NUSANTARA BIOSCIENCE
Vol. 14, No. 2, pp. 166-171
November 2022

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n140206

Fruit development and capsaicin content of hot pepper (*Capsicum annuum*) plant cultivated in different soil salinity stress

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Manuscript received: 17 June 2022. Revision accepted: 22 August 2022.

Abstract. *Purnama PC, Sumardi I, Nugroho LH. 2022. Fruit development and capsaicin content of hot pepper (*Capsicum annuum*) plant cultivated in different soil salinity stress. Nusantara Bioscience 14: 166-171.* Land scarcity for cropping at Java Island is a challenge for scientists to look for alternative cropping land. The use of saline land for cropping needs to have further discussed. Red pepper (*Capsicum annuum* L.) can be used as a plant model because, aside from being used as a vegetable, it is also used as natural medicine because of its secondary metabolite, capsaicin. A harsh environment could induce changes in the primary metabolism, which leads to secondary metabolite decomposition. For example, plants respond to stress, such as salt stress, by synthesizing flavonoids and phenolic acid as defense systems to reduce damage. However, the total sugar level and organic acids are decreased. This research aimed to study the fruit development and capsaicin content of hot pepper grown on various coastal soil sand to know whether or not different growth medium affects the size of each part of the fruit. The design of this research was a Completely Randomized Block Design (CRBD). In this research, five different salinity mediums were used, they were A. 15.20 dS/m, B. 5.70 dS/m, C.1.10 dS/m, and D. 2.85 dS/m obtained from Pandansimo and E. 3.25 dS/m obtained from Sleman, Yogyakarta, Indonesia, as comparation. Seedlings were transferred to the polybag after having four truly expanded leaves. Fruit development was observed every week, starting from the first day after flowering (DAF) to 35 DAF. Pericarpium and placenta thickness, fruit diameter, number, length, and width of the giant cell were recorded appropriately from the slides prepared using the paraffin method. Capsaicin content was determined at 14 and 35 DAF, performed with Gas Chromatography-Mass Spectrometry (GC-MS). The results show structural changes in the exocarpium; on the first day after flowering, there was only one layer of epidermis cells, but at 7 DAF, there was one layer of epidermis cells and one layer of collenchyma cells. Next, at 14 DAF, one layer of epidermis cells and two layers of collenchyma cells are observed. The structure of the mesocarpium, endocarpium, and placenta were not changed. The capsaicin content of the green fruit (14 DAF) was lower than the mature one (35 DAF) in all survival mediums. The highest capsaicin content at 14 and 35 DAF was obtained from a plant grown at medium C. Different growing mediums affected pericarpium and placenta thickness, number, length, and width of the giant cell fruit diameter.

Keywords: Capsaicin, *Capsicum annuum*, development, GC-MS, saline

INTRODUCTION

Agricultural land scarcity in Java, Indonesia, is a challenge for researchers to find alternative agricultural land; one way is to utilize available marginal lands, for example, peatland, saline land, and swamps. In 1974, Massoud (1974) estimated that the saline land in Indonesia had reached 13,213 thousand ha, which had expanded due to the increase of invasive fertilization, which caused higher salt levels in the soil. Moreover, seawater intrusion that pollutes groundwater sources used for irrigation is also the cause of excess salt in the soil, especially in coastal areas (Rhoades and Miyamoto 1990). Too much salt in the soil inhibits plant growth by preventing nutrients and water from being absorbed by the roots. In the case of *Sapium sebiferum* (L.) Dum. Cours. grown on the soil closer to the coast, with its growth further slowing down (Barrileaux and Grace 2000).

Aside from influencing growth, environmental factors also affect the secondary metabolites content of plants, as in the case of *Capsicum annuum* L. (jalapeno) grown on sand medium with the addition of nitrogen showing an increase in growth, yield, and capsaicin content (Johnson and Decoteau 1996). In other cases, NaCl solution added in the planting medium of the *C. annuum* (jalapeno) cultivar is known to affect capsaicin content. Proving that environmental conditions and genetic factors can affect capsaicin content (Arrowsmith et al. 2012).

Some plants are also known to have a tolerance to high salt levels, for example, corn, chili, cassava, long beans, and soybeans which are widely cultivated in the coastal areas of Pandansimo, Yogyakarta, Indonesia. The *C. annuum* is one of the chili varieties grown in Indonesia. (Solichatun et al. 2022). Moreover, national demand increased yearly; in 2000, the total consumed reached 427,018 tons (Rukmana and Oesman 2002). The food industry is the principal user of chili fruits. It is often used as a coloring and flavoring agent in sauce, soup, processed meat, snacks, candies, soft drinks, and alcoholic beverages. In addition to their sensory features, oleoresin is extracted from pepper fruits and used as an ingredient in numerous commercial products such as insect repellent or selfdefense sprays. *Capsicum* fruits can also be employed in medicinal applications since they are an important source of bioactive compounds that provide health benefits to consumers (Baenas et al. 2018). It is known that the content of secondary metabolites of chili peppers is widely used to overcome obesity, cardiovascular diseases, and gastrointestinal diseases (Sharma et al. 2013). Chili fruit is also reported to contain antioxidants (Loizzo et al. 2017), hypoglycemic activity (Tundis et al. 2012), suppress intracellular accumulation of triglycerides (Feng et al. 2014), and have anticancer properties (Corson and Crews 2007).

Capsicum fruit varies in size, shape, color, flavor, and pungency (from nonspicy varieties to the hottest species). The pungency in chili peppers is known to come from its secondary metabolite compound known as capsaicin, which is the component of capsaicinoids, along with four other compounds, namely nordihydrocapsaicin, dihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin. About 90% of the spiciness in chili peppers is determined by capsaicin and dihydrocapsaicin (Barbero et al. 2014). In addition to capsaicin, chili peppers contain proteins, fats, carbohydrates, minerals (calcium, phosphorus, iron), vitamins (A, B1, B2, B3, C), essential flavon oils, and carotenoids. The known fatty acid content is palmitic, stearic, oleic, and linoleic by 76% (Jarret et al. 2013).

Capsaicinoids are the compounds responsible for chili pungency, and it is also valued as an excellent source of natural pigments and antioxidant compounds (de Sa Mendes and Gonzales 2020). Capsaicinoids are found in different amounts in *Capsicum* fruits, depending mainly on the variety. The color of the chili can vary as green, yellow, or white (for the immature fruit), orange as semi-ripe, and it becomes red, dark red, brown, and sometimes almost black in the ripe stage. These colors originate from the carotenoids produced in the fruit during its ripening stages. Once the fruits are harvested, they change mainly in color, but changes also happen in the aroma, flavor, and antioxidant activity (Manikharda et al. 2018). *Capsicum* fruit, especially in its dehydrated form, is an excellent source of tocopherols which are recognized for their high resistance to lipid oxidation; in such a way, these compounds could serve as protective agents for the antioxidant capacity in the pericarp (Wildman et al. 2017).

The capsaicinoids increase during maturation until they reach the maximum content and immediately decrease, presenting a degradation greater than 60%. On the other hand, it has been reported that the activity of peroxidases that degrade these compounds increases at the same time that the content of capsaicinoids is reduced (Cisneros-Pineda et al. 2017). Therefore, both external factors and the maturity state of chili may have generally affected the secondary metabolites production.

It is remained unclear until now the compartmentalization of each metabolite in the hot pepper fruit because the placenta, pericarp, and seed produce the enzyme important for capsaicinoid production. However, according to Zamljen et al. (2021) placenta is the main synthesis point of capsaicinoid, and the other two produces in smaller amounts. But some compounds known to be found in a certain part of the fruits, such as pericarpium, placenta, and seed, for example, capsaicin, are mainly synthesized in the placenta (Gamboa-Becerra et al. 2015), while anthocyanins are described as being accumulated in pericarpium during fruit development (Aza-Gonzales et al. 2013).

This study aims to determine the development and capsaicin content of hot pepper fruit in different coastal soil planting mediums and whether different planting mediums affect the size of the constituent components of red chili fruit.

MATERIALS AND METHODS

All experiments are conducted in the greenhouse. The seeds used are *C. annuum* North Red Star variety produced by PT Sang Hyang Seri (Persero), which is transferred into polybags that have been filled with soil medium obtained from Pandansimo beach, Bantul, namely A. 15.20 dS/m, B. 5.70 dS/m, C.1.10 dS/m, and D. 2.85 dS/m and as a comparison medium E with salt content 3.25 dS/m was obtained from Sleman, Yogyakarta, Indonesia. Transfer of seedlings to polybags is carried out after the seedlings have 4 leaves. Each treatment has five replications separated into different polybags. Observations were carried out on 1, 7, 14, 21, 28, and 35 DAF to determine the development of fruits preserved with preparations using the single staining paraffin method. The parameters observed were: pericarpium thickness, placenta thickness, number, length, and width of giant cells, as well, as fruit diameter. Gas Chromatography-Mass Spectrometry technique (Agilent GC 6890N 5975B MSD) was used to analyze capsaicin content in fruits aged 14 and 35 DAF. The capillary column is an Agilent 19091S-433 model, HP-5MS 5% Phenyl Methyl Siloxane. Capsaicin standard was obtained from Sigma Chemical Co. Single-stain paraffin embedding method (Ruzin 1999) was applied in the preparation of microscopic slides for examination of fruit development. The quantity of capsaicin compound was analyzed with Gas Chromatography-Mass Spectrometry using capsaicin as standard injected simultaneously with the samples. The experimental design used was CRBD (Completely Randomized Block Design) followed by Variance Analysis (ANOVA), which continued with the LSD-Least Significance Difference with a 5% significance level.

RESULTS AND DISCUSSION

Response of flowering to the growing medium

The results of planting *C. annuum* on growing mediums with various salt levels, namely A (15.20 ds/m), B (5.70 ds/m), C (1.10 ds/m), D (2.85 ds/m), and E (3.25 ds/m), was plants cannot survive more than two weeks in planting mediums A and D. All of them died suffered from high salinity and drought since its texture is 100% sand so that the growing medium cannot hold water. Conversely, in planting mediums B, C, and E, plants can survive, produce flowers, and eventually bear fruit (Table 1).

Table 1. The speed of chili fruits flowering on various growing medium

Planting medium	A	B	C	D	E
Flowering speed (day)	X^{7}	59.6^{b}	55.8^{b}	X^{9}	45.8^{a}

Notes: The value followed by the same character showed no significant differences among each other based on LSD analyses at 5 % significance. X^{7}: Die on day 7th, X^{9}: Die on day 9th

Anatomy of fruit development

Pericarpium thickness

Pericarpium is the further development of ovary walls that are not rapidly differentiated before. At the time of anthesis, most of it is composed of parenchyma, transport tissue, and the epidermis layer, which has a cuticle at the outermost part.

The pericarpium thickness of the red pepper fruit grown on planting mediums B, C, and E began to differ markedly in fruits aged 7 to 35 DAF. Whereas at the age of 21 and 28 DAF in medium C and E, there is no noticeable difference, indicating the increase in pericarpium thickness is the same. At 35 DAF of the three mediums, the lowest pericarpium thickness is at medium B (Table 2, Figure 1).

Placenta thickness

Chili seeds are attached to the placenta, composed of parenchymal cells similar to the ones found in mesocarpium (Figure 2). The stele type observed in the placenta is bicolateral, the xylem is situated between the outer and inner phloem, and the cambium is found.

Capsaicin content

In all survival growing mediums, the capsaicin content of green fruits (14 DAF) is lower than that of brownish-green fruits (35 DAF) (Table 4). The capsaicin content of green and brownish-green fruits in medium B (5.7 dS/m) was lower than in medium C (1.1 dS/m), both mediums coming from a coastal region. The capsaicin content of green and brownish-green fruits in medium E (3.25 dS/m) as the control medium is lowest compared to the other two mediums, probably due to the texture of the medium, which in the form of *geluhan* sand and also the source of the salt which are not coming from the coastal area but unknown salt from the ground completely far away from the shoreside, approximately 25 km.

Table 2. Pericarpium thickness of chili on various growing media

Day	Pericarpium thickness (µm)		
	B	C	E
1	249.6^{aq}	249.6^{ap}	348.0^{aq}
7	428.4^{bq}	393.0^{bp}	526.5^{br}
14	531.0^{cp}	747.0^{cq}	778.5^{cr}
21	688.5^{dp}	976.5^{dq}	987.0^{dq}
28	773.5^{ep}	1155.0^{eq}	1125.0^{eq}
35	802.5^{ep}	943.5^{dq}	1018.5^{dr}

Notes: The value followed by the same character showed there were no significant differences among each other based on LSD analyzes at 5 % significance

Table 3. The placenta thickness of chili fruit grown on a different medium

Day	Placenta thickness (µm)		
	B	C	E
1	438.0^{aq}	216.6^{ap}	210.0^{ap}
7	713.4b^{cp}	468.0^{br}	526.5^{bq}
14	819.0^{dp}	1065^{cq}	1158^{cr}
21	913.5^{cp}	1209^{dq}	2058^{dq}
28	765.0^{cdp}	1552.5^{eq}	1156.5^{cr}
35	670.5^{bp}	1063.5^{cq}	1306.5^{dr}

Notes: The value followed by the same character within one column (a, b, c) and one row (p, q, r) showed there were no significant differences among each other based on LSD analyses at 5 % significance

Table 4. The relative content of capsaicin (%) of chili grown on different growing mediums

Planting medium	14DAF	35DAF
B	23.97	58.78
C	77.99	100
E	17.17	32.27

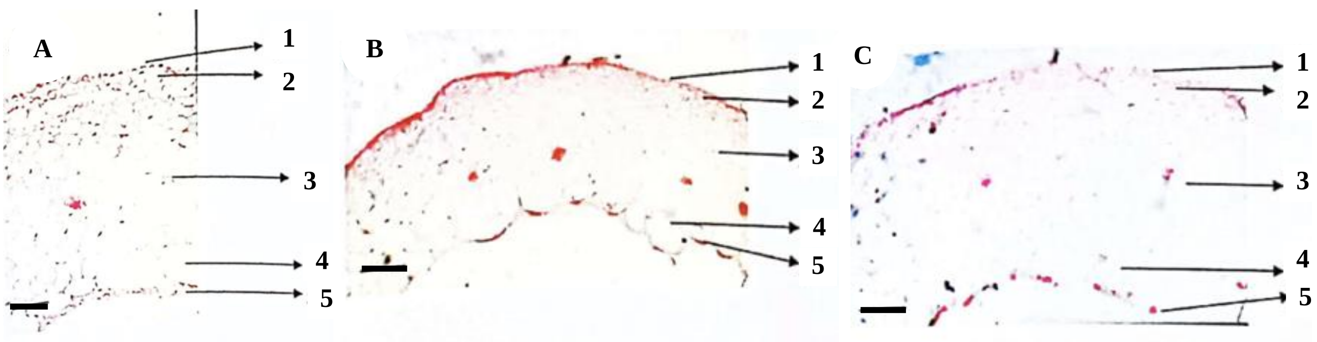

Figure 1. Cross section of chili pericarpium fruit aged 35 DAF on the growing medium: A. 5.7 dS/m, B.1.1 dS/m, C. 3.25 dS/m observed under Nikon light microscope: 1. Epidermis with cuticle, 2. Collenchyma, 3. Parenchyma, 4. Giant cell, 5. Sclerenchyma. 1 Bar A= 25 µm, 1 Bar B, C= 50 µm

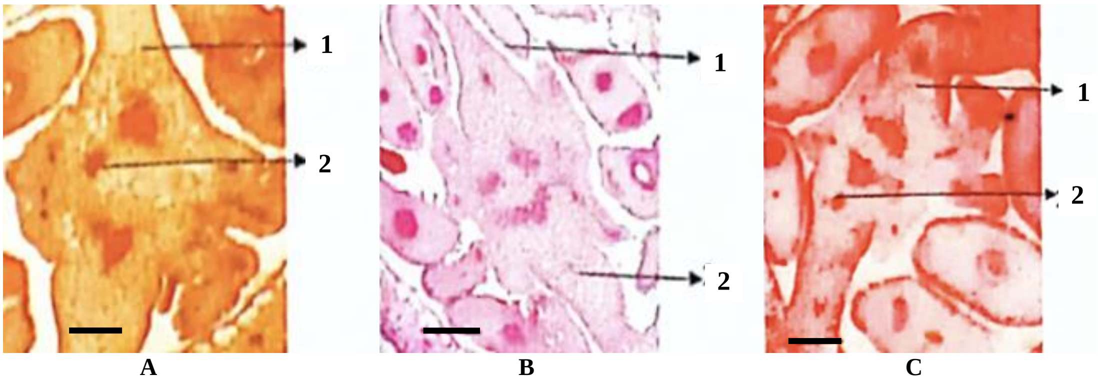

Figure 2. Cross-section of chili fruit through placenta aged 7 DAF grown on planting medium: A. 5.7 dS/m, B. 1.1 dS/m, C. 3.25 dS/m observed under Nikon light microscope: 1. Parenchyma, 2. Stele in the placenta. 1 Bar = 50 µm

Discussion

From planting hot pepper in mediums A, B, C, D, and E, none of the plants could survive in mediums A and D, and the plants died on days 7th and 9th, respectively (Table 1). It could be that in medium A the P content is 10.43 ppm (low), the total N is 0.0056% (very low), the K is 0.63 ppm (medium), and the salt level is far above the normal limit of 4 dS / m which is 15.20 dS/m (Wilkinson 1994). Therefore, the texture is 100% sand which cannot hold water; as a result of which, plants cannot get water. Likewise, in medium D, although the salt content is 2.85 dS/m, the texture is 100% sand, and the nutrient content is low. On the other hand, in medium B (5.70 dS/m), the texture is in the form of *geluhan* sand, allowing the roots to absorb water and nutrients, the P content is 20.79 ppm, the total N is 0.015%, and the K available is 1.45 ppm relatively high. In medium C (1.1 dS/m), the texture is *geluh*, the P content is 51.76 ppm (very high), the N total is 0.11%, and the K available is 0.12 ppm (low). While E (3.25 dS/m), soil texture is in the form of *geluhan* sand, the P is 41.26 ppm, the N total is 0.025%, and the K is 1.56 ppm (high). Thus, it is clear that plants can not survive at medium A and D due to their salt level, which is beyond the normal level, and their texture which is 100% sand.

As one of the site productions of capsaicinoid, pericarpium is important to measure growth. The results from pericarpium measurement showed that the pericarpium at medium B is less developed compared to the other two planting medium, C and E, mainly due to the salt level of growing medium B (5.70 dS/m) being high. Salt inhibits the growth of plants by affecting their osmotic potential. As a result, cell turgor decreases and inhibits the elongation of plant cells. The high level of Na+ causes K+, which plays a role in activating enzymes involved in pyruvate synthesis, and protein translation cannot be absorbed (Manchanda and Garg 2008). K also acts as a cofactor in protein synthesis, functioning to maintain water balance and the movement of the stomata. The low K causes the closed stomata so that the rate of photosynthesis is reduced, as a result of which the energy for growth decreases (Salisbury and Ross 1995).

The placenta is the site of capsaicinoids, of which 90% consist of capsaicin and dihydrocapsaicin (Topuz and Ozdemir 2017). Though the production site is at the placenta, epidermal cells accumulate them in the vacuoles and eventually excrete them in the seeds and on the inner surface of the pericarp (Cisneros-Pineda et al. 2017). In the placenta thickness measurement, at day 35 DAF overall showed a reduction in the decreasing nutrient supply in the growing medium. Therefore, the placenta of hot pepper at medium B is less developed than in the other two growing mediums, C and E (Table 3, Figure 2).

Several researchers have confirmed that in the epidermal cells of the placenta, where capsaicinoids (mainly capsaicin and dihydrocapsaicin) are synthesized, a higher concentration of capsaicin is found. They are secreted into the cell wall and finally accumulate within the structures named vesicles located at the surface of the placenta. It is worth mentioning that the placenta was the one that presented the highest content of capsaicin, but in turn, it was the one that conserved less bioactive compounds, similar to the pericarp (Palma-Orozco et al. 2021).

The results of capsaicin measurement showed that the content at 35 DAF is higher than 14 DAF (Table 4). That indicates that the older the fruit, the higher the capsaicin content. Changes during fruit ripening include modification of cell walls that become softer, conversion of carbohydrates to sugars, increased susceptibility to pathogens, raised production of aromas and volatile compounds, changes in biosynthesis, and accumulation of pigments. The softening of the cell wall is in line with the decrease in lignin content which reduces the formation pathway so that precursors (phenylalanine) will be more widely used to form alkaloids, namely capsaicin (Diaz et al. 2004). Among all, the capsaicin concentration at medium C is the highest. That is likely because the C growing medium is collected from Pandansimo, which has salt naturally from the coastal region and has a texture in the form of a *geluh* so that it has enough fine particles to provide a larger water absorption surface and more nutrients attached to these particles. In addition, the medium also contains P 51.76 ppm, N total 0.11%, and K

0.12 ppm. Potassium can increase pigment biosynthesis and affect pungency, and N can increase chili production (Johnson and Decoteau 1996). It is the finding that the content of bioactive compounds is influenced by climatic conditions, maturation time, genotype, and cultivation techniques (Aza-Gonzales et al. 2013).

As we noticed, the pericarpium and the placenta thickness at medium C are higher than in B, which were 943.5 µm and 1,063.5 µm, respectively. Moreover, the capsaicin concentration of red pepper fruits grown at medium C is higher than B. Thus, in line with what Materska (2014) reported, the pericarpium and placenta is the most prominent site of metabolites production found in chili. He reported the comparison of the pericarpium and placenta in chili and found that the placenta was richest in flavonoids while the pericarpium presented larger diversity in glycosylated compounds.

The degradation of capsaicin in the fruit of plants treated with high salt concentration may be due to peroxidase enzymes. Studies in capsicum show the involvement of peroxidases in the degradation of capsaicinoids in the capsicum cultivar (Fujiwake et al. 1980). In addition to that, Contreras-Padilla and Yahia (1998) also revealed that peroxidases are most likely candidates for capsaicinoid degradation. That may also be the possible reason for the degradation of capsaicin in the high salt-exposed plant. These results suggest that the degradation of capsaicin in these fruits could be a response to salt stress conditions and needs further investigation to understand this process properly. It is known that the component of capsaicinoids is not only capsaicin, thus could be the deposition is not only in the form of capsaicin. According to Castro-Concheat et al. (2014), capsaicinoids were considered part of the phenol content of various fruits. Therefore, the content in all parts of chili was much higher than capsaicin, as found in their work, 3 to 6 times higher. Moreover, the right method to extract also highly influence the yield; for example, the Folin Ciocalteu method, despite being easy, sensitive, and precise, can be affected by the presence of aromatic amines, carbon dioxide, ascorbic acid, and other reducing compounds and thus interfering results (Prior et al. 2015). It has been reported that capsaicinoids are synthesized in the superficial cells of the placenta, and these are specialized as parts that secrete these compounds (González-Estrada et al. 2018)

The increase in salt affects primary metabolism, plant growth, and development due to ion toxicity, which induces nutrient and water deficiencies and oxidative stress. Plant cells survive in dealing with osmotic pressure by adjusting the osmotic, accumulating compounds to maintain osmotic pressure, regulating oxidative stress, inducing the formation of certain proteins, and altering some physiological adaptations such as modification of stem and root growth and transpiration. In addition, it also affects the production of secondary metabolites, which physiologically play a role in dealing with or tolerating stress. Most secondary metabolites are produced through intermediates from primary carbon metabolism via phenylpropanoids, shikimate pathways, mevalonates, or MEPs (non-mevalonate pathways). The most studied secondary metabolites include chlorophyll, carotenoids, and phenols. Increased synthesis of secondary metabolites is used to protect cellular structures from oxidative damage (Machanda and Garg 2008). Thus, the possibility that occurs why the capsaicin content of green and brownish-green fruits in medium B is lower than C is that even though in terms of salt levels of growing medium B are higher than C, there is a possibility of an increase in secondary metabolites collected not only as capsaicin but also in the form of flavonoids or other phenol compounds. These phenol compounds are produced to increase antioxidant activity that can prevent the formation of free radical fats. In general, increasing secondary metabolites is expected to increase the internal concentration of cells to prevent the release of fluid from inside plants to the external environment, whose concentration is more concentrated.

In principle, water loss can cause losses in chili's sensory and nutritional quality; such losses are related to the change in color and textures and a decrease in the content of vitamins and other bioactive compounds (Arslan and Ozcan 2019). Therefore, it can be concluded that the accumulation among the different varieties differs, and the difference could be due to environmental and cultivation conditions (Ruiz-Lau et al. 2017).

In conclusion, the anatomical features observed (pericarpium, placenta, parenchyma, giant cell, and stele) are not changed by the saline soil medium; indeed, it affects its size. Overall the thickness of the pericarpium and placenta decreased at 35 DAF, likely due to the depletion of nutrients in the medium. The capsaicin content of green fruits (14 DAF) is lower than that of brownish-green fruits (35 DAF) in all growing mediums. The saline soil influenced the capsaicin content, proving that both hot pepper fruits grown in the coastal saline soil had higher capsaicin contents than the control soil. For improvement, more experiments should be conducted to separate each part of the anatomical features and analyze the capsaicin content to understand its production site.

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