Revista Ciência Agronômica, v. 54, e20207665, 2023
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Study of physiological maturity of melon seeds by enzymatic changes[1]

Estudo da maturidade fisiológica de sementes de melão através das alterações enzimáticas

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ABSTRACT - Plant species have different adaptation mechanisms regarding seed survival to desiccation, preventing cellular destruction during the water loss. The knowledge of these mechanisms is of great importance for the understanding of how the seed formation/maturation processes and the processes involved in germination occur. Thus, this study aimed to determine physiological and enzymatic changes during the maturation process of melon seeds of the cultivar BRS Anton, obtained from fruits at different maturation stages and subjected to post-harvest storage, by the action of total proteins and the enzymes catalase, peroxidase, and superoxide dismutase. Melon fruits were grown in a greenhouse at Embrapa Vegetables and harvested at five different times: 30, 45, 60, 75, and 90 days after anthesis (DAA). Thirty fruits were harvested in each season and 15 fruits had their seeds extracted immediately after harvest and the other 15 fruits were stored for 15 days. After processing and drying, the seeds were submitted to the following analyses: germination, first count, protein profile analysis, and activity of antioxidant enzymes (total proteins, peroxidase, catalase, and superoxide dismutase). The activity of the antioxidant enzymes from 60 DAA under storage showed a more orderly behavior, reaffirming the results of physiological tests. It shows that the seeds at this point of physiological maturation are well-formed and drying caused no damage to their cell membranes.

Key words: Protein profile. Antioxidant enzymes. Vigor. Germination.

RESUMO - As espécies vegetais apresentam diferentes mecanismos de adaptação no que diz respeito à sobrevivência das sementes à dessecação, prevenindo a destruição celular durante a perda de água. O conhecimento desses mecanismos é de grande importância para o entendimento de como ocorre os processos de formação/maturação de sementes e dos processos envolvidos na germinação. Dessa forma, o objetivo deste trabalho foi determinar as alterações fisiológicas e enzimáticas durante o processo de maturação das sementes de melão da cultivar "BRS Anton", obtidas de frutos em diferentes estádios de maturação e submetidas ao armazenamento pós-colheita, por meio da ação das proteínas totais e das enzimas catalase, peroxidase e superóxido dismutase. Os frutos de melão foram cultivados em casa de vegetação na Embrapa Hortaliças e colhidos em cinco épocas distintas: aos 30, 45, 60, 75 e 90 dias após a antese (DAA). Em cada época, foram colhidos 30 frutos, sendo que 15 frutos tiveram suas sementes extraídas imediatamente após a colheita e 15 frutos foram armazenados por 15 dias. Após o beneficiamento e secagem, as sementes foram submetidas às seguintes análises: germinação, primeira contagem, análise do perfil de proteínas e atividade de enzimas antioxidantes (proteínas totais, peroxidase, catalase e superóxido dismutase). Observou-se que as atividades das enzimas antioxidativas a partir dos 60 DAA sob armazenamento, apresentou um comportamento mais ordenado reafirmando os resultados dos testes fisiológicos. Demostrando que as sementes neste ponto de maturação fisiológica, encontra-se bem formadas e que a secagem não provocou danos em suas membranas celulares.

Palavras-chave: Perfil proteico. Enzimas antioxidantes. Vigor. Germinação.

DOI: 10.5935/1806-6690.20230026
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Received for publication 22/10/2020; approved on 21/09/2021
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INTRODUCTION

Melon (*Cucumis melo* L.) is a very popular vegetable crop in the world; currently, it is one of the main products exported by Brazil (NASCIMENTO *et al.*, 2012). It is cultivated in several regions of the world due to its adaptation to different types of soil and climate (RAY *et al.*, 2013). There is a high demand for melon production and marketing, and Brazil has a high potential to meet this demand, with an area of approximately 23,200 hectares destined for its cultivation (INSTITUTO BRASILEIRO DE GEOGRAFIA E STATÍSTICA, 2016).

Seed is one of the most expensive items during the melon production, corresponding to 31.6% of the effective operating costs (ASSOCIAÇÃO BRASILEIRA DO COMÉRCIO DE SEMENTES E MUDAS, 2014). The search for new knowledge, such as the determination of the physiological maturity point of the seed, is necessary to obtain its better performance in the field. The determination of physiological maturity is strategic for defining the ideal harvest time, contributing to the production of seeds with high physiological and sanitary quality.

The seed deterioration process begins from the physiological maturity point, causing a reduction in vigor and viability (FREITAS, 2009). Defense mechanisms are essential for conserving seed quality. These mechanisms are known as enzymatic systems and are involved in the antioxidative response to neutralize singlet oxygen and other free radicals formed under stress conditions (CONTRERAS-PORCIA *et al.*, 2010), such as the drying process.

The increase in respiratory rate and activation of hydrolytic respiratory enzymes is an indication of increased metabolism, which occurs after seed hydration (MARCOS-FILHO, 2015). Thus, understanding the changes that occur in the seeds at different development stages as they lose water is of paramount importance for the development of adequate methodologies for drying seeds harvested with high water contents. Seeds become more tolerant to higher drying temperatures with progressive water loss after physiological maturity. Desiccation tolerance refers to the ability of seeds to withstand dehydration, decrease their metabolic activity, and survive in this state, maintaining their longevity (LI *et al.*, 2011).

Sensitivity to desiccation is a complex physiological phenomenon and involves a series of deleterious and/or protective mechanisms depending on the desiccation conditions. As enzymes, antioxidant molecules act to neutralize free radicals such as hydrogen peroxides (H_2O_2), singlet oxygen, and hydroxyl radical (OH^-), which are toxic to cells and capable of damaging cellular constituents, such as proteins, DNA, and membranes. These radicals accumulate because free radical scavenging systems are not effective in dehydrated organisms. Some free radical scavenging enzymes such as superoxide dismutase, catalase, and peroxidase can reduce the toxic products resulting from the free radical attack. The knowledge of these mechanisms is essential to monitor changes resulting from deterioration (VIDIGAL *et al.*, 2009).

In this context, this study aimed to determine biochemical changes during the maturation process of melon seeds of the cultivar BRS Anton under different maturation stages and storage conditions of the fruits. The cultivar BRS Anton was chosen in this study as a model, but the obtained results could be used in some aspects of seed production technology for other melon cultivars.

MATERIAL AND METHODS

Parents 114-5 (female parent) and 76-2 (male parent) were cultivated in a greenhouse at Embrapa Vegetables, located at longitude 805584.23 mE and latitude 8236617.15 mS, Zone 22, Zone L, according to the coordinates UTM, in Brasília/DF, from April to July 2018. Crossing the accessions gave rise to the cultivar BRS Anton, studied in this research.

The experiment was carried out in a greenhouse, covered with transparent plastic, measuring 5.0 m (height), 7.0 m (width), and 30.0 m (length). Seedlings were transplanted into 480 plastic pots (1 seedling/pot) with a capacity of 5 liters, 400 for the female parent (114-5) and 80 for the male parent (76-2). A total of 2400 kg of sterilized soil and 150 kg of Rohrbacher® substrate, mixed with 1.95 kg of ammonium sulfate, 9.5 kg of single superphosphate, 3.9 kg of limestone, and 65 kg of poultry manure, were used. The seedlings were staked using string and irrigated by a drip system.

Hand pollination was performed before flower anthesis during the first hours of the day between 7:30 and 10:00 h after verifying the presence of flower buds. After pollination, the flowers were protected with aluminum foil to avoid contamination with exogenous pollen and identified using labels.

The fruits were harvested at five different times: 30, 45, 60, 75, and 90 days after anthesis (DAA). Thirty fruits were harvested in each season, 15 of them had their seeds extracted immediately after harvest and the other 15 fruits were stored. The 15 stored fruits were placed in identified plastic harvest boxes for a period of fifteen days at room temperature in an airy place. The 30 fruits were submitted to the same procedures of extraction, washing, and drying of seeds.

After extraction, the seeds were placed in plastic buckets (5 liters) for a period of 24 hours for fermentation and mucilage removal. Running water was added for washing. Subsequently, the seeds were submitted to the drying procedure for a period of 48 hours at a temperature of 32 °C.

The dried seeds were submitted to tests of germination, first count, total protein analysis, and the enzymes superoxide dismutase, catalase, and peroxidase.

Germination: carried out with four replications of 50 seeds in a germination paper roll moistened with distilled water at the proportion of 2.0 times the dry paper mass in a germinator with an alternating temperature of 20 °C (16 h, dark) and 30 °C (8 h, light). Counts were carried out eight days after the test installation and evaluations were performed according to criteria established by the Rules for Seed Testing (BRASIL, 2009).

First count: was performed together with the germination test by counting the number of normal seedlings present on the fourth day after the beginning of the test. The results were expressed as a percentage (BRASIL, 2009).

Enzymatic analyses: initially, a crude enzymatic extract was obtained from the seeds to determine the protein content and the activity of the enzymes superoxide dismutase, catalase, and peroxidase. The extract was obtained by homogenizing, in a mortar, 0.3 g of seeds from each treatment in 2.0 mL of 0.1 M potassium phosphate buffer pH 6.8, supplemented with 50 mg of PVPP (polyvinylpolypyrrolidone). Subsequently, the homogenate was centrifuged at 12,000 xg (approximately 10,000 rpm) for 15 minutes at 4 °C and the supernatant was collected and stored in an ice bath. **1 – Superoxide dismutase (SOD) enzyme activity:** The determination of SOD activity considered the enzyme's ability to inhibit the photoreduction of NBT (nitro blue tetrazolium chloride). The activity was determined by adding 50 µL of crude extract from the seeds to a solution containing 13 mM methionine, 75 µM NBT, 100 nM EDTA, and 2 µM riboflavin in 3.0 mL of 50 mM potassium phosphate buffer pH 7.8. A wavelength of 560 nm was used for reading in a Thermo Scientific™ UV-Vis GENESYS 10S analytical spectrophotometer. The reaction started with the lighting of the tubes in a chamber composed of tubular fluorescent lamps (15W) at 25 °C. The lamps were turned off after five minutes of incubation and the reaction was stopped. The blue compound formed (formazan) by the photoreduction of NBT was determined by reading in a spectrophotometer at 560 nm. The tubes considered blank for analysis received the same reagents but were kept covered with aluminum foil to protect them from light. A unit of SOD is defined as the amount of enzyme required for 50% inhibition of NBT photoreduction. The percentage of inhibition, the sample volume, and the protein concentration in the sample (µg μL^{-1}) were considered to calculate the specific enzyme activity. **2 – Catalase (CAT) enzyme activity:** CAT enzyme activity was defined by the amount of enzyme required to catalyze H_2O_2 decomposition. For the test, 950 µL of 50 mM potassium phosphate buffer pH 7.0 supplemented with hydrogen peroxide at a final concentration of 12.5 mM was placed in quartz cuvettes and the reaction started by adding 50 µL of crude extract. The variation of absorption (ΔE) was calculated by the difference of readings at 240 nm at an interval of 80 seconds. The enzyme activity was calculated using the molar extinction coefficient of 39.4 mM^{-1} cm^{-1} and the specific activity (µKat µg $Prot^{-1}$) was determined considering the concentration of soluble protein in the sample. **3 – Peroxidase (POX) enzyme activity:** The spectrophotometric method, in which the guaiacol oxidation in the presence of hydrogen peroxide is measured at a wavelength of 420 nm, was adopted. The results were expressed in peroxidase units. Each peroxidase unit corresponded to a variation of 0.001 in the absorbance value at 470 nm per minute of reaction per milligram of total protein. **4 – Total soluble protein content:** The quantity of soluble proteins in the samples was determined using the Coomassie brilliant blue G-250 dye method to bind to negative charges of the polypeptide chain, whose reaction converts it to the blue form. The analysis was performed in triplicates, using a test tube with 5 mL of the reagent, and adding 100 µL of the crude extract. Then, it was stirred, and the absorbance was read at 595 nm after 15 minutes. The bovine serum albumin (BSA, Sigma) solution was used as a standard to obtain a standard curve at a concentration range between 0 and 1 mg mL^{-1}, whose protein concentration in the samples was determined by interpolating the standard curve.

Statistical analysis: the data were subjected to analysis of variance according to the F-test at a 5% probability and the mean comparison test by Scott & Knott ($p \leq 0.05$), using the SISVAR software (Version 5.6). The data that did not present normal distribution were transformed into arcsec $(x/100)^{1/2}$ to meet the assumption of normality of distribution and then subjected to analysis of variance using for the F-test the 5% probability level. The means were grouped by the Scott & Knott test using the SISVAR software (Version 5.6).

RESULTS AND DISCUSSION

Table 1 shows the summary of the analysis of variance for biochemical parameters of melon seeds from fruits harvested at different maturation stages and submitted or not to storage. Almost all the variables showed significant differences both for the factors alone and for the interaction between them, except for the protein content, which was not significant in the storage factor.

Most of the variables subjected to regression analysis were adjusted to the tested models (Table 2), with the choice of models being based on the significance of the parameters and the coefficient of determination (R^2). The protein content of seeds from non-stored fruits did not adjust to linear and quadratic models, while CAT activity did not adjust to any of the models. CAT activity of seeds from stored fruits did not adjust to the cubic model.

Figures 1 and 2 show that seeds obtained from fruits harvested at 30 days after anthesis (DAA) and not stored had the lowest percentage of germination. However, storage for 15 days favored germination, increasing this percentage. Storage also favored the germination of seeds from fruits harvested at 45 and 60 DAA. Fruit storage apparently did not influence seed germination at 75 DAA. Moreover, seeds from fruits harvested at 90 DAA showed a decrease in the percentage of germination both in seeds from stored and non-stored fruits.

According to Vidigal *et al.* (2009), some studies on fleshy fruit species have shown that seeds kept for a period stored in the fruit after harvest reach maximum levels of germination and vigor, continuing with the maturation process. The study found that germination was null for pepper seeds from fruits harvested at 40 DAA and not stored, results very similar to those found for melon fruits of the cultivar BRS Anton harvested at 30 DAA and not stored, in which seed germination was close to zero. Silva *et al.* (2017) found a similar result for pumpkin seeds, with some stability in vigor from 60 DAA, this period being considered as a possible indication of the physiological maturity of these seeds.

An oscillation was observed in the course of the maturation process, presenting a maximum value in the concentration of total proteins for seeds harvested at 30 DAA, regardless of fruit storage or not (Figure 3). For seeds extracted from fruits harvested at 30, 45, 60, 75,

Table 1 - Summary of analysis of variance for biochemical changes in yellow melon seeds of the cultivar BRS Anton from fruits harvested at 30, 45, 60, 75, and 90 days after anthesis and submitted or not to storage

SV	DF	Mean squares			
		TP	SOD	CAT	POX
Time	4	1.409*	0.018*	359.535*	0.084*
Storage	1	0.440^{ns}	0.004*	432.067*	0.086*
Time* Storage	4	0.947*	0.017*	525.568*	0.304*
Residual	20	0.081	0.0004	4.623	0.001
CV (%)		7.00	9.45	7.28	6.03

SV – sources of variation; TP – total protein (mg mL^{-1}); SOD – superoxide dismutase (U min^{-1} mg^{-1} of protein); CAT – catalase (nmol min^{-1} mg^{-1} of protein); POX – peroxidase (nmol min^{-1} mg^{-1} of protein); ^{ns} – not significant, * – significant at 5% by the F-test

Table 2 - Summary of the regression analysis for total protein (TP) and superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) enzymes of yellow melon seeds of the cultivar BRS Anton from fruits harvested at 30, 45, 60, 75 and 90 days after anthesis and submitted or not to storage

Storage	Parameter	Mean squares		
		Linear	Quadratic	Cubic
0 days	PT	114.130^{ns}	71.313^{ns}	19.579*
	SOD	74.007**	17.533**	24.368**
	CAT	10824.100^{ns}	6471.500^{ns}	2016.400^{ns}
	POX	1.225**	0.875**	0.900**
15 days	PT	10.726**	26.788**	1.940*
	SOD	5.186*	18.924*	2.089**
	CAT	2788.900*	2885.786**	656.100^{ns}
	POX	3.025**	2.161**	8.100**

^{ns} – not significant); ** and * – significant at 1 and 5% by the t-test, respectively

and 90 DAA and stored for 15 days. The total protein content of yellow melon of the cultivar BRS Anton showed higher concentrations at 45 and 75 DAA for stored seeds and 45 and 90 DAA for non-stored seeds. Similarly, Silva *et al.* (2017) observed that pumpkin seeds from fruits stored for 20 days had a higher protein concentration up to 60 DAA, stabilizing up to 75 DAA.

Seeds extracted from melon showed different behavior regarding the SOD enzyme activity according to the storage or not of the fruits (Figure 4), increasing up to 45 DAA in seeds from stored fruits and up to 60 and 75 DAA in fruits not stored, decreasing from that point. Nakada *et al.* (2011) observed that SOD concentration increased in cucumber seeds only up to 40 DAA, while Silva *et al.* (2017) observed a decrease in SOD concentration in pumpkin seeds during their maturation stages, especially those from stored fruits. In our study, the formation of free radicals may have occurred in immature melon seeds during drying, activating SOD as a cellular repair mechanism to promote the removal of molecular oxygen (O_2) from the seeds. Defense mechanisms are essential for seed quality conservation. They are involved in the antioxidant response to neutralize singlet oxygen and other free radicals formed under stress conditions (CONTRERAS-PORCIA *et al.*, 2010), such as the drying process.

CAT activity in seeds extracted from stored fruits increased from 45 DAA, reaching a maximum value at 60 DAA and decreasing afterward (Figure 5). CAT activity did not change significantly in seeds obtained from non-stored fruits. Nakada *et al.* (2011) observed an increase of this enzyme up to 40 DAA in cucumber seeds, probably due to the seed immaturity. Thus, the drying process is considered a stress factor, in which there is the activation of the formation of free radicals due to higher tolerance to desiccation, which explains the higher CAT activity.

SOD and CAT enzymes must present similar behaviors so that the germination mechanism is in agreement and optimal functionality, as one finishes the work of the other in the defense mechanism. SOD works by canceling the reactive oxygen forms, producing hydrogen peroxide (H_2O_2), while CAT prevents the formation of other reactive compounds, converting hydrogen peroxide

Figure 1 - Percentage of germination of yellow melon seeds of the cultivar BRS Anton from fruits harvested at 30, 45, 60, 75, and 90 days after anthesis and submitted or not to storage (0 and 15 days)

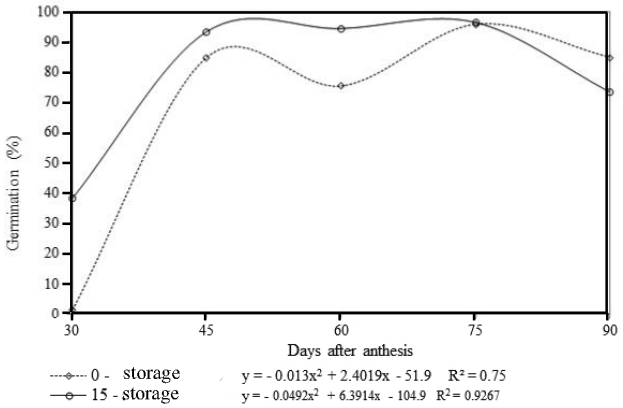


Figure 2 - Percentage of first germination count of yellow melon seeds of the cultivar BRS Anton from fruits harvested at 30, 45, 60, 75, and 90 days after anthesis and submitted or not to storage (0 and 15 days)

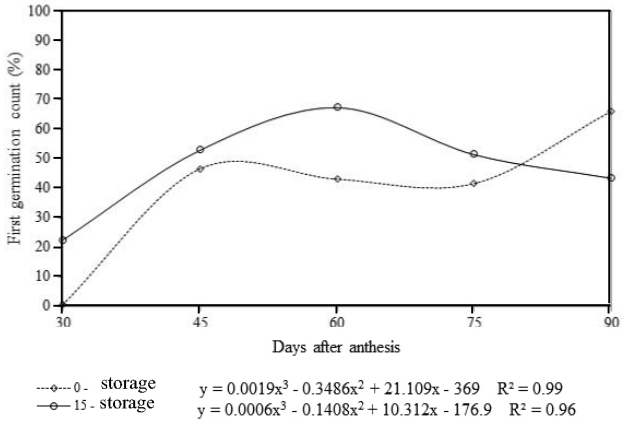


Figure 3 - Total protein (PT) concentration of yellow melon seeds of the cultivar BRS Anton from fruits harvested at 30, 45, 60, 75, and 90 days after anthesis and submitted or not to storage (0 and 15 days)

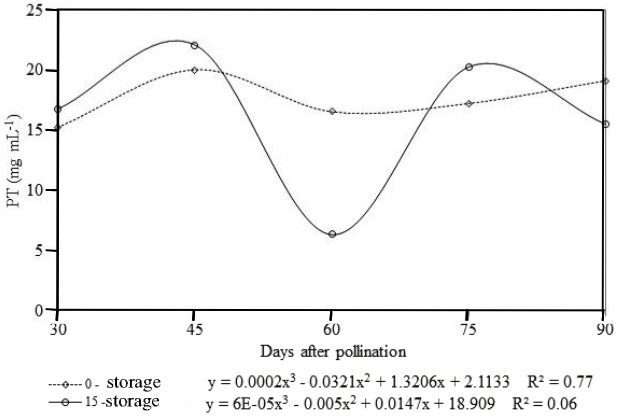


Figure 4 - Superoxide dismutase (SOD) enzyme activity in yellow melon seeds of the cultivar BRS Anton from fruits harvested at 30, 45, 60, 75, and 90 days after anthesis and submitted or not to storage (0 and 15 days)

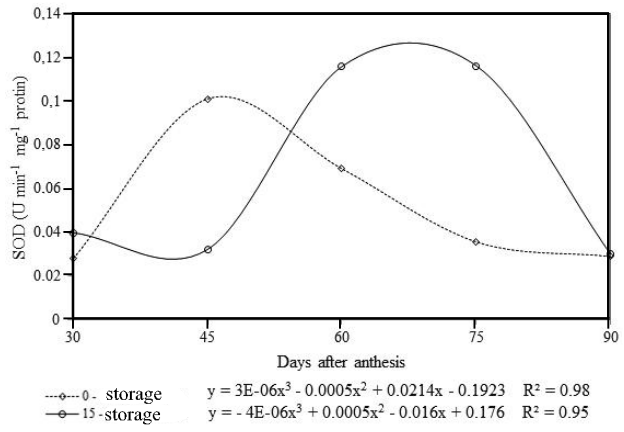


Figure 5 - Catalase (CAT) enzyme activity in yellow melon seeds of the cultivar BRS Anton from fruits harvested at 30, 45, 60, 75, and 90 days after anthesis and submitted or not to storage (0 and 15 days)

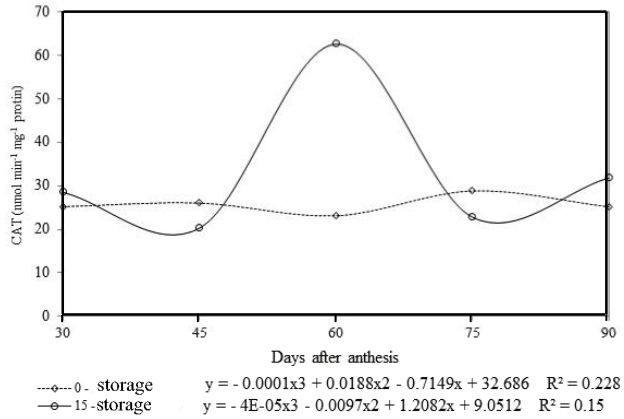
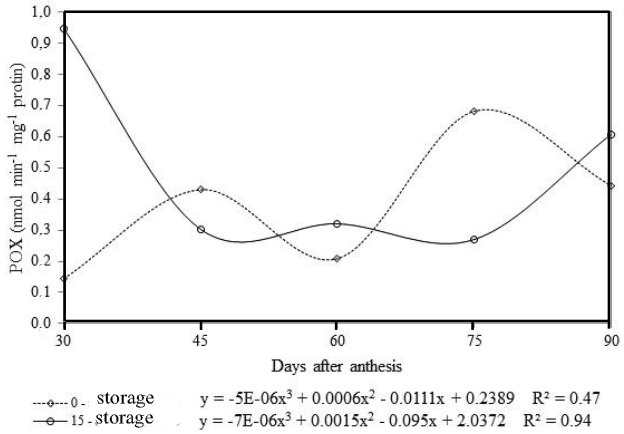


Figure 6 - Peroxidase (POX) enzyme activity in yellow melon seeds of the cultivar BRS Anton from fruits harvested at 30, 45, 60, 75, and 90 days after anthesis and submitted or not to storage (0 and 15 days)



into water and oxygen, that is, non-reactive species (CONTRERAS-PORCIA *et al*., 2010). Thus, the activity of these enzymes must be analogous and, consequently, complementary, a result observed in the analysis of the seeds of the cultivar BRS Anton.

The pattern of POX activity was lower in seeds at the beginning of their maturation process, that is, from 30 DAA of seeds from non-stored fruits, showing a more constant activity from 45 DAA. Seeds from fruit storage showed a higher concentration of the enzyme after 30 DAA (Figure 6). Similarly, Carvalho *et al*. (2014) observed a decrease in POX enzyme activity in seeds stored under uncontrolled conditions.

In this case, seeds from fruits that underwent a resting period had a longer time to complete their maturation, while mature seeds have their physiological quality preserved because they remain in osmotic balance inside the fruit (ABUD *et al*., 2013).

CONCLUSION

In general, the activity of antioxidant enzymes in seeds of the cultivar BRS Anton from 60 DAA under storage showed a more orderly behavior, reaffirming the results of the physiological tests, germination test, and first count, which showed better quality in seeds at 60 DAA from stored fruits. It demonstrates that seeds at this point of physiological maturation are well-formed and drying did not cause damage to their cell membranes. Thus, the proper use of the storage technique for 15 days post-harvest of the fruits before seed extraction demonstrated that seeds not yet fully mature complete their maturation inside the fruits.

REFERENCES

ABUD, H. F. *et al*. Qualidade fisiológica de sementes das pimentas malagueta e biquinho durante a ontogênese. **Pesquisa Agropecuária Brasileira**, v. 4, p. 1546-1553, 2013.

ASSOCIAÇÃO BRASILEIRA DO COMÉRCIO DE SEMENTES E MUDAS. **2º levantamento de dados socioeconômicos da cadeia produtiva de hortaliças no Brasil**. 2014. Disponível em: http://www.abcsem.com.br/imagens_noticias/Apresenta%C3%A7%C3%A3o%20completa%20dos%20dados%20da%20cadeia%20produtiva%20de%20hortali%C3%A7as%20-%2029MAIO2014.pdf. Acesso em: 10 fev. 2017.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. **Regras para análise de sementes**. Brasília, DF: Mapa/ACS, 2009. 399 p.

CARVALHO, E. R. *et al.* Enzyme activity in soybean seeds produced under foliar application of manganese. **Ciência e Agrotecnologia**, v. 38, p. 317-327, 2014.

CONTRERAS-PORCIA, L. *et al.* Tolerance to oxidative stress induced by desiccation in *Porphyra columbina* (Bangiales, Rhodophyta). **Journal of Experimental Botany**, v. 62, p. 1815-1829, 2010.

FREITAS, A. R. Deterioração e armazenamento se sementes de hortaliças. *In*: NASCIMENTO, M. W. (ed.). **Tecnologia de sementes de hortaliças**. Brasília: Embrapa Hortaliças, 2009. p. 155-182.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. Sistema IBGE de Recuperação Automática - SIDRA. **Agropecuária**: Produção Agrícola Municipal. 2016. Disponível em: http://www.sidra.ibge.gov.br/bda/tabela/listabl. asp?z=t&o=1&i=P&e=l&c=1612>. Acesso em: 5 fev. 2017.

LI, X. *et al*. Highly efficient in vitro regeneration of the industrial oilseed crop *Crambe abyssinica*. **Industrial Crops and Products**, v. 33, p. 170-175, 2011.

MARCOS-FILHO, J. **Fisiologia de sementes de plantas cultivadas**. 2. ed. Londrina: ABRATES, 2015. 660 p.

NAKADA, P. G. *et al.* Desempenho fisiológico e bioquímico de sementes de pepino nos diferentes estádios de maturação. **Revista Brasileira de Sementes**, v. 33, p. 113-122, 2011.

NASCIMENTO, N. J. R. *et al*. Formas de aplicação e doses de nitrogênio para o meloeiro amarelo no litoral do Ceará. **Irriga**, v. 17, p. 364-375, 2012.

RAY, D. K. *et al.* Yield trends are insufficient to double global crop production by 2050. **Plos one**, v. 8, p. 1-9, 2013.

SILVA, P. P. *et al.* Biochemical changes in hybrid pumpkin seeds at different stages of maturation. **Revista Ciência Agronômica**, v. 48, n. 3, p. 558-564, 2017.

VIDIGAL, D. S. *et al*. Alterações fisiológicas e enzimáticas durante a maturação de sementes de pimenta (*Capsicum annuum* L.). **Revista Brasileira de Sementes**, v. 31, n. 2, p. 129-136, 2009.