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Understanding the Regular Biological Mechanism of Susceptibility of Tomato Plants to Low Incidences of Blossom-End Rot

Mostafa Abdelkader [1,*], Fathia A. Elkhawaga [2], Ahmed A. Suliman [3], Mikhail Puchkov [4], Kristina Nikolaevna Kuranova [4], Mohamed H. Mahmoud [5] and Mohamed F. M. Abdelkader [6]

1 Horticulture Department, Faculty of Agriculture, Sohag University, Sohag 82524, Egypt
2 Botany Department, National Research Centre, Giza 12622, Egypt
3 Agricultural and Biological Research Institute, National Research Center, Giza 12622, Egypt
4 Laboratory of Selection and Seed Production, Astrakhan State University, 414056 Astrakhan, Russia
5 Department of Biochemistry, College of Science, King Saud University, Riyadh 12372, Saudi Arabia
6 Department of Plant Production, College of Food and Agriculture, King Saud University, Riyadh 12372, Saudi Arabia
* Correspondence: m.abdelkader@agr.sohag.edu.eg

Abstract: Blossom-end rot (BER) is a physiological disorder caused by calcium deficiency, often paired with moisture stress and irregular traditional watering practices. To understand the biological mechanism affecting the incidence of BER, different tomato genotypes were cultivated in sandy soil and were traditionally irrigated. Nine tomato genotypes were investigated to assess their susceptibility to BER, and the incidence rate varied from 7.1% in "Voskhod" to 57.6% in "Majnat" genotypes. This study also comprehensively analyzed various physiological and biochemical parameters to elucidate their correlation with BER incidence in tomato plants. Our findings revealed a range of parameters positively correlated with BER incidence, including the relative water content of roots, the number of fruits per plant, vitamin C content, and potassium content. Parameters related to fruit quality, such as titratable acidity, peroxidase content, and firmness degree, also showed positive correlations with BER incidence. Conversely, parameters such as total yield per hectare, chlorophyll content, average plant height, and calcium content demonstrated strong negative correlations with BER incidence, suggesting potential protective effects against this disorder. This study highlights that genotypes with higher marketable yields, such as 'Voskhod' (65 tons/ha), showed lower BER incidence, underscoring the importance of selecting the appropriate genotype in improving yield and reducing blossom-end rot in tomato fields. Conversely, cultivars with high BER sensitivity, such as 'Chelnok' (26.3 tons/ha), require specially designed cultivation management to avoid production declines.

Keywords: tomato; abiotic stress; antioxidant activity; macronutrient; fruit quality

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1. Introduction

Vegetable production faces numerous challenges, from both biological and environmental factors, often resulting in significant losses. With the global population expanding, there is a growing demand for reliable food sources [1]. However, abiotic stresses, particularly exacerbated by climate change, are increasingly prevalent. Climate changes, characterized by extreme temperatures, erratic rainfall patterns, and more frequent and intense natural disasters, are becoming more common [2,3]. These changes are anticipated to heighten the incidence of stress-related disorders. Furthermore, climate change's short- and long-term effects are projected to exacerbate these extreme weather conditions, further threatening food stability and crop productivity [4–6].

Tomato (*Solanum lycopersicum* L.) is the second most cultivated vegetable crop globally, trailing only behind potatoes. The leading producers in the Mediterranean basin are Turkey, Egypt, Italy, Spain, and Morocco [7]. Egypt stands as one of the leading tomato producers

globally. Nonetheless, over half of the tomatoes cultivated in the country go to waste during various stages of the production, harvesting, retail, and wholesale processes, resulting in substantial reductions in quantity and quality [8]. Blossom-end rot (BER) is a century-old problem and the most devastating physiological disorder [1] to affect various crops such as tomato, pepper, watermelon, and eggplant [9,10]. BER and related disorders primarily affect the fruit, but other parts of the plant can also suffer, such as leaves and flowers [11,12]. BER is a physiological disorder caused by inconsistent watering [13], a notion that has been held up until today. Early studies also showed that BER is of critical concern, as it was linked to significant crop losses due to increased transpiration rates caused by the exceeding applications of nitrogen fertilizers [14]. The presence of BER on tomatoes renders that fruit unmarketable and can cause substantial yield losses [9,15]

Symptoms of BER in tomatoes manifest as small, light-coloured, water-soaked spots on the end of the fruit, a condition linked to cell plasmolysis and membrane permeability issues [16]. Symptoms first appear externally on the pericarp at the distal end, although affected areas may also be present internally in the distal placenta tissue without visible external signs [17]. The affected areas often expand and transform into brown necrotic regions, covering a considerable portion of the fruit and occasionally affecting the entire fruit. Sometimes, BER-affected regions may fail to expand, leading to their disappearance. These areas are often susceptible to invasion by pathogens [18].

Most studies have shown that calcium deficiency promotes BER incidence [19,20]. During fruit growth, the instability and fluctuating calcium concentrations correlate to BER's appearance [21]. Also, water stress, often caused by waterlogged soils, is typically the initial trigger for blossom-end rot (BER), and short periods of water stress (less than one hour) can lead to BER in highly susceptible genotypes. Traditional irrigation systems, such as shallow watering and flooding, promote shallow root systems. Sandy soil with fluctuating moisture levels can also exacerbate moisture problems [22]. Other factors that have been found to favor this physiological disorder include early planting in cold soils, poor fruit setting, and waterlogged soils. In addition, investigations have indicated that high levels of nitrogen in the soil lead to BER development by affecting calcium uptake [23]. As mentioned above, most previous studies focused on the environmental reasons that cause BER, but few investigated the physiological and biochemical mechanisms of BER incidence. So, the hypothesis of this study is to examine the ability of various tomato genotypes to the susceptibility of BER incidence by studying the morphological and physio-chemical attributes of tomato leaves and fruits to reach a complete vision of how the genotype can be a vital factor in BER alleviation.

2. Materials and Methods

2.1. Experimental Conditions

The experiment was conducted on newly reclaimed sandy soil at the experimental station of the Faculty of Agriculture, Sohag University, Egypt. Seeds of eight genotypes (Chelnok, Blagodatny, Dubok, Majnat, Severyanka, Meteor-F1, Cegnom-F1, and Voskhod) were supplied by The Federal Scientific Vegetable Centre (FSBSI), Moscow, Russia, while 023-F1 was provided by the Horticulture Department, Sohag University, Egypt. Firstly, The seeds were sown on plastic trays, and over six weeks tomato seedlings were kept under greenhouse conditions in temperatures ranging from 17 to 22 °C at 60–65% relative air humidity with good ventilation to avoid diseases [24]. Afterward, seedlings (200 seedlings in each genotype) with 4–5 true leaves were transplanted under open-field conditions at the beginning of November. A traditional flooding irrigation (2 times/week) system was applied, and the plants were fertilized with ammonium nitrate at 200 kg/ha divided into three doses. The leaf samples were collected after the flowering stage, while fruits were collected at the fruit-ripening stage. These samples were implemented in various physio-cultivations that have been linked to increased incidences of BER. Furthermore, research indicates that excessive nitrogen fertilizer levels in the soil can contribute to disease development by negatively impacting plant calcium uptake [23].

Measuring phenotypical attributes: phenotypical characteristics of cultivated tomato genotypes were determined at the beginning of the flowering stage, such as plant height (cm), the number of leaves/plant (Pcs), leaf area (cm^2), the fresh and dry weights of leaves and roots (g), and root length (cm).

2.2. Determination of Physio-Biochemical Attributes

Photosynthetic pigments and carotenoids: chlorophyll a and b and carotenoids were measured calorimetrically using acetone (80%) [25].

Proline: proline was extracted from fresh plant leaves and measured at 520 nm using Shimadzu 240 UV/VIS from Japan [26].

Total phenolics: the phenolic contents were determined in tomato plant leaves and fruits by the Folin–Ciocalteau reagent method using spectrophotometry [27].

Total flavonoids: flavonoids were determined in plant leaves and fruits at 415 nm using a spectrophotometer [28].

Total antioxidant activity: the total antioxidant activity was determined in tomato plant leaves and fruits using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) method [29].

Enzyme extraction and assay: antioxidant enzymes were extracted from fresh leaf material (0.5 g) and measured with a spectrophotometer (Shimadzu 240 UV/VIS, Kyoto, Japan).

Peroxidase assay (POD): The assay mixture was performed using a pyrogallol and H_2O_2 mixture, which was prepared according to the Sigma Manual. The change in absorbance was continuously monitored at 420 nm. One unit of enzyme activity was calculated as a 0.001/min change in absorbance [30].

Polyphenol oxidase assay (PPO): The assay mixture was performed using a potassium phosphate buffer (pH 6.0) containing 1.3 mM of pyrogallol and 100 µL of crude extract at room temperature. The increase in absorbance was monitored continuously for 3 min at 430 nm. One unit was determined as the amount of the enzyme that caused a change of 0.001/min in absorbance [30].

Superoxide dismutase assay (SOD): Enzyme activity was determined by measuring the inhibition in photo reduction of nitroblue tetrazolium (NBT) by the SOD enzyme. The absorbance was recorded at 560 nm using a spectrophotometer. One unit (U) of SOD activity was defined as the amount of enzyme causing a 50% inhibition of photochemical reduction of NBT under assay conditions [30].

TSS, lycopene, firmness, and ascorbic acid: The total soluble solids (TSS) percentage in tomato fruits was determined using a Chinese-made digital refractometer, model TD-45 [31]. Lycopene was extracted from fresh tomato samples using a hexane-acetone-ethanol (2:1:1, v:v:v) mixture [32,33]. The lycopene concentration was calculated using its specific extinction coefficient (E 1% 1 cm) of 3450 in hexane. Tomato fruit firmness was measured using a Chinese-made digital fruit hardness tester (model Gy-4). Fruit hardness is the universally accepted measure of fruit ripeness. The fruit Penetrometer accurately measures fruit hardness by measuring the force required to push a plunger tip into the fruit. Ascorbic acid content (vitamin C). Ascorbic acid was measured via methods described by the Association of Analytical Communities, particularly the 2, 6-dichlorophenol indophenol titration method, and expressed as mg·100 g^{-1} FW [34].

Macro-nutrient contents: For estimating N, P, K, and Ca contents in tomato fruits, samples were dried at 70 °C until a constant weight was reached and they were digested [35]. After that, nitrogen content was determined using the modified micro Kjeldahl method [36]. Phosphorus content was estimated by a spectrophotometric method at 650 nm [35]. A flame photometer method was used to estimate potassium and calcium contents [37].

Yield and its components: the average fruit weight (g), number of fruits/plant (Pcs), plant yield (Kg), total yield (ton/ha), incidence rate (%), and marketable yield (ton/ha) were recorded and calculated.

Statistical analysis: The experimental design was a randomized complete block design with four replicates. The obtained data were analyzed using R programming software (V. R-4.4.0). A one-way analysis of variance (ANOVA) was applied to determine statis-

tically significant differences among the studied genotypes. The data were presented as mean ± SD and were compared using the Tukey test ($p < 0.05$).

3. Results

Phenotypical attributes: The data in Table 1 indicated that most cultivated genotypes had medium-to-high growth characteristics, except for Chelnok, which had a compacted growth. Chelnok registered the smallest plant height (<47 cm), number of leaves (<17), and average leaf area (<14 cm^2). The results showed that the above-ground shape and form positively correlated to the root distributions, whereas the root length and weight reduction, as in the Chelnok genotype, may have restricted plant growth. On the other hand, Majnat and Cegnom F1 showed the highest growth and development compared to the other cultivars. By checking the obtained data, we noticed that the most extended plant height (>69 cm) and root length (>35 cm) were produced by Meteor F1. The relative water contents (RWCs) of the leaves did not statistically differ among all studied cultivars and ranged between 90 and 93%, while these percentages were reduced to less than 70% in the roots (Table 1).

Table 1. Phenotypical parameters of the evaluated tomato genotypes for their susceptibility to blossom-end rot.

Parameter	Plant Height, cm	Number of Leaves/Plant	Leaf Area, cm^2	Leaf Fresh Weight, g	Leaf Dry Matter, g	RWC of Leaves, %	Root Fresh Weight, g	Root Dry Matter, g	RWC of Root, %
Chelnok	46.8 ± 1.69 e	16.8 ± 1.69 e	21.2 ± 1.69 f	13.63 ± 0.32 b	1.485 ± 0.04 b	91.07 ± 2.16 a	17.6 ± 0.56 e	5.65 ± 0.20 d	67.8 ± 2.15 a
Blagodatny	57.15 ± 1.62 d	24.1 ± 1.27 bc	35.8 ± 1.13 b	17.19 ± 0.49 a	1.817 ± 0.08 a	91.5 ± 2.12 a	27.9 ± 0.14 b	8.44 ± 0.223 bc	61.45 ± 2.77 c
Dubok	61.7 ± 1.83 c	26.7 ± 0.98 b	26.9 ± 0.14 e	16.84 ± 0.20 a	1.231 ± 0.08 d	92.75 ± 0.35 a	22.65 ± 0.49 c	8.00 ± 0.27 c	64.67 ± 1.46 b
Majnat	69.35 ± 1.90 a	31.15 ± 1.62 a	39.25 ± 1.06 a	17.8 ± 0.42 a	1.58 ± 0.057 b	91.25 ± 0.35 a	35.7 ± 0.42 a	9.59 ± 0.29 b	53.66 ± 2.47 d
Severyanka	60.65 ± 2.33 c	24.85 ± 1.20 bc	25.7 ± 0.84 e	14.05 ± 0.35 b	1.32 ± 0.058 c	90.15 ± 1.20 a	20.7 ± 0.56 d	7.96 ± 0.22 c	61.51 ± 2.14 c
Meteor-F1	54.3 ± 1.83 d	21.6 ± 1.97 d	33.6 ± 0.56 bc	16.45 ± 0.49 a	1.29 ± 0.066 cd	91.35 ± 0.91 a	25.15 ± 0.49 c	12.40 ± 0.42 a	65.50 ± 3.69 ab
Cegnom-F1	66.1 ± 1.27 b	26.35 ± 1.90 b	29.7 ± 0.98 d	15.13 ± 0.89 ab	1.27 ± 0.045 cd	90.5 ± 0.70 a	22.95 ± 0.35 c	10.40 ± 0.27 b	54.65 ± 3.48 d
Voskhod	54.25 ± 1.76	23.55 ± 2.05 cd	22.7 ± 0.89 f	15.5 ± 0.42 ab	1.16 ± 0.07 e	91.7 ± 0.42 a	29.45 ± 0.77 b	12.92 ± 0.10 a	56.54 ± 1.77 d
023-F1	61.85 ± 1.48 c	31.7 ± 2.40 a	32.75 ± 1.06 c	14.95 ± 0.63 b	1.37 ± 0.08 c	91.02 ± 0.59 a	22.7 ± 0.56 c	8.57 ± 0.45 bc	62.23 ± 1.08 bc

Different letter(s) in the same column indicate significant variance between the studied genotypes ($p \leq 0.05$).

Photosynthetic and chlorophyll pigments: At the flowering stage, chlorophyll a content in the evaluated cultivars ranged from 1.70 to 3.46 mg g^{-1} (Table 2). The highest values were found in the leaves of Majnat, without significant differences from Severyanka, Meteor, and Cegnom, respectively. Chlorophyll b values ranged between 1.18 and 1.84 mg g^{-1} (Table 2).

The highest chlorophyll b content values were observed in Majnat leaves. The range of carotenoid content was from 0.62 to 1.53 mg g^{-1} (Table 2). As observed in chlorophyll, the highest values of carotenoids were found in Majnat and Meteor F1.

Superoxide dismutase, peroxidase, polyphenol oxidase, and proline content: Values of superoxide dismutase enzyme (SOD) contents in tomato plant leaves ranged from 79.4 to 130.3 U min g^{-1} (Table 3). The highest value of SOD was found in Meteor F1, while Majnat came in second position without significant differences from Severyanka, Cegnom F1, and Chelnok. Peroxidase enzyme (POD) values ranged from 34.9 to 80.7 U min g^{-1} (Table 3). Meteor F1 recorded the highest significant value of POD, followed by Majnat and Severyanka, with significant differences between them. The lowest significant content of POD was found in Blagodatny. The polyphenol oxidase enzyme (PPO) range was between 6.9 and 17.8 U min g^{-1} (Table 3). The highest content of PPO was found in Meteor F1, as found in SOD and POD results, followed by Majnat and Severyanka, with significant differences among them. Blagodatny represented the lowest value of the PPO enzyme (Table 3). Regarding proline content, it ranged from 30.9 to 576.6 μg g^{-1}. The highest significant value of proline was recorded in Meteor F1, followed by Majnat, Severyanka, and

Cegnom F1, respectively, with significant differences among them. The lowest significant proline content was found in Blagodatny (Table 3).

Table 2. Photosynthetic leaf pigments of the evaluated tomato genotypes for their susceptibility to blossom-end rot.

Tomato Cultivar	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
Chelnok	2.74 ± 0.03 cd	1.30 ± 0.03 cd	1.12 ± 0.10 d
Blagodatny	2.70 ± 0.25 cd	1.20 ± 0.05 de	0.98 ± 0.04 de
Dubok	2.39 ± 0.04 d	1.11 ± 0.08 e	0.94 ± 0.03 e
Majnat	3.46 ± 0.05 a	1.84 ± 0.01 a	1.53 ± 0.02 a
Severyanka	3.38 ± 0.02 ab	1.74 ± 0.03 a	1.34 ± 0.03 b
Meteor-F1	3.30 ± 0.05 ab	1.77 ± 0.06 a	1.44 ± 0.04 ab
Cegnom-F1	3.27 ± 0.19 ab	1.50 ± 0.01 b	1.15 ± 0.06 cd
Voskhod	2.93 ± 0.22 bc	1.42 ± 0.08 bc	1.29 ± 0.04 bc
023-F1	1.70 ± 0.07 e	1.18 ± 0.05 de	0.62 ± 0.07 f

Different letter(s) in the same column indicate significant variance between the studied genotypes ($p \leq 0.05$).

Table 3. Levels of superoxide dismutase (SOD), peroxidase (POD), polyphenol oxidase (PPO), and proline content in the leaves of the evaluated tomato genotypes for their susceptibility to blossom-end rot.

Tomato Cultivar	SOD (U min g^{-1} FW)	POD (U min g^{-1} FW)	PPO (U min g^{-1} FW)	Proline (μg g^{-1} FW)
Chelnok	99.2 ± 4.57 bc	50.9 ± 0.87 d	8.4 ± 0.25 f	52.7 ± 1.14 g
Blagodatny	79.4 ± 0.39 e	27.7 ± 0.66 g	6.9 ± 0.45 g	30.9 ± 1.37 h
Dubok	84.8 ± 2.34 de	51.1 ± 0.32 d	8.7 ± 0.09 f	95.9 ± 5.42 e
Majnat	105.8 ± 2.81 b	57.7 ± 0.61 b	14.5 ± 0.39 b	550.5 ± 2.12 b
Severyanka	100.2 ± 0.28 bc	53.9 ± 1.01 c	13.2 ± 0.17 c	533.1 ± 10.1 c
Meteor-F1	130.3 ± 3.57 a	80.7 ± 0.78 a	17.8 ± 0.19 a	576.6 ± 5.89 a
Cegnom-F1	96.8 ± 3.16 bc	34.9 ± 0.17 f	12.2 ± 0.18 d	317.9 ± 8.03 d
Voskhod	91.0 ± 1.67 cd	48.6 ± 0.43 e	9.8 ± 0.18 e	90.4 ± 2.05 ef
023-F1	87.4 ± 2.64 de	47.0 ± 0.55 e	12.2 ± 0.39 d	86.4 ± 5.39 f

Different letter(s) in the same column indicate significant variance between the studied genotypes ($p \leq 0.05$).

Phenolics, flavonoids, and antioxidant activity in leaves: Total phenolics in tomato plant leaves ranged between 13.73 and 26.10 mg g^{-1} (Table 4). The highest value was found in Meteor F1, followed by Majnat, Severyanka, and Cegnom F1, respectively, with significant differences. The lowest phenolic content was recorded in Voskhod. The total flavonoid content ranged from 1.75 to 6.48 mg g^{-1} (Table 4). The highest significant values were found in Meteor F1, followed by Majnat and Severyanka, with significant differences. The Voskhod cultivar represented the lowest significant value of total flavonoids. Total antioxidant activity ranged from 51.9 to 150.0 μMol Trolox g^{-1} (Table 4). Meteor-F1 recorded the highest significant values of antioxidant activity, as found in the phenolics and flavonoid results, while Majnat came in second position without significant differences from Severyanka and Cegnom F1.

Table 4. Levels of total phenolic, total flavonoids, and total antioxidant activity in the leaves of the evaluated tomato genotypes for their susceptibility to blossom-end rot.

Tomato Cultivar	Total Phenolics (mg g^{-1} DW)	Total Flavonoids (mg g^{-1} DW)	Total Antioxidant Activity (µMol Trolox g^{-1} DW)
Chelnok	17.71 ± 0.33 ef	2.39 ± 0.03 de	64.2 ± 3.72 d
Blagodatny	17.20 ± 0.38 f	2.12 ± 0.01 ef	111.1 ± 2.06 c
Dubok	15.90 ± 0.36 g	2.07 ± 0.06 f	51.9 ± 3.50 e
Majnat	23.85 ± 0.25 b	3.83 ± 0.07 b	133.5 ± 4.72 b
Severyanka	21.22 ± 0.70 c	2.67 ± 0.18 cd	123.1 ± 4.44 b
Meteor-F1	26.10 ± 0.11 a	6.48 ± 0.10 a	150.0 ± 0.78 a
Cegnom-F1	20.42 ± 0.53 d	2.49 ± 0.05 d	127.1 ± 3.88 b
Voskhod	13.73 ± 0.33 h	1.75 ± 0.13 g	58.2 ± 1.66 de
023-F1	18.00 ± 0.57 e	2.85 ± 0.05 c	105.5 ± 5.60 c

Different letter(s) in the same column indicate significant variance between the studied genotypes ($p \leq 0.05$).

Phenolics, flavonoids, antioxidant activity, soluble sugars, and lycopene contents: At the red-ripening stage of tomato fruit, total phenolics ranged between 22.8 and 36.8 mg g^{-1} (Table 5). The highest significant values were found in "Voskhod". "Severyanka" came in second position, followed by "Cegnom F1", with significant differences. The lowest phenolic contents were recorded in Dubok and 023 F1, without significant differences.

Table 5. Levels of total phenolics, total flavonoids, total antioxidant activity, total soluble sugars, and lycopene content in the fruits of the evaluated tomato genotypes for their susceptibility to blossom-end rot.

Tomato Cultivar	Total Phenolics (mg g^{-1} DW)	Total Flavonoids (mg g^{-1} DW)	Total Antioxidant Activity (µMol Trolox g^{-1} DW)	Total Soluble Sugars (mg g^{-1} DW)	Lycopene (mg 100 g^{-1} FW)
Chelnok	30.4 ± 0.47 d	2.77 ± 0.07 c	164.8 ± 2.05 b	59.1 ± 0.98 b	2.78 ± 0.05 c
Blagodatny	27.8 ± 0.97 e	2.38 ± 0.01 c	94.1 ± 0.89 f	55.7 ± 0.88 bc	2.62 ± 0.02 cd
Dubok	23.3 ± 0.44 g	2.44 ± 0.03 c	118.4 ± 2.27 d	57.4 ± 1.42 bc	2.54 ± 0.05 cd
Majnat	36.8 ± 1.09 a	3.92 ± 0.01 b	167.4 ± 1.88 b	78.5 ± 1.41 a	3.30 ± 0.05 b
Severyanka	34.3 ± 0.05 b	3.11 ± 0.01 bc	124.7 ± 2.38 c	57.6 ± 3.22 bc	2.66 ± 0.023 cd
Meteor-F1	26.4 ± 0.89 f	3.22 ± 0.15 bc	106.3 ± 1.50 e	56.7 ± 0.65 bc	2.59 ± 0.03 cd
Cegnom-F1	31.8 ± 0.98 c	3.69 ± 0.13 b	93.3 ± 2.44 f	52.5 ± 0.76 c	2.44 ± 0.03 de
Voskhod	37.4 ± 0.82 a	5.46 ± 0.06 a	199.7 ± 1.00 a	78.7 ± 1.07 a	3.59 ± 0.06 a
023-F1	22.8 ± 1.63 g	2.76 ± 0.03 c	73.6 ± 1.72 g	47.2 ± 1.42 d	2.20 ± 0.08 e

Different letter(s) in the same column indicate significant variance between the studied genotypes ($p \leq 0.05$).

Total flavonoid content ranged from 2.38 to 5.46 mg g^{-1} (Table 5). The highest significant value was found in Meteor F1. Majnat came in second position, without significant differences from Severyanka, Cegnom F1, or Voskhod. Total antioxidant activity in fruits ranged from 73.6 to 199.7 µMol Trolox g^{-1}. Voskhod recorded the highest significant value of antioxidant activity, as found in phenolics and flavonoid results, while Majnat came in second position, without significant differences from Chelnok. Soluble sugar contents in tomato fruits ranged from 47.2 to 87.7 mg g^{-1} (Table 5). Meteor F1 and Majnat recorded the highest significant contents, while 023 F1 represented the lowest value of total soluble sugars (Table 5). Lycopene contents in tomato fruit ranged from 2.20 to 3.59 mg 100 g^{-1} (Table 5). The highest significant value of lycopene was found in Meteor F1, followed by

Majnat, with significant differences between them. The lowest value of lycopene was found in 023 F1 (Table 5).

Macro-nutrient contents in tomato fruits: At the red-ripening stage of tomato fruit, nitrogen content values in the evaluated cultivars ranged from 2.5 to 5.0% (Table 6). The highest values were found in "Voskhod" and Meteor F1. The "023 F1" cultivar represented the lowest values of N, without significant differences between them.

Table 6. Levels of macro-nutrients (nitrogen, phosphorus, potassium, and calcium) in the evaluated tomato genotypes for their susceptibility to blossom-end rot.

Cultivar	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)
Chelnok	3.4 ± 0.09 c	0.22 ± 0.01 ab	3.86 ± 0.04 ab	0.32 ± 0.02 b
Blagodatny	3.0 ± 0.12 d	0.15 ± 0.02 cd	4.16 ± 0.02 a	0.24 ± 0.01 c
Dubok	3.2 ± 0.12 cd	0.20 ± 0.02 a–c	3.81 ± 0.04 ab	0.23 ± 0.01 c
Majnat	2.6 ± 0.12 e	0.18 ± 0.02 b–d	3.40 ± 0.06 bc	0.33 ± 0.01 ab
Severyanka	4.6 ± 0.12 b	0.19 ± 0.01 a–c	3.28 ± 0.06 bc	0.36 ± 0.01 ab
Meteor-F1	4.9 ± 0.06 a	0.22 ± 0.01 ab	3.36 ± 0.04 bc	0.35 ± 0.02 ab
Cegnom-F1	4.5 ± 0.09 b	0.19 ± 0.01 a–c	3.39 ± 0.06 bc	0.34 ± 0.02 ab
Voskhod	5.0 ± 0.09 a	0.25 ± 0.01 a	3.11 ± 0.04 c	0.39 ± 0.01 a
023-F1	2.5 ± 0.09 e	0.13 ± 0.01 d	3.86 ± 0.04 ab	0.25 ± 0.01 c

Different letter(s) in the same column indicate significant variance between the studied genotypes ($p \leq 0.05$).

The values of phosphorus ranged from 0.13 to 0.25%. The highest phosphorus content was found in Voskhod, without significant differences from Severyanka, Dubok, Meteor F1, and Cegnom F1 (Table 6). The lowest value of phosphorus was found in the 023 F1 cultivar, and its result was statistically similar to that obtained from Blagodatny and Majnat genotypes. The potassium range was between 3.28 and 4.16% (Table 6). Blagodatny recorded the highest level of potassium content, without significant differences from Chelnok, Dubok, and 023 F1. Calcium contents in tomato fruits ranged from 0.23 to 0.39% (Table 6). The highest value of Ca was found in Majnat, as were in N, P, and K values, without significant differences from Severyanka, Meteor F1, Cegnom F1 and Voskhod. The lowest content of Ca was found in 023 F1, without significant differences from Blagodatny and Dubok.

Yield and its components: A significant ($p < 0.05$) difference in tomato yield and its components was recorded in response to differences among cultivated genotypes. Average fruit weights ranged from small sizes (68.4 g) in Chelnok to large sizes (110.5 g) in Majnat. All other studied genotypes produced medium fruit weights (83–104 g). Plant yield ranged from 1.85 kg to 2.58 kg, and, as expected, Chelnok produced the lowest yield, while 023 F1 gave the maximum yield per plant. Incidence rates varied among cultivars depending on their susceptibility to BER (Table 7). The lowest incidence rate (7.1%) was observed in the Voskhod cultivar, while the maximum rate (57.7%) was registered in the "Majnat" genotype, which reflected on the marketable yield per hectare, whereas it reached 65 tons in Voskhod, and decreased by 26.3 ton in Chelnok (Table 7).

TSS, lycopene, firmness, and ascorbic acid: The total soluble solids (TSS) produced by tomato plants were significantly affected by the cultivars. TSS content values in the evaluated cultivars ranged from 3.0 to 5.8% at the red stage of tomato fruit ripening. It was found that the TSS produced by tomato plants was significantly affected by the cultivars, as the difference was significant between the TSS values. Severyanka recorded the highest value (5.8).

This was followed by Chelnok, which recorded (5.5) a significant difference between the Blagodatny, Majnat, Meteor F1, Cegnom F1, Voskhod, and 023 F1 cultivars. Dubok recorded the lowest value among the varieties under study (3.0%). Acidity is one of the most critical chemical characteristics of fruits. In Table 8, we find a significant difference

between the varieties under study in terms of the degree of acidity of the fruits. Where Chelnok recorded the highest value (3.84), it was followed by Dubok, which recorded a value of 3.71, and Majnat recorded the lowest value. Tomatoes are considered one of the fruits rich in vitamins, especially vitamin C.

Table 7. Yield components of the evaluated tomato genotypes for their susceptibility to blossom-end rot.

Parameter	Fruit Weight, g	Number of Fruits/Plant	Plant Yield, kg	Incidence Rate, %	Marketable Yield, ton/ha
Chelnok	68.4 ± 2.33 d	27.04 ± 1.145 b	1.85 ± 0.141 e	52.7 ± 3.67 b	26.3 ± 4.04 f
Blagodatny	102.5 ± 3.5 b	21.35 ± 0.774 e	2.19 ± 0.154 c	12.4 ± 1.34 e	57.5 ± 3.18 c
Majnat	92.5 ± 3.53 c	24.07 ± 1.302 cd	2.22 ± 0.035 c	57.7 ± 3.46 a	28.2 ± 2.76 f
Dubok	110.5 ± 4.9 a	20.02 ± 0.50 f	2.21 ± 0.16 a	9.90 ± 1.20 e	62.0 ± 5.46 a
Severyanka	87.5 ± 3.53 c	23.20 ± 2.95 d	2.02 ± 0.176 d	11.4 ± 1.83 e	53.7 ± 3.58
Meteor-F1	90.1 ± 4.45 c	30.73 ± 0.420 a	2.77 ± 0.09 a	42.2 ± 2.75 c	47.9 ± 0.57 e
Cegnom-F1	92.5 ± 3.53 c	25.83 ± 0.070 b	2.39 ± 0.084 b	11.8 ± 2.19 e	63.1 ± 0.67 b
Voskhod	83.1 ± 4.10 c	26.20 ± 0.60 b	2.16 ± 0.056 c	7.10 ± 2.12 f	65.1 ± 0.20 bc
023-F1	104.3 ± 5.16 b	24.38 ± 0.204 c	2.57 ± 0.10 a	33.0 ± 3.11 d	51.7 ± 0.27 d

Different letter(s) in the same column indicate significant variance between the studied genotypes ($p \leq 0.05$).

Table 8. Fruit quality of the evaluated tomato genotypes for their susceptibility to blossom-end rot.

Tomato Cultivar	T.S.S %	Acidity mg/100 g	Vitamin C mg/100 g	Firmness kg/cm^2
Chelnok	5.5 ± 0.01 b	3.84 ± 0.01 a	20.23 ± 0.04 a	4.2 ± 0.02 c
Blagodatny	3.5 ± 0.03 g	3.24 ± 0.01 b	16.51 ± 0.02 bc	4.1 ± 0.02 d
Dubok	3.0 ± 0.02 d	3.71 a ± 0.01	18.72 ± 0.04 ab	4.2 ± 0.03 c
Majnat	5.3 ± 0.01 c	2.19 ± 0.02 cd	17.25 ± 0.04 b	4.2 ± 0.01 c
Severyanka	5.8 ± 0.03 a	2.31 ± 0.02 e	19.19 ± 0.06 a	4.3 ± 0.02 c
Meteor-F1	5.0 ± 0.01 d	2.22 ± 0.01 c	16.62 ± 0.04 bc	6.5 ± 0.03 a
Cegnom-F1	4.1 ± 0.03 f	3.46 ± 0.01 c	16.06 ± 0.06 bc	4.2 ± 0.02 c
Voskhod	4.4 ± 0.02 e	3.17 ± 0.02 d	17.83 ± 0.06 b	4.0 ± 0.03 d
023-F1	5.2 ± 0.01 c	3.08 ± 0.01 b	17.25 ± 0.04 b	6.0 ± 0.03 b

Different letter(s) in the same column indicate significant variance between the studied genotypes ($p \leq 0.05$).

Therefore, acidity is considered one of the chemical characteristics preferably evaluated in fruits. The results presented in Table 8 show that Chelnok recorded the highest value, followed by Severyanka. Meanwhile, there was no significant difference between Majnat and Meteor F1. As shown in Table 8, it is clear that the highest value for fruit hardness was recorded for Meteor F1, followed by 023 F1, while Voskhod recorded the lowest value for fruit firmness.

The relationship between incidence rate and the measured parameters: The correlation analysis (Table S1) revealed significant associations between various physiological and biochemical parameters and the incidence rate of blossom-end rot in tomatoes. Root water content (RWC) exhibited a strong positive correlation (r = 0.841) with BER incidence, indicating that higher RWCs in roots may contribute to increased susceptibility to BER. Similarly, parameters such as the number of fruits per plant (r = 0.495), RWC of leaves (r = 0.475), vitamin C content (r = 0.453), and potassium content (r = 0.437) showed positive correlations with BER incidence (Figure 1). Other factors, such as titratable acidity, peroxidase content, and firmness degree, also exhibited positive correlations, although to a lesser extent. Conversely, parameters like total yield per hectare (r = −0.925), chlorophyll a content

(r = −0.463), and average plant height (r = −0.453) showed strong negative correlations with BER incidence, suggesting potential protective effects against this physiological disorder.

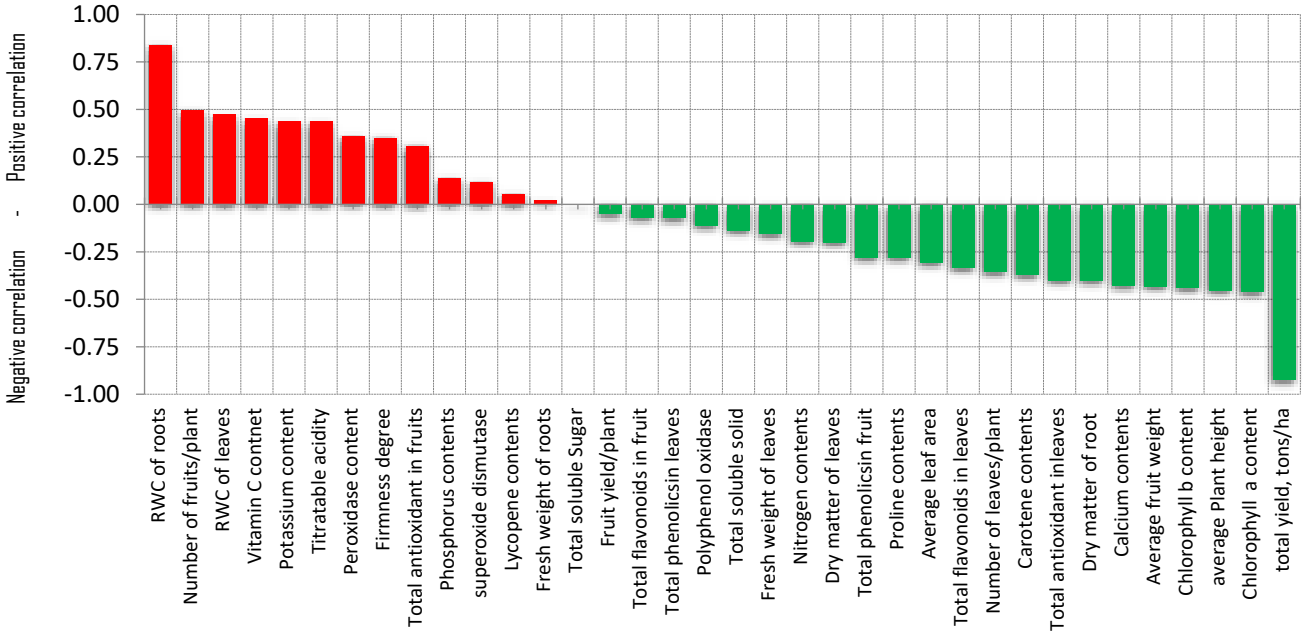


Figure 1. Pearson correlation between the incidence rate of blossom-end rot and the biological characteristics of the evaluated tomato genotypes.

4. Discussion

Investigation into the underlying reasons behind blossom-end rot in tomatoes has been extensively documented in the scientific literature over recent decades [16,38]. BER affects tomatoes cultivated in both fields and greenhouses, with no documented occurrences reported in wild species [16].

BER occurrence seems to be independent of plant growth rate itself, as it can either hinder or promote plant growth. However, it does seem to correlate with the rate of fruit growth and/or potential fruit size across different cultivars, indicating a genetic influence on susceptibility to BER [39,40]. In our study, the cultivated tomato genotypes varied in morphology characteristics such as plant height, the number of leaves, and root length. These phenotypical differences can affect various physiological processes within the plant. Chlorophyll, for instance, is one of the essential molecules in plants, as it is responsible for photosynthesis and for converting solar light into chemical energy [41,42]. The variation in morphological traits among the genotypes can potentially impact chlorophyll content and distribution, thereby affecting the overall photosynthetic efficiency and growth performance of the plant. In this study, Majnat was superior in photosynthetic pigments, i.e., chlorophyll a, b, and carotenoids (3.46, 1.84, and 1.53 mg g^{-1}, respectively), compared to Meteor F1 (Table 2). Numerous studies have reported that different tomato genotypes varied in chlorophyll content. Other studies are consistent with the findings of our investigation: that chlorophyll content was negatively correlated with blossom-end rot in tomatoes [43].

Nutrient deficiency is considered a kind of abiotic stress affecting plants [44]. Calcium deficiency is the leading cause of blossom-end rot in tomatoes [45]. Calcium deficiency results in membrane degradation caused by lipid peroxidation and the production of reactive oxygen species [43]. Antioxidant compounds have defensive properties that protect plants from oxidative damage from free radicals [46]. Antioxidants stimulate the plants to withstand various stress conditions. Antioxidants could be enzymatic, such as superoxide dismutase, peroxidase, and polyphenol oxidase [47], or non-enzymatic, such as vitamin C, lycopene, flavonoids, and phenolics [48]. In this study, Meteor F1 was superior in SOD, POD, and PPO contents (130.3, 80.7, and 17.8 U min g^{-1}, respectively), as shown in Table 3.

Our results also showed that the highest total phenolics, flavonoids, and antioxidants in tomato plant leaves were in Meteor F1 (26.1, 6.48 mg $g^{-1,}$ and 150 µMol Trolox g^{-1}, respectively) (Table 4). Consequently, Meteor F1 was superior in total phenolics content (37.4 mg g^{-1}) in tomato fruits compared to Majnat (36.8 mg g^{-1}), as presented in Table 4. Meteor F1 also had the highest flavonoids and total antioxidants (5.46 mg g^{-1} and 199.7 µMol Trolox g^{-1}, respectively) in tomato fruits (Table 5). These results are in harmony with the results of [47], which evaluated different tomato varieties and reported a significant difference between them in SOD, POD, PPO, total phenolics, and antioxidants content. Also, the depletion of Ca increased the blossom-end rot in tomato and bell pepper plants, and was accompanied by a decrease in SOD and POD content [49,50]. The high total phenolics, antioxidants, lycopene, and vitamin C contents were related to Ca concentrations in tomato plants [51].

Proline, an amino acid, is highly beneficial in plants exposed to various stress conditions. Besides acting as an excellent osmolyte, proline plays significant roles during stress as a metal chelator, an antioxidative defense molecule, and a signaling molecule [52]. The current study found the highest proline content in Meteor F1, followed by Majnat and Severyanka (576.6, 550.0, and 533.1 µg g^{-1}, respectively). Several researchers recorded different proline contents in various tomato cultivars [47,53,54]. Generally, proline was negatively correlated with BER incidence in our experiment, and a large variation among cultivars has been observed. In our study, Meteor F1 exhibited the highest proline content, followed by Majnat and Sereryanka. However, despite Meteor F1 and Majnat having maximum proline contents, they showed high BER percentages. Conversely, Sereryanka genotypes, which also had elevated proline contents, demonstrated notably lower BER incidences. This discrepancy suggests that while proline accumulation is generally associated with stress tolerance, its presence alone may not be sufficient to mitigate BER occurring, and other physiological or genetic factors may be at play.

Total soluble sugars is one of the main components contributing to the taste of tomato fruit [55]. Our results indicated that Meteor F1 and Majnat were superior in total soluble sugar content (78.7 and 78.5 mg g^{-1}, respectively). These results agree with other scientists' findings [56–58], who evaluated different tomato varieties and reported variations in soluble sugar levels in each variety. The most crucial carotenoid in tomato fruits is lycopene. Lycopene is a red compound with intense antioxidant activity [59]. This work found the highest lycopene values in Meteor F1, followed by Majnat (3.59 and 3.30 mg 100 g^{-1}, respectively). These results agree with those of [60,61].

Nitrogen (N) is a valuable macronutrient in plants. It is necessary for building proteins, amino acids, nucleic acids, phospholipids, and several secondary metabolites [45]. The data presented in Table 5 indicate that Majnat and Meteor F1 were superior in N content (5.0 and 4.9%, respectively), followed by Severyanka and 7 (4.6 and 4.5%, respectively). Phosphorus (P) is also a vital macronutrient. It is the critical component of many biological processes, such as energy metabolism (ATP) and the synthesis of nucleic acids, cell membranes, phospholipids, and amino acids [45]. In this study, the highest level of P in tomato fruits was recorded in Majnat (0.25%), as well as in Meteor F1 (0.22%), Dubok (0.20), Severyanka (0.19%), and Cegnom F1 (0.19%), as shown in Table 5. Potassium (K) is the third most essential macronutrient and a richly absorbed cation that plays a significant role in meristematic development and in carrying out functions such as gas exchange and water regulation in plants, protein synthesis, enzyme activations, photosynthesis, and carbohydrate transport [62]. Our results show that Blagodatny was superior in K content (4.16%), in addition to Chelnok (3.86%), 023 F1 (3.86%), and Dubok (3.81%).

Calcium (Ca) is a secondary messenger of shock during stress events. It stabilizes the normal functioning of cell membranes, where a concentration of free Ca is maintained in the cytoplasm at sub-micromolar levels to facilitate intracellular signaling. In the current study, the highest value of Ca was found in Majnat (0.39%), as well as in Severyanka (0.36%), Meteor F1 (0.35%), Cegnom F1 (0.34%), and Voskhod (0.33%) as shown in Table 5. These results agree with those that indicated that the mineral content of tomato fruits (N, P,

and K) was significantly affected by cultivars [57]. Calcium deficiency is the leading cause of blossom-end rot in tomatoes. A high concentration of K can exacerbate blossom-end rot. This is because an excess of K can interfere with Ca uptake by plants, leading to Ca deficiency even if there is enough Ca in the soil [43].

Generally, the data demonstrated that Voshod, Severyanka, and Cegnom F1 had the highest nitrogen (N) and calcium (Ca) contents, coupled with the lowest BER incidences, which supports the established theory that a sufficient calcium content is critical in preventing BER, while the Meteor F1 genotype did not follow the same trend and showed a high BER incidence. This exception highlights the complexity of BER susceptibility and may lead back to genotypic factors. Potassium levels can inhibit calcium accumulation. In our study, this antagonistic effect was also observed. The results indicated that varieties with higher potassium contents, such as Chelnock and Dubok, have higher rates of BER. This due to the fact that excess potassium can interfere with calcium absorption, leading to calcium deficiency and increased susceptibility to BER.

Yield data highlight significant differences in tomato yield and its components among different cultivated genotypes. These differences are evident in various parameters, such as average fruit weight, plant yield, incidence rates of BER, and marketable yield per hectare. The differences in BER incidence rates directly impact the marketable yield per hectare. "Voskhod" demonstrates the highest marketable yield (65 tons), followed by other genotypes, while "Chelnok" shows decreased marketable yields (26.3 tons) due to higher BER incidence rates. Our data underscore the vital impact of genotype selection in tomato cultivation to optimize yield and minimize losses caused by blossom-end rot. Cultivars like "Voskhod", with a medium fruit size and lower BER incidence rates, can contribute significantly to higher marketable yields, whereas cultivars such as "Chelnok" may require additional management strategies to mitigate the yield losses associated with BER (Figure 2). This is in harmony with those who found that the occurrence of blossom-end rot is related to metabolic disorders regulated with gene expression in stressed plants [63].

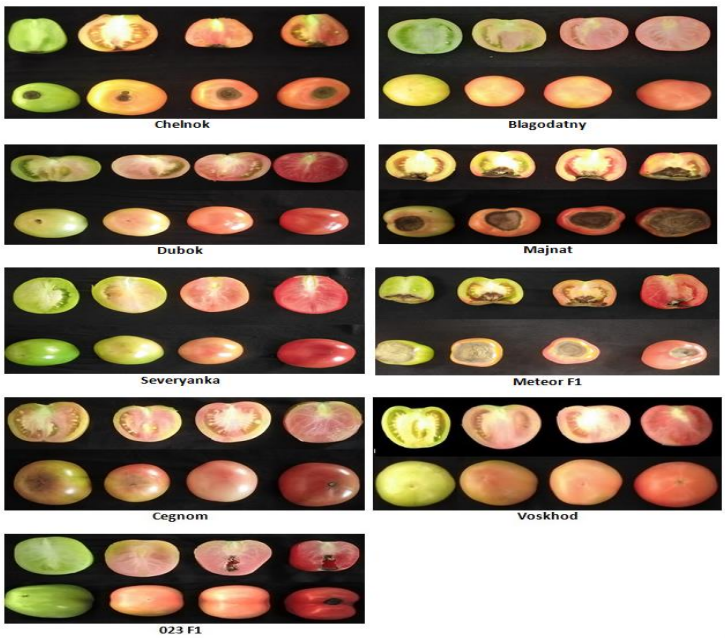


Figure 2. The development of blossom-end rot symptoms in the cultivated tomato genotypes.

The analysis revealed a range of physiological and biochemical parameters significantly correlated with the incidence rate of BER in tomato plants. Notably, parameters such as RWC, the number of fruits per plant, the RWC of leaves, vitamin C content, and potassium content showed positive correlations with BER incidence, indicating potential factors contributing to increased susceptibility to this disorder. Additionally, parameters related to fruit quality, including titratable acidity, peroxidase content, and firmness degree,

exhibited positive correlations with BER incidence, albeit to a lesser extent. Conversely, parameters such as chlorophyll a content demonstrated strong negative correlations with BER incidence, suggesting potential protective effects against this physiological disorder. These findings shed light on the complex interplay between physiological and biochemical factors influencing BER susceptibility in tomato plants, providing valuable insights for future research and management strategies in tomato cultivation.

5. Conclusions

This study provides crucial insights into the dynamics of blossom-end rot (BER) in tomato fields, highlighting the significant role of genotypic variability in susceptibility to BER. By investigating a range of physio-biochemical parameters in diverse tomato genotypes, we identified critical factors contributing to BER incidence, such as water content, number of fruits, vitamin C content, potassium levels, titratable acidity, peroxidase content, and fruit firmness. These findings not only advance the scientific understanding of BER in tomatoes, but also lay the groundwork for targeted interventions to improve tomato production. In addition, the correlation between specific morphological and physiological characteristics and BER incidence will enable researchers to better predict BER rates in tomato genotypes. Utilizing this knowledge and adopting good farming practices can enhance productivity by reducing losses due to BER, thereby promoting sustainable tomato farming globally.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10060648/s1, Table S1: Coefficients of correlation for the different pairs of studied parameters of tomato genotypes (Pearson correlation matrix analysis).

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