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PHYSIOLOGICAL QUALITY IN SEEDS FROM HAPLOID INDUCERS IN MAIZE

Abstract – The objective of the present work was to evaluate the physiological quality in seeds of haploidy inducers of different types and origins. To this end, between December 2015 and March 2016, a series of physiological quality assessments were conducted on nine genotypes inducing haploidy in corn, namely: the gymnogenetic inducers Stock 6, TAIL P1 and TAIL P2; the hybrid between the TAIL P1 x TAIL P2 inductors; the hybrid TAIL P2 x TAIL P1, analogous to the previous hybrid, but with inversion of the female parent at the crossing; and androgenetic inducers W23, 90109 igig, 91202 igig and 91207 igig. The tests carried out were germination, accelerated aging, emergence in a bed, emergence index, fresh and dry mass, length of aerial part and root and the ratio between these characteristics, the humidity and the weight of 100 seeds. The results indicated that the physiological quality was improved throughout the selection for greater haploid induction. The haploidy induction system (andorgenetic or gymnogenetic) does not interfere with physiological quality, however the results obtained reinforce the need for care in the multiplication and conservation of haploidy inducing seeds in tropical conditions for their use in breeding.

Keywords: *Zea mays* L.; Haploids induction; Tropicalization; Germintion and vigor.

QUALIDADE FISIOLÓGICA EM SEMENTES DE INDUTORES DE HAPLOIDIA DE MILHO

Resumo - O objetivo do presente trabalho foi avaliar a qualidade fisiológica em sementes de indutores de haploidia de diferentes tipos e origens. Para tanto, entre dezembro de 2015 e março de 2016, fram conduzidas uma série de avaliações de qualidade fisiológica em nove genótipos indutores de haploidia em milho, a saber: os indutores gimnogenéticos Stock 6, TAIL P1 e TAIL P2; o híbrido entre os indutores TAIL P1 x TAIL P2; o híbrido TAIL P2 x TAIL P1, análogo ao híbrido anterior, mas com inversão do genitor feminino no cruzamento; e os indutores androgenéticos W23, 90109 igig, 91202 igig e 91207 igig. Os testes efetuados foram de germinação, envelhecimento acelerado, emergência em canteiro, índice de emergência, massa fresca e seca, comprimento de parte aérea e raiz e a razão entre essas características, a umidade e o peso de 100 sementes. Os resultados indicaram que a qualidade fisiológica foi aprimorada ao longo da seleção para maior indução de haplóides. O sistema de indução de haploidia (andorgenético ou gimnogenético) não interfere na qualidade fisiológica, porém os resultados obtidos reforçam a necessidade de cuidados na multiplicação e conservação das sementes de indutores de haploidia em condições tropicais para seu uso em melhoramento.

Palavras-chave: *Zea mays* L.; Indução de haploidia; tropicalização; germinação e vigor.

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The doubled haploid (DH) technology has been increasingly used in maize breeding programs because it allows inbred lines to be obtained in even three generations, combining reduced time and reduced cost in developing new cultivars (Ren et al., 2017; Trindade et al., 2017)

The doubled haploid technology is based on obtaining haploid maize plants, with the basic chromosome number known for species (n =10 for maize). Haploid plants have extremely reduced vigor and difficulties for reproduction due to the impossibility of normal meiosis in the reproductive cells, with frequent male sterility. Treatment of haploid seedlings with mitosis inhibitors promotes chromosome duplication, which results in fertile plants that are called doubled haploid lines, since for each chromosome that the haploid plant had previously, it comes to have an exact copy, which provides it with complete homozygosity and the same number of chromosomes as a diploid plant (Prigge & Melchinger, 2011).

To obtain haploids in maize, the protocol most used at this time is *in vivo*, which crosses source-genotypes with haploid inducers (Prasanna et al., 2012). Haploid inducers consist of genotypes that 1) have a haploid identification system based on phenotypic markers, the most used markers being based on seed pigmentation by anthocyanin (expression of the R1-navajo gene) and 2) when crossed with a source-genotype, induce the emergence of haploid seeds in the ear (Trindade et al., 2017).

All the genotypes currently used as haploid inducers are derived from Stock 6 lines, which induce the formation of gynogenic haploids (act as a male parent), and W23, which generates androgenic haploids (act as a female parent) (Hu et al., 2016). Thus, the haploid inducers now in use were developed in temperate climate locations, and their agronomic performance is hurt in tropical environments, requiring characterization of different phenological aspects of the plant for their efficient use under tropical conditions.

The physiological quality of a seed is constituted by the set of all the genetic, physiological, physical, and health parameters that affect its ability to give rise to high-yielding plants. The production of quality seeds is essential in breeding programs and for plant production as a whole, since seeds are responsible for initial establishment of the crop and they significantly contribute to maintaining a good final stand, resulting in satisfactory yields (Reis et al., 2011; Henning et al., 2011). The main characteristics desirable in seeds are longevity, germination power, and vigor (Andrade et al., 2001; Pereira et al., 2005).

Considering the presence of temperate climate germplasm in the genetic base of the haploid inducers now in use, it can be hypothesized that there is a difference in the physiological quality of seeds from haploid inducers in accordance with genetic characteristics, the origin and type of inducer, and its adaptability to a tropical environment. Thus, it becomes important to evaluate the physiological quality of seeds from these materials, with a view toward their

use and conservation within breeding programs that use the doubled haploid technology to obtain superior lines.

Considering the lack of information regarding germination potential in haploid inducers, the aim of this study was to evaluate the physiological quality in seeds from haploid inducers in maize of different types and origins.

MATERIALS AND METHODS

All the studies were conducted in the Seed Analysis Laboratory and in experimental areas of Embrapa Milho e Sorgo in the municipality of Sete Lagoas, Minas Gerais, from December 2015 to March 2016. Nine haploid-inducing genotypes for maize were evaluated, namely, the gynogenic inducers Stock 6, TAIL P1, and TAIL P2; the hybrid between the TAIL P1 × TAIL P2 inducers; the TAIL P2 × TAIL P1 hybrid, analogous to the previous hybrid, but with inversion of the female parent in the cross; and the androgenic inducers W23, 90109 igig, 91202 igig, and 91207 igig.

In relation to the origin of each inducer evaluated, Stock 6 (gynogenic inducer) and W23 (androgenic inducer) are inducer lines adapted to a temperate climate, with a difference only for the induction system, from which all the haploid inducers currently in use were derived (Hu et al., 2016). The TAIL (Tropically Adapted Inducer Lines) lines are haploid inducer lines adapted to tropical climate through backcrossing of the inducers UH400 and RWS with tropical maize lines (Kebede et al., 2011; Prasanna et al., 2012). The androgenic inducers 90109igig, 91202igig, and 91207igig are genotypes arising from the cross between the W23 line and tropical genotypes (Rabel et al., 2008).

All the seeds evaluated were obtained in a multiplication field set up at Embrapa Milho e Sorgo from January to June 2015, and seeds were obtained by manual crosses between plants from the same inducer. Approximately 15 viable ears were obtained from each inducer, resulting in quantities of seeds ranging from 500 to 2000 grams per inducer.

After harvesting the ears from each inducer, they were kept in a shed until the seeds reached 13% moisture, at which time they were threshed. After classification of the seeds, they were labeled and stored in kraft paper bags and kept in climate-controlled cold storage with temperature at around 10ºC and humidity around 15%, where they were stored for four months.

After that period, homogeneous samples of each haploid inducer were selected for evaluation of physiological quality based on the 11 laboratory and field tests described below.

I) Germination (GER): carried out with four replications of 50 seeds placed in rolls of germitest (germination testing) paper. The sheets of paper were previously moistened with water in the amount of 2.5 times the weight of the dry paper. After sowing, the rolls of paper were placed in a seed germinator at a temperature of 25˚C. The percentage of normal seedlings was calculated at six days after sowing according to

the description in the Rules for Seed Analysis (Regras para Análise de Sementes) (Brasil, 2009).

II) Abnormal and dead seedlings (ADS): The percentage of abnormal and dead seedlings was calculated in a single reading together with GER described above.

III) Accelerated aging (AA): test performed in a gerbox (plastic germination box), containing 40 mL of NaCl saturated solution. The seeds were distributed on an aluminum screen fastened in the closed gerbox and placed under the temperature of 42˚C in BOD for 96 hours. After that period, the germination test was set up with four replications of 20 seeds in rolls of paper; normal seedlings were counted at four days after sowing.

IV) Seedbed emergence (SE): for this test, seeds from each inducer were sown in furrows of 1 m length and 5 cm depth at a spacing of 10 cm in seedbeds. Four replications of 50 seeds were used, with a single count at 14 days after sowing.

V) Emergence Speed Index (ESI): evaluation was carried out by daily counting of emerged plants beginning on the fourth day after sowing up to 14 days. After that, this vigor index was determined through the sum of the number of emerged seedlings on each day divided by the number of days that had passed from sowing to emergence, according to the following formula:

$$ESI = E1/N1 + E2/N2 + ... En/Nn$$

where: ESI = emergence speed index; E1, E2,... En = number of normal seedlings counted in the first count, in the second count, and in the last count; and N1, N2,... Nn = number of days from sowing to the first, second, and last count.

VI) Seedling fresh matter weight (FM): total weight of normal seedlings from each replication, checked soon after the germination test in a roll of paper by weighing on a balance with precision of 0.001 g.

VII) Seedling dry matter weight (DM): total dry matter of the normal seedlings from each replication, which passed through the germination test in a roll of paper, after drying in a forced air laboratory oven at 65ºC. Weight was determined on a balance with precision of 0.001 g.

VIII) Shoot length (SL): mean of the length of the shoots of all the normal seedlings from each replication after the germination test, measured in centimeters with the aid of a graduated ruler.

IX) Root length (RL): mean of the length of the roots of all the normal seedlings from each replication after the germination test, measured in centimeters with the aid of a graduated ruler.

X) Root/shoot length ratio (RL:SL): ratio between the root length and shoot length of seedlings that passed through the germination test in a roll of paper.

XI) One hundred seed weight (100SW): four replications of 100 seeds were used (Brasil, 2009) in which the seeds were placed in a metallic

crucible which had previously been weighed on a balance with 0.001 g precision to determine weight. Then the samples were weighed and the weight of the sample was determined by the difference between the weight of the container and the total weight of the container with the sample.

XII) Seed moisture content (SM): four replications of 25 seeds were used, which were weighed on a balance with 0.001 g precision. After that, the seeds were placed under a temperature of 105ºC in a forced air circulation oven for 24 hours. After that period, the containers were placed in a seed desiccator for 30 minutes and then weighed once more on a precision balance. The moisture content of the seeds was determined by the difference between the weighings.

For analysis of the data obtained from the evaluations, a completely randomized design was considered, with four replications and treatments composed by the nine genotypes under evaluation. Analysis of variance was initially performed on th e data, and the degrees of freedom were decomposed for evaluation of the data through orthogonal contrasts, established based on the origin and type of system of haploid induction (gynogenesis or androgenesis). The following orthogonal contrasts were compared: i) Stock 6 versus gynogenic inducers; ii) W23 versus androgenic inducers; and iii) gynogenic inducers versus androgenic inducers.

After analysis of variance, the genotypes were compared to each other by the Scott-Knott cluster test. Finally, correlation analysis was performed among the parameters under study, and the direct and indirect effects on germination were evaluated through path analysis. All analyses were carried out with assistance of the SISVAR (Ferreira, 2010) and Genes (Cruz, 2013) statistical programs.

RESULTS AND DISCUSSION

All the parameters had values of the coefficient of variation (CV%) below 18% (Table 1), which was expected, considering that most of the analyses were conducted in the laboratory under controlled conditions, but this denotes the reliability of the evaluations made. There were significant values of genotype for all the parameters evaluated, except for DM (Table 1), indicating that the genotypes evaluated are different from each other regarding the physiological quality of their seeds.

Decomposition of the effects of genotype by type of inducer (Table 1) shows that for the gynogenic inducers, there were significant differences for ADS, AA, FM, SL, RL, 100SW, and SM, which are characteristics linked to seed germination, vigor, and quality. The androgenic inducers, in turn, exhibited differences for SE, ESI, FM, RL, 100SW, and SM.

In relation to the orthogonal contrasts among types of inducers regarding physiological quality (Table 1), significant differences were observed between gynogenic inducers and androgenic inducers only for shoot length (SL) and 100 seed weight (100SW). The fact that

Table 1 – Estimate of mean squares, general mean and contrasts, upper (UL) and lower (LL) limits and genotypic determination coefficient (H^2) for 12 traits evaluated in seeds of nine haploidy inducers.

Sources of variation	DF	GER	ADS	AA	SE	ESI	FM
Genotype	8	253.94**	3.97**	614.00**	249.03**	22.01**	13.79**
GI	4	227.30	4.72**	651.70**	66.30	15.66	18.25**
AI	3	308.67	3.36	495.33	575.22**	36.19**	11.12*
GI vs AI	1	196.35	2.87	819.20	1.33	4.92	4.02
Stock 6 vs GI	1	819.20**	17.71**	768.80**	231.20**	53.48**	12.73**
W23 vs AI	1	432.00**	4.57**	901.33**	1333.52**	97.64**	21.65**
Error	27	39.67	0.45	66.78	31.44	2.41	1.10
General Mean		78.61	21.33	71.17	88.03	26.42	6.16
Stock 6 mean		93.5	6.0	54.5	95.0	30.0	4.9
W23 mean		67.0	33.0	63.5	72.0	21.8	3.8
GI mean		80.7	19.2	66.9	88.2	26.8	6.5
AI mean		76.0	24.0	76.5	85.8	26.0	5.8
UL		98.00	46.00	94.00	100.00	31.90	11.00
LL		54.00	2.00	28.00	64.00	19.20	3.20
CV%		8.01	13.45	11.48	6.37	5.87	17.02
H^2 (%)		82.54	90.47	89.75	52.59	84.63	93.97
Sources of variation	**DF**	**DM**	**SL**	**RL**	**RL:SL**	**100SW**	**SM**
Genotype	8	0.06	5.85**	45.29**	0.05	79.98**	3.90**
GI	4	0.07	8.14**	67.37**	0.05	38.67**	3.32*
AI	3	0.07	1.65	29.51**	0.06	19.84**	5.65**
GI vs AI	1	0.03	9.28**	4.30	0.04	425.67**	0.98
Stock 6 vs GI	1	0.02	14.62**	152.68**	0.10**	0.11	2.02*
W23 vs AI	1	0.13**	0.00	66.22**	0.17**	25.33**	12.85**
Error	27	0.01	0.44	2.78	0.01	0.29	0.44
General Mean		0.255	7.33	14.04	1.98	17.83	11.72
Stock 6 mean		0.21	5.2	8.2	1.66	21.0	12.5
W23 mean		0.07	7.9	10.9	1.42	11.8	10.0
GI mean		0.3	6.9	13.7	2.1	20.9	11.9
AI mean		0.2	7.9	14.4	1.9	14.0	11.5
UL		0.7	10.50	21.50	4.00	25.10	13.10
LL		0.03	4.80	7.30	1.20	10.90	8.30
CV%		45.95	9.14	11.88	6.11	3.01	5.68
H^2 (%)		80.86	94.48	95.87	75.07	99.25	86.67

*,** significant at the 5 and 1% levels of probability by the F test. DF = Degrees of freedom. GER= germination percentage; ADS= percentage of abnormal and dead seedlings; AA = germination percentage after accelerated aging; SE = Seedbed emergence; ESI = emergence speed index; FM = Seedling fresh matter weight; DM = seedling dry matter weight; SL = shoot length; RL = root length; RL:SL = root/shoot length ratio; 100SW = One hundred seed weight; SM = seed moisture content. GI = gymnogenetic inducers; AI = Androgenetic inducers; CV% = coefficient of variation.

there were differences among inducers within each group but there were no differences between the groups is an indication that the variations regarding seed physiological quality in haploid inducers is determined not according to the haploid induction system but rather according to the intrinsic characteristics of the genotype in question. Physiological quality is a determining factor for germination under adverse conditions and can vary according to management practices in production, harvest, and storage. Physiological quality should be evaluated in a detailed manner, considering the variations among cultivars and the conditions of obtaining the seed (Pereira et al., 2005; Carvalho et al., 2010).

The contrasts of Stock 6 versus gynogenic inducers and W23 versus androgenic inducers showed significant differences for all the characteristics evaluated, except for DM and 100SW in the contrast between Stock 6 and gynogenic inducers, and for SL in the contrast between W23 versus androgenic inducers (Table 1). The breeding process in haploid inducers requires both increase in the haploid induction rate and improvement in the agronomic performance of the inducers in relation to the parents (Prasanna et al., 2012; Hu et al., 2016), which certainly contributed to gains from selection in seed physiological quality, resulting in variations for the characteristics under study. In the Stock 6 versus gynogenic inducer contrast, the Stock 6 inducer had significantly higher mean values for GER, SE, ESI, and SM, whereas the other gynogenic inducers had greater ADS, AA, FM, SL, RL, and RL:SL. The W23 inducer, in turn, had mean values that were significantly lower than all the inducers derived from it in all the characteristics evaluated, except for SL.

In addition to that observed above, the coefficients of genotypic determination (H^2) estimated for the characteristics under study ranged from 52.59 to 99.25, denoting that the values observed are due to the genotypic variability inherent to the haploid inducers under evaluation, and that there is potential for increasing these values through selection.

Application of the Scott-Knott cluster test regarding germination for the inducers evaluated resulted in three groups – one group formed by the Stock 6 inducer, which had GER of 93.5%, followed by the TAILP1 × TAILP2, 90109igig, and 91202igig inducers (Table 2). The other inducers had GER below 80%, and the W23 inducer had the lowest germination percentage (67%). It should be noted that regulations (Instrução Normativa n° 45) of the Brazilian Ministry of Agriculture (Brasil, 2013) indicate that commercial maize seeds should have at least 75% germination. In an analogous manner, the ADS was greater for the genotypes TAILP1, TAILP2, TAILP2 × TAILP1, W23, and 91207igig, which showed lower germination (Table 2).

The accelerated aging test resulted in variations between 86.5% and 75.0% in the group with greatest AA, with the 90109igig inducer standing out (Table 2). The SE parameter ranged from 97.0% to 85.0%, with the genotype

Table 2 – Means of 12 traits associated to the physiological quality of seeds of nine haploidy inducers in maize.

Inducers	Traits											
	GER (%)		ADS (%)		AA (%)		SE (%)		ESI		FM (g)	
Stock6	93.5	a	6.0	c	54.5	b	95.0	a	30.0	a	4,9	c
TAILP1	78.5	c	21.5	a	75.0	a	86.0	a	25.4	b	5.6	c
TAILP2	77.0	c	23.0	a	51.5	b	88.5	a	27.0	a	4.9	c
TAILP1xTAILP2	80.5	b	19.5	b	77.5	a	87.0	a	26.2	b	6.9	b
TAILP2xTAILP1	74.0	c	26.0	a	76.0	a	84.5	a	25.1	b	10.0	a
W23	67.0	c	33.0	a	63.5	b	72.0	b	21.8	c	3.8	c
90109igig	82.5	b	18.0	b	86.5	a	89.0	a	28.0	a	7.4	b
91202igig	84.5	b	15.0	b	85.0	a	97.0	a	28.3	a	6.9	b
91207igig	70.0	c	30.0	a	71.0	a	85.0	a	26.0	b	5.1	c
Inducers	Características											
	DM (g)		SL (cm)		RL (cm)		RL:SL		100SW (g)		SM (%)	
Stock6	0.21	c	5.2	d	8.2	d	1.66	b	21.0	c	12.5	a
TAILP1	0.20	c	6.7	c	12.2	c	1.81	b	22.6	b	11.4	b
TAILP2	0.19	c	6.9	c	13.8	b	2.07	a	17.1	e	11.9	b
TAILP1xTAILP2	0.30	b	6.5	c	15.0	b	2.26	a	25.0	a	10.7	c
TAILP2xTAILP1	0.51	a	9.1	a	19.5	a	2.55	a	18.7	d	13.0	a
W23	0.07	c	7.9	b	10.9	c	1.42	b	11.8	g	10.0	c
90109igig	0.33	b	8.8	a	17.5	a	2.05	a	15.6	f	11.7	b
91202igig	0.33	b	7.3	c	14.7	b	2.09	a	16.2	f	12.9	a
91207igig	0.17	c	7.6	b	14.5	b	1.96	a	12.3	g	11.6	b

Means with the same letter belong to the same group according to the Scott-Knott test at 5%. GER= germination percentage; ADS= percentage of abnormal and dead seedlings; AA = germination percentage after accelerated aging; SE = Seedbed emergence; ESI = emergence speed index; FM = Seedling fresh matter weight; DM = seedling dry matter weight; SL = shoot length; RL = root length; RL:SL = root/shoot length ratio; 100SW = One hundred seed weight; SM = seed moisture content.

91202igig standing out, and with the W23 inducer showing the lowest SE (72.0%). Application of the Scott-Knott test to the ESI, which indicates the speed at which the seedling emerges after sowing, led to formation of three groups, with the inducers Stock6, TAILP2, 90109igig, and 91202igig standing out. The AA, SE, and ESI are characteristics that indicate seed response to stresses and allow estimation of the physiological potential of the seeds under a broad range of environmental conditions (Bittencourt et al., 2012).

The FM and DM had an analogous response, in which the TAILP2 × TAILP1 inducer had the highest value of FM and DM (10.0 and 0.51 g, respectively), forming a single group, and the W23 inducer had the lowest value for FM and DM (3.8 and 0.07 g, respectively).

The TAILP2 × TAILP1 and 90109igig inducers expressed the highest values of SL (9.1 and 8.8 cm, respectively) and RL (19.5 and 17.5 cm, respectively). Consequently, the highest RL:SL ratio was obtained by the genotype TAILP2 × TAILP1 (2.55). The fresh and dry matter of the seedling and rapid growth of the root system are indications of the potential of the genotype for an "initial take-off", which is the rapid emergence and establishment of the seedling, and which allows the inducer to better tolerate biotic and abiotic stresses in the initial stages of development (Jorge et al., 2005; Carvalho et al., 2010). Thus, the TAILP2 × TAILP1 inducer shows considerable potential for establishment in the field, which is a favorable characteristic in isolated lots for haploid induction.

The TAILP1 × TAILP2 inducer had the highest value of 100SW (Table 2), followed by the TAIL P1 inducer. Considering that the female parent of the TAILP1 × TAILP2 inducer hybrid is the TAIL P1 inducer line, this 100SW value denotes that there is a relationship among the genotypes for this characteristic. According to Gaspar & Nakagawa (2002), seed germination and vigor are affected by size – larger seeds had better germination and vigor results than smaller ones. Vanzolini & Nakagawa (2007) evaluated seed vigor in popcorn seedlings and reported in studies that smaller seeds germinated rapidly; however, larger seeds gave rise to seedlings of greater size and weight.

The moisture content of the seeds ranged from 10% to 13% among the inducers evaluated, with the highest value of SM for the TAILP2 × TAILP1 inducer hybrid, with differences of 4% between the most extreme values of SM. Zucareli et al. (2014) studied the physiological quality of sweet corn seeds and reported that the moisture content after the accelerated aging test varied significantly among the genotypes evaluated. However, the differences were not lower than 1%. The same authors measured mean moisture values from 28.7% to 34.11% in the genotypes studied, which did not interfere in the seed physiological processes. However, under low moisture contents, seed metabolic activity is reduced (Borges & Rena, 1993; Castro & Hilhorst, 2004), and moisture content in seeds is an important factor for their storage, viability, and germination.

Correlation analyses indicated high, positive, and significant correlations between GER and the SE and ESI characteristics (Table 3), denoting that the germination potential evaluated in the laboratory reliably corresponds to what occurs under field conditions. In the latter case, selection based on germination would have a considerable effect on those two characteristics, which are parameters for determination of seed vigor.

However, GER had correlations ranging from -0.999 to 0.249 with ADS, FM, DM, SL, RL, and RL:SL. The high negative correlation of GER with ADS is related to the nature of the latter characteristic, which represents the seeds that did not germinate or that resulted in plants without viability, representing exactly

Table 3 – Phenotipic correlations between 12 traits associated to phisyologic quality of seeds in nine haploid inducers in maize.

Traits	Traits											
	GER	ADS	AA	SE	ESI	FM	DM	SL	RL	RL:SL	100SW	SM
GER	1.000											
ADS	-0.999*	1.000										
AA	0.006	0.006	1.000									
SE	0.851**	-0.853**	0.149	1.000								
ESI	0.891**	-0.888**	0.007	0.946	1.000							
FM	0.107	-0.098	0.621	0.255	0.135	1.000						
DM	0.249	-0.242	0.559	0.395	0.279	0.985**	1.000					
SL	-0.584	0.597	0.528	-0.415	-0.455	0.554	0.443	1.000				
RL	-0.278	0.293	0.643	0.007	-0.090	0.841**	0.777*	0.803**	1.000			
RL:SL	0.032	-0.025	0.439	0.323	0.193	0.879**	0.879**	0.404	0.850**	1.000		
100SW	0.542	-0.539	0.036	0.378	0.326	0.273	0.315	-0.520	-0.076	0.314	1.000	
SM	0.407	-0.396	-0.185	0.245	0.476	-0.527	-0.464	-0.244	-0.428	-0.587	-0.318	1.000

GER= germination percentage; ADS= percentage of abnormal and dead seedlings; AA = germination percentage after accelerated aging; SE = Seedbed emergence; ESI = emergence speed index; FM = Seedling fresh matter weight; DM = seedling dry matter weight; SL = shoot length; RL = root length; RL:SL = root/shoot length ratio; 100SW = One hundred seed weight; SM = seed moisture content. *,** significant at the 5 and 1% levels of probability by the t test.

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the opposite of germination. In contrast, the low magnitude correlations between GER and characteristics that show the initial development of the seedling, such as FM, SL, RL, and RL:SL, would indicate that larger sized plants would have more delayed germination compared to seedlings of lower initial size, a characteristic called the initial take-off of the plants. The other characteristics evaluated (AA, DM, 100SW, and SM) had low magnitude correlations with germination, ranging from -0.47 to 0.27. Considering the group of genotypes evaluated, the correlation results obtained indicate little effect of important characteristics, such as moisture content, fresh matter weight, and 100 seed weight (indicative of seed dimensions and, consequently, seed reserves) with germination, which would be the main characteristic of the seed. Thus, the correlations indicate the relationships among the characteristics, but do not express the direct and indirect effects that the characteristics studied have on the germination of seeds from the haploid inducers. In this context, path analysis is an alternative for evaluation of the direct and indirect effects of a set of characteristics on a main variable (Cruz et al., 2014), serving for an understanding of the interrelations among the traits under study. The path analysis methodology (Wright, 1923) is a modification of multiple regression in which the phenotypic correlations among characteristics are decomposed into direct and indirect effects on a main variable, allowing understanding of the relationship between the traits and reliable selection of parameters for indirect selection. Measurement of direct and indirect effects is dependent on the set of traits studied, which is normally established by previous knowledge of the researcher and of possible knowledge of interrelations expressed in path diagrams (Rios et al., 2012).

The direct effects of the characteristics on germination are gathered on the diagonal in bold print in Table 4. Assuming the possibility of collinearity among the characteristics, since they are highly correlated, and the linear relationship among the variables, we chose to use the collinearity diagnosis and the methodology proposed by Carvalho (1994), ridge path analysis, for estimation of the parameters.

Considering the direct effects of all the characteristics evaluated on GER, the estimates of ADS indicate a direct negative effect, but of lower magnitude, on germination (-0.504 - Table 4) with highly negative correlation, and that this characteristic negatively interferes in the effect of the others. In contrast, the SE and ESI characteristics, which had high direct correlation with germination, show that their direct effect was not so high and was dependent on other characteristics, including ADS. The same occurs for characteristics related to plant vigor and physiological quality, such as SL, 100SW, and SM (Table 4). The importance of the FM and DM characteristic is also noteworthy. The FM indirectly and positively contributed to the DM, SL, RL, and RL:SL characteristics, indicating that the fresh weight of the plant is an important

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Table 4 – Estimates of direct (diagonal in bold) and indirect (no bold) effects of eleven traits on seed germination of haploid inducers in maize obtained via path analysis under multicollinearity.

TRAITS	ADS	AA	SE	ESI	FM	DM	SL	RL	RL:SL	100SW	SM
ADS	**-0.504**	-0.003	0.430	0.448	0.097	0.240	-0.592	-0.291	0.025	0.534	0.393
AA	0.001	**0.014**	0.002	0.001	-0.012	-0.011	-0.010	-0.013	-0.009	-0.001	0.004
SE	-0.079	0.014	**0.093**	0.088	0.002	0.003	-0.003	0.001	0.002	0.003	0.002
ESI	-0.116	0.001	0.124	**0.131**	-0.011	-0.023	0.038	0.008	-0.016	-0.027	-0.040
FM	-0.011	0.068	0.028	0.015	**0.298**	0.293	0.165	0.250	0.262	0.081	-0.157
DM	-0.048	0.111	0.078	0.055	-0.183	**-0.186**	-0,082	-0.144	-0.164	-0.059	0.086
SL	-0.049	-0.043	0.034	0.037	-0.079	-0.063	**-0.143**	-0.114	-0.058	0.074	0.035
RL	-0.037	-0.081	-0.001	0.011	0.063	0.058	0.060	**0.075**	0.064	-0.006	-0.032
RL:SL	-0.003	-0.049	-0.036	-0.022	-0.006	-0.006	-0.003	-0.006	**-0.007**	-0.002	0.004
100SW	-0.070	0.005	0.049	0.042	-0.006	-0.007	0.012	0.002	-0.007	**-0.023**	0.007
SM	-0.066	-0.031	0.046	0.079	-0.055	-0.049	-0.026	-0.045	-0.061	-0.033	**0.105**
TOTAL	**-0.999****	**0.006**	**0.851****	**0.891****	**0.107**	**0.249**	**-0.584**	**-0.278**	**0.032**	**0.542**	**0.407**
DETERMINATION COEFFICIENT											**0.97719**
k VALUE USED IN ANALYSIS											**4.49438^{e-02}**
EFFECT OF THE RESIDUAL VARIABLE											**0.15101**
DETERMINANT OF THE CORRELATION MATRIX BETWEEN EXPLANATORY VARIABLES											**4.73365^{e-07}**

GER= germination percentage; ADS= percentage of abnormal and dead seedlings; AA = germination percentage after accelerated aging; SE = Seedbed emergence; ESI = emergence speed index; FM = Seedling fresh matter weight; DM = seedling dry matter weight; SL = shoot length; RL = root length; RL:SL = root/shoot length ratio; 100SW = One hundred seed weight; SM = seed moisture content. *,** significant at the 5 and 1% levels of probability by the t test.

parameter for evaluation and that it is related, even if indirectly, to other characteristics, and may even replace them in some situations.

Physiological quality is a fundamental parameter for indicating the success of a sowing and the potential for storage of a seed (Scheeren et al., 2010; Espíndola et al., 2018); the greater the physiological quality, the greater the tolerance of the seed to storage. The results obtained in the present study regarding the haploid inducers evaluated reinforce the temperate origin of these genotypes and the need for care in multiplication and conservation of their seeds under tropical conditions for their use in research and breeding. It should also be emphasized that the data obtained indicate the need for new introgression of tropical genetics in the inducers evaluated in this study, through backcrosses, in order to improve the adaptation of these genotypes to tropical conditions. This process will result in improvement in agronomic performance, in inductive potential, and in the physiological quality of the seeds from the haploid inducers.

CONCLUSIONS

The physiological quality of the seeds from the gynogenic and androgenic inducers evaluated was superior to the Stock 6 and W23 inducers, from which the other inducers were derived, indicating advancement in this parameter together with selection for greater induction of haploid plants.

The physiological quality of seeds in haploid inducers did not vary according to the induction system (androgenic or gynogenic) but rather according to the genetic characteristics and the conditions of obtaining and storing the seeds.

The results obtained in the present study reinforce the need for care in the multiplication and conservation of the seeds from haploid inducers under tropical conditions for their use in research and breeding, due to their temperate climate origin.

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