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The quality of Pachino tomato. The soilless tomato cultivation with salty water

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Indice

Abstract

Tomato is one of the most consumed and traded crops in the world. This is due to its high productivity and its composition rich of nutrient, in particular antioxidants. Among these, the most present are polyphenols and carotenoids, mainly lycopene and β-carotene, vitamin A provitamin. The production of tomato is widespread almost all over the world, however it may have some critical issues, which include soil acidity and alkalinity. The pH represents one of the main soil characteristics for all crops and can affect the absorption of ions and the osmotic balance of the plant inducing water stress, oxidative stress and other problems that decrease plant production yield. Soil alkalinization is a spreading problem affecting more and more planet cultivation soil. In this study, two tomato genotypes with opposing responses to alkaline pH were analyzed: the Moresco genotype, sensitive to alkaline pH, and the Blue genotype, naturally tolerant to alkaline pH. Both genotypes were grown with hydroponic technique under different pH conditions, from optimal (pH 5.2) to more alkaline (pH 7.2, 8.2, and 9.2). The aim of this study was to investigate the consequences of alkalinity in the cultivation of the two genotypes, analyzing their composition and evaluating the response mechanisms used by the plant to deal with alkaline stress. The cultivation lasted eight days, after which leaf samples were taken and the content of proline, polyphenols, flavonols, condensed tannins, chlorophyll and carotenoids were analyzed. The results showed that the contents of the amino acid proline, polyphenols and flavonols compounds are influenced by elevated pH only in the Blue genotype, in which they increased in relation to the increase in pH; the content of condensed tannins is not affected by pH for either genotypes; the content of leaf pigments, that means chlorophyll and carotenoids, varies depending on the genotype, in fact it was higher in the Moresco genotype, but was not affected by pH. The increase in proline and antioxidants polyphenols and flavonols, may indicate that the Blue genotype develops a mechanism of response to the stress that makes the plant more tolerant. In fact, proline, polifenols and flavonols, are involved in signaling and response pathways of tomato against stress. This information has important implications for cultivation in alkaline environments and may be useful for studies about the use of stress in improving nutritional quality of crops.

Riassunto

Il pomodoro è una delle coltivazioni più consumate e commerciate al mondo. Questo è dovuto alla sua alta produttività e la sua composizione ricca di nutrienti, in particolare antiossidanti. Tra questi, i più presenti sono polifenoli e carotenoidi, principalmente licopene e β-carotene, provitamina della vitamina A. La produzione di pomodoro è diffusa in tutto il mondo, tuttavia può avere delle criticità, tra cui acidità e alcalinità del suolo. Il pH rappresenta una delle caratteristiche più importanti del suolo per tutte le coltivazioni e può influenzare l'assorbimento di ioni e l'equilibrio osmotico della pianta inducendo stress idrico, stress ossidativo e altri problemi che diminuiscono la resa di produzione della pianta. In particolare l'alcalinizzazione del suolo è un problema in diffusione che influisce su sempre più suolo terrestre. In questo studio sono stati analizzati due genotipi di pomodoro con opposta risposta al pH alcalino: il genotipo Moresco, sensibile al pH alcalino, e il genotipo Blue, naturalmente resistente al pH alcalino. Entrambi i genotipi sono cresciuti con tecnica idroponica in differenti condizioni di pH, da ottimale (pH 5.2) a maggiormente alcalino (pH 7.2, pH 8.2, pH 9.2). Lo scopo di questo studio è di investigare sulle conseguenze dell'alcalinità nella coltivazione dei due genotipi, analizzando la loro composizione e cercando i meccanismi di risposta usati dalla pianta per affrontare lo stress alcalino. La coltivazione è durata otto giorni, dopo i quali sono stati analizzati i contenuti di prolina, polifenoli, flavonoli, tannini condensati, clorofilla e carotenoidi da campioni fogliari prelevati. I risultati hanno mostrato che i contenuti dell'aminoacido prolina, di polifenoli e di flavonoli sono influenzati dal pH basico solo nel genotipo Blue, nel quale sono aumentati in relazione all'innalzamento del pH; il contenuto di tannini condensati non è influenzato dal pH per nessuno dei due genotipi; il contenuto di pigmenti fogliari, cioè clorofilla e carotenoidi, varia a seconda del genotipo, infatti era più alto nel genotipo Moresco, ma non è influenzato dal pH. L'incremento in prolina e antiossidanti polifenoli e flavonoli, può indicare che il genotipo Blue ha sviluppato meccanismi di risposta allo stress che permettono alla pianta di resistere. Infatti, prolina, polifenoli e flavonoli, sono coinvolti in meccanismi di segnalazione e risposta allo stress nel pomodoro. Queste informazioni hanno importanti implicazioni per la coltivazione in ambienti alcalini e possono risultare utili per studi sull'uso dello stress nel miglioramento della qualità nutrizionale delle coltivazioni.

1. Introduction

The cultivated tomato (*Solanum lycopersicum* L.) is an annual herbaceous dicotyledonous belonging to the Solanaceae family, which includes over 3000 species from all over the world, among which eggplant, bell pepper, and potato. Tomato is native to the Center and South America (Bergougnoux, 2013). The original domestication area might be southern Mexico or Peru, but it remains still uncertain (Razdan & Matoo, 2006). The crop arrived in Europe after the discovery of America, leading to its transportation to Spain and then its diffusion in other Mediterranean countries. Tomato arrived in Italy from Spain in 1548, initially in the ports of Naples and Sicily, and then spread throughout the country along with other plants from the American continent, i.e. potato.

Compared to its ancestors and thanks also to the work of man, the tomato has undergone numerous genetic changes over time, which have also induced the domestication syndrome: the adaptation of a species to the cultivated state. One of the most obvious examples of the tomato domestication syndrome is the change in composition and color of the berry from green to pigmented (Angelini, 2010). This occurs with the ripening of the fruit that is accompanied by loss of chlorophyll and synthesis of carotenoid pigments (β-carotene and lycopene) and flavonoids. At the cellular level, the change occurs with the transformation of photosynthetic organelles, chloroplasts, into chromoplasts, organelles of accumulation of secondary metabolites. Fruit ripening is regulated by transcription factors, the plant hormone ethylene, and the last studies also mention DNA methylation (Liu et al., 2022).

Southern Europe represents a suitable climatic situation for tomatoes, which at first were used as ornamental plants and not as food, but over time their potential was discovered making them the protagonist of many of the institutional dishes of the area (Angelini, 2010). Through the years tomato has become an important component of the traditional Mediterranean diet. The popularity of the fruit and its numerous uses make the tomato one of the most important sources of vitamins, minerals, and fiber in the human diet. On the other hand, many tomato-based processed products (e.g., ketchup), don't have the same nutritional characteristics, but come with high supplements of sugars and salt (Erba et al., 2013). Composition is an important variable for the processing of tomatoes, in terms of sugar and acid content. But fresh-market tomatoes are selected also for color, size, and homogeneity of the berry (Bhandari et al., 2023).

Tomato fruit is a berry composed of 95% of water. Sugars and organic acids account for 60% of the dry matter, these soluble solids represent the main variable in the processing industries. The proportion of these compounds is a key factor in flavor quality and intensity (Grandillo et al., 2004). A high acid and a low sugar content is responsible for a tart taste, while a high sugar and low acid content will result in too sweet and no tasty tomato. The most desirable and favorable taste is due to a high acid and a high sugar content (Todevska et al., 2023).

The nutritional quality of tomatoes depends on genetics, cultivation practices, environmental factors, harvesting period, and storage (Todevska et al., 2023). Anyway, tomato berry presents several useful nutritional compounds: minerals, vitamins, essential amino acids, monounsaturated fatty acids, carotenoids, and phytosterols, and comes with low-calorie content. These nutrients help in constipation prevention, reduction of high blood pressure, stimulation of blood circulation, maintenance of lipid profile and body fluids, detoxification of body toxins, and maintaining bone structure and strength (Ali et al., 2021; Todevska et al., 2023). Moreover, many of these compounds, such as carotenoids, ascorbic acid (vitamin C), and tocopherol (vitamin E), have antioxidant properties (Frusciante et al., 2007; Erba et al., 2013). Tomato fruit is also a good source of fiber: its peel and seeds are composed of on average 30% of fiber, which resists from small intestine digestion and absorption, making a partial or complete fermentation by intestinal bacteria in the large intestine. Fibers produce many beneficial effects on the digestive tract such as the regulation of intestinal function, improvement of the tolerance to glucose in diabetics, and prevention of chronic diseases such as colon cancer (Herrera et al., 2010). Moreover, traits like appearance (shape, color), consistency (variating on the ripeness), and palatability (determined sugar, acids, and volatile compounds content), are considered when determining the quality of the tomato. The shelf life of fruits is also important for the market, as a longer shelf life contributes to lower economic losses and less fruit waste (Todevska et al., 2023).

Its characteristics make tomato one of the most produced and consumed fruits all over the world. The main producing countries in the world are China (making around 20% of the world production, more than 62million tons/year on 779.703 hectares), followed by India (10% world production, 19 million tons/year), and United States (8% world production, with 12 million tons/year). In Europe, the main producing countries are Italy and Spain. Over the past decade, trends have shown a steady increase in both production and consumption.

While the global population increase is driving a growing demand for horticultural products, urbanization, and climate change are causing a loss of arable land. Tomato cultivation is carried on even in areas not suitable climate for plant growth, for example, areas with unsuitable pH. Irrespective of the crop and cultivation system, soil pH is a key variable for plant productivity and yield.

Soil pH is a determinant factor in agriculture as it influences the availability of nutrients and, therefore, the bioavailability of these nutrients for the plants. This availability depends on the chemical form of the elements, influenced by the soil's ongoing pedogenetic processes. Nitrogen fertilizers, decomposition of the organic matter, soil type and land use, rainfall, and weathering of minerals altogether contribute to determining soil pH (Oshunsanya S, 2019). Consequently, soil and crop productivity are linked to the soil pH value (Minasny et al., 2016). The optimal pH for the survival of most crops is between 5.5 and 7.5. However, some crops have adapted to different soil pH values because each region has its characteristics of pH, texture, porosity, and temperature, which in turn affect the content of basic and acidic ions (Oshunsanya, 2019). In soil, acidic pH can be increased by using finely ground agricultural lime (limestone or chalk), while alkaline pH can be lowered by using acidifying fertilizers or organic materials (Oshunsanya S, 2019).

Both acidic soils (pH <7) and alkaline soils (pH > 7) influence the growth and development of crops and their productivity (Oshunsanya, 2019). Changes in soil pH can result from natural causes or human-induced factors. Decreasing pH can occur due to various processes such as heavy precipitation, crop growth, fertilizer use, acid rain (increasingly common due to climate change), and oxidative atmospheric agents (Neina, 2019). An increase in soil pH can be caused, for example, by the erosion of mineral compounds containing Na^+, Ca^{+2}, Mg^{+2}, and K^+, such as silicate minerals, aluminosilicates, and carbonates. These minerals typically enter the soil through the deposition of sediments eroded by water or wind (Neina, 2019).

Plant productivity decreases when pH drops below 5.5: in this situation, toxic amounts of aluminum and manganese are soluble and are released into the soil solution. Soluble aluminum is toxic to the roots of many crops and therefore limits their access to soil, water, and nutrients. Low soil pH also decreases microbial activity and makes nutrients such as phosphorus, magnesium, calcium, and molybdenum gradually unavailable to plant roots in the soil. In these conditions, fertilizers become less effective, and agricultural production can be significantly reduced. Crops grown on acidic soils may suffer from aluminum, hydrogen, and manganese toxicity, as well as calcium and magnesium deficiencies. Aluminum toxicity is the most widespread problem deriving from acidic soils, occurring when the element is present in the ionic form Al3+. This form is the most soluble of aluminum form at pH < 5. Aluminum is not a plant nutrient but in its ionic form can enter plant roots passively through the osmosis process, inhibiting root growth and development by interfering with the uptake and transport of essential nutrients, cell division, cell wall formation, and enzyme activity (Oshunsanya S, 2019).

On the other hand, alkaline soils can also negatively influence crop growth and development. Alkaline soils are characterized by slow infiltration, reduced hydraulic conductivity, and low water retention capacity, making water stress the main implication of alkaline soils, together with ion toxicity and high pH. (Oshunsanya S, 2019). Moreover, alkali stress has complex effects on plant metabolism, specifically root physiology, causing a reduction of root length and area (Kang et al., 2011). The problem is particularly important, for example, in northeastern China, where salt-alkalinized grassland covers more than 70% of the land, and this area is expanding (Wang et al., 2015).

In tomato, high pH interferes with Sodium (Na^+) transport, causing a rise of this element to a toxic level. This interferes with ionic balance in tissues. As a cascade, physiological functions, like water and ion uptake in roots, are affected, and leaf water potential decreases as well as stomatal conductance and net photosynthetic rates (Oshunsanya S, 2019; Khan et al., 2022). The decrease of the stomatal conductance causes a reduction in leaf development and a decrease in fresh weights and leaf area of the shoots (Kang et al., 2011). In addition, alkaline pH interferes with Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) uptake (Wang et al., 2011; Kang et al., 2011). Under alkali stress, the concentration of inorganic anions decreases. In this situation, tomato synthesizes organic acids to compensate for the inorganic anion shortage. To cope with the damages caused by saline-alkaline stress the levels of Abscisic Acid (ABA), involved in stress response, increase. The response of ABA to environmental stresses includes the enhancement of reactive oxygen species (ROS), followed by antioxidant enzyme activities (Xu et al., 2022).

In this study, the behavior of two different tomato genotypes was evaluated in response to different alkaline pH. The “Moresco” genotype, sensible to high pH, and the “Blue” genotype, tolerant to high pH, were used to quantify different metabolites related to the stress response.

2. Materials and methods

2.1. Plant material and growing conditions

Two tomato (*Solanum lycopersicum* L.) genotypes were used in this study: Moresco and Blue. The seeds were soaked in 3% hydrogen peroxide for 30 mins and rinsed with distilled water. Subsequently seeds were moved into boxes containing germination paper and kept in the dark, at 25°C until germination. After three days, the sprouted seeds were transferred to 500 ml glass pots for hydroponic growth.

Plants were grown hydroponically at four different pH conditions: control group, and 3 levels of alkali stress obtained by adjusting the pH of the Hoagland solution using NaOH solution (5NM): (I) control Hoagland solution at pH at 5.2-5.5; (II) solution at pH 7.2; (III) at pH 8.2 and (IV) at pH 9.2, obtained by adding 140 µl, 195µl, and 265µl of NaOH solution per 1000 ml of Hoagland, respectively. The pH of each solution was measured using a digital pH-meter Crimson BASIC 30 (Crimson instruments, Barcelona, Spain). 10 plants per pot for a total of 4 replicas per pH condition were used. The pH was adjusted according to Hoagland and Arnon (1950) nutrient solution modified for tomato.

After 10 days of growth into 500ml glass pots, plants have been moved into bigger containers with the same experimental conditions. After 30 days of growth plants have been sampled for biochemical analyses and amino acids profiling.

The study was conducted in growth chamber at 25C with a 16 h photoperiod.

2.2. Determination of proline content

The proline content was estimated according to the method of Bates et al., (1973) (Quagliata et al., 2023). Briefly, 0.100 g fresh weight (FW) of tomato leaves were homogenized with 2 mL of 3% (w/v) 5-sulfosalicylic acid dihydrate. After a centrifugation step at 5000 rpm for 10 min, an aliquot (0.5 mL) of the supernatant was added to reaction tubes containing an equal volume of glacial acetic acid and acid-ninhydrin reagent (previously prepared by dissolving 1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL 6 M phosphoric acid). The reaction was conducted at 100 °C for 1 h and stopped by cooling the samples in ice. The reaction mixture was extracted with 1.5 mL toluene and shaken vigorously for 20 sec. Subsequently, the chromophore containing toluene was separated from the aqueous phase and the absorbance read at 520 nm with an Agilent UV-Vis 8453 spectrophotometer (Santa Clara, CA, USA), using toluene as a blank. Calibration was done with 2 – 600 µL of a 1 mM L-proline (98.5 - 101.0%, pharma grade, PanReac AppliChem ITW Reagents S.R.L., Monza, Italy) stock solution, and the results were expressed as µmol g^{-1} FW. Measurements were taken from 31 different plants.

2.3. Determination of total phenolic, total flavonoid compounds, and condensed tannins content

The contents of total phenolics (TPC), flavonoids (TFC), and condensed tannins were determined in the extracts of tomato leaves, previously dried in the dark, according to Wakeel et al., (2019) with some modifications. A total of 34 plants were considered for these measurements. For the extraction, 1 g DW of leaf material was soaked in 10 mL of 80% (v:v) methanol. The samples were placed on an orbital shaker (ASAL VDRL mod. 711, Cernusco s/N, Milano, Italy) for 30 min and then incubated in the dark at 4 °C. After 48 h of incubation, the samples were filtered through Whatman filter paper no. 1 and the filtrates were used for TPC, TFC, and condensed tannin assays.

The TPC was quantified using the Folin-Ciocalteu method (Al-Duais et al., 2009). Briefly, 0.125 mL of leaf extract was added to 2 mL of water, followed by the addition and mixing of 0.125 mL of the Folin-Ciocalteu's reagent. The samples were left for 3 min in the dark and then 1.250 mL of 7% (w:v) Na2CO3 and 1 mL of distilled H2O were added and shaken vigorously followed by 90 min incubation in the dark. Then, the absorbance of the blue solutions was read at 760 nm with an Agilent UV-Vis 8453 spectrophotometer (Santa Clara, CA, USA). The amount of the extract was substituted by the same amount of 80% (v/v) methanol in the blank. Gallic acid (98%, Thermo Fisher Scientific Inc., Rodano, Milano, Italy) (in the 5 – 300 µg mL^{-1} concentration range) was the standard of choice and the results were expressed as gallic acid equivalent (GAE) mg g^{-1} DW of extract.

The TFC was quantified with an aluminum chloride colorimetric method (Chang et al., 2002). Briefly, 0.250 mL of leaf extract were mixed with 0.075 mL of 5% (w:v) NaNO2 and 5 min later with 0.075 mL of 10% (w:v) AlCl3. The samples were shaken and after 5 min of incubation in the dark were neutralized with 0.500 mL of 1 M NaOH solution. The mixtures were left in the dark for 15 min and then the readings were taken at 415 nm with an Agilent UV-Vis 8453 spectrophotometer (Santa Clara, CA, USA) against a blank of 80% (v:v) methanol. Quercetin (≥ 95%, Merck KGaA, Darmstadt, Germany) (in the 12.5 – 150 µg mL^{-1} concentration range) was the standard of choice and the results were expressed as quercetin equivalent (QE) mg g^{-1} DW of extract.

The condensed tannin content was quantified using the acidified vanillin method (Broadhurst and Jones, 1978). Briefly, 0.500 mL of leaf extract were mixed with 3 mL of 4% vanillin in methanol and 1.5 mL of concentrated HCl. The mixtures were incubated in the dark for 20 min and then read at 500 nm with an Agilent UV-Vis 8453 spectrophotometer (Santa Clara, CA, USA) against a blank of 80% (v/v) methanol. Tannic acid (ACS reagent, Merck KGaA, Darmstadt, Germany) (in the 12.5 –

900 µg mL^{-1} concentration range) was the standard of choice and the results were expressed as tannic acid equivalent (TAE) mg g^{-1} DW of extract.

2.4. Determination of leaf pigments content

The content of pigments (chlorophyll a, chlorophyll b, and carotenoids) was measured in leaves of tomato plants sampled 48 h after the chilling exposure, following the method of Prodhan et al., (2017) with slight modifications. Four mL of chilled methanol were added to 0.050 g FW of leaf material. The mixture was homogenized and incubated for 30 min in the dark at 4 °C. Afterwards, the samples were centrifuged (PK110 centrifuge, Alc International S.r.l., Cologno Monzese, MI, Italy) at 3500 rpm for 20 min. The absorbance of supernantants were measured at 470, 653 and 666 nm with an Agilent UV-Vis 8453 spectrophotometer (Santa Clara, CA, USA). The specific absorption coefficient in methanol was used to calculate chlorophyll a and b and total carotenoid contents in leaves. The results were expressed as mg g^{-1} FW (Lichtenthaler and Wellburn, 1983).

3. Results

3.1. Proline content is affected by both the pH treatment and the genotype

The proline content shows different value trends for the two varieties with increasing pH. In Moresco the proline medium content shows an initial increase (+7.72%) from the standard pH 5.2 to pH 7.2, and then a decrease from the standard value to pH 8.2 (-26.56%) and pH 9.2 (-37.67%) (**Figure 1**). In the Blue genotype, the proline medium content shows an initial increase (+8.88%) from the standard pH 5.2 to pH 7.2 and keeps increasing significantly at pH 8.2 (+17.4%), and pH 9.2 (+36.92%) (**Figure 1**). There are no significant differences between the proline content in the two genotypes at pH 5.2, 7.2, or 8.2. There is a significant difference in the proline content between the two genotypes at pH 9.2 (**Figure 1**).

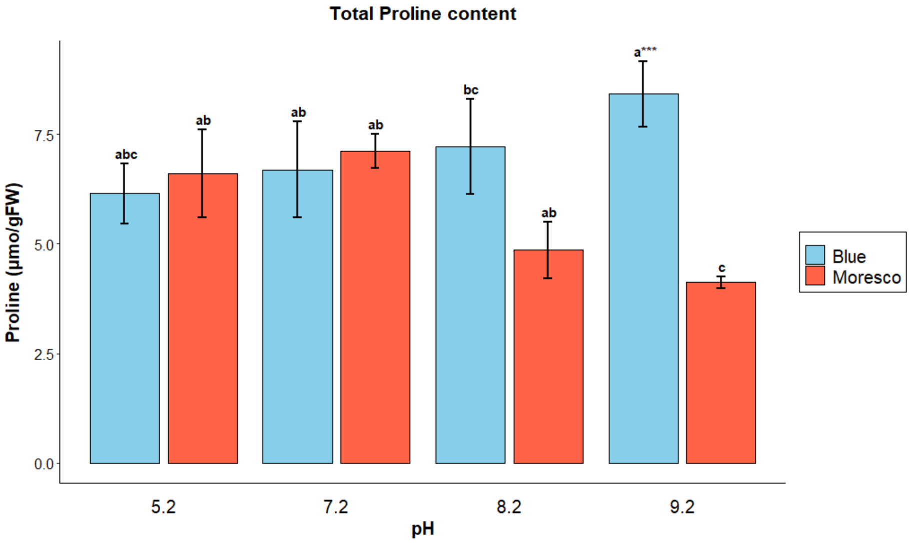

Figure 1. Changes in the content of proline. Proline content in the leaves of tomato plants at the four pH (5.2, 7.2, 8.2, 9.2) sampled after 8 days of growth. Colors show the two genotypes (Blue and Moresco). Significance is based on Wilcoxon's test: *** p-value ≤ 0.01.

3.2. Polyphenols and flavonoids are influenced by the pH in the Blue genotype

The polyphenols content in the Moresco genotype shows an irregular trend concerning the pH. After an initial increase in the polyphenols content from the standard pH 5.2 to 7.2 (+30.27%), there is a decrease to pH 8.2 (-1.85%) and pH 9.2 (-33.06%) (**Figure 2**). The polyphenol content in the Blue genotype shows an increase with the increase of the pH. After an initial decrease in the polyphenols content from the standard pH 5.2 to 7.2 (-2.19%), there is an increase to pH 8.2 (+58.81%) and pH 9.2 (+96.42%) (**Figure 2**). The differences between the average polyphenols content in Moresco and Blue are significant at pH 5.2, 7.2, and 9.2 (**Figure 2**).

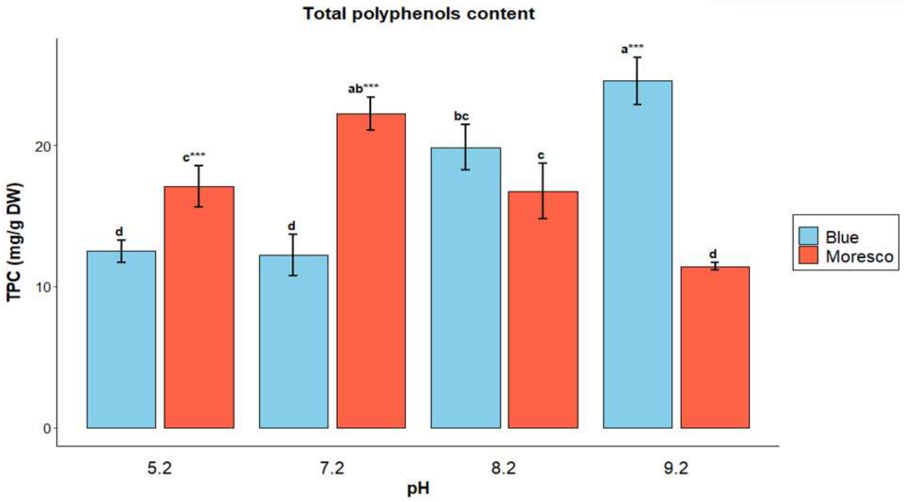

Figure 2. Changes in the content of polyphenols. Polyphenols content in the leaves of tomato plants at the four pH (5.2, 7.2, 8.2, 9.2) sampled after 8 days of growth. Colors show the two genotypes (Blue and Moresco). Significance is based on Wilcoxon's test: *** p-value ≤ 0.01.

In the Moresco genotype, the average flavonoid content shows again an irregular trend, with an increase from the standard pH 5.2 to 7.2 (+1.37%), and pH 8.2 (+26.17%) and a decrease to 9.2 (-8.22%) (**Figure 3**). In the Blue genotype, the average flavonoid content shows again an increase with the increase of the pH. After an initial decrease in the flavonoid content from the standard pH 5.2 to 7.2 (-1.71%), there is an increase to pH 8.2 (+26.17%) and pH 9.2 (+30.56%) (**Figure 3**). The average content of flavonoids is generally lower in the Blue genotype than in the Moresco one. This difference is highly significant at pH 5.2 and significant at pH 8.2 **(Figure 3).**

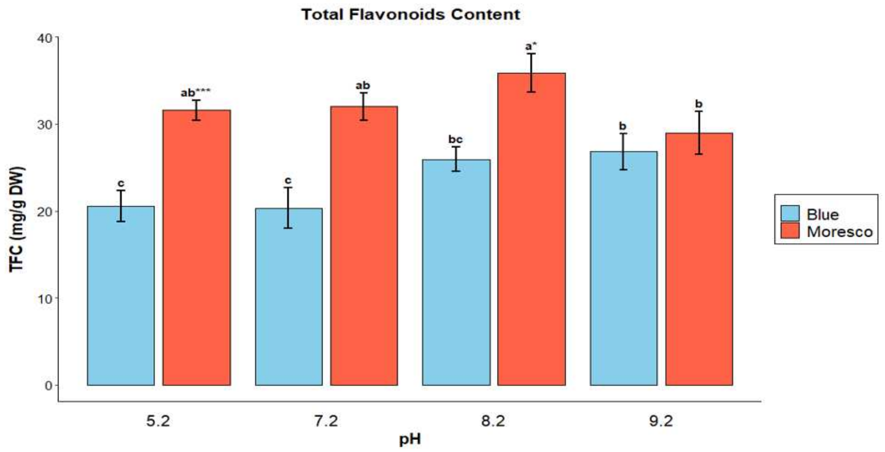

Figure 3. Changes in the content of flavonoids. Flavonoids content in the leaves of tomato plants at the four pH (5.2, 7.2, 8.2, 9.2) sampled after 8 days of growth. Colors show the two genotypes (Blue and Moresco). Significance is based on Wilcoxon’s test: * p-value ≤ 0.1, ** p-value ≤ 0.05, *** p-value ≤ 0.01.

3.3. Tannins content is not affected either by the genotype or the treatment

Condensed tannin content in the two genotypes seems not affected by the changing of the pH. In the Moresco genotype the condensed tannins content increase from pH 5.2 to 7.2 (+17.07%), then has a little decrease at pH 8.2 (+15.15%) and pH 9.2 (+12.15%) (**Figure 4**). The Blue genotype shows a similar trend. From pH 5.2 to 7.2 there is an increase of the tannin content (+4.34%). The content compared to the standard pH 5.2 increase also at pH 8.2 (+16.59%). Lastly, there is a decrease at pH 9.2 (+2.88) (**Figure 4**). Generally, the tannin content in the Blue genotype is higher than the Moresco, the difference between the values is highly significant just at pH 5.2 (**Figure 4**).

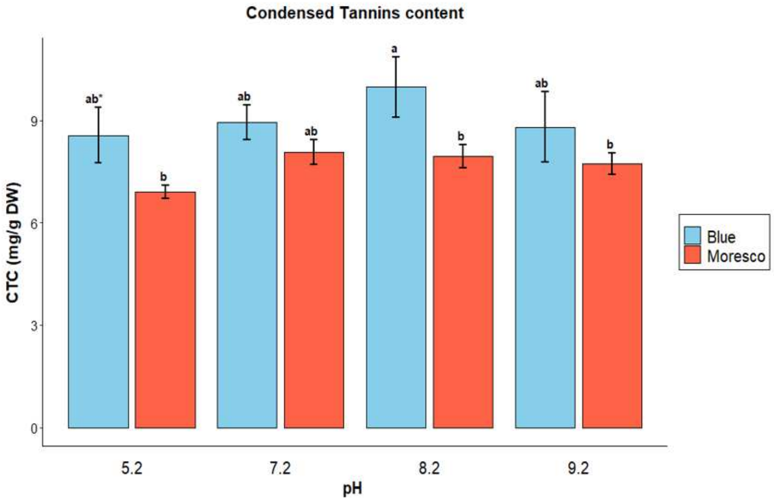

Figure 4. Changes in the content of condensed tannins. Condensed tannins content in the leaves of tomato plants at the four pH (5.2, 7.2, 8.2, 9.2) sampled after 8 days of growth. Colors show the two genotypes (Blue and Moresco). Significance is based on Wilcoxon's test: * p-value ≤ 0.1, ** p-value ≤ 0.05, *** p-value ≤ 0.01

3.4. Leaf pigments content is affected by the genotype but not by the treatment

The chlorophyll content seems not affected by the pH for the two genotypes and shows irregular trends. In the Moresco genotype, the content increases from pH 5.2 to 7.2 (+9.86%), then decreases under the standard pH 5.2 value at pH 8.2 (-8.95%) and at pH 9.2 increases again compared to the standard pH value (+34.57%) (**Figure 5A**). In the Blue genotype, the chlorophyll content decreases significantly from pH 5.2 to 7.2 (-27.62%), then at pH 8.2 compared to the standard pH there is a rise but the value remains under the standard value (-18.99%). Lastly, at pH 9.2, there is another decrease compared to the pH 5.2 value (-39.61%). At every pH the chlorophyll content is higher in the Moresco genotype, these differences are all highly significant (**Figure 5A**). The carotenoid content seems not affected by the pH too. The Moresco genotype shows a little increase from pH 5.2 to 7.2 (+2.78%), then a decrease at pH 8.2 (-19.08%), and at pH 9.2 another little increase compared to the standard pH 5.2 content (-7.93%) (**Figure 5B**). The Blue genotype content decreased from pH 5.2 to 7.2 (-9.59%) and at pH 8.2 had another decrease (-21.73%). Compared to the standard value at pH 9.2 there is a slight increase (-14.88%) (**Figure 5B**). Differently from the treatment, the genotype seems to influence the carotenoid content, which is higher for the Moresco genotype in all pH situations, these differences also are highly significant (**Figure 5B**).

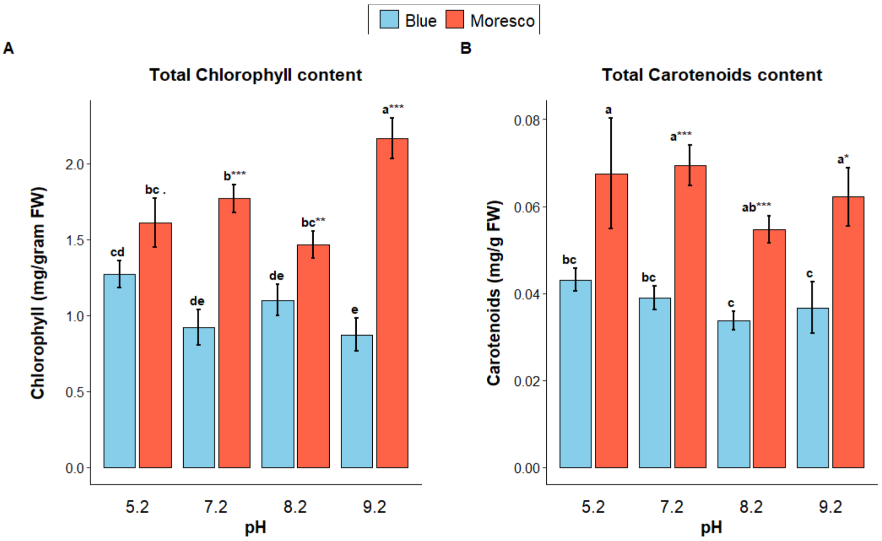

Figure 5. Changes in the content of chlorophyll (A) and carotenoids (B). Chlorophyll content and carotenoids content in the leaves of tomato plants at the four pH (5.2, 7.2, 8.2, 9.2) sampled after 8 days of growth. Colors show the two genotypes (Blue and Moresco). Box plots show medians. Significance is based on Wilcoxon's test: * p-value ≤ 0.1, ** p-value ≤ 0.05, *** p-value ≤ 0.01.

4. Discussion

This study aimed to investigate the effects of alkaline pH on tomatoes. The treatment lasted 8 days, after which samples were taken and analyzed. The composition of the tomato plant in proline, polyphenols, flavonoids, tannins, chlorophyll, and carotenoids was evaluated since these compounds can be used as a marker for the health status of the plant.

Proline is an amino acid with the function of osmolyte in case of osmotic stress like water stress, saline stress, or pH stress. Its content is related to the water content of leaves thus, the accumulation of this osmolyte in response to stress improves the capacity of the cell to maintain turgor at low water potential (Claussen, 2004; Lv et al., 2015). Since many plants accumulate large quantities of proline in response to various environmental stresses, its concentration is considered an indicator of the environmental stress imposed on plants (Montesinos-Pereira et al., 2014). In the present study, the amino acid proline was quantified in two genotypes with opposite responses to alkaline stress. In the Moresco genotype, sensitive to high pH, there was no correlation between the quantity of proline and the pH. On the other side, the Blue genotype, tolerant to alkaline pH, showed an increased quantity of proline with the increase of the pH (**Figure 1**). This result may lead to the wrong assumption that Moresco was less stressed than Blue, especially considering that the difference in the proline content was particularly significant at pH 9.2 (maximum stress for Moresco and optimum situation for Blue). Instead, considering the role that proline plays in osmotic adjustment, we can assume that the high quantity of a compound so closely related to stress is indeed the reason behind the high performance of Blue at high pH. In fact, from a visual analysis, Blue did not look more stressed than Moresco (data not shown). Nevertheless, in virtue of the different roles that proline plays in response to different stresses, its physiological importance in plant stress tolerance and its function as a stress indicator remains controversial (Poustini et al., 2007).

phenols are part of a heterogeneous group of bioactive compounds with different functions, the most important one is the antioxidant capacity that shields the cell membrane from free radicals in the plant. Moreover, they present numerous useful functions in human health, for example are widely known for their use as nutraceutical compounds in medicine against some modern diseases including cancer (Dere et al., 2022; Garg et al., 2019). The structure of phenolic compounds varies widely, although their common feature is the presence of one (simple phenolics) or more (polyphenols) hydroxyl substituents, attached directly to one or more aromatic or benzene rings. According to the structure, they are divided into flavonoids, phenolic acids, stilbenoids, and lignans (Šamec et al., 2021).

AREB1/ABF2, *ABF3,* and *AREB2/ABF4* are the genes induced both by high salinity and drought that are key regulators of Abscisic acid (ABA) signaling in response to stress. Drought, is one of the consequences of high pH, induced by the osmotic imbalance caused by ions in the vacuoles of plant dermal tissue (Klunklin & Savage, 2017; Dere et al., 2022). In tomato, two AREB transcription factors (*SlAREB1* and *SlAREB2*) were identified, and both were significantly induced by ABA. *SlAREB1* is proven to be mainly involved in the regulation of stress response-related gene expression, with an increase of the tolerance to saline–alkaline stress and especially the antioxidant capacity (Xu et al., 2022). The polyphenols and the flavonoid content in the Blue genotype are affected by the pH: both increased with the pH. On the other side, the genotype Moresco doesn't show any pattern relative to the pH. The raised antioxidant capacity, which includes polyphenols and flavonols, as a response to the stress may be the motivation for the increase of polyphenols and flavonoids in the Blue genotype, tolerant to alkaline pH.

Condensed tannins, also known as proanthocyanidins, are a group of polyphenolic compounds, secondary plant metabolites that have a defense function against insect pests by the property of denaturing and binding proteins, antimicrobial nature and are also oxidatively active (Sieniawska & Baj, 2016; Constabel et al., 2014). The condensed tannins content doesn't show a correlation with the pH either in the Blue or in the Moresco genotype. The synthesis of these compounds depends on the CO_2 concentration in the surrounding atmosphere. Water full availability and a deficiency of CO_2 decrease the synthesis of tannins and, thus, their concentration, and vice versa. Elevated pH can cause water stress and also a high concentration of CO_2 creating the ideal situation for the synthesis (Białczyk & Lechowski, 1999). The reason why there wasn't an increase of condensed tannins may be because in this study plants have experienced water stress caused by the osmotic imbalance induced by pH, but without the necessary CO_2 concentration to promote the synthesis and increase of condensed tannins compounds in the two genotypes.

Chlorophylls are a group of compounds with very related structures, they are necessary for their photoreceptors function in plants' photosynthesis (Katz et al., 1978). In the case of Blue and Moresco, the content of chlorophyll is genotype-dependent: Moresco has higher chlorophyll values than Blue at every pH. Thus, the chlorophyll content seems not related to the pH.

A lot of studies about high pH influence on tomato cultivation show that it affects the chlorophyll content causing an initially slight increase to afterwards decrease considerably (Li et al., 2015). The decrease may be caused by a lowering enzymatic activity of protochlorophyllide reductase and α-aminolevulinic acid dehydratase, enzymes involved in the biosynthesis of chlorophyll. Also, the enhancement of oxidative stress has a contribution to causing chloroplast injury (Khan et al., 2019).

Carotenoids are a group of compounds with many functions, for example, antioxidant function or dissipating excess heat in chloroplast during drought stress. They are also important in the human diet being associated with a reduction in the risk of prostate and other cancers, as well as protection against cardiovascular disease. The main carotenoids in tomato are lycopene (80–90% of total carotenoids) and β-carotene (Atkinson et al., 2011). Tomato carotenoid content may change because of drought stress which in this study is one of the consequences of alkaline pH. This change is controversial because in some studies drought induces an increase, while in others a decrease. This difference may be due to the different intensity and time of the stress exposition but also to an interaction of ABA and ethylene. Ethylene is an important plant hormone crucial in regulating carotenoid accumulation, while ABA is produced in response to drought and osmotic stress handling the response pathway. Their different pathways are known to inhibit one another, this may be the reason why in this study the carotenoid content seems not affected by alkaline stress but just by the different genotypes (Atkinson et al., 2011; Patanè et al., 2021).

5. Conclusion

This study focused on the consequences of alkaline pH on two tomato genotypes: Moresco, sensitive to high pH, and Blue, tolerant to high pH. Results show that the Blue genotype activates response mechanisms to tolerate stress by increasing levels of prolines, polyphenols, and flavonoids, while the Moresco genotype shows no response to the stress. Blue tomato genotype shows an interesting response for possible breeding programs in an alkaline environment. In addition, its response may be interesting for the valuable tool proposed by some last studies with the imposition of stress during cultivation for producing high-quality vegetables, even if the stress may hurt the yield and fruit ripening time.

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