

Effect of ordinary and nano calcium on some quality traits of tomato lines and their individual hybrids in unheated greenhouses

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Abstract

The study was conducted during the agricultural seasons from August 2022 to June 2024 at the Baqubah Central Nursery affiliated to the Diyala Agriculture Directorate to study the genetic dimension of pure lines of colored tomatoes and evaluate the performance of their individual hybrids under the effect of spraying with two different calcium solutions on some quality traits. The first season included conducting a genetic fingerprint test and determining the genetic dimension of the twenty pure tomato lines imported from the Tomato Genetics Center at the University of California in Davis, USA (TGRC) CM Rick Tomato Genetics Center). Using ten primers, by applying SSR (Simple Tandem Sequencing) technology. Based on the results of the genetic fingerprint test and the evaluation of the genetic dimension by means of the cluster diagram, 6 pure lines with the highest genetic dimension were selected (LA3695, LA2804, LA4355, LA4449, LA2970, and LA0806). They were planted in the greenhouse and entered into a half-crossing program to produce hybrids, while the second season included comparing the genetic compositions (6 parents + 15 hybrids) under the effect of spraying with two different attempts of calcium (no spraying, spraying with normal calcium 2 g L⁻¹, spraying with nano-calcium (2 g L⁻¹) using protected cultivation according to the (RCBD) design and using the split-split plot design system more than once (Split-Split Plot Design) and with three replicates by using the main plot (genetic compositions) and the subplot (calcium spraying). The results were as follows: Parent 1 excelled in total fruit acidity (0.919%), Parent 2 in fruit sugar percentage (6.796%), and Parent 3 in beta-carotene content (11.309 mg/100g). Parent 4 was superior in anthocyanin content (31.034 mg/100g), while Parent 5 excelled in fruit hardness (3.547 kg/cm²), total soluble solids (6.242%), and fruit sugar percentage (6.736%). Parent 6 surpassed others in vitamin C content (12.455 mg/100g) and lycopene content (6.544 mg/100g). The hybrid 1×4 excelled in anthocyanin content in fruits (37.984 mg/100g). The hybrid 1×6 was superior in vitamin C content (18.217 mg/100g) and total acidity (1.185%). The hybrid 2×3 excelled in beta-carotene content (13.904 mg/100g), while the hybrid 2×5 was superior in fruit sugar percentage (12.799%). The hybrid 3×5 outperformed others in total soluble solids (9.155%), and the hybrid 4×6 excelled in lycopene content (6.544 mg/100g). Lastly, the hybrid 5×6 was superior in fruit hardness (5.888 kg/cm²). Spraying the genetic compositions with calcium resulted in significant differences across all studied traits. The nano-calcium treatment achieved the highest significant values in fruit hardness (5.092 kg/cm²), total soluble solids (7.043%), vitamin C (12.912 mg/100g), fruit acidity (0.739% and 0.860%), beta-carotene (8.746 mg/100g), lycopene (6.013 mg/100g), fruit sugars (9.720%), and anthocyanin content (17.547 mg/100g), outperforming the control and ordinary calcium treatments.

Keyword. Tomato lines, individual hybrids, calcium

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown, produced, and consumed vegetable crops in the world. It is an herbaceous plant belonging to the Solanaceae family. Its importance in the nutritional value of its fruits, which are used in cooking, making paste, tomato salad, and juice. It is an important source of vitamins A, C, and E, potassium, folic acid, lycopene, and fiber [32]. Tomatoes exhibit a wide diversity in color, shape, and taste due to natural variation or hybridization. They appear in colors such as red, pink, orange, yellow, green, purple, and black. The red color is primarily caused by lycopene, while beta-carotene contributes to yellow and orange hues when lycopene is absent. Green tomatoes retain their color upon ripening due to the absence of red pigments. Black tomatoes owe their dark color to anthocyanin, an antioxidant, with its intensity influenced by sunlight exposure [27,28]. At the beginning of the last century, tomato breeding and improvement programs began, primarily through the selection of good genetic compositions resulting from natural and artificial pollination, or through genetic mutations. These programs led to the development of new varieties and hybrids, which were characterized by early maturity, high productivity, fruit quality, multiple colors, and resistance to environmental and biological stresses. Determining the genetic dimension between strains is one of the requirements of plant breeders in implementing their research in order to develop distinctive hybrids by crossbreeding these strains. Therefore, estimating the genetic dimension and its degree is positively correlated to the development of distinctive hybrids, and the

diversity of genetic sources for strains or varieties provides basic information for plant breeders and benefits them in designing genetic breeding and improvement programs [24]. Reciprocal crossbreeding is one of the best mating designs used for hybridization between different genetic compositions, whether they are pure strains or open-pollinated varieties, as it ensures the discovery of all possible hybrid combinations and enables the researcher to determine which parents are most likely to be compatible with each other by guessing a set of genetic parameters. This way, the best hybrids with the desired traits are found [10]. Environmental factors like temperature, lighting, and nutrition can complicate the identification of genetic relationships in plant breeding. However, advancements in molecular genetics, particularly DNA analysis, have improved traditional breeding programs. Biotechnologies such as Simple Sequence Repeats (SSR) provide accurate and rapid tools for identifying genetic variation, creating genetic fingerprints, and mapping genetic compositions, significantly enhancing breeding efficiency [2,38]. The use of nano-applications in the agricultural process leads to improving production in quantity and quality, as it contributes to increasing the productive efficiency of the cultivated area when sprayed on the green part by increasing the activity of carbon metabolism processes, increasing crop resistance to diseases, maintaining the required characteristics of agricultural crops, and increasing the active substances in the plant [5]. Currently, there are more than 800 nano-fertilizer products worldwide, the active substance of which is produced with nano-specifications to cover the needs of plants [8]. Calcium is a major nutrient that has many

physiological functions that are important for plant growth and development in both stressful and non-stressful conditions [44]. It has a dual function, as it is an essential component of the structure, cell walls, and membranes. In addition to its structural role, its main function is its ability to act as a second messenger in a variety of physiological processes, such as growth and fertilization of pollen grains within cells, absorption, distribution, and storage in the plant. Therefore, calcium must be available to the plant in sufficient quantities to regulate these processes [29,30,31,46]. Calcium deficiency symptoms often occur in young tissues such as leaves and young fruits due to decreased resupply from old to young tissues, which leads to deficiency symptoms in young leaves and meristematic tissues, and deficiency of this element can cause blossom end rot disease of tomato fruits. The objective of the study was to evaluate the effect of genetic compositions and calcium on some growth traits of tomatoes.

Materials and methods

The study was implemented at the College of Agriculture, University of Diyala, for the period from August 2022 to June 2024, as genetic tests were carried out in the laboratories of the private Wahj Al-Dana Company, Baghdad, Karrada, to determine the six plants required to carry out the hybridization process (genetic dimension) through the genetic fingerprint of the studied samples (pure tomato lines) and drawing the cluster diagram. While field experiments were carried out in the fields of the government nursery affiliated with the Diyala Agriculture Directorate, Baqubah District, Diyala Governorate for the two seasons. The study involved three essential stages, starting with studying the genetic dimension to determine

the six pure lines, then hybridization to produce hybrids, and finally comparing the hybrids and spraying them with calcium of both types.

Sources of pure tomato lines

The twenty pure tomato parental lines were imported from the CM Rick Tomato Genetics Center (TGRC), University of California in Davis, USA (<http://tgrc.ucdavis.edu>). The study included finding the genetic fingerprint and determining the genetic dimension of the 20 pure tomato lines studied: LA2374 (1), LA3695 (2), LA0797 (3), LA2973 (4), LA2972 (5), LA1996 (6), LA4026 (7), LA4657 (8), LA2804 (9), LA4355 (10), LA4449 (11), LA0279 (12), LA2970 (13), LA0030 (14), LA0806 (15), LA2377 (16), LA2971 (17), LA2661 (18), LA4345 (19), LA4452 (20).

First agricultural season (2022) reciprocal-half-crossing between pure tomato lines

In light of the results of the DNA test (genetic fingerprint) that was previously conducted, six pure genetically distant lines were selected bearing the following codes: LA3695 (2), LA2804 (9), LA4355 (10), LA4449 (11), LA2970 (13), and LA0806 (15). These selected lines will be coded to represent the parents involved in the hybridization (P1, P2, P3, P4, P5, P6). This selection based on genetic variation allows for high genetic diversity in plant breeding programs. The seeds of the selected pure lines were planted based on the genetic dimension between them in a private nursery on 15/10/2022 in sterile cork plates. After all the seeds germinated and were verified, service operations of irrigation, fertilization, and control were applied until 5-6 true leaves of the plant (seedling) appeared, and then they were transferred to the permanent field (a plastic house with a width of 9 meters and a height of 3.20 meters)

located in the Baqubah Governmental Nursery on 15/11/2022. After preparing the soil of the plastic house by plowing, smoothing, and making terraces, it was divided into six terraces. The one terrace contains two planting lines; the distance between one line and another is 40 cm, and between one plant and another is also 40 cm within one terrace. Chemical and organic fertilizers and preventive pesticides (fungicide and insecticide) were added to the soil according to the recommended quantities, and the soil was covered with black polyethylene [36,9]. The seedlings were planted on lines alongside the GR irrigation pipes in the shape of a duck's paw, with two irrigation lines for each terrace. Each pure line was planted with 30 plants. We continued to provide the plants with balanced fertilizers, nutrients, and humic, in addition to amino acids, according to scientific recommendations, while carrying out continuous control operations for the whitefly. When the plants reached the flowering stage, they were introduced into a reciprocal-half-crossing program according to the second method of [19] to produce generation hybrids as shown in Table (1), by

transferring pollen grains from the anther of a flower as a father that is open and fully developed physiologically to the stigma of another flower as a mother before it opens and is in the cream color stage, and after completing the neutering process on them (the neutering process of the flower of the mother plant) by removing the male parts (anthers), and then they were wrapped in tin bags or paper bags to ensure that pollen grains from another plant were not mixed, with marks indicating the parents involved in the hybridization (name of father and mother). This process continued daily for all the parents involved in the hybridization (the strains used as father and mother) and in one direction until the completion of the crossings. After ensuring the success of the hybridization and fruit setting process and monitoring the fruits until fully ripe, all the seeds were extracted and placed in paper bags, and all the information related to the hybridization was recorded on them. This process continued until a sufficient quantity of seeds was obtained. They were stored at a suitable temperature and humidity until used in the following season.

Table 1. Reciprocal-half crosses between pure lines of tomato

Parents	P1	P2	P3	P4	P5	P6
P1	+	*	*	*	*	*
P2		+	*	*	*	*
P3			+	*	*	*
P4				+	*	*
P5					+	*
P6						+

Second season (2023) comparing hybrids with their parents under the influence of two types of calcium fertilizer

The experiment was carried out in the plastic houses of the Baqubah Government Nursery, which is affiliated with the Diyala Agriculture

Directorate. The second-season experiment included two factors: comparing the genetic compositions of the parents with their hybrids (the first factor) and the influence of spraying with two types of calcium fertilizer (the second factor). As for the foliar fertilization factor with calcium, there were the following

levels: comparison (spraying with distilled water only), spraying with nano-calcium at a concentration of 2 g L⁻¹ (recommendation of the producing company) provided by the Iranian company, and spraying with ordinary calcium at a concentration of 2 g L⁻¹ (recommendation of the producing company) provided by the Spanish Disper company.

Agriculture

The seeds of the parents and hybrids were planted in cork plates on 9/8/2023 in one of the private nurseries in the Khan Bani Saad area. After reaching the stage of four to five true leaves, they were transferred to plastic houses on 17/10/2023. The area of the experimental unit was 3.584 meters with dimensions of 2.8 x 1.28 m, with 7 plants in the experimental unit, under the GR drip irrigation system. The field was irrigated before planting the seedlings to moisten the soil, and the seedlings, which were 10-15 cm long and 4-3 mm in diameter, were planted next to the irrigation pipes in the middle of the terrace, according to the split-plot system with

a complete random block design (RCBD). To ensure ease of spraying, calcium fertilization levels (ordinary and nano) will occupy the main terraces, while the genetic compositions will be placed in the secondary terraces. The genetic compositions will consist of 21 (15 single hybrids with 6 parents), resulting in 63 treatments. Each treatment will be replicated three times, resulting in a total of 189 experimental treatments. Soil samples were taken before the planting experiment from different locations in the field at a depth of 30 cm and were representative of the field soil and mixed well. It was analyzed in the Laboratory of Agricultural Research, Iraq (Table 2). The plants' spraying process began before the flowers appeared on the plant and continued for three sprays, with ten days between each spray. The plant service operations continued after planting, including irrigation and weeding as needed at regular times. A preventive program was followed to protect against insects and diseases until the fruits were harvested.

Table 2. The physical and chemical properties of soil

Measurements	Value	Unit of measurement
Texture of soil	Sandy loam	-
Clay	156.70	%
Silt	284.30	%
Sand	559	%
Ph	7.49	-
Ec	1.41	ds.m ⁻¹
N	18.13	mg. kg ⁻¹
P	14.10	mg. kg ⁻¹
K	174.89	mg. kg ⁻¹
S	11.00	mg. kg ⁻¹
Organic matter	1.53	%
Caco3	237.10	g. kg ⁻¹

Studied

Fruit hardness (kg cm²)

It was measured using the pressure hardness device from the shoulder area of the fruit and for seven fruits from each experimental unit [4].

Total soluble solids (T.S.S(%))

It was estimated using the hand refract meter by taking a drop of fruit juice [21].

Concentration of vitamin C in fruits (mg/100 ml-1 juice)

The fruit juice filtrate was calibrated with dye (2-6, dichlorophenol indophenols), and then vitamin C was extracted from the juice [21].

Total acidity in fruits(%)

It was calculated by taking a random sample of the fruits of each experimental unit and squeezing them, then filtering the juice and bleaching its color using charcoal, and taking 10 ml of the juice and using a burette with sodium hydroxide (N 0.1) after adding 1 ml of phenolphthalein reagent, and the results were estimated on the basis that the dominant acid is citric [35].

Estimation of beta-carotene and lycopene pigments in fruits (mg 100 g⁻¹ fresh weight) (According to the method mentioned by [17], by taking 1 g of the plant sample and, after crushing it in 10 ml of ethanol, then filtering it using filter paper and reading the lycopene and beta-carotene dyes on wavelengths of 450 nm and 470 nm, respectively, by the spectrometer. By applying the following equations, we obtain the concentrations of the two pigments (lycopene and beta-carotene pigments), respectively:

$$\times 1000000 \div 3450 \times 100X \text{ (mg)} = Y \text{ (ml)}$$

$$\times 1000000 \div 2592 \times 100X \text{ (mg)} = Y \text{ (ml)}$$

$$\text{Volume of ethanol used } Y = \text{(ml)}$$

$$\text{Reading of the device for sample filtrate} = A$$

$$\text{Total sugar content in juice (\%)} =$$

traits

It was estimated by taking 1 ml of diluted juice and adding 1 ml of phenol solution (5%) and 5 ml of concentrated sulfuric acid with continuous shaking, then the absorption reading of the spectrophotometer was recorded at a wavelength of 550 nm, and the percentage of sugars in the fruits was calculated by dropping the readings onto the standard curve prepared using glucose [22]. Then the following equation was applied:

$$\text{Sugars (\%)} = \frac{\text{Concentration from the standard curve} \times \text{dilution}}{\text{Volume of the juice taken}} \times 10000 \times 100$$

Anthocyanin pigment in the fruits (mg 100 g⁻¹)

Anthocyanin in the fruits were estimated according to the method of [35], where 5 ml of the clear juice was taken for each sample and placed in a 10 ml test tube and 5 ml of the extraction solution was added to it (ethanol 9 and hydrochloric acid 1.5 N at a ratio of 15:85 for each, respectively), then it was centrifuged for 3 minutes at a speed of 3000 rpm and the precipitate was excluded. The filtrate was taken and completed to 10 ml with the extraction solution (acidified alcohol), and the light absorption was read by a spectrophotometer at a wavelength of 535 nm. The following equation was applied, taking into account the dilution for samples with high concentration, according to the following equation :

$$\text{Anthocyanin (100 mg/ml)} = \frac{\text{device reading} \times \text{solution volume}}{\text{sample volume}} \times 98.2 \times \text{dilution} \times 100\%$$

Statistical Analysis

The data were analyzed according to the split-plot design and the complete randomized design (RCBD). The Statistical Analysis System (SAS) program was used to analyze the data according to Duncan's multiple test to

compare the means at the probability level (0.05). Then, the genetic analysis for the reciprocal-half cross was conducted by dividing the square means of the coefficients. Figure 1 presented a cluster analysis diagram, also known as a relative tree, based on SSR-P markers for pure tomato lines.

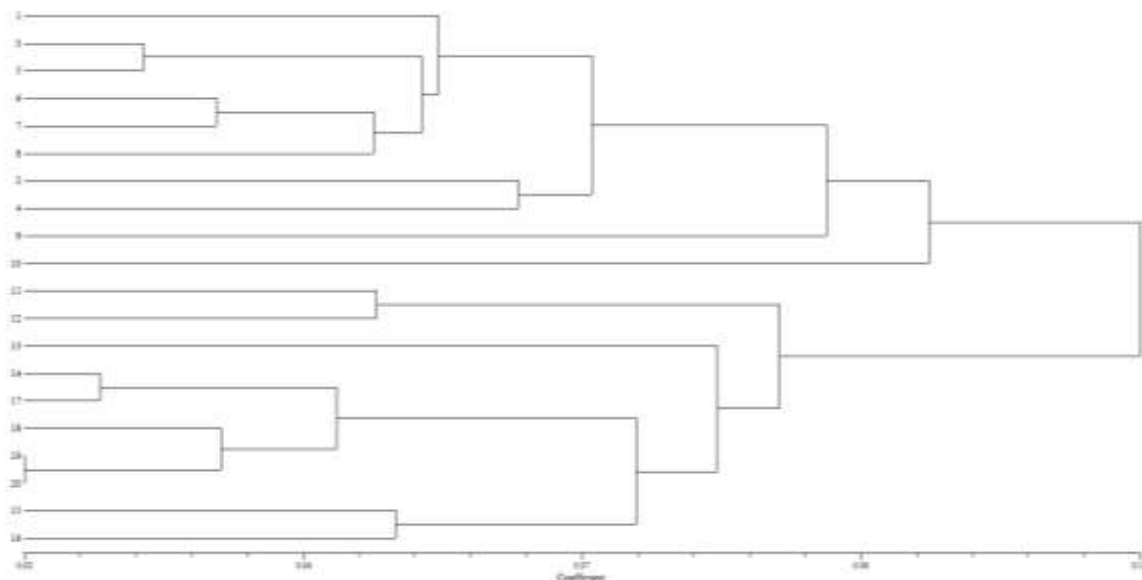


Figure 1. Cluster analysis diagram (relative tree) of pure tomato lines according to SSR-P markers

Fruit hardness (kg cm²)

Table 3 showed that a significant effect of genetic compositions on the fruit hardness trait, as parent 5 was significantly distinguished by the best fruit hardness of 3.547 kg cm² and it did not differ significantly from parent 6, which reached 3.517 kg cm², while it decreased to 2.611 kg cm² in the fruits of parent 4. The hybrid 5×6 excelled with the best fruit hardness of 5.888 kg cm², compared to the fruits of the hybrid 2×4, which gave the lowest hardness of 2.834 kg cm². The fruits of plants sprayed with nano-calcium were distinguished by the highest hardness of 5.092 kg cm², compared to the lowest hardness of the fruits of the control

into their components from the data for all measured traits [16.]

Results

plants, which was 2.789 kg cm². The interaction between genetic compositions and spraying with calcium had a significant effect on fruit hardness. Spraying with nano calcium and parent 5 recorded the highest fruit hardness of 4.737 kg cm², compared to 1.697 kg cm² for fruits of parent 4 treated with distilled water only, while fruit hardness in the hybrids ranged between 6.879 kg cm² for hybrid 5×6 under spraying with nano calcium to 1.527 kg cm² for hybrid 2×4 in the control treatment.

Total soluble solids (TSS(%))

The results in Table 4 indicated that parent 5 was superior with the highest percentage of total soluble solids, reaching 6.242%, and parent 1 gave the lowest percentage, reaching

3.677%. While the percentage of total soluble solids ranged between 9.155% in the 3×5 hybrid and 4.896% for the 4×6 hybrid. The plants treated with nano-calcium outperformed with the best percentage of total soluble solids, reaching 7.043%, compared to the percentage of total soluble solids for the control plants, which reached 5.747%. As for the interaction between genetic compositions and calcium quality, the plants of parent 5 with nano-calcium treatment gave the highest percentage of total soluble solids, reaching 6.885%, while the plants of parent 1 with control treatment recorded the lowest percentage, reaching 3.071%. The hybrids 3×5 and 2×5 under the nano-calcium treatment gave the highest percentage of soluble solids, reaching 9.818% and 9.7753%, respectively, while it decreased in the hybrid 4×6 to 4.439% in the control treatment.

Concentration of vitamin C in fruits (mg 100 g-1)

Parent 6 was distinguished by the highest concentration of vitamin C in the fruits, reaching 12.455 mg 100 g-1, while parents 2 and 3 gave the lowest values, reaching 8.490 and 8.465 mg 100 g-1, respectively (Table 5). The hybrid 1×6 was distinguished by the highest amount of vitamin C, reaching 18.217 mg 100 g-1, while this percentage decreased to 8.372 mg 100 g-1 in the hybrid 2×3. The highest value of vitamin C was recorded for the nano-calcium spray treatment, reaching 12.912 mg 100 g-1, while the fruits of the control treatment gave the lowest value, reaching 11.488 mg 100 g-1. The interaction between genetic compositions and calcium fertilizer had a significant effect on the trait, as the fruits of parents 5 and 6 with the nano-calcium spray treatment were distinguished by the highest content of vitamin C, reaching 12.634 and 12.686 mg 100 g-1, respectively,

and the fruits of parent 3 in the control treatment gave the lowest value, reaching 8.015 mg 100 g-1. As for the hybrids, the fruits of the hybrid 1×6 in the nano-calcium spray treatment were distinguished by the highest value, reaching 18.894 mg 100 gm-1, while the fruits of the hybrid 2×3 in the control treatment recorded the lowest value of 8.080 mg 100 gm-1.

Total acidity in fruits(%)

The fruits of parent 1 were significantly superior with the highest acidity content compared to all parents, which reached 0.919%, while the parent 3 recorded the lowest acidity content, which reached 0.345%. The hybrid 1×6 was significantly superior to all hybrids, which reached 1.184% compared to the hybrids 1×2 and 1×3, which gave the lowest acidity content, reaching 0.462% and 0.453% respectively (Table 6). The nano-calcium spray treatment was significantly superior, as it gave the highest rate for this trait, which reached 0.739% compared to the control treatment, which gave the lowest rate, which reached 0.613%. The interaction between the two study factors affected the trait, as parent 1 in the nano-calcium treatment gave the highest acidity content of 1.023%, while parent 3 in the control treatment recorded the lowest acidity content of 0.234%. The hybrid 1×6 in the nano-calcium treatment was distinguished by the highest acidity content of 1.322% compared to the hybrids 1×3 and 2×3 in the control treatment, which amounted to 0.412% and 0.410%, respectively.

Beta-carotene pigment in fruits (mg 100 g-1 fresh weight)

The results of Table 7 indicate that there are significant differences in the genetic compositions in the content of beta-carotene pigment in the fruits, as parent 3 showed the

highest ratio of 11.309 mg 100 g⁻¹, while parent 1 recorded the lowest content of beta-carotene of 3.528 mg 100 g⁻¹, which reflected this difference in the performance of the parents significantly on the performance of their hybrids, as the hybrid 2×3 recorded the highest ratio of 13.904 mg 100 g⁻¹, while the hybrid 2×6 gave the lowest ratio of 4.815 mg 100 g⁻¹. The pigment in the fruits increased significantly with the use of nano-calcium, which reached 8.746 mg 100 g⁻¹, while it decreased to 6.852 mg 100 g⁻¹ in the control treatment. The interaction between genetic compositions and calcium fertilizer had a significant effect on the trait, as the plants of parent 3 under the nano-calcium treatment outperformed with the highest content of 12.782 mg 100 g⁻¹, compared to the plants of parent 1, which recorded the lowest content of 2.567 mg 100 g⁻¹ with the control treatment. The hybrid 1×3 under the nano-calcium treatment was distinguished by the highest content of 14.364 mg 100 g⁻¹, while it did not differ significantly from the hybrid 2×3, which reached 14.228 mg 100 g⁻¹, while it decreased to 3.256 mg 100 g⁻¹ in the hybrid 4×6 with the control treatment.

Lycopene pigment in fruits (mg 100 g⁻¹ fresh weight)

The results of Table 8 showed significant differences between the genetic compositions in the lycopene pigment in fruits, as parent 6 achieved the highest pigment content of 6.544 mg 100 g⁻¹, while parent 1 gave the lowest value of 1.364 mg 100 g⁻¹, and the difference between parents in the pigment content of the fruits affected the resulting hybrids, as the hybrid 4×6 was significantly superior with the highest pigment content of 8.963 mg 100 g⁻¹, while the hybrid 1×2 recorded the lowest value of 2.549 mg 100 g⁻¹. The ordinary and nano calcium types had a significant effect on

the trait, as the nano-calcium treatment gave the highest pigment content of 6.013 mg 100 g⁻¹, compared to the control treatment, which recorded the lowest value, reaching 5.529 mg 100 g⁻¹. The interaction between the study factors had a significant effect on the trait, as the use of calcium in its two types, ordinary and nano, led to a significant increase in the trait values for the studied genetic compositions, as the plants of parent 6 outperformed with the nano-calcium treatment by giving the highest pigment content of 6.728 mg 100 g⁻¹, while the plants of parent 1 in the control treatment recorded the lowest value of 1.224 mg 100 g⁻¹. The hybrid plants 4×6 and 5×6 in the nano-calcium treatment achieved the highest values, which were 9.546 and 9.576 mg 100 g⁻¹ respectively, while the hybrid plants 1×2 in the control treatment gave the lowest value of 2.254 mg 100 g⁻¹.

Total sugar content in juice (%)

The results of Table 9 showed that there are significant differences in the fruit content of total sugars between the studied genetic compositions, as parents 2 and 5 gave the highest increase in the content of total sugars, reaching 6.796% and 6.736%, respectively, while parent 1 recorded the lowest value, reaching 3.729%. The difference between parents also had a significant effect on the values of the hybrids for the same trait, as the hybrid 2×5 achieved the highest increase, reaching 12.799%, while the hybrid 4×6 recorded the lowest value, reaching 6.443%. Calcium, both ordinary and nano, had a significant effect on the trait, as the plants of the nano-calcium treatment gave an increase in the percentage of total sugars, reaching 9.720%, compared to the plants of the control treatment, which recorded the lowest value, reaching 7.791%. The interaction between the study factors had a significant effect on the

trait, as the use of both ordinary and nano calcium led to a significant increase in the trait values for the studied genetic compositions, as the plants of parent 2 in the nano-calcium treatment outperformed in the highest percentage of total sugars, reaching 7.427%, while the plants of parent 1 in the control treatment recorded the lowest value, reaching 2.653%. The hybrid plants 2×5 and 3×5 in the nano-calcium treatment recorded the highest percentage of total sugars, reaching 13.427% and 13.223%, respectively, while they did not differ significantly from the hybrid 2×3, which gave 13.127%, while the hybrid plants 4×6 in the control treatment gave the lowest value, reaching 5.577%.

Anthocyanin pigment in the fruits (mg 100 g⁻¹)

The parent 4 was significantly superior in the anthocyanin pigment content in the fruits, as it gave 31.034 mg 100 g⁻¹, while parent 2 recorded the lowest value for the trait, reaching 2.409 mg 100 g⁻¹. The hybrid 1×4 was significantly distinguished by the highest pigment content, reaching 37.984 compared to the hybrid 1×2, which recorded the lowest value, reaching 4.064 mg 100 g⁻¹ (Table 10). The nano-calcium treatment was significantly superior in the highest content of anthocyanin pigment, reaching 17.547 mg 100 g⁻¹, while it decreased to 14.969 mg 100 g⁻¹ in the control treatment. The interaction between the study factors had a significant effect, as the interaction between parent 4 and nano-calcium spray achieved the highest pigment content in the fruits, reaching 33.887 mg 100 g⁻¹, while parent 2 under the control treatment gave the lowest value, reaching 2.276 mg 100 g⁻¹, and the hybrid 1×4 in the nano-calcium treatment was distinguished by the highest value, reaching 40.579 mg 100 g⁻¹, compared to the

hybrid 1×2 in the control treatment, which gave 3.431 mg 100 g⁻¹.

Discussion

The tables revealed significant differences among the genetic compositions (parents and resulting hybrids) in the crop's qualitative characteristics. The superiority of one genetic composition over another arises from complex interactions between the unique genetic composition of each genetic composition and environmental conditions. Gene-environment interactions indicate that genes alone do not determine plant traits; instead, they interact with the prevailing environmental conditions. For example, the same gene may produce large fruits in a nutrient-rich environment but small fruits in a nutrient-poor environment. This variation can be attributed to genetic diversity, as each hybrid inherits a distinct genetic composition resulting from the hybridization of parents with different traits. This genetic composition determines various inherited characteristics, including the quantitative and qualitative properties of the fruits. These findings align with those of [15] and are consistent with studies by [11,25,14,20,26,23,18].

Similarly,[1]demonstrated in tomato plants that hybridization, particularly with wild varieties, resulted in F1 hybrids outperforming their parents in several quantitative and qualitative traits, including total yield, fruit count, fruit sugar content, total soluble solids, vitamin C, and pigments such as beta-carotene and lycopene. The results of this study, in addition to many previous studies, confirm the importance of genetic diversity in plant breeding programs. The process of identifying the pure lines necessary for hybridization is based on the availability of variations in physiological and morphological traits. The differences in traits between the studied

parents increase the chances of obtaining hybrid plants with the desired traits. Therefore, assessing genetic diversity is the first and most important step in any tomato breeding and improvement program. The results of the genetic dimension study showed the difference in the parental genotypes used in this experiment, which led to obtaining hybrid plants with good quantitative and qualitative traits. Genetic diversity is the fuel that drives the agricultural improvement process, as it provides the raw material needed to develop new varieties with higher productivity and better quality. These results are consistent with previous studies [15,41,12,3,40,37]. [13,39] reported similar differences in the number of fruits per plant among the studied tomato hybrids. The results showed that spraying the genetic compositions (parents and their hybrids) with regular and nano calcium improved the qualitative characteristics of the crop. This is attributed to the role of calcium in improving the vegetative growth characteristics and increasing the leaf content of nutrients, in addition to its effect in increasing the efficiency of the genetic compositions in representing carbon dioxide and increasing the percentage of manufactured carbohydrates in the leaves. These factors combined contribute to enhancing plant growth and increasing the yield, thus improving the quality of the fruits [33]. Perhaps the reason is due to its role in increasing the various metabolic activities responsible for the division processes in the plant cell, in addition to its role in activating many enzymes. It is also considered an important carrier within cells in metabolism, a mediator of morphological responses and increasing the absorption of the necessary elements to activate enzymes, which work to decompose organic compounds and encourage

the respiration process and contribute to the construction of protoplasm, DNA and RNA nucleic acids, as well as the process of regulating and transporting nutrients across cell membranes, which led to improving the qualities of the crop in terms of quantity and quality [43,34]. These results confirm what was reached by [7]. Since nano-fertilizers increase the activity of carbon metabolism processes, increase crop resistance to diseases, maintain the necessary characteristics of agricultural crops, and increase the amount of active substances in the plant, they help to increase the productive efficiency of the cultivated area, which in turn improves both the quantity and quality of production [5.]

Increasing the calcium content of the leaves, both regular and nano, led to a significant increase in the firmness of the fruits. This is due to the transfer of calcium from its production sites in the leaves to the fruits, where it combines with pectic acid to form calcium pectate, the main component of the middle lamella that connects the cell walls together, which increases the firmness of the fruits. In addition, calcium contributed to increasing the acidity of the fruits, increasing the percentage of total soluble solids, and increasing the vitamin C in the fruits. This is due to calcium improving the efficiency of the photosynthesis process in the leaves, which led to an increase in the production of essential nutrients, which in turn are transferred to the fruits, which improves the quality of the fruits [42,45.]

Conclusion

The pure lines of colored tomatoes used in the study are characterized by a genetic dimension, and thus they can be used as parents in other breeding programs to improve growth and productivity traits of tomatoes. The use of parents and their hybrids and nano-

calcium at a concentration of 2 g L⁻¹ improved most of the quality growth traits.

Table 3. Effect of genetic compositions, calcium and their interaction on fruit hardness (kg cm²)

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	1.999 \bar{f}	3.113 z	3.798 qr	2.970 N
2	1.887 \bar{g}	2.738 \bar{b}	3.897 pq	2.841 O
3	2.188 \bar{e}	3.387 x	4.148 mn	3.241 M
4	1.697 \bar{h}	2.639 \bar{bc}	3.497 vw	2.611 P
5	2.387 \bar{d}	3.517 uv	4.737 i	3.547 JK
6	2.417 \bar{d}	3.518 uv	4.617 j	3.517 K
1×2	2.379 \bar{d}	3.617 tv	4.749 i	3.582 J
1×3	3.119 z	4.627 j	5.767 d	4.505 F
1×4	1.977 \bar{fg}	3.318 xy	4.320 l	3.205 M
1×5	3.677 st	4.898 h	6.027 c	4.867 D
1×6	4.082 no	5.552 ef	6.599 b	5.411 C
2×3	2.127 \bar{e}	3.397 wx	4.457 k	3.327 L
2×4	1.527 \bar{i}	2.989 \bar{a}	3.985 op	2.834 O
2×5	3.137 z	4.327 l	5.467 f	4.310 G
2×6	3.277 y	4.477 k	5.649 e	4.468 F
3×4	2.719 \bar{b}	4.477 k	5.616 e	4.271 G
3×5	4.241 lm	5.754 d	6.693 b	5.563 B
3×6	3.510 uv	4.737 i	5.838 d	4.695 E
4×5	2.567 \bar{c}	3.757 rs	4.927 h	3.751 I
4×6	2.717 \bar{b}	4.118 n	5.267 g	4.034 H
5×6	4.942 h	5.842 d	6.879 a	5.888 A
Mean of calcium	2.789 C	4.038 B	5.092 A	

Table 4. Effect of genetic compositions, calcium and their interaction on total soluble solids (T.S.S. %)

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	3.071 \bar{r}	3.991 \bar{p}	3.971 \bar{p}	3.677 S
2	4.170 $\bar{n}\bar{o}$	5.220 \bar{h}	6.772 s	5.387 O
3	5.146 $\bar{i}\bar{j}$	6.279 x	6.753 s	6.059 K
4	3.250 \bar{q}	4.129 \bar{o}	5.136 \bar{j}	4.171 R
5	5.378 \bar{g}	6.465 v	6.885 r	6.242 J
6	4.037 \bar{p}	4.207 \bar{n}	4.999 \bar{k}	4.414 Q
1×2	6.679 t	7.739 jk	8.825 d	7.747 E
1×3	7.579 mn	8.449 f	8.716 e	8.248 D
1×4	4.538 \bar{l}	5.643 \bar{e}	5.875 \bar{a}	5.352 O
1×5	7.246 o	7.238 o	7.549 n	7.344 G
1×6	5.515 \bar{f}	5.770 $\bar{b}\bar{c}$	5.825 $\bar{a}\bar{b}$	5.703 N
2×3	7.871 i	8.128 h	9.169 c	8.389 C
2×4	5.171 $\bar{h}\bar{i}\bar{j}$	5.717 $\bar{c}\bar{d}$	6.667 t	5.851 M
2×5	7.922 i	8.375 g	9.753 a	8.683 B
2×6	5.311 \bar{g}	5.983 z	6.349 w	5.901 L
3×4	7.567 mn	7.697 kl	7.777 j	7.680 F
3×5	8.112 h	9.535 b	9.818 a	9.155 A
3×6	6.250 x	6.547 u	6.790 s	6.529 I
4×5	6.171 y	7.115 p	7.638 lm	6.974 H
4×6	4.439 \bar{m}	4.580 \bar{l}	5.671 $\bar{d}\bar{e}$	4.896 P
5×6	5.210 $\bar{h}\bar{i}$	5.449 \bar{f}	6.979 q	5.879 LM
Mean of calcium	5.747 C	6.393 B	7.043 A	

Table 5. Effect of genetic compositions, calcium and their interaction on concentration of vitamin C in fruits (mg/100 ml-1 juice)

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	10.022 \bar{l}	11.356 \overline{ef}	11.879 z	11.085 N
2	8.114 \bar{u}	8.433 \bar{r}	8.924 \bar{n}	8.490 R
3	8.015 \bar{v}	8.654 \bar{p}	8.727 \bar{o}	8.465 R
4	11.115 \bar{h}	11.433 \bar{d}	11.924 z	11.490 K
5	10.017 \bar{l}	11.237 \bar{g}	12.634 u	11.296 L
6	12.247 x	12.433 w	12.686 u	12.455 G
1×2	8.137 \bar{u}	8.654 \bar{p}	10.321 \bar{j}	9.037 P
1×3	8.237 \bar{t}	8.556 \bar{q}	10.127 \bar{k}	8.973 Q
1×4	13.389 q	14.237 p	14.756 n	14.127 F
1×5	15.269 m	15.843 k	16.218 j	15.776 D
1×6	17.603 e	18.154 c	18.894 a	18.217 A
2×3	8.080 \bar{u}	8.360 \bar{s}	8.678 \overline{op}	8.372 S
2×4	8.311 \bar{s}	9.683 \bar{m}	9.714 \bar{m}	9.236 O
2×5	10.012 \bar{l}	11.680 \bar{b}	11.759 \bar{a}	11.150 M
2×6	11.399 \overline{de}	12.432 w	13.174 s	12.335 H
3×4	11.579 \bar{c}	12.525 v	13.252 r	12.452 G
3×5	10.512 \bar{i}	12.115 y	13.347 q	11.991 I
3×6	11.321 \bar{f}	11.639 \bar{b}	12.772 t	11.910 J
4×5	14.278 p	14.689 o	15.347 l	14.771 E
4×6	16.580 i	17.218 g	17.689 d	17.162 C
5×6	17.014 h	17.547 f	18.348 b	17.636 B
Mean of calcium	11.488 C	12.232 B	12.912 A	

Table 6. Effect of genetic compositions, calcium and their interaction on total acidity in fruits (%)

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	0.823 l	0.911 j	1.023 g	0.919 E
2	0.404 \overline{ef}	0.463 \overline{cd}	0.490 \overline{ab}	0.452 N
3	0.234 \overline{g}	0.396 \overline{f}	0.406 \overline{ef}	0.345 O
4	0.421 \overline{e}	0.462 \overline{cd}	0.486 \overline{b}	0.456 N
5	0.462 \overline{cd}	0.522 wxy	0.612 tu	0.532 L
6	0.485 \overline{b}	0.672 r	0.681 qr	0.612 J
1×2	0.420 \overline{e}	0.456 \overline{d}	0.512 xyz	0.462 N
1×3	0.412 \overline{ef}	0.452 \overline{d}	0.497 \overline{zab}	0.453 N
1×4	0.875 k	0.963 i	1.010 gh	0.949 D
1×5	1.044 f	1.089 e	1.272 b	1.135 B
1×6	1.112 d	1.118 d	1.322 a	1.184 A
2×3	0.410 \overline{ef}	0.462 \overline{cd}	0.499 \overline{zab}	0.457 N
2×4	0.622 st	0.678 r	0.710 op	0.670 H
2×5	0.563 v	0.672 r	0.700 pq	0.645 I
2×6	0.562 v	0.600 u	0.634 s	0.598 K
3×4	0.483 \overline{bc}	0.508 $\overline{yz\bar{a}}$	0.534 w	0.508 M
3×5	0.572 v	0.636 s	0.715 nop	0.641 I
3×6	0.508 $\overline{yz\bar{a}}$	0.531 wx	0.577 v	0.538 L
4×5	0.726 no	0.730 no	0.759 m	0.738 G
4×6	0.735 n	0.873 k	0.922 j	0.843 F
5×6	1.002 h	1.088 e	1.172 c	1.087 C
Mean of calcium	0.613 A	0.680 B	0.739 A	

Table 7. Effect of genetic compositions, calcium and their interaction on beta-carotene pigment in fruits (mg/g-1 fresh weight)

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	2.567 \bar{l}	3.710 \overline{hij}	4.306 \overline{fg}	3.528 Q
2	9.101 op	9.861 m	12.193 fg	10.385 E
3	10.130 lm	11.016 j	12.782 e	11.309 D
4	3.396 \overline{jk}	3.644 \overline{ij}	5.164 \bar{e}	4.068 P
5	7.201 t	8.435 r	9.466 n	8.367 H
6	3.579 \overline{ijk}	4.227 \overline{fg}	6.027 $yz\bar{a}$	4.611 O
1×2	11.024 j	11.697 hi	12.014 gh	11.578 C
1×3	12.363 f	13.134 d	14.364 a	13.287 B
1×4	4.435 \bar{f}	5.471 \overline{cde}	7.244 t	5.717 K
1×5	7.694 s	8.523 r	9.186 nop	8.468 H
1×6	5.171 \bar{e}	5.366 \overline{de}	5.653 \overline{bcd}	5.397 L
2×3	13.577 c	13.907 b	14.228 ab	13.904 A
2×4	5.354 \overline{de}	6.134 xy	6.697 uvw	6.062 J
2×5	9.396 no	9.900 m	11.405 i	10.234 E
2×6	3.878 \overline{hi}	4.024 \overline{gh}	6.544 vw	4.815 N
3×4	5.640 \overline{bcd}	6.435 wx	6.889 tuv	6.321 I
3×5	7.040 tu	10.354 kl	10.601 k	9.332 F
3×6	5.760 $z-\bar{c}$	6.106 xyz	7.003 tu	6.290 I
4×5	8.755 qr	8.996 pq	9.207 nop	8.986 G
4×6	3.256 \bar{k}	5.907 $y-\bar{b}$	5.922 $y-\bar{b}$	5.028 M
5×6	4.572 \bar{f}	5.699 $\overline{a-d}$	6.774 uvw	5.682 K
Mean of calcium	6.852 C	7.740 B	8.746 A	

Table 8. Effect of genetic compositions, calcium and their interaction on lycopene pigment in fruits (mg/g-1 fresh weight

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	1.224 \bar{v}	1.342 \bar{u}	1.528 \bar{t}	1.364 T
2	2.009 \bar{s}	2.083 \bar{r}	2.061 $\bar{r}\bar{s}$	2.051 S
3	3.270 \bar{n}	3.238 \bar{n}	3.405 \bar{m}	3.304 Q
4	5.299 $\bar{e}\bar{f}$	5.409 \bar{c}	5.419 \bar{c}	5.375 N
5	5.689 \bar{b}	5.717 \bar{b}	5.917 \bar{a}	5.774 L
6	6.328 vw	6.578 u	6.728 s	6.544 I
1×2	2.254 \bar{q}	2.485 \bar{p}	2.909 \bar{o}	2.549 R
1×3	3.613 \bar{l}	3.822 \bar{k}	3.887 \bar{j}	3.774 P
1×4	5.280 \bar{f}	6.028 z	6.907 pq	6.071 K
1×5	6.705 s	6.843 r	6.885 qr	6.811 H
1×6	6.377 v	7.121 m	7.443 j	6.980 F
2×3	4.050 \bar{i}	4.507 \bar{h}	4.571 \bar{g}	4.376 O
2×4	5.395 $\bar{c}\bar{d}$	5.350 $\bar{d}\bar{e}$	5.708 \bar{b}	5.484 M
2×5	6.637 t	7.028 n	6.987 no	6.884 G
2×6	6.949 op	7.220 l	7.458 j	7.209 E
3×4	6.097 y	6.215 x	6.309 w	6.207 J
3×5	7.085 m	7.177 l	7.330 k	7.197 E
3×6	7.841 g	8.062 f	8.087 f	7.996 C
4×5	7.554 hi	7.518 i	7.609 h	7.560 D
4×6	8.301 d	9.043 b	9.546 a	8.963 A
5×6	8.154 e	8.692 c	9.576 a	8.807 B
Mean of calcium	5.529 C	5.784 B	6.013 A	

Table 9. Effect of genetic compositions, calcium and their interaction on total sugar content in juice (%)

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	2.653 \bar{d}	3.654 \bar{c}	4.881 z	3.729 R
2	6.134 wx	6.827 tu	7.427 rs	6.796 M
3	4.427 \overline{ab}	5.136 z	7.127 st	5.563 O
4	4.077 \bar{b}	5.005 z	6.377 vw	5.153 P
5	5.827 xy	7.238 s	7.144 st	6.736 M
6	3.653 \bar{c}	4.454 \bar{a}	5.681 y	4.596 Q
1×2	10.753 l	12.277 efg	12.834 bc	11.954 C
1×3	10.727 l	11.944 g-j	12.433 def	11.701 D
1×4	9.877 mn	10.816 l	11.652 jk	10.781 F
1×5	7.977 q	9.745 n	10.877 l	9.533 I
1×6	6.577 uv	7.254 s	8.777 p	7.536 L
2×3	12.105 f-i	12.559 cde	13.127 ab	12.597 AB
2×4	9.627 n	10.560 l	11.327 k	10.504 G
2×5	12.206 e-h	12.766 bcd	13.427 a	12.799 A
2×6	8.552 p	10.162 m	11.800 ij	10.171 H
3×4	10.778 l	11.548 jk	11.877 hij	11.401 E
3×5	11.713 ij	12.427 def	13.223 a	12.451 B
3×6	7.777 qr	8.427 p	9.827 mn	8.677 J
4×5	6.477 uvw	7.777 qr	9.153 o	7.802 K
4×6	5.577 y	6.327 vw	7.427 rs	6.443 N
5×6	6.127 wx	6.657 uv	7.727 qr	6.837 M
Mean of calcium	7.791 C	8.740 B	9.720 A	

Table 10. Effect of genetic compositions, calcium and their interaction on the content of anthocyanin pigment in the fruits (mg 100 ml⁻¹)

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	13.003 \bar{d}	14.164 \bar{a}	14.836 y	14.001 K
2	2.276 \bar{y}	2.412 \bar{x}	2.541 \bar{w}	2.409 T
3	5.060 \bar{s}	6.145 \bar{r}	6.486 \bar{q}	5.897 Q
4	29.156 l	30.060 k	33.887 h	31.034 D
5	7.650 \bar{n}	8.225 \bar{l}	8.793 \bar{j}	8.222 O
6	15.275 w	16.830 u	18.824 p	16.976 I
1×2	3.431 \bar{v}	4.109 \bar{u}	4.653 \bar{t}	4.064 S
1×3	5.068 \bar{s}	7.956 \bar{m}	10.025 \bar{h}	7.683 P
1×4	36.097 e	37.276 c	40.579 a	37.984 A
1×5	11.848 \bar{e}	13.078 \bar{cd}	15.164 wx	13.363 M
1×6	17.115 t	17.933 s	18.300 r	17.782 H
2×3	4.720 \bar{t}	6.072 \bar{r}	6.618 \bar{p}	5.803 R
2×4	31.078 j	32.276 i	35.282 f	32.878 C
2×5	7.067 \bar{o}	8.611 \bar{k}	9.092 \bar{i}	8.256 O
2×6	16.214 v	19.978 no	20.060 n	18.750 F
3×4	34.549 g	36.820 d	38.793 b	36.720 B
3×5	10.152 \bar{g}	10.830 \bar{f}	13.003 \bar{d}	11.328 N
3×6	13.181 \bar{c}	13.848 \bar{b}	14.390 z	13.806 L
4×5	14.836 y	15.127 x	18.298 r	16.087 J
4×6	18.756 pq	19.911 o	20.182 m	19.616 E
5×6	17.830 s	17.918 s	18.689 q	18.145 G
Mean of calcium	14.969 C	16.170 B	17.547 A	

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