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Physiological and biochemical alterations during germination and storage of habanero pepper seeds

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The objective of this study was to evaluate physiological and biochemical alterations during the development and storage of habanero pepper seeds with a view toward determining the time of harvest. Seeds were manually extracted from the fruit at three stages of development: E1 (fruit with first signs of yellowing), E2 (mature fruit) and E3 (mature fruit submitted to seven days of rest). After drying, seeds with 8% water content were stored at 10°C for 0, 4 and 8 months, and their quality evaluated by means of germination and vigor tests. Activities of the enzymes α-amylase, endo-β-mannanase, esterase, Superoxide Dismutase (SOD), malate dehydrogenase (MDH) and alcohol dehydrogenase (ADH) were evaluated during germination at 0, 48, 96 and 144 h after seeding. A randomized block design was used in a 3 × 3 factorial design (stages of development × storage) with 4 replications. Lower germination and vigor values were observed for the E1 stage seeds at all storage periods. In recently stored seeds, greater germination and vigor values were observed for the E3 stage seeds. Dormancy was observed principally in recently stored seeds and this was overcome at four months of storage. In summary, the physiological tests and activity of the enzymes evaluated indicated that the habanero pepper should be harvested at the E3 stage for a higher seed quality.

Key words: *Capsicum chinense*, seed maturation, seed quality.

INTRODUCTION

The demand for high quality seeds has grown substantially in recent years, which requires that seed companies adopt advanced technologies during production, processing and storage processes. The adoption of new seed technologies requires knowledge of the factors that influence physiological quality on the one hand and on the physiological and biochemical alterations that occur during the germination and storage processes on the other hand. Studies related to maturation and harvest of seeds is important since seeds reach their maximum quality in the field. Such knowledge is necessary for seed producers to determine the ideal time for harvest that would minimize seed quality deterioration caused by a prolonged period in the field, and increase seed production, by preventing a too early harvest since this may result in a large proportion of immature seeds (Vidigal et al., 2009). In species with fleshy fruit, it has been observed that seeds maintained for a certain period of time in the fruit after harvest complete the maturation process, reaching maximum levels of germination and vigor (Barbedo et al., 1994; Vidigal et al., 2006; Dias et al., 2006). Therefore, post-harvest storage of the fruit before seed extraction may be advantageous because it allows early harvest, and avoid exposure to possible unfavorable conditions that may deteriorate seed quality (Barbedo et al., 1994;

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Table 1. Mean values of maximum (Tx), minimum (Tn), and mean (Tm) temperatures, relative humidity (UR), total rainfall (Pt) and insolation (I) of the months corresponding to the crop development period of the 2007 harvest.

Months	Tx (ºC)	Tn (ºC)	Tm (ºC)	UR (%)	Pt (mm)	I (h)
January	27.6	18.7	21.9	85.7	554.7	3.1
February	28.9	18.1	22.6	73.1	151.3	7.5
March	30.9	18.1	23.6	66.5	35.4	9.0
April	28.1	17.2	21.8	72.4	35.6	7.3
May	25.7	12.8	18.1	70.6	30.4	7.5
June	25.8	11.1	17.3	66.3	5.9	8.8
July	25.4	11.1	17.1	66.8	17.6	7.7

Source: Agrometeorology Sector of the Engineering Department – UFLA.

Dias et al., 2006).

Deterioration is a process determined by a series of physiological, biochemical, physical and cytological alterations that occur in a progressive manner, leading to lower quality and culminating in death of the seed (Freitas, 2009). The main alterations related to the deterioration process are degradation and inactivation of enzymes, reduction of respiratory activity and loss of integrity of cellular membranes (Copeland and McDonald, 2001). The speed of this deterioration process is influenced by storage conditions. During seed storage, it is necessary to maintain adequate temperature and humidity conditions in the attempt to preserve quality. In this research, physiological and biochemical alterations during development of yellow habanero pepper (*Capsicum chinense*) seeds were evaluated, with a view toward determination of the best time for harvest. In addition, enzymatic alterations were evaluated during the germination process of the seeds processed at different stages of maturity and storage.

MATERIALS AND METHODS

The research was conducted in the experimental area and in the Central Seed Laboratory (Laboratório Central de Sementes) of the Agriculture Department (Departamento de Agricultura) of the Federal University of Lavras (Universidade Federal de Lavras - UFLA), in Lavras, MG. The city located in the Southern Region of Minas Gerais, latitude 21° 14' S and longitude 40° 17' W and at 918.8 m altitude. The annual average temperature is 19.4°C and rainfall is distributed principally from October to April, with annual amounts of 1529.7 mm. In a first stage of research, yellow habanero pepper (*C. chinense*) seedlings were formed for installation of the experiment in the field. Seeds were sown in "styrofoam" trays with 72 cells containing the commercial substrate Plantmax® - hortaliças and 5 ml of 2000 ppm solution of ammonium sulfate per cell. Transplanting of the seedlings was performed 45 days after seeding to an experimental area of the olericulture sector of the Agriculture Department in an area with a Dark Red Latosol/Oxisol and clayey texture; prepared conventionally. Tests were installed in a randomized complete block design with four replications. Each plot consisted of 2 rows of 5 m length with 5 plants per meter and spacing of 1.5 m between rows. Plant cultivation was performed in accordance with Filgueira (2005). Temperature and relative air humidity data during plant development are presented in Table 1. Seeds were manually extracted from many fruits at three stages of development: E1 (fruit with first signs of yellowing), E2 (mature fruit) and E3 (mature fruit submitted to seven days of rest). Then the seeds were dried in a laboratory oven with air circulation at 35°C until reaching 8% water content. The seeds corresponding to each stage of development of the fruit were packed in airtight plastic packages and stored in a walk-in cooler at 10°C and 50% relative humidity for periods of 0, 4 and 8 months after drying. At the end of each storage period, seed quality was evaluated by means of germination tests; emergence tests (Brazil, 2009); emergence rate index (Maguire, 1962); electrical conductivity (Vidigal et al., 2008) and accelerated aging (Bhering et al., 2006). Furthermore, the activity of the enzymes esterase, superoxide dismutase (SOD), malate dehydrogenase (MDH), alcohol dehydrogenase (ADH) and endo-β-mannanase (Downie et al., 1994) was evaluated.

The activities of the enzymes α-amylase (Alfenas et al., 1991), endo-β-mannanase, esterase, MDH and ADH were evaluated during the germination process of the seeds at 0, 48, 96 and 144 h after seeding. The seeds were ground in the presence of PVP (polyvinylpyrrolidone) and liquid nitrogen in a mortar over ice and afterwards stored at a temperature of -86°C. The experimental design used was randomized complete blocks in a 3 × 3 factorial design, with the factors being: stage of development of the fruit (E1, E2 and E3) and storage periods (0, 4 and 8 months). Analysis of variance was performed for all tests using the statistical program Sisvar (Ferreira, 2000). For comparison among the means, the Scott-Knott test was used at the 5% probability level.

RESULTS

It is observed from the results in Table 2, the interaction between the factors stage of development of the fruit (E1, E2 and E3) and storage periods (0, 4 and 8 months) was significant by F test for all the variables analyzed. Lower germination values were observed for the habanero pepper seeds processed in the first stage of development (E1), in all storage periods (Table 2). At 0 and 8 months of storage, there was no difference between the germination values of the seeds processed at the E2 and E3 stages of development, whereas at 4 months of storage, greater germination values were observed for E3 stage seeds. An increase in germination was observed for E2 and E3 stage seeds at the 4th month of storage

Table 2. Percentage of germinated seedlings (%), percentage of seedling emergence (%), emergence rate index and vigor obtained by the accelerated aging test and electrical conductivity of habanero pepper seeds gathered at different stages of development throughout the storage period.

Storage periods			
Stages	**0**	**4**	**8**
Germination			
E1	1^{Ba}	3^{Ca}	7^{Ba}
E2	25^{Ac}	41^{Bb}	58^{Aa}
E3	32^{Ab}	50^{Aa}	53^{Aa}
Emergence			
E1	5^{Cb}	35^{Ba}	30^{Ba}
E2	60^{Bb}	85^{Aa}	84^{Aa}
E3	75^{Ab}	86^{Aa}	87^{Aa}
Emergence rate index			
E1	0^{Cb}	5^{Ba}	5^{Ca}
E2	8^{Bc}	23^{Ab}	29^{Ba}
E3	13^{Ac}	25^{Ab}	34^{Aa}
Accelerated aging			
E1	1^{Cb}	3^{Cb}	23^{Ca}
E2	20^{Bc}	68^{Ab}	87^{Ba}
E3	43^{Ab}	47^{Bb}	95^{Aa}
Electrical conductivity			
E1	825^{Cb}	580^{Bb}	634^{Ca}
E2	750^{Bc}	535^{Ab}	463^{Ba}
E3	654^{Ac}	511^{Ab}	406^{Aa}

(1) Means followed by the same capital letter in the column and small letter in the row do not differ among themselves by the Scott-Knott test at the 5% probability level.

(Table 2). These values were maintained in E3 stage seeds but increased in E2 stage seeds after eight months of storage. As for E1 stage seeds percentage of germinated seedlings were very low and did not differ significantly during storage. The results obtained in the emergence test and emergence rate index, under greenhouse conditions, are presented in Table 2. Lower plantlet emergence values were observed in seeds processed in the E1 development stage at all storage periods. At 4 months of storage, there was an increase in the percentage of seedling emergence from seeds processed in different stages of development (Table 2). The results of the emergence rate index indicated less vigor in the E1 stage seeds and greater vigor values in the E2 and E3 stage seeds stored for 4 months. However, at 8 months, there was vigor increase for E3 and E2 seeds and maintenance for E1 stage seeds. The results of the accelerated aging test (Table 2) showed greater means of seed vigor in E1, E2, and E3 stage seeds stored for 8 months. At the beginning of storage, the seeds processed in the E3 stage were more vigorous for other stages. Regardless of the storage period, less vigor was observed in habanero seeds extracted at the E1 stage. By the electrical conductivity test (Table 2), greater vigor was observed in E3 seeds that were either recently stored, or stored for 8 months. There was no significant difference in the conductivity values observed in E2 and E3 stages seeds stored for 4 months. In the same way, greater leaching was also observed at 8 months of storage (Table 2). Regardless of the stage of development of habanero pepper seeds, at the end of 8 months of storage, there were lower means of electrical conductivity.

Considering the electrophoretic analyses, the enzymatic profile of esterase (Figure 1) +showed a greater activity of this enzyme for E1 stage seeds at the three storage periods, with this being attributed to greater immaturity of these seeds but also to seed quality deterioration throughout the storage period. Regarding enzyme SOD (Figure 1), there was an increase in its

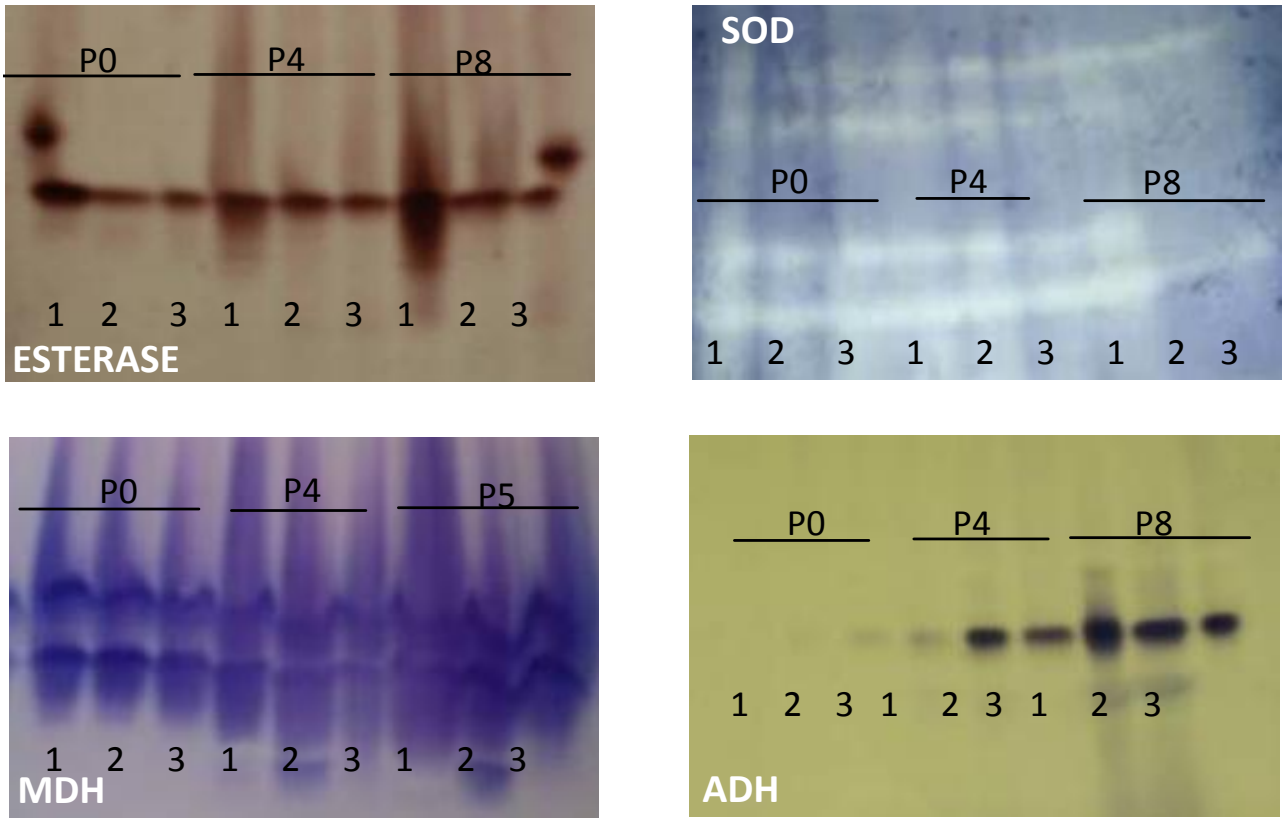

Figure 1. Esterase enzyme profiles, SOD, MDH and ADH of habanero pepper seeds processed in the E1 (1), E2 (2) and E3 (3) stages in the three storage periods 0 (P0), 4 (P4) and 8 (P8) months of storage.

activity at 4 months of storage for all the three stages of development. At 8 months of storage, less activity was observed in E2 and E3 seeds, while in E1 stage seeds there was an increase in the activity of this enzyme. The enzyme MDH (Figura 1) had increased activity in all three stages of development at 8 months of storage. Regarding the enzyme ADH (Figure 1), greater activity in E2 stage seeds was observed at 4 and 8 months. The profile of the enzyme endo-β-mannanase showed an increase in activity (Figure 2) for seeds processed in the most advanced stages of development. Moreover, less activity of this enzyme was observed in recently stored seeds regardless of the maturity stage. In the present research, greater germination and vigor values were observed after storage of the seeds in E2 and E3 stage, leading to the supposition of breaking of dormancy of the seeds during storage (Table 2). Considering the electrophoretic analyses of the enzymatic patterns during germination, there was no difference in the activity of the enzymes in the dry seeds in relation to those soaked for 48 h in all the enzymatic patterns, with the exception of that observed for the enzyme endo-β-mannanase (Figure 3). Variations in the MDH enzyme patterns in habanero pepper seeds (Figure 4) were verified, with less activity in the period of 144 h of soaking in seeds processed in the different stages of development and different storage times. In relation to the patterns observed for the enzyme ADH, it may be observed that with the advance of the soaking period, the activity of the enzyme ADH diminished in the three storage periods (Figure 4). It was also verified that activity of the enzyme increased throughout the storage period in seeds soaked for 48 h. Like MDH, there was variation in the activity of the enzyme ADH in seeds processed at different stages of maturity, in terms of the storage period and of the soaking period. In habanero seeds (Figure 4), greater activity of the ADH enzyme was observed in E2 and E3 seeds after 48 h of soaking at 0 and 8 months of storage, whereas at 4 months of storage, greater activity was observed in seeds of all three stages of development after 48 h of soaking.

Regarding the enzyme α-amylase, Figure 4 showed that there was variation in the activity of this enzyme in terms of the stage of development at which the seeds were processed, storage period and duration of soaking. The activity of the enzyme α-amylase may become evident through the clearer bands in a bluish background, where the starch was hydrolyzed. Greater activity of the enzyme α-amylase was observed at 4 months of storage compared to 0 and 8 months of storage for all stages of

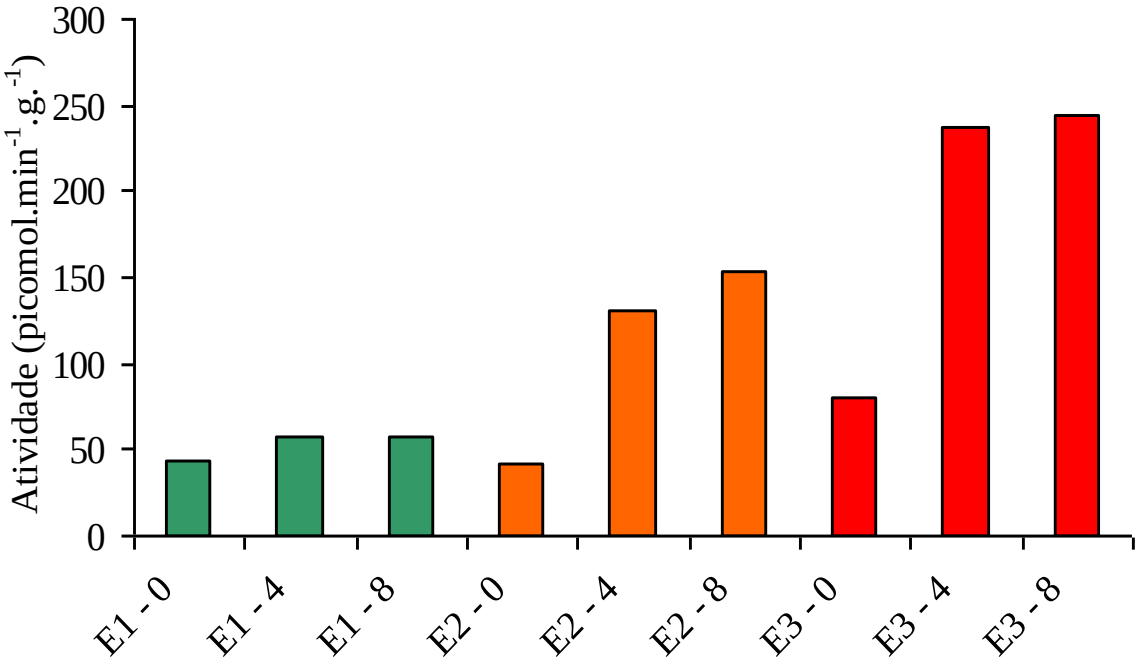

Figure 2. Activity of the enzyme endo-β-mannanase in habanero pepper seeds processed in the E1, E2 and E3 development stages in three storage periods of 0, 4 and 8 months of storage.

seed development. E1 seeds presented greater activity of this enzyme than E2 and E3 seeds at the different soaking times and storage periods, except for recently stored seeds soaked for 48 h and those stored for 8 months and soaked for 96 h.

DISCUSSION

Barbedo et al. (1994) also verified that better quality eggplant seeds were obtained from fruit harvested 50 days after anthesis and submitted to 15 days of post harvest storage. According to Sanchez et al. (1993), green pepper seeds should remain in the mature fruit (50 days after anthesis) after harvest from 7 to 14 days so that maximum germination potential is reached. According to Nascimento et al. (2006), immature fruit, of green color, generally produces seeds with low vigor and germinating power or even poorly formed seeds. The low germination percentage at the beginning of storage may be related to the presence of dormancy in the seeds which was broken throughout the storage period. These results corroborate those found by Bosland and Votava (1999), in which dormancy was observed in recently gathered seeds of species of the genus *Capsicum* (Table 2). Thus, it may be inferred that in recently stored seeds and in those stored for 4 months, aerobic respiration is greater at the beginning of the germination process. It may be observed that the greatest percentage of germination is after 4 months of storage. According to Nascimento et al. (2006), the sowing of pepper seeds recently extracted from the fruit may represent a risk for obtaining uniform stands, contributing to increased seed expenses. These seeds are induced dormancy to preserve the perpetuation of the species. Since this seed peppers should be stored before being sown. The increase in seedling test emergency seed processed in different stages of development in relation to germination emphasizes that in spite of the reports regarding the occurrence of dormancy in pepper seeds (Bosland and Votava, 1999) (Table 2). This dormancy can be broken down by microorganisms in the substrate, when the seeds of determined cultivars are extracted from completely mature fruit and seeded thereafter (Bolsland and Votava, 1999).

Randle and Honma (1981) verified in work with different cultivars of the genus Capsicum, that the genotype and the age of the fruit influence the intensity of dormancy of the seeds. The authors reported that seeds extracted from mature fruit with days of rest before seed extraction germinate more rapidly, younger fruits being more prompt to increased seed dormancy. According to Barbedo et al. (1994), by the emergence rate index, it is possible to detect small existing differences in the physiological quality of cucumber seeds extracted from fruits harvested 15 to 45 days after anthesis (DAA) and without storage. Valdes and Gray (1998), upon harvesting tomato fruits of differing maturity stages and without post harvest storage, observed that the mean germination time of the seeds differed significantly among

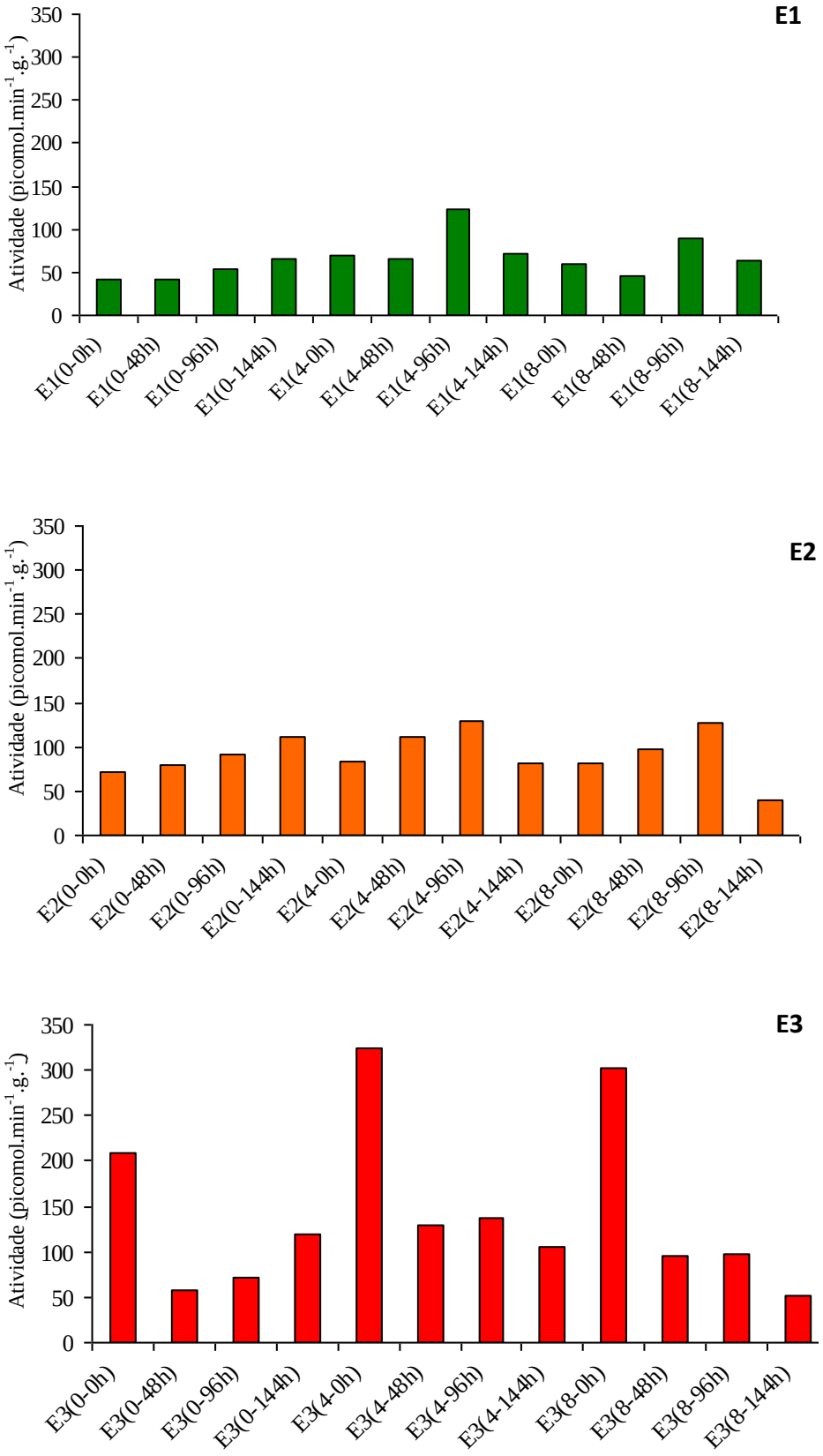

Figure 3. Activity of the enzyme endo-β-mannanase in habanero pepper seeds during 0, 48, 96 and 144 h of germination in the E1, E2 and E3 stage in the three storage periods of 0, 4 and 8 months of storage.

maturity stages, greater germination time being observed in the less mature seeds. Our findings on electrical conductivity were similar to those observed by Vidigal et al. (2006) and Dias et al. (2006) for tomato seeds extracted from fruits with different stages of maturity submitted to post-harvest storage (Table 2). Greater leaching of exudates was observed in immature seeds consistent with less structuring of the organelle and cellular membrane system. Nevertheless, the high conductivity value observed for E1 seeds suggests destructuring of the system of membranes, probably because of their immaturity (Albuquerque et al., 2009), and this fact is also reinforced by the results of the other physiological tests.

As observed by Bhering et al. (2006), the accelerated aging test was efficient to evaluate the effect of pepper seeds, checking statistical difference between the different treatments (Table 2). Esterase is an enzyme that participates in membrane hydrolysis of esters. This fact shows greater lipid peroxidation since this enzyme is involved in ester hydrolysis reactions, being directly connected to lipid metabolism (Santos et al., 2004). Many of these lipids are constituents of membranes, whose degradation increases with deterioration (Figure 1). The enzyme endo-β-mannanase being involved in the degradation of the endosperm in seed germination. In lettuce and coffee seeds, this enzyme is considered as key in the germination process, being involved in mannanase degradation at the time of germination, resulting in weakening of the cell walls of the endosperm (Silva et al., 2004; Veiga, 2005) (Figure 2). Vidigal et al. (2009) observed a small increase in the activity of SOD in chili peppers obtained from fruits harvested 50 DAA and stored for 6 days, greater physiological quality as evaluated by the germination and vigor tests (Figure 1). Enzyme ADH activity reported here was also found by Vidigal et al. (2009) (Figure 1). This enzyme is related to anaerobic respiration, promoting reduction of the acetaldehyde to ethanol. Acetaldehyde accelerates seed deterioration (Buchanan et al., 2005). With the increase of ADH activity, the seeds are more protected against the deleterious action of this compound, which is greater when compared to that of ethanol. In the 8th month of storage, through the fact of the seeds being in more advanced stages of deterioration, there is greater respiratory intensity and consequently greater demand of activity from the enzyme MDH (Figure 1). MDH enzyme patterns varied according to storage and soaking periods and seed maturity stages. In research undertaken by Taiz and Zeiger (2004), no difference in MDH activity was observed in seeds during the maturation process. Nevertheless, the authors reported that the reserve organs in development need greater energy supply and, therefore, respiratory activity in these plant tissues is more intense (Figure 4). These results with MDH and ADH may be associated with the germination and vigor data, in which the seeds processed in the E2 and E3 stages have better quality than the seeds of the E1 stage (Table 2).

According to Nedel et al. (1996), within a group of enzymes, the α and β - amylases are involved in the main starch degradation system. Development of the amylase activity constitutes an important event and may be detected at the beginning of germination with its main role being the making of substrates for the plantlet nutrition until it becomes photosynthetically self-sufficient. A large number of types of seed dormancy arise from blocking the action of α-amylase. The α-amylase present in the dormant seeds is found in small quantities. The activity of this enzyme increases to the extent that the dormancy of rice seeds is overcome during the storage period (Vieira et al., 2008).

In this study, the presence of dormant seeds was observed, principally at the E1 stage of development and in recently stored seeds (Table 2). In these seeds, high activity of α-amylase was observed, which confirms the importance of this enzyme in the germination process of pepper seeds. In E1, E2 and E3 stage seeds and recently stored seeds, there was an increase in the activity of the enzyme endo-β-mannanase (Figure 3) to the extent that the soaking period of the seeds was increased during the germination process. The greatest activity of this enzyme was observed at 144 h of soaking, which coincided with the occurrence of root protrusion. Seeds of the three stages of development stored for 4 and 8 months germinated 96 h after soaking. These data may be correlated with the greater activity of this enzyme in seeds submitted to these treatments. Greater activity of the endo-β-mannanase, in absolute values, was verified in the E2 and E3 stage seeds at all the storage and soaking periods during the germination process. The lowest germination and vigor values were observed in E1 seeds and in recently stored seeds. In these seeds, the activity of the enzyme endo-β-mannanase was, the lowest, indicating the importance of this enzyme in the germination of pepper seeds. The greatest level of activity of this enzyme was observed in seeds stored for 4 months and soaked for 96 h, and this was true for all the three stages of development (Figure 3). The results of the tests used for evaluation of physiological quality, showed an increase in the germination and vigor values for 4 month-stored seeds (Table 2). Based on these results, the presence of dormancy in recently harvested seeds is inferred. This dormancy has probably been overcome in the 4th month of storage as shown by a greater activity of the enzyme endo-β-mannanase.

Conclusions

By means of the physiological tests and the activity of the enzymes evaluated, it was observed that habanero pepper seeds should be extracted from E3 stage fruits, which ensures production of better quality seeds. Seed

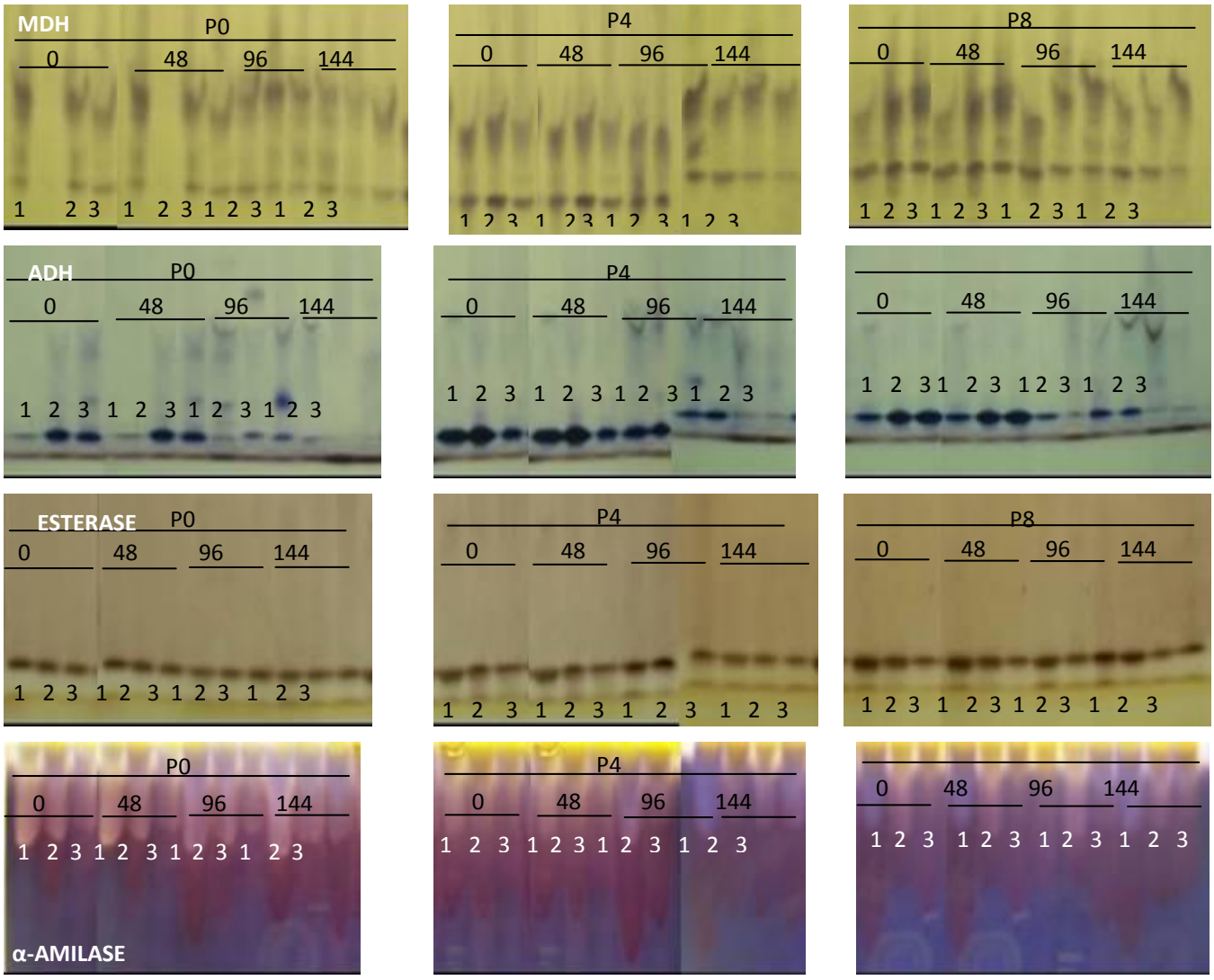

Figure 4. Electrophoretic patterns of the enzyme MDH, ADH, esterase and α-amylase observed in habanero pepper seeds during germination: 0, 48, 96 and 144 h in the E1 (1), E2 (2) and E3 (3) stages with 0 (P0), 4 (P4) and 8 (P8) months of storage.

extraction from fruits harvested at E1 stage must be avoided because of lower physiological quality due to seed dormancy and immaturity. Seed physiological maturity in the species studied does not coincide with the maximum germination and vigor values due to the incidence of dormancy.

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