

# Overexpression of Some Stress Tolerance Genes Confers Enhanced Pesticide Tolerance in 5-Aminolevulinic Acid Treated Bean Seedlings

Murat Aydin (✉ [maydin@atauni.edu.tr](mailto:maydin@atauni.edu.tr))

Ataturk Universitesi Ziraat Fakultesi <https://orcid.org/0000-0003-1091-0609>

**Esra Arslan**

Ataturk University: Ataturk Universitesi

**Guleray Agar**

Ataturk University: Ataturk Universitesi

**Mahmut Sinan Taspinar**

Ataturk University: Ataturk Universitesi

---

## Research Article

**Keywords:** ALA, antioxidant genes, deltamethrin, QPCR, REMAP, SAP gene

**Posted Date:** April 6th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-354198/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

Overdose of pesticides leads to a decrease in yield and quality of plants such as bean. The unconscious use of deltamethrin, one of the synthetic insecticides, increases the amount of reactive oxygen species (ROS) by causing oxidative stress in plants. In this case, plants tolerate to stress by activating the antioxidant defense mechanism and many genes. 5-Aminolevulinic acid (ALA) improves tolerance to stress by external administration in low doses. There are many gene families that are effective in the regulation of this mechanism. In addition, one of the response mechanisms at the molecular level against environmental stressors in plants is retrotransposon movements. In this study, the expression levels of SOD, GPX, CAT and SAP genes were determined by Q-PCR in deltamethrin (0.5 ppm) and various doses (20, 40 and 80 mg/l) of ALA treated bean seedlings. It was determined that while deltamethrin increased the expression of these genes, 20 and 40 mg/l ALA gradually increased the expression of genes at levels close to control, but 80 mg/l ALA increased almost the same level as deltamethrin. In addition, REMAP was performed to determine the polymorphism caused by retrotransposon movements. While deltamethrin treatment has caused to decrease of genomic template stability (GTS), ALA treatments have prevented this decline. Collectively, these findings demonstrated that ALA gives the utility for alleviating pesticide stress effects on bean.

## Introduction

Dry bean is a warm season plant, that rich in protein and vitamins and can easily grow in almost any type of soil. Also it is an important legume in terms of having the most cultivation area and production (28.9 million tons, FAO 2019) among edible legumes in the world. However, the richness of the bean's protein ratio increases its susceptibility to diseases and harmful insects. In this sense, it becomes necessary to use modern agricultural techniques and inputs in order to increase the yield and quality of agricultural products and to combat diseases and pests.

The use of pesticides is a form of agricultural struggle in order to protect agricultural products from the damage of diseases, pests and weeds. Deltamethrin [(S) - $\alpha$ -cyano-3-phenoxybenzyl (1R, 3R) -cis-2,2-dimethyl-3-(2,2-dibromovinyl) -2,2-cyclopropanecarboxylate] is a kind of the synthetic pyrethroid and a broad spectrum insecticide (Sayeed et al. 2003). It is known that deltamethrin has been used successfully to control aphid infestation in fields where important crops such as beans are grown (Johnstone 1984). However, many sections of plants such as cell (Mukhopadhyay et al. 2006) genome (Chauhan et al. 2007; Ansari et al. 2009; Aylward et al. 2011) and chromosomes (Marques et al. 2014) are negatively affected due to the accumulation and non-degradation of insecticides by forming insoluble bonds in agricultural products (Bashir et al. 2007). In this case, many genes are activated to protect crops from the effects of pesticide stress (Kishimoto et al. 2002; Tian et al. 2013). The expression of antioxidant enzyme genes [superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR)] increase in order to avoid the harmful effects of ROS, whose amount increases against oxidative stress that occurs during pesticide stress. Furthermore, stress-associated proteins (SAPs) are known as response factors to abiotic and biotic stresses and it confer stress tolerance to plants (Wang et al. 2020).

One of the response mechanisms created at the molecular level against environmental stressors is retrotransposon movements in plants. Although they are inactive during normal growth and development, they are activated during stress and increase the mutation rate and also cause methylation change in the genome. It has been determined that Ttd1a retrotransposon activated when exposed to salt and light stress, located next to a resistance gene and thus protected the wheat against stress (Woodrow et al. 2010). Retrotransposon-based markers have a key role in determining retrotransposon movements induced by stress. REMAP is one of these markers that amplify the DNA region between retrotransposon and simple sequence repeats regions (Kalendar et al. 2011).

Plants activate many plant growth regulators (PGRs) as well as antioxidant defense mechanisms genes and retrotransposon movements in order to tolerate damage on their metabolism. Also the exogenously use of PGRs has a positive effect on increasing stress tolerance in stressed plants (Ali et al. 2013). One of these regulators, 5-aminolevulinic acid (ALA), is a precursor molecule involved in the biosynthesis of porphyrins such as chlorophyll (Chl), vitamin B12 and heme in plants (Balestrasse et al. 2010; Ali et al. 2013). The exogenous application of ALA is an effective antistress agent under optimum conditions that plays a role in the development of plant tolerance. It has been demonstrated many stress studies such as low temperature (Balestrasse et al. 2010), high temperature (Zhang et al. 2012), low light (Sun et al. 2009), excessive salinity (Naeem et al. 2011), heavy metal stress (Ali al. 2013) and herbicide stress (Zhang et al. 2008).

In this study, we aimed to determine the expression levels of the SOD, CAT, GPX and SAP genes which are induced by the activation of the antioxidant mechanism against the oxidative damage caused by deltamethrin when used in excessive doses. In addition, it was determined whether ALA, which has been stated to have a healing role in our previous study (Taspinar et al. 2017), caused a change in the expression of these genes when applied with deltamethrin. Also retrotransposon mobility and the rate of polymorphism was determined by using REMAP technique.

## Material And Methods

### Plant material

*Phaseolus vulgaris* L. cv Elkoca seeds used as plant material provided by Ataturk University Faculty of Agriculture.

### Growth conditions and ALA, and deltamethrin treatments

The seeds used in experiment were selected based on equal sizes and seterilized with 5% hypochlorite solution for 5 min and rinsed three times with distilled water. Then, they were germinated in a hydroponic system at 25°C for 16 light/8 dark hours in plastic boxes (Arslan 2021) containing Hoagland solution (Sigma H2395-10 L) (Hoagland and Arnon 1938). Ten seedlings were obtained in equal growth period by selection in 7-day-old plants. These seedling were kept in same conditions. The experiment was carried out completely random design with three replications. 20-day-old seedling leaves were sprayed ALA

solutions (Sigma, A3785) [0 (control), 20, 40 and 80 mg/l] (Beyzaei et al. 2015). Afterwards 5 days of ALA treatment, 0.5 ppm deltamethrin solution (Sigma, 45423) was sprayed on the leaves (Duran et al. 2015). Bulk sample strategy was applied for molecular analysis. Leaf samples were harvested 5 days after deltamethrin application from five randomly selected plants for each replication of treatments and were stored at -80°C.

## Total RNA Extraction, cDNA Synthesis, and Gene Expression

Total RNA of 100 mg leaves were extracted with RNeasy Plant Mini Kit (Qiagen) according to suppliers' instructions. RNA purity and concentrations were assessed by determining the spectrophotometric absorbance of the samples with NanoDrop-1000 spectrophotometer ( $OD_{260/230} > 2$ ). RNA integrity was evaluated on 1.2% agarose gel, staining with ethidium bromide and visualisation with UV light. First-strand cDNA synthesis was performed with RevertAid First Strand cDNA Synthesis kit (Thermo Scientific) as described Arslan et al. (2021). The Quantitative PCR was performed with SYBR Green/ROX qPCR Kit (Thermo Scientific) according to manufacturer's protocol and following genes were amplified: SOD, GPX, CAT and SAP.  $\beta$ -actin was preferred as housekeeping gene. To design the primers of genes it was used databases related to bioinformatics studies conducted in *Phaseolus vulgaris* genome (Table S1). Accession numbers of genes were found using the Pfam (pfam.xfam.org) database. Then, Phytozom (<https://phytozome.jgi.doe.gov/>) database, which is the plant genomic source, was used. Finally, primers were created using Primer3 (<http://frodo.wi.mit.edu/>) program from selected base sequences. The Q-PCR reactions were run in a Qiagen Rotor-Gene and the cycling conditions consisted of initial denaturation at 95°C for 10 min, followed by 40 cycles of amplification at 95°C for 15 s, at 56–65°C for 30 s and extension at 72°C for 30 s. The relative gene expression levels which were determined using the  $2^{-\Delta\Delta Ct}$  equation were calculated to get the expression fold change (Livak and Schmittgen 2001). Each sample was analyzed in three technical replicates. One Way ANOVA was performed to evaluate the effect of treatments on gene expression. Duncan's multiple range test ( $P \leq 0.05$ ) was performed to compare the mean values. Data were analyzed using SAS 9.3 software for windows.

## Genomic DNA extraction

Total DNA was extracted from 0.1 g leaves of each treated group by CTAB method of Taspinar et al. (2018). Integrity and quality of DNA was evaluated by electrophoresis on 1% agarose gel.

## REMAP

The REMAP reactions were based on previously published method (Yigider et al. 2020). For amplification the IRAP primers [Nikita-E2647, Stowaway, Sukkula and Bare 1(0)] were combined with ISSR primers (8081, 8082) (Table S2). Amplifications were carried out in a Thermo Scientific™ Arktik™ Thermal Cycler with following PCR programs: an initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 1 min, distinct temperatures for each primer for 1 min and 72°C for 2 min; and a final extension at 72°C for 15 min. PCR products were run by electrophoresis on 2% agarose gel in 0.5×TBE buffer stained with ethidium bromide and photographed in DNR Minibis Gel Documentation System (USA).

# REMAP data analysis

The gel images obtained were evaluated with the TotalLab TL120 program. Genomic template stability (GTS) (%) for each primer was calculated using the formula  $100 - (100 * a/n)$  according to Atienzar et al. (1999). “a” in the formula indicates the REMAP polymorphic profiles determined for each treated sample, and “n” indicates the total number of DNA bands obtained in the negative control group with the relevant primer. The polymorphism observed in the REMAP profiles of the treatment groups includes the emergence of a new band or the disappearance of an existing band compared to the negative control group. For all treatments, a binary matrix was generated based scored as 1 (present) or 0 (absent) for each primer. The following calculations were carried out with the use of NTSYSpc 2.11f software. The Jaccard’s similarity coefficient was calculated by using the SIMQUAL module. The similarity coefficients were then used to construct dendrograms, by using the UPGMA (unweighted pair group method with arithmetic averages) employing the SAHN. The goodness of dendrogram was verified in MXCOMP program by using Jacard’s similarity matrix and co-phenetic value matrix. The three-dimensional PCoA was performed based on the similarity matrix.

## Results And Discussion

### Gene expression profile of some antioxidant enzymes and stress protein under deltamethrin and ALA treatments

Unfavorable environmental conditions and insect infestations are the strongest factors limiting yield in beans (Gogo et al. 2014). Pesticides such as deltamethrin, are frequently used in agricultural lands to reduce the effects of harmful organisms. Overdose of deltamethrin causes oxidative damage in plants by activating ROS (Bashir et al. 2007). ROS play a dual role in plant responses to abiotic stress, both as toxic by-products of stress metabolism and as an important signal transduction molecule in complex metabolic processes responsible for the emergence of stress responses based on calcium, hormone and protein phosphorylation (Miller et al. 2010). The uncontrolled oxidation obtained when ROS are overproduced leads to cellular damage and ultimately cell death. In order to prevent the plant from being damaged by this situation, the current antioxidant mechanism should keep active oxygen under control (Bose et al. 2014). When these removal mechanisms are not damaged by stress, ROS are rapidly destroyed by antioxidant mechanisms (Ahmad et al. 2010). Among the regions targeted by ROS, such as protein and DNA that are difficult to repair, result in genetic damages. Genetic studies in seedlings of *Phaseolus vulgaris* also suggest a link between pesticide stress and oxidative stress, since deltamethrin induces the expression of several genes that are also induced by oxidative stress. In this study, expression levels of SOD, CAT and GPX genes, which are antioxidant genes were determined. According to our results, all of these genes were upregulated in deltamethrin stress.

SOD is the first line of defense against ROT during abiotic stress in a plant cell. Control of SODs in both expression and activity of ROS contributes to the regulation of stress tolerance (Forman 2007; Liu et al.

2008). According to the results of SOD gene expression analysis, the rate of gene expression in deltamethrin treatment alone was about two fold more compared to control (1.819) (Fig. 1). Similarly, GPX and CAT genes upregulated in deltamethrin treatment approximately two and three fold according to none treated seedlings, respectively (Fig. 2 and Fig. 3). To scavenged the ROS efficiently, the activity of APX and SOD must be high to remove the H<sub>2</sub>O<sub>2</sub> produced by superoxide ion dismutation (Pospíšil 2012). Therefore, in our study the high expression level of both SOD, CAT and GPX genes could be for the removal of ROS. In a similar study, Sharma et al. (2015) investigated the expression levels of CAT, SOD, APX and GR enzyme genes in salt and pesticide stress applied to rice, and found that all genes were highly upregulated in both stresses.

As well as antioxidant genes, SAP gene was highly upregulated (2.511) in deltamethrin stress (Fig. 4). Proteins in the SAP family contain the A20/AN1 zinc finger domain and are known to be important determinants of stress responses in plants (Vij and Tyagi 2006). Similar results were obtained in different plants during different abiotic stresses. that SAP genes (OSISAP) found in rice in particular are induced by abiotic stress (Vij and Tyagi 2006). Overexpression of the OSISAP1 gene in tobacco (*Nicotiana tabacum*) increase the tolerance to cold, drought and salt stress (Mukhopadhyay et al. 2004). Similarly, the OSISAP8 gene, which is transferred to the tobacco plant, is thought to have a role in the development of tolerance against abiotic stress (Kanneganti and Gupta 2008). Giri et al. (2011) determined that the OSISAP11 gene transferred to transgenic Arabidopsis interacts with OSIRLCK253, a receptor-like cytoplasmic kinase, providing tolerance to drought and salt stress as well as TaSAP5 gene in wheat (Zhang et al. 2017). Studies have associated SAP proteins with roles such as ubiquitination, redox detection, and regulation of gene expression under abiotic stress (Ströher et al. 2009; Ben-Saad et al. 2012; Kang et al. 2013). However, the mechanism by which SAP proteins play the main role mechanically in stress responses has not been fully elucidated.

In recent studies, plant growth regulators have also been reported to have roles in the regulation of the plant defense system against various stresses (Zhang et al. 2008; Balestrasse et al. 2010; Naeem et al. 2011; Zhang et al. 2012; Ali et al. 2013). In addition, it has been proven that high concentrations of ALA play a role as a herbicide or insecticide (Rebeiz et al. 1984). As a matter of fact, in our study, ALA in low doses (20 and 40 mg/l) is beneficial in creating stress tolerance by increasing the expression of antioxidant genes. In 80 mg/l ALA application, it was determined that SOD gene expression was higher than deltamethrin treatment and the difference between these two treatments had been significant (Fig. 1). On the other hand, CAT and GPX gene expressions in 80 mg/l ALA application were close to the result obtained from deltamethrin application (Fig. 2 and Fig. 3). In addition, when ALA and deltamethrin were treated together, 40 mg/l ALA increased the expression of these genes more. This situation may be related to ALA protecting the cell against the destructive effect of ROS by removing H<sub>2</sub>O<sub>2</sub> (Ali et al. 2015). Sharma et al. (2015) found that the SOD gene was more induced in brassinosteroid applications than salt or pesticide stress alone in rice. Exogenously applied high concentrations of ALA, accumulates in excessive amounts in cells, causing an increase in the amount of ROS by being exposed to both enolisation and aerobic oxidation with metal catalysis (Reyter and Tyrrell 2000). In this case, the enzyme

called heme oxygenase degrades the free heme group and converts bilirubin into iron and carbon monoxide (Shekhawat and Verma 2010) and causes a decrease in oxidative stress in the plant (Grochot-Przeczek et al. 2012). While the mechanism for oxidative stress and degradation of the heme group remains unclear, it is thought to be an evolutionary protection mechanism given by the plant cell to counteract the destructive effect of the free heme group (Kumar and Bandyopadhyay 2005). Noriega et al. (2012) determined that the cadmium increased the ALA content in the root, leaf and nodule parts of the soybean and the plant was exposed to more oxidative stress. At the same time, it was found that cadmium or ALA applications both inhibited antioxidant enzyme activities and caused a significant decrease in SOD and guaiacol peroxidase expression.

Our experiment results also indicated that SAP gene was gradually upregulated in ALA treatments. However, the highest expression rate was 80 mg/l ALA + deltamethrin and the lowest was in the application of 20 mg/l ALA. ZFP185, a A20/AN1 zinc finger protein, is linked to abscisic acid and gibberellic acid, which regulate the cell growth and stress response mechanism (Zhang et al. 2016). Thus, it can be assumed that the SAP gene works in conjunction with these hormones in establishing stress tolerance. In our study, it is thought that 80 mg/l ALA application has a more damaging effect with deltamethrin by acting as an insecticide, it may have a role in the formation of stress tolerance by linking with signal molecules such as abscisic acid and gibberellic acid, and leads to a greater increase in the expression of these genes.

## **Changes in REMAP pattern under deltamethrin and ALA treatments**

Another effect of various environmental stressors at the genome level is retrotransposon mobility. In our study, the retrotransposon polymorphism caused by deltamethrin was determined by REMAP. A total of eight primer pairs were tried for REMAP analysis and 113 bands were obtained and 98 of them were determined as polymorphic bands. All of the primers were determined as polymorphic. Maximum number of polymorphic bands counted in Bare 1 (0) + ISSR 8081 and minimum in Stowaway + ISSR 8081 primer pairs (Table S3). The polymorphic information content (PIC) value of the primer pairs used to determine the molecular effects of the treatments varied between 0.365-0.427 and the average was 0.382 (Table S3). Maximum PIC value for dominant markers is 0.5. Because two alleles are assumed per locus and both are affected by the number and frequency of alleles. In this respect, the Stowaway + ISSR 8081 primer pair had the highest PIC value (Table S3). On the other hand, the discriminating power (D) parameter used in the evaluation of the primers shows the efficiency of the primers in the identification of individuals. The D value of the primers varied between 0.408–0.867 (Table S3). The Bare 1 (0) + ISSR 8081 primer pair, which has both the discrimination power and the highest polymorphic band content, was determined as the most distinctive primers.

With the treatment of deltamethrin, 73% polymorphism ratio was occurred. When examining the effects of applications in terms of GTS ratio, the lowest GTS value was obtained in deltamethrin treated seedlings compared to the control. While the molecular weights of the missing bands of applications compared to

the control are between 50-1440 bp, the newly formed bands were between 224–1581 bp (Table S4). When the effects of different doses of ALA were examined compared to the control, it was determined that 40 and 80 mg/L ALA doses caused a decrease in the GTS values, depending on the dose increase. On the other hand, 40 mg/L ALA reduced the negative effect of deltamethrin on GTS (Fig. 5).

The similarity index of the applications varied between 0.38–0.73. The highest similarity with the control occurred between 20 mg/L ALA, while the least similarity was deltamethrin treatment (Table S5). This result showed that deltamethrin caused a significant change induced by retrotransposon in genome. On the other hand, when the similarity indexes of ALA applications against deltamethrin application with control were evaluated, it was determined that 40 mg/L ALA application against deltamethrin had the highest similarity between these applications with control (Table S5). Furthermore, genetic similarity values were used for cluster analysis through UPGMA, resulting in a dendrogram (Fig. 6). The cophenetic correlation coefficient was calculated to evaluate goodness of dendrogram. This value was determined as 0.84. and indicated a good fit (Rholf 1993). The UPGMA analysis indicated clearly differences among treatments. The treatments were grouped into five clusters, with cluster I containing control and 20 mg/L ALA, cluster II containing 80 mg/L ALA and 80 mg/L ALA + DM, cluster III containing 40 mg/L ALA, cluster IV containing DM and Cluster V containing 20 mg/L ALA + DM and 80 mg/L ALA + DM (Fig. 6). The results of PCoA supported result from obtained cluster analysis obtained through UPGMA (Fig. 7). While there are many studies on increasing retrotransposon mobility and polymorphism with stress, there is no about deltamethrin stress in the literature. Evrensel (2011) investigated the mobility of Nikita and BARE-1 retrotransposons in barley (*Hordeum vulgare* L.) under plant tissue culture conditions using the IRAP molecular marker technique and reported that the polymorphism that occurs in callus of different ages is due to the movements of Nikita and BARE-1 retrotransposons. Yigider (2020) determined the polymorphism resulting from the movement of some retrotransposons by heavy metal stress in maize using IRAP and REMAP techniques. In our previous study where we determined the effect of deltamethrin and ALA applications on DNA methylation changes (Taspinar et al. 2017), the high level of DNA methylation polymorphism caused by deltamethrin decreased to lower values with ALA. Furthermore, a change in the GTS rate was observed at all doses of ALA in this study. This may be due to epigenetic change. Taspinar et al. (2017) indicated that that ALA caused changes in DNA methylation in *Phaseolus vulgaris*. Reinders et al. (2009) and Mirouze and Paszkowski (2011) reported that the epigenetic situation changes may promote the movement of DNA transposons and retroelements which are abundant in the plant genome.

## Conclusion

Overall, our results unequivocally establish that SOD, CAT, GPX and also SAP genes induced by the activation of the antioxidant mechanism against the oxidative damage caused by deltamethrin. In addition, it was determined that ALA caused a change in the expression of these genes when applied together with deltamethrin. Thus, an important step of the plant's response mechanism against stress has been elucidated. At the same time, the retrotransposon mobility caused by deltamethrin stress and the effect of ALA on this mobility and its polymorphism ratio were determined by using REMAP

technique. In this respect, it is thought that it is the first study conducted on this subject and the results obtained as a result of this study will lead to the cultivation of the bean plant, which has an important place in the world, in lands exposed to intense pesticide stress and to lead other studies in this field.

## Declarations

**Ethics approval and consent to participate:** Not applicable.

**Consent for publication:** Not applicable.

**Availability of data and materials:** All data generated or analysed during this study are included in this published article (and its supplementary information files).

**Competing interests:** The authors declare that they have no competing interests.

**Funding:** This study was funded by Atatürk University, Scientific Research Projects (FBA-2018-6712)

**Authors' contributions:** MA analyzed all statistic materials, EA performed the analyses, MST and GA contributed in writing manuscript. All authors read and approved the final manuscript.

**Acknowledgements:** The authors are grateful to the Atatürk University, Scientific Research Projects (FBA-2018-6712)

## References

1. Ahmad P, Jaleel CA, Salem MA, Nabi G, Sharma S (2010) Roles of enzymatic and non enzymatic antioxidants in plants during abiotic stress Crit Rev Biotechnol 30:161-175
2. Ali B, Tao QJ, Zhou YF, Gill RA, Ali S, Rafiq MT (2013) 5-Aminolevulinic acid mitigates the cadmium-induced changes in *Brassica napus* as revealed by the biochemical and ultra-structural evaluation of roots. Ecotox Environ Safe 92:271-280.
3. Ali B, Gill RA, Yang S, Gill MB, Farooq MA, Liu D, Daud MK, Ali S, Zhou W (2015) Regulation of cadmium-induced proteomic and metabolic changes by 5- aminolevulinic acid in leaves of *Brassica napus* L PLoS One 10:e0123328
4. Ansari RA, Kaur M, Ahmad F, Rahman S, Rashid H, Islam F, Raisuddin S (2009) Genotoxic and oxidative stress-inducing effects of deltamethrin in the erythrocytes of a fresh water biomarker fish species, *Channa punctata*. Bloch Environ Toxicol 24:429-436.
5. Arslan E, Agar G, Aydın M (2021) Humic acid as a biostimulant in improving drought tolerance in wheat: the expression patterns of drought-related genes. Plant Mol Biol Rep <https://doi.org/10.1007/s11105-020-01266-3>
6. Atienzar FA, Conradi M, Evenden AJ, Jha AN, Depledge MH (1999) Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability

- with key fitness parameters in *Daphnia magna* exposed to benzo [a] pyrene Environ Toxicol Chem 18:2275-2282.
7. Aylward LL, Krishnan K, Kirman CR, Nong A, Hays SM (2011) Biomonitoring equivalents for deltamethrin. Regul Toxicol Pharmacol 60:189-199.
  8. Balestrasse KB, Tomaro ML, Batlle A, Noriega GO (2010) The role of 5- aminolevulinic acid in the response to cold stress in soybean plants. Phytochemistry 71:2038-2045.
  9. Bashir F, Siddiqi MTO, Iqbal M (2007) The antioxidative response system in *Glycine max* (L) Merr exposed to deltamethrin, a synthetic pyrethroid insecticide. Environ Pollut 147:94-100.
  10. Ben-Saad R, Ben-Ramdhan W, Zouari N, Azaza J, Mieulet D, Guiderdoni E, Ellouz R, Hassairi A (2012) Marker-free transgenic durum wheat cv Karim expressing the AISAP gene exhibits a high level of tolerance to salinity and dehydration stresses. Mol Breed 30:521-533.
  11. Beyzaei Z, Averina NG, Sherbakov RA (2015) Involvement of nitrate reductase in the ameliorating effect of 5-aminolevulinic acid on NaCl-stressed barley seedlings. Acta Physiol Plant 37:1-8.
  12. Bose J, Rodrigo-Moreno A, Shabala S (2014) ROS homeostasis in halophytes in the context of salinity stress tolerance. J Exp Bot 65:1241-1257.
  13. Chauhan LK, Kumar M, Paul BN, Goel SK, Gupta SK (2007) Cytogenetic effects of commercial formulations of deltamethrin and/or isoproturon on human peripheral lymphocytes and mouse bone marrow cells. Environ Mol Mutagen 48:636-643.
  14. Duran RE, Kilic S, Coskun Y (2015) Response of maize (*Zea mays* L saccharata Sturt) to different concentration treatments of deltamethrin. Pesticide Biochem Physiol 124:15-20.
  15. Evrensel C, Yilmaz S, Temel A, Gozukirmizi N (2011) Variations in BARE-1 insertion patterns in barley callus cultures. Department of Molecular Biology and Genetics, Faculty of Scienc 10:980-987.
  16. FAO (2019) Food outlook. Biannual Report on Global Food Markets. <http://www.fao.org/3/ca6911en/ca6911en.pdf>. Accessed 22 March 2021.
  17. Forman HJ (2007) Use and abuse of exogenous H<sub>2</sub>O<sub>2</sub> in studies of signal transduction. Free Radic Biol Med 42:926-932.
  18. Giri J, Vij S, Dansana PK, Tyagi AK (2011) Rice A20/AN1 zinc-finger containing stress-associated proteins (SAP1/11) and a receptor-like cytoplasmic kinase (OsRLCK253) interact via A20 zinc-finger and confer abiotic stress tolerance in transgenic Arabidopsis plants. New Phytol 191:721-732.
  19. Gogo EO, Saidi M, Ochieng JM, Martin T, Baird V, Ngouajio M (2014) Microclimate modification and insect pest exclusion using agronet improve podyield and quality of French bean. Hortscience 49:1298-1304.
  20. Grochot-Przeczek A, Dulak J, Jozkowicz A (2012) Haem oxygenase-1: noncanonical roles in physiology and pathology. Clinical Sci 122:93-103.
  21. Hoagland DR, Arnon DI (1938) Growing plants without soil by the water-culture method. Circ Calif Agric Exp St 1938:16.

22. Johnstone GR (1984) Control of primary infections of subterranean clover red leaf virus, a luteovirus, in a broad bean crop with the synthetic pyrethroid deltamethrin Australas. *Plant Pathol* 13:55-56.
23. Kalendar R, Flavell AJ, Ellis THN, Sjakste T, Mois C, Schulman AH (2011) Analysis of plant diversity with retrotransposon-based molecular markers. *Heredity*, 106:520-530.
24. Kang M, Abdelmageed H, Lee S, Reichert A, Mysore KS, Allen RD (2013) AtMBP-1, an alternative translation product of LOS2, affects abscisic acid responses and is modulated by the E3 ubiquitin ligase AtSAP5. *Plant J* 76:481-493.
25. Kanneganti V, Gupta AK (2008) Overexpression of OsiSAP8, a member of stress associated protein (SAP) gene family of rice confers tolerance to salt, drought and cold stress in transgenic tobacco and rice. *Plant Mol Biol* 66:445-462.
26. Kishimoto K, Nishizawa Y, Tabei Y, Hibi T, Nakajima M, Akutsu K (2002) Detailed analysis of rice chitinase gene expression in transgenic cucumber plants showing different levels of disease resistance to gray mold (*Botrytis cinerea*). *Plant Sci* 162:655-662.
27. Kumar S, Bandyopadhyay U (2005) Free heme toxicity and its detoxification systems in human *Toxicol Lett* 157:175-188.
28. Liu B, Chen Y, StClair DK (2008) ROS and p53: a versatile partnership. *Free Radic Biol Med* 44:1529-1535.
29. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using realtime quantitative PCR and the 2(-Delta Delta C(T)) Method *Methods*. 2:402-408.
30. Marques A, Custodio M, Guilherme S, Gaivao I, Santos MA, Pacheco M (2014) Assessment of chromosomal damage induced by a deltamethrin-based 41 insecticide in fish (*Anguilla anguilla* L) e a follow-up study upon exposure and postexposure periods. *Pestic Biochem Phys* 113:40-46.
31. Miller GAD, Suzuki N, Ciftci-Yilmaz S, Mittler RON (2010) Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environ* 33:453-467.
32. Mirouze M, Paszkowski J (2011) Epigenetic contribution to stress adaptation in plants. *Curr Opin Plant Biol* 14:267-274.
33. Mukhopadhyay A, Vij S, Tyagi AK (2004) Overexpression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco. *Proc Natl Acad Sci USA*, 101:6309-6314.
34. Mukhopadhyay I, Siddique HR, Bajpai VK, Saxena DK, Chowdhuri DK (2006) Synthetic pyrethroid cypermethrin induced cellular damage in reproductive tissues of *Drosophila melanogaster*. Hsp70 as a marker of cellular damage. *Arch Environ Contam Toxicol* 51:673-680.
35. Naeem MS, Rasheed M, Liu D, Jin ZL, Ming DF, Yoneyama K (2011) 5- Aminolevulinic acid ameliorates salinity-induced metabolic, water-related and biochemical changes in *Brassica napus* L. *Acta Physiol Plant* 33:517-528.
36. Noriega G, Caggiano E, Lecube ML, Santa Cruz D, Batlle A, Tomaro M, Balestrasse KB (2012) The role of salicylic acid in the prevention of oxidative stress elicited by cadmium in soybean plants. *Biometals* 25:1155-1165.

37. Pospíšil P (2012) Molecular mechanisms of production and scavenging of reactive oxygen species by photosystem II. *Biochim Biophys Acta* 1817:218-231.
38. Rebeiz CA, Montazer-Zouhour A, Hopen HJ, Wu SM (1984) Photodynamic herbicides: 1 Concept and phenomenology. *Enzyme Microb Technol* 6:390-396.
39. Reinders J, Wulff BB, Mirouze M, Marí-Ordóñez A, Dapp M, Rozhon W, Paszkowski J (2009) Compromised stability of DNA methylation and transposon immobilization in mosaic *Arabidopsis* epigenomes. *Genes Develop* 23:939-950.
40. Reyter SW, Tyrrel RM (2000) The heme synthesis and degradation pathways: role in oxidant sensitivity Heme oxygenase has both pro- and antioxidant properties. *Free Rad Biol Med* 28:289-309.
41. Rohlf FJ (1993) NTSYS-pc: numerical taxonomy and multivariate analysis system, version 180. Applied Biostatistics, Setauket, New York, USA
42. Sayeed I, Parvez S, Pandey S, Bin-Hafeez B, Haque R, Raisuddin S (2003) Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. *Ecotox Environ Safe* 56:295-301.
43. Sharma I, Bhardwaj R, Pati PK (2015) Exogenous application of 28-homobrassinolide modulates the dynamics of salt and pesticides induced stress responses in an elite rice variety Pusa Basmati-1. *J Plant Growth Regul* 34:509-518.
44. Shekhawat GS, Verma K (2010) Haem oxygenase (HO): an overlooked enzyme of plant metabolism and defence. *J Exp Bot* 61:2255-2270.
45. Ströher E, Wang XJ, Roloff N, Klein P, Husemann A, Dietz KJ (2009) Redoxdependent regulation of the stress-induced zinc-finger protein SAP12 in *Arabidopsis thaliana*. *Mol Plant* 2:357-367.
46. Sun YP, Zhang ZP, Wang LJ (2009) Promotion of 5-aminolevulinic acid treatment on leaf photosynthesis is related with increase of antioxidant enzyme activity in watermelon seedlings grown under shade condition. *Photosynthetica* 47:347-354.
47. Taspinar MS, Aydin M, Arslan E, Yaprak M, Agar G (2017) 5-Aminolevulinic acid improves DNA damage and DNA Methylation changes in deltamethrin-exposed *Phaseolus vulgaris* seedlings. *Plant Physiol Biochem* 118:267-273.
48. Taspinar MS, Aydin M, Sigmaz B, Yagci S, Arslan E, Agar G (2018) Aluminum-induced changes on DNA damage, DNA methylation and LTR retrotransposon polymorphism in maize. *Arab J Sci Eng* 43:123-131.
49. Tian DQ, Pan XY, Yu YM, Wang WY, Zhang F, Ge YY, Shen XL, Shen FQ, Liu XJ (2013) De novo characterization of the *Anthurium* transcriptome and analysis of its digital gene expression under cold stress. *BMC Genom* 14:827.
50. Vij S, Tyagi AK (2006) Genome-wide analysis of the stress associated protein (SAP) gene family containing A20/AN1 zinc-finger(s) in rice and their phylogenetic relationship with *Arabidopsis* *Mol Genet Genomics* 276: 565-575.
51. Wang Z, Kuang J, Han B, Chen S, Liu A (2020) Genomic characterization and expression profiles of stress-associated proteins (SAPs) in castor bean (*Ricinus communis*) *Plant Div*

52. Woodrow P, Pontecorvo G, Fantaccione S, Fuggi A, Kafantaris I, Carillo P (2010) Polymorphism of a new Ty1-copia retrotransposon in durum wheat under salt and light stresses. *Theor Appl Genet* 121:311-322.
53. Yigider E, Taspinar MS, Aydin M, Agar G (2020) Humic acid effects on retrotransposon polymorphisms caused by zinc and iron in the maize (*Zea mays* L) genome. *Cereal Res Commun* <https://doi.org/10.1007/s42976-020-00111-3>.
54. Zhang J, Li DM, Gao Y, Yu B, Xia CX, Bai JG (2012) Pretreatment with 5-aminolevulinic acid mitigates heat stress of cucumber leaves. *Biol Plantarum* 56:780-784.
55. Zhang N, Yin Y, Liu X, Tong S, Xing J, Zhang Y, Hu Z (2017) The E3 ligase TaSAP5 alters drought stress responses by promoting the degradation of DRIP proteins. *Plant Physiol* 175:1878-1892.
56. Zhang WF, Zhang F, Raziuddin R, Gong HJ, Yang ZM, Lu L et al (2008) Effects of 5- aminolevulinic acid on oilseed rape seedling growth under herbicide toxicity stress. *J Plant Growth Regul* 27:159-169.
57. Zhang Y, Lan H, Shao Q, Wang R, Chen H, Tang H, Zhang H, Huang J (2016) An A20/AN1-type zinc finger protein modulates gibberellins and abscisic acid contents and increases sensitivity to abiotic stress in rice (*Oryza sativa*). *J Exp Bot* 67:315-326.

## Figures

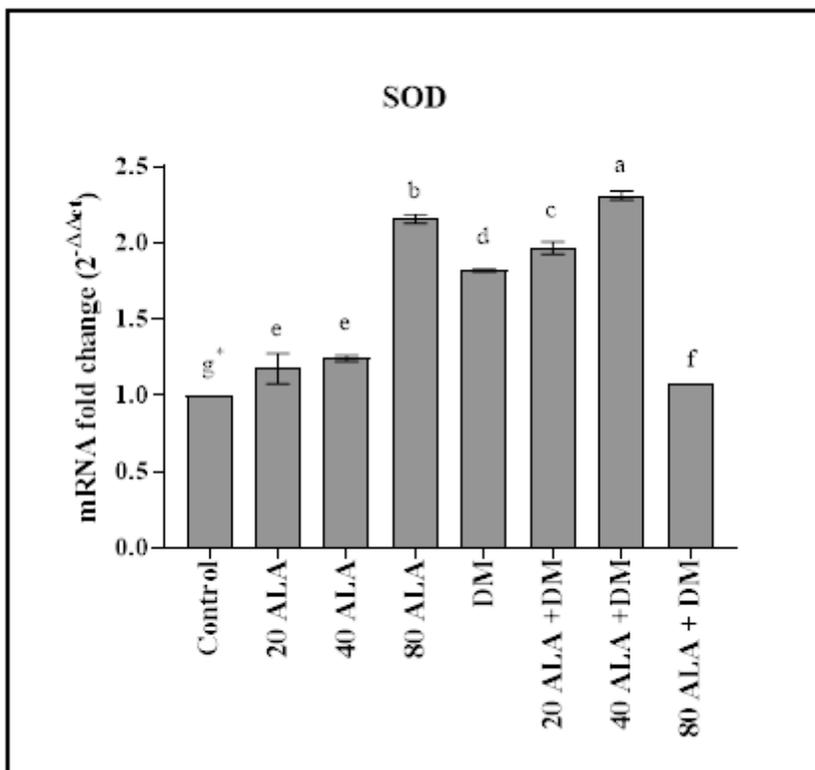
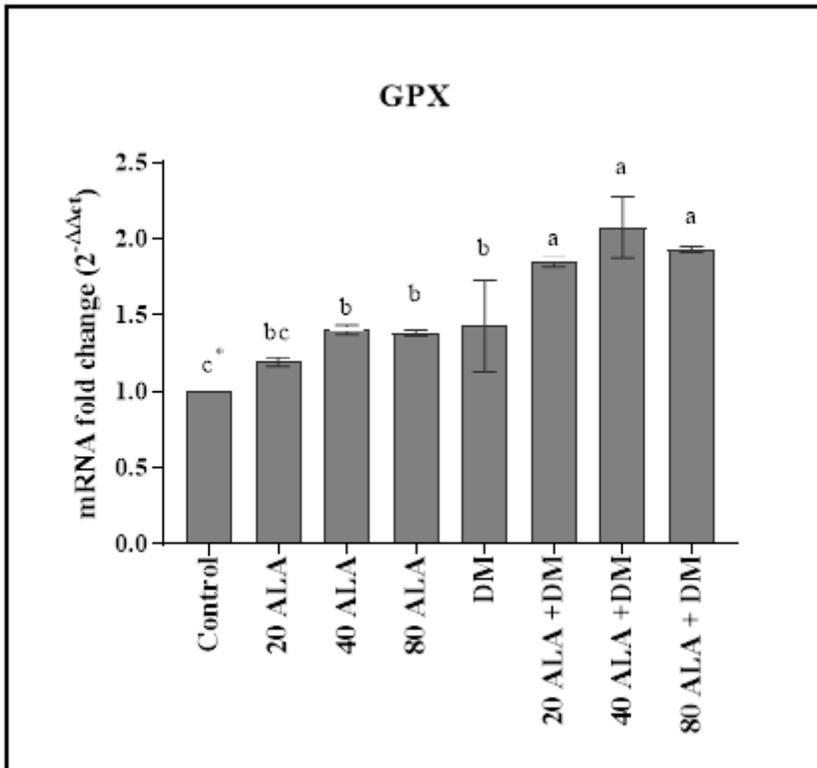


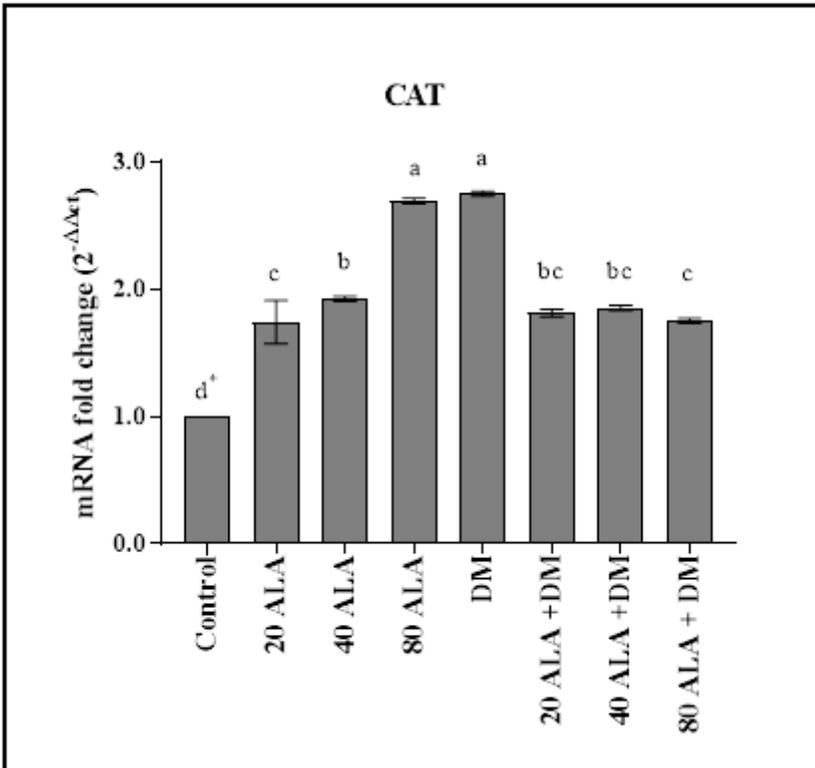
Figure 1

The expression patterns of SOD gene under ALA and deltamethrin treatments. Data represent the means  $\pm$ SD of three replications. The Different letter on the graph indicate significant differences based on Duncan's multiple range test ( $P \leq 0.05$ ).



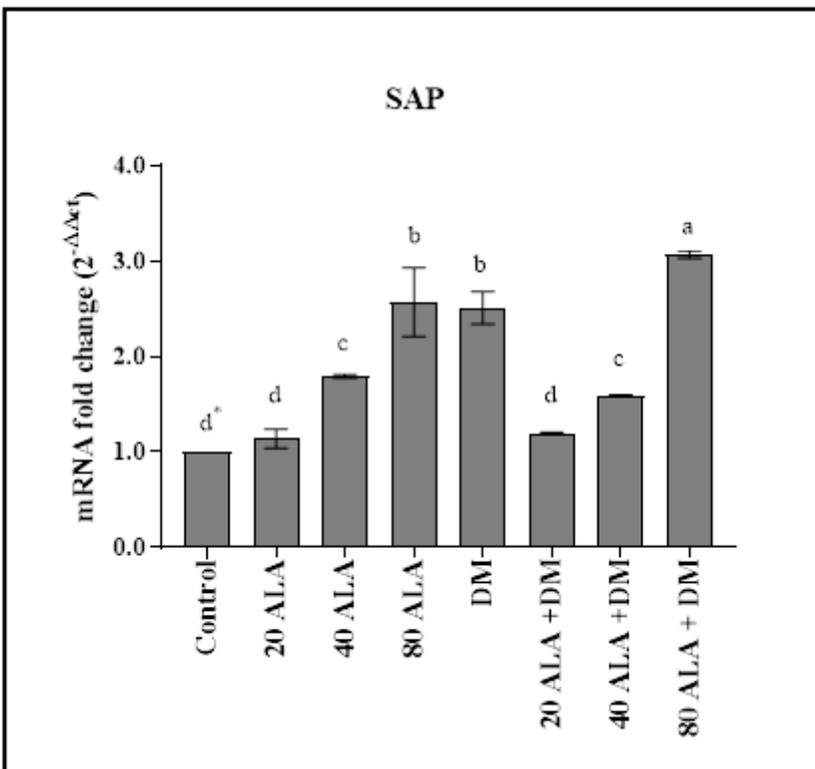
**Figure 2**

The expression patterns of GPX gene under ALA and deltamethrin treatments. Data represent the means  $\pm$ SD of three replications. The Different letter on the graph indicate significant differences based on Duncan's multiple range test ( $P \leq 0.05$ ).



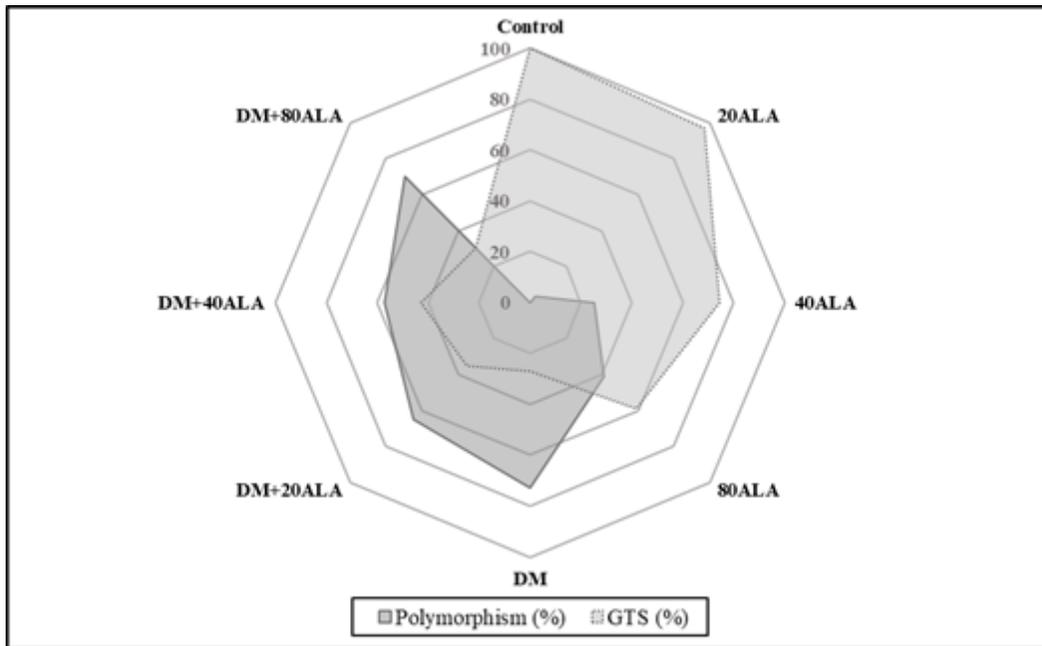
**Figure 3**

The expression patterns of CAT gene under ALA and deltamethrin treatments. Data represent the means  $\pm$ SD of three replications. The Different letter on the graph indicate significant differences based on Duncan's multiple range test ( $P \leq 0.05$ ).



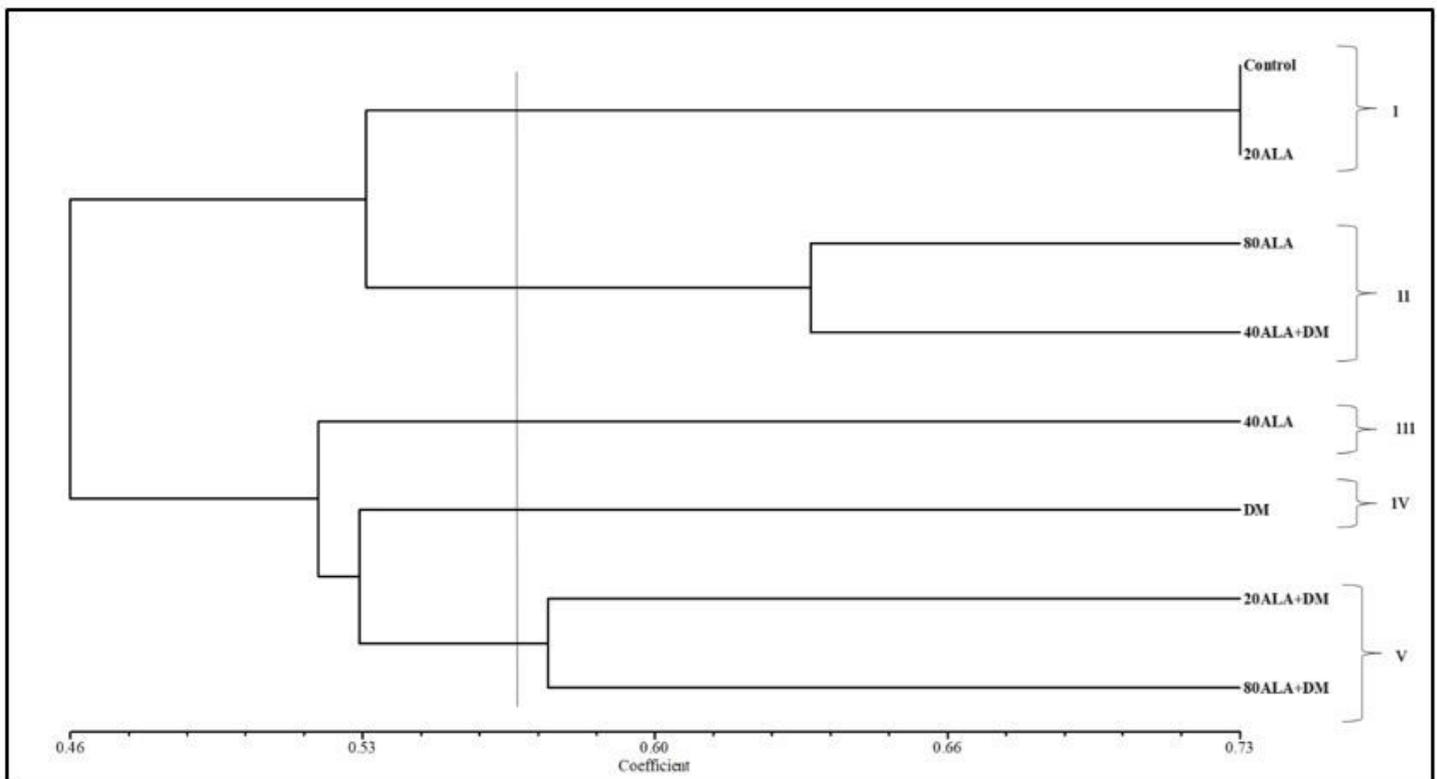
**Figure 4**

The expression patterns of SOD gene under ALA and Deltamethrin treatments. Data represent the means  $\pm$ SD of three replications. The different letter on the graph indicate significant differences based on Duncan's multiple range test ( $P \leq 0.05$ ).



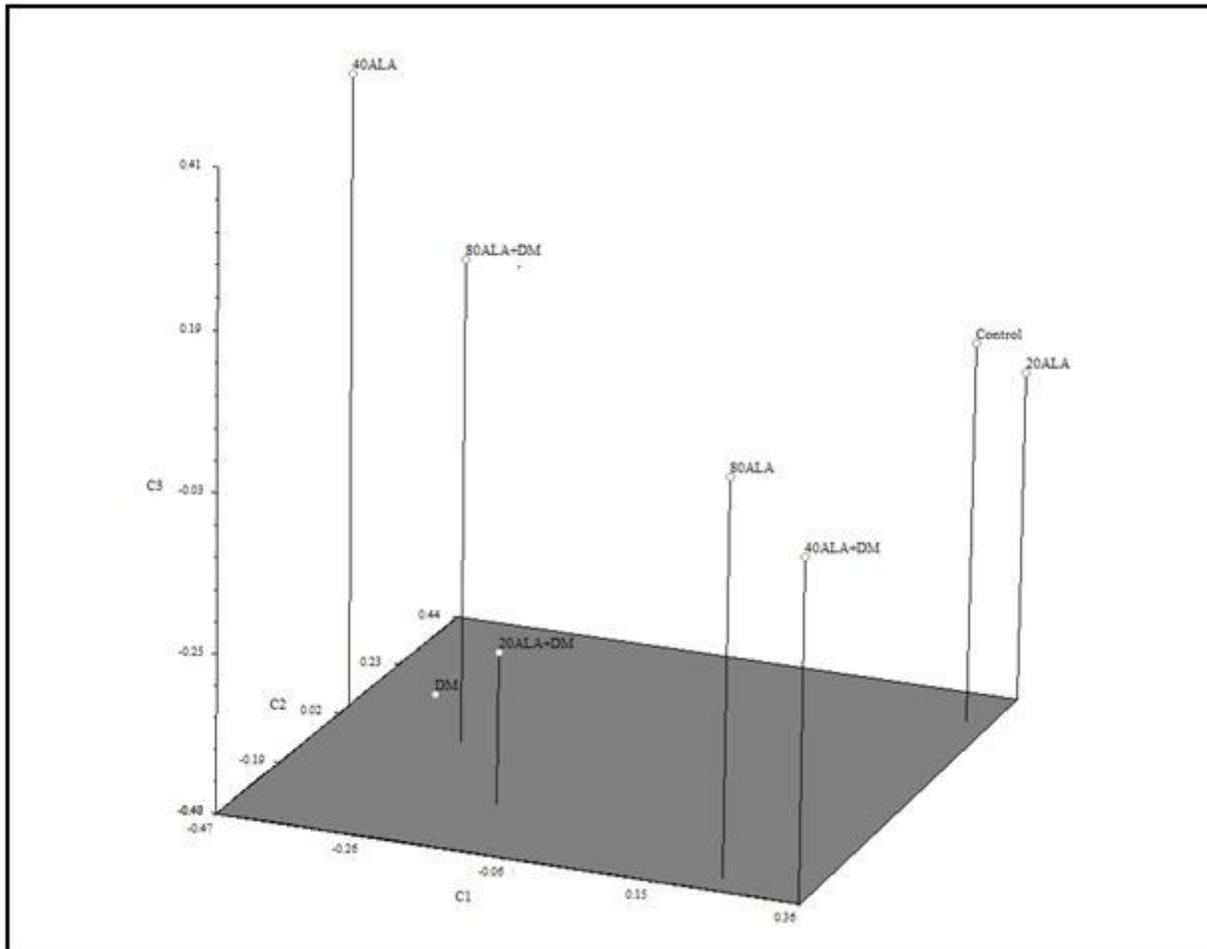
**Figure 5**

Changes in GTS and polymorphism value under ALA and deltamethrin treatments.



**Figure 6**

The dendrogram obtained from REMAP data using Jaccard's coefficients of similarity and UPGMA clustering.



**Figure 7**

Distribution of treatments by 3-dimensional principal coordinate analysis using Jaccard's similarity

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformationsFiles.docx](#)