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Metabolomic profiling of drought-tolerant little millet (*Panicum sumatrense* L.) genotype in response to drought stress

Dhawale RN, Padhiyar Shital M, Kheni Jasminkumar and Tomar RS

Abstract
Metabolomics research in plant systems measures all or a set of metabolites present in a specified sample during a particular time. Metabolites can be regarded as the ultimate response to environmental changes**.** Several cellular metabolites are altered during drought stress, such as soluble sugars, organic acids, phenolics, amino acids, fatty acids, nucleotides, peptides, cofactors, and secondary metabolites. The current study was aimed to metabolic *profiling* in leaves and roots of drought-tolerant *little millet* genotype "OLM-203/Tarini" to discover putative metabolites responsible to drought stress at the seedling stage. By gas chromatography-mass spectrometry (GC-MS) 60 untargeted metabolites, 31 metabolic pathways were identified in little millet genotype (OLM-203/Tarini) samples (leaves and root) were investigated under control and water stress conditions at the 23 and 32 days seedling stage. The principal component analysis (PCA), partial least-squares discriminant analysis (PLS-DA), heat map, and cluster analysis were applied to the metabolomics data obtained by the GC−MS technique to determine the important metabolites for drought tolerance. Twenty five metabolic pathways, namely, galactose metabolism, fatty acid metabolism, starch and sucrose metabolism, vitamin K metabolism, pentose phosphate pathway, beta oxidation of very long chain fatty acids, citric acid cycle, fructose and mannose metabolism, alpha linoleic acid and linoleic acid metabolism, butyrate metabolism, homocysteine degradation, lactose degradation, glycerolipid metabolism, Warburg effect, malate-aspartate shuttle, glycerol phosphate shuttle, ketone body metabolism, glucose alanine cycle, gluconeogenesis, mitochondrial electron transport chain, Valein-leucine and isoleucine degradation, catecholamine biosynthesis, vitamin B6 metabolism, riboflavin metabolism, and lactose synthesis were significantly affected by water-deficit conditions. This study provides valuable information about the metabolic response of OLM-203/Tarini genotype to drought stress and metabolites identified, which encourages further study by transcriptome and proteomics to improve drought tolerance in OLM-203/Tarini.

Keywords: OLM-203/Tarini, metabolism, metabolites, GC-MS, metabolic profiling

Introduction
Small millets are warm-season cereals largely grown in the semi arid tropical regions of Asia and Africa, under rainfed farming systems (Rai *et al.*, 2008) [19]. Small millets includes finger millet (*Eleusine coracana*), kodo millet (*Paspalum scrobiculatum*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), little millet (*Panicum sumatrance*) and barnyard millet (*Echinocloa frumentacea*). Millets are important crops of Asia and Africa (especially in India, Nigeria and Niger), with 97% of millet production in developing countries (McDonough *et al.*, 2000) [18]. Millets, considered as important food staples in human history. They have been in cultivation in East Asia for the last 10,000 years. India is the world's largest producer of millet (Lu *et al.*, 2009) [17]. The total area of small millets in India is around 1.92 m ha, of which finger millone occupies 1.19 m ha. Small millets are a group of six crops comprising of finger millet, kodo millet, little millet, foxtail millet, barnyard millet and proso millet. They are considered as Nutri-cereals and are source of food, feed and fodder.

The crops are grown in a variety of agro-ecological situations including plains, coast and hills as well as in diverse soils and varying rainfall. They are known for resilience and drought enduring capacity and are relatively less prone to major pests and diseases. The richness in calcium, dietary fiber, polyphenol and protein contents in millets make them unique among the cereals. Generally, millets show significant amounts of amino acids like methionine and cystine and also have high fat content than rice and maize. Small millets grains are rich in dietary energy, vitamins, several minerals (especially micronutrients such as iron, calcium and zinc), insoluble dietary fiber and phytochemicals with antioxidant properties (Bouis, 2000) [4] and are considered as "Nutri-cereals".

They are rich in compounds that help against several chronic diseases like isthemic strokes, cardiovascular diseases, cancers, obesity and Type II diabetes (Jones *et al.*, 2000, Jones, 2006) [10, 11]. Little millet is rich in cholesterol, when consumed increases good cholesterol in the body, suitable for growing kids and strengthens the body. Its complex carbohydrate digests slowly which is very helpful for diabetic patients (Gayatri**,** 2015). It contains high phosphorous (220 mg/100 g) and iron (9.3 mg/100 g). It is especially good for people have low body mass. This presents importance of small millets in relation to nutritional and health aspects to enhance cultivation and consumption of health food.

Probiotics are "living microorganisms" which when administered in adequate amounts confer a health benefit on the host (Abd El-Salam *et al.*, 2012) [1]. Fermented millet products act as a natural probiotic treatment for diarrhea in young children (Lei *et al.*, 2006) [15]. In Africa, millet *Koko* is prepared in the form of fermented millet porridge and drink (Lei and Jacobsen M. 2004) [14]. Prebiotics are non digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon (Laminu *et al.*, 2011) [12]. Millets whole grain also shows prebiotic activity, which helps to increase the population of bacteria's that plays a key role to promote digestion. Malting reduces important beneficial biochemical changes in the millet grain.

Metabolomics research in plant systems is progressing; it measures all or a set of metabolites present in a specified sample during a particular time. Metabolites can be regarded as the ultimate response to environmental changes **(**Arora *et al.*, 2018 and Lu *et al.*, 2013) [3, 16]**.** The current study was aimed to metabolic profiling in leaves and roots of drought-tolerant and control little millet genotype when subjected to drought stress at the seedling stage.

The current study was aimed to compare metabolic changes in leaves and roots of drought-tolerant and -sensitive peanut genotypes when subjected to drought stress at the seedling stage. For putative identification of drought specific metabolites, we employed a gas chromatography-mass spectrometry (GC-MS)-based untargeted metabolomics approach. The metabolic content of little millet (*Panicum sumatrance*) genotype "OLM-203/Tarini" was compared to reveal the effects of drought stress on the metabolomic level. These results provide insights into metabolites involved in the mechanisms of plant drought tolerance, which can eventually contribute to the future metabolomics studies of domesticated crops. To our best knowledge, this is the first time that a metabolic comparison has been made in cultivated OLM-203/Tarini in leaf and root samples via GC-MS analysis.

Materials and Methods

Experiment conduct in June season and little millet drought tolerant genotype OLM-203 used as experimental material and conduct at Department of Biotechnology, J.A.U., Junagadh. Seeds will be sown in 2 kg plastic bag under small greenhouse and mud pots filled with equal weight soil mixture of sand, warmi compost and FYM in ratio of (40:40:20) and 25 to 30 seeds sown per polytheen bag with three replication of one genotype to comparative study with control and water stress (or water withhold) (Figure 1). During experiment in green house condition an average maximum temp. 36 ˚C -37 ˚C in day and minimum temp. (24 ˚C -25 ˚C) in night with relative humidity an average 84% - 86% in day and 49% -51% in night. Seedlings were maintained by thinning after 10 days of sowing. 23 days old seedling was used for drought stress. Regular irrigation interval of one day (alternate day) up to 23 days. After 23 days water withhold for seven days or (drought stress) so, total 32 days old seedlings were used for analysis. So, total 24 sample**s** was used for metabolomics study of control leaves and roots (with regular watering) and treated leaves and roots with water stress treatment up to 32 days (Figure 2).

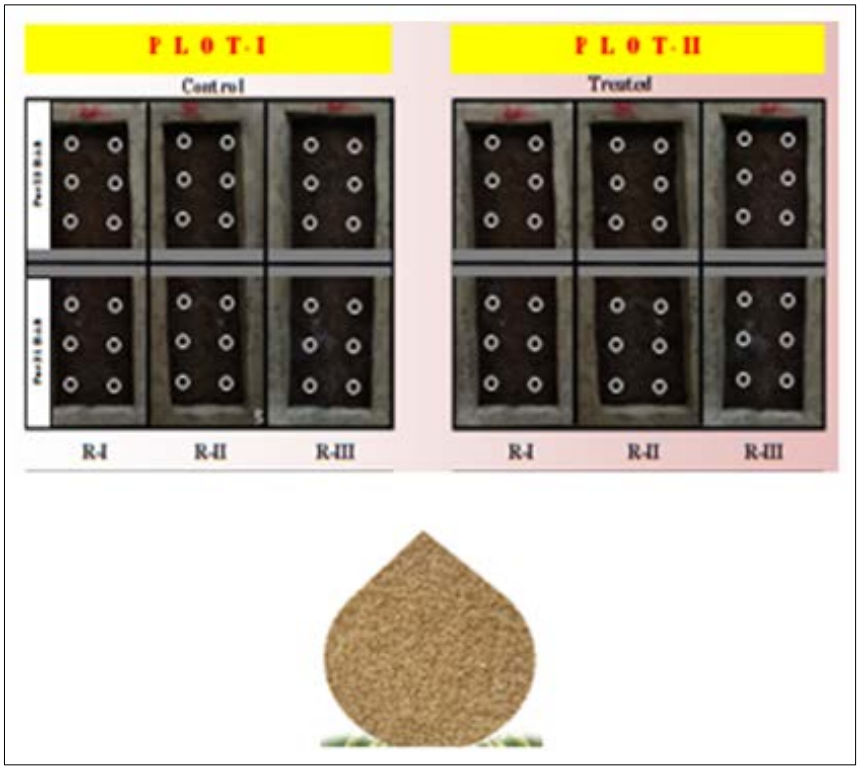

Fig 1: Three replication of one genotype

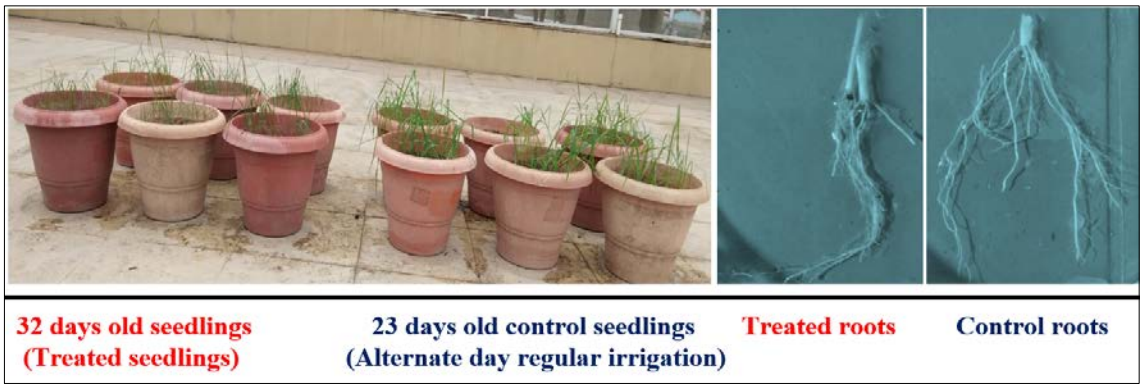

Fig 2: *Control* leaves and roots (with regular watering) and treated leaves and roots with water stress treatment up to 32 days

Fig 3: Isolated metabolites analysed by high performance GCMS-QP2010 SE-Shimadzu, *Japan*

The derivatized samples was analyzed by a GC-MS (gas chromatography-mass spectrometry, Shimadzu QP2010Plus, Japan) instrument was connected to a mass selective detector (Shimadzu GC−MS-QP2010 SE, Japan) (Figure 3) and operated according to the manufacturer's instructions. The derivatize extract (1µl) was injected into a column capillary (DB-17 MS, 30 m × 0.25 mm) using a splitless injection (230°C, 1.5 min). The inlet temperature and ion source temperature was set at 280 °C and 230 °C, respectively. Helium gas (99.99% purity) was be used at a flow rate of 1ml min−1 as a carrier gas. The electron ionization of 70eV was used in the full scan mode (50-1000 Da, m/z).

Results and Discussion

The quantitative and qualitative compositions of plant metabolomes reflect their responses to biotic and abiotic stimuli, genome, and physiological status, thus serving as a connecting link between genotypes and phenotypes. It makes a significant contribution to the research of stress biology by recognizing various compounds such as byproducts of stress metabolism, stress signal transduction molecules, and molecules that are part of the plant acclimation process (Ramalingam, A. *et al*., 2015 and Lanzinger, A. *et al*., 2015) [20, 13]. Metabolites can be regarded as the ultimate response to environmental changes (Arora, N. *et al*., 2018 and Lu, Y**.** *et al*., 2013) [3, 16]. Several cellular metabolites are altered during drought stress, such as soluble sugars, organic acids, phenolics, amino acids, fatty acids, nucleotides, peptides, cofactors, and secondary metabolites (Das, A. *et al*., 2017) [6]. Many of these metabolites are vital components of the plant's defense system (Dixon, R. A. (2001) [7]. Polyamines and phenolic compounds (phenolic acids and flavonoids) are substantial groups of plants secondary metabolites that impart tolerance and are described as a new kind of biostimulants under environmental stress, especially drought stress conditions (Chen, D. *et al*., 2019 and Aninbon, C. *et al*., 2016) [5, 2].

Untargeted Metabolites

The current study carried out to understand the metabolic alteration in different parts (leaves and roots) of the plant at the seedling stage that could provide a more precise indication of stress tolerance in plants. In the present study, a total of 46 and 29 metabolites were accumulated in leaf and root extracts of peanut, respectively, as determined from the chromatogram. Using the NIST library, metabolites were identified. The total number of metabolites was identified in each samples are given in Figure 4 and Table 1.

Functional classification of metabolites

All the metabolites were classified into six groups namely amino acid, fatty acid, organic acid, sugar, sugar alcohol, sterol, polyamines and other classes. The number of compounds identified in GC-MS and their classification given in Table 2.

Table 1: Total number of metabolites identified in metabolomics study analysis used by using GC-MS.

Amino acid and derivatives	**40**	**Serine, Threonine, Homocysteine, and Proline (41%)**
Organic acids	09	Pentonic acid, Propanoic acid, Nonanoic acid, Malic acid, Trihydroxybutyric acid, Hydroxy glutaric acid, Ribonic acid, Allonic acid and Mannonic acid (9.3%)
Sugar	18	Xylulose, Galactose oxime, Ribose, Xylose, Fructose, Glucose oxime, Glucose, Ribofuranose, Glucopyranoside, Mannose, Galactopyranoside, Turanose, Deoxygalactose, Melibiose and Lactose (18.7%)
Sugar acids	03	Xylonic acid, Glucaric acid and Glucuronic acid (3.1%)
Sugar alcohol	02	Arabinitol and Galactitol (2%)
Fatty alcohol	01	Octacosanol (1%)
Fatty acids	14	Butanoic acid, Dodecanoic acid, Hexanoic acid, Tetradecanoic acid, Pentanoic acid, Hexadecanoic acid, Palmitelaidic acid, Octadecanoic acid, Linoleic acid, Linolenic acid, Octadecatrienoic acid, Heptadecanoic acid, Oleic acid and Eicosanoic acid (14.5%)
Organic compounds	06	Butanedioic acid, Erythrose, Pentaric acid, Hexanedioic acid, Gluconic acid and Gulonic acid (6.2%)
Inorganic compounds	01	Acetamide 96-100 (1%)
Phenol	02	Thymol and Hydroquinone (2%)

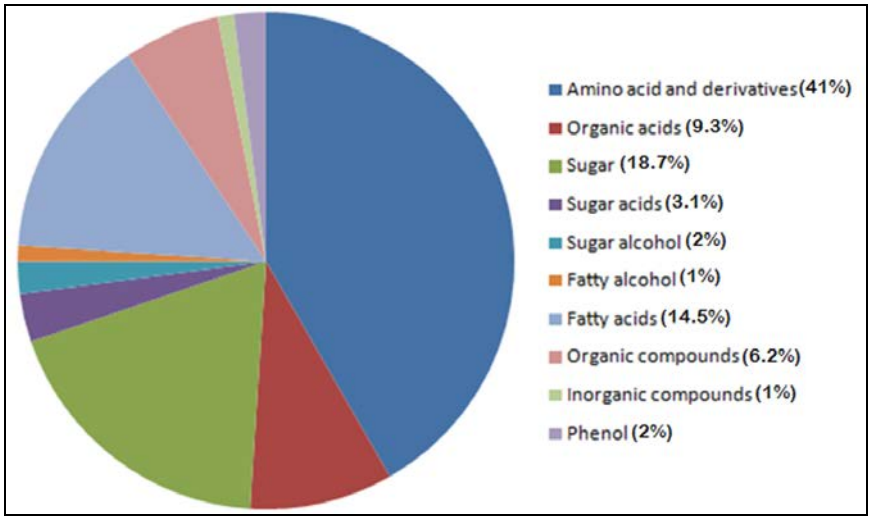

Fig 4: Pie charts shows the classification of metabolites

The current study carried out to understand the metabolic alteration in different parts (leaves and roots) of the little millets at the 32 days seedling stage that could provide a more precise indication of stress tolerance in plants. In the present study, a total of 50, 52 metabolites were accumulated in control and treated leaf samples respectively whereas, a total of 50, 56 metabolites were accumulated in control and treated root samples respectively, as determined from the chromatogram. Using the NIST library, metabolites were identified as sugars, sugar alcohols, sugar acids, fatty acids, and others such as dicarboxylic acid, diterpene alcohol, organic acid, and sugar amine. The detailed of total number of sixty (60) metabolites produced in each sample are given in the Table 2.

Table 2: Detailed of total number of metabolites produced in each sample

	23Days Control Leaf Samples	**32Days Treated Leaf Samples**	**23Days Control Root Samples**	**32Days Treated Root Samples**
Acetamide	+	+	+	+
Butanoic acid	+	-	+	+
Butanedioic acid	+	+	+	+
Serine	+	+	+	+
Erythrose	+	+	+	+
Threonine	-	+	+	+
Pentonic acid	+	-	-	-
Propanoic acid	-	+	+	+
Nonanoic acid	+	+	+	+
Malic acid	+	+	+	+
Trihydroxybutyric acid	+	+	+	+
Homocysteine	-	-	+	+
Glucohexodialdose	+	+	-	+
Arabinitol	+	+	+	+
Hydroxy glutaric acid	+	+	+	+
Ketoglucose	-	-	+	+
Proline	-	+	+	+
Dodecanoic acid	+	+	+	+

Ribonic acid	+	+	-	+
Allonic acid	+	-	+	+
Mannofuranose	+	+	+	+
Hexanoic acid	+	+	+	+
Xylulose	+	+	+	+
Mannonic acid	+	+	+	+
Galactose oxime	+	+	+	+
Pentaric acid	+	+	+	+
Xylonic acid	-	-	+	+
Ribose	+	+	+	+
Hexanedioic acid	-	+	+	+
Tetradecanoic acid	+	+	+	+
Gluconic acid	+	+	+	+
Xylose	+	+	-	+
Gulonic acid	+	+	+	+
Pentanoic acid	+	+	+	+
Glucaric acid	+	-	+	+
Hexadecanoic acid	+	+	+	+
Fructose	+	+	+	+
Glucose oxime	+	+	+	+
Galactitol	+	+	+	+
Glucose	+	+	+	+
Palmitelaidic acid	+	+	+	+
Octadecanoic acid	+	+	+	+
Linoleic acid	+	+	+	+
Ribofuranose	-	+	+	+
Linolenic acid	-	-	+	+
Octadecatrienoic acid	-	+	-	-
Glucuronic acid	+	+	+	+
Heptadecanoic acid	+	-	+	-
Oleic acid	+	+	+	+
Eicosanoic acid	+	+	+	+
Glucopyranoside	+	+	+	+
Mannose	+	-	+	+
Galactopyranoside	+	+	+	+
Turanose	+	+	+	+
Thymol	+	+	+	+
Deoxygalactose	+	+	+	+
Melibiose	+	+	+	+
Octacosanol	+	+	+	+
Lactose	+	+	+	+
Hydroquinone	-	-	+	+
Total No. of metabolites	50	52	50	56

Different metabolites of tolerant and sensitive genotypes were also determined using variable importance in projection (VIP) measure of partial least-squares discriminant analysis (PLS-DA) and metabolites with VIP score > 1 were explained)Wang, Y. *et al*., 2015) [21]. PLS-DA is a chemometric method used to optimize the separation between different groups (Gromski, P. S. *et al*., 2015) [9]. The VIP score (Figure 5A) of control samples showed higher intensity of stress-specific metabolites, such as hexadecanoic acid, linolenic acid, mannonic acid, galactose oxime, dodecanoic acid, glucohexodialdose, and malic acid in the OLM-203 drought tolerant genotype while threonine, fructose, serine, homocysteine, xylonic acid, ketoglucose, acetamide and hydroxyglutaric acid the treated by drought condition. In the mean decrease accuracy, metabolites were linolenic acid, mannonic acid, hexadecanoic acid, pentonic acid, glucopyranosid, glucohexodialdose, and malic acid were present in higher concentrations in the OLM 203 genotype. In treated samples (Figure 5B), glucose, threonine, acetamide, serine, oleic acid, fructose glucose oxime, and hydroquinone were intensively observed, while in control sample, linolenic acid, mannonic acid, hexadecanoic acid, pentonic acid, glucopyranosid, glucohexodialdose, and malic acid were observed in OLM 203.

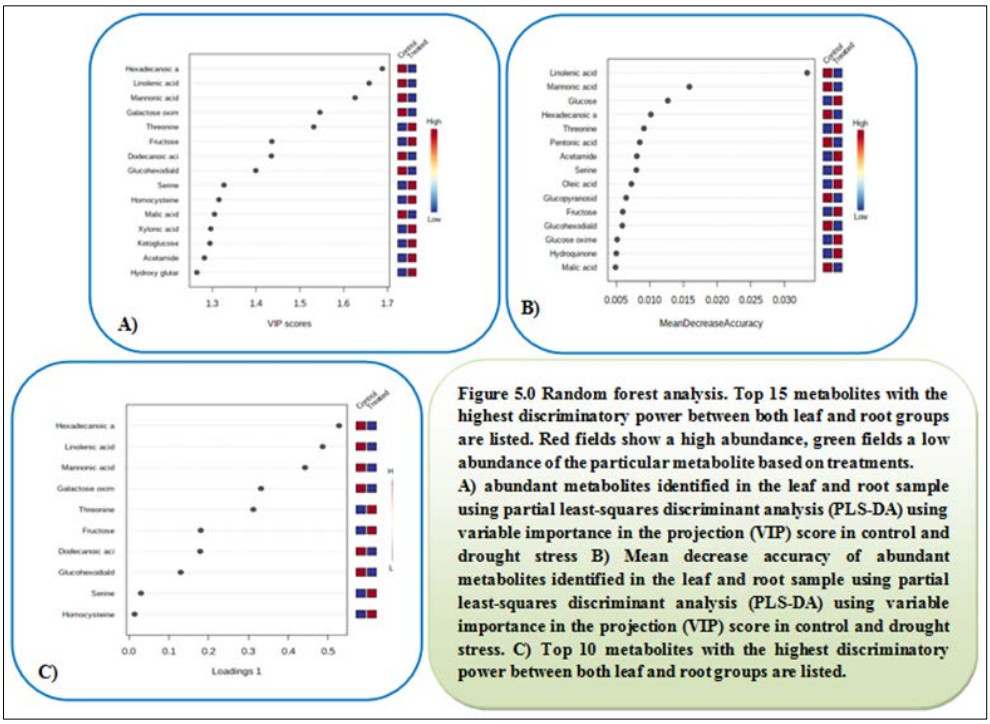

Fig 5: Random forest analysis. top 15 metabolites with the highest discriminatory power between both leaf and root groups are listed. Red fields show a high abundance, green fields a low abundance of the particular metabolite based on treatments

Hierarchical clustering analysis further classified these genes into 2 major groups based on their expression pattern (Figure 6). More than half of these genes exhibit differential expression between control and drought growth conditions. Therefore, sample number 6 and 7 are 23 days control leaf and 23 days control root samples respectively and representing 100 per cent similar to each other. In cluster first, there are 14 samples clustered with each other. Sample number 11 (23 days control root) is clustered with remaining 13 samples (18, 7, 6, 12, 2, 3, 19, 21, 24, 8, 10, 9, and 5). Five samples number 2, 3, 7, 8, and 9 depicting the 23 days control leaf samples (regular irrigation) and are clustered with sample numbers 18, 6, 12, 19, 21, 24, and 10 among them sample number 18 and 24 are the 32 days treated root samples also clustered in this main cluster.

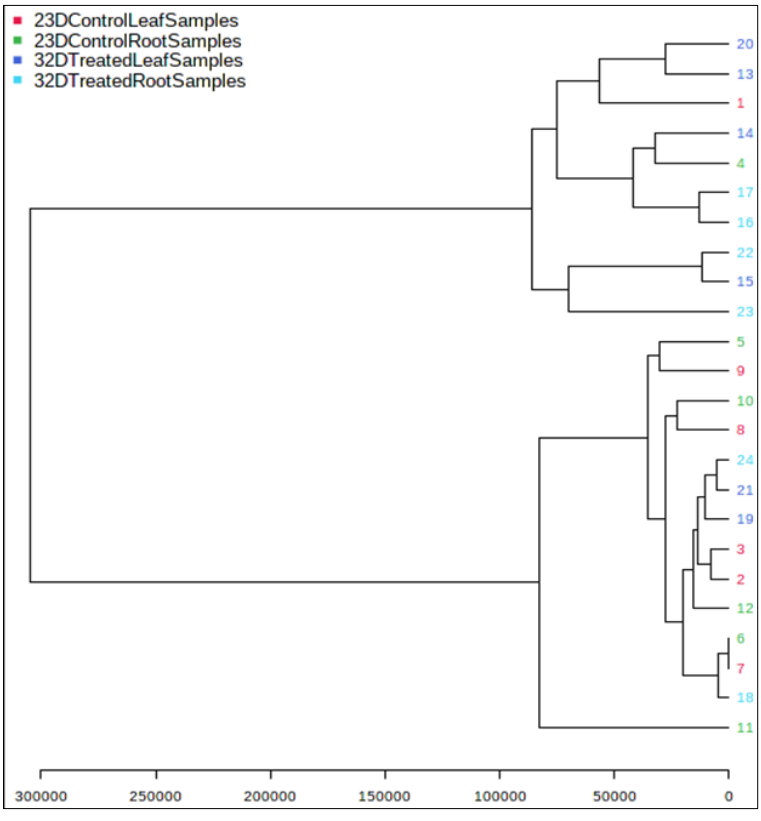

Fig 6: Hierarchical clustering analysis further classified these genes into 2 major groups based on their expression pattern

Five sample number 6, 12, and 10 are the 23 days control root samples (regular irrigation) and sample numbers 19 and 21 are the 32 days treated leaf samples are clustered in a single cluster. In case of cluster second, all types of samples also present. Most of these the differentially expressed genes (DEGs) were commonly expressed in control root as well as control leaf samples. However, to the best of our knowledge, metabolomic profiling in various genotypes of little millet have not been reported. There is no single report of metabolomic profiling of little millet in drought tolerance. In the present study metabolome profiling of little millet genotype having drought tolerance trait was carried out by GC-MS platform from single genotype described in materials and methods.

To quantitatively compare the SP metabolites of each ejaculate-portion and the EE, 50 of the 63 identified spectral peaks (which corresponded to 11 different metabolites) were included in the statistical analysis. This selection was based on the signal intensity and peak isolation in order to achieve enough signal-to noise and to avoid spectral overlapping. Then, data were normalized to DSS and Pareto-scaled **(**Figure 7) for further analysis with Metabo Analyst 5.0.

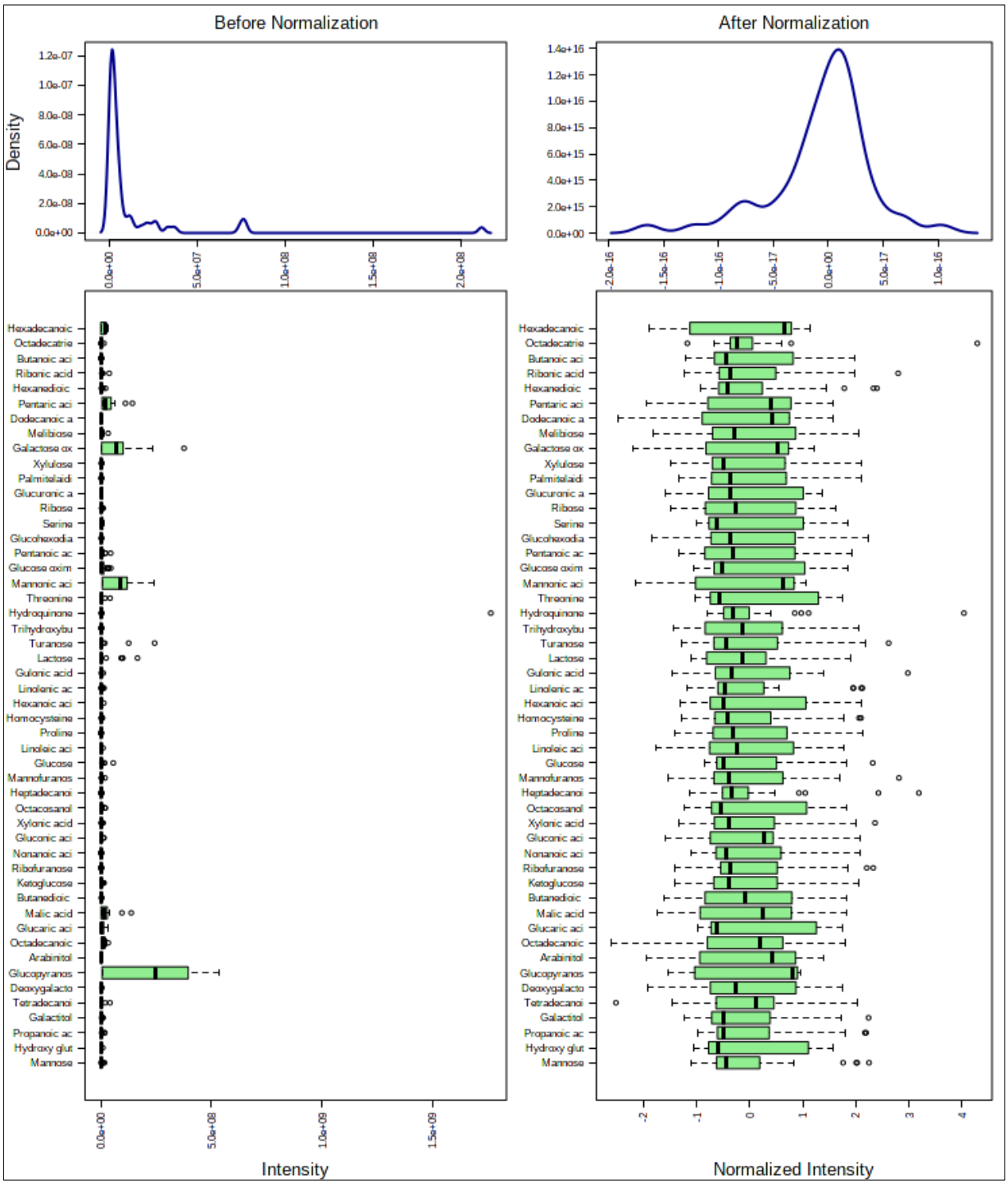

Fig 7: Selected metabolites' spectral bin data ormalization to DSS of little millet (OLM 203), drought tolerant genotype generated using Metabo Analyst mannonic acid to exanedioic acid. Total fifty metabolites are considered for normalization and was performed with Pareto-scaling and is shown on the left side. Empty dots in box-plots represent the outlier values both before and after normalization. Values were classified as outliers when their value was lower than 1.5 times the 25th percentile or higher than 1.5 times the 75th percentile.

Metabolites profilling of drought tolerance genotype OLM-203 (Tarini) by Heat map

As shown in (Figure 8A), all the sixty metabolites samples were classed into four main groups. The control sample (T0) of DT was clearly divergent from all the samples under drought stress treatment. Interestingly, samples of the single genotype from stress stage were clustered together. Whereas, when the stress becomes more severe, the samples from the same genotype were clustered together and all samples from cluster first is also clustered with remaining two clusters, supporting the great difference of drought tolerance.

Heat map analysis showing abundance of metabolites during control and drought stress in leaves and roots of the tolerant (OLM 203 / Tarini) genotype (Figure 8B). Heat map analysis of all metabolites in the 23 days regular watering leaf and root samples of OLM 203 revealed a high accumulation of sugars such as glycine, proline, D-ribose, β-D-galactopyranoside, α-D-glucopyranose, ribose, xylose, glucohexodialdose, mannofuranose, erythrose, galactose, galactose oxime, and xylulose, melibiose, deoxygalactose, ribofuranose, β-D-galactopyranose respectively. Metabolites such as octacosanol, thymol, acetamide, allonic acid, linoleic acid, pentonic acid, xylonic acid (sugar acid), glucaric acid, eicosanoic acid, butanoic acid, palmitelaidic acid, nanonoic acid, heptadecanoic acid were detected only in the leaf sample and serine, proline, oleic acid, linolenic acid, butanedioic acid, arabinitol, dodecanoic acid, glucoronic acid, pentaric acid, ribonic acid, hydroxyglutaric acid, octadecatrienoic acid and galactitol were detected in roots of OLM 203 respectively.

In the case of 32 days water stress (treated) tolerant genotype leaf and root samples of OLM 203, sugars such as D-fructose, glucose, mannose, lactose, ribose, melibiose and ketoglucose, organic acids such as 2,3,4-trihydroxybutyric acid and xylonic acid, and gluconic acids such as glucaric acid and thymol and glucose oxime (sugar amines), and propanoic acid (fatty acid), proline and homocysteine, while there are 43 other important compounds were accumulated under water stress were present in leaf samples.

In treated root samples, fructose, lactose, erythrose and ketoglucose were observed, while, propanoic acid, acetamide, palmitelaidic acid, nonanoic acid, butanoic acid, eicosanoic acid, serine, oleic acid, hydroxyglutaric acid, homocysteine, glucaric acid, threonine, trihydroxybutyric acid, hydroquinine, proline, mannofuranose, pentanoic acid, gulonic acid and octadecanoic acid were also present prominently and as in additional metabolites in treated root samples, total 36 metabolites such as arabinitol, propanoic acid, lactose, glucose oxime, ketoglucose, fructose, erythrose, melibiose and allonic acid were present, while palmitic acid, mannonic acid, malic acid, linoleic acid, and gulonic acid were present in OLM 203.

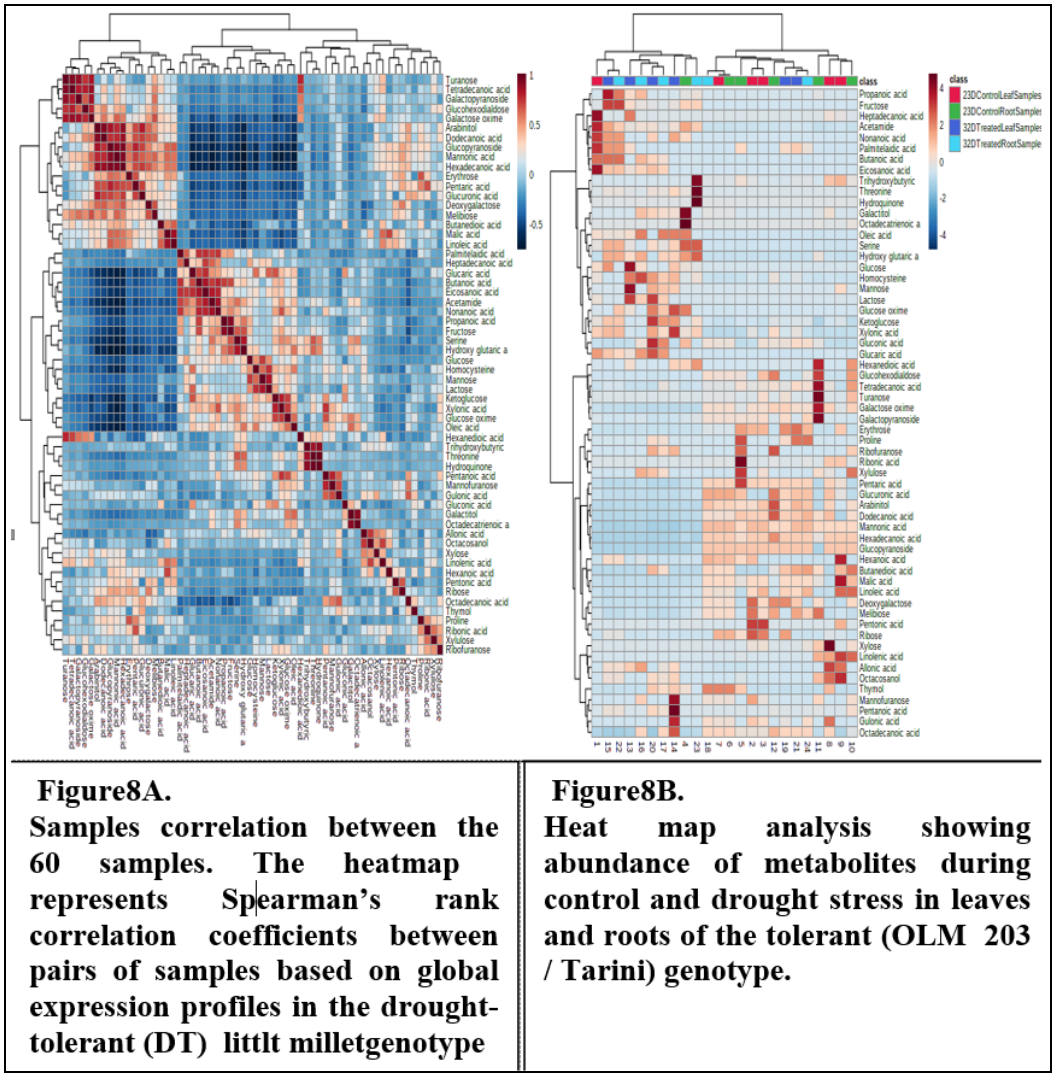

Figure8A.
Samples correlation between the 60 samples. The heatmap represents Spearman's rank correlation coefficients between pairs of samples based on global expression profiles in the drought-tolerant (DT) littlt milletgenotype

Figure8B.
Heat map analysis showing abundance of metabolites during control and drought stress in leaves and roots of the tolerant (OLM 203 / Tarini) genotype.

Fig 8: Heat map analysis

Conclusions

The VIP score of control samples showed higher intensity of stress-specific metabolites, such as hexadecanoic acid, linolenic acid, mannonic acid, galactose oxime, dodecanoic acid, glucohexodialdose, and malic acid in the OLM-203 drought tolerant genotype while threonine, fructose, serine, homocysteine, xylonic acid, ketoglucose, acetamide and hydroxyglutaric acid the treated by drought condition. In the mean decrease accuracy, metabolites were linolenic acid, mannonic acid, hexadecanoic acid, pentonic acid, glucopyranosid, glucohexodialdose, and malic acid were present in higher concentrations in the OLM 203 genotype. In treated samples, glucose, threonine, acetamide, serine, oleic acid, fructose glucose oxime, and hydroquinone were intensively observed, while in control sample, linolenic acid, mannonic acid, hexadecanoic acid, pentonic acid, glucopyranosid, glucohexodialdose, and malic acid were observed in OLM 203.

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References

1. Abd El-Salam, Hippen MH, Salem AR, Assem MM, El-Aassar MFM. Survival of probiotic Lactobacillus casei and Enterococcus fecium in Domiati cheese of high conjugated linoleic acid content. Emiratus Journal of Food and Agriculture. 2012;24(2):98-104.
2. Aninbon C, Jogloy S, Vorasoot N, Nuchadomrong S, Senawong T, Holbrook CC, *et al*. Effect of mid-season drought on phenolic compounds in peanut genotypes with different evels of resistance to drought. Field Crops Res. 2016;187:127-134.
3. Arora N, Dubey D, Sharma M, Patel A, Guleria A, Pruthi PA, Kumar D, *et al*. NMR-based metabolomic approach to elucidate the differential cellular responses during mitigation of arsenic (III; V) in a green microalga. ACS Omega. 2018;3:11847-11856.
4. Bouis HE. Enrichment of food staples through plant breeding: a new strategy for fighting micronutrient malnutrition. Nutrition. 2000;16:701-4.
5. Chen D, Shao Q, Yin L, Younis A, Zheng B. Polyamine Function in Plants: Metabolism, Regulation on Development, and Roles in Abiotic Stress Responses. Front. Plant Sci. 2019;9:1975.
6. Das A, Rushton PJ, Rohila JS. Metabolomic Profiling of Soybeans (*Glycine max* L.). Reveals the Importance of Sugar and Nitrogen Metabolism under Drought and Heat Stress. Plants. 2017;6:21.
7. Dixon RA. Natural products and plant disease resistance. Nature. 2001;411:843-847.
8. Gayathri A. Little millet nutrition; health benefits and facts you must know, 2015.
9. Gromski PS, Muhamadali H, Ellis DI, Xu Y, Correa E, Turner ML, *et al*. A tutorial review: Metabolomics and partial least squares-discriminant analysis-a marriage of convenience or a shotgun wedding. Anal. Chim. Acta. 2015;879:10-23.
10. Jones JM. Grain-based foods and health cereals. Cereal Foods World. 2006;51:108-113.
11. Jones PJH, Raeini-Sarjaz M, Ntanios FY, Vanstone CA, Feng JY, Parsons WE. Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. Journal of Lipid Research. 2000;41:697-705.
12. Laminu HH, Modu S, Numan AI. Production; *In vitro* protein digestibility; phytate content and acceptability of weaning foods prepared from pearl millet (Pennisetum typhoideum) and cowpea (Vigna unguiculata). International Journal of Nutrition and Metabolism. 2011;3(9):109-113.
13. Lanzinger A, Frank T, Reichenberger G, Herz M, Engel KH. Metabolite profiling of barley grain subjected to induced drought stress: responses of free amino acids in differently adapted cultivars. J Agric. Food Chem. 2015;63:4252-4261.
14. Lei V, Jacobsen M. Microbial characterization and probiotic potential of Koko and Koko sour water; African spontaneously fermented millet porridge and drink. Journal of Applied Microbialogy. 2004;96:384-397.
15. Lei V, Friis H, Michealsen KF. Spontaneously fermented finger millet product as a natural probiotic treatment for diarrhea in young children: An intervention study in Northern Ghana. International Journal of Food Microbiology. 2006;110:246-253.
16. Lu Y, Lam H, Pi E, Zhan Q, Tsai S, Wang C, *et al*. Comparative metabolomics in Glycine max and Glycine Soja under salt stress to reveal the phenotypes of their offspring. J Agric. Food Chem. 2013;61:8711-8721.
17. Lu H, Zhang J, Liu KB, Wu N, Li Y, Zhou K, *et al*. Earliest domestication of common millet (Panicum miliaceum) in East Asia extended to 10,000 years ago. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(18):7367-7372.
18. McDnough, Cassandrea M, Rooney Lloyd W, Serna–Saldivar, Sergio O. The Millets. Food Science and Technology: Hand book of Cereal Science and Technology 2nd CRC Press. 2000;99:177-210.
19. Rai KN, Gowda CL, Reddy BVS, Sehgal S. Adaptation and potential uses of sorghum and pearl millet in alternative and health foods. Comprehensive Reviews in Food Sciences. 2008;7:340-352.
20. Ramalingam A, Kudapa H, Pazhamala LT, Weckwerth W, Varshney RK. Proteomics and metabolomics: two emerging areas for legume improvement. Front. Plant Sci. 2015;6:1116.
21. Wang Y, Xu L, Shen H, Wang J, Liu W, Zhu X, *et al*. Metabolomic analysis with GC−MS to reveal potential metabolites and biological pathways involved in Pb & Cd stress response of radish roots. Sci. Rep. 2015;5:18296.