Physiological maturity of eggplant seeds[1]

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ABSTRACT – Determination of seed physiological maturity and ideal moment for harvesting fruits to extract their seeds are important aspects to produce seeds with high quality. To identify the best period for harvesting eggplant fruits, associated with ideal resting period of the fruit for extracting seeds, an eggplant production field was installed in municipality of Ijaci, in the State of Minas Gerais, Southwestern Brazil. The fruits were harvested at periods of 49, 56, 63, 70, and 77 days after pollination (DAP). The seeds of fruits harvested in each period were manually extracted immediately after harvest or after a post-harvest resting period of seven days, under a shed. The physiological quality of seeds was assessed by tests of: germination percentage; germination and emergence speed indexes; and electrical conductivity; which were carried out in the Central Seed Laboratory, Federal University of Lavras. Electrophoretic analyses of isoenzymes: catalase (CAT); esterase (EST); superoxide dismutase (SOD); and peroxidase (PO), were also therein performed. Results of germination and vigor of seeds have showed that the best period for harvesting the fruit is around 70 DAP; and that seeds should be extracted immediately after harvest. Electrophoretic analysis of enzymes has showed immaturity for eggplant seeds, harvested after 49 DAP.

Index terms: *Solanum melongena,* harvest, germination, resting period.

Maturidade fisiológica de sementes de berinjela

RESUMO - A determinação da maturidade fisiológica das sementes e do momento ideal de colheita dos frutos para extraí-las são aspectos importantes para a produção de sementes com alta qualidade. Para identificar a melhor época de colheita dos frutos de berinjela e o tempo ideal de descanso do fruto para extração das sementes, foi instalado um campo de produção, no município de Ijaci, MG. Os frutos foram colhidos aos 49, 56, 63, 70 e 77 dias após a polinização (DAP). As sementes dos frutos colhidos, em cada época, foram extraídas manualmente, imediatamente após a colheita, ou então após repouso pós-colheita, por sete dias, em galpão. A qualidade das sementes foi avaliada por meio dos testes de: germinação; emergência; índices de velocidade de germinação e emergência; e condutividade elétrica; e também a análise eletroforética das isoenzimas catalase (CAT), esterase (EST), superóxido dismutase (SOD) e peroxidase (PO) foram conduzidas no Laboratório Central de Sementes, Universidade Federal de Lavras. Os resultados de germinação e vigor das sementes revelaram que a melhor época para a colheita dos frutos é de, aproximadamente, 70 DAP; e que as sementes devem ser extraídas imediatamente após a colheita. A análise eletroforética das enzimas evidenciou imaturidade das sementes aos 49 DAP.

Termos para indexação: *Solanum melongena*, colheita, germinação, período de repouso.

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Introduction

The species *Solanum melangena* L. is native from warm regions of India, Myanmar (former name of Burma), and China. The eggplant crop was introduced into the Iberian Peninsula by the Arabians, during the Middle Ages, and was afterwards disseminated to other European countries. In Brazil, the crop was introduced by the Portuguese settlers, in the Sixteenth Century. The crop is more intensely cultivated in the states of São Paulo, Rio de Janeiro, and Paraná, and its consumption is larger in the former state cited (Filgueira, 2003). It is a horticultural product, which has been widely used in the market, due to the increasing interest of population in consuming vegetal origin products as well as by its medicinal use.

For some plant species, the seed physiological maximum quality is reached simultaneously with the moment in what the seed has its maximum dry mass; while for other species such event may occur before or after that moment. Once the maximum quality is reached, the deterioration process is initiated leading to a progressive reduction in the physiological quality of the seeds; since that from the physiological maturity, the seed is practically disconnected from mother-plant, and thus no more receives photo-assimilated compounds (Demir and Ellis, 1992). Such process can be a threat to physiological quality of seeds, once these may be exposed to weathering, besides pest attack and infection by microorganisms (Carvalho and Nakagawa, 2000).

Therefore, an important aspect of seed production is the correct determination of physiological maturity and the ideal harvest moment, in order to reduce the losses caused by the previously mentioned factors. This manner, maturation process and its relations with recommendable period for harvest should be perfectly known (Marcos-Filho, 2005).

Some studies have demonstrated the positive effect of the post-harvest storage of eggplant fruits on the improvement of quality of their seeds. Barbedo et al. (1994) have found that better quality of eggplant seeds were obtained from fruits harvested at 50 days after anthesis (DAA) and subjected to 15 days post-harvest storage; the same, however, has not occurred when the resting period was applied to fruits harvested in 70 DAA.

The use of seeds with high levels of germination and vigor leads to a higher probability of success in the horticultural production; and this fact is of great importance due to high cost of seeds of the new cultivars available in the market (Nascimento, 2005). There are many constraints in relation to unevenness of maturation of fruits among plants of the same species and the methodologies for extracting their seeds have represented large barriers to the advances of scientific research (Miranda et al., 1992).

Studies on the right point of physiological maturation of seeds and the determination of the best harvest time, as well as their practical implications on seed production, are very important. Therefore, there is still the need in developing technologies for seed production of horticultural plants, once the information concerning changes on the activity of enzymes during their maturation are still scarce.

Variations on profile of enzymes, mainly in those processes related to respiration, lipids peroxidation, and removal of free radicals, may be efficient tools in monitoring biochemical changes resulting from the deterioration that occurs in seeds (Chauhan et al., 1985). Seed deterioration is a process regulated by a series of changes of physiological, physical, and cytological that is initiated starting from the physiological maturity; what will imply in drop of their quality (Marcos-Filho, 2005).

Within this context, this study has aimed at identifying the best harvest period for eggplant fruits and the adequate storage time of the fruit for extraction of its seeds.

Material and Methods

The tests for assessing physiological quality of seeds were carried out in the Central Laboratory for Seed Analyses, Federal University of Lavras, municipality of Lavras, State of Minas Gerais, Southeastern Brazil.

Seeds of two breeding lines of eggplant, for production of hybrid GÊNOVA, provided by the company HortiAgro Sementes Ltda (HortiAgro seeds Ltd.) headquartered in the municipality of Ijaci, State of Minas Gerais, were used in the experiments. This municipality is located in southern Minas Gerais (21º10’ S latitude; 44º55’ W longitude; and altitude of circa 832 m).

The sowing for obtaining seedlings was performed into a seed germinator, in the laboratory, and then transplanted to their definitive place, under greenhouse conditions. The seedlings were planted with 1.20 m spacing between the rows and 1.0 m spacing between plants within the row, in a completely randomized experimental design. In conducting the crop, the technical recommendations of Filgueira (2005) were followed. It has been necessary to provide individual support with a stake to each plant; once these were not able to withstand the weight of fruits.

The production of eggplants hybrid seeds was performed with the emasculation of the floral buds of one of the breeding line, followed by manual pollination of the emasculated floral bud; what was performed through introduction of its stigma into a small vial containing pollen collected from the other parental. The flower buds, which had already been pollinated, were then identified with dated strips or tags, to control the days after pollination, since the procedure was not carried out on the same day in all plants previously selected.

The fruits were harvested with 49, 56, 63, 79, and 77 days after pollination (DAP) aiming at characterizing the point of physiological maturity of seeds and 12 fruits were randomly harvested at each programmed date. Fruits collected in each harvest date were submitted to two post-harvest storage periods: zero (before storage): and seven days, in a shed, under natural environmental conditions. Seed were extracted immediately after harvest (0 day) or after a resting period of seven days; and afterwards rinsed under tap water. After washing, the seeds were dried in the shade, under natural environment, during a period of two days to attain the adequate moisture content for storage (circa 8%).

The seeds were then subjected to the following tests:

Germination: was performed with four replications of 50 seeds each, for each treatment. Seed of each replication were evenly distributed onto two sheets of Germitest® paper, covered with another sheet of the same paper, all of which had been moistened with a 0.2% potassium nitrate (KNO_3) solution, in a volume equivalent to 2.5 times the mass o dry substrate, and the set (paper + seeds) were turned into rolls. These rolls were then placed into a seed germinator, under alternate temperatures of 20 ºC and 30 ºC, and photoperiod of 8/16 h (light/dark), respectively. Additional lighting was supplied during the 8 h period, when temperature was 30 ºC. First count of germination test was performed at the seventh day after starting the test and final assessments of emergence were performed at the fourteenth day after sowing, by computing the mean percentage of normal seedling emerged, according to Rules for Seed Testing (Brasil, 2009).

Percentage of germination and germination speed index: four replications of 50 seeds each, for each treatment were used in this test. For this, seeds of each treatment and replication were sown into plastic trays containing the commercial Plantmax® substrate. After the sowing, which was performed, the trays were maintained into a growth chamber, at 25 ºC. The emergence speed index was assessed by daily counting the number of normal seedlings emerged, according to Maguire (1962). The total number of normal seedlings emerged was also determined by counting the number of normal seedlings until stabilization of germination, at the fourteenth day.

Electrical conductivity: for this test, the mass conductivity method was used and performed according to the methodology proposed by the Vigor Committee of the International Seed Testing Association (ISTA, 1995). Four subsamples of 50 seeds each, with known mass, were immersed into 50 mL of distilled water, into plastic cups, and then maintained under constant temperature of 25 ºC, during 24 h. After such period, the electrical conductivity was determinated, with the aid of a conductivimeter, and results were expressed in $\mu S.cm^{-1}.g^{-1}$ of seeds.

Biochemical analyses: the seeds were stored into deep-freezer at - 84 ºC, until the moment of be macerated into a porcelain mortar containing liquid nitrogen and Polyvinylpyrrolidone (PVP) (antioxidant).

Electrophoretic analyses of isoenzymes: two samples, of 100 mg each, of the macerated material, were used for each treatment, to which were then added extraction buffer (Tris HCL 0.2 M, pH 8), in a volume equivalent to 2.5 times the mass of each sample, and 0.1% β-Mercaptoethanol (BME). The material was placed overnight into a refrigerator and afterwards centrifuged at 14,000 rpm, at 4 ºC, during 30 min. Of the liquid supernatant, 50 µL were applied to polyacrylamide gel and the electrophoresis was run during 4 h, at 150 V. The gels were then stained for the enzymes: esterase (EST); catalase (CAT); peroxidase (PER); and superoxide dismutase (SOD), according to (Alfenas, 1998).

The assessment of results of electrophoresis gels was performed, by considering the presence or lack of bands of similar molecular weight and the number and intensity of bands for each treatment.

A completely randomized experimental design was used in the experiment, with the treatments arranged in a 2 x 5 factorial scheme {2 periods for seed extraction (with and without post-harvest resting period) x 5 periods for fruit harvesting (49, 56, 63, 70, and 77 days after pollination)}. For the tests of: germination; electrical conductivity; and emergence, data were subjected to ANOVA, and the means were compared by F test, at 5% probability. For comparing the factors: resting period of fruits after harvest and harvesting periods, the regression analysis was performed.

Results and Discussion

After ANOVA, data have shown that there have not been statistically significant differences among values of: percentage of germination; germination speed index; emergence speed index; percentage of emergence; and electrical conductivity, when seeds were extracted soon after harvest or after a resting period of seven days for the fruits in a shed, under natural environmental conditions; thus indicating that quality of seeds does not depend on resting period for extraction of seeds (Figures 1 to 5).

In studies performed with castor bean (*Ricinus communis* L.) seeds, Silva et al., (2009) have observed that for obtaining higher germination percentage, the seeds should be harvested in 86 DAA, and that the post-harvest resting did not influence that parameter. In contrast, Martins et al. (2006), in a study carried out with papaya (*Carica papaya* L.) seeds, to assess the influence of post-harvest storage of fruits on changes of the physiological quality of seeds, have observed that the resting period of fruits for 10 days, at 25 ºC, significantly increased germination and vigor of seeds, for both genotypes studied.

In Figure 1, the results of germination speed index are presented. Through the graph it is possible to observe that for seeds harvested in the interval between 63 and 70 DAP; the germination was faster than for the seed harvested in the remaining periods. Marrocos et al. (2011) have observed that seeds of Summer squash (*Cucurbita pepo* L.) have attained physiological maturity in a period between 50 to 60 DAA, what correspond to the points of maximum germination and vigor of its seeds.

In Figure 2, it is shown that percentage of seeds harvested in 63 and 70 DAP, germinated in higher percentage. Seeds harvested in 49 DAP; have already presented germination capacity, although in low percentage. Oliveira et al. (1999), have obtained similar results, in a study with seeds of pepper (*Capsicum annuum* L.), and have found high values for germination and vigor of seeds extracted from older fruits, harvested starting from 55 DAA, and that the best seed quality was found for fruits harvested between 60 and 70 DAA; Vidigal et al. (2006), in studies with seeds of tomato (*Lycopersicon esculentum* Mill. syn. *Solanum esculentum* L.) have observed that high quality seeds are obtained from newly-harvested fruits with 50 to 60 DAA; and Miranda et al. (1992), studying eggplant seeds have found that the seeds of fruits harvested in 50, 60, and 70 DAA, in the lower third of the plant, did not present difference in the physiological quality.

Through results achieved in the vigor tests, which are presented in Figures 3 and 4, it was possible to verify the best results when the seeds were extracted from fruits harvested in 63 and 70 DAP. Such results were similar to the results observed in the germination test.

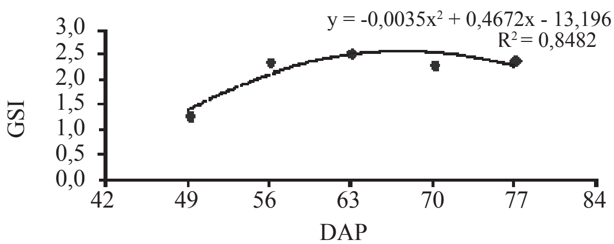

Figure 1. Germination speed index (GSI) of *Solanum melongena* seeds, in function of harvest period.

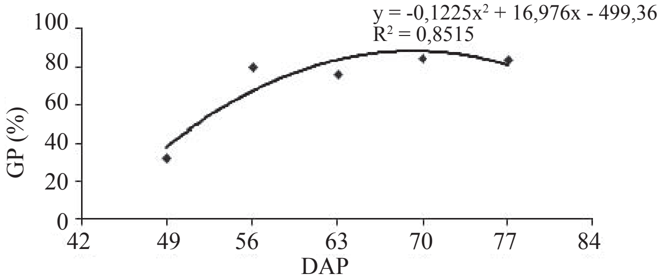

Figure 2. Germination percentage (GP) of *Solanum melongena* seeds, in function of harvest period.

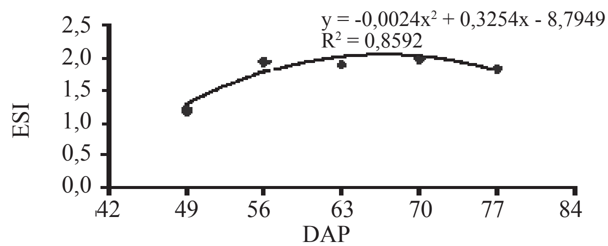

Figure 3. Emergence speed index (ESI) of *Solanum melongena* seeds, in function of harvest period.

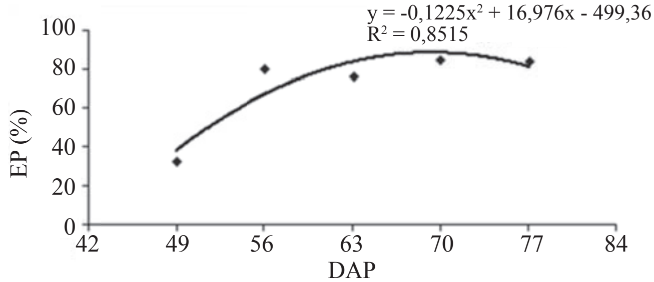

Figure 4. Emergence percentage (EP) of *Solanum melongena* seeds, in function of harvest period.

In Figure 5 it can be observed high values for electrical conductivity of seeds harvested at 49 DAP, due to destruction of their membrane systems, probably because the seeds were still immature. Therefore, as in the remaining tests of vigor, there has been lower electrical conductivity, thus indicating higher vigor of seeds extracted from fruits harvested between 63 and 70 DAP. Similar results were found by Vidigal et al. (2009), who have found that pepper seeds obtained at 40 DAA are still immature; while seeds extracted from fruits harvested between 60 to 70 DAA, have their membranes well structured, therefore not affecting results of electrical conductivity test.

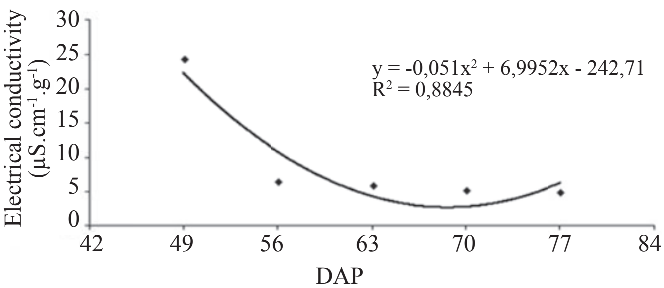

Figure 5. Electrical conductivity ($\mu S.cm^{-1}.g^{-1}$) of *Solanum melongena* seeds, in function of harvest period.

The enzymes catalase (CAT) and superoxide dismutase (SOD) constitute efficient mechanisms of detoxification, acting on removal of free radicals (McDonald, 1999). By analyzing the activity of enzyme CAT (Figure 6), it is can be observed that there were not differences statistically significant between the intensity and size of electrophoretic bands of seeds with and without rest time for the fruits, independently of harvest period. However, the progressive reduction on the efficiency of the enzyme CAT, as the harvest period increases, can be indeed observed. It seems that a progressive reduction on the efficiency of the enzyme CAT occurs; and therefore prevention of damages caused by oxidation, mainly after 70 DAP, also happens. Such observation corroborates results of physiological quality assessments, where reduction on seed quality is verified when the harvest is performed at later periods.

For the enzyme SOD (Figure 7) it is perceivable that higher activity occurred in seed extracted from fruits harvested at 49 DAP (with and without rest); and that starting from 56 DAP a constant activity of this enzyme occurred, with a discrete increase for the seeds extracted from fruits harvested at 70 DAP, and without resting period. A more intense activity of enzyme SOD, during early or late phases of maturation process of seeds is an indicator that its defense action in reducing peroxide, and the consequente reduction of free radicals formation, have been most required on stages in which the seeds were not completely formed (49 DAP), i.e., already initiating the process of deterioration (from 70 DAP on). Such fact was also verified on behavior of the enzyme CAT.

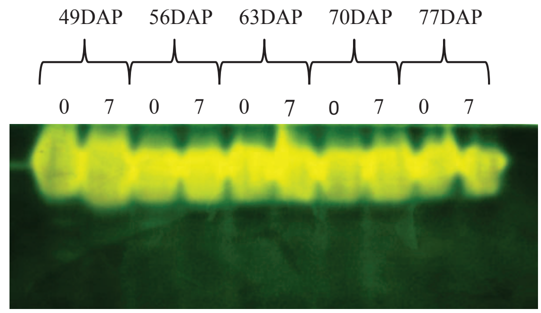

Figure 6. Electrophoretic profiles of the catalase enzyme (CAT) in *Solanum melongena* seeds, extracted from fruits harvested at 49, 56, 63, 70, and 77 days after pollination (DAP) and stored during zero and seven days.

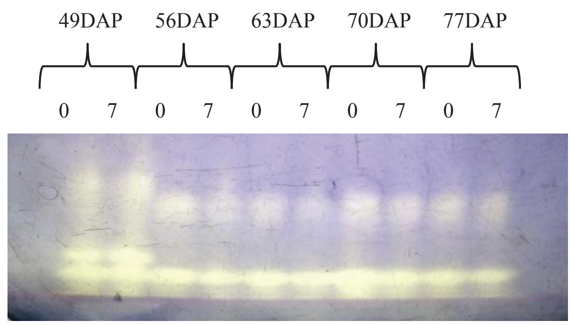

Figure 7. Electrophoretic profiles of the enzyme superoxide dismutase (SOD) in *Solanum melongena* seeds, extracted from fruits harvested at 49, 56, 63, 70, and 77 days after pollination (DAP) and stored during zero and seven days.

In studies with pepper seeds, Vidigal et al. (2009) have found that there has been a small increase on intensity of SOD enzyme bands in the seeds extracted from fruits harvested starting from 50 DAA, and afterwards stored during six days. The SOD is an enzyme that acts against reactive forms of oxygen, prevents the action of the radical superoxide (O2-), and catalyzes reactions of electron transferences to produce hydrogen peroxide (H2O2) (McDonald, 1999).

The enzyme EST (Figure 8) is associated to reactions of the hydrolysis of esters and is directly linked to metabolism of lipids and to degenerative processes of cell membranes (Santos et al., 2004). Increased activity of enzyme EST has been verified in seeds extracted from fruits harvested in 49 DAP (with and without resting period for the fruits); and starting from 56 DAP (without rest period) was perceived reduction on the activity of this enzyme, which returns on presenting high activity around 70 DAP (with and without resting period for the fruits), and again reducing its activity in seeds extracted from fruits harvested at 77 DAP (with and without rest). The changes verified in the patterns of the enzyme EST are evidences of occurrence of degenerative processes. Therefore, results achieved in this study can be validated by the observations verified in this enzymatic system. It is possible that the membrane protection system of cells is less efficient in the early stages, when these membranes are not still well constituted; or on more advanced stages of development, i.e., when the processes of deterioration of membranes initiates.

The enzyme PO (Figure 9) uses the hydrogen peroxide to oxidize a large variety of hydrogen compounds donors, such as: groups with aromatic rings; diamines; ascorbic acid; amino acids; and some inorganic ions (Nkang, 1996). For the enzyme PO, it was observed higher activity in seeds extracted from fruits at younger maturation stages. Its activity keeps on diminishing with the advance of maturation stages; except for seed extracted from fruits harvested in 70 DAP (with storage), when the enzyme PO increases its activity and then decreases again. The activity of this enzyme varies with type of tissue and developmental stage of plant; and is inversely proportional to the its growth.

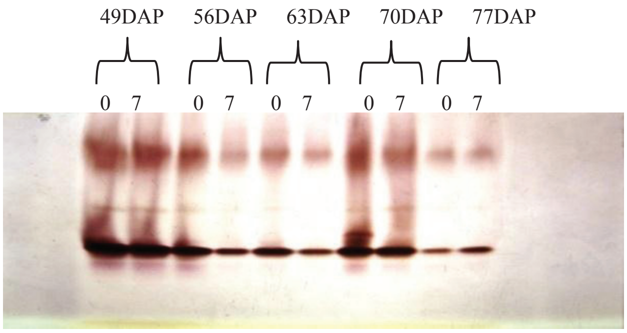

Figure 8. Electrophoretic profiles of the esterase enzyme (EST) in *Solanum melongena* seeds, extracted from fruits harvested at 49, 56, 63, 70, and 77 days after pollination (DAP) and stored during zero and seven days.

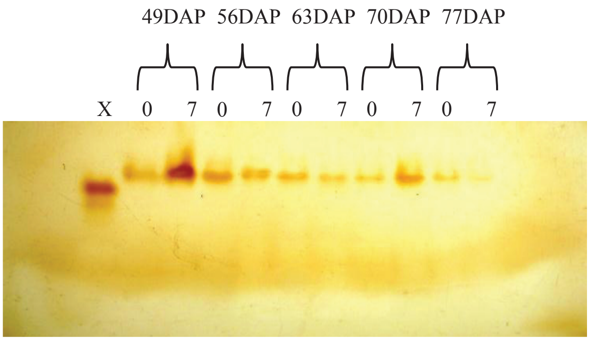

Figure 9. Electrophoretic profiles of the peroxidase enzyme (PO) in *Solanum melongena* seeds, extracted from fruits harvested at 49, 56, 63, 70, and 77 days after pollination (DAP) and stored during zero and seven days. The letter x represents the corn standard sample.

At the 70 DAP; it seems that a specific stress occurs, which may be reduced with the increase of intensity of bands of the enzymes EST and PO. From the data on physiology, it is noticeable that at this point of development of the seeds, occurs the maximum physiological quality. The natural disconnection of seeds or fruits from the mother-plant may be the event that triggers the increase of oxidation, which is compensated by the increase on the activity of enzyme PO; in addition to the increase on activity of enzyme EST in hydrolysis of lipids. The hydrogen peroxide, formed as a sub-product of enzyme SOD activity, although less reactive, becomes toxic in high concentrations, once it may react forming hydroxyls, which cause peroxidation of lipids (Bowler et al., 1992).

By the results obtained it is possible to observe that quality of seeds increases with evolution of development and that 70 DAP is the initial point of deterioration process. Although some enzymatic systems, that are typical of deterioration process, start to be perceived exactly in that same point; once is in it that occur the highest viability as well as the highest vigor of seeds; what may be confirmed by the physiological quality assessments.

Conclusions

The ideal point for harvesting eggplant fruits for extraction of seed with high physiological quality occurs at 70 days after pollination.

The post-harvest storage period of the fruits, before the extraction of seeds does not alter physiological quality of eggplant seeds.

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