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Changes in redox status of tomato fruit play a role in ripening-behaviour of tomato varieties

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Abstract

Tomato fruit ripening, being an oxidative phenomenon, is regulated by anti-oxidants. In this study, role of non-enzymatic anti-oxidants was studied during the course of tomato fruit ripening to ascertain their role in determining postharvest ripening behaviour among the four varieties that are contrasting in terms of ripening. Ripening index (%) of fruits confirmed fast-ripening behaviour for varieties Pusa Ruby and Pusa Sadabahar and slow-ripening behaviour for varieties Pusa Gaurav and Roma. Tomato fruit ripening began with degradation of chlorophylls and accumulation of carotenoids, lycopene, vitamin C, total soluble phenols (TSP), total flavonoids (TF) and glutathione (total and reduced). Higher (55.6 to 86.6%) and lower (34.7 to 38.2%) degradation of chlorophyll *b* along with higher (by 9.5 to 11.8-fold) and lower (by 3.9 to 6.2-fold) accumulation of lycopene fraction (%) in fast- and slow-ripening varieties, respectively indicated that changes in the levels of anti-oxidative pigments have role in ripening behaviour. Progressive increase in TSP, TF and vitamin C was observed in tomato fruits during storage. Fine regulation of ripening behaviour with modulation of glutathione redox status confirmed the differential onset of ripening in fast- and slow-ripening varieties. Early onset of ripening at around green-mature stage of harvesting was observed in fast-ripening varieties while it began at around 5 days after harvest in slow-ripening varieties. Further, strong associations of ripening index (%) with the levels of non-enzymatic anti-oxidants during storage period also suggested their role in imparting fast- and slow-ripening behaviour to tomato fruits of different varieties.

Key words: Antioxidants, glutathione, pigments, ripening, storage, tomato

Introduction

Tomato is one of the most widely consumed vegetables and it occupies an important position worldwide in various culinary preparations. Tomato fruit is not only nutritionally rich but also a source of nutraceuticals and immune-boosting bioactive compounds. Despite being beneficial to health and high in demand, postharvest losses up to 40% are reported for tomato fruits (Macheka *et al*., 2018). This is primarily due to highly perishable nature of tomato fruits in view of climacteric ripening behaviour. It is therefore that understanding of ripening-related changes occurring during fruit development and postharvest storage become crucial. Tomato fruit is in fact considered as a model system for development and climacteric ripening of fruit (Ghorbani *et al*., 2025). Development of tomato fruit is primarily controlled by hormones in addition to several other endogenous and exogenous factors while, ripening of tomato fruit is a specialized senescence process that leads to programmed cell death and marks the final stage of fruit's maturity. Tomato fruit exhibits climacteric ripening behaviour and it is primarily governed by gaseous plant hormone ethylene and its auto-catalytic production *via* system 2 of ethylene production (Paul and Pandey, 2013).

Survey of literature indicates that besides ethylene, tomato fruit ripening is also influenced/regulated by various other internal and external cues in addition to preharvest & postharvest factors particularly; mineral composition (Ramesh *et al*., 2021), internal atmosphere (Paul and Pandey, 2016) and morphological & anatomical features of tomato fruit (Paul *et al*., 2010). More recently, tomato fruit ripening has also been found to be associated with oxidative stress in terms of generation of reactive oxygen species (ROS). Since anti-oxidants counteract the buildup of ROS and therefore, they can also play a key role in maintaining the balance between the generation and removal of ROS during the period of ripening (Wei *et al*., 2025). Redox homeostasis during fruit ripening is not only modulated by the action of enzymatic anti-oxidants but also by non-enzymatic anti-oxidants (Zheng *et al*., 2025). Better shelf-life of purple tomato fruits being rich in anthocyanins (with strong anti-oxidant activity) further indicated for the role of anti-oxidants in ripening and ripening-related changes (Sharma *et al*., 2024).

Role of non-enzymatic low molecular weight anti-oxidants has been discussed in detoxification of ROS but, their role in tomato fruit ripening-induced oxidative stress is not elaborated so far. The present study thereby attempts to find out alterations in content of anti-oxidants (pigments such as chlorophylls and carotenoids and non-enzymatic anti-oxidants such as total soluble phenols, total flavonoids, vitamin C and of glutathione status) during the course

of tomato fruit ripening to ascertain their role in determining ripening behaviour among the varieties that are contrasting in terms of ripening.

Materials and methods

Plant materials, experimental set up, sampling and determination of ripening index (RI %): Seeds of tomato varieties namely; Pusa Ruby, Pusa Sadabahar, Pusa Gaurav and Roma were procured from Division of Vegetable Science, ICAR-Indian Agricultural Research Institute (IARI), New Delhi. Seeds were surface sterilized and sown on raised soil bed. Seedlings at 5 leaf stage were transplanted and grown in experimental plots of Division of Plant Physiology, ICAR-IARI, New Delhi. Standard package of practices as recommended for the region were followed. Healthy and uniform size of tomato fruits (35 to 40 g) at immature and green-mature (GM) stages (as per the description of Jones, 2008) were harvested, washed gently under tap water followed by rinsing with distilled-water and allowed to air-dry at room temperature. From the plant harvested lot, forty fruits at GM stage (representing one replication) were stored in a well-ventilated plastic basket in dark room maintained at 22 ±1.0 °C and 90 ±5.0 % RH up to 14 days after harvest (DAH) in three independent replications. To judge the progress of ripening at periodic intervals from GM stage to 5, 10 and 14 DAH during the storage, ripening index (RI %) was calculated in a non-destructive way as per the procedure of Sharma *et al*. (2020). Tomato fruits are usually harvested at GM stage for postharvest storage and ripening purposes. At each sampling three fruits, each representing one replication and specified stage, were used for various biochemical estimations. Outer pericarp of tomato fruit was used to determine pigments and non-enzymatic anti-oxidants at GM stage and 5, 10 and 14 DAH. Estimations were also done for fruits that were harvested at immature stage. This was done to gain a comparative insight for the differences that occur in fruits as they move from plant-attached immature stage to GM stage (when the fruits were harvested).

Non-enzymatic antioxidants

Chlorophylls: Estimated using the method described by Paul *et al*. (2017). Following formulae were used: chlorophyll *a* = (11.24 x $A_{661.6}$) - (2.04 x $A_{644.8}$), chlorophyll *b* = (20.13 x $A_{644.8}$) - (4.19 x $A_{661.6}$) and total chlorophylls = (7.05 x $A_{661.6}$) + (18.09 x $A_{644.8}$). Wherein, A is absorbance at given wavelength. Chlorophylls were then expressed in terms of µg g^{-1} fresh weight (FW).

Total carotenoids and lycopene: Estimation was carried out following the protocol of Wang and Ying (2005). Calculated using the formulae: total carotenoids (µg mL^{-1}) = 4.0 x A_{450} and total lycopene (µg mL^{-1}) = 3.12 x A_{503}. Wherein, A is absorbance at given wavelength. Values were expressed in terms of µg g^{-1} FW. Fraction of lycopene out of total carotenoids was also derived and presented as lycopene fraction (%).

Total soluble phenols (TSP): Estimation was done by following the procedure of Toor and Savage (2005). Absorbance of the supernatant was recorded at 760 nm. Standard curve was prepared by using pure gallic acid. TSP content was presented as µg (gallic acid equivalent) g^{-1} FW.

Total flavonoids (TF): Determination was also done following the method of Toor and Savage (2005). Absorbance of supernatant was recorded at 510 nm. Standard curve was prepared using pure rutin. TF content was expressed as µg (rutin equivalent) g^{-1} FW.

Vitamin C: Estimated by following the procedure of Kang *et al.* (2005). The absorbance of the supernatant was recorded at 243 nm. Standard curve was prepared by using pure vitamin C and the content was expressed as µg g^{-1} FW.

Glutathione and redox status: Procedure of Loggini *et al.* (1999) was followed for estimation of total glutathione (TG) and oxidized glutathione (GSSG). Reduced glutathione (GSH) was then calculated as the difference between TG and GSSG. Contents were expressed in terms of nmoles g^{-1} FW. Derived parameters representing the glutathione status in terms of redox ratio *i.e.,* reduced glutathione/oxidized glutathione (GSH/GSSG) and oxidized glutathione/reduced glutathione (GSSG/GSH) were also calculated.

Statistical analysis: The replicated data were statistically analyzed using two factors complete randomized design. Factor one was variety and factor two was ripening/storage stage. Ranking of mean values was also carried out using Duncan's Multiple Range Test. Correlation analysis was carried out between RI (%) and content or ratio values for different biochemical parameters separately at 5, 10 and 14 DAH. Statistical analysis was done using R statistical software.

Results and discussion

Ripening index (RI) % showed differences in ripening behaviour of tomato fruits of four varieties: The RI (%), an indicator of ripening behaviour, was measured in terms of percentage for all the four varieties (Fig. 1). Pusa Ruby and Pusa Sadabahar had RI values of 43.3 and 35.8%, respectively at 5 days after harvest (DAH) whereas Pusa Gaurav and Roma had value of 0%. At 14 DAH, the RI was 31.7% for Pusa Gaurav as well as Roma while, it was 100 and 98.3% for Pusa Ruby and Pusa Sadabahar, respectively. The data (Fig. 1) confirmed that Pusa Ruby and Pusa Sadabahar are fast-ripening type and Pusa Gaurav and Roma are slow-ripening type confirming their most contrasting ripening behaviour. Henceforth fast-ripening

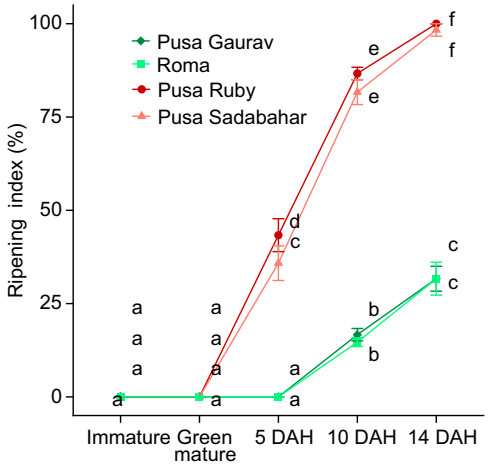

Fig. 1. Postharvest ripening behaviour in terms of ripening index (RI) % as recorded at different stages of tomato fruit ripening in four varieties.

Immature and green-mature (GM) are stages at which tomato fruits were directly harvested from the plant. Whereas 5, 10 and 14 days after harvest (DAH) are the postharvest stages of tomato fruits harvested at GM stage and stored in a dark well-ventilated room maintained at 22 ±1.0 °C and 90 ±5.0 % RH. Vertical line on each point (mean value) is ± standard error of mean (± SEM). Mean values followed by different alphabetic letter/s are significant over one another at $P \leq 0.05$

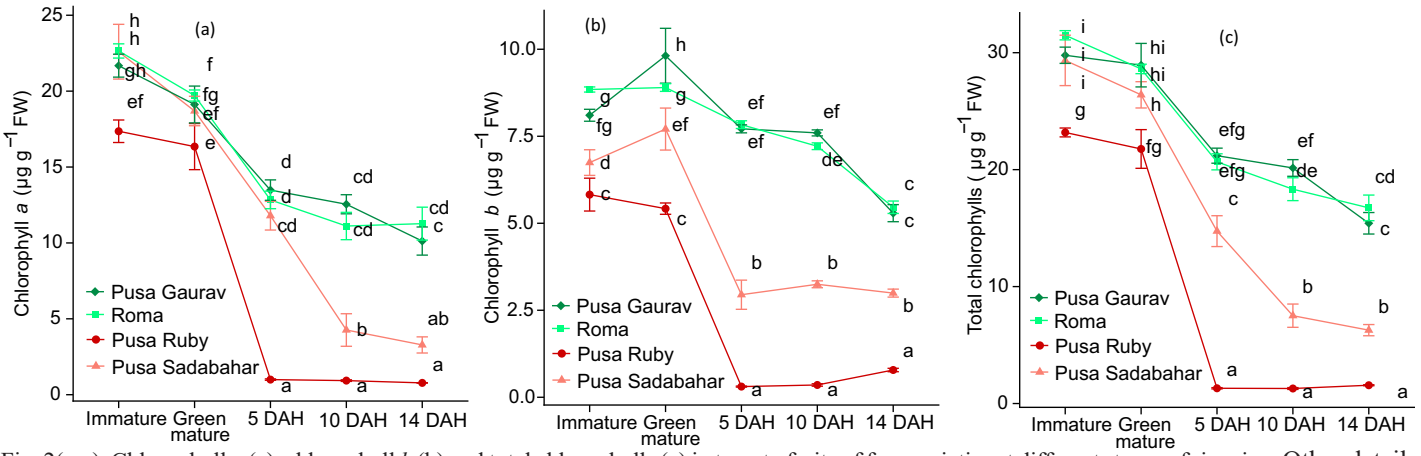

Fig. 2(a-c). Chlorophyll *a* (a), chlorophyll *b* (b) and total chlorophylls (c) in tomato fruits of four varieties at different stages of ripening. Other details are same as given in foot notes of Fig. 1

varieties and slow-ripening varieties will be abbreviated as FRV and SRV, respectively.

Rate of changes in chlorophylls of tomato fruits during initial stages of ripening can influence the rate of ripening: A gradual decrease in chlorophyll *a*, chlorophyll *b* and total chlorophylls was observed with the progress of ripening from GM stage and onwards (Fig. 2a-c). Decrease in chlorophyll *a* from GM stage to 14 days after harvest (DAH) was more prominent in FRV like Pusa Ruby (95.2%) and Pusa Sadabahar (82.5%) in contrast to SRV like Pusa Gaurav (47.1%) and Roma (42.8 %) (Fig. 2a). The decline in chlorophyll *b* was comparatively less than chlorophyll *a,* particularly in SRV with the progress of ripening. Chlorophyll *b* was reduced by 86.6% in Pusa Ruby, 55.6% in Pusa Sadabahar, 34.7% in Pusa Gaurav and 38.2% in Roma (Fig. 2b) suggesting for better stability of chlorophyll *b* during the course of ripening. The role of chlorophyll *b* in fruit ripening was reported in apple by Gorfer *et al*. (2022). In this study, a sharp fall in chlorophyll *b* content of FRV after GM stage indicated for the early onset of oxidative stress. Since chlorophyll *b* is a crucial part of light harvesting complex II (LHC II), it plays role in maintaining the basic structural integrity of associated proteins and overall photosynthetic system and apparatus under oxidative stress (Gorfer *et al*., 2022). Thus, retention of relatively higher levels of chlorophyll *b* in SRV of tomato must have assisted in maintenance of photosynthetic activity and thereby delay in onset of ripening.

With the progress of ripening from GM stage to 14 days after harvest (DAH), total chlorophylls content reduced by 93.3% in Pusa Ruby, 78.6% in Pusa Sadabahar, 48.3% in Pusa Gaurav and 46.9% in Roma (Fig. 2c). Decline in total chlorophylls was rapid and maximum at initial stages of ripening from GM stage (21.77 µg g^{-1} FW) to 5 DAH (1.30 µg g^{-1} FW) in Pusa Ruby than during later stages of ripening (Fig. 2c). Correlation analysis between levels of chlorophylls (chlorophyll *a*, chlorophyll *b* and total chlorophylls) and indicator of ripening rate *i.e.,* RI (%) showed negative but significant associations at 5, 10 and 14 DAH (Table 1). Based on results, it can be stated that rate of chlorophyll degradation surpassed the rate of chlorophyll biosynthesis much faster in FRV compared to SRV. Breakdown of chlorophylls in fruits basically mimics the chlorophyll catabolism in leaves. Fruit ripening, being a specialized senescence process, dynamic alterations in pigments are witnessed during this process (Kapoor *et al*., 2022). Chlorophyll catabolic pheophorbide *a* oxygenase (PAO)/phyllobilin pathway (a major route for chlorophyll breakdown in plants) and stay-green (SGR) genes play an important role in tomato fruit ripening and leaf senescence (Guyer *et al*., 2014). A stay-green gene 2 (SGR2) regulates the de-greening of green- and gold-fleshed kiwi fruits differentially during ripening. Transcription of SGR2 and other chlorophyll degradation genes was higher in gold-fleshed fruits across the developmental stages compared to green-fleshed kiwi fruits (Pilkington *et al*., 2012). Similar modulation of chlorophyll catabolic genes can also be expected in differential regulation of FRV and SRV of tomato. Moreover, senescence induced oxidative stress is accompanied with disintegration of photosynthetic machinery and thus reduction in photosynthetic capacity well below a threshold level also further accelerates senescence during ripening (Paul *et al*., 2005). So, further investigations involving chlorophyll biosynthesis and breakdown genes may clearly elucidate the role of chlorophylls and its threshold limits in tomato fruit ripening.

Changes in the levels of total carotenoids and lycopene in tomato fruits during initial phase of ripening are linked with ripening behaviour: Content of total carotenoids and lycopene increased with the progress of ripening as a part of ripening-related co-regulated activity. With the progress of ripening from GM stage to 14 days after harvest (DAH) during storage, FRV (especially Pusa Ruby) showed significantly higher content of total carotenoids in comparison to SRV (particularly Roma) (Fig. 3a). The total carotenoids showed maximum increase (9.3-fold) in Pusa Ruby from 8.62 µg g^{-1} FW at GM stage to 80.09 µg g^{-1} FW at 14 DAH while, minimum increase (1.6-fold) was found in Roma from 9.9 µg g^{-1} FW at GM to 16.04 µg g^{-1} FW at 14 DAH (Fig. 3a). As per Cordoba *et al*. (2003), besides being influenced by environmental and genetic factors carotenoids accumulation was also linked with the phases and progress of tomato fruit ripening. In the present study, during early stages of ripening from GM to 5 DAH, variety Pusa Ruby showed higher accumulation of carotenoids (2.4-fold change) and variety Roma showed lower accumulation of carotenoids (only 1.1-fold change) indicating for their fast- and slow-ripening types, respectively (Fig. 3a).

Among total carotenoids in tomato fruits, lycopene is the major one and it increased rapidly in FRV namely Pusa Ruby (20.9-fold, from GM stage to 5 DAH) and Pusa Sadabahar (13.1-fold, from GM stage to 5 DAH) but slowly or with

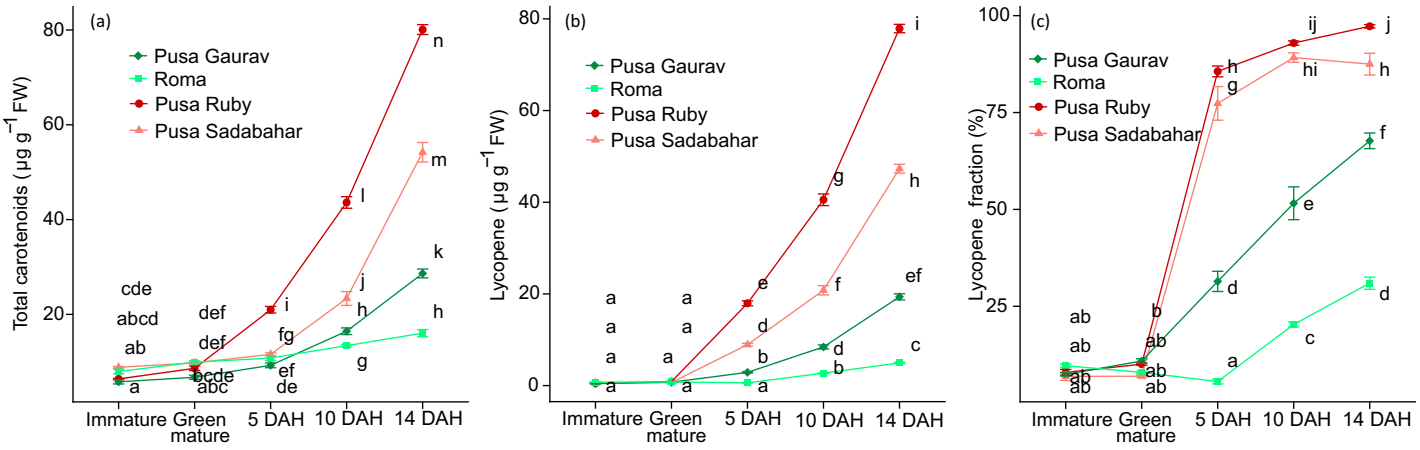

Fig. 3(a-c). Total carotenoids (a), lycopene (b) and lycopene fraction % (c) in tomato fruits of four varieties at different stages of ripening. Other details are same as given in foot notes of Fig. 1

no change in SRV namely Pusa Gaurav (3.9-fold, from GM stage to 5 DAH) and Roma (no change, from GM stage to 5 DAH) (Fig. 3b). While all the varieties had similar lycopene content at immature and GM stage, Pusa Ruby had maximum lycopene contents at 5 DAH (17.95 µg g^{-1} FW) and later at 14 DAH (76.55 µg g^{-1} FW). This was followed by Pusa Sadabahar at 5 DAH (8.92 µg g^{-1} FW) and later at 14 DAH (44.0 µg g^{-1} FW), Pusa Gaurav at 5 DAH (2.89 µg g^{-1} FW) and later at 14 DAH (19.37 µg g^{-1} FW) and lastly Roma at 5 DAH (0.7 µg g^{-1} FW) and later at 14 DAH (4.95 µg g^{-1} FW) (Fig. 3b). Maximum and distinct differences in lycopene content were for Pusa Ruby and Roma (Fig. 3b), two highly contrasting varieties with respect to ripening behaviour (Fig. 1). In comparison to GM stage, increase in lycopene content at 5 DAH was 20.9-fold in Pusa Ruby and nil in Roma while, remaining two varieties were in-between with 13.1-fold increase for Pusa Sadabahar and 3.9-fold increase for Pusa Gaurav (Fig. 3b). Later during 5 DAH to 10 DAH, least increase (3.9-fold) in Roma indicated for delayed commencement of ripening and ripening-related changes.

The lycopene fraction (%) showed no change from immature stage to GM stage but distinct increase was noticed from GM to 14 DAH in all the varieties (Fig. 3c). Fruits of Pusa Ruby showed highest increase in lycopene fraction % (9.5-fold) while Roma showed minimum increase (3.9-fold) by 14 DAH over GM stage. This further established relationships between changes in lycopene with the ripening behaviour of tomato fruits in different varieties. Lenucci *et al.* (2006) reported that lycopene, being the predominant carotenoid, comprises 80 to 90% of total carotenoids in ripened tomato fruits. Our study pointed out for higher lycopene fraction, up to 81.8 to 95.6 %, in FRV and here it gets accumulated early during the ripening *i.e.,* from GM to 5 DAH. While, lycopene fraction comprised only to lesser extent, 31.0 to 69.0 %, in SRV and here the accumulation was delayed towards later phase of fruit ripening (from 5 DAH and onwards) (Fig. 3c). Further, strong and positive associations of total carotenoids, lycopene and lycopene fraction (%) were obtained with RI (%) at 5, 10 and 14 DAH (Table 1) confirming for the role of these pigments in regulation of ripening and thereby the ripening behaviour of tomato fruits. Kapoor *et al.* (2022) reported that progress of tomato fruit ripening was linked with higher oxidative stress (indicated by higher level of ROS) and this was also accompanied with rise in the levels of lycopene and β-carotene. Carotenoids and lycopene, being potent quenchers of singlet oxygen species, tried to lower down ROS and thereby they were tightly associated with maturation and ripening process of tomato fruit (Wei *et al.*, 2025).

Changes in total soluble phenols (TSP) and total flavonoids (TF) in tomato fruits during ripening coincide with ripening behaviour: Increase in TSP during the storage period from GM to 14 days after harvest (DAH) was higher in FRV compared to SRV (Fig. 4a). Significant differences between FRV and SRV were maintained at any of the ripening stages (Fig. 4a). Pusa Ruby showed highest and Roma showed lowest TSP at 14 DAH whereas, Pusa Sadabahar was closer to Pusa Ruby and Pusa Gaurav was closer to Roma (Fig. 4a). Thus, the data on TSP showed variety dependent increase in during ripening with positive and highly significant

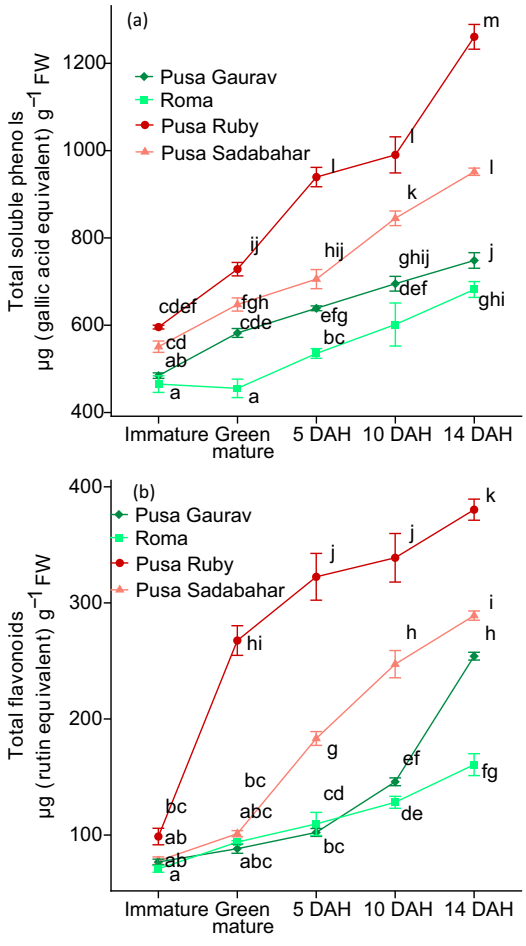

Fig. 4(a-b). Total soluble phenols (a) and total flavonoids content (b) in tomato fruits of four varieties at different stages of ripening. Other details are same as given in foot notes of Fig. 1

associations at 5, 10 and 14 DAH with RI (Table 1). Changes in TF during initial phase from GM to 5 DAH were 20.5% for Pusa Ruby, 81.1% for Pusa Sadabahar, 15.9% for Pusa Gaurav and 16.7% for Roma (Fig. 4b). The data indicated that FRV showed early and prominent increase in TF, during immature to GM stage for Pusa Ruby and during GM to 5 DAH for Pusa Sadabahar. While, SRV showed late and slight increase in TF, during 5 DAH to 10 DAH for Pusa Gaurav and Roma (Fig. 4b). Positive and significant association of TF with RI (Table 1) indicated for the association of changes in TF in tomato fruits with the progress of ripening.

As per many reports, tomato fruits enhance endogenous level of TSP and TF to scavenge ROS that are generated during ripening (Ilahy *et al*., 2011). For different varieties, trend obtained for TSP and TF (Fig. 4a-b) matched with the trend of rate of ripening (Fig. 1) suggesting that rate of ripening is linked with the extent of increase in the production of ROS in tomato fruits. Phenolics being anti-oxidants, serve as a part of coping up mechanism against the built up of oxidative stress due to the initiation and progress of ripening in a variety-specific manner. This gets the support from the report that TSP and TF along with carotenoids play major role during ripening and oxidative stress and they acted efficiently in quenching ROS and try to maintain redox status during ripening of tomato fruits (Decros *et al*., 2023).

Changes in vitamin C of tomato fruits during ripening are linked with ripening behaviour: Vitamin C in tomato fruits increased with the progress of ripening in a variety dependent manner. By 14 days after harvest (DAH), fruits of fastest ripening variety, Pusa Ruby, accumulated maximum vitamin C while fruits of slowest ripening variety, Roma, accumulated minimum vitamin C (Fig. 5). In comparison to GM stage, 14 DAH recorded higher accumulation of vitamin C in FRV than SRV. The data also showed that increase in vitamin C was higher and earlier (from immature to GM stage and after that) in FRV while the increase was lesser and delayed (5 DAH to 10 DAH and after that) in SRV (Fig. 5). Fruits of most contrasting tomato varieties with respect to ripening behaviour *i.e.,* fastest (Pusa Ruby) and slowest (Roma) showed the most contrasting trend for vitamin C. It was important to note that the second most fast-ripening variety (Pusa Sadabahar) showed the trend similar to Pusa Ruby while the second most slow-ripening variety (Pusa Gaurav) showed the trend similar to Roma (Fig. 5). Highly significant

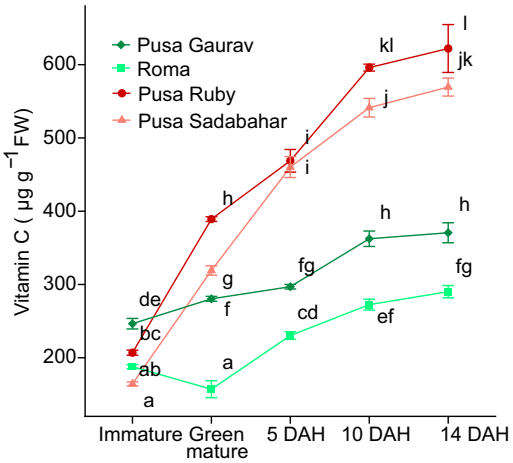

Fig. 5. Vitamin C in tomato fruits of four varieties at different stages of ripening. Other details are same as given in foot notes of Fig. 1

and positive association of vitamin C with RI (Table 1) further supported for the involvement of vitamin C in rate of tomato fruit ripening. Production of vitamin C was reported to get enhanced at the onset of fruit ripening with definite role in modulation of oxidative stress in chloroplast/chromoplast and mitochondria (Arabia *et al*., 2024). It can thereby be inferred that tomato fruits of a variety with higher rate of ripening produce higher levels of oxidative stress quite early and this in turn cause higher induction and production of vitamin C at early stage (immature to GM). On the other hand, tomato fruits of a variety with slower rate of ripening produce lower level of oxidative stress that too little late and this in turn causes lower induction and production of vitamin C at little later stage (5 to 10 DAH).

Changes in redox state of tomato fruits are related with slow- and fast-ripening behaviour: Total glutathione **(**TG) and reduced glutathione (GSH) (Fig. 6a-b) showed distinct pattern of change for FRV and SRV. A continuous rise in TG and GSH was observed up to 14 days after harvest (DAH) for FRV, while, SRV showed increase up to 5 DAH and then decline. TG and GSH increased progressively from immature stage to 14 DAH by 3.4-fold and 4.9- fold, respectively in Pusa Ruby and 2.6-fold and 3.8-fold, respectively in Pusa Sadabahar (Fig. 6a-b). On the other hand, TG increased by 2.7-fold in Pusa Gaurav from immature stage to 5 DAH followed by 0.60-fold decline (from 5 to 14 DAH) while, Roma showed 2.8-fold increase in TG from immature to 10 DAH followed by 0.78-fold decline (from 10 to 14 DAH) (Fig. 6a). Similarly, GSH showed increase from immature to 5 DAH in Pusa Gaurav and Roma by 2.8-fold and 2.6-fold, respectively followed by decline from 5 to 14 DAH by 0.38-fold and 0.62-fold, respectively (Fig. 6b). It was interesting to note that TG and GSH increased in fruits of all the varieties during the transition from immature to 5 DAH with similarity in pattern for both fast as well as slow-ripening varieties (Fig. 6a, b). The association of TG and GSH with RI was initially negative (5 DAH) but it changed to positive at later stage/s (10 and 14 DAH) (Table 1) indicating the importance and role of glutathione in modulation/balancing the levels of oxidative stress during fruit ripening. Glutathione is already known as an important non-enzymatic anti-oxidant that plays important and regulatory role in maintaining cellular redox homeostasis *via* balancing the oxidative stress in tomato plant (Ding *et al*., 2022) and mangofruit (Zhou *et al*., 2023).

Redox ratio (reduced glutathione to oxidized glutathione, GSH/GSSG) from immature to 5 DAH was lesser in FRV and higher in SRV with contrasting difference among these two groups of varieties (Fig. 6c). This trend was however got reversed at 10 and 14 DAH, as the ratio became significantly higher in FRV and lower in SRV. Indicated that GSH was less during initial phase of ripening (immature to 5 DAH) in FRV and this may be due to higher utilization of GSH due to higher level of oxidative stress during this phase (Fig. 6c). On the other hand, higher GSH in FRV during late ripening phase (at 10 and 14 DAH) (Fig. 6b) may be due to lower utilization of GSH as higher level of oxidative stress at early phase had already induced the ripening and ripening-related changes in an irreversible way (Fig. 6c). The reverse of above trend was observed for SRV. In this way, contrasting group of varieties showed opposite trends for changes in redox ratio (Fig. 6c) supporting the fact that higher oxidative stress occurred early (immediately after immature stage and up

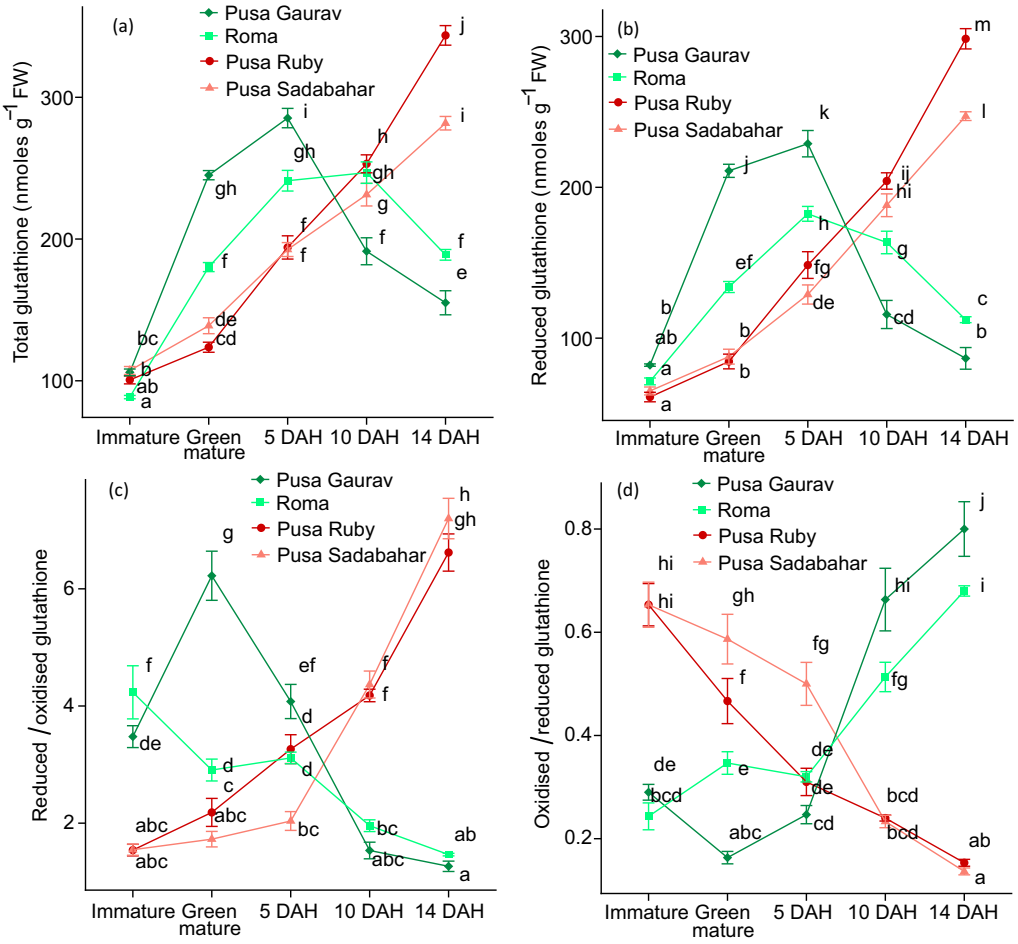

Fig. 6(a-d). Total glutathione (a), reduced glutathione (b), redox ratio (reduced/oxidized glutathione) (c) and oxidized/reduced glutathione (d) in tomato fruits of four varieties at different stages of ripening. Other details are same as given in foot notes of Fig. 1

to 5 DAH) in FRV and delayed (immediately after 5 DAH and up to 14 DAH) in SRV (Fig. 6c). This was further confirmed by positive and highly significant correlations between the values of redox ratio at 10 and 14 DAH and RI (Table 1). The ratio GSSG to GSH (Fig. 6d) was just opposite of redox ratio so it followed the trend just opposite of redox ratio. This was also evident from significant negative association of GSSG/GSH at 10 and 14 DAH with RI (Table 1). The data thereby highlighted the role of cellular redox status of tomato fruits of different varieties in relation to their ripening behaviour.

Table 1. Association (in terms of Pearson's correlation coefficient, r value) between a given parameter and corresponding ripening index (%) at a given day after harvest (DAH) across the tomato fruits of different variety. 5 days after harvest (DAH), 10 DAH and 14 DAH are the postharvest storage period of tomato fruits harvested from the plants at GM stage and kept in a dark well-ventilated room maintained at 22 ±1.0 °C and 90 ±5.0 % RH. The value of r was calculated using replicate-level observations across the four varieties at a given stage; n = 12. *: Indicates that r value is significant at $P \leq 0.05$; **: Indicates that r value is significant at $P \leq 0.01$; NS: Means non-significant

Parameter	5 DAH	10 DAH	14 DAH
Chlorophyll *a*	-0.73*	-0.92**	-0.94**
Chlorophyll *b*	-0.92**	-0.92**	-0.90**
Total chlorophylls	-0.84**	-0.93**	-0.94**
Total carotenoids	0.76**	0.77**	0.88**
Lycopene	0.91**	0.85**	0.87**
Lycopene fraction (%)	0.92**	0.91**	0.76**
Vitamin C	0.89**	0.93**	0.91**
Total soluble phenols	0.84**	0.87**	0.85**
Total flavonoids	0.87**	0.91**	0.75**
Total glutathione	-0.86**	NS	0.95**
Reduced glutathione	-0.80**	0.77**	0.97**
Redox ratio (reduced glutathione/ oxidised glutathione)	NS	0.92**	0.99**
Oxidised glutathione/reduced glutathione	NS	-0.89**	-0.99**

Earlier studies have shown that GSH levels and the ratio of GSH to GSSG are important players in maintaining redox balance under oxidative environment that prevails during the induction and progression of fruit ripening in tomato and other fruits (Yan *et al*., 2022). Our results showed that FRV are marked with maximum oxidative stress at around GM stage of fruits as evident in terms of higher ratio of GSSG to GSH (Fig. 6d). It was documented by García-Quirós *et al*. (2020) that lower redox ratio (GSH/GSSG) accelerated flower senescence in *Arabidopsis*. Since, fruit ripening is also a senescence process and therefore fast-ripening tomato fruits with high oxidative stress at GM stage exhibited lower GSH/GSSG (or higher GSSG/GSH) in comparison to SRV (Fig. 6c-d). The involvement of GSH in regeneration of vitamin C and in quenching of ROS and thereby maintaining cellular integrity and regulation of ripening in tomato was reported by Yan *et al*. (2022). In mango fruit, Zhou *et al*. (2023) reported an increase in GSH and vitamin C along with anti-oxidant enzymes in coping up with lipid peroxidation and oxidative damage during the progress of ripening.

Tomato fruit ripening is a complex metabolic process associated with oxidative stress and modulation of several anti-oxidative activities/metabolites to cope up with this stress. This study established that alterations in the levels of pigments (chlorophylls, carotenoids and lycopene) and non-enzymatic anti-oxidants (phenols, flavonoids, vitamin C, and glutathione) are linked with the postharvest ripening behaviour of varieties. The progressive degradation of chlorophylls and accumulation of lycopene in tomato fruits was higher in FRV compared to SRV. Most importantly, retention of chlorophyll b in slow-ripening varieties suggested for a possible association with delayed ripening of tomato fruits. Early increase in non-enzymatic anti-oxidants (including lycopene) in fast-ripening fruits serves as an acclimatization response against early onset of ripening (at green-mature stage). Interestingly, this onset was slightly delayed to 5 days after harvest (DAH) or beyond in SRV. The glutathione-based redox status of tomato fruits was modulated to impart ripening behaviour. Overall, slow-ripening behaviour was

conferred to tomato fruits by slower degradation of chlorophyll *b*, reduced accumulation of carotenoids, lycopene, vitamin C, TSP and TF and altered redox ratio (higher at green-mature stage to 5 DAH and lower at 5 DAH to 14 DAH). It can be inferred that apart from ethylene, timing and response shown by anti-oxidants towards the oxidative stress that accompany with initiation of ripening also play regulatory role in imparting fast- and slow-ripening behaviour to tomato fruits of different varieties

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