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PGPR MEDIATED BIO-FORTIFICATION OF TOMATO FRUIT METABOLITES WITH NUTRITIONAL AND PHARMACOLOGICAL IMPORTANCE

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ABSTRACT

There is a growing interest in producing food plants with increased nutritional and medicinal values. The purpose of this study was biofortification of nutritionally and pharmacologically important metabolites of tomato fruits by using a PGPR strain "*Pseudomonas aeruginosa* PM12". Tomato plants were co-cultivated in the presence of this bacterial strain and changes in fruit metabolites were discovered. Metabolites were quantified and identified by performing GC/MS analysis. The fruit extracts from the tomato plants receiving bacterial strains showed significant up-regulations in quantities of sugars, flavonoids, terpenoids, carotenoids, and total phenolic contents. Principal component analysis well separated both treatment groups to show their significant effects. In support of these findings, metabolomic changes were incorporated in primary metabolic pathways to show that upon exposure to this beneficial bacterial strain, extensive re-modulations were induced in the bio-synthesis pathways of sugars, phenolics, carbohydrates and amino acids. These results suggest that application of *Pseudomonas aeruginosa* PM12 can induce plants for increased production of various bioactive compounds.

Keywords: Tomato, *Pseudomonas aeruginosa*, bio-fortification, GC/MS, metabolomics

INTRODUCTION

Tomato is considered one of the most widely cultivated and consumed fruits all-over the world. According to an estimate more than 150 million metric ton tomato fruit is produced annually and its processed products are consumed widely. Tomato fruit is a source of many beneficial products in human diet such as antioxidants, vitamins and minerals. Along with its economic importance, tomato is taken as a model plants to conduct studies on several aspects including, plant physiology, fruit development and plant genetics. Plant growth promoting rhizobacteria (PGPR) were first defined by Kloepper and Schroth (1978). PGPR mainly colonize plant roots and establish an ecological niche in rhizospheric vicinity (Lynch and Whipps, 1990). These beneficial bacterial microbes can act as biofertilizer or phyto-stimulant by various modes of actions e.g. nitrogen-fixation, stimulating the availability of minerals and secretion of phytohormones, such as auxin and gibberellins (Pingand Boland, 2004).

Plant's secondary metabolites are considered an important source of high-value chemicals that exhibit many important pharmacological properties. These compounds are difficult to synthesize chemically and are mainly isolated from natural biological sources. Tomato fruit is a source of protein, high-quality fiber, pigments, vitamins and also delivers bioactive secondary metabolites (Hanif, 2014). A significant number of these studies are available describing nutritional and medicinal properties of tomato plants and its fruit. Tomato fruit extracts have been previously shown to possess antimicrobial, antiviral and anticancer properties (Friedman *et al.*, 2005). Some phenolic acids of tomato fruits have antioxidant properties and can reduce cardiovascular diseases (Hertog *et al*., 1995). Some tomato fruit metabolites which are termed as stilbenes, play an important role in the prevention of atherosclerosis (Pace-Asciak *et al*., 1995) and carcinogenesis (Jang *et al*., 1997).

Plant metabolites are essential components of human diet along with the roles in plant physiological processes. Thus, there is an important biotechnological interest in increasing production of nutritionally and pharmacologically important biochemicals in tomato fruit and other crop plants to improve consumer health and organoleptic quality traits. Current study was conducted to explore the bio-fortification potential of *Pseudo-monas aeruginosa* PM12. For that purpose, tomato fruit metabolites bio-fortification was studied in tomato plants co-cultivated with this beneficial bacterial strain. In this study, GC/MS approach was implemented to discover extensive re-modulations in biosynthesis pathways leading to enhanced metabolites biosynthesis in tomato fruits. The data presented here is a valuable and a timely resource to fully capitalize for improving fruit nutrition quality by PGPR mediated bio-fortification process.

MATERIALS AND METHODS

Plant material: Tomato seeds of variety "Rio Grande" were purchased from market. These were surface sterilized by standard sodium hypochlorite method. Sterilized seeds were sown in 14-inch diameter plastic pots containing sterilized potting mix. Plants were kept in shade house and provided with distilled water whenever needed.

Bacterial treatment preparation: Bacteria stain *Pseudomonas aeruginosa* PM12 was isolated from tomato rhizosphere growing in vegetable garden of University of the Punjab Lahore, Pakistan. Bacterial cells were mass cultivated in LB broth media. After 24hour growth, bacterial cells were pelleted by centrifugation at 6000rpm for 20 minutes. Bacterial inoculum was prepared by re-suspending cells in distilled sterilized water and inoculum concentration was adjusted to 10^{7-8} cells/mL by taking Optical density of 1.0 at 600nm spectrophotometrically.

Greenhouse experiment: After 15 days of emergence, each pot was provided with 100mL of previously prepared bacterial inoculum by soil drench method. Plants were kept in shade house for incubation. After 60 days, harvest was taken upon fruit development. Plants were provided with distilled sterilized water whenever needed. Control plants were provided with 100 mL of distilled sterilized water. Five replicate pots were included in each treatment.

Sample preparation: Tomato fruits were peeled and pericarp area was separated for extraction procedure. Five mature fruits were selected from each treatment randomly for biological replication. Five gram of fruit pericarp tissues were used for extraction process. The peel was extracted with chloroform-methanol-water solvent system overnight at room temperature and ribitol was added as an internal standard. Supernatant was dried in clean GC/MS glass tubes. The dried material was derivatized by MOX and MSTFA reagents by adopting method of Roessner *et al*., (2006).

GC/MS analysis: GC/MS analysis was performed in Perkin Elmer GC/MS system according to method of Roessner-Tunali et al., (2003). One uL of sample volume was injected in GC HP-5MS capillary column for metabolite analysis. GC/MS temperature conditions were followed as adopted by Roessner-Tunali et al. (2003). Here helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. Turbomass system was used to deconvolute and to identify chromatographic peaks. Mass spectra library "NIST 2005" was used for comparison of fragmentation patterns of the obtained mass spectra.

Statistical analysis: The relative quantities of all the metabolites were calculated after normalizing the respective peak areas by the internal standard. All the data were analyzed by ANOVA and DNMRT by using computer aided software DSASTAT. Principal component analysis was performed by using online tool "Clustvis". Heatmap was also drawn by using "Clustvis" tool. Pathway analysis was performed by Excel soft-ware by manually adding the entries.

RESULTS

The comparative study of extracted metabolites identified more than 40 metabolites in tomato fruits comprising sugars, sugar alcohols, amino acids, organic acids, phenolics and flavonoids (Figure 1and 3).

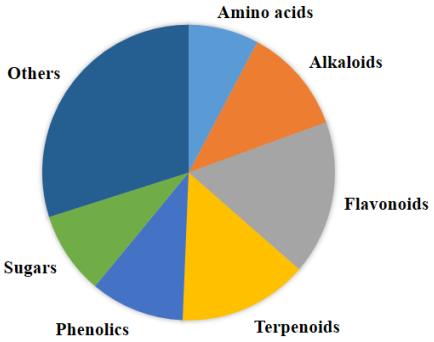

Figure 3. Category wise distribution of up-regulated biochemicals in fruits of tomato plants grown under influence of *Pseudomonas aeruginosa* PM12.

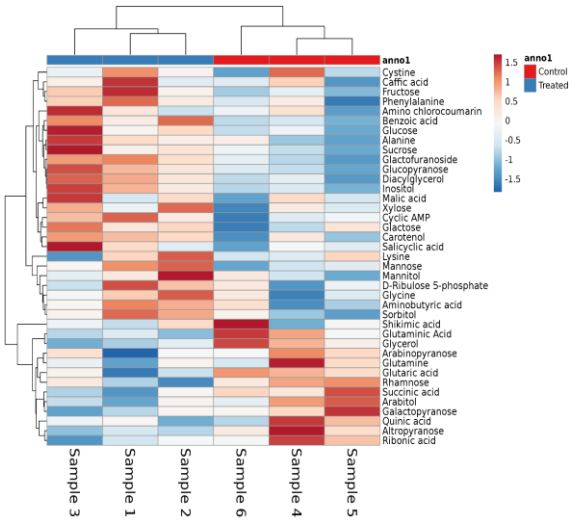

Fig. -4: Heatmap analysis of change in metabolite levels of fruits of tomato fruit plants cultivated in the presence (treated) and absence of *Pseudomonas aeruginosa* PM12 (control).

Relative quantities of metabolites were calculated by comparing with internal standard ribitol. For the ace of representation, GC/MS results were also presented in the form of heatmap, to describe changes in levels of different metabolites in the form of varying color scale (Figure 4).

Data presented here provided extensive positive re-modulatory potential of "*Pseudomonas aeruginosa*PM12" on metabolite profile of tomato fruits. In comparison to the control plants, several metabolites were significantly increased in fruits of tomato plants co-cultivated with *Pseudomonas aeruginosa* PM12 (Figure 1 and 3). Principal component analysis was applied on combined metabolite data set of both treatments (Figure 2). Here two distinctive cluster of both treatment data were observed representing statistical significance of treatment applications (Figure 2).

GC/MS analysis showed that sugars contents were significantly increased in tomato fruits under influence of bacterial inducer *Pseudomonas aeruginosa* PM12 (Figure 1). Among some significantly up-regulated sugars were included arabinopyranose, altropyranose, fructose, glactole, glucose and sucrose (Figure 1). Seemly in case of organic acids, aminoadipic acid, benzoic acid, glutaric acid nanoic acid, ribonic acid shikimic aicd were up-regulated significantly as compared to control plants (Figure 1). Same type of significantly up-regulated entries was seen among metabolites belonging to amino acids groups. Among some other up-regulated metabolites were belonging to alkaloids, flavonoids, terpenoids and phenolic groups (Figure 1 and 3).

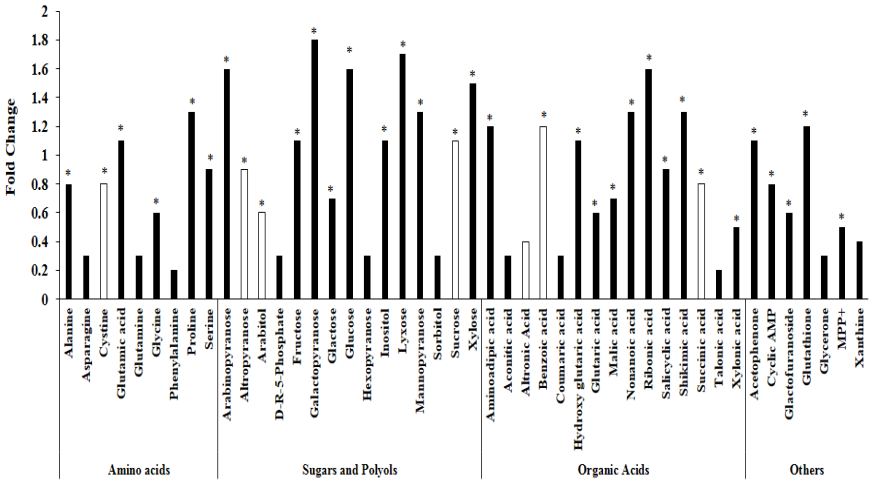

Figure 1. Changes in metabolite levels of fruits of tomato plants grown under influence of *Pseudomonas aeruginosa* PM12. Metabolite levels were normalized with internal standard and compared with fruits of control plants. (*) represents data differ significantly as governed by ANOVA at p=0.05.

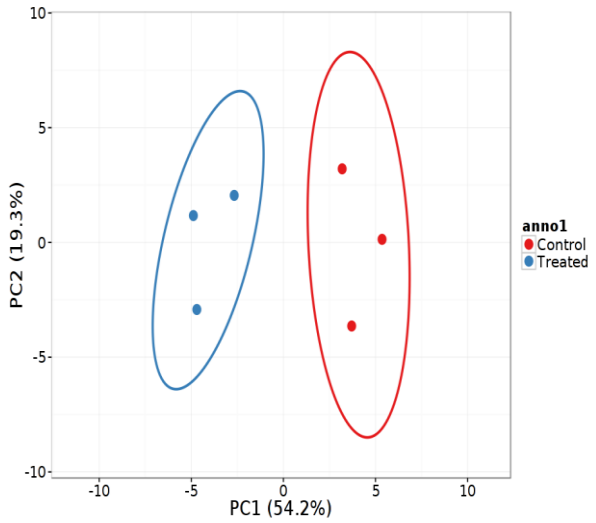

Figure-2: Differences between metabolic profiles of tomato fruit detected by principal component analysis (PCA). Metabolic profiles were obtained by GC/MS analysis of fruits of tomato plant co-cultivated under influence of *Pseudomonas aeruginosa* PM12 and control plants (n = 3).

Furthermore, GC/MS results were incorporated in plant metabolic pathway overview to provide evidence for increased production of these biochemicals. Biosynthetic routes were indicated with lines. Extensive re-programming was observed in tomato plants treated with bacterial inducer as compared to non-treated plants (Figure 5). Globally, precursors of sugar metabolism (sucrose, maltose, manitol, myo-inositol, fructose), TCA (malate, fumerate, succinate) and phenylpropenoid pathway (shikimic acid, tryptophane, tyrosine, phenylalanine) were significantly up-regulated (Figure 5).

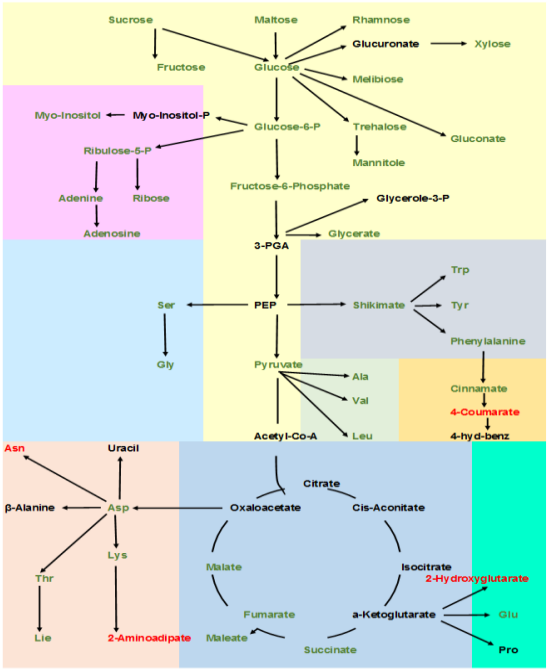

Fig.-5: Alterations in the metabolomics pathway as detected in the fruit extracts of tomato plants grown under influence of *Pseudomonas aeruginosa* PM12. Metabolites were quantified after normalizing with internal standard and compared with control treatment. Green and red colors represent significant up- and down-regulation respectively as governed by ANOVA.

DISCUSSIONS

Fruit ripening is a complex process resulting in intense changes in color, texture, flavor, and aroma of the fruit (Giavonnani, 2007). Owing to the medicinal, nutritional and economic importance of fruit commodities, the ripening process is continued to be extensively studied at the biochemical level. In the last couple of decades, an array of studies has been developed including procedures for the evaluation of changes in metabolomics. These types of analysis have enabled us a step ahead to utilize metabolite profile for improving crop quality. GC/MS is considered as a high-throughput technique for the study of metabolomics in plants.

Tomato fruits has a beneficial role in human health, therefore attention was focused on biofortification of fruit metabolites of tomato by using a PGPR bacterial strain. Tomato plants were cultivated in the presence of bacterial strain in the rhizoshpere. Upon ripening, whole metabolome of both treated and control plants was analyzed by GC/MS technique to track the changes induced by this bacterium in biochemical profile of tomato fruit. Chemicals were grouped in their respected classes and changes in their quantities were represented in different graphical ways. Here up-regulated metabolites were mainly belonging to sugars, sugar alcohols, alkaloids, terpinoids, flavonoids and organic acids. Along with that, the changes were incorporated in plant metabolic pathways to get an overview of coordinated re-programming responsible for alternation in levels of these biochemicals.

Plant sugars including monosaccharaides, polysaccharides and polyols are important components of human diet and have great drugs potential. These carbohydrates are essential components of natural products, important source of energy and of great therapeutic importance (Cao *et al*., 2011). Some glycosylated sugars are candidates of antimicrobial and anti-cancer drug candidates (Cao *et al*., 2011). In current investigation, numerous sugars and sugar alcohols were increased significantly in fruits of treated tomato plants as compared to control plants. These mainly included fructose, manitol, inositol, malibiose, fructose, galactose and glucose.

Likewise, sugars, carotenes and flavonoids are important health-promoting substances because of their anti-oxidative, anti-cancer, anti-diabetes and cardiovascular protective effects (Knekt *et al*., 2002; Ross and Kasum, 2002; Sato *et al*., 2008). In the same way, alkaloids are biochemical products of plants that present a variety of pharmacological and nutritional benefits to humans (Taveira*et al*., 2012; Choi*et al*., 2013). Alkaloids of carboline origin possess antimicrobial, anti-HIV and anti-parasitic activities (Bouayad*et al*., 2012). Here application of bacterial inducer significantly increased production of some carotenoids, flavonoids and steroidal alkaloidsin tomato fruits such as amino chlorocoumarin, carotenone and artanol. Phenolics are plant origin biochemicals composed of aromatic ring. Some polyphenolic compounds are therapeutic tools, used to cure inflammatory diseases, cardiovascular disorders, and diabetes (Raiola*et al*., 2014). These properties are impaired to these biochemicals due to their ability to interact with an array of cell signaling molecules in human and animals (Raiola*et al*., 2014). In current research work, some phenolic acids increased significantly including aminobutyric acid, benzoic acid, shikimic acid and quinic acid.

Lastly, GC/MS results were incorporated in plant metabolic pathways to get a real glimpse of re-programing responsible for alterations in these biochemicals. Data indicate that alterations in individual biochemicals could be an essential element for bacterial induced re-programing in biosynthesis of these fruit metabolites. Increased precursors activities of these pathways enhanced the accumulation of certain biochemicals observed in current research work. This is a logical assum-

ption that remarkably resulted in enhanced nutriational quality of tomato fruits under influence of a beneficial bacterial strain.

In conclusion, our results reported here shed some light on a novel approach of microbes mediated biofortification of food commodities. This approach will be useful for development of framework that would provide more detailed observation on biotechnological importance of beneficial microbes. This approach can be very useful for detection and increased recovery of medicinally important phytochemicals from plant commodities. It will also raise some new questions behind the mechanism of microbial mediated biofortification process.

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