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PHYSIOLOGICAL QUALITY OF *Capsicum chinense* 'ADJUMA' SEEDS DURING DEVELOPMENT

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Abstract
Once “bode” pepper plants (Capsicum) have continuous development and fructification, it is not possible to determine a specific season for seed physiologic maturation and harvest. This research aimed to evaluate physiological quality of “bode” pepper (*Capsicum chinense* 'Adjuma’) during fruit development and enzymes expression on seed germination. Manually extracted seeds were submitted to physiological tests being evaluated germination at first count (FC), final (G), and germination speed index (GSI), additionally, enzymatic analyses were carried. The lowest values for the physiological tests were observed for initial development stages. The greatest for FC, G and IVG were observed for seeds from fruits 70 days after anthesis (DAA). Superoxide dismutase enzyme has the highest values on seeds harvested at 49 DAA while malate dehydrogenase has more expression at 70 DAA. Catalase, alcohol dehydrogenase, and esterase have the higher expression at 63 DAA. The maturation stage influences bode pepper seed physiological quality, being seeds harvested at 70 DAA those with the better results on the evaluated parameters, thus, considered physiologically mature and the indicated time for harvest.

Keywords: Enzymes. Germination. Pepper. Seed maturation.

1. Introduction

Originated in Americas, peppers from the *Capsicum* genus have been consumed from Brazilian native tribes since before the Portuguese arrival. According to Carvalho et al. (2006), peppers are consumed by at least a quarter of world population. They are used as appetite stimulants, antioxidants and sources from vitamins C, E, and carotenoids. Also, in its constitution, proteins, carbohydrates, lipids, and minerals can be found (Saleh et al. 2018).

Pepper crop have been gaining ground on pharmaceutical, food, and cosmetic industries due to its many ways it can be used, as consumption of fresh fruits, jams, sauces, and even on medicines (Saleh et al. 2018). In Brazil, peppers are grown in all regions, mainly at center-west and northeast, being Minas Gerais, São Paulo, Ceará, and Bahia the major producers (Rebouças et al. 2013).

As any sexually propagated plant species, pepper crop depends on the use of high-quality seeds. However, the main limitation is the low offer of good quality seeds, due to the fruit maturation process for this species being uneven, with continuous growing, flowering, and fructification (Gonçalves et al. 2018). Thus, it is possible to find in a plant fruits and seeds at distinct stages of development and physiological maturation. This difficult the determination of a season which seeds would be physiologically mature, consequently preventing to define an ideal moment for harvest and obtainment of high-quality seeds (Justino et al. 2015).

When physiologically mature, seeds have the highest values of dry matter and capacity to produces enzymes. Antioxidant enzymes as superoxide dismutase (SOD) and catalase (CAT) are important for cell structure, protecting against damages by removing the excess of reactive oxygen free radicals and reducing the oxidative damage (Li et al. 2017).

Considering this, physiological quality analyses during pepper seed development are important for the establishing of high productivity plants on field. Thus, this study aimed to evaluate the physiological quality of "bode" pepper seeds (*Capsicum chinense* 'Adjuma') during different development stages and the enzyme expression during germination.

2. Material and Methods

Seed production

All experiments were conducted at HortiAgro Sementes S/A experimental area, Palmital farm in Ijaci-MG municipality (21° 9'24'' S 44° 55'34'' W) and in Federal University of Lavras (UFLA) / Central Laboratory for Seed Research (LCPS) / Sector of Seeds at Lavras-MG, Brazil.

Seedling production for this research's seed production was carried on nursery being used for seeds from the commercial producer Topseed®. *Capsicum chinense* seeds were sown in polystyrene trays with 72 cells filled by commercial substrate Carolina®. Trays were kept over concrete benches covered by 30% shading screen Sombrite® to reduce the sun radiation. Irrigation was carried according to necessity and 45 days after germination seedlings were transplanted to 10-liter vases filled by mix of soil, sand, and organic compost at 2:1:1 proportion. During the plant growth, until the seed harvest, pulverization, fertilization, and irrigation were carried according to the crop needs.

During the flowering period, flowers were labeled on the anthesis day and daily monitored until the fruit quantity enough for all the experimental analysis was reached. Fruits were harvested at 28, 35, 49, 63, and 70 days after anthesis (DAA). Seed extraction was carried manually being open using a scalpel and washed with water. After extraction, seeds were washed on 1% sodium hypochlorite solution for 1 minute and rinsed running water. After that, seeds have the initial weight measured and submitted to slow drying at 35°C on oven with forced air circulation until the weight equivalent to 8% water content was reached (Queiroz et al. 2011), being carried water content test to confirm that this value was reached. After that, seed quality tests were carried.

Physiological tests

For germination tests, seeds were placed on Gerbox type boxes over a germination paper previously moistened with volume of distilled water at 2.5 times heavier than substrate. Were considered as treatment seeds harvested after 28, 35, 49, 63, and 70 days after anthesis (DAA) and for each one was established four replicates with 50 seeds each. Gerbox boxes were placed in B.O.D. germination chamber with alternating temperatures and light being 30°C with light for 8h and 20°C with dark for 16h. Daily germination counts were carried (Brasil 2009) to calculate the germination speed index (GSI), also was calculated the germination percentage at first count at 7 days (FC) and final (G) at 14 days. Was considered germinated the seed which developed all healthy structures of leaves, stem, and radicle (normal seedlings).

Enzymatic analysis

For each treatment, 100 grams of seeds was stored in deep freezer at -86°C. For extraction, seeds were macerated in porcelain crucible with liquid nitrogen and polyvinylpyrrolidone and stored again at -86°C until the experiments were realized.

Enzymes were extracted by using buffer 0.2 M Tris HCl pH 8.0 with addition of 0.1% betamercaptoethanol at rate of 250 µL buffer for 100 mg seed. Samples were homogenized on vortex and kept in fridge by 12h, when were centrifuged at 11700 g for 60 minutes at 4°C. Electrophoresis was conducted on discontinuous acrylamide gels of 7% (concentrator) and 4.5% (separator). Gel electrode system used was the Tris-glycine pH 8.9. Was applied 50 µL of the supernatant in the gel and electrophoresis was conducted at 150 volts for 6 hours being temperature maintained from 3 to 4°C by keeping the system inside a fridge during the process.

Gels were revealed for the enzymes catalase (CAT), esterase (EST), superoxide dismutase (SOD), alcohol dehydrogenase (ADH), and malate dehydrogenase (MDH) according to recommended by Alfenas *et al.* (2006). Bands measuring was carried by using the software ImageJ (Rasband, 2016), resulting in an average of density for each treatment measured by the pixels^2 amount. From each treatment was established three replicates.

Data analysis

For both seed physiological quality and enzymatic activity, completely random design was used. Data were analyzed through analysis of variance (ANOVA) and Tukey's test (5% error probability). All statistical analysis was carried using the software R for Windows 3.1.3 (2015).

3. Results

Physiological Analysis

Results for first and final germination counts, and germination speed index can be observed at Figure 1. According to the results, is possible to conclude that for all parameters tested, lower results are observed for the initial development stages. Highest values for germination at first (FC) and final (G) count, and germination speed index (GSI) were observed for seeds extracted from fruits at 70 days after anthesis.

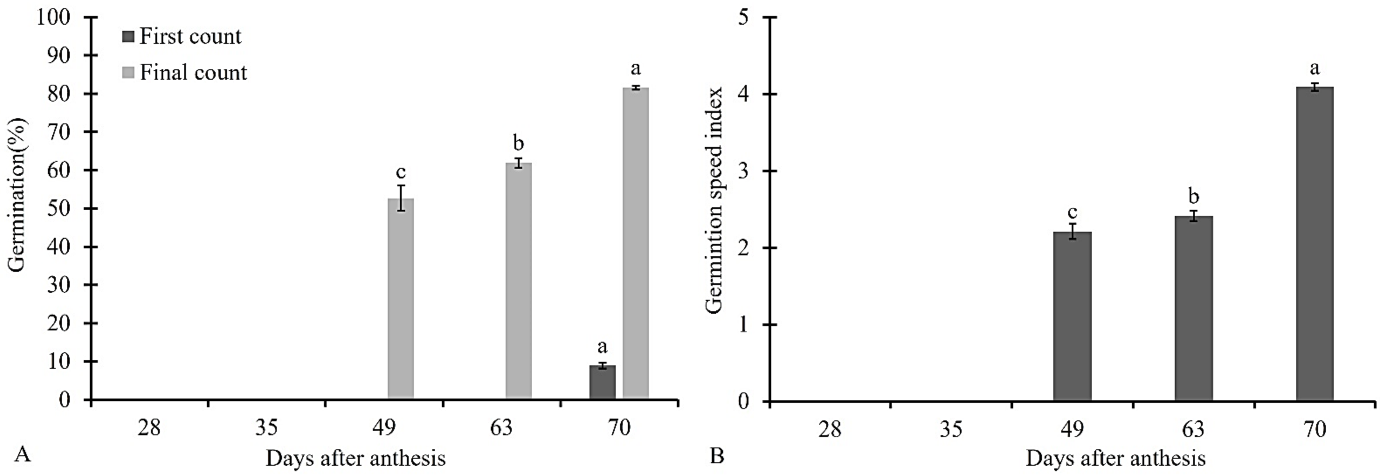

Figure 1. A - Germination and B - germination speed index (GSI) of bode pepper (*Capsicum chinense*) developing seeds. Same letters indicate absence of differences among treatments according to Tukey's test at 5% probability.

Enzymatic analysis

Regarding the enzymatic quantification of superoxide dismutase (SOD) for bode pepper seeds during development can be observed in Figure 2A. Higher expression rates were observed in seeds after 49 days after anthesis. After 63 and 70 days after anthesis, SOD expression was lower than 49 days and no difference between these points was observed. Enzyme catalase (CAT), have higher expression for seeds extracted from fruits at 63 days after anthesis compared to those collected at 70 days (Figure 2B). No CAT expression was detected before 63 days after anthesis on the conditions of the experiments here carried.

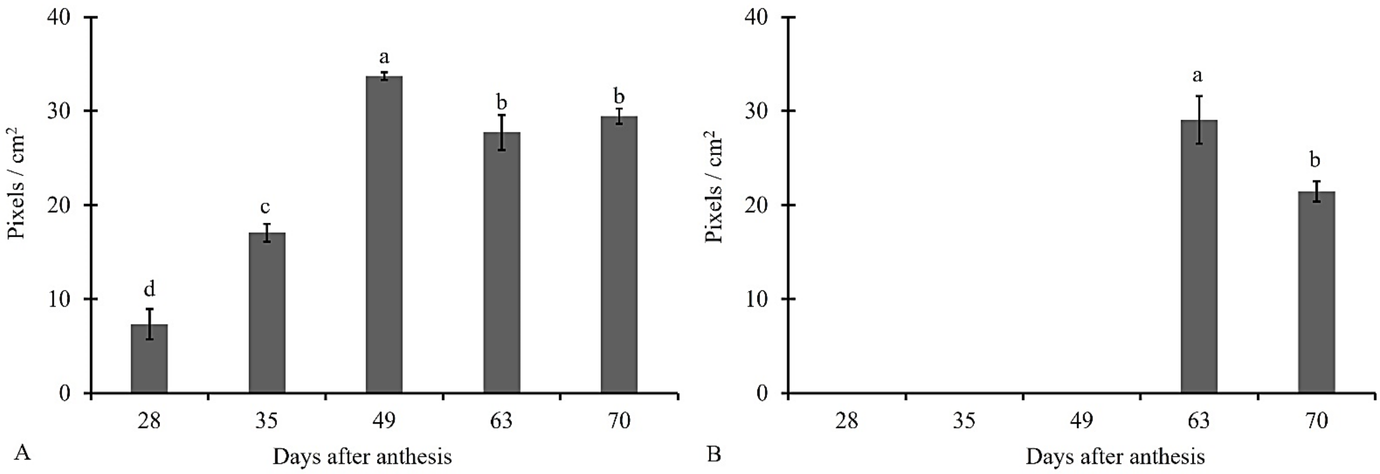

Figure 2. A - Expression of enzymes Superoxide Dismutase and B - Catalase in bode pepper (*Capsicum chinense*) developing seeds. The values of Pixels/cm^2 refer to the band sizes observed on the gels after electrophoresis. Same letters indicate absence of differences among treatments according to Tukey's test at 5% probability.

The malate dehydrogenase (MDH) expression during bode pepper seed development can be observed on Figure 3A. Higher enzyme expression was found in seeds harvested at 70 days after anthesis, followed by seeds at 63 days. No differences were found between 28 and 49 days after harvest and the lowest values were found at 35 days.

Regarding the enzyme alcohol dehydrogenase (ADH) expression in seeds during development (Figure 3B), it is possible to observe higher expression in seeds at 63 days after anthesis, followed by those harvested in seeds at 49 and 70 days after anthesis, which has statistically equivalent results. The lowest expression was found in seeds harvested after 28 and 35 days after anthesis.

Esterase enzyme (EST) activity during pepper seed development can be observed on Figure 3C. Higher expression was observed for seeds harvested after 63 days after anthesis, like the results observed for ADH ant CAT.

4. Discussion

The physiological seed maturity point is generally defined by the maximum dry matter accumulation and the moment which seed has the complete physiological apparatus necessary for the germination to happen (Bareke et al. 2018). As can be observed in Fig 1, seeds at 28 and 35 days after anthesis (DAA), probably have not reached the physiological maturity, which can explain the no germination result for these treatments (Gonçalves et al. 2015). Premature seeds from the *Capsicum* genus may have physiological dormancy, which also justify the low seed germination and vigor, as observed for *Capsicum chinense* Jacq. var. Bode Vermelha (Gonçalves et al. 2015) and for *Capsicum baccatum* (Alves et al. 2020).

The highest physiological performance observed for seeds extracted from fruits at 70 days after anthesis corroborates to those observed by Figueiredo et al. (2017), for *Capsicum baccatum* var. *pendulum*. The authors reported that the lowest values on water content and dry matter accumulation were observed at 70 days after anthesis, indicating this as the moment which seed reached the physiological maturity.

Both catalase and superoxide dismutase act favor the seed physiological quality once they act on defense mechanisms against reactive oxygen species, which can avoid on damage on cellular structure and consequently help on seed development (Li et al. 2017). SOD is the first enzyme to acts by neutralizing superoxide, catalyzing two electrons transference resulting on hydrogen peroxide. Following this process, catalase prevents other reactive components formation by converting the hydrogen peroxide into water and oxygen, non-reactive species (Araújo et al. 2018).

For an antioxidant mechanism acts efficiently, both CAT and SOD need to have their expression correlated, once one continues the process initiated for the other (Araújo et al. 2018). Thus, possibly, seeds at 63 days after anthesis may have a more efficient antioxidant mechanism, consequently, higher physiological quality.

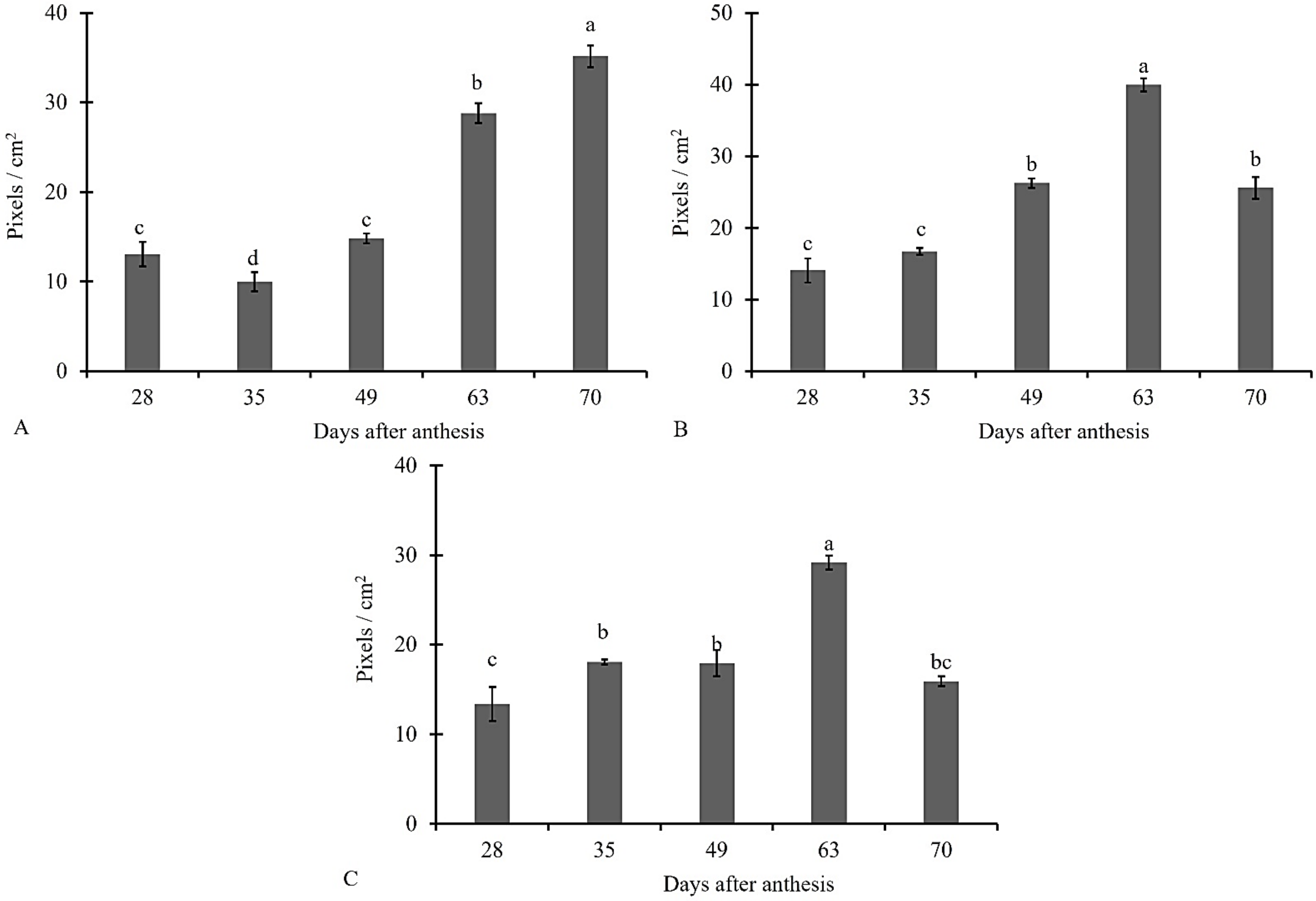

Figure 3. A - Expression of enzymes Malate Dehydrogenase, B - Alcohol Dehydrogenase, and C - Esterase in bode pepper (*Capsicum chinense*) developing seeds. The values of Pixels/cm^2 refer to the band sizes observed on the gels after electrophoresis. Same letters indicate absence of differences among treatments according to Tukey's test at 5% probability.

In tobacco seeds, those from fruits at the initial development stages have the lower expression of MDH. However, this enzyme activity increases during seed physiological maturation (Andrade et al. 2018). Malato dehydrogenase acts as a catalyzer for the reaction which converts malato in oxaloacetate for the NADH production during Krebs Cycle. Decrease on this enzyme expression promotes the mitochondria membranes rupture, prejudicing the ATP production and oxygen absorption (Andrade et al. 2018).

The ADH activity occurs during anaerobic respiration, being responsible for the metabolism of ethanol into acetaldehyde. This compost accelerates seed deterioration, thus, with ADH activity increase, seeds are more protected against the harmful action of this compost and so have more vigor (Andrade et al. 2018).

Enzymes ADH, CAT, and EST have higher expression at 63 days after anthesis, followed by 70 days. At these points, highest values were found for the physiological parameters, which indicate that from 63 to 70 days after anthesis, seeds reach the physiological maturity. Considering this, alcohol dehydrogenase, catalase, and esterase can be used as markers to predict pepper seed physiological quality, once the enzymatic profile of these coincides with the values of germination percentage and speed index.

5. Conclusions

For the conditions of this research, the enzymes catalase, alcohol dehydrogenase and esterase increase with the proximity of the physiological maturity point.

From 63 to 70 days after anthesis, *Capsicum chinense* seeds have the highest percentage of germination, indicating this point as the physiological maturity.

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References

ALFENAS, A.C. Eletroforese e marcadores bioquímicos em plantas e microrganismos. 2. ed. Viçosa, UFV, 2006.

ALVES, A.S., et al. Influência dos diferentes estágios de maturação do fruto na germinação de sementes de pimenta de cheiro. *Brazilian Journal of Animal and Environmental Research*. 2020, **3**, 1197-1201. https://doi.org/10.34188/bjaerv3n3-038

ANDRADE, D.B., et al. Morphological, physiological, and biochemical indicators of quality in tobacco fruits and seeds. *Genetics and Molecular Research.* 2018, **17**, 1-16. https://doi.org/10.4238/gmr18058

ARAÚJO, R.F., et al. Alterações fisiológicas e atividade de enzimas antioxidantes em sementes de pimentas *Capsicum chinense* Jacq e *Capsicum frutescens* L. durante o processo de maturação. *Revista Ceres*. 2018, **65**, 534-545. https://doi.org/10.1590/0034-737X201865060009

BAREKE, T. Biology of seed development and germination physiology. *Advances in Plants & Agricutlure Research.* 2018, **8**, 336-46. https://doi.org/10.15406/apar.2018.08.00335

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. 2009. Regras para análise de sementes. Secretaria de Defesa da Agropecuária – MAPA/ACS.

CARVALHO, S.I.C., et al. Pimentas do gênero Capsicum no Brasil. Brasília: Embrapa Hortaliças-Documentos, 2006.

FIGUEIREDO, J.C., et al. Maturação de sementes de pimenta em função de épocas de colheita dos frutos. *Scientia Agraria.* 2017, **18**, 1-7. http://dx.doi.org/10.5380/rsa.v18i3.51324

GONÇALVES, L.S., et al. Potencial fisiológico da semente de pimenta dedo-de-moça em relação aos estádios de maturação e períodos de descanso dos frutos. *Horticultura Brasileira*. 2018, **36**, 486-491. https://doi.org/10.1590/S1806-66902014000400011

GONÇALVES, V.D., et al. Maturação fisiológica de sementes de pimenta 'Bode Vermelha'. *Revista Caatinga.* 2015, **28**, 137-146. https://doi.org/10.1590/1983-21252015v28n316rc

JUSTINO, E.V., et al. Determinação da maturidade fisiológica de sementes de pimenta dedo de moça *Capsicum baccatum* var. *pendulum*. *Horticultura Brasileira.* 2015, **33**, 324-33. https://doi.org/10.1590/S0102-053620150000300008

LI, R., et al. Enhancing iodine content and fruit quality of pepper (*Capsicum annuum* L.) through biofortification. *Scientia Horticulturae.* 2017, **214**, 165-173. https://doi.org/10.1016/j.scienta.2016.11.030

QUEIROZ, L.A.F. and VON PINHO, E.V.R. Época de colheita e secagem na qualidade de sementes de pimenta Habanero Yellow. *Revista Brasileira de Sementes.* 2011, **33**, 472-481. https://doi.org/10.1590/S0101-31222011000300010

RASBAND, W.S. Imagej, U.S. National Institutes of Health. Maryland, Bethesda, 2020. https://imagej.nih.gov/ij/docs/faqs.html

REBOUÇAS, T.N.H., VALVERDE, R.M.V. and TEXEIRA H.L. Bromatologia da pimenta malagueta in natura e processada em conserva. *Horticultura Brasileira.* 2013, **31**, 163-165. https://doi.org/10.1590/S0102-05362013000100026

SALEH, B.K., OMER, A. and TEWELDEMEDHIN, B. Medicinal uses and health benefits of chili pepper (*Capsicum* spp.): a review. *MOJ Food Process Technology.* 2018, **6**, 325-328. http://dx.doi.org/10.15406/mojfpt.2018.06.00183

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