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Chemical pregerminative promoters in *Zea mays* L. seed

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ABSTRACT

Objective: Plant life begins at germination. Stimulating germination with chemical methods can be advantageous. The pregerminative treatment of four promoters was determined in two maize genotypes (G1, G2).

Design/methodology/approach: Sixty seeds per Petri dish were used as experimental unit with three repetitions, organized in a completely randomized 2×4 factorial design. Two factors were taken into consideration: A) Genotypes (G1, G2); and B) four pregerminative promoters. The genotypes were Antelope G1 and yellow Antelope G2. Meanwhile, the pregerminative promoters were salicylic acid ($C_7H_6O_3$) (SA), citrulline ($C_6H_{13}N_3O_3$) (CI), humic substances derived from leonardite (HS), and tap water (TW), in 1000-ppm concentrations. The following variables were evaluated: germination percentage (GP)/days^{-1}, radicle diameter (RD), radicle length (RL), and number of lateral seminal roots (NSR). An analysis of variance and Tukey tests ($\alpha \leq 0.05$) were performed.

Results: The germination promoters were highly significant in both genotypes, as well as during the promoter-genotype interaction. G1 and G2 means showed a higher growth and development for humic substances (HS) during germination in the NSR.

Study Limitations/Implications: Germination can be inhibited, if the promoters are overweighted.

Findings/Conclusions: The best genotype and germination promoter (G2) had a 94% effectiveness and HS at 1000 ppm. CI and SA registered the lowest GP.

Keywords: Seed, biostimulant, seminal roots.

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INTRODUCTION

Maize (*Zea mays* L.) is the third most consumed food by humans and animals worldwide, after wheat and rice; therefore, it is important to study the physiological mechanisms of germination, growth, and development (Assem, 2015). Germination includes a series of processes —from seed imbibition to radicle emergence (Doria, 2010). Some of the visual indicators used to assess these processes include: radicle emergence, coleoptile emergence, mesocotyl elongation, and emergence of lateral seminal root (Sáenz and Cassab, 2021). Visual indicators have

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different sequences and are influenced by temperature, germination, and emergence. They were recorded earlier in warm soils conditions than in cold ones (Nielsen, 2016); however, the seed germination and vigor of maize is related to its metabolic activity during the first hours (El-Maarouf-Bouteau H., 2022).

Several pregerminative treatments —such as biostimulants and phytohormones— are used to promote germination. Biostimulants include humic substances, microorganisms, plant and animal products, microbial inoculations, etc. which improve germination and root growth (Calvo *et al.*, 2014; Hasanuzzaman and Responses, 2019). Root growth and water absorption are enhanced by humic substances (HS) (Olk *et al.*, 2018, Canellas *et al.*, 2015; du Jardin, 2015). Furthermore, Sarropoulou *et al.* (2016) mention that metabolism and transport of citrulline has been observed in *Arabidopsis thaliana* and *Cucurbitaceae* crops. In studies performed with cherry slides, there were direct effects on *in vitro* rooting. Phytohormones do not only provide control and protect germination under stress processes, they also enhance the tolerance of germination under low-temperature environments (Steven *et al.*, 2019). Dawood *et al.* (2019) and Raskin (1992) mention that salicylic acid (SA) is a phenolic compound that acts as an endogenous regulator of physiological and biochemical processes in the germination and seedling stages. SA increases root size in maize seedlings and, at low concentrations, increases germination in beans (Haas *et al.*, 2015, Rodríguez-Larramedi *et al.*, 2017, Dawood *et al.*, 2019).

Several maize species found in the La Laguna region are affected by genotype and environment. In the last 30 years, climate change has caused a persistent statistical deviation with extreme temperature variations and limited water resources (Inzunza-López *et al.*, 2011; CONAGUA, 2022). The interaction of two maize genotypes and promoters will favor germination (Ortiz-Timoteo *et al.*, 2018). This study was developed to assess four germination promoters in two maize genotypes.

MATERIALS AND METHODS

Study area

The experiment was conducted in October 2019, at Universidad Autónoma Agraria Antonio Narro-Unidad Laguna, located in the City of Torreón, Coahuila (25° 31' 11" N and 103° 25' 57" W, 1123 m.a.s.l.). According to the Köppen climate classification (as modified by García (2004)), the area has a BWh, warm desert climate, with a maximum temperature of 40 °C, minimum of 6 °C, and average rainfall of 250 mm.

Establishment of the experiment

Sixty maize (*Zea mays* L.) seeds of genotype (G1) white Antelope and genotype (G2) yellow Antelope Y were used per Petri dish. Damaged seeds were discarded. Subsequently, they were disinfected using 10 mL/L of sodium hypochlorite at 1% and left to settle for 15 minutes. Then they were washed with drinking water (FAO, 2001) and the promoters were sprayed three consecutive days, along with SA, CI, HS, and leonardite by-products in 1000 Mg kg^{-1} concentrations and running water as control solution (Table 1), until the germination percentage was observed.

Table 1. Physical and chemical characteristics of the tap water used in the experiment under greenhouse conditions.

Chemical elements	Mg kg^{-1}
Iron Fe^{3}	0
Zinc Zn^{+}	0.02
Copper Cu^{+}	0.03
Manganese Mn^{+}	0.01
Boron B^{+}	0.99
Sodium Na^{+}	120
Potassium K^{+}	13
Calcio Ca^{+}	288
Magnesium Mg^{+}	29
Nitrates NO_3	23.03
Phosphate phosphorus	0.08
Phosphorus Diacino H_2PO_4	0.25
Sulfate of SO_4	643.6
Carbonates CO_3	0
Bicarbonates HCO_3	170.83
Chlorides Cl	198.52
Physical parameters	
pH	7.80
Electrical conductivity mS/cm	2.21
Sodium absorption ratio	1.8
Interchangeable sodium (%)	0.38

Variables assessed

The following variables were measured: germination percentage (GP) (Moreno *et al.*, 2018; Caroca *et al.*, 2016), radicle diameter (RD), radicle length (RL), and number of lateral seminal roots (NSR). Regarding germination percentage (GP), seed with a ≥2 mm radicle length were considered to be germinated seeds. PG was calculated using the following formula (Caroca *et al.*, 2016):

$$GP = \left[\left(No.\ of\ germinated\ seeds\right) / \left(No.\ of\ sown\ seeds\right)\right] \times 100$$

A vernier Truper CALDI-6MP with a 30 cm rule was used to estimate the radicle diameter (RD) and the radicle length (RL), and the number of secondary seminal roots was counted.

Statistical analysis

A completely randomized 2×4 factorial design with 3 replications was used. Each Petri dish represented an experimental unit. The study factors were the G1 and G2 genotypes, factor A and its promoters, Factor B (SA, CI, HS, and TW). An analysis of variance and

a comparison of means (Tukey, P<0.05) were performed, both using the SAS statistical package (SAS, 2009).

RESULTS AND DISCUSSION

Germination behavior

The analysis of variance (Table 2) shows the effects of genotype (G1, G2) and of pregerminative treatments (promoters) on the germination percentage, where it was highly significant for the three days of % GP1, % GP2, and % GP3 ($p<0.0001$) with ($p\leq0.05$). On their part, the pregerminatives (promoters) were significant only for % GP1 and % GP3 days, while they were significant for all three days of the G1 and G2 × promoters interaction. A coefficient of variation of 41, 8, and 6% was recorded for % GP1, % GP2 and % GP3 days, respectively. In the case of GP, RD, RL, and NSR, they were highly significant only for G1 and G2 × promoters and for RD and RL with one ($p<0.0001$) and only for promoters in RL ($p<0.0001$), and between G1 and G2 with NSR ($p<0.0001$). Moreover, they were significant for G1 and G2 in GP (0.0001), RL ($p<0.0028$), and promoters in NSR ($p<0.0019$), as well as for G1 and G2 × promoters interaction for GP (0.0005) ($p<0.0007$) with coefficients of variation of 6% for GP, RD, and RL and 17% for NSR.

Behavior of the germination variable means of genotypes 1 and 2

The behavior of means with respect to genotypes G1 and G2 germination in the double-entry Table 3 on day GP1 was statistically different for G2. Meanwhile, G1 was statistically different on days GP2 and GP3, with respect to G1 and G2. However, with CI, HS, and AL, these promoters had outstanding results on day GP1, while with SA and HA they behaved similarly on day GP2 and day GP3, respectively. On their part, GP matched G1 and HS promoters. Regarding the RD variable, the mean is statistically the same for genotypes G1, G2, as well as for the promoters. In the case of RL, the mean between genotypes G1 and G2 belonged to G2 and, in the case of the promoters, it matched CI and

Table 2. Analysis of variance (mean squares and statistical significance) for the germination variables of days 1, 2, and 3 (% GP1, % GP2, % GP3), radicle diameter (RD), radicle length (RL), and lateral seminal roots (SR).

VS	GL	Germinal variables						
		% GP1	% GP2	% GP3	GP	RD	RL	NSR
Genotypes	1	782**	630**	551**	551**	0.010	21*	5**
Promoter	3	42	36	99	99	0.012	57**	0.685*
Genotypes * Promoter	3	40*	105*	228*	228*	0.124**	53**	0.852*
Error	16	7	9	22	22	0.007	1.69	0.087
Total	23	1142	1203	1894	1894	0.536	379	11
CV (%)		41	8	6	6	6	6	17

VS=variation sources; Germination percentage of day 1, 2, and 3=% GP1, % GP2, % GP3; germination percentage=GP; radicle diameter=RD; radicle length=RL; and number of lateral seminal roots=SR. ** Highly significant * Significant, Coefficient of variation (CV) (Tukey, 0.05).

TW. In relation to NSR, the G1 and G2 genotypes mean belonged to G2 and, in the case of promoters, it was HS.

Figure 1 shows the behavior of the germination percentages of genotypes G1 and G2 on days 1, 2, and 3, when no significant difference was recorded for G1 with regard to promoters on day 1; meanwhile, in the case of G2, CI, HS, and TW promoters were statistically the same. Moreover, on day 2, promoters were the same for both genotypes. However, on day 3, genotypes G1 and G2 were the same only for the HS promoter, while CI had the lowest result.

Germination percentage

The findings of this experiment suggest that the highest germination percentage for varieties belongs to G1 and that, when they were exposed to germination promoters, HS achieved a higher germination for both G1 and G2 (Table 2 and 3). These results match the findings of authors such as Rodrigues *et al.* (2017) and Šerá and Novák (2011), who assessed the effect of treating maize seeds with a humic acid-based commercial product and found that humic acid promotes a greater growth of seedlings, in addition to greater dry

Table 3. Behavior of G1 and G2 means during germination.

Variable		SA	CI	HS	TW	Average
GP1	G1	0.66 a	1.33 a	0.66 a	0.66 a	0.83 b
	G2	4.66 b	13.66 a	16.76 a	14.00 a	12.25 a
	Average	2.66 b	7.50 a	8.66 a	7.33 a	
GP2	G1	38.66 b	42.33 ab	47.33 a	40.66 b	42.25 a
	G2	39.66 a	25.66 c	30.66 bc	32.00 b	32.00 b
	Average	39.16 a	34.00 b	39.00 ab	36.33 ab	
GP3	G1	78.33 c	87.66 ab	94.33 a	82.00 bc	85.58 a
	G2	83.00 a	65.00 b	78.00 a	77.66 a	76.00 b
	Average	80.83 ab	76.33 b	86.16 a	79.83 ab	
GP	G1	78.33 c	87.66 ab	94.33 a	82.00 bc	85.58 a
	G 2	83.00 a	65.00 b	78.00 a	77.66 a	76.00 b
	Average	80.83 ab	76.33 b	86.16 a	79.83 ab	
RD	G1	1.36 b	1.43 b	1.66 a	1.46 b	1.48 a
	G2	1.46 a	1.60 a	1.20 b	1.50 a	1.44 a
	Media	1.41 a	1.51 a	1.43 a	1.48 a	
RL	G1	14.73 b	25.03 a	24.13 a	25.96 a	22.46 b
	G2	24.8 b	25.46 ab	19.93 c	27.16 a	24.34 a
	Average	19.77 c	25.25 a	22.03 b	26.56 a	
NSR	G1	0.50 c	1.36 b	2.20 a	1.23 b	1.32 b
	G2	2.26 a	2.03 a	2.20 a	2.43 a	2.23 a
	Average	1.38 b	1.70 b	2.20 a	1.83 ab	

Germination percentage of days 1, 2, and 3=% GP1, % GP2, and % GP3; Genotype 1 and 2=G1 and G2; Salicylic Acid=SA; Citrulline=CI; Humic Substances=HS; Tap water=TW; Germination percentage=GP; Radicle diameter=RD; Radicle length=RL; and Number of lateral seminal roots=SR.; Means with the same letter in each column (a, b) or per row (a, b, and c) are not different (Tukey, 0.05).

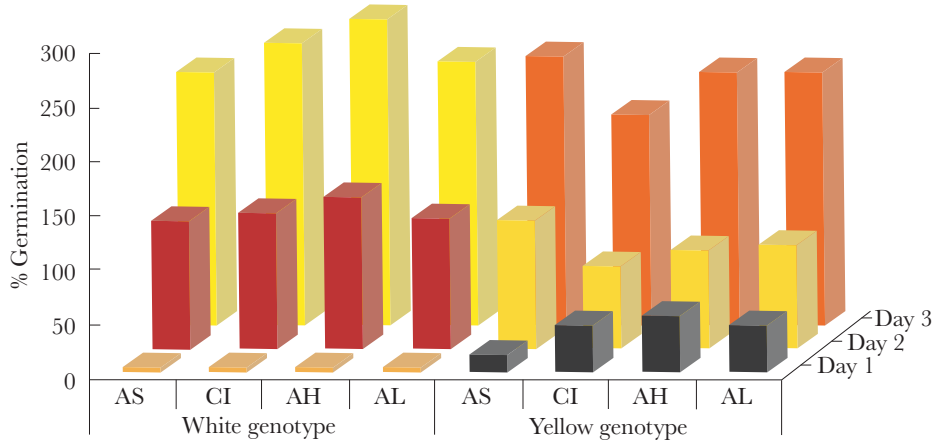

Figure 1. Germination behavior of G1 and G2 with regard to promoters, salicylic acid (SA), citrulline (CI), humic acid (HA), and tap water (TW), throughout days 1, 2, and 3.

mass of maize sprouts. These results have a positive influence on the emergence rate index, up to doses of 158 mL 100 kg^{-1} seeds. For their part, Šerá and Novák (2011) used seeds of Lamb's Quarters (*Chenopodium album* agg.) and determined that the main differences in germination and length of sprouts occurred during the first days of the experiment.

Radicle diameter

No statistical differences in radicle diameter were found between either genotypes G1 and G2 (Table 3). El-Mergawi (2019) and Rodríguez-Larramedi *et al.* (2017) mention that high doses (20 mM) with salicylic acid can inhibit germination, affecting growth and root development. For their part, Pertuit *et al.* (2001) mentioned that high doses of leonardite-based humic substances also inhibited root growth and sprouts.

Radicle length

Table 3 shows a greater development of the radicle length in G2 and, in the case of germination promoters, citrulline (CI) and tap water (TW) were the same. Sarropoulou *et al.* (2016) carried out studies with cherry slides which had direct effects on *in vitro* rooting, as well as in the accumulation of proline in the leaves and roots.

Number of seminal roots

The largest number of seminal roots shows that the promoter with humic substances (HS) is statistically different from the other promoters; in the case of genotypes, it belonged to G2 (Table 3). Authors like Olk *et al.* (2018) mention that humic acids favor root development. Qin and Leskovar (2020) mention that, in studies made with tomato, pepper, lettuce and watermelon cultivars, the biomass of roots, leaves, and sprouts was higher after transplantation, improving the growth rate at a faster rate.

CONCLUSIONS

When pregerminative treatments were applied and a promoter × genotype interaction was carried out, germination was highly significant in promoters and between two

genotypes. For the GP and NSR variables, the leonardite-based HS obtained a greater response for germination and root development (in the case of G1 and G2), while it decreased for GP, RL, and NSR when salicylic acid and citrulline were used. Therefore, we conclude that the HS pregerminative treatment with G2 favors germination by 94%, when exposed to a 1000 mg kg^{-1} stimulus. This increases the number of seminal roots and the germination percentage in three days. This result will be of interest for further studies where pregerminative treatments can be manipulated to ensure higher GP on certified seeds.

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