Insights Into Role of STP13 in Sugar Driven Signaling That Leads to Decrease in Photosynthesis in Dicot Legume Crop Model (*Phaseolus Vulgaris* L.) Under Fe and Zn Stress.

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Abstract

Mineral (Fe/Zn) stress significantly affects fundamental metabolic and physiological responses in plants that results in reduction of plant growth and development. In the present study, common bean variety; Shalimar French Bean-1 (SFB-1) was used as an experimental material and were grown under *in vitro* condition on 4 different MGRL media i.e. normal MGRL medium (Control), MGRL without Fe (0-Fe), MGRL without Zinc (0-Zn) and MGRL with excess Zn (300-Zn) for 21 days under optimum conditions. Shoot and root tissue from all the treatments were harvested and further, subjected to estimation of total chlorophyll, total sugar and extraction of total RNA for differential gene expression of sugar transporter 13 (STP13). We observed significant decrease in total chlorophyll content in stressed plants. However, the concentration of total sugar and fold expression of STP13 gene was significantly higher in shoots of Fe/Zn stressed and in roots of 300-Zn plants. We observed higher accumulation of sugar under stress condition that correlated with high expression of sugar transporter 13 (STP 13). Further, we observed decrease in the chlorophyll content under stress conditions. Based on these findings, we propose the role of sugar driven signaling in decreasing photosynthesis in case of common bean. The decrease in photosynthesis is, confirmed by observing significant decrease in chlorophyll content in stressed plants.

Introduction

Mineral stress is affecting multiple vital functions in plant cells that are must for the growth and development of a plant. Among various abiotic stresses, mineral deficiency or toxicity is one of the major stress. Among minerals, iron (Fe) and Zinc (Zn) represent the most important micronutrients that are critical for plant growth and crop yields [1]. These micronutrients are directly involved in multiple metabolic pathways and physiological responses, catalyzes the reduction-oxidation reactions, chlorophyll synthesis and photosynthesis, cofactor of many enzymes, playing a key role in transcription and cellular functioning, as are critical for proper growth and development of plants [2]. Both Fe and Zn stress leads to chlorosis in plants that reduces photosynthetic activity [3, 4]. The impact of deficiency of these micronutrients are well characterized that includes deviations in protein synthesis, primary and secondary metabolism and carbohydrate partitioning between source and sink tissues resulting in the inhibition of photosynthesis [5]. Evidences also show that sugar accumulation in leaves play role in many physiological and metabolic processes that include metabolism and synthesis of important macromolecules, osmotic homeostasis, stabilization of membranes, carbon partitioning, seed germination, flowering, senescence, lower photosynthetic activity and induces chlorosis [6, 7]. In case of Arabidopsis, under mineral stress conditions sugars were found getting accumulated in the shoots due to reduction in phloem unloading in sink tissue that have affected the expression levels of proteins involved in photosynthesis as such decrease in the photosynthetic activity [8, 9]. In the present study, we focused on expression of STP13 under Fe/Zn stress and correlated it with photosynthetic activity. Here we used common bean variety SFB-1 as a dicot legume crop model.

Material Methods

In vitro growth of *Phaseolus vulgaris* L. plants

The experimental material used for this study was Shalimar French Bean-1 (SFB-1) variety. Seeds were surface-sterilized and were germinated on 4 different sterile MGRL media i.e. normal MGRL medium (Control), without Fe (0-Fe), without Zinc (0-Zn) and with 300 μM ZnSO_4 (300-Zn) i.e. excess Zn prepared by using minerals (3 mM KNO_3 , 2 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 1.75 mM $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 1.75 mM $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 1.5 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.5 mM K_2SO_4 , 67 μM $\text{Na}_2\text{-EDTA} \cdot 2\text{H}_2\text{O}$, 30 μM H_3BO_3 , 10.3 μM $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$, 8.6 μM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 130 nM $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, and 24 nM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 2.3 mM MES-KOH (pH 5.7), 1.0% (w/v) sucrose, and 1.2% purified agar [3] in phytajars. The seeds in phytajars were grown for 3 weeks under constant temperature ($24 \pm 2^\circ\text{C}$) and photoperiod (16 h light/8 h dark cycle). After 7 days cotyledons were cut and the plants were continued to grow for next 2 weeks. After 3 weeks growth, Chlorophyll content was estimated from shoots of plants grown under different conditions (normal, 0-Fe, 0-Zn and Excess Zn). Further, total RNA was also

extracted and total sugars were measured from the shoots and roots of the plants grown under all 4 conditions as detailed above.

Estimation of chlorophyll

Chlorophyll content was estimated from shoots of plants grown under different conditions (normal, 0-Fe, 0-Zn and 300 Zn) by 100% dimethyl formamide method [10, 11, 12]. The total chlorophyll content was calculated by the following Arnon's formula.

Estimation of total sugars

Total sugars were estimated by Anthrone method [13, 14] for both tissues root and shoot of the plants grown under different conditions (normal, 0-Fe, 0-Zn and 300 Zn). Optical density of samples was measured at 620 nm by spectrophotometer (Thermoscientific). The content of soluble sugar is calculated according the standard curve.

RNA extraction and quantification

Total RNA was extracted from both shoot and root tissues of of SFB-1 grown *in vitro* under 4 different conditions as detailed above, using Trizol method as per the manufacturer's instructions. The quantity and quality of isolated RNA was checked at 260 and 280 nm with Nanodroplite (Thermoscientific) and UV–Visible spectrophotometer (Thermoscientific). Prior to reverse transcription, isolated total RNA samples were run on a 1% agarose gel. To rule out DNA contamination DNase treatment was given by using DNase kit (Sigma Aldrich, USA).

cDNA synthesis and amplification of STP13 gene

cDNA synthesis was performed with equal concentration of RNA (1 µg) in all the samples using Thermo Scientific RevertAid First Strand cDNA Synthesis Kit using oligo dT primers as per manufacturer instruction. The integrity of cDNA synthesis was checked by conventional PCR using cDNA as template and Actin and Tubulin as primers that have amplified 175 bp and 120 bp gene fragments respectively. For the validation of STP13 gene, cDNA as template and the forward and reverse primers of STP13 given in **Table 1** were used that have amplified 135bp amplicon by PCR based approach. The amplified PCR product was electrophoretically separated on 2% agarose gel. The primers sequences of all genes are given in **Table 1**. Reported primers were used for Actin gene [15] and primers used for Tubulin and STP13 were designed by using PRIMER 3 Plus program.

Relative quantification by qRT-PCR

Total RNA was extracted from both shoot and root tissues of plants grown under 4 different conditions (control, 0-Fe, 0-Zn, 300Zn). cDNA was synthesized as mentioned above. Actin and Tubulin was used as internal control for normalization of all the reactions. Primers used for normalization (ACT-F, ACT-R and TUBF, TUBR) and expression analysis of STP13 gene (STP13-F and STP13-R) are given in Table 1. qRT-PCR reactions were performed on a LightCycler 480 (Roche) using KAPA SYBR® FAST qPCR Master Mix kit as per manufactures instruction. Amplicon dissociation curve was also recorded at the end of the PCR cycles. All reactions were run in triplicate and repeated twice. Relative expression of gene was analyzed by using Livak and Schmittgen method [16].

Statistical analysis

The results obtained are the mean values of 12 and 6 biological replicates for biochemical and expression analysis respectively each with three technical replicates for all four treatments. The data was analysed using one-way analysis of variance (ANOVA) followed by post hoc test (Multiple Comparisons) using SAS software (statistical analysis software institute, Cary, NC, USA) to analyze significant differences among multiple samples at 0.05 levels.

Results And Discussion

We observed higher expression of sugar transporter 13 (STP 13) in shoots of plants (**Table 2 & Supplementary Fig 1**) grown under 0-Fe (4.826 fold), 0-Zn (7.9 fold) and 300Zn (8.503 fold) stress conditions and in roots of plants grown under 300-Zn (7.295 fold) compared to control (1.000 fold). Further, higher expression of STP 13 in plants under stress conditions, correlated with higher accumulation of sugar in shoots for 300-Zn ($3.322 \mu\text{M g}^{-1}$) 0-Fe ($2.837 \mu\text{M g}^{-1}$) and 0-Zn ($2.466 \mu\text{M g}^{-1}$) treatments (**Supplementary Table 1 & Supplementary Fig 1**). This gives insights about the role of sugar driven signaling in decreasing photosynthesis which was further, confirmed by observing significant decrease in chlorophyll content in leaves of plants grown in 0-Fe ($176.970 \mu\text{g gFW}^{-1}$), 0-Zn ($186.330 \mu\text{g gFW}^{-1}$) and 300-Zn ($93.590 \mu\text{g gFW}^{-1}$) (**Supplementary Table 1 & Supplementary Fig 1**) conditions compared to control ($262.070 \mu\text{g gFW}^{-1}$). We observed that under Fe/Zn stress condition, there was higher expression of sugar transporter STP 13 as well as higher sugar concentration in shoots. As such, Fe/Zn stress leads to accumulation of sugars in shoots, as synthesis and utilization of these sugars were not properly managed. This study revealed that the regulation of STP 13 expression, total sugar and total chlorophyll content are interrelated and enhances our current understanding of STP 13 function in *Phaseolus vulgaris* under Fe/Zn stress conditions. STP 13 is known to have its role in source and sink tissues and is transcriptionally regulated by many abiotic or biotic stresses, like wounding, drought, high salinity, pathogens, or programmed cell death [8, 17, 18, 19] and mineral stress. Zargar et al. [3] observed higher expression levels of STP 13 levels in shoots of Arabidopsis under Fe-deficient conditions. We also found higher expression of STP 13 gene under excess Zn (300 Zn) conditions in roots of *Phaseolus vulgaris* because under extreme conditions like heavy metal stress root glycolytic [20] and fermentation [21] activities are enhanced to degrade starch into sugars in plants as higher sugar levels are required to maintain fundamental metabolic processes and STP 13 is involved in the distribution of these sugars in root cells [19]. STP 13 was also found to mediate the uptake of monosaccharide like glucose, mannose, galactose and fructose at a significant rate [18] in roots of Arabidopsis under high salinity and is the only osmotic- and salt-stress inducible gene [19]. These monosaccharides act as a signaling molecule in response to many biotic and abiotic stresses in plants [7]. These results suggests that under Fe/Zn stress conditions higher expression of sugar transporter 13 might have a role in sugar accumulation in shoots to maintain fundamental processes. Further, Fe/Zn stress conditions might interfere in the export of sugars in sink tissues due to which sugars get accumulated in the shoots. This sugar accumulation in shoots due to Fe/Zn stress conditions in turn suppresses the expression levels of genes involved in photosynthesis. Moreover, sugars might act as a signaling molecule to decrease the photosynthetic activity in plants under stress conditions. This study suggests, cells under Fe/Zn stress conditions induces sugar signaling involved in the inhibition of photosynthesis, which might have role in decreasing expression of proteins involved in photosynthesis [8]. However, it is worth mentioning that the expression of genes involved in photosynthesis under all these conditions, needs to be studied for further validation.

Conclusion

Mineral stress conditions lead to decrease in photosynthesis by inducing sugar signaling, which might have role in decreasing expression of proteins involved in photosynthesis. Substantial amount of research will be required to study and link various physiological and biochemical processes with sugar signaling and regulation of genes involved in functions of various enzymes; carbohydrate metabolism, transport and partitioning. Therefore, it is believed that further extensive studies on accumulation of sugars to confer tolerance against the mineral stress by modulating several physiological processes is an emerging field of research to be explored.

Declarations

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Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Osmolovskaya, N., Shumilina, J., Bureiko, K., Chantseva, V., Bilova, T., Kuchaeva, L., Laman, N., Wessjohann, L.A., & Frolov, A. (2019). Ion Homeostasis Response to Nutrient-Deficiency Stress in Plants. In Cell Growth (Vikas B and Michael Fasullo M.,ed.), IntechOpen, doi: 10.5772/intechopen.89398.
2. Yruela, I. (2013). Transition metals in plant photosynthesis. *METALLOMICS* 5, 1090-1109.
3. Zargar, S.M., Kurata, R., Inaba, S., Oikawa, A., Fukui, R., Ogata, Y., Agrawal, G.K., Rakwal, R., & Fukao, Y. (2015a). Quantitative proteomics of Arabidopsis shoot microsomal proteins reveals a cross talk between excess Zinc and Iron deficiency. *Proteomics* 0,1-6.
4. Hafeez, B., Khanif, Y.M., & Saleem, M. (2013). Role of Zinc in Plant Nutrition- A Review. *American Journal of Experimental Agriculture* 3(2), 374-391.
5. Hermans, C., Hammond, J.P., White, P.J., & Verbruggen, N. (2006). How do plants respond to nutrient shortage by biomass allocation. *Trends in Plant Science* 11, 610-617.
6. Shi, H., Wang, B., Yang, P., Li, Y., & Miao, F. (2016). Differences in Sugar Accumulation and Mobilization between Sequential and Non-Sequential Senescence Wheat Cultivars under Natural and Drought. *Plos One* 11(11), e0166155.
7. Sami, F., Yusuf, M., Faizan, M., Faraz, A., & Hayat, S. (2016). Role of sugars under abiotic stress. *Plant Physiology and Biochemistry* 109,54-61.
8. Zargar, S.M., Agrawal, G.K., Rakwal, R., & Fukao, Y. (2015b). Quantitative proteomics reveals role of sugar in decreasing photosynthetic activity due to Fe deficiency. *Frontiers in Plant Science* 6,592.
9. Lemonnier, P., Gaillard, C., Veillet, F., Verbeke, J., Lemoine, R., Coutos-Thevenot, P., & Camera, S.L. (2014). Expression of Arabidopsis sugar transport protein STP13 differentially affects glucose transport activity and basal resistance to Botrytis cinerea. *Plant Molecular Biology* 85,473-484.
10. Sibley, J.L., Eakes, D.J., Gilliam, C.H., Keever, G.J., Dozier, W.A., & Himelrick, D.G. (1996). Foliar SPAD-502 meter values, nitrogen levels, and extractable chlorophyll for red maple selections. *Hortscience* 31(3), 468-470.
11. Inskeep, W.P., & Bloom, P.R. (1985). Extinction coefficients of chlorophyll a and B in n, n-dimethylformamide and 80% acetone. *Plant Physiology* 77(2), 483-485.
12. Aono, M., Kubo, A., Saji, H., Tanaka, K., & Kondo, N. (1993). Enhanced tolerance to photooxidative stress of transgenic *Nicotiana tabacum* with high chloroplastic glutathione reductase activity. *Plant and Cell Physiology* 34(1), 129-135.
13. Mandre, M., Tullus, H., & Kloseiko, J. (2002). Partitioning of carbohydrates and biomass of needles in Scots pine canopy. *Zeitschrift für Naturforschung* 57(3-4), 296-302.
14. Jin, Z.M., Wang, C.H., Liu, Z.P., & Gong, W.J. (2007). Physiological and ecological characters studies on Aloe vera under soil salinity and seawater irrigation. *Process Biochemistry* 42 (4), 710-714.
15. Chen, J.B., Wang, S.M., Jing, R.L., & Mao, X.G. (2009). Cloning the PvP5CS gene from common bean (*Phaseolus vulgaris*) and its expression patterns under abiotic stresses. *Journal of Plant Physiology* 166, 12-19.
16. Livak, K.J., & Schmittgen, T.D. (2001). Analysis of relative gene expression data using realtime quantitative PCR and the 2(-Delta Delta C (T)) Method. *Methods* 25(4), 402-408.
17. Buttner, M. (2010). The Arabidopsis sugar transporter (AtSTP) family: an update. *Plant Biology* 12(1), 35-41.
18. Norholm, M.H., Nour-Eldin, H.H., Brodersen, P., Mundy, J., & Halkier, B.A. (2006). Expression of the Arabidopsis high-affinity hexose transporter STP13 correlates with programmed cell death. *FEBS Letters* 580(9), 2381-2387.

19. Yamada, K., Kanai, M., Osakabe, Y., Ohiraki, H., Shinozaki, K., & Yamaguchi-Shinozaki K (2011). Monosaccharide absorption activity of Arabidopsis roots depends on expression profiles of transporter genes under high salinity conditions. *Journal of Biological Chemistry* 286, 43577–43586.
20. Jelali, N., Wissal, M.S., Dellorto, M., Abdelly, C., Gharsalli, M., & Zocchi, G. (2010). Changes of metabolic responses to direct and induced Fe deficiency of two *Pisum sativum* cultivars. *Environmental and experimental botany* 68, 238–246.
21. Thimm, O., Essigmann, B., Kloska, S., Altmann, T., & Buckhout, T.J. (2001). Response of Arabidopsis to iron deficiency stress as revealed by microarray analysis. *Plant Physiology* 127, 1030–1043.
22. Urwat, U., Zargar, S.M., Manzoor, M., Ahmad, S.M., Ganai, N.A., Murtaza, I., Khan, I., Nehvi, F.A. (2019). Morphological and biochemistry responses of *Phaseolus vulgaris* L. to mineral stress under in-vitro conditions. *Vegetos* 32, 431-438

Tables

Table 1: List of designed primers

S.No.	Primer	Primer sequence (5'→3')	Tm °C	Size of amplicon	Gene Accession No.	Reference
1.	ACTF	GAAGTTCTCTTCCAACCATCC	61.5 °C	175bp	KF033666.1	Chen et al. 2009
	ACTR	TTTCCTTGCTCATTCTGTCCG	66.3 °C			
2.	TUBF	TCGTGGGTTTCAGCAGTAT	56.71 °C	120bp	KF569615.1	This study
	TUBR	GCAAAGGCAGTCAAGTATCG	55.96 °C			
3.	STP13F	ACACCCCCAACAGTCTCATC	56.9 °C	135bp	MK911717.1	This study
	STP13R	TGACCTCTTTAGCCACACGA	55.9 °C			

Tm = Melting Temperature. °C = Degree Celsius. ACT = Actin. TUB = Tubulin. STP13 = Sugar transporter 13

Table 2: Differential expression of STP13 gene in response to Fe/Zn stress in *Phaseolus vulgaris* L.

Treatment	SHOOT				ROOT			
	Mean Ct	ΔCt	ΔΔCt	2 ^{-ΔΔCt}	Mean Ct	ΔCt	ΔΔCt	2 ^{-ΔΔCt}
Control	24.440 ^a ±0.465	4.640 ^a ±0.465	0.000	1.000	23.262 ^a ±0.431	1.262 ^a ±0.431	0.000	1.000
0-Fe	22.169 ^b ±0.284	2.369 ^b ±0.284	-2.271	4.826	24.524 ^b ±0.199	2.524 ^b ±0.199	1.262	0.417
0-Zn	21.458 ^b ±0.206	1.658 ^b ±0.206	-2.982	7.9	26.397 ^c ±0.300	4.397 ^c ±0.300	3.135	0.114
300-Zn	21.352 ^b ±0.073	1.552 ^b ±0.073	-3.088	8.503	20.395 ^d ±0.275	-1.605 ^d ±0.275	-2.867	7.295

The results are means of six biological replicates with three technical replicate for each treatment and are presented as mean±SE. Different superscript (a,b,c,d) denotes significant differences among treatments (P ≤ 0.05).

Supplementary Files

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