International Journal of Agricultural Technology 2023 Vol. 19(6):2465-2476
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Seed Physio-bichemical quality of chilli (*Capsicum frutescens*) in different seed maturity

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Hairani, P. M., Sudjatmiko, S., Setyowati, N., Diaguna, R. and Triyostin. W. (2023). Effect of fruit color on the physiological and biochemical quality of chili seeds (*Capsicum frutescens*). International Journal of Agricultural Technology 19(6):2465-2476.

Abstract Result showed that mature red chili peppers exhibited the greatest seed vigor. Specifically, these peppers demonstrated a seed vigor index percentage of 62.67% and a seed growth rate of 40.91% etmal^{-1}. There was a positive correlation coefficient observed between the seed vigor parameter of mature red chili peppers and the parameters of fruit chlorophyll and carotenoid content. In contrast, it was shown that the chlorophyll content of the fruit had a negative association with both the germination rate and seed vigor index in fruits of both second mature red and first immature red fruits. The fruit's carotenoid concentration shown negative associations with the seed vigor index in both second mature red and first immature red fruits, as well as negative associations with the germination rate of first immature red fruits.

Keywords: Fruit ripening, Seed quality, Seed viability, Seed vigor

Introduction

Chili peppers (*Capsicum frutescens*) are widely cultivated and utilized spices across the world. The chili pepper is a widely used spice that holds considerable importance due to its culinary appeal and nutritional benefits (Azlan *et al.,* 2022). The production, added value, and competitiveness of Indonesian chili face many obstacles in terms of quantity, quality, and continuity (Saptana *et al.*, 2022). Seeds play a crucial role as a primary constituent and valuable resource for cultivating superior crops, especially in the context of vegetable cultivation (Yildirim *et al.*, 2021).

According to Yildirim *et al.* (2021), chili plants exhibit a continuous blooming pattern, resulting in the potential for seed production at different stages of fruit maturation. The identification of an optimal harvest timing for seeds with high moisture content during their developmental stage is of utmost importance in ensuring the production of seeds with superior quality (Kaiser *et al.*, 2016). The moisture retention of Pepper (*Capsicum annuum*

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L.) seeds during maturity has been observed (Demir and Ellis, 1992). It has been found that a high germination percentage cannot be solely attributed to continued flowering on the mother plant (Yildirim *et al*., 2021). The seed quality of pepper seeds is influenced by the optimal timing of fruit maturity, leading to implications for high-quality pepper transplant production (Demir and Samit, 2001; Nogueira *et al*., 2017; Vidigal *et al*., 2011; Yildirim *et al*., 2021).

According to Kaiser *et al.* (2016), ripening is the result of a sequence of morphological, biochemical, and physiological changes that take place from fertilization to seed independence from the parent plant and are directly influenced by genetic and environmental factors. The synthesis and accumulation of reserves serve as the primary distinguishing features of the modifications, which cover a sequence of early stages in the germination process (Marcos Filho, 2015). Forest seed harvesting often relies on many physical factors to determine the ripeness of fruits and seeds. These criteria include changes in color, size, and odor, as well as the presence of predators, dispersers, and fruit dehiscence (Piña-Rodrigues and Aguiar, 1993).

Changes in fruit color have been the most widely used indicator of these traits, as many fruit species alter their color as they ripen (Dranski *et al*., 2010; Herzog *et al*., 2012; Lopes *et al*., 2014; Oro, 2012; Srimathi, 2013). This study sought to determine the physio-biochemical quality of chili (*Capsicum frutescens*) seeds at various stages of maturation.

Materials and methods

The experiment was carried out at the Research Group on Crop Improvement Laboratory, Post-Harvest Laboratory, and Plant Reproductive Biology Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University.

Planting materials

The chili pepper (*Capsicum frutescens*) fruit and seeds at three different stages of ripeness are used as shown in Figure 1.

Top Paper method for viability and vigor test

The experiment employed a completely randomized design with two factors. The first factor was fruit ripeness, namely, mature red (MR), second-immature red (SIR), and first-immature red (FIR) (Figure 1), and the second was soaked (without soaking and with soaking in KNO_3). Each treatment was replicated three times, resulting in 18 experimental units.

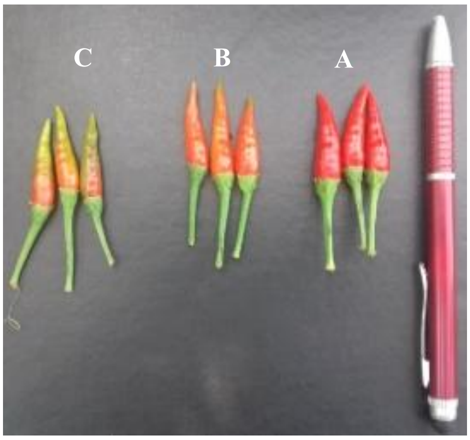

Figure 1. Chili pepper with three levels of maturity based on color, i.e. A) Mature red (MR); B) Second-immature red (SIR); C) First-immature red (FIR)

First, the seeds of the fruit are obtained the day after harvesting. Manual extraction requires slicing chilies, removing the seeds with forceps, and then detaching the seeds from the funiculus. The seeds are then placed in a disc-shaped container and air-dried for three days in a room with air conditioning. The dried seeds are sealed in airtight plastic and preserved in a plastic jar for one week in a room with air conditioning to prevent limited germination (McCormack, 2005). The seeds are then refrigerated at a constant temperature of approximately 40°C. The stored seeds are examined to determine their water content, seed viability, vigor index, and seed growth rate.

The seed quality assessment employed in this study was tested on paper (ISTA, 2015). Prior to conducting the assessment, a preliminary procedure was performed whereby the seeds were immersed in a 0.2% KNO_3 solution for a duration of 24 hours. This treatment was employed to disrupt the dormancy of the seeds. In order to compare treatments, it was ensured that seeds subjected to the same test procedure were not subjected to KNO_3 soaking.

To assess the seed viability and vigor index, a total of 900 seeds were used which divided into three replications of 50 seeds each at different maturity levels. In terms of measuring seed growth rate, a total of 450 seeds were used, divided into three sets of 25 seeds each, in order to ensure accurate and reliable results.

The seeds are sown in a moist medium. The media consisted of three layers of modified straw paper in the form of a transparent box. The planted seeds are then securely covered and kept at room temperature for 14 days. On days 7 and 14, seed viability and vigor index were measured. Observations of growth rate were conducted from day 5 to day 14 in the meantime. The media's relative humidity was constantly maintained until the observation procedure is complete.

The collected data was analyzed for variance at the 5% level, and if it showed that there was a treatment effect, it was further examined using Duncan's Multiple Range Test at the 5%. Regression and correlation analyses were performed to examine the relationship between carotenoids and chlorophyll, as well as other indicators of seed physiological development.

The physiological quality of seeds was studied, including seed viability, seed vigor index, seed growth rate, seed water content, and dry weight of seeds and fruit.

Analysis of chlorophyll and carotenoid content

The experiment was conducted using a one-factor arranged in completely randomized design. The first factor was level of seed maturation (mature red (MR), second immature red (SIR), and first-immature red (FIR), Each treatment was replicated three times to generate 18 experimental units.

Total chlorophyll, anthocyanins, and carotenoid analysis of seeds and flesh of fruits

The prepared 0.3 g of chili seeds are crushed, put in a mortar, and then 1 ml of acetone is added. Instead of crushing the seeds, the maceration method cuts them into two pieces, adds one 1mL of acetone, and repeats the process. After the seeds were chopped and ground, 2 mL of acetone are combined and thoroughly blended. After the liquid was placed in a tube, the sample was shaken, filtered, and centrifuged for ten minutes at 1000 rpm. The supernatant was placed in a reaction tube. After adding 2 mL of acetone in total, a marble-sized ball was placed on top. The supernatant was diluted eight times before being measured using a spectrometer. To determine the absorption values of chlorophyll a and b, the supernatant was measured at wavelengths of λ 470 nm, 537 nm, 647 nm, and 663 nm using a UV 1201 spectrometer.

Total chlorophyll and carotenoid are calculated using the following formula:

Total chlorophyll = 7.15 x A 663 – 18.71x A 647, Chlorophyll a = 0.01375 x A663-0.000897 x A537-0.003046 x A647, Chlorophyll b = 0.02405 x A647-0.004305 x A537-0.005507 x A663, Anthocyanin = 0.08173 x A537-0.00697 x A647-0.002228 x A663 and Carotenoid (µmol/ml) = [A470-{17.1x(Chlorophyll a + Chlorophyll b)-9.475 x Anthocyanin] 119.26.

Notes: A 470 = Wavelength absorbance 470 nm, A 537 = Wavelength absorbance 537 nm, A 663 = Wavelength absorbance 663 nm and A 647 = Wavelength absorbance 647 nm.

Results

The findings indicated that there was no interaction effect between level of fruit ripeness and soaking treatment on all variables measured. However, fruit ripeness had an effect on both the vigor index ($p<0,0001$) and seed growth rate ($p<0,0001$). In addition, the seed vigor index was also affected by the soaking treatment ($p<0,025$).

The ability of seeds to germinate is the ability of seeds to grow normally under optimal environmental conditions. One of the factors used to determine seed viability is germination capability. Result displayed the average germination capacity value of chili seeds, which illustrated that the percentage of germination capacity value of chili seeds increased with maturity level (Table 1). The percentage of germination capacity in the seed soaking treatment with KNO_3 was not substantially different from the percentage of germination capacity in the unsoaked seeds.

Table 1. Average germination capacity (%) of chili pepper seeds with different levels of maturity and soaking

Level of maturity	Soaking		
	without	Soaking with KNO_3	
MR	88,00 ± 5,03	90,00 ± 1,15	89,00 ± 2,35
SIR	89,33 ± 1,33	92,67 ± 3,71	91,00 ± 1,91
FIR	70,00 ± 8,08	89,33 ± 7,69	79,67 ± 6,60
	82,44 ± 4,17	90,67 ± 2,54	

Note: MR=mature red; SIR=second immature red; FIR=first-immature red.

The percentages of vigor index and seed growth rate were used to describes the seed vigor. The ability of seeds to grow normally in substandard conditions is referred to as seed vigor. It revealed that chili seeds from mature red fruit were the highest vigor index (62.67%), whereas chili seeds from first-immature red fruit was the lowest index vigor percentage (15%). Seed soaking with KNO_3 improved the index vigor percentage to 36.22% when compared to no soaking (Table 2).

Table 2. Average index vigor (%) of chili pepper seeds with different levels of maturity and soaking

Level of maturity	Soaking		
	without	Soaking with KNO_3	
MR	58,67 ± 0,67	66,67 ± 1,76	62,67 ± 1,98 a
SIR	22,00 ± 4,00	25,33 ± 1,76	23,67 ± 2,09 b
FIR	13,33 ± 1,76	16,67 ± 2,67	15,00 ± 1,61 c
	31,33 ± 7,06 b	36,22 ± 7,78 a	

Note: MR=mature red; SIR=second immature red; FIR=first-immature red.

Seed growth rate is calculated by measuring the increase in the percentage of normal seedlings every 24 hours which is one of the parameters of seed vigor. The results revealed that mature red fruit has the maximum seed growth rate, at 40.91% $etmal^{-1}$ (Table 3). Low seed growth rate (28.91–29.03% $etmal^{-1}$) is produced by first-immature red fruits (28.91–29.03% $etmal^{-1}$).

Table 3. Average seed growth rate (% $etmal^{-1}$) of chili pepper seeds with different levels of maturity and soaking

Level of maturity	Soaking		
	without	Soaking with KNO_3	
MR	40,46 ± 0,79	41,36 ± 2,56	40,91 ± 1,21 a
SIR	27,03 ± 1,10	31,02 ± 1,97	29,03 ± 1,35 b
FIR	28,64 ± 2,39	29,18 ± 2,40	28,91 ± 1,52 b
	32,04 ± 2,26	33,85 ± 2,22	

The average values for the dry weight of seeds (g), dry weight of fruit (g), and weight of 1000 seeds (g) were consistent across all treatments. Nevertheless, it is notable that seeds derived from orange-green fruit was substantially elevated water content reaching 14% (Figure 2).

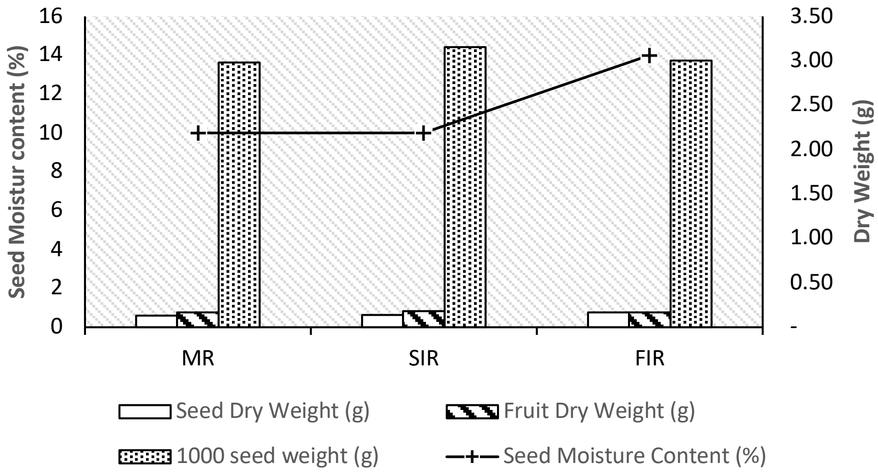

Figure 2. Average values of seed dry weight, fruit dry weight, 100 seed weight, and seed moisture content at three maturity levels

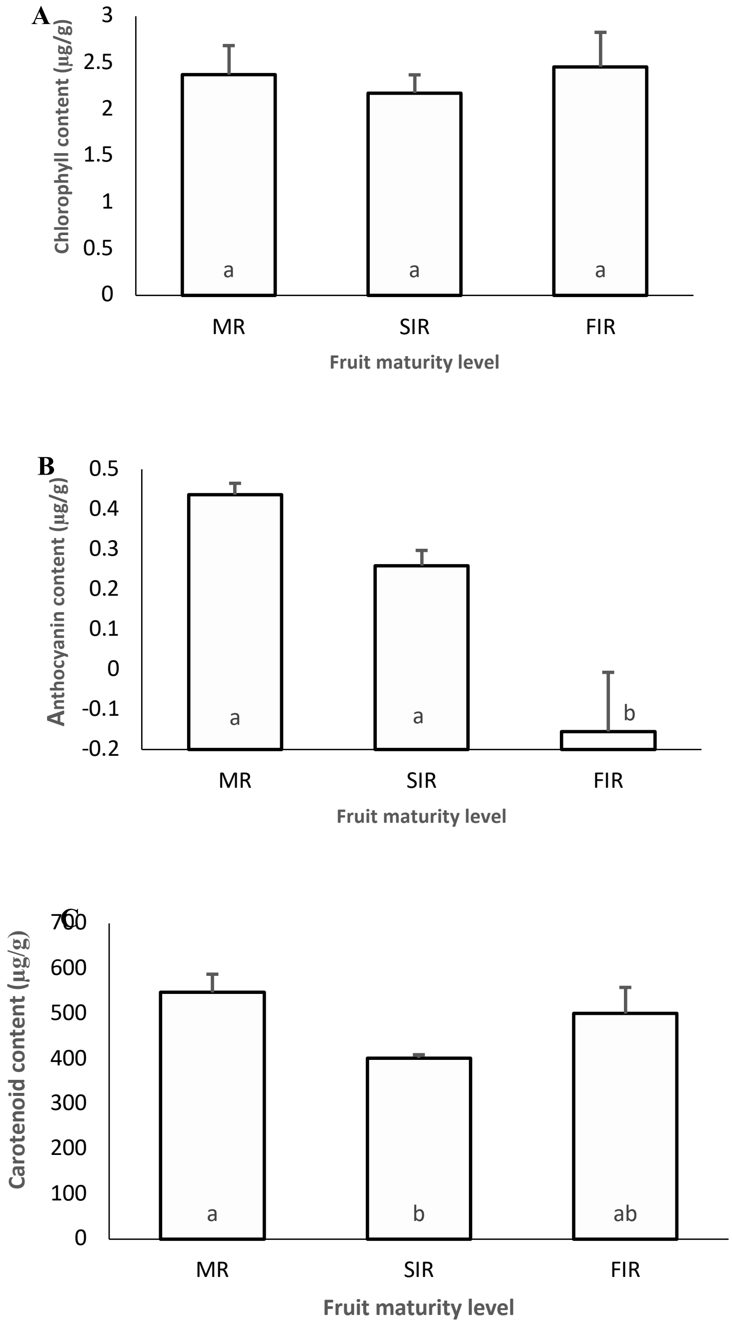

Figure 3. The total amount chlorophyll (A), anthocyanins (B), and carotenoids (C) found in chili pepper flesh fruits at different stages of ripeness

Table 4. Correlation among seed viability and vigor, total chlorophyll, anthocyanins, and carotenoids of cayenne pepper fruit

Level of maturity	Seed viability and vigor	Fruit chlorophyl contents	Fruit antocyanin content	Fruit caratenoid content
MR	Growth rate	0,44	-0,06	0,51
	Vigor index	0,00	0,16	0,16
	Viability	0,70	-0,55	0,36
SIR	Growth rate	-0,32	0,30	0,36
	Vigor index	0,22	-0,20	-0,41
	Viability	-0,56	0,56	0,38
FIR	Growth rate	-0,62	0,29	-0,59
	Vigor index	-0,39	0,68	-0,52
	Viability	0,12	-0,16	0,15

Discussion

The estimation of the time of harvest often depends on fruit color, which provides a measure of fruit ripeness. This experiment aimed to assess the viability and vigor of chili pepper seeds across three different maturation levels. The findings indicate that the degree of maturity strongly affected the seed vigor, but it did not have much of an impact on the viability indicators. This is in agreement with Alan and Eser (2008), who found that the germination rate of chili was best at full ripe-red color, but when the fruit was overripe, it caused a significant decline in seed quality.

The method of seed priming has been widely recognized for its ability to enhance both the germination rate and seed vigor. One strategy that might be used is the use of potassium nitrate (KNO_3). The purpose of the KNO_3 soaking treatment is to stimulate dormant chili seeds. As a preliminary treatment, this soaking adheres with ISTA (2015). The results shows that the percentage of seed vigor index was significantly impacted by the seed soaking treatment with KNO_3. According to Agustiansyah *et al.* (2021), the vigor index of chili seeds subjected to KNO_3 treatment exhibited a greater value compared to seeds that were not immersed in KNO_3. Furthermore, in a study conducted by Kabilan *et al*. (2022), it was claimed that the application of GA_3 resulted in the most favorable germination outcomes, followed by the use of KNO_3.

Variations in the maturity level of chili pepper seeds have a significant impact on the vigor index and seed development speed, which are measures of seed vigor, but have no effect on the percentage of seed germination, which is a measure of viability. This demonstrates that germination testing is insufficient on its own to assess the physiological quality of a seed lot. Germination capability, according to Ilyas (2012), is actually the result of two calculations: the first produces seeds that germinate regularly, while the second calculates the amount of time required for weaker seeds to germinate normally. Thus, the total of the strong and weak seedlings determines the germination percentage. Weak seedlings rarely grow into healthy plants in the field due to stress from the environment.

According to McCormack (2005), chilies with a full red fruit color produce good physiological quality seeds, while those harvested too young produce low quality. It is shown the same in that seeds originating from mature fruit was a high vigor. Sadjad (1999) stated that seed vigor is the primary reference for judging seed quality. High vigor seeds can generate vigorous plants even if the field conditions or growing environment are not ideal.

The weight of 100 seeds, fruit dry weight, seed dry weight, and seed water content were not statistically examined. Nonetheless, the water content of the seeds indicated a tendency for seeds with first immature red fruit to be high water content. Darmawan *et al*. (2014) confirmed the above, indicating that a high seed water content is achieved during the green (young) phase of chili fruit development and diminishes as the fruit ages (turns red).

There is a correlation between fruit maturation and an increase in the dry weight of seeds (Blay *et al.,* 1999). In this experiment, the dried weight of seeds and fruit tended to be high in first-immature red, which affected the weight of one thousand seeds. This could be due to the original fruit samples were selected at random from branches. According to Ritonga (2013), competition amongst chilies for photosynthetic absorption affects the variations in traits of each branch. In addition, Wijaya (2014) found that quantitative fruit characteristics, such as length, diameter, weight, and seed weight, will decrease as the branch is higher.

In addition to evaluating physiological parameters, this study examined the biochemistry of chili pepper, including its chlorophyll, carotenoid, and anthocyanin content. The results revealed that the chlorophyll content was extremely low, with a few samples having a negative value. It is believed that the chlorophyll content of chili pepper seeds and flesh is so low that it cannot be detected. The chili fruit used for the first-immature red stage is dominated by a yellowish orange hue, which is an expression of the carotenoids. It also caused the seeds and fruit of chili pepper to contain a higher concentration of carotene than chlorophyll.

The carotene content of chili pepper seeds and fruit tends to increase as the fruit matures. According to Giuliano *et al.* (1993), during tomato fruit growth, chlorophyll turns into chromoplasts, and during fruit ripening, the color of the pericarp layer changes from green to red. Carotenoids are indications of seed physiological maturity. When sweet corn seeds were physiologically ripe, their total carotenoids reached their maximum (Prasetyaningsih, 2006).

The carotene content of chili pepper seeds is positively correlated with fruit; however, there was no significant correlation between seed viability and seed carotene. Even though most correlation values were positive, not all correlation values were significantly affected. It indicated that while the overall carotene content of fruit and seeds were not affected on one another, it had an impact on the viability and vigor of seeds. Total carotenoids can be utilized in predicting the viability and vigor of chili pepper.

Acknowledgements

We would like to express our sincere appreciation to the management of the Research Group on Crop Improvement Laboratory, Post-Harvest Laboratory, and Plant Reproductive Biology Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, as well as those who assisted with this research.

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(Received: 8 October 2023, Revised: 9 November 2023, Accepted: 14 November 2023)