

Seed reserves reduction rate and reserves mobilization to the seedling explain the vigour of maize seeds¹

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ABSTRACT – Understanding how the seed reserve dynamics occurs during germination and seedling formation is determinant for advancements on seed technology. The aims of this study were: to verify which accelerated ageing temperature is the most effective to separate the vigour levels of maize seeds and to evaluate the reserves dynamics during germination and seedling formation process. Seven maize cultivars were submitted to the germination rate, accelerated ageing, thousand seed weight, total seedling length, shoot and root length, dry matter of seed and seedling, remaining dry matter in the endosperm, seed reserves reduction rate, conversion efficiency of reserves, reserves mobilization rate to the seedling and energy expenditure using the completely randomized statistical design. The reserves dynamics and seedling formation depends on the genotype and the initial seed vigour. Accelerated ageing at 45 °C for 72 hours is the most efficient combination to segregate vigour levels. Genotypes with higher seed reserve utilisation efficiency have higher vigour, producing seedlings with higher dry matter, higher total, shoot and root length, regardless of seed weight. The two rates evaluated prompt us to conclude that they explain the maize seed vigour and can be used in quality control programs to select high physiological quality cultivars.

Index terms: *Zea mays* L., seed reserves utilization, germination, physiological quality.

Taxa de redução de reservas da semente e mobilização de reservas para a plântula explicam o vigor de sementes de milho

RESUMO – Compreender como a dinâmica de reserva ocorre durante a germinação e formação de plântulas é determinante para avanços na tecnologia de sementes. Objetivou-se com este trabalho verificar qual temperatura de envelhecimento acelerado é mais eficaz para separar níveis de vigor de sementes de milho e avaliar a dinâmica de reservas durante o processo de germinação e formação de plântulas. Sete cultivares de milho foram submetidas ao teste de germinação, envelhecimento acelerado, peso de mil sementes, comprimento total de plântulas, parte aérea e raiz, massa seca de sementes, plântulas e remanescente no endosperma, taxa de redução de reservas, eficiência de conversão, taxa de mobilização de reservas para a plântula e gasto energético utilizando delineamento inteiramente casualizado. A dinâmica das reservas e formação de plântulas dependem do genótipo e vigor inicial das sementes. O envelhecimento acelerado 45 °C por 72 horas é a combinação mais eficaz para segregar níveis de vigor. Genótipos com maior eficiência de utilização de reservas têm maior vigor, produzindo plântulas com maior massa seca, comprimento total, de parte aérea e raiz, independente do peso da semente. As taxas avaliadas explicam o vigor de sementes de milho e podem ser usadas em programas de controle de qualidade para selecionar cultivares com alta qualidade fisiológica.

Termos para indexação: *Zea mays* L., utilização de reservas das sementes, germinação, qualidade fisiológica.

Introduction

Seeds when taken to the field are subject to adverse environmental conditions, making it essential to use seeds

with high physiological quality, which is directly related to the potential of germination and vigour and affected by the genotype (Oliveira et al., 2013; Nerling et al., 2013; Prazeres et al., 2016a). When seeds of different genotypes are

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submitted to ideal conditions of temperature and humidity, the germination potential can be expressed, with the normal seedlings formation. However, when these same cultivars are exposed under stressful conditions, the normal seedlings formation can be impaired according to the differences in the vigour levels of the cultivars (Marcos-Filho, 2015; Finch-Savage; Bassel, 2015).

Researches on maize in recent years are usually specific for the relationship between physiological quality and the factors that affect it such as storage components (Nerling et al., 2018; Abreu et al., 2016; Prazeres; Coelho, 2016a), enzymes (Oliveira et al., 2015; Santos et al., 2016; Silva-Neta et al., 2015; Lopes et al., 2017; Diniz et al., 2018), heterosis (Nerling et al., 2013; Oliveira et al., 2015; Prazeres; Coelho, 2016a; Prazeres; Coelho, 2016b; Abreu et al., 2018) and image analysis (Pinto et al., 2015; Dias et al., 2015; Castan et al., 2018; Medeiros et al., 2018).

However, no research has been found evaluating how the process of dynamic reserves and seedling formation occurs and how this may explain the seed vigour. Researches of this nature may contribute to the understanding of the seedling formation process from seeds with different levels of initial vigour, considering that higher vigour seeds are expected to produce more vigorous seedlings (Egli; Rucker, 2012). Thus, the characteristics of use of seed reserves, such as the reduction of seed reserves and the mobilization for formation of new seedling tissues are important parameters of vigour evaluation (Soltani et al., 2006; Cheng et al., 2015). The studies found in the literature generally evaluate seed vigour or seedling vigour separately and in that sense, have been reported in chickpea, wheat, rice, soybean and sweet corn (Soltani et al., 2002; Soltani et al., 2006; Mohammadi et al., 2011; Cheng et al., 2013; Cheng et al., 2015; Pereira et al., 2015; Cheng et al., 2018).

Soltani et al. (2006) described that seedling growth could be measured by the weight of mobilised seed reserves (in mg.seedlings^{-1}) and conversion efficiency of mobilised seed reserve to seedling tissue (mg.mg^{-1}). Based on this principle, the study of the dynamics of seed reserves for seedling formation could be studied as an alternative method of seed vigour determination. Pereira et al. (2015), in a study about the dynamics of seed reserves of soybean cultivars, concluded that there is a correlation between dry matter of seeds, seed reserves reduction and dry matter of seedling. For the results of reduction of seed reserves, conversion efficiency of reserves and dry matter of seedling, the same authors found a negative correlation between the variables.

The selection of cultivars with higher vigour is essential in the management practices to obtain crops with high

productivity, due to the higher capacity to overcome the adverse conditions in field conditions (Marcos-Filho, 2015; Finch-Savage; Bassel, 2015) and due to stand uniformity (Egli; Rucker, 2012; Fromme et al., 2019). Also, the understanding of the mechanisms of how the dynamics of the reserve components during germination and seedling formation is determinant for the contribution to the advancements of seed technology (Soltani et al., 2006; Egli; Rucker, 2012).

In this work we propose the understanding of the two concepts in an associated way through the evaluation of seedling performance parameters to help in the understanding of the behaviour of different genotypes and with that, to help the internal quality control programs. The hypotheses tested here were: (I) there is a difference in tolerance of hybrid maize genotypes to accelerated ageing temperatures; (II) the ability of the embryo to remove nutrients from reserve tissues and transform them into seedlings is determined by the initial seed vigour. Therefore, the objectives of this study were to verify which accelerated ageing temperature is the most effective in the separation of the vigour levels of simple hybrids and to evaluate the dynamics of the reserves during germination and the seedling formation process in maize seeds with different vigour levels.

Material and Methods

All the experiment was developed at the Laboratory of Seed Analysis of the *Universidade do Estado de Santa Catarina* (UDESC). Seven cultivars of simple hybrid maize from the 2016/2017 crop were harvested, processed and stored in a controlled chamber (45 ± 5 % RH and 10 ± 2 °C) until the beginning of the analyses. The mean samples of 1.000 g of each cultivar were homogenised and divided to obtain 4 replications of 250 g using a sample divider (Coelho et al., 2010).

To determine the germination rate (GR), we used eight replications of fifty seeds, which were distributed in a germitest paper roll, moistened with distilled water 2.5 times the dry paper weight, according to the Rules for Seed Testing (Brasil, 2009). The rolls were packed in plastic bags, carried to the Mangelsdorf germinator vertically and kept at 25 ± 2 °C for seven days. The percentage of germination is the average result of the number of normal seedlings (Brasil, 2009).

To determine the vigour by accelerated ageing test (AA), seeds were distributed in a single layer on an aluminium screen, which were placed in boxes of polystyrene crystals (gerbox) containing 40 mL of distilled water (Marcos-Filho, 1999). The boxes were closed and placed in accelerated ageing chamber at 43 °C for 72 hours (AOSA, 1983) and at 45 °C for 72 hours (Bittencourt; Vieira, 2006). After this period, the seeds were submitted to the germination test, as previously described

(Brasil, 2009). The results were expressed in percentage.

Seed moisture degree (MD) was determined using the oven method at 105 ± 3 °C, transferring a 4.5 ± 0.5 grams of seeds to aluminium capsules using two replications. After 24 hours, the capsules were weighed and the mean moisture percentage was obtained (Brasil, 2009). The thousand seed weight (TSW) was performed using eight replications of one hundred seeds. Each replication was weighed and the mean was multiplied by ten, with the final result expressed in grams as indicated in the Rules for Seed Testing (Brasil, 2009).

To determine the dry matter of seed (DMS), three replications of twenty seeds were weighed and the results were multiplied by a thousand and subtracted by the moisture degree of each sample, expressed in mg seed⁻¹ (Soltani et al., 2006; Pereira et al., 2015). The same twenty seed samples obtained to determine the dry weight of seeds for each hybrid were used to total seedling length (TSL), root length (RL) and shoot length (SL), distributed on a line drawn in the upper third of the germitest paper moistened with distilled water in the ratio 2.5 times the weight of the dry paper. However, the TSL, RL and SL were measured in ten normal seedlings per replicate, taken at random, with a digital calliper in millimetres. The evaluations were performed 120 hours (five days) after the beginning of the test. The mean TSL, RL and SL were divided by the number of normal seedlings (ten), with results expressed in mm seedling⁻¹ (Nakagawa, 1999).

To determine the dry matter of seedling (DMSL), the ten seedlings obtained from the seedling length test described above were used. For this, the embryos (embryonic axis + scutellum) were separated from the endosperms, weighed and dried in an oven at 80 °C for 24 hours. The samples were weighed again and the results in mg were divided by the number of normal seedlings obtained in the rolls (ten) and the dry matter of seedlings was expressed in mg seedling⁻¹, according to Nakagawa (1999) by the following expression:

$$DMSL = \frac{(\text{Initial weight of the embryo} - \text{Final weight of the embryo})}{10} \times 1000$$

As described previously, the endosperms were separated from the embryos, weighed and dried in an oven at 80 °C for 24 hours to determine the remaining dry matter in the endosperm (RDME) after 120 hours. The samples were weighed again and the results in mg were divided by the number of normal seedlings obtained in the rolls and the dry matter remaining in the endosperm was expressed in mg endosperm⁻¹, according to Nakagawa (1999).

$$RDME = \frac{(\text{Initial weight of the endosp} - \text{Final weight of the endosp})}{10} \times 1000$$

To determine the reduction of seed reserves (RSR), we subtracted the dry matter of seed (mg.seed⁻¹) by the remaining dry matter in the endosperms (mg.endosperm⁻¹), as a following expression. The results were expressed in mg.mg⁻¹ (Soltani et al., 2006; Pereira et al., 2015).

$$RSR = DMS - RDME$$

To determine the conversion efficiency of seed reserves (CESR), we divided the dry matter of the seedlings by the reduction of seed reserves to determine how much of dry matter the seed had at the beginning of the test and how much of that mass was converted to dry matter of seedling, according to Soltani et al. (2006) and Pereira et al. (2015). The results were expressed in mg.mg⁻¹.

$$CESR = \frac{DMSL}{RSR} \quad \text{or} \quad CESR = \frac{DMSL}{(DMS - RDME)}$$

Seed reserves reduction rate (SRRR) was also determined. This relation allows the identification of the cultivars that mobilised the most dry mass during the period of 120 hours (five days), evaluating the real reduction of reserves, being a more reliable and comparable variable, since it is not influenced by external factors such as the dry mass of the seed. The rate was calculated by the following expression, according to Soltani et al. (2006) and Pereira et al. (2015):

$$SRRR = \frac{RSR}{DMS} \times 100 \quad \text{or} \quad SRRR = \frac{(DMS - RDME)}{DMS} \times 100$$

To calculate the reserves mobilization rate to the seedling (RMRS), we divided the dry matter of seedling by the dry matter of seed. The results were expressed in percentage.

$$RMRS = \frac{DMSL}{DMS} \times 100$$

To determine energy expenditure (EE) after 120 hours of germination (five days), we subtracted the values of remaining dry matter in the endosperm (RDME) and dry matter of seedling (DMSL) from dry matter of seed (DMS). Energy expenditure obtained in mg were transformed as a percentage.

$$EE = \frac{(DMS - RDME - DMSL)}{DMS} \times 100$$

Data were analysed in software R (R Core Team, 2019) using scripts developed by the research group itself. The normality of the data was tested by the Shapiro-Wilk test and the homogeneity of the variances was tested by the Levene test before analysis of variances. The test of means comparisons used was Scott-Knott (1974) at 5% probability. The percentage data (GR and AA) were transformed using $\sqrt{x}/100$ to meet the theoretical assumptions of the F test

(normal distribution of error and homogeneity of variances), although the results were presented in the original scale (%). Pearson correlations were obtained amongst the evaluated characteristics at 1% and at 5% probability. Multivariate analyses of principal component analysis and hierarchical cluster analysis were used to better visualise the discrimination of the high and low vigour groups.

Results and Discussion

The results of Table 1 and 2 showed differences among the hybrids by the Scott-Knott test at 5% probability for almost all the response variables, except for the conversion efficiency of seed reserves (CESR). For the moisture degree (MD), values from 11% to 13% were observed, indicating that all hybrids presented similar conditions of initial moisture for the experiment. In spite of the difference between the moisture levels of the cultivars, this was not considered compromising the results of the tests, since it is recommended that the difference between the cultivars does not exceed 2% to avoid increasing the deterioration intensity and to reduce the chemical, physiological and sanitary changes in seeds (Marcos-Filho, 1999; Barrozo et al., 2014; Dias et al., 2015). The moisture degree determination was used only as a method to control the initial conditions of the seeds used in the experiment.

Apart from that, all hybrids presented germination rate (GR) higher than 90% (from 94% to 99%), that is, despite the existence of a statistically significant difference between them, all presented a high germination potential (Table 1). This potential is important in establishing crops because it determines the plant stand and the final productive potential of the crop until the seedling ceases

to depend on seed reserves and becomes autotrophic (Bewley et al., 2013; Finch-Savage; Bassel, 2015).

It was observed with our results that there were differences in the physiological potential (germination and vigour) among the hybrid maize genotypes used in the experiment (Table 1). In study carried out by Nerling et al. (2013) with crosses among maize varieties, it was observed that there was genotype effect on germination and seed vigour, where these authors verified the importance of analysing the physiological quality to define the potential of the genotype and its crosses due to the presence of heterosis.

When the seeds were submitted to stress by accelerated ageing, it was observed that at 43 °C (AA43) was possible to separate the hybrids in two levels of vigour by the Scott-Knott test ($p < 0.05$), with values ranging from 81% to 96% (Table 1). Four of the seven hybrids evaluated showed higher vigour (32R48VYHR, DKB230PRO3, 30R50VYH and P2866H), while the other three hybrids presented lower vigour (30F53VYH, P1630H and 30F53VYHR).

However, for studies on the understanding of the expression of seed vigour, it is important to use cultivars that are contrasting in this characteristic, but which present high germination potential (Dias et al., 2015). Thus, it was necessary to use another stress condition in order to establish greater contrasts amongst the hybrids and to allow the selection of the most distinct cultivars possible. In the evaluation of the physiological quality of the seeds, it is important to use vigour determination methods capable of identifying the differences between the individuals, especially when the germination power between them is similar (Marcos-Filho, 1994).

When the seeds were submitted to stress by accelerated

Table 1. Averages of moisture degree (MD), germination rate (GR), accelerated ageing test at 43 °C (AA43), accelerated ageing test at 45 °C (AA45), thousand seeds weight (TSW), total seedling length (TSL), shoot length (SL) and root length (RL) of seven maize hybrid.

Hybrids	MD	GR	AA43	AA45	TSW	TSL	SL	RL
	%	%	%	%	g	mm.seedling ⁻¹	mm.seedling ⁻¹	mm.seedling ⁻¹
32R48VYHR	13	99 a ¹	96 a	76 b	293.0 c	244.2 b	76.5 a	167.7 b
30F53VYH	13	98 a	81 b	24 d	274.3 d	205.9 b	58.2 b	147.8 b
DKB230PRO3	11	97 a	95 a	93 a	259.3 e	230.7 b	61.8 b	168.9 b
30R50VYH	12	97 a	94 a	55 c	333.1 a	228.0 b	58.3 b	169.7 b
P1630H	13	97 a	88 b	40 d	272.7 d	226.3 b	61.6 b	164.7 b
P2866H	13	95 b	95 a	81 b	276.5 d	294.4 a	79.3 a	215.1 a
30F53VYHR	13	94 b	86 b	35 d	324.2 b	226.9 b	66.4 b	160.4 b
Means	13	97	91	61	294.9	236.6	66.0	170.6
C.V.	–	5.57	4.78	12.79	1.53	5.16	5.80	7.62

¹Means followed by the same letter in each column belong to the same group according to the Scott-Knott grouping criterion at 5% probability ($p < 0.05$).

ageing at 45 °C (AA45), it was possible to separate the hybrids in more efficient and contrasting levels of vigour by the Scott-Knott test, forming four groups, varying from 24% to 93% of vigour (Table 1). The most vigorous hybrid by this test (AA45) was the DKB230PRO3, with 93% and the less vigorous were the 30F53VYH, P1630H and 30F53VYHR, with values of 24%, 40% and 35% of vigour, respectively. These same hybrids were the ones that showed low vigour when the temperature was less severe, indicating the higher sensitivity of them to this stress.

For the condition of our experiment, AA45 was the most effective in the sense of separation of vigour levels and it was reported by other authors. Bittencourt; Vieira (2006) tested the combinations of two temperatures (42 and 45 °C) and two exposure periods (72 and 96 h) to ageing stress on maize and concluded from their results that the combination of 45 °C for 72 h was the one that promoted segregation more advantageous related to vigour levels, especially when the lots have similar germination potential, such as those that occurred in this present study. Nerling et al., (2013) and Prazeres; Coelho (2016b) defined accelerated ageing stress at 45 °C for 72 h as the variable that contributed the most to define the physiological potential of maize varieties and hybrids.

In relation to the thousand seeds weight (TSW), significant differences were observed amongst the cultivars, with values varying from 259.3 g to 333.1 g (Table 1). It was observed that the hybrid with the highest vigour (DKB230PRO3) was the one with the lowest TSW, indicating absence or relation between seed size and vigour. Moreover, for the results of dry matter of seed (DMS), we observed the same separation of means by Scott-Knott test found for TSW, where the hybrid

of highest vigour (DKB230PRO3) was the one that presented the lowest DMS value (224.5 mg.seed⁻¹) (Table 2).

The influence of maize seed size on vigour has not yet been fully elucidated by scientific research. In this study, the results showed that vigour did not depend on the size of the seed evaluated by the weight of one thousand seeds and the dry matter of seeds. These same results were reported by Molatudi; Mariga (2009), who tested sowing of large and small seeds at different depths and concluded that seed size has no effect on seedling emergence and vigour, although higher depths significantly affect these parameters.

In addition, Sulewska et al. (2014), who evaluated the growth of seedling, alpha-amylase enzyme activity and yield of maize seeds of different sizes (different one thousand seeds weight) for three years. These authors concluded that smaller seeds present higher germination, higher activity of the alpha-amylase enzyme, lower seedling growth rate and higher productivity. On the other hand, other authors argue that when maize seed size is larger, seedling vigour is increased, especially when sowing depth is higher (El-Abady, 2015).

The fact that lower weight seeds present high vigour may be explained by the higher seed reserves reduction rate (SRRR) that these hybrids presented, where there were greater reduction of seed reserves (RSR), lower remaining dry matter in the endosperm (RDME) and, consequently, higher availability of nutrients to be mobilized for the growth and development of seedlings. This mobilization was evaluated by the reserve mobilization rate to the seedling (RMRS), which represents the ability of the cultivar to remove nutrients from reserve tissues and convert them to seedlings, although the seed reserve conversion efficiency (CESR) has been similar.

Table 2. Averages of dry matter of seed (DMS), dry matter of seedling (DMSL), remaining dry matter in the endosperm (RDME), reduction of seed reserves (RSR), conversion efficiency of seed reserves (CESR), seed reserves reduction rate (SRRR), reserves mobilization rate to the seedling (RMRS) and energy expenditure (EE) of seven maize hybrids.

Hybrids	DMS	DMSL	RDME	RSR	CESR	SRRR	RMRS	EE
	mg.seed ⁻¹	mg.seedling ⁻¹	mg.endo ⁻¹	mg	mg.mg ⁻¹	%	%	%
32R48VYHR	260.3 c	95.4 a	138.7 c	121.5 a	0.79 a	46.7 a	36.6 a	10.1 a
30F53VYH	240.2 d	74.2 c	148.4 b	91.8 d	0.81 a	38.2 b	30.9 b	7.3 b
DKB230PRO3	224.5 e	81.9 b	121.3 e	103.2 c	0.79 a	46.0 a	36.5 a	9.5 a
30R50VYH	286.2 a	83.9 b	184.8 a	101.3 c	0.83 a	35.4 b	29.3 b	6.1 b
P1630H	237.4 d	65.1 d	151.8 b	85.5 d	0.76 a	36.0 b	27.4 b	8.6 a
P2866H	243.3 d	84.4 b	130.9 d	109.4 b	0.78 a	45.5 a	35.2 a	10.4 a
30F53VYHR	277.8 b	88.6 b	177.2 a	100.6 c	0.88 a	36.2 b	31.9 b	4.3 b
Means	252.4	81.9	150.4	101.9	0.81	40.6	32.5	8.0
C.V.	1.34	5.21	3.56	5.72	5.48	5.19	5.66	25.47

Means followed by the same letter in each column belong to the same group according to the Scott-Knott grouping criterion at 5% probability ($p < 0.05$).

During seedling formation, the dry matter removed from the endosperm is hydrolysed and directed to the embryonic axis. However, not everything that is removed from the endosperm is transformed into a seedling because energy is expended to maintain cellular metabolism (Bewley et al., 2013). In this context, the energy expenditure (EE) amongst the hybrids showed that the more vigorous hybrids have higher energy expenditure to form seedlings than the low-vigour hybrids, which means that they had a more active and more effective metabolism, because despite the higher energy expenditure, there was no reduction in vigour (Table 2).

The seed reserves reduction rate (SRRR) is the ratio between the reduction of seed reserves (RSR) and the dry matter of seed (DMS), in other words, is the amount of mass that has been removed from the reserve tissue for maintenance of metabolism and for the development of the seedling. On the other hand, the reserves mobilization rate to the seedling (RMRS) represents how much was actually used for the growth and development of the embryonic axis during germination, which means the ability that a hybrid has possessed in using what has been removed from the seed to form a normal seedling during the evaluation period (after 120 hours of sowing).

We calculated the seed reserves reduction rate (SRRR) and the reserves mobilization rate to the seedling (RMRS) and we observed that the hybrids with higher vigour (DKB230PRO3, 32R48VYHR and P2866H) were those that presented the highest rates, with values of 46.0, 46.7 and 45.5% of SRRR,

respectively. The values of RMRS were 36.5, 36.6 and 35.2%, respectively. That is, they have a greater ability to use the endosperm reserves to form the seedling, even though the conversion efficiency of seed reserves of the hybrids has been similar. With these analyses used in the experiment, we elucidate what occurs physiologically with seed reserves during germination and seedling formation in hybrids with different vigour levels.

The highest RMRS were found in the most vigorous hybrids. This result was found by Ehrhardt-Brocardo; Coelho (2016) in common bean seeds (*Phaseolus vulgaris*). These authors evaluated the seedling performance test and its relation with the physiological quality of common bean seeds and found that seeds with greater potential for converting cotyledon reserves to seedling formation were the ones with the best vigour by accelerated ageing and longer seedling length, corroborating with the results found in this present study. Seeds that present a lower rate of mobilization of reserves for the formation of seedlings gave rise to smaller seedlings (Ehrhardt-Brocardo; Coelho, 2016), corroborating to our results with maize seeds.

According to the results obtained in this study, there were significant correlations between initial seed vigour by accelerated ageing (AA45) and seedling performance variables (TSL, SL, RL, RDME, RSR, SRRR and RMRS) (Table 3). When vigour by accelerated ageing was higher (AA45), total seedling length was higher ($r = + 0.60$), as

Table 3. Results of the Pearson correlation between the fifteen variables evaluated based on the mean of the hybrids.

	GR	AA43	AA45	OTSW	TSL	SL	RL	DMS	DMSL	RDME	RSR	CESR	SRRR	RMRS	EE
GR	–	0.02	0.09	-0.29	-0.25	-0.21	-0.23	-0.22	-0.14	-0.20	-0.02	-0.24	0.10	-0.01	0.12
AA43	–	–	0.70**	0.01	0.44*	0.46*	0.37	0.01	0.43	-0.26	0.52*	-0.15	0.47*	0.46*	0.30
AA45	–	–	–	-0.30	0.60**	0.48*	0.56**	-0.30	0.38	-0.61**	0.60**	-0.35	0.77**	0.66**	0.53*
OTSW	–	–	–	–	-0.12	-0.08	-0.12	0.98**	0.40	0.88**	0.13	0.51*	-0.51*	-0.30	-0.57**
TSL	–	–	–	–	–	0.73**	0.96**	-0.13	0.33	-0.40	0.51*	-0.25	0.55*	0.46*	0.45*
SL	–	–	–	–	–	–	0.52*	-0.04	0.58**	-0.40	0.66**	-0.07	0.62**	0.64**	0.41
RL	–	–	–	–	–	–	–	-0.15	0.20	-0.34	0.37	-0.29	0.44*	0.32	0.40
DMS	–	–	–	–	–	–	–	–	0.45*	0.85**	0.22	0.45*	-0.45*	-0.27	0.01
DMSL	–	–	–	–	–	–	–	–	–	-0.02	0.85**	0.37	0.50*	0.74**	-0.51*
RDME	–	–	–	–	–	–	–	–	–	–	-0.32	0.52*	-0.85**	-0.66**	-0.73**
RSR	–	–	–	–	–	–	–	–	–	–	–	-0.17	0.77**	0.74**	0.45*
CESR	–	–	–	–	–	–	–	–	–	–	–	–	-0.42	0.08	-0.77**
SRRR	–	–	–	–	–	–	–	–	–	–	–	–	–	0.87**	0.73**
RMRS	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.39
EE	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

GR – Germination Rate; AA43 – Accelerated Ageing test at 43 °C; AA45 – Accelerated Ageing test at 45 °C; TSW – Thousand Seeds Weight; TSL – Total Seedling Length; SL – Shoot Length; RL – Root Length; DMS – Dry Matter of Seed; DMSL – Dry Matter of Seedling; RDME – Remaining Dry Matter in the Endosperm; RSR – Reduction of Seed Reserves; CESR – Conversion Efficiency of Seed Reserves; SRRR – Seed Reserves Reduction Rate; RMRS – Reserves Mobilization Rate to the Seedling; EE – Energy Expenditure.

** , *Significant at 1% ($p < 0.01$) and 5% ($p < 0.05$) of probability by the t test, respectively.

was shoot and root length ($r = +0.48$, $r = +0.56$) (Table 3). We evaluated the TSL, SL and RL variables and the results showed that there was a significant difference in total seedling length (TSL), shoot length (SL) and root length (RL) by comparison among the hybrids, indicating the difference in vigour between them.

There was a high correlation between the vigour test by accelerated ageing (AA45) and the seedling performance test for total seedling length (TSL), shoot length (SL) and root length (RL). Sena et al. (2017), evaluating the sensitivity of different seedling performance tests for vigour evaluation in twenty maize seed lots, found that shoot length and root length were the most sensitive variables for vigour classification at different levels, which did not corroborate to our results.

On the other hand, the remaining dry matter in the endosperm was lower ($r = -0.61$) in higher vigour hybrids, because the reduction of seed reserves was higher ($r = +0.60$). Thus, the seed reserves reduction rate was improved ($r = +0.77$) and the reserves mobilization rate to the seedling ($r = +0.66$) was higher in these cultivars (32R48VYHR, DKB230PRO3 and P2866H).

For a better understanding of relationships between variables that were not clearly identified and in order to filter and define the most important relationships found in Pearson

correlation analysis, we applied two multivariate analysis tools in a complementary way. For the principal component analysis (PCA), the total variance explained by the two main components was 76.95%, with 50.53% of the variance explained by PC1 and 26.42% explained by PC2 (Figure 1).

The loading values showed that the hybrid DKB230PRO3 was grouped in PC1+/PC2+ by the germination rate (GR), energy expenditure (EE) and seed reserves reduction rate (SRRR) variables. The others high vigour hybrids 32R48VYHR and P2866H were grouped in PC1+/PC2- by the variables of accelerated ageing (AA43 and AA45), reserves mobilization rate to the seedling (RMRS), root length (RL), shoot length (SL) and total seedling length (TSL), reduction of seed reserves (RSR) and dry matter of seedling (DMS). However, the hybrids 30R50VYH and 30F53VYHR were grouped in PC1-/PC2- by the variables of remaining dry matter in the endosperm (RDME), conversion efficiency of seed reserves (CESR), thousand seeds weight (TSW) and dry matter of seed (DMS). Finally, the other two hybrids P1630H and 30F53VYH were clustered into PC1-/PC2+. The results observed in the PCA reaffirm the hypothesis that more vigorous hybrids (DKB230PRO3, 32R48VYHR and P2866H) were better able to remove nutrients from reserve tissues (TRRS) and transform them into seedlings (RMRS), although they spent more energy

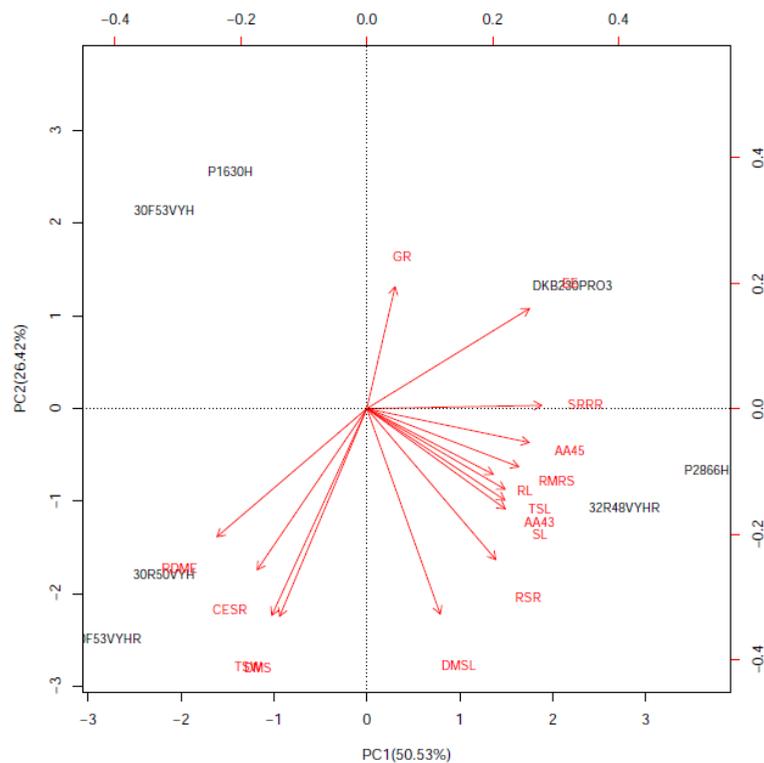


Figure 1. Scores of the first and second Principal Components (PC1 and PC2) for seedling performance variables in seven cultivars of hybrid maize with different levels of vigour.

(EE) in the seedling formation process.

The heat map of Hierarchical Cluster Analysis (HCA) shows that there were two main groups, where the highest vigour cultivars (32R48VYHR, DKB230PRO3 and P2866H) were in the same group, while the lower vigour cultivars (30R50VYH, 30F53VYHR, 30F53VYH and P1630H) were in another group (Figure 2), indicating that there was dissimilarity between the samples. The dissimilarity between the groups was calculated by the cophenetic correlation coefficient between the observations that were grouped. Thus, a dendrogram is an appropriate summary of the data if the correlation between original distances and cophenetic distances is high (Uarota et al., 2014). A cophenetic correlation coefficient of 89% was found. It was observed that the germination rate (GR) variable was grouped separately from the other variables and in the intermediate area of the cluster, since it was important for all the hybrids.

It was observed that the cultivars classified as higher vigour were grouped by PCA and HCA (Figure 1 and Figure 2) in a different group of the cultivars classified as lower vigour, where the variables that contributed the most

to this separation were the same ones found in the Pearson correlation positively correlated with vigour by accelerated ageing (RMRS, SRRR, DMSL, RSR, RL, TSL and SL). On the other hand, for the hybrids of lower vigour, the variables that contributed the most to the separation were those that did not correlate significantly with the vigour (OTSW, DMS, CESR), or that there was a negative correlation (RDME). Thus, the parameters of stored reserves dynamics and seedling formation evaluated in this study explained the vigour in hybrid maize seeds and can be used as a method of evaluation of the physiological quality.

The results of this study suggest that more detailed research regarding the study of enzymatic activity and/or reserve components involving the seed reserve reduction rate and the reserves mobilization rate to the seedling should be done, mainly using the two most contrasting cultivars for the level of vigour by accelerated ageing. These efforts should be done mainly to understand and elucidate metabolically why the differences on vigour exist, identifying the mechanism that reflects the expression of vigour in seeds of hybrid maize, since the physiological changes occurred in these cultivars

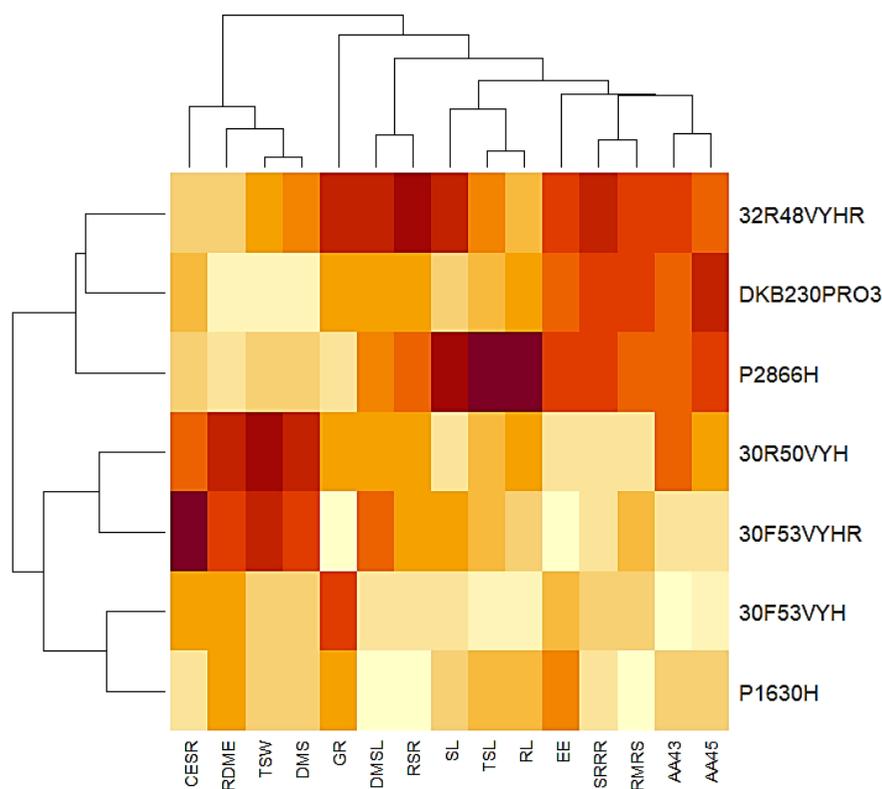


Figure 2. Heat Cluster Map* (HCA) for the dynamics of seedling formation and physiological quality of seven maize seeds, with a cophenetic correlation coefficient of 89%.

*The higher colour intensity of the dendrogram indicates the greater importance of the variable for the sample under analysis.

were presented and discussed in this article.

Conclusions

Accelerated ageing at 45 °C for 72 hours (AA45) is the most efficient combination to segregate vigour levels of hybrid maize seeds.

Genotypes with higher seed reserve utilisation efficiency (TRRS and RMRS) have higher vigour, producing seedlings with higher dry matter, higher total length, shoot length and root length, regardless of seed weight.

Seed reserves reduction rate and seedling reserve mobilization explain the vigour of hybrid maize seeds and can be used in internal quality control by programs to select cultivars with high physiological quality.

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