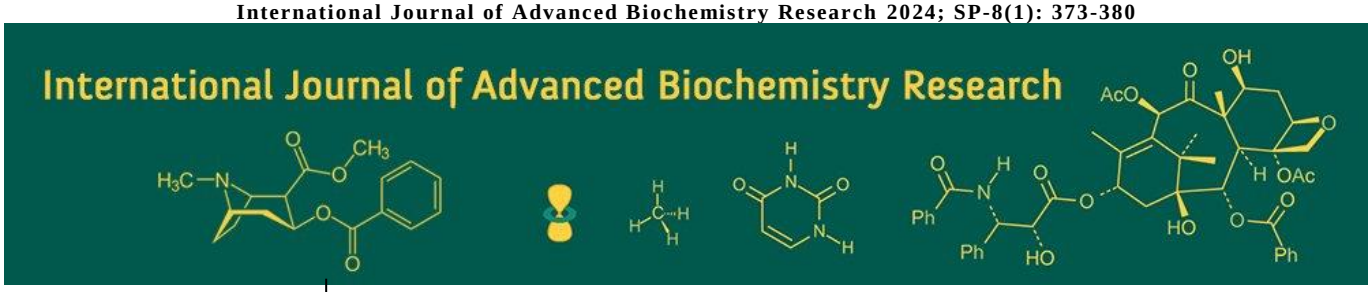

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The effect of Gibberellic acid, Abscisic acid and salicylic acid on Metabolome profiling in wheat (*Triticum aestivum* L.) irrigated with saline water

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Abstract
The goal of the current experiment was to find out how salicylic acid, abscisic acid, and gibberellic acid affected wheat that was watered with salt water. The leaves of wheat treated with different concentrations of growth regulators [T_1(control) and T_2(Sprayed with GA_3 @ 100 ppm + SA@ 100 ppm + ABA @ 100 ppm)] grown in a pot irrigated with two different concentration of saline water [I_1 (1.15 EC) and I_2(4 EC)] from the two varieties GW-496 and KRL-210 (V_1 and V_2). The analytical profiling method known as "metabolomics" allows for the measurement and comparison of a vast array of metabolites found in biological materials. Metabolomics provides a window into metabolic pathways by combining multivariate data analysis with high throughput analytical chemistry. The growth, development and production of plant are usually affected by various environmental conditions including salinity like abiotic stress. The most frequent stress that can encourage the build-up of antioxidants and suitable osmolytes is salinity. Plant species produce primary metabolites, such as fatty acids, amino acids, and nucleotides, which are crucial to the life cycle of plants.

Keywords: Wheat (*Triticum aestivum* L.), saline water, metabolome profiling, gibberellic acid, abscisic acid and salicylic acid

Introduction
Wheat originated from South East Asia, has global significance as for millions of people, it is the main source of nutrition. It became the first domesticated cereal crop, maximum consumed and a trade crop worldwide. Based on productivity, wheat is the third most commonly grown cereal after maize and rice. It is one of the important cereal crops that provide high protein and calorie content i.e. nearly twenty percent of nutrition to the world population (Chaves *et al*., 2013) [6]. It provides over 20% of the calories and proteins in human nutrition (Bhutto *et al*., 2016) [3].

Abiotic and biotic stresses of many kinds are encountered by agricultural crops. Abiotic stresses that seriously endanger agriculture and worsen the environment include salinity, drought, high temperatures, chemical toxicity, and oxidative stress. The main factor causing crop loss globally is abiotic stress, which lowers average yields for the majority of important agricultural plants by more than 50%. (Bray *et al*., 2000) [4]. Among the abiotic stresses, Salt affecting soil is a world-wide problem, soil salinity adversely influences the crop production.

This study used hydrochloric acid-based hydrolysis and trimethylsilyl derivatization to design a standardized, high-throughput, and impartial technique for GC-MS metabolomic profiling of free and conjugated phenolics and organic acids of whole-grain cereals. The study shows how to derivatize grain metabolites using a unique trimethylsilylation technique based on trimethylsilyl cyanide (TMSCN) for the first time. The new protocol offers a more objective and broad-spectrum derivatization of metabolites in comparison to previous commonly used derivatization methods. It can also produce repeatable metabolomics profiles of complicated biological materials. (Khakimov *et al*., 2013) [7]. The obtained raw GC-MS data of cereals were processed by a semi-automated multi-way decomposition method, PARAFAC2 (Bro *et al*., 1999) [5]. The PARAFAC2 processing of the raw GC-MS data lead to unambiguous deconvolution of elusive peaks such as, overlapped, retention time shifted and low s/n peaks and enable an automatic estimation of relative concentrations of detected peaks (Amigo *et al*., 2008) [2].

Growth hormone gibberellic acid promotes blooming, improves fruit set, and increases fruit size. It responds favorably to increasing the fruits and vegetables shelf life and quality standards. Potassium improved defenses against worms, bacteria, viruses, and fungi.

An essential plant hormone that controls how the body reacts to salt stress is abscisic acid. Increased tolerance is the outcome of ABA buildup brought on by stresses like salinity. Numerous lines of evidence have demonstrated that ABA causes H_2O_2 to accumulate, and that this is crucial for ABA signaling, which aids in plants ability to adapt and endure in these harsh conditions. (Abdelaal, 2015) [1].

Plants produce salicylic acid (SA), a phenolic molecule of hormonal origin that is vital in responding to various abiotic stresses and pathogen invasion. Salicylic acid is a phenolic molecule that protects plants from biotic and abiotic challenges like salt and can control plant growth.

Materials and Methods

Metabolome profiling

1. Exraction of metabolites

100 mg of flour was crushed in liquid nitrogen in a precooled mortar pestle till fine powder forms. add 2 ml of (methanol/H_2O = 3:1 + 0.1% Formic acid) and transfer to a 5 ml Eppendorf tube. Add 1 ml pre- chilled chloroform. vortex for 10 seconds. Add 1 ml of water (4 °C) and vortex for 10 seconds. Centrifuge it at 7000 RPM for 5 minutes to separate the phases. Separate the polar and non-polar phases by collecting in different tubes. Dry the polar phase tube in vacuum evaporator till the water dries completely Lisec *et al*. (2006) [8].

2. Derivitization of metabolites

Dry completely in vacuum evaporator for 20 minutes. add 50 µl pyridine and redissolve by sonicating for 10 minutes. Add 100 µl of methoxyamine-HCl (20 mg/ml in pyridine) and gently mix it. Sonicate it again for 5 minutes and incubate at 37 °C with constant agitation for 90 minutes. For trimethylsilation step, add 100 µl of MSTFA and seal with paraffin. Vortex for 30 seconds. Incubate the mixture at 37 °C for 60 minutes with constant agitation. Collect supernatants and spin down in minispin to properly mix the two phases and proceed for GC-MS analysis.

Results and Discussion

The systematic study of endogenous small molecule metabolites is the goal of the omic method known as "metabolomics." Discovering the metabolic oscillations of important plant compounds and the metabolic fluxes connected to several study fields, particularly in growth phases and tissue compound identification, has been made possible by the promising analytical technique known as metabolomics. This strategy, which combines the benefits of targeted and untargeted approaches, has been applied to the investigation of the metabolic profiles of diverse tissue samples. (Liu *et al*., 2017) [9].

Number of metabolites unique elements identified through GC-MS in wheat treated under different treatment conditions. The total number of compounds (141) identified in two varieties of wheat: salt susceptible (GW-496) and salt tolerant (KRL-210) with tap water and 4 EC saline water. Treatments like: $T_1V_1I_1$=Salt susceptible variety + Tap water [Control (water spray)], $T_8V_1I_1$= Salt susceptible variety + Tap water (Sprayed with GA_3 @ 100 ppm + SA@ 100 ppm + ABA @ 100 ppm), $T_1V_2I_1$= Salt tolerant variety + Tap water [Control (water spray)], $T_8V_2I_1$= Salt tolerant variety + Tap water (Sprayed with GA_3 @ 100 ppm + SA@ 100 ppm + ABA @ 100 ppm) Saline water, $T_1V_1I_2$=Salt susceptible variety + Saline water [Control (water spray)], $T_8V_1I_2$= Salt susceptible variety + Saline water (Sprayed with GA_3 @ 100 ppm + SA@ 100 ppm + ABA @ 100 ppm), $T_1V_2I_2$=Salt tolerant variety + Saline water [Control (water spray)], $T_8V_2I_2$=Salt tolerant variety + Saline water (Sprayed with GA_3 @ 100 ppm + SA@ 100 ppm + ABA @ 100 ppm).

Table 1: Number of metabolites unique elements identified through GC-MS in wheat treated under different treatment conditions

Sr. no.	List names	Number of elements	Number of unique elements
1	$T_1V_1I_1$	23	23
2	$T_1V_1I_2$	44	44
3	$T_1V_2I_1$	59	59
4	$T_1V_2I_2$	34	33
5	$T_8V_1I_1$	51	51
6	$T_8V_1I_2$	58	58
7	$T_8V_2I_1$	54	53
8	$T_8V_2I_2$	37	37
Overall number of unique elements			141

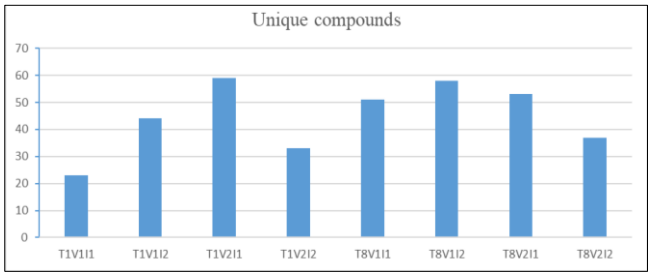

Fig 1: Graphical representation of identified compounds in particular treatments of salt susceptible (GW-496) and salt tolerant (KRL-210) varieties of wheat

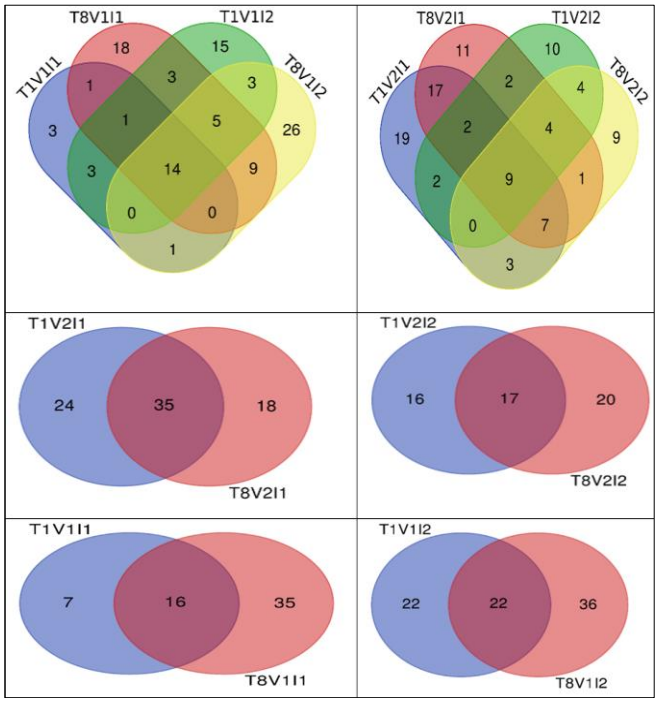

Fig 2: Venn diagram showing relationship between metabolites of salt susceptible (gw-496) and salt tolerant (krl-210) varieties of wheat grown under different treatments condition

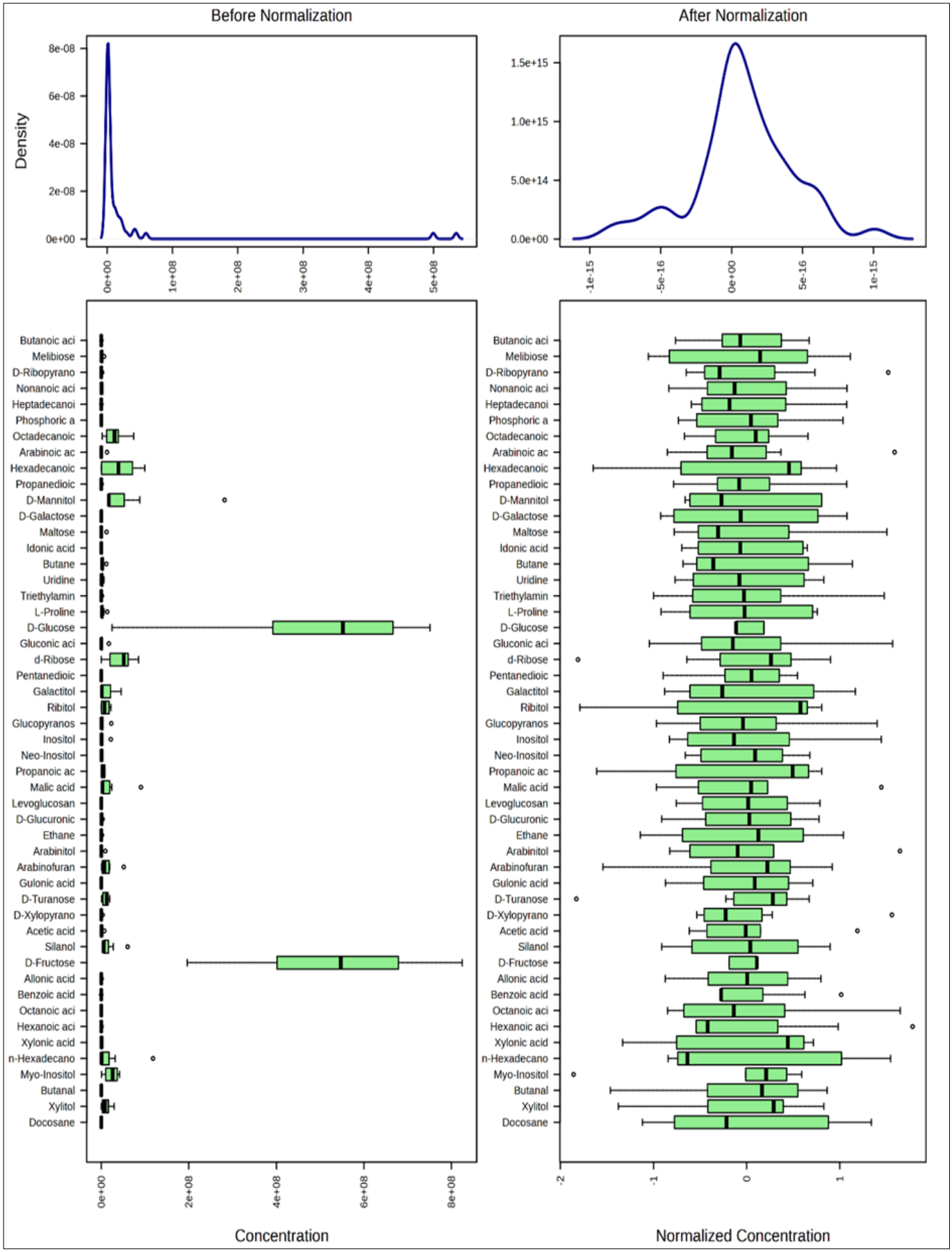

Fig 3: Data normalization of metabolites before analysis using MS spectra (Selected methods: Row wise normalization quantile normalization; Data transformation log transformation; and data scaling Pareto scaling)

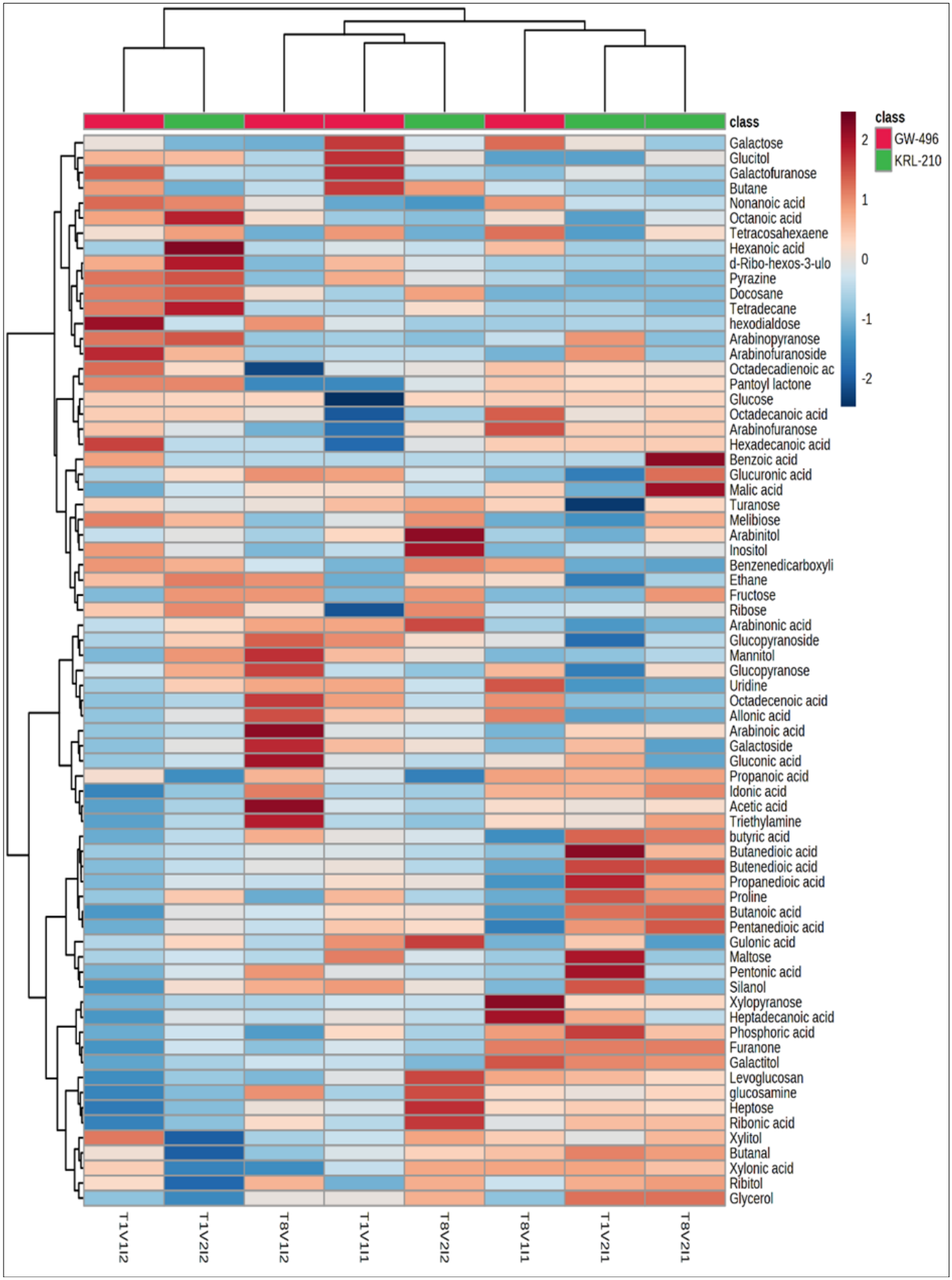

Fig 4: Heat map of metabolites particular to treatments of salt susceptible (GW-496) and salt tolerant (KRL-210) varieties of wheat

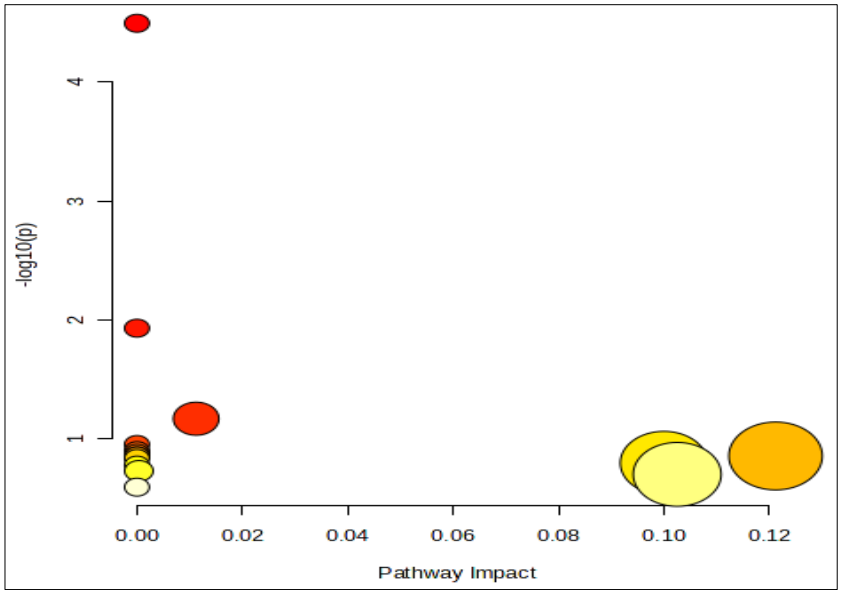

Fig 5: Summary plot for Pathway analysis showing metabolite set population of $T_1V_1I_1$

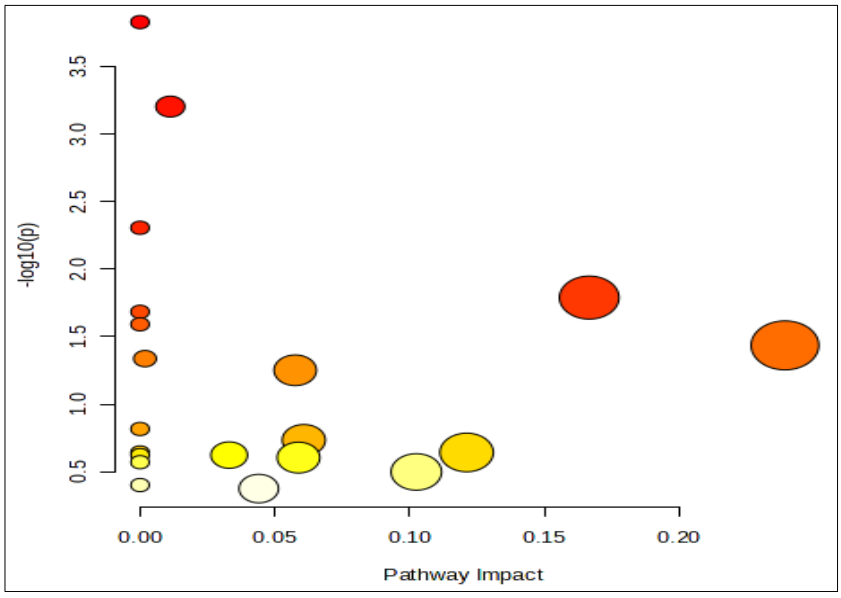

Fig 6: Summary plot for Pathway analysis showing metabolite set population of $T_8V_1I_1$

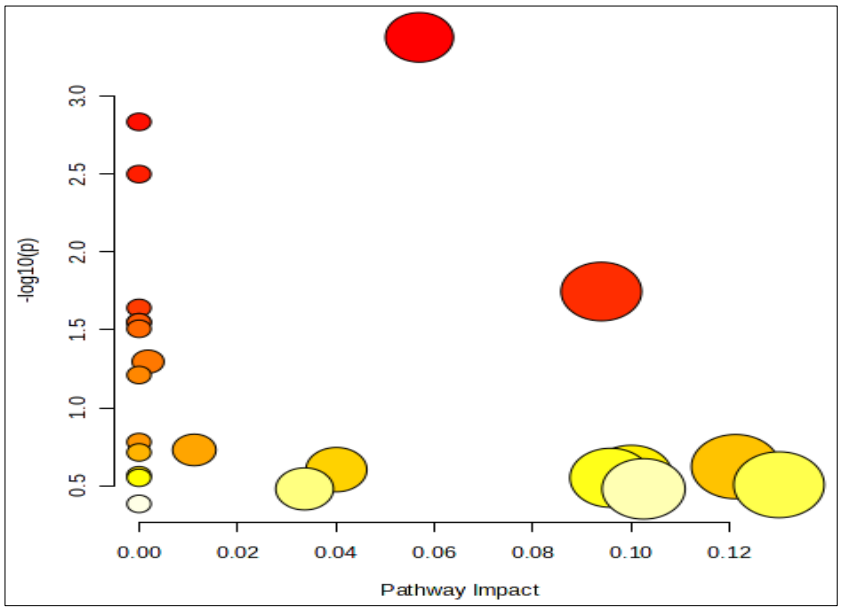

Fig 7: Summary plot for Pathway analysis showing metabolite set population of $T_1V_2I_1$

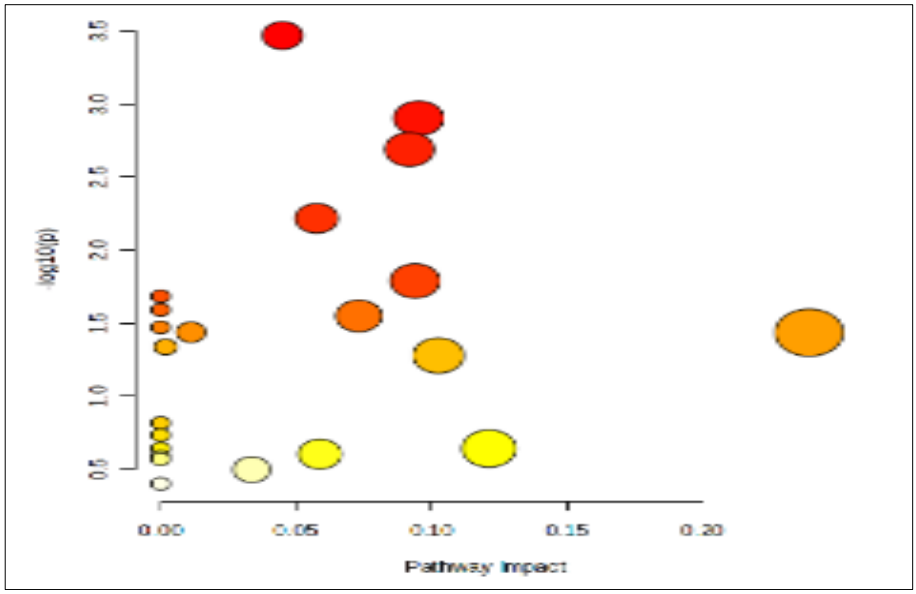

Fig 8: Summary plot for Pathway analysis showing metabolite set population of $T_8V_2I_1$

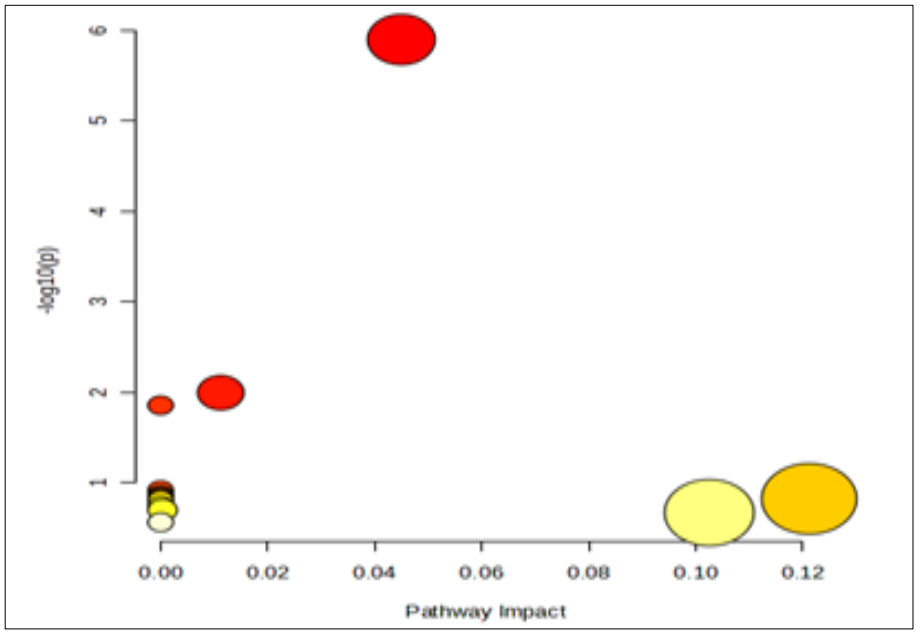

Fig 9: Summary plot for Pathway analysis showing metabolite set population of $T_1V_1I_2$

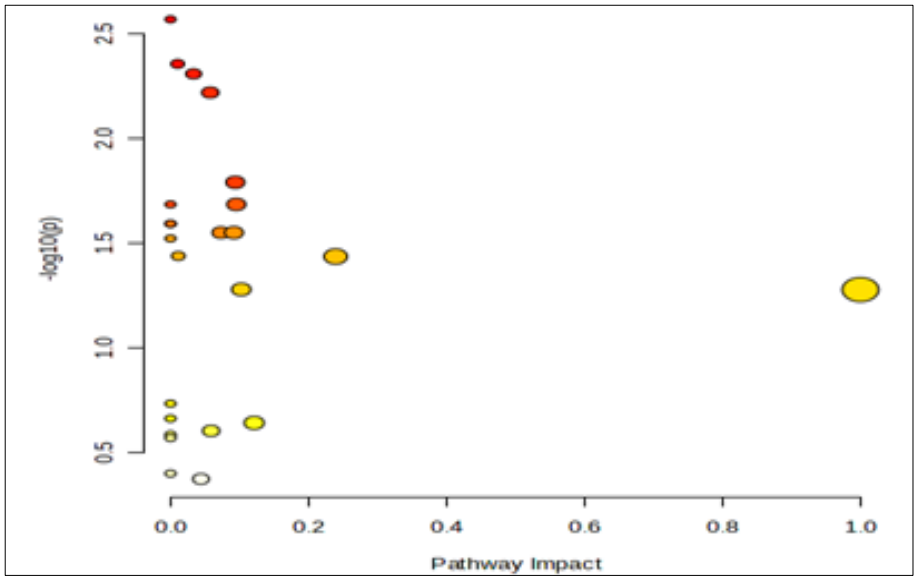

Fig 10: Summary plot for Pathway analysis showing metabolite set population of $T_8V_1I_2$

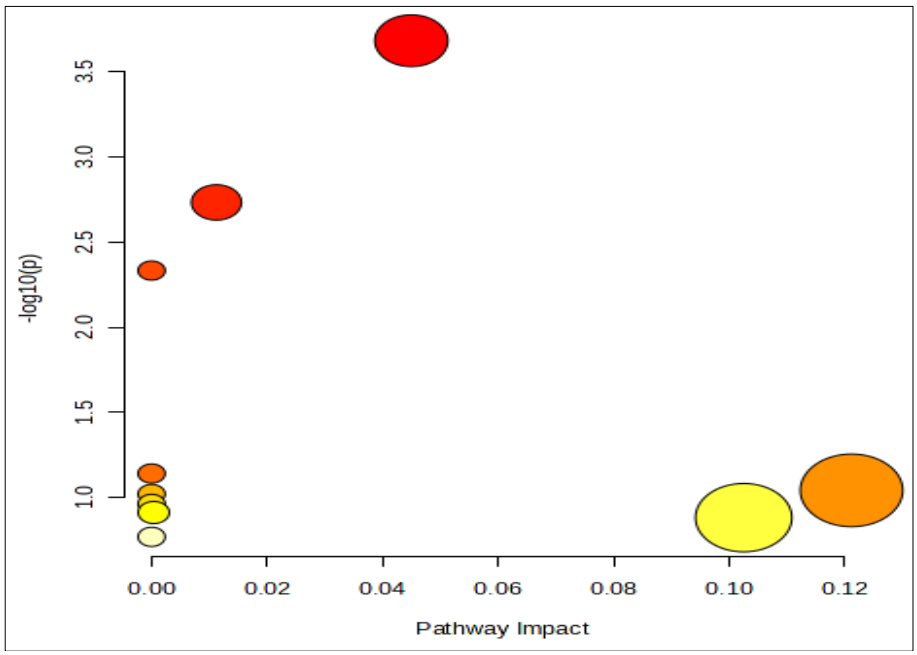

Fig 11: Summary plot for Pathway analysis showing metabolite set population of $T_1V_2I_2$

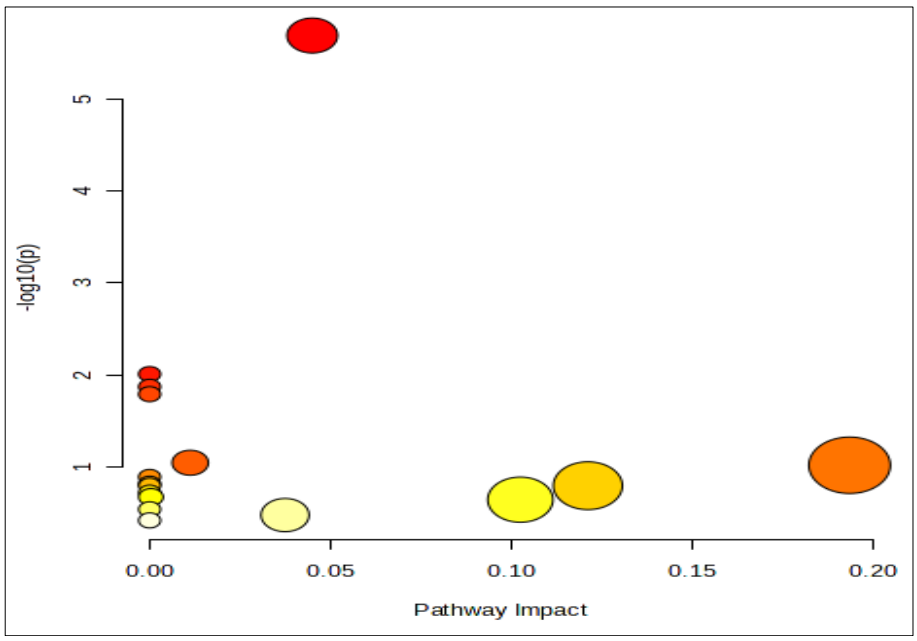

Fig 12: Summary plot for Pathway analysis showing metabolite set population of $T_8V_2I_2$

Treatment at $T_1V_1I_1$ analysis showing metabolite set in the population 14 Pathway. Galactose metabolism followed by Fatty acid biosynthesis and Biosynthesis of unsaturated fatty acids (Fig. 5). Treatment at $T_8V_1I_1$ analysis showing metabolite set in the population 22 Pathway. Biosynthesis of unsaturated fatty acids followed by Fatty acid biosynthesis and Galactose metabolism (Fig. 6). Treatment at $T_1V_2I_1$ analysis showing metabolite set in the population 25 Pathway. Galactose metabolism followed by Pentose and glucuronate interconversions and Fatty acid degradation (Fig.7). Treatment at $T_8V_2I_1$ analysis showing metabolite set in the population 24 Pathway. Biosynthesis of Galactose metabolism followed by Pentose and glucuronate interconversions and Ascorbate and aldarate metabolism (Fig. 8). Treatment at $T_1V_1I_2$ analysis showing metabolite set in the population 14 Pathway. Galactose metabolism followed by Fatty acid biosynthesis and Biosynthesis of unsaturated fatty acids (Fig. 9). Treatment at $T_8V_1I_2$ analysis showing metabolite set in the population 26 Pathway. Biosynthesis of unsaturated fatty acids followed by Glycolysis / Gluconeogenesis and Galactose metabolism (Fig. 10). Treatment at $T_1V_2I_2$ analysis showing metabolite set in the population 10 Pathway. Galactose metabolism followed by Fatty acid biosynthesis and Biosynthesis of unsaturated fatty acids (Fig.11).Treatment at $T_8V_2I_2$ analysis showing metabolite set in the population 16 Pathway. Galactose metabolism followed by Pentose and glucuronate interconversions and Ascorbate and aldarate metabolism (Fig. 12).

Conclusion

It is a profiling technique for measuring and comparing a large number of metabolites present in biological samples. The current study could provide an insight on the expression

of different classes of metabolites by the application of iron oxide nanoparticles in the treated and control samples. The total number of compounds (141) identified in two varieties of wheat: salt susceptible (GW-496) and salt tolerant (KRL-210) with tap water and 4 EC saline water. Leaves are also enriched with metabolites like: monosachharides, sugar acids, sugar alcohols and dicarboxylic acids.

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