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Evaluation of some Tomato (*Solanum Lycopersicum* L.) Genotypes for Blossom-End Rot Incidences through Physiological Measurements and ISSR-Markers

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Abstract
Irregular traditional watering practices paired with moisture stress and calcium deficiency cause Blossom-end rot (BER) in tomatoes. As with other abiotic stresses and physiological disorders, BER affects the growth of tomato plants phenotypically and physiologically. In this study, we measured some physiological parameters as a response to BER in nine tomato genotypes, indicating the health and strength of plants and fruit yield. Genotypes varied in the studied traits; Severyanka genotype recorded the highest results in maturity and fruit taste, while Dubok was the lowest in these two traits. While the 023-F1 as a locally grown genotype recorded good Chl/Car ratio results. Also, we used ISSR PCR technique to detect molecular markers for tomato genotypes that give 76.07% polymorphism, 0.363 PIC (Polymorphic Information Content), 0.479 of estimated He (Expected Heterozygosity) and 4.72 for EMR (Effective Multiplex Ratio). Based on the ISSRs data analysis with the UPGMA method, the nine-tomato genotypes were divided into two main groups according to their similarities. Six positive ISSR markers were found related to the Chl/Car ratio for the locally grown 0.23-F1 genotype, and by drawing the kinship tree, we found that it belongs to the group of some genetically superior genotypes, such as Severyanka and Majnat. The locally grown 023-F1 genotype was very similar in the studied traits to western genotypes and can be introduced into breeding programs to improve its traits for BER resistance further.

Keywords: Tomato, Abiotic stress, Blossom-end rot, Physio-biochemical traits, ISSRs molecular markers

1. Introduction

Tomato (*Solanum lycopersicum* L.) is an annual herbaceous plant in the Solanaceae family. Tomatoes are the second most consumed and commercially significant vegetable farmed globally. It also plays a major role in the agricultural sector. It is well known that tomato output has significantly expanded globally [1].

Numerous biotic and abiotic conditions make it challenging to produce vegetables, frequently leading to a significant output loss throughout each growth cycle. Blossom-end rot (BER) is one of the most destructive physiological disorders that affect a variety of crops, including watermelon (*Citrullus lanatus* (Thunb.), eggplant (*Solanum melongena* L.), pepper (*Capsicum annuum* L.), and tomato (*Solanum lycopersicum* L.). Multiple research investigations examining the physiological features of the illness have shown that irregular watering circumstances in accessions that are mostly cultivated and disrupted calcium (Ca^{2+}) homeostasis are linked to the underlying causes of BER [2, 3].

Several physiological and biochemical parameters were positively correlated with BER incidence, including vitamin C content, potassium content, roots' relative water content, and the number of fruits per plant. Also, parameters related to fruit quality, such as firmness degree, peroxidase content and titratable acidity showed positive correlations with BER incidence. On the contrary, calcium content, average plant height, total yield per hectare and chlorophyll content strongly demonstrated negative correlations with BER incidence, which reflect potential protective effects against this disorder [4].

Genetic studies, especially molecular markers, are considered powerful tools that have revolutionized plant improvement strategies and enabled breeders to select plants with desirable traits at an early stage. These markers are DNA sequences that can pinpoint the genome sequences in plants that oversee critical characteristics, including drought tolerance, disease resistance, and yield potential. The effectiveness and precision of molecular markers have significantly increased due to technological advancements, making them a vital tool in plant breeding initiatives [5].

As a microsatellite assay variation, inter simple sequence repeat markers (ISSRs) combine the benefits of various marker types such as AFLP, RAPD, and SSR. Despite being a dominant marker, it has been widely used for studies of genetic diversity, phylogeny, genomics, and evolutionary biology. Additionally, because the sequences to be amplified show high

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polymorphism in the material, it is more reproducible than RAPD and has a simpler technical design. The quick preliminary characterization of breeding material and the supply of locus-specific molecular markers can be achieved with ISSRs [6, 7]. In this work, we employed the ISSR markers to screen the genetic diversity of tomato (*Solanum lycopersicum* L.) genotypes, both exotic and locally cultivated, to determine resistance and susceptibility to blossom end rot.

2. Experimental (Materials and Methods)

2.1. Plant materials and field experiment

At the experimental station of the Faculty of Agriculture, Sohag University, Egypt, the study was carried out on recently reclaimed sandy soil. Eight tomato genotypes were provided by the Federal Scientific Center for Vegetables (FSBSI), Moscow, Russia; their names were Chelnok, Blagodatny, Dubok, Majnat, Severyanka, Meteor-F1, Cegnom-F1, and Voskhod, whereas 023-F1 was provided by the Horticultural Department, Sohag University, Egypt. At first, tomato seedlings were planted on plastic trays and raised in a greenhouse for six weeks, with good ventilation and temperatures between 17 and 22°C and 60 to 65 % relative humidity, to prevent disease, according to [4].

Then, at the start of November 2023, seedlings with four to five true leaves (200 seedlings per genotype) were transplanted in an open field at Sohag University experimental station, Sohag, Egypt. A conventional flooding irrigation system was used twice a week, and the plants received three doses of 200 kg/ha of ammonium nitrate fertilizer. Fruit samples were taken during the fruit-ripening stage, whilst leaf samples were taken following the flowering stage. These specimens were employed in diverse physio-cultivations associated with elevated Blossom-end rot (BER) occurrences.

2.2. Physio-biochemical and fruit measurement

The leaf dry matter (LDM) and the root dry matter (RDM) were estimated. Chlorophyll: chlorophyll a and b and carotenoids were measured calorimetrically using acetone (80%) [8]. Marketable yield (ton/acre), fruit maturity degree and fruit taste index were measured calorimetrically using acetone (80%) according to [4, 9-11].

2.3. Isolation of genomic DNA & ISSR-PCR reactions

Genomic DNA was extracted from leaves according to the modified CTAB protocol of [12], which has some modifications according to [13]. The quality and quantity of DNA were assessed spectrophotometrically using the Bio-Rad SmartSpec 3000 UV/V spectrophotometer through 0.8% agarose gel electrophoresis for 30-min at 100 volts. The DNA concentration was adjusted to 10ng/µL.

Fifteen ISSR primers were used to characterize the nine tomato samples (Table 1), manufactured by Macrogen company (Korea) and were used for PCR amplification [6, 14]. All PCR reactions were performed in a total volume of 25 µL using a thermocycler (Applied Biosystems™4375305). The PCR reaction mixture contained 12.5 µL of COSMO PCR RED Master Mix DNA Polymerase (WF10203001 Co., Ltd.), 2.0 µL of genomic DNA (10 ng/µL), 2.0 µL of each primer (10 M), and 6.5 µL of distilled water to adjust the final volume of the PCR product.

The ISSRs-PCR program included an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 48°C for 1 minute, and extension at 72°C for 2 min. After the last cycle, a final step of 3 minutes at 72°C to complete the final extension. ISSR amplified products were separated by 40 min electrophoresis at 80 V on a 1.5% agarose gel and visualized using ethidium bromide (10 mg/mL) in 1X TBE buffer. After electrophoresis, the DNA profiles were visualized on a UV transilluminator in a gel documentation system and photographed for analysis. The DNA marker used in this investigation was GeneRulerTM 100bp DNA ladder (Fermentas, Thermo Scientific™ Catalog Number SM0241, SM0242).

2.4. Allele scoring and diversity analysis

The gel images were analyzed using the GelAnalyzer3 (http://www.gelanalyzer.com) to determine the molecular sizes of the amplified fragments. The amplified fragments were scored as present (1) or absent (0). Polymorphic Information Content (PIC), Expected Heterozygosity (He), and Effective Multiplex Ratio (EMR) values were determined using the online program (https://irscope.shinyapps.io/iMEC/) according to [15]. Cluster analysis was conducted using NTSYSpc software with the UPGMA algorithm to construct the dendrogram of similarities and relationships between tomato genotypes [16].

2.5. Statistical analysis

Physio-biochemical traits data were statistically analysed using analysis of variance (ANOVA) by the SPSS software (version 17). Then calculated the ratio (% percentage). of each trait which is more efficient to express the incidence in the nine genotypes according to BER.

Table 1: List of ISSR primer and sequences

No.	Primer Name	Sequence	No.	Primer Name	Sequence
1	ISSR-1	(CAC)3GC	9	ISSR-10	DBD(CA)7
2	ISSR-3	(TC)8A	10	ISSR-19	(AG)8T
3	ISSR-4	(AC)8C	11	ISSR-21	(GA)8T
4	ISSR-5	(CA)6AC	12	ISSR-22	(CT)8G
5	ISSR-6	(AG)8YT	13	ISSR-23	(AG)8YT
6	ISSR-7	(AGC)4YT	14	ISSR-26	(AC)8
7	ISSR-8	(CA)8R	15	ISSR-27	T(AG)9
8	ISSR-9	(GA)8YG			

Where is Y= (T, C) and R= (A, G)

3. Results and discussion

3.1. Physio-biochemical determination attributes

Leaves dry matter (LDM) varied across the cultivated tomato genotypes (Fig. 1A), with 'Severyanka' exhibiting the highest LDM at 9.85%, followed closely by 'Cegnom-F1' at 9.50% (Table 2). 'Dubok' showed the lowest LDM at 7.25%. These variations indicate differences in the dry matter accumulation in the leaves among the genotypes. Roots Dry Matter (RDM) also displayed significant variation, with 'Cegnom-F1' having the maximum RDM at 4**6**.35%, followed by 'Majnat' at 4**5**.34%. The least RDM was observed in 'Chelnok' at 32.20%. This suggests a significant genotypic influence on roots biomass accumulation (Fig. 1B).

Table 2: Physio-biochemical and fruit parameters of the nine tomato genotypes for their susceptibility to blossom-end rot

	Genotype	Leaf dry matter (LDM %)	Root dry matter (RDM %)	Total chlorophyll (mg g^{-1} FW)	chl a/b ratio	Chl/Car ratio	Marketable yield, ton/feddan	Fruit maturity degree	Fruit taste index
1	Chelnok	8.93	32.2	4.04	2.11	3.61	11.06	13.69	1.079
2	Blagodatny	8.5	38.55	3.9	2.25	3.98	24.16	10.80	0.864
3	Dubok	7.25	35.33	3.5	2.15	3.72	11.86	8.09	0.775
4	Majnat	8.75	46.34	5.3	1.88	3.46	29.84	24.20	1.429
5	Severyanka	9.85	38.49	5.12	1.94	3.82	22.59	25.11	1.486
6	Meteor-F1	8.65	34.5	5.07	1.86	3.52	20.13	22.52	1.348
7	Cegnom-F1	9.5	45.35	4.77	2.18	4.15	26.54	11.85	0.938
8	Voskhod	8.3	43.46	4.35	2.06	3.37	25.29	13.88	1.011
9	023-F2	8.98	37.77	2.88	1.44	4.65	21.72	16.88	1.152

Total chlorophyll content (Fig. 1C) ranged from 2.88 mg g−1 FW in '023-F1' to 5.30 mg g−1 FW in 'Majnat'. High chlorophyll content in 'Majnat' and 'Severyanka' (5.3 and 5.12 mg g^{-1} FW) indicates better photosynthetic capacity than other cultivated genotypes such as 'Dubok' and '023-F1'.

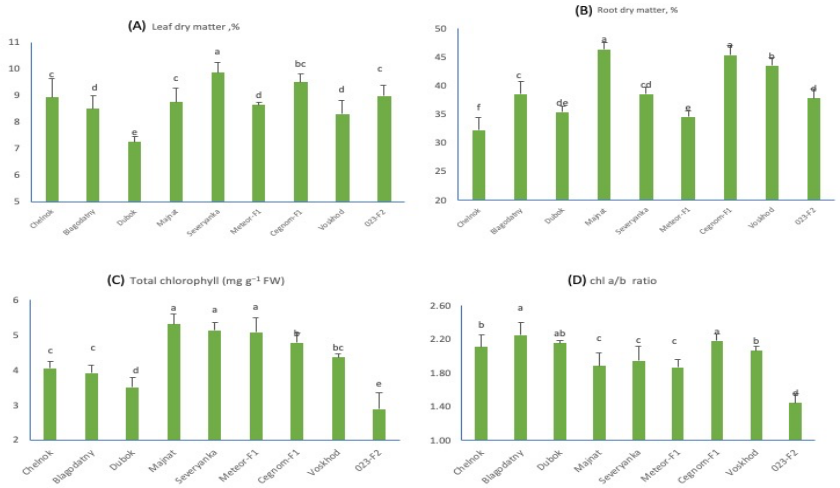

Fig. 1: Leaves dry matter (A), Roots dry matter (B), Total leaf chlorophyll contents (C), and Chlorophyl a/b ratio (D) of the investigated tomato (Solanum Lycopersicum L.) genotypes (Chelnok, Blagodatny, Dubok, Majnat, Severyanka, Meteor-F1, Cegnom-F1, Voskhod, and 023-F1) for evaluating BER incidences by studying physio-biochemical parameters

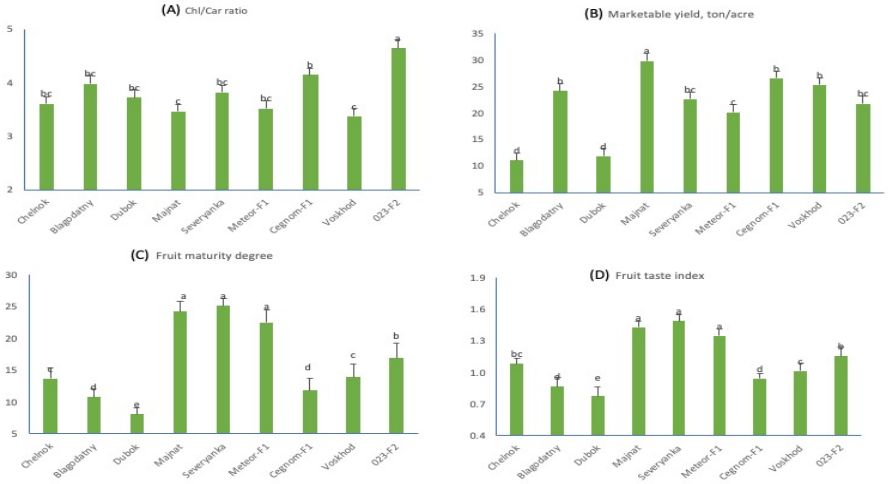

Fig. 2: Chlorophyll /carotene ratio (A), marketable yield per acre (B), fruit maturity degree (C), and fruit taste index (D) of the investigated tomato (Solanum Lycopersicum L.) genotypes (Chelnok, Blagodatny, Dubok, Majnat, Severyanka, Meteor-F1, Cegnom-F1, Voskhod, and 023-F1) for evaluating BER incidences by their studying physio-biochemical parameters.

'Cegnom-F1' has the highest chlorophyll ratio at 2.18, indicating a balanced chlorophyll composition for efficient photosynthesis (Fig. 1D). The minimum ratio was observed in '023-F1' at 1.44, suggesting potential differences in light absorption efficiency among tomato genotypes. The Chl/Car ratio ranged from 3.37 in 'Voskhod' to 4.65 in '023-F1'. Higher ratios in these genotypes suggest better photoprotection mechanisms (Fig. 2A). Genotypes with higher total chlorophyll content, such as 'Majnat' (5.30 mg g^{-1} FW) and 'Severyanka' (5.12 mg g^{-1} FW), are likely to have better photosynthetic efficiency, leading to more robust growth and better fruit development. An optimal Chl a/b ratio, such as in 'Cegnom-F1' (2.18), provides a balanced chlorophyll ratio, which could reduce BER by ensuring adequate energy for fruit development and reducing stress impact that precipitates BER.

'Majnat' genotype exhibited the highest marketable yield at 29.84 tons/acre (Fig. 2B), followed by 'Cegnom-F1' (26.54 tons/acre). 'Chelnok' had the lowest tomato yield at 11.06 tons/acre. These results highlight vital variation in tomato yield potential among the genotypes. The degree of fruit maturity varied, with 'Severyanka' reaching the maximum maturity degree at 25.11, indicating the early ripening stage (Fig. 2C). In contrast, 'Dubok' had the lowest maturity degree at 8.09. The fruit taste index ranged from 0.78 in 'Dubok' to 1.49 in 'Severyanka'. Genotypes like 'Majnat' and 'Severyanka' indicated better taste profiles (Fig. 2D).

3.2. Analysis of ISSR-PCR molecular markers

Fifteen ISSR primers were used for the PCR amplification to characterize the nine tomato genotypes. Fig. (3) illustrates an example of an ISSR-PCR pattern created by primer ISSR-1. The total number of amplified fragments was 149, ranging from 4 bands with ISSR-3 to 15 bands with ISSR-7, with an average of 9.93 for all primers. Polymorphism percentages ranged from 42.86% with primer ISSR-6 to 100 % primers ISSR-9 and ISSR-27 with an average of 76.07%. The molecular size of the

produced bands ranged from 242 bp to 3426 bp. The average value of PIC was 0.363 which reflects a reasonable percentage of diversity between different genotypes of tomato, as a higher PIC value indicates a greater level of polymorphism and diversity among the genotypes studied. Primers ISSR-5, ISSR-6, ISSR-7, ISSR-22 and ISSR-27 reveal the highest degree of 0.5 for the expected heterozygosity. Moreover, primer ISSR-21 gives the highest ratio of EMR, as shown in Table 3.

Twenty-three unique bands are supposed to be markers related to specific characters. For example, primer ISSR-1 and primer ISSR-5 give a positive marker with 'Blagodatny', which may relate to the Chl a/b ratio. Also, primer ISSR-21 gives a positive marker with 'Cegnom-F1' assumed to be related to root dry matter (RDM%), Chl a/b ratio, Chl/Car ratio and marketable yield, ton/acre. Whereas primers ISSR-4, 6, 8, 12, 22 and ISSR-23 produce a positive marker with 023-F1 genotype related to Chl/Car ratio.

3.3. Phylogenetic relationships between tomatoes genotypes

Based on the 0:1 data of ISSR-PCR markers, the NTSYSpc method was used to measure the similarities between tomato genotypes considered in this research. According to the phylogeny tree, the nine genotypes of tomato are divided into two main groups: I and II as shown in Figure 4. Group I consists of two clusters; Cluster I

contains (((Meteor-F1 and Severyanka) with Majnat) with Cegnom-F1) whereas Cluster II includes Voskhod and 023-F1. Group II includes ((Blagodatny and Dubok) with Chelnok).

Plants with higher dry matter content are mainly more resilient to environmental stresses, including water stress, which is a contributing factor to blossom-end rot (BER) due to their enhanced ability to maintain cellular integrity and function under various environmental stresses [17, 18]. Higher Chl/Car ratios suggest a better photoprotective capacity, which helps mitigate oxidative stress and reduces susceptibility to BER, as oxidative damage is one of the physiological triggers for this disorder. In this study, the results also showed that the varieties containing a higher percentage of dry matter and chlorophyll were the

Table 3: Results of ISSR-PCR amplification and polymorphism.

No.	Name	BS "bp"	AB	Poly%	UB	PIC	He	EMR
1	ISSR-1	297-2585	11	81.82	2	0.36	0.47	4.22
2	ISSR-3	720-1389	4	50	1	0.37	0.49	2.22
3	ISSR-4	316-1265	8	50	1	0.34	0.43	5.44
4	ISSR-5	296-2102	10	80	2	0.37	0.5	4.56
5	ISSR-6	247-1752	7	42.86	2	0.37	0.5	3.78
6	ISSR-7	242-1644	15	86.67	2	0.37	0.5	8.89
7	ISSR-8	471-3358	12	66.67	4	0.33	0.42	3.67
8	ISSR-9	260-1453	12	100	-	0.36	0.47	4.56
9	ISSR-10	276-1650	10	90	1	0.37	0.48	4.11
10	ISSR-19	630-3387	8	87.5	1	0.37	0.49	3.56
11	ISSR-21	291-1801	11	81.82	1	0.37	0.49	6.44
12	ISSR-22	440-3426	11	90.91	1	0.37	0.5	5.11
13	ISSR-23	617-1775	10	60	2	0.37	0.48	5.89
14	ISSR-26	468-1461	11	72.73	3	0.36	0.47	4.22
15	ISSR-27	425-1258	9	100	-	0.37	0.5	4.11
Total			149	-	23	-	-	-
Average			9.933	76.07	1.769	0.363	0.479	4.719

***BS= Bands size; AB= Amplification Bands; UB= Unique band; Poly%= Polymorphism %; PIC= Polymorphic Information Content; EMR= Effective multiplex ratio; He= Expected Heterozygosity.

ones that resisted infection with this physiological disease, and this is consistent with the previous studies referred to [19, 20, 26, 27]. The results of ISSRs showed the efficiency of the markers to measure polymorphism between different genotypes in the same species and also that there is a specific marker that may be associated with an important character or phenotype as we obtain with the Chl/Car ratio where the locally grown variety 023-F1 showed its superiority by the appearance of unique markers at the level of 6 ISSR markers and this agreed with [1, 6, 21, 22].

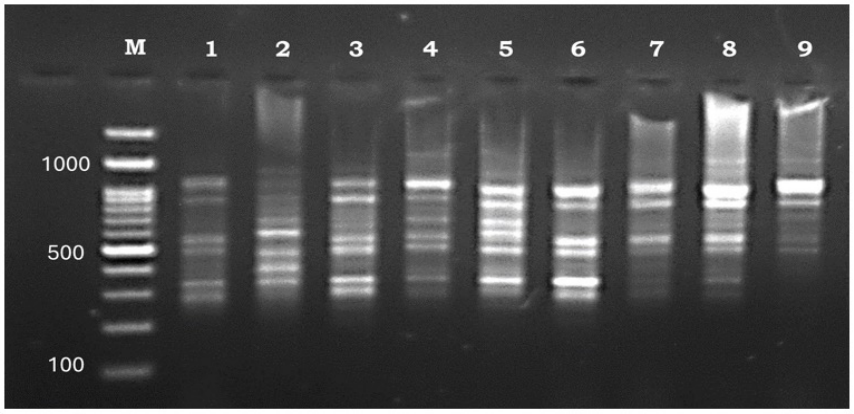

Fig. 3: PCR amplification profiles of nine Tomato genotypes obtained with primer ISSR-1, M: 100 bp ladder.

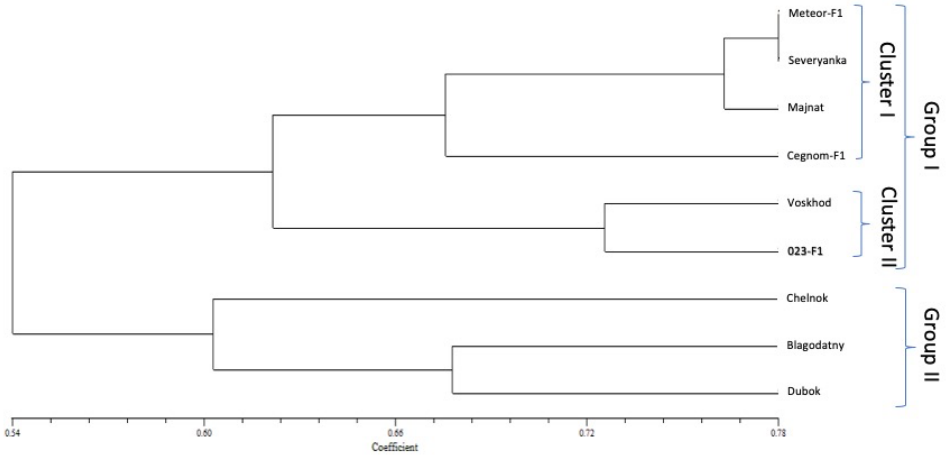

Fig. 4: Phylogenetic tree of nine Tomato genotypes based on the similarity's distances coefficient.

Higher yielding marketable genotypes are generally more resilient. However, high yielding cultivars should be monitored for BER as they may also exhibit a higher incidence of disorder if rapid fruit growth exceeds calcium uptake, a known cause of BER. Tomato genotypes with higher maturity, such as 'Severyanka', may be less susceptible to BER if they have a well-coordinated system of development and nutrient transport. Fruit taste index, with higher values, indicates better fruit quality, which is often associated with optimal nutrient levels, which may lead to a lower incidence of BER [23, 24]. Tomato fruit firmness is considered one of the genetic traits related to genotype. It is one of the traits that increase the ability of tomatoes to be stored and transported. It also increases their price in the markets.

Looking at table 2, Severyanka is one of the hardest and most tolerant fruits for transport. In contrast, Dubok fruits were less hard. These results are generally consistent with [18] studies. By studying the taste of the fruits and their edibility, it was found that this test is one of the most crucial fruit tests through which the ability of the fruits to be marketed is determined. By examining the genotypes under study, it was found that Majnat and Severyanka are among the best genotypes in terms of edibility for the consumer, unlike Dubok, which obtained the lowest value in this test [25]. We used the results of the 0-1 data matrix obtained from ISSR-PCR for genetic diversity to separate the genotypes into groups and the locally grown variety 023-F1 was in the same group as Severyanka which is superior in traits such as degree of maturity and fruit taste according to its similarity to [6, 7]. Finally, we found that the Severyanka genotype performed better than the others regarding fruit firmness and consumer edibility, while the Dubok genotype scored lowest after performing several physiological tests on the tomato genotypes under study. In terms of genetically connecting these features, we discovered six markers related to Chl/Car ratio indicators that set the locally cultivated 023-F1 genotype apart through (ISSR-4, 6, 9, 13, 23 & 24). This genotype is a member of Group I, which also contains Majnat and Severyanka since these genotypes share many favourable genetic characteristics.

4. Conclusion

For genetic diversity, ISSR-PCR was used to measure percentages of polymorphism and to determine the specific markers related to an essential character in the production of tomato. Also, we can depend on the unique bands to be sequenced and used later in other techniques like SCAR-PCR for future studies. According to the stability of the ISSR-PCR reaction, it's a powerful tool for measuring the similarities between very closed genotypes and lines in the same species and obtaining the phylogeny tree or relationship diagram among them.

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