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# INFLUENCE OF FRUIT RIPENING STAGE, POST-HARVEST SEED DEVELOPMENT, AND GENOTYPIC VARIATION ON THE MASS OF 1000 SEEDS IN EGGPLANT (*SOLANUM MELONGENA* L.)

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**Abstract:** This study examined the effects of fruit ripening stage and post-harvest seed development on the mass of 1000 seeds in eggplant (*Solanum melongena* L.) across three morphologically distinct varieties (Serbian, Chinese, and Italian). Field experiments were conducted over three years (2017–2019) at the Institute of Field and Vegetable Crops in Novi Sad, Serbia. Fruits were harvested at unripe, semi-ripe, and fully ripe stages, then stored for 10 or 20 days at 20–25°C prior to seed extraction. Seeds were obtained following a three-day fermentation process and evaluated according to ISTA Rules. Fruit ripening stage was the predominant factor, explaining over 97% of the variation in seed mass. Seeds from fully ripe fruits consistently exhibited the highest mass, up to 68% greater than those from unripe fruits. Post-harvest storage increased seed mass across all ripening stages, indicating continued seed filling after harvest. Seeds extracted after 20 days showed significantly higher mass than those extracted after 10 days. The Serbian variety achieved the highest mass of 1000 seeds at full ripeness, while the Italian variety reached peak values at the semi-ripe stage following both storage durations. In contrast, the Chinese variety consistently exhibited the lowest values, highlighting genotypic differences in seed development potential. These results confirm that fruit physiological ripening and post-harvest storage significantly influence seed quality. Delaying seed extraction is an effective strategy to enhance the mass of 1000 and seed vigor, offering practical implications for breeding and seed production.

**Key words:** seed filling, post-harvest storage, physiological ripening, seed extraction timing, seed quality.

## Introduction

Eggplant (*Solanum melongena* L.) is one of the globally important vegetable crops in the Solanaceae family, valued for both its fruit yield and seed quality, which directly influence the mass of 1000 seeds, germination capacity, seedling

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vigour, and subsequent plant performance. Eggplant seeds are small, flattened, and kidney-shaped, typically concentrated in the lower half of the fruit (Gvozdenović et al., 2011).

Most researchers agree that, for seed production purposes, fruits should be harvested at advanced ripening stages to ensure optimal seed quality. For example, Popović et al. (2022) have demonstrated that the mass of 1000 seeds significantly increases in fully ripe fruits compared to earlier ripening stages. Under standard seed production conditions, the mass of 1000 seeds ranges from 4.0 to 5.3 g (Passam et al., 2010), reaching its maximum when fruits are harvested at later developmental stages and after a postharvest ripening period. Similar findings were reported by Ranaweera et al. (2021), who have shown that delaying seed extraction (via post-harvest ripening) from early-harvested fruits improves germination percentage and seed vigour. In contrast, seeds extracted too early (prior to full physiological ripening) may perform poorly. Franquiera (2015) has found that physiological seed ripening in eggplant occurs around 50 days after anthesis, when dry seed mass peaks before declining. Extraction timing after harvest also plays a critical role: Tetteh et al. (2023) observed that waiting 4 to 6 days after harvest before seed extraction improved the mass of 1000 seeds, seed vigour, and emergence across several cultivars.

The mass of 1000 seeds is considered a key indicator of seed quality. It plays a critical role in determining sowing rates and is closely related to seed vigour. Seeds with a higher mass of 1000 seeds typically contain more nutrient reserves, germinate more quickly and uniformly, are more tolerant to environmental stresses, and tend to produce more robust seedlings. Consequently, such seeds are also more suitable for long-term storage.

Despite existing knowledge on seed ripening in eggplant, there is still a lack of integrative and quantitative analyses addressing the combined and interactive effects of fruit ripening stage, post-harvest storage duration, and genotypic variation on the mass of 1000 seeds. This gap limits both the physiological understanding of seed development and the optimisation of seed production practices. Therefore, this study was designed to provide a systematic and comparative evaluation of these factors across three morphologically distinct eggplant genotypes under controlled experimental conditions. It was hypothesised that delayed fruit harvest and extended post-harvest storage would enhance seed mass through continued seed filling, with genotype-specific responses. The findings are expected to contribute to improved seed production strategies and to provide a basis for optimising harvest timing and post-harvest handling in eggplant seed systems.

## Material and Methods

Field experiment

The field experiment was conducted over a three-year period (2017–2019) at the experimental station of the Institute of Field and Vegetable Crops, situated in Rimski Šančevi near Novi Sad, Serbia (E45°19'52.2"N, 19°50'10.4"E). This site, characterised by a temperate continental climate and fertile loamy soils, was used consistently throughout the trial period to ensure uniform agroecological conditions. Seeds were sown in a controlled greenhouse at the beginning of April each year. Transplanting to open-field conditions took place in the second half of May, following a randomised block design with five replicates. Each variety was represented by 250 plants (50 plants per replicate), arranged in rows spaced 70 cm apart, with 50 cm between plants within rows.

Plant material

Three morphologically distinct eggplant varieties were used in the study: Domaći srednje dugi, Chinese snake-like (Chinese variety) and Violeta lunga di Romagna (Italian variety).

1. Domaći srednje dugi (Serbian variety) – Fruits are oval-shaped, dark purple to bluish in colour, with green calyx lobes. The average fruit length is 18.6 cm, width is 8.5 cm, and the mean weight of unripe fruits is 243 g (Figure 1).

Figure 1. Domaći srednje dugi (Serbian variety).
Source: Authors' own photograph.

2. Chinese snake-like (Chinese variety) – The fruit is elongated, glossy, and dark purple to black in colour. The average fruit length is 29.3 cm, width is 6.5 cm, and the mean fruit weight is 228 g (Figure 2).

Figure 2. Chinese snake-like (Chinese variety).
Source: Authors' own photograph.

3. Violeta lunga di Romagna (Italian variety) – The fruit is large, elongated, and purple to dark purple in colour, with green calyx lobes. The average fruit length is 21.3 cm, width is 7.4 cm, and average fruit weight is 315 g (Figure 3).

Figure 3. Violeta lunga di Romagna (Italian variety).
Source: Authors' own photograph.

Seed extraction and determination of the mass of 1000 seeds

To assess the mass of 1000 seeds of eggplant (*Solanum melongena* L.), fruits were harvested at three physiological ripening stages: unripe (fully developed with dark purple epidermis), semi-ripe (light purple skin), and fully ripe (brownish-yellow skin indicating physiological ripening) (Figure 4).


Figure 4. Three stages of fruit ripening.
Source: Authors' own photograph.

Immediately after harvest, fruits were divided into two treatment groups based on post-harvest storage duration before seed extraction:

Group 1: Fifty fruits from each ripening stage were stored in a shaded warehouse at ambient temperature (20–25°C) for ten days after harvest.

Group 2: Another fifty fruits per ripening stage were stored under identical conditions for twenty days after harvest.

Following the designated storage period, all fruits were manually chopped into smaller pieces to facilitate pulp breakdown. The macerated fruit material was then placed in sterile plastic containers and left to ferment for three days at room temperature (22–25°C). Fermentation promotes the enzymatic degradation of the fruit pulp, enabling easier detachment of seeds. After fermentation, seeds were manually separated by thoroughly rinsing with tap water over a fine-mesh sieve (1 mm), ensuring the removal of any adhering pulp or mucilage. Cleaned seeds were air-dried under ambient laboratory conditions (22–25°C, 40–60% relative humidity) for seven days, avoiding direct sunlight to prevent thermal damage. Once completely dry, seeds were stored in paper envelopes in a dry, dark room for 60 days before analysis, to ensure physiological stabilisation.

Measurement of the mass of 1000 seeds

The mass of 1000 seeds was determined following the guidelines of the International Rules for Seed Testing (ISTA Rules, 2017). The procedure was as follows: For each sample (ripening stage × storage duration × variety), eight replicates of 100 seeds were counted manually using an optical seed counter to ensure accuracy and avoid mechanical damage. Each replicate of 100 seeds was weighed using a calibrated precision analytical balance (Mettler Toledo, ±0.001 g accuracy), previously zeroed and tested with certified calibration weights. The arithmetic mean of the eight replicate measurements was expressed in grams per

100 seeds (g) and reported as the mass of 1000 seeds. During measurement, ambient humidity and temperature were monitored and kept stable to avoid fluctuations in seed moisture content. This method ensured accurate and repeatable evaluation of the mass of 1000 seeds, allowing for statistically robust comparisons between treatments and varieties.

Statistical analysis

The statistical analysis of experimental data was conducted using the Statistica 13 software package (2015), developed by StatSoft, Inc., Tulsa, OK, USA. The results were processed using appropriate statistical procedures, including analysis of variance (ANOVA) at significance levels of α =0.01 and α=0.05, Fisher's Least Significant Difference (LSD) test at α=0.01 and α=0.05, and Tukey's Honestly Significant Difference (HSD) test at α=0.01. Data are presented in both tabular and graphical formats and served as the basis for drawing conclusions relevant to the research problem.

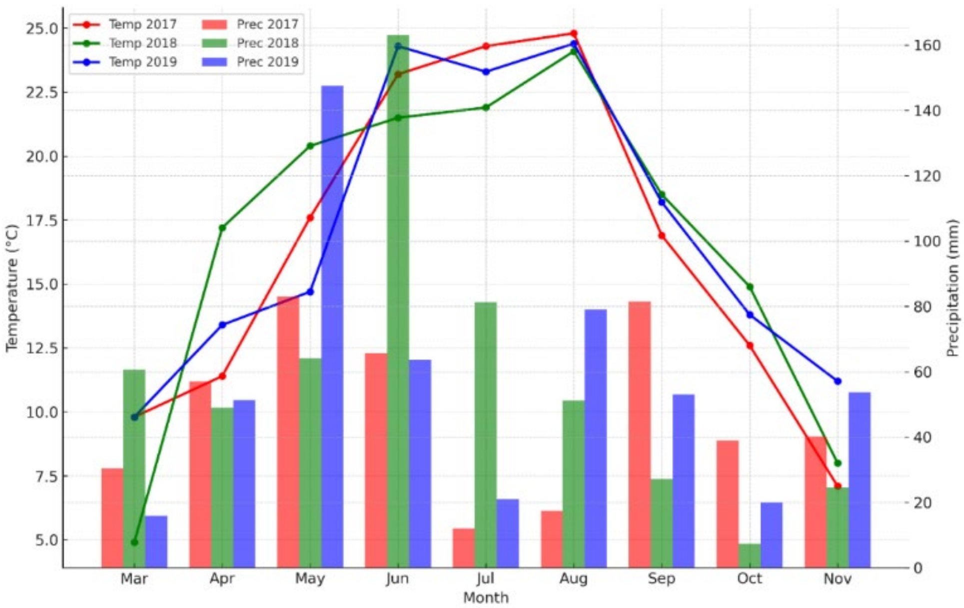


Figure 5. Climatic conditions at Rimski Šančevi during eggplant (*Solanum melongena* L.) growing seasons (2017–2019), represented by a Walter-style diagram of monthly temperature and precipitation.
Source: Meteorological data on air temperature and precipitation were obtained from the Republic Hydrometeorological Service of Serbia (2025).

Meteorological data

Meteorological data on air temperature and precipitation for the study period were obtained from the Republic Hydrometeorological Service of Serbia (Republic Hydrometeorological Service of Serbia, 2025) and are presented in Figure 5, which displays monthly mean values for temperature and precipitation across the three growing seasons (2017–2019). Average air temperatures exhibited relatively consistent patterns over the observed years, with August being the warmest month in all seasons (mean: 24.4°C). In contrast, precipitation levels were more variable, with 2017 recording notably lower rainfall overall, while June 2018 and May 2019 had the highest precipitation within their respective years.

## Results and Discussion

The evaluation of the mass of 1000 seeds in three eggplant varieties – Serbian, Chinese, and Italian, across different ripening stages and two post-harvest extraction times revealed clear and consistent developmental patterns (Figure 6; Tables 1 and 2). Seeds extracted from unripe fruits displayed the lowest mass, while those from fully ripe fruits reached the highest levels, confirming the expected physiological progression of seed filling during fruit ripening. On the tenth day after harvest, mean values ranged from 1.76 to 2.17 g in unripe fruits, from 3.08 to 3.28 g in semi-ripe fruits, and from 4.39 to 4.69 g in fully ripe fruits (Figure 6).

When extraction was delayed to the twentieth day, all values increased, reaching 2.39–2.66 g, 3.41–3.64 g, and 4.58–4.88 g for unripe, semi-ripe, and fully ripe fruits, respectively (Figure 6). These increments demonstrate that seed development continues during post-harvest storage, even without a vascular connection to the mother plant. Such post-harvest increases were especially pronounced in seeds from unripe fruits, whose mass rose from 2.00 to 2.54 g (Table 1), whereas seeds from fully ripe fruits showed smaller yet still significant gains (4.55 to 4.76 g; Table 1), reflecting their proximity to physiological ripening.

Variance analysis further underscored the dominant role of ripening stage in determining the mass of 1000 seeds. In both extraction periods (Table 2), ripening accounted for more than 97% of total variability, while the effects of variety and production year were statistically significant but comparatively minor.

Interactions among factors were generally non-significant, except for the strong combined effects of extraction time and ripening stage observed in the integrated analysis (Table 1), which confirmed that the magnitude of post-harvest seed filling depends heavily on whether fruits were harvested at an early, intermediate, or advanced stage. Among varieties, the Chinese genotype consistently produced seeds with significantly lower mass than the Serbian and

Italian varieties, regardless of ripening stage or extraction time (Figure 6; Tables 1 and 2). This pattern suggests intrinsic genotype-specific differences in assimilate allocation and seed-filling efficiency.

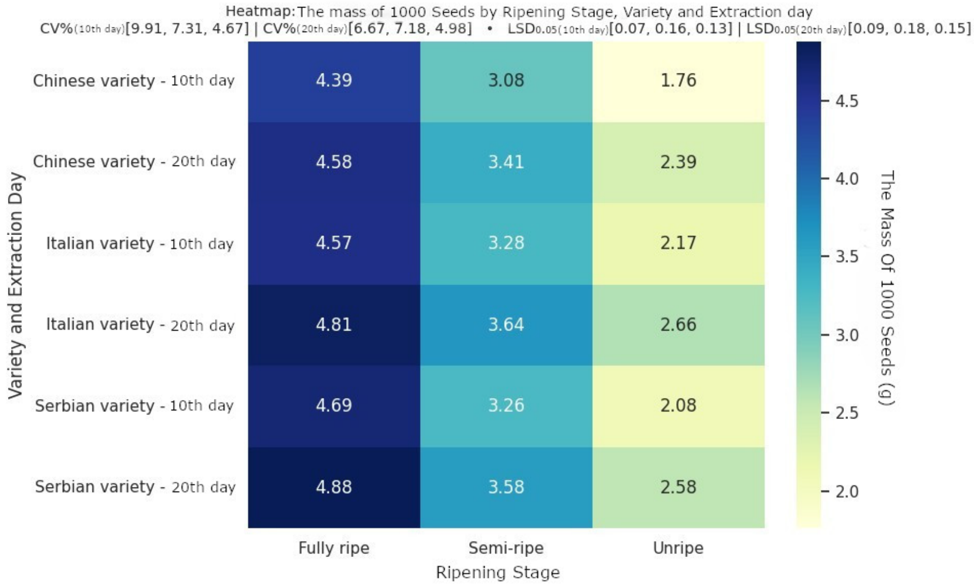


Figure 6. Variation in the mass of 1000 seeds of eggplant (*Solanum melongena* L.) in relation to fruit ripening, extraction time, and variety.

*Values represent means ± standard error (SE). Data were collected for three eggplant varieties (Serbian, Chinese, and Italian) at three fruit ripening stages (unripe, semi-ripe, fully ripe), and two seed extraction times (10$^{th}$ and 20$^{th}$ day after fruit harvest). Coefficients of variation (CV%) and least significant differences ($LSD_{0.05}$) are provided for each combination to aid in the interpretation of statistical differences. Source: Authors' own data

The biological interpretation of these results aligns well with established concepts of seed ripening. Fully ripe fruits produced seeds that were approximately 68% heavier than those from unripe fruits on the tenth day after harvest, indicating extensive reserve accumulation and advanced embryonic development. These findings correspond with earlier observations in eggplant and other Solanaceae crops, where progressive ripening enhances dry matter deposition in seeds (Passam et al., 2010; Takač et al., 2015). Furthermore, the continued increase in seed mass during storage supports the concept of late post-harvest ripening, as described by Nonogaki et al. (2010), whereby biochemical ripening and reserve stabilisation persist after fruit detachment. This pattern of continued filling until a physiological plateau is reached reflects the classical model of mass ripening and transition into desiccation tolerance (Bewley et al., 2013).

Table 1. Analysis of variance of the effect of seed extraction timing on the mass of 1000 seeds. Tukey's honestly significant difference (HSD) test (α=0.01).

| Ripening stage | Extraction time | Mass of 1000 seeds (g) |
|---|---|---|
| Unripe | 10th day after harvest | 2.00 b |
| | 20th day after harvest | 2.54 a |
| Semi-ripe | 10th day after harvest | 3.20 b |
| | 20th day after harvest | 3.54 a |
| Fully ripe | 10th day after harvest | 4.55 b |
| | 20th day after harvest | 4.76 a |
| Extraction time (ET) | | ** |
| Ripening stage (RS) | | ** |
| Variety (V) | | ** |
| ET x RS | | ** |
| ET x V | | ns |
| RS x V | | ** |
| ET x RS x V | | ns |

Source: Authors' own data.

Similar dynamics have been reported in sweet pepper, where a seven-day fruit resting period significantly improved seed mass and protein content (Colombari et al., 2022), and in zucchini, where delayed extraction from 3 to 12 days enhanced seed dry weight and vigour (Aguilar et al., 2024). Studies on pumpkin (Pattar et al., 2025) and cereals, such as *Kengyilia melanthera* (Ling et al., 2023) and durum wheat (Jawad et al., 2025), provide broader evidence that delayed harvest or delayed extraction favours seed quality across taxa.

Table 2. Analysis of variance of main effects and their interactions on the mass of 1000 seeds at two post-harvest extraction periods (10th and 20th day).

| Source of variation | Degrees of freedom | 10th day | | | 20th day | | |
|---|---|---|---|---|---|---|---|
| | | MS# | SS | SS (%) | MS | SS | SS (%) |
| Total | 14 | 10.72 | 150.03 | 100.00 | 8.16 | 114.17 | 100.00 |
| RS* | 2 | 73.17** | 146.33 | 97.53 | 55.47** | 110.94 | 97.17 |
| V | 2 | 1.06** | 2.13 | 1.42 | 0.82** | 1.63 | 1.43 |
| Y | 2 | 0.53** | 1.06 | 0.71 | 0.58** | 1.16 | 1.02 |
| RS×V | 4 | 0.08 | 0.32 | 0.21 | 0.03 | 0.12 | 0.11 |
| V×Y | 4 | 0.05 | 0.19 | 0.13 | 0.08 | 0.32 | 0.28 |
| Error | 120 | 0.02 | 2.43 | - | 0.03 | 3.35 | - |

**(p<0.01) # Variability explained as percentage of sum of squares (SS %); mean square (MS); *Ripening stage (RS); Variety (V); Year (Y).
Source: Authors' own data.

Recent studies further illuminate the physiological mechanisms underlying these trends. Tetteh et al. (2023) demonstrated that delaying seed extraction by 4–6 days in eggplant significantly improved seed weight, vigour, and germination.

Ranaweera et al. (2021) have confirmed that post-harvest storage of fruits improves seed quality, especially in less ripe fruits, closely paralleling the strong responses observed in unripe and semi-ripe fruits (Table 1). In addition, newly published research highlights the influence of post-harvest environmental conditions, namely Özmen and Kenanoğlu (2024) have shown that temperature regimes during fruit storage markedly affect seed viability and antioxidant composition, suggesting that the benefits of delayed extraction might be further optimised by controlling storage conditions.

Beyond physiology, emerging genetic evidence provides new context for interpreting varietal differences. QTL mapping in eggplant has recently identified several loci associated with seed dormancy and late-ripening traits, including dr1.1, dr2.1, and dr6.1, with the WRKY-domain gene Smechr0201082 proposed as a candidate regulator influenced by ABA and GA signalling (Ai et al., 2024). Although seed mass was not explicitly mapped in that study, the hormonal pathways involved overlapped substantially with those regulating seed filling and desiccation, suggesting potential genetic bases for the consistently lower mass observed in the Chinese variety in our study (Figure 6). A recent synthesis on eggplant seed biology has also emphasised that genotype-driven differences in dormancy and seed-filling potential represent essential targets for future breeding efforts (Popović et al., 2022). Integrating genetic markers with physiological strategies such as delayed extraction could therefore represent an effective dual approach to improving seed quality.

Environmental conditions across production years, although accounting for less than 2% of the variation (Table 2), still exerted a significant influence, consistent with observations by Rahman et al. (2017), who reported seasonal effects on seed mass and yield in Bangladesh. Given that temperature and radiation affect fruit photosynthesis and assimilate partitioning, even small year-to-year fluctuations may translate into measurable differences in seed development.

Altogether, the results of this study demonstrate that both fruit ripening stage and post-harvest extraction time were decisive for determining seed mass in eggplant, with ripening stage being overwhelmingly dominant but extraction time providing a practical means to enhance seed quality. The consistency between our findings and recent research across Solanaceae, Cucurbitaceae, and cereals reinforces the universality of the process in which seeds continue to accumulate reserves after harvest while still enclosed within the fruit. From a practical perspective, storing fruits for 10–20 days before seed extraction represents a low-cost, efficient strategy to improve the mass of 1000 seeds, particularly when fruits are harvested before reaching full physiological ripening. When combined with informed genotype selection and optimised post-harvest storage conditions, this approach offers a robust pathway toward improving seed quality and overall reproductive performance in eggplant.

## Conclusion

This study demonstrates that fruit ripening stage is the primary determinant of the mass of 1000 seeds in eggplant, with fully ripe fruits producing the heaviest seeds across all varieties. Furthermore, post-harvest storage of fruits for up to twenty days enables continued seed filling, significantly increasing seed mass regardless of initial ripening. These findings emphasise the critical roles of both physiological ripening and post-harvest handling in optimising seed development. The demonstrated capacity for continued nutrient accumulation after harvest presents a practical opportunity to refine seed extraction protocols, improve seed vigour, and enhance overall seed yield. Together, these insights contribute to a deeper understanding of eggplant seed biology and provide valuable guidance for breeders and seed producers seeking to improve seed quality and crop productivity. Based on these results, it is recommended to harvest fruits at full physiological ripening and delay seed extraction for at least 20 days after harvest, storing them in a shaded warehouse at ambient temperature (20–25°C), particularly for less ripe fruits and genotypes with lower seed-filling potential, in order to maximise seed mass and vigour.

### Acknowledgements

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# UTICAJ FAZE ZRELOSTI PLODA, DOZREVANJA SEMENA NAKON BERBE I GENOTIPSKE VARIJABILNOSTI NA MASU 1000 SEMENA KOD PLAVOG PATLIDŽANA (*SOLANUM MELONGENA* L.)

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R e z i m e

Ova studija ispitivala je uticaj faze zrelosti ploda i dozrevanja semena nakon berbe na masu 1.000 semena plavog patlidžana (*Solanum melongena* L.) kod tri morfološki različite sorte (srpska, kineska i italijanska). Tokom trogodišnjeg perioda (2017–2019), poljski ogledi su izvedeni na Institutu za ratarstvo i povrtarstvo u Novom Sadu. Plodovi su brani u fazama nedozrelosti, poluzrelosti i pune zrelosti, a zatim čuvani 10 ili 20 dana na 20–25°C pre ekstrakcije semena. Seme je izdvajano nakon trodnevne fermentacije i analizirano prema ISTA pravilima. Rezultati su pokazali da je zrelost ploda ključni faktor, sa više od 97% učešća u varijabilnosti mase semena. Seme iz potpuno zrelih plodova imalo je najveću masu, i do 68% veću u odnosu na seme iz nedozrelih plodova. Dozrevanje nakon berbe povećalo je masu semena u svim fazama, potvrđujući nastavak njegovog razvoja. Seme izdvojeno nakon 20 dana imalo je značajno veću masu u poređenju sa semenom izdvojenim nakon 10 dana. Najveće vrednosti mase 1.000 semena zabeležene su kod srpske sorte u punoj zrelosti, dok je italijanska sorta postigla najviše vrednosti u poluzreloj fazi nakon dozrevanja. Kineska sorta imala je najniže vrednosti u svim tretmanima, što ukazuje na genotipske razlike. Rezultati potvrđuju značaj zrelosti ploda i dozrevanja nakon berbe za kvalitet semena. Odlaganje ekstrakcije predstavlja efikasnu meru za povećanje mase i vitalnosti semena, pružajući značajne smernice oplemenjivačima i proizvođačima semena u cilju unapređenja kvaliteta semena plavog patlidžana.

**Ključne reči:** nalivanje semena, skladištenje nakon berbe, fiziološka zrelost, vreme izdvajanja semena, kvalitet semena.

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