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Chemical treatments in maize seeds to improve germination in acidic soils

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ABSTRACT

Objective: To evaluate the effect of different germination promoters on three maize genotypes grown in Dystric Cambisol soils, since germination problems are linked to latency and restrict agronomic management.

Design/Methodology/Approach: We conducted an experiment at the Instituto Tecnológico Superior de Juan Rodríguez Clara using a split-plot design with a factorial treatment arrangement. The large plot contained genotypes (GEN) G1=MS-405, G2=Arlequin, and G3=MS-404; while the small one comprised promoter (PROMO) HS=humic substance, CI=citrulline, and SA=salicylic acid. We evaluated the following variables: germination speed (GS), emergence percentage (EMERG), stem and leaf volume (S&LV), root volume (RV), chlorophyll (CHL), secondary roots (SECR), stem diameter (DMT), number of leaves (NL), foliar area (FA), root length (RL), and plant height (PH). Then, we conducted a variance analysis and Tukey's tests ($\alpha \leq 0.05$).

Results: For each promoter, we observed main effects in EMERG, CHL, and PH for CI; S&LV, NL, FA, and PH for HS; and RL for SA. In genotypes G2 and G3, variables GS, EMERG, NL, and PH were statistically equivalent, DMT varied only in G2, and there were no statistical differences for S&LV, RV, CHL, SECR, FA, and RL. We observed some simple effects in combinations with CI: GS and PH varied in G3, EMERG in G2 and G3, CHL in G1 and G3, DMT in G1 and G2, and S&LV in G2.

Study limitations/Implications: Soaking corn for one hour in the solution and weighing the correct amount properly are required, since weighing too much may inhibit germination.

Findings/Conclusions: Promoter CI at a dose of 1,000 ppm accelerates the emergence speed of genotypes G2 and G3 in acidic soils.

Keywords: Abscisic acid; Amino acids; Krebs cycle; Emergence.

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INTRODUCTION

Knowing the germination processes is crucial, mainly because of the alterations brought by climate change. When the embryo develops, the endosperm transfers nutrients (Debouza *et al.*, 2021), and once the mitochondria become active, they enter the glycolysis process and then the Krebs cycle (Ali and Elozeiri, 2017). With the testa rupture, cellular respiration increases, and reserves, glycosides, proteins, lipids, hormones,

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enzymes, carbohydrates, and phytins are mobilized in the endosperm (Gómez-Maqueo and Gamboa-de Buen, 2016).

Using pre-germination chemical treatments stimulates germination and decreases abscisic acid (ABA) (Bautista-Rodríguez *et al.*, 2017). Moreover, these treatments remove tissues such as coleorhiza (Anh *et al.*, 2019). At the beginning of the Krebs cycle, using (non-protein) amino acids such as citrulline (CI) (Song *et al.*, 2020) improves nitrogen balance and eliminates hydroxyl radicals. With biostimulants such as humic substances (HS) (Veobides-Amador *et al.*, 2018), the transport of organic and inorganic molecules and the absorption of proteins, amino acids, and ionic nutrients becomes more efficient (Popa *et al.*, 2022). These same processes can be balanced with hormones such as gibberellin (GA) (Tuan *et al.*, 2018) or with salicylic acid (SA). The latter reduces oxidative damage (ROS) due to excess or intoxication (Huang *et al.*, 2021).

Mexico has areas of tropical climate, where soils are acidic. In the state of Veracruz, in municipalities such as Juan Rodríguez Clara and Isla, soils contain scarce Dystric Cambisol organic matter, which limits crop productivity (Tosquy-Valle *et al.*, 2020). With pre-germination chemical treatments, promoters boost maize seed germination in acidic soils. This study assessed the effects of different germination promoters in three maize genotypes.

MATERIALS AND METHODS

We conducted the experiment on June 11, 2021 —during the spring and summer cycle—, on a zero tillage 1,800 m^2 plot at the Instituto Tecnológico Superior (ITS) Juan Rodríguez Clara. The ITS is located in the Municipality of Juan Rodríguez Clara, Veracruz, Mexico (18° 01" 6.1' N, 95° 94" 1.7' W, 133 masl). According to Köppen —modified by García (2004)— the climate is warm subhumid (AW0), with an average temperature of 24.5 °C and an average annual precipitation of 1,100 mm. The soil in the area is classified as Dystric Cambisol by the Norma Oficial Mexicana de la Clasificación Agronómica PROY-NOM-021-RECNAT-2000 (Tosquy-Valle *et al.*, 2020). We soaked the three maize genotypes in the treatments (SA=salicylic acid, CI=citrulline, HS=humic substances) for one hour at a concentration of 1,000 ppm. After sowing, we applied atrazine as herbicide.

Assessed variables

We evaluated emergence percentage (EMERG); germination speed (GS) (Sobarzo-Bernal *et al.*, 2021); stem and leaf volume (S&LV) —using the buoyancy technique by submerging the roots in a cylinder full of water and measuring the displaced liquid in cm^3 (Angulo-Castro *et al.*, 2017)—; root length (RL) in cm; foliar area (FA) in cm^3; chlorophyll (CHL); and number of leaves (NL). We measured EMERG daily (PE) starting the third day after sowing. To determine seedling germination (TE) we followed the formulas suggested by Sharma *et al.*, (2022):

$$PE = \frac{Number\ of\ emerged\ seeds \times 100}{Total\ number\ of\ seeds}$$

$$TE = \frac{N1 * T1 + N2 * T2 + N3 * T3 + ...Nn * Tn}{Total\ number\ of\ emerged\ seeds}$$

Where N=number of particles appearing consecutively and T=time elapsed from the beginning of the test to the end of the measurement period.

For S&LV, we used a 50 ml submerged tube. For RL, we used a measuring tape in cm. We counted the number of roots. To obtain the foliar area (FA), we measured leaf length and width (Berdjour *et al.*, 2020). To measure leaf CHL contents, we used a chlorophyll meter (FT Green LLC®, USOS) (Mendoza-Tafolla *et al.*, 2022).

Statistical analysis

We chose a split-plot design in a 3×3 factorial treatment arrangement with three replications. Factor A covered GEN (G1=MS-405, G2=Arlequin, and G3=MS-404), and factor B comprised PROMO (SA=salicylic acid, CI=citrulline, and HS=humic substances). With the data obtained, we conducted a variance analysis and a comparison of means using Tukey's test ($p<0.05$). To process the data, we resorted to the SAS statistical package (SAS, 2009).

RESULTS AND DISCUSSION

Emergence in acidic soils

Due to the hydration of pre-germination treatments, the seeds showed an increase in germination speed (GS) and emergence percentage (EMERG) (Escobar-Álvarez *et al.*, 2021). In the variance analysis (Table 1), we can observe high significance ($p<0.05$) for GEN in germination speed (GS), emergence percentage (EMERG), stem and leaf volume

Table 1. Variance analysis (mean squares and statistical significance) for the following variables: germination speed (GS), emergence percentage (EMERG), stem and leaf volume (S&LV), chlorophyll (CHL), secondary roots (SECR), stem diameter (DMT), number of leaves (NL), leaf area (LA), and root length (RL), plant height (PH).

		Gen	Block	B* Gen	Promo	Gen *Promo	Error	Total	CV (%)
VARIABLES	DF	2	2	4	2	4			
	GS	486.96**	14.44	58.55	7.8	97.02*	6.17	738.4	13
	EMERG	3880.07**	190.29	192.59	492.74*	880.81*	42.7	6148.96	8
	S&LV	25.35**	0.24	0.33	4.16**	2.34*	0.06	33.17	4
	CHL	283.05*	32.39	19.63	530.63*	468.79*	16	1526.62	9
	SECR	1.73	7.16	5.52	8.85	10.21	1.97	56.9	13
	DMT	0.19*	0.039	0.019	0.052*	0.20*	0.0081	0.6	13
	NL	11.87**	0.008	0.016	3.52*	6.36*	0.26	24.9	8
	LA	364.28	791.41*	1090.74*	1007.03*	3885.94*	78.13	8077.04	15
	RL	465.87*	65.34	143.27	363.81*	820.41*	21.9	2121.61	17
	PH	25.80*	0.06	1.71	8.52	4.7	0.66	48.78	10

DF: Degrees of freedom, Gen: genotype, Block, B*Gen: Bloc * Gen: Gen*promo: genotype*promoter, CV: Coefficient of variation. ** Highly significant * Significant (Tukey, 0.05).

(S&LV), and number of leaves (NL), with coefficients of variation (CV) of 13, 8, 4, and 8%, respectively. Regarding PROMO, high significance is only observed for S&LV. Moreover, there is significance ($p<0.0001$) when promoter * genotype interaction (Promoter * Gen) occurs. Dago *et al.* (2021) mention that using biostimulants promotes germination and improves stem length and diameter.

The behavior of means in the double entry table (Table 2) shows main effects of PROMO as follows: CI in variables EMERG, CHL, and PH; HS in S&LV, NL, FA, and PH; and SA in RL. Regarding germination in GEN (G1, G2, and G3), G2 and G3 were statistically equivalent in GS, EMERG, NL, and PH. Only G2 varied in DMT. There was no statistical difference for variables S&LV, RV, CHL, SECR, FA, and RL. We observed some simple effects in combinations with CI: GS and PH varied in G3, EMERG in G2 and G3, CHL in G1 and G3, DMT in G1 and G2, and S&LV in G2. Song *et al.* (2020) mention that citrulline eases nitrogen assimilation, thus increasing the content of chlorophyll and stem and leaf tissue. HS produces variations in EMERG and S&LV in G2 and G3, and in NL, FA, and PH in G3. Bijanzadeh *et al.* (2019) mention that humic substances produce biochemical effects, promote potassium absorption, improve photosynthesis, increase cellular respiration, and improve seedling growth in maize (Figure 1). HS are also related to the amino acid and Krebs cycle metabolism (Popa *et al.*, 2022). As for SA, it produces

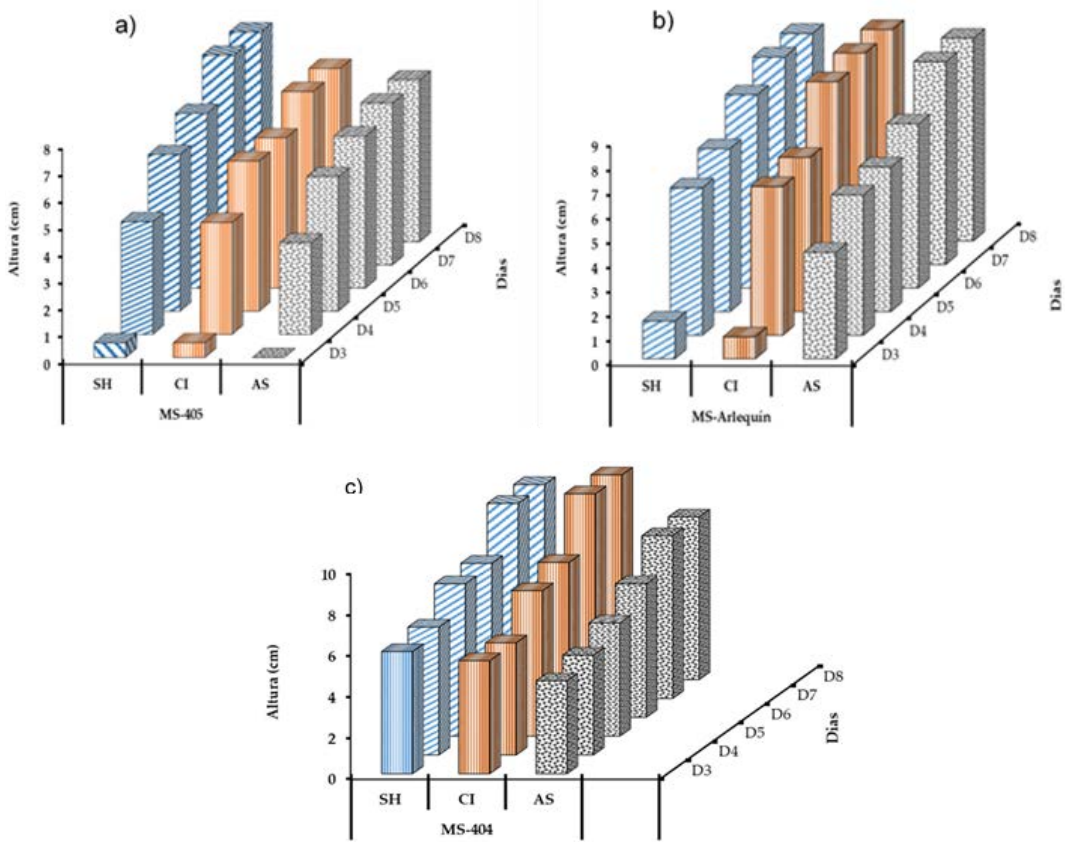

Figure 1. Height of genotypes MS-405, Arlequin, and MS-404, measured for eight days after emergence, considering controllers SH (HS)=humic substance, CI=citrulline, and AS (SA)=salicylic acid.

Table 2. Means for promoter effects (HS=humic substances, CI=citrulline, and SA=salicylic acid) on maize genotypes G1, G2, and G3, and interaction regarding germination speed (GS), emergence percentage (EMERG), stem and leaf volume (S&LV), chlorophyll (CHL), stem diameter (DMT), number of leaves (NL), foliar area (FA), root length (RL), and plant height (PH).

VAR	GEN	Promovedor			
		SH	CI	AS	MEDIA
VG	G1	13.75 cd	15.66 bcd	9.17 d	12.86 b
	G2	18.87 abc	18.21 abc	22.54 ab	19.87 a
	G3	22.63 ab	24.06 a	22.38 ab	23.02 a
	MEDIA	18.41 a	19.31 a	18.03 a	
EMERG	G1	60 b	84 a	60 b	68 b
	G2	90 a	93 a	100 a	94 a
	G3	90 a	94 a	94 a	93 a
	MEDIA	80 b	90 a	85 ab	
VTYH	G1	10.1 ab	12.7 ab	6.2 b	9.6 a
	G2	15.0 a	15.0 a	12.0 ab	14.0 a
	G3	15.0 a	7.0 b	9.0 ab	10.3 a
	MEDIA	13.4 a	11.6 ab	9.1 b	
CLFI	G1	45.6 ab	51.9 a	44.0 ab	47.2 a
	G2	40.1 ac	46.7 ab	53.6 a	44.1 a
	G3	31.9 c	51.5 a	37.0 bc	40.1 a
	MEDIA	39.2 c	50.1 a	44.9 b	
DMT	G1	0.54 bc	0.77 a	0.40 c	0.57 b
	G2	0.70 ab	0.83 a	0.80 a	0.78 a
	G3	0.70 ab	0.60 abc	0.70 ab	0.67 ab
	MEDIA	0.65 a	0.73 a	0.63 a	
NH	G1	6.1 b	6.0 b	4.7 c	5.6 b
	G2	7.0 ab	7.0 ab	7.0 ab	7.3 a
	G3	8.0 a	6.0 b	7.0 ab	7.0 a
	MEDIA	7.0 a	6.3 b	6.2 b	
AF	G1	61.2 abc	63.7 abc	45.5 bc	56.8 a
	G2	51.2 abc	78.2 ab	61.9 abc	74.0 a
	G3	81.9 a	40.5 c	43.5 c	55.3 a
	MEDIA	64.8 a	60.8 ab	50.3 b	
LR	G1	20.2 b	25.7 b	23.0 b	23.0 a
	G2	21.0 b	33.7 ab	25.0 b	28.6 a
	G3	27.0 b	25.0b	47.0 a	33.0 a
	MEDIA	22.7 b	28.1 ab	31.7 a	
ALT	G1	7.81abc	6.46 bc	6.03 c	6.77 b
	G2	8.31 ab	8.67ab	7.88 abc	8.29 a
	G3	9.52 a	9.98 a	7.94 abc	9.15 a
	MEDIA	8.55 a	8.37 a	7.28 b	

† The variables VG (GS), EMERG, VTYH (S&LV), CLFI (CHL), DMT, NH (NL), AF (FA), LR (RL), and ALT (PH) presented statistical differences between promoters (SH / HS, CI, AS / SA) and genotypes (G1, G2, and G3) ($p \leq 0.05$). a,b,c: Mean values per column with different letters are statistically different ($p \leq 0.05$). VG (GS)=%, EMERG=%, VTYH (S&LV)=mm, CLFI (CHL)=nm, DMT=mm, AF (FA)=cm^2, LR (RL)=cm, and ALT (PH)=cm.

variations in EMERG for G2 and G3, in CHL and DMT for G2, and in RL for G3. Bijanzadeh *et al.* (2019) and Dzib-Ek *et al.* (2022) mention that SA at concentrations of 1 and 0.01 μM significantly promotes root length and secondary root formation ($p<0.05$), regulates plant growth and development processes, including seed germination, and improves vigor (Chitnis *et al.*, 2014).

CONCLUSIONS

Pre-germination treatment by soaking in citrulline at 1,000 ppm in the MS-404 and Arlequin maize genotypes showed positive effects on germination speed (94%) and percentage (93%), as well as on the development of seedlings in acidic soils during a 15-day monitoring period. Other studies can follow up on the effects of pre-germination treatments and establish whether their application impacts production completion.

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