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# **Research Note**

# Initial development of maize in response to different periods of seed immersion in humic acid (HA)

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## Abstract

Currently, there is a higher demand for improved seeds, associated with the treatment with fungicides, insecticides, and recently inoculants and biostimulants. Treating corn seeds with humic acid (HA) triggers physiological and metabolic processes that can increase productivity. However, the effects of seed immersion time on plant performance are unknown. Therefore, this study aimed to test different periods of corn seed immersion in HA (10 mmol L<sup>-1</sup> of C) and the effects on seedling development characteristics. The treatments consisted of soaking seeds for 00:00; 00:17; 01:00; 02:00; 04:00; 08:00; 16:00 and 24:00 hours. The experimental design was completely randomized, with four replicates. At 30 days after sowing, two plants per replicate were collected to determine the shoot, root, and total fresh and dry masses of the plants. The plants grown from seeds soaked for 8 hours had the strongest response among the plants analyzed in this study.

Keywords: Zea mays; Biostimulant; Plant production.

#### Introduction

The projected 2019-2020 corn harvest indicates that Brazil will become the second largest exporter of this cereal grain worldwide (Food and Agriculture Organization of the United Nations [FAO], 2019). However, the average corn yield of the country (4.90 t/ha) is lower than that of the global average (5.65 t/ha) (Contini et al., 2019). To increase the current productivity levels and reduce production costs, new technologies have been incorporated into production systems, including biostimulants (Baldotto et al., 2019).

Among the most explored biostimulants in agriculture, humic acids (HA) are complexes that promote plant hormonal balance, favoring the expression of plant metabolism and increasing the use efficiency of light, water, and nutrients (Canellas et., 2014).

HA primarily affects plants through biostimulating the mechanisms of substances similar to growth regulators, such as auxins (Trevisan et al., 2010). Auxins are key regulators of many aspects of plant growth and development, including cell division, cell elongation, differentiation, tropisms, apical dominance, senescence, abscission, and flowering (Teale et al., 2006).

Treating corn seeds with biostimulants, such as HA, is a promising strategy for increasing productivity. This method has been increasingly used and has been gaining relevance in recent years. However, the effects of seed immersion time on plant performance are unknown. Baldotto et al. (2019) observed a  $\sim$  15% increase in corn yield when immersing seeds in HA for 16 hours. Lonhienne et al. (2017), immersing seeds corn in humic substances for 5 minutes found no effects on germination after 5 days of incubation, however observed an increase in root (100%), shoot (54%) area and seedling

vigour (100%). Eyheraguibel et al. (2008), after treating corn seeds with humic substances on germitest paper, also found no effects on germination, except for an increase in radicle length.

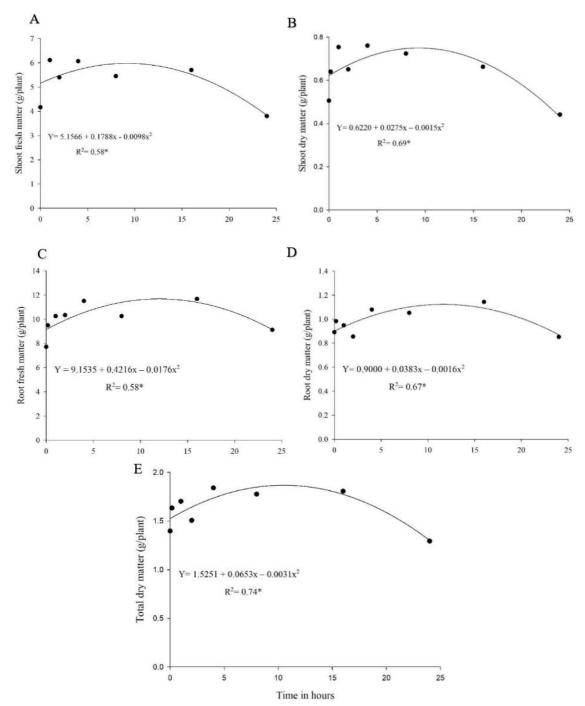
The hypothesis of this study is that the contact time of seeds with the HA solution can affect plant performance, because the effects of HA on plants are partially derived from hormonal changes (Trevisan et al., 2010). In addition, the seed immersion time may preclude mechanical sowing. The high moisture content of seeds impairs their distribution in implements, causing planting failures (Mantovani et al., 1999).

Based on the above, this study aimed to assess the effects of different periods of corn seed immersion in humic acid solutions on the initial seedling development.

#### **Results and Discussion**

Significant (p < 0.05) differences were found for all growth variables evaluated when fitting the regression equations (Figure 1). The quadratic curves of the study variables, as a function of time (Figure 1), suggest that the initial growth of corn seedlings depends upon the seed immersion time in a solution of humic acids (HA) isolated from cattle manure at a concentration of 10 mmol L<sup>-1</sup> of C.

The regression model adjusted for the growth variables suggests time-dependent response in seeds treatment with HA (Figure 1). The treatment of seeds with HA was favorable for the growth characteristics evaluated. The findings of this study are in line with others that have demonstrated increased initial corn growth. After seed immersion in a



**Figure 1.** Shoot fresh matter (A), shoot dry matter (B), root fresh matter (C), root dry matter (D), and total dry matter (E) of AG 1051 corn plants grown for 30 days after different seed immersion times (00:00; 00:17; 01:00; 60 02:00; 04:00; 08:00; 16:00 and 24:00 hours) a solution of humic acids isolated from cattle manure at a concentration of 10 mmol L<sup>-1</sup> of C. \* indicates significance at the 5% probability level, according to the F test.

solution containing HA isolated from cattle manure for 16 hours, Melo et al. (2015), observed increase in the corn growth (45 days). Similarly, by treating the seeds for 16 hours, Baldotto et al. (2019) successfully reproduced the effects observed in a greenhouse in their field study. In this study, the strongest response occurred when the seeds were soaked for approximately 8 hours, and prolonging this time to 16 hours stabilized the effects. However, longer periods tended to inhibit growth (Figure 1).

Other authors have observed that longer contact times with seed bioregulators limit the growth-promoting effects (maximum point). Therefore, exceeding a certain limit, seed bioregulators have negative physiological effects on plant growth and development. Santos et al. (2013) observed that soaking sunflower seeds for 4 hours with the bioregulator Stimulate<sup>®</sup> (0.009% kinetin; 0.005% gibberellic acid and 0.005% indolebutyric acid) increases seedling germination and vigor, and that prolonged periods of seed immersion increased the percentage of abnormal seedlings. The authors attributed the positive effects to hormonal changes in the seeds and the negative effects to the anaerobic conditions to which the seeds were exposed and the phytotoxic effects of the biostimulant.

Considering the results, corn seeds should not be soaked for longer than 16 hours. Marcos Filho (2015) suggested that, when hydrating dry seeds, the repair mechanisms of cellular components naturally damaged by dehydration act during seed maturation. The anaerobic conditions caused by prolonged seed immersion (over 16 hours) may have impaired the mechanisms of membrane repair.

The inhibitory effect on plant growth when seeds were emerged for over 16 hours also suggests that a hormonal imbalance may have occurred, given the extended seed exposure to the biostimulant. Taiz et al. (2015) postulate that the exogenous application of hormones, such as auxins, may increase growth. However, depending upon the hormone concentration or exposure time, they may have inhibitory or phytotoxic effects. Alves Netto et al. (2019) noted that increasing the concentration and time of corn seed exposure to the bioregulator Stimulate<sup>®</sup> decreased radicle development, which was also attributed to hormonal disorders.

The results from this study support the hypothesis that HA primarily have hormonal effects on plants (Trevisan et al., 2010). Melo et al. (2015) observed a quadratic variation when treating corn seeds with increasing doses of HA isolated from cattle manure. The authors suggested that initial seedling growth depended upon the applied doses, attributing this observation to the hormonal effects of HA on seeds.

The variation in the initial growth of corn seedlings as a function of the seed immersion time in humic acid solution indicates that the contact time with HA should be calibrated for each variety and/or management system. The moisture content of seeds treated for 8 hours (maximum response time of growth variables) (Figure 1) also precludes mechanical sowing with the current implements available. Therefore, higher doses of HA and different periods of seed exposure should be tested, to generate data enabling the use of HA solutions in corn seed treatments at a commercial scale.

#### **Materials and Methods**

#### Experimental design

The experiment was conducted in greenhouse at the Federal University of Viçosa, Florestal campus - Minas Gerais (19º52'16.3"S, 44º25'26.1"W; 750 meters altitude). Corn seeds of the cultivar AG 1051 were immersed, for either 00:00; 00:17; 01:00; 02:00; 04:00; 08:00; 16:00 and 24:00 hours, in HA isolated from cattle manure at a concentration of 10 mmol L<sup>-1</sup> of C. The HA were isolated according to the recommendations from the International Humic Substances Society (IHSS, 2017). The seeds were immersed in plastic cups, containing enough solution to submerge them. Five seeds were sown in pots containing 1 dm<sup>3</sup> of soil corrected and fertilized according to Ribeiro et al. (1999). After emergence, three plants were removed and the other two were left for the remainder of the experiment. The pots were irrigated daily to preserve the field capacity between 80 and 100%. The experimental design was completely randomized, with four replicates.

#### **Experimental evaluations**

At 30 days after sowing, the following variables were determined: shoot (SFM) and root (RFM) fresh matter weight. Before drying, the fresh matter of the shoots and roots were placed in previously labeled paper bags and transferred to a convection air oven, under a constant temperature of  $60 \, ^{\circ}C$ , where they remained until reaching a constant weight. After removing the samples from the greenhouse, the shoot (SDM) and root (RDM) dry matter weights were determined, and the

total dry matter (MST) was calculated as the sum of the SDM and RDM.

### Statistical analysis

The variables were analyzed according to their distribution through Shapiro Wilk's test for normality. In sequence, the results were subjected to an analysis of variance using R Software version 4.0.0. In addition, polynomial regression curves were fitted to measure the effects of different seed immersion times in HA.

#### Conclusions

We concluded that periods of corn seed immersion in humic acids (10 mmol  $L^{-1}$  of C) isolated from cattle manure longer than 8 hours and shorter than 16 hours positively affect the quantitative characteristics of seedlings.

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