Hydrogen peroxide in seed germination and initial development of maize seedlings (*Zea mays* L.)

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ABSTRACT

Objective: the objective of this study was to evaluate the effect of hydrogen peroxide (H_2O_2) on seed germination and initial development of maize seedlings (*Zea mays* L.) based on water stress or irrigation.

Design/Methodology/Approach: seeds of seven maize materials were treated with four doses of H_2O_2 (150, 100, 50 and 0 mM). The elongation of the mesocotyl, coleoptile and other characters of maize seedlings were evaluated. Individual and combined analyzes of variance were performed with SAS® v. 9.1 for Windows (SAS® Institute, Inc., 2002). A comparison of means was performed with the Tukey test ($p \leq 0.05$), as well as principal components analysis (PCA).

Results: the effect of H_2O_2 in the seed of native and hybrid maize showed positive effects for the rate and percentage of emergence, length of mesocotyl and coleoptile and dry matter production in both humidity environments. The analysis of dispersion on principal components allowed us to visualize that the doses of 50 and 100 mM were superior for Xnuc Naal and San Pableño genotypes, both in irrigation and drought.

Limitations/Implications of the study: this study was limited to evaluate seedlings. It is suggested to extend evaluations to the reproductive stage.

Findings/Conclusions: doses of H_2O_2 at 50 and 100 mM showed a positive effect on seed germination and initial development of maize seedlings.

Keywords: water stress, ecophysiology, biomass.

INTRODUCTION

Hydrogen peroxide (H_2O_2) has been of interest in agronomic research due to effects on plant growth and development under conditions of water stress (Černý *et al.*, 2018). Plants usually generate reactive oxygen species (ROS), such as H_2O_2, which is a highly reactive oxygen molecule that acts as a regulator in plant growth as a response to biotic and abiotic stress (Choudhury *et al.*, 2017). Excessive accumulation of H_2O_2 can be harmful to plants

by causing oxidative damage, so the importance of managing an adequate dose of H_2O_2 is highlighted (Guo *et al.*, 2023).

Some studies have shown that the use of H_2O_2 as a treatment in seeds could mitigate the adverse effects of water stress by modulating various physiological and biochemical processes (Cetinel *et al.*, 2021). Guo *et al.* (2021) indicated that H_2O_2 improves seedling vigor, so it could be useful as a promoter of germination and emergence of maize seedlings in irrigation or drought. In addition, it has been observed that H_2O_2 can induce antioxidant responses, and regulate the expression of stress-related genes in plants exposed to drought conditions (Khedia *et al.*, 2019). Drought regularly affects the emergence, elongation of mesocotyl and coleoptile of seedlings, and development of adult maize plants (Villalobos *et al.*, 2024).

The mesocotyl is the region that connects the plumule (top of the seedling) to the radicle (at the bottom). Whereas the coleoptile is the protective sheath that surrounds the plumule emerging from the seed during germination (Niu *et al.*, 2020). The study of these structures could have important implications for acclimatization under conditions of water stress and deep seeding of maize seedlings (Zhao *et al.*, 2021; Villalobos *et al.*, 2024). The objective of this study was to evaluate the effect of H_2O_2 on seed germination and initial development of maize seedlings (*Zea mays* L.), with water stress and irrigation as evaluation conditions.

MATERIALS AND METHODS

Location of the study and genetic material

The experiment was established in greenhouse conditions at the Chiná Technological Institute located in Chiná, Campeche, Mexico, in the 2024 spring-summer cycle. Seeds of seven maize genotypes were used: one variety (Chichen Itza), five native races (San Pableño, Xnuc Naal, Dzib Bacal, Pix Cristo, Chaac Mejen Naal), and one commercial hybrid (Zarco) as the control.

Establishing conditions of the experiment

A series of seedbeds were established with soil known in the region as kan-kab or luvisol. Seeds of all genotypes were treated with four concentrations of H_2O_2 150, 100, 50 and 0 mM for a period of eight hours. Subsequently, seeds were sown in the seedbeds at a depth of 15 cm, and they were distributed at a distance of 5 cm between plants and 10 cm between rows. A randomized complete blocks design was used and the experimental unit consisted of 20 seeds with two replicates. The seedbed measurements were1.5 m$\times$0.70 m$\times$0.20 m (length$\times$width$\times$height), after sowing irrigation to saturation was applied for all treatments. For the irrigation treatment, constant watering was implemented in order to keep the substrate moist and for the drought treatment only that irrigation to saturation was applied. The variables were recorded seven days after planting.

Variables and statistical analysis

The percentage of emergence (PE) was determined by counting the number of seedlings emerged at the end of the trial related to the number of seeds sown per experimental unit. The emergence velocity rate (VE) was calculated by counting the seedlings that emerged per day in the days following the appearance of the first seedling. The units of measurement were seedlings per day (Maguire, 1962).

$$PE = \left[\left(Number\ of\ seedlings\ emerged \right) / 25 \right] \times 100 \quad (1)$$

$$VE = \sum_{i=1}^{n} \frac{ei}{i} \quad (2)$$

Where, X_i: number of seedlings emerged on the *i*-th day. N_i: number of days after sowing until the *i*-th day.

The following variables were recorded in each seedling, but reported as averages. Mesocotyl length (LOM, cm) was measured from the seed junction to the base of the coleoptile; choleptile length (LOC, cm) from the base of the coleoptile to its apex, with a ruler. The total dry weight of the biomass (TDW, mg) composed by the dry weights of mesocotyl (DWM, mg), coleoptile (transparent sheath) (DWC, mg) and aerial portion of the plant (developing green leaves) (DWA, mg). All dry weights were obtained by drying those structures in an oven (MEMMERT®) at 70 °C for 72 h, then weighed on an analytical scale (METLER®).

With the data obtained, individual and combined analyses of variance were performed, as well as the comparison of means with the Tukey test ($p \leq 0.05$) in SAS® v. 9.1 for Windows. A principal component analysis (PCA) was performed from the matrix of correlations between the characters, with the SAS® princomp procedure. Likewise, a graph of dispersion on principal components, with the first two of them as vectors in each case, was developed to explain the variation due to moisture, genotype, and dose of H_2O_2.

RESULTS AND DISCUSSION

Some interactions such as dose×moisture, dose×genotype, moisture×genotype, and dose×moisture×genotype were significant ($p \leq 0.05$) in specific characteristics shown (Table 1). That is, there was genetic diversity in the variables under study, for genotypes effect in each dose of H_2O_2, and based on soil moisture content. The absence of an interaction humidity×genotype in LOM and dry weight of seedling structures implies that, although water stress reduces those in regard to the irrigation condition, the effect was proportional and parallel for all genotypes.

Soil moisture. LOM and DWM, DWC, DWA, and TDW were positively associated with the irrigation condition (Figure 1A). Likewise, VE and PE did not present a favorable association under drought conditions; that is, drought reduced both rate and percentage of seed emergence (Figure 1A, quadrant 3). In a similar study, Li *et al.* (2017) associated such differences with water stress, which led to a reduction in water absorption, seed moisture content, seed germination and vigor index, as well as seedling growth and DWA. Zhao *et al.* (2021) indicated that among maize varieties there is a variability in the amount of water required to initiate the germination and emergence processes of seedlings. Length of coleoptile was not associated with any of the other variables studied.

Table 1. Results of the analysis of variance of mesocotyl length and other characteristics of maize with different doses of H_2O_2 under irrigation or drought during the 2024 spring-summer cycle.

FV	DF	VE	EP	LOM	LOC	DWM	DWC	DWA	TDW
RE	1	0.04^{**}	17.6^{**}	0.07^{ns}	0.2^{ns}	2.36^{*}	0.53^{ns}	108.9^{ns}	91.5^{ns}
M	1	1.45^{**}	5516.0^{**}	17.4^{**}	4.2^{**}	4.21^{**}	1.80^{ns}	395.3^{**}	541.5^{**}
G	6	1.10^{**}	3585.7^{**}	18.5^{**}	11.5^{**}	1.83^{**}	1.27^{*}	196.4^{**}	214.3^{**}
D	3	3.00^{**}	7849.6^{**}	10.4^{**}	0.7^{*}	22.2^{**}	6.41^{**}	546.5^{**}	930.5^{**}
D*M	3	0.03^{**}	313.4^{**}	1.5^{**}	0.6^{*}	0.12^{ns}	0.19^{ns}	7.1^{ns}	8.2^{ns}
D*G	18	0.28^{**}	662.9^{**}	4.5^{**}	2.0^{**}	1.16^{**}	0.99^{*}	58.0^{*}	66.1^{*}
M*G	6	0.08^{**}	235.9^{**}	0.4^{ns}	0.7^{*}	0.06^{ns}	0.09^{ns}	5.2^{ns}	6.4^{ns}
D*M*G	18	0.04^{**}	91.7^{**}	0.5^{*}	0.5^{*}	0.13^{ns}	0.10^{ns}	11.4^{ns}	12.3^{ns}
Error		0.01	0.69	0.31	0.22	0.69	0.50	32.3	35.23
Total									
C.V. %		0.07	1.32	8.89	12.01	8.76	12.33	23.35	15.61

FV: variation factor; DF: degrees of freedom; RE: replicate; M: moisture; G; genotype; D: dose; CV: coefficient of variation; VE: emergence rate (seedlings per day); EP; emergence percentage (%); LOM: length of mesocotyl, cm; LOC: length of coleoptile, cm; DWM: dry weight of mesocotyl, mg; DWC: dry weight of coleoptile, mg; DWA: dry weight of the aboveground portion, mg; and TDW: total dry weight of seedling, mg. Significant at *$p \leq 0.05$; **$p \leq 0.01$; ns: non-significant.

Genotype. Maize genotypes Xnuc Naal and San Pableño were positively associated with LOM, DWM, DWA, and TDW (Figure 1B, second quadrant). Sáenz and Cassab (2021) indicated that some genotypes have developed long mesocotyls that allow plant to emerge at greater depths than others, such as Xnuc Naal and San Pableño. Likewise, LOC was positively associated with PE, VE and DWC (Figure 1B, third quadrant). The maize variety Chichen Itza and the Zarco (hybrid) showed a lower association in regard to all the characteristics studied compared to the native races.

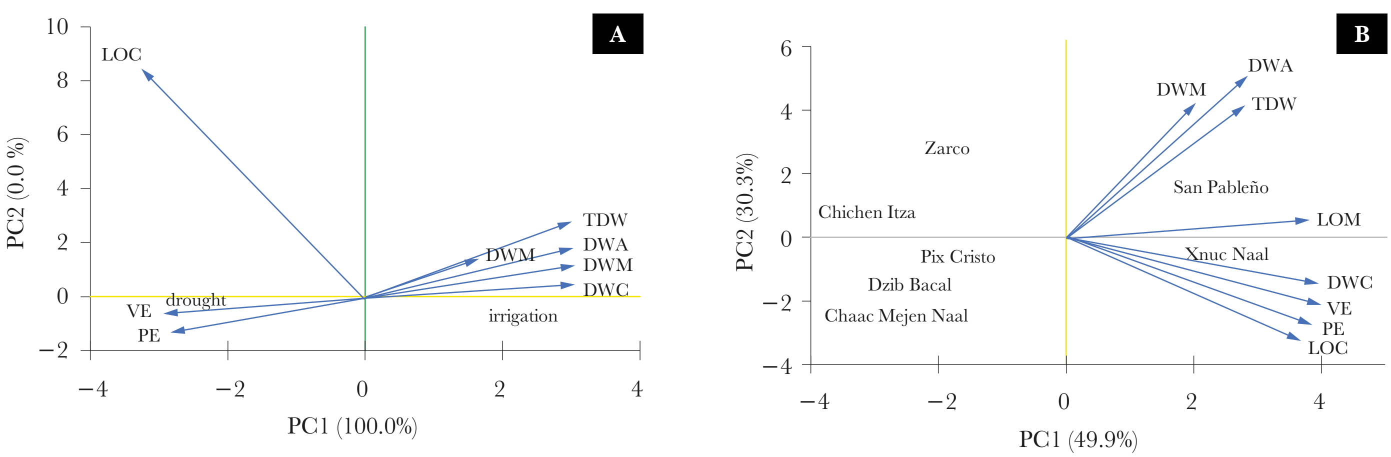

Figure 1. Dispersion of means of A: characteristics and B: genotypes of maize in irrigation and drought based on the first two principal components (2024 spring-summer cycle). VE: emergence rate (seedlings emerged per day); PE: percentage of emergence; LOM: length of mesocotyl, cm; LOC: length of coleoptile, cm; DWM: dry weight of mesocotyl, mg; DWC: dry weight of coleoptile, mg; DWA: dry weight of the aboveground portion, mg; and TDW: total dry weight of seedling, mg.

Hydrogen Peroxide dose. Based on the projection and angles of the vectors, TDW, DWA, DWM, LOC and LOM were positively associated with the dose 50 mM of H_2O_2 (Figure 2, quadrant 2). Likewise, when the seeds did not contain H_2O_2, there was no positive association in any characteristic (Figure 2, quadrant 3).

At a high dose (150 mM) of H_2O_2 there was a lower effect on the characteristics of maize seedlings (Figure 3, quadrant 4). Both VE and PE were positively associated with the 100 mM dose of H_2O_2 (Figure 2, quadrant 1); that is, H_2O_2 could be useful as a promoter of seed germination and plant emergence (Guo *et al.*, 2021) of maize seedlings in irrigation or drought.

Interaction dose×genotype×moisture. Principal component analysis (PCA) indicated that the first two components (PC1 and PC2) together accounted for, 76.6% in drought (Figure 3A), and 77.5% in irrigation (Figure 3B), of the total variation observed among H_2O_2 doses, genotype, and moisture. Cargnelutti and Toebe (2021) indicated that at least two main components are sufficient to adequately represent the variation of the evaluated characteristics.

In this study, for the first component (PC1) the most important variables in regard to dose of H_2O_2 were LOC, DWM, TDW and DWA in drought (Figure 3A) and irrigation (Figure 3B). For the second component (PC2), the origin variables with superior weight were PE, VE, LOM and DWC in both moisture conditions (Figure 3A, B). Other studies reported that the mesocotyl-coleoptile elongation contributes to improve crop establishing in maize, which leads to a higher percentage of emergence and population density. In such conditions, re-sowing is not necessary and this impact positively the production costs of maize crop (Reed *et al.*, 2022).

In this context, based on the PCA (PC1 and PC2) shown in Figure 3, it can be observed that the 50 mM dose of H_2O_2 in drought and irrigation was the most determinant at PC1; this is, the characteristic of greater relevance in the explanation of the variation observed. This means that, the lower the concentration of H_2O_2, the better response was obtained on the characteristics of the maize seedlings (Černý *et al.*, 2018).

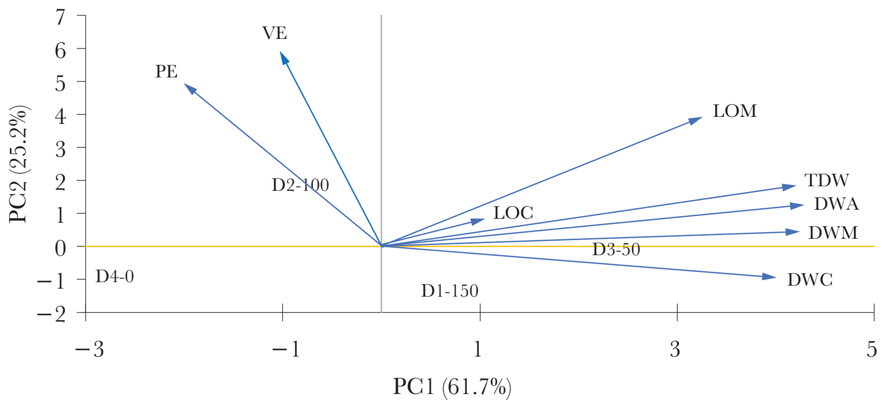

Figure 2. Dispersion of H_2O_2 doses based on the first two principal components (2024 spring-summer cycle). VE: emergence rate (seedlings emerged per day); PE: percentage of emergence; LOM: length of mesocotyl, cm; LOC: length of coleoptile, cm; DWM: dry weight of mesocotyl, mg; DWC: dry weight of coleoptile, mg; DWA: dry weight of the aboveground portion, mg; and TDW: total dry weight of seedling, mg. D1, D2, D3 and D4: doses 150, 100, 50 and 0 mM of H_2O_2.

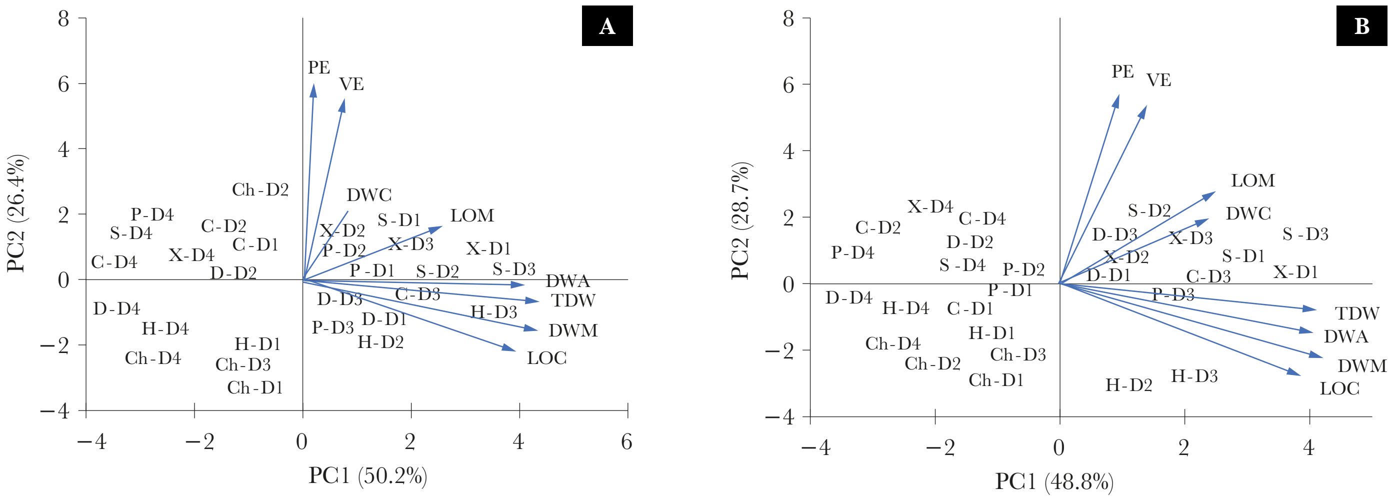

Figure 3. Dispersion of genotypes based on the dose of H_2O_2 in A: drought and B: irrigation, for the first two principal components (2024 spring-summer cycle). VE: emergence rate (seedlings emerged per day); PE: percentage of emergence; LOM: length of mesocotyl, cm; LOC: length of coleoptile, cm; DWM: dry weight of mesocotyl, mg; DWC: dry weight of coleoptile, mg; DWA: dry weight of the aboveground portion, mg; and TDW: total dry weight of seedling, mg. D1, D2, D3 and D4: doses 150, 100, 50 and 0 mM of H_2O_2. S: San Pableño; X: Xnuc Naal; D: Dzib Bacal; P: Pix Christ; C: Chaax Mejen Naal; H: Zarco (control).

CONCLUSIONS

The effect of hydrogen peroxide on native and hybrid maize seeds showed positive effects on rate and percentage of emergence, length of mesocotyl, length of coleoptile and dry matter production in both moisture environments. The dispersion on principal component analysis allowed us to observe that the 50 and 100 mm doses were paramount through the Xnuc Naal and San Pableño genotypes, in irrigation and drought.

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