



# Journal of Biological Sciences

ISSN 1727-3048

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## Effect of Activity Ceasing of Acetyl CoA-carboxylase on Growth and Antioxidant System in Seedling Stage of Barley

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**Abstract:** In order to the better understanding and to compensate against environmental factors affecting on yield, it is necessary to know patterns of plant physiological behaviors and association between biosynthetic pathways of biomolecules. Therefore, Barley landrace of 'Siah' was planted and activity of acetyl CoA-carboxylase (ACoC) enzyme was stopped using sethoxydim in 3-4 leaf old stage. Sampling was done from barley seedlings at various stages and were analyzed the parameters of dry matter production, membrane stability index, relative water content, activities of scavenging enzymes of hydrogen peroxide, hydrogen peroxide accumulation and malondialdehyde. Results showed that growth and development of the seedlings were inhibited by activity ceasing of ACoC. It has been made by inhibition of necessary fatty acid biosynthesis to generate new cells and to increase the cells sizes. During activity ceasing of ACoC, there was significant changes in activity scavenging enzymes of hydrogen peroxide except to guaiacol peroxidase. Activity of the enzymes altogether were resulted in accumulation of hydrogen peroxide in plants with ceased activity of ACoC. The accumulation of hydrogen peroxide also caused to increase lipids peroxidation. The obtained results displayed that activity ceasing of ACoC caused inhibition of plant growth and disruption of plant defense mechanisms.

**Key words:** Acetyl-coA carboxylase, fatty acids, oxidative stress, antioxidant enzymes

### INTRODUCTION

The fatty acids in plants are usually straight-chain carboxylic acids having an even number of carbon atoms (Heldt, 2004). They both free and as part of complex lipids, play key roles in storage, transport of energy, as essential components of all membranes and gene regulators (Rustan and Drevon, 2005). Fatty acids are used in structure of compounds as well as lipids, waxes and carotenoids (Gunning and Steer, 1996). Lipids are used for both energy and storage. They are a structurally diverse group of hydrophobic compounds that are soluble in organic solvents (Ohlrogge and Browse, 1995). Lipids are important for plant structure and function that make up plant membranes (Bhat and Panstruga, 2005). Waxes which make up the protective cuticle that reduces water loss from exposed plant tissues (Von Wettstein-Knowles, 1995) and Carotenoids involved in photosynthesis (Heldt, 2004).

Fatty acids biosynthesis involves the cyclic condensation of two-carbon units derived from acetyl-coa (Rawsthorne, 2002). The first committed step in the pathway is the synthesis of malonyl-coa from acetyl-CoA

and CO<sub>2</sub> by the enzyme acetyl-coa carboxylase (Sasaki *et al.*, 1995). The tight regulation of acetyl-coa carboxylase appears to control the overall rate of fatty acid synthesis (Ohlragge and Jaworski, 1997). So ceasing activity of acetyl-coa carboxylase enzyme as regulator point of fatty acids biosynthesis can make up disruption of fatty acids anabolism (Murphy and Walker, 1982). In other hand, activity ceasing of acetyl-coa carboxylase prevents growth of plant because there is not necessary fatty acids to contribute in membranes structure (Shaner, 2004).

Fatty acids biosynthesis carry out in chloroplasts and plastids of leaf cells that hydrogen potential for this process is provided by light reactions of photosynthesis (Givan, 1983). Ceasing of fatty acid biosynthesis can decrease redox potential of chloroplast and so increase production of Reactive Oxygen Species (ROS) (Mittler, 2002). The O<sub>2</sub> molecule is a free radical, as it has two impaired electrons that have the same spin quantum number. This spin restriction makes O<sub>2</sub> prefer to accept its electrons one at a time, leading to the generation of the so called ROS, which can damage the cells. ROS are also produced continuously as by products of various

metabolic pathways that are localized in different cellular compartments such as chloroplast, mitochondria and peroxisomes (Gill and Tuteja, 2010). Reactive oxygen species have a tendency for reacting with biomolecules as well as lipids, proteins and nucleic acids. It makes up lipid peroxidation, scrappy of proteins and mutation in DNA structure (Mittler, 2002). Aggregation of these injuries makes up cell death a long metabolic disorder (Esfandiari *et al.*, 2007).

The lipids peroxidation is considered as the most damaging process known to occur in every living organism. Membrane damage is sometimes taken as a single parameter to determine the level of lipid destruction under various stresses. Now, it has been recognized that during lipids peroxidation, create polyunsaturated precursors which include small hydrocarbon fragments such as ketones, MDA and compounds related to them (Gill and Tuteja, 2010). Malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acids hydroperoxides, has been utilized very often as a suitable biomarker for lipid peroxidation (Baillly *et al.*, 1996) which is an effect of oxidative damage (Sofa *et al.*, 2004).

Reactive oxygen species are produced in both stressed and unstressed plant (Hennouni *et al.*, 2008). Plants have an advanced defense system against ROS involving enzymatic and non-enzymatic means. Catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) are antioxidant enzymes which play a capital role in keeping hydrogen peroxide levels harmless and therefore contribute to protect the plant from ROS damages (Ahmad *et al.*, 2008). CATs are tetrameric heme containing enzymes with the potential to directly dismutate  $H_2O_2$  into  $H_2O$  and  $O_2$  and is indispensable for ROS detoxification during stressed conditions (Garg and Manchanda, 2009). APX is thought to play the most essential role in scavenging ROS and protecting cells in higher plants, algae, euglena and other organisms. APX is involved in scavenging of  $H_2O_2$  in water-water and ASH-GSH cycles and utilizes ASH as the electron donor (Gill and Tuteja, 2010). GPXs are a large family of diverse isozymes that use GSH to reduce  $H_2O_2$  and organic and lipid hydroperoxides and therefore help plant cells from oxidative stress (Hennouni *et al.*, 2008). The plant glutathione transferases, formerly known as glutathione S-transferases are a large and diverse group of enzymes which catalyse the conjugation of electrophilic xenobiotic substrates with the tripeptide glutathione (GSH; g-glucyseygly). Plant GSTs are known to function in herbicide detoxification, hormone homeostasis, vacuolar sequestration of anthocyanin, tyrosine metabolism, hydroxyperoxide detoxification, regulation of apoptosis and in plant responses to biotic and abiotic

stresses (Dixon *et al.*, 2002). So, GST is a kind of defense enzymes that play critical roles in the detoxification of xenobiotics and the protection of tissues against oxidative stress that was accomplished by quenching reactive molecules with the addition of reduced glutathione (Zhao and Zhang, 2006).

Nowadays it is necessary to recognize patterns of plant physiological behaviors and effects of biosynthesis pathways on plant biomolecules such as better understanding their tracing on plant growth that it is beneficial to overcome on ingredient of lessening plant yield. Activity ceasing of acetyl-coa carboxylase enzyme were not studied on plant growth, scavenging enzymes of peroxide hydrogen and biological compounds. So, this research is performed with activity ceasing of acetyl-coa carboxylase at seedling stage in barely plant and studied enzymes activity of defense mechanisms, oxidative stress and physiological parameters.

## MATERIALS AND METHODS

Seeds of Barely (*Hordeum vulgare*) landrace "Siah" were planted with intervals of 15 cm between rows and 1 CM on them in a soil with light texture under controlled conditions. Seedlings were grown in a growth chamber at 16 h light: 8 h dark photoperiod, 25°C, relative humidity of 65% and with a light intensity of 2500 lux. The source of light inside the growth chamber was a combination of yellow and white fluorescent lamps. For activity ceasing of ACoC, were used Setoxydim solution with 3% concentration and were sprayed on the seedlings. Leaf samples were collected in days of 0, 1, 3, 5 and 7 after ceasing activity of ACoC and immediately placed in liquid nitrogen. Leaf samples were kept at -20°C and used to measure activity of enzymes and biological markers of oxidation. In addition membrane stability index (MSI), Relative Water Content (RWC) and dry matter were measured in sampling times.

**Enzyme extraction:** For CAT, GST and GPX extraction, leaf samples (0.5 g) were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA with pre-chilled pestle and mortar. Each homogenate was transferred to centrifuge tubes and was centrifuged at 4°C in Beckman refrigerated centrifuge for 15 min at 15000×g. The supernatant was used for enzyme activity assay (Esfandiari *et al.*, 2007).

For APX extraction, leaf samples (0.5 g) were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA, 2 mM ascorbate (AsA) and 5% polyvinylpyrrolidin (PVP 6000) with pre-chilled pestle and mortar. The other stages were similar to the extraction of other enzymes (Esfandiari *et al.*, 2007).

**Enzyme activity assay:** CAT activity was measured according to Aebi (1984). Reaction mixture contained 100 mM potassium phosphate buffer (pH 7), 75 mM H<sub>2</sub>O<sub>2</sub>, enzyme extract and distilled water. Reaction started by adding H<sub>2</sub>O<sub>2</sub> and the decrease in absorbance was recorded at 240 nm for 1 min. Enzyme activity was computed by calculating the amount of H<sub>2</sub>O<sub>2</sub> decomposed.

APX activity was measured according to Yoshimura *et al.* (2000) by monitoring the rate of ascorbate oxidation at 290 nm. The reaction mixture contained 25 mM phosphate buffer (pH 7), 0.1 mM EDTA, 1 mM H<sub>2</sub>O<sub>2</sub>, 0.25 mM reduced ascorbate (AsA) and the enzyme sample. No change in absorption was found in the absence of AsA in the test medium.

GPX activity was measured according to Panda *et al.* (2003). Reaction mixture contained 100 mM potassium phosphate buffer (pH 7), 0.1 mM EDTA, 5 mM guaiacol, 15 mM H<sub>2</sub>O<sub>2</sub> and enzyme sample. The enzyme produced a colorful product by using H<sub>2</sub>O<sub>2</sub> and guaiacol as substrates. The absorbance of the product was monitored at 470 nm and peroxidase activity was expressed as units mg<sup>-1</sup> protein min.

Glutathione-S-transferase activity was measured following the method of Habig *et al.* (1974). One milli liter of reaction mixture contained 50 µL of 100 mM sodium phosphate buffer (pH 6.5), 400 µL double distilled water and 1.0 mM reduced glutathione (GSH). The reaction was initiated by adding 1.0 mM 1-chloro-2,4-dinitrobenzene (CDNB), as the substrate, dissolved in ethanol. The change in absorbance at 340 nm was measured for 1 min.

MDA was measured by colorimetric method. 0.5 g of leaf samples were homogenized in 5 mL of distilled water. An equal volume of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) solution was added and the sample incubated at 95°C for 30 min. The reaction stopped by putting the reaction tubes in an ice bath. The samples were then centrifuged at 10000×g for 30 min. The supernatant was removed, absorption read at 532 nm and the amount of nonspecific absorption at 600 nm read and subtracted from this value. The amount of MDA present was calculated from the extinction coefficient (Stewart and Bewley, 1980).

Hydrogen peroxide levels were determined according to Sergiev *et al.* (1997). Leaf tissues (0.5 g) were homogenized in ice bath with 5 mL 0.1% (w/v) TCA. The homogenate was centrifuged at 12000×g for 15 min and 0.5 mL of the supernatant was added to 0.5 mL 10 mM potassium phosphate buffer (pH 7.0) and 1 mL 1 M KI. The absorbency of supernatant was read at 390 nm. The content of H<sub>2</sub>O<sub>2</sub> was given on standard curve.

Protein content of samples was determined by method of Bradford (1976). Bovine serum albumin was used as a standard.

Membrane Stability Index (MSI) was determined by recording the electrical conductivity of leaf ions leaching in double distilled water (Sairam *et al.*, 2002). Leaf samples (0.1 g) were taken in test tubes containing 10 mL of double distilled water in two sets. One set was kept at 40°C for 30 min and another set at 100°C in boiling water bath for 15 min and their respective electrical conductivities, C1 and C2, were measured by a pH-EC meter. Membrane stability index was calculated by the following formula:

$$MSI = \frac{1-C_1}{C_2} \times 100$$

Relative Water Content (RWC) was estimated by taking leaf sample (0.5 g) in 100 mL distilled water. Leaf samples were placed in kraft's bag and oven dried at 70°C for 48 h. On concordant dry weight achievement, the weight of sample was noted:

$$RWC = \frac{\text{Fresh weight-dry weight}}{\text{Turgid weight-dry weight}}$$

Dry matter was estimated by placing seedling in kraft's bag and oven dried at 70°C for 48 h.

**Statistical analysis:** All physiological and biochemical parameters were recorded with five replications. The data were analyzed with SPSS V.17 software. Mean comparison were carried out by LSD method.

## RESULTS AND DISCUSSION

The results showed that levels of malondialdehyde and hydrogen peroxide were significantly increased in activity ceasing condition of ACoC only on the seventh day. Activity of guaiacol peroxidase did not change in activity ceasing condition of ACoC than control plants on the third and seventh days. Amount activity of ascorbate peroxidase significantly decreased in activity ceasing condition of ACoC compared to the control in the seventh day that was completely in contrast of catalase activity. Activity of glutathione S-transferase were significantly decreased in activity ceasing condition of ACoC.

The results indicate that damaging to lipids, even occur in desirable conditions of environmental (Fig. 1a) while exist equilibrium between active oxygen species and other toxic compounds with present of defenses mechanisms of cellular that prevent from metabolic disorders (Shao *et al.*, 2008). Increased amount of malondialdehyde suggests the occurrence of oxidative

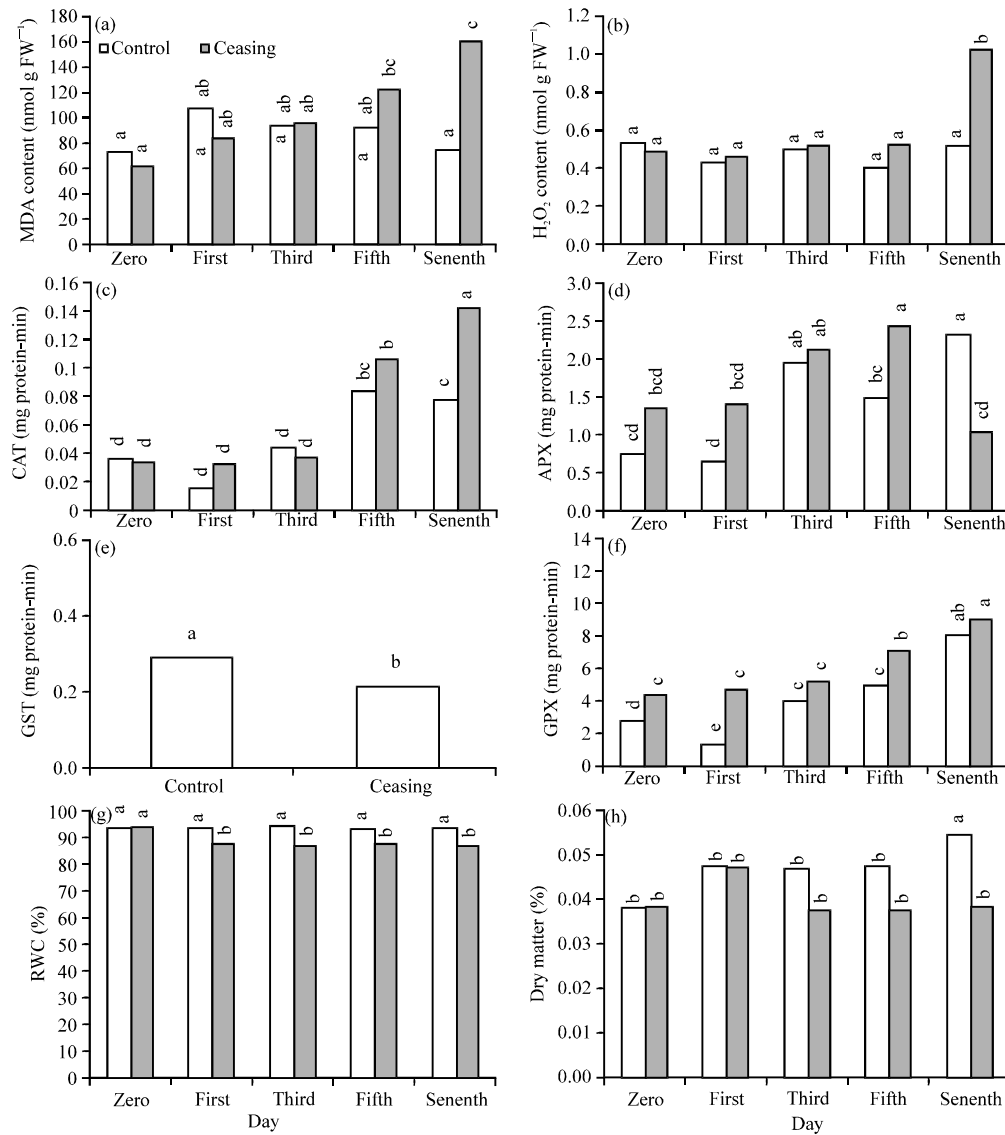


Fig. 1(a-h): Effect of activity ceasing of ACoC on (a) MDA content, (b) H<sub>2</sub>O<sub>2</sub> content, (c) Activity of enzymes CAT, (d) APX, (e) GST, (f) GPX, (g) Growth parameters as RWC and (h) Dry matter, Bars with different letters are significantly different

stress and damaging to the membrane of organelles and Plasmalma (Sofo *et al.*, 2004) in activity ceasing condition of ACoC in the seventh day (Fig. 1a). The fact that the production of reactive oxygen species overcame on the defense mechanisms and oxidative stress was occurred in this condition (Ahmad *et al.*, 2008).

Process amount of hydrogen peroxide is the same as malondiadehyde (Fig. 1b). Hydrogen peroxide is produced in the metabolism of the vital processes of cell organelles (Mittler, 2002). The univalent reduction of O<sub>2</sub><sup>-</sup> produces H<sub>2</sub>O<sub>2</sub>. H<sub>2</sub>O<sub>2</sub> is moderately reactive and has relatively long half-life (1 m sec) whereas, other ROS such as O<sub>2</sub><sup>-</sup>, OH<sup>-</sup> and <sup>1</sup>O<sub>2</sub>, have much shorter half-life. H<sub>2</sub>O<sub>2</sub> is starting to be

accepted as a second messenger for signals generated by means of ROS because of its relatively long life and high permeability across membranes (Quan *et al.*, 2008). It has been well established that excess of H<sub>2</sub>O<sub>2</sub> in the plant cells leads to the occurrence of oxidative stress. H<sub>2</sub>O<sub>2</sub> may inactivate enzymes by oxidizing their thiol groups (Gill and Tuteja, 2010). This compound is highly toxic to cells. Accumulation as the key points of damage to cells will cause metabolic disorders. Including how to stop some of the Calvin cycle enzymes such as ribolose-phosphat kinase and phosphatase (Foyer and Noctor, 2009; Yamazaki *et al.*, 2003; Mittler, 2002). Levels of hydrogen peroxide suggests increased production of this

oxidant than defense mechanism of its collecting in terms of ceasing activity of ACoC than the control. So, it is created imbalance between produced and collected amount of hydrogen peroxide in a short time after ceasing activity of ACoC and caused oxidative stress. Rajaeian *et al.* (2011) have reported that levels of Hydrogen peroxide and malondialdehyde increased in treated maize plants with methyl-viologen as receptor-mediated electron and transferring on the oxygen. Scarpeci *et al.* studied the methyl violgen (MV, O<sub>2</sub> propagator in the light) induced generation of O<sub>2</sub><sup>·-</sup> in *Arabidopsis thaliana* chloroplasts during active photosynthesis and suggests that O<sub>2</sub><sup>·-</sup> generated in photosynthetically active chloroplasts leads to the activation of genes involved in signaling pathways (Gill and Tuteja, 2010).

Comparing the activity of enzymes catalase, ascorbate peroxidase and Guaiacol peroxidase on the seventh day showed that reducing the activity of ascorbate peroxidase was participant with increased of catalase activity while activity of Guaiacol peroxidase has been no change (Fig. 1c-f). Ascorbate peroxidase enzyme converts hydrogen peroxide into water and plays importance role in Glutathione-ascorbat and Mahler defense cycles (Asada, 2000). The catalase enzyme transmutes Toxic metabolite of hydrogen peroxide to water and oxygen (Blokhina and Fagerstedt, 2010). Guaiacol peroxidase which is pertained for a large family of varied isozyme, hold down the amount of hydrogen peroxide, lipid and organic hydroperoxidases (Dixon *et al.*, 2002). Increased hydrogen peroxide and lipid peroxidation in the seventh day suggest that increased of catalase activity cannot compensate decreased activity of ascorbate peroxidase and no change activity of guaiacol peroxidase and thus production of active oxygen species surpassed on the collecting mechanisms and increased lipid peroxidation and injuries on membranes. Decreased activity of glutathione S-transferase (Fig. 1e) from collecting enzymes of toxic metabolites was server in continuing of damages on biomoleculars (Mohammadi *et al.*, 2000). Hassan *et al.* (2005) ala has reported decreased activity of APX and non-significant activity of GPX in terms of oxidative stress. Increased activity of CAT were reported by some researchers as Seekin *et al.* (2010) and Yamazaki *et al.* (2003). Srivastava *et al.* (2005) reported a decrease in CAT activity in *A. doliolum* under NaCl and Cu<sup>2+</sup> stress. Simova-Stoilova *et al.* (2010) reported increased CAT activity in wheat under drought stress but it was higher especially in sensitive varieties. Contrarily, Pan *et al.* (2006) studied the combined effect of Salt and drought stress and found that it decreases the CAT activity in *Glycyrrhiza uralensis* seedlings.

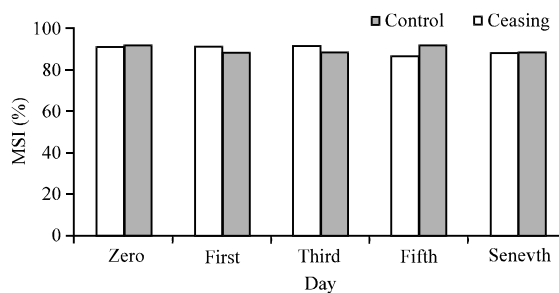


Fig. 2: Effect of ceasing activity of ACoC on MSI (%)

Due to increasing in amount of hydrogen peroxide and malondialdehyde lipid peroxidation and damaging to biological membranes, was expected slaking of membrane stability index that based on measuring the electrical conductivity of electrolytes leaking from the cells of intact leaves to solution (Azizpour *et al.*, 2010), but these were not significantly reduced (Fig. 2). This index expressed the amount of damaging to Plasmalma (Almeselmani *et al.*, 2006). It may be damaged the membranes of intracellular organelles without injuring structure of Plasmalma, so it caused to be prevented from leaking the elements and metabolites. Therefore, electrolyte leakage was reduced and membrane stability index have been no significant changes. Effective factors in reducing the relative water content of the leaves are involved by reduced root growth and increased evapotranspiration of the plant community (Tarumingkeng and Coto, 2003). Necessary turgor pressure is provided for cell growth and expansion of cellular wall, by the increased relative water content of leaves (Yamasaki and Dillenburg, 1999), which contributed in growth and development of plant. Thus reducing the relative water content of the leaves impaired plant growth and development in terms ceasing activity of ACoC (Fig. 1g). Costa-Franca *et al.* (2000) have reported decreased RWC in dry stress in *Phaseolus vulgare*. It is also considerable that amount of dry matter significantly increased in control plants in seventh day than past days. While there was none significant change in dry matter of treated plants in different days of sampling (Fig. 1h). This trend shows that growth and development of plant has been stopped in terms of ceasing activity of ACoC. Hatata and Abdel-Aal have related that dry matter decreased in cadmium stress in sunflower.

## CONCLUSION

ACoC Enzyme is a key enzyme in path of fatty acids biosynthesis. Fatty acids are one of the major components in the plasma membrane structure. Plasma membranes include many roles in cell that can mention their role in cell division and thus growth and

development plant. So no change in dry matter in terms of ceasing activity of ACoC enzyme is indicative of disorder in the synthesis of fatty acids and established their key roles in cell structure and metabolism and growth of plant.

#### ACKNOWLEDGMENT

The reported study was supported and financially backed by research chancellorship at University of Maragheh.

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