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# Maize seed coatings and seedling sprayings with chitosan and hydrogen peroxide: their influence on some phenological and biochemical behaviors

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**Abstract:** Objective: To evaluate the effect of chitosan (CH) and hydrogen peroxide ($H_2O_2$) seed coatings and seedling sprinklings on two different maize varieties by measuring their phenology, the $H_2O_2$ presence, the catalase (CAT) activity, and the protein quantity. Methods: Seven groups of ten seeds for each maize variety were treated with CH (2% (20 g/L) and 0.2% (2 g/L)) or $H_2O_2$ (8 mmol/L) by coating, sprinkling, or both. Germination and seedling growth were measured. One month after germination, the presence of $H_2O_2$ in seedlings in the coated seed treatments was evaluated. Protein content and CAT activity were determined under all treatments. Results: $H_2O_2$ seed coating enhanced the germination rate and increased seedling and stem length in the quality protein maize (QPM) variety. Seedlings had a higher emergence velocity under this treatment in both varieties. CH and $H_2O_2$ sprinklings did not have an effect on seedling phenology. Exogenous application of $H_2O_2$ promoted an increase of endogenous $H_2O_2$. CH and $H_2O_2$ seedling sprinkling increased the protein content in both maize varieties, while there was no significant effect on the CAT activity of treated seeds and seedlings. Conclusions: CH and $H_2O_2$ enhance some phenological and biochemical features of maize depending on their method of application.

**Key words:** *Zea mays*, Chitosan, Hydrogen peroxide, Phenology, Catalase, Protein
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## 1 Introduction

Worldwide cereal crops are currently threatened by adverse environmental conditions and pathogen attacks which impede optimum seed germination, diminishing significantly seedling growth and productivity. Specifically, these conditions can be observed in the production of maize (*Zea mays*), one of the most important crops in the world. Both maize production and nutritional quality have been seriously affected. According to the Food and Agriculture Organization, during 2010, 844405181 tons of maize were produced globally, a mere 3.01% increase over 2009 production levels (FAOSTAT, 2010). With these threats to human and animal food security in mind, researchers have been looking for new methods to enhance maize production and improve its quality.

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In order to increase seed germination and plant phenology, the application of some chemical and biochemical substances has been tested in different crops, focusing on the use of salicylic acid (Khodari, 2004), abscisic acid (Sarath *et al*., 2007), and more recently, hydrogen peroxide ($H_2O_2$). Although $H_2O_2$ has been used for years to disinfect seeds prior planting (Piernas and Guiraud, 1997; Weissinger and Beuchat, 2000; Miché and Balandreau, 2001), it has recently been found that the exogenous application of vital cellular component to seeds and plants has positive effects over them. $H_2O_2$ treatment increases seed germination rates (Korystov and Narimanov, 1997; Amjad *et al*., 2004; Çavusoglu and Kabar, 2010), coleoptile emergence percentages, radicle and coleoptile elongation, and fresh weights of the seedlings (Çavusoglu and Kabar, 2010). $H_2O_2$ is coupled with important functions in metabolism, homeostasis of plants and reactive oxygen species (ROSs) generation too. It has been reported that soaking seeds in $H_2O_2$ induces a pronounced increase in enzymes activity levels, including catalase (CAT), ascorbate peroxydase (APX), and superoxide dismutase (SOD) (Gondim *et al*., 2010; Li *et al*., 2011). These enzymes play an important role on naturally over-expressing stress responses and signal activation at biochemical level.

Some authors have reported negative aspects in the use of $H_2O_2$. For example, exogenous $H_2O_2$ poisons seedlings and reduces seed germination due to excessive activation of ROSs (Edwards and Sutherland, 1979; James and Genz, 1981; Pernezny *et al*., 2002).

Chitosan (CH) is another chemical that has recently been used in plant protection. This biopolymer is a large cationic polysaccharide mainly obtained from waste materials from seafood processing (Guan *et al*., 2009), with antiviral, antibacterial, and antifungal properties (El-Hadrami *et al*., 2010). When CH is applied to plant seeds, their germination index is enhanced, the mean germination time is reduced, shoot height, root length, and seedling vigor are increased (Bhaskara Reddy *et al*., 1999; Ruan and Xue, 2002; Shao *et al*., 2005; Guan *et al*., 2009; Kananont *et al*., 2010; Ziani *et al*., 2010; Zeng *et al*., 2012), vegetative growth is increased, time to flowering is reduced (Ohta *et al*., 1999), and fresh weight is increased (Asghari-Zakaria *et al*., 2009). CH has been applied not only to seeds but also to seedlings. CH foliar application on strawberry crops increases plant height and number of leaves, augments leaf fresh and dry weights, and enhances the number and weight of seedlings (Abdel-Mawgoud *et al*., 2010). It has been reported that CH has effects not only at the phenological level but also at the enzymatic level. This is evidenced by peroxidase (POD) and CAT activity level increases in the protein extract of some edible cultivars like tomato, guava, and sweet wormwood that were treated with CH (Ortega-Ortíz *et al*., 2007; Guan *et al*., 2009; Lei *et al*., 2011; Hong *et al*., 2012). The inhibition of CAT activity (Zeng *et al*., 2010) is important in plant development, defense, aging, and senescence too (Yang and Poovaiah, 2002). Although, there are multiple reports of CH application in food production, there are not reports for the effect of CH application on crop nutritional value.

Research into the effects of CH and $H_2O_2$ has targeted application at seed and seedling levels, because in these early stages most of the biochemical and enzymatic mechanisms are initiated, having continuing effects on growth as the plant continues to develop.

The aim of this investigation was to evaluate the effects of CH and $H_2O_2$ seed coatings, and CH and $H_2O_2$ sprinklings on seedlings of two different varieties of maize by measuring their phenology, $H_2O_2$ presence, enzymatic activity, and protein quantity under both chemical treatments. To carry out this investigation, we evaluated in vivo, during short periods of time and under greenhouse conditions, the effect of coating corn seedlings with CH and $H_2O_2$ on their germination and growth (thickness of stems, total length of the leaves and stems) of seedlings. After one month of growing, the presence of $H_2O_2$ was evaluated in treated plants in order to determine if the use of $H_2O_2$ and CH enhance $H_2O_2$ production. Then, CAT activity was determined in seedling leaves from treated plants. Finally, plant proteins were quantified in order to know if the application of CH and $H_2O_2$ increased protein content.

## 2 Materials and methods

### 2.1 Materials

Two maize seed varieties were used in this

experiment: a normal variety (N-279, with a moisture content of 11.5% and a germination rate of 100%), and a high quality protein maize variety (QPM-374, with a moisture content of 11.7% and a germination rate of 100%) obtained from the Maize Breeding Program of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) based in the Campo Experimental del Bajío, México. CH for maize treatment was obtained from chitin shrimp exoskeletons, with a molecular weight of 125 kDa and a deacetylation degree of 92%, and it was produced and characterized in our laboratories without further purification (Miranda, 2000). $H_2O_2$ (30%) of reagent grade was obtained from Fermont, Productos Químicos de Monterrey, SA de CV, Monterrey, NL, Mexico, with a molecular weight of 34.01 g/mol.

### 2.2 Preparation of coating and sprinkling solutions

A 2% (20 g/L) CH solution was prepared, dissolving 10 g of the biopolymer in 500 ml of water acidified with acetic acid (Meyer Chemical SA de CV, Tlahuac, DF, Mexico), and kept under constant agitation for 24 h before its pH was adjusted to 5.0 with a 12% (120 g/L) sodium hydroxide solution (JT Baker, Xalostoc, Edo., de Mexico, Mexico). An 8 mmol/L $H_2O_2$ solution was prepared, diluting 4.074 ml of commercial reagent in 500 ml of distilled water, reaching a pH of 7.8. For seedling sprinkling, a 0.2% (2 g/L) CH solution was prepared, dissolving 1 g of the biopolymer in 500 ml of acidified water, under the same conditions as the CH seed coating preparation. The same $H_2O_2$ seed coating preparation was used for seedling sprinkling.

### 2.3 Seed and seedling treatments

Fourteen groups were formed (each one of ten seeds, sowing one seed per pot): seven groups of the normal variety and seven groups of QPM maize. The first group was considered to be a control (with no treatment); in the second group, seeds were coated with CH; in the third group seedlings were sprinkled with CH; the fourth group was submitted to a combined treatment (seed coating and seedlings sprinkled with CH); in the fifth group seeds were coated with $H_2O_2$; in the sixth group seedlings were sprinkled with $H_2O_2$; and the seventh group was submitted to a combined treatment (seeds coating and seedling sprinkling with $H_2O_2$).

CH treated seeds were soaked in the 2% CH solution for 12 h and then they were dried in an oven at 29 °C for 24 h to counteract the effects of excessive moisture on the seeds by treatment with the biopolymer solution. $H_2O_2$-treated seeds were soaked in the 8 mmol/L solution for 12 h and were not dried. Maize seedlings were sprinkled with the CH and $H_2O_2$ solutions, finely the entire seedling canopy.

The study was conducted in the greenhouse of the Grains and Seeds Research Unit in Facultad de Estudios Superiores, Cuautitlán, UNAM, which was previously sanitized in order to carry out the planting under protected conditions (ISTA, 1993; Albajes *et al*., 1999; Sonneveld and Voogt, 2009) to avoid interference in the experimentation results. The substrate on which seeds were seeded was prepared using Sunshine mixture #3® (Sun Gro Horticulture Inc., Canada CM, Ltd.) enriched with perlite Hortiperl® (Termolita SA de CV, México) in a proportion of 10:1. Seeds were sown at a depth of 3 cm, and pots were irrigated with municipal tap water.

### 2.4 Evaluation of maize seedling germination

All seeds were sown at the same time. Germination was evaluated until seeds reached the final stages of germination (Monasterio *et al*., 2007).

### 2.5 Evaluation of maize seedling growth

First, seedling emergence velocity was evaluated nine days after sowing. Different numbers were given to each emergency phase: 0 for non germinated seeds, 1 for coleoptiles, 2 for plumules, 3 for seedlings with one leaf seedlings, and 4 for seedlings with two leaves. Then, seedling growth was assessed in two stages: 19 d after planting (where the seeds were only coated but not sprinkled with CH and/or $H_2O_2$), and on the 13th day (because at this stage seeds were coated and seedlings were sprinkled 5 d before). Stem length, sheet length, overall seedling length, and stem diameter were measured.

### 2.6 Diaminobenzidine (DAB) staining for determining the presence of $H_2O_2$

This test was carried out only with seedlings grown from coated seeds. The DAB solution was prepared by homogeneously mixing 25 mg of

3,3'-diaminobenzidine and 25 ml of 3-(*N*-morpholino) propanesulfonic acid (MOPS) 10 mmol/L, and adjusting it to pH=3.8. The plant material (maize leaves) was cut into short pieces and immersed in DAB solution for 8 h and then, faded in several washes of methanol in a heated water bath. Once faded, the plant material was stored in 50% glycerol for preservation, and the presence of $H_2O_2$ was determined according to Thordal-Christensen *et al*. (1997).

### 2.7 Quantification of total proteins

Maize leaf samples were ground in liquid nitrogen with a mortar pre-cooled to 4 °C, obtaining a fine tissue dust, which was transferred to Falcon tubes in order to clean the sample with acetone powders, adding 5 volumes of acetone per volume of tissue. The system was stirred by inversion and stored for 24 h at 4 °C. After this time, the system was transferred to Eppendorf tubes and was centrifuged at 4000 r/min for 15 min to ensure tissue precipitation. The supernatant (which contained chlorophyll and other organic compounds) was discarded. Then, the tissue pellet was resuspended in 0.05 mol/L phosphate buffer at pH 7, and was centrifuged at 13000 r/min for 15 min to ensure the protein extraction; the supernatant (enzyme extract) was recovered (Castro-Rivera *et al*., 2006; Ortega-Ortíz *et al*., 2007). Protein quantification was made by the Bradford method, adjusting it to micro-plate technique. Each reaction mixture was prepared using 200 µl of 1:5 Bradford (1976) reagent and 10 µl of the enzyme extract, carrying them directly into the micro-plate wells. The blank was prepared with 200 µl of Bradford reagent 1:5 and 10 µl of phosphate buffer used for extraction. Samples and blank were incubated during for 5 min at room temperature and the corresponding absorbance values were read on a spectrometer (Spectronic 20D$^+$ Digital, Sargent-Welch) at a wavelength of 620 nm. The calibration curve was prepared from solutions of bovine serum albumin (Sigma Aldrich, Saint Louis, Missouri, USA) of known concentrations. Determination was made by triplicate.

### 2.8 Determination of total CAT

The activity of CAT was assayed as described by Ortega-Ortíz *et al*. (2007) with the following modifications: the reaction mixture was prepared using 6 ml of the enzyme extract and 1 ml of 0.022 mol/L $H_2O_2$. The phosphate buffer solution was used as a blank. Sample absorbance values were read immediately (to prevent oxidation of $H_2O_2$) in an UV-Vis spectrophotometer (Spectronic 20D$^+$ Digital, Sargent-Welch) at a wavelength of 240 nm. The calibration curve was prepared from reagent grade $H_2O_2$ solutions (Fermont, Productos Químicos de Monterrey, SA de CV, Monterrey, NL, Mexico) of known concentrations. Determination was made by triplicate.

### 2.9 Data analyses

Differences between treatments were analyzed by analysis of variance (ANOVA) for experiments with one factor and a completely randomized design, with a post hoc analysis by Tukey test using the computer program JMP™ Release 5.0.1.2 (SAS Institute Inc., USA). Mean values with statistical difference of $p<0.05$ were considered to be significant.

## 3 Results

### 3.1 Effect of coatings on seed germination

A percentage of 98.57% of planted seeds reached their final phase of germination nine days after sowing. As shown in Fig. 1, it was found that there was a significant difference between germination percentages by maize variety, namely, normal

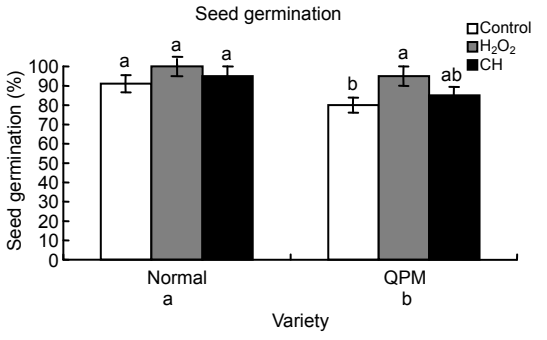



**Fig. 1 Germination percentage means by variety and seed coating**

Different letter(s) above the bars indicate significant difference ($p$=0.05, Tukey) among treatments within the same variety. Different letter(s) below the bars indicate significant difference ($p$=0.05, Tukey) among varieties regardless of treatment

variety seeds had higher germination rates ($p$=0.0017). It was found that $H_2O_2$ was an effective seed coating in QPM variety seeds, promoting a higher germination percentage than the control ($p$=0.0028). There were no significant differences in germination percentages among coatings in the normal variety.

### 3.2 Effect of coatings on seedling emergence velocity

Seeds from both varieties under $H_2O_2$ coating emerged quicker than either those coated with CH or the control group ($p$<0.0001). The behavior of the control and the CH-coated seedlings was the same. According to varieties, normal seedlings emerged significantly quicker than the QPM ones ($p$=0.6576) (Fig. 2).

### 3.3 Phenological seedling evolution under CH and $H_2O_2$ treatments

Nineteen days after planting but before sprinkling, seedlings from coated seeds were measured. There were no significant differences in the response variable measurements irrespective of treatment for the normal variety (Table 1). In the QPM variety, $H_2O_2$ coating promoted increased stem length when compared with CH coating and the control. Similarly, the length of seedlings from $H_2O_2$ coating was greater than the corresponding control. QPM leaf length and stems thickness did not present significant differences by treatment.

Phenological measurements performed on Day 30 were necessary to determine whether the seedling applications of CH and $H_2O_2$ coatings and sprinkling promoted a change in physical characteristics. As shown in Table 2, neither normal variety nor the QPM one present significant differences between dimensions in any of the growth responses measured.

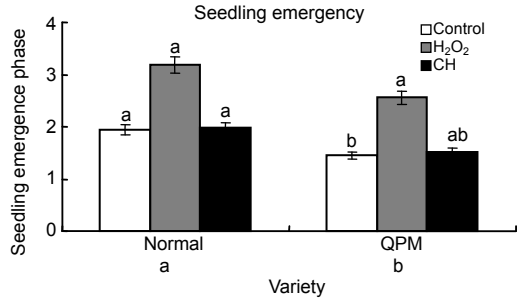



**Fig. 2 Emergence means of seedlings from coated seeds**

Different letter(s) above the bars indicate significant difference ($p$=0.05, Tukey) among treatments within the same variety. Different letter(s) below the bars indicate significant difference ($p$=0.05, Tukey) among varieties regardless of treatment

### 3.4 Presence of $H_2O_2$

In this qualitative test, the presence of $H_2O_2$ was manifested by a red-brown coloration in leaves. $H_2O_2$ was positive in all groups. As described in Table 3, the variety with the highest presence of $H_2O_2$ was QPM because the brownish color resulting from DAB coloration was evident in the cleavage site forming a wider colorful perimeter than the corresponding normal sample. The normal variety showed more coloration under $H_2O_2$ treatment, followed by CH and finally the control. QPM showed more presence of $H_2O_2$ in the control, followed by $H_2O_2$ and then those treated with CH.

### 3.5 Seedling protein content

This assessment, performed on tissue samples taken from seedlings 30 d after planting, showed that the highest protein content was present in the leaves of plants sprinkled with CH or $H_2O_2$ independent of the variety used (Table 4).

**Table 1 Effect of seed coating with CH and $H_2O_2$ on the phenological variables, 19 d after planting in the greenhouse**

| Variety | Coating treatment | Seedling length (cm) | Leaf length (cm) | Stem length (cm) | Stem thickness (mm) |
|---|---|---|---|---|---|
| Normal | Control | 38.19±2.35 a | 28.46±2.31 a | 9.73±0.22 a | 5.25±0.14 a |
| | CH | 42.66±2.88 a | 32.99±2.83 a | 9.67±0.27 a | 5.54±0.17 a |
| | $H_2O_2$ | 40.00±2.86 a | 29.83±2.81 a | 10.17±0.27 a | 5.76±0.17 a |
| QPM | Control | 36.97±0.57 b | 27.26±0.48 a | 9.71±0.19 b | 5.96±0.14 a |
| | CH | 37.40±0.61 ab | 27.84±0.59 a | 9.56±0.23 b | 5.91±0.17 a |
| | $H_2O_2$ | 39.25±0.89 a | 28.75±0.60 a | 10.49±0.23 a | 5.81±0.18 a |

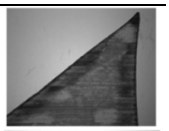
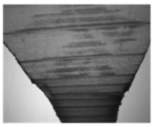
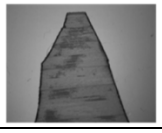
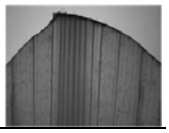
Values are expressed as mean±standard error (SE). Different letter(s) following the values indicate significant difference ($p$=0.05, Tukey) among treatments within the same variety

**Table 2 Effect of seed coating and seedling sprinklings on the phenological variables, 30 d after planting in the greenhouse**

| Variety | Coating/sprinkling treatment | Seedling length (cm) | Leaf length (cm) | Stem length (cm) | Stem thickness (mm) |
|---|---|---|---|---|---|
| Normal | Control | 65.68±1.90 a | 49.57±1.36 a | 16.11±2.84 a | 6.45±1.21 a |
| | CH/none | 65.96±1.76 a | 49.64±1.36 a | 16.32±1.85 a | 6.68±1.21 a |
| | None/CH | 69.53±1.73 a | 52.89±1.34 a | 16.64±2.47 a | 6.71±1.19 a |
| | CH/CH | 67.16±1.73 a | 51.08±1.34 a | 16.07±3.43 a | 6.69±1.19 a |
| | $H_2O_2$/none | 67.36±1.73 a | 51.08±1.34 a | 16.27±2.18 a | 9.71±1.19 a |
| | None/$H_2O_2$ | 67.45±1.73 a | 51.05±1.34 a | 16.40±2.00 a | 7.11±1.19 a |
| | $H_2O_2$/$H_2O_2$ | 71.63±1.73 a | 54.47±1.34 a | 17.16±2.76 a | 7.27±1.19 a |
| QPM | Control | 67.53±1.60 a | 50.76±1.34 a | 16.76±0.43 a | 7.52±0.26 a |
| | CH/none | 67.47±1.60 a | 49.86±1.34 a | 17.61±0.43 a | 7.34±0.26 a |
| | None/CH | 69.78±1.60 a | 52.59±1.34 a | 17.19±0.43 a | 7.48±0.26 a |
| | CH/CH | 68.40±1.63 a | 51.56±1.37 a | 16.84±0.43 a | 7.57±0.26 a |
| | $H_2O_2$/none | 69.51±1.69 a | 52.34±1.42 a | 17.17±0.45 a | 7.64±0.27 a |
| | None/$H_2O_2$ | 67.83±1.60 a | 50.85±1.34 a | 16.98±0.43 a | 7.55±0.26 a |
| | $H_2O_2$/$H_2O_2$ | 68.75±1.60 a | 50.99±1.34 a | 17.76±0.43 a | 7.17±0.26 a |

Values are expressed as mean±standard error (SE). Different letter(s) following the values indicate significant difference ($p$=0.05, Tukey) among treatments within the same variety

**Table 3 Qualitative analyses of $H_2O_2$ presence in maize leaves from CH and $H_2O_2$ treated seedlings by DAB technique**

| Normal group | Stereoscopic observation | | OPM group | Stereoscopic observation | |
|---|---|---|---|---|---|
| Control | Presence of $H_2O_2$ in the ribs and in the middle of the sheet |  | Control | Presence of $H_2O_2$ in the entire sheet with great intensity mainly in ribs and cleavage |  |
| $H_2O_2$ | Presence of $H_2O_2$ in the whole leaf, mainly in ribs and cleavage site, with an intense color |  | $H_2O_2$ | Presence of $H_2O_2$ in secondary veins, but mainly in cleavage sites. The midrib acquired subdued colors |  |
| CH | Slight presence of $H_2O_2$ in the main rib; little coloration in the edges, despite the cleavage |  | CH | Same manifestations of presence of $H_2O_2$ in secondary veins, midrib and cleavage sites |  |

Dark areas in the leaf images indicate the presence of endogenous $H_2O_2$

### 3.6 Specific activity of CAT

There was no statistically significant difference in terms of CAT specific activity in any treatment or in any variety, indicating that this enzyme does not enhance its activity when either CH or $H_2O_2$ is applied to maize seeds as coatings (Table 5).

## 4 Discussion

Germination is a key step when sowing seeds, because if we do not have a good germination, productivity will decrease since the beginning. In this experiment, although maize seed technical data indicated that the germination rate was 100% for both varieties, experimentally and under greenhouse conditions, we found that the germination rate was 85% in all cases, indicating good quality seeds in general. In the case of QPM maize seeds, the application of $H_2O_2$ coating promoted an increased germination when compared with the control; nevertheless, the normal variety did not show improvement on germination rates under any seed coating regime. These results match the pioneer reports of Hameed *et al*. (2004) and Msanga and Maghembe (1989), which showed that exogenous application of $H_2O_2$ to seeds enhanced germination. Normal

**Table 4 Effect of seed coating and seedling sprinkling with CH and $H_2O_2$ on the protein content 30 d after planting in the greenhouse**

| Variety | Coating/sprinkling treatment | Protein content (μg) |
|---|---|---|
| Normal | Control | 21.317±3.538 c |
| | CH/none | 16.104±3.538 c |
| | None/CH | 51.470±3.538 a |
| | CH/CH | 40.440±3.538 a |
| | $H_2O_2$/none | 24.610±3.538 bc |
| | None/$H_2O_2$ | 42.281±3.538 a |
| | $H_2O_2$/$H_2O_2$ | 37.484±3.538 ab |
| QPM | Control | 14.761±4.595 b |
| | CH/none | 34.213±4.595 ab |
| | None/CH | 41.483±4.595 a |
| | CH/CH | 31.469±4.595 ab |
| | $H_2O_2$/none | 15.831±4.595 b |
| | None/$H_2O_2$ | 42.535±4.595 a |
| | $H_2O_2$/$H_2O_2$ | 39.334±4.595 a |

Values are expressed as mean±standard error. Different letter(s) following the values indicate significant difference ($p$=0.05, Tukey) among treatments within the same variety

**Table 5 Effect of seed coating and seedling sprinkling with CH and $H_2O_2$ on the CAT activity 30 d after planting in the greenhouse**

| Variety | Coating/ sprinkling treatment | CAT activity (μmol of destroyed $H_2O_2$/ (min·mg protein)) |
|---|---|---|
| Normal | Control | 1720.640±447.96 a |
| | CH/none | 2819.153±447.96 a |
| | None/CH | 2834.457±447.96 a |
| | CH/CH | 3203.963±447.96 a |
| | $H_2O_2$/none | 1879.146±447.96 a |
| | None/$H_2O_2$ | 2804.873±447.96 a |
| | $H_2O_2$/$H_2O_2$ | 3280.392±447.96 a |
| QPM | Control | 3985.62±47741 a |
| | CH/none | 3109.18±47741 a |
| | None/CH | 3266.59±47741 a |
| | CH/CH | 3728.48±47741 a |
| | $H_2O_2$/none | 3774.02±47741 a |
| | None/$H_2O_2$ | 128795.27±47741 a |
| | $H_2O_2$/$H_2O_2$ | 2906.41±47741 a |

Values are expressed as mean±standard error. Different letter(s) following the values indicate significant difference ($p$=0.05, Tukey) among treatments within the same variety

variety germination rates were higher than those for the QPM variety. CH coating did not promote higher seedling germination rates. This result contradicts some findings, because it has been reported that maize seed priming with CH enhances germination index; nevertheless, this finding was made under low temperatures (about 15 °C) using CH concentration from 0.25% (2.5 g/L) to 0.75% (7.5 g/L), and in previous studies the characteristics of CH have not been reported (Guan *et al*., 2009). It also contradicts what has been found in other cultivars, because germination ability is improved in wheat when priming seeds with CH at a concentration of 2–8 mg/ml (Bhaskara Reddy *et al*., 1999), peanuts (Zhou *et al*., 2002) and rice seeds coated with 1.5% (15 g/L) CH solution (Ruan and Xue, 2002). We chose to use a 2% (20 g/L) CH solution for coating seeds because at this concentration, CH not only enhances maize phenological characteristics, but also protects the crop against biotic and abiotic stresses (Lizárraga-Paulín *et al*., 2011a; 2011b). Variations on the response of CH on seed germination can be due to the biopolymer concentrations used, the specific seed and crop features, the conditions under which the cultivar is produced (specially temperature) and even the characteristics of CH, such as, the molecular weight or the deacetylation degree, which can vary drastically depending on the biopolymer extraction method or even the prime matter from which it is obtained.

Seedlings from $H_2O_2$-coated seeds emerged quicker than the control and the CH-coated ones (between which there were no significant differences in emergence). It could be because $H_2O_2$ stimulates seed germination and sprouts growth due to the oxidative stress caused by ROS at cellular level (Korystov and Narimanov, 1997).

A large portion of the maize plants grown in the field is destined to become forage (stubble), and is the basis of animal alimentation; that's why researchers are looking for producing a larger amount of the cereal. According to the phenological variables evaluated in this experiment, we found that 19 d after planting, QPM seedlings from $H_2O_2$-treated seeds showed greater physical growth than the others. Principally, this was demonstrated by stem length, because stems from $H_2O_2$-treated seeds were higher than the other treatments and the other variety. Thirty days after planting, no significant differences were found between treatments, even when seeds were not only coated, but their seedlings were also sprinkled with the corresponding treatments. This indicates that CH and $H_2O_2$ sprinklings do not favor phenological

characteristics; only seed coating alters some physical characteristics on the plant. We expected to find a response when applying by a foliar way the CH or $H_2O_2$ solutions, because it has been reported that this treatment affects the photosynthetic rate of some crops, increases the stomatal conductance and transpiration rate, and promotes plant development, specially leaves length (Khan *et al*., 2002). Maybe sprinkling solution concentrations need to be increased in order to promote a higher conductance inside the plant.

ROSs are involved in many important plant processes, principally in those involved in defending plants against stress. $H_2O_2$ can be produced either directly or as a result of superoxide dismutation. It can diffuse into cells activating some enzymes, especially CAT and POD (Apel and Hirt, 2004). Evaluating the presence of $H_2O_2$ in plants, (qualitatively POD), it was found that when applying $H_2O_2$ by seed coating, endogenous $H_2O_2$ was produced in the leaves, particularly in stressed sites (cleavages sites). The application of CH by seed coating promoted the endogenous manifestation of $H_2O_2$, too. Nevertheless, it was qualitatively lesser than the coloration acquired by $H_2O_2$. The respective controls showed almost no coloration. This indicates that the POD activity increases when applying exogenous $H_2O_2$ to the seeds, making the plant able to resist pathogens attacks and allowing it to initiate defense mechanisms against stresses (Bradley *et al*., 1992; Camarena-Gutiérrez and de la Torre-Almaráz, 2007), and generating major concentrations of ROSs.

CH and $H_2O_2$ sprinkling favored the production of proteins in maize seedlings, turning this treated cereal into a high quality food for animal consumption. Both varieties showed an increase in protein content according the treatments, because independently of being coated or not, those seedlings which were sprinkled with any of the substances, promoted more protein generation. In the case of QPM seedlings, sprinkling treatments with $H_2O_2$ and CH are encouraging, because they have the potential to turn this crop into an even higher quality "QPM", with not only excellent lysine and triptophane levels, but also a high protein content. There are still no reports of the effect of sprinklings on cereals for enhancing their protein quantity. Digestibility and well-use tests must be performed in order to give a better understanding of the benefits of this enriched cereal for animals.

According to some reports, it was expected that CAT activity would increase with the application of $H_2O_2$ and CH as reported by Ortega-Ortíz *et al*. (2007), Guan *et al*. (2009), Lei *et al*. (2011), and Hong *et al*. (2012). However, no significant differences were found when evaluating the specific activity of CAT in all treated plants. CH and $H_2O_2$ applications do not enzymatically favor plant development, so other treatments need to be purposed to improve maize defense, aging, and senescence.

Application of CH and $H_2O_2$ to maize plants, enhance some characteristics at seed and seedling levels, generate good quality individuals in the germination stage, during growth, in terms of their peroxide production and their nutritional quantity. Despite these advances, more research is needed to produce cultivars with improved characteristics that allow for the use of growth-improving treatments harmless to both the plant and the consumer.

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