# Mineral deficiencies influence on tomato leaves: pigments, hydrogen peroxide and total phenolic compounds contents

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**Abstract**

The detection of hydrogen peroxide, chlorophyll pigments, anthocynin, carotenoids, total phenolic compounds and lipid peroxidation levels as potential stress signaling molecules in tomato (*Lycopersicon esculentum* Mill.) leaves in response to specific mineral deficiency were studied. The stress signaling molecules were measured in the plant leaves at different growth time points cultured in specific mineral deficient nutrient solutions. The results showed that hydrogen peroxide was significantly increased after 48 and 72h of growth in $NO_3^-$ and S deficient nutrient solutions. While the significant accumulation of $H_2O_2$ in the plant leaves was observed after 72h and 96h of growth in $K^+$ deficient nutrient solution. In the case of $Mg^{2+}$ and $Fe^{2+}$ deficiency the significant accumulation was observed after 72h cultivation. The only significant reduction in chlorophyll *a* content was detected after 96h cultivation under $Mg^{2+}$ specific deficiency with no significant changes due to $NO_3^-$, $SO_4^{2-}$, $Fe^{2+}$, $K^+$, $Ca^{2+}$ and $PO_4^{2-}$ deficiencies. Meanwhile, chlorophyll *b* contents were significantly reduced in the plant leaves grown for 48h under $Mg^{2+}$ and $Ca^{2+}$ specific mineral deficiency. Longer cultivation in $Mg^{2+}$ dificient hydroponic culture for 96h resulted in 215% increment in chlorophyll *b* content. No significant changes in chlorophy *b* contents were detected in hydroponic cultures dificient in $NO_3^-$, $SO_4^{2-}$, $Fe^{2+}$, and $PO_4^{2-}$. Impose of minerals deficiencies on anthocyanins and carotenoids contents were showed parallel changes. Both increased significantly in response to $PO_4^{2-}$ and $NO_3^-$ specific deficiencies after 48h and in the case of $Mg^{2+}$ deficiency after 72h. Meanwhile, $SO_4^{2-}$ deficiency caused the significant increase of both after 72h and 96h. Regarding TPC the results clarified that $Mg^{2+}$, $Ca^{2+}$ and $K^+$ specific deficiencies caused significant reductions that appeared after 48h. In contrast, S deficiency caused significant increase in TPC values after 72h. On the other hand, the estimated levels of MDA showed significant increment under $Ca^{2+}$ and $K^+$ and $PO_4^{2-}$ specific deficiencies at all time points while in the case of $Mg^{2+}$ and Fe deficiencies the increment was first reported after 48h and with the later one (Fe) the increment continues up to 96h. These results indicate that some of presented metabolites could be used as stress markers. These results support the possible role of anthocyanins, carotenoids, hydrogen peroxide, total phenolic compounds contents and MDA as early signaling metabolites in tomato plants under specific mineral deficiency.

**Keywords:** Abiotic stresses; Acclimation; Antioxidants; Mineral deficiency; Signals.
**Abbreviations:** Chl_chlorophyll; FW_fresh weight; GAE_gallic acid equivalents; MDA_malondialdehyde; ROS_ Reactive oxygen species; TCA_trichloroacetic acid; TPC_Total phenolic compounds.

**Introduction**

At global scale, crop plants affected by the continuous deterioration of soil properties since it influence the form, amount, retention and movement of soil nutrients. Therefore, poor cultivation soil causes decrease in global food production and in particular in the developing countries. At the physiological and molecular levels, deficiencies in essential minerals like other abiotic stresses experienced by plants in many different ways. Eventually, adaptation or acclimation occurs over different time scale affected by the dose of exposure, plant development stage and combinations of different stresses which are a critical determinant for plant productivity (Wang et al., 2002; Wang & Wu, 2010). When essential mineral nutrients are scarce, several molecular and physiological events such as photosynthesis rate and appearance of chlorosis are responsible for sensing and signaling mineral resources limitation. Therefore, their ultimate effects on plant development and biomass allocation need for further investigation. In particular, the information's about upstream molecular targets which are limited until

recently (Hermans et al., 2006; Kehr, 2013; Verbruggen & Hermans, 2013). Phenolic compounds are secondary metabolites that found in leaves, flowers and fruits. Metabolically, their chemical precursor is the amino acid phenylalanine. Phenolic compounds play an important role in plant metabolism as plant hormone controllers and protectant of plants against stresses. They play a major role in the process of plant interaction with their environment. Hence, they tend to accumulate in dermal tissues where they introduce their ability as a potential protecting agent against different abiotic and biotic stresses. In addition, they act as attractants in fruit dispersal, as structural materials and showed signaling properties (Garcia-Salas et al., 2010; Sakihama et al., 2002; Tsao & McCallum, 2009). In accordance, anthocyanins are group of phenolic compounds where their production and accumulation might be influenced by several abiotic factors (Chon et al., 2012; Do & Cormier 1991; Kobayashi et al., 1993; Mori & Sakurai 1994;

Nakajima et al., 1989; Wellmann et al., 1976; Zhang et al., 1997; Zhong et al., 1991).

In relation, hydrogen peroxide accumulation in response to stress conditions reported to play a dual role in plants according to the accumulation level in plant cells. At low accumulation levels it can act as a signal molecule triggering acclimation response to different biotic and abiotic stresses. Where at high accumulation levels it provokes programmed cell death (Gao et al., 2010; Quan et al., 2008; Orozco-Cárdenas et al., 2001; Prasad et al., 1994).

Recently, metabolome analyses have involved addressing the internal environment adjustment of the plant in response to minerals deficiencies. In this study, the analyzed metabolome were limited to the pigments (chlorophyll, anthocyanins and carotenoids), hydrogen peroxide, total phenolic compounds contents and lipid peroxidation level in tomato (*Lycopersicon esculentum* Mill.) leaves at different growth time points on specific mineral ($Mg^{2+}$, $Ca^{2+}$, Fe, $K^+$, P, N, S) deficiencies.

## Results

### Hydrogen peroxide accumulation

It have been reported that reactive oxygen species (ROS) tend to accumulate in response to different stress conditions where they act as signaling molecules (Sharma et al., 2012; Trachootham et al., 2008; Tuteja & Sopory, 2008). In agreement, the results in Fig.1 showed that early $H_2O_2$ significant accumulation were detected in tomato leaves grown in hydroponic cultures deficient in $NO_3^-$ and $SO_4^{2-}$ after 48h of incubation reaching 1.6 and 1.4 folds, respectively. The results also revealed that these significant increments were continue and reached high levels of 4.1 and 3.7 folds after 72hs which later on (after 96hs cultivation) sharply decreased, as acclimation response, to nonsignificant levels compared to the control treatment. In the case of $Mg^{2+}$ and $Fe^{2+}$ deficiencies the earliest significant accumulation of $H_2O_2$ were appeared at 72h and reached 2.2 and 1.5 folds, respectively. Similar to $NO_3^-$ and $SO_4^{2-}$ treatments, longer cultivation (96h) of tomato in hydroponic cultures deficient in $Mg^{2+}$ and $Fe^{2+}$ leads to reduction of $H_2O_2$ amounts to nonsignificant levels as compared to the complete culture. In the case of $K^+$ deficiency, the significant reduction in $H_2O_2$ production was measured at 72 and 96h (0.9 and 0.75, respectively).

### Chlorophyll a and b contents

In this experiment, chlorophyll content as indirect tool for estimation of bioproductivity and to understand the photosynthetic regime of plant under specific mineral deficiencies were studied. The results (Fig. 2A, B and C) showed that the only significant change (49% reduction) in chlorophyll *a* content was detected in *Lycopersicon esculentum* Mill. leaves after 96h cultivation under $Mg^{2+}$ specific deficiency. On the other hand, no significant changes in chlorophy *a* contents were detected in tomato leaves grown in hydroponic cultures dificient in $NO_3^-$, $SO_4^{2-}$, $Fe^{2+}$, $K^+$ and $PO_4^{2-}$ compared to the tomato leaves of the plant grown in complete culture. However, the analysis of chlorophyll *b* content in the plant leaves grown under $Mg^{2+}$ and $Ca^{2+}$ specific mineral deficiency revealed that a significant reduction of 56 and 47% in chlorophyll *b* content were measured in tomato leaves after 48h incubation. Meanwhile, longer cultivation (72 and 96h) of tomato in $Mg^{2+}$ dificient hydroponic culture caused contineous increament in chlorophyll *b* content reached to signifcant levels of 215%

after 96h cultivation in comparsion to that grown in complete nutreint solution. On the other hand, no significant changes in chlorophy *b* contents were detected in tomato leaves grown in hydroponic cultures dificient in $NO_3^-$, $SO_4^{2-}$, $Fe^{2+}$ and $PO_4^{2-}$ compared to the tomato leaves of the plant grown in complete culture.

### Anthocyanins contents

Figure 3. showed that the effect of specific mineral deficiencies on anthocyanins content appeared as time dependent and mineral specific response. The results showed that the significant increment of 2.5 and 2.9 folds in anthocyanins content recorded after 48h cultivation apeared in $PO_4^{2-}$ and $NO_3^-$ specific depleted nutreint solutions, respectively. While after 72h the significant increment of 8.7 and 2.1 folds in anthocyanins content recorded in $Mg^{2+}$ and $SO_4^{2-}$ specific depleted nutreint solutions, respectively. However, eventhough longer cultivation of tomato in $SO_4^{2-}$ specific depleted nutreint solution up to 96h resultede in reduction of anthocyanins content as compared to anthocyanins content recorded at 72h the magnitude of anthocyanins content still significantly higher (1.3 folds) than the control experiment. On the other hand, no significant changes in anthocyanins content were detected in tomato leaves grown in hydroponic cultures dificient in $Ca^{2+}$, $K^+$ and $Fe^{2+}$ at all cultivation times studied compared to the tomato leaves of the plant grown in complete culture.

### Carotenoids contents

In agreements with the anthocyanins content results (Fig 3.) the results of carotenoids content (Fig 4.) also showed no significant changes recorded in tomato leaves when the plant grown in hydroponic cultures dificient in $Ca^{2+}$, $K^+$ and $Fe^{2+}$ at all cultivation times studied compared to the tomato leaves of the plant grown in complete culture. Meanwhile, carotenoids contents were significantly increased and reached 2.1, 2.4 and 1.9 folds in the plant leaves when cultivated for 48h in $PO_4^{2-}$, $NO_3^-$ and $SO_4^{2-}$ specific depleted nutreint solutions, repsectively. In the case of $Mg^{2+}$ and $SO_4^{2-}$ dificient hydroponic cultures the significant increment (7.7 and 1.8 folds, respectivelly) in carotenoids content were recorded after 72h cultivation. After 96h cultivation, only tomato leaves of tomato plants grown in $SO_4^{2-}$ specific depleted nutreint solution recorded significantly higher carotenoids contents compared to the leaves of the control experiment.

### Total phenolic contents

The results shown in Fig 5. showed that the earliest response (at 48h) in total phenolic compounds content as significant decrease (0.6, 0.72 and 0.63 folds) were appeared in the tomato leaves of the plants that grown on $Mg^{2+}$, $Ca^{2+}$ and $K^+$ specific depleted nutreint solutions, respectively. Eventhough the values of the total phenolic compounds content in the tomato leaves taken from the plants grown in $Fe^{2+}$, $PO_4^{2-}$, $NO_3^-$ and $SO_4^{2-}$ dificient cultures were less than that reported with the control experiment the differences were nonsignificant. In contrast, at 72h cultivation time, the analysis of total phenolic compounds content in tomato leaves of the $Mg^{2+}$, $Ca^{2+}$, $K^+$, $Fe^{2+}$ and $NO_3^-$ deficient cultures showed nonsignificant increment except with $PO_4^{2-}$ deficient nutrient solution which showed nonsignificant reduction of total phenolic compounds contents. The results (Fig 5.) also showed that the only significant increment (2.8 folds) in total phenolic compounds content at72h cultivation time was

**Table 1.** Management of particular mineral deficiency and the replacement with other salt with the final concentration for each one as describe in the materials and methods.

| Deficient nutrient | Replacement | Purpose of replacement |
|---|---|---|
| Complete medium | All minerals are | Presents |
| Mg/ $MgSO_4.7H_2O$ | $NaSO_4$ | Maintain the supply of sulfur |
| Ca/$Ca(NO_3)_2.4H_2O$ | $NaNO_3$ | Maintain the supply of nitrogen |
| Fe/ Fe–$Na_2$EDTA | -------- | ------- |
| P/ $KH_2PO_4$ | KCl | Maintain the supply of potassium |
| K/ $KH_2PO_4$ | $NaNO_3$ | Maintain the osmoticum |
| N/ $KNO_3$ and $Ca(NO_3)_2.4H_2O$ | KCl and $CaCl_2$ | Maintain the supply of potassium and calcium |
| S/ $MgSO_4.7H_2O$ | $MgCl_2$ | Maintain the supply of magnesium |
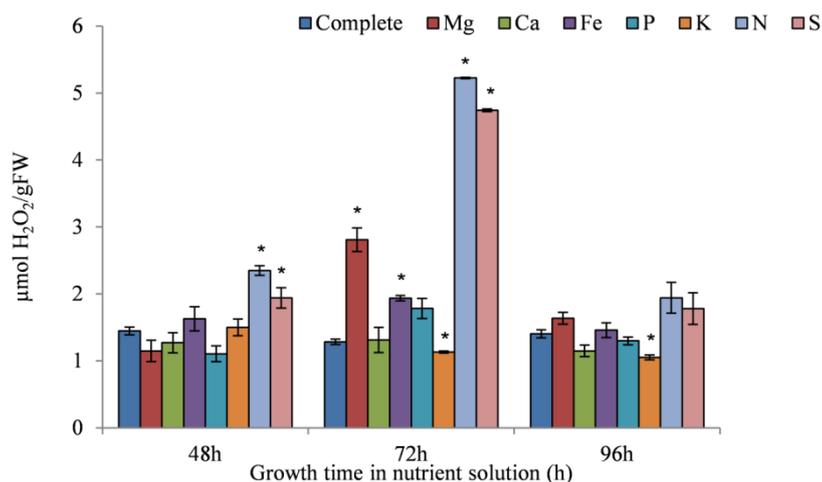


**Fig 1.** Hydrogen peroxide accumulation level in *L. esculentum* Mill. leaves in response to specific mineral deficiencies in comparison to complete nutrient solution grown plants at time dependent exposure ($*P \leq 0.05$).

recorded in the tomato leaves of the plants that grown in $SO_4^{2-}$ specific depleted nutreint solution. However, subsequent depletion in total phenolic compounds content in test cultures at 96h was observed.

*Lipid peroxidation level*

Figure 6. shows that lipid peroxidation levels were significantly increased and reached 5.4, 8.3, 29, 12 and 9 folds in tomato leaves after 48h cultivation of tomato plants in $Mg^{2+}$, $Ca^{2+}$, $Fe^{2+}$, $PO_4^{2-}$ and $K^+$ specific depleted nutreint solutions. On the other hand, the icrement in lipid peroxidation levels were nonsignificant in the case of $NO_3^-$ and $SO_4^{2-}$ dificient cultures. At 72h cultivation, the analysis results showed that lipid peroxidation levels were further increased in tomato leaves of $Ca^{2+}$, $PO_4^{2-}$ and $K^+$ deficient cultures. Meanwhile, in the other mineral deficient cultures slight to high reduction in lipid peroxidation levels were observed. After 96h cultivation, lipid peroxidation levels were reached 2.6, 3.6 and 4.1 folds increment in tomato leaves of $Fe^{2+}$, $PO_4^{2-}$ and $K^+$ specific depleted nutreint solution compared to the plants grown in complete nutrient solution.

**Discussion**

Metabolomics currently is a significant tool used to study plant acclimation to abiotic stress. Metabolome analysis showed that plant responses to abiotic stresses are dynamic and complex; they are both elastic and plastic (Cramer et al., 2011). However, wide ranges of primary and secondary metabolites are involved in the stress tolerance (Arbona et al.,

2013; Ramalingam et al., 2015; Rodziewicz et al., 2014). Under different treatments, anthocyanin accumulation is considered as a positive response to oxidative stress (Diaz et al., 2006; Hughes et al., 2010; Misyura et al., 2012). In addition, the accumulation of carotenoids is regarded to suppress the oxidation (Cao et al., 2015). Phenolic compounds may act as radical scavengers for enhancement of tolerance to oxidative stress (Kondo & Kawashima, 2000; Michalak, 2006).

It was reported that high sugar accumulation in leaves as consequences of $Mg^{2+}$ deficiency has a negative effects on transcription of photosynthesis genes in particular repress *Cab2* expression. In addition, it might affect photosynthetic activity by reducing chlorophyll pigments concentration (Hermans & Verbruggen, 2005; Liu et al., 2008). In both effect, might be depend on period of exposure beside the severity of $Mg^{2+}$ limitation (Fig. 2). However, the effect was clearly after 24h and 96h, $Mg^{2+}$ remobilization can only prevent a drastic drop in leaves $Mg^{2+}$ concentrations for a limited period following $Mg^{2+}$-withdrawal as acclimation response (Fig. 2B). The increment in carotenoids/ chlorophylls ratio in Mg-deficient plants at 72h which was reached over 5folds may be an adaptive response to the elevation of $H_2O_2$ generation (Fig.1), to detoxify ROS. The reduction of total phenolic compounds in Mg-deficient plants at 48h might be involved directly to scavenge ROS, oxidized by peroxidase and to inhibit lipid peroxidation (Fig. 6) by trapping the lipid alkoxyl radical. Reduction of phenolic compounds strictly prevents diffusion of free radicals and restricts peroxidative reactions such as membrane damage and ions leakage (Kondo & Kawashima, 2000; Michalak, 2006; Sharma et al., 2012).

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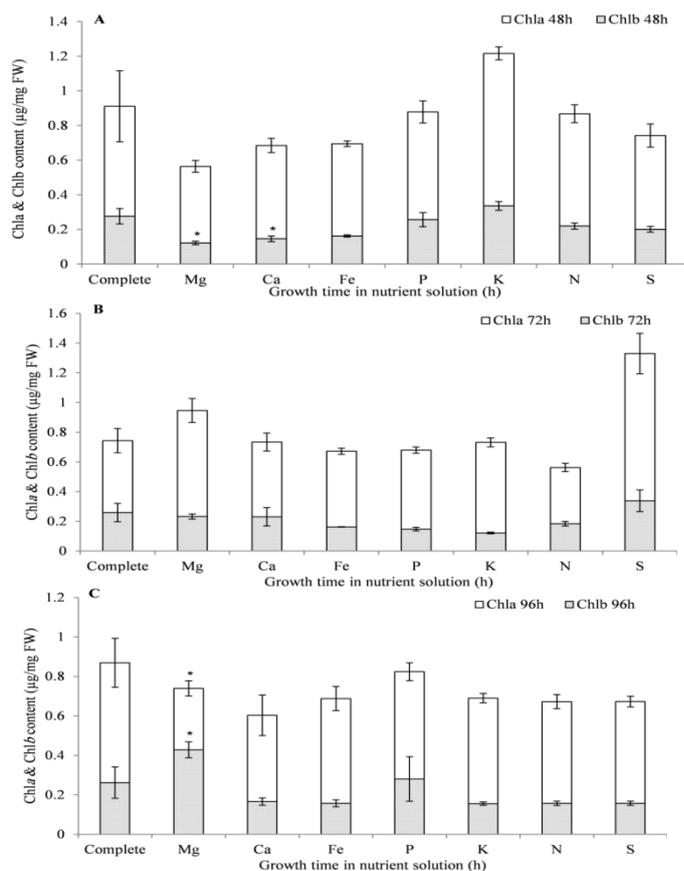
**Fig 2.** Chlorophyll *a* and *b* contents in *L. esculentum* Mill. leaves in response to specific mineral deficiencies in comparison to complete nutrients solution grown plants at time dependent exposure (A) 48h, (B) 72h and (C) 96h (*$P \leq 0.05$).
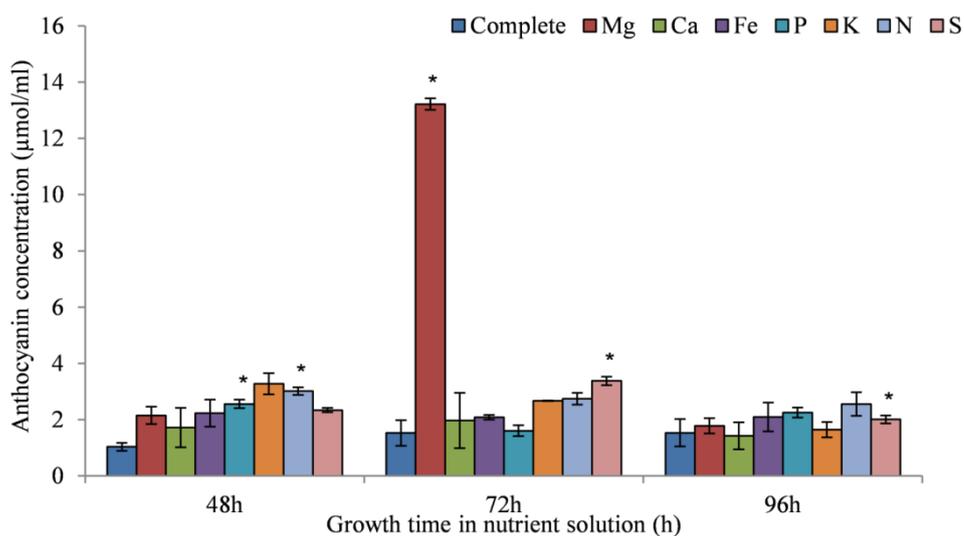


**Fig 3.** Anthocyanin's accumulation level in *L. esculentum* Mill. leaves in response to specific mineral deficiencies in comparison to complete grown plants at time dependent exposure (*$P \leq 0.05$).
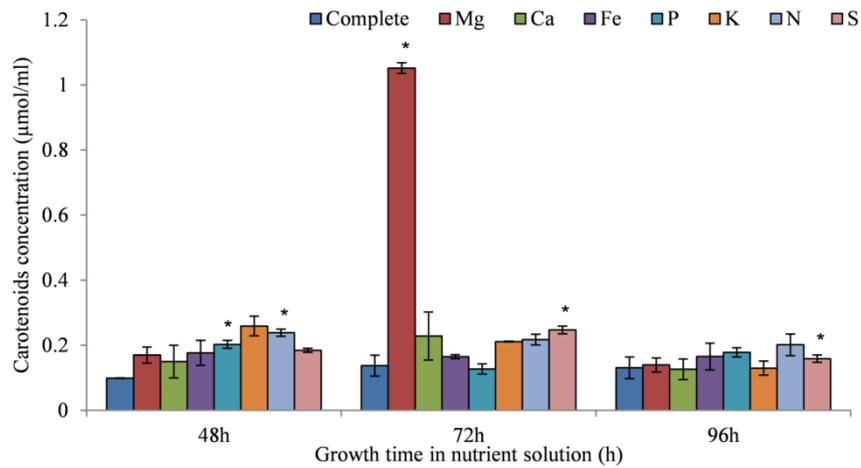
**Fig 4.** Carotenoids accumulation level in *L. esculentum* Mill. leaves in response to specific mineral deficiencies in comparison to complete grown plants at time dependent exposure (*$P \leq 0.05$).
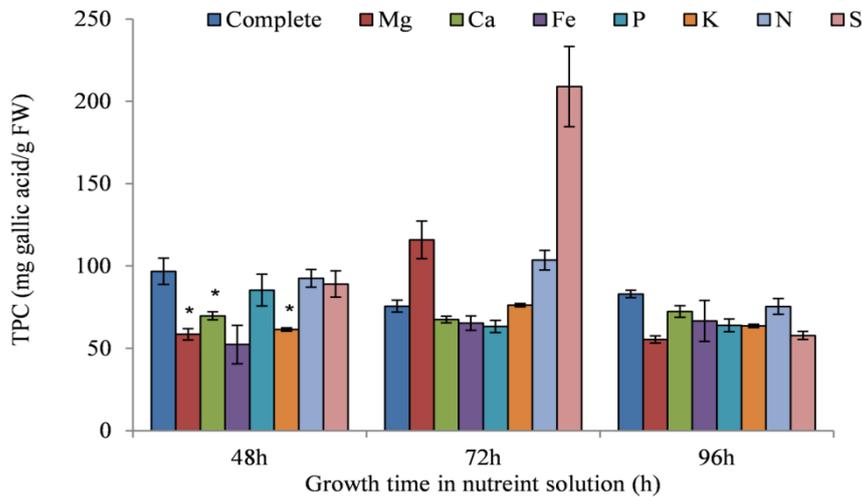


**Fig 5.** Total phenolic compounds (TPC) accumulation level in *L. esculentum* Mill. leaves in response to specific mineral deficiencies in comparison to complete grown plants at time dependent exposure (*$P \leq 0.05$).
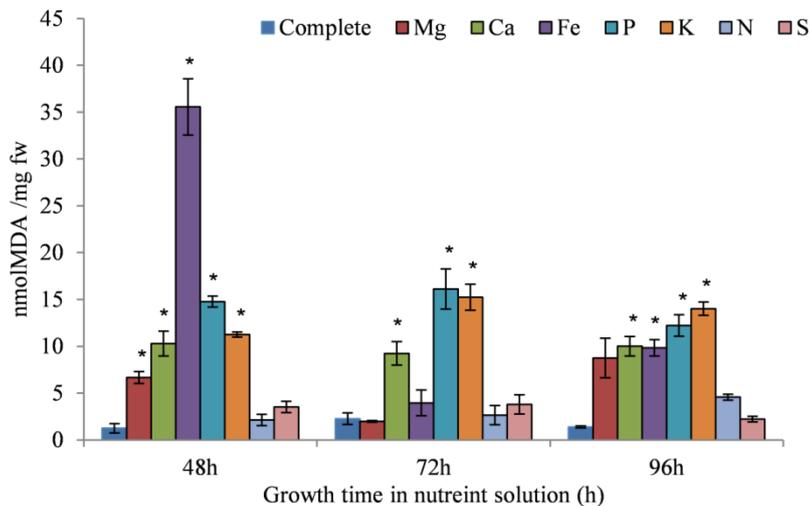


**Fig 6.** Lipid peroxidation level (MDA content) in *L. esculentum* Mill. leaves in response to specific mineral deficiencies in comparison to complete grown plants at time dependent exposure (*$P \leq 0.05$).

The symptoms of $Ca^{2+}$ deficiency in plants were early discussed (Simon, 1978). Oxidative degradation seems to proceed at elevated rates of photosynthesis of Ca-deficient tomato leaves and resulted in an increased malondialdehyde content in the plant tissue (Fig.7). Therefore, oxidation-prone chlorophyll in particular Chl *b* which was significantly reduced at 48h. This oxidation was resulting in formation of $^1O_2$ and other oxygen species. In consequence, the interaction between $^1O_2$ and membrane lipids which probably generating free radicals and decomposing membrane lipids in a chain reaction (Schmitz-Eiberger et al., 2002).

Castañeda and Pérez (1996) reported the role of $Ca^{2+}$ in the synthesis of phenols. This role was not directly caused by $Ca^{2+}$ ions (Ruiz et al., 2003), since $Ca^{2+}$ is involved in many signal transduction pathways in plants for defense against various stresses (Tuteja & Mahajan, 2007). Where high levels of $Ca^{2+}$ ions promoted more oxidation than synthesis of phenolic compounds (Ruiz et al., 2003) as in Fig. 5 after 48h of Ca-deficient tomato leaves, which might be through the $Ca^{2+}$ ions redistribution to tomato leaves especially via apoplastically to cope his role in signal transduction pathways (Gilliham et al., 2011; Tuteja & Mahajan, 2007).

The response of $Fe^{2+}$ deficient plant leaves was previously reported by Ranieri et al., (2001) through the determination of chlorophyll, carotenoids and $H_2O_2$ content. In $Fe^{2+}$ deficient tomato leaves, the $H_2O_2$ content underwent increase following the iron deficiency with significant content after 72h then reduced to the control level after 96h. The increment of $H_2O_2$ content suggests that $Fe^{2+}$ deficiency may induce both a decreased capacity to $H_2O_2$ scavenging and/or an active production of $H_2O_2$ with a consequent rise of oxidative stress (Ranieri et al., 2001). The triggering of Fenton reaction which may explain the oxidative stress which resulted in an increased malondialdehyde content in the $Fe^{2+}$ deficient plant tissues significantly after 48h and 96h of iron starvation (Fig.5). However, it was reported that iron deficiency differently affects peroxidase isoforms (Ranieri et al., 2001). $PO_4^{2-}$ deficient tomato leaves showed significant accumulation of anthocyanins and carotenoids contents after 48h of phosphorus starvation which act as indicator of P-deficiency stress (Awad et al., 1990; Raese, 2002; Steyn et al., 2002). This accumulation may be to increased tolerance to P-deficiency (Gaume et al., 2001). It was reported that leaves lipid peroxidation level was increased when the $PO_4^{2-}$ was deficient (Chen et al., 2015) as also this study was recorded (Fig. 6). This elevation in lipid peroxidation was explained by $PO_4^{2-}$ deficiency induced the production of $O_2^{\bullet-}$ which can produce more toxic ROS, including the hydroxyl radical ($OH^\bullet$) and perhydroxyl radical ($HO_2^\bullet$) that can initiate lipid peroxidation (Chen et al., 2015).

Potassium is essential for many physiological processes. Hence, the plants sensitivity to K-deficient was depend on the period of exposure and severity of deficiency. $K^+$ deprivation was accompanied by increases in ROS accumulation; $H_2O_2$ production (Shin & Schachtman, 2004). Cakmak (1994) has been reported that K-deficient plants showed increase in peroxidases activity (ascorbate peroxidase and guaiacol peroxidase) that detoxify and utilize $H_2O_2$. While Liu et al., (2013) indicated that $K^+$ deficiency protects plant due to recognizable enhancement of antioxidant enzyme activities. Thereby, the significant reduction of $H_2O_2$ content after 72h and 96h in this study may as result of the increase in peroxidases activity. In contrast, K-deficient plants lipid peroxidation level was significant increased among all periods of exposure might be caused through an increase in NADPH dependent $O_2^{\bullet-}$ generation as detected before (Cakmak, 2005). The impact of $K^+$ deprivation on chlorophyll contents was not significant effective. However, the progress in contents changes was met with those results of Cakmak (1994) that explained by ROS accumulation causing chlorophyll destruction. The biochemical responses of tomato growing in $K^+$ deprivation nutrient solution at the level of anthocyanins accumulation suggesting that $K^+$ starvation disturbed carbon storage and transport (Battie-Laclau et al., 2014) and carotenoids content was not significant change and met with results of Breś et al., (2016). The reduction in total phenolic compounds accumulation caused by $K^+$ deprivation at all the period of exposure suggesting a strong trade-off in nutrients channeled toward the production of total phenolics for their antioxidant potential (Haukioja et al., 1998; Mudau et al., 2007; Venkatesan & Ganapathy, 2004). Deficiency of $NO_3^-$ caused markedly increasing of hydrogen peroxide in tomato leaves (Tewari et al., 2007) which was significant increase after 48h and 72h. This increasing still higher than that grown in complete nutrient solution after 96h which may as increasing in activity of antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase and glutathione reductase (Tewari et al., 2007). In the same time, this accumulation was in parallel with lipid peroxidation increasing (Tewari et al., 2007). The reduction of Chl contents caused by $NO_3^-$ deprivation was related to chlorophyll breakdown (Diaz et al., 2006; Feild et al., 2001). The protective role for anthocyanins appeared by increasing the level significantly after 48h and still higher than that grown in complete nutrient solution after 72h and 96h as a stress response (Chalker-Scott & Scott 2004; Diaz et al., 2006). It has been found that total phenolic compounds contents was increased to $NO_3^-$ deprivation in tomato leaves (Løvdal et al., 2010) in particular levels of flavonoids and caffeoyl derivatives with pronounced increased levels of anthocyanin. These responses for $NO_3^-$ deprivation were mediated by enhancement of expression of related genes at least partly be mediated through *ANT1* and *SlJAF13* (Løvdal et al., 2010). However, the $NO_3^-$ deprivation effect on accumulation of phenolic compounds contents was restricted to the period of exposure (8 days as reported by Løvdal et al., 2010) which might explain the slight change in total phenolic compounds contents in this study. The present study shows that sulfur stress markedly increase in $H_2O_2$ concentration in tomato leaves which in consonance with reports of Chandra and Pandey (2014) and Tewari et al., (2004). Impose of $SO_4^{2-}$ deprivation was in parallel with that of $NO_3^-$ deprivation on tomato leaves. This suggest that $H_2O_2$ may be considered as a potential signaling molecule which play dual roles by induced antioxidative systems, resulting in mitigated oxidative damage (rising in MDA to nonsignificant levels). In the same time, elevate allocation of $NO_3^-$ and $SO_4^{2-}$ to leaf resulting in enhanced $NO_3^-$ and $SO_4^{2-}$ assimilation (Khan et al., 2016). Reduced chlorophyll contents were observed under sulfur deficiency (Ferreira & Teixeira, 1992; Lunde et al., 2008). Chlorophyll contents after 72h of exposure to $SO_4^{2-}$ deprivation showed opposite result to that of 48h and 96h, this may be explained by sulfur remobilization to cope the $SO_4^{2-}$ deprivation (Khan et al., 2016). Where with continuous deprivation, sulfur transporters will be target of the modification and finally the $SO_4^{2-}$ deprivation symptoms as chlorosis are clearly appeared (Hawkesford, 2000).

A significant increase in anthocyanins content and the accumulation of $H_2O_2$ indicated that $SO_4^{2-}$ deprivation also provokes an oxidative stress in tomato leaves (D'Hooghe et al., 2013).

The $SO_4^{2-}$ deprivation grown plants rising carotenoids contents may be an acclimate response to the increased ROS

generation, as carotenoids are known to detoxify ROS (Pérez-Gálvez & Mínguez-Mosquera, 2002). Among key biological functions of carotenoid is sensing and signaling the oxidative stress condition through nonenzymatic oxidation by ROS (Havaux, 2014; Ramel et al., 2012).

The study suggests that, the biosynthesis of these compounds is tightly regulated by environmental conditions such as nutrient availability. The low chlorophyll of mineral deficient leaves was specific mineral dependent then on the severity of the deficiency. The later one was nonspecific for mineral which deficient. The level of $H_2O_2$ was specific mineral dependent and on the severity of the deficiency as appeared in response to $NO_3^-$ and $SO_4^{2-}$ deficient leaves which was early after 48h and 72h and in K-deficient leaves which was after 72h and 96h.

Anthocyanin accumulation in response to nutrient availability was demonstrated by screen of anthocyanin content change with time dependent manner which indicated it strongly influenced by early deprivation of $K^+$, $PO_4^{2-}$ and $NO_3^-$ and then longer with $Mg^{2+}$ and $SO_4^{2-}$.

## Materials and Methods

### Plant material and growth conditions

Tomato (*Lycopersicon esculentum* Mill.) seeds (BURPEE TOMATO, Super Beefsteak) placed in 9 cm petri dishes containing two layers of filter papers moistened with 6 ml of sterile distilled water and incubated at 25°C in dark. Seedling transferred to grown for two weeks on soil mixture of peatmoss, perlite and vermiculite in prepared in the ratio of 2:1:1, respectively. The seedlings were incubated under controlled conditions of 14h light with ~80 μmol photon m$^{-2}$ s$^{-1}$, and 21 °C/ 10 h dark and 20 °C, ~55 % relative humidity).

Fourteen days old plantlets were transplanted to 100 ml jars containing aerated nutrient solution having the chemical composition of: 10 mM $KNO_3$, 10 mM $Ca(NO_3)_2.4H_2O$, 5 mM $MgSO_4.7H_2O$, 2.5 mM $KH_2PO_4$, 0.05 mM KCl, 0.05 mM Fe–$Na_2$EDTA, 5.0 mM $MnSO_4$, 0.5 mM $CuSO_4.5H_2O$, 2μM $ZnSO_4.7H_2O$, 0.115 mM $H_3BO_3$, 0.1 mM $H_2MoO_4.2H_2O$. The pH of the nutrient solution was maintained at 6.5 ±0.2. Deficiency of a particular mineral nutrient was created and the replacement with equivalent moles as shown in Table 1.

Samples of tomato leaves collected at different time points directly in liquid nitrogen and stored at -80°C until the time of analysis.

### Measurement of H₂O₂

The leaf hydrogen peroxide content of treated and control samples were assayed as described by Christou et al. (2013). Frozen leaf material (~0.1g) was homogenized on ice with 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was then centrifuged at 15,000 *g* for 15 min. at 4 °C. The supernatant was transferred to a new tube and 0.5 ml of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI and mixed gently. The absorbance of the assay mixture was read at 390 nm and the content of $H_2O_2$ was calculated based on a standard curve of known concentrations of $H_2O_2$.

### Quantification of chlorophylls, anthocyanins and Carotenoids

For chlorophylls measurement, extraction and quantification were performed according to Porra (2002). In briefly, 20 mg of the leaf samples were ground in 1 ml cold 80% acetone followed by 1h darkness incubation then centrifugation at 13000 rpm at 4 °C for 10 min, and the supernatant was read on 646.6 and 663.6 nm. For anthocyanins and carotenoids quantification the method of Sims and Gamon (2002) was used. The procedure in principle is similar to the procedure of chlorophyll measurements except in the extraction step were extraction of anthocyanins and carotenoids performed in methanol/HCl/water (90:1:1, vol:vol:vol) mixture.

### Determination of total phenolic content

Total phenolic compounds content were determined using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965) and as described by Lu et al., (2011). In brief, 100 μl of 70% methanolic leaves extract was reacted with 750 μl of the diluted Folin–Ciocalteu reagent for 10 min at room temperature. Then, 750 μl of 2% sodium carbonate was added. The mixture was allowed to stand in the dark (ca. 20 °C) for 45 min before measuring the absorbance at 765 nm against a blank, containing deionized water instead of sample extract. TPC values were determined from a standard curve prepared of gallic acid. Results are expressed as mg of gallic acid equivalents/g fresh weight (mg GAE/g FW).

### Lipid peroxidation assay

Leaf tissue (50 mg) was homogenized in 1 mL of 80% (v/v) ethanol on ice. The homogenate was centrifuged at 16,000g for 20 min at 4 °C. The supernatant (0.5 mL) was mixed with 0.5 mL of 20% (w/v) trichloroacetic acid containing 0.65% (w/v) thiobarbituric acid, followed with direct incubation at 95°C for 30 min. then immediately cooled on ice. The absorbance of the supernatant was measured, after centrifugation at 10,000g for 10 min., at 532 nm, subtracting the value for nonspecific absorption at 600 nm. The level of lipid peroxidation was measured through calculating malondialdehyde (MDA) concentration from the extinction coefficient 155 mM $^{-1}$ cm$^{-1}$ (Hodges et al., 1999).

### Statistical analysis

For all experiment, samples of the plant leaves were analyzed and all the assays were carried out in triplicate for each of three independent biological samples. The results were expressed as mean± SD. The comparison between two samples was performed by student's *t*-test and the *P* values of ≤0.05 were considered as significant.

## Conclusion

These metabolome readjustment of plant cells internal environment reflects plant plasticity for acclimation to different abiotic stress factors, in particular mineral deficiencies. However, extended study to include other possible <u>factors</u> such as the antioxidant enzymes (in particular total ascorbate peroxidases, polyphenol oxidase, and phenylalanine ammonia lyase) to understand the process of *Lycopersicon esculentum* Mill. leaves acclimation in response to mineral deficiencies is required.

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