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Class III Peroxidase Activity in the Scutellum as an Indicator of the Final Germination Phase to the Post-Germination in Maize Embryos of the Conic Complex

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Abstract

Mexico is the place of origin of maize. Currently, fifty-nine races of maize have been described, classified into seven groups, and for this study, maize grains from the Conical group were used. The main components of the grain are the pericarp, aleurone, starchy endosperm and embryo; the embryo comprises the scutellum and embryonic axis. The scutellum is essential for germination; its metabolism is associated with the generation of reactive oxygen species; thus, it is protected by various antioxidants, including class III peroxidases (POX). Accordingly, the objectives of this research are to demonstrate that POX is a biochemical indicator of germination, and to relate the germination morphometric parameters with POX activity in the scutellum that was subjected to imbibition using phosphate solutions at different concentrations and pH. The results revealed that POX is inducible and is an indicator of the embryo germination/postgermination phase. Morphometric changes during germination and their relationship with POX activity are similar in embryos of the Conic group from different locations and harvest dates; therefore, this parameter can be used as a marker between grains from the same maize group.

Keywords

Maize, Conic Group, Class III Peroxidase, Scutellum, Germination

1. Introduction

Global population growth is exponential, and the current population exceeds 8

billion people which is estimated to exceed 9 billion people by 2040. In recent years, agricultural productivity has become maximum and remains stable over several crop cycles, but one factor that could slow this growth is the decrease in arable land. Statistics indicate that to satisfy the food demand of the estimated population in 2050, a 70% increase in food production is needed. Several problems, including events associated with climate change, the accelerated increase in the cost of fuels, problems in supply chains and an accelerated decrease in food reserves after the food crisis of 2008 exist in the realization of this goal [1] [2].

Among the most cultivated cereals are rice and wheat along with maize, which is a crop of relevant importance from both economic and food perspectives [3]. This versatile crop is cultivated in approximately 135 countries and is consumed mostly as dry grain, although in some regions, it is consumed as tender grain [4]. Its value as a food resource extends from human nutrition to animal feed and aquaculture, as well as to the industrial production of starch, proteins, oils, sweeteners, alcoholic beverages and fuel. It is also used as fodder in cattle feed for both meat and milk production [5]. In addition, maize exhibits an impressive capacity for adaptation and development under diverse agroclimatic conditions.

Mexico is the place of origin of maize, and the production in 2024 declined for the third time consecutively to 23.7 million tons, approximately 12% below the average of the previous five years, mainly because of severe weather conditions in the producing regions during the planting period. This reduced production is associated with a decrease in international prices [6] [7]. However, during the period of 2025-2026, with a slight increase in production, the country contributed 25.7 million metric tons, representing 2% of the global maize production ranking eighth among maize producing countries, which included the United States, China, Brazil, the European Union and Argentina [8].

Of the 220 maize races in Latin America [9], 64 have been identified and described in Mexico [10]-[14], of which 59 can be considered native and have been grouped into seven groups or racial complexes [9] [14] [15]. In the central plateau of Mexico, maize plants develop cone-shaped or pyramidal ears categorized into the Conical Complex, which are widely used to make tortillas, tamales, pozole and popcorn.

The maize scutellum has a high metabolism that is strongly associated with different processes that occur during germination, seedling growth and in the transition period between these stages. Initially, the scutellum provides nutrients, which are products of the hydrolysis of its own reserves that are transformed *in situ* into substances that are subsequently translocated to the rest of the embryo/seedling to sustain its growth. Some of these hydrolyzed products are converted into starch, which temporarily accumulates in this organ and is subsequently metabolized to maintain and sustain the growth and establishment of the seedling [16]-[19]; scutellum secretes phytohormones into the surrounding endosperm and activates the aleurone layer, which was initially demonstrated in rice and barley [20]-[22]. During the early post-germination stage of Chalqueño

maize, the epidermal cells of the scutellum are transformed into a columnar type that secrete enzymes into the endosperm; some of these enzymes permeabilize the fibrous layer, while other enzymes hydrolyze the reserves of the endosperm around the embryo that are then incorporated and mobilized for seedling growth [19] [23]. During this period, the metabolism of this organ is very high and is accompanied by the production of reactive oxygen species (ROS), which must be controlled and maintained at a low concentration by various antioxidant mechanisms to avoid causing tissue damage [19].

In seedlings, ROS are generated as a product of various metabolic pathways during the catabolism and transformation of reserves. The pathways that result in the production of higher levels of ROS include the electron transport chain in the mitochondria and the β-oxidation of lipids in the glyoxysome, in addition to other reactions that occur in the cytoplasm and in the apoplast. ROS are formed by the sequential reduction of an O_2 molecule that receives electrons to form the superoxide radical anion ($O_2 \cdot^-$), hydrogen peroxide (H_2O_2) or the hydroxyl radical (^{o}OH) or when the electrons of an oxygen molecule are excited to form the oxygen singlet ($^1O_2$) [24].

Plants use low or moderate levels of ROS as signaling molecules, and they are involved in the mechanisms underlying the perception of changes in endogenous and environmental conditions that impact development and growth processes, such as the remodeling of cell wall architecture or the activation of stress response mechanisms [25] [26]. To maintain cellular redox homeostasis under adverse conditions, plants implement complex antioxidant mechanisms for the removal of excessive concentrations of ROS.

There are two antioxidant systems in plants, namely, the enzymatic and the non-enzymatic systems, both of which regulate the balance between the production and elimination of ROS in cells. The enzymatic mechanisms involve several enzymes, such as peroxidases, which reduce the intra and extracellular concentrations of ROS. Peroxidases belong to a superfamily of antioxidant enzymes that possess sulfhydryl groups and are divided into two groups based on the presence or absence of a heme group. Peroxidases that contain a heme group are further divided based on their presence or absence in animals; peroxidases in the latter subgroup are classified as class I, II or III. These three classes have similar three-dimensional structures and a similar organization of the heme group, which consists of a protoporphytin with an iron III molecule. Class III enzymes, which are specific oxido-reductase enzymes, include the peroxidases secreted by plants [27]. Class II and III peroxidases contain disulfide bonds and calcium binding sites, which contribute structural stability and functional importance. Class III peroxidases (POX) play important roles in the regulation of plant development, growth and physiology, especially in terms of protection against adverse environmental conditions [28].

POX are present in the apoplast [29] [30], integral in plasma membrane [31] [32], and vacuole [33] [34]. In higher plants, class III peroxidases (Guaiacol pe-

roxidase, EC1.11.17) are heme oxido-reductases, which are known as secreted enzymes because they are exported to the apoplastic space [35] [36] or they are in vacuoles [34]. Because POX have several isoforms with low specificity for their substrates and redundant functions, exploring the individual function of each of the 119 genes in the maize genome is difficult because of the high variability of the isoforms [29] [37]. POXs function by oxidizing a wide range of organic and inorganic substrates in the presence of H_2O_2 [38] [39]. The oxidation of phenolic monomers results in the conversion to oligomers or phenolic polymers accompanied by a decrease in ROS levels, making this group of antioxidant enzymes highly efficient [40].

POX activity is associated with different stages of plant growth and development [40]-[43]. At the tissue level, POX activity is involved in tissue remodeling, such as the formation of aerenchyma under hypoxic conditions [44]. At the cellular level, POX catalyzes the oxidation of auxin which can control cell elongation [45]. Alternatively, it participates in the relaxation/softening of the cell wall (CW) and facilitates cell expansion [46]-[52]; for a review, see Kärkönen *et al.* [25]. POX activity in the CW can result in the establishment of cross-links between components such as extensins, determining its cytoarchitecture [53], increasing rigidity through the cross-linking of its constituents [54]-[59] or generating high stiffness through deposition of lignin by polymerizing phenolic alcohols and their cross-linking with other cell wall compounds [60]-[62]; for more details see Delmer *et al.* [63].

POX, in addition to contributing to antioxidant mechanisms, is associated with defense against abiotic stresses, such as drought [64]-[66], salinity [65]-[67], low temperatures [68] and the presence of toxic substances such as arsenic [30]. Moreover, when exposed to biotic stress [69] [70], POX increases the degree of lignification of the CW and strengthens its rigidity [30] [69] [71] or decreases pathogens resistance when invading the plant [72].

The objective of the present study is to correlate the activity of the class III peroxidases present in the scutellum with the morphometric parameters of the terminal phase of germination in isolated maize embryos subjected to imbibition for 24 h in phosphate media at different concentrations and pH. Another aim of this study is to confirm that the class III peroxidase activity in the scutellum is a biochemical indicator of the final stage of germination and the transition toward the start of seedling growth in of maize of the Conical Complex.

2. Materials and Methods

2.1. Biological Material, Storage Conditions and Reagents Used

In this study, two varieties of maize grains from the Conical Group were used, purchased from two different locations and harvest years as follows: a) grains of plants grown in the Chalco Valley (CH), State of Mexico (19˚15'00"N 98˚50'00"O/19.25, −98.83333) during the 2016 planting cycle, and b) plants grown in the Municipality of Milpa Alta (MA) in Mexico City (19˚11'39"N 99˚01'17"W/19.19405,

−99.02149) and obtained during the 2018 sowing period. The mature grains were acquired from producers in the local market. The grains were transported to the laboratory, cleaned to remove residues, placed in airtight plastic containers and stored in darkness at 7˚C and 40% relative humidity until use [23]. For the study, it was ensured that the grains of each race used had similar weights (from 0.5 to 0.6 g).

The chemical reagents, substrates, inhibitors, and solvents used in this study were obtained from Sigma-Aldrich, Merck, Baker (USA).

2.2. Conditions for Embryo Imbibition

The embryos were manually dissected from the grains with similar weight of each race with no damage and then were superficially disinfected with a 3% NaClO solution for 5 min and washed three times with sterile water. Five embryos with similar dry weight were placed per germinator, ensuring that the scutella have no damage; the embryo were placed into direct contact with the base of the germinator. Each germinator consisted of a Petri dish with two layers of filter paper on both the base and the cover. To each germinator, 10 ml of the corresponding imbibition medium was added at the base to moisten it while ensuring that there was no excess. The imbibition medium used was H_2O (pH 6.8) and was contrasted with four sodium phosphate (Pi) media at a concentration of 1 mM or 10 mM at pH 6.8 or pH 4.5. Sodium phosphate media were calculated using the Henderson-Hasselbalch equation and adjusted on a potentiometer with concentrated NaOH or HCl in case of needing.

The embryos were incubated for 24 h in darkness at 25˚C - 26˚C in a Riossa bacteriological incubator (NY, USA). Once the imbibition time was completed, the fresh weight of the embryo was determined, and the length of the radicle from the base of the scutellum to its apex was measured.

2.3. Enzymatic Extraction and Quantification of Class III POX Activity

At the end of the 24 h of imbibition on H_2O (pH 6.8) or sodium phosphate media (1 mM or 10 mM at pH 6.8 or pH 4.5), from each germinator four of the most vigorous embryos were selected, and their fresh weight and radicle length were determined; from these embryos, the scutella were dissected and the fresh weight is obtained. The scutella were placed on ice bed until use; the embryonic axis was removed to rule out the high activity of class III peroxidases in the expanding radicle [73]. To obtain the enzymatic extract, the procedure of Corona-Carrillo *et al.* [19] was followed. Briefly, each enzymatic extract consisted of 4 scutella that were homogenized in 3 ml of a 100 mM sodium phosphate buffer (pH 6.8). The homogenate was centrifuged at 10,000 × g for 30 min at 4˚C in a Sorvall RC-5 refrigerated centrifuge (Waltham, MA, USA). The resulting supernatant was the enzymatic extract and was stored for short time at 4˚C until use [19] [23] [74].

The activity of class III peroxidase (POX) was quantified by the method de-

scribed by Corona-Carrillo *et al.* [19] [23]. Briefly, two reaction mixtures were used: a) guaiacol peroxidase (POXgua) in a reaction medium consisting of 870 µl of a 50 mM sodium phosphate buffer (pH 6.8), 10 µl of enzymatic extract and 10 µl of 1 M guaiacol; to start the reaction, 9 µl of 3% H_2O_2 was added, and the absorbance was determined at 475 nm every 20 s for 2 min; and b) catechin peroxidase (POXcat) in a reaction mixture consisting of 440 µl of a 50 mM sodium phosphate buffer (pH 6.8), 20 µl of enzymatic extract, and 440 µl of 20 mM (+) catechin; the reaction was initiated by adding 4 µl of H_2O_2 at 3%, and the absorbance at 475 nm was recorded every minute for 5 min.

The effect of KCN as an inhibitor of POX activity was determined [19] [73], and 10 µl of 0.1 M KCN was added to each reaction mixture removing the same volume of the 50 mM sodium phosphate buffer (pH 6.8). The absorbances were recorded using a Mecasys Optizen Pop UV/Vis spectrophotometer (Korea). The enzymatic activity results are expressed as the change optical density (OD) for minute in each scutellum (OD/min scutellum).

2.4. Statistical Analysis

The quantitative results are expressed as the mean ± standard error of 6 to 11 independent test, each test consists of four scutella. Results were analyzed as a factorial experiment, using maize race, phosphate concentration and pH as factors. A one-way analysis of variance (ANOVA) was conducted, followed by a Tukey-Kramer multiple comparison test, with a significance level of $p < 0.05$ for parametric data, and a Kruskal–Wallis one-way ANOVA on ranks followed by a Bonferroni multiple comparison test with a significance value of $p < 0.05$ was conducted for nonparametric data. The statistical analyses were performed using the NCSS 2020 software.

3. Results

3.1. Characterization of the Embryos/Scutellum of Maize Grains from the Chalco Valley and Milpa Alta

To compare the grains from the two locations, grains weighing between 0.5 and 0.6 g that exhibited high vigor (data not shown) were selected. The embryos of both grains were imbibed in five different media for 24 h. At the end of this time, the embryos were found to be in the germination phase and in transition to seedling growth, associated with the beginning of radicle elongation. It is important to note that the imbibition was performed using isolated embryos, which favored homogeneous imbibition through direct diffusion of the imbibition medium without any restrictions due to the removal of impermeable pericarp and the endosperm. Thus, the real-time of the germination was synchronized under the different imbibition conditions and allow association with the of scutellum/embryonic axis characteristics of the race from which they originated.

The fresh weight of the scutellum isolated from the embryos following imbibition for 24 h in the different imbibition media varied (**Figure 1(A)**). The average

fresh weight of scutellum (FWS) of the grains of plants grown in the Chalco Valley (CH) imbibed in the different media was 0.183 ± 0.003 g, while the fresh weight of the scutellum of the grains from the Municipality of Milpa Alta (MA) was 0.170 ± 0.003 g, resulting in an average difference of 7.1%. With respect to imbibition in H_2O (pH 6.8; H_2O/6.8) resulted in a slight increase in the FWS of the CH compared with that of the MA (0.177 ± 0.007 g and 0.164 ± 0.007 g, respectively) resulting in a difference of 7.92%. The imbibition of CH maize embryo in various phosphate imbibition media produced a differential increase in scutellum fresh weight from 1.81% in 10 mM phosphate medium (pH 4.5; Pi 10/4.5) to 8.66% with 1 mM phosphate medium (pH 4.5; Pi 1/4.5) compared to the H_2O/6.8 imbibition medium (**Figure 1(A)**). In comparison, the fresh weight of the MA scutellum was slightly lower than that of CH scutellum, although the changes were not significant. In comparison, the scutellum of the MA maize embryo imbibed in various phosphate media produced an increase from 10.7% to a slight decrease of 0.3% in fresh weight (Pi1/6.8 and Pi10/4.5 respectively) compared to imbibition in H_2O/6.8 (**Figure 1(A)**). This variation in the results shows very small changes and does not differ significantly between the different imbibition media or between the two types of maize from different locations (**Figure 1(A)**). This implies that the scutellum of the grains from the two locations exhibits similar characteristics with respect to imbibition in different media. Furthermore, the scutellum represents approximately 80% of the embryo mass in both grains.

The maize grains used in the present study have high viability, vigor and germination capacity. In most embryos, radicle elongation had begun, while in others, the expansion was very small or not yet evident (**Figure 1(B)**). The radicle length of embryos from grains from the Chalco Valley (CH) imbibed in H_2O/6.8 for 24 h was 1.53 ± 0.27 mm which was intermediate compared with the values obtained under other conditions; however, radicle elongation had not yet been initiated in a proportion of the embryos, while the radicles of very few embryos reached a maximum length of 5 mm. When the embryos were imbibed in Pi 1/6.8, the average length of the radicle was 0.7 ± 0.15 mm, with a greater percentage of embryos that had not yet initiated radicle elongation or that were very small for this race. An increase in radicle length was observed when the embryos were imbibed in Pi 10/6.8 with the average length being 1.86 ± 0.30 mm. The difference in radicle length of embryos imbibed in Pi 10/6.8 was only 1.21 times greater with respect to H_2O/6.8 or 2.6 with respect to Pi 1/6.8. Whereas the size difference implies only 0.45 times when comparing the radicle length in imbibition with Pi 1/6.8 with respect to that of H_2O/6.8. Therefore, at a pH of 6.8, the radicle length was statistically different between embryos imbibed in 1 and 10 mM phosphate media. In Pi 10/6.8 medium a higher number of embryos having elongated radicles and one embryo that achieved the maximum length for this race (**Figure 1(B)-(D)**). The radicle of the embryos imbibed in two different concentrations of phosphates (pH 4.5) was similar, with a length of 1.0 ± 0.19 or 1.06 ± 0.25 mm when the embryos were imbibed in 1 mM phosphate or 10 mM phosphate medium re-

spectively (Figure 1(B)). Imbibition in phosphate media at a pH of 4.5 resulted in inferior radicle growth compared with radicle length of embryos imbibed in H_2O (pH 6.8). However, it is important to note the variation in the radicle length since very few embryos achieved a radicle length of 5 to 6 mm, and several embryos had not yet initiated radicle elongation (Figure 1(C), Figure 1(D)). Thus, for the CH embryos, imbibition in Pi 10/6.8 favored the asynchronous transition from germination to seedling growth. The smallest radicle length was observed for embryos imbibed in Pi 1/6.8 indicating that these embryos were at the end of the germination stage.

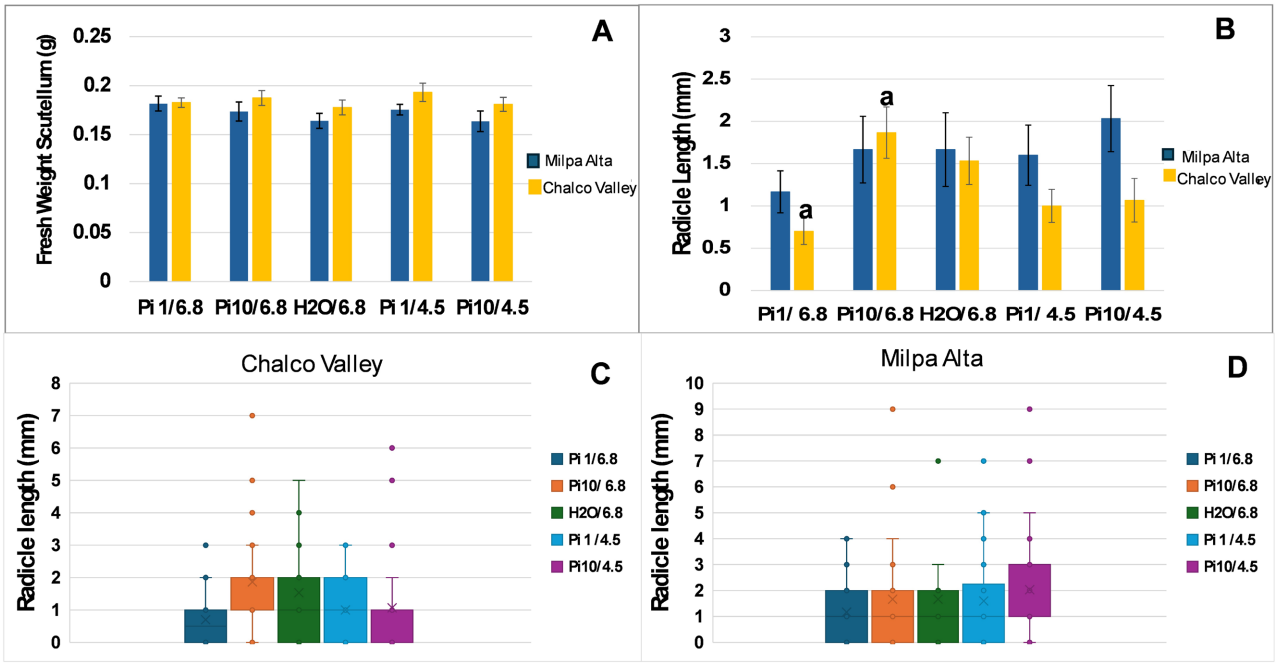

Figure 1. Morphometric parameters of the embryo in different imbibition media. Embryos from grains from the Chalco Valley or the municipality of Milpa Alta were incubated in different imbibition medium: H_2O (pH 6.8; H_2O/6.8), 1 mM phosphate medium (pH 6.8; Pi 1/6.8), 10 mM phosphate medium (pH 6.8; Pi 10/6.8), 1 mM phosphate medium (pH 4.5, Pi 1/4.5), or 10 mM phosphate medium (pH 4.5; Pi 10/4.5), at 25˚C - 26˚C for 24 h. (A) Fresh weights of the scutellum. (B) Radicle length of the embryos. (C) Radicle length and its dispersion in Chalqueño embryos. (D) Radicle length and its dispersion in Cónico embryos. In (A), the bars represent the mean ± standard error of 6 to 11 independent tests; 4 scutella were used for each test to determine the fresh weight of the scutellum; (B)-(D), the bars represent the mean ± standard error of 30 independent measurements to determine radicle length. The values represent the mean ± standard error of 150 total embryos. A one-way ANOVA was performed, followed by a Tukey-Kramer test, with a value of $p < 0.05$ considered to indicate statistical significance, the lowercase letters denote statistically significant changes.

When the MA embryos (Figure 1(B)) were imbibed for 24 h in H_2O (pH 6.8) the radicle length was 1.66 ± 0.43 mm, which indicates that the length is slightly greater than the radicle length under the same imbibition condition of the CH embryo. The shortest radicle length is induced in the Pi 1/6.8 medium (1.16 ± 0.24 mm), which is 1.65-fold greater in relation to the same imbibition medium in the CH embryo.

Radicle length was similar between CH embryos imbibed in a 10 mM phosphate

medium (pH 6.8) compared to MA embryos imbibed in H_2O/6.8 (1.66 ± 0.39 mm and 1.66 ± 0.43 mm MA vs CH). A similar length in the MA radicle when imbibed in Pi10/6.8, H_2O/6.8 and Pi1/4.5 (1.66 + 0.39 mm, 1.6 + 0.35 mm respectively). However, imbibition of embryos in Pi10/4.5 induced the greatest radicle length, representing the maximum length values of the entire study with an average value of 2.03 ± 0.39 mm, with a marked decrease in the number of embryos that have not yet begun radicle elongation, while other embryos have radicles that reached lengths of 7 to 9 mm (**Figure 1(C)**, **Figure 1(D)**). The increase in radicle length induced by this imbibition medium Pi 10/4.5 between the two races was 1.96 higher in the MA than CH. However, the differences in the values were not significant, but the results revealed that some of the MA embryos exhibited accelerated germination at 24 h and were already in the seedling growth phase (**Figure 1(C)**, **Figure 1(D)**). Accordingly, the imbibition medium that resulted in the greatest radicle elongation in the embryos of MA maize race was Pi 10/4.5, while the smallest radicle elongation was observed for embryos imbibed in Pi 1/6.8 (**Figure 1(B)**).

The larger length of the radicle of the MA embryos imbibed in Pi 10/4.5 or of the Chalqueño embryo imbibed in Pi 10/6.8 compared with that of embryos imbibed in H_2O/6.8 revealed small differences between 1.22 to 1.21-fold respectively, although this increase was not significant. The imbibition of the embryos in 10 mM phosphate medium tended to accelerate the germination process and with it, radicle elongation, but this effect was associated with the pH of the imbibition medium and the maize race; thus, a pH of 6.8 better induced radicle elongation in the CH embryos, whereas a pH of 4.5 better induced radicle elongation in the MA embryos. In contrast, imbibition of the embryos in Pi 1/6.8 tended to produce the smallest elongation of the radicle for both races.

The degree of radicle expansion for both races indicated that after 24 h of imbibition, the embryos were at the end of the germination stage toward the transition to the beginning of seedling growth. Although in some, the size of the radicle indicates that they were properly in the seedling growth phase (**Figure 1(C)**, **Figure 1(D)**).

3.2. Effect of the Imbibition Media on Class III Peroxidase (POX) Activity in the Scutellum

The use of catechin and H_2O_2 as substrates allowed the determination of POXcat activity in the scutellum of the two maize races. POXcat activity was higher in the CH scutellum than in that of the MA scutellum when the embryos were imbibed in the different imbibition media (**Figure 2(A)**). The POXcat activity in the CH scutellum induced by the different imbibition media exhibited the following trend: Pi 1/6.8 > Pi 1/4.5 > H_2O/6.8 > Pi 10/6.8 > Pi 10/4.5. The difference in activity was significant between the first and the last imbibition media. For the same concentration of phosphates, there is a tendency to induce greater activity at pH 6.8 compared to pH 4.5 and 1 mM phosphates are approximately 1.4-fold more

potent than 10 mM phosphate at the same pH. When the embryos of the MA maize race were imbibed for 24 h in the different media, the POXcat activity in the scutellum fluctuated slightly, with small differences that were not significant, as follows: Pi 1/4.5 > Pi 1/6.8 > Pi 10/6.8 > H_2O/pH 6.8 > Pi 10/4.5. The POXcat activity when the embryos were imbibed in 10 mM phosphate medium tended to be lower than that when the embryos were imbibed in 1 mM phosphate medium, but no association was found between POXcat activity and the pH of the imbibition media, showing that the small differences are not statistically significant (**Figure 2(A)**). There are significant differences when comparing the activity of POXcat from imbibition in two media; in Pi1/6.8, and in H_2O, in which the activity in the MA scutellum represents 56.6% or 55.85% of the activity of the CH scutellum respectively. The POXcat activity in the CH scutellum was higher than in the MA scutellum in the other imbibition media, where the proportional difference (CH *vs* MA) was less than 1.3 to 1.5 times, indicating that the changes were not significant.

The addition of KCN to the reaction mixture caused a significant decrease in POXcat activity for each imbibition condition in both maize (**Figure 2(B)**). The residual POXcat activity with respect to the total POXcat activity without KCN in the of the CH scutellum represented between 2.12 ± 0.19 to 3.56 ± 0.78% when the embryos were imbibed in the different media. Similarly, the residual POXcat activity in the MA scutella fluctuated between 2.77 ± 0.58 to 3.69 ± 0.43% in the different media used. These results show that KCN generated an inhibitory effect greater than 96%; this effect was replicated under all imbibition conditions and in both races. This decrease was significant compared with the total POXcat activity under the same imbibition conditions (**Figure 2(B)**); this indicates that the observed activity is the result of catechin peroxidation.

A tendency towards an inversely proportional relationship was observed between POXcat activity induced by the type of imbibition medium and incipient radicle elongation. Under the conditions of imbibition at the extremes of this trend for the CH scutellum (**Figure 2(C)**), imbibition at Pi 1/6.8 resulted in the highest POXcat activity with the shortest radicle length, while the lowest POXcat activity was observed when embryos were imbibed at Pi 10/6.8 with a tendency to develop longer radicles. Although the differences in POXcat activity and radicle length in MA embryos do not show a statistically difference among them, there is a similar propensity (**Figure 2(D)**) for greater POXcat activity was observed in embryos imbibed at Pi 1/4.5 and that had shorter radicles, conversely, the lowest POXcat activity was observed in scutellum of embryos imbibed in Pi 10/4.5 having larger radicles (**Figure 2(D)**). For both embryos, an inverse correlation is maintained between the POXcat in the scutellum and the radicle length.

The POX activity in the CH scutellum in the presence of guaiacol and H_2O_2 (POXgua) tended to be slightly higher under 4 of the 5 imbibition conditions compared with the activity of the MA scutellum (**Figure 3(A)**). Imbibition of the CH maize embryos in H_2O/6.8 for 24 h resulted in a POXgua activity of 50.25 ± 8.08

OD/min scutellum, representing the highest value across all the imbibition conditions and between the two scutella from different locations. Under the same imbibition conditions, the POXgua activity in the MA scutella was 31.86 ± 3.23 OD/min scutellum, indicating that, on average, POXgua activity was 1.57-fold greater in the CH scutellum than in that of MA scutellum under the same imbibition conditions (Figure 3(A)).

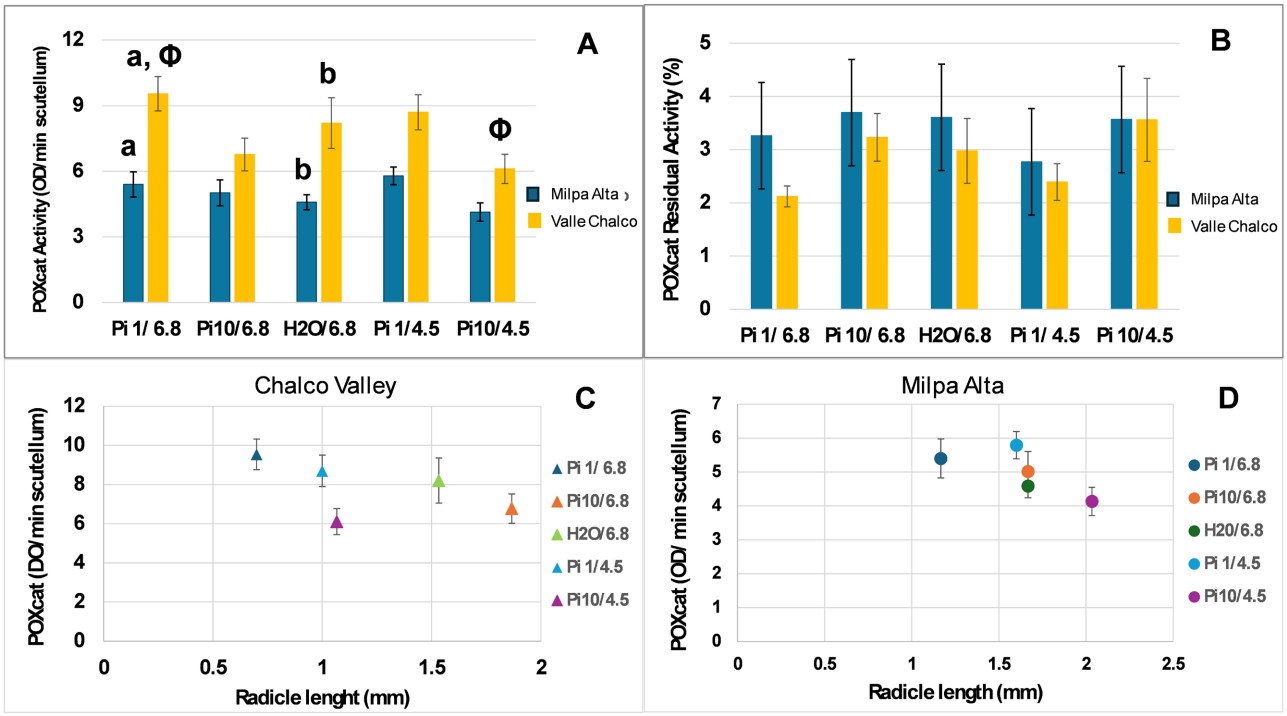

Figure 2. Effect of the imbibition medium on class III peroxidase activity in the scutellum when catechin + H_2O_2 was used as substrate. (A) POXcat activity in CH scutellum and MA scutellum extracted from embryos imbibed for 24 h in different media: H_2O (pH 6.8; H_2O/6.8), 1 mM phosphate medium (pH 6.8; Pi 1/6.8), 10 mM phosphate medium (pH 6.8; Pi 10/6.8), 1 mM phosphate medium (pH 4.5; Pi1/4.5), or 10 mM phosphate medium (pH 4.5; Pi 10/4.5). (B) Residual activity of POXcat upon addition of KCN to the reaction mixture. The results represent the percentage of residual activity compared to the total POXcat activity from the same enzyme extract without the addition of KCN. (C) Relationship between POXcat activity in the scutellum and radicle length of CH embryos imbibed for 24 h in different media. (D) Relationship between POXcat activity in the scutellum and radicle length of MA embryos imbibed for 24 h in different media. The data represents the mean ± standard error of 6 to 11 independent tests; for each test, 4 scutella were used. An ANOVA was performed, followed by a Tukey-Kramer multiple comparison test, with a value of $p < 0.05$ considered to indicate statistical significance. The results reveal significant changes in POXcat activity in the scutellum induced by the different imbibition media at 24 h between the two maize races, statistically significant difference between the two imbibition conditions in the same race is represented by Φ; the lowercase letters denote statistically significant changes under the same imbibition condition between the two grains.

POXgua activity in the CH scutellum imbibed in different phosphate media varied from a maximum of 42.86 ± 6.18 OD/min scutellum for Pi 10/6.8 to a minimum of 32.49 ± 4.01 OD/min scutellum for Pi 1/4.5. The activity trend between the different imbibition conditions was as follows: H_2O/6.8 > Pi 10/6.8 > Pi 10/4.5 > Pi 1/6.8 > Pi 1/4.5. POXgua activity in the MA embryo fluctuated from a maximum of 40.23 ± 6.35 OD/min scutellum when imbibed in Pi 10/6.8 to a min-

imum of 30.96 ± 3.35 OD/min scutellum when imbibed in Pi 1/6.8, with a decreasing trend in the following order: Pi 10/6.8 > Pi 1/p4.5 > Pi 10/4.5 > H_2O/6.8 ≥ Pi 1/6.8. The differences in the POXgua activities under the different imbibition conditions and between the two embryos were not significant (**Figure 3(A)**). However, in the presence of phosphates, there was a tendency in both grains to exhibit the maximum POXgua activity in the scutellum when the embryos were imbibed in Pi 10/6.8, in contrast, the scutellum of the embryos imbibed in Pi 1/6.8 tended to exhibit the lowest POXgua activity. The proportional difference in the POXgua activity for the same imbibition condition between the scutella of the two embryos, considering the activity for each imbibition based on the MA scutellum, ranged from a maximum of 1.57-fold in the imbibition medium in H_2O/pH 6.8 to minimum of 0.85-fold that in Pi 1/4.5 medium. Moreover, the trend in the proportional change in the POXgua activity according to the imbibition condition between the races was as follows: H_2O/6.8 > Pi 1/6.8 > Pi 10/6.8 > Pi 10/4.5 > Pi 1/4.5.

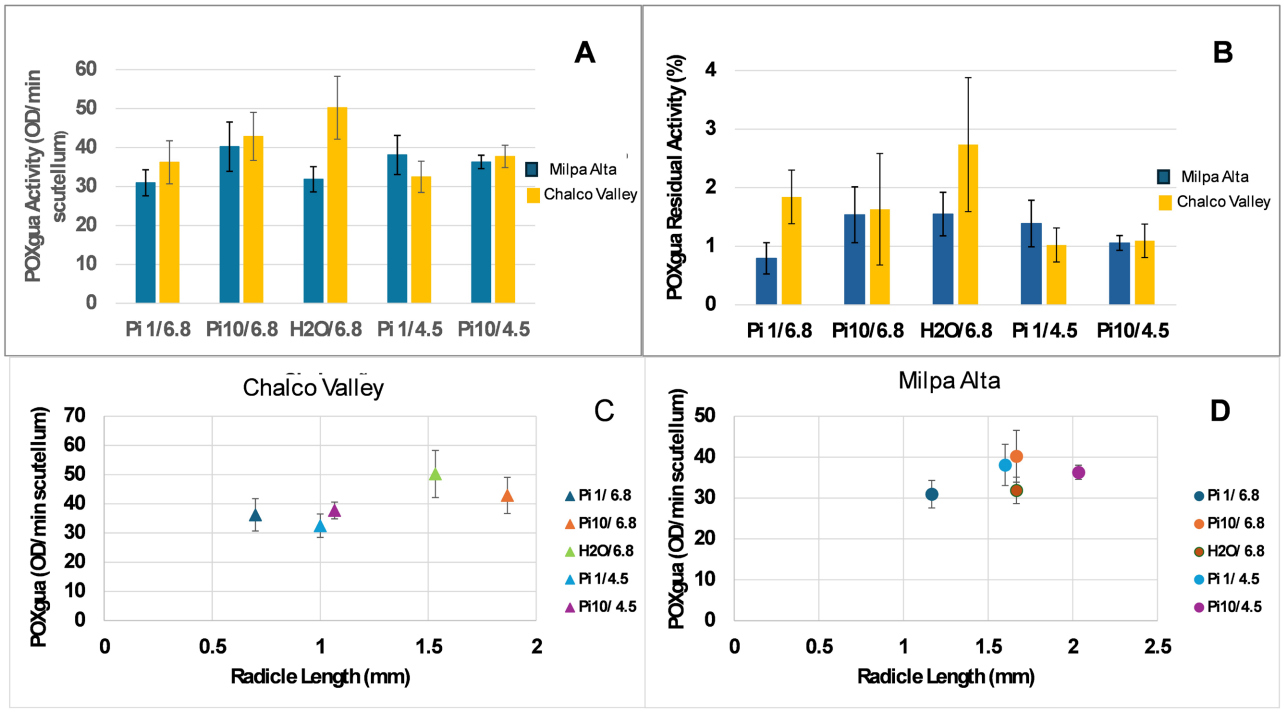

Figure 3. Effects of the imbibition medium on class III peroxidase activity in the scutellum when guaiacol + H_2O_2 was used as substrate (POXgua). (A) POXgua activity in scutellum extracts of the CH and MA embryos imbibed for 24 h in different media: H_2O (pH 6.8; H_2O/6.8), 1 mM phosphate medium (pH 6.8; Pi 1/6.8), 10 mM phosphate medium (pH 6.8, Pi 10/6.8), 1 mM phosphate medium (pH 4.5, Pi1/4.5) or 10 mM phosphate medium (pH 4.5, Pi 10/4.5). The differences in POXgua activity under the various imbibition conditions in both races were not significant. (B) Residual POXgua activity upon addition of KCN to the reaction mixture. The results represent the percentage of residual activity compared to the activity of POXgua from the same enzyme extract without the addition of KCN. (C) Correlations between the POXgua activity in the scutellum and the radicle length of CH embryos imbibed for 24 h in different media. (D) Correlations between the POXgua activity in the scutellum and the radicle length of MA embryos imbibed for 24 h in different media. The data represents the mean ± standard error of 6 to 11 independent tests; for each test, 4 scutella were used. A Kruskal-Wallis one-way ANOVA on ranks was conducted followed by a Bonferroni multiple comparison test with a value of $p < 0.05$ considered to indicate statistical significance. No significant differences were found in the quantification of this biochemical parameter.

The residual POXgua activity in the presence of KCN in the CH scutellum fluctuated on average from 2.73 ± 1.14% when imbibed in H_2O/6.8 to 1.02 ± 0.28% when imbibed in Pi 1/4.5. In contrast, the residual POXgua activity in the MA scutellum varied from 1.55 ± 0.37% when imbibed in H_2O/6.8 to 0.79 ± 0.26% when imbibed in Pi 1/6.8. This indicated an inhibition of greater than 96%. These results confirm the presence of a class III peroxidase in the scutellum of both maize races that is strongly inhibited in the presence of KCN (**Figure 3(B)**).

When the radicle length and the POXgua activity for each imbibition condition for the two races were correlated (**Figure 3(C)** and **Figure 3(D)**), it was found that a difference in radicle length did not lead to a significant increase in POXgua activity. Thus, CH embryos imbibed in H_2O/6.8 showed the highest POXgua activity in the scutellum but had intermediate radicle lengths, similarly in Cónico race, the embryo shows an intermediate radicle length with the maximum POXgua activity in the scutellum imbibed in Pi 10/6.8.

3.3. Proportional Differences between POXgua and POXcat Activities

The POX activity index (AI) between the two substrate mixtures was determined by obtaining the ratio between the POXgua activity and the POXcat activity in the scutellum (POXgua/POXcat) after 24 h of imbibition in different media. Imbibition in H_2O/6.8 resulted in an AI value of 5.45 ± 1.18 for CH scutellum and 7.28 ± 0.91 for MA scutellum indicating a 1.33-fold increase between the two races (**Figure 4(A)**).

The AI value when the CH scutellum was imbibed in phosphate media ranged from a minimum of 3.84 ± 0.38 for Pi 1/4.5 to a maximum of 6.33 ± 0.38 for Pi 10/4.5, with the following trend: Pi 10/4.5 > Pi 10/6.8 ≥ H_2O/6.8 ≥ Pi 1/6.8 > Pi 1/4.5 (**Figure 4(A)**). In contrast, for MA scutellum, the observed AI tended to be higher than for the CH scutellum (**Figure 4(A)**), and this difference was not significant for the same imbibition condition between races. Therefore, the AI value varied between 6.42 ± 0.49 to 9.15 ± 0.97, for Pi 1/6.8 and Pi 10/4.5 respectively; however, the difference was not statically significant. The trend observed for the different media was as follows: Pi 10/4.5 > Pi 10/6.8 ≥ H_2O/6.8 > Pi 1/4.5 ≥ Pi 1/6.8 (**Figure 4(A)**). In the scutella of both grains, the differences in AI were due mainly to the discrepancy in the POXcat activity under different imbibition conditions; and only the difference in AI values was statically significant between the MA scutellum imbibed in Pi 10/4.5 compared with CH scutellum imbibed in Pi 1/4.5 or with Pi 1/6.8.

The residual activity index (RAI) when the CH embryo was imbibed in H_2O/6.8 was 4.66 ± 1.34 and the lowest RAI of 2.1 ± 0.52 was achieved when the embryos were imbibed in Pi 10/4.5. Thus, the trend of the RAI for this maize was as follows: H_2O/6.8 > Pi 10/6.8 ≥ Pi 1/6.8 > Pi 1/4.5 ≥ Pi 10/4.5. The RAI in the MA scutellum was maximum when the embryos were imbibed in Pi 10/6.8, with a proportion achieving a value of 3.86 ± 1.6, and the minimum value of 2.20 ± 0.35 when the

embryos were imbibed in Pi 1/4.5. The trend of the RAI for this scutellum was as follows: Pi 10/6.8 ≥ H_2O/6.8 > Pi 10/4.5 > Pi 1/6.8 ≥ Pi 1/4.5. The differences in RAI between the imbibition conditions for the same race or between races were not significant (Figure 4(B)).

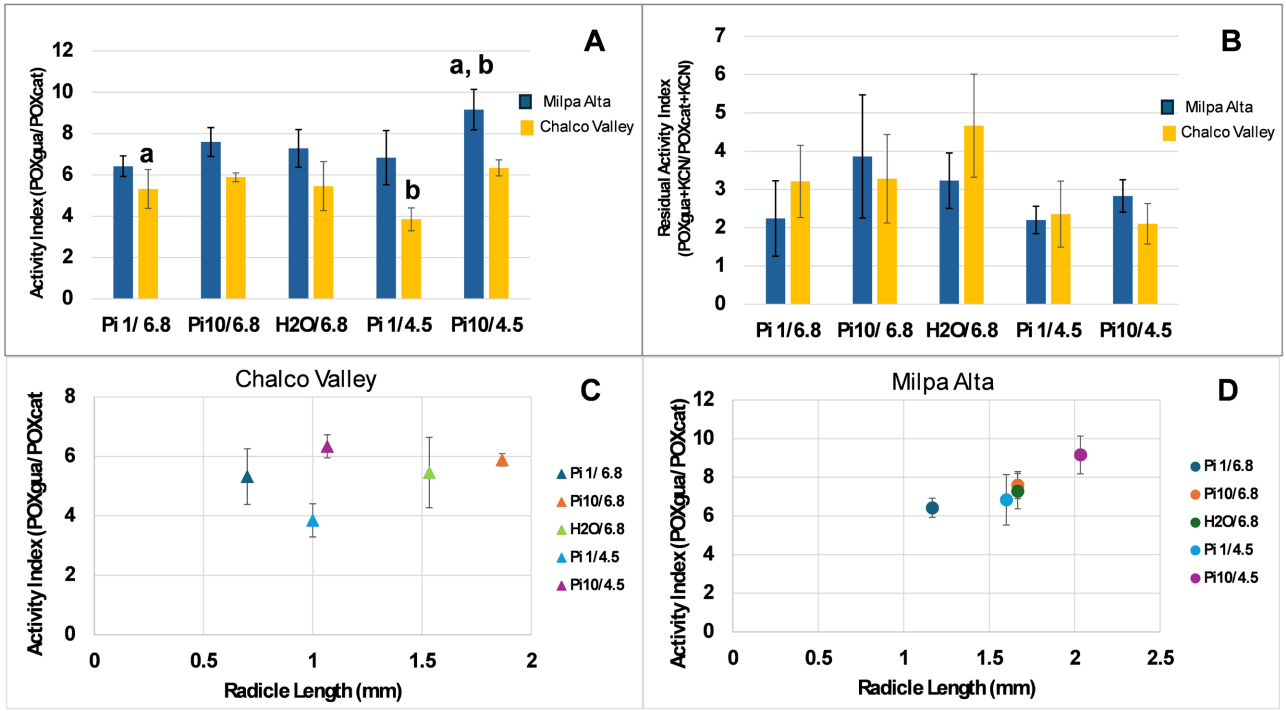

Figure 4. POX activity index (AI) of the scutella. The data represents the AI values calculated from the ratio between the activity of POXgua/POXcat for the scutellum of embryos imbibed for 24 h in different media. (A) The activity index for the scutellum of grains of different locations at 24 h after imbibition in different media. (B) The residual activity index for the scutellum at 24 h, involving the addition of KCN to the activity assay. The scutellum used were obtained from embryos of each race after being imbibed for 24 h in different media: H_2O (pH 6.8, H_2O/6.8), 1 mM phosphate medium (pH 6.8; Pi 1/6.8), 10 mM phosphate medium (pH 6.8, Pi 10/6.8), 1 mM phosphate medium (pH 4.5, Pi1/4.5), or 10 mM phosphate medium (pH 4.5, Pi 10/4.5). (C) Correlations between the POX activity index of the scutellum and the average radicle length of the CH embryos imbibed for 24 h in different media. (D) Correlations between the POX activity index of the scutellum and the radicle length of the MA embryo imbibed for 24 h in different media. The data represents the mean ± standard error of 6 to 11 independents tests, with 4 scutella used for each test. A Kruskal-Wallis one-way ANOVA on ranks was conducted followed by a Bonferroni multiple comparison test with a value of $p < 0.05$ considered to indicate statistical significance. Bars with the same letter indicate a statistically significant difference.

In terms of the activity index, the scutella of CH maize presented a fluctuating baseline value at 24 h after imbibition across the different imbibition conditions independent of the radicle length (Figure 4(C)). For the MA scutella, the observed values of the AI in relation to the radicle length fluctuated around a basal value for 4 of the 5 conditions, whereas for Pi 10/4.5, the highest AI corresponding to a greater radicle length was obtained (Figure 4(D)).

4. Discussion and Conclusions

Phosphate not only plays a vital role in energy transfer and metabolic regulation but also is an important macronutrient for the synthesis of phospholipids [75] that

constitute plant membranes [76], proteins and nucleic acids [75] used in the energy metabolism of plants as ATP. The growth and development of seedlings or plants are particularly dependent on the availability of phosphate [77]. Recently, Mohamed *et al.* [78] published a review reporting that phosphate solutions (KH_2PO_4) are used for halopriming, which is a technique that involves the imbibition of seeds in inorganic salts prior to sowing. Exposure to dilute saline solutions such as NaCl, KNO_3, $CaCl_2$, or KH_2PO_4 increases the physiological readiness of seeds for rapid and synchronized germination while increasing their tolerance to salt stress, drought and extreme temperatures. Halopriming can increase the concentration of compatible osmolytes in seeds and improve the activity of antioxidant enzymes, such as peroxidases, catalases and superoxide dismutase ([78] and the references therein). Therefore, this study focuses on determining the effect of imbibition of maize embryos under different imbibition conditions in the presence of phosphates.

Importantly, the pericarp and the endosperm of the embryos were removed, and the scutellum were exposed to the imbibition medium to allow the direct diffusion of the imbibition medium into the embryo. This allowed a more precise determination of the time of onset of imbibition and the determination of the time at which radicle elongation occurred, permitting the establishment of the period of germination and the transition to seedling growth. The embryos from both locations are viable and have high vigor according to the results of the MTT tests (vigor assays with thiazolyl blue tetrazolium), which were similar to the findings of previous studies [23].

Due to the differences in grain size observed between the plants in both locations, it was decided to use grains of similar weight between them, with a variation of 500 and 600 mg. Thus, the CH grains in this study are lighter than the grains in previous studies by the authors. The range of the weights used was defined based on the highest weight of the grains from the locality of Milpa Alta.

The imbibition of the embryos of both localities for 24 h shows that they are still at the end of germination, as most have not yet begun radicle elongation, with a radicle length of 0 to 1 mm (60% - 70%). Meanwhile, the number of embryos that have begun radicle elongation, indicating they are transitioning to seedling growth, ranges from 25.5% to 30%, with a radicle length of 2 to 4 mm. Between 4.6% and 9.33% of these embryos are clearly undergoing seedling growth. The CH embryos imbibed in Pi 10/6.8 and the MA embryos imbibed in Pi 10/4.5 induce an increase in radicle lengths (**Figure 1(C)**, **Figure 1(D)**). Imbibition of the embryos in H_2O/6.8 resulted in similar radicle lengths for both embryos, which were lower than those reported for larger embryos in past studies. The smallest radicle length was observed when the embryos of both races were imbibed in Pi 1/6.8, which was more notable for the CH embryos. However, a small number of embryos (4 of 150 embryos) imbibed in four of the five imbibition media (except for Pi 1/6.8) had radicle lengths between 5 and 7 mm, and very few embryos (2 of 150 embryos) achieved a radicle length of 9 mm (**Figure 1(C)**, **Figure 1(D)**). The embryos with the longest radicle lengths are found in the MA embryo. In a parallel

study conducted by our research group using smaller grains than those used in the present study, the time at which the majority of the embryos are observed to be in the accelerated radicle elongation phase occurred between 26 and 28 h of imbibition (in H_2O at a pH of 6.8); at this time, a radicle length greater than 4 mm are reached and the embryos are already transitioned from the germination phase to the initial phase of seedling growth (not shown). Thus, it was confirmed that in the present study, the majority of the embryos imbibed for 24 h were in the final phase of germination and/or in the transition/beginning to early post-germination phase, unlike the CH embryos from previous studies, which had a higher weight, they were clearly in the initial phase of seedling growth or skotomorphogenesis at 24 h [79]. The high metabolism of the scutellum is associated with different simultaneous processes that occur during germination, seedling growth or the transition between the two stages. This organ provides nutrients, which are the products of the initial hydrolysis of its stored lipid reserves which are catabolized and converted into substances that are translocated to the embryonic axis to sustain its growth, to the rest of the embryo/seedling [16]-[19].

During the final phase of germination and the initial phase of seedling growth, the scutellum consolidates the development of epithelial and vascular tissues to favor the efficient transport of nutrients, initially from the scutellum itself to the rest of the embryo and subsequently from the endosperm through the scutellum to the growing seedling [17] [23]. Thus, efficient communication between the scutellum and the embryonic axis is established, which initially impacts the coordinated development first of the embryo and subsequently of the seedling. The experimental design implemented in this study allowed the placement of the embryo in the germinator with the scutellum in intimate contact with the imbibition medium during the 24 h of imbibition, allowing the scutellum to be the first structure exposed to the imbibition medium with subsequent diffusion to the rest of the embryo. Thus, the scutellum perceives and responds to the composition of the imbibition medium.

Thus, the maize grains from Chalco Valley in the present study had a lower weight than those used in previous studies of the authors. Therefore, a decrease was observed between the fresh weight of the CH scutellum at 24 h after imbibition in the present study and those previously imbibed under similar conditions in H_2O/6.8 or in Pi 10mM/4.5. The fresh weight of the scutellum in this study was 85 to 90% that of the fresh weight of the scutella in a previous study [19] [23] [79]. Therefore, the fresh weight of the scutella at 24 h after imbibition non-significantly around a basal value among the different imbibition conditions, indicating that the imbibition medium did not modify the wet weight of the scutella and thus allowed it to be used as a parity basis in the morphometric comparison and in the comparison of the POXcat and POXgua activities between the scutellum of the embryos from the two localities.

Peroxidases are encoded by several genes and thus they have several isoforms that are involved in a wide range of cellular processes. Class III peroxidases act on many substrates and oxidize organic and inorganic compounds in the presence of

H_2O_2 [38] [39] [80] [81]. They also reduce the intracellular levels of H_2O_2 by oxidizing the phenolic monomers with the production of oligomers and phenolic polymers. Therefore, peroxidases constitute a group of very efficient antioxidant enzymes [40]. POX activity is associated with morphogenesis since it plays an important role in different stages of plant growth and development [40] [41]. Thus, it participates in processes such as germination and seedling establishment [42] [43]. POX activity is a biochemical parameter associated with the metabolism of the scutellum, which can be used as an indicator of the transition from germination to seedling growth [19] [23] [79]. It participates in tissue morphogenesis in the lignification of the secondary wall of vessel elements and facilitates efficient transport in the embryo [74] [79]. The establishment of functional vascular tissue allows the scutellum to provide nutrients to the rest of the embryo/seedling and simultaneously receive water and other compounds from the radicle of the embryo/seedling to efficiently coordinate growth.

Class III peroxidases use a wide variety of phenolic compounds as substrates [80] [81], in this study guaiacol or catechin were used as substrates in the presence of H_2O_2 to determine peroxidase activity. Guaiacol is one of the most usual substrates as reported in the literature and it is used to determine class III POX activity and characterize the products of the peroxidation reaction [82]-[85]. In comparison, catechin has been used as a substrate to a much lesser extent, with studies reporting the characterization of products of peroxidation *vs* oxidation of catechin-forming compounds with different degrees of polymerization [86]-[88]. In the CH scutellum and MA scutellum, POX activity was detected only when guaiacol or catechin was in the presence of H_2O_2, and the oxidation of catechin in the absence of H_2O_2 was not detected, which excludes the presence of a polyphenol oxidase in the extracts and confirms previously published findings [19].

KCN is a potent inhibitor of hemoperoxidases, such as class III peroxidases, by acting on the Fe^{3+} of the heme group, blocking its ability to react with H_2O_2 and inhibiting the oxidation of the co-substrate; therefore, it is a broad-spectrum inhibitor in which various phenols are used as co-substrates. Therefore, the use of KCN is an inhibitor of POXcat and POXgua activity, and its presence in the assay results in very low residual activity, indicating that the studied enzyme is inhibited by more than 96% and that no activity is detected in the absence of H_2O_2 in the scutellum of both embryos from different locations. These results confirm that the quantified activity is due to class III peroxidase.

The results revealed differences in the induction of POXcat activity in the scutellum between the grains from the two locations, in which the values are higher, although not statistically significant, in the CH scutellum compared to the MA scutellum. The POXcat activity in the MA scutellum was much more similar under four of the five imbibition conditions. The difference in activity between CH *vs* MA scutellum is 1.78-fold higher when they are imbibed in H_2O/6.8 and 1.76-fold higher when they are imbibed in Pi 1/6.8. There is a significant change in POXcat activity in the CH scutellum when imbibed in Pi 10/4.5 compared to the

higher activity in Pi 1/6.8. The difference in the proportion is only 1.16-fold under the other conditions. POXcat activity in the scutellum of both grain types tends to be higher in presence of 1 mM phosphates compared to 10 mM phosphates at pH 6.8 or pH 4.5, and the activity of POXcat in H_2O/6.8 is intermediate between the two phosphate conditions (1 mM *vs* 10 mM). In the scutella of the maize from of both races, a trend is observed in the correlation between the highest POXcat activity (Pi 1/6.8) and the shortest radicle length, whereas a trend towards greater radicle length is associated with lower POXcat activities in the scutellum in CH embryos when imbibed in Pi 10/6.8 or in the MA embryo imbibed in Pi 10/4.5.

The results revealed no statistical differences in the induction of POXgua activity in the scutellum in the different imbibition media between the grains from the two locations, with the highest values corresponding to the CH scutellum compared to the MA scutellum. The maximum POXgua activity occurs when the CH embryos are imbibed in H_2O/6.8, whereas the lowest POXgua activity occurs when the embryos are imbibed in Pi 1/4.5, indicating 0.85-fold less activity than that of the MA embryos imbibed in the same medium. For CH maize embryos, the tendency towards longer radicle lengths is recorded when they are imbibed in Pi 10/6.8 and H_2O/6.8 and correlates with high POXgua activity in the scutellum; while the tendency towards a smaller radicle is obtained when the embryos are imbibed in Pi 1/6.8 and correlates with a decrease in POXgua activity, although not significant. In MA maize, the correlation between greater radicle length and greater POXgua activity is less clear; embryos imbibed in Pi10/4.5 tend to have a longer radicle, but while POXgua activity is present, considering the dispersion in the group with the highest activity, it on average ranks third. A trend toward a shorter length when the embryos are imbibed in Pi 1/6.8 is associated with a lower average POXgua activity. However, as the changes as a function of the imbibition time did not exhibit significant differences, they can be considered only a trend and should be corroborated by testing with additional imbibition times.

The activity index of POX (AI) is lower in the CH scutellum than in the MA scutellum. In the CH maize, the AI ranged from 3.84 ± 0.55 to 6.33 ± 0.38. The greater tendency in radicle length in the presence of Pi 10/6.8 in the CH embryo is related to an AI that is in the middle of the interval for this type of maize. However, in the MA scutellum, the activity indices were higher than those found in the CH scutellum, ranging from 6.42 ± 0.4 to 9.15 ± 0.97. The highest activity index in the Pi 10/4.5 of the MA scutellum coincided with the greatest radicle length. This was found to be a difference between embryos from the two locations. Whereas in MA embryo imbibed at Pi 1/6.8 there is a tendency towards a shorter radicle length that correlates with a lower value in AI. A comparison of the activity index of the CH scutellum with those previously published by the authors [79] revealed that the activity index of the scutellum after 24 h of imbibition in this study imbibed in H_2O/6.8 is similar to that reported previously in H_2O/4.5 and inferior to the AI of H_2O/6.8 in the previously publishing; which is closer to the AI in H_2O/6.8 obtained in the MA scutellum. Moreover, when the CH embryos

are imbibed in Pi 10/4.5, the IA of the scutellum is also similar to that previously published, and under both conditions, the activity index of the MA scutellum is higher. It is important to mention the high AI obtained when the MA embryo is imbibed at Pi 10/4.5, the highest value in this study, and which is in the interval of IA when stress is generated as previously published [79], probably to counteract oxidative stress. It is observed that the IA varies for the same imbibition time according to the medium used in this process and that as the imbibition time the values decrease, indicating that during the seedling growth the values are lower than in the final phase of germination, indicating that the AI varies by imbibition condition and the time [79].

A relationship exists between embryo germination morphometric parameters and class III peroxidase activity after 24 h imbibition in water or phosphate solutions at different concentrations and pH levels. POXcat and POXgua activities in the scutellum are induced during and after germination and can be a good biochemical indicator associated with the activity index (POXgua/POXcat), indicating the stage of embryo development or seedling growth in Conical Complex embryos.

In this context, the results of the parameters quantified in the present study exhibit great similarity between the embryos or scutella of maize from different locations. The differences in the parameters studied between the embryos of the two races mostly did not represent statistically significant changes, suggesting that the two races studied, which belong to the Conical Complex, are closely related from their origin. Differences in growth conditions, year, and location of cultivation had less influence. It has been established that the most stable and reliable descriptors of racial diversity, which faithfully reflect the genotype, are morphological traits, such as the size of the grain (e.g., the length, width, and mass), the size of the ear (e.g., the length and width), and the number of rows. These characteristics are less influenced by variations in environmental conditions; thus, they constitute the primary and most reliable descriptors used for classification [89]. The grains from both locations belong to the Conical Complex, although the characteristics of the grains/cobs from Chalco Valley indicate that they belong to the Chalqueño race, which has been used in previous studies by the research group. These grains come from a nearby but distinct location within Valley. However, the morphological characteristics of the grains from Milap Alta indicate that they belong to the Conical Complex, but with characteristics of the Conical race. There is a phylogenetic relationship between both these maize races because the Chalqueño race originated in the colonial era in central Mexico and was probably derived from the crossing of the Cónico and the Tuxpeño maize races [11]. And the Chalqueño maize is considered a highly developed form of Cónico maize.

It has been described that maize races exhibit morphological overlaps between different taxonomic groups, for example, in the Chalqueño and Cónico races. This confirms that the races are not discrete entities but rather form part of the Pyramidal or Cónico Complex, with a continuum of variations connecting several races

in the Altiplano Central [89] [90]. This implies that the results of the present study, therefore, the grains from both locations induce similar responses with small variations in biochemical and morphometric parameters; both types of grains are highly related.

Author Contributions

D.M.D-P., J.I.C-C., D.A.L-R., C.L-V. and L.L-V. carried out the experiments and participated in the data analysis. D.M.D-P. and J.I.C-C. drafted the manuscript, designed and coordinated the study, evaluated the data, and wrote the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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