedlings should contain all the essential structures, allowing the analyst to judge the possibility of that seedling to continue its development and become a productive plant.

One of the reasons that a maize seedling may be considered abnormal is the size of the primary leaf compared to the coleoptile length. If the primary leaf is less than half the coleoptile length by the time of the assessment, the seedling is considered abnormal (Brasil, 2009). In some genetic backgrounds, it has been observed that seedlings exhibit delayed growth of aerial structures, specifically the primary leaf, which leads them to be classified as abnormal seedlings and adversely affects germination test results. These results, however, do not always reflect the performance of seed lots in the field.

The growth rates of primary leaf and coleoptile may vary in some cases; they are both genetically controlled and influenced by environmental conditions during germination (Kurtyka, Burdach, & Karez, 2011). This study aims to investigate the effects of genotype, water, light, temperature and chemical treatment on the differential growth of primary leaf in relation to coleoptile length in maize seedlings.

Material and methods

Seeds from two simple hybrids and their reciprocal crosses were used. All demonstrated slow primary leaf growth. Simple hybrid 1 was the product of Line A x Line B, and hybrid 4 was its reciprocal cross (Line B x Line A); simple hybrid 2 was the product of Line A' x Line C, and hybrid 3 was its reciprocal cross (Line C x Line A'). Lines A and A' were from the same genetic background but carried different trans genes. The seeds to be evaluated (classified in sieve 22) were obtained from the four hybrids at the same time and under the same climate conditions.

Assay 1: A germination test was used to assess the effect of water, light, temperature and hybrid origin. The seeds were placed on paper towel substrate (Germitest) moistened with a volume of distilled water in an amount equal to 2.0, 2.5, and 3.0 times the paper weight at temperatures of 20°C, 25°C, and 30°C and in a 20-30°C range. The seeds were subjected to two treatments, one dark and the other with 12 hours of dark and 12 hours light. The factorial design used was 4 x 3 x 4 x 2 (4 hybrids, 3 volumes of water, 4 temperatures, and 2 light conditions) in a completely randomized design with four replicates of 25 seeds per treatment. Evaluations were performed four, five, six and seven days after sowing. The data collected were the number of normal seedlings (at four and seven days), the number of seedlings with primary leaf growth less than 50% of coleoptile length, and the number of seedlings with primary leaf growth between 50% and 75% of coleoptile length. The data were evaluated for data normality and homogeneity of variance among treatments. A normal data distribution was found only for the normal seedlings. Then, a constant value equal to 3 was added (Lucio, Couto, Lopes, & Storck, 2011; Yakamura, 1999), and the Box-Cox transformation was performed (Box & Cox, 1964). After processing, the results were subjected to analysis of variance and means compared with a significance level of 5% by the Scott-Knott test using SISVAR® software (Ferreira, 2011).

Assay 2: A germination test was performed in sand (TGA). The test was carried out in germination chambers with 25°C temperature and a photoperiod of 12 hours light and 12 hours lightless. Seeds of the four hybrids were sown in trays with sand that had been sieved, washed and sterilized (autoclaved to one atmosphere and 120°C for sixty minutes) with water equivalent to 60% of field capacity in a completely randomized design with four replicates of 25 seeds per treatment. Evaluations were performed at three, five and seven days. The data collected were the number of normal seedlings and the number of seedlings with differential primary leaf growth in relation to coleoptile length. The data were evaluated for data normality and homogeneity of variance among treatments and then subjected to analysis of variance and means compared with a significance level of 5% by the Scott-Knott test using SISVAR® software (Ferreira, 2011).

Assay 3: Seed pericarp thickness was assessed. Sections were made in the middle region of the seeds of each hybrid and reciprocal cross using a microtome (Olympus CBL, model BX60). Five replicate sections of approximately 20 microns were obtained for each material. The sections were dyed for 60 seconds with three drops per replicate of1% Saflablau (0.9% Astra + 0.1% Safran). Images of the pericarp were obtained using a stereoscopic microscope adapted with a camera. The pericarp thickness was measured with Image J® software (Rasband, 2012). The measurements obtained were submitted to analysis of variance and means compared with a significance level of 5% by the Scott-Knott test using SISVAR® software (Ferreira, 2011).

Assay 4: Histochemical analysis was performed to evaluate lignin deposition on the coleoptile wall. Seedlings with four days of growth were sectioned transversely at the transition region between the coleoptile and first leaf in a table microtome and stained with phloroglucinol solution (1 g 100 ml^{-1}) for 5 minutes. An unstained section was used as control. The slides were evaluated in a stereoscopic microscope. Evaluation of lignin deposition in the coleoptile wall was visually performed in all five replicates.

Assay 5: The expression of α-amylase and β-amylase enzymes was evaluated using the following samples for all hybrids: ten seeds soaked for 72 hours, ten dry seeds and ten seedlings with four days of growth. The seeds and seedlings were homogenized with liquid nitrogen and stored in a freezer at -81°C until analysis. Extraction of β- and α-amylase and gel development were performed according to Alfenas (2006).

Results and discussion

The classes of maize seedlings observed in the germination assessments are shown in Figure 1. Slow development of the primary leaf in relation to the coleoptile is shown in Figure 1A, with primary leaf growth less than 50% of coleoptile length.

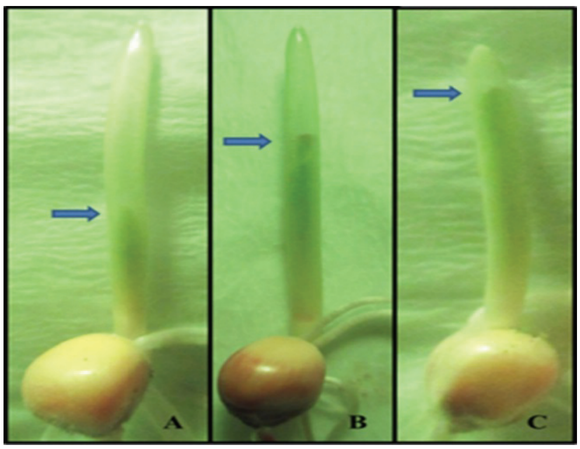

Figure 1. Maize seedlings after five-day germination test. A) abnormal seedling with primary leaf growth less than 50% of coleoptile length; B) normal seedling with primary leaf growth between 50 and 75% of coleoptile length; C) normal seedling.

Assay 1: The 'normal seedling' group showed significant interaction effects of hybrid identity and temperature in the germination test at the fourth day. Lower germination rates were observed at 20°C for hybrids 2 and 3 (Table 1). According to Zucarelli, Cavariani, Oliveira, and Nakagawa (2011), temperatures below the optimum recommended for maize seed germination slow down the imbibition process. Lower temperatures also enable difficulties in the reorganization of cell membranes and consequently cause delays in germination (Carvalho, Sediyama, Reis, Dias, & Moreira, 2009), influenced by physiological quality and the genotype (Grzybowski, Vieira, & Panobianco, 2015).

Table 1. Percentage of normal maize seedlings assessed at the fourth day after sowing.

Hybrid	Temperature			
	20-30°C	20°C	25°C	30°C
1	98 aA	95 aA	99 aA	99 aA
2	96 aA	87 bB	96 aA	98 aA
3	96 aA	85 bB	96 aA	97 aA
4	98 aA	92 aB	98 aA	97 aA
	CV(%) = 4.45			

Means followed by the same lowercase letter (in columns) or the same capital letter (in rows) do not differ by the Scott-Knott test at 5% of probability.

There was also a triple interaction among hybrid identity, temperature and light conditions for seedlings with primary leaf growth less than 50% of coleoptile length. In Table 2, the seeds of hybrid 4 showed more seedlings with primary leaf growth less than 50% of coleoptile length when subjected to temperatures of 30°C or a range from20-30°C under lightless conditions. The results contradict the findings of Deng, Yang, Zhang, and Li (2015) and Zucarelli, Cavariani, Portugal, and Nakagawa (2008) that the temperature of 30°C was more suitable for germination and growth of maize seedlings.

Table 2. Percentages of maize seedlings with primary leaf growth less than 50% of coleoptile length, assessed at the fourth day after sowing.

		Temperature			
Hybrid	Light	20-30°C	20°C	25°C	30°C
1	Light	0 aA	0 aA	0 aA	0 aA
2	Light	0 aA	0 aA	0 aA	0 aA
3	Light	0 aA	0 aA	0 aA	0 aA
4	Light	0 aA	0 aA	0 aA	0 aA
1	Lightless	0 bA	0 aA	0 aA	0 bA
2	Lightless	0 bA	0 aA	0 aA	0 bA
3	Lightless	0 bA	0 aA	0 aA	0 bA
4	Lightless	4 aA	0 aB	0 aB	4 aA
		CV(%) = 1.78			

Means followed by the same lowercase letter (in columns) or the same capital letter (in rows) do not differ by the Scott-Knott test at 5% of probability.

In the 'seedlings with primary leaf growth between 50 and 75% of coleoptile length' group, there was a significant interaction between hybrid identity and light conditions. In lightless conditions, hybrid 3 presented the lowest percentage of seedlings with primary leaf growth between 50% and 75% of coleoptile length. Under light, only hybrid 4 showed any seedlings with this characteristic (Table 3). Lima, Santos, and Melo (2010) found that maize plants grown in light less conditions developed an elongated mesocotyl and coleoptile and had primary leaves rolled inside the coleoptile.

Table 3. Percentages of maize seedlings with primary leaf growth between 50 and 75% of coleoptile length, assessed at the fourth day after sowing.

Hybrid	Light	Lightless
1	0 bB	5 aA
2	0 bB	4 aA
3	0 bA	0 bA
4	4 aA	4 aA
	CV (%) = 2.87	

Means followed by the same lowercase letter (in columns) or the same capital letter (in rows) do not differ by the Scott-Knott test at 5% of probability.

In the 'seedlings with primary leaf growth less than 50% of coleoptile length' group after five days, only hybrid identity was a significant factor, indicating the influence of genotype on uneven growth. The highest percentage of seedlings with primary leaf growth less than 50% of coleoptile length at five days was observed for hybrid 4 (Table 4). According to Mertz et al. (2009) and Nerling, Coelho, and Nodari (2013), the physiological qualities of seeds are genetically inherited from their parents, so different hybrids of the same species may show varying vigor, germination and emergence in the same production conditions.

Table 4. Percentages of maize seedlings with primary leaf growth less than 50% of coleoptile length, assessed at the fifth day after sowing.

Hybrid	Seedling
1	0 b
2	0 b
3	0 b
4	2 a
CV (%) = 1.51	

Means followed by the same letter do not differ by the Scott-Knott test at 5% probability.

In the seedlings with primary leaf growth between 50% and 75% of coleoptile length' group after five days, there was a significant quadruple interaction among hybrid identity, water, temperature and light conditions. A higher percentage of seedlings with primary leaf growth between 50% and 75% of coleoptile length was observed for hybrid 4 in lightless conditions (Table 5). Because the percentage of seedlings with primary leaf growth between 50% and 75% of coleoptile length varied on the fifth day after sowing, it was not possible to determine what temperature or volume of water had the best effect on germination.

Table 5. Percentages of maize seedlings with primary leaf growth between 50% and 75% of coleoptile length, assessed at the fifth day after sowing.

			Temperature			
Hybrid	Water	Light conditions	20-30°C	20°C	25°C	30°C
1	2,0	Lightless	0 aB	4 aA	5 aA	3 aA
2	2,0	Lightless	0 aB	0 bB	3 bA	4 aA
3	2,0	Lightless	2 aA	2 bA	2 bA	0 bA
4	2,0	Lightless	2 aB	7 aA	7 aA	0 bB
1	2,0	Light	2 aA	0 bA	2 bA	0 aA
2	2,0	Light	0 aB	3 aA	0 bB	0 aB
3	2,0	Light	0 aA	0 bA	0 bA	0 aA
4	2,0	Light	2 aB	0 bB	4 aA	0 aB
1	2,5	Lightless	2 bA	3 aA	2 b A	3 aA
2	2,5	Lightless	6 aA	4 aA	4 aA	0 bB
3	2,5	Lightless	2 bA	2 b A	0 b A	0 bA
4	2,5	Lightless	5 aA	3 aA	5 aA	0 bB
1	2,5	Light	0 aB	0 bB	3 aA	0 aB
2	2,5	Light	0 aB	2 bB	4 aA	0 aB
3	2,5	Light	0 aA	0 bA	2 bA	0 aA
4	2,5	Light	0 aB	3 aA	0 bB	0 aB
1	3,0	Lightless	2 bB	0 bB	4 bA	2 aB
2	3,0	Lightless	0 bB	4 aA	3 bA	0 aB
3	3,0	Lightless	2 bA	3 aA	2 cA	2 aA
4	3,0	Lightless	3 aB	3 aB	8 aA	2 aB
1	3,0	Light	0 aA	0 bA	0 bA	0 aA
2	3,0	Light	0 aA	2 bA	2 bA	0 aA
3	3,0	Light	0 aA	2 bA	0 bA	0 aA
4	3,0	Light	0 aB	3 aA	3 aA	0 aB
CV (%) = 1.43						

Means followed by the same lowercase letter (in columns) or the same capital letter (in rows) do not differ by the Scott-Knott test at 5% of probability.

In the 'seedlings with primary leaf growth less than 50% of coleoptile length' group after six days, there was a significant triple interaction among hybrid identity, temperature and water. Hybrid 4 presented the highest percentage of seedlings with primary leaf growth less than 50% of coleoptile length at a temperature of 25°C or range of 20°C to 30°C and water volume 3.0 times the paper weight (Table 6). This result differs from the findings of Lipchinsky, Sharova, and Medvedev (2013), who reported that decreased temperature caused an instant stretching of the coleoptile's cell wall.

Table 6. Percentages of maize plants with primary leaf growth less than 50% of coleoptile length, assessed at the sixth day after sowing.

		Temperature			
Hybrid	Water	20-30°C	20°C	25°C	30°C
1	2,0	0 aA	0 aA	0 aA	0 aA
2	2,0	0 aA	0 aA	0 aA	0 aA
3	2,0	0 aA	0 aA	0 aA	0 aA
4	2,0	0 aA	0 aA	0 aA	0 aA
1	2,5	0 aA	0 aA	0 aA	0 aA
2	2,5	0 aA	0 aA	0 aA	0 aA
3	2,5	0 aA	0 aA	0 aA	0 aA
4	2,5	0 aA	0 aA	0 aA	0 aA
1	3,0	0 bA	0 aA	0 bA	0 aA
2	3,0	0 bA	0 aA	0 bA	0 aA
3	3,0	3 aA	0 aB	0 bB	0 aB
4	3,0	3 aA	0 aB	2 aA	0 aB
CV(%) = 2.33					

Means followed by the same lowercase letter (in columns) or the same capital letter (in rows) do not differ by the Scott-Knott test at 5% of probability.

In the 'seedlings with primary leaf growth between 50% and 75% of coleoptile length' variable was a significant interaction between temperature and light. The highest percentage of seedlings with primary leaf growth between 50% and 75% of coleoptile length occurred at 20°C in the absence of light, six days after sowing (Table 7). Sbrussi and Zucarelli (2014) reported that temperatures below 20°C caused the absence of normal seedlings across different lots of maize seeds due to delayed germination. Zucarrelli et al. (2008) observed that a temperature of 20°C reduced the development of the aerial structures of maize plants when compared with seedling development at 30°C.

Table 7. Percentages of maize plants with primary leaf growth between 50% and 75% of coleoptile length, assessed at the sixth day after sowing.

	Temperature			
Light conditions	20-30°C	20°C	25°C	30°C
Lightless	0 aB	3 aA	0 aB	0 aB
Light	0 aA	1 bA	0 aA	0 aA
CV(%) = 1.96				

Means followed by the same lowercase letter (in columns) or the same capital letter (in rows) do not differ by the Scott-Knott test at 5% of probability.

In the 'normal seedling' group after seven days, hybrid identity showed a significant effect. The percentage of normal seedlings was lower for hybrid 2 (Table 8).

Table 8. Percentage of normal maize seedlings, assessed at the seventh day after sowing.

Hybrid	Average
1	100 a
2	97 b
3	99 a
4	98 a
CV (%) = 2.29	

Means followed by the same letter do not differ by the Scott-Knott test at 5% probability.

At seven days after sowing, there were no seed lings with primary leaf growth less than 50% of coleoptile length.

For the 'seedlings with primary leaf growth between 50% and 75% of coleoptile length' group there was a significant quadruple interaction among hybrid, temperature, water and light. The highest percentage of seedlings with primary leaf growth between 50% and 75% of coleoptile length was observed in hybrid 4,followed by hybrids 2 and 3, which had a higher percentage of this seedling type than hybrid 1 (Table 9) under light less conditions with water quantity 2.0 to 3.0 times paper weight. This was also observed by Ávila, Braccini, and Scapim (2007), who reported a reduction in the length of maize plants when water availability was reduced. However, according to Yap (1981), an excess of water is also detrimental to the development of maize seedlings. It was not possible to determine which temperature showed the largest percentage of seedlings with this feature, since such seedlings were observed at all temperatures.

According to the Brasil (2009) germination test for maize seeds, normal seedling evaluation is performed between the fourth and the seventh day after sowing. Due to the results observed in this test, it is recommended that an evaluation at seven days be performed for hybrids with differential growth, in order to prevent inaccurate reports. It is noteworthy that most seed companies perform only one count at five days which could affect the test results.

Assay 2: In the sand germination test (TGA) three days after sowing, the lowest percentage of normal seedlings was observed for hybrid 3. However, at the fifth and the seventh day there was no difference in the percentage of normal seedlings among all hybrids (Table 10). In sand substrate, 100% of seedlings had emerged at seven days, and in this test no seedlings with abnormal primary leaf growth in relation to the coleoptile were observed. This fact shows that although these seeds presented delays in aerial organ growth, those results do not reflect the performance of the hybrids in the field, where despite the delay they produce healthy plants. Conceição et al. (2012) also failed to differentiate maize seed lots through the sand germination test, emphasizing the need for adequate choice of substrate in the germination test, especially for differential growth materials.

Table 9. Percentage of maize seedlings with primary leaf growth between 50% and 75% of coleoptile length, assessed at the seventh day after sowing.

			Temperature			
Hybrid	Water	Light conditions	20-30°C	20°C	25°C	30°C
1	2,0	Lightless	0 aA	0 bA	0 aA	2 cA
2	2,0	Lightless	0 aA	0 bA	0 aA	2 cA
3	2,0	Lightless	0 aB	0 bB	1 aB	8 aA
4	2,0	Lightless	0 aB	5 aA	0 aB	4 bA
1	2,0	Light	0 aA	0 b A	0 bA	0 aA
2	2,0	Light	0 aB	3 aA	0 bB	0 aB
3	2,0	Light	0 aA	0 bA	0 bA	0 aA
4	2,0	Light	0 aB	0 bB	3 aA	0 aB
1	2,5	Lightless	0 aB	3 aA	0 bB	0 aB
2	2,5	Lightless	0 aB	0 bB	3 aA	0 aB
3	2,5	Lightless	0 aA	0 bA	0 bA	0 aA
4	2,5	Lightless	1 aB	0 bB	5 aA	0 aB
1	2,5	Light	0 aA	0 bA	0 aA	0 aA
2	2,5	Light	0 aA	0 bA	0 aA	0 aA
3	2,5	Light	0 aA	1 bA	1 aA	0 aA
4	2,5	Light	0 aB	3 aA	0 aB	0 aB
1	3,0	Lightless	0 aA	0 aA	0 cA	0 aA
2	3,0	Lightless	0 aA	0 aA	2 bA	0 aA
3	3,0	Lightless	0 aB	0 aB	5 aA	1 aB
4	3,0	Lightless	1 aB	1 aB	8 aA	1 aB
1	3,0	Light	0 aA	0 bA	0 bA	0 aA
2	3,0	Light	0 aA	1 bA	0 bA	0 aA
3	3,0	Light	0 aA	2 aA	0 bA	0 aA
4	3,0	Light	0 aB	1 bB	3 aA	0 aB
CV(%) = 1.07						

Means followed by the same lowercase letter (in columns) or the same capital letter (in rows) do not differ by the Scott-Knott test at 5% of probability.

Table 10. Percentage of normal maize seedlings in the sand germination test, assessed at three to seven days after sowing.

Hybrid	3 days	5 days	7 days
1	16 a	100 a	100 a
2	12 a	96 a	100 a
3	4 b	100 a	100 a
4	16 a	100 a	100 a
CV(%)	38.50	5.12	2.25

Means followed by the same letter in columns do not differ by the Scott-Knott test at 5% probability.

Assay 3: In the histological analysis, differences were observed in the pericarp thickness of hybrid seeds. Hybrid 1 showed greater thickness (Table 11). When working with reciprocal maize crosses, José, Von Pinho, Von Pinho, Ramalho, and Silva (2005) also reported differences among the pericarp structures, corroborating the results obtained in this experiment. According to Tracy and Galinat (1987), the thickness and density of the cell walls in the pericarp vary among maize genotypes, affecting seed quality and germination.

However, the hybrid with the highest pericarp thickness was not the hybrid that showed slow primary leaf growth. There was no difference in the pericarp thickness of the hybrid 4 that could explain its greater susceptibility to differential primary leaf growth compared to the coleoptile. Thus, it was found that the pericarp did not present resistance to the germination of hybrid maize seed and did not influence differential primary leaf growth compared to the coleoptile.

Table 11. Pericarp thickness of hybrid maize seeds and their reciprocal crosses.

Hybrid	Pericarp thickness (µm)
1	203.12 a
2	132.79 b
3	138.13 b
4	148.62 b
	CV (%) = 10.65

Means followed by the same letter do not differ significantly by the Scott-Knott test at 5% probability.

Assay 4: In the histochemical test, it was observed that the four hybrids showed a small amount of lignin in the cell wall of the coleoptile (Figure 2).

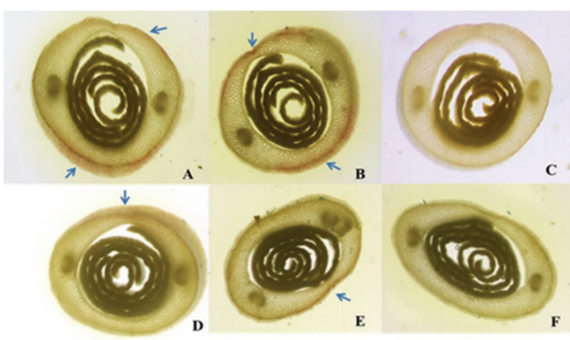

Figure 2. Histochemical test in maize seedlings showing the presence of lignin. A) Hybrid 1; B) hybrid 4; C) unstained control (Hybrid 1 and 4); D) hybrid 2; E) hybrid 3; F) unstained control (hybrids 2 and 3). Magnified 200x.

Musel et al. (1997) also observed the presence of lignin in the coleoptile cell wall of maize seedlings and considered its presence a potential regulator of coleoptile growth. However, Hohl, Greiner, and Schopfer (1995) observed that the elongation of the coleoptile was not affected by lignification of the wall. In this work, despite the finding of lignin in the hybrid seedlings' coleoptile walls, it was not possible to say that the presence of lignin was responsible for differential growth of primary leaf in relation to the coleoptile of maize seedlings, because lignin was present in all hybrids.

Assay 5: As shown in Figure 3, dry seeds possessed low expression of α-amylase in all hybrids. However, the expression of this enzyme was observed in soaked seeds. According to Silva-Neta et al. (2015), α-amylase activity is higher after root protrusion, because it increases the available reserves to promote seedling growth. Soaked seeds from hybrid 1 showed the lowest expression of α-amylase and soaked seeds from hybrid 3 and 4 showed the highest expression of this enzyme. According to Oliveira et al. (2013), genotypes with greater expression of α-amylase enzyme can easily provide carbohydrates to the embryo, resulting in higher germination and vigor. However, in this study, hybrid 4, despite having the highest activity of α-amylase enzyme, was also the hybrid with the highest percentage of seedlings with slow growth of aerial organs. In seedlings with four days of growth, the expression of α-amylase enzyme was lower than in soaked seeds. Seedlings from hybrid 1 had the highest expression of this enzyme, and seedlings from hybrid 4 had the lowest expression.

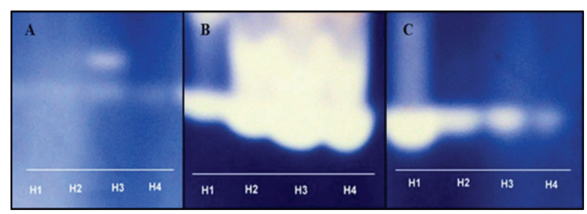

Figure 3. Enzymatic profiles for α-amylase enzyme (E.C. 3.2.1.1) from maize simple hybrids and their reciprocal crosses. A) Dry seeds; B) soaked seeds; C) seedlings with four days of growth.

Enhanced expression of β-amylase enzyme occurs in dry seeds (Figure 4), where this enzyme is preexisting. The expression of this enzyme was greater in dry seeds of hybrid 3 than in the remaining hybrids. Seeds from hybrid 4 showed very little expression of β-amylase, even when dry. In soaked seeds, the expression of β-amylase was lower than in dry seeds, but the enzyme was still present. In soaked seeds from hybrids 1, 2, and 3 the expression of this enzyme was higher than in hybrid 4. As expected, there was no expression of β-amylase enzyme in seedlings with four days of growth; the β-amylase enzyme is preexisting in dry maize seed and assists in immediate starch hydrolysis after soaking, until α-amylase is synthesized again.

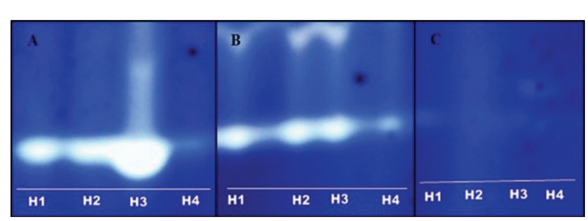

Figure 4. Enzymatic profiles for β-amylase (E.C. 3.2.1.2) enzyme from maize simple hybrids and their reciprocal crosses. A) Dry seeds; B) soaked seeds; C) seedlings with four days of growth.

Conclusion

There are differences among hybrid maize genotypes regarding the initial growth of primary leaf in relation to coleoptile, especially under lightless germination conditions.

Water availability and temperatures within the optimal range (20° to 30°C) do not affect the response of these hybrids in relation to differential growth of the primary leaf.

Pericarp thickness, lignin content and differences in enzyme expression among different genotypes are not related to changes in the relationship of primary leaf growth to coleoptile length during the maize seed germination test.

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References

Alfenas, A. C. (2006). *Eletroforese de iso enzimas e proteínas afins: fundamentos e aplicações em plantas e microorganismos*. Viçosa, MG: UFV, 2006.

Ávila, M. R., Braccini, A. L., & Scapim, C. A. (2007). Teste de comprimento de plântulas sob estresse hídrico na avaliação do potencial fisiológico das sementes de milho. *Revista Brasileira de Sementes*, *29*(2), 117-124. doi: 10.1590/S0101-31222007000200016.

Box, G. E. P., & Cox, D. R. (1964). An analysis of transformation. *Journal of the Royal Statistical Society*, *26*(2), 211-252.

Brasil. (2009). Ministério da Agricultura, Pecuária e Abastecimento *Regras para análise de sementes*. Brasília, DF: MAPA.

Brasil. (2003). *Lei n° 10.711, de 5 de agosto de 2003. Dispõe sobre o Sistema Nacional de Sementes e Mudas e dá outras providências*. Brasília, DF: MAPA.

Brasil. (2013). *Instrução Normativa n° 45, de 17 de setembro de 2013. Padrões para produção e comercialização de sementes de milho (Zea mays L.).* Brasília, DF: MAPA.

Carvalho, L. F., Sediyama, C. S., Reis, M. S., Dias, D. C. F. S., & Moreira, M. A. (2009). Influência da temperatura de embebição da semente de soja no teste de condutividade elétrica para avaliação da qualidade fisiológica. *Revista Brasileira de Sementes*, *31*(1), 9-17. doi: 10.1590/S0101-31222009000100001.

Conceição, P. M., Sediyama, C. A. Z., Vieira, R. F., Galvão, J. C. C., Corrêa, M. L. P., & Conceição, P. S. (2012). Estimativa do vigor de sementes de milho através da avaliação do sistema radicular de plântulas. *Ciência Rural*, *42*(4), 600-606. doi: 10.1590/S0103-84782012000400004.

Deng, B., Yang, K., Zhang, Y., & Li, Z. (2015). The effects of temperature on the germination behavior of white, yellow, red and purple maize plant seeds. *Acta Phisiologiae Plantarum*, *37*(174), 1-11. doi: 10.1007/s11738-015-1937-1.

Ferreira, D. F. (2011). Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia*, *35*(6), 1039-1042. doi: 10.1590/S1413-70542011000600001.

Grzybowski, C. R. S., Vieira, R. D., & Panobianco, M. (2015). Teste de estresse na avaliação do vigor de sementes de milho. *Revista Ciência Agronômica*, *46*(3), 590-596. doi: 10.5935/1806-6690.20150042.

Hohl, M., Greiner, H., & Schopfer, P. (1995). The cryptic-growth response of maize coleoptiles and its relationship to H202- dependent cell wall stiffening. *Physiology Plant*, *94*(3), 491-498. doi: 10.1111/j.1399-3054.1995.tb00959.x.

José, S. C. B. R., Von Pinho, E. V. R., Von Pinho, R. G., Ramalho, M. A. P., & Silva Filho, J. L. (2005). Características físicas do pericarpo de sementes de milho associadas com tolerância à alta temperatura de secagem. *Revista Brasileira de Sementes*, *27*(1), 125-131. doi: 10.1590/S0101-31222005000100015.

Kurtyka, R., Burdach, Z., & Karez, W. (2011). Temperature dependence of growth in maize seedlings and excised coleoptile segments. *Polish Journal of Environmental Studies*, *20*(5), 1221-1225.

Lima, O. R. F., Santos, G. G., & Melo, M. B. (2010). Influência da luz na germinação do milho. *Arte e Ciência*, *1*(1), 1-8.

Lipchinsky, A., Sharova, E. I., & Medvedev, S. S. (2013). Elastic properties of the growth-controlling outer cell walls of maize coleoptile epidermis. *Acta Physiologiae Plantarum*, *35*(7), 2183-2191. doi: 10.1007/s11738-013-1255-4.

Lucio, A. D., Couto, M. R. M., Lopes, S. J., & Storck, L. (2011). Transformação box-cox em experimentos com pimentão em ambiente protegido. *Horticultura Brasileira*, *29*(1), 38-42. doi: 10.1590/S0102-05362011000100007.

Mertz, L. M., Henning, F. A., Cruz, H. L., Meneghello, G. E., Ferrari, C. S., & Zimmer, P. D. (2009). Diferenças estruturais entre tegumentos de sementes de soja com permeabilidade contrastante. *Revista Brasileira de Sementes*, *31*(1), 23-29. doi: 10.1590/S0101-31222009000100003.

Musel, G., Schindler, T., Bergfeld, R., Ruel, K., Jacquet, G., Lapierre, C., … Schopfer, P. (1997). Struture and distribution of lignin in primary and secondary cell walls of maize coleoptiles analyzed by chemical and immunological probes. *Planta, 201*(2), 146-156.

Nerling, D., Coelho, C. M. M., & Nodari, R. O. (2013). Genetic diversity for physiological quality of seeds from corn (*Zea mays L.*) intervarietal cross breeds. *Journal of Seed Science*, *35*(4), 449-456. doi: 10.1590/S2317-15372013000400006.

Oliveira, G. E., Von Pinho, R. G., Andrade, T., Von Pinho, E. V. R., Santos, C. D., & Veiga, A. D. (2013). Physiological quality and amylase enzyme expression in maize seeds.

Ciência e Agrotecnologia, *37*(1), 40-48. doi: 10.1590/S1413-70542013000100005.

Rasband, S. W. (2012). ImageJ - Image Processing and Analysis in Java. *Astrophysics Source Code Library, 1*(1), 6013.

Sbrussi, C. A. G., & Zucarelli, C. (2014). Germinação de sementes de milho com diferentes níveis de vigor em resposta à diferentes temperaturas. *Semina: Ciências Agrárias*, *35*(1), 215-226. doi: 10.5433/1679-0359.2014v35n1p215.

Silva-Neta, I. C., Von Pinho, E. V. R., Veiga, A. D., Von Pinho, R. G., Guimarães, R. M., Caixeta, F., Santos, H. O., & Marques, T. L. (2015). Expression of genes related to tolerance to low temperature for maize seed germination. *Genetics and Molecular Research*, *14*(1), 2674-2690. doi: 10.4238/2015.March.30.28.

Tracy, W. F., & Galinat, W. C. (1987). Thickness and cell layer number of the pericarp of sweet corn and some of its relatives. *Horticultural Science*, *22*(4), 645-647.

Yakamura, K. (1999). Transformation using (x + 0.5) to stabilize the variance of populations. *Journal Researches on Population Ecology*, *41*(3), 229-234. doi: 10.1007/s101440050026.

Yap, S. K. (1981). Collection, germination and storage of dipterocarp seeds. *Malasyan Forester*, *44*(2/3), 281-300.

Zucarelli, C., Cavariani, C., Portugal, G., & Nakagawa, J. (2008). Potencial fisiológico de sementes de milho hidratadas pelo método do substrato de papel toalha. *Revista Brasileira de Sementes*, *30*(3), 122-129. doi: 10.1590/S0101-31222008000300016.

Zucarelli, C., Cavariani, C., Oliveira, E. A. P., & Nakagawa, J. (2011). Métodos e temperaturas de hidratação na qualidade fisiológica de sementes de milho. *Revista Ciência Agronômica*, *42*(3), 684-692. doi: 10.1590/S1806-66902011000300015.

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