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Germination under stress simulation and image analysis as tools for water deficit phenotyping of maize

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ABSTRACT: Faster phenotyping tools are crucial for field progeny selection. We conducted research comparing two water deficit simulation methods on contrasting cultivars for water deficit tolerance. In a first step, we conducted two experiments: one for seed germination in sand at 10% and 70% water retention capacity, being analyzed seedling emergence and growth; other for seed germination in stress simulation by moistening the germination paper with PEG solution at -0.6 MPa, being analyzed seed germination. These experiments were used to distinguish characteristics of two maize lineages with different response to drought, being 57 – sensitive and 91 – tolerant. After that, we produced hybrid progenies from these lines at generations F_1, F_2, and $F_{2:3}$ and applied the stress simulation by moistening the germination paper with -0.6 MPa of PEG solution. The seedling size was analyzed trough image analysis by the GroundEye® system. We could distinguish both lines and its hybrids through the stress simulation and image analysis. The results indicate that maize cultivars can be phenotyped for water deficit tolerance either 5 days postsowing through stress simulation or via image analysis of root length from seedlings germinated under -0.6 MPa. This method provides faster, more accurate, and more cost-effective methods for assessing water deficit tolerance in maize cultivars.

Index terms: abiotic stress, phenotype, seedling development, *Zea mays* L.

Resumo: Ferramentas rápidas de fenotipagem são cruciais para a seleção de progênies em programas de melhoramento genético. Esta pesquisa foi conduzida com o objetivo de avaliar a possibilidade de uso da simulação de estresse em laboratório como forma de distinguir fenótipos de milho quanto à tolerância à seca. Foram conduzidos inicialmente dois experimentos: um em que avaliou-se a emergência de plântulas e seu crescimento em areia sob diferentes capacidades de campo; e um segundo com o uso de solução de PEG a -0.6 Mpa para umedecer o papel de germinação. Estes experimentos foram usados para distinguir características de duas linhagens contrastantes quanto à tolerância à seca: linhagem 57 – sensível e 91 – tolerante. No próximo experimento, foram produzidas sementes hibridas a partir do cruzamento de ambas as linhagens nas gerações F_1; F_2 e $F_{2:3}$ as quais foram colocadas para germinar em papel umedecido com solução de PEG a -0.6 MPa com posterior avaliação do crescimento das sementes através da análise de imagens com o sistema GroundEye®. Pelos resultados dessa pesquisa foi possível distinguir tanto as características das linhagens parentais quanto as progênies hibridas quanto à tolerância à seca. Neste sentido, pode-se concluir que a simulação do estresse associada à análise de imagens pode ser uma ferramenta útil para fenotipagem de milho.

Termos para indexação: estresse abiótico, fenótipo, desenvolvimento da plântula, *Zea mays* L.

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INTRODUCTION

Considering not only changes in environmental conditions but also the possibility of establishing crops in new areas with lower water availability, stress tolerance is important to understand. During their whole developmental stage, plants are subjected to water deficit conditions, requiring different levels of tolerance. According to Cooper et al. (2014), stress tolerance physiological mechanisms may be related to existing genetic variation, aiming to use those mechanisms for breeding. These authors mention that the main steps of maize breeding, with stress tolerance objectives, are germplasm selection, phenotyping, and selection.

Through phenotyping, interesting characteristics are observed, and progenies are selected, the accuracy of which is fundamental to the process (Setter, 2012). The use of characteristics that are strongly correlated with stressing conditions, as drought tolerance is essential, especially those that may be analyzed in the laboratory once the field is complex and longer (Meeks et al., 2013; Pace et al., 2014).

Considering this, the germination under stress simulation in laboratory may be a faster alternative. This can be observed based on the studies of Abreu et al. (2017; 2019), which used germination tests in laboratory as indirect methods to phenotype maize lines. Marques et al. (2019; 2020) also studied seed, seedling, and reserve tissue gene expression under stress conditions, with significant differences among genotypes regarding the response to water deficit. In addition to the use of different substrates and watering conditions, the use of osmotic molecules such as polyethylene glycol (PEG) can be a faster and more practical method, as evidenced by Viçosi et al. (2017) using mannitol ($C_6H_{12}O_6$).

Although water deficit is a complex characteristic, it must be understood for improving plant breeding (Cattivelli et al., 2008). Guedes et al. (2014) indicated that for obtaining superior genotypes, the use of diverse characteristics can be a better tool. It has also been indicated for reducing costs and labor, with accurate results (Sousa et al., 2015). Additionally, evaluating seedling growth by image analysis can be interesting because it is accurate, faster, and less expensive, as is an automated procedure (Kapadia et al., 2017; Medeiros et al., 2018).

The objective of this research to establish fast and low-cost methods for characterizing maize regarding water deficit tolerance. For this purpose, we assessed the use of germination in sand over different field capacity and use of polyethilenglycol for simulating this stress and used image analysis to measure the characteristics of the seedling size.

MATERIAL AND METHODS

Study area

The study was conducted at the Central Laboratory of Seed Research (LCPS) of the Agriculture Department of the *Universidade Federal de Lavras* (UFLA). For lineage selection, we based in the research from Abreu et al. (2019), Marques et al. (2019; 2020), and Santos et al. (2021). Two lineages contrasting in water deficit tolerance were chosen: 91 as tolerant and 57 as sensitive. Seeds from both groups were replicationd through self-fecundation in the Agriculture Department experimental area under the same edaphoclimatic conditions. Cobs were collected when the water content reached 35% and were dried on low-scale experimental dryers as described by Navratil and Burris (1982). Drying was performed at 45 °C by mass until a 12% water content was reached, at which point the seeds were manually threshed to avoid mechanical damage. Samples from these seeds were collected for experimental installation to evaluate two phenotyping methods for water deficit tolerance, using characteristics related to seed germination and seedling development. Seeds were separated on oblong sieves to remove round seeds and then on round sieves. We used seeds retained on sieve 20. Subsequently, the seeds were treated with the fungicide Vitavax/Thiram® 200 SC at a dosage of 300 mL.100 kg^{-1}, to avoid fungal contamination during the tests.

Line 57 and 91 phenotyping

Sand germination conditions: seeds from both lineages were sown on trays with sand as the substrate. To simulate water deficit, a retention capacity of 10% was used, and as a control, 70% retention was used (Abreu et al., 2019). The trays were kept in a growth chamber at 30 °C, and water repositioning under both conditions was conducted daily for both conditions based on the initial weight of each tray. After 4, 5, and 6 days, we evaluated seedling emergence and remaining reserve tissue (EMERG). Using these data, the emergence speed index (ESI) was calculated. After the 7th day, the plants and reserve tissues were collected, washed under running water, and manually measured for aerial part (CPA) and root (CR) length, seminal root number (NR), dry weight of aerial part (PSPA) and root (PSR), and weight gain from the aerial part (PVPA) and root (PVR) according to methods from Abreu et al. (2019). Were used 9 replications of 10 seeds for each genotype under each condition in accordance with a completely randomized design.

Polyethylene glycol water deficit simulation: it was conducted using a PEG 6000 solution at -0.6 MPa, which is 2.5 times heavier than the paper weight. The solution was used to moisten the substrate for the seed germination test (Abreu et al., 2014). The experiment was carried out using four replications of 50 seeds each under a completely randomized design. In the control treatments, water was used to moisten the paper roll. Tests were carried out in a B.O.D. chamber at a constant light and temperature of 30 °C. On the third day after sowing, seeds with radicle protrusions were counted, and these values were considered to indicate germination. The germination speed index was subsequently calculated (Maguire, 1962). Additionally, the values were evaluated daily, from the third to the seventh day after sowing.

From these results, germination, Emissions I and II and data from the 4th and 7th days after sowing were considered, and the Emissions I and II speed indices were calculated considering the daily count and equation from Maquire (1962). After nine days, the aerial parts, roots and remaining reserve tissues of both lineages were weighed green and dry. The data were statistically analyzed by analysis of variance, and the means were compared by the Scott–Knott test at 5% probability. The analyses were performed using R® software (R Core Team, 2020).

$F_{2:3}$ phenotyping

$F_{2:3}$ seed production: Based in the differences observed in the stress simulation in both sand and PEG 6000 for lines 57 and 91, the use of stress simulation in PEG 6000 germination test was used to phenotype the $F_{2:3}$ progeny.

Firstly, the $F_{2:3}$ progeny seeds were obtained by seeds by planting a simple hybrid produced by crossing lines 91 and 57. Crosses to obtain F_1 hybrids were performed manually between parental plants, and plastic bags were placed over the ears before the emergence of silk to protect them. When silk receptivity was observed, the anthers of the pollen-donating plants were covered with a paper bag to prevent genetic contamination, and crossing was performed the next day. Plants from lines 91 and 57 were also self-pollinated to produce seeds. The soil was conventionally prepared, and corrections were made according to chemical analysis. A spacing of 0.8 m between rows and 7 plants per linear meter were used. Fertilization, as well as other cultural and phytosanitary practices, was conducted according to the needs of the crops.

At 60 days after sowing, ears were sampled weekly to assess seed moisture content, and harvesting was carried out when the seeds reached 25% moisture content. Ears from self-pollinated lines and crosses were manually harvested, dried in an ear dryer at 35 °C until they reached 13% moisture content, and then manually threshed. The seeds were classified by size using circular sieve screens, and seeds retained on 20 screens were used. Subsequently, the seeds were stored in a cold room at 10 °C. These seeds (from both lines and from F_1 and reciprocal hybrids) were obtained by Abreu et al. (2017) and used in this study.

Seeds from the inbred lines and hybrids, as well as those from the F_2 (harvest/2016) and $F_{2:3}$ (harvest 2018/2019) generations, were produced in an experimental area of the Department of Agriculture/UFLA, as previously described.

Seed germination in PEG 6000: prior to the installation of the experiments, seeds from the $F_{2:3}$ progenies were classified using oblong sieves and then classified using round sieves. Seeds from 203 $F_{2:3}$ progenies retained in sieve 20

were selected. The seeds were treated with Vitavax/Thiram® 200SC fungicide at a dosage of 300 mL.100 kg^{-1}. After that, the stress simulation in PEG 6000, as described before was caried out. Thirty seeds from each progeny were used in two replications of 15 each. The evaluations were conducted based on two criteria. At five days after the experiment was initiated, the plants were counted, and the remaining reserve materials with at least 3 cm of primary root (Emergence I) were removed. At seven days after the experiment was initiated, seedlings and remaining reserve materials with at least 3 cm of primary root, presence of aerial part and at least 3 secondary roots were counted (Emergence II). The progenies were classified according to their tolerance to water deficit based on the following criteria: tolerant (80% to 100%), intermediate (79% to 50%), and intolerant (below or equal to 49%), based on the results observed at 5 and 7 days according to the criteria described above. This classification was carried out based on the research developed by Salgado et al. (2008).

Image analysis: it was used for $F_{2:3}$ progeny phenotyping. Of the 203 progenies, images were captured of 108 seedlings and tissues remaining from the reserve material seven days after sowing using GroundEye S800® equipment. This equipment consists of a capture module that has an acrylic tray, a high-resolution camera and integrated software for evaluation. Two replications of 10 seedlings and the remaining tissues of reserve material per progeny were used. In the configuration of the analysis, for the calibration of the background color, the CIELab color model was used, with a luminosity index ranging from 0 to 100, dimensions "a" ranging from -120.0 to 120.0 and dimensions "b" ranging from -120.0 to -25.5.

The methods used for phenotyping the $F_{2:3}$ progeny followed a completely randomized design. The data were statistically analyzed through analysis of variance, and the means were compared by the Scott–Knott test at the 5% level with the aid of the R® program (R Core Team, 2020). The analyses were performed according to the statistical model in equation 1A. For estimating variance components, the data were analyzed using the mixed model approach obtained by the restricted maximum likelihood method (REML). The significance of the components was measured using the likelihood-ratio test (LRT). For this analysis, the same model presented above was used. The experimental quality was measured through estimation of the experimental coefficient of variation (CV%) and proposed selective accuracy, according to equation 1B. Herdability estimates were obtained through the estimator in equation 1C. Spearman's correlation coefficients were also obtained between the rankings of the progenies in the evaluations carried out at 5 and 7 days (PEG), between the characters evaluated in the image analysis (length of the primary root and aerial part and total seedling size) and between both experiments.

Equation 1A	Equation 1B	Equation 1C	Equation 1D
$y_{ij} = \mu + p_i + e_{ij}$	$CV\ (\%) = \frac{\sqrt{\sigma_e^2}}{\overline{Y}} * 100$	$CV\ (\%) = \frac{\sqrt{\sigma_e^2}}{\overline{Y}} * 100$	$h^2 = \frac{\sigma_p^2}{\sigma_p^2 + \frac{\sigma_e^2}{r}}$
where: y_{ij} = observation of progeny i in repetition j μ = general constant associated on all observation p_i = fixed effect of progeny i, $p_i \sim (0, \sigma_p^2)$ e_{ij} = random experimental error associated with observation y_{ij}, $e_{ij} \sim e_{ij} \sim N\ (0, \sigma_e^2)$.	Where: σ_e^2 = residual variance component $\overline{Y}$ = general phenotipic average $r_{gg'} = \sqrt{1 - \left(\frac{PEV}{\sigma_p^2}\right)}$ PEV = BLUPS predicted variance of error σ_p^2 = progeny variance component	Where: σ_e^2 = residual variance component $\overline{Y}$ = general phenotipic average $r_{gg'} = \sqrt{1 - \left(\frac{PEV}{\sigma_p^2}\right)}$ PEV = BLUPS predicted variance of error σ_p^2 = progeny variance component	Where σ_p^2 = progeny variance component σ_e^2 = residual variance component r = number of replicates

Equation 1. Models used for data analyses.

RESULTS AND DISCUSSION

Maize seedling emergence from lineage 91 was higher than 57 at evaluations from 4, 5, and 6 days from sowing (Table 1), with the same results observed for the ESI analysis (Table 1).

At four, five and six days, the values of seedling emergence and tissue remaining from the reserve material were greater for lineage 91 than for the other lineages, which was also observed for IVE (Table 1). For the variables shoot length, main root length and number of seminal roots (Table 1), the highest values were observed for the seedlings and tissues remaining from the reserve material of line 57.

Higher germination speeds at four days according to the germination speed index (IVG), emergence speed indices I and II (IVE1 and IVE2, respectively), seedling emergence and remaining tissues of reserve material II at four and seven days were observed for the seeds and seedlings, and tissues remaining from the reserve material of line 91 germinated and developed in the presence of PEG 6000 solution (Table 2). At four and seven days after sowing, the values of emergence II on paper moistened with water did not significantly differ between the evaluated lines. In the presence of the PEG 6000 solution, strain 91 was superior (Table 3). This was also observed for the ESI_2 variable.

Regarding the results observed for the variables green weight of roots and green and dry weight of shoots (Table 3), no significant differences were observed when seedlings and tissues remaining from the reserve material of strains 91 and 57 were developed in the presence of a solution of PEG 6000. However, when the seedlings and tissues remaining from the reserve material were developed on paper moistened with water, higher values were observed for strain 91. For the root dry weight variable, the interaction between the factors was not significant. Higher values were observed for strain 91 and when the paper was moistened with water (Table 4).

Table 1. Values of emergence percentage (4 to 6 days) and speed index (ESI), and seedling size from maize lines, at 7 days, developed in sand with 10 and 70% of water retention capacity.

Emergence Percentage			
Time (days)	Lineages		CV (%)
	91	57	
4	66 a	39 b	50.06
5	98 a	89 b	8.84
6	99 a	94 b	6.17
Emergence Speed Index			
	Lineages		CV (%)
	57	91	
ESI	5.28	4.32	17.19
Seedling size			
Lines	Length		Seminal root number
	Aerial part	Main root	
91	9.91 B	12.87 B	3.22 B
57	10.82 A	15.205 A	6.0 A
CV (%)	9.91	15.67	6.76

*Significant at the 5% probability level. * Means followed by the same lowercase letter on the line, do not differ statistically by Tukey's test at the 5% probability level.

Table 2. Values for germination, emergence I, and II of maize lines germinated on paper moistened with a solution of PEG 6000 and water.

Lineages	Germination				Emergence I			
	4 days		GSI		4 days		7 days	
	Water	PEG	Water	PEG	Water	PEG	Water	PEG
91	100 Aa	98 Aa	66 Aa	61 Ab	98 Aa	0 b	98.5 Aa	71.5 Ab
57	98 Aa	57 Bb	64 Aa	42 Bb	73 Ba	0 b	96.5 Aa	30.5 Bb
CV	5.14		3.2		8.21		9.87	

Emergence speed index I values

Lineages	ESI I	Environment	ESI I
91	31.25 A	Water	45.55 A
57	25.9 B	PEG	11.6 B
CV	8.39	CV	8.39

Lineages	Emergence II				ESI_2	
	4 days		7 days			
	Water	PEG	Water	PEG	Water	PEG
91	99 Aa	69 Ab	99 Aa	99 Aa	24.88 Aa	22.1 Ab
57	94 Aa	10 Bb	97 Aa	83 Bb	23.99 Aa	18.35 Bb
CV	15.61		3.65		5.86	

*Significant at the 5% probability level. * Means followed by the same uppercase letter in the column and lowercase in the row, do not differ statistically by Tukey's test at the 5% probability level.

Table 3. Values of dry weights of shoots (PSPA) and green weights of shoots and roots (PVPA, PVR) of seedlings and remaining tissues of reserve material of maize lines developed on paper moistened with a PEG 6000 solution and water.

Lineages	PSPA		PVR		PVPA	
	Water	PEG	Water	PEG	Water	PEG
91	2.5 Aa	0.12 Ab	12.32 Aa	2.10 Ab	29.81 Aa	0.615 Ab
57	2.0 Ba	0.19 Ab	8.13 Ba	1.84 Ab	18.47 Ba	0.690 Ab
CV (%)	7.72		19.48		5.93	

PSR

Lineages	Average	Environments	Average
91	0.76 A	Water	1.09 A
57	0.69 B	PEG	0.36 B
CV (%)	11.62	CV (%)	11.62

*Significant at the 5% probability level. * Means followed by the same uppercase letter in the column and lowercase in the row, do not differ statistically by Tukey's test at the 5% probability level.

Table 4. Estimates of variance components obtained by the restricted maximum likelihood method (REML) and correlation for Emergence I, II, and seedling length of $F_{2:3}$ progenies and maize.

Parameter	Emergence I (5 days)	Emergence II (7 days)	Length		
			Primary root	Aerial part	Total
$\hat{\sigma}^2_{Prog}$	503.94***	470.14***	4.10***	0.18***	5.41***
$\hat{\sigma}^2_e$	130.99	166.78	2.41	0.31	3.31
$\hat{H}^2$	0.89	0.85	0.97	0.92	0.97
$r_{gg'}$	0.94	0.92	0.99	0.96	0.99
CV (%)	17.02	22.94	20.36	82.50	21.89
Minimum	13,93	14.43	4.05	0.06	4.37
Maximum	96,23	90.44	11.05	2.24	12.29
Average	67,24	56.29	7.63	0.68	8.30
Variation amplitude	82,3	76.01	7.00	2.18	7.92
Spearman correlation	0.74***			...	

[1/] Progeny variance ($\hat{\sigma}^2_{Prog}$), residual variance ($\hat{\sigma}^2_e$), broad (h^2) heritability and selective accuracy ($\hat{r}_{gg'}$). [2/] Magnitudes of average BLUPs. Significance by Likelihood Ratio Test - 0 '***' 0.001 '**' 0.01 '*'. Source: From the author (2021).

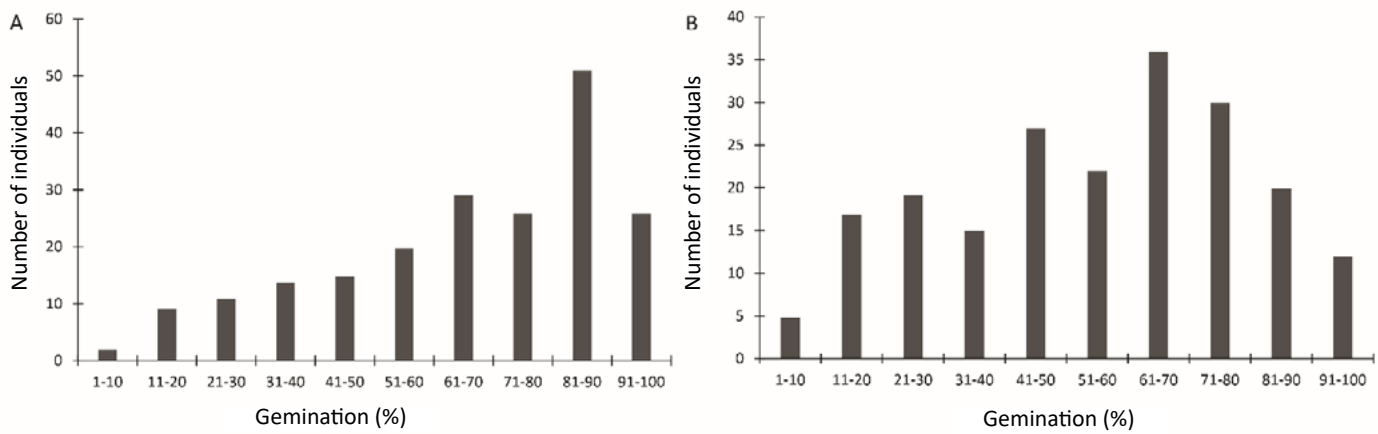

Figure 1. Distribution of the F_2 population of the 91x57 hybrid combination after 5 (A) and 7 (B) days, in 10 phenotypic classes, from the results of germination under water deficit, PEG 600, -0.6MPa, of the F2:3 seeds.

Figure 1 shows the distribution of individuals from the F_2 population according to the results of germination under water deficit, carried out on the seeds of the 203 $F_{2:3}$ progenies of individuals from the F_2 population at 5 and 7 days. Figure 1A shows a greater percentage of progenies with seed germination between 81 and 90%, followed by those with germination between 61 and 70%, when evaluated on the fifth day after sowing.

At 7 days (Figure 1B), a greater number of progenies in which was observed a germination from 61 to 70% was observed, followed by those with 71 to 80% germination. At 7 days, a lower percentage of germination data was observed in the extreme classes. This was not observed after five days. When analyzing the data, it should be considered that at five days, seedlings, and remaining tissues of reserve material with at least 3 cm of main root were considered to have emerged, and on the seventh day, seedlings, and remaining tissues of reserve material with at least 3 cm were considered to have emerged. cm of main root, 3 seminal roots and aerial part present.

The component of genetic variance (progeny) was highly significant for all evaluated traits and for both phenotyping methods and manual and image analysis, revealing the existence of genetic variability between the $F_{2:3}$ progenies (Tables 4 and 5). The low magnitude of the residual variance component in both phenotyping methods confirmed the low environmental variation when using PEG 6000 (-0.6 MPa).

When considering the heritabilities (Table 4), which are related to the characteristics evaluated through image analysis, primary root length, shoot length and total size of the seedlings and remaining tissues of the reserve material (97%, 92% and 97%, respectively), it is possible to observe that more than 90% of the total variation observed in all the traits is of genetic origin, which decreases the probability of selection errors. Thus, considering that heritability refers not only to the characteristic of tolerance to water deficit but also to the genotypes and the environmental conditions to which the individuals were subjected, it is concluded that there was little environmental variation.

Santos et al. (2021), when evaluating different maize genotypes in terms of tolerance to water deficit under the same conditions evaluated in this study, observed differences between genotypes 91 and 57 through the variables seedling emergence and tissue remaining from reserve material and root length, with the highest values observed for lineage 91. The same author did not observe differences between these materials in terms of shoot length. Marques et al. (2019; 2020) observed a significant interaction between the genotypes evaluated for water deficit tolerance and the environment and between sand with 10 and 70% water retention capacity and the variables root length and shoot length.

From the results observed in this study, in which characteristics related to the germination and development of seedlings and remaining tissues of reserve material were evaluated, it can be inferred that water restriction using a PEG 6000 solution at -0.6 MPa was efficient for phenotyping contrasting materials in terms of water deficit. Setter (2012) reported that the identification of characteristics related to stress tolerance is essential for phenotyping. Durães et al. (2004) mentioned that the choice of crop development phase that expresses tolerance to water stress is one of the most important requirements for phenotyping.

In the present research, characteristics related to seed germination and the development of seedlings and tissues remaining from reserve material under water deficit were evaluated. During seed germination, a series of hydrolysis reactions and syntheses of substances occur, which are dependent on water (Marcos-Filho, 2015). Water deficiency during these processes can influence the percentage of germination, the germination speeds of the seeds and the emergence of seedlings and tissues remaining from the reserve material, which can compromise the establishment of the culture in the field (Fancelli and Neto, 2000).

There are few studies in which seeds, seedlings and remaining tissues of reserve material are used for phenotyping for tolerance to water deficit. According to Kranner et al. (2010), seeds are highly vulnerable to the incidence of stress during development or germination, which results in loss of vigor or viability, making this organ an attractive model for studies related to tolerance to abiotic stresses, such as water deficit. These findings can be confirmed by the results of this research. Similar results to those observed in this research were also observed by Abreu et al. (2014, 2019), Baccini et al. (2004), Marques et al. (2019; 2020) and Santos et al. (2021), this indicates that the methods here tested may be applied in maize phenotyping.

It is known that phenotyping is an important phase in breeding programs aimed at water deficit tolerance and that this must be performed using traits that must reflect gene expression for the trait of interest. Thus, it is understood that the characteristics selected for this purpose must replication the results for the safe selection of genotypes.

Considering the results obtained in other studies with different objectives, lineage 91 was shown to be related to drought tolerance, while lineage 57 was shown to be intolerant. Considering the results obtained in the present research, it is inferred that evaluating the characteristics related to seed germination and development of seedlings and remaining tissues of reserve material subjected to water deficit when using a PEG 6000 solution (0.6 MPa) can be considered safe for the phenotyping of genotypes for drought tolerance. Under these conditions, the characteristics through which the seedlings and remaining tissues of reserve material had the greatest length of main root, at least 2 cm, and more than two seminal roots were considered suitable for phenotyping after 4 days.

Salgado et al. (2008) also observed a nonnormal distribution in seed vigor data, evaluated by the accelerated aging test when phenotyping of $F_{2:3}$ progenies was performed in a study of tolerance to high drying temperatures in corn seeds. The authors reported that this behavior may be indicative of oligogenic inheritance. When considering the segregation of a major gene and assuming that the phenotypic distribution of each genotype of the major gene is normal, the resulting distribution is generally nonnormal, which does not imply the presence of a major gene.

The estimated accuracy values, i.e., correlation between the evaluated characteristics and for both phenotyping methods, manual and through image analysis, were great, above 90%, demonstrating the low probability of selection error (Tables 5 and 6).

This statement became clearer when observing the correlation between the rankings of the progenies at 5 and 7 days after sowing (74%) (Table 5). Furthermore, when analyzing these data, progenies classified as tolerant on the fifth day were not classified as intolerant on the seventh day and vice versa. Thus, the progenies not classified as tolerant or susceptible on the fifth or seventh days of evaluation behaved as intermediates in relation to tolerance to water deficit.

The practical implication of this, in the selection processes for water deficit tolerance using traits related to the development and growth of seedlings and remaining tissues of reserve material for phenotyping, is the fact that there is no risk of selecting a genotype as tolerant or even being intolerant and vice versa. These results also reinforce root development and growth in response to water deficit during seed germination and the emergence of seedlings and tissues remaining from reserve material.

Albacete et al. (2014) and Yang et al. (2012) referred to abscisic acid as a stress signal from the root to the aerial parts of plants that induces stomatal closure and consequently transpiration. Hu and Xiong (2014) reported that plants can increase root growth, with the aim of increasing water absorption in deep soils. These authors mentioned the ability to modify the architecture of the root system in relation to length, weight, volume, and density.

It is important to consider the benefits of image analysis for phenotyping in breeding programs. This tool has been used for species distinction (Marques et al., 2019) and for seed vigor studies (Abud et al., 2017). For maize, computerized analysis has also been used for seed vigor determination (Medeiros et al., 2018), highlighting the potential applications of this tool in phenotyping and reducing the time and costs of program procedures by decreasing the probability of human error, among other advantages.

Sousa et al. (2015) emphasized that phenotyping through image analysis has advantages over traditional methods due to the automation of the process, which saves time and labor, in addition to greater precision. The same authors

Table 5. Correlations of spearman ranks between the characteristics evaluated at 5 and 7 days after sowing, environment under stress condition, and the characteristics evaluated through image analysis.

Characteristics	Length		
	Root	Aerial part	Total size
PEG - 5 days	0,89***	0,70***	0,90***
PEG - 7 days	0,70***	0,56***	0,64***

Significance by Likelihood Ratio Test - 0 '***' 0.001 '**' 0.01 '*'.

Table 6. Correlations of spearman ranks between the characteristics evaluated through image analysis.

Characteristics	Length	Total
	Aerial part	
Primary Root	0,69***	0,99***
Aerial part		0,78***

Significance by Likelihood Ratio Test - 0 '***' 0.001 '**' 0.01 '*'. Source: From the author (2021).

reinforced that this has been possible under controlled conditions in phenotyping aimed at prospecting, discovering and validating genes, both in model plants and in cultivated plants. In the image analysis, when the sizes of the main roots, secondary roots and shoot length (Total Size) were considered, a high correlation was observed between the highest values for this variable and the data through which the progenies were classified as tolerant, that is, at five and seven days after sowing. According to Basu et al. (2016), the presence of secondary roots is associated with the adaptive strategy of plants to increase water absorption.

Zhang et al. (2009) observed greater root growth in transgenic tobacco plants tolerant to water deficit associated with the overexpression of the APX gene ascorbate peroxidase. It is known that this enzyme is one of the main enzymes involved in the detoxification of reactive oxygen species (ROS) in plants. Based on these results, it is possible to infer that the initial development of roots of plants and tissues remaining from reserve material is an important characteristic for the selection of genotypes tolerant to water deficit. Based on the results obtained in this research, phenotyping for tolerance to water deficit, either through image analysis or manually, can be performed five days after sowing, considering the length of the main root, under water deficit conditions. simulated with the PEG 6000 solution (-0.6 MPa), with the advantage that phenotyping by image analysis is faster and requires less labor.

CONCLUSIONS

The use of a PEG 6000 solution (-0.6 MPa) to simulate water restriction during the processes of seed germination and development of seedlings and tissues remaining from reserve material is indicated for the phenotyping of maize genotypes for water deficit tolerance.

For the phenotyping of maize genotypes for tolerance to water deficit, evaluating the length of the main root of the seedlings and the tissues remaining from the reserve material five days after sowing is recommended, both by the image analysis technique and by traditional methods. However, the image analysis technique requires less time and workforce.

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