

Limiting Maize seed vigor and its response under salt stress conditions

Vigor de sementes em milho e a sua resposta sobre condições de estresse salino

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Resumo:

O estande de plântulas é primordial para o rendimento da cultura do milho, e este pode ser afetado por diferentes estresses, entre eles o estresse salino. Dessa forma, são necessários estudos mais aprofundados quanto aos mecanismos de tolerância ao estresse salino em genótipos comerciais de milho híbrido. O objetivo do trabalho foi determinar a importância do uso de sementes de milho com alto vigor para a superação do estresse salino durante a germinação e formação de plântulas. Quatro híbridos foram utilizados, sendo quatro lotes com alto vigor e quatro lotes com baixo vigor, submetidos a cinco níveis de salinidade (0, 50, 100, 150 e 200 mmol L⁻¹ de NaCl). Foram avaliadas a germinação, plântulas anormais, comprimento da parte aérea, sistema radicular e total da plântula, além de suas respectivas massas secas, número de raízes e taxa de mobilização das reservas da semente. Todos os parâmetros avaliados apresentaram redução com o aumento do nível do estresse e as plântulas originadas por sementes de alto vigor apresentaram melhor desempenho fisiológico. O estresse salino interfere negativamente no desempenho das sementes independente da sua qualidade fisiológica e sementes de alto vigor proporcionam maior capacidade de superação das condições de estresse salino quando comparadas com sementes de baixo vigor. A condição testada permite segregar genótipos ou lotes visando a tolerância a esses estresses.

Palavras-Chave: estresse iônico, estresse osmótico, qualidade fisiológica, fisiologia do estresse

Abstract:

Seedling stands are essential for good maize yield, which can be affected by different stresses, including salt stress. Thus, more in-depth studies are needed on salt tolerance mechanisms in commercial hybrid maize genotypes. The aim of this study was to determine the importance of using high vigor maize seeds to overcome salt stress during germination and seedling formation. Four hybrids were used, four high vigor (HV) and four low vigor (LV) seed lots submitted to five levels of salt stress (0, 50, 100, 150 and 200 mmol L⁻¹ of NaCl). Germination, abnormal seedlings, shoot, root system and total seedling length and their respective dry masses, number of roots and seed reserve mobilization rate were evaluated. All the variables declined with an increase in stress level and the high vigor seedlings exhibited better physiological performance. Salt stress negatively affects seed performance regardless of their physiological quality, and high vigor seeds can better overcome salt stress when compared to their low vigor counterparts. The tested condition allows segregating genotypes or seed lots, aimed at salt tolerance.

Keywords: ionic stress, osmotic stress, physiological quality, physiology of stress

1. Introduction

Maize crops have physiological characteristics that can affect their productive performance, such as low prolificacy and high interspecific competition (Alkharabsheh *et al.*, 2021; Sangoi *et al.*, 2016). Thus, to achieve satisfactory yields, it is essential to establish uniform seedling stands in terms of spatial and temporal distribution, especially under adverse environmental conditions that can affect seedling establishment (Waterworth *et al.*, 2015). The physiological quality of seeds is a determining factor for a successful production system. Vigor allows seeds to overcome abiotic stresses during seedling emergence (Marcos-Filho, 2015). Thus, the use of high-vigor seeds may be a relevant strategy to reduce problems related to seedling emergence and development, even under adverse environmental conditions.

Among abiotic stresses, arable soil salinity affects agriculture globally and significantly compromises crop establishment and development. Agricultural crops are highly sensitive to stressors during seedling emergence, especially salt stress (Alkharabsheh *et al.*, 2021). In general, salt stress reduces germination and emergence rates, as well as root growth and dry mass (Konuskan *et al.*, 2017) due to the initial osmotic stress, which hinders water uptake. In addition, toxicity caused by Na⁺ and Cl⁻ ions further exacerbates the situation (Panuccio *et al.*, 2018).

Biotic and abiotic stress tolerance combined with higher yields are extremely important to sustainably meet the needs of maize crops. In this respect, salt tolerance is mainly dependent on morphology and physiology (Kakar *et al.*, 2019). Seed vigor is a physiological attribute that enhances germination, seedling formation, stand establishment, and tolerance to environmental factors (Dwivedi *et al.*, 2021). Thus, understanding the interaction between vigor, field factors and salinity remains a challenge.

According to Padilha *et al.* (2022), the use of high vigor (HV) bean seeds contributed to overcoming salt stress, generating better seedlings. However, Silva *et al.* (2016) showed that seed vigor did not influence germination under salt stress conditions. Given the above, in-depth studies are needed that compare the vigor maize seed lots to determine their response to salt stress during germination and seedling formation, since HV seeds may contribute to overcoming this abiotic stress.

As such, the aim of the present study was to analyze the physiological response of high and low vigor (LV) hybrid maize seed lots under salt stress conditions, seeking to identify the physiological differences between them in germination and seedling formation.

2. Materials and Methods

The study was carried out at the Official Seed Analysis Research Laboratory (LASO) of the State University of Santa Catarina (UDESC), at the Center for Agro-Veterinary Sciences (CAV), located in the municipality of Lages, in Santa Catarina state, Brazil.

The study used seeds of four commercial simple hybrids (AG 9025 PRO3, 32R22VYHR, 30F53VYH and DKB 230 PRO3), produced during the 2019/2020 growing season. The choice of hybrids was based on the production ceiling, precocity, grain quality and main genotypes marketed in the current harvest. After acquisition, the seed lots were stored in a dry chamber at a temperature of 10°C and relative humidity of 45% ± 5%, until they were used.

To obtain LV seed lots, the four lots used were deteriorated in an aging chamber under high temperature and humidity. The seeds were placed on a metal screen in 11x11x3.5 transparent plastic germination boxes, known as "Gerboxes[®]", containing 40 mL of distilled water (Marcos-Filho, 1999) and kept at 45°C for 72 hours (Aosa, 1983; Bittencourt & Vieira, 2006). The seeds were removed from the plastic boxes and dried at 30°C in an air circulation oven until reaching 12% moisture content.

Thus, unaged seed lots (HV) were coded as L1 (AG 9025 PRO3), L2 (32R22VYHR), L3 (30F53VYH) and L4 (DKB 230 PRO3); and artificially aged seed lots (LV) L5 (AG 9025 PRO3), L6 (32R22VYHR), L7 (30F53VYH) and L8 (DKB 230 PRO3).

Physiological characterization

The physiological quality of the lots was characterized by germination, first germination count, and accelerated ageing.

To determine germination percentage, four repetitions of 50 seeds were used, distributed on paper towel moistened with distilled water and weighing 2.5 times that of the dry paper, then evaluated at four (first germination count) and seven days (germination) (Brasil, 2009).

To carry out the accelerated aging test, the seeds were placed in an accelerated aging chamber at 45°C for 30 hours, according to previous tests. Next, the germination test was conducted as previously described.

Vigor response under salt stress conditions

Salt stress during germination was simulated with different sodium chloride (NaCl) solutions at concentrations of 0, 50, 100, 150 and 200 mmol. L⁻¹, which were used to moisten the germination paper. The water potential of each NaCl concentration was measured using the Van't Hoff equation ($\Psi_{os} = RTC$), where Ψ_{os} = osmotic potential; R = the general constant for perfect gases (8.32 J mol⁻¹ K⁻¹); T = temperature (K) = 273 + T (°C); and C = concentration (mol. L⁻¹) (Salisbury and Ross, 1991).

The electrical conductivity of the NaCl solutions was measured with a conductivity meter at 25°C, for three minutes of electrode submersion, in order to categorize the salt stress level used. Salt stress was classified as proposed by Richards (1954) (Table 1).

Table 1. NaCl concentrations used to simulate salt stress.

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Tabela 1. Concentrações de NaCl utilizadas para simular o estresse salino

NaCl mmol L ⁻¹	NaCl g L ⁻¹	CE dS m ⁻¹	Classification (Richards, 1954)
0	0	0,0	Non-saline
50	2.92	5.2	Moderately saline
100	5.84	9.7	Highly saline
150	8.77	14.6	Highly saline
200	11.69	19.4	Severely saline

Once the solutions were determined, the germination percentage, first germination count and seedling performance under different salt stress levels were evaluated.

To calculate germination percentage, both normal and abnormal seedlings were considered. Four replicates of 50 seeds were used, distributed on a roll of "Germitest" paper moistened at 2.5 times its dry weight with solutions of 0, 50, 100, 150 and 200 mmol L⁻¹ of NaCl (Table 1). For the first germination count, normal seedlings obtained at four days were considered (Brasil, 2009).

For seedling performance, 20 seeds were distributed on the upper portion of the Germitest® paper (Krzyzanowski *et al.*, 2020), moistened at 2.5 times its dry weight with solutions of 0, 50, 100, 150 and 200 mmol L⁻¹ of NaCl (Table 1). These rolls were packed in transparent plastic bags to prevent moisture loss and kept in germinators at a controlled temperature of 25°C and 12-hour photoperiod for seven days.

At seven days, the following were measured: root system length (RSL), shoot length (SL), total seedling length (TSL), root system dry mass (RSM), shoot dry mass (SDM), total seedling dry mass (TSDM) (Krzyzanowski *et al.*, 2020), the physiological rate of mobilization of seed reserves (RMSR) and the number of roots (NR). The RMSR is the seedling dry mass divided by the seed dry mass multiplied by 100 (Andrade *et al.*, 2019). The lengths were measured with a digital caliper and

expressed in millimeters per seedling (mm pl⁻¹). The seedlings were then dried in an oven 80°C for 24 hours and weighed to determine dry mass, expressed in milligrams per seedling (mg pl⁻¹) (Krzyzanowski *et al.*, 2020).

Statistical analysis

The experimental design for physiological characterization was completely randomized with 8 treatments (lots) and four replications. To determine the physiological response of vigor under salt stress conditions, the experiment used a 2x5 factorial design, with two vigor groups (high and low) and five salt stress conditions (0, 50, 100, 150 and 200 mmol L⁻¹ of NaCl). Means were compared by Tukey's test at 5% probability. Comparison of means and regression analysis were performed using Sisvar® statistical software (Ferreira, 2011).

3. Results and discussion

Physiological characterization

Analysis of variance (ANOVA) showed that the lots exhibited significant difference for the variables germination (G), first germination count (GC) and accelerated aging (AA) (Table 2).

Table 2. Analysis of variance framework for vigor segregation and grouping into high and low vigor lots for the variables germination (G), first germination count (GC) and accelerated aging (AA). Source of variation (SV), Degrees of freedom (DF). *,**significant difference according to the test F at 1% ($p < 0.01$) and 5% ($p < 0.05$), respectively, and "ns": not significant

Tabela 2. Resumo do quadro de análise de variância para a segregação do vigor e agrupamento em lotes de alto vigor e baixo vigor para as variáveis germinação (G), primeira contagem germinação (GC) e envelhecimento acelerado (AA). Fonte de variação (SV), Graus de liberdade (DF). *,**diferença significativa pelo teste F a 1% ($p < 0,01$) e 5% ($p < 0,05$), respectivamente e "ns": não significativo

SV	DF	GC	G	AA
Lot (F1)	7	*	*	*
Error	24			
Total	31			
CV.		3.84	3.98	1.91

The germination results demonstrate differences in the physiological quality of the lots if maize seeds are used, where the germination of lots belonging to the non-aged seed groups was generally superior to that of the aged seeds. Lots L1, L2, L3 and L4 obtained first germination counts of 98, 92, 96 and 92%, respectively, with no difference between the groups. On the other hand, L5, L6, L7 and L8 exhibited lower first germination count percentages (17, 31, 73 and 73% respectively), demonstrating that artificial aging resulted in batch deterioration and loss of vigor (Table 3).

Table 3. Germination (G), first germination count (GC) and accelerated aging (AA) of the hybrid maize lots used. Means followed by the same letter in the column did not differ statistically according to Tukey's test at 5% ($p < 0.05$) probability

Tabela 3. Germinação (G), primeira contagem de germinação (GC) e envelhecimento acelerado (AA) dos lotes de milho híbrido utilizados. Médias seguidas da mesma letra na coluna não diferem estatisticamente entre si pelo teste de Tukey a 5% ($p < 0,05$) de probabilidade

Seed Lot	Hybrid	GC (%)	G (%)	AA (%)
L1	AG 9025 PRO3	98 a	99 a	98 a
L2	32R22VYHR	92 a	96 a	94 a
L3	30F53VYH	96 a	98 a	98 a
L4	DKB 230 PRO3	92 a	98 a	95 a
L5	AG 9025 PRO3	17 d	56 c	45 d
L6	32R22VYHR	31 c	53 c	48 d
L7	30F53VYH	73 b	84 b	69 c
L8	DKB 230 PRO3	73 b	94 a	85 b

The AA results corroborated with those obtained from the GC. Lots L1, L2, L3 and L4 showed superior results (98, 94, 98 and 95% respectively), and the aged seed lots (L5, L6, L7 and L8) obtained 45, 48, 69 and 85% respectively, demonstrating lower vigor. Of the vigor tests used, the first germination count and accelerated aging are among the most widely applied and are efficient in determining seed lot vigor (Marcos-Filho, 2015).

These results show that the process of reducing artificial vigor was effective, with L1, L2, L3 and L4 belonging to the HV group, and L5, L6, L7 and L8 to its LV counterpart, which were grouped to assess the response to salt stress.

Vigor response to salt stress: analysis of variance revealed a vigor effect for all variables evaluated, indicating that the difference in the physiological quality of the seed lots affects performance during germination (Table 4).

Table 4. Analysis of variance for first germination count (GC), germination (G), abnormal seedlings (AS), shoot length (SL), root system length (RSL), total seedling length (TSL), shoot dry mass (SDM), root system dry mass (RSDM), total seedling dry mass (TSDM), number of roots (NR), and seed reserve mobilization rate (RMR). Source of variation (FV), Vigor factor (F1), Salt stress factor (F2), Degrees of freedom (DF). *, **: significant difference according to the F test at 1% ($p < 0.01$) and 5% ($p < 0.01$), respectively, and "ns": not significant

Tabela 4. Resumo do quadro de análise de variância para primeira contagem de germinação (GC), germinação (G), plântulas anormais (AS), comprimento da parte aérea (SL), comprimento do sistema radicular (RSL), comprimento total da plântula (TSL), massa seca da parte aérea (SDM), massa seca do sistema radicular (RSDM), massa seca total da plântula (TSDM), número de raízes (NR) e taxa de mobilização das reservas da semente (RMR). Fonte de variação (FV), Fator vigor (F1), Fator estresse salino (F2), Graus de liberdade (DF). *, **: diferença significativa pelo teste F a 1% ($p < 0,01$) e 5% ($p < 0,05$), respectivamente e "ns": não significativo

SV	DF	GC	G	AS	SL	RSL	TSL	SDM	RSDM	TSDM	NR	RMR
F1	1	*	*	*	*	*	*	*	*	*	*	*
F2	4	*	*	*	*	*	*	*	*	*	*	*
F1 x F2	4	*	*	ns	**	*	*	*	ns	*	ns	*
Error	150											
Total	159											
CV.		27.75	9.14	36.06	8.13	6.53	5.47	6.83	7.52	5.27	17.34	7.57

The first germination count at the different salt stress levels showed a decreasing linear response in both the LV and HV groups. However, the LV lots obtained significantly lower percentages, differing at all concentrations, except for 200 mmol L⁻¹ of NaCl, thus demonstrating that under severe salt stress conditions, vigor does not increase germination speed (Table 5).

Table 5. First germination count (GC), germination (G) and abnormal seedlings (AS) of high (HV) and low vigor (LV) seed lots under different salt stress conditions. Means followed by the same lowercase letter in the column and uppercase in the row do not differ statistically according to Tukey's test at 5% ($p < 0.05$) probability. Var: Variable

Tabela 5. Primeira contagem de germinação (GC), germinação (G) e plântulas anormais (AS) de lotes de sementes com alto (HV) e baixo vigor (LV) sob diferentes condições de estresse salino. Médias seguidas da mesma letra, minúscula na coluna (nível de vigor) e maiúscula na linha (concentração salina) não diferem estatisticamente entre si pelo teste de Tukey a 5% ($p < 0,05$) de probabilidade

Var.	Vigor	NaCL (mmol L ⁻¹)				
		0	50	100	150	200
GC (%)	HV	94 aA	81 aA	51 aB	15 aC	2 aD
	LV	49 bA	32 bB	13 bC	5 bD	1 aD
G (%)	HV	98 aA	97 aA	96 aA	94 aA	87 aA
	LV	72 bA	77 bA	76 bA	68 bA	49 bB

The poor first germination count results are due to the decline in seed water potential, where increased stress from the significant accumulation of sodium chloride in the substrate results in less

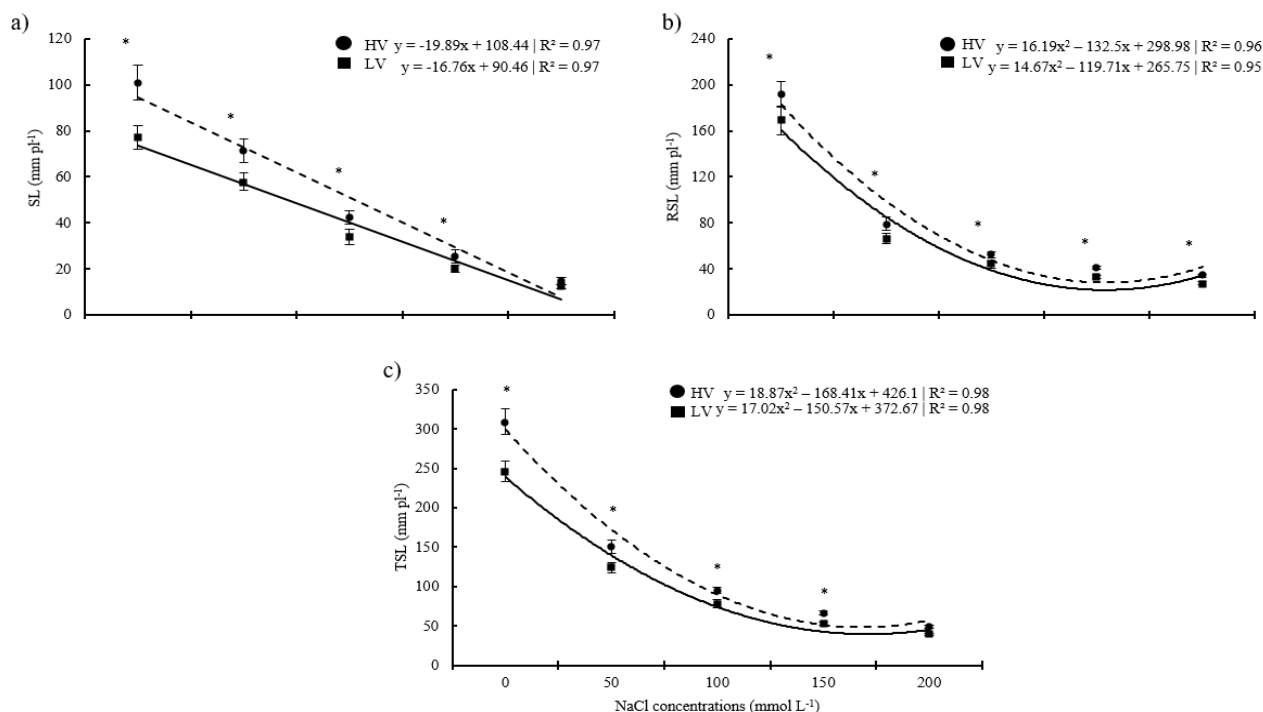
seed water absorption (Nunes *et al.*, 2020) and the need to produce other components to perform osmotic regulation (Nadeem *et al.*, 2019; Padilha *et al.*, 2022). Thus, germination is slowed by the difference in water potential at each stress level, reducing the GC regardless of seed lot vigor.

Saline stress reduced the germination percentage. The groups demonstrated that germination differed at all points, where LV seed lots were superior at all saline stress levels. This is due to the poor physiological quality of the LV group seeds. In addition, this difference increased significantly as the NaCl level and/or concentration increased, demonstrating the importance of vigor in overcoming salt stress and in normal seedling formation. In general, LV seeds are physiologically compromised due to deterioration, which requires more time for their physical and biochemical restructuring both under optimal environmental conditions and in situations of stress, associated with lipid peroxidation, loss of seed protection capacity, damage to genetic material and consumption of reserve material to withstand stress (Andrade *et al.*, 2019; Ebone *et al.*, 2019). Thus, sowing with poor quality (low vigor) seeds can compromise seed performance in the field, especially in areas where salt pans reduce germination speed, emergence uniformity and seedling establishment, posing serious risks to the producer (Ahmad *et al.*, 2023).

Shoot length (SL) declined significantly as the stress level increased, and seed lot vigor directly interferes with this growth. Thus, vigor influences seedling shoot length (Figure 1a). However, this relationship decreases as stress level rises, and under severe stress conditions, such as 200 mmol L⁻¹ NaCl, vigor no longer influences this parameter. The importance of vigor during abiotic stress conditions was observed by Padilha *et al.* (2022), where HV bean seed lots performed better under imposed salt stress, exhibiting greater length.

Figure 1. Shoot length (SL) (a), root system length (RSL) (b) and total seedling length (TSL) (c) evaluated in high (HV) and low vigor (LV) seed lots under salt stress conditions (NaCl). *: significant difference according to Tukey's test at 5% ($p < 0.05$) probability

Figura 1. Comprimento da parte aérea (SL) (a), comprimento do sistema radicular (RSL) (b) e comprimento total da plântula (TSL) (c) avaliados em lotes de sementes de alto vigor (HV) e baixo vigor (LV) sob condições de estresse salino (NaCl). *: indica diferença significativa pelo teste de Tukey a 5% ($p < 0,05$) de probabilidade



Root system length (RSL) was the most susceptible to stress. At the highest stress level (200 mmol L⁻¹), root length declined by 83.56 and 92.81% in the HV and LV group, respectively, exhibiting better performance in the former. Another factor was the abrupt reduction in root length at

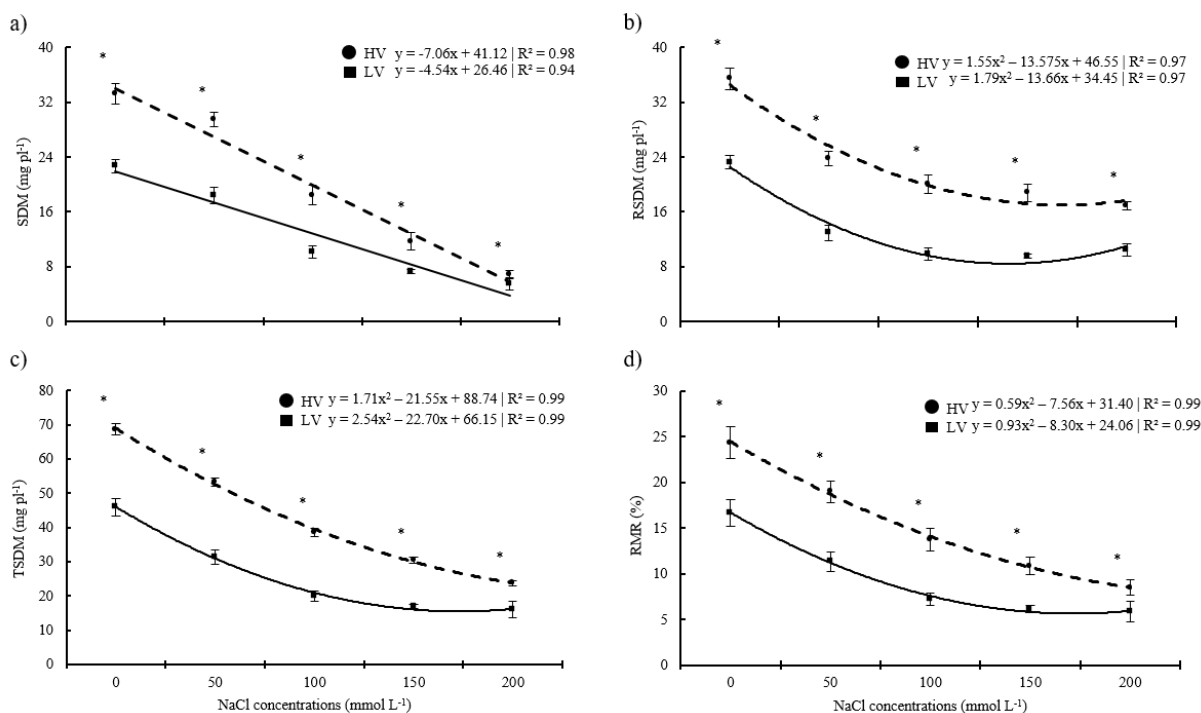
50 mmol L⁻¹ of NaCl (Figure 1b). According to Maia *et al.*, (2015), this decrease in RSL under salt stress is associated with the death of the root apical meristem, preventing division, elongation, cell maturation and consequently differentiation and growth. This relationship is more evident in seedlings produced by LV seeds. Given that seeds and seedlings are susceptible to numerous types of stress in the field, investment in vigorous seeds is economically profitable, since sowing with poor quality seeds may lead to premature seedling death because they could not withstand the stress period, thereby requiring reseeded.

Thus, total seedling length (TSL) exhibited the same response to stress in relation to SL and RSL, and a decrease with the imposed stress, where HV seeds performed better up to 150 mmol L⁻¹ (Figure 1c). The SL, RSL and TSL results indicate that under stress conditions above 100 mmol L⁻¹, the differences in length are smaller when compared to the control (Fig. 1a-c). Thus, in real field conditions, vigorous seeds can better withstand high salt stress, since the maximum level tested here (200 mmol L) already exceeds that observed in the field.

Shoot dry mass (SDM) declined linearly with the addition of stress. HV seeds showed the formation of shoots with greater mass regardless of stress level. These results demonstrate that seed lot vigor favors the formation of seedlings with better shoot performance even under abiotic stress (Figure 2a). The formation of a vigorous and well-structured shoot is extremely important for seedling stand formation, as and to ensure satisfactory yields under salt stress conditions. In the early stages of maize development, a probable strategy is to invest in shoots for subsequent photosynthesis (Silva *et al.*, 2016).

Figure 2. Shoot dry mass (SDM) (a), root system dry mass (RSDM) (b), total seedling dry mass (TSDM) (c), and reserve mobilization rate (RMR) (d) evaluated in high (HV) and low vigor (LV) seed lots under salt stress conditions (NaCl). *: significant difference according to Tukey's test at 5% ($p < 0.05$) probability

Figura 2. Massa seca de parte aérea (SDM) (a), massa seca de sistema radicular (RSDM) (b), massa seca total de plântula (TSDM) (c), taxa de mobilização de reservas (RMR) (d) avaliadas em lotes de sementes com alto vigor (HV) e baixo vigor (LV) em condições de estresse salino (NaCl). *: indica diferença significativa pelo teste de Tukey a 5% ($p < 0,05$) de probabilidade



Root system dry mass (RSDM) was negatively affected by salt stress; however, HV seeds showed greater accumulation of dry matter regardless of the stress level imposed. Both RSL and RSDM declined as salt stress increased (Figure 2b). In general, a similar decrease in root system dry

mass of maize (Konuskan *et al.*, 2017) and rice (Cavalcante *et al.*, 2019) was observed in different osmotic potentials caused by the addition of NaCl, demonstrating that salinity is detrimental to seedling performance and, according to Silva *et al.* (2016), the root system is the most affected by this stress. Thus, HV seeds produced seedlings with greater root system length and mass, showing that they are better able to overcome salt stress imposed during germination and seedling formation.

The decrease in SL, RSL, TSL, SDM, RSDM, and TSDM demonstrates the severity of salt stress in the plant's biological organism. In general, HV seed lots formed more vigorous seedlings due to their overcoming stress during germination and seedling formation. According to Andrade *et al.* (2019), seeds that mobilize reserves quickly and efficiently perform better, which is associated with seed lot vigor.

The reserve mobilization rate (RMR) is the percentage of reserves that were actually mobilized to form the seedling. A comparison between the HV and LV groups showed that, regardless of salinity level, the HV group mobilized more reserves for seedling formation (Figure 2d). HV seeds in the absence of stress obtained a seed reserve mobilization rate of 24.35%, decreasing quadratically up to 8.47% under the most severe salt stress. In the control situation, the LV group exhibited an initial mobilization rate of 16.67%, decreasing to 5.83% at the last stress level (Figure 2d).

In this respect, the seed reserve mobilization rate showed a similar response to the growth parameters (RSL, SL, TSL, SDM, RSDM and TSDM), reserve mobilization declined with increased salt stress, resulting in poorer seedling performance (Figure 2d). However, HV seeds obtained higher RMR, which explains the better performance observed, even under stress conditions. This association is observed for bean (Padilha *et al.*, 2022) and maize seeds (Andrade *et al.*, 2019), with greater vigor resulting in higher TMR and better-performing seedlings.

RMR depends on the hydrolysis capacity of the reserve components and must therefore occur quickly during germination to provide soluble sugars for metabolism. Thus, given that starch is the main reserve in maize seeds, Mieog *et al.* (2017) alpha-amylase activity during germination is a determining factor for the embryonic axis to have enough energy to perform metabolic functions quickly from the available soluble sugars for seedling formation. The higher alpha-amylase activity under salt stress conditions favors the formation of better-performing seedlings. This association was highlighted by Liu *et al.* (2018), where greater alpha-amylase activity during germination under salt stress favored rice seed germination.

In this respect, Sommer *et al.* (2024) tested HV and LV maize seeds, demonstrating that the former exhibited greater total protein hydrolysis and better seedling performance when compared to LV seeds. This can be explained by the HV seed reserves that are hydrolyzed and mobilized more quickly, providing low molecular weight compounds and promoting greater and faster energy supply for seedling development.

4. Final Considerations

Increasing salt stress levels negatively affect the physiological performance of seeds and seedling formation.

High vigor seed lots can better overcome salt stress and mobilize seed reserves when compared to their low vigor counterparts.

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Conflict of Interest

The authors declare no conflict of interest.

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