Comparative metabolic profile of maize genotypes reveals the tolerance mechanism associated under combined stresses.

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Abstract:

Drought, heat, high temperature, waterlogging, low-N stress, and salinity are the major environmental constraints that limit plant productivity. In tropical regions maize grown during the summer rainy season, and often faces irregular rains patterns, which causes drought, and waterlogging simultaneously along with low-N stress and thus affects crop growth and development. The two maize genotypes CML49 and CML100 were subjected to combination of abiotic stresses concurrently (drought and low-N / waterlogging and low-N). Metabolic profiling of leaf and roots of two genotypes was completed using GC-MS technique. The aim of study to reveal the differential response of metabolites in two maize genotypes under combination of stresses and to understand the tolerance mechanism. The results of un-targeted metabolites analysis show, the accumulated metabolites of tolerant genotype (CML49) in response to combined abiotic stresses were related to defense, antioxidants, signaling and some metabolites indirectly involved in nitrogen restoration of the maize plant. Alternatively, some metabolites of sensitive genotype (CML100) were regulated in response to defense, while other metabolites were involved in membrane disruption and also as the signaling antagonist. Therefore, the present study provides insight into the molecular mechanisms of tolerance under various stresses of maize plants, that governed on the regulation of cell wall remodeling, maintain metabolic homeostasis, defense against the pathogen, proper signaling, and restore growth under stress conditions.

Keywords: Combine stresses; GCMS; Metabolomics; drought; low-N stress; waterlogging stress; and Tolerant. Vs Sensitive

Abbrevations:

GC-MS- Gas chromatography mass spectrometry

Low-N- Low nitrogen stress

DAS- Days after sowing

PCA-Principal component analysis

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1. Introduction

Crop faces various stresses in their life span including abiotic and biotic. Several abiotic stresses that affect crop growth, and development are drought, waterlogging, low nutrient availability, salinity, high temperature and heat. The predicted changes in the global climate has accentuate in the frequency and intensity of abiotic factors, most of the abiotic stresses occur in combination, for instant, in tropical area's terminal drought and waterlogging occurs simultaneously during summer-rainy season. Although on many occasions the stresses are for shorter duration and relatively mild in nature, but have a significant impact on plant growth and development. Therefore, co-occurrence of abiotic stresses are more destructive to crops than their separate occurrence at different crop growth stages **(Mittler 2006; Prasad et al 2011)**. Besides, the composite responses of plants due to various concurrent stresses rely on signals, these varied and contrast signals may interact with each other to enhanced or obstruct one another **(Vile et al. 2012; Suzuki et al. 2014)**. Maize is the most important cereal, grown at the wide geographical ranges of latitude and longitude. In South Asia, particularly in tropical and subtropical environment maize is largely grown during summer-rainy season, in marginal areas which often faces extreme water availability such as waterlogging or water scarcity in a form of terminal drought. Correspondingly, co-occurrence of both the stresses often headed to the depletion in nitrogen content, thus causing low-N stress in the soil. However, studies on combined stresses by **Rizhsky et al (2004)** revealed that the response of plant to combined stresses is unique and cannot be extrapolated from the response of plant to each individual stress. Though, plants belonging to the same genus showed different molecular response in combined stresses **(Aprile et al 2013).** Therefore, the nature of the interaction is an important aspect to fully identify the influences of combined abiotic stresses on crop plants. Among omics approach is metabolomics that has been neglected in crop improvement programs **(Kumar et al 2017).** It has not be fully explored due to its complexity. Though, metabolites plays important role in plant metabolism that influences the plant biomass and architecture **(Turner et al 2016).** Metabolomics study enable to identify wide variety of metabolites that gives the comprehensive view of the cellular process and physiological condition that display in a particular stress condition. Besides, the metabolites assign the functional gene and impact of the particular gene on the metabolic pathway, and that provides information of regulation as well interruptions of linked pathways **(Wen et al 2015).** Further, metabolomics study enable to improve breeding resources through selecting the remarkable traits **(Zivy et al 2015).** Therefore, the aim of metabolomics profiling is to compare the combined stress response in two maize inbred that differed in their stress adaptability and to unrevealed the complex molecular mechanism of tolerance at metabolomics levels in different stress conditions applied simultaneously.

2. Materials and Methods

Plant Material and Growth conditions

In the present work two maize inbred (CML49 & CML100) were selected that showed different adaptability to various combine stress treatments on this basis we have categorized them as 'tolerant and sensitive.' The selected two inbred (CML49 and CML100) plants with 60 pots each were grown in natural conditions in greenhouse up to 30 days. 30 DAS they were subjected to various stress treatment, the plants were grown in low nitrogen, only 25% of the recommended dose of urea was applied to the treated pots. First 30 pots of each inbred were exposed to a drought stress for 10 days, while 30 control pots were supplied with full nutrient and water. After re-watering for two days normally, the same plants were exposed to waterlogging stress for up to 7 days, to maintain the water level 2-3cm above the soil surface of the pots plants were watered day and night. The roots samples from stressed and control replicates plants of each inbred (CML49 & CML100) were kept on the last day of combined stresses for extracting metabolites. Samples were quickly frozen in liquid nitrogen after removing from the plant and then kept at -80^0 C for further analysis.

Sample Preparations and Metabolites extraction

The leaves and roots samples from three independent biological replicates each from control and treated maize plants (CML49 & CML100) was used for metabolite profiling. Harvested plant samples immediately frozen in liquid Nitrogen in a plastic bag (resistant to liquid N_2). Frozen samples were stored at -80^{0}C. The leaves and roots were homogenized separately with liquid Nitrogen in a mortar and pestle into fine powder. One gram powder of each sample was extracted three times with 5 ml of methanol at room temperature and sonicated for 15 min and centrifuged. The extract was concentrated to 1 ml by Speed Vac. The supernatant filter through Whatman filter paper (No. 4), extracts collected and stored at 4 ^{0}C for further use. The prepared extracts of both leaves and roots of the control and treated plants were subjected to gas chromatography–mass spectroscopy (GC–MS) analysis. Samples were analyzed using GC-MS for the analysis of untargeted metabolites. For derivatization, methoxylamine in pyridine (10µl) and *N*-methyl-*N*-trimethylsilyltrifuoroacetamide MSTFA (90µl) were added.

Gas Chromatography and Mass spectrometry

The GC-MS analysis was performed with a GC-MS QP-2010 ULTRA equipped with an auto sampler (AOC-20i + s) from Shimadzu (Japan), using Equity-5 column, 30.0 m × 0.25 um × 0.25 mm for separation, and helium was used as a carrier gas at a constant flow rate of 1.0 mL/min. The 1µL sample was injected using 1/100 split-mode injections at a temperature of 260°C. The oven temperature program was initially set at 100°C and held for 2 min. The temperature was gradually increased to 250°C at a rate of 10°C/min, and 300°C at 15°C/min. Ions were generated by a 70ev electron beam at an ionization current of 2.0mA. Total ion chromatogram spectra were recorded in the mass range of 40–900 m/z at the rate of 2.5 spectra s^{-1}. For metabolite identification and annotation, peaks were matched against customized reference spectrum databases including the National Institute of Standards and Technology NIST and the Wiley Registry, internal libraries and

further confirmed with ChEBI (http://www.ebi.ac.uk/chebi/init.do) and ChemSpider (http://www.chemspider.com/). Data obtained was then uploaded to the web-based tool Metabo Analyst for high throughput analysis.

3. Results

Comparison of metabolite levels of two inbred in combined abiotic stress condition

Metabolomics profiling using GC-MS was conducted of tolerant (CML49) and sensitive (CML100) genotypes under various combined stresses. Total 132 compounds were identified in two genotypes that includes, fatty acids, (long, medium and short chains), fatty acid derivatives, fatty aldehydes, fatty alcohols, carboxylic acids, terpenoids, glucosinolates, heterocyclic compounds, halohydrogens, alkaloids, phenolic acids, steroids and vitamins. Univariate analysis of two genotypes (CML49 and CML100) was performed by fold change analysis, t-test and volcano plots. Fold change showed the comparison between the control group metabolites with that of treated group metabolites and important features selected by t-test with threshold ($p<0.05$) level, the results was plotted in log 2 scale. Thus, same fold change have the same distance from zero base line**.** The tolerant and (CML49) and sensitive (CML100) genotypes were subjected to drought, waterlogging and low-N stresses. Tolerant genotypes contains saturated fatty acids, unsaturated fatty acids, fatty alcohols, various terpenoids, carboxylic acids, methyl esters, halocarbons, heterocyclic compounds, Strikingly, in both genotypes an overlap of Furan's, Tiglic acid and some antioxidant compounds Tocopherol acetate, squalene and Isophytol was observed in leaves and roots. Sensitive genotype leaves shows higher accumulation of saturated and unsaturated fatty acids mostly, fatty alcohols, fatty anhydride and higher alkanes level was presented in greater amount **(Table 1)**.

Table 1. Metabolic profile of leaf and roots of tolerant and sensitive maize plants after exposure to combined abiotic stresses.

TOLERANT GENOTYPE (CML49)			SENSITIVE GENOTYPE (CML100)		
Metabolites	Class	Fold Change	Metabolites	Class	Fold Change
LEAVES Metabolites					
			LEAVES Metabolites		
Octanoic acid	Saturated F A	-2.003 ns	Nonyl Iodide	Liner alkane	-2.0774**
Erythrodiol	Triterpenoid	-1.8924 ns	Docosane	Linear Alkane	-1.59**
9-Decen-1-Ol	Fatty Alcohol	-1.9768 ns	Nonayl Bromide	Organic compound	-1.4869**
Oleic acid	Saturated F A	-1.3913**	Isophytol	Terpenoid	-1.3326**
Linalool	Monoterpenoid	-1.1993 ns	Citronellyl acetate	Fatty alco ester	-1.3162**
N-Pentadecane	Alkane hydrocarbon	-1.0197***	Tocopherol acetate	Steroid	-1.2953**
ROOTS Metabolites			Carvon	Terpenoid	-1.2777**
Furan carboxylic acid	CA	-1.4828**	Heptyl hexyl ether	Ether	-1.2772**
Acetic acid	carboxylic acid (CA)	-1.4511**	Octadecanal	long chain aldehyde	-1.2726**
Pelargonic acid	Saturated F A	-1.4303**	Napthalene acid	Organic compound	-1.2717**
Oxalic acid	CA	-1.4271**	Squalene	Steroid	-1.2623**
Caprylic acid	saturated F A	-1.4134**	Phthalate	Ester	1.0616 ns

The metabolomics profile of two different genotypes suggested that the response of maize plants to various stresses applied simultaneously was shared as well as also distinctive. However, tolerant genotype roots metabolite profile display large variance in metabolites accumulation, (mostly are halohydrocarbons, heterocyclic compounds, organic cations and saturated and unsaturated fatty acids, carboxylic acids, esters) compared to roots of sensitive genotype which have less diversity in metabolite accumulation. To reduce the multivariate data complexity, to highlight the similarities and difference patterns between samples, PCA was performed for metabolites concentration data of control and treated leaves and roots of tolerant and in sensitive plants. Also, to verify the difference between the metabolic profiles of the control and treated tissues statistically and to identify the main

metabolites responsible for the differences. A score scatter plots of tolerant leaf showed the good separation of metabolites between treated and control plants. In PC1 the variance is greater (70.1%) and PC2 showed variance of (19.3%). Similar trend of metabolites separation was observed of sensitive genotype leaves. The treated plants exhibited higher variances in PC1 (91.2%) and control plants variance is less PC2 (5.2%) **Fig 1a, b.**

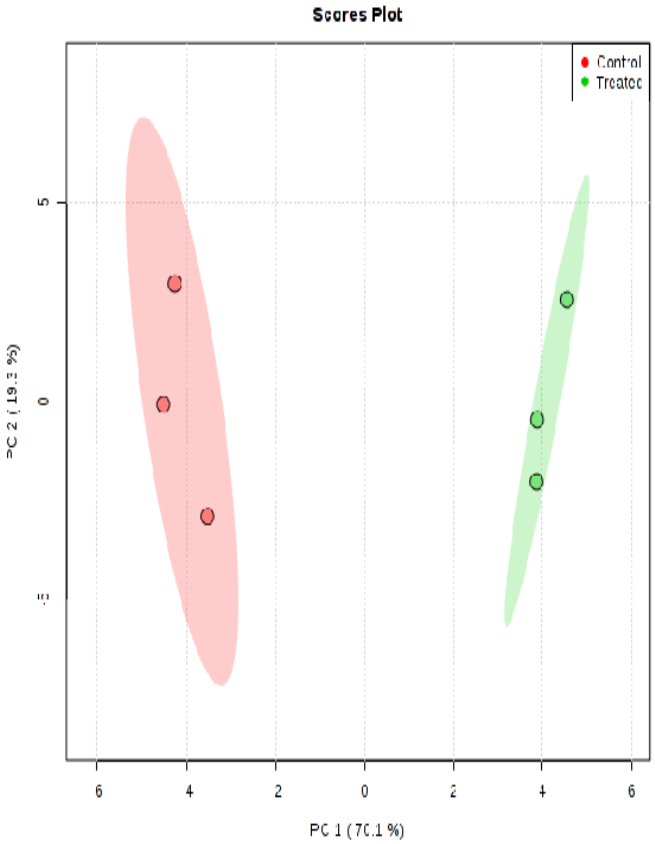

Fig 1a. Score plot of tolerant leaf shows higher variations in metabolites of PC1 than PC2

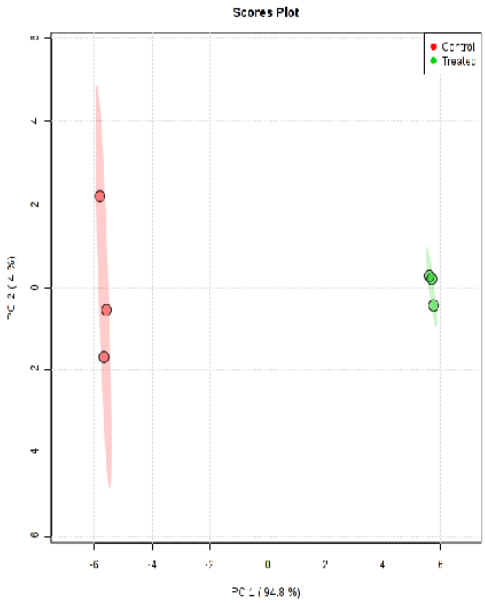

Fig 1b. Score plot of Sensitive leaf showing variations in metabolites of control and treated leaf. PC1 have higher variation than PC2.

However PCA of roots the trend was opposite for both genotypes, the score plot for PC1 (94.8%) and PC2 (4%) demonstrate clear separation between control (root metabolites) and treated (root metabolites) it may be due to the presence of many saturated and unsaturated fatty acids and halo-hydrocarbon in the samples. Correspondingly, sensitive (CML100) genotype PC1 (94%) and PC2 (5.3%) **Fig 2a, b**, control root metabolites were well separated and treated roots metabolites patterns shows less variance in separation because presence of similar metabolites like, 2-Furan carboxylic acid and Furan anhydride.

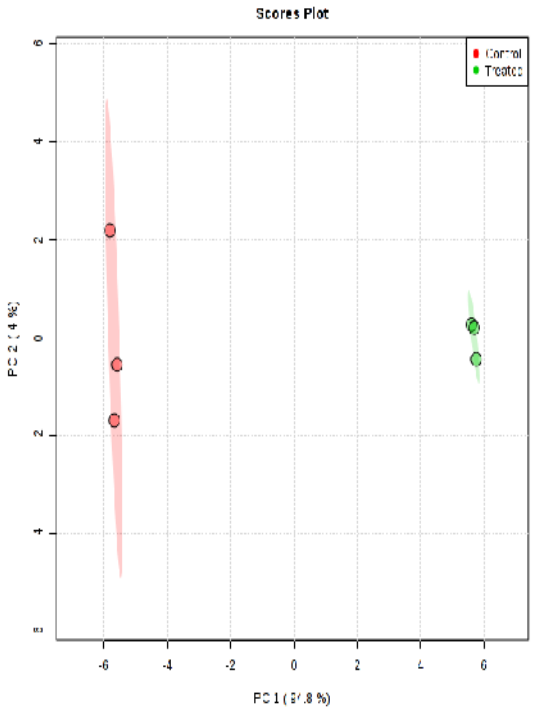

Fig 2a. Score plot of tolerant roots. Control roots shows larger variation in metabolites compared to treated roots

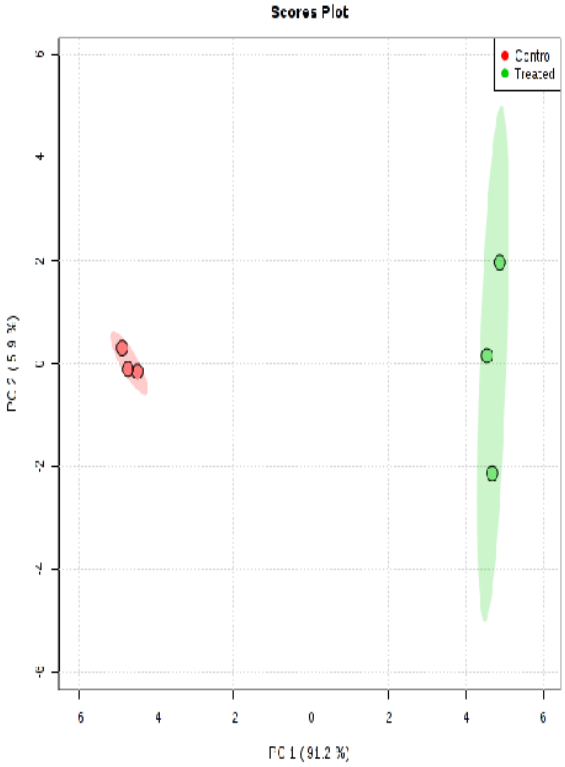

Fig 2 b. Score plot of sensitive roots. Control roots shows less diversity in metabolites compare to treated roots.

The combine data of tolerant genotype of leaf and roots shows higher fold change in octanoic acid, that are involved in fatty acid biosynthesis, 9-Decen-1-ol related to alcohol dehydrogenase and Erythrodiol an antioxidant. In roots, fold change was high of carboxylic acids (Pelargonic acid, Valeric acid, oxalic acid, sebacic acid, acetic acid) most of them have unpleasant odor might plays defensive role against various biotic stresses. Therefore it seems that metabolites of tolerant genotype are more uniform, shows coherent pattern. While, sensitive genotype leaves fold change was higher in Compounds like Iodononane, Nonyl Bromide, Docosane, Isophytol, Citronellyl, Tocopherol acetate, Carvon, Heptyl hexyl ether, 1-Napthalene acetic acid, Squalene, Phthalate. Most of them organic compound, aldehydes and terpenoids with unpleasant odor. Also Octodecanal (Stearaldehyde) which is pheromone. Whereas, roots of sensitive genotype contains Tiglic acid, Furan carboxylic acid, Valeric acid, Furan anhydride, Butyl glyoxalate, are carboxylic acids that are highly volatile and toxic secreted in response to various insects, bacteria. Caryophyllene is sesquiterpine, non-steroidal help to attracts insects. However, metabolites such as Nitropropane, 2-Nonane have mild fruity, sweet odor may help to attract insects that help in pollination. Hence sensitive genotypes in response to multiple stress release various metabolites with random pattern.

Comparative metabolomics analyses of two combined stresses response have highlighted a number of metabolites involved in diverse metabolic pathways. The Pathway analysis of the tolerant leaf and roots showed biosynthesis of unsaturated fatty acids, Glyoxalate and dicarboxylate metabolism, linoleic metabolism, fatty acid metabolism, Nicotinate and nicotinamide metabolism, Sulfur metabolism, pyruvate metabolism, and Glycolysis and gluconeogenesis metabolism. However, the pathway impact is highest of Nicotinate and Nicotinamide metabolism, sulfur metabolism and Pyruvate metabolism. Whereas, Sensitive genotype leaf and roots the number of pathways are more compared to tolerant genotype. Mainly the impact was high of the following pathways, Steroid biosynthesis, Sulfur metabolism, Panthothenate and Co-enzyme biosynthesis, Sesquiterpine and terpenoids biosynthesis, Arginine and Proline metabolism, Valine, leucine and isoleucine degradation, While, Aminoacyl t-RNA synthesis, Glucosinolate biosynthesis and Valine, Lucin and Isoleucine synthesis had no impact **(Supplemetary Table S1)**,.

Heat map and correlation studies of metabolites

The heat map leaf and roots metabolites of tolerant genotype shows unique pattern. Although, the leaf metabolites of the tolerant genotypes differed with root metabolites. Almost all metabolites tolerant genotype leaf have higher concentration (=1), and some metabolites of treated leaf have concentrations above one (>1) like, Octanoic acid, 9-Decen1-ol, Erythediol. The concentration >1 of the following metabolites 1,16 dichloro hexadecane, 1-Pentanol, Eicosternoic acid, Lenolenic acid and ethyl Nonane. On the contrary, treated roots metabolites have very high concentrations the same metabolites in control roots shows v low concentration. Therefore the roots and leaf metabolites have no correlation with each other **Fig 3.**

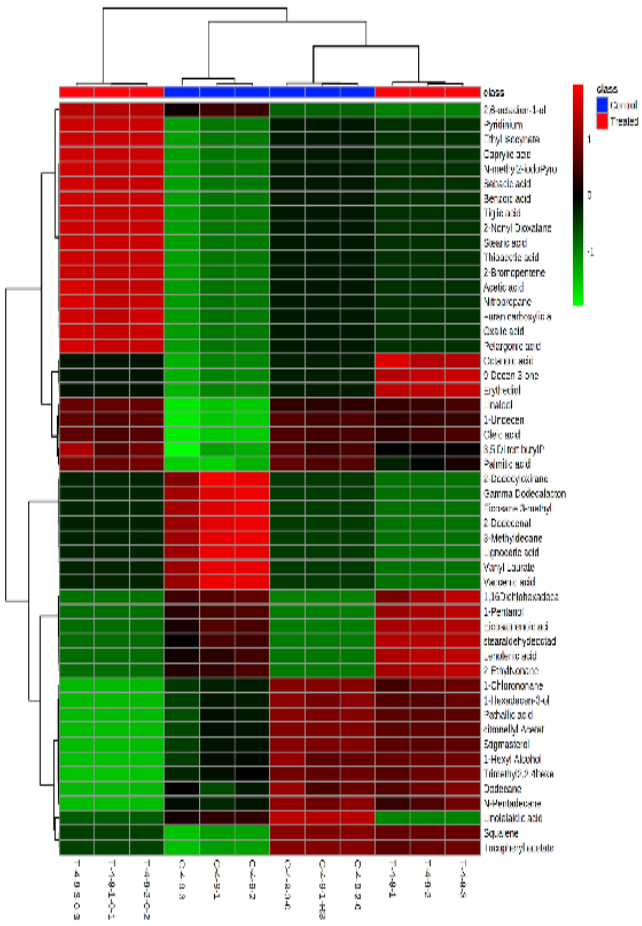

Fig 3. Heat map and Hierarchical clustering showing correlation between leaf and roots metabolites of tolerant genotype in response to combined abiotic stresses. The Red colors scale represents the high and Green - low concentrations of metabolites in control and treated plants.

Whereas, leaf and roots metabolites of sensitive genotype categorized into low and high concentrations **Fig 4.**

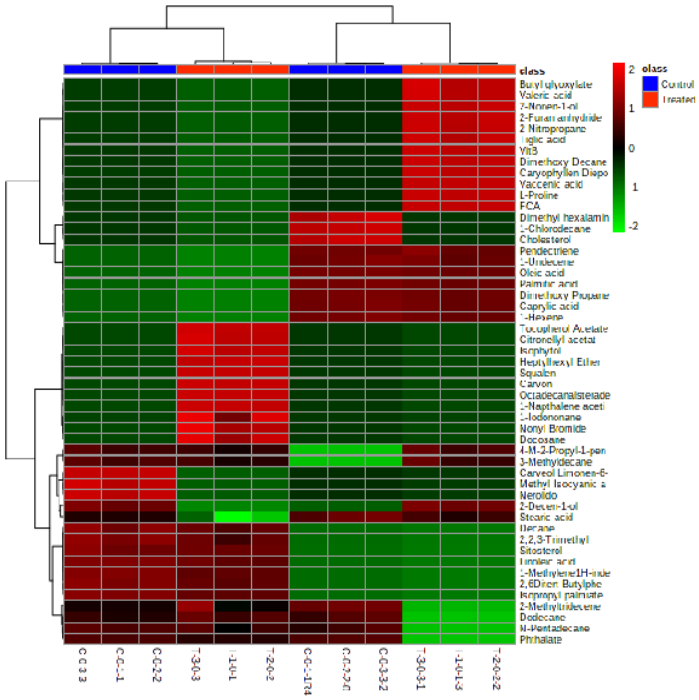

Fig 4. Heat map and Hierarchical clustering displays correlation between roots and leaf of sensitive genotype in response to combined abiotic stresses in control and treated. Red represent the –high concentration of metabolites and green represent the low concentration of metabolites.

Some of the metabolites of control roots are correlated with control and treated leaf (Dodecane, N-Pentadecane and Pthalate) shows higher concentration. Besides, Pentadectrine, 1-Undecen, oleic acid, palmatic acid, Dimethoxy propane, caprylic acid and 1-Hexene are common metabolites in control and treated roots with high concentrations. Therefore, in sensitive genotypes the leaf, roots metabolites are co-related or common. Thus there was less variations of metabolites in sensitive genotypes.

Data analysis

The GC-MS data was analyzed using MetaboAnalyst (www.metaboanalyst.ca) web server. Peak areas of identified metabolites in triplicate in a form of comma separated excel file (.CVS format) were uploaded in this software. The software, check the data integrity of the uploaded files automatically. MetaboAnalyst replaces zero values with a small positive value (the half of the minimum positive number detected in the data). Data filtering, and data normalization was done by pooling controlled groups. The internal data structure was transformed to a table with each row represents a tissue sample (root, leaf) from tolerant genotype and sensitive genotypes and each column representing a feature (a metabolite). The data structure in this format, we performed a row-wise data normalization. The purpose of Row-wise normalization was to normalize each sample (row) so that they are comparable to each other. In Metaboanalyst, correlation analysis shows the

correlation between different features. Further, hierarchical clustering analysis was done with the squared Euclidean distance and the average linkage method (between groups).The clustering result in the form of a heatmap. The values of the metabolites mean abundances were used to cluster the metabolomic data.

4. Discussion

Role of tolerant genotype metabolites in multiple stresses

In the present work untargeted metabolites were quantified by GCMS technique and differentially regulated metabolites of tolerant (CML49) and sensitive (CML100) were compared with their respective controls. The major observation was differences in metabolite levels in treated versus untreated plants of both genotype, though less metabolites differed in treated leaves and roots of sensitive genotype comparative tolerant one respectively, these variations caused by simultaneous exposure of genotypes to various stresses and it depend on plants response also. The leaves metabolites of tolerant genotype shows fold increase in terpenoids linalool (monoterpen) and erythrodiol (triterpein) the main role of isoprenoid family have been associated in protection against oxidative and other abiotic stresses, especially volatile isoprenoids (Monoterpen) protective in photosynthetic processes during thermal and oxidative stresses (**Claudia Vikers et al. 2009**). Its role in membrane stabilization **(Sharkey and Singsaas 1995)** a hypothesis supports terpenes behave directly as an antioxidant, scavenges ROS by reactions through the conjugated double bond system **(Loretu and Velikova 2001; Affeck and Yakir 2002)**. Similarly, Erythrodiol is a pentacyclic triterpenes, leaf surface contains triterpenoids as constituents of waxes being involved in different roles such as maintenance of leaves structure, provide water, permeability, and plant insect interactions **(Bauer et al 2004; Mintz-Oron et al 2008; Wilson et al 2008).** Correspondingly, non-volatile isoprenoid like Tocopherol acts as lipid soluble redox buffer and important scavenger of singlet oxygen species **(Foyer et al 2005)**. Also function as an antioxidants, in transgenic tobacco leaves its deficiency led to an enhancement in the lipid peroxidation **(Abbasi et al 2009).** Majority of the metabolites accumulated in roots of tolerant genotype were belongs to saturated and unsaturated fatty acids, FAs and their derivatives directly involved in providing resistance against the bacterial (C16) and fungal pathogen (18:2 and 18:3) hence responsible for plants basal immunity **Kachroo and Kachroo (2009)**. Moreover, indirect and direct role of 18:0, 18:1, 18:2, and 18:3 plant defense signaling. In parsley, fungal infection rapidly induces the transcription of the 18:3-synthesizing omega-3 FA **(Kirsch et al 1997)**. Significant role of Oleic acid as a stimulator of the signaling enzyme phospholipase D (PLD) that function in anti-death cell **(Zhang et al. 2003).** However, free fatty acids not only regulate abiotic stress tolerance, but also involve defenses against wound-induced responses and insect/herbivore feedings **(Upchurch 2008)**. In our studies, most of the metabolites of tolerant were involved in Phosphotidiyal and sphingolipid metabolism. Further work of **Yozo Okazaki and Kazuki Saito (2014)** confirms that membrane lipids has specific signal mediator and sensor of various biotic and abiotic stresses. Hence activating defense reactions at

transcriptional level. As the lipids are stress mitigators, presence of polyunsaturated fatty acids that reduced cell damage expose to stress by scavenging reactive oxygen species. Therefore reducing the intensity of environmental stresses **(Mene-Saffrane et al 2009).** Also, there are reports on membrane lipid remodeling in response to various stresses like, freezing, dehydration and nutrition depletion **(Moellering et al 2010; Gasulla et al 2013; Okazaki et al 2013b)**, and this remodeling contributes to the uphold membrane integrity and membrane- bound protein functions. While, Benzoic acid related to salicylic acid, (phenolic phytohormone) involve as an endogenous signal mediating plant defense responses against pathogens. Besides, it influence responses to abiotic stresses and related to aspects of plant growth and development **(Vlot et al 2009; Rivas-San Vicente and Plasencia 2011)**. The presence of haloalkanes (2-Bromopentene, ethyl isocynates) heterocyclic compound (2-Nonyl-1,3-dioxalane), hetrocyclic aromatic compounds (N-Methyl-2-Iodopyrrol), Methyl ester (Thioacetic acid) and Methyl ketone (3-Nitropropane) all these compound processes strong, unpleasant and odor may involve in repelling herbivory insects, pest and bacteria, fungi, nematodes (**Lamberth et al 2012; Francis et al 2013).** The role of low molecular weight heterocyclic compounds derivatives of pyrimidine, pyrazole and isoflavones as a stimulators of seed germination and growth of maize seedlings **(Tsygankova et al 2016).** Likewise, N-methyl-Δ-^{1}Pyrrolinium is a cation combined with nicotinic acid to forms the alkaloid (S)-nicotine in roots of tobacco plant **(Katoh et al. 2005)** that inhibits a wide variety of herbivores **(Rosenthal G A 1986)**. *Trichoderma harzianum*, used as biocontrol agent, it produced Nonanoic acid (NA) that was very inhibitory to spore germination and mycelial growth of two cacao pathogens, Crinipellis perniciosa Stahel and Moniliophthora roreri Cif **(Aneja et al 2005).** The antibiotic effect of acetic acid on some bacteria, including *Salmonella typhimurium*, *Escherichia coli* O157, and *Listeria monocytogenes* **(Delaquis et al 1999).** The soluble oxalic acid is a strong acid, besides being toxic and lethal to grazing animals **(Von Burg 1994)**. It showed inhibitory effects on sucking insects' planthopper and aphids **(Yoshihara et al 1980; Massonie 1980).** Therefore we concluded that most of the roots metabolites were involved in providing stability to membranes and plant defense.

Role of sensitive genotypes metabolites in multiple stresses

In sensitive leaf and root shows the accumulation of various metabolites, belongs to different classes, like terpenoids, Steroids, Ether lipids, Hydroquinone lipids, fatty aldehyde, fatty alcohol ester, fatty acyl conjugates, cyclic anhydrides, carboxylate, and linear alkanes. The leaves shows the emission of main terpenoids limnonen-oxide (carvon) and citronell. They are allelopathic in nature thus restricting seed germination and growth of nearby plants **(Maffei and Gertsch 2011).** Moreover, plants emitted volatiles, also perceive or recognize them in inter- and intra-plant communications, so that plants under herbivore attack can alert neighbouring plant species, and priming their chemical defenses **(Heil 2014).** The below ground emission of volatiles is an efficient trait: in maize roots the sesquiterpene (E)-β-caryophyllene is necessary to attract entomopathogenic

nematodes to roots damaged by the ferocious maize pest Diabrotica virgifera. Maize varieties that lack this signal have been shown to be far more vulnerable to maize pest **(Rasmann et al 2005; Rasmann and Turlings 2007).** However, there were some reports, that indicate due to lipophilic nature of monoterpenes the physical arrangement of phospholipids in the membranes were distorted and thus affect membrane functioning **(Zunino and Zygadlo 2004)**. Further, **Kaur et al (2010)** showed that citronellol generates ROS that induced lipid peroxidation, leakage of membrane and altered the membrane structure and permeability in wheat. Markedly as reported **Takshak and Agarwal (2015)** in UV-B treated leaf of *W somnifera* accumulated higher amount of phyto-constituents like Isophytol, trans-squalene, nonacosane and these plants were effective in scavenging free radicals. Furan fatty acids and their role in plants have been reviewed in detail **(Mowlong et al 2014)**. F-acids are mainly bound to phospholipids, they substitute for PUFAs and their strong capability (F-acids) to serve as radical scavengers suggests that plants use them to defend against oxidative stress **(Spiteller 2005).** Moreover, the insecticidal activity of some 2-carboxylbenzofurans and their coumarin precursors against sweet potato weevil, *Cylas formicarius elegantulus* first demonstrated by **Williams et al (1994)** and later insecticidal properties of Benzofuran 2-carboxylic acid derivatives was reported **Jackson et al (1998).** Water deficit led to produced various compounds in plants **Carmo-Silva et al (2009)** observe that in C4 grasses the amount of 5-HNV(2-amino-5- hydroxypentanoic acid (5-hydroxynorvaline, 5-HNV) in leaves of, C. dactylon and Z. japonica, increased under water deficit condition. However, any benefits from this unusual non-protein amino acid for drought resistance should be further explored to see that if it plays an important role in stress defensive mechanisms.

5 Conclusion

The metabolomics study of leaf and roots in response to combined abiotic stresses of two maize genotypes tolerant and sensitive showed the varied metabolic profile. Metabolomic profile of tolerant genotype have uniform pattern mostly involved in the biosynthesis of fatty acid metabolism, biosynthesis of saturating and unsaturated fatty acids, sulfur metabolism, thereby, regulated signaling, re-fix soil nitrate metabolism and defense against the wide variety of pathogens. Therefore, enhancing the tolerant mechanism of the genotype. In contrast, sensitive plant metabolites mostly involved in leaking or disrupting the membranes that disrupt signaling between various organelles and also for various stresses, defense related metabolites were accumulated in random pattern. either defense against the particular pathogens or pollination attracters. In present roots metabolomics study we can say, the metabolites somehow help to survive the sensitive genotype in multiple stress conditions but most of them are the causes of susceptibility. On the basis of the above observation, we can conclude that tolerant genotype plants by remodeling of the cell wall, maintain metabolic homeostasis, and proper signaling thus able to tolerate multiple stress conditions. Therefore, the study provides a comprehensive analysis of tolerance mechanism.

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Table 1. Metabolic profile of leaf and roots of tolerant and sensitive maize plants after exposure to combined abiotic stresses.

TOLERANT GENOTYPE (CML49)			SENSITIVE GENOTYPE (CML100)		
Metabolites	Class	Fold Change	Metabolites	Class	Fold Change
LEAVES Metabolites			LEAVES Metabolites		
Octanoic acid	Saturated F A	-2.003 ns	Nonyl Iodide	Liner alkane	-2.0774**
Erythrodiol	Triterpenoid	-1.8924 ns	Docosane	Linear Alkane	-1.59**
9-Decen-1-Ol	Fatty Alcohol	-1.9768 ns	Nonayl Bromide	Organic compound	-1.4869**
Oleic acid	Saturated F A	-1.3913**	Isophytol	Terpenoid	-1.3326**
Linalool	Monoterpenoid	-1.1993 ns	Citronellyl acetate	Fatty alco ester	-1.3162**
N-Pentadecane	Alkane hydrocarbon	-1.0197***	Tocopherol acetate	Steroid	-1.2953**
ROOTS Metabolites			Carvon	Terpenoid	-1.2777**
Furan carboxylic acid	CA	-1.4828**	Heptyl hexyl ether	Ether	-1.2772**
Acetic acid	carboxylic acid (CA)	-1.4511**	Octadecanal	long chain aldehyde	-1.2726**
Pelargonic acid	Saturated F A	-1.4303**	Napthalene acid	Organic compound	-1.2717**
Oxalic acid	CA	-1.4271**	Squalene	Steroid	-1.2623**
Caprylic acid	saturated F A	-1.4134**	Phthalate	Ester	1.0616 ns

Thioacetic acid	Methyl ester	-1.4076**	ROOTS Metabolites		
Tiglic acid	Fatty acyl	-1.401**	Tiglic acid	Fatty acyl	-1.6161***
Sebacic acid	saturated carbon	-1.3988**	Butyl glyoxylate	carboxylic acid	-1.5969***
Benzoic acid	benzoic derivative	-1.3973**	Valeric acid	Carboxy acid	-1.5837***
Oleic acid	Unsaturated FA	-1.3913**	2-Nitropropane	Halocarbon	-1.5753***
Stearic acid	saturated FA	-1.3889**	Furan anhydride	Furans	-1.5637***
N-methyl Idopyrol	Hetrocyclic	-1.417**	2-Nonane	---------	-1.5538***
2-Bromopentene	Halocarbon	-1.339**	Caryophyllen	Sesquiterpenoid	-1.535***
Nonayl dioxalane	Hetrocyclic	-1.3986**	Dimethoxy Decane	----------	-1.5271***
Ethyl Isocynate	Halohydrocarbon	-1.3945**	NAKIJIQUINONE B	Hydroquinine lipids	-1.526***
Cyclohexanediol	----------	-1.3916**	L-Prolin	----------	-1.5062***
Pyridinum	Organic cation	-1.3869**	Furan carboxy acid	Carboxylic acid	-1.4969***
Linalool	Monoterpenoid	-1.993 ns	Vaccenic acid	Trans fatty acid	-1.4738***

-

Table represented the Fold change in metabolic profile of leaves and roots of tolerant (CML49) and sensitive (CML100) genotypes. The values are the means of the normalized data analyzed by Metaboanalyst software. All the metabolites are statistically significant.
**Statistically significant (<0.05)
*** Statistically significant (0.0001)
ns- Non Significant