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AP Dwivedi
Principal Scientist (Agronomy), ICAR-Indian Institute of Sugarcane Research, Lucknow, Uttar Pradesh, India

Sunil G Dalvi
Plant Tissue Culture Section, Vasantdada Sugar Institute, Manjari (Bk.), Pune, Maharashtra, India

Kamini Kumari
Professor Lovely Professional University, Phagwara, Punjab, India

KK Singh
Principal Scientist (Agronomy), ICAR-Indian Institute of Sugarcane Research, Lucknow, Uttar Pradesh, India

MK Tripathi
Principal Scientist (Agronomy), ICAR-Indian Institute of Sugarcane Research, Lucknow, Uttar Pradesh, India

Corresponding Author:
AP Dwivedi
Principal Scientist (Agronomy), ICAR-Indian Institute of Sugarcane Research, Lucknow, Uttar Pradesh, India

Responses of foliar application of low molecular weight derivatives gamma radiated chitosan (Bio stimulator) in sugarcane under irrigated condition in Subtropical India

AP Dwivedi, Sunil G Dalvi, Kamini Kumari, KK Singh and MK Tripathi

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Abstract
Chitosan, being one of the most promising biological macromolecules, has an immense scope in agriculture to boost crop growth and defense mechanism responses resulting enhanced crop yield. In this study, chitosan was exposed to gamma rays in order to obtain a low molecular weight derivative. Viscometric characterization showed a sharp decrease in molecular weight and FTIR based analysis confirmed retention of structural integrity of the polymer upon gamma irradiation. Assessments of various physiological and biochemical attributes were carried out on sugarcane crop to study, that were subjected to progressive for better growth and development of sugarcane crops. The irradiated chitosan was found to be better response for growth and development of sugarcane due to chitosan through positive modulation of various gas exchange parameters alongside significant improvement in relative tissue water content, SOD activity, soluble sugars and adenine energetics. Furthermore, application of irradiated chitosan significantly reduced cell membrane damage, lipid peroxidation, H_2O_2 and free-proline accumulations. This is also reported on the use of gamma irradiated chitosan to boost in bio-physiological functions in sugarcane. Overall comparative assessments showed that differential plant responses were triggered upon foliar application of normal and gamma irradiated chitosan in sugarcane crop grown under irrigated conditions.

Keywords: Chitosan, sugarcane, irrigation, antioxidant enzymes adenine energetics biostimulant

1. Introduction
There is a need to develop appropriate agronomic, breeding, and genomic strategies to enhance growth and development in sugarcane through effective physio-biochemical process with higher productivity and input use efficiency. While conventional production techniques & breeding and transgenic methods are responsible for low productivity and time consuming, use of bioregulators for enhancing crop productivity has attracted much attention. Various chemical and hormonal based bio regulators are exogenously applied to boost the plant signaling to enhance growth and crop yield is most vulnerable able to input efficiency. Input efficiency is one of the limiting factors to sugarcane yield and sugar productivity. Developmental growth stages, the tillering and grand growth phases are considered the most critical contributing to N80% of sugarcane yield and it is at these stages. Input efficiency is one of the limiting factors to sugarcane yield and sugar productivity. Chitosan, derived from chitin which is one of the most abundant biopolymers, next to cellulose, has non-toxic, non-allergenic, biodegradable and biocompatible properties and imparts multiple stress tolerance in plants. Therefore, it has been immensely exploited as a versatile bioactive substance with superior material and functional properties. Much of the chitosan is derived from different waste materials, chiefly wastes of fishery and sea food industries which have abundant renewable resource and alterative for waste management. Being a potential bioactive substance, chitosan and its derivatives have found immense applications in diverse fields that include cosmetics, pharmaceuticals to agriculture. Biological effects of chitosan include antimicrobial (against bacteria, fungi and viruses) and antioxidant (to encounter oxidative damages caused due to adverse conditions) activities beside its growth promoting properties.

Exogenous application of chitosan has potential to alleviate adverse effects of salinity and drought stresses. Despite earlier reports on the use of chitosan against biotic and abiotic stresses, studies on the use of gamma irradiated chitosan in crop plant are limited. Plant cellular responses to exogenously supplied chitosan differ based on the type of chitosan (high/low MW), degree of acetylation, availability of functional group etc. Different approaches to obtain low molecular weight chitosan derivatives are shown to increase biological potential (in food, medicine/pharmaceuticals, agriculture, biotechnology, material science) over the native chitosan. Chitosan nanoparticles were shown to induce innate immune response in plants through up-regulation of defense related genes including that of several antioxidant enzymes as well as elevation of total phenolics. Low molecular weight chitosan can be obtained by various ways but these processes, although effective for desired recovery of the final product, are associated with flaws such as lengthy treatment time, low productivity and selectivity, high processing cost (enzymatic method) and formation of toxic chemical by-products. However, irradiation of chitosan with gamma rays is very efficient and without any of these drawbacks and the product formed can be directly used for downstream applications without any further processing. In this study, we explored if normal chitosan and its gamma irradiated low molecular weight derivative could differentially increase input efficiency in sugarcane, which is a commercial crop grown in many parts of the world. To the best of our knowledge, exogenous foliar application of gamma irradiated chitosan to increase water use efficiency has not yet been reported in sugarcane. With this aim, a comparative study was carried out to assess the effects of normal CSN (NL-CSN) with gamma irradiated CSN (IR-CSN) on physiological and biochemical attributes of sugarcane plants grown under irrigated condition.

2. Materials and Methods

A Field experiment was conducted entitled "Management of Bioresources for Enhancing Sugarcane Productivity and Soil Health" during spring 2019-20 to 2022-2023 with the 12 different treatment combinations *viz,* T_1: 100% N:P:K (Control-Recommended dose of fertilizer), T_2 : 75% N:P:K, T_3 : T_1 + Use of low molecular weight derivatives gamma radiated chitosan (Bio stimulator) -drenching @ 2.5 ml/l of water *, T_4: T_3 + Drenching (*Gluconacetobacter diazotrophicus*), T_5: T_4 + Denching (*Bacillus subtilis*), T_6: T_5 + Drenching (*Bacillus cereus*), T_7: T_6 + foliar application of GA_3 @ 35 ppm at 90, 120 and 150 DAP, T_8: T_2 + Use of low molecular weight derivatives gamma radiated chitosan (Bio stimulator) -drenching @ 2.5 ml/l of water*, T_9: T_8 + Drenching of *Gluconacetobacter diazotrophicus*, T_{10}: T_9 + Drenching of (*Bacillus subtilis*), T_{11}: T_{10} + Drenching of (*Bacillus cereus*), T_{12}: T_{11} + foliar application of GA_3 @ 35 ppm at 90, 120 and 150 DAP. The design of experiment was Randomized Block Design with three replications. The variety taken in experiment was CoPk5191. The soil type of experimental field was loamy soil.

2.1. Sugarcane plant husbandry and growth conditions: Sugarcane settlings (of popular commercial cultivar CoLk 05191 derived from single eye buds were raised at the nursery of the ICAR-Indian Institute of Sugarcane Research, Lucknow, 226002, India (18° 31′ 38.9244″ N, 73° 58′ 20.568″ E, 549 m above MSL). For this purpose, individual eye buds setts were excised out and pre-treated with fungicide and insecticide as per the recommended package of practices. The single eye bud setts were raised in polybags and grown under ambient conditions in a shade. Polybags were filled by mixture of soil and FYM. After 30 days, healthy uniformly grown settlings were transplanted in open field.

2.2. Preparation of low molecular weight derivatives gamma radiated chitosan (Bio stimulator): Two and half ml/litre of water solution of chitosan (extracted from shrimp shells having degree of deacetylation 85%) was prepared in 1% (v/v) glacial acetic acid in water and continuously stirred for couple of hours. This homogenous, highly viscous solution was then packaged in polythene bags of appropriate dimension for gamma radiation treatment. Gamma irradiation (^{60}Co) was carried out using and provide by Plant Tissue Culture Section, Vasantdada Sugar Institute, Manjari (Bk.), Pune 412 307, India coordinated with Food Technology Division, Bhabha Atomic Research Centre (BARC), Mumbai, India and this low molecular weight derivatives gamma radiated chitosan (Bio stimulator) provided by Vasantdada Sugar Institute, Manjari (Bk.), Pune 412 307, India for conductance of field trial at ICAR-India Institute of Sugarcane Research, Lucknow, India The gamma irradiation dose of 100 kGy was chosen based on earlier reports and worked out to be optimal based on our optimization studies

2.3. Characterization of normal and gamma irradiated chitosan: Sprays of respective solutions were carried out thrice at 60, 90 and 120 days interval after transplanting of settling during early hours on the day using a handheld mist sprayer to get fine mist of solution on both the abaxial and adaxial surfaces of the leaves. Twelve (12) number of treatments including control and plants were sprayed with equal volume of water (containing 2.5 ml/l) alone instead of chitosan solutions. With the commencement of first foliar spray, all chitosan sprayed plants till third spray. Foliar application of GA 3 were sprayed in same manner with concentration of 35 ppm as per protocol of the experiment. Bio fertilizers (*Gluconacetobacter diazotrophicus*, *Bacillus subtilis*, *Bacillus cereus*) were applied as a sett treatment for half an hour as per recommended procedure.

2.4. The initial soil analysis: The initial soil analysis were also done for chemical and biological properties of soil and values are as 0-15 cm depth; Organic Carbon-0.59%, pH-7.73, ECe-0.12/m, N-254.01 Kg/ha, P_2O_5-29.12 Kg/ha, K_2O-202.16 Kg/ha; however at 15-30 cm depth the values are as; Organic Carbon-0.37%, pH-7.87, ECe-0.37 ds/m, N-205.93 Kg/ha, P_2O_5-18.39 Kg/ha, K_2O-188.94 Kg/ha. The biological analysis report of these samples are Bacteria-$8.8*10^6$, Actynomycies -$3.74*10^4$, Fungi-$9.31*10^4$.

3. Discussion

Sugarcane (*Saccharum* spp.) is the world's highest biomass producing crop with immense industrial importance mainly due to its major (70–80%) share in the global sugar production. Commercial cultivation of sugarcane is challenged by adverse environmental calamities and reducing input efficiency especially, nutrient use efficiency and water use efficiency, extreme low and high temperatures that limit sugarcane productivity. Since sugarcane crop is grown in the tropical and subtropical regions, the crop experiences sudden and erratic changes in climatic milieu for some or other time during the cropping cycle. Moreover, sugarcane is considered as water guzzler, water and nutrient intensive crop as its cultivation demands frequent and large quantity of water for irrigation, nutrients and pesticide. Sugarcane known for input responsive

crop in both conventional and advanced perspectives, radiation processing is an area of active research having diverse applications (from agriculture to pharmaceuticals). In particular, radiation processing of naturally occurring polymers such as chitosan has immense implications. Radiation processing has become a method of choice for modification of polysaccharides for being safer, environmental friendly and easier method to modify polymers. Considering the recent advancements in this area to utilize chitosans for enhancing nutrient use efficiency and water productivity diverse agricultural applications, ranging from resistance against diseases to protection, it is imperative to study Sugarcane crop responses to foliar application of low molecular weight derivatives gamma radiated chitosan (Bio stimulator) in sugarcane under irrigated condition in Sub-tropical India. With this view, the present study was aimed to attempt a assess the efficacy of Bio stimulator (chitosans) and Bio fertilizers on Growth, Yield and Quality of Sugarcane and assess the efficacy of Bio stimulator and Bio fertilizers on beneficial Soil Microbes and Nutrients Status of Soil for their influences on various physiological and biochemical attributes in sugarcane plants. For this purpose the very first noticeable observation upon effect of bioresources (Bio-stimulator-Chitoson, *Gluconacetobacter diazotrophicus*, *Bacillus subtilis*, *Bacillus cereus* and GA-3, was the drastic reduction in viscosity of the CSN polymer. Reduction in the viscosity upon gamma irradiation has been proven to have direct relation with the molecular weight of chitosan polymer. Similar observations were already reported by Bano *et al*. and Garcia *et al*. where in lobster shell chitosan showed drastic reduction in viscometric. The studies reported that treatment T_7 (100% N.P.K. + Use (Drenching) of low molecular weight derivatives gamma radiated chitosan (Bio stimulator) @ 2.5 ml per litre of water drenching with *Gluconacetobacter diazotrophicus* + drenching with *Bacillus subtilis* and *Bacillus cereus* and foliar application of GA-3 @ 35 ppm at 90, 120 at 150 days after transplanting have been found best treatment for cane yield (73.64 t/ha) and significantly influenced over other treatment combinations followed by T_{11} (72.34 t/ha) and T_9 (71.92 t/ha.). The no. of tillers, NMC, cane weight and other growth parameters were also found in similar trends.

Table 1: Effect of Bioresources on Germination, No. of Tillers.

Treatment	Germination (%) 2019-20	Germination (%) 2020-21	Germination (%) 2021-22	Mean	No. Tillers at 120 DAP (000) 2019-20	No. Tillers at 120 DAP (000) 2020-21	No. Tillers at 120 DAP (000) 2021-22	Mean
T_1: 100% N:P:K (Control)	29.3	51.67	55.34	45.74	59.0	213.67	55.34	109.54
T_2: 75% N:P:K (Control)	31.4	42.67	55.29	43.42	53.33	213	55.29	107.41
T_3: T_1 + Use of Bio stimulator derivative @ 2.5 ml/l of water*	32.3	57.67	53.63	48.17	90.00	232.67	53.63	125.63
T_4: T_3+ sett Treatment (*Gluconacetobacter diazotrophicus*)	33.1	59.33	62.50	51.94	63.33	235	62.50	120.48
T_5: T_4 + sett Treatment (Bacillus *subtilis*)	33.2	51.67	59.88	48.55	60.0	217	59.88	112.49
T_6: T_5 + sett Treatment (*Bacillus cereus*)	32.8	55.00	61.38	50.03	60.0	220.33	61.38	114.10
T_7: T_6 + Foiliar Application of GA3 @ 35 ppm at 90, 120 and 150 DAP	33.9	75.33	72.09	60.74	120.0	262	72.09	151.56
T_8: T_2- Use of Bio stimulator derivative @ 2.5 ml/l of water*	33.2	61.67	62.09	52.62	63.33	244.67	62.09	123.56
T_9: T_8 + sett Treatment (*Gluconacetobacter diazotrophicus)*	33.1	67.67	63.09	54.92	76.66	242.67	63.09	127.67
T_{10}: T_9 + sett Treatment (*Bacillus subtilis*)	32.8	54.67	59.92	49.43	60.00	218.67	59.92	113.06
T_{11}: T_{10} +sett Treatment (*Bacillus cereus*)	34.1	66.33	64.88	55.40	83.33	247.67	64.88	132.16
T_{12}: T_{11} + Foiliar Application of GA3 @ 35 ppm at 90, 120 and 150 DAP	32.1	61.33	62.88	52.40	66.66	239	62.88	123.05
CD at 5%	NS	8.47	7.18	7.21	43.44	19.35	7.18	7.27

Table 2: Effect of Bioresources on Germination, No. of Tillers, NMC and Cane Yield.

Treatment	NMC (000/ha.) 2019-20	NMC (000/ha) 2020-21	NMC (000/ha.) 2021-22	Mean	Cane yield (t/ha.) 2019-20	Cane yield (t/ha.) 2020-21	Cane yield (t/ha.) 2021-22	Mean
T_1: 100% N:P:K (Control)	64.00	62.81	115.70	81.04	61.98	65.71	55.61	71.26
T_2: 75% N:P:K (Control)	60.00	60.73	112.73	78.02	53.89	64.84	53.24	65.24
T_3: T_1 + Use of Bio stimulator derivative @ 2.5 ml/l of water*	78.00	58.02	110.39	82.34	72.38	64.05	51.38	74.2
T_4: T_3+ sett Treatment (*Gluconacetobacter diazotrophicus*)	74.00	68.08	121.78	88.15	72.94	70.15	65.68	79.46
T_5: T_4 + sett Treatment (*Bacillus subtilis*)	72.00	63.17	116.66	84.14	63.02	66.87	57.86	72.39
T_6: T_5 + sett Treatment (*Bacillus cereus*)	66.75	67.38	120.45	85.06	72.38	67.78	61.33	74.72
T_7: T_6 + Foliar Application of GA3 @ 35 ppm at 90, 120 and 150 DAP	86.66	74.51	135.74	99.17	89.05	75.08	73.64	90.23
T_8: T_2- Use of Bio stimulator derivative @ 2.5 ml/l of water*	73.50	65.87	120.49	86.82	83.81	68.57	63.98	75.4
T_9: T_8 + sett Treatment (*Gluconacetobacter diazotrophicus)*	75.58	72.63	124.93	91.25	86.19	72.21	71.92	80.4
T_{10}: T_9 + sett Treatment (*Bacillus subtilis*)	66.66	66.35	117.28	83.63	68.41	66.67	59.41	73.18
T_{11}: T_{10} + sett Treatment (*Bacillus cereus*)	76.08	73.50	126.27	92.15	84.76	73.57	72.34	80.96
T_{12}: T_{11} + Foiliar Application of GA3 @ 35 ppm at 90, 120 and 150 DAP	74.50	70.25	124.44	89.93	80.00	71.26	69.89	79.84
CD at 5%	21.09	6.48	6.30	7.2	16.12	3.98	5.15	8.9

Table 3: Effect of Bioresources on Brix (%)

Treatment	Brix (%) 2019-20	Brix (%) 2020-21	Brix (%) 2021-22	Mean
T_1: 100% N:P:K (Control)	19.44	19.22	17.253	18.64
T_2: 75% N:P:K (Control)	19.69	19.71	16.403	18.60
T_3: T_1 + Use of Bio stimulator derivative @ 2.5 ml/l of water*	19.95	19.21	16.183	18.45
T_4: T_3+ sett Treatment (*Gluconacetobacter diazotrophicus*)	19.60	18.68	16.4	18.23
T_5: T_4 + sett Treatment (*Bacillus subtilis*)	19.51	18.783	16.207	18.17
T_6: T_5 + sett Treatment (*Bacillus cereus*)	19.28	19.62	16.843	18.58
T_7: T_6 + Foiliar Application of GA3 @ 35 ppm at 90, 120 and 15 DAP	19.73	18.933	17.107	18.59
T_8: T_2- Use of Bio stimulator derivative @ 2.5 ml/l of water*	19.72	19.04	16.667	18.48
T_9: T_8 + sett Treatment (*Gluconacetobacter diazotrophicus*)	19.44	18.177	16.547	18.05
T_{10}: T_9 + sett Treatment (*Bacillus subtilis*)	19.81	19.197	16.35	18.45
T_{11}: T_{10} +sett Treatment (*Bacillus cereus*)	19.15	18.773	16.373	18.10
T_{12}: T_{11} + Foiliar Application of GA3 @ 35 ppm at 90, 120 and 150 DAP	20.11	19.363	17.103	18.86
CD at 5%	NS	NS	0.564	0.56
SE(m)	0.20	0.445	0.191	0.28

Table 4: Effect of Bioresources on purity (%)

Treatment	Purity (%) 2019-20	Purity (%) 2020-21	Purity (%) 2021-22	Mean
T_1: 100% N:P:K (Control)	90.13	87.27	81.2	86.20
T_2: 75% N:P:K (Control)	90.31	87.21	76.3	84.61
T_3: T_1 + Use of Bio stimulator derivative @ 2.5 ml/l of water*	90.06	83.957	79.66	84.56
T_4: T_3+ sett Treatment (*Gluconacetobacter diazotrophicus*)	90.46	85.83	80.94	85.74
T_5: T_4 + sett Treatment (*Bacillus subtilis*)	89.96	87.787	80.133	85.96
T_6: T_5 + sett Treatment (*Bacillus cereus*)	90.50	87.25	80.027	85.93
T_7: T_6 + Foiliar Application of GA3 @ 35 ppm at 90, 120 and 15 DAP	90.68	85.537	80.904	85.71
T_8: T_2- Use of Bio stimulator derivative @ 2.5 ml/l of water*	90.34	85.757	78.973	85.02
T_9: T_8 + sett Treatment (*Gluconacetobacter diazotrophicus*)	67.11	87.13	79.567	77.94
T_{10}: T_9 + sett Treatment (*Bacillus subtilis*)	90.14	84.45	80.65	85.08
T_{11}: T_{10} +sett Treatment (*Bacillus cereus*)	90.21	87.423	80.603	86.08
T_{12}: T_{11} + Foiliar Application of GA3 @ 35 ppm at 90, 120 and 150 DAP	90.52	87.41	81.5	86.48
CD at 5%	NS	NS	NS	NS
SE(m)	5.39	1.774	1.135	2.77

Table 5: Effect of Bioresources on Sucrose (%)

Treatment	Sucrose (%) 2019-20	Sucrose (%) 2020-21	Sucrose (%) 2021-22	Mean
T_1: 100% N:P:K (Control)	17.52	16.77	14.01	16.10
T_2: 75% N:P:K (Control)	17.78	17.19	12.52	15.83
T_3: T_1 + Use of Bio stimulator derivative @ 2.5 ml/l of water*	17.96	16.14	12.90	15.67
T_4: T_3+ sett Treatment (*Gluconacetobacter diazotrophicus*)	7.72	16.02	13.27	15.67
T_5: T_4 + sett Treatment (*Bacillus subtilis*)	17.55	16.49	13.08	15.71
T_6: T_5 + sett Treatment (*Bacillus cereus*)	17.45	17.13	13.48	16.02
T_7: T_6 + Foiliar Application of GA3 @ 35 ppm at 90, 120 and 15 DAP	17.89	16.20	13.83	15.97
T_8: T_2- Use of Bio stimulator derivative @ 2.5 ml/l of water*	17.81	16.33	13.16	15.77
T_9: T_8 + sett Treatment (*Gluconacetobacter diazotrophicus*)	17.58	15.84	13.17	15.53
T_{10}: T_9 + sett Treatment (*Bacillus subtilis*)	17.85	16.20	13.19	15.75
T_{11}: T_{10} +sett Treatment (*Bacillus cereus*)	17.27	16.41	13.20	15.63
T_{12}: T_{11} + Foiliar Application of GA3 @ 35 ppm at 90, 120 and 150 DAP	18.20	16.93	13.94	16.36
CD at 5%	NS	NS	0.79	0.79
SE(m)	0.18	0.57	0.27	0.34

4. Conclusion

The effect of bioresources (Bio-stimulator-Chitoson, *Gluconacetobacter diazotrophicus*, *Bacillus subtilis*, *Bacillus cereus* and GA-3, was the drastic reduction in viscosity of the CSN polymer. Reduction in the viscosity upon gamma irradiation has been proven to have direct relation with the molecular weight of chitosan polymer. Similar observations were already reported by Bano *et al*. and Garcia *et al*. where in lobster shell chitosan showed drastic reduction in viscometric. The studies reported that treatment T_7 (100% N.P.K. + Use (Drenching) of low molecular weight derivatives gamma radiated chitosan (Bio stimulator) @ 2.5 ml per litre of water drenching with *Gluconacetobacter diazotrophicus* + drenching with *Bacillus subtilis* and *Bacillus cereus* and foliar application of GA-3 @ 35 ppm at 90, 120 at 150 days after transplanting have been found best treatment for cane yield (73.64 t/ha) and significantly influenced over other treatment combinations followed by T_{11} (72.34 t/ha) and T_9 (71.92 t/ha.).The no. of tillers, NMC, cane weight and other growth parameters were also found in similar trends.

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