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Germination and vigor of maize hybrids seeds submitted to water stress

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Abstract: The water stress is the most limiting factor for germination of maize seeds, in this sense, to know more tolerant hybrids to this factor is essential for productive sustainability of the culture. The objective of this study was to evaluate the seed germination and seedling growth of maize hybrids submitted to water stress. The study was conducted in a completely randomized design in a factorial arrangement 4 x 5, with 8 repetitions. The first factor was composed of hybrids AS 1551, AG 5011, AG 8011 and CD 384. The second factor was the osmotic potential of 0.0, -0.4, -0.8, -1.2 and -1.6 MPa, obtained from KCl solutions applied to the seeds in order to simulate the water stress. It was determined the hard and dead seeds percentages, normal and abnormal seedlings, besides the length and dry mass of aerial and radicular part. The osmotic potential of -0.8 MPa completely inhibited the formation of normal seedlings in all hybrids. The reduction of osmotic potential of 0.0 to -0.4 MPa caused a reduction in the number of normal seedlings and dry mass production of aerial part. However, it increased the dry mass production of roots. In descending order of classification the hybrids that presented higher tolerance to water stress are AS 1551> AG 5011> CD 384> AG 8011.

Keywords: KCl, osmotic potential, salinity, *zea mays L*.

Germinação e vigor de sementes de híbridos de milho submetidas ao déficit hídrico e salino

Resumo: O déficit hídrico é o fator mais limitante à germinação das sementes de milho, nesse sentido, conhecer híbridos mais tolerantes a este fator é essencial para a sustentabilidade produtiva da cultura. Objetivou-se com este estudo avaliar a germinação das sementes e o crescimento de plântulas de híbridos de milho submetidos ao estresse hídrico e salino. O estudo foi conduzido em delineamento inteiramente casualizado em arranjo fatorial 4 x 5, com 8 repetições. O primeiro fator foi constituído pelos híbridos AS 1551, AG 5011, AG 8011 e CD 384. O segundo fator foram os potenciais osmóticos de 0,0, -0,4, -0,8, -1,2 e -1,6 Mpa, obtidos a partir de soluções de KCl, aplicados nas sementes visando simular a ocorrência de déficit hídrico. Foram determinadas as percentagens de sementes duras e mortas, plântulas normais e anormais, além do comprimento e massa seca de parte aérea e radicular. O potencial osmótico de -0,8 MPa inibiu totalmente a formação de plântulas normais em todos os híbridos. A redução do potencial osmótico de 0,0 para -0,4 MPa causou redução no número de plântulas normais e na produção de massa seca de parte aérea, no entanto, elevou a produção de massa seca radicular. Em ordem decrescente de classificação os híbridos que apresentam maior tolerância ao déficit hídrico e salino são o AS 1551 > AG 5011 > CD 384 > AG 8011.

Palavras-chave: KCl, potencial osmótico, salinidade, zea may

Introduction

The water stress is the most limiting factor for seed germination and maize seedlings growth (BERGAMASCHI et al., 2004; OUDA et al., 2008.). The maize tolerance to water stress is variable according to the crop phase and the type of hybrid genotype (ANDRIOLI and SENTELHAS, 2009). The germination and emergence is one of the stadiums in which the plant appears more sensitive to the occurrence of water stress (QUEIROZ and CAZETTA, 2016). This is due to the damage being irreversible, as well as the delay of these processes end up exposing the seeds to the action of microorganisms, resulting in the reduction of plants stand (ALMANSOURI et al., 2001) and therefore the final grain yield (VAZ-DE-MELO et al., 2012).

The soil salinization can promote the indirect occurrence of water stress on crops (MORTELE et al., 2006). This phenomenon occurs due to intrinsic of location, as low rainfall, or extrinsic related to errors in the irrigation and fertilizer management (TÔRRES et al., 2004; SILVA et al., 2016). The high concentration of salts in the soil reduces the osmotic potential, remaining the water retained to salts, and making it consequently less available to plant absorption (RIBEIRO et al., 2001), resulting in increased concentration of ions in the plasma of plant tissues (AMORIM et al., 2002).

Due to the difficulty in assessing the isolated effect of water stress on seed germination in field experiments (QUEIROZ and CAZETTA, 2016), it has been used other methods to determine the tolerance of genotypes to this factor. Being the sowing seeds on substrates with different osmotic potentials obtained by using salts or polyethylene glycol (KAPPES et al., 2010; QUEIROZ and CAZETTA, 2016), in a controlled environment widely used. Due to the reduction in grain yield caused by water and saline stress, the elucidation of the tolerance ability to these factors, for different hybrids is essential for yield stability of maize (VAZ-DE-MELO et al., 2012), and in view of the economic importance of culture in the world (FAO, 2014) and for making part of the food base of the human population (QUEIROZ and CAZETTA, 2016).

The hypothesis tested in this study is that the maize hybrids have different levels of tolerance to water stress. In this sense, this study aimed to evaluate the seed germination and seedling growth of maize hybrids submitted to water stress.

Material and Methods

The study was conducted in a laboratory bench of seeds technology at the Federal University of Santa Maria *campus* Frederico Westphalen during September to October, 2014.

The experimental design was a randomized blocks in a factorial arrangement of 4 x 5 (hybrids x osmotic potential), with eight replications. The first factor was composed of maize hybrids: triple hybrid CD 384 (Codetec), triple hybrid AG 8011 (Agroceres), triple hybrid AG 5011 (Agroceres) and simple hybrid AS 1551 (Agroeste). The hybrids were acquired in trading companies of their seeds. Before the experiment implantation it was measured the values of the characteristic Weight of 100 seeds (P100) with eight repetitions, moisture and temperature of genotypes, which are described in Table 1.

Table 1. Weight of 100 seeds, moisture (%) and average temperature (°C) of maize hybrids seeds

Hybrids	Weight of 100 seed (g)	Moisture (%)	Temperature (°C)
CD 384	30.18	12.50	23.20
AG 8011	25.86	12.50	23.10
AS 1551	22.63	12.50	23.20
AG 5011	34.96	13.20	23.10

The second factor of variation was constituted of the osmotic potential of 0.0, -0.4, -0.8, -1.2 and -1.6 MPa, used to simulate the occurrence of water stress. These levels were selected in order to provide the optimal conditions for seeds germination (Potential of 0 MPa) until gradually similar the occurrence of a strong water stress that practically inhibited the seeds germination (Potential of -1.6 MPa). Similar levels of this study have been used in several studies with this same purpose (CONUS et al., 2009; KAPPES et al., 2010; VAZ-DE-MELO et al., 2012). To obtain the different osmotic potentials it was used KCl salt, in the solutions formulation prepared with deionized water, being the salt concentration in each solution calculated according to the Van't Hoff equation cited by Salisbury and Ross (1992).

$$Yos = - RTC \qquad \text{(equation 1)}$$

Where: Yos = osmotic potential (MPa); R = general gas constant (0.082 atm.mol L^{-1} K^{-1}); T = temperature (K); C = concentration (mol L^{-1}); mol L^{-1} x KCl molar mass = g L^{-1} and T (K) = 273 + (ºC).

The seed germination test was performed in accordance with the Rules for Seed Analysis (RAS) (MAPA, 2009). It was used 400 seeds in each treatment, divided into eight replications. The seeds were placed on two sheets of paper "germitest", covering them with another sheet for the making of "rolls. The paper sheets were moistened with solutions in the ratio of 2.5 times the mass of dry paper, and the rolls wrapped in plastic bags and kept in camera like BOD at 25 ± 2 °C.

The evaluated variables were: the first count at four days after sowing (DAS), the percentage of normal and abnormal seedlings, hard seeds and dead at seven DAS, where it was adopted the following criteria: in the evaluation of the first count were computed all the seeds that presented some tissue (germinated) both root to the aerial part. Normal seedlings: seedlings that had root and aerial part in good development (> 25 mm). Abnormal seedlings: with weak development, or with aerial or root part less than 25 mm; Hard seeds: seeds that remained without absorbing water and dead seeds: the seeds that do not germinate at the end of the test, that are not hard, and usually, are softened, attacked by microorganisms and show no sign of germination.

After the evaluation of germination, it was performed a seedling development analysis, separating random 80 normal seedlings per treatment. Initially the seedlings were divided at aerial and radicular part, where it was determined the length using a graduated ruler (cm). Following, the seedlings were placed in bags of paper and dried at a temperature of 45 ± 2 °C until the constant weight be obtained from biomass, to determine the dry mass of aerial part (DMAP) and roots (DMR) (g)

The obtained data were submitted to analysis of variance by F test ($p \leq 0.05\%$). When there was a significant interaction between the factors, it was proceeded a multiple comparison of average by Tukey test ($p \leq 0.05\%$) for the different hybrids and adjustment regressions for water potential. For this, analyzes were performed using the *Statistical Analysis System* software - SAS 8.0.

Results and Discussion

From the results of analysis of variance, it was found a significant interaction between the sources of variation for all the variables. It was obtained normal seedlings only until the osmotic potential of -0.4 MPa, while lower potential completely inhibited the formation of well-developed seedlings (Table 2). This result disagrees with those obtained by Conus et al. (2009) evaluating different osmotic potentials to the same culture, where the authors concluded that the reduction in the osmotic potential did not affect the germination of the crop. One of the factors that may have been decisive for the difference in results between the studies is that the authors used only BRS 3003 cultivar, which may have high resistance to water stress. Mortele et al. (2006) studying the resistance of popcorn cultivars observed cultivars with germination capacity only until the potential of -0.6 MPa, corroborating with the present study.

In the osmotic potential of 0.0 MPa, all the hybrids had high germination (> 90%) (Table 2), confirming with Kappes et al. (2010), who studying five different hybrids observed high percentage of normal seedlings (> 95%). The hybrid AG 8011 presented the highest normal seedlings values (97.26%), but did not differ from AG 5011 (96%). The lower value of normal seedlings in this potential was observed for the hybrid AS 1551 with 90.76%, what indicates that the seeds used in the study under optimum conditions had less germination potential than the other hybrids.

The hybrid AG 8011 that presented the highest percentage of normal seedlings in the potential 0.0 Mpa, was the one that had the highest sensitivity to water stress presenting a percentage of only 84.5% of normal seedlings in the osmotic potential of -0.4 MPa (Table 3). Other hybrids did not differ and had percentage values between 88 and 92%. These attributes evaluated in germination and vigor tests, the percentage of normal seedlings is the most important, because it represents the portion of plants that effectively showed ability to reach its production potential.

Table 2. Average values of normal and abnormal seedlings, dead and hard seeds of maize hybrids submitted to different osmotic potentials

Hybrids	Osmotic potential (MPa)				
	0.00	-0.40	-0.80	-1.20	-1.60
			Normal seedlings (%)		
CD 384	93.76 BC	91.26 A	0.00 NS	0.00 NS	0.00 NS
AG 8011	97.26 A	84.50 B	0.00	0.00	0.00
AS 1551	90.76 C	89.76 A	0.00	0.00	0.00
AG 5011	96.00 AB	88.50 A	0.00	0.00	0.00
			Abnormal seedlings (%)		
CD 384	5.76 NS	8.50 NS	98.50 NS	90.26 NS	61.14 B
AG 8011	2.50	12.52	98.00	91.72	45.76 C
AS 1551	5.26	6.50	99.50	96.50	92.26 AB
AG 5011	2.00	9.76	96.00	89.76	66.50 B
			Hard seeds (%)		
CD 384	0.26 NS	0.00 NS	1.50 NS	9.50 A	38.28 B
AG 8011	0.26	1.26	1.76	5.76 AB	49.26 A
AS 1551	1.26	1.76	1.26	2.26 A	6.00 D
AG 5011	0.76	1.00	2.50	6.50 A	27.26 C
			Dead seeds (%)		
CD 384	0.26 B	0.26 NS	0.00 NS	0.26 C	0.58 B
AG 8011	0.00 B	1.00	0.26	2.76 AB	5.00 A
AS 1551	2.76 A	2.00	1.76	1.00 BC	1.76 B
AG 5011	1.26 B	0.76	1.76	3.76 A	6.26 A

Means followed by the same letter in the column do not differ by Tukey test at the level of 5% of error probability.

In the percentage of abnormal seedlings there was significant difference among treatments only in the osmotic potential of -1.6 MPa, in which the hybrid AS 1551 was the one that presented the highest value of 92.22% (Table 2), higher value from the others that were 66.50%, 61.14% and 45.76% for hybrids AG 5011, CD 384 and AG 8011, respectively. The variable percentage of abnormal seedlings in lower potential is extremely important to detect which hybrid has the higher resistance, because even the seedling not being considered normal, it was able to issue radicle and/or aerial part, proving to be more tolerant than the others without embryonic growth. Kappes et al. (2010) were also able to differentiate the resistance among the genotypes for variable abnormal seedlings only in lower potential of -0.9 and -1.2 MPa.

The hard seeds, that showed no water absorption after 7 days of sowing, were significant differences among the hybrids in the potentials below of -1.2 MPa (Table 2), and in both potential (-1.2 and -1.6) the hybrid AG 8011 showed the major averages. Among the other hybrids, again the AS 1551 had the lowest value of hard seeds (only 6%). Water is involved directly or indirectly in all metabolic stages of germination (URSULINO et al., 2016). For embryonic growth of endosperm seeds it is necessary a water content close to 40% (CARVALHO and NAKAGAWA, 2012). This way, it can be inferred that in the higher potentials where the water stress was severe, the seeds could not absorb the amount of water required, and consequently did not present germination.

For dead seeds, in other words, the seeds that soaked water, but were attacked by microorganisms resulting in the non-germinative development, the hybrid AS 1551 was the one that presented the highest average at 0.0 MPa. The other hybrids were not significantly different. For the osmotic potential of -1.2 MPa, the AG 5011 was the one that presented the highest value, since the lowest percentage of dead seeds occurred in the hybrid CD 384. In the potential of -1.6 MPa, the hybrids AG 5011 (6.35%) and AG 8011 (5%) showed the highest values, differentiating from the others.

For the variable percentage of normal seedlings, according to the water potential in each hybrid, it was observed a similar behavior to the hybrids, and all of them increased as quadratic function associated with osmotic potential (Figure 1a). For the variable percentage of abnormal seedlings, the results are practically the opposite of those described previously (Figure 1b), and it was obtained the percentage of low values (near 0%) in the higher osmotic potentials, and to the extent that it was decreased the osmotic potential, the percentage of abnormal seedlings raised to values close to 100%. The hybrids had third degree equation adjusted for this variable, in other words, the values tended to rise until a maximum value that occurred near the osmotic potential of -1.2 MPa, from that point, the values showed substantial decrease. This reduction of the number of abnormal seedlings from the potential of -1.2 MPa in all the hybrids is due to high values of hard seeds at potential of -1.6 MPa.

At analysis of hard seeds proportion, it’s possible to visualize that hybrids had behavior which followed quadratic function, and by the reduction of osmotic potential there was a tendency to increase the percentage of hard seeds (Figure 1c). When the seeds are submitted to very negative potentials (> 1.2 MPa), the water present in the substrate is maintained with the salt, and even the seed can be dehydrated, so that the seed cannot initiate the germination process (RIBEIRO et al., 2001). For the variable dead seeds, reduction of osmotic potential did not affected hybrids CD 384 and AG 5011, and the average values were of 0.27 and 2.76%, (Figure 1d). Lower osmotic potential resulted at quadratic decrease of dead seeds from hybrid AS 1551. On the other hand, dead seeds from hybrid AG 8011 increase linearly according to the reduction of osmotic potential.

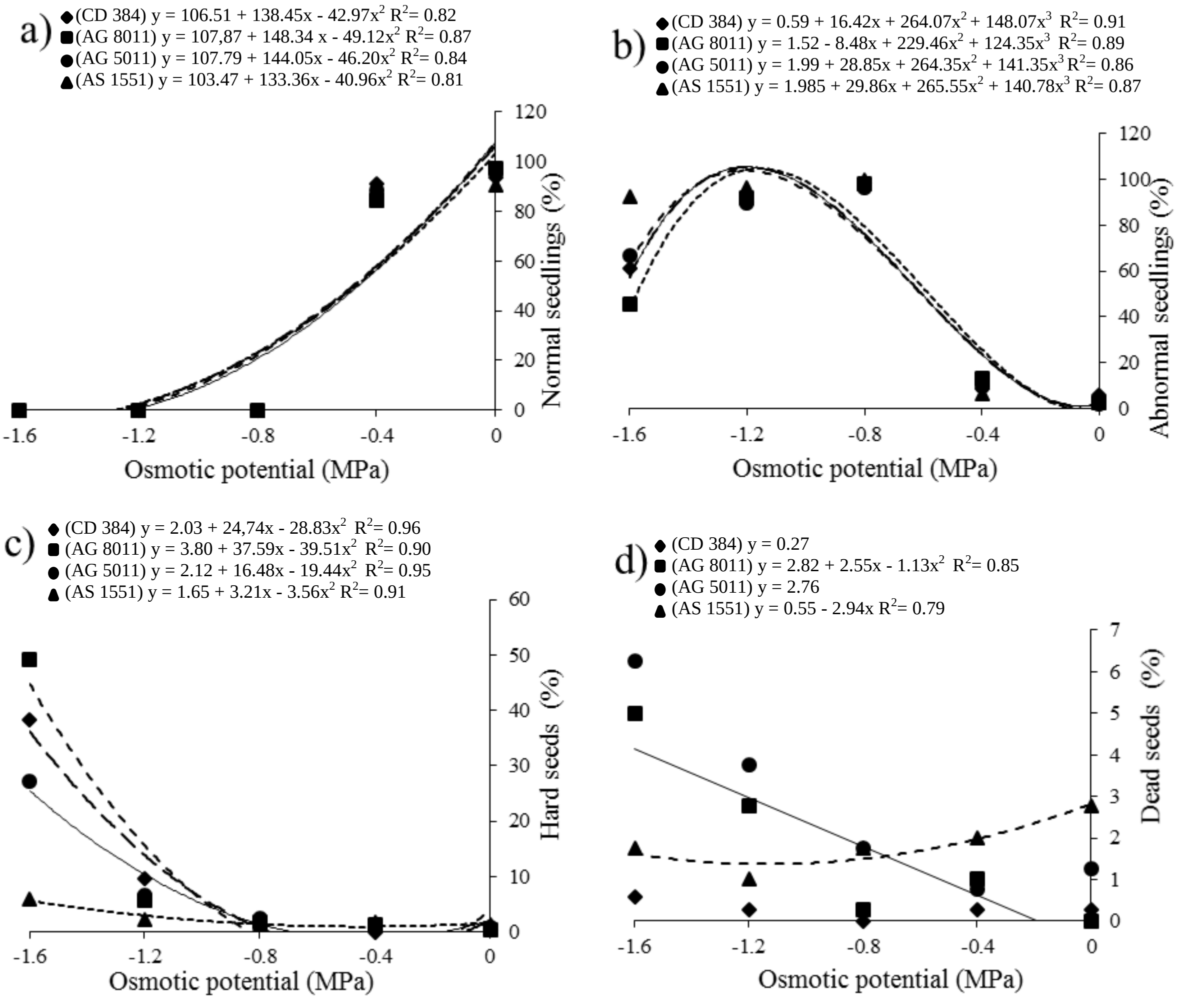

Figure 1. Regression analysis of percentages of normal seedlings (a), abnormal seedlings (b), hard seeds (c) and dead seeds (d) of maize hybrids submitted to different osmotic potentials.

Percentage of normal seedling is more efficiently evaluated at first count after germination. The result represents as an indicative to the vigor of seeds (NAKAGAWA, 1999). Here, we noted a statistical difference among the hybrids in the osmotic potential of -1.2 MPa, where the hybrid AS 1551 was the one that presented the highest value 53.50%, which did not differ from the AG 5011 with 50.26% (Table 3), and, low vigor (26.75%) was found in the hybrid AG 8011. In a study conducted by Kappes et al. (2010) evaluating the resistance of 6 maize genotypes, did not observe differences for this variable, however, the average results in each osmotic potential were similar to the present study.

The height of aerial part of seedlings in the potential of 0.0 MPa was higher in the hybrid AG 8011, where it was achieved an average length of 96.7 mm. The lowest values are observed for the hybrids CD 384 and AS 1551 (65.0 and 49.5 mm, respectively). In the osmotic potential of -0.4 MPa, the height of aerial part was affected, mainly in hybrids that had presented the higher values in the potential of 0.0 MPa. The higher length at -0.4 MPa occurred to the hybrid CD 384 (43.1 mm), and lower value was found to the AG 5011 (27.3 mm). Conus et al. (2009) also observed reduction in the height of aerial part under decrease of osmotic potential.

Table 3. Average values of first count, height of aerial part, root length, aerial and root dry mass of maize hybrids submitted to different osmotic potentials

Hybrids	Osmotic potential (MPa)				
	0.00	-0.40	-0.80	-1.20	-1.60
			First count (%)		
CD 384	99.26 NS	99.50 NS	93.26 NS	39.76 B	0.00 NS
AG 8011	99.76	98.00	92.50	26.75 C	0.00
AS 1551	96.25	95.76	96.26	53.50 A	0.00
AG 5011	97.76	97.76	91.72	50.26 A	0.00
			Height of aerial part (mm)		
CD 384	65.00 B	43.10 A	0.00 NS	0.00 NS	0.00 NS
AG 8011	96.70 A	41.60 A	0.00	0.00	0.00
AS 1551	49.50 C	36.70 B	0.00	0.00	0.00
AG 5011	70.80 B	27.30 C	0.00	0.00	0.00
			Root length (cm)		
CD 384	15.37 B	8.65 C	0.00 NS	0.00 NS	0.00 NS
AG 8011	15.29 B	9.70 B	0.00	0.00	0.00
AS 1551	20.80 A	12.72 A	0.00	0.00	0.00
AG 5011	14.67 B	10.32 B	0.00	0.00	0.00
			Aerial dry mass (g)		
CD 384	0.147 A	0.081 A	0.00 NS	0.00 NS	0.00 NS
AG 8011	0.147 A	0.061 B	0.00	0.00	0.00
AS 1551	0.116 C	0.082 A	0.00	0.00	0.00
AG 5011	0.134 B	0.054 C	0.00	0.00	0.00
			Root dry mass (g)		
CD 384	0.962 B	1.128 B	0.00 NS	0.00 NS	0.00 NS
AG 8011	0.804 C	0.811 C	0.00	0.00	0.00
AS 1551	0.578 D	0.782 C	0.00	0.00	0.00
AG 5011	1.155 A	1.258 A	0.00	0.00	0.00

Means followed by the same letter in the column do not differ by Tukey test at the level of 5% of error probability.

In osmotic potential of 0.0 and -0.4 MPa, the hybrid AS 1551 presented higher root length (20.80 and 12.72 cm, respectively, Table 3). On the other hand, lower values were observed for the hybrid AG 5011 (14.67 cm at 0.0 MPa) and the hybrid CD 384 (8.65 cm at -0.4 MPa). The dry mass of aerial part of seedlings in the potential of 0.0 MPa was lower in the hybrid AS 1551 (0.116 g plant^{-1}). However, at -0.4 MPa this showed the highest value (0.082 g plant^{-1}), demonstrating higher tolerance.

In the potential of -0.4 MPa, the hybrid that had the lower dry mass production of aerial part was the AG 5011 (0.054 g plant^{-1}). The production of root dry mass at 0.0 and -0.4 MPa were higher in hybrid AG 5011 (1.155 g plant^{-1} and 1.258 g plant^{-1}, respectively). The lower root dry mass was obtained in hybrid AS 1551 with 0.782 g plant^{-1}. We observed that reduction of osmotic potential resulted in increase of root dry mass due to development of secondary roots. It's about a plant defense mode, where it searches to explore a larger surface area in the demand of water. These results corroborate with those reported by Pereira et al. (2013) where they affirm that hypocotyl of the seedlings is more affected by water stress than the root system.

It is possible visualize that first count of hybrids decreased as a quadratic proportion according to the reduction of osmotic potential (Figure 2a), and their behavior are similar to each other (except for AS 1551 that showed higher values, especially at -1.2 MPa). The hybrids responded in a quadratic form to the reduction of osmotic potential for the variable height of aerial part (Figure 2b), it can be highlighted in this variable, the hybrid AG 8011 in the potential of 0.0 MPa by the highest value. However, at -0.4 MPa, the AG 8011 not achieved apparent difference related to the others. This result shows that the hybrid AG8011 presented a high vigor only in the absence of water stress. For root length, the hybrids showed second-degree equations, deserving highlight for the hybrid AS 1551 that showed higher values when compared to the other (Figure 2c). Kappes et al. (2010) also noted high reduction of root length with the reduction of osmotic potential, reducing around 4 cm from the potential of -0.3 to 0.0 MPa. The water deficit causes a reduction in the germination velocity of seeds, as well as, produces influences on physiological and biochemical processes, thus all the biomass production of the seedlings are influenced negatively (URSULINO et al., 2016).

For aerial dry mass production of seedlings, the results were quite similar to those mentioned above, characterized by a quadratic function to the reduction of osmotic potential (Figure 2d). The root dry mass of hybrids genotypes showed third-degree equations, and with the application of the deficit of -0.4 MPa there was an increase in dry mass produced. In -0.8 MPa condition, there was no production of normal seedlings, because the seeds not absorbed the amount of water required to development adequate root and aerial part. The curves that showed higher increase in root dry mass due to the reduction of the osmotic potential were the hybrids AG 5011 and CD 384 (Figure 2e). This increase in the root dry mass production in the potential of -0.4 in relation to the control treatment, occurs due to the plants under water stress conditions direct their reserves to the root system in order to maintain their development and provide a higher water and nutrients absorption (TAIZ and ZEIGER, 2009). However, when the water stress becomes too severe, the plants can not maintain a normal metabolism activity, that leads to decrease of growth rate.

The present study confirms the findings described by other authors such as Andrioli et al. (2009), Kappes et al. (2010), Silva et al. (2016) in which they contacted different indexes of water stress tolerance among maize hybrids. The tolerance to the stress is dependent on the genotypic characteristics of the hybrid, highlighting the characteristics of density and length of the root system, index of leaf area, leaf slope, plant height and physiological regulation (ANDRIOLI et al. 2009). Therefore, tolerant hybrids identification is fundamental for many places where corn is cultivated under common water stress condition (VAZ-DE-MELO et al., 2012). As well as, concomitantly using other management practices as seed with high vigor and emergence (SILVA et al., 2016), to sow the crop on the dates recommended for the region, to deposit the seeds at a

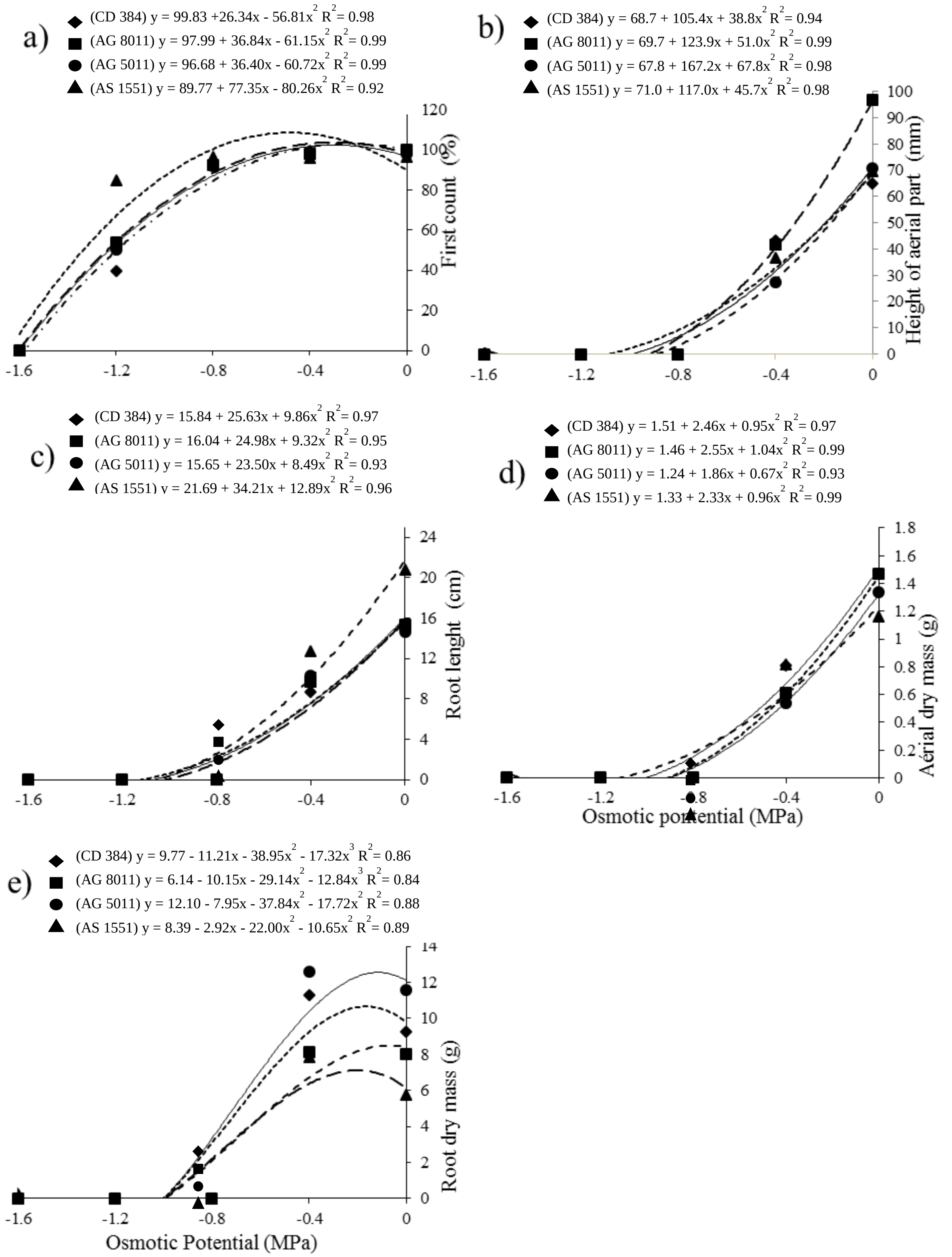

Figure 2. Regression analysis of the first count values (a), height of aerial part (AP) (b), root length (c), aerial dry mass and root (e) of maize hybrids submitted to different osmotic potentials.

suitable depth according to the soil moisture (WEIRICH NETO and LOPES, 2012). The maintenance of a good layer of straw on the soil is fundamental to reduce the variation of soil temperature and to promote that the moisture present remains for a longer period of time (FURLANI et al., 2008). All these techniques may assist to achieve an appropriate percentage of germination and emergence.

Conclusion

The osmotic potential of -0.8 MPa completely inhibited the formation of normal seedlings in all the hybrids. In this osmotic potential, the seeds not absorbed the amount of water required to development adequate root and aerial part.

The reduction of osmotic potential from 0.0 to -0.4 MPa decreased the emergence of normal seedlings and production of aerial dry mass. However, increased the root dry mass production due to development of secondary roots, where the seedling seek to explore a larger surface area in the search by water.

In descending order of classification, the hybrids that presented higher tolerance to water and saline stress are AS 1551> AG 5011> CD 384> AG 8011. In this sense, should be given preference to the hybrids more tolerant in soils with high salinity and/or high probability to occurrence of water stress.

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