Australian Journal of Crop Science AJCS
AJCS 9(11):1010-1015 (2015) ISSN:1835-2707

Determination of harvest maturity in *Capsicum baccatum* L. seeds using physiological and biochemical markers

Maria Isabel Lima Silva[1], Eduardo Luiz Voigt[2*], Leilson Costa Grangeiro[1], Elizângela Emídio Cunha[2], Cristiane Elizabeth Costa de Macêdo[2], Salvador Barros Torres[3]

[1]Universidade Federal Rural do Semi-Árido, Avenida Francisco Mota, 572, 59625-900, Costa e Silva, Mossoró, RN, Brazil
[2]Universidade Federal do Rio Grande do Norte, Campus Universitário Lagoa Nova, 59072-910, Lagoa Nova, Natal, RN, Brazil
[3]Empresa de Pesquisa Agropecuária do Rio Grande do Norte, Caixa Postal 137, 59625-900, Costa e Silva, Mossoró, RN, Brazil

***Corresponding author: elvoigt@cb.ufrn.br**

Abstract

This work aimed to determine the harvesting time of pepper (*Capsicum baccatum* L.) seeds, cv. Dedo-de-moça, based on physiological markers and patterns of metabolite consumption and reserve deposition. Seeds were harvested at different maturation stages from 10 to 90 days after anthesis and seed quality was assessed according to water content, dry weight, germinability, electrolyte leakage, content of starch, neutral lipids, soluble proteins, total soluble sugars, non-reducing sugars, and total free amino acids. Physiological maturity was reached at 40 days after anthesis, when seeds displayed maximal dry weight and 60% of water loss; however, harvesting maturity was established 50 days after anthesis, taking into account maximal germinability, minimal electrolyte leakage, reserve deposition, and non-reducing sugar accumulation. Our results show that biochemical markers are very useful to characterize the developmental phases and harvesting maturity during *C. baccatum* seed formation.

Keywords: metabolite consumption; non-reducing sugar accumulation; pepper seed development; reserve deposition; seed maturation.
Abbreviations: BOD_biochemical oxygen demand; BSA_bovine serum albumin; CV_coefficient of variation; DAA_days after anthesis; DW_dry weight; ERI_emergence rate index; FW_fresh weight; GRI_germination rate index; LEA_late embryogenesis abundant; NL_neutral lipids; NRS_non-reducing sugars; RFO_raffinose family oligosaccharides; ROS_reactive oxygen species; SDS-PAGE_sodium dodecyl-sulphate polyacrylamide gel electrophoresis; SP_soluble proteins; SSP_seed storage proteins; TFAA_total free amino acids; TSS_total soluble sugars.

Introduction

Seed development starts immediately after double fertilization in angiosperms. During histodifferentiation, cell division allows embryo development from the zygote and endosperm formation from the primary endosperm cell. After the establishment of apical-basal and radial patterns in the embryo, food reserves are synthesized and deposited into the storage tissues over the course of seed expansion. In orthodox seeds, desiccation tolerance is acquired during maturation drying, which involves the accumulation of late embryogenesis abundant (LEA) proteins and non-reducing sugars (Bewley et al., 2012). Physiological maturity is reached when the seed displays maximal dry weight accumulation and considerable water loss. If maximal germination capacity and vigor are also reached at physiological maturity, it is likely to carry out seed harvest (Patrick and Offler, 2001); thus, the investigation of seed maturation in crop species is fundamental to determine harvesting maturity in order to avoid productivity losses due to seed deterioration in the field (Vidigal et al., 2011). In the majority of studies on seed maturation, harvesting maturity has been determined based on physiological markers, including dry weight and water content (Eskandari, 2012), germination percentage and emergence rate index (Ghassemi-Golezani and Hosseinzadeh-Mahootchy, 2009), accelerated aging and electrical conductivity (Samarah and Abu-Yahya, 2008; Vidigal et al., 2011), fruit and seed color (Yang et al., 2004). Recently, biochemical techniques have also been employed to assess seed quality throughout late maturation, in order to determine the activity of enzymes involved in cell respiration (Ramya et al., 2012) and reserve mobilization (Oliveira et al., 2013). In spite of these efforts, seed quality and harvesting maturity during late seed development have been poorly characterized in terms of metabolite consumption and reserve deposition. Thus, the aim of this work is to characterize seed quality and harvesting maturity during the development of *Capsicum baccatum* L. seeds by physiological markers and biochemical techniques, assessing the patterns of metabolite consumption and reserve deposition. The results obtained by biochemical techniques are compared with those determined by physiological markers in order to identify new markers for the determination of harvesting maturity during late seed maturation.

Results and discussion

Physiological characterization of seed development

In the course of *C. baccatum* seed development, changes in fresh weight (FW), dry weight (DW), and humidity

percentage (Fig 1) followed the patterns previously described for typical orthodox seeds (Bewley et al., 2012). The FW content (Fig 1A) was adjusted to the fourth-order polynomial model (R^2=0.9309), increasing 52% from 10 to 50 days after anthesis (DAA) and then diminishing 37% from 50 to 90 DAA. According to the fourth-order polynomial model (R^2=0.9784), the DW content (Fig 1B) increased 9-fold from 10 to 40 DAA and remained unchanged until the last harvest (90 DAA). Concomitantly, the water content, expressed as humidity percentage (Fig 1C), progressively decreased during the experiment as described by the sixth-order polynomial model (R^2=0.9912); in fact, the humidity percentage was 90% at the first harvest and diminished to 15% at the end of the experiment. It is widely accepted that seeds reach physiological maturity when DW content is maximal and the water content is considerably reduced throughout the developmental process (Bewley et al., 2012). Taking into account only these physiological markers, *C. baccatum* seeds were physiologically mature at 40 DAA, as DW accumulation was almost accomplished and water content was only 30%. In agreement with these results, physiological maturity based on DW accumulation was also reached before seed desiccation has been completed in *Crotalaria pallida* (Yang et al., 2004), chickpea (Samarah and Abu-Yahya, 2008), and cowpea (Eskandari, 2012).

The germinability of *C. baccatum* seeds was evaluated during the maturation process in terms of emergence percentage and emergence rate index (ERI), which fitted to the fourth-order (R^2=0.9177) and the sixth-order polynomial models (R^2=0.9701), respectively (Fig 2A, B). Accordingly, seedling emergence was not detected until 30 DAA (ERI=0.00), increased from 39.5% (ERI=1.26) at 40 DAA to 73.5% (ERI=2.76) at 50 DAA, and was not significantly altered until the last harvest. Some studies have assessed emergence percentage and ERI instead of germination percentage and germination rate index (GRI) as markers to determine when seeds become germinable. As in *C. baccatum*, seedling emergence was also verified from 40 DAA in *Capsicum annuum* (Vidigal et al., 2011) and cowpea (Eskandari, 2012).

The results obtained by the electrical conductivity test using *C. baccatum* seeds harvested at different moments after anthesis were adjusted to the fifth-order polynomial model (R^2=0.9843) (Fig 2C), and diminished 95% from 10 to 50 DAA and then remained almost unchanged until the end of the experiment. The electrical conductivity of seed leachate has been adequately employed as an indirect marker of seed vigour during natural aging, given that electrolyte leakage indicates membrane damage and consequent cell death (Bewley et al., 2012); however, during early seed development, electrolyte leakage may be related to the intense release of ions and amino acids in the seed apoplast from the phloem of the mother plant (Patrick and Offler, 2001). In this manner, membrane integrity was mainly verified to *C.baccatum* seeds between 50 and 70 DAA. Electrical conductivity measurements also progressively declined at late seed development in faba bean (Ghassemi-Golezani and Hosseinzadeh-Mahootchy, 2009), *C. annuum* (Vidigal et al., 2011), and onion (Ramya et al., 2012)..

Deposition of food reserves

The variation of the neutral lipid (NL) content during *C. baccatum* seed development was adjusted to the sixth-order polynomial model (R^2=0.7957) (Fig 3A). NL content increased 14-fold between 10 and 30 DAA, diminished 78% from 30 to 60 DAA, and doubled from 60 to 80 DAA. The starch content showed the opposite trend as described by the fifth-order polynomial model (R^2=0.9458) (Fig 3B). Starch content decreased 67% at the first harvest until 30 DAA, increased 23% from 30 to 60 DAA, and remained almost unchanged until the end of the experiment. Consistent with these results, it seems that starch played a part as a temporary carbon storage during early seed development, probably contributing to embryo formation and reserve biosynthesis, as previously described in *Arabidopsis thaliana* (Baud et al., 2002), *Medicago truncatula* (Djamel et al., 2005), and soybean (Saldivar et al., 2011). In accordance with the sixth-order polynomial model (R^2=0.7897) (Fig 3C), the soluble protein (SP) content in *C. bacatum* seeds increased 66% from 10 to 40 DAA and then remained almost unchanged until the last harvest. Among this protein pool, SDS-PAGE profile (Fig 4) revealed polypeptide bands showing molecular weight of 79, 64, 48, 43, 38, 27, 25, and 12 kDa, which are compatible with the chains of different seed storage proteins (SSP). 7S (vicilin) and 11S (legumin) globulins were previously identified in *C. annuum* cultivars by SDS-PAGE (Vladova et al., 2000). It is reasonable that the 79- and 64-kDa bands may be vicilin-like chains; the 48-, 43-, and 38-kDa bands seem legumin-like acidic subunits; the 27- and 25-kDa bands may correspond to legumin-like basic subunits; and the 12-kDa band is compatible with a 2S albumin-like chain (Bewley et al., 2012). The intensity of all of these polypeptide bands increased progressively over the course of pepper seed development and picked at 50 DAA (Fig. 4). In addition, the legumin-like subunits were mainly accumulated, as previously reported (Vladova et al., 2000).

Metabolite profile during seed development

The content of total soluble sugars (TSS) (Fig 5A) and total free amino acids (TFAA) (Fig 5C) was abruptly decreased during *C. baccatum* seed development, as shown by the third-order (R^2=0.9369) and the fifth-order polynomial models (R^2=0.9852), respectively. Since the first harvest until 30 DAA, the TSS and TFAA content reduced by 80 and 60%, respectively. After this period, a gradual decrease was observed in the content of both metabolites, reaching less than 5% of the initial amounts. As expected, the TSS and TFAA content decreased in parallel with the accumulation of NL (Fig 3A) and SP (Fig 3C), indicating the involvement of these metabolites as precursors of carbon and nitrogen reserves, respectively. In *A. thaliana* (Baud et al., 2002) and *M. truncatula* (Djamel et al., 2005), the decline in hexose pool also correlated with oil deposition as well as amino acid consumption took place when SSP were synthesized. The variation of the non-reducing sugar (NRS) content along *C. baccatum* seed development fitted to the fifth-order polynomial model (R^2=0.8617) (Fig 5B). Accordingly, the NRS content increased 5-fold between 10 and 60 DAA and then reduced 40% until the last harvest. As NRS picked at 60 DAA, following NL (Fig 3A) and TSS consumption (Fig 5A), it is possible that NL and TSS fueled early seed development and were partially interconverted to NRS. It is well established that NRS, including sucrose and raffinose family oligosaccharides (RFO), are accumulated during maturation drying and play a part in desiccation tolerance. According to the water-substitution hypothesis, NRS may bind to hydrophilic head groups of membrane lipids, replacing water and stabilizing cell membranes as seeds undergo desiccation (Bewley et al., 2012).

Physiological and biochemical characterization of developmental phases

Although it is difficult to define precisely when each phase took place throughout *C. baccatum* seed development, the

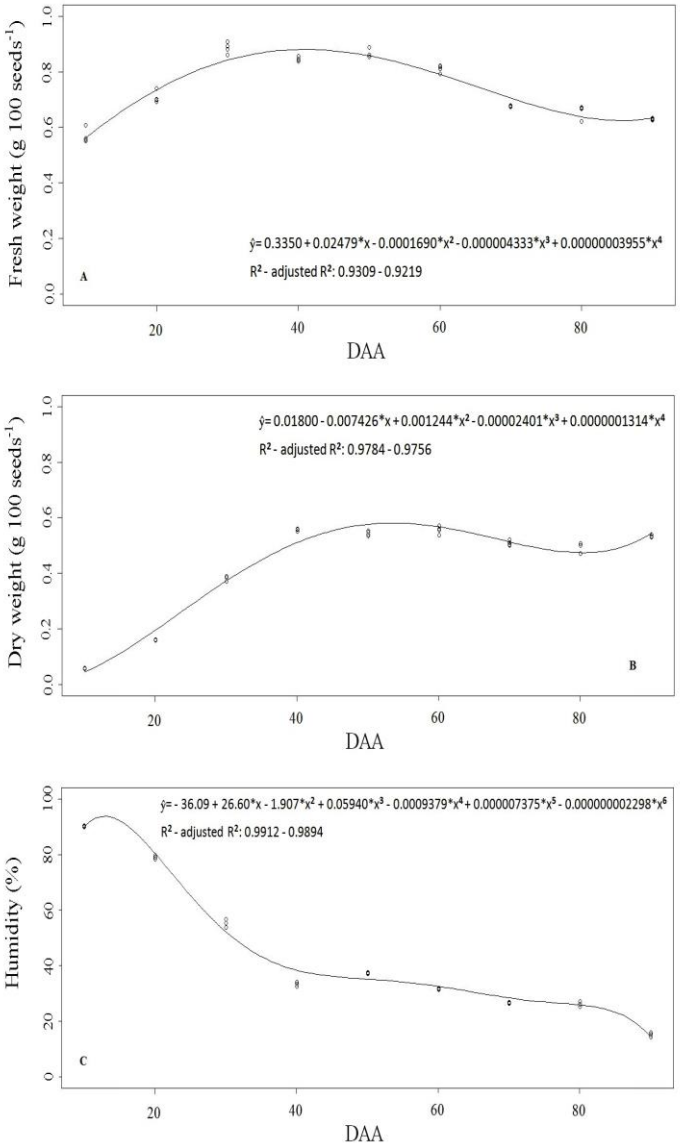

Fig 1. Fresh weight (A), dry weight (B), and humidity percentage (C) during seed development in pepper (*Capsicum baccatum* L.), cv. Dedo-de-moça. Dots represent individual observations in the regression analysis; adjusted to the most appropriate model.

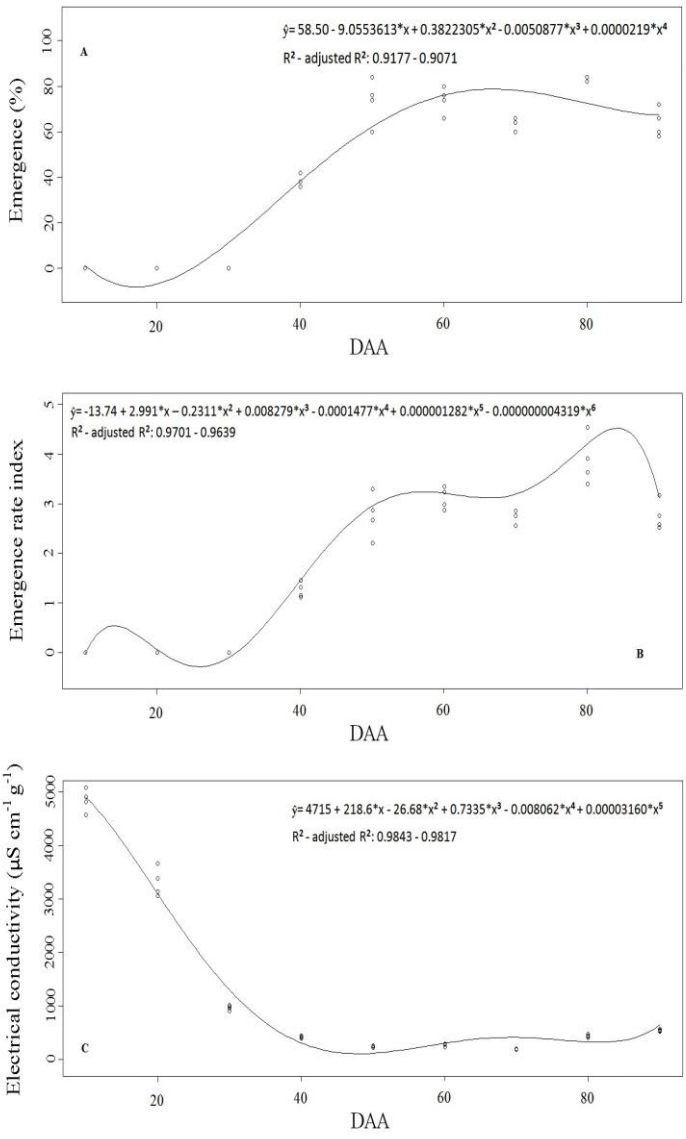

Fig 2. Emergence percentage (A), emergence rate index (B), and electrical conductivity (C) during seed development in pepper (*Capsicum baccatum* L.), cv. Dedo-de-moça. Dots represent individual observations in the regression analysis; adjusted to the most appropriate model.

biochemical markers assessed in this work provide some clues.

From 10 to 20 DAA, seeds showed low DW content (Fig 1B) and high humidity (Fig 1C), were poor in lipid and protein reserves (Fig 3A and C), rich in metabolites (Fig 5A and C), and unable to germinate (Fig 2A), characterizing histodifferentiation. Reserve deposition was intensified between 20 and 50 DAA, as a parallel increase in DW (Fig 1B), NL, starch, and SP (Fig 3) content was associated with a drastic decrease in humidity (Fig 1C). Since 50 DAA, seeds progressively lost water (Fig 1C), were able to germinate (Fig 2A), and accumulated NRS (Fig 5B), evidencing maturation drying. In *A. thaliana* (Baud et al., 2002), *M. truncatula* (Djamel et al., 2005) and *Vicia faba* (Patrick and Stoddard, 2010), similar biochemical markers were also utilized to characterize the different phases of seed development.

According to our results, it is reasonable to propose that *C. baccatum* seeds reached physiological maturity at 40 DAA, but harvesting maturity was established at 50 DAA, when maximal germinability (Fig 2A) and minimal electrolyte leakage (Fig 2C) were accompanied by reserve deposition (Fig 3) and NRS accumulation (Fig 5B). Using this approach, biochemical markers were very helpful to characterize the developmental phases and harvesting maturity during *C. baccatum* seed formation.

Materials and Methods

Plant materials

Pepper (*Capsicum baccatum* L.) seeds, cv. Dedo-de-moça, were utilized in this study.

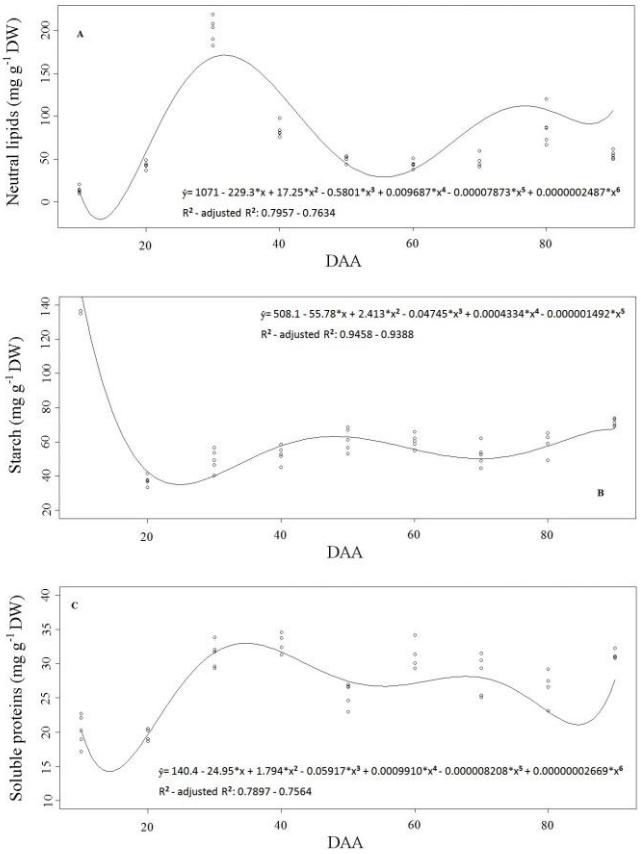

Fig 3. Content of neutral lipids (A), starch (B), and soluble proteins (C) during seed development in pepper (*Capsicum baccatum* L.), cv. Dedo-de-moça. Dots represent individual observations in the regression analysis; adjusted to the most appropriate model.

This genotype was chosen as an experimental model as it is mainly cultivated and consumed in Brazil in comparison with other cultivars of the *Capsicum* genus. Moreover, *C. baccatum*, cv. Dedo-de-moça, also shows high economical relevance in Brazil in terms of family-based farming and integration between small farmers and industry (Carvalho et al., 2009). Plants were cultivated in an ultisol at the experimental field of Universidade Federal Rural do Semi-Árido, Mossoró, RN, Brazil (5°11´South 37°20´ West) from January to October 2012. Soil analysis revealed the following characteristics: pH (water)=6.40; P=324.8 $mg.dm^{-3}$; K=111.3 $mg.dm^{-3}$; Ca=4.30 $cmol.dm^{-3}$; Na=24.0 $mg.dm^{-3}$, and Mg=0.80 $cmol.dm^{-3}$. Seeds were sown in a nursery and seedlings were transferred to the experimental field when they displayed four to six leaves or 150 mm of height according to Ozores-Hampton et al. (2000). Seedlings were planted in a 1.0 x 0.6 m disposal, irrigated by drip system, and cultivated as described by Bowen and Frey (2002). Flowers were marked at anthesis and fruits were harvested from 10 to 90 DAA. Seeds were removed manually from fruits and washed in running water. Samples utilized for physiological determination were dried at room temperature for 24 h and those employed for biochemical determination were frozen and maintained at -20 °C.

Physiological determinations

Eight samples of 100 seeds were immediately removed from fresh fruits and weighed to determine FW.

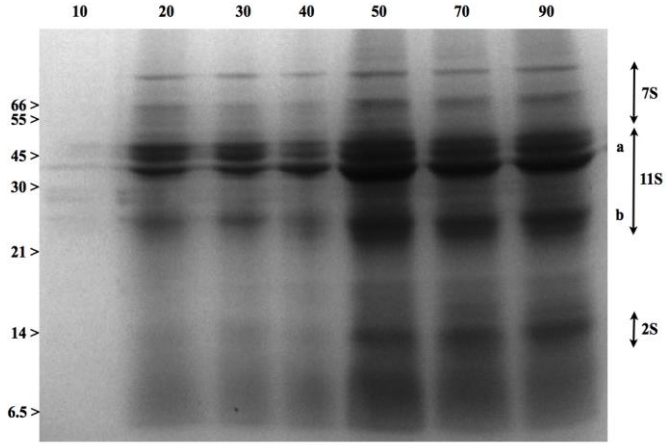

Fig 4. SDS-PAGE profile of seed soluble proteins from pepper (*Capsicum baccatum* L.), cv. Dedo-de-moça, during seed development. SP were extracted with 100 mM Tris-HCl buffer pH 7.0 containing 500 mM NaCl and 2 mM 2-mercaptoethanol, separated in 12% (w/v) polyacrylamide gels and stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 in 40% (v/v) methanol and 10% (v/v) acetic acid. Molecular mass is indicated in kDa. 7S are vicilin-like chains, 11S are legumin-like chains, composed by acidic (a) and basic (b) subunits, and 2S are putative albumin bands.

These samples were dried at 105±3 °C for 24 h and weighed again to assess DW. Water content was then calculated and expressed as humidity percentage, in accordance with the Brazilian Seed Analysis Rules (Brasil, 2009). Seeds submitted to emergence tests were previously treated with Thiram fungicide in the proportion of 3 g of product per 1 kg of seeds. Four samples of 25 seeds were sown in coconut fibre, moisturized with distilled water, and maintained at a greenhouse for 30 days. Samples were irrigated and observed daily; the number of normal seedlings was recorded. At the end of the experiments, emergence percentage was assessed and emergence rate index (ERI) was calculated as described by Maguire (1962).

In order to perform the electrical conductivity tests, four samples of 50 seeds were immersed in 25 mL of distilled water and maintained at a Biochemical Oxygen Demand (BOD) incubator at 25 °C for 24 h (Vidigal et al., 2011). Following, the electrical conductivity of the solution was assessed and expressed as µS cm^{-1} g^{-1}.

Biochemical quantifications

The content of NL was determined by gravimetry. Samples of 200 mg of dry seeds were macerated for 5 min and NL were extracted with 8 mL of n-hexane at 60 °C over 5 h. The supernatants were transferred to plastic tubes whose mass was previously determined. After evaporation of n-hexane at 80 °C, the NL content was determined as the difference between initial and final mass of the tubes and expressed as mg g^{-1} DW. For SP extraction, samples of 200 mg of fresh seeds were macerated with 1.5 mL of 100 mM Tris-HCl buffer pH 7.0 containing 500 mM NaCl and 2 mM 2-mercaptoethanol for 5 min. After centrifugation at 10,000 xg for 10 min, the supernatants were collected and the pellets were re-extracted twice with 1 mL of extraction buffer. The supernatants were combined to yield a 3.5 mL protein extract per sample.

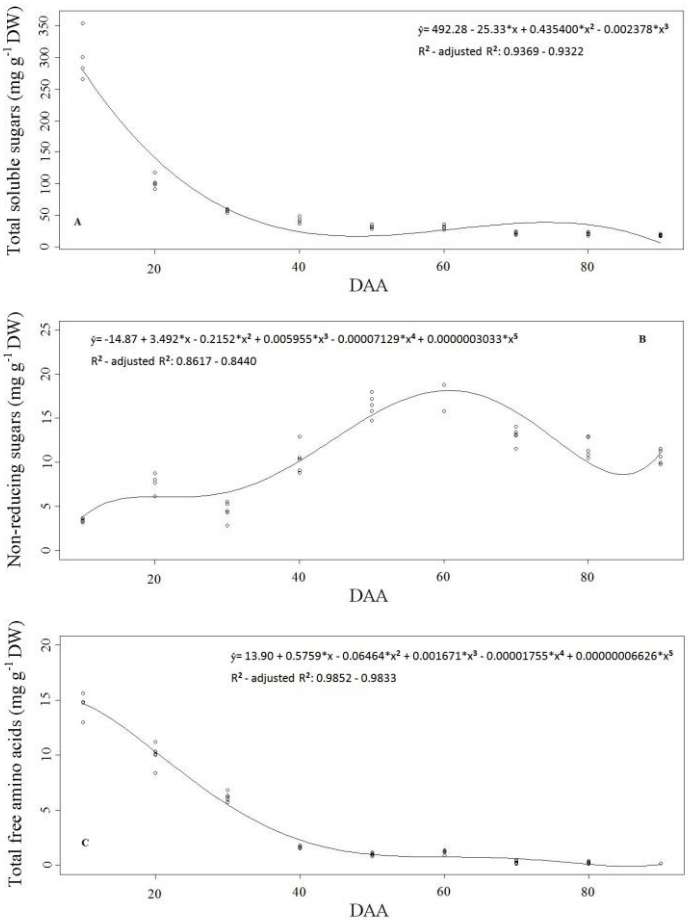

Fig 5. Content of total soluble sugars (A), non-reducing sugars (B), and total free amino acids (C) during seed development in pepper (*Capsicum baccatum* L.), cv. Dedo-de-moça. Dots represent individual observations in the regression analysis; adjusted to the most appropriate model.

SP content was determined by the Bradford (1976) assay using bovine serum albumin (BSA) as standard and expressed as mg g^{-1} DW. The SP extracts were submitted to sodium dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 2-mercaptoethanol according to Laemmli (1970). Then, SP were separated in 12% (w/v) polyacrylamide gels under 200 V and 50 mA for 80 min. Gels were stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 in 40% (v/v) methanol and 10% (v/v) acetic acid during 30 min, washed with 40% (v/v) methanol and 10% (v/v) acetic, and digitalized using an image capture system.

TSS, NRS, and TFAA were extracted from 200 mg of fresh seeds that were cut into 2x2 mm fragments, transferred to 5 mL of 80% (v/v) ethanol in glass tubes, and incubated at 60 °C for 30 min. The supernatants were collected and the pellets were re-extracted with 5 mL of 80% (v/v) ethanol under the same conditions; a 10 mL extract was obtained per sample and the pellets were stored to determination of starch. TSS content was quantified according to the Dubois et al. (1956) method employing a D-glucose standard curve. NRS determination was carried out as described by Van Handel (1968) utilizing the anthrone reagent (Morris, 1948; Yemm and Willis, 1954) and a sucrose standard curve. TFAA content was determined by the ninhydrin assay (Peoples et al. 1989) using L-glutamine as standard. All metabolites were expressed as mg g^{-1} DW.

To assess the content of starch, the pellets obtained from the extraction of low molecular weight soluble compounds (TSS, NRS, and TFAA) were macerated with 1.5 mL of 30% (v/v) $HClO_4$ during 5 min. After centrifugation at 10,000 xg for 10 min, the supernatants were collected and the pellets were re-extracted twice with 1 mL of 30% (v/v) $HClO_4$. The supernatants were then combined to yield a 3.5 mL starch extract. Starch content was determined as described by McCready et al. (1950) utilizing D-glucose as standard and expressed as mg g^{-1} DW.

Experimental design and statistical analysis

Samples were harvested randomly in five replicates for each determination procedure. Results of dependent variables were analyzed through linear regression fitting the data to polynomial models (first to sixth orders) sequentially. As recommended by Littell et al. (1991), we adopted the following criteria for selecting the best model: type I sum of squares and the probability value for the F test associated with this sum of squares**;** the coefficient of multiple determination (R^2 and adjusted R^2 for degrees of freedom) and the coefficient of variation (CV). All statistical analyses were performed using R version 2.13.0 software (R Development Core Team, 2011).

Conclusions

C. baccatum seeds, cv. Dedo-de-moça, reach physiological maturity at 40 DAA based on maximal DW accumulation and 60% of water loss; however, harvesting maturity is established at 50 DAA considering maximal germinability, minimal electrolyte leakage, reserve deposition, and NRS accumulation. Biochemical markers, including reserve and metabolite content, are helpful to characterize the developmental phases and harvesting maturity during *C. baccatum* seed formation.

Acknowledgements

Thanks to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the fellowships and Banco do Nordeste do Brasil for funding.

References

Baud S, Boutin J-P, Miquel M, Lopiniec L, Rochat C (2002) An integrated overview of seed development in *Arabidopsis thaliana* ecotype WS. Plant Physiol Biochem. 40:151-160.

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2012) Seeds: physiology of development, germination and dormancy. 3rd edn. Springer, New York.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. Anal Biochem. 72:248-254.

Brasil (2009) Ministério da Agricultura, Pecuária e Abastecimento - Secretaria de Defesa Agropecuária - Regras para análise de sementes. Mapa/ACS, Brasília.

Bowen P, Frey B (2002) Response of plasticultured bell pepper to staking, irrigation frequency, and fertigated nitrogen rate. Hortic Sci. 37:95-10.

Carvalho SIC, Ribeiro CSC, Henz GP (2009) "BRS Mari": nova cultivar de pimenta dedo-de-moça para processamento. Hortic Bras. 27:571-573.

Djemel N, Guedon D, Lechevalier A, Salon C, Miquel M, Prosperi JM, Rochat C, Boutin JP (2005) Development and composition of the seeds of nine genotypes of the *Medicago truncatula* species complex. Plant Physiol Biochem. 43:557-566.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem. 28: 248-254.

Eskandari H (2012) Seed quality changes in cowpea (*Vigna sinensis*) during seed development and maturation. Seed Sci Technol. 40:108-112.

Ghassemi-Golezani K, Hosseinzadeh-Mahootchy A (2009) Changes in seed vigour of faba bean (*Vicia faba* L.) cultivars during development and maturity. Seed Sci Technol. 37:713-720.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227:680-685.

Littell RC, Freud RJ, Spector PC (1991) SAS® System for linear models. 3rd edn. SAS Institute Inc., Cary.

Maguire JD (1962) Speeds of germination: aid selection and evaluation for seedling emergence and vigor. Crop Sci. 2:176-177.

McCready RM, Guggolz J, Silveira V, Owens HS (1950). Determination of starch and amylose in vegetables. Anal Chem. 22:1156-1158.

Morris DL (1950) Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Anal Chem. 22:1156-1158.

Oliveira GE, Pinho RG, Andrade T, Pinho EVR, Santos CD, Veiga AD (2013) Physiological quality and amylase enzyme expression in maize seeds. Cienc Agrotec. 37:40-48.

Ozores-Hampton M, Stansly PA, Obreza TA (2000) Biosolids and soil solarization effects on bell pepper (*Capsicum annuum*) production and soil fertility in a sustainable production system. Hortic Sci. 35:443.

Patrick JW, Offler CE (2001) Compartmentation of transport and transfer events in developing seeds. J Exp Bot. 52:551-564.

Patrick JW, Stoddard FL (2010) Physiology of flowering and grain filling in faba bean. Field Crop Res. 115:234-242.

Peoples MB, Faizah AW, Reakasem BE, Herridge DF (1989) Methods for evaluating nitrogen fixation by nodulated legumes in the field. Australian Centre for International Agricultural Research, Camberra.

R Development Core Team R. (2011) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available in http://www.R-project.org/, access on May12th 2014.

Ramya M, Yogeesha HS, Bhanuprakash K, Gowda RV (2012) Physiological and biochemical changes during seed development and maturation in onion (*Allium cepa* L.). Veg Sci. 39:157-160.

Saldivar X, Wang Y-J, Chen P, Hou A (2011) Changes in chemical composition during soybean seed development. Food Chem. 124:1369-1375.

Samarah NH, Abu-Yahya A (2008) Effect of maturity stages of winter- and Spring-sown chickpea (*Cicer arietinum* L.) on germination and vigour of the harvested seeds. Seed Sci Technol. 36:177-190.

Van Handel E (1968) Direct microdetermination of sucrose. Anal Biochem. 22:280-283.

Vladova R, Pandeva R, Petcolicheva K (2000) Seed storage proteins in *Capsicum annuum* cultivars. Biol Plantarum. 43:291-295.

Vidigal DS, Dias DCFS, Dias LAS, Finger FL (2011) Changes in seed quality during fruit maturation of sweet pepper. Sci Agric. 68:535-539.

Yang QH, Yin SH, Song SQ, Ye WH (2004) Development of desiccation tolerance and germination physiology of *Crotalaria pallida* Ait. seeds. Seed Sci Technol. 32:99-111.

Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J. 57:508-514.