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SCoT MARKER-BASED ANALYSIS AND BIOCHEMICAL TRAITS EVALUATION OF TOMATO VARIETIES

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Abstract

This study evaluates the genetic diversity of nine tomato genotypes using SCoT (Start Codon Targeted polymorphism) molecular markers and assesses their acidity and Vitamin C levels to determine nutritional properties. Among the primers analyzed, 11 generated 118 bands, of which 78 were polymorphic, with polymorphic band percentages ranging from 60% to 80%. The average polymorphic information content (PIC) was 0.29, with the highest value for SCoT 10, while the mean diversity index (H) and marker index (MI) were 0.360 and 3.40, respectively. Hierarchical Cluster Analysis (HCA) divided the genotypes into two main groups and revealed a moderate-high level of similarity between the analysed varieties clustered in the same group. Acidity levels ranged from 0.21% in Kumato Nero cv. to 0.73% in Claudia cv., while Vitamin C content varied between 11.03 mg/100 g (Kumato Nero cv.) and 17.11 mg/100 g (Matilda cv.). Kumato Nero exhibited the lowest Vitamin C and acidity levels, suggesting high consumer acceptance. This research highlights the importance of assessing genetic diversity and also nutritional value, providing valuable insights for future breeding programs to improve tomato traits.

Key words*: Solanum lycopersicum L., acidity, vitamin C, molecular marker, tomatoes.*

INTRODUCTION

The tomato (*Lycopersicon esculentum* L.) is a widely cultivated vegetable with diverse applications worldwide. As one of the most important fruit crops globally, tomatoes are a rich source of nutrients, particularly vitamin C and essential minerals like phosphorus, iron, and calcium. Due to the rising demand for high-quality produce throughout the year, tomato cultivation in controlled environments has become increasingly popular, allowing growers to maximize productivity and quality regardless of seasonal changes. The tomato is recognized not only for its global agricultural importance but also for its nutritional profile, including significant levels of vitamin C and soluble solids that contribute to its taste, shelf life, and health benefits (Oliveira et al., 2013; Turhan et al., 2011; Dorais et al., 2008). The through assessment of biochemical cahracteristcs is a crucial component of breeding programs since these qualitative features are important for both fresh consumption and processing. In an effort to find accessions with better nutritional and organoleptic qualities, recent efforts have concentrated on assessing spontaneous variation within tomato germplasm (Jia et al., 2011; Tripodi et al., 2021; Tripodi et al., 2024). Considering its capacity to provide insight into cultivar variability and contribute to selecting

desired characteristics, genetic diversity analysis has emerged as a key component of contemporary plant breeding programs (Shahlaei et al., 2014). The capacity of Start Codon Targeted (SCoT) markers to identify polymorphisms associated with functional genes, as well as their cost-effectiveness and repeatability, have made them renowned amongst the multitude of the molecular markers that are now readily available (Collard and Mackill, 2008; El-Fiki et al., 2021). By effectively defining genetic diversity in tomato accessions, studies using SCoT markers have paved the road for precision breeding techniques that combine molecular information with phenotypic quality metrics (El-Fiki et al., 2021; Abdein et al., 2018; EL-Mansy, et al., 2021).

Furthermore, a through method of tomato advancement is provided by the evaluation of both genetic variation and quality attributes such as soluble solids and vitamin C content. It is well known that soluble solids, which signify sugars and specific metabolites brought on by stress, can change depending on environmental and genetiv variables (Jia et al., 2011) Comparably, the vitamin C level of tomato fruits plays a crucial role in their antioxidant defence system, impacting not just human health, but also their shelf life and postharvest quality (Constantino et al., 2022). The interplay between these traits highlights the potential of integrating biochemical characterization with molecular marker-assisted selection; a strategy increasingly advocated accelerating the breeding process (Jia et al., 2011; Constantino et al., 2022).

In light of the above, this study focuses on a detailed genetic diversity analysis of tomato varieties using SCoT markers while concurrently evaluating soluble solids, titratable acidity and vitamin C content. This approach is anticipated to facilitate the identification of genetically diverse and nutritionally superior tomato varieties, reinforcing breeding programs aimed at developing cultivars with enhanced quality characteristics and environmental adaptability (El-Fiki et al., 2021; Constantino et al., 2022). The integration of these methodologies not only strengthens the understanding of the genetic basis underlying quality traits but also promises to streamline the selection process in tomato breeding programs, ensuring that high nutraceutical value and consumer preferences are met.

MATERIALS AND METHODS

Plant material

The experiment was carried out in the greenhouse of the Horticultural Research Station UASVM Cluj-Napoca. The biological material was represented by nine tomato genotypes from Buzău Plant Genetic Resources Bank: Matilda (T1), Măriuca (T2), Kumato Nero (T3), Claudia (T4), Vitamina (T5), Serena (T6), Siriana (T7), Kristinica (T8) and Darsirius (T9).

DNA extraction

DNA was isolated from the tomato leaves using the CTAB (Cetyltrimethylammonium bromide) technique, based on the procedure published by Lodhi et al. (1994). The extracted DNA was quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) to ascertain the amount and purity of each evaluated sample.

Genetic analyses using SCoT markers

The molecular markers SCoT were employed in the present investigation as based on the amplification of conservative and particular areas flanking the start codon (ATG) of the methionine amino acid on both sides (Table 1). The components of the PCR reaction mixes were as follows L Green Master Mix (Promega), 20 mM Tris-HCl, 1.5 mM MgCl2, 50 mM KCl, 0.24 mM dNTPs, Taq polymerase (0.5 U) (Promega), primer (0.8 µm), and template DNA (25 ng). With minor adjustments, amplification was carried out according to the procedure outlined by Collard and Mackill (2008): denaturation for three minutes at 94°C, followed by 35 cycles of 94°C, 50°C, and 72°C for one minute each, and a final extension at 72°C for five minutes. A 1K bp DNA ladder (Bioline, Inc. USA) was used to separate the PCR amplification products. The separation was performed on agarose gels (2%) (Promega, Madison, Wisconsin, USA) in 1 x TAE, at 0.29 V/cm^2 for 1.5 hours, and detected by EtBr staining

(Sigma-Aldrich GmbH, Darmstadt, Germany). PCR amplifications were carried out in duplicate and visualized using the UVP Biospectrum AC Imaging System (UVP BioImaging Systems, Germany). Segments of SRAP and SCoT that were easily reproducible were assessed as either present (1) or absent (0).

Table 1. The SCoT primer sequences used for the assessment of genetic relationships of tomato varieties

SCoT primer	Sequence (5′-3′)	Tm (°C)	GC content (%)
SCoT 1	CAACAATGGCTACCACCA	52	50
SCoT 3	CAACAATGGCTACCACCG	53.7	56
SCoT 4	CAACAATGGCTACCACCT	51.8	50
SCoT 5	CAACAATGGCTACCACGA	52	50
SCoT 6	CAACAATGGCTACCACGC	54.3	56
SCoT 9	CAACAATGGCTACCAGCA	58.3	50
SCoT 10	CAACAATGGCTACCAGCC	57.5	56
SCoT 11	AAGCAATGGCTACCACCA	62.8	50
SCoT 12	ACGACATGGCGACCAACG	59.5	61
SCoT 13	ACGACATGGCGACCATCG	58.3	61
SCoT 14	ACGACATGGCGACCACGC	62.7	67

Determination of TSS, TA and Vitamin C

Total Soluble Solids (TSS%) was determined by the index of refraction measured with a portable refractometer. Titratable Acidity (TA), expressed as % of citric acid (CA), was determined by titration of the sample with sodium hydroxide, using phenolphthalein as indicator. Vitamin C (mg/100 g) was determined by redox titration with potassium iodate in the presence of potassium iodide.

All analyses were performed in triplicate for the nine cultivars.

Statistical Analysis

The results of the study were analyzed using statistical software R4.4.2 ("Pile of Leaves") (R Core Team, 2024). The significance level was set at $p < 0.05$. The normality of the data was tested using the Shapiro-Wilk test and descriptive statistics.

The data showed normal distribution and were described as mean ± standard deviation (SD). The One-Way ANOVA test, followed by the Tukey post-hoc, was used to verify the existence of significant differences between cultivars. Pearson correlations and linear regressions were used to test for a link between the studied variables. The data were analyzed by IBM SPSS Statistics 19.0., ORIGINPRO 2021 v.9.8 and PAST 4.1.1. software.

RESULTS AND DISCUSSIONS

Genetic diversity by SCot markers

Molecular characterization of tomato varieties was evaluated using selected SCoT markers that produced clearly reproducible DNA bands (Figure 1). Among the evaluated primers, 11 generated higher polymorphism, with a total of 118 bands, out of which 78 proved to be polymorphic. The polymoprhic bands (PB) per selected primer ranged between 2 (SCoT 11) and 12 (SCoT 4 and SCoT 14). PB percentage varied between 80.0% and 60.0% with an average of 52.18%. The tested markers' polymorphisms and discriminatory ability were assessed using indicators including polymorphic information content (PIC), diversity index (H), marker index (MI) (Table 2).

Table 2. Description of the SCoT primers selected for the assessment of genetic relationships of tomato varieties

Primers	No. AB	No. PB	Polymorphism (%)	H	PIC	E	MI	D	R
SCoT 1	10	6	60	0.06	0.06	0.44	1.89	1.00	0.89
SCoT 3	12	9	75	0.30	0.26	2.78	6.21	0.97	3.78
SCoT 4	17	12	70.59	0.38	0.31	3.11	1.11	0.93	4.00
SCoT 5	10	6	60	0.33	0.27	3.11	7.58	0.96	2.67
SCoT 6	16	10	62.50	0.44	0.35	5.00	1.65	0.89	2.89
SCoT 9	5	4	80	0.33	0.27	3.11	7.58	0.96	4.00
SCoT 10	3	2	66.67	0.50	0.37	7.22	2.67	0.77	2.89
SCoT 11	10	6	60	0.33	0.23	3.22	6.53	0.91	3.22
SCoT 12	12	7	58.33	0.40	0.32	4.11	1.21	0.93	4.00
SCoT 13	8	5	62.50	0.38	0.31	3.78	1.05	0.94	1.78
SCoT 14	16	12	75	0.49	0.37	8.56	3.11	0.68	2.22
Total	119	79	-	-	-	-	-	-	-
Mean	9	5.64	52.18	0.36	0.29	4.12	3.41	0.90	2.91

Note: AB = amplified bands; PB = polymorphic bands; H = genetic diversity; PIC = polymorphic information content; E = effective multiple ratio; MI = marker index; D = diversity index; R = resolving power.

The mean value of PIC was 0.29, with the highest in SCoT 10, whereas the average values of H and Mi were 0.360 and 3.40, respectively. The HCA organized the varieties in two major groups. The primary cluster comprised Matilda (T1) and Mariuca (T2) with the highest similarity. The following cluster positioned the variety Serena (T6), which proved to be distinct from the others and closely grouped with Siriana (T7). The following sub-cluster positioned Claudia (T4) and Vitamina (T5) tomato varieties into the same group. The last sub-cluster organized variaties Darsirius (T9)

and Kumato Nero (T3) which were closely grouped with Kristiana (T8). In a different study, the genetic diversity and relationships among 64 accessions were analyzed using 26 SCoT markers which generated 294 polymorphic bands and a PIC value of 0.84 (Rasul et al., 2022). Shahlahei et al. (2014) reported that out of the used markers, 10 generated a total of 83 bands, out of which 30 proved to be polymorphic.

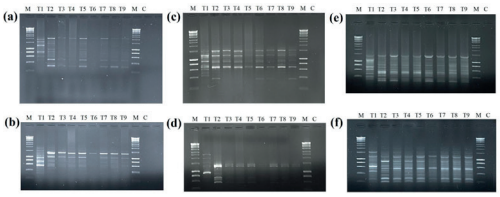

Figure 1. Amplification profiles of SCoT 4 (a), SCoT 5 (b), SCoT 6 (c), SCoT 8 (d), SCoT 12 (e), and SCoT 14 (f). Lanes T1-T9 represent different tomato varieties; M-1Kb ladder (Bioline); C- negative control

As shown in Figure 2 the HCA organized the varieties in two major groups. The primary cluster comprised Matilda (T1) and Mariuca (T2) with the highest similarity. The following cluster positioned the variety Serena (T6) which proved to be distinct from the others and closely grouped with Siriana (T7). The following sub-cluster positioned Claudia (T4) and Vitamina (T5) tomato varieties into the same group. The last sub-cluster organized varieties Darsirius (T9) and Kumato Nero (T3), which were closely grouped with Kristiana (T8).

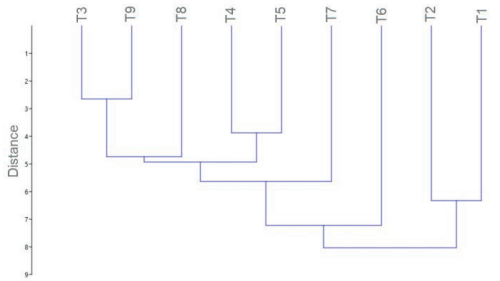

Figure 2. The HCA depicting the genetic relationship among the evaluated tomato varieties from the SCoT analysis (Euclidean distance, R = 0.84).

The genetic improvement of tomato varieties increasingly relies on an integrated approach that couples molecular marker analysis with the assessment of key fruit quality attributes such as titratable acidity, soluble solids, and vitamin C content.

Recent studies demonstrated that SCoT markers allowed for the clear differentiation of tomato accessions, validating their utility in comprehensive diversity studies (Shahlaei et al., 2014). Al-Ghufaili et al. further supported this conclusion by showing that cluster analyses based on SCoT data effectively divided tomato genotypes into distinct phylogenetic groups, providing insights into the underlying genetic relationships essential for breeding strategies (Al-Ghufaili et al., 2023). Furthermore, according to the HCA a maximum coefficient of 0.8 has been observed in tomato accessions according to their area of collection, namely AC36 (Rozh) and AC40 (Roma), respectively. These tomato accessions in the SCoT dataset may be further used in future breeding strategies (Rasul et al., 2022). In a different study, the use of SCoT markers generated 9 polymoprhic bands, with an average of 5 bands per primer and the highest polymorphism of 50% detected for SCoT 2 and 15. Furthermore, the highest PIC (0.31) and MI (0.47) has been observed in SCoT 2, whereas the highest Rp (13.1) has been detected in SCoT 12. The HCA separated the tomato accessions in two groups, with G1, G3 and G5 positioned in the same cluster (EL-Mansy et al., 2021).

Evaluation of TSS, TA and Vitamin C

In parallel with the molecular investigations, the assessment of biochemical markers, including titratable acidity, soluble solids, and vitamin C content, is essential for evaluating the nutritional and organoleptic qualities of tomato fruits. Phenotypic evaluations of these traits provide critical information regarding the fruit quality and consumer acceptability of different cultivars. Recent studies have highlighted that biochemical parameters are often correlated with underlying genetic differences. For example, research incorporating phenotypic trait analysis with molecular marker data has underscored the potential of using such an integrated approach to identify and select superior genotypes that combine both desirable fruit quality traits and robust genetic backgrounds (Radzevičius et al.,

2024). This dual approach is particularly useful for breeding programs focused on enhancing flavor, nutritional quality, and stress resilience, as it allows for the integration of molecular fingerprints obtained via SCoT markers with biochemical profiles (Radzevičius et al., 2024).
Statistically significant differences ($p<0.05\%$) were revealed in the present study among the studied cultivars in terms of acidity, vitamin C and total soluble solids content of tomato fruits (Table 3).
Total soluble solid (TSS) is one of the most important quality factors for tomato (Agbemavor et al., 2014), being represented by water-soluble components, mostly consisted of sugars (glucose, sucrose and fructose), organic acids and water-soluble proteins (pectin) (Astuti et al., 2018). Since sugars are the main constituents of total soluble solids, representing 65 to 70%, the measurement of TSS content fairly estimates the sugar level in tomato fruit Malundo et al., 1995; Saliba-Colombani et al., 2001; Agbemavor et al., 2014. The flavour of tomatoes, which is most likely to match the consumer perception of the internal quality, is dependent on both high sugar and acids content, meaning that high acids and low sugars will produce a sour tomato, while high sugars and low acids will result in a tasteless tomato (Astuti et al., 2018; DeBruyn et al., 1971). Thus, soluble solids and titratable acidity are the main components responsible for tomato flavour (DeBruyn et al., 1971).
Total soluble solids content (Table 3) showed significant statistical differences ($p<0.05\%$), ranging from 2.88% ('Darsirius') to 6.71% ('Matilda') in the fruit of the studied cultivars. Arazuri et al. (2007), in a study which evaluated fruits of some breeding lines of tomatoes, stated that total soluble solid of 4.80 to 8.80% indicates the high quality of tomato. Citric acid (CA) is the most dominant organic acid in tomatoes, which besides malic acid, are the organic acids that contribute to the flavour of tomatoes (Agbemavor et al., 2014). The titratable acidity (TA) content in tomato fruit of the studied cultivars ranged from 0.35% to 0.88%. The highest acidity values were recorded at 'Claudia' (0.73%), followed by 'Vitamina' (0.21%) and 'Darsirius' (0.68%) cultivars. The results are in line with those reported by Agbemavor et al., 2014.Tomato supply important antioxidants for health, such as vitamin C (Bui et al., 2010). The vitamin C content in the fruit of the studied cultivars ranged between 11.0 mg/100 g in 'Kumato Nero' and 17.1 mg/100 g in 'Matilda' cultivar, which were in accordance with those reported by Bui et al., 2010; Kaur et al., 2006.
Sugars, acids, and their interactions are important to sweetness, sourness, and overall flavor intensity in tomatoes (DeBruyn et al., 1971). Regarding the vitamin C and total soluble solids content, 'Matilda' cultivar showed the highest values, with an average of 17.1% vitamin C and 6.71% soluble solids, respectively (Table 3).

Table 3. Chemical characteristics of the fruit in nine tomato cultivars

Cultivars (cod)	Titrable acidity (TA) (%CA)	Vitamin C (mg/100 g)	Total Soluble Solids (TSS) (%)
Matilda (T1)	0.63 ± 0.03^{bc}	17.1 ± 0.49^{a}	6.71 ± 0.10^{a}
Măriuca (T2)	0.49 ± 0.02^{d}	12.3 ± 0.19^{c}	5.01 ± 0.10^{d}
Kumato Nero (T3)	0.21 ± 0.02^{e}	11.0 ± 0.70^{d}	3.41 ± 0.10^{e}
Claudia (T4)	0.73 ± 0.01^{a}	13.2 ± 0.65^{c}	6.11 ± 0.20^{b}
Vitamina (T5)	0.69 ± 0.03^{c}	15.1 ± 0.11^{b}	6.20 ± 0.10^{b}
Serena (T6)	0.60 ± 0.01^{c}	13.5 ± 0.49^{c}	5.70 ± 0.10^{c}
Siriana (T7)	0.62 ± 0.02^{c}	15.3 ± 0.50^{b}	5.30 ± 0.10^{d}
Kristinica (T8)	0.47 ± 0.01^{d}	12.8 ± 0.22^{c}	3.50 ± 0.10^{e}
Darsirius (T9)	0.68 ± 0.03^{ab}	13.1 ± 0.19^{c}	2.88 ± 0.12^{f}

Note: The data presented are means±S.D. The letters in common indicate no significant differences between tomato cultivars within the same fruit chemical characteristics according to Tukey's HSD test at $p<0.001$.

Among the evaluated tomato fruits, the identified biochemical traits were shown to have statistically significant outcomes (Table 4).
Tomato acidity content was strongly impacted by the presence and identified levels of vitamin C and total soluble solids. Furthermore, it has been observed that the vitamin C levels of tomato fruits was significantly impacted by the total soluble solids amount.

Table 4. The relationship between the chemical characteristics of tomato fruit in nine tomato cultivars

	Titratable Acidity (% CA)	Vitamin C (mg/100 g)	Total Soluble Solids (%)
Acidity (% Citric Acid)	-		
Vitamin C (mg %)	0.622**	-	
Soluble solids Content	0.534**	0.695***	-

Note: *p < 0.05, ** p < 0.01, *** p < 0.001.

According to the relationships between the acidity and vitamin C levels, it has been observed that the fruit's acidity and vitamin C were shown to be positively correlated. Approximately 38% of the fruit's acidity is responsible for the differences in the amount of vitamin C content (Figure 3).

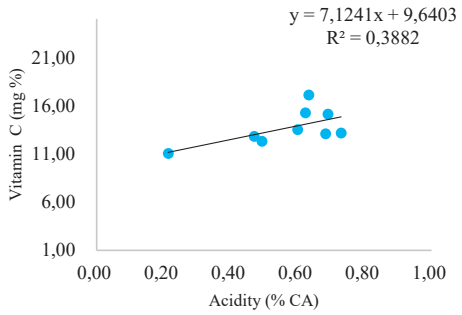

Figure 3. The association between the vitamin C content and acidity level in tomato fruits

Following, the fruit's acidity and total soluble solids content were also shown to be positively correlated. Approximately 28% of the total soluble solids prove to be responsible for the fluctuations in acidity levels (Figure 4).

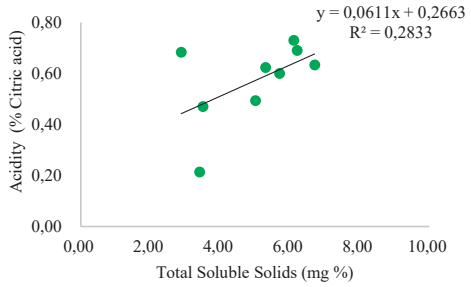

Figure 4. The association between acidity level and the vitamin C content in tomato fruit

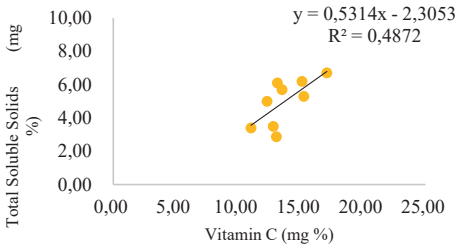

Figure 5. The association between total soluble solids and the vitamin C content in tomato fruit

Total soluble solid content and vitamin C were shonw to be positively associated, with the vitamin C concentration accounting for nearly 48% of the variance in the total soluble solids content (Figure 5).

Regarding the acidity concentration, the highest amount was identified in genotypes T4, T5 and T9, varied between 0.7-0.68% citric acid/100 g and the lowest was registered in the T3 genotype (0.21% citric acid/100 g). Results revealed significant variations among cultivars, with vitamin C content ranging from 11.03 mg/100 g to 17.11 mg/100 g. The Kumato Nero cultivar exhibited the lowest vitamin C and acidity level indicating potential for improved consumer acceptance. These results highlight the importance of cultivar selection in optimizing both nutritional quality and genetic diversity, providing valuable insights for breeding programs aimed at enhancing tomato quality.

Moreover, studies that have combined SCoT marker-based genetic analyses with evaluations of fruit quality traits have clarified that the observed genetic variability can be statistically correlated with variations in titratable acidity, soluble solids, and vitamin C (Abdeldym et al., 2020). The hypothesis that alterations in genetic profiles, as identified by SCoT markers, are complemented by quantificable alterations in fruit quality variables is partially supported by the work of El-Fiki and colleagues (2021), even though their primary focus is on generated genetic variability through irradiation and molecular marker screening. According to these results, breeding projects aiming at creating superior tomato cultivars may be more successful whether SCoT marker data and biochemical evaluations are combined for marker-assisted selection (El-Fiki et al., 2021).

CONCLUSIONS

The present investigation emphasizes how crucial it is to combine thorough phenotypic assessments of important quality attributes as soluble solids, vitamin C content, and titratable acidity with SCoT marker-based analysis for genetic diversity.

A high polymorphism ranging from 60% to 80% was obtained by 11 of the primers that were analysed, which generated 78 polymorhic bands. PIC's mean value was 0.29, and out of the evaluated primers SCoT 10 had the highest PIC value.

Acididty levels oscillated between 0.21% in Kumato Nero cultivar to 0.73% in Claudia cultivar, whilst the vitamin C content varied between 11.03 mg/100 g (Kumato Nero cv.) to 17.11 mg/100 g (Matilda cv.). Therefore, among the evaluated cultivars, Kumato Nero presented the lowest levels of vitamin C and acidity.

In addition to improving our knowledge of the genetic makeup of tomato germplasm, this all-encompassing approach makes it easier to pot for genotypes that satisfy consumer quality demands and agronomic performance requirements. The association shown in research using both molecular and chemical characteristics represents an achievable approach to improve tomato breeding initiatives and guarantee the generation of genetically viable and nutritionally superior varieties.

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