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# Corn Seedlings Have Tolerance to Water Deficit

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**Abstract**

The present work aimed to evaluate the germination and seedling growth of two corn cultivars under water deficit conditions induced with manitol solutions, associated with doses of humic substances. The experiment was carried out at the Seed Laboratory of the Federal University of Tocantins - UFT, in Gurupi, Tocantins. Seeds of two cultivars were used: M274 and UFT Cerrado; and the product based on humic substances: Humix. For each cultivar, the factorial experiment (5×3) was mounted in a completely randomized design, and the treatments were composed of five osmotic potentials, with three doses of Humix. Germination percentage, primary root and epicotyl length, seedling dry mass and absolute growth rate were evaluated. The results were submitted to the F test by analysis of variance with probability of 5%, and when the effect of the doses was significant, the data were submitted to regression analysis. It is concluded that the biostimulant Humix had no expressive effect on germination and that the reduction of osmotic potential does not affect germination itself, but affects seedling growth, as osmotic potential becomes more negative.

***Keywords:*** Cerrado; corn; biostimulant; drought; germination; seedlings.

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## 1. Introduction

Corn (*Zea mays* L.) is highlighted as one of the leading cereals grown and consumed in the world, providing widely used products for human, animal and raw material to the industry [1,2]. Brazil is the 3rd largest producer of corn and 2nd largest exporter of grain [3]. According to data from the Brazilian Supply Company - CONAB [4], the harvest of the 2018/19 should exceed 95.2 mi of ton, concentrating most of the production in the second harvest. Corn cultivation at this time increases risks due to the lower rainfall index in the most critical stages of culture, and in the Tocantins it is common to occur these abiotic stresses, which negatively interfere in the establishment and development of culture [5,6,7]. Water plays an essential role in germination, as it initiates the germination process through soaking, which will trigger metabolic activation resulting in embryo growth [8]. The occurrence of water deficit at the beginning of germination can affect it visibly. For the management of agriculture, different alternatives of cultivation can be adopted to enable a more productive agriculture, but less impactful to the environment [9], among these alternatives the use of biostimulants in seeds, which estimulate the germination [10,11]. With emphasis on these, based on humic substances, which increase the number of mitosis and lateral roots of corn seedlings [12]. In view of the above, considering the importance of water for germination and the beneficial interactions between growth and the addition of humic substances, this work aimed to evaluate the germination and growth of seedlings of two maize cultivars, under conditions of water deficit associated with doses of humic bio-stimulant, and its effects on the vigor and initial development of the crop.

## 2. Material and methods

The experiment was carried out at the Laboratory of Plant Ecophysiology and Seed Analysis Laboratory of the Federal University of Tocantins (UFT), Gurupi University Campus. The manitol solutions ($C6H14O6$) were used to obtain the desired osmotic potential, the concentrations used were calculated by Van't Hoff's formula, according to the equation:

$$Yos = R * T * C \qquad (1)$$

Where:

Yos=Osmotic potential (MPa);

R=general Constant of perfect gases (0,082 $MPa.dm^3.mol.^{-1}.K^{-1}$);

T=temperature (°K);

and C=concentration ($mol.dm^{-3}$).

The evaluations were carried out with seeds of two corn cultivars: UFT Cerrado (2019) of the company Pioneiro Ltda., and the cultivar M274 (2017) of the company Pioneira®. In the treatment of seeds, Humix biostimulants were used, it was composed of: (Humic acid: 25%, Fulvic acid: 5%, Potassium: 3% and pH 8.5).

To evaluate the response of seeds under water stress associated with the use of humic substances, for each cultivar, the was conducted factorial experiment (5x3) in the entirely randomized design. The treatments were composed by the combination of five osmotic potentials – 0 (control), -0.25, -0.50, -0.75 and -1.0 MPa – simulated with manitol solutions, and three doses of the humic substance, Humix – 0.0 (control), 1.0 ml and 2.0 ml/100 seeds. The Humix was applied directly on the seeds within a beaker, with the use of a graduated pipette. After application, the beaker containing the seeds was stirred for 1 minute, aiming to homogenize the product on the seeds. The germination test was performed using four replicates with 50 seeds for each treatment. The seeds were sown between three sheets of germitest paper, using 2.5 times the weight of dry paper for soaking with distilled water, containing pre-established amounts of manitol, to provide the determinated levels of osmotic potential, and one treatment only with distilled water, the control. Rollers tied with rubber bands were made, which were placed in plastic bags, positioned vertically inside the germinator, with a constant temperature of 25 ° C, remaining there for seven days. The evaluations were made on the fourth and seventh day. On the fourth day, the first germination count was performed for the percentage of germinated seedlings and for vigor evaluation. And on the seventh day, the percentage of normal seedlings and abnormal seedlings was computed. To evaluate the seedling length and vigor one test was conducted with four repetitions (ten corn seeds one each) [13]. The seeds were manually distributed in the middle of sheets of germitest paper, and placed to germinate under the same conditions as the germination test. The length evaluation was done with a graduated ruler in the fourth and seventh days, where only seedlings that had above 0.5 cm of primary root were measured. The mean data were expressed in cm seedling$^{-1}$. This data was used for determining the absolute growth rate (RGA) [14], using the following formula:

$$RGA = \frac{(W2-W1)}{(t2-t1)} = g.day \qquad (2)$$

Being:

RGA= absolute growth rate

W1 e W2 are the lengths of the fourth and seventh day

t1 e t2 are the time in days.

To determine the dry seedling mass, after seven days, the substrate was removed, seed debris were removed. The roots and aerial parts were divided and packed in paper bags and taken to dry in a drying Chamber with forced air circulation, under a temperature of 60°C per 24h. The bags were removed from the drying Chamber and the masses were weighed in scale with an accuracy of 0.0001 g [15,16]. Thus, determining the weight (g) of dry primary root mass, epicotyl dry mass and total dry mass. The data obtained were submitted to the F test by analysis of variance at 5% probability, and when significant, they were submitted to regression analysis. The analyzes were performed using the R software. Because it was a quantitative factor, the average of osmotic potential levels was compared by regression analysis ($p < 0.05$). SigmaPlot software was used for the preparation of the graphics.

## 3. Results

The results of the mass of a thousand kernels and the tetrazolium test are described in table 1. It was observed that cultivars present different values on average for the mass of a thousand kernels. The cultivar M274 had a higher weight because of it larger seed size. The tetrazolium test both cultivars obtained the same percentage of viable seeds.

**Table 1:** Thousand-seed weight in grams and tetrazolium test in percentage from two corn cultivars. Gurupi, Tocantins, 2019.

| Cultivars | One thousand kernels | Tetrazolium test (%) | |
|---|---|---|---|
| | | Viable | Infeazible |
| M274 | 415,9 | 88 | 12 |
| UFT Cerrado | 265,3 | 88 | 12 |

For the germination percentage the cultivar M274 presents a significant difference ($p<0.05$) for the interaction Osmotic Potential and Humix (OPxH) at the Tukey test level 5%. The cultivar UFT Cerrado has a significant effect ($p<0.01$) for osmotic potential and ($p<0.05$) for Humix doses. It was observed that in the OPxH interaction of the cultivar M274, the effect of Humix within each osmotic potential obtained a significant difference only in the control (Figure 1A), with lower germination rate in the dose of 2 ml Humix and with 1 ml in the osmotic potential -1.0 MPa resulted in a lower germination rate, compared to the others. The effect of osmotic potentials for Humix doses obtained a significant difference with the dose of 2 ml of Humix and the osmotic potential at the dose of 1 ml.

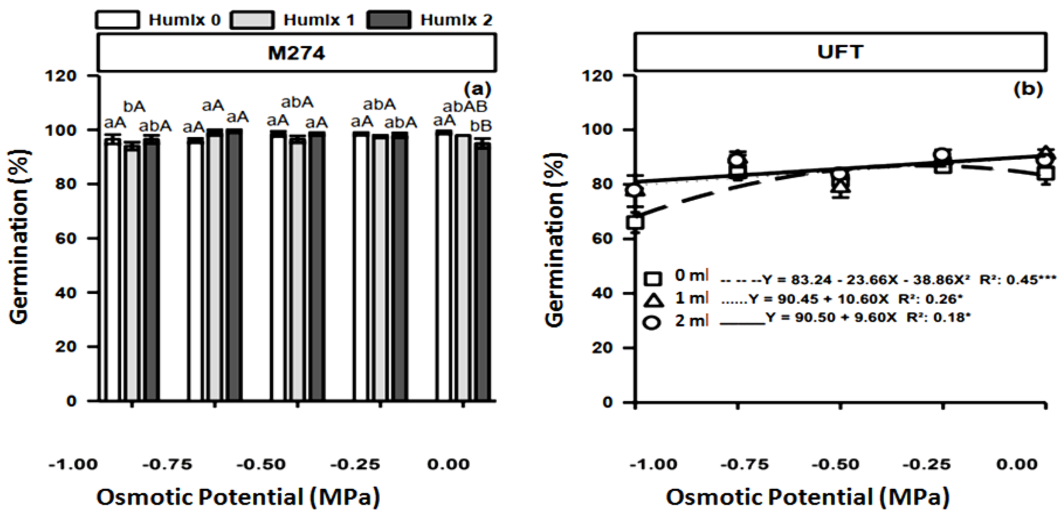


**Figure 1AB:** Germination percentage from two corn cultivars (M274 and UFT Cerrado) in different osmotic potentials and doses of Humix. Gurupi, Tocantins, 2019. Uppercase letters compare the effect of Humix within each osmotic potential, while lowercase letters compare the effect of osmotic potential for each dose of Humix (Tukey test 5%).

There was no significant decrease in germination percentage for the isolated osmotic potential levels. According to Kappes and his colleagues [17], corn is tolerant to water deficit, admitting potential of up to -1.2 MPa, without a large decrease in germination percentage. For the cultivar UFT Cerrado, there was a significant difference ($p<0.05$) between Humix doses, it is observed that seeds treated with Humix obtained a higher germination percentage compared to the control (figure 1B). The reduction in the germination rate of the cultivar UFT Cerrado as the osmotic potential decreases was observed. This can be attributed to the lower mass of a thousand kernels (Table 1) at the beginning of seedling development, seeds with smaller masses may, therefore, have less reserve which results in less force [17].

A significant difference ($p<0.01$) due to osmotic potential in the percentages of normal seedlings was observed (Figure A and B). The number of normal seedlings decreases and the number of abnormal seedlings increases with the decrease of osmotic potential (figure 2). There was no significant difference with Humix doses tested.

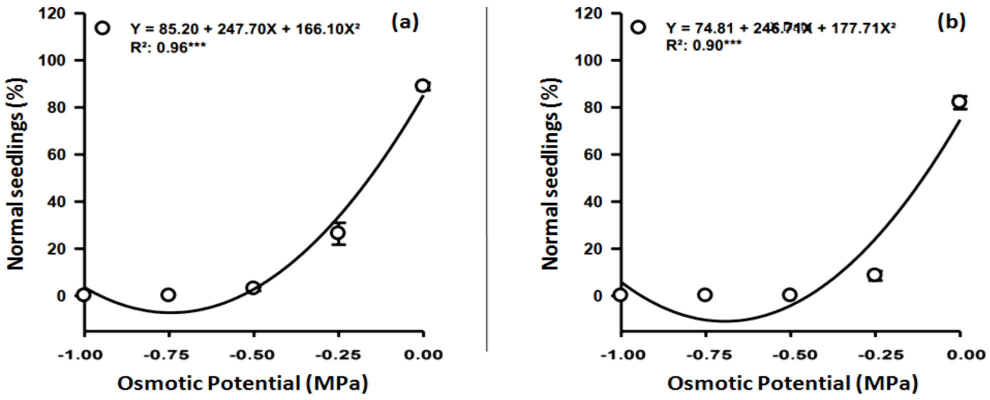


**Figure 2:** Percentage of normal seedlings from two corn cultivars (M274 and UFT Cerrado) in different osmotic potentials and doses of Humix.

Osmotic potentials below -0.50 MPa reduced abruptly the germination of normal seedlings of cultivars, presenting values close to zero. The normal seedling formation was observed up to the potential -0.25 MPa. Avilla and his colleagues (2007) also found a reduction in germination (normal seedlings) of canola seeds subjected to water stress, corroborating with the results observed in this work. In a comparison of germination, normal and abnormal seedlings (data not shown), it can be affirmed that water stress abruptly affected seedling development, more than the percentage of germination itself. Teixeira and his colleagues [18] working with crambe seeds, found the formation of normal seedlings up to the potential of -0.6 MPa, emphasizing that the seeds obtained primary root up to the potential of -1.0 MPa, but there was no formation of normal seedlings. The figure 3 represents the initial length means (4 days) of the primary root and epicotyl and aerial part. It is noted that as the osmotic potential decreases, the growth of the primary root and epicotyl for cultivars is also reduced. The cultivar UFT Cerrado presented lower epicotyl length, it is linked with the lower seed weight (Table 1).

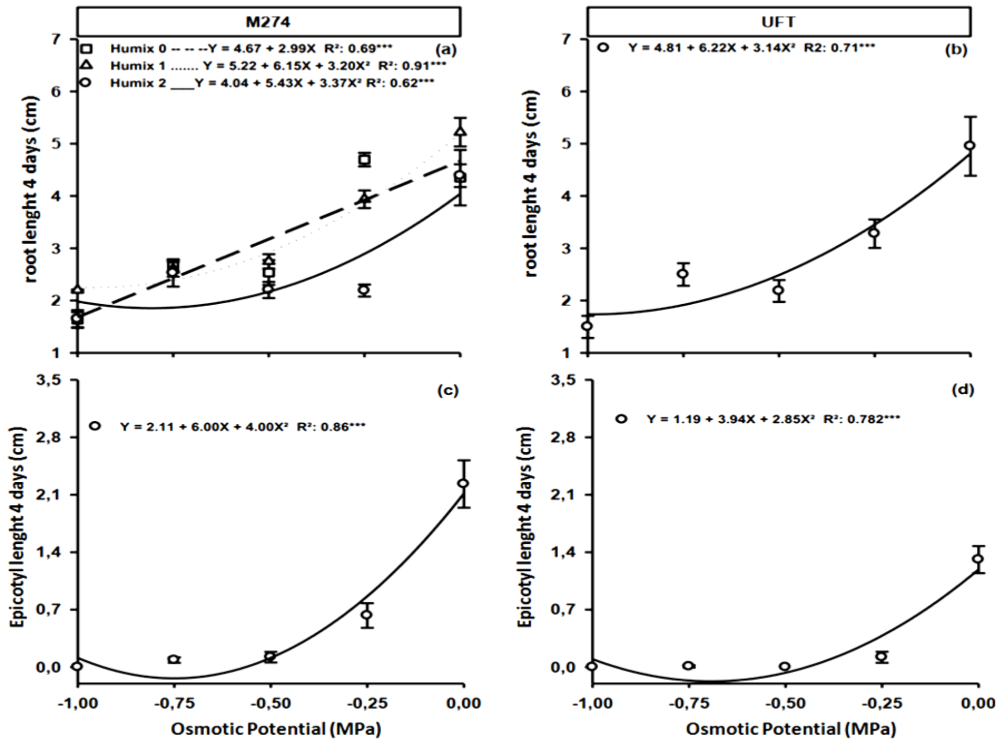


**Figure 3:** Initial length (4 days) of primary root (AB) and epicotyl (CD) for cultivars of (M274 and UFT Cerrado) in different osmotic potentials associated with doses of Humix.

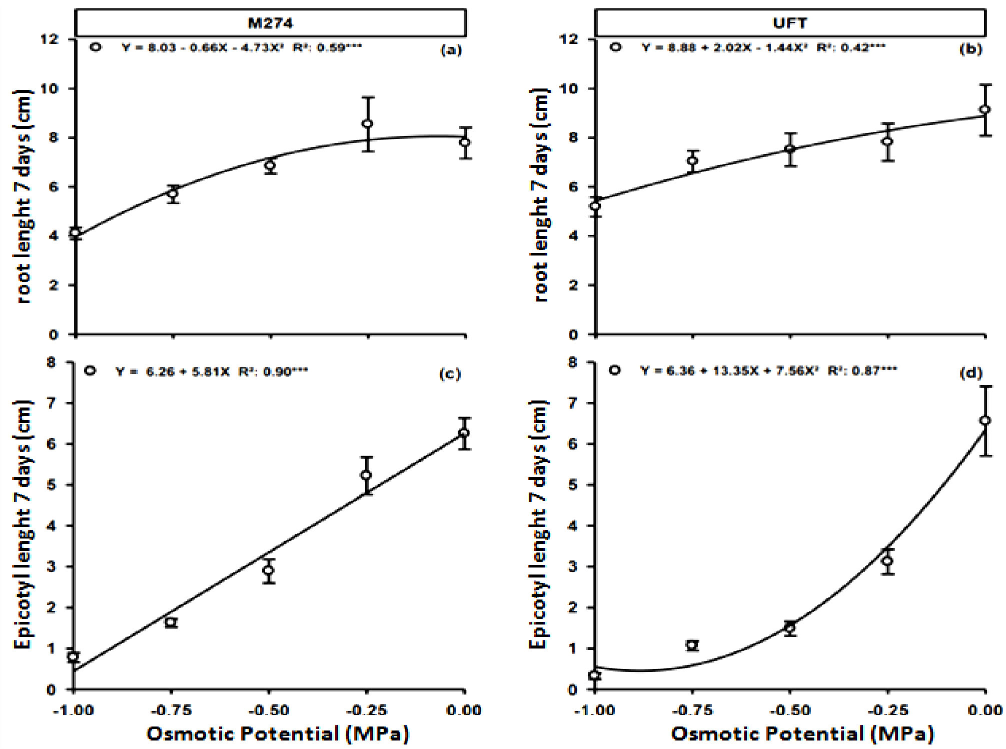


**Figure 4:** Final length (7days) of the primary root (AB) and epicotyl (CD) for corn cultivars (M274 and UFT Cerrado) in different osmotic potentials associated with doses of Biostimulant Humix.

The cultivar M274 showed a significant difference ($p<0.05$) for Humix doses tested, it is verified that seeds treated with biostimulant, at the dose of 1ml/100 seeds obtained an initial growth of the primary roots higher in relation to the control. The cultivar UFT Cerrado did not differ significantly between its doses (Fig.3AB). Both cultivars demonstrate, on average, the same values of primary root length. (Fig.3CD) Comparing the initial growth of primary roots and epicotyl, it is observed that when the osmotic potential decrease, it is a trend of greater primary root growth in relation to the epicotyl, reaching values equal to zero growing by epicotyl in potentials smaller than 0.50 MPa. This effect is because seedlings submitted to negative osmotic potentials levels present greater development of the root system in relation to the air part of the plant due to explore the soil at greater depths to find water [19,20].

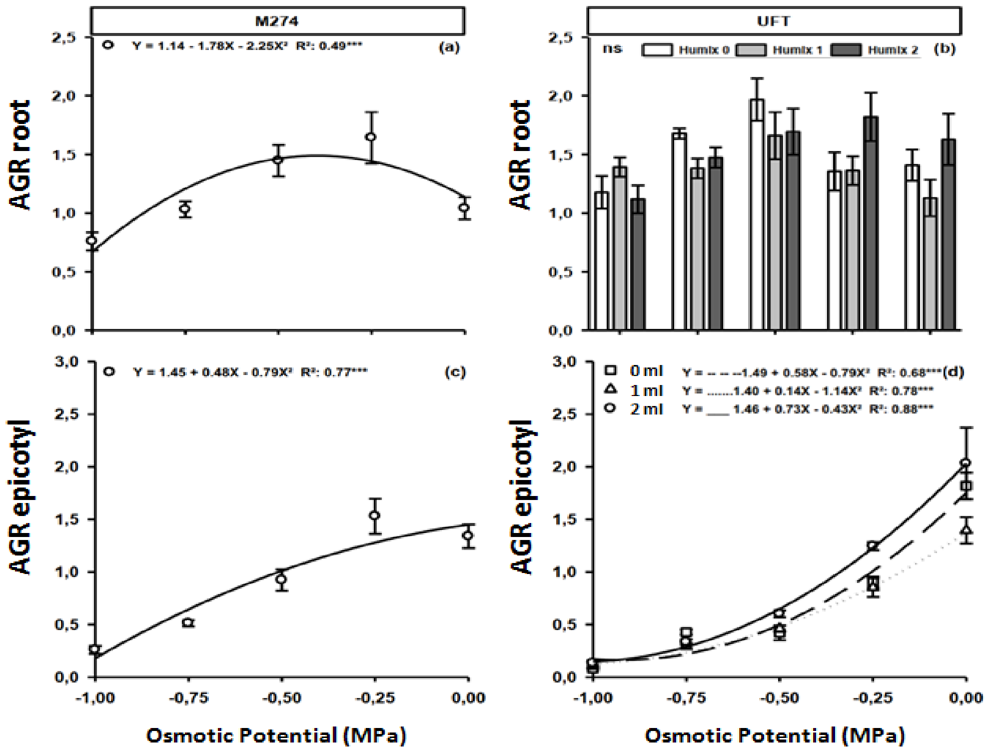


**Figure 5:** Absolute growth rate of root (AB) and epicotyl (CD) of two corn cultivars (M274 and UFT Cerrado) in different osmotic potentials associated with the dose of Humix.

There was a significant difference ($p<0.01$) for the length of the primary roots and the epicotyl for two cultivars in relation to the osmotic potentials tested (Fig. 4 ABCD), but there was no significant difference for Humix doses. The biostimulant Humix doses, regardless of osmotic potential, not influenced the growth of the primary roots and the epicotyl of seedlings at the final length. Similar results were found by Silva and his colleagues [21], who evaluated the effects of biostimulant via seed treatment on the initial development of cotton seedlings, and Kolling and his colleagues [22] who evaluated the effects on corn seedlings, did also not find significant differences in root and the epicotyl length. Related to the primary root growth, the cultivar UFT Cerrado has good performance in lower osmotic potential levels and also the increase of growth the primary roots are positive ($\beta$=+2.02). In cultivar M274 that increase is negative ($\beta$= -0.66) (Fig. 4AB). Similar results were observed for the epicotyl length in the final evaluation (Fig. 4CD). Considering these variables is possible to say the cultivar UFT Cerrado probably will have a better answer water stress in the field. The results for the absolute growth rate of corn seedlings submitted to water stress are shown in Figure 5. A significant difference ($p<0.01$) was observed for both cultivars with osmotic potentials. The cultivar M274 showed no significant difference for humix doses, but presented a higher absolute growth rate of primary root and epicotyl at the osmotic potential level of -0.25 MPa (Figure 5AC).

The cultivar UFT Cerrado obtained a significant difference ($p<0.05$) for humix doses, and the Interaction OPxH in the absolute growth rate (AGR) to epicotyl lenght. (Fig. 5D) It is noted that the use of biostimulant Humix, obtains a higher epicotyl growth rate compared to the control, without water stress (Figure 5D). For this cultivar, no significant difference was observed between osmotic potentials or between Humix doses for root AGR (fig. 5B).

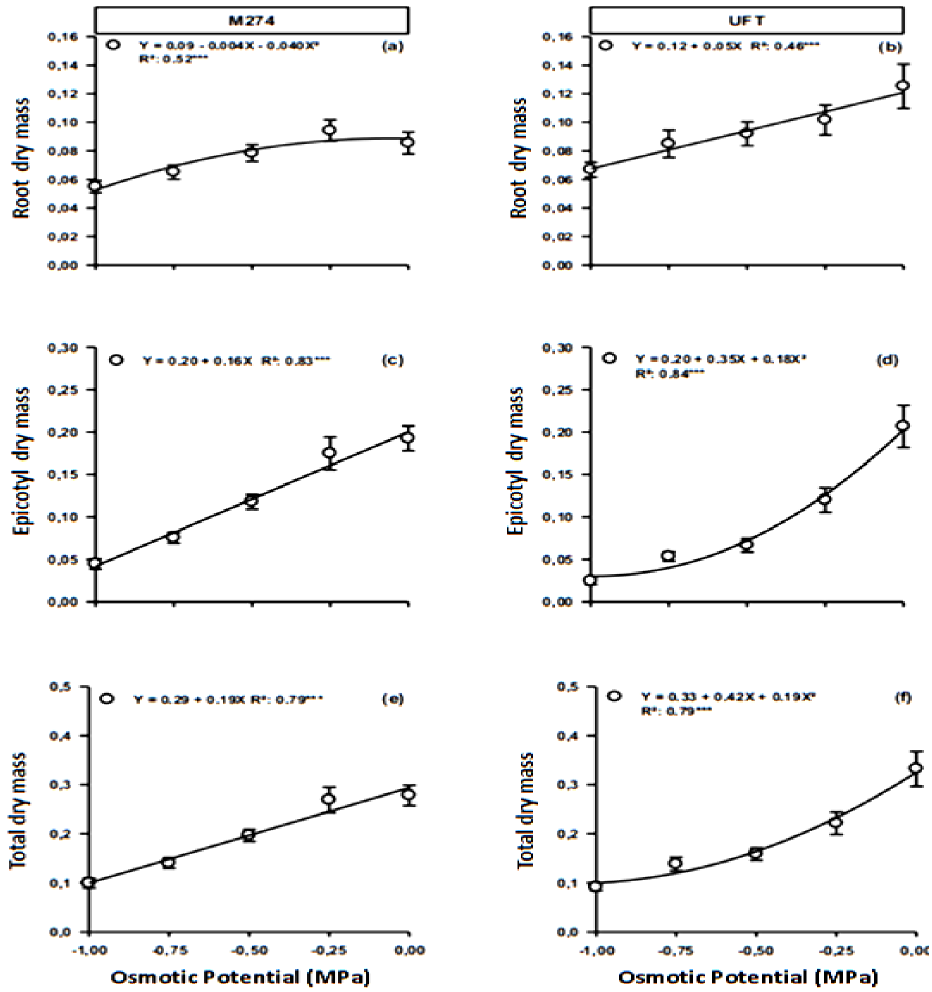


**Figure 6:** Primary root dry mass (AB), epicotyl (CD) and total (EF) dry mass of two corn cultivars (M274 and UFT Cerrado) submitted to different osmotic potentials associated with the dose of Humix

There was a significant effect ($p<0.01$) only for the osmotic potentials for two cultivars. The cultivar M274 presented better values of dry root mass in osmotic potential -0.25 MPa comparing with the control, but in the general UFT Cerrado presented a better increase rate ($\beta$=+0.05) and higher root dry mass (Fig. 6A,B). Epicotyl dry mass answer was similar for both cultivars (Fig. 6 CD). It is noted that the total dry mass of seedlings decreases with the reduction of osmotic potentials. Similar results were obtained by [23] and Tatto and his colleagues [24] in soybeans, Mortele and his colleagues [25] in corn, and Nunes and his colleagues [26] in sunflower, both found that there was a progressive reduction in the dry mass of seedlings as it decreases the osmotic potential of the solution. According to [27] and Rosseto and his colleagues [28], the reduction of dry biomass, as well as seedling growth, can be explained by the decrease in seed metabolism, due to lower availability of water for the digestion of reserves and translocation of metabolized products. As observed in this study, as osmotic potential becomes more negative there is a reduction in seed performance, becoming critical for seedling development. The results reveal the importance of studies of the water potentials of the substrate considered critical for the germination and development of seeds, thus being able to indicate the minimum and optimal conditions of water potential in the soil for germination and seedling growth.

## 4. Conclusions

Humix biostimulant had no significant effect on the germination variable. Furthermore, the reduction in osmotic potential did not affect the germination of the seeds, but it did affect the growth of the seedlings and consequently the establishment of an ideal initial crop population in the field. The Cerrado UFT cultivar showed the best response to the hydric stress induced by the osmotic potential, presenting a higher absolute growth rate of the epicotyl.

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