Revista Ciência Agronômica, v. 52, n. 4, e20207534, 2021
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Anatomical, histochemical and physiological changes during maturation of chickpea (*Cicer arietinum* L.) seeds[1]

Alterações anatômicas, histoquímicas e fisiológicas durante a maturação de sementes de grão-de-bico (*Cicer arietinum* L.)

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ABSTRACT - The maturation stage of seeds affects their physiological quality soon after harvest. The aim of this study was to evaluate the anatomical, histochemical, and physiological changes in chickpea 'BRS Aleppo' seeds obtained from pods in different maturity stages. Pods were collected in five maturity stages: green, yellow-green, yellow, golden-yellow, and brown. For anatomical and histochemical characterization, sections were stained with toluidine blue, Lugol's solution, Xylidine-Ponceau (XP), and ruthenium red. For evaluation of physiological changes, seeds were dried to 12% (wet basis) water content. The following tests were performed: seed water content, germination, percentage and speed of seedling emergence, and electrical conductivity. A completely randomized experimental design was used, with four replications. As the chickpea seeds develop, the innermost layers of the seed coat collapse. Accumulation of starch and protein were already observed in the seeds of green pods and pectin was prominent compound of the seed coat sclerids. In general, chickpea seeds from yellow, golden-yellow and brown pods have greater germination and vigor.

Key words: Anatomy. Histochemistry. Physiological quality. Pulse. Seed development.

RESUMO - O estádio de maturação das sementes influencia a sua qualidade fisiológica logo após a colheita. O objetivo do trabalho foi avaliar as alterações anatômicas, histoquímicas e fisiológicas em sementes de grão-de-bico 'BRS Aleppo' obtidas de vagens em diferentes estádios de maturação. Foram colhidas vagens em cinco estádios de maturação: verde, verde-amarelo, amarelo, amarelo-dourado e marrom. Para caracterização anatômica e histoquímica os cortes foram corados com Azul de Toluidina, Lugol, Xylidine Ponceau (XP), Vermelho de Rutênio. Para avaliação das alterações fisiológicas, as sementes foram secas até 12% de umidade. Foram realizados os seguintes testes: grau de umidade, germinação, porcentagem e índice de velocidade de emergência e condutividade elétrica. Utilizou-se o delineamento inteiramente casualizado com quatro repetições. Verificou-se que com o desenvolvimento das sementes de grão-de-bico as camadas mais internas do tegumento sofrem colapso. O acúmulo de amido e proteína já está em andamento nas sementes de vagens verdes. A pectina se destaca dentre os compostos constituintes de parede celular dos esclereídes do tegumento. Em geral, sementes obtidas de vagens com coloração externa amarela, amarelo-dourado e marrom apresentam maior germinação e vigor.

Palavras-chave: Anatomia. Histoquímica. Qualidade fisiológica. Pulse. Desenvolvimento de semente.

DOI: 10.5935/1806-6690.20210048
Editor-in-Chief: Prof. Salvador Barros Torres - sbtorres@ufersa.edu.br
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Received for publication 08/08/2020; approved on 31/01/2021
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INTRODUCTION

Pulses, edible dry seeds of legumes such as beans, peas, lentils, and chickpeas, have assumed an important role in debates regarding human nutrition, due to their nutritional value. Chickpea (*Cicer arietinum* L.) holds a prominent position among pulses as the second most consumed legume in the world (SWAMY; RAJA; WESLEY, 2020). The chickpea crop has been expanding in the world and, consequently, this fact affects the demand for high quality seeds. In this context, studies related to seed production and processing are important to make high quality seeds available to the market.

The process of seed development involves a series of changes that begin with ovule fertilization and extend to physiological maturity, characterized by maximum dry matter content, when plant-seed translocation ends (BEWLEY *et al*., 2013). At that time, maximum dry matter content may or not coincide with maximum seed physiological quality, which can occur a little earlier or later depending on the species (SILVA *et al*., 2019). In *Vignia sinensis* seeds, Eskandari (2012) observed that maximum seed physiological quality was reached after physiological maturity. Maximum germination and vigor was also observed after physiological maturity in sunn hemp (*Crotalaria juncea* L.) seeds (ARAÚJO *et al*., 2018).

Determining the maturity stage, i.e., at which seed physiological quality is highest is essential, especially for species with indeterminate growth habit, such as chickpea. This aspect is important to combine high seed performance and optimum harvesting season (CONCEICAO *et al*., 2020). However, the number of days from anthesis, flowering, or fructification to maximum germination potential can vary among cultivars and environmental conditions (BARBEDO; CENTENO; FIGUEIREDO-RIBEIRO, 2013).

Thus, whenever possible, physical/morphological traits of the plant, fruit, and/or seed should be precisely identified and associated with seed physiological quality and used to monitor both seed physiological maturity and the time for seed harvesting (VIDIGAL *et al.*, 2011). According to Samarah and Abu-Yahya (2008) chickpea seeds reached physiological maturity when pod color became yellow, but germination and vigor were at maximum level after that stage, when the pods became brown. Also, Shaheb *et al*. (2015) found common bean (*Phaseolus vulgaris* L.) seeds can be harvested from 75 to 85 days after seedling emergence, when the pods are of yellowish and brown color. Lima Cruz *et al*. (2019) observed physiological maturity of cowpea (*Vigna unguiculata* L. Walp.) seeds occurred from 15 to 21 days after anthesis, and they recommend harvest when the pods have a light green color.

Another important aspect in studies on seed maturation is related to anatomical and histochemical changes that occur from the time of fertilization of the ovule up to seed maturity. In chickpea, using light microscopy and scanning electron microscopy, the seed coat, cotyledons as well as seed chemical compounds such as starch grains, and protein bodies could be observed (WOOD; KNIGHTS; CHOCT, 2011). Nevertheless, those studies only evaluated histochemical characteristics and not related these modifications to other important changes as dry matter, germination and vigor that occur throughout seed development. The analysis of all these information is relevant in studies on maturation with the purpose to obtain high physiological potential seeds.

Therefore, the main objective of this study was to evaluate and associate the anatomical, histochemical, and physiological changes in chickpea seeds from pods in different stages of maturation.

MATERIAL AND METHODS

The study was conducted in the Plant Science Department of the Universidade Federal de Viçosa (Brazil) using chickpea cv. BRS Aleppo seeds. The seed production field was set up in an experimental area of the Universidade Federal de Viçosa, located in the district of São José do Triunfo, Viçosa city, Brazil. Viçosa is located in the Zona da Mata region of Minas Gerais (Brazil), at 689.7 m altitude and geographic coordinates of 20°45’ S and 42°51’ W. Monthly climate data for the period between sowing and seed harvests indicated that relative humidity was around 80% and mean temperatures were from 22.9 °C to 12.0 °C (INSTITUTO NACIONAL DE METEOROLOGIA, 2017).

Planting was performed on 06 April 2017 after conventional soil tillage, in 20 m length furrows spaced 0.50 m each, with a total experimental area of 50 m^2 and density of 12 plant/linear m of plant furrow. Before sowing, fertilization was performed based on the results of soil chemical analysis, employing 250 $kg.ha^{-1}$ of simple superphosphate, 160 $kg.ha^{-1}$ of potassium chloride and 60 $kg.ha^{-1}$ of nitrogen. Topdressing was done 30 days after sowing with ammonium sulfate at a dose of 10 $kg.ha^{-1}$, following the recommendation of Nascimento, Artiaga and Suinaga (2016). Crop cultural practices were carried out according to research recommendation for chickpeas (NASCIMENTO; ARTIAGA; SUINAGA, 2016) with spray irrigation provided whenever necessary.

The flowers were marked at the time of anthesis to determine the number of days after anthesis (DAA) necessary to obtain pods with outside colors of green, yellow-green, yellow, golden-yellow, and brown. Pods were manually collected in these five maturity stages.

After harvest, pods samples of each treatment were manually threshed, and the seeds were separated for anatomical and histochemical characterization. Seeds were fixed in FAA_{70} (formaldehyde: acetic acid: ethyl alcohol) for 48 h under vacuum conditions and stored in 70% alcohol (JOHANSEN, 1940). After that, they were dehydrated in an increasing graded ethylic series and embedded in methacrylate (Historesin – Leica) according to manufacturer's recommendations. The material was then cut in cross sections of 5 μm thickness in a rotary microtome (RM2265 – Leica).

For anatomical characterization, the sections were stained with toluidine blue (O'BRIEN; FEDER; MCCULLY, 1964), and mounted on glass slides with synthetic resin (Permount). For histochemical analyses, the sections were placed under the following reagents/stains: Toluidine blue with Lugol's reagent for detection of starch (JOHANSEN, 1940); Xylidine Ponceau (XP) for detection of total proteins (VIDAL, 1977), and ruthenium red for detection of pectin (JOHANSEN, 1940). Images (anatomical/structural part and histochemical) were obtained and observed in a photomicroscope (AX70 TRF, Olympus Optical, Tokyo, Japan) with the U-PHOTO system connected to a digital camera (AxionCan, Carl Zeiss, Gena, Germany) and to a microcomputer. Descriptive analysis was performed for results in reference to anatomical characterization and for histochemical analyses.

The other subsample of pods were dried under laboratory conditions for one week. Then, the pods were manually threshed, and the seeds were naturally dried until they reached water content of approximately 12%. After that, seeds were evaluated for physiological quality by the tests described below. Seeds were treated with fungicide [Carbendazim + Thiram] (Protreat SC) at the rate of 150 mL of commercial product / 100 kg of seeds before the tests, except for seed water content, seed dry matter, 1000-seed weight, and electrical conductivity.

Seed water content: this was determined by the oven method at 105 $\pm$ 3 °C for 24 hours using four replications of 25 seeds each (BRASIL, 2009), and results were expressed in percentage (fresh weight basis).

Seed dry matter: this was determined together with seed water content (BRASIL, 2009), consisting of the final mean weight of the four replications of 25 seeds after drying. Results were expressed in g/100 seeds.

1000-seed weight: eight replications of 100 seeds each with water content near to 12% were weighed on a balance (precision 0.001 g), and results were expressed in g/sample (BRASIL, 2009).

Germination: was performed with four replications of 50 seeds each sown in rolls of paper towel moistened with water in the amount of 2.0 times the weight of the dry paper. The rolls were kept in a seed germinator regulated to 20 °C under 16 h dark/8 h light (NASCIMENTO; ARTIAGA; SUINAGA, 2016). Germination was counted on the fifth (germination first count) and eighth day after sowing (BRASIL, 2009) and results were expressed in percentage of normal seedlings.

Seedling emergence and emergence speed index (ESI): was conducted with four replications of 50 seeds each in a greenhouse. Seeds were distributed at a depth of 1.0 cm in a 1:1 mixture of soil and sand contained in polystyrene trays. Counting was performed daily after emergence of the first seedling up to stabilization of the number of seedlings, which occurred at 15 days after sowing. The mean percentage of emerged seedling and the ESI were calculated according to Maguire (1962).

Electrical conductivity: four replications of 50 seeds each were weighed on a balance with precision of 0.001 g and placed in plastic cups containing 250 mL of distilled water (KHAJEH-HOSSEINI; REZAZADEH, 2011). They were then maintained in a Biochemical Oxygen Demand (B.O.D.) incubator at 25 °C for 24 h. After this period, electrical conductivity was determined in a conductivity meter Digimed CD 21 and results were expressed in $\mu S.cm^{-1}.g^{-1}$ of seeds (VIEIRA; KRZYZANOWSKI, 1999).

A completely randomized experimental design was used, with four replications. Normality and homogeneity tests were performed on the errors obtained for each variable. Analysis of variance was then performed on the data, processed on the program R (R CORE TEAM, 2019). For each test, the mean values obtained for the treatments were compared by the Tukey test ($p \leq 0.05$). Descriptive analysis was carried out for the results in reference to anatomical characterization.

RESULTS AND DISCUSSION

Figure 1A shows the pods collected at different maturity stages, characterized by their outside color and number of days after anthesis. At 30, 55, 65, 75, and 85 days after anthesis (DAA), the pods reached green, yellow-green, yellow, golden-yellow, and brown outer color, respectively. Figure 1B shows the chickpea seeds obtained from the pods at each maturation stage after natural drying.

Chickpea seed structure comprises a seed coat and embryo, without the presence of the endosperm and, as such, are exalbuminous. These seeds have a seed coat with more external macro- and osteosclereids, followed by parenchyma layers that collapse with seed development

(Figure 2G, 2H, 2J, 2K, 2M, and 2N), and may exhibit a vascular tissue system (Figure 2G and 2J). Some studies show that the inner part of the seed coat is compressed and absorbed during seed development (SMÝKAL *et al*., 2014), as may have occurred in the chickpea seeds in the present study.

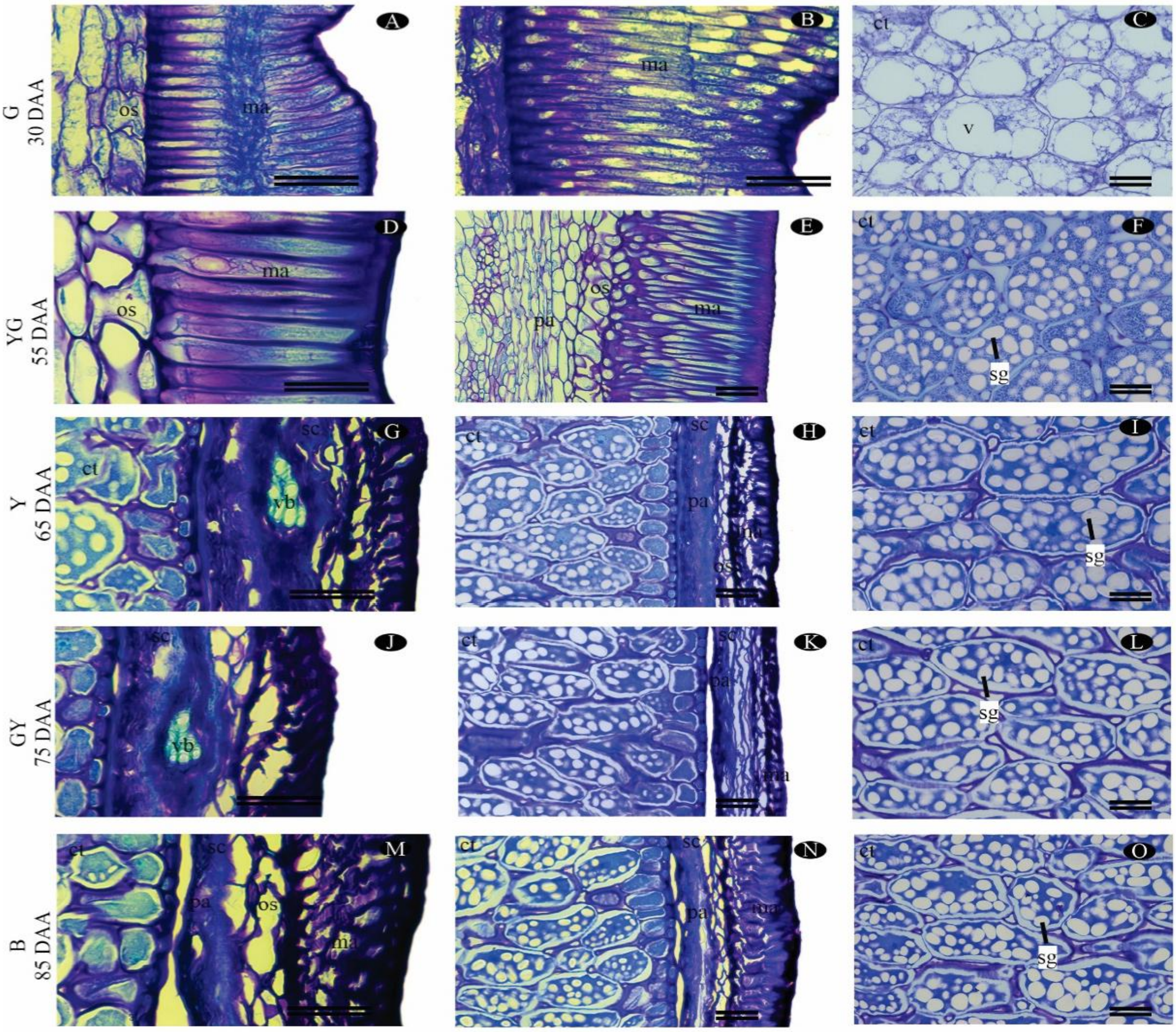
The macrosclereids have an irregularly dense wall that is more slender in the middle part, which sometimes results in a compressed portion (Figures 2A and 2B). There are regions of the seed coat where these macrosclereids may be of various sizes (Figures 2A and 2B) or may be more regular (Figures 2D and 2E). The predominance of pectins and cellulose in the constitution of these cell walls is evident, due to the reaction with toluidine blue, ranging from purple to blue in these layers (Figures 2A, 2B, 2D, 2E, 2G, 2H, 2J, 2K, 2M, and 2N). This outermost layer of the seed coat is followed by a second layer of osteosclereids that have a similar cell wall constitution (Figures 2A, 2D, and 2E). Below the osteosclereids, there are various layers of a parenchymatous nature (Figures 2E, 2H, 2K, and 2N).

Furthermore, Figure 2 shows that the parenchyma cells of the cotyledon reserve are well vacuolated in the green stage (Figure 2C). In the yellow-green stage, the reserves, mainly composed of starch, are already quite visible; however, the reserve parenchyma cells in the green and yellow-green stages are a little smaller than in the stages that follow - yellow (Figure 2I), golden-yellow (Figure 2L), and brown (Figure 2O).

The results of reaction with Lugol's reagent plus toluidine blue show that deposition of reserves is already in process in the cotyledons of seeds from pods in the green stage when small grains of starch are found (Figure 3A). These starch grains increase in size beginning at the yellow-green stage (Figure 3E), stabilizing from this stage on (Figures 3I and 3J). In seeds from pods in the green stage, small starch grains were also observed in the parenchyma layer below the seed coat sclereids (Figure 3B); however, they were temporary and are not observed in seeds obtained from pods in the yellow-green stage on (Figure 3F). Those observations indicates carbohydrate transport kinetics, initially through the phloem to a parenchyma region, which remains metabolically active in the seed coat of seeds in development (BEWLEY *et al*., 2013). After that, considering the absence of vascularization

Figure 1 - Appearance of the chickpea pods and seeds collected at different maturation stages (A) and of the seeds after natural drying (B). green (G); yellow-green (YG); yellow (Y); golden-yellow (GY) and brown (B). Scale bar = 1 cm

Figure 2 - Cross sections of chickpea seeds collected in the green (A to C), yellow-green (D to F), yellow (G to I), golden-yellow (J to L), and brown (M to O) stages, stained with toluidine blue. G, green; YG, yellow-green; Y, yellow; GY, golden-yellow; and B, brown. ct, cotyledon; DAA, days after anthesis; ma, macrosclereids; os, osteosclereids; pa, parenchymal cells; sc, seed coat; v, vacuole; vb, vascular bundle. Scale bar = 50 µm



between the seed coat and embryo in development, the carbohydrates and other reserves are transported through the apoplastic pathway or suspensor pathway to the cotyledons of seeds in development, where starch and other reserves are deposited (BEWLEY *et al*., 2013).

The result of reaction with ruthenium red confirmed the presence of a wall rich in pectin and with unequal thickness, more evident in seeds obtained from pods in the green stage (Figure 3C). There is an increase in wall thickness in seeds obtained from pods in the yellow-green stage (Figure 3G); however, it is insufficient to avoid partial collapse of this layer in seeds obtained from pods in the yellow stage on (Figure 3K). The presence of pectin and cellulose confers a hydrophilic characteristic to the seed coat walls, easing water absorption by the seed. The primary function of these components is to retain imbibed water, thus contributing to the germination process (YANG *et al*., 2012).

Protein deposition is small in the reserve parenchyma of cotyledons of seeds obtained from pods in the green stage (Figure 3D), though small marking of proteins is observed in the sclereid layer and parenchyma layer present in the seed coat of this same stage. In seeds developed from pods at the yellow-green stage, marking of the XP reagent reduces in the seed coat region; it is restricted to the innermost layers of the seed coat in contact with the cotyledons, where the presence of proteins is clear (Figure 3H). Marking of the XP reagent declines in the seed coat region, is more intense in the cotyledon cells of seeds obtained from pods in the yellow stage (Figure 3L), and stabilizes from this stage on.

The main reserve substances in the seeds are carbohydrates, proteins, and lipids. During germination, these substances are mobilized and their products from degradation are used for different purposes throughout seedling development (ZHAO *et al*., 2018). According to Bewley *et al*. (2013), the carbohydrates pre-formed in the seed are used as substrates for respiration during the pre-germination period. Proteins, for their part, are the main sources of nitrogen and sulfur, elements necessary for synthesis of new proteins, nucleic acids, and secondary compounds in seedlings in development (BEWLEY e*t al*., 2013).

The water content determined soon after harvest of chickpea seeds declined over the period of development (Figure 4A). The seeds obtained from pods in the green, yellow-green, and yellow stages had the highest water contents, 82%, 62%, and 47%, respectively, i.e. higher than those from the fruits at the golden-yellow and brown stages (27% and 24%, respectively), which did not differ significantly (Figure 4A). Drying was performed before threshing to minimize damage in the seeds caused by a fast loss of water. During the maturation process, cells may be more sensitive to the speed of dehydration than the reduction in the amount of water lost. Thus, removal of seeds from the pods and their rapid drying may lead to loss of viability especially in the intolerant to dehydration phase (BARBEDO; CENTENO; FIGUEIREDO-RIBEIRO, 2013). It is important to emphasize that the germination test was performed for fresh (data not showed) and dried seeds from green pods and the values were similar for both indicating that premature slow drying did not caused damage.

The lowest value of seed dry weight was observed from pods at the green stage (Figure 4A), in which the seeds had a higher percentage of water and smaller size in relation to the further stages, as can be observed in Figure 1B. In this stage, the seeds had a greenish color and the 1000-seed weight (Figure 4B) was significantly lower than those from the other fruit stages.

At the beginning of seed development, water content is high, as the cell division and expansion processes prevail, which are highly dependent on water. After that phase, seeds slowly lose water, which is replaced by synthesized reserves (SILVA *et al*., 2011). This pattern is well documented for several crops as described in *Vigna sinensis* by Eskandari (2012), and in cowpea (*Vigna unguiculata*) by Lima Cruz *et al*. (2019). Thus, as the maturation process advanced, there was a reduction in the seed water content and an

Figure 3 - Cross sections of chickpea seeds stained with toluidine blue and Lugol’s solution in the green (A and B), yellow-green (E and F), yellow (I), and brown (J) stages. Cross sections of seeds stained with ruthenium red in the green (C), yellow-green (G), and yellow (K) stages. Cross sections stained with XP in the green (D), yellow-green (H), and yellow (L) stages. ct, cotyledon; ma, macrosclereids; pa, parenchyma cells; pb, protein body; sc, seed coat; sg, starch grain. Scale bar (A, D, E, H, I, J and L) = 160 µm; Scale bar (B, C, F and G) = 150 µm

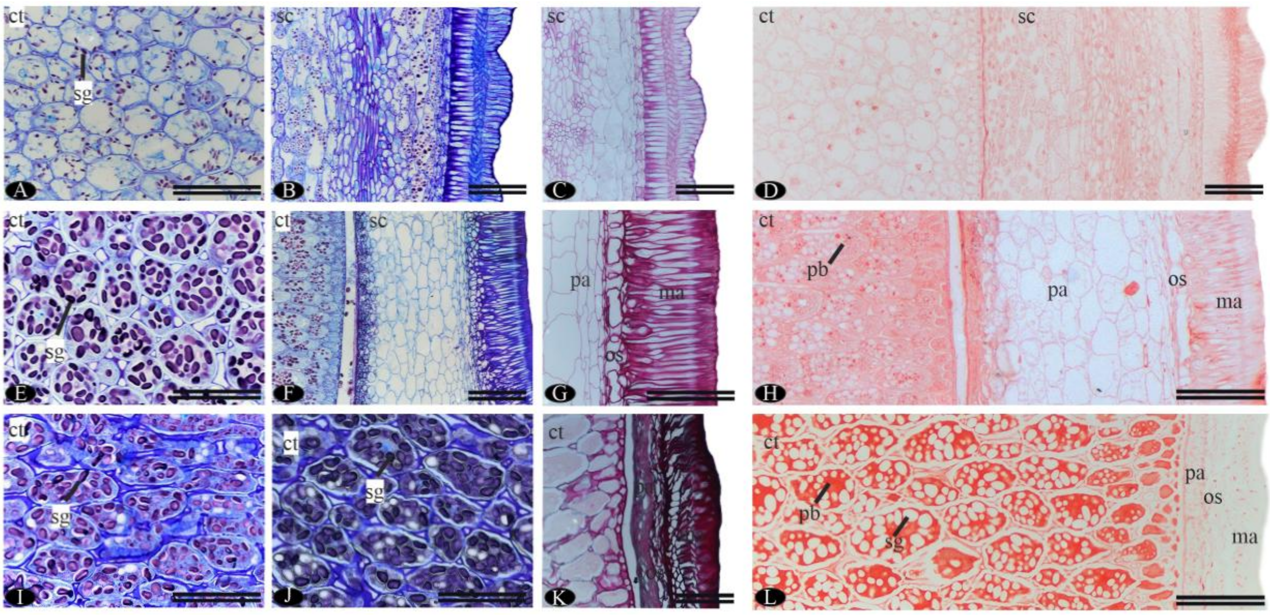
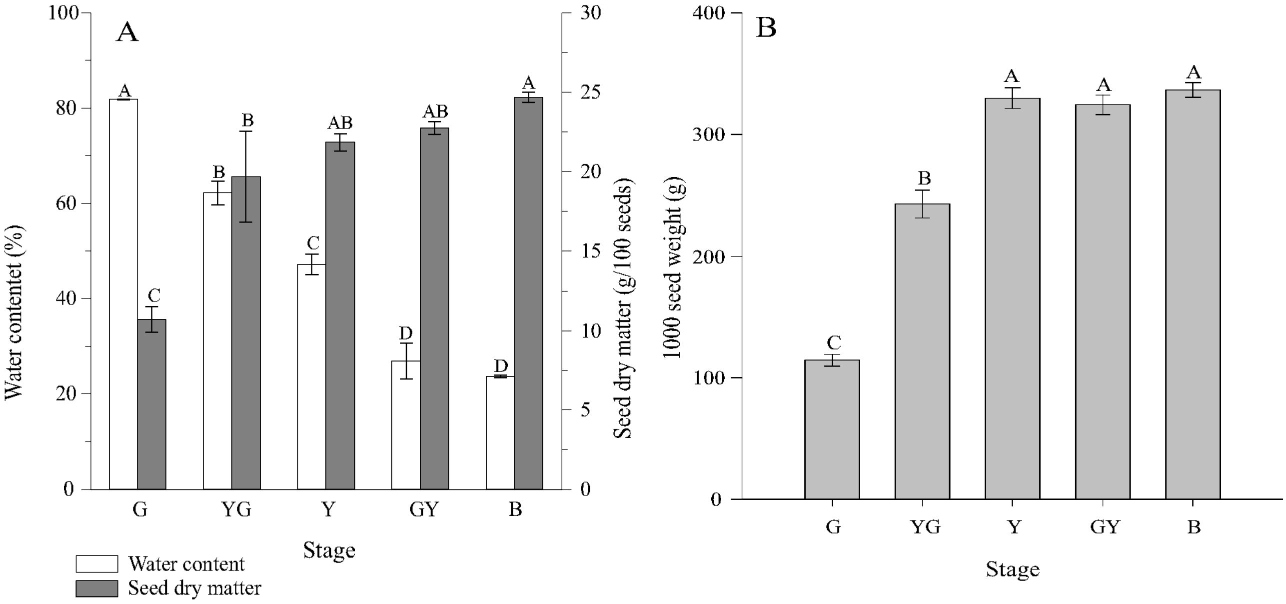


Figure 4 - Seed water content (A), seed dry matter (A) and 1000-seed dry weight (B) of chickpea from pods at different maturation stages. Mean values followed by the same letters do not differ by the Tukey test ($p \leq 0.05$). Green (G), yellow-green (YG), yellow (Y), golden-yellow (GY) and brown (B)



increase in dry matter weight (Figure 4A). The highest values of dry matter were observed in seeds in the yellow, golden-yellow, and brown stages.

Maximum accumulation of dry matter is an indication that seeds reached physiological maturity, when there is no more translocation of assimilates from the plant to the seeds (MARCOS-FILHO, 2015). From the yellow stage on, there was no significant increase in dry matter, indicating that the seeds probably reached physiological maturity at that time. During this period, seeds are exposed to unfavorable weather, which may compromise their physiological quality. These results are in agreement with Samarah and Abu-Yahya (2008), where chickpea seeds reached physiological maturity with 40%-50% water content and yellow pods. In the present study, the seeds obtained from yellow pods also had water content in this range, i.e., around 47% (Figure 4A). Associating plant and/or fruit and seed characteristics with the physiological maturity is important for obtaining high physiological quality seeds, because it is known that as of that point, seeds will remain in the field until reach a water content compatible with harvest, and they are prone to deterioration (BEWLEY *et al*., 2013). The cycle of the cultivar BRS Aleppo is around 120 days (NASCIMENTO; ARTIAGA; SUINAGA, 2016). Avelar *et al*. (2018) detected dry matter content reduction in seeds of this cultivar harvested from 121 days after sowing, indicating a beginning of the deterioration process.

During the seed development process, there was accumulation of reserve substances, especially starch and proteins (Figure 3). In seeds obtained from pods at the green stage, the presence of small starch grains was observed on the cotyledon cells. However, in that phase, seed dry matter was still low (11 g/100 seeds). In this phase, cell division and increase in cell volume establish the spatial limits for containing transfer of reserves that determine the amount accumulated and final seed size (MARCOS-FILHO, 2015). Beginning at the yellow-green stage, in addition to starch, protein bodies were evident on the cells of the reserve parenchyma (Figures 3E and 3K). These findings are according to Bewley *et al.* (2013) observations, where accumulation of carbohydrates precedes that of lipids and proteins in seeds. Accumulation of these reserve substances intensified in the seeds of the yellow stage, without evident changes in the following stages (Figures 3I, 3J, and 3L). These observations confirm the data obtained for dry matter content, which practically did not increase significantly from the yellow stage on (Figure 4A). At physiological maturity, seeds contain significant amounts of two or three reserve molecules during seed storage reserve synthetized during development (MARCOS-FILHO, 2015) as observed by our results. These compounds are essentials for seed germination and seedling establishment in the field.

Figure 4B shows the values regarding 1000-seed weight during development of chickpea seeds, which allow to establish positive relationship with seed size, maturation stage (Figure 1) and reserves accumulation (Figure 4A). In general, the highest seed weight is related to greatest reserves accumulation during maturation process, as observed for dry matter weight (Figure 4A), in which the seeds from the yellow, golden-yellow, and brown stages had higher values of 1000-seed weight (330, 324, and 337 g, respectively) in relation to the other fruit stages, with a lower value for seeds from the green stage (115 g). These results indicated that seeds from green pods are still begining the filling process as observed in Figures 2 and 3. In common bean (*Phaseolus vulgaris* L.) seeds, Shaheb *et al*. (2015) observed higher values of 1000-seed weight in seeds from golden-yellow and brown pods, whereas lower values were found in those obtained from green pods. As emphasized above, during seed development there is a period of rapid cell division and expansion at beginning of embryo formation causing a metabolic sink that receives assimilates translocated from mother plant. Subsequently, an increase is seen in the seed size and weight as a consequence of cell expansion and accumulation of storage reserves until yellow-green stage when the maximum dry matter content is reached (i.e.) physiological maturity.

Figure 5A shows there was an increase in seed germination as maturation advanced, with lower values for seeds from the green stage and higher values for seeds from the yellow, golden-yellow, and brown stages, which did not differ from each other. Samarah and Abu-Yahya (2008) observed that germination of chickpea seeds increased as maturation advanced, reaching a maximum value when the pods were brown, but in the present study, this maximum value occurred already for the seeds obtained from yellow pods (Figure 5A). Figure 4A shows that beginning at the yellow stage, the maximum seed dry weight was also greater. Histochemical analyses (Figure 3) indicate that seeds from the yellow, golden-yellow, and brown stages already had high deposition of starch and proteins in relation to seeds from the green and yellow-green stages, indicating greater availability of reserve substances for seed germination and seedling growth.

The low quality of seed collected at an early stage can be explained by the presence of immature seeds, according to Shaheb *et al*. (2015). This was also observed in the present study for seeds from the green and yellow-green stages and confirmed by seed dry matter (Figure 4A), 1000-seed weight (Figure 4B), and histochemical analyses (Figure 3); seeds from these two stages had low starch and protein content. Seeds obtained from green and yellow-green pods did not achieve the minimum standard of germination established for the species, which is 80% (Figure 5A) (BRASIL, 2012).

The germination first count can be used as an indication of vigor, because it is based on the supposition that more vigorous seeds germinate more rapidly, exhibiting a higher percentage of germination on the date established for first count of the test. Greater speed of germination was obtained for seeds from brown pods, followed by seeds from golden-yellow pods (Figure 5B). The worst performance was for seeds from green pods, which did not differ from those from the yellow-green stage, which can be attributed to the lower dry matter content and lower weight of these seeds (Figure 4A and 4B). The maturation process is interrupted in seeds collected from more unripe fruit, which results in seeds with low physiological potential (GONÇALVES *et al*., 2018).

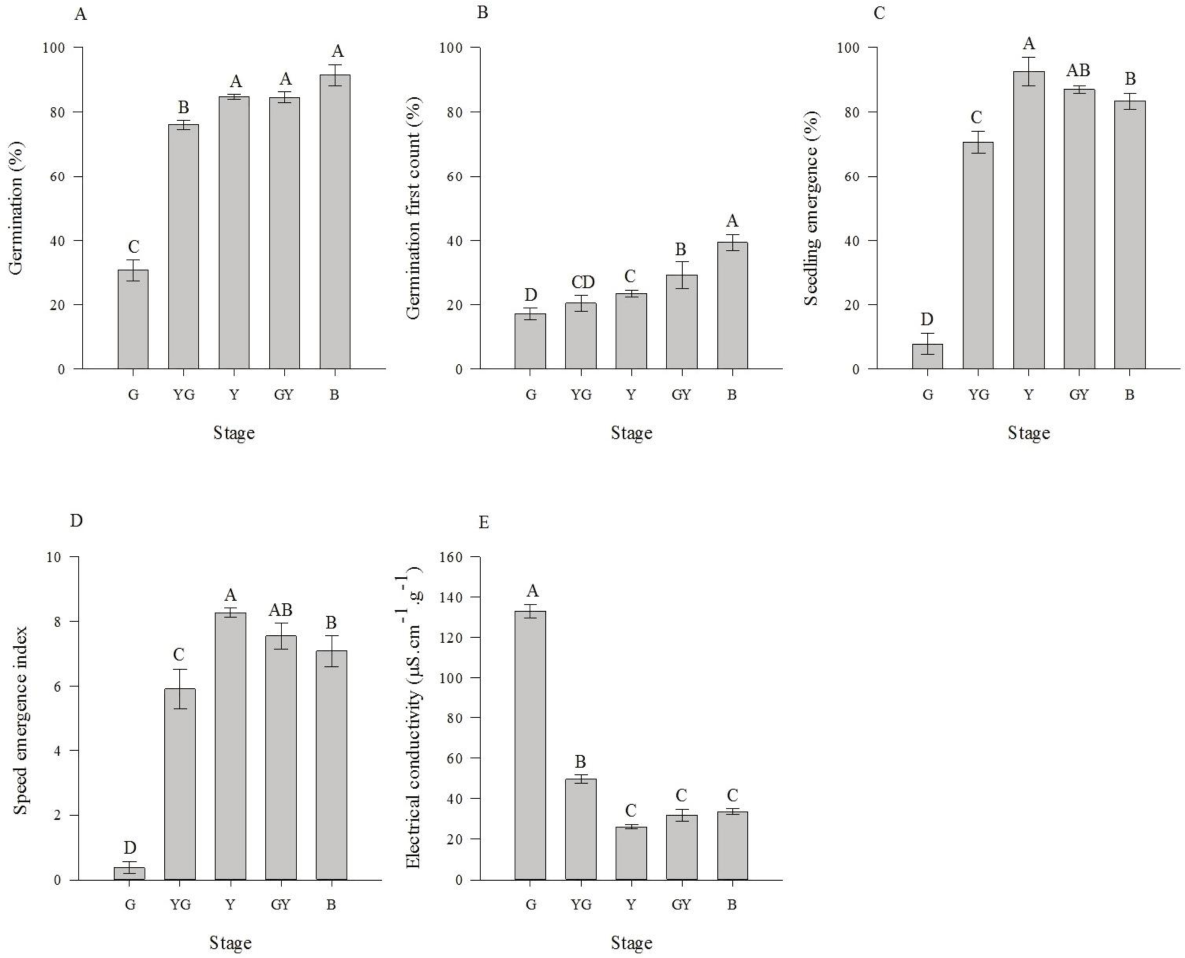
The results of percentage of seedling emergence and emergence speed index (Figures 5C and 5D, respectively) were similar, with lower values for seeds obtained from green pods, followed by those from yellow-green pods, and both were significantly lower to those from the other maturity stages. In general, the highest values were obtained for seeds from yellow pods, which were significantly higher than those from other fruit stages; these values did not differ only from seeds from golden-yellow pods. In general, in numerical terms, seedling emergence was 93% (yellow pods), 87% (golden-yellow pods), and 83% (brown pods).

The electrical conductivity test evaluates the amount of leachates present in the seed imbibition solution and is related to the integrity of the cell membranes (VIDIGAL *et al*., 2011). High vigor seeds have rapid reorganization of membranes during imbibition and, consequently, lower ions leachate and lower electrical conductivity readings. Higher values of electrical conductivity, indicating lower vigor, were observed in seeds from the green stage, decreasing for seeds from the yellow-green stage, which had higher values than those from the yellow stage (Figure 5E). From that stage on, there was stabilization of conductivity, with no significant difference in vigor of seeds from the yellow, golden-yellow, and brown stages (Figure 5E).

These results indicate that the cell membrane system of seeds from the initial green and yellow-green pod maturity stages did not have ideal organization. As maturation proceeds, membrane repair occurs, which can be perceived by reduction in leaching of solutes by seeds from the yellow, golden-yellow, and brown pod stages (Figure 5E). Some studies report reduction in electrical conductivity, indicating an increase in seed vigor during the maturation process (GONÇALVES *et al*., 2018; SILVA *et al*., 2019; VIDIGAL *et al*., 2011).

The final phase of the orthodox seed development process is characterized by seed dehydration, as can be seen by the results obtained for water content (Figure 4A). Before that phase, there is intense accumulation of reserves, until reaching a maximum value that characterizes seed physiological maturity (BEWLEY *et al.*, 2013). During this phase of reserve deposition, there is accumulation of potentially protective molecules, especially late

Figure 5 - Germination percentage (A), germination first count (B), seedling emergence (C), emergence speed index (D), and electrical conductivity (E) of chickpea seeds obtained from pods in different maturation stages. Mean values with the same letters do not differ by the Tukey test ($p \leq 0.05$) and compare the maturation stages. Green (G), yellow-green (YG), yellow (Y), golden-yellow (GY) and brown (B)



embryogenesis abundant (LEA) proteins and soluble sugars, such as sucrose, raffinose, and stachyose that act to prevent damage caused to membranes through removal of water from cells (BEWLEY *et al*., 2013). Possibly the seeds obtained from green and yellow-green pods were in the reserve deposition phase and still in preparation for the desiccation stage. Therefore, the greater amount of soluble solids and of water, and the lack of repair mechanisms and of reserves contributed to greater electrical conductivity and, indirectly, to lower vigor of seeds from the green and yellow-green stages.

Lower values of electrical conductivity were found by Samarah and Abu-Yahya (2008) in chickpea seeds obtained from pods in the yellow and brown stages, i.e., greater vigor, in relation to seeds from the green and yellow-green stages. These authors emphasize that low values of electrical conductivity may be related with later stages of harvested, when the seeds have greater dry weight compared with earlier harvested seed. Furthermore, they observed that seeds from brown pods also had higher germination and seedling dry weight.

In summary, greater germination and vigor generally occurred in seeds from the yellow, golden-yellow, and brown pod stages (Figure 5). In these stages, there were significant increases in seed dry matter, indicating that physiological maturity was reached (Figure 4A). Furthermore, these results show that seeds collected before physiological maturity, as observed in seeds obtained from green and yellow-green pods, generally had worse performance, as also reported by Samarah and Abu-Yahya (2008) in chickpea and by Lima Cruz *et al*. (2019) in cowpea.

In order to obtain high quality seeds, it is important to establish parameters (such as morphological characteristics of the plant and of the fruits/seeds and days after anthesis) which make it possible to characterize the physiological maturity of the seeds, and also to determine the stage of maturation at which the seed quality is maximum (VIDIGAL *et al*., 2011). As chickpea seed harvest is mechanized, the present study can offer assistance in establishing criteria based on outside pod color to define the ideal point for harvest, and to obtain seeds with high germination and vigor.

CONCLUSIONS

1. Chickpea seeds obtained from pods of green color are in the cell expansion stage and, despite reduced dry matter, starch and proteins were found in the cells of the seed coat and cotyledons. These reserve substances become more evident in the cells of the reserve parenchyma of the cotyledons as of yellow-green stage, stabilizing at the yellow, golden-yellow, and brown pod stages. As the chickpea seeds develop, there is an accumulation of pectic substances in the macrosclereids present in the outermost region of the seed coat;
2. Maximum seed size and dry weight were attained in the pods of yellow color. In general, seeds obtained from pods with yellow, golden-yellow, and brown outside color have greater germination and vigor.

ACKNOWLEDGEMENT

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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