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Enzymatically hydrolysed sago bagasse improves physiological, biochemical and molecular attributes of *Solanum lycopersicum*

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ABSTRACT

The present study was aimed to investigate the plant biostimulant properties of sago bagasse (SB), an abundant by-product of sago industry. Sago bagasse hydrolysate (SBH) was produced by enzymatic hydrolysis and characterized by X-ray powder diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), particle size analyzer and Scanning electron microscopy (SEM) analysis. Further, plant growth promoting ability of the SBH was confirmed by seed germination and greenhouse experiments. In seed germination experiment, SBH treatment registered improved seed germination traits and also accelerated protein (3.13%) and sugar (9.53%) content as compared to control. Moreover, SBH treatment significantly increase expression of carbon assimilating enzymes like malate dehydrogenase (5.0%), citrate synthase (11.47%) isocitrate dehydrogenase (8.08%). Similarly, nitrogen assimilating enzymes such as nitrate reductase (15.14%) and glutamate synthase (10.98%) were also higher in SBH the treated plants. In addition, qPCR analysis demonstrated the efficiency of SBH by up-regulating carbon and nitrogen assimilating genes responsible for plant growth. Thus, the present finding strongly suggest that SBH with plant growth promoting properties could be utilized for the agricultural productivity as a low-cost ecofriendly biofertilizer.

1. Introduction

Over the past decade, chemical fertilizers are widely used in agriculture and this has resulted in environmental pollution and food security issues. Extensive use of chemical fertilizers causes soil pollution and their residues are toxic to all form of living organisms. Perhaps, it may be reduced by an alternative bio-product known as plant biostimulant (Calvo et al., 2014). 'Plant biostimulant' is defined as the active substances used to stimulate plant growth, yield and quality, regardless of its nutrient content. Due to their multi-spectrum activity, biostimulants are gaining more attention and its global market expected to increase by $ 2241 million by 2018 (Anonymous, 2013). Biostimulants are largely produced from the protein-rich agro-industrial by-products, legume seeds and vegetable substrates, which has the ability to increase seed germination, productivity and quality of various plants when applied in the small quantities. About 60 bioactive substances have been named as biostimulants, including seaweed extract, chitosan, amino acids, and peptides (Jardin, 2015; Kauffman et al., 2007; Kolomaznik et al., 2012).

However, only a few carbohydrates have been recognized for plant growth promoting activities. Hence, extending the evaluation of other polysaccharides might kindle various features of plant growth promotion. The Role of carbohydrate on plant growth and development are diverse; carbohydrates regulate cell volume increase, cell division, and developmental based on the plant energy status (Rolland et al., 2006). Generally, adequate carbohydrate content might regulate various metabolic processes, consequently, it increase overall plant growth, development and defence responses. Hence, incorporating sugars in agricultural formulations provide new opportunities for improving general plant growth, biomass accumulation and plant yield. Based on the above reasons, in our study Sago bagasse (SB), a by-product from sago industry was preferred over the other agro- industrial waste due to its well balanced nutritional composition, carbon acquisition efficiency on plants and its easy availability. To the best of our knowledge, SB has

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never been evaluated for plant biostimulant activity. Based on the facts stated above, our present study aimed to produce eco-friendly biostimulant from SB through enzymatic hydrolysis and evaluate its plant growth promoting ability with *S. lycopersicum* (as a test crop).

2. Materials and methods

2.1. Chemicals and reagent

All the required basic chemicals used in this study were of analytical grade and obtained from HiMedia, Merck (India). For qRT-PCR analysis, cDNA conversion kit was optioned from Takara (Takara Bio India Pvt. Ltd) and QuantiNova SYBR Green PCR kit was purchased from QIAGEN (USA). Sago bagasse was obtained from Sago serve, Salem, Tamil Nadu, India. Commercial plant biosurfactant was gifted by the Victas Laboratory Pvt. Ltd, Coimbatore, Tamil Nadu, India.

2.2. Enzymatic hydrolysis of SB

Sago starch (1%) was prepared in water and the pH was adjusted to 7.0 using sodium acetate buffer. Then the dispersion was sterilized and 0.08 g (2000 U) of amylase enzyme was added. Enzymatic hydrolysis was performed at 42 °C for 3 h and samples were collected at each 1 h interval. Eventually, the amylase enzyme activity was stopped by heating the suspension at 80 °C for 20 min. Based on the hydrolysis time, sago bagasse hydrolysates have been named as SBH1 (1 h), SBH2 (2 h) and SBH3 (3 h), respectively.

2.3. Physiochemical characterization

For characterization studies, SB and SBH's were lyophilized at a pressure of 4000 mbar (Lyodel, Delvac Pumps India) under −40 °C for 12 h. For FTIR analysis, SB and SBH's were scanned between 500 and 4000 cm^{-1} at a resolution of 4 cm^{-1} in a Bruker Tensor FT-IR spectrometer (TENSOR II Flyer, India) (Pal et al., 2008). X-ray diffraction patterns were performed using CuKα radiation for 5–70° (2θ) at a step scan rate of 0.02° θ s^{-1} (Jiang et al., 2011). Particle size distribution of the SB and SBH's were characterized using an integrated laser light scattering instrument (Malvern Mastersizer MSS, Malvern Instruments Ltd., U.K). Relative refractive index and absorption were set to be 1.38 and 0.100, respectively. The morphological changes after the enzymatic hydrolysis were investigated using SEM for SB and SBH3 (Final hydrolyzate). Further, SB and SBH's Carbohydrate content was quantified by the method of Miller (1959); Starch content was quantified Chrastil (1987) method. Organic content and protein content of SB and SBH's were estimated by De Nicola et al. (2013).

2.4. Seed germination and greenhouse study

For seed germination experiment, SBH3 was considered as 100% formulation and it was diluted to 0.5%, 1% and 1.5% using sterile distilled water and subjected for seed germination experiment with *S. lycopersicum* seeds (PKM). Healthy and uniform size seeds were placed evenly in petri dish lined with cotton and whatman No.1 filter paper. About 15 ml SBH dilutions was added to each petri dish and subsequently sealed with para-film. Control seeds were treated with sterile distilled water. After seven days of treatment, seedling were analyzed for their morphology, germination percentage (GP), germination index (GI) and vigor index (VI) (Guan et al., 2009).

The root hairs were observed by toluidine blue staining under the light microscope as suggested by D'Haeze et al. (2000). The roots were excised from *S. lycopersicum* and placed on the clean slide and stained with toluidine blue (0.05%) for 1 min. The excess stains were removed by washing and tissues observed under a light microscope (Olympus) at 50X magnification.

2.5. Greenhouse experiment

Further, biostimulant efficiency of SBH was studied in a greenhouse condition (28–36 °C for day-time and 20–28 °C for night-time with 16 h/8 h light/dark). One-month-old *S. lycopersicum* seedlings were selected and replanted in the pot containing garden soil, then allowed to grow for 7 days at greenhouse condition for acclimatization. After acclimatization, plants were treated with SBH3 at 5%, 10% and 15% through foliar application along with a surfactant. Control plants were sprayed with a mixture of water and surfactant equivalent to the final volume. A surfactant was used for the uniform spreading of SBH on plant foliage. After 7 DAT (day after treatment), seedlings were harvested and subjected for further studies.

2.6. Leaf photosynthetic pigments, protein and starch quantification

Total chlorophyll and carotenoid content were quantified as described by Kupper et al. (2000). Leaf soluble protein content was quantified as described by Bradford (1976) using bovine serum albumin (BSA) as standard. Moreover, leaf starch contents were estimated by both quantitative (McCready et al., 1950) and qualitative (Sehnke et al., 2001) analysis.

2.7. Measurement of nitrogen assimilating enzymes

In order to estimate the expression pattern of nitrogen assimilating enzymes, 200 mg of leaves were homogenized with 5 mM $MgCl_2$, 100 mM Hepes-NaOH pH 7.5 and 1 mM dithiothreitol (DTT). Extracts were filtered and then centrifuged under 4 °C at 20,000 rpm for 15 min. The supernatant was collected and subjected for Nitrate reductase (NR; EC 1.6.6.1) and Glutamine synthase (GS; EC 6.3.1.2) enzyme estimation (Schiavon et al., 2008).

2.8. Measurement of Carbon assimilating enzymes

For carbon assimilating enzyme estimation, about 200 mg leaves were grounded using 100 mM Tris HCl buffer (pH 8.2), 5 mM b-mercaptoethanol, 1 mM Na_2 EDTA, and 10% glycerol. Leaf homogenates were centrifuged and supernatants were subjected for Citrate synthase (CS EC 1.11.1.6), Isocitrate dehydrogenase (EC 1.1.1.42) and malate dehydrogenase (1.1.1.37) enzyme estimations as described by Schiavon et al. (2008).

2.9. qRT-PCR analysis

Total RNA was extracted using TRIzol reagent and the residual DNA was removed by DNase I (Himedia) treatment. Integrity of RNA samples were verified by 1.5% gel and the quantity was measured using NanoDrop 1000 (Thermo Scientific). An aliquot of 5 µg of RNA was used to synthesis cDNA with a Primscript RT Reagent kit (Takara). qRT-PCR experiment was performed in ABI step one plus thermal cycler using QuantiNova SYBR Green PCR kit (QIAGEN) with following temperature profile, heat activation 95 °C for 2 min, followed by 40 cycles of 95 °C for 30 s, 52 °C for 20 s and 72 °C 30 s. Relative transcript abundance was calculated using the $2^{-\Delta\Delta Ct}$ method and expression was normalized by *GAPDH* (Glyceraldehyde-3-phosphate dehydrogenase) gene as an internal standard. Primers used in the qRT-PCR were listed (Table 1).

2.10. Statistical analysis

All the experiments were performed in triplicate (n = 3) and the data were recorded. Seed germination data was analyzed by the Kruskal-Wallis test, plant growth promoting activities as well as greenhouse experiments were analyzed by one-way ANOVA and means were compared with the Tukey's test, using the SPSS software (version

Table 1
List of primers used in the q-PCR study.

S.no	Gene	Primer
1.	ICDH	F: CGCAAGCAAATACCCTGGGA R: CACATTGCCTCCTGGCATAAC
2.	MDH	F: CCAGCAAAGAACTTCCACGC R: ACACCACCTCTCTTTTGGACTT
3.	NR	F: GCGGAAGCTTGGTGGTACAAA R: TCGAGTGACCAAAAGCACCA
4.	CS	F: GTGTTGGTCTCGAGGGTTCA R: GTTGGGAGCCTTGTGGAAGT
5.	GS	F: CCACTTAGTTGGTTAGGAGGTGA R: AGCAGCTGTTCCACTCTGTT
6.	GAPDH	F: CAGCTCATTTGAAGGGTGGC R: TCAACGGTCTTCTGAGTGGC

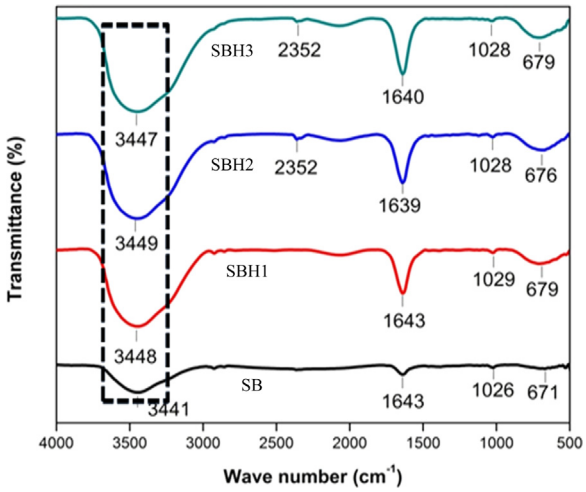

Fig. 1. FTIR spectra of SB and SBH1, SBH2 and SBH3. The selected regions in the FTIR spectra representing the OH stretch variation between SB and SBH's (SBH1 to SBH3).

20, SPSS Inc., www. spss.com).

3. Results and discussion

The FT-IR spectra of native SB and SBH's (SBH1, SBH2, SBH3) have been exhibited in Fig. 1. A broad peak in SB and SBH has been observed at 3441 cm^{-1} corresponds to the hydroxyl group. This peak has also been observed in hydrolyzed SBH's with slight variation in wave number. Intensity of these peaks was gradually increased with the increased hydrolysis time. This makes clear that the hydrolysis of SB led to produce more free OH groups. Additionally, the peak at 2352 cm^{-1} was exhibited only in the SBH2 and SBH3, which is attributed to the CH stretch. Thus, it may postulate that the addition of amylase displayed both OH and CH stretching in their spectra, which may indicate the starch fragmentation. The peak intensity has increased in the region of 1026 cm^{-1} during the enzymatic hydrolysis attributed to the C-OH stretch. The same trend of FTIR spectra was observed in the starch constitution by Kacurakova and Wilson (2001). Peak from 1639 to 1643 cm^{-1} might probably a feature of the water molecule in the starch. The major FTIR peaks like 3441, 1643, 1026 and 671 cm^{-1} were consequently increased when increasing the hydrolysis time. In overall FTIR analysis, SBH not exhibited any new wave numbers compared to the SB. Hence, it has been proposed that amylase enzyme increased the starch degradation ratio without affecting any other functionalities of SBH.

In X-ray diffraction patterns studies, native SB exhibited diffraction peaks at 2θ angle (22.8°, 15.2° and 17.4°) representing a typical A-type crystalline arrangement (Fig. 2). The relative crystallinity of SBH1,

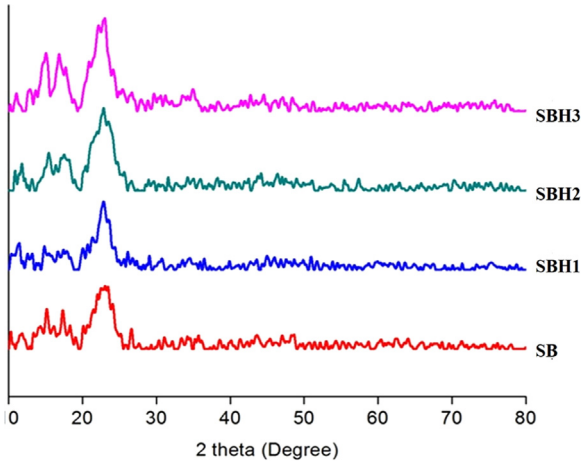

Fig. 2. X- Ray diffraction pattern of SB and SBH1, SBH2 and SBH3.

SBH2 and SBH3 were increased based on the hydrolysis time. The increased crystallinity of SBH's reflects the fact that, enzymatic hydrolysis influenced the degree of crystallinity. This could be justified as, the hydrolysis of SB producing terminal carboxylate and quaternary ammonium ions. Thus, compared with the SB, ionic nature of the SBH's was greater as a consequence of increasing polarity. Therefore the increased peak intensity of SBH's has been considered as a vital support for the preferential hydrolysis of starch on the starch domains (inter and intra chain hydrogen bonds in the starch). These crystalline modifications may expect to change amylopectin length and shape of SBH, these results are consistent with previous reports (Jiang et al., 2011). Based on the FTIR and XRD analysis, SBH3 offers more advantage in the terms of polymer fragmentation compare to the SBH2 and SBH1. Hence, SBH3 has been selected for the further studies.

Particle size of SB and SBH were evaluated through particle size analyzer. The particle size of SB was 615–712 nm, whereas, SBH shown different particle sizes from 6 to 396 nm (Fig. 3A and B). The particle size distributions between the SB and SBH were not identical, notably, the particle size corresponding to 615–712 nm of SB was gradually decreased in the SBH3 (Fig. 3A and B). This reduction in particle size reveals the degrading behaviour of amylase, and SBH particle size ranges from 10 to 150 nm are assumed as short length oligosaccharide. While some wide particles (> 300) in the SBH might probably referring as starch or complex of starch and proteins. SBH could be suitable for foliar application as well as soil application because of the smaller particle size (6–396 nm). In foliar application, molecules transportation have been varying based on the particle size, because which decides molecule's penetration, mobility, and transport. There have been two main pathways for foliar absorption of molecules via leaves, i.e., stomatal and cuticular pathways. The cuticular pathway limits the particle size below 5 nm, because of its small sizes of cuticular pores. However, the stomatal mediated pathways allow the penetration of micrometer size range particle (Eichert et al., 2008). In our study, even though particle size of SBH was smaller (6–396 nm), the polar nature of the sugars and polysaccharides can't be transported via lipophilic upper epidermis. Moreover polar solute transportation via cuticle is not clear (Fernández and Eichert, 2009). Thus, foliar application of SBH along with the non – ionic surfactants may improve the transport efficiently. SBH consisted with the various lengths of polysaccharides have more possibility to enter the plant cell through the stomatal mediated pathway and larger oligomers of SBH might be degraded later by plant amylase. In soil application, physiochemical constitutions of biostimulants are widely affected by the various environmental factors and microbial communities. The lower particle size of SBH (6–396 nm) might easily absorbed by the plant roots. Moreover, oligomers and polymers of SBH could be degraded by the soil microbes thus improve

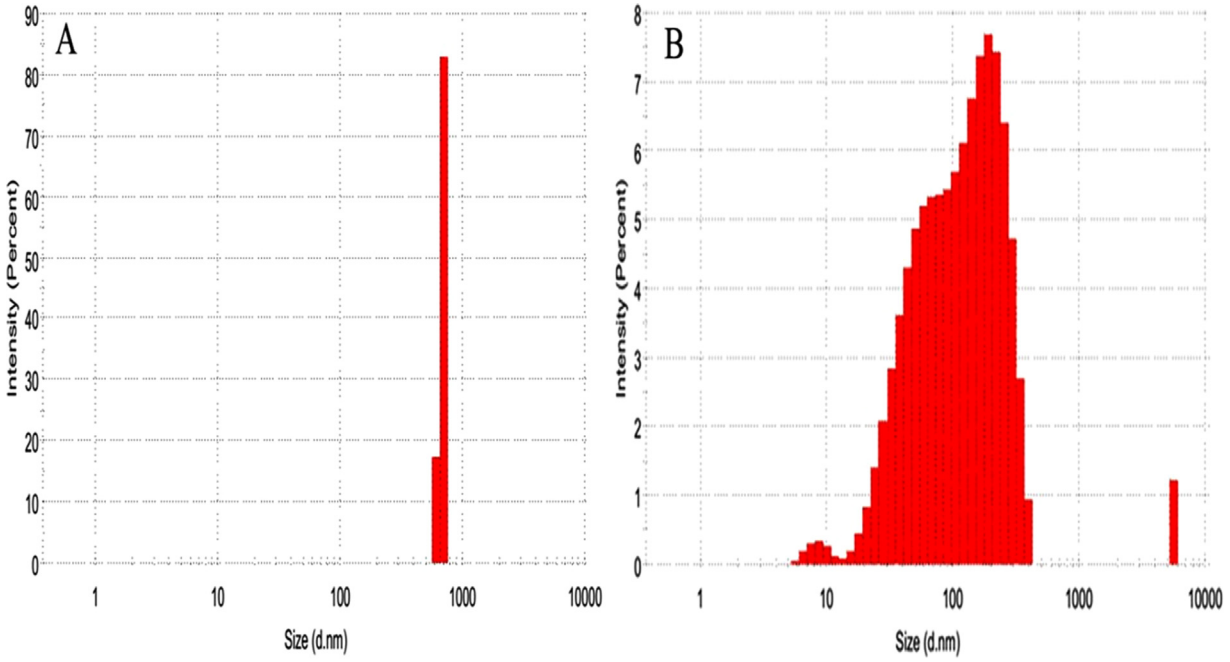

Fig. 3. Symmetric distribution chart of native SB (A) and the SBH3 (B).

the bioavailability of carbon source for plants. In addition, polysaccharide present in the SBH could enhance the water holding capacity of the soil.

The structural modification SB and SBH's were also evaluated by SEM and those results were again ensured the starch degradation (Fig. 4). Based on the SEM and particle size analysis, the smaller particle of SBH might contain higher specific surface area and this can be expected for the higher biological activity. Further, nutritional characteristics of the native SB and SBH were studied and summarized in Table 2. Maximum carbohydrate content was observed in SBH3 (308.35%) compared to the SB.In fact, organic substances have an important role in the biostimulant compositions due to their plant

Table 2
Chemical characterization of SB and SBH3.

Components	SB	SBH3
Organic matter %	198.3	201.54
Proteins (mg/ml)	44.56	45.68
Sugars (mg/ml)	48.99	357.34
Starch %	11.03	8.23

Data was reported as the mean of three measures ± SD.

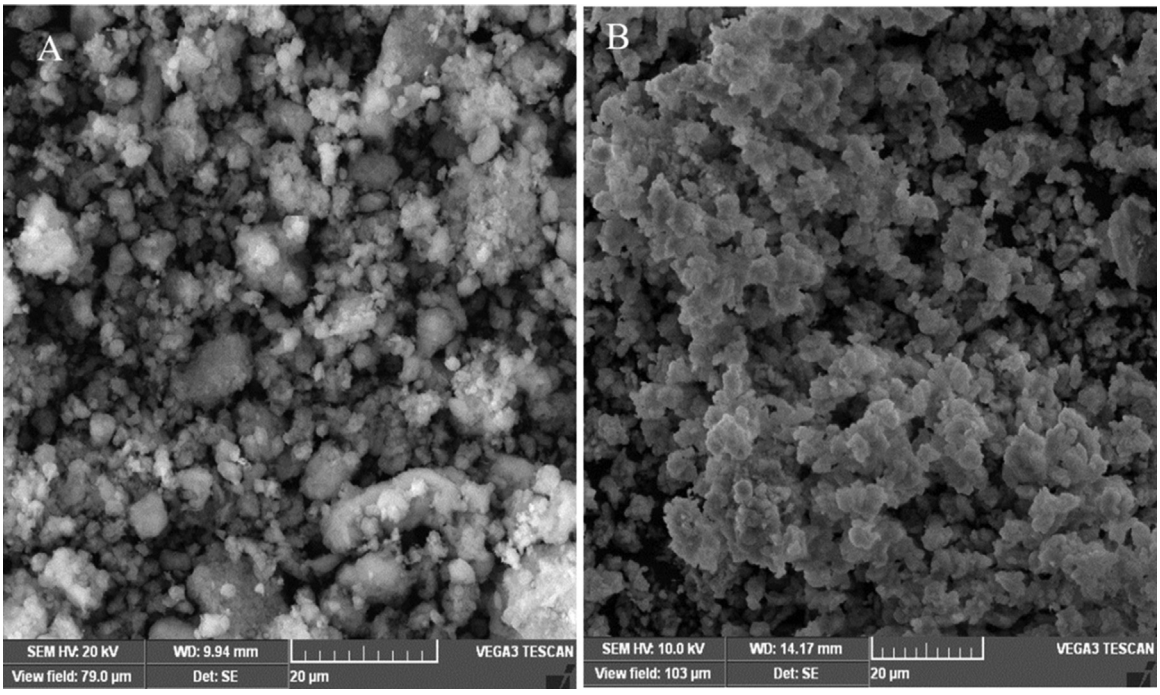

Fig. 4. SEM micrographs of SB (A) and SBH3 (B).

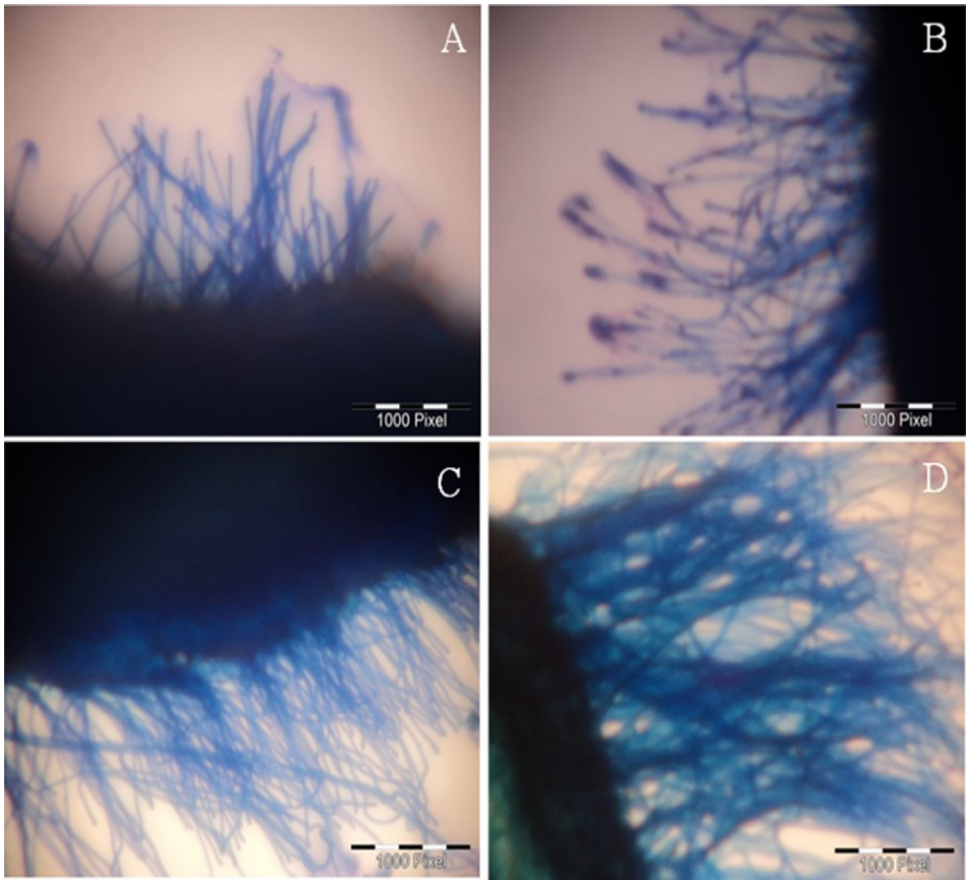

Fig. 5. Effect of SBH on S. *lycopersicum* root hairs development. Control (A), SBH 0.5% (B), SBH 1% (C), SBH 1.5% (D).

growth promoting activities (Naeem et al., 2015). Sugar represented in the SBH3 was reducing sugar, which is well known as an energy source and precursor for various plant metabolisms (Van-den-Ende, 2014). Hence, application of SBH could provide a readily available carbon source to plants. Sweet immunity is an emerging concept in plant stress physiology, which is centrally controlled by the plant sugars (Van den Ende and Valluru, 2009). Therefore, in our study SBH also explored for stress- alleviating effects and this study is under investigation with preliminary promising results (data not shown). In overall characterization studies, SBH3 offers several advantages over the SB in terms of particle size, chemical constitution and other functional properties. Moreover, further optimization studies on enzymatic hydrolysis (pH, temperature and substrate pre-treatment) may increase the potential molecular functionalities of SBH for agricultural uses.

Germination study is the prominent method to determine the plant growth promoting the efficiency of the biostimulant. As shown in the Fig. 5 and Table 3 SBH was exerted a significant improvement in the shoot and root length of *S. lycopersicum*. Among the treatments, the maximum root 4.2 cm and shoot 6.26 cm length were observed in 1.5% of SBH treatment. Similarly, germination traits such as germination percentage (86.66%), germination index (3.85) and vigor index (907.9) were higher in the 1.5% SBH treated seeds. During seed germination, embryonic cells are metabolically active due to accelerated glycolysis process (Yu et al., 2014). Similarly, Seed vigor also depends on seed reserved energy. Considering the above reasons, it has been suspected that sugar and other core components of SBH might enhance the seedling growth through the activation of glycolysis pathways.

Similarly, SBH treatment significantly improve the growth of root hairs. Among the treatments, the maximum number of root hairs were observed in the 1.5% of SBH treatment (Fig. 5). In recent years, organic substances have been considered as an important component in agriculture due to the plant growth promotion, root hair acceleration (Canellas et al., 2010). The abundant root hairs and elongated roots have been considered as an important trait in sustainable crop production. Moreover, root hairs have been playing an important role in enhancement of root surface area, substrate anchorage and nutrient uptake (Brown et al., 2012).

Interestingly, SBH treatments improve the biochemical parameters of *S. lycopersicum*. The total sugar content was gradually increased in the SBH 5% (2.76%), SBH 10% (6.23%) and SBH15% (9.53%) treatments compared to the control. Similarly, protein content also markedly increased in the SBH treated plants. Maximum protein content was

Table 3
Effect of SBH on seed germination traits of *S.lycopersicum*.

Treatments	Germination (%)	Germination index (GI)	Vigor index (VI)	Shoot length (cm)	Root length (cm)
Control	72.19 ± 1.09^{c}	3.09 ± 0.04^{c}	481.9 ± 0.87^{d}	5.267 ± 0.14^{c}	1.6 ± 0.05^{d}
SBH 0.5%	82.22 ± 1.11^{b}	3.49 ± 0.04^{b}	650.8 ± 0.62^{c}	5.4 ± 0.15^{bc}	2.433 ± 0.17^{c}
SBH 1%	86.66 ± 0.33^{a}	3.75 ± 0.04^{ab}	789.3 ± 0.43^{b}	5.6 ± 0.11^{b}	3.5 ± 0.11^{b}
SBH 1.5%	86.66 ± 1.92^{a}	3.85 ± 0.08^{a}	907.9 ± 1.03^{a}	6.26 ± 0.05^{a}	4.2 ± 0.11^{a}

Note: Results were expressed as the means of three replicates ± SE. Values followed by different letters were significantly different at $P < 0.05$.

Table 4
Bio-stimulating effect of SBH on plant growth and biochemical expression of *S. lycopersicum*.

Treatments	Shoot length (cm)	Root length (cm)	Chlorophyll (mg g^{-1})	Carotenoids (mg g^{-1})	Soluble protein (mg g^{-1})	Sugars (μg g^{-1})	Starch (μg g^{-1})
Control	15.32 ± 0.18^{c}	5.5 ± 0.11^{c}	1.63 ± 0.12^{c}	1.70 ± 0.13^{c}	7.17 ± 0.1^{c}	16.4 ± 0.173^{d}	42.9 ± 0.12^{d}
SBH 5%	20.41 ± 0.22^{b}	6.2 ± 0.08^{bc}	4.01 ± 0.10^{b}	2.27 ± 0.09^{b}	8.28 ± 0.13^{b}	19.16 ± 0.145^{c}	48.6 ± 0.12^{c}
SBH 10%	21.94 ± 0.36^{b}	6.8 ± 0.08^{b}	5.74 ± 0.11^{a}	3.68 ± 0.09^{a}	9.74 ± 0.08^{ab}	22.63 ± 0.145^{b}	55.12 ± 0.11^{b}
SBH 15%	25.56 ± 0.28^{a}	7.51 ± 0.14^{a}	5.33 ± 0.14^{a}	3.43 ± 0.12^{a}	10.3 ± 0.17^{a}	25.93 ± 0.120^{a}	59.03 ± 1.4^{a}

Note: Results were expressed as the means of three replicates ± SE. Values followed by different letters were significantly different at $P < 0.05$.

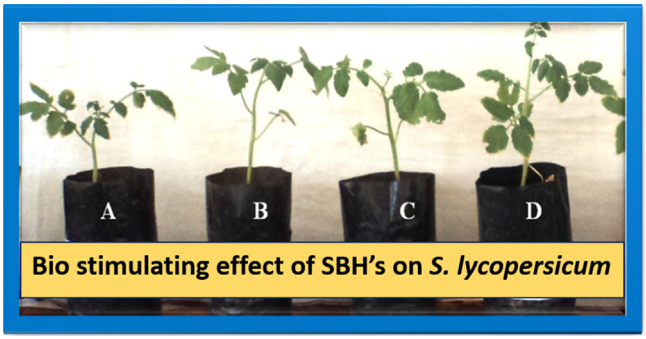

Fig. 6. Bio stimulating effect of SBH's on *S. lycopersicum*: Control (A), 5% SBH (B), 10% SBH (C) and 15% SBH (D).

observed in 15% of SBH treatment (3.13%) followed by SBH 10% (2.57%) and 5% (1.11%) (Table 4). Sufficient quantity of C in leaves might induce higher protein content, which might be due to the higher uptake of N from the soil. These results justify to our hypothesis that, higher costs of carbohydrates in SBH treated plants may result in improved protein synthesis, which might be achieved by the coordinated regulation of carbon and nitrogen metabolism. (Fig. 6)

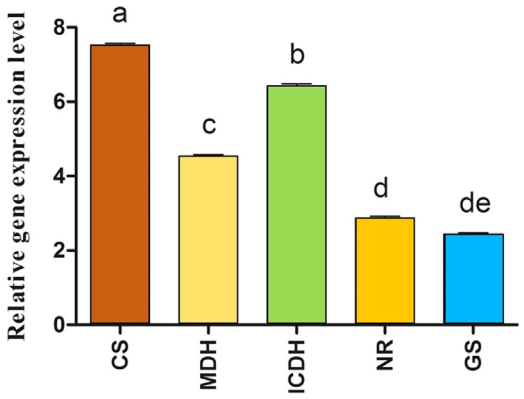

Fig. 8. Effect of SBH on the genes encoding C and N assimilating enzymes. Results are mean ± SD of three replicates. Values with different letters indicate significant differences at P < 0.05 according to Tukey test.

Assimilated carbon is the main precursor for the synthesis of starch; photosynthetically produced glucose is either used or stored in plant leaves depend on the physiological status of the plant. In our study, starch quantification and Lugol's staining showed consistently increased

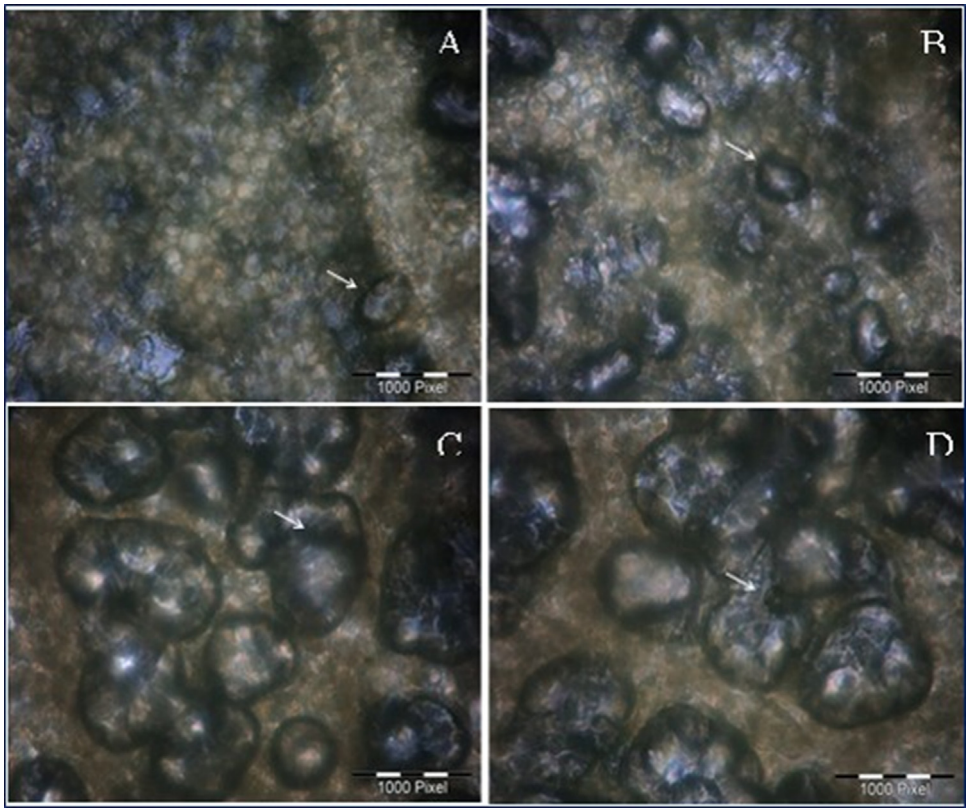

Fig. 7. Effect of SBH on starch accumulation: (A) control, (B) 5% SBH, (C) 10% SBH and (D) 15% SBH. The white arrows are indicating the higher starch accumulation in the SBH treatments.

Table 5
Effect of SBH on nitrogen and carbon assimilating enzymes of *S. lycopersicum*.

Treatments	Nitrogen assimilating enzymes (μmol $^{-1}$ min $^{-1}$ mg $^{-1}$ protein)			Carbon assimilating enzymes (μmol $^{-1}$ min^{-1} mg $^{-1}$ protein)	
	ICDH	MDH	CS	NR	GS
Control	6.2 ± 0.084^{d}	4.16 ± 0.03^{d}	11.69 ± 0.04^{d}	15.34 ± 0.18^{d}	12.87 ± 0.06^{d}
SBH 5%	9.46 ± 0.19^{c}	6.24 ± 0.06^{c}	17.89 ± 0.05^{c}	22.12 ± 0.04^{c}	16.57 ± 0.07^{c}
SBH 10%	11.64 ± 0.22^{b}	7.11 ± 0.03^{b}	20.16 ± 0.05^{b}	26.62 ± 0.04^{b}	19.17 ± 0.05^{b}
SBH 15%	14.28 ± 0.08^{a}	9.17 ± 0.03^{a}	23.16 ± 0.05^{a}	30.48 ± 0.06^{a}	23.85 ± 0.07^{a}

Note: Results were expressed as the means of three replicates ± SE. Values followed by different letters were significantly different at $P < 0.05$.

starch accumulation in all the treated leaves (Fig. 7 and Table 4). Starch synthesis and degradation are essential to maintain optimal carbohydrate balance to avoid energy stress (Stitt and Zeeman, 2012). Based on the biochemical results, it can be described that increased photosynthesis by SBH treatment might lead to improve plant growth, protein and starch contents. Since all these biochemical mechanisms are highly interlinked with plant energy status.

In the enzymatic analysis, SBH treated plants showed higher carbon assimilating enzymes expression. Among the treatment, the maximum CS (11.47%), MDH (5.01%) and ICDH (8.08%) were observed in 15% of SBH treatment. Similarly, the gene expression pattern of CS (7.48 fold increase), MDH (4.5 fold increase) ICDH (6.36 fold increase) were also higher in the SBH 15% treated plants than the control (Fig. 8). These results have been consistent with previous studies (Schiavon et al., 2008). Carbon assimilating enzymes, CS and ICDH are crucial enzymes in energy metabolism. The sugar content of SBH (Table 2) might trigger the expression of ICDH and CS enzymes. ICDH is a precursor for the production of 2- oxoglutarate, which is an important enzyme that connects carbon and nitrogen metabolism. In the earlier study, improved C assimilation has been reported by González et al. (2014) dealing with the biological activity of oligo carrageenans derived from the seaweeds.

Nitrogen assimilating enzymes, namely NR and GS provoked in all the SBH treated seedlings (Table 5). The maximum NR activity was observed in SBH 15% (15.14%), SBH10% (11.28%) and SBH 5% (6.78%) respectively. In the GS assay, SBH 5, 10, 15% treated plants showed 10.98%, 6.3% and 3.7% improvement than the control. These results were consistent with the data of (Pego et al., 2000) who described that NR activity was closely related with concentration of glucose in the nutrient medium. To explore such responses, we attempted to study the expression of N assimilating genes through qRT-PCR. In gene expression analysis, nitrate reductase gene 2.84-fold increase and glutamate synthase 2.41-fold increase were observed in SBH treated plants compared to control (Fig. 8). Various biostimulants treatments have shown to stimulate N and C assimilating genes (Calvo et al., 2014; Ertani et al., 2013).

As mentioned above, N assimilation is always dependent the C assimilation because nitrogen assimilation requires a continuous supply of energy and carbon skeletons. Hence, SBH treated plants may in turn to activate N assimilating genes through increased energy status. Based on these results, we are proposing that SBH rich in the carbohydrates may have two major prospects to enhance the plant growth. The prospects are (i) Increased photosynthesis in the SBH treatment might activate C assimilation by MDH, CS, ICDH enzymes (ii) Higher C assimilation greatly influences the uptake of N and this leads to synthesis of N containing organic compounds through NR, GS enzymes. Moreover, both C and N metabolism intertwined many ways in plants: (i) transamination of glyoxylate to glycine process (ii) assimilation of ammonia to amino acid (iii) ammonia to glycine by decarboxylation and (iv) transamination of glyoxylate with serine to yield hydroxyl pyruvate and glycine. Hence, assimilated products from C and N metabolism and its interlined pathway could facilitate the plant growth and development.

4. Conclusions

The present study demonstrated the biostimulating efficiency of SBH through germination and greenhouse studies. A higher dosage of SBH resulted in substantial improvement in plant physiology and biochemical attributes. These results were consistent with our hypothesis that the higher carbohydrate may improve or balance the plant C and N assimilation responsible for plant growth. Hence, SBH might expect to serve as prospective biostimulant to improve plant growth and yield of various crops. Nevertheless, further studies should be addressed to evaluate its biostimulating ability in the field condition.

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