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. Deficiency of these micronutrients leads to inhibition of photosynthesis by having impact on various crucial biological processes like protein synthesis, primary and secondary metabolism and carbohydrate partitioning between source and sink tissues. In the present study, common bean variety Shalimar French Bean-1 (SFB-1) plants were used as an experimental material and were grown under in vitro condition on four 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 tissues 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 samples harvested from mineral stress 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.

Keywords Sugar signaling · Photosynthesis · *Phaseolus vulgaris* L. · In vitro · Iron · Zinc

Introduction
Mineral stress affects multiple vital functions in plant cells that are crucial 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, catalyze the reduction–oxidation reactions, chlorophyll synthesis and photosynthesis, they are cofactor of many enzymes, playing a key role in transcription and cellular functioning, and are critical for proper growth and development of plants [2]. Both Fe and Zn deficiency leads to chlorosis in plants that reduces photosynthetic activity [3, 4]. The impact of deficiency of these micronutrients leads to 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 a role in many physiological and metabolic processes that include 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 thus causing a 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. STP 13 which is a sugar transporter, was selected based on our earlier evidences of observing higher accumulation of sugar in plant tissues under mineral stress compared to plants grown under normal condition (MGRL media). Further, in the present study we selected a released common bean variety Shalimar French Bean-1 (SFB-1) as a dicot legume crop model.

Materials and methods

In vitro growth of Phaseolus vulgaris L. plants

The experimental material used for this study was SFB-1 variety of common bean. Seeds were surface-sterilized and were germinated on four different sterile MGRL media i.e. normal MGRL medium (Control), without Fe (0-Fe), without Zinc (0-Zn) and with 300 μM ZnSO₄ (300-Zn) i.e. excess Zn prepared by using minerals 3 mM KNO₃, 2 mM Ca(NO₃)₂·4H₂O, 1.75 mM NaH₂PO₄·2H₂O, 1.75 mM Na₂HPO₄·12H₂O, 1.5 mM MgSO₄·7H₂O, 1.5 mM K₂SO₄, 67 μM Na₂-EDTA·2H₂O, 30 μM H₂BO₃, 1.5 mM MnSO₄·5H₂O, 8.6 μM FeSO₄·7H₂O, 1 μM CuSO₄·5H₂O, 130 mM CaCl₂·6H₂O, and 24 mM (NH₄)₆Mo₇O₂₄·4H₂O, 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 ± 2 °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 in 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 four conditions as detailed above.

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 (Thermo scientific). 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 SFB-1 grown in vitro under four 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 (Thermo scientific). 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 135 bp amplicon by PCR based approach. The amplified PCR product was electrophoretically separated on 2% agarose gel. The primer 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 four 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 manufacturer’s 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 300-Zn (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 μM/g) 0-Fe (2.837 μM/g) and 0-Zn (2.466 μM/g) 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 μg gF/W), 0-Zn (186.330 μg gF/W) and 300-Zn (93.590 μg gF/W) (Supplementary Table 1 & Supplementary Fig. 1) conditions compared to control (262.070 μg gF/W). 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 regulated. This study revealed that the regulation of STP 13 expression, total sugar and total chlorophyll content are interrelated and enhances our current understanding of STP13 function in *Phaseolus vulgaris* under Fe/Zn stress conditions.

STP13 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, 17–19] and mineral stress. Zargar et al. [3] observed higher expression levels of STP13 levels in shoots of *Arabidopsis* under Fe-deficient conditions. We also found higher expression of STP13 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.

### Table 1 List of designed primers

| S. No. | Primer | Primer sequence (5′ → 3′) | Tm (°C) | Size of amplicon (bp) | Gene Accession No. | References |
|--------|--------|---------------------------|---------|----------------------|--------------------|------------|
| 1      | ACTF   | GAAGTTCTCTTCCAACCATCC     | 61.5    | 175                  | KF033666.1         | Chen et al. [15] |
|        | ACTR   | TTTCTGCTTCATTTCTGTCG      | 66.3    |                      |                    |            |
| 2      | TUBF   | TCGTGGTTTGCAGCTATAT      | 56.71   | 120                  | KF569615.1         | This study  |
|        | TUBR   | GCAAAAGCAGTCAGTATCG      | 55.96   |                      |                    |            |
| 3      | STP13F | ACACCCCAAGACGTCTCAC        | 56.9    | 135                  | MK911717.1         | This study  |
|        | STP13R | TGACCTCTTTAGCCACAGA       | 55.9    |                      |                    |            |

*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±0.465 | 4.640±0.465 | 0.000 | 1.000 | 23.262±0.431 | 1.262±0.431 | 0.000 | 1.000 |
| 0-Fe      | 22.169±0.284 | 2.369±0.284 | –2.271 | 4.826 | 24.524±0.199 | 2.524±0.199 | 1.262 | 0.417 |
| 0-Zn      | 21.458±0.206 | 1.658±0.206 | –2.982 | 7.9 | 26.397±0.300 | 4.397±0.300 | 3.135 | 0.114 |
| 300-Zn    | 21.352±0.073 | 1.552±0.073 | –3.088 | 8.503 | 20.395±0.275 | –1.605±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)
to degrade starch into sugars in plants as higher sugar levels are required to maintain fundamental metabolic processes and STP13 is involved in the distribution of these sugars in root cells [19]. STP13 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 suggest 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 that decreases the photosynthetic activity in plants under stress conditions. This study suggests, that Fe/Zn stress conditions induces sugar signaling which might have a 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.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-021-06295-z.

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Author contributions UU: Did experimental work and wrote first draft of the manuscript. SMZ: Conceived the idea, guided in all experimental work and finalized the draft. SMA: Guided in expression analysis and edited the final manuscript. ANG: supervised all the research and helped in bioinformatics work.

Declarations

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

Consent to participate All authors had agreed to participate.

Consent to publish All authors had agreed to publish the work presented in the manuscript.

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