



ISSN: 0144-8617

## Carbohydrate Polymers

A Journal Devoted to Scientific and Technological Aspects of Industrially Relevant Polysaccharides

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### Editors-in-Chief > Editorial board



Manuel Coimbra



Kevin J. Edgar

**Carbohydrate Polymers** is a major journal within the field of glycoscience, and covers the study and exploitation of polysaccharides which have current or potential application in areas such as bioenergy, bioplastics, biomaterials, biorefining, chemistry, drug delivery, food, health, nanotechnology, packaging, paper, pharmaceuticals, medicine, oil recovery, textiles, tissue engineering and wood, and other aspects of glycoscience.

The role of the well-characterized carbohydrate polymer must be the major proportion of the work reported, not a peripheral topic. At least one named carbohydrate polymer must be cited and be the main focus of the paper and its title. Research must be innovative and advance scientific knowledge.

**Characterization** - For all polysaccharides, including those obtained from a supplier, essential structural information which will affect their behavior in the subsequent work should be given, along with a description of how that information was ascertained. Examples of such essential information include molecular weight, mannuronate/guluronate ratio for alginates, degree of esterification for pectin, degree of deacetylation for chitosan. Editors are unlikely to send papers for formal review with a statement such as "sodium alginate was purchased from XXX Inc." unless additional information is supplied. For papers involving synthesis, polysaccharide derivatives must also be well-characterized. For papers describing identity or

application of newly-discovered polysaccharides, purity and monosaccharide composition are essential; some molecular size and linkage information is highly desirable.

**Hypotheses** - Nearly all scientific papers benefit from inclusion of a statement of hypothesis. Such statements should be concise, declarative, and should describe the one or more key hypotheses that the studies upon which the manuscript is based were intended to confirm or refute. Inclusion of a hypothesis statement makes it simple to contrast the hypothesis with the most relevant previous literature and point out what the authors feel is distinct about the current hypothesis (novelty). It also permits the authors to describe why they feel it would be important to prove the hypothesis correct (significance).

**Topics of interest to the journal:**

- structure-property relationships
- analytical methods
- chemical, enzymatic and physical modifications
- biosynthesis
- natural functions
- interactions with other materials

**Topics not of interest to the journal:**

- biological, physiological and pharmacological aspects of non-carbohydrate molecules attached to, or mixed with, carbohydrate polymers, unless the polysaccharide has a relevant and specific role
- materials science of biocomposites where there is no mention of any specific carbohydrate polymer, or the role of the carbohydrate polymer is not the major proportion of the study
- polyalkanoates, polylactic acid, or lignin
- routine studies of extraction yields without characterisation of the extracted polysaccharide under the different conditions
- routine studies of complexation of a drug with a single cyclodextrin
- studies of newly discovered natural polysaccharides or new polysaccharide derivatives where the structure of the polysaccharide (derivative) is unknown
- production and isolation of enzymes which act on polysaccharides (studies on the mode of action of an enzyme on a polysaccharide are within the journal scope)
- carbohydrate oligomers where the degree of polymerization is less than four
- treatments of cotton fabrics and cellulose-based paper where the research is largely not about the component cellulose itself
- use of carbohydrate polymers as a support material (e.g. in enzyme immobilization, chromatography, etc.) where there is no specific involvement of the chemistry of the carbohydrate polymer
- production of chars from polysaccharides, regardless of the application to which the char will be used. Such manuscripts are out of scope since they do not focus on the science of well-characterized polysaccharides

Carbohydrate Polymers has an open access companion journal, [Carbohydrate Polymer Technologies and Applications](#), which is devoted to scientific and technological aspects and applications of polymers and oligomers containing carbohydrate.

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OA ISSN: 2666-8939

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6th EPNOE Conference

Papers from ICPNB 2019

Century of Cell

Tatiana Budtova, Kevin Edgar

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## Special Issue: 7th International Polysaccharide Conference

Guest editors: Tatiana Budtova - Submission deadline: 1 March 2022

🕒 November 23, 2021

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## Change to submission requirements (Graphical Abstract and Highlights)

🕒 August 15, 2021

## John Mitchell obituary

🕒 January 19, 2021

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Full editorial board

61 editors and editorial board members in 28 countries/regions

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## Gender Diversity Distribution of the Editors ⓘ

Based on 86% responding editors

● 42% woman ● 58% man ● 0% non-binary or gender diverse ● 0% prefer not to disclose

## Benchmark Gender Diversity distribution across Food Science portfolio Editors ⓘ

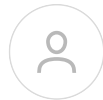
● 29% woman ● 71% man ● 0% non-binary or gender diverse ● 0% prefer not to disclose

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Editor-in-Chief  
[Manuel Coimbra](#)



Editor-in-Chief  
[Kevin J. Edgar](#)

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## Conferences

### EuroSense 2022 | A Sense of Earth

📅 September 13 – 16, 2022 📍 Turku, Finland

### 11th International Colloids Conference

📅 June 12 – 15, 2022 📍 Altis Grand Hotel, Lisbon, Portugal

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## Mendeley Datasets

### Data for: Effect of Nanocellulose Fiber Hornification on Water Fraction Characteristics and Hydroxyl Accessibility during Dehydration

1 file (2018) Song Zeng

### Data for: Origin of Vacuum-Assisted Chiral Self-Assembly of Cellulose Nanocrystals

2 files (2021) Jianming Zhang

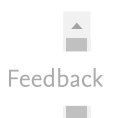
### Data for: NMR and electrophoresis data in Change of network structure in agarose gels by aging during storage studied by NMR and Electrophoresis

1 file (2021) shingo matsukawa

[> View all Mendeley datasets](#)

## Tweets by @ELSfoodscience

**Elsevier FoodScience**  
@ELSfoodscience



BAd-CRISPR: inducible gene knockout in interscapular brown adipose tissue of adult mice Romanelli et al.'s data suggest that BAd-CRISPR is an efficient tool to speed discoveries in [#adiposetissue](#) biology — [@umichmedicine](#) [@UMPhysiology](#) [#CRISPR](#) [spkl.io/6o194PiUV](#)

5:34 PM - Jan 17, 2022

**Elsevier FoodScience**  
[@ELSfoodscience](#)

Preparation and characterisation of organic UV filters based on combined PHB/liposomes with natural phenolic compounds [#openaccess](#) Journal of Biotechnology [spkl.io/6o194PqkD](#)

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## Abstracting and Indexing

This journal has been indexed in:

- BIOSIS Citation Index
- Polymer Contents
- Science Citation Index

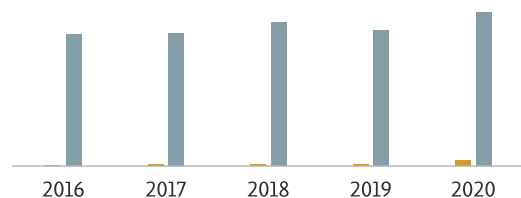
and 9 more

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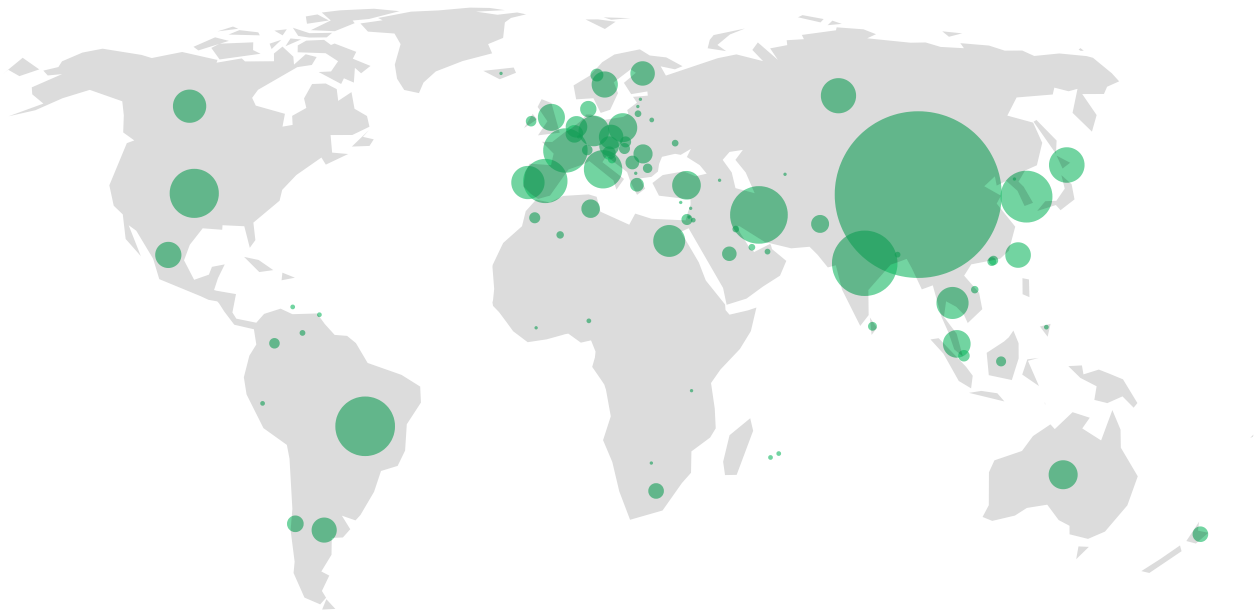
A total of 1,455 articles were published in 2020.

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## Authorship

The primary corresponding authors at country/region level in the last five years.



	Top countries/regions	Authors
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#2	IN India	387
#3	BR Brazil	321

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Carbohydrate Polymers is a well reputed journal and helps contribute to the scientific community

– Dr. Muthu Mohamed, India

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NO Norway (2) ZA South Africa (2) GB United Kingdom (2) AR Argentina FI Finland HK Hong Kong  
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TH Thailand TN Tunisia

#### Gender Diversity Distribution of the Editors <sup>①</sup>

Based on 86% responding editors

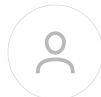
● 42% woman ● 58% man ● 0% non-binary or gender diverse ● 0% prefer not to disclose

#### Benchmark Gender Diversity distribution across Food Science portfolio Editors <sup>①</sup>

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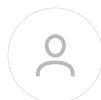
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#### Manuel Coimbra

University of Aveiro Chemistry Department, 3810-193, Aveiro, Portugal

Carbohydrate polymers

[➤ Contact Manuel Coimbra](#)

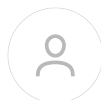
#### Kevin J. Edgar

Virginia Polytechnic Institute and State University, Virginia Tech, 230A Cheatham Hall, 310 West Campus Drive, VA 24061, Blacksburg, United States of America

Polysaccharide chemistry, Synthesis, Derivatization, Structure-property relationships, Drug delivery, Hydrogels, Copolymers, Degradable and recyclable plastics

[➤ Contact Kevin J. Edgar](#)

### Editors



#### Tatiana Budtova

Mines ParisTech, Center for Materials Forming, Paris, France

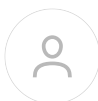
Polysaccharides, Solutions, Gels, Aerogels, Composites

**Cristina De Castro**

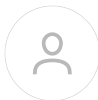
- University of Napoli Federico II Department of Agriculture, Portici, Italy
- Capsular polysaccharide, Carbohydrate structure, GC-MS, Lipopolysaccharide, Molecular modeling, NMR, Viral autonomous glycosylation

**Sushil Dhital**

- Monash University Department of Chemical Engineering, Clayton, Victoria, Australia
- Dietary fiber, hydrocolloids, enzyme kinetics, food structure

[View more](#)**Gloria Huerta-Angeles**

- Contipro a s, Dolni Dobrouc, Czechia
- Hyaluronan, medical devices, chemical characterization of polysaccharides.

[View more](#)**Marcello Iacomini**

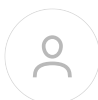
- Federal University of Parana, Curitiba, Brazil
- Chemical characterization of polysaccharides, the Chemistry of Polysaccharides such as glucans, Galactans, Manogalactans, Mannans, Xylans, Xylomannans or Heteropolysaccharides in general and their biological activities, Heparins, Chondroitins sulfate, Hyaluronic acid and Heparinoids

**Hiroshi Kamitakahara**

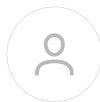
- Kyoto University, Kyoto, Japan
- End-functionalization, Methylcellulose, Regioselective functionalization, Surface activity, Thermo-reversible supramolecular hydrogels

**Simon McCarthy**

- HemCon Medical Technologies Inc, Portland, Oregon, United States of America
- Biomedical, Characterization, Chitin, Chitosan, Modification


**Fwu-Long Mi**

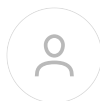
- Taipei Medical University, Department of Biochemistry and Molecular Cell Biology, School of



**Rekha S. Singhal**


 Institute of Chemical Technology, Mumbai, India

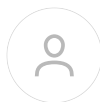
 Starch modification, Hydrocolloids, Chemical modification, Microencapsulation, Microbial polysaccharides, and Food technology applications



**Carlos Stortz**


 University of Buenos Aires, Buenos Aires, Argentina

 Polysaccharides, Carrageenans, Fucoidans, Cell wall polysaccharides, Molecular modeling of carbohydrates, DFT of carbohydrates



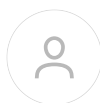
**Run-Cang Sun**

 Dalian Polytechnic University Center for Lignocellulose Science and Engineering School of Light Industry and Chemical Engineering, Dalian, China


 Carbohydrate Polymers, hemicelluloses, lignin, cellulose, biomaterials

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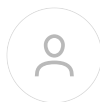
**Cláudia Nunes**

 University of Aveiro Department of Materials and Ceramic Engineering, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

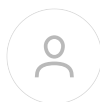
 Polysaccharides structure, Biomaterials, Food packaging, Biomedical devices, Marine

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**John Blanchard†**



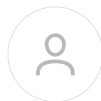
**John F. Kennedy**





**John R. Mitchell†**

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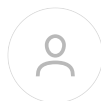


**Carmen Alvarez-Lorenzo**

University of Santiago de Compostela, Santiago de Compostela, Spain

Cellulose derivatives, Drug carriers, 3D printing, Electrospinning, Drug-device combination products

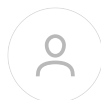
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Purdue University, West Lafayette, Indiana, United States of America

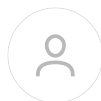
Starch, Hydrocolloids, Chemistry, Functionality, Applications



**Emiliano Bedini**

University of Naples Federico II Department of Chemistry Sciences, Napoli, Italy

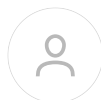
Glycosaminoglycans, Semi-synthesis, Sulfation, Glycosylation



**Eric Bertoft**

University of Minnesota Department of Animal Science, St. Paul, Minnesota, United States of America

Starch, Amylopectin, Amylose, Structure, Function

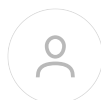


**Sami Boufi**

University of Sfax Faculty of Sciences of Sfax, Sfax, Tunisia

Biobased nanoparticles (nanocellulose, starch nanoparticles, nanochitin, lignin), Nanocomposites and composites based on lignocellulosic fillers and nanofillers, Biobased particles as colloidal stabilizer, Hybrid material based on cellulose and inorganic NPs

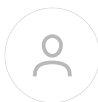
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**H Cheng**

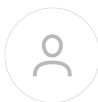
USDA-ARS Southern Regional Research Center, New Orleans, Louisiana, United States of America

Biobased Materials, Polysaccharides, Chemical and Enzymatic Modifications, Packaging Films,



**Annie Chimphango**

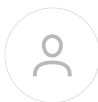
- Stellenbosch University Department of Process Engineering, Stellenbosch, South Africa
- Biomaterials (extraction, modification, and application), Nanomaterials (extraction, modification, and application), Biocomposites, Integrated biorefineries, Bioactive substances delivery devices



**Bjørn Christensen**

- Norwegian University of Science and Technology, Trondheim, Norway
- Polysaccharides, Structure-function relationships, Stability and degradation, gelation, biomaterials, chemical modification

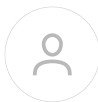
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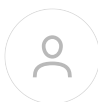
- University of Limerick, Limerick, Ireland
- Hyaluronic acid, Lignin, Tissue Engineering, Sustainable polymers, Carbon Fiber

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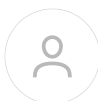
**Lucimara M. C. Cordeiro**

- Federal University of Paraná Department of Biochemistry and Molecular Biology, Curitiba, Brazil
- Dietary fiber, Complex carbohydrates, Pectin, Human health



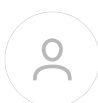
**Ilídio J. Correia**

- University of Beira Interior, Covilha, Portugal
- Biomaterials, Drug Delivery, Nanotechnology, Tissue Engineering, Wound healing

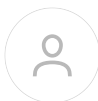


**Hongbing Deng**

- Wuhan University, Wuhan, China
- Biopolymers, Chitin and chitosan, Cellulose, Nanofibers, Biomaterials




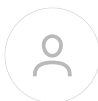
**Kevur Desai**



**Jacques Desbrieres**


 University of Pau and Pays de l'Adour, Pau, France

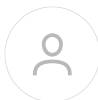
 Polysaccharides, Physico-chemistry (rheology, interfaces), Chemical modification, Smart materials, Applications (cosmetics, food, wastewater treatment)




**Alain Dufresne**


 International Graduate School of Paper Print Communication and Biomaterials, St Martin d'Heres, France

 Polymer nanocomposites, Nanocellulose, Starch nanocrystals, Cellulose nanocrystals

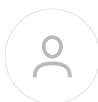


**Andre R. Fajardo**

 Federal University of Pelotas, Pelotas, Brazil

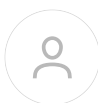
 Polysaccharide-based materials, Hydrogels, Biomaterials, Polymer composites, Polysaccharide chemistry

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**Pedro Fardim**

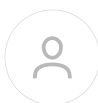
 Åbo Akademi University Physics, ABO, Finland



**Gil Garnier**

 Monash University Department of Chemical Engineering, Clayton, Victoria, Australia

 Cellulose and Nanocellulose, Biodiagnostics, Biomaterials, Colloids and Interfaces, Biorefinery, Green Chemistry, Sustainable process



**Kailash C. Gupta**



 Indian Institute of Technology Roorkee, Uttarakhand, India

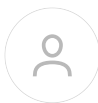
 Biopolymers, Drug delivery, polymeric Gels, Biosensors, and Tissue engineering






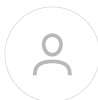
**Jovin Hasjim**

 Roquette Freres, Lestrem, France  
 Carbohydrates, Starch, Amylose and Amylopectin





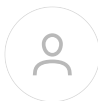
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 The University of British Columbia, Vancouver, British Columbia, Canada





**Seunho Jung**

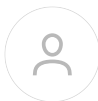
 Konkuk University Department of Systems Biotechnology, Gwangjin-gu, South Korea  
 Polysaccharide Engineering, Hydrogel Application, Cyclodextrin Technology, Microbial Exopolysaccharide





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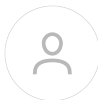
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 Surface science, Polysaccharide derivatives, Biomaterial, Polymer processing

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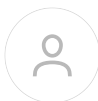
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 Friedrich Schiller University Jena, Jena, Germany  
 Polysaccharides, Functionalization, Structure analysis, Structure-property-relationships, Regioselectivity, ,



**Xiaoxi Li**

 South China University of Technology, Guangzhou, China  
 Starch structural modification and functionality regulation, Delivery systems/carrier materials and their release behaviors, Starch-based food packaging material, and plasticizer migration from packaging to foods



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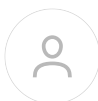


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 National Metal and Materials Technology Center, Advanced Polymer Technology Research Group, Khlong Nuengi, Thailand


 Polysaccharides, Structure, Pectin, Extraction

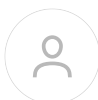
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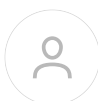


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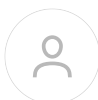


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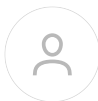
 Starch chemistry and technology, Starch digestibility, Cereal carbohydrates, Dietary fiber



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 Victoria University of Wellington Ferrier Research Institute, Wellington, New Zealand

 Polysaccharides, Oligosaccharides, Structure, Methylation, Analysis



**Andrej Sinica**

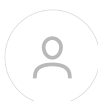
 University of Chemistry and Technology Prague, Praha, Czechia



**Maciej Skotak**


 New Jersey Institute of Technology, Newark, New Jersey, United States of America

 Chitosan, Biomaterials, Electrospinning, Neurotrauma, Drug delivery

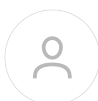


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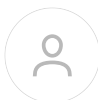


**Karin Stana Kleinschek**

 Graz University of Technology Institute of Chemistry and Technology of Biobased Systems, Graz, Austria


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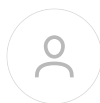
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**Eduardo Jaime Vernon-Carter**

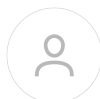
 Metropolitan Autonomous University Iztapalapa, Iztapalapa, Mexico

 Emulsion science, Food rheology, Biopolymers and Biomaterials, Interface structuring, Stability of dispersed phase systems



**Manuel Vilanova**

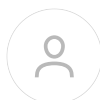
University of Porto, Porto, Portugal



**Qi Wang**

Agriculture and Agri-Food Canada Guelph Research and Development Centre, Guelph, Ontario, Canada

Microencapsulation, Polysaccharide, Bioactivity, Antibacterial substances

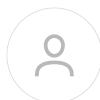


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Wrexham Glyndwr University, Wrexham, United Kingdom

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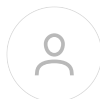
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The Hong Kong Polytechnic University, Hong Kong, Hong Kong

Functional foods, Gut microbiota, Medicinal fungi, Polysaccharides, Fermentation

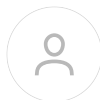


**David Fengwei Xie**

University of Warwick International Institute for Nanocomposites Manufacturing, Warwick, United Kingdom

Polysaccharides; Bioplastics; Polymer composites; Polymer engineering: Structure-property relationships

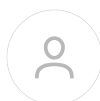
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Starch, Dietary fiber, Gut microbiota, Digestion kinetics, Food structure





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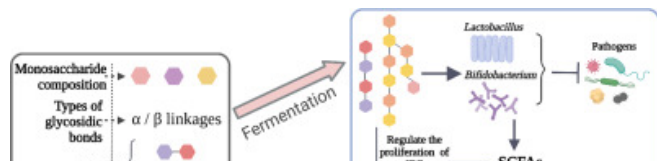
Na Zhang, Mingliang Jin, Kaiming Wang, Zhihong Zhang, ... Hua Wei

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## Fungal exopolysaccharides: Properties, sources, modifications, and biomedical applications

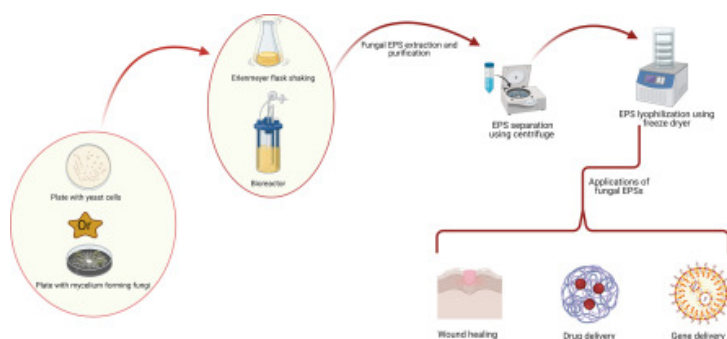
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## Preparation of chromium (III) ion-imprinted polymer based on azo dye functionalized chitosan

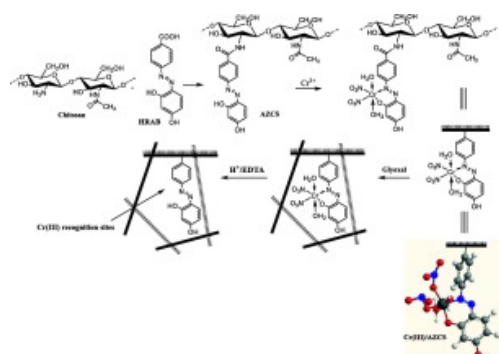
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## One-step synthesis of glycogen-type polysaccharides from maltooctaose and its structural characteristics

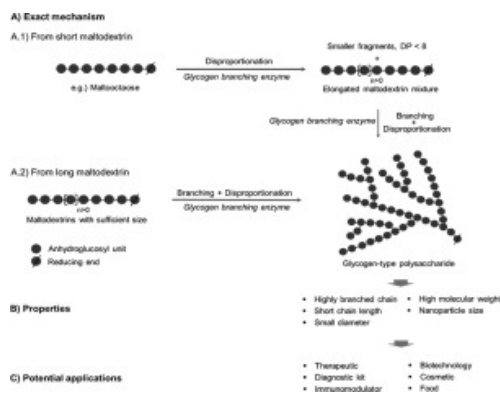
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## Rheological analysis of physical states of cellulose nanocrystal suspension and synergetic effect of aligned gel state

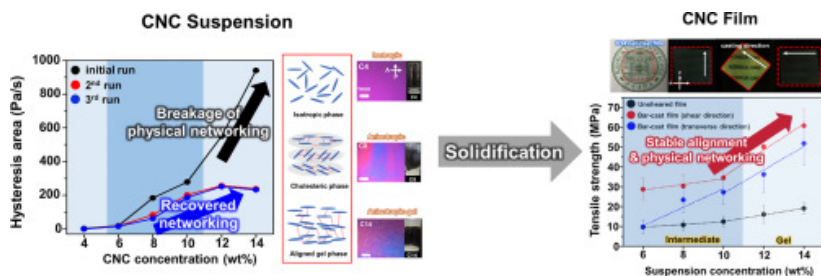
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## Effects of wet-media milling on multi-scale structures and *in vitro* digestion of tapioca starch and the structure-digestion relationship

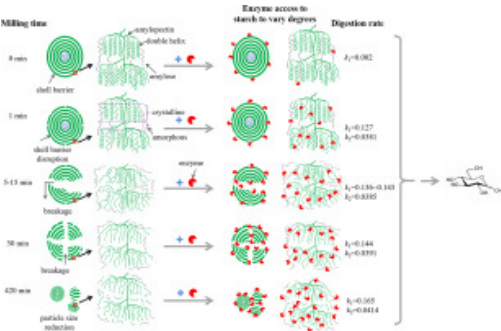
Xuxu Li, Xinran Yue, Qilin Huang, Binjia Zhang

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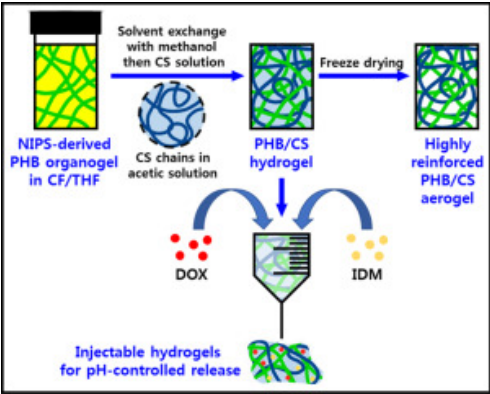
Chitosan-reinforced PHB hydrogel and aerogel monoliths fabricated by phase separation with the solvent-exchange method

Jiseon Kang, Seok Il Yun

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Remediation and resource utilization of chromium(III)-containing tannery effluent based on chitosan-sodium alginate hydrogel

Xiaoyu Guan, Bingyuan Zhang, Dongping Li, Mengyan He, ... Jinming Chang

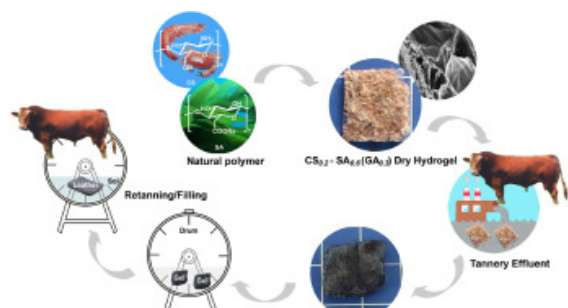
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$CS_{0.2}-SA_{0.6}(GA_{0.3})$  was successfully synthesized with a porous and layered three-dimensional structure, realizing the integration of chromium (III) containing tanning effluent treatment and retanning/filling resource utilization of adsorbent/adsorbate.



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Effect of  $\beta$ -cyclodextrins on the physical properties and anti-staling mechanisms of corn starch gels during storage

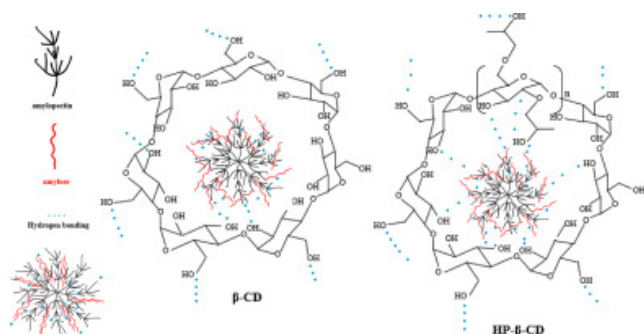
Ziyang Jia, Yun Luo, Francisco Jose Barba, Yan Wu, ... Yang Fu

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Pickering emulsions stabilized by biodegradable dextran-based nanoparticles featuring enzyme responsiveness and co-encapsulation of actives

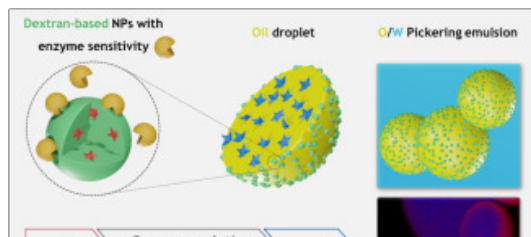
Valentin Maingret, Coraline Chartier, Jean-Luc Six, Véronique Schmitt, Valérie Héroguez

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## Hydrophobically modified polysaccharides and their self-assembled systems: A review on structures and food applications

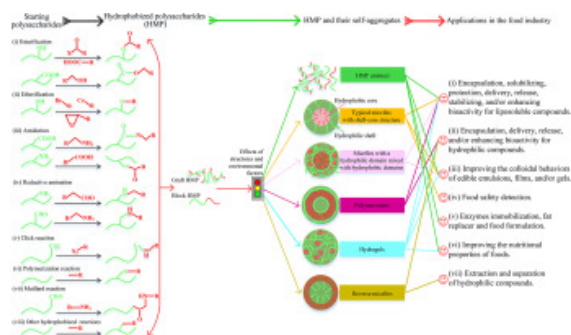
Zhen Wu, Hong Li, Xiaowan Zhao, Fayin Ye, Guohua Zhao

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## Stabilizing emulsions using high-amylose maize starch treated by solvothermal process

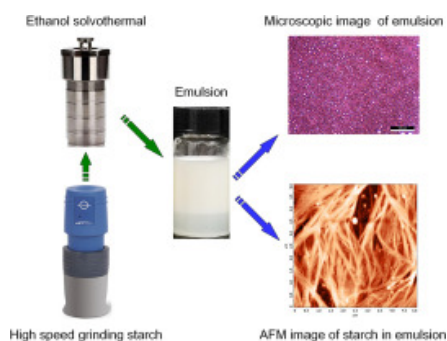
Yanhong Chen, Yu Liu, Huitao Liu, Yuan Gao

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## Ultrafast improvement of cellulose accessibility via non-dissolving pretreatment with $\text{LiBr} \cdot 3\text{H}_2\text{O}$ under room temperature

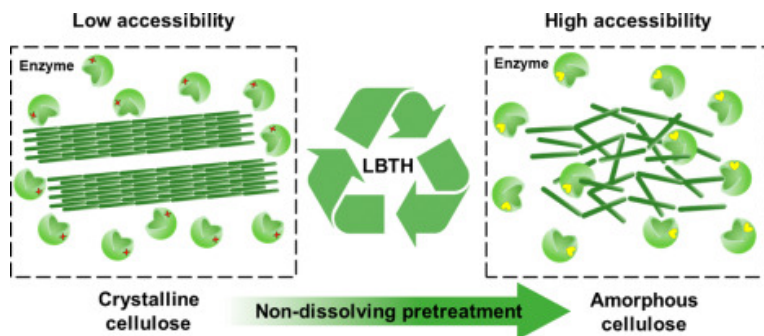
Zhonglei Huang, Guang Yu, Chao Liu, Meiyang Wu, ... Hui Peng

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## Selective xyloglucan oligosaccharide hydrolysis by a GH31 $\alpha$ -xylosidase from *Escherichia coli*

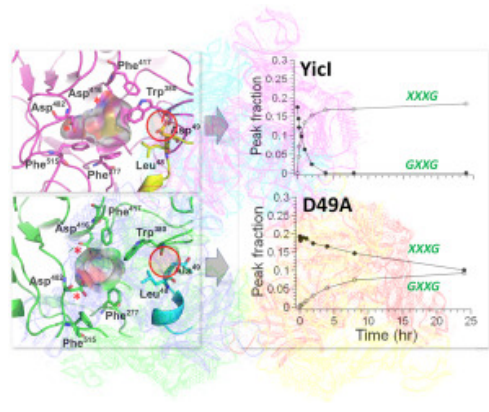
Lara Aparecida Buffoni de Campos Carneiro, Carlos Alessandro Fuzo, Luana Parras Meleiro, Sibeli Carli, ... Richard John Ward

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## One-dimensional nanohybrids based on cellulose nanocrystals and their SERS performance

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### Abstract

Controllable and uniform loading of gold nanospheres (AuNSs) on cellulose nanocrystal (CNC) were first achieved by electrostatic adsorption self-assembly. By adjusting the size, Zeta potential, and the loading ratio of AuNSs and CNC, the particle spacing of AuNSs on CNC surface was successfully regulated. This strategy provides an easy and efficient approach to construct one dimensional (1D) "hot spots", which is a key to improve the performance of surface enhanced Raman scattering spectroscopy (SERS). When used as SERS probe, the nanohybrid of CNC@AuNSs is able to detect Rhodamine 6G at the low concentration of  $5 \times 10^{-8}$  g/L. Because AuNSs are fixed on CNC to form stable "hot spots", the SERS reproducibility of CNC@AuNSs is significantly improved compared to that of colloidal gold nanoparticles, which generally form unstable "hot spots" via irreversible aggregation. This type of multifunctional nanoprobes based on CNC has potential applications in the field of sensing detection.

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## Injectable, self-healable and adhesive hydrogels using oxidized Succinoglycan/chitosan for pH-responsive drug delivery

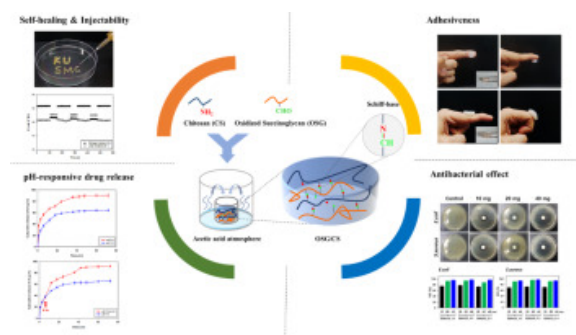
Yohan Kim, Yiluo Hu, Jae-pil Jeong, Seunho Jung

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## Synthesis, characterization, and biological evaluation of novel selenium-containing chitosan derivatives

Wanwen Chen, Hao Cheng, Lingyun Chen, Xiaobei Zhan, Wenshui Xia

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## Abstract

Though great efforts have been made to develop selenium polysaccharides with unique properties using  $\text{HNO}_3\text{-Na}_2\text{SeO}_3$  methods, the Se content is still low due to the poor esterification efficiency of  $\text{H}_2\text{SeO}_3$ . Herein, selenodiacetic acid (SA) was introduced into chitosan (CS) to synthesize *O*-selenodiacetyl chitosan (OSAC) and chitosan-ammonium selenodiacetate (CASA) by covalent and non-covalent interaction, respectively. The obtained CS derivatives were characterized by UV-vis, FTIR,  $^1\text{H}$  NMR, XPS, TGA, and XRD spectra, and the OSAC and CASA showed high Se content up to  $15,720 \pm 475$  and  $26,363 \pm 698$   $\mu\text{g/g}$ . The OSAC and CASA demonstrated increased antioxidant activities compared to the CS in DPPH and ABTS free radical scavenging assays. Moreover, they exhibited a potent anticancer effect on HepG2 cells with the  $\text{IC}_{50}$  values of 0.918 and 1.459  $\mu\text{g/mL}$ . Taken together, this study provides a promising strategy for the design of novel selenium polysaccharides with high Se content and greater biological activity

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Targeted delivery of methotrexate by modified yeast  $\beta$ -glucan nanoparticles for rheumatoid arthritis therapy

Huanhuan Chen, Ying Sun, Xiaojuan Xu, Qifa Ye

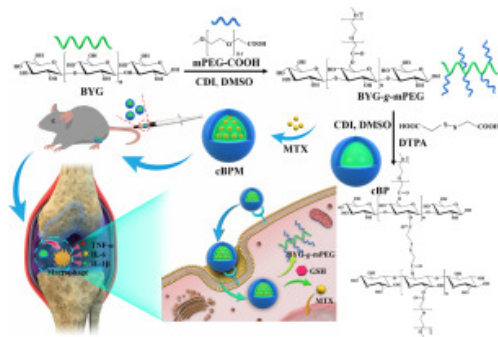
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Scheme 1. Preparation of BYG-based nanoparticle and its targeting therapy of rheumatoid arthritis.



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## Microgels based on Infernan, a glycosaminoglycan-mimetic bacterial exopolysaccharide, as BMP-2 delivery systems

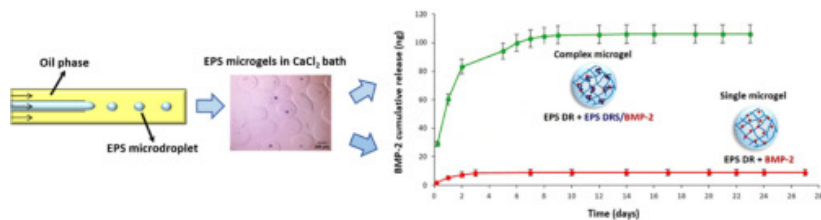
Perrine Gélébart, Stéphane Cuenot, Corinne Sinquin, Boris Halgand, ... Agata Zykwinska

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## Non-radical synthesis of chitosan-quercetin polysaccharide: Properties, bioactivity and applications

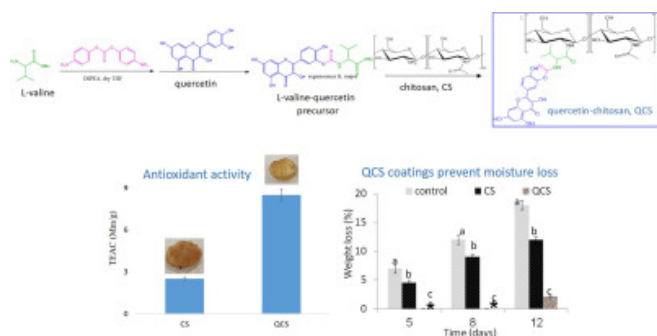
Yevgenia Shebis, Alexander Laskavy, Anat Molad-Filosof, Hadar Arnon-Rips, ... Elena Poverenov

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## Role of sulfated polysaccharides from seaweeds in bone regeneration: A systematic review

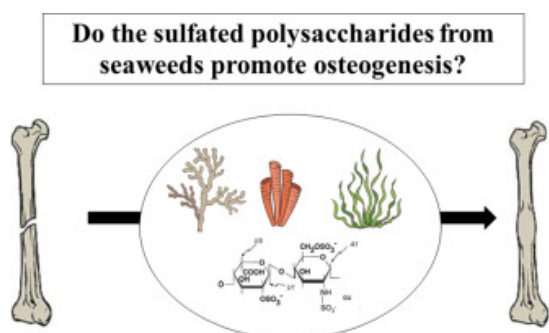
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## Functionalized chitosan/spherical nanocellulose-based hydrogel with superior antibacterial efficiency for wound healing

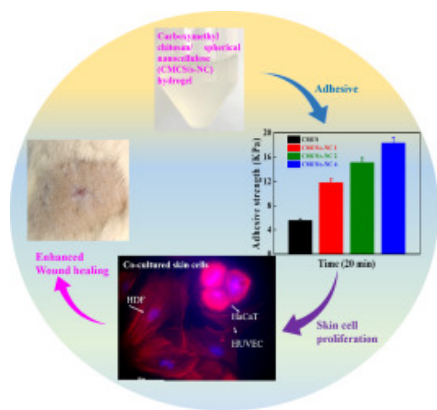
Dinesh K. Patel, Keya Ganguly, Jin Hexiu, Sayan Deb Dutta, ... Ki-Taek Lim

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## Lipase induced highly hydrophobic nanofibrillated cellulose film for strain sensor application

Yingchao Wang, Qiang Wang, Shanshan Liu, Xingxiang Ji, ... Jiachuan Chen

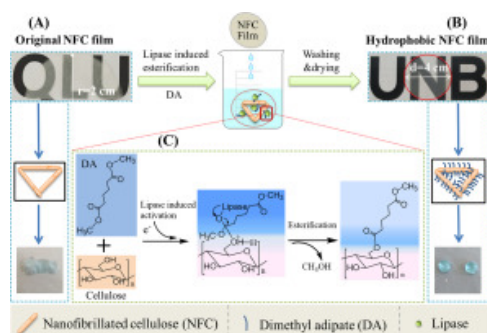
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Schematic representation of lipase induced highly hydrophobic NFC film.



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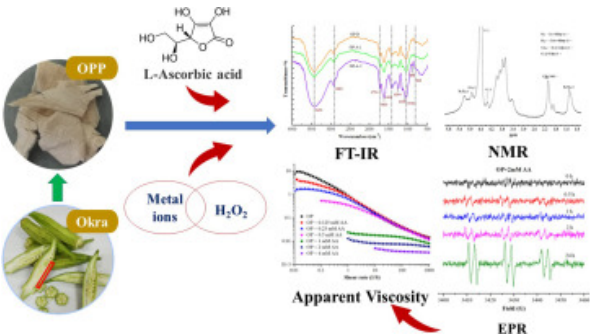
## Mechanism of viscosity reduction of okra pectic polysaccharide by ascorbic acid

Xiumei Zhu, Jinyin Chen, Hui Wang, Zongcai Tu, ... Shaoping Nie

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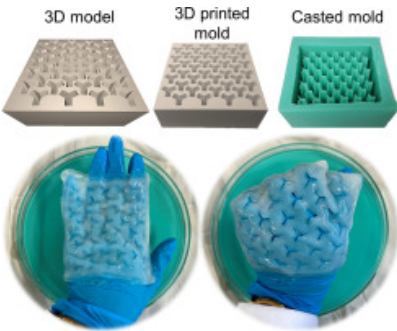
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Bacterial nanocellulose enables auxetic supporting implants

Rubina Ajdary, Roozbeh Abidnejad, Janika Lehtonen, Jani Kuula, ... Orlando J. Rojas

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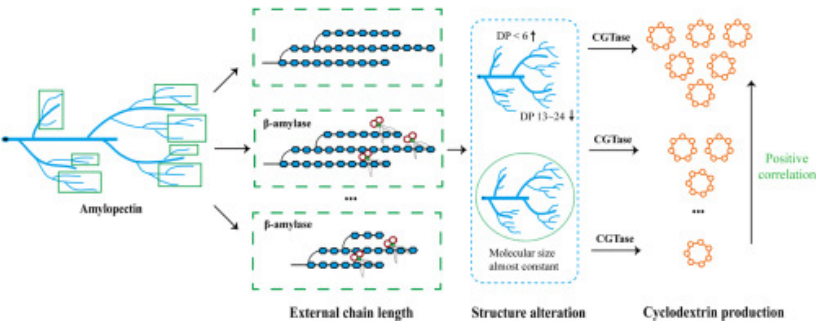
Deciphering external chain length and cyclodextrin production with starch catalyzed by cyclodextrin glycosyltransferase

Hangyan Ji, Yuxiang Bai, Yixi Liu, Yanli Wang, ... Zhengyu Jin

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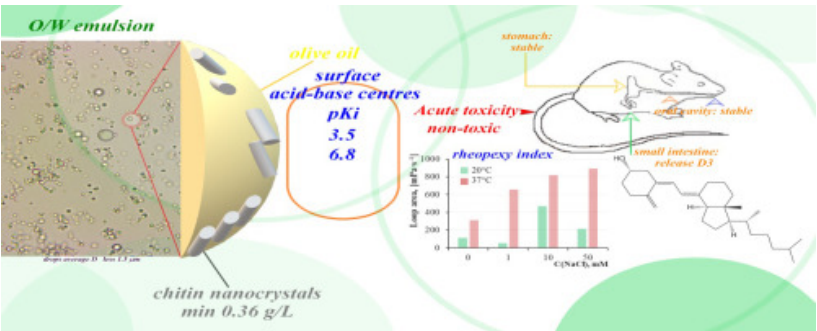
Surface, rheopexy, digestive stability and toxicity of olive oil emulsions stabilized by chitin nanocrystals for vitamin D3 delivery

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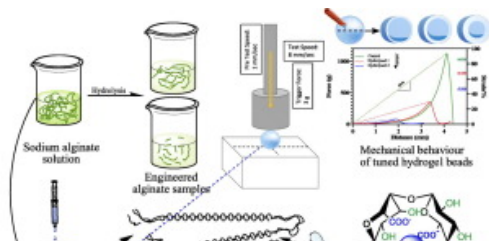
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## Chitin/corn stalk pith sponge stimulated hemostasis with erythrocyte absorption, platelet activation, and $\text{Ca}^{2+}$ -binding capabilities

Hao Cheng, Xin Pan, Zhe Shi, Xusheng Huang, ... Zhanjun Shi

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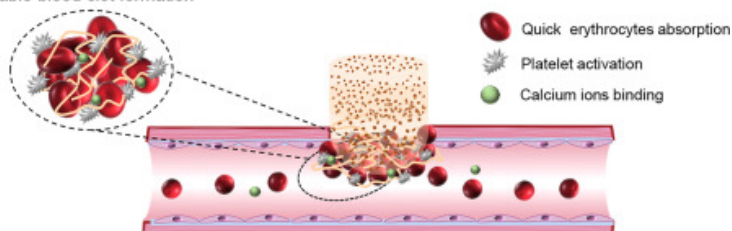
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#### Chitin/Corn Stalk Pith Sponge Stimulated Hemostasis

Efficient hemostasis and stable blood clot formation



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## Chitosan, chitosan nanoparticles and modified chitosan biomaterials, a potential tool to combat salinity stress in plants

Sri Renukadevi Balusamy, Shadi Rahimi, Johan Sukweenadhi, Sneha Sunderraj, ... Haribalan Perumalsamy

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Abstract

### Abstract

Chitosan being non-toxic, biocompatible, and biodegradable gained considerable interest among agriculturists. Our research review discusses about the role of Cs, chitosan nanoparticles (CsNPs), and modified chitosan biomaterials (CsBMs) under salt stress to improve growth parameters such as plant height, weight, stem width, fruit yield, pigments such as chlorophyll *a*, *b*, total chlorophyll, and carotenoid contents, as well as antioxidant and non-antioxidative enzymes. Upon Cs treatment and salt stress, total aminoacids (TAA), glutamic acids, and gamma-aminobutyric acid (GABA) were increased. Furthermore, Cs activated SOS1 pathway and increased various gene transcripts involved in sodium compartmentalization, proton motive force, energy

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production, and phenol metabolism. On the other hand, CsNPs and modified CsBMs treated plants under salinity stress increased indole terpene alkaloid metabolism, defense related genes, decreased ROS production by enhancing JA signaling,

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## Improved production of gamma-cyclodextrin from high-concentrated starch using enzyme pretreatment under swelling condition

Hao Wu, Xiaoxiao Li, Hangyan Ji, Birte Svensson, Yuxiang Bai

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## Deep eutectic solvents boosting solubilization and Se-functionalization of heteropolysaccharide: Multiple hydrogen bonds modulation

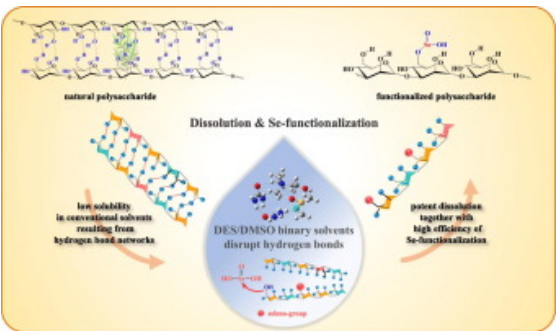
Yaxu Sun, Xiaoyan Jia, Ru Yang, Xiaojie Qin, ... Junlong Wang

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## Chitosan-gum arabic embedded alizarin nanocarriers inhibit biofilm formation of multispecies microorganisms

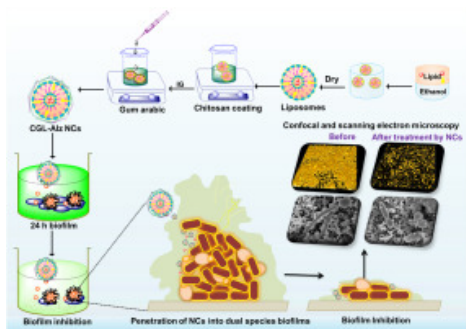
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## Alginate oligosaccharides: The structure-function relationships and the directional preparation for application

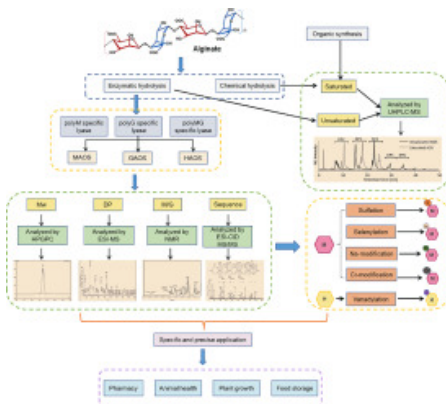
Shuang Lu, Kai Na, Jiani Wei, Li Zhang, Xiaohua Guo

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## Co-delivery of triamcinolone acetonide and verapamil for synergistic treatment of hypertrophic scars via carboxymethyl chitosan and *Bletilla striata* polysaccharide-based microneedles

Nan Zhang, Lingping Xue, Ayesha Younas, Fenfen Liu, ... Yongxing Zhao

Article 119219

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## Abstract

### Abstract

Hypertrophic scar (HS) is a frequently diagnosed skin disease that is difficult to treat. HS is usually associated with itching and pain and causes both physical and psychological issues. In this study, a safe, convenient, and efficient therapy for HS is developed. Carboxymethyl chitosan (CMCH) and *Bletilla striata* polysaccharide (BSP) are used to prepare microneedles (MN) via a micro-molding method. Hydroxypropyl  $\beta$ -cyclodextrin (HP- $\beta$ -CD) is used to encapsulate triamcinolone acetonide (TA) and the obtained inclusion is co-loaded with verapamil (VRP) to MN. The MN is then attached to an Ethyl cellulose (EC) base layer to obtain a MN patch. The MN patch has uniform needles, sufficient mechanical strength, good penetration and dissolution in skin, and low cytotoxicity. It also significantly decreases the thickness of HS, and hydroxyproline (HYP) and transforming growth factor-beta 1 (TGF- $\beta$ 1) expression in HS, improves collagen fiber arrangement, and reduces dermis congestion and hyperplasia.

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A universal construction of robust interface between 2D conductive polymer and cellulose for textile supercapacitor

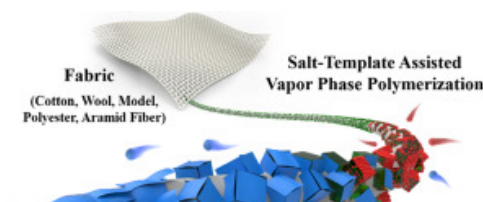
Jun Wan, Rong Hu, Junfeng Li, Shiyun Mi, ... Weilin Xu

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A universal and scalable construction of robust interface between two-dimensional conductive polymer and traditional cellulose fibers are realized by a salt-template assisted vapor phase polymerization method. The prepared 2D polypyrrole@ cotton electrode displays high specific capacitance and good cycling stability during bending. This work provides a new strategy for the robust interface between functional materials and various cellulose fibers, and has great potential for industrial manufacture.



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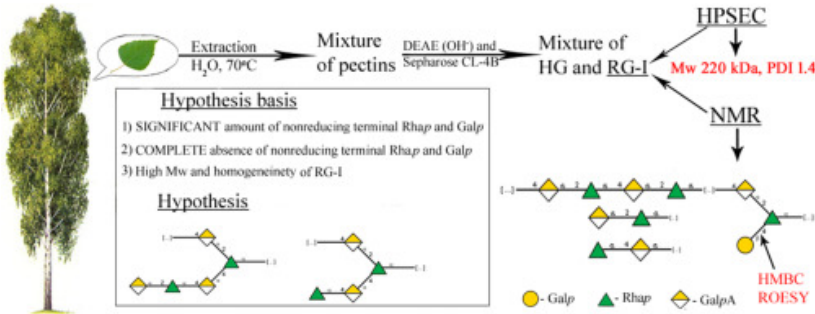
Pectin from leaves of birch (*Betula pendula* Roth.): Results of NMR experiments and hypothesis of the RG-I structure

Victoria V. Golovchenko, Victor A. Khlopin, Olga A. Patova, Liubov S. Feltsinger, ... Alexander S. Shashkov

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Hierarchical surface wrinkles and bumps generated on chitosan films having double-skin layers comprising topmost carrageenan layers and polyion complex layers

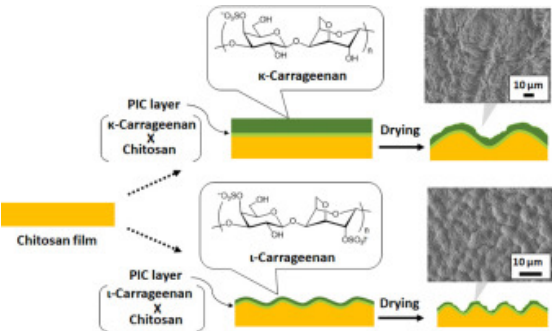
Hironori Izawa, Tomoe Yonemura, Yumi Nakamura, Yuta Toyoshima, ... Shinsuke Ifuku

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Fast and highly efficient adsorption of cationic dyes by phytic acid crosslinked β-cyclodextrin

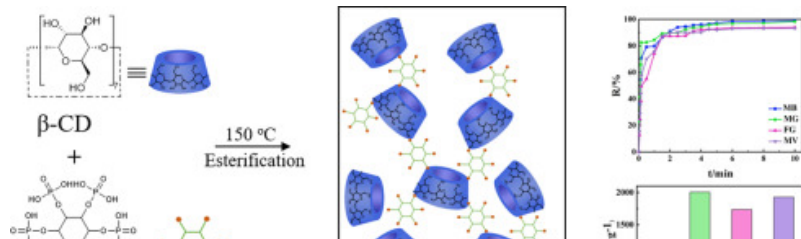
Yao Li, Erlei Yu, Suning Sun, Wenbo Liu, ... Liang Xu

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## Disease resistance and growth promotion activities of chitin/cellulose nanofiber from spent mushroom substrate to plant

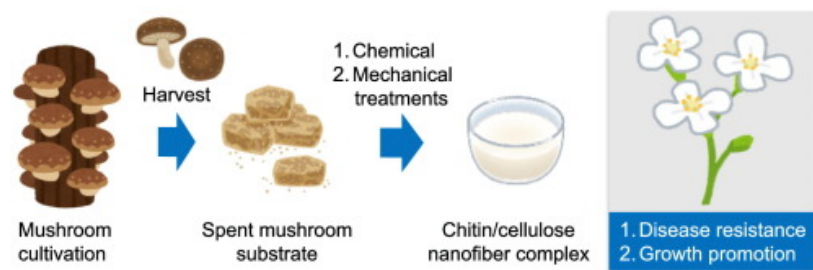
Hujun Li, Saori Yoshida, Naofumi Mitani, Mayumi Egusa, ... Shinsuke Ifuku

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## An environmentally tolerant, highly stable, cellulose nanofiber-reinforced, conductive hydrogel multifunctional sensor

Miao Li, Dong Chen, Xia Sun, Zesheng Xu, ... Feng Jiang

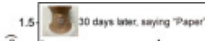
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Physically cross-linked organohydrogels with good stretchability, environmental tolerance, and long-term stability were prepared from cellulose nanofibers for multifunctional sensors.



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## Review

# Chitosan, chitosan nanoparticles and modified chitosan biomaterials, a potential tool to combat salinity stress in plants

Sri Renukadevi Balusamy<sup>a,\*</sup>, Shadi Rahimi<sup>b</sup>, Johan Sukweenadhi<sup>c</sup>, Sneha Sunderraj<sup>d</sup>,  
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Chitosan nanoparticles in plant stress tolerance  
Functionalized chitosan nanoparticles in plant stress  
Polymers in secondary metabolites under plant stress

## ABSTRACT

Chitosan being non-toxic, biocompatible, and biodegradable gained considerable interest among agriculturists. Our research review discusses about the role of Cs, chitosan nanoparticles (CsNPs), and modified chitosan biomaterials (CsBMs) under salt stress to improve growth parameters such as plant height, weight, stem width, fruit yield, pigments such as chlorophyll *a*, *b*, total chlorophyll, and carotenoid contents, as well as antioxidant and non-antioxidative enzymes. Upon Cs treatment and salt stress, total aminoacids (TAA), glutamic acids, and gamma-aminobutyric acid (GABA) were increased. Furthermore, Cs activated SOS1 pathway and increased various gene transcripts involved in sodium compartmentalization, proton motive force, energy production, and phenol metabolism. On the other hand, CsNPs and modified CsBMs treated plants under salinity stress increased indole terpene alkaloid metabolism, defense related genes, decreased ROS production by enhancing JA signaling, increased essential oil, anthocyanins, membrane stability, alkaloids, and diterpene glycosides. This is the first review that specifically brings insights about the physiological and biochemical parameters of the plants by comparing Cs/CsNPs/modified CsBMs treatment options under salt stress and encourages the use of CsNPs and modified CsBMs compared to Cs for better plant function under salinity stress.

## 1. Introduction

Soil salinity is one of the main problems in arid and semiarid areas that affect the growth and productivity by interfering with the nutritional status of plants through complex interaction mechanisms. The typical salt challenged plants to exhibit reduced nutrient absorption, poor transport of nutrients from roots to shoots, decreased dry leaf and stunted root growth, degradation of leaf pigments like chlorophyll and carotenoids affecting photosynthetic machinery, and causing a reduction in overall growth (Ashour, Esmail, & Kotb, 2020; Mosavikia, Mosavi, Seghatoleslami, & Baradaran, 2020; Safikhani, Khoshbakht, Chaichi, Amini, & Moteszarehadeh, 2018; Sen, Chouhan, Das, Ghosh, & Mandal, 2020; Zayed, Elkafafi, Zedan, & Dawoud, 2017). Reactive

oxygen species (ROS) are reactive molecules that cause oxidative stress, damage to proteins and nucleic acids, produce excess malondialdehyde (MDA) by inactivating antioxidant enzymes and ultimately causes cell death under salt stress (Sheikhalipour et al., 2021). They also play an important role in cell signaling and tissue homeostasis (Su et al., 2019). Further, during salt stress, plants also use non-enzymatic compounds such as phenolic compounds and flavonoids, ascorbate (vitamin C), chlorophylls, carotenoids, glutathione constitute, and  $\alpha$ -tocopherols to remove ROS while protecting cells from oxidative stress (Gerami, Majidian, Ghorbanpour, & Alipour, 2020; Sen et al., 2020; Sheikhalipour et al., 2021). Additionally, salt stress could activate the modulation of nitric oxide (NO) on gene expression and protein function, inducing the  $H^+$ -ATPase activity, chlorophyll biosynthesis, osmolyte

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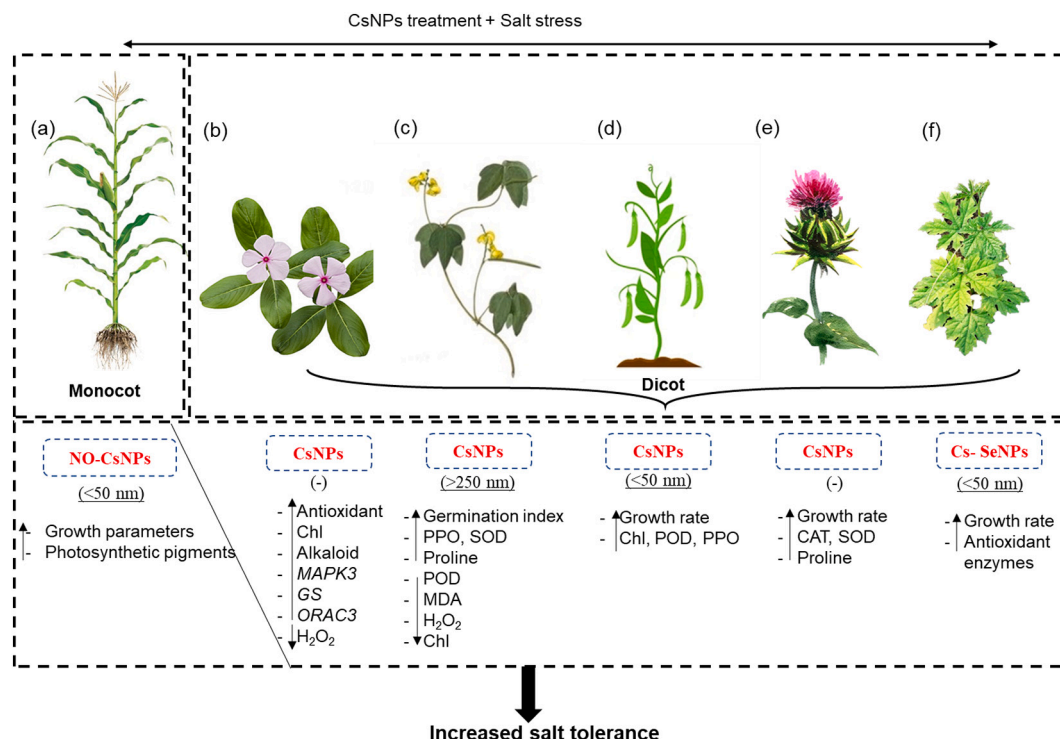
E-mail addresses: [renubalu@sejong.ac.kr](mailto:renubalu@sejong.ac.kr) (S.R. Balusamy), [harirai2004@hanyang.ac.kr](mailto:harirai2004@hanyang.ac.kr) (H. Perumalsamy).

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**Fig. 1.** CsNPs/ modified CsBMs treatment under salinity stress improved overall plant performance in various plant species. (a) maize, (b) periwinkle, (c) mung bean, (d) bean, (e) milk thistle, (f) bitter melon. Biomaterials with different sizes used to combat salt stress of different plant species were listed. Chl- chlorophyll, MAPK3- mitogen activated protein kinase; GS- geissoschizine synthase, ORAC3-terpene indole alkaloid transcription factor; H<sub>2</sub>O<sub>2</sub>- hydrogen peroxide; PPO-polyphenol oxidase; SOD-superoxide dismutase; MDA- malondialdehyde; POD-peroxidase.

accumulation, and secondary metabolites (Oliveira, Gomes, Pelegrino, & Seabra, 2016). Altogether, significant destructive effects of salt stress can occur on plant growth, development, differentiation rate as well as primary and secondary metabolism (Gerami et al., 2020; Hassan, Ali, Gaber, Fetouh, & Mazrou, 2021).

Chitosan (Cs) has a wide application scope in various biological fields including agriculture, thanks to its amine and -OH groups which make it with nucleophilic behavior and easily available for various chemical reaction (Rabêlo et al., 2019). The application of Cs in the right manner and concentration can effectively reduce the harmful effects of high salinity levels and increase plant growth and/or the yields (Safikhani et al., 2018), as it reported on maize (Turk, 2019), mung bean (Sen & Mandal, 2016), rice (Garude, Vemula, & SRM, 2019), safflower (Golkar, Taghizadeh, & Yousefian, 2019), stevia (Gerami et al., 2020), sunflower (Bakhoun, Sadak, & Badr, 2020), and tomato (Hernández-Hernández et al., 2018; Ullah et al., 2020) plants. Those studies also showed that Cs under saline stress alleviated effect at gene expression level as well as in biochemical level. Additionally, usage of Cs is preferred due to several properties such as antioxidant (Jabeen & Ahmad, 2013; Safikhani et al., 2018), anti-microbial (Gerami et al., 2020), biodegradable (Ullah et al., 2020), bioactive (Bakhoun et al., 2020; Turk, 2019), biocompatible (Hassan et al., 2021; Safikhani et al., 2018), water-soluble (Golkar et al., 2019) and non-toxic (Sen et al., 2020; Zayed et al., 2017). They can be applied through foliar spray or soil application to induce stress tolerance and improve plant performance by activating several stress-related enzymes. It also can be applied as seed coating (Garude et al., 2019) and solid matrix priming (SMP) or matric conditioning. In SMP, the seeds are mixed with solid or semi-solid materials with a certain amount of water and take advantage of the chemical and physical characteristics of the solids to limit the water absorption of the seeds (Sen & Mandal, 2016).

Nanotechnology exploits the unique characteristics of nanoscale particles with a diameter of less than 100 nm is a promising new field of biotechnology in various fields, including agriculture. The application of

nanomaterials is a relatively new and emerging field of study that has great potential for mitigating plant stress, both biotic and abiotic. The advantage of chitosan biomaterials (CsBMs) over Cs is, CsBMs possess interface and surface effects, and their smaller size makes CsBMs effective over bulk Cs (Divya & Jisha, 2018). In fact, various applications of Cs-NPs have been found to increase salinity tolerance in plants (Sen et al., 2020; Sheikhalipour et al., 2021; Zayed et al., 2017). It is even more interesting to see the composition of CsBMs in mitigating the deleterious effect of salt stress on bitter melon (Sheikhalipour et al., 2021), maize (Oliveira et al., 2016), milk thistle (Mosavikia et al., 2020), and periwinkle (Hassan et al., 2021). Despite using CsBMs alone to improve the productivity and quality of several crops, the use of modified CsBMs gained more advantage. However, information is still scarce concerning the abiotic stress tolerance effects of CsBMs. Previous reviews have focused on the importance of Cs in both abiotic and biotic stress conditions but lack information on physiological and biochemical parameters of Cs, CsBMs/modified CsBMs (Hidangmayum, Dwivedi, Katiyar, & Hemantaranjan, 2019; Pongprayoon, Siringam, Panya, & Roytrakul, 2020). For the first time, this review summarizes the beneficial effect of CsBMs alone or in the modified form compared to Cs, specifically under salt stress to improve overall performances (Fig. 1). This study gives a comprehensive insight from the synthesis of Cs nanomaterials to the mechanism of transport and function of Cs/CsBMs/modified CsBMs under salinity conditions. It broadly discusses the physiological changes by Cs/CsBMs/modified CsBMs, including changes in primary metabolites, JA signaling, antioxidant activity, secondary metabolites and membrane permeability, leading to improved photosynthesis and growth under salinity conditions.

## 2. Synthesis of CsNPs/modified CsBMs for salt stress mitigation

CsBMs have been reported to be prepared by emulsion droplet coalescence, reverse micellar method, ionic gelation, precipitation, sieving, and spray drying therefore, widely used in various biomedical

**Table 1**

Common characteristics features of CsBMs/modified CsBMs synthesis used to combat salt stress.

Botanical name	Common name	Molecular weight/deacetylation percentage	Chitosan nano/microparticles (CsNPs/CsMPs)	Methods used	Size	Shape	Poly dispersity index (PDI)	Zeta potential	References
<i>Zea mays L</i>	Maize	~87.4% deacetylation	NO releasing Cs-NPs	Iontropic gelatin	38.81 ± 18.10 nm	–	0.300 ± 0.010	17.7 ± 0.1 mV	(Oliveira et al., 2016)
<i>Momordica charantia</i>	Bitter melon	75–85% deacetylation	Cs-Se NPs	–	50 nm	Spherical	–	–	(Sheikhalipour et al., 2021)
<i>Phaseolus vulgaris L.</i>	Bean	100–300 kDa / –	CsNPs	Polymerization	46.32 nm	–	–	–	(Zayed et al., 2017)
<i>Catharanthus roseus (L.) G. Don</i>	Periwinkle	Medium molecular weight/80% deacetylation	CsNPs	Ionic gelatin of Cs with triphosphate anion	–	–	–	–	(Hassan et al., 2021)
<i>Vigna radiata</i>	Mung bean	–/–	CsNPs	Iontropic gelatin	254 nm	Sphere	0.501	+103 mV	(Sen et al., 2020)
<i>Silybum marianum (L.) Gaertn.</i>	Milk thistle	–/–	CsNPs	–	–	–	–	–	(Mosavikia et al., 2020)
<i>Solanum lycopersicum</i>	Tomato	1531 ± 372 kDa / –	Cs-MPs	Ionic gelatin	2.10 µm ± 0.78 µm	–	0.14, 1.95 ± 0.32	–	(Colman et al., 2019)

applications (Agnihotri, Mallikarjuna, & Aminabhavi, 2004; Alonso, Calvo, & Remun, 1997; Calvo, Remunan-Lopez, Vila-Jato, & Alonso, 1997; Mitra, Gaur, Ghosh, & Maitra, 2001). However, chitosan nanoparticles (CsNPs) used in the salt stress tolerance were prepared using ionotropic gelatin method (Oliveira et al., 2016). The use of ionotropic gelation method benefits Cs to encapsulate various molecules, produce nanoparticles in a wide range of sizes, ability to encapsulate drugs with medium to high efficiency, and to produce stable nanoparticles indicating the versatility of the technique for using it in biomedical field (Sacco et al., 2021). However, the importance of CsNPs synthesized using ionotropic gelation method in plants was not elucidated till date. It is well known that the size of the prepared nanoparticles depends on the molecular weight, chemical structure, and degree of deacetylation of Cs (Chan & Kwok, 2011; Luangtana-anan et al., 2005). For instance, the higher the Cs molecular weight, the larger size of CsNPs were observed irrespective of whether the plant is monocot or dicot. In bitter melon, molecular weight of Cs 310–375 kDa were used to synthesize chitosan-selenium based nanoparticles (Cs-SeNPs) showed 75–85% deacetylation and produced 50 nm size nanoparticles. Whereas, in case of bean plants the molecular weight of Cs is between 100 and 300 kDa, and therefore the size of the synthesized CsNPs is recorded as 46.32 nm. However, this study lacks the deacetylation percentage (Table 1). The effect of Cs and CsNPs/modified CsBMs on plant defense response against salt stress were summarized in Tables 2 & 3. Nevertheless, the correlation between molecular weight, size, and surface charge of CsNPs and plant salt stress defense response are still in the preliminary stage and therefore, research should be conducted in this area to unravel many interesting facts.

### 3. Effect of Cs/CsNPs/ modified CsBMs on plant growth under salinity stress

Considering the effect of Cs and CsNPs on plant growth, we described the effect of Cs and CsNPs on growth parameters, including plant height, number of leaves, root length, fruit quality in this section.

#### 3.1. Improved plant growth upon Cs treatment

A foliar spray of 1000 mg L<sup>-1</sup> Cs under 5844 mg L<sup>-1</sup> NaCl to maize seedlings improved root length by 13.7%, plant height by 10.7% compared to salt stressed maize seedlings and considered to be the critical parameters in determining plant productivity (Turk, 2019). The growth promoting effect of Cs was likely due to the ability of Cs to activate primary metabolic pathways such as photosynthesis, glycolysis,

and glucose assimilation (Zhang, Li, Xing, Liu, & Li, 2017). A foliar application of Cs at 150 mg L<sup>-1</sup> to control non-stressed tomato plants showed the maximum plant height value, 63.75 cm. When tomato plants were treated with 8766 mg L<sup>-1</sup> of salt stress, average of 11.99 number of leaves were found. Whereas, foliar application of 150 mg L<sup>-1</sup> Cs under 8766 mg L<sup>-1</sup> salt stress significantly increased the leaf numbers up to 20.07, which was increased 19.07% from the stressed one.

Similarly, the leaf area of tomato plants decreased significantly up to 5.4% compared to control, with increased salt stress concentration up to 8766 mg L<sup>-1</sup>. However, the 150 mg L<sup>-1</sup> Cs application reversed the effect of salt stress by significant increase up to 9.8% in tomato plants' leaf area compared to control non-treated plants. Interestingly, compared to salt stressed plants, all Cs concentrations induced a substantial increase in the leaf area of salt stressed plants. When 150 mg L<sup>-1</sup> Cs was treated under salinity stress, the maximum stem diameter was observed compared to salt stress treated plants.

Salinity stress also affected the fruit weight (15.83 g), and number of fruits (55.43) compared to control plants. However, foliar application of Cs 150 mg L<sup>-1</sup> under salinity stress 61.6% increased number of fruits and 16.1% increased fruit weight of tomato plants, compared to salt stressed plants. The yield of tomato per plant also decreased significantly during salinity stress compared to control non-treated plants, which was 17.7% smaller. In comparison, foliar application of Cs showed a maximum yield of 1.38 kg compared to salt treated plants. Like leaf area, the yield was also increased in all Cs concentrations, compared to salt stress plants. Fruit firmness and fruit juice were also affected by salinity stress in tomato plants, valued 2.78 kg<sup>-2</sup> and 4.93 of pH, respectively. However, Cs application under salt stress increased the fruit firmness up to 29.5% and fruit juice up to 4.7% compared to salt stressed plants (Ullah et al., 2020). Overall, growth parameters such as plant height, root length, number of leaves, stem diameter, fruit quality etc., were reversed upon Cs application during salinity stress. Thus, Cs can be a potential tool to alleviate salt-induced detrimental effects in plants.

The application of Cs foliar spray on marigold also resulted in the same positive effect on growth parameters such as plant height, leaf area, number of branches and plants, dry weight by reducing salinity stress (Abdel-Mola & Ayyat, 2020). The sunflower seeds soaked with different concentrations of Cs 25, 50, and 75 mg L<sup>-1</sup> under different 4000 and 8000 mg L<sup>-1</sup> salinity levels could enhance the growth indices of sunflower plants. Compared to 8000 mg L<sup>-1</sup> of salinity stress and Cs treatment, 50 mg L<sup>-1</sup> Cs under 4000 mg L<sup>-1</sup> salinity stress recorded the highest shoot length, stem circumference, number of leaves, fresh weight, and dry weight (Bakhoun et al., 2020). Therefore, 50 mg L<sup>-1</sup> Cs

**Table 2**  
Effect of chitosan and its application under salinity stress of plants.

Plants	Parts	Growth conditions	Cs	Treatment	Salt	Treatment	Methods studied	References
<i>Helianthus annuus</i> L. (Sunflower)	Seeds	<i>In vivo</i>	25, 50, 75 mg L <sup>-1</sup>	Soaking	4000 & 8000 mg L <sup>-1</sup>	Irrigation	Photosynthetic pigments, shoot length, a number of leaves, stem circumference, fresh and dry weight of plants, IAA, phenol content, free aminoacids, proline, TSS, seed weight, oil, yield, protein.	(Bakhoun et al., 2020)
<i>Zea mays</i> cv. Arifiye (Maize)	Seedlings	<i>In vivo</i>	1000 mg L <sup>-1</sup>	Foliar	5844 mg L <sup>-1</sup>	Foliar	Reactive oxygen species, antioxidant content, mitochondrial respiration rate, root length, plant height	(Turk, 2019)
<i>Silybum marianum</i> (L.) Gaertn (Milk thistle)	Plant	<i>In vivo</i>	0.01, 0.05 & 0.1% (dw/dw)	Mixed with dry soil	2560, 6400, 9600 mg L <sup>-1</sup>	Irrigation	Root and shoot growth, leaf chlorophyll content, soluble sugars, proline content, antioxidant enzymes, H <sub>2</sub> O <sub>2</sub>	(Safikhan et al., 2018)
<i>Agrostis stolonidera</i> (Creeping bentgrass)	Plants	<i>In vivo</i>	100, 200, 500, 1000 & 2000 mg L <sup>-1</sup>	Dissolve with Hoagland solution	5.84 mg L <sup>-1</sup> for 4 days; 8.76 mg L <sup>-1</sup> for 4 days & 11.68 mg L <sup>-1</sup> for 16 days	Irrigation	Chlorophyll content, sugars, amino acids, antioxidant enzyme activities, Na <sup>+</sup> /K <sup>+</sup> antiporter gene expression, PA, MDA	(Geng, Li, Hassan, & Peng, 2020)
<i>Carthamus tinctorius</i> (L.) (Safflower)	Callus	<i>In vitro</i>	25 & 50 mg L <sup>-1</sup>	<i>In vitro</i> media	15,000 mg L <sup>-1</sup>	Tissue culture	Callus growth, flavonoids, flavonols. Anthocyanin, phenolics, DPPH radical scavenging	(Golkar et al., 2019)
<i>Vitex trifolia-Purpurea</i> (Arabian Lilac)	Plants	<i>In vivo</i>	30, 60, 90 mg L <sup>-1</sup>	Spray	1000, 2500 & 5000 mg L <sup>-1</sup>	Irrigation	Chlorophyll content, total carbohydrates, K <sup>+</sup> % and Ca <sup>2+</sup> %, Na <sup>+</sup> , K <sup>+</sup> ratio, proline, phenol content	(Ashour et al., 2020)
<i>Carthamus tinctorius</i> L. (Safflower)	Seeds	<i>In vitro</i>	2.5, 5, 7.5 mg L <sup>-1</sup>	Priming	2176, 4880, 6880 & 8640 mg L <sup>-1</sup>	Tissue culture	Germination, shoot length, root length, proline, POD, catalase, MDA	(Jabeen & Ahmad, 2013)
<i>Helianthus annuus</i> L. (Sunflower)	Seeds	<i>In vitro</i>	2.5, 5, 7.5 mg L <sup>-1</sup>	Priming	2176, 4880, 6880 & 8640 mg L <sup>-1</sup>	Tissue culture	Germination, shoot length, root length, proline, POD, CAT, MDA	(Jabeen & Ahmad, 2013)
<i>Triticum aestivum</i> L. (Wheat)	Seeds	<i>In vivo</i>	250, 500, 750 mg L <sup>-1</sup>	Soaking	2922, 5844, 8766 & 11,688 mg L <sup>-1</sup>	Irrigation	Growth parameters, antioxidant enzymes, MDA, proline	(Peykani & Sepehr, 2019)
<i>Zea maize</i> L. (Corn)	Seeds	<i>In vivo</i>	250, 500, 750 mg L <sup>-1</sup>	Soaking	2922, 5844, 8766 & 11,688 mg L <sup>-1</sup>	Irrigation	Growth parameters, antioxidant enzymes, MDA, proline	(Peykani & Sepehr, 2019)
<i>Vigna radiata</i> (Mung bean)	Seeds	<i>In vitro</i>	1, 2, 5 mg L <sup>-1</sup>	Priming	2560, 4800, 6400 & 9600 mg L <sup>-1</sup>	Soaking	Plant height, root length, germination rate	(Sen & Mandal, 2016)
<i>Solanum lycopersicum</i> (Tomato)	Seeds	<i>In vitro/ Salt stress -in vivo</i>	15 mg L <sup>-1</sup> chitosan immobilized with bacterium		2.9 × 10 <sup>-3</sup> and 5.8 × 10 <sup>-3</sup> mg L <sup>-1</sup>	Irrigation	Plant biomass, chlorophyll pigments, proline content, MDA, H <sub>2</sub> O <sub>2</sub> , antioxidant enzymes, nutrient accumulation	(Chanratana et al., 2019)
<i>Solanum lycopersicum</i> Mill. (Tomato)	Plants	Green house	1 g	Soil mixture	5844 mg L <sup>-1</sup>	Mixed in steiner nutrient solution	Plant height, stem diameter, number of leaves, number of clusters, SOD, CAT, GPX, PRI, JA gene expression	(Hernández-Hernández et al., 2018)
<i>Solanum lycopersicum</i> Mill. (Tomato)	Plants	<i>In vivo</i>	76,325, 152,650 & 228,975 mg L <sup>-1</sup>	Foliar	2922, 5844 & 8766 mg L <sup>-1</sup>	Irrigation	Leaf chlorophyll content, plant height, leaf area, stem diameter, number of fruits, fruit firmness and fruits juice pH, plant yield	(Ullah et al., 2020)
<i>Calendula officinalis</i> L. (Marigold)	Seedlings	<i>In vivo</i>	100, 200 & 400 mg L <sup>-1</sup>	Foliar	1000, 2000, 3000, 4000 & 5000 mg L <sup>-1</sup>	Irrigation	Vegetative growth parameters, floral parameters, chlorophyll content, proline, 3-carotene, free proline, Na, Cl %	(Abdel-Mola & Ayyat, 2020)
<i>Triticum aestivum</i> (Wheat)		Field	10 & 20 mg L <sup>-1</sup>	Foliar			Chlorophyll, carotenoids, vegetative parameters, antioxidant enzymes, MDA, phenols, flavonoids, beta-carotene, anthocyanin, lycobine	(Abdallah, Ramadan, El-Bassiouny, & Bakry, 2020)
<i>Silybum marianum</i> (Milk thistle)	Termina cuttings	Field	250 & 500 mg L <sup>-1</sup>	Foliar	2922 & 5844 mg L <sup>-1</sup>	Irrigation	Growth parameter, biochemical parameter, essential oil yield	(Helaly, Farouk, Arafa, & Ambhimmid, 2018)
<i>Ocimum basilicum</i> L. (Sweet Basil)	Seedlings	<i>In vivo</i>	200 mg L <sup>-1</sup>	Foliar	1461, 2922, 5844 & 8766 mg L <sup>-1</sup>	Irrigation	Chlorophyll, phenols, PAL, CVOMT gene expression	(Rashidi, Khavari-Nejad, Ramak, &

(continued on next page)

Table 2 (continued)

Plants	Parts	Growth conditions	Cs	Treatment	Salt	Treatment	Methods studied	References
<i>Stevia rebaudiana</i> Bertoni (Stevia plant)	Seeds	Pots	200, 400, 600 mg L <sup>-1</sup>		$2.9 \times 10^{-3}$ , $5.8 \times 10^{-3}$ & $8.7 \times 10^{-3}$ mg L <sup>-1</sup>	Spray method	Chlorophyll and carotenoids, diterpene glycosides, antioxidant enzymes -CAT, POX, lipid peroxidation	Saadatmand, 2020) (Gerami et al., 2020)

**Abbreviations:** ROS- Reactive oxygen species, IAA- Indole acetic acid; DPPH- 2,2-diphenyl-1-picrylhydrazyl, PAL-phenylalanine ammonia-lyase, CVOMT- Chavicol O-methyltransferase, SOD-Superoxide dismutase, CAT- Catalase, GPX- Glutathione peroxidase, MDA- Malondialdehyde, PA- Polyamine, PR1-Pathogen related protein-1, JA-Jasmonic acid, POX- Peroxidase, ABTS- 2,2'-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonic acid.

Table 3

Effect of CsNPs and its modified form on defense related pathways in plants under salt stress and normal conditions.

NPs	Plants	Parts treated	Growing conditions	Chitosan BM's concentration	Treatment	Salt	Treatments	Methods studied	References
Nitric oxide-releasing chitosan nanoparticles (NO-CsNPs)	<i>Zea mays</i> L.	Seeds	<i>In vivo</i>	76.32 & 152.65 mg L <sup>-1</sup>	Directly treated to sand	17,532 mg L <sup>-1</sup>	Directly treated to sand	Photosynthetic pigments, growth analysis, S- nitrosothiol content	(Oliveira et al., 2016)
Chitosan-Selenium nanoparticles (Cs-SeNPs)	<i>Momordica charantia</i>	Seeds	Growth chamber	10 & 20 mg L <sup>-1</sup>	Foliar	2922 & 5844 mg L <sup>-1</sup>	Hoagland nutrient supplemented with NaCl	Antioxidant enzymes, proline, essential oil from fruit, relative water content & K <sup>+</sup> , MDA, H <sub>2</sub> O <sub>2</sub> , Na aggregation studies	(Sheikhalipour et al., 2021)
Chitosan microparticles (CsMPs)	<i>Solanum lycopersicum</i>	Seeds	<i>In vitro</i>	0.1 and 1 mg mL <sup>-1</sup>	Foliar	–	–	Germination, vigor index, root and shoot biomass, ROS, antioxidant enzymes, defense related protein markers, auxin and cytokinin pathways	(Colman et al., 2019)
Chitosan nanoparticles (CsNPs)	<i>Phaseolus vulgaris</i> L.	Seeds	Trays	1, 2 and 3 mg L <sup>-1</sup>	Soaking	$2.9 \times 10^{-3}$ , $5.8 \times 10^{-3}$ & $8.7 \times 10^{-3}$ mg L <sup>-1</sup>	Hoagland nutrient solution mixed with different concentrations of salt	Membrane stability index, relative water content, antioxidant enzyme activity, PPO activity, proline	(Zayed et al., 2017)
Chitosan nanoparticles (CsNPs)	<i>Catharanthus roseus</i> (L.) G. Don	Plants	Pots	10 mg L <sup>-1</sup>	Foliar	8766 mg L <sup>-1</sup>	Irrigation	Plant height, shoot and root fresh and dry weights, chlorophyll content, MDA, H <sub>2</sub> O <sub>2</sub> , antioxidant enzymes, diterpene, MAPK3, GS, ORCA3, rBCL genes	(Hassan et al., 2021)
Chitosan nanoparticles (CsNPs)	<i>Vigna radiata</i>	Seeds	Tissue culture	2 mg L <sup>-1</sup>	Priming	2560 & 6400 mg L <sup>-1</sup>	Solid matrix priming	Chlorophyll, carotenoids, reducing sugar, protein content, phenol, flavonoids, vitamin c, proline, MDA, H <sub>2</sub> O <sub>2</sub> , DPPH, ABTS, CAT, POD, PPO, APX	(Sen et al., 2020)
Chitosan nanoparticles (CsNPs)	<i>Silybum marianum</i> (L.) Gaertn.	Seeds	Tissue culture	2.5, 5 & 10 mg L <sup>-1</sup>	Priming	$2.9 \times 10^{-3}$ , $5.8 \times 10^{-3}$ & $8.7 \times 10^{-3}$ mg L <sup>-1</sup>	Mixed with Van't Hoff formula	Germination rate, chlorophyll, CAT, proline content	(Mosavikia et al., 2020)

**Abbreviations:** ROS- reactive oxygen species, PAL-phenylalanine ammonia-lyase, SOD-superoxide dismutase, CAT- catalase, GPX- glutathione peroxidase, MDA-malondialdehyde, APX- ascorbic acid, PPO-polyphenol oxidase, DPPH-2,2- diphenyl-1-picrylhydrazyl, GS- gissoschizine synthase, MAPK3- mitogen-activated protein kinase 3, ORAC3- terpene indole alkaloid transcription factor.

was considered the most effective concentration and can be used to mitigate sunflower plants' salinity stress of 4000 mg L<sup>-1</sup> in concentration.

1 g of Chitosan-polyvinyl alcohol (Cs-PVA) was mixed with different parts of soil mixture under 5844 mg L<sup>-1</sup> saline stress showed increased plant height, stem diameter, number of leaves at 28- and 42-days after

transplantation (DAT) compared to Cs treated non-stressed and non-treated control tomato plants. Thus, proving Cs-PVA can be utilized to actuate the resilience of plants to salt pressure (Hernández-Hernández, Juárez-Maldonado, et al., 2018) by providing positive and synergistic role of Cs and PVA to produce salt tolerant tomato plants. When 0.5 mg L<sup>-1</sup> Cs was applied to milk thistle plants under 2560 mg L<sup>-1</sup> of salinity



stress, showed increasing plant height. Moreover, when the severity of salinity stress increased to  $6400 \text{ mg L}^{-1}$ , maximum plant height under  $0.5$  and  $0.1 \text{ mg L}^{-1}$  Cs application was observed. However, the maximum plant height at  $9600 \text{ mg L}^{-1}$  salinity level was observed at  $0.1 \text{ mg L}^{-1}$  Cs application. Thus, a lower percentage of Cs would be needed to reach a maximum height at an increased salinity level. Nevertheless, salinity stress of  $9600 \text{ mg L}^{-1}$  decreased root dry weight by  $55.3\%$  compared to control milk thistle plants. The salinity stress caused serious cellular damage, decreased biomass, root growth, water status, and photosynthesis. The application of Cs at all concentrations increased all the parameters, and thus improved tolerance to salt stress of milk thistle plants. This might be because of Cs adjusting root morphology to strengthen its absorption and survival by increasing root length and root surface area. Although the shoot dry weight was also increased in all tested concentrations of Cs, the maximum shoot dry weight was achieved in  $2560 \text{ mg L}^{-1}$  salt treatment with  $1 \text{ mg L}^{-1}$  Cs. The exact mechanism of action of Cs on growth is unclear, it was found that Cs may induce plant hormones such as gibberellins, enhance growth, and the developmental process by activating signaling linked to auxin biosynthesis. The different salinity stress concentrations recorded different biomass yields in milk thistle plants. For instance,  $1 \text{ mg L}^{-1}$  of Cs under  $9600 \text{ mg L}^{-1}$  salt treatment resulted in  $37.41\%$  higher plant biomass than non-treated control plants. Whereas, when the salt concentration of  $2560 \text{ mg L}^{-1}$  and  $6400 \text{ mg L}^{-1}$  applied along with  $0.5 \text{ mg L}^{-1}$  and/or  $0.1 \text{ mg L}^{-1}$  Cs exhibited the maximum biomass compared to  $9600 \text{ mg L}^{-1}$  salinity level and no Cs treatment (Safikhani et al., 2018). The combined treatment of zeolite-Cs ( $8 \text{ g kg}^{-1}$  and  $250 \text{ mg kg}^{-1}$ , respectively) by mixing in soil alleviated the depressing effects of salt stress by increasing plant growth, declined sodium and chloride concentration in both roots and shoots of rosemary plants (Helaly et al., 2018). In summary, the application of different concentrations of Cs under salt or non-stressed conditions improved the overall performance of plants by stimulating the growth effects of various plants.

### 3.2. Improved plant growth parameters upon CsNPs/modified CsBMs treatment

CsNPs play a potential role in increasing the growth parameters of various plants under abiotic stresses, particularly salt stress. Selenium (Se) is a beneficial nutrient, fertilizer for plants that can improve growth, yield of the plants and salt tolerance (Diao et al., 2014; Iqbal et al., 2015; Moore, Mahmoudkhani, Sanders, & Durand, 2012). A foliar spray of  $10$  and  $20 \text{ mg L}^{-1}$  of Cs-SeNPs nanoparticles improvised the bitter melon to tolerate salinity by increasing growth parameters, and better yield of essential oils. The treatment of  $20 \text{ mg L}^{-1}$  Cs-SeNPs significantly improved shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, stem height, and relative water content under  $2922 \text{ mg L}^{-1}$  and  $5844 \text{ mg L}^{-1}$  NaCl concentration compared to both  $10 \text{ mg L}^{-1}$  Cs-SeNPs treated and control plants. However, the more prominent effect was found when plants treated with  $20 \text{ mg L}^{-1}$  Cs-SeNPs against  $5844 \text{ mg L}^{-1}$  NaCl concentration. Moreover, essentially expanded fruit yield, fruit weight, and number of fruits under  $2922$  and  $5844 \text{ mg L}^{-1}$  NaCl (Sheikhalipour et al., 2021), indicating that  $20 \text{ mg L}^{-1}$  Cs-SeNPs could improve both fruit, and growth rate of the bitter melon plants under salinity stress by suggesting its synergistic role. When glycol-Cs and selenium nanoparticles (SeNPs) combined at optimum concentration, they can act synergistically to improve quality of ginseng by reduced oxidative stress, and increased ginsenoside production (Abid et al., 2021). The maize plants subjected to  $17,532 \text{ mg L}^{-1}$  NaCl stress under  $85.28 \text{ mg L}^{-1}$  and  $170.57 \text{ mg L}^{-1}$  nanoencapsulated S-nitroso-mercaptoposuccinic acid (MSA) CsNPs resulted in a complete arrest of salt induced alterations in fresh and dry weights of shoots and roots. Compared with  $8.96 \text{ mg L}^{-1}$ ,  $17.92 \text{ mg L}^{-1}$  free S-nitroso-MSA and  $170.57 \text{ mg L}^{-1}$  nanoencapsulated S-nitroso-MSA- CsNPs,  $85.28 \text{ mg L}^{-1}$  nanoencapsulated S-nitroso-MSA-CsNPs exhibited the highest salt tolerance to maize plants (Oliveira et al., 2016), indicating that the salt tolerant

effect is dependent on concentration of nanoencapsulated S-nitroso-MSA-CsNPs. It is also noteworthy to speculate from reported studies in this review that CsNPs itself is capable of exhibiting salt tolerant effect other than its role as nanocarriers. The synthesis of Cs-microparticles (CsMPs) and foliar treatment of  $0.01 \text{ mg L}^{-1}$ , and  $0.1 \text{ mg L}^{-1}$  CsMP's promoted a statistically significant increase in germination rate compared to bulk Cs and control tomato plants without salt stress. Moreover, treatment of  $0.01 \text{ mg L}^{-1}$  and  $0.1 \text{ mg L}^{-1}$  CsMPs enhanced the vigor index in 11-day old seedlings by  $29.3\%$  and  $38.1\%$  compared to control plants. Surprisingly, the vigor index of both bulk Cs and CsMPs treated plants showed similar improvement. However, bulk Cs showed reduced biomass, whereas CsMPs at  $0.01 \text{ mg L}^{-1}$  and  $0.1 \text{ mg L}^{-1}$  concentrations increased leaf area, root dry weight, and biomass (Colman et al., 2019). This study suggests the use of CsBMs over bulk Cs to improve the growth of plants thus can be used as a plant growth stimulator.

During the vegetative (V) and flowering stage (F), plant height, branch numbers, also fresh and dry weights of shoots and roots significantly reduced periwinkle plants exposed to  $8766 \text{ mg L}^{-1}$  salt. However, a foliar spray of  $10 \text{ mg L}^{-1}$  CsNPs under  $8766 \text{ mg L}^{-1}$  salinity stress reversed the effect of salt stress on fresh and dry weights of shoots and roots. However, the highest growth parameters were recorded in the CsNPs alone treated plants without salt stress (Hassan et al., 2021), indicating that  $10 \text{ mg L}^{-1}$  CsNPs is effective as a plant growth regulator under non-salinity conditions. The germination index (GI), co-efficient velocity (CV) was significantly improved under salinity stress in both Cs and CsNPs treatment by priming method with respect to that of control. However, there were some differences in GI and CV were observed between Cs and CsNPs treatments of mung bean seedlings. At low salinity stress (no salinity), GI of CsNPs treated seedlings increased by  $22.3\%$  compared to Cs alone treated seedlings. Seedlings treated with CsNPs increased CV by  $19.6\%$  compared to salt stressed seedlings. On the other hand, the significant lower mean germination time (MGT) was recorded in the CsNPs primed seedlings compared to Cs alone treated seedlings. However, CsNPs treated seedlings MGT increased by  $97\%$  with respect to control untreated seedling (no salinity). The germination stress tolerance index (G.S.I) of Cs and CsNPs was recorded as the highest at low salinity stress (no salinity) compared to hydro-primed, and control seedlings (Sen & Mandal, 2016). Treatment of  $1$ ,  $2$ , and  $3 \text{ mg L}^{-1}$  CsNPs, and  $5844 \text{ mg L}^{-1}$  salt to study salt tolerance in bean plants by measuring advanced salt tolerance index (S.T.I), seed germination and radical length.  $3 \text{ mg L}^{-1}$  CsNPs treatment under salt stress showed the highest S.T.I for germination after  $24$  h,  $48$ , and  $72$  h compared to salt treated plants. In addition to that, when  $3 \text{ mg L}^{-1}$  of CsNPs treated to bean plants resulted in increased S.T.I of radical length after  $24$ ,  $48$ , and  $72$  h. Further, there is an increase in plant height, leaf area, fresh and dry weight of shoot and root (Zayed et al., 2017). A seed priming technique is used to treat milk thistle seeds with  $2.5$ ,  $5$ , and  $10 \text{ mg L}^{-1}$  CsNPs and  $2922$ ,  $5844$ , and  $8766 \text{ mg L}^{-1}$  of salt. When seeds were subjected to  $8766 \text{ mg L}^{-1}$  salt stress, there was a significant reduction as much as  $49.12\%$  in germination percentage and  $50.07\%$  reduction of seedling length compared to control group. Among all different concentrations of CsNPs treated, priming with  $2.5 \text{ mg L}^{-1}$  CsNPs positively affected germination parameters, and physiological attributes such as  $40.4\%$  increased germination percentage,  $74.0\%$  increased seedling length, and  $3.7\%$  increased seedling dry weight compared to that of  $8766 \text{ mg L}^{-1}$  salt stressed milk thistle seedlings parameters (Mosavikia et al., 2020). Cs-NPs even at low concentration increased plant productivity, and growth in milk thistle plants.

In summary, for the improvement of physiological status of plants through Cs/CsBMs/ modified CsBMs application, seed priming is considered as the most effective method compared to other methods including foliar spray, *in vitro* application, and direct application to soil. Interestingly, as a finding from this review, seed priming requires only low concentration of Cs/CsNPs/modified CsBMs compared to other treatment methods to exhibit a significant difference in physiological

parameters in salt treated or untreated plants. However, that is not the case when other methods are used for Cs/CsBMs/ modified CsBMs application. From the above studies, it is clearly understood that the effect of Cs/CsNPs are species-specific, it is always important to optimize concentration for the maximum effect of Cs/CsBMs to diminish salt stress in plants.

#### 4. Effect of Cs/CsNPs/ modified CsBMs on photosynthesis under salinity stress

##### 4.1. Effect of Cs treatment on photosynthesis

The most significant environmental factor for plants is light, which provides energy for the photosynthesis process, and is considered as a regulator for all aspects of the vital process as far as plants are concerned. During the photosynthesis process of higher plants, pigments such as carotenoids, chlorophylls (chl a, b), and phycobilins are considered as the major pigments and greatly affected by various stresses including salt stress (Franklin & Whitelam, 2004; Jackson & Jenkins, 1995; Lima et al., 2017). Salt stress is a typical abiotic stress that antagonistically influences plant development and improvement. Foliar application of Cs at 250 and 500 mg L<sup>-1</sup> to rosemary plants reduced the chl a and b- during 2nd season, and increased during 1st season under 2922 and 5844 mg L<sup>-1</sup> salinity stress indicating that the application of Cs showed considerable increase in photosynthetic pigment, content, and composition of carotenoids by preventing chlorophyll from oxidation (Helaly et al., 2018). Application of 2 mg L<sup>-1</sup> Cs by foliar method to control and 1461 mg L<sup>-1</sup> NaCl treatments reduced the total chlorophyll content. Whereas 2 mg L<sup>-1</sup> Cs treatment under 2922 mg L<sup>-1</sup> and 5844 mg L<sup>-1</sup> salinity stress increased chlorophyll compared to that of control treated plants indicating that the ability of 2 mg L<sup>-1</sup> to increase or decrease the chl content depends on salt concentration (Rashidi et al., 2020). The increase in salt irrigation from 1000 to 5000 mg L<sup>-1</sup> resulted in decreased total chlorophylls and carotene content. The maximum reduction was observed in 5000 mg L<sup>-1</sup> in flowers marigold compared to control. Among different foliar application concentrations, 400 mg L<sup>-1</sup> Cs is the best concentration to improve the chlorophyll and carotene content by arresting salinity stress (Abdel-Mola & Ayyat, 2020). In tomato, foliar application of Cs alone or in combination with salt stress affects chlorophyll content significantly. The chlorophyll content was substantially reduced with increasing salinity levels. However, when 150 mg L<sup>-1</sup> Cs was treated under 2922, 5844 and 8766 mg L<sup>-1</sup> of salinity stress showed dose dependent decrease in chlorophyll content (higher the salt stress, lower the chlorophyll content) (Ullah et al., 2020). In Arabian lilac, there was also a similar trend recorded as tomato. During increase in salinity stress from 1000, 2500, 5000 mg L<sup>-1</sup> under 30, 60 and 90 mg L<sup>-1</sup> Cs treatments reduction in chlorophyll content was recorded in a dose dependent decrease manner. Whereas when Cs alone were treated to arabian lilac plants, the chlorophyll content was increased with increased concentration of Cs treatment compared to salt stressed and control plants (Ashour et al., 2020), indicating that Cs acts as an elicitor to increase chlorophyll content in plants.

In stevia, Cs directly sprayed on seedlings with unique concentrations of 200, 400, and 600 mg L<sup>-1</sup> and under 2922, 5844 and 8766 mg L<sup>-1</sup> of salinity stress reversed chlorophyll, and MDA content but activates antioxidant enzyme activity and increased stevia glycosides. Therefore, Cs increased adaptive factors in stevia plant to tolerate NaCl stress (Gerami et al., 2020). Two wheat cultivars namely Sakha 94\*, and Gemmeza 9\* were soaked with 10 and 20 mg L<sup>-1</sup> concentrations of Cs for 12 h and sowed in saline soil. Both Cs concentrations showed significant increase in chl a and b, carotenoids, and total pigments in saline condition. Compared to 10 mg L<sup>-1</sup> Cs, 20 mg L<sup>-1</sup> Cs treatment showed a significant increase in chl a and b, carotenoids, and total pigments indicating that high concentration of Cs could effectively combat salt stress in wheat plants (Abdallah et al., 2020). The soaking with different

concentrations of 25, 50, and 75 mg L<sup>-1</sup> Cs vs 4000 and 8000 mg L<sup>-1</sup> salt stress were compared, and photosynthetic pigments were evaluated in sunflower plants. Compared to all Cs and salt concentrations tested individually, 50 mg L<sup>-1</sup> Cs and 4000 mg L<sup>-1</sup> salt stress showed significant increase in photosynthetic pigments such as chl a, b, and carotenoids compared to all other treatments (Bakhoun et al., 2020).

When the salinity stress is increased to 6400 and 9600 mg L<sup>-1</sup>, decrease in chl a, b and total chlorophyll contents were noticed in milk thistle plants. Conversely, 0.1 mg L<sup>-1</sup> Cs application in soil showed increased trends in chl a, and total chlorophyll content, and maximum content of chl b was recorded in 0.5 mg L<sup>-1</sup> of Cs treated milk thistle plants. These results confirmed that a lower concentration of 0.1 mg L<sup>-1</sup> Cs increased chl a/ total chlorophyll whereas chl b was increased at 0.5 mg L<sup>-1</sup> which is the highest concentration of Cs treatments (Safikhani & Chaichi, 2021). However, this study didn't monitor the combined effect of salinity stress and Cs treatment on photosynthetic pigments, and therefore, future studies are warranted.

The changes in chl content, net photosynthesis rate (Pn), photochemical efficiency (Fv/Fm), and performance index on absorption basis (PIABS) in leaves of creeping grass were studied under 100, 200, 500, 1000 and 2000 mg L<sup>-1</sup> of Cs and 5844, 8766, 11,688 mg L<sup>-1</sup> salt stress treatment. When creeping grass is subjected to salt stress alone, there was a significant decline in chlorophyll content, Pn, Fv/Fm, and PIABS. Applying salt stress to Cs pre-treated seedlings alleviated the negative effects caused by salt stress by increasing chl a, chl b, total chl contents of leaves, and Pn compared to untreated plants at 12 or 24 d of salt stress. Interestingly, Fv/Fm and PIABS were not affected by Cs at 12, 24 d of normal condition but showed a significant increase of both parameters under Cs treatment at 12, 24 d of salt stress (Geng et al., 2020). This study suggests that creeping grass was sensitive to salt stress, but Cs application under salt stress improved tolerance to creeping grass by increasing chl, Pn and (Fv/Fm).

The photosynthetic response of tomato plant was monitored after treating Cs-immobilized aggregated *M. oryzae* CBMB20 (T5) and Cs-immobilized non-aggregated *M. oryzae* CBMB20 (T6) under salt stress treatments. The results showed that both T5 and T6 showed significant accumulation of chlorophyll and carotenoid content compared to plants inoculated with liquid formulation, control plants under normal and salt stress conditions (Chanratana et al., 2019). The effect of 200 mg L<sup>-1</sup> Cs on chl content was evaluated in sweet basil subjected to 1461, 2922, 5844, and 8766 mg L<sup>-1</sup> salinity stress. The application of 400 and 600 mg L<sup>-1</sup> Cs under 2922 mg L<sup>-1</sup> NaCl resulted in significant increase of chl a, b and total chl contents. However, 400 mg L<sup>-1</sup> Cs exhibited highest amount of all chl contents under 5844 mg L<sup>-1</sup> NaCl treatments. Among chl b and total chl, chl a increased during 400 mg L<sup>-1</sup> Cs and 8766 mg L<sup>-1</sup> NaCl treatment. Surprisingly 200 mg L<sup>-1</sup> Cs didn't show significant increase in all photosynthetic contents in all levels of NaCl treatment (Gerami et al., 2020) indicating that low concentration of Cs didn't show any positive effect on chlorophyll contents under salinity stress in sweet basil plants.

##### 4.2. CsNPs/modified CsBMs treatment on photosynthesis

CsBMs are non-toxic in nature and they are used to fight off salt stress in plants by enhancing plant growth stimulators and secondary metabolites (Hernández-Hernández, Juárez-Maldonado, et al., 2018; Safikhani et al., 2018). *Catharanthus roseus* plants were exposed to 18,766 mg L<sup>-1</sup> NaCl treatment and 10 mg L<sup>-1</sup> CsNPs concentration by foliar spray method exhibited reduction in chlorophyll and activated antioxidant enzymes such as CAT, APX, and glutathione reductase (GR). Accordingly, Cs-NPs eased the oxidative pressure by lowering MDA and H<sub>2</sub>O<sub>2</sub> levels, membrane function retention, and thus improving salt resilience (Hassan et al., 2021). Under soil salinity stress, photosystem II maximum quantum yield and relative electron transport rates are negatively affected indicating that photosynthesis are compromised under salt stress. The use of nitric oxide have shown to improve plant growth and

	0%				>200%
Shoot FW (%)					
Total chlorophyll (%)					
NaCl Concentrations	Chitosan (Cs), Chitosan nanoparticles (CsNPs) and modified CsBMs				
Chitosan (Cs)	0.001-0.0025%	0.0025%-0.1%	0.1-0.5%	0.5-1%	>10%
<i>Helianthus annuus</i> L., 4000 mg/L (Bakhom et al., 2020)					
<i>Helianthus annuus</i> L., 4000 mg/L (Bakhom et al., 2020)					
<i>Helianthus annuus</i> L., 8000 mg/L (Bakhom et al., 2020)					
<i>Helianthus annuus</i> L., 8000 mg/L (Bakhom et al., 2020)					
<i>Vitex trifoliapurpurea</i> , 1000 ppm (Ashour et al., 2020)					
<i>Vitex trifoliapurpurea</i> , 1000 ppm (Ashour et al., 2020)					
<i>Vitex trifoliapurpurea</i> , 1000 ppm (Ashour et al., 2020)					
<i>Vitex trifoliapurpurea</i> , 2500 ppm (Ashour et al., 2020)					
<i>Vitex trifoliapurpurea</i> , 2500 ppm (Ashour et al., 2020)					
<i>Vitex trifoliapurpurea</i> , 2500 ppm (Ashour et al., 2020)					
<i>Vitex trifoliapurpurea</i> , 5000 ppm (Ashour et al., 2020)					
<i>Vitex trifoliapurpurea</i> , 5000 ppm (Ashour et al., 2020)					
<i>Vitex trifoliapurpurea</i> , 5000 ppm (Ashour et al., 2020)					
<i>Lycopersicum esculentum</i> , 50 mM (Ullah et al., 2020)					
<i>Lycopersicum esculentum</i> , 50 mM (Ullah et al., 2020)					
<i>Lycopersicum esculentum</i> , 50 mM (Ullah et al., 2020)					
<i>Lycopersicum esculentum</i> , 100 mM (Ullah et al., 2020)					
<i>Lycopersicum esculentum</i> , 100 mM (Ullah et al., 2020)					
<i>Lycopersicum esculentum</i> , 100 mM (Ullah et al., 2020)					
<i>Lycopersicum esculentum</i> , 150 mM (Ullah et al., 2020)					
<i>Lycopersicum esculentum</i> , 150 mM (Ullah et al., 2020)					
<i>Lycopersicum esculentum</i> , 150 mM (Ullah et al., 2020)					
<i>Cadlenda officinalis</i> L. 1000 ppm (Abdel-Mola & Ayyat, 2020)					
<i>Cadlenda officinalis</i> L. 1000 ppm (Abdel-Mola & Ayyat, 2020)					
<i>Cadlenda officinalis</i> L. 1000 ppm (Abdel-Mola & Ayyat, 2020)					
<i>Cadlenda officinalis</i> L. 2000 ppm (Abdel-Mola & Ayyat, 2020)					
<i>Cadlenda officinalis</i> L. 2000 ppm (Abdel-Mola & Ayyat, 2020)					
<i>Cadlenda officinalis</i> L. 2000 ppm (Abdel-Mola & Ayyat, 2020)					
<i>Cadlenda officinalis</i> L. 3000 ppm (Abdel-Mola & Ayyat, 2020)					
<i>Cadlenda officinalis</i> L. 3000 ppm (Abdel-Mola & Ayyat, 2020)					
<i>Cadlenda officinalis</i> L. 3000 ppm (Abdel-Mola & Ayyat, 2020)					
<i>Stevia rebaudiana Bertoni</i> 50 mM (Gerami et al., 2020)					
<i>Stevia rebaudiana Bertoni</i> 50 mM (Gerami et al., 2020)					
<i>Stevia rebaudiana Bertoni</i> 50 mM (Gerami et al., 2020)					
<i>Stevia rebaudiana Bertoni</i> 100 mM (Gerami et al., 2020)					
<i>Stevia rebaudiana Bertoni</i> 100 mM (Gerami et al., 2020)					
<i>Stevia rebaudiana Bertoni</i> 100 mM (Gerami et al., 2020)					
<i>Stevia rebaudiana Bertoni</i> 150 mM (Gerami et al., 2020)					
<i>Stevia rebaudiana Bertoni</i> 150 mM (Gerami et al., 2020)					
<i>Stevia rebaudiana Bertoni</i> 150 mM (Gerami et al., 2020)					
<i>Ocimum basilicum</i> L. 25 mM (Rashidi et al., 2020)					
<i>Ocimum basilicum</i> L. 50 mM (Rashidi et al., 2020)					
<i>Ocimum basilicum</i> L. 100 mM (Rashidi et al., 2020)					
<i>Rosmarinus officinalis</i> L. Mild salinity, EC 0.53 ds/m (Helaly et al., 2018)					
<i>Rosmarinus officinalis</i> L. Severe salinity, EC 0.56 ds/m (Helaly et al., 2018)					
<i>Ocimum basilicum</i> L. 150 mM (Rashidi et al., 2020)					
<i>Solanum lycopersicum</i> , 50 mM (Chanratana et al., 2019)					
<i>Solanum lycopersicum</i> , 100 mM (Chanratana et al., 2019)					
<b>Chitosan+immobilized aggregated <i>M. oryzae</i> CBMB20</b>					
<i>Solanum lycopersicum</i> , 50 mM (Chanratana et al., 2019)					
<i>Solanum lycopersicum</i> , 100 mM (Chanratana et al., 2019)					
<b>Chitosan+immobilized non-aggregated <i>M. oryzae</i> CBMB20</b>					
<i>Solanum lycopersicum</i> , 50 mM (Chanratana et al., 2019)					
<i>Solanum lycopersicum</i> , 100 mM (Chanratana et al., 2019)					
<b>Chitosan nanoparticles/modified CsBMs (CsNPs/CsBMs)</b>					
<i>Momordica charantia</i> 50 mM (Sheikhalipour et al., 2021)					
<i>Momordica charantia</i> 50 mM (Sheikhalipour et al., 2021)					
<i>Momordica charantia</i> 100 mM (Sheikhalipour et al., 2021)					
<i>Momordica charantia</i> 100 mM (Sheikhalipour et al., 2021)					
<i>Phaseolus vulgaris</i> , 100 mM (Zayed et al., 2017)					
<i>Phaseolus vulgaris</i> , 100 mM (Zayed et al., 2017)					
<i>Phaseolus vulgaris</i> , 100 mM (Zayed et al., 2017)					
<i>Catharanthus roseus</i> (L.) G. Don, no salt, vegetative stage (Hassan et al., 2021)					
<i>Catharanthus roseus</i> (L.) G. Don, 150 mM, vegetative stage (Hassan et al., 2021)					
<i>Catharanthus roseus</i> (L.) G. Don, no salt, flowering stage (Hassan et al., 2021)					
<i>Catharanthus roseus</i> (L.) G. Don, 150 mM, flowering stage (Hassan et al., 2021)					
<i>Vigna radiata</i> , 4 dS/m (Sen et al., 2020)					
<i>Vigna radiata</i> , 4 dS/m (Sen et al., 2020)					
<i>Vigna radiata</i> , 8 dS/m (Sen et al., 2020)					
<i>Vigna radiata</i> , 8 dS/m (Sen et al., 2020)					
<i>Silybum marianum</i> (L.) Gaertn., 50 mM (Mosavikia et al., 2020)					
<i>Silybum marianum</i> (L.) Gaertn., 50 mM (Mosavikia et al., 2020)					
<i>Silybum marianum</i> (L.) Gaertn., 50 mM (Mosavikia et al., 2020)					
<i>Silybum marianum</i> (L.) Gaertn., 100 mM (Mosavikia et al., 2020)					
<i>Silybum marianum</i> (L.) Gaertn., 100 mM (Mosavikia et al., 2020)					
<i>Silybum marianum</i> (L.) Gaertn., 100 mM (Mosavikia et al., 2020)					
<i>Silybum marianum</i> (L.) Gaertn., 150 mM (Mosavikia et al., 2020)					
<i>Silybum marianum</i> (L.) Gaertn., 150 mM (Mosavikia et al., 2020)					
<i>Silybum marianum</i> (L.) Gaertn., 150 mM (Mosavikia et al., 2020)					

Fig. 2. Impact of different concentrations of Cs/CsBMs on total chlorophyll and shoot FW (%) under salinity conditions. The higher concentration of Cs could enhance chlorophyll biosynthesis, as shown in *Solanum lycopersicum* but still, the effect of Cs concentration is species-and salt stress specific as shown by *Vigna radiata*.



**Table 4**

Optimum concentration of Cs/CsNPs/ modified CsBMs and their biological effect in plants under salinity stress.

Botanical name	Common name	Parts treated	Chitosan (Cs)/ CsNPs/modified CsBMs tested	Salt conc. Tested	Optimum conc. of Chitosan-Cs/ CsBMs	Optimum Salt conc.	Effect	References
Chitosan (Cs)								
<i>Helianthus annuus</i> L.	Sunflower	Seeds	25, 50 and 75 mg L <sup>-1</sup>	4000 and 8000 mg L <sup>-1</sup>	50 mg L <sup>-1</sup>	4000 mg L <sup>-1</sup>	Highest shoot length, stem circumference fresh weight and dry weight	(Bakhoum et al., 2020)
<i>Zea mays</i> cv. Arifiye	Maize	Seedlings	10 mg L <sup>-1</sup>	5844 mg L <sup>-1</sup>	10 mg L <sup>-1</sup>	5844 mg L <sup>-1</sup>	Maximum AOX 1 transcript level recorded, Decreased superoxide anion, H <sub>2</sub> O <sub>2</sub> and lipid peroxidation	(Turk, 2019)
<i>Silybum marianum</i> (L.) Gaertn	Milk thistle	Plant	0.01, 0.05 & 0.1 mg L <sup>-1</sup>	2560, 6400, 9600 mg L <sup>-1</sup>	0.1 and 0.5 mg L <sup>-1</sup>	9600 mg L <sup>-1</sup>	Highest plant height, higher biomass, highest peroxidase activity, low H <sub>2</sub> O <sub>2</sub> accumulation	(Safikhani et al., 2018)
<i>Carthamus tinctorius</i> (L.)	Safflower	Callus	25 & 50 mg L <sup>-1</sup>	15 mg L <sup>-1</sup>	25 mg L <sup>-1</sup>	15 mg L <sup>-1</sup>	Highest content of total phenolics and total flavonoids	(Golkar et al., 2019)
<i>Lycopersicon esculentum</i>	Tomato	Plant	50, 100, & 50 mg L <sup>-1</sup>	2922, 5844 and 8766 mg L <sup>-1</sup>	150 mg L <sup>-1</sup>	8766 mg L <sup>-1</sup>	Maximum plant height, average number of compound leaves, leaf area, stem diameter, number of fruits plant, fruit firmness, leaf chlorophyll content, total soluble solids and yield plant with minimum fruit juice pH	(Ullah et al., 2020)
<i>Vitex trifolia-Purpurea</i>	Arabian Lilac	Plant	30, 60, 90 mg L <sup>-1</sup>	1000, 2500 & 5000 mg L <sup>-1</sup>	60 and 90 mg L <sup>-1</sup>	1000–5000 mg L <sup>-1</sup>	Increased plant growth parameters, chlorophyll content, carbohydrates, decreased phenol and Na <sup>+</sup> and Cl <sup>-</sup> ions	(Ashour et al., 2020)
<i>Helianthus annuus</i> L.	Sunflower	Seed	2.5, 5 and 7.5 mg L <sup>-1</sup>	2176, 4880, 6880, 8640 mg L <sup>-1</sup>	2.5–5 mg L <sup>-1</sup>	With increasing salt stress	Increased germination rate	(Jabeen & Ahmad, 2013)
<i>Carthamus tinctorius</i> L.	Safflower	Seed	2.5, 5.0 and 7.5 mg L <sup>-1</sup>	2176, 4880, 6880, 8640 mg L <sup>-1</sup>	2.5–7.5 mg L <sup>-1</sup> 2.5 mg L <sup>-1</sup> 7.5 mg L <sup>-1</sup> 2.5 mg L <sup>-1</sup> 2.5–5.0 mg L <sup>-1</sup>	All salinity levels All salinity levels 8640 mg L <sup>-1</sup> All salinity levels With increasing salt stress	Decreased proline Decreased catalase Increased MDA Increased protein Increased germination rate	(Jabeen & Ahmad, 2013)
<i>Triticum aestivum</i> L.	Wheat	Seed	250, 500 and 750 mg L <sup>-1</sup>	2922, 5844, 8766 and 11,688 mg L <sup>-1</sup>	250, 500 and 750 mg L <sup>-1</sup> 250 mg L <sup>-1</sup> 750 mg L <sup>-1</sup>	2922 mg L <sup>-1</sup> 11,688 mg L <sup>-1</sup> 11,688 mg L <sup>-1</sup>	Increased shoot and root length Increased catalase, proline, and MDA Decreased proline	(Peykani & Sepehr, 2019)
<i>Zea maize</i> L.	Maize	Seed	250, 500 and 750 mg L <sup>-1</sup>	2922, 5844, 8766 and 11,688 mg L <sup>-1</sup>	250, 500 mg L <sup>-1</sup> 750 mg L <sup>-1</sup> 500 mg L <sup>-1</sup> 250 mg L <sup>-1</sup> 750 mg L <sup>-1</sup>	2922 mg L <sup>-1</sup> 2922 mg L <sup>-1</sup> 2922 mg L <sup>-1</sup> 11,688 mg L <sup>-1</sup> 11,688 mg L <sup>-1</sup>	Increased shoot length Increased root length Increased seedling weight Increased catalase, proline, and MDA Decreased proline	(Peykani & Sepehr, 2019)
<i>Vigna radiata</i>	Mung bean	Seed	1, 2 and 5 mg L <sup>-1</sup>	2560, 4800, 6400 and 9600 mg L <sup>-1</sup>	2 mg L <sup>-1</sup>	4800 mg L <sup>-1</sup>	Improved germination index, coefficient of velocity, shoot and root length, maximum threshold level of shoot phytotoxicity	(Sen & Mandal, 2016)
<i>Solanum lycopersicum</i> Mill.	Tomato	Seed	15 mg L <sup>-1</sup> chitosan immobilized with bacterium	2922 and 5844 mg L <sup>-1</sup>	15 mg L <sup>-1</sup>	2922 mg L <sup>-1</sup> 5844 mg L <sup>-1</sup>	Increased plant dry weight, low amount of electrolyte leakage, increased CAT activity, improved nitrogen uptake Increased SOD, CAT, GR activity, increased nitrogen uptake	(Chanratana, Joe, Anandham, et al., 2019)
<i>Calendula officinalis</i> L.	Marigold	Seedlings	100, 200 & 400 mg L <sup>-1</sup>	1000, 2000, 3000, 4000 & 5000 mg L <sup>-1</sup>	400 mg L <sup>-1</sup>	1000–5000 mg L <sup>-1</sup>	Significant augmentation in all vegetative parameters, higher increase of plant height, highest value of total chlorophylls, lowest value of proline, sodium, and chloride (400 mg L <sup>-1</sup> ). Highest mean values of free proline content, decrease in total chlorophyll and carotene, highest value of chlorine and sodium contents (5000 mg L <sup>-1</sup> salt)	(Abdel-Mola & Ayyat, 2020)
<i>T. aestivum</i>	Wheat	–	–	–	–	–	–	

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Table 4 (continued)

Botanical name	Common name	Parts treated	Chitosan (Cs)/CsNPs/modified CsBMs tested	Salt conc. Tested	Optimum conc. of Chitosan-Cs/CsBMs	Optimum Salt conc.	Effect	References
			10 and 20 mg L <sup>-1</sup>		10 and 20 mg L <sup>-1</sup> 10 mg L <sup>-1</sup> 20 mg L <sup>-1</sup>		Enhanced growth parameters, increased photosynthetic pigments, maximum increase in proline and free amino acid, decreased MDA content, increased carbohydrates and protein content (10 and 20 mg L <sup>-1</sup> ) Increased CAT and POX activities Increased SOD activities, increased antioxidant compounds, increasing N, P and K contents Enhanced growth parameters	(Abdallah et al., 2020)
<i>Rosmarinus officinalis</i> L.	Rosemary	Termina cuttings	250 & 500 mg L <sup>-1</sup>	2922 and 5844 mg L <sup>-1</sup>	250 mg L <sup>-1</sup>	2922 mg L <sup>-1</sup>		(Helaly et al., 2018)
<i>Ocimum basilicum</i> L.	Sweet basil	Seedling	200 mg L <sup>-1</sup>	1461, 2922, 5844 and 8766 mg L <sup>-1</sup>	200 mg L <sup>-1</sup>	8766 mg L <sup>-1</sup>	Increased shoot fresh weight, leaf area, chlorophyll, decreased total phenol, expression of <i>PAL</i> gene Increased plant height, stem diameter, elevated expression of <i>SOD</i> and <i>JA</i> genes, activates antioxidant defense mechanisms	(Rashidi et al., 2020)
<i>Solanum lycopersicum</i> Mill.	Tomato	Plant	1 g	5944 mg L <sup>-1</sup>	1 g	5944 mg L <sup>-1</sup>	Increasing shoot length, root length, dry weight, chlorophyll content, increased SOD, CAT, POD and reduced MDA content	(Hernández-Hernández, González-Morales, et al., 2018)
<i>Triticum aestivum</i> L.	Wheat	Seedling	Cs-0.625 mg L <sup>-1</sup>	8766 mg L <sup>-1</sup>	0.625 mg L <sup>-1</sup>	8766 mg L <sup>-1</sup>	Highest germination percentage, improved seedling length, increased hypocotyl length	(Ma et al., 2011)
<i>Lens culinaris</i>	Lentil	Seed	1000, 3000 and 6000 mg L <sup>-1</sup>	2922, 5844, 11,688 and 17,352 mg L <sup>-1</sup>	3000 mg L <sup>-1</sup>	–		(Al-Tawaha & Al-Ghzawi, 2013)
<i>Stevia rebaudiana Bertoni</i>	Stevia	Seed	200, 400 and 600 mg L <sup>-1</sup>	2922, 5844 and 8766 mg L <sup>-1</sup>	400 and 600 mg L <sup>-1</sup>	2922–8766 mg L <sup>-1</sup>	High carotenoid content, Increased chlorophyll a, enhancement of protein content, elevated CAT and POX activity, highest amount of stevioside and rebaudioside A, lower MDA content	(Gerami et al., 2020)
<i>Zea mays</i> cv.	Maize	Seedlings	1 mg L <sup>-1</sup>	5844 mg L <sup>-1</sup>	1 mg L <sup>-1</sup>	5844 mg L <sup>-1</sup>	Enhanced plant growth, increased total respiration rate, cytochrome pathway, alternative respiration and enhanced antioxidant activities	(Turk, 2019)
Chitosan nanoparticles (CsNPs) and modified chitosan biomaterials (CsBMs)								
<i>Zea mays</i> L.	Maize	Seed	2.92 and 5.84 mg L <sup>-1</sup>	17,532 mg L <sup>-1</sup>	2.92 and 5.84 mg L <sup>-1</sup>	17,532 mg L <sup>-1</sup>	Higher leaf S-nitroso thiol content, prevented salt induced effects on photosynthetic pigments, prevented negative effects of salinity on fresh and dry weight of root	(Oliveira et al., 2016)
<i>Silybum marianum</i> (L.) Gaertn.	Milk thistle	Seed	2.5, 5 and 10 mg L <sup>-1</sup>	2922, 5844 and 8766 mg L <sup>-1</sup>	2.5 mg L <sup>-1</sup> 10 mg L <sup>-1</sup>	5844 mg L <sup>-1</sup> 8766 mg L <sup>-1</sup>	Improved physiological traits, largest germination rate Increased proline content, CAT and SOD	(Mosavikia et al., 2020)
<i>Vigna radiata</i>	Mung bean	Seed	2 mg L <sup>-1</sup>	2560 and 6400 mg L <sup>-1</sup>	2 mg L <sup>-1</sup>	6400 mg L <sup>-1</sup>	Decreased chlorophyll, increased protein content, increased proline, highest SOD, PPO, decreased POD, reduction of MDA and H2O2 content	(Sen et al., 2020)
<i>Phaseolus vulgaris</i> L.	Bean	Seed	1, 2 and 3 mg L <sup>-1</sup>	2.92 × 10 <sup>-3</sup> , 5.84 × 10 <sup>-3</sup> and 8.76 × 10 <sup>-3</sup> mg L <sup>-1</sup>	3 mg L <sup>-1</sup>	5.84 × 10 <sup>-3</sup> mg L <sup>-1</sup>	Highest percentage of salt tolerance index, increased leaf area, increased fresh and dry weight of shoot and root, increased relative water index, chlorophyll content, PPO and POD	(Zayed et al., 2017)
<i>Catharanthus roseus</i> (L.) G. Don	Periwinkle	Plant	10 mg L <sup>-1</sup>	8766 mg L <sup>-1</sup>	10 mg L <sup>-1</sup>	8766 mg L <sup>-1</sup>	Increased chlorophyll, antioxidant enzymes activities, reduced MDA and H2O2, higher alkaloid accumulation, elevated expressions of <i>MAPK3</i> , <i>GS</i> and <i>ORAC3</i> genes	(Hassan et al., 2021)
<i>Momordica charantia</i>	Bitter melon	Seed	10 and 20 mg L <sup>-1</sup>	2922 and 5844 mg L <sup>-1</sup>	20 mg L <sup>-1</sup>	2922 and 5844 mg L <sup>-1</sup>	Increased plant growth, improved relative water content, increased antioxidant enzyme activity, increased proline concentration, increased K <sup>+</sup> , increased	(Sheikhalipour et al., 2021)

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Table 4 (continued)

Botanical name	Common name	Parts treated	Chitosan (Cs)/CsNPs/modified CsBMs tested	Salt conc. Tested	Optimum conc. of Chitosan-Cs/CsBMs	Optimum Salt conc.	Effect	References
							chlorophyll and carotenoid content, decreased MDA, H2O2 and Na <sup>+</sup>	

**Abbreviations:** CVOMT- Chavicol O-methyltransferase, SOD-Superoxide dismutase, CAT- Catalase, GPX- Glutathione peroxidase, MDA- Malondialdehyde, JA- Jasmonic acid, POX- Peroxidase, SOD-superoxide dismutase, CAT- catalase, GPX- glutathione peroxidase, MDA- malondialdehyde, APX- ascorbic acid, PPO- polyphenol oxidase, POD-peroxidase, GS- geissoschizine synthase, MAPK3- mitogen-activated protein kinase 3, ORAC3- terpene indole alkaloid transcription factor.

development, plant response to abiotic stress especially salt stress by activating nitric acid related genes and proteins, inducing antioxidant systems, chlorophyll biosynthesis, photosynthesis etc., (Ahmad et al., 2016; Delledonne, Xia, Dixon, & Lamb, 1998; Simontacchi, Galatro, Ramos-Artuso, & Santa-María, 2015). Salt stressed seedlings supplemented with nitric oxide-releasing CsNPs containing 85.28 mg L<sup>-1</sup> S-nitroso-MSA-CsNPs did not significantly alter photosystem II maximum quantum yield and relative electron transport rate, chlorophyll contents compared to control stressed plants. However, 170.57 mg L<sup>-1</sup> concentration of S-nitroso-MSA-CsNPs, both free and encapsulated S-nitroso-MSA prevented negative effects of maize plants under salinity stress on photosynthetic parameters and chl content. Although this research didn't study whether the alleviation of salt stress is solely dependent on the active component or due to the synergistic role. Further research are warranted to prove this effect.

In bitter melon, severe salinity stress declined the amount of chl a and b, total chl, and carotenoids. However, under saline conditions of 2922 mg L<sup>-1</sup> and 5844 mg L<sup>-1</sup>, 20 mg L<sup>-1</sup> of CsNPs significantly increased chl a and chl b, total chl, carotenoid compared to control plants sprayed with NPs alone. Later, application of 20 mg L<sup>-1</sup> Cs-SeNPs treated plants using foliar spray increased chl a, chl b, total chl, and carotenoid content. Moreover, severe salinity decreased Pn by 24.37% compared to non-saline conditions. Treatment of 20 mg L<sup>-1</sup> of Cs-SeNPs increased Pn even in non-saline condition indicating that Cs-SeNPs showed positive effect even under non saline condition. Whereas, plants treated with 2922 mg L<sup>-1</sup> and 5844 mg L<sup>-1</sup> NaCl, and 20 mg L<sup>-1</sup> Cs-SeNPs indicate that Cs-SeNPs can reverse negative effects caused by salinity stress of bitter melon plants (Sheikhalipour et al., 2021). The salinity stress of 2560 and 6400 mg L<sup>-1</sup> decreased the chl and carotenoids contents of mung bean plants. However, application of CsNPs using solid matrix priming (SMP) increased in total chl content relative to control plants under salinity stress. As expected compared to Cs, CsNPs showed slightly increased chl and carotenoid contents in both saline and non-saline conditions (Sen & Mandal, 2016) indicating that CsNPs are readily available for the plants compared to Cs alone. In tested concentration, salinity stress of milk thistle plants decreased photosynthetic pigments chl a, b and total chl. The increased photosynthetic pigments were recorded in the absence of salt stress and by 10 mg L<sup>-1</sup> CsNPs priming treatment and decreased pigments were found upon non-application of CsNPs under 8766 mg L<sup>-1</sup> NaCl treatment. Additionally, there was a dose-dependent decrease in photosynthetic pigments when there is an increase in salinity stress in the absence of CsNPs treatment (Mosavikia et al., 2020). The sunflower seeds were soaked in different concentrations of Cs 25, 50 and 75 mg L<sup>-1</sup> and seeded under exceptional salt levels of 4000 and 8000 mg L<sup>-1</sup>, fundamentally upgraded photosynthetic pigments contents. Particularly, seeds treated with 50 mg L<sup>-1</sup> Cs under 4000 mg L<sup>-1</sup> salinity level recorded the most important growth factors, photosynthetic pigments, and IAA (Bakhoun et al., 2020).

In summary, both Cs and CsBMs application through soaking and pre-treatment of seedlings increased physiological parameters such as total chl, chl a and chl b, and carotenoids under salinity stress (Fig. 2, Table 4). Whereas foliar spray and the direct spray of Cs/CsBMs reduced physiological parameters under salinity stress indicating that soaking and pre-treated method is advantageous over foliar spray and direct

spray to the soil. Further, CsNPs tend to be easily uptaken by plants as Cs in nano-form showed increased bioavailability and thus reflected increased chl and carotenoid content compared to that of Cs itself.

## 5. Effect of Cs/CsNPs/ modified CsBMs on primary metabolites under salinity stress

### 5.1. Cs treatment increases aminoacids responsible for osmotic regulation

Cs induced significant differences in amino acids of various plants including safflower, maize, and *Triticum aestivum* seedlings under stressed and non-stressed conditions (Jabeen & Ahmad, 2013; Peykani & Sepehr, 2019). Besides osmotic regulation, proline has many differential roles such as enzyme protection and increased membrane stability (Babu & Devaraj, 2008). The increase of proline, an amino acid, can modulate the negative effects of salinity stress and increase tolerance to salt stress. The role of Cs and its positive impact was studied in milk thistle plant in a pot experiment with different concentration of Cs 0.01, 0.05, and 0.1% (DW/DW) by blending in with dry soil and salt stress. During salt stress of 2560, 6400, and 9600 mg L<sup>-1</sup>, Cs treatment, decreased adverse effects recorded by measurement of physiological attributes. The increase of soluble sugars and proline was accomplished by Cs application across all salt stress levels. Similarly, plants treated with Cs alone also showed increased proline content. Interestingly a synergistic effect on proline content was seen under salt stress and Cs application. The highest level of proline was accumulated in plants grown under 9600 mg L<sup>-1</sup> salt stress and 10 mg L<sup>-1</sup> Cs treatment (Safikhani et al., 2018).

Like salt stress and Cs application synergistic effect, treatment of 400 µl L<sup>-1</sup> Cs increased proline content in thyme plants grown under drought stress (Emami Bistgani, Siadat, Bakhshandeh, Ghasemi Pirbalouti, & Hashemi, 2017). Salt stress 5844 mg L<sup>-1</sup> for 4 days, 8766 mg L<sup>-1</sup> for 4 days, and 11,688 mg L<sup>-1</sup> for 16 days increased total amino acids content (TAA), free proline, and glutamic acid, except GABA. Nevertheless, 500 mg L<sup>-1</sup> Cs application showed 19.84% increased of TAA, 6.79% increased of glutamic acid, and 29.48% increased of GABA, compared to untreated plants under salt stress (Geng et al., 2020) indicating that amino acids play crucial role in the osmotic adjustment and energy metabolism. 500 mg L<sup>-1</sup> Cs application under salt stress decreased free proline content in creeping bent grass indicating that Cs alleviated the increase of proline due to salt stress and decreased stress damage caused by salt stress (Geng et al., 2020). *Triticum aestivum* L. and *Zea mays* L. plants are delicate to salinity stress that has been a significant issue among farmers today. Seeds of these plants were covered with 250, 500 and 750 mg L<sup>-1</sup> Cs solutions before they were planted and subjected to 2922-, 5844-, 8766-, and 11,688 mg L<sup>-1</sup> salinity for 7 days. Salinity stress at 11688 mg L<sup>-1</sup> highly accumulated proline content of wheat and maize plants, and 750 mg L<sup>-1</sup> Cs at higher concentration decreased proline accumulation. However, 250 mg L<sup>-1</sup> Cs, 11,688 mg L<sup>-1</sup> NaCl, and no Cs treatment under 11,688 mg L<sup>-1</sup> increased proline content (Peykani & Sepehr, 2019), indicating the dependency effect on the fluctuation of proline upon the differences in salt and Cs concentration.

## 5.2. Regulation of proline content by CsNPs/modified CsBMs treatment

The treatment of nanoparticles ( $\text{SiO}_2$ ) improves proline accumulation under salinity stress in *Curcubita pepo* L (Siddiqui, Al-Whaibi, Faisal, & Al Sahli, 2014). Although exact mechanism of  $\text{SiO}_2$  involvement is poorly understood, it is thought that  $\text{SiO}_2$ NPs were involved in maintaining critical physiological and biochemical attributes under stress condition. When *P. vulgaris* were treated with 1, 2, and 3  $\text{mg L}^{-1}$  of CsNPs under 5844  $\text{mg L}^{-1}$  salt stress, 1  $\text{mg L}^{-1}$  of CsNPs increased proline content up to 287.63% whereas CsNPs at 2  $\text{mg L}^{-1}$  and 3  $\text{mg L}^{-1}$  under 5844  $\text{mg L}^{-1}$  salt stress decreased proline content to 117.34% and 158.46%, indicating that low concentration of CsNPs is crucial for mitigating salt tolerance (Zayed et al., 2017). This study proves that increase of proline can overcome salt stress. Mung bean seedlings treated with Cs and CsNPs under salt stress (2560 dan 6400  $\text{mg L}^{-1}$ ) showed a considerable increase in proline content, but it was more pronounced under CsNPs treatment than unprimed and salt treated seedlings (6400  $\text{mg L}^{-1}$ ). The proline content was highly accumulated under higher salinity 6400  $\text{mg L}^{-1}$  and CsNPs treatment than non-treated control and hydro-primed seedlings. However, there was an increasing trend in the accumulation of proline content in CsNPs untreated seedlings but it was lesser than CsNPs treated seedlings under high salinity, indicating that CsNPs under higher salinity increased proline content confers increased tolerance to hyper osmotic stress (Sen et al., 2020). Seed priming technique was adapted to treat 2.5, 5, and 10  $\text{mg L}^{-1}$  CsNPs onto milk thistle seedlings under salinity stress. The interaction between salinity stress and CsNPs priming showed significant increase in free proline content under all concentrations of CsNPs treatment compared to control (no salt and no CsNPs) plants. Specifically, when 10  $\text{mg L}^{-1}$  CsNPs treated to plants exposed to 8766  $\text{mg L}^{-1}$  NaCl showed higher free proline accumulation, which is 286% compared to control plants (Mosavikia et al., 2020). Proline, accumulation was increased by 38.61% under salinity condition in bitter melon. Interestingly 20  $\text{mg L}^{-1}$  Cs-SeNPs treatment resulted in further increase in free proline accumulation in all groups: 12.3% increased on no salt treated plants, 10.08% increased on 2922  $\text{mg L}^{-1}$  of NaCl and 8.86% increased on 5844  $\text{mg L}^{-1}$  NaCl, compared to control (Sheikhalipour et al., 2021). Despite of osmolytes function, proline can give enzyme protection and increase membrane stability under various stress condition (Abdallah et al., 2020; Zayed et al., 2017).

In summary, high concentration of Cs (50, 250, and 750  $\text{mg L}^{-1}$ ) under salinity stress showed decreased proline accumulation whereas, CsNPs at low concentrations (0.02, 1, 2, 2.5, 3, 5, and 10  $\text{mg L}^{-1}$  concentrations) increased proline accumulation under salt stress indicating that both Cs/CsNPs combat salinity stress by regulating proline contents in the opposite ways to improve salt tolerance in plants. Moreover, proline content was significantly higher in CsNPs treated plants compared to Cs alone treated mung bean plants under salinity stress. These results indicate that treatment of CsNPs is preferred over Cs to regulate proline and improve salt tolerance in various plants by preventing salt induced deleterious effect. On the other hand, when creeping bent grass treated with 500  $\text{mg L}^{-1}$  Cs showed increased total amino acids, glutamic acids, and GABA content under salinity stress. However, this is the only one study that investigated other amino acids than proline, thus more studies are warranted in this direction.

## 6. Effect of modified Cs/CsNPs under salinity stress

### 6.1. Cs-PVA and CuNPs increases enzymatic defense mechanism

Several studies have shown to improve growth and increase in secondary metabolites of plants when chitosan poly-vinyl hydrogel (Cs-PVA) and copper nanoparticles (CuNPs) combined (Hernández-Fuentes et al., 2017; Hernández-Hernández, González-Morales, et al., 2018; Juárez-Maldonado, Ortega-Ortiz, Pérez-Labrada, Cadenas-Pliego, & Benavides-Mendoza, 2016; Pinedo-Guerrero et al., 2017). Cs-PVA +

CuNPs treatment improved the salinity stress by increasing the content of phenols,  $\beta$ -carotene, vitamin C, and lycopene contents. Similarly, Cs-PVA+CuNPs increased the defense gene expression, enzyme activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and phenylalanine amino lyase (PAL) under salinity stress (Hernández, Benavides-Mendoza, Ortega-Ortiz, Hernández-Fuentes, & Juárez-Maldonado, 2017; Hernández-Fuentes et al., 2017; Hernández-Hernández, González-Morales, et al., 2018; Hernández-Hernández, Juárez-Maldonado, et al., 2018). Application of Cs hydrogel along with CuNPs improved tomato growth and quality compared to Cs hydrogel or CuNPs treatment (Juárez-Maldonado et al., 2016). Cs-PVA and CuNPs absorbed on Cs-PVA showed different biochemical response under salinity stress. Cs-PVA alone treated tomato plants under salinity stress increased growth, chlorophyll a, b, total chlorophyll, carotenoids, and SOD. The combined treatment of Cs-PVA and CuNPs increased enzymatic defense mechanism in plants (Hernández-Hernández, González-Morales, et al., 2018). From this study, it can be concluded that Cs-PVA and Cs-PVA + CuNPs can exhibit totally different functions and therefore, choice of the material should depend upon the purpose of study.

### 6.2. Cs-based SeNPs improves plant growth

A functionalized synthesis of Cs-based Se nanomaterials prevents the potential harmful effects on plants and consumers of plant products. The CsNPs used for encapsulation and delivering bioactive components act as a shield and prevent the damage of biomolecules from temperature, light, and pH. Therefore, it has a potential interest in the use of agriculture (Mujtaba et al., 2020). Foliar application of 10  $\text{mg L}^{-1}$  Cs-SeNPs is effective for increasing the essential oil content under salinity stress whereas higher concentration of 20  $\text{mg L}^{-1}$  Cs-SeNPs increased vitamin C and K level in tomato plants under 2922- and 5844  $\text{mg L}^{-1}$  salt stress and it decreased Na level by 21.28% and 20.29% under 2922- and 5844  $\text{mg L}^{-1}$  salt stress (Sheikhalipour et al., 2021). However, the effect was more pronounced and highly favored during salt stress conditions indicating that Cs-SeNPs effectively combat salt stress and improve plant performance under salinity stress.

### 6.3. MSA encapsulated CsNPs for sustain release of NO

NO is a signaling molecule engaged with plant reactions to different abiotic stresses. Due to its instability, it cannot be used as a potential application in agriculture (Oliveira et al., 2016). For the sustained release of this signaling molecule in plants, CsNPs are used. Through the ionic gelation process, NO donor was encapsulated within CsNPs to form S-nitro-MSA-CsNPs. The encapsulated NO donor CsNPs showed higher leaf S-nitrosothiols content (an indicator of NO bioavailability) in salt-stressed maize plants than free MSA. Soil salinity stimulated leaf chlorosis, anthocyanin accumulation and necrosis. At the same time, MSA encapsulated CsNPs reversed all the detrimental effects such as photosystem II activity, chlorophyll content, growth of maize plants and NO bioactivity in salt stressed maize plants (Oliveira et al., 2016). Altogether, CsNPs combined with NO signaling molecule improved plant growth parameters under salt stress. Few studies have been studied this area and more studies are warranted in the future.

### 6.4. Cs-immobilized with *Methylobacterium oryzae* improved salt stress

The application of plant growth promoting bacteria as bioinoculant to alleviate salt stress is defendable and eco-friendly strategy in the field of agriculture. The use of Cs-immobilized aggregated *M. oryzae* CBMB20 as a bioinoculant improved tomato plant performance by improving plant dry weight, nutrient uptake (N, P, K and  $\text{Mg}^{2+}$ ), photosynthetic efficiency, and decreased electrolyte leakage under salt stress. Compared to Cs-immobilized non-aggregated *M. oryzae* CBMB20 strain, Cs-immobilized aggregated *M. oryzae* CBMB20 elevated salt stress



tolerance, increased antioxidant enzymes, enhanced proline content, decreased  $\text{Na}^+$  influx into plant cells, and thus decreasing  $\text{Na}^+/\text{K}^+$  ratio under saline conditions (Chanratana, Joe, Roy Choudhury, et al., 2019). Therefore Cs can be efficiently used as a carrier material to promote plant growth under salt stress.

## 7. Exogenous/endogenous elicitors upon Cs/CsNPs treatment under salinity stress

### 7.1. Effects of Cs and SA under salt stress

Various research works reported the potential effects of SA in the growth of plants under salinity stress (Abdallah et al., 2020; Golkar et al., 2019). The endogenous plant bioregulator SA was combined with Cs to study the alleviating effects of salinity stress in wheat cultivars. To study the salt tolerance effect, two wheat cultivars (*Triticum aestivum* L.) namely Sakha 94 and Gemmeiza 9 were grown in saline soil for 75 days treated with 10 and 20  $\text{mg L}^{-1}$  Cs and 25 and 50  $\text{mg L}^{-1}$  SA individually using seed priming and spraying methods, resulted in increased physiological characteristics (plant height, leaves number, tiller, tiller fresh and dry weight, water content), photosynthetic pigments (chl a, chl b, carotenoids and total pigments), and osmoprotectant substances (total soluble sugars (TSS), proline and free amino acids) in both wheat cultivars compared to the control plants. Furthermore, Cs or SA treatment decreased MDA and increased antioxidant enzymes in both the cultivars. However, the Cs treatment (20  $\text{mg L}^{-1}$ ) showed the highest decrease in the accumulation of MDA content and increase of POX and CAT enzymes compared to both concentrations of SA treatment of Sakha 94 cultivars and control plants, suggesting the use of Cs over SA to alleviate salt stress by activating antioxidant enzymes. On the other hand, Gemmeiza cultivars highly decreased MDA content at SA 50  $\text{mg L}^{-1}$  treated plants and increased antioxidant enzymes such as POX and CAT, specifying the unique role of SA to activate antioxidants and downregulate MDA content. This study suggests that Cs is highly effective in Sakha 94 cultivars than Gemmeiza 9 cultivars. Seed priming of Cs or SA improvised yield parameters in saline stressed wheat cultivars compared to control plants. When Sakha 94 treated with 25  $\text{mg L}^{-1}$  SA resulted in increased plant height, number of leaves per tiller, water content whereas, 50  $\text{mg L}^{-1}$  SA increased tiller fresh weight, tiller dry weight, root fresh weight, root dry weight compared to control and Cs treatments. Furthermore, Gemmeiza 9 wheat cultivar treated with 20  $\text{mg L}^{-1}$  Cs showed increase in water content, and 25  $\text{mg L}^{-1}$  SA showed increased plant height, leaves number/tiller, tiller fresh weight, tiller dry weight whereas 50  $\text{mg L}^{-1}$  SA increased water content of Gemmeiza 9 cultivars when compared to non-treated control plants. Interestingly at 20  $\text{mg L}^{-1}$  Cs treatment root fresh and dry weight was significantly increased among all treatments and control plants of Gemmeiza 9 cultivars. Nevertheless, when treated individually, both Cs and SA increased all physiological criteria in both cultivars compared to control plants (Abdallah et al., 2020). The effect of two elicitors, 25 and 50  $\text{mg L}^{-1}$  Cs, and 50 and 100  $\text{mg L}^{-1}$  SA on the secondary metabolite accumulation and cell reinforcement action of safflower callus under 15  $\text{mg L}^{-1}$  salt stress indicated that the lower concentrations of 25  $\text{mg L}^{-1}$  Cs accumulated total phenolics content (TPC) more than 50  $\text{mg L}^{-1}$  Cs under salinity stress. Whereas the highest callus growth rate, highest content of total flavonols and the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity were observed under elicitation by 50  $\text{mg L}^{-1}$  of SA under salinity stress (Golkar et al., 2019). These results indicate that the lower concentration of Cs 25  $\text{mg L}^{-1}$  treatment is optimal for increasing secondary metabolites in safflower plants, while SA at 50  $\text{mg L}^{-1}$  treatment is essential to increase callus growth, antioxidants, and secondary metabolites. It will be interesting to explore the combined effect of Cs/CsNPs and SA on systemic induced resistance in plants under biotic/abiotic stress for improved plant resistance.

### 7.2. Cs/CsNPs treatment regulates JA signaling

Jasmonic acid (JA) is meant to be a well-known salt stress marker in plants by regulating both osmotic and oxidative stress due to the ability of JA to provide systemic physiological alteration instead of by simply controlling osmotic stress (Zhao et al., 2014). Cs activates the octadecanoid pathway of jasmonates and protects the plants from salt stress by regulating cell ion concentration (Liu et al., 2008; Pichyangkura & Chadchawan, 2015). The treatment of chitosan-polyvinyl alcohol hydrogels + copper nanoparticles (Cs-PVA + Cu-NPs) and the Cs-PVA caused 75% and 66% diminished activation of JA transcript expression compared to salt alone treated plants. Additionally, Cs-PVA + Cu-NPs or Cs-PVA could also induce the transcripts of JA in the absence of salt stress. The decreased expression of JA under Cs-PVA + Cu-NPs and the Cs-PVA treatment indicated that both treatments mitigate salt stress (Hernández-Hernández, Juárez-Maldonado, et al., 2018). This is possibly due to the action of JA as a signaling molecule to regulate oxidative and ionic stress by activating antioxidant defense system of the tomato plants, and through the mediation of octadecanoid pathway of the jasmonates which was reflected in an improvement of growth parameters of tomato plants. The role of several nanoparticles such as  $\text{CeO}_2$ , Cs-PVA + CuNPs, nano- $\text{SiO}_2$  in the salt stress showed significant improvement by regulating cell ions were also reported previously in several plants (Farhangi-Abriz & Torabian, 2018; Hernández-Hernández, González-Morales, et al., 2018; Rossi, Zhang, Lombardini, & Ma, 2016; Wu, Shabala, Shabala, & Giraldo, 2018).

## 8. Effect of Cs/CsNPs on antioxidant activity, secondary metabolites, and membrane permeability under salt stress

Several antioxidants and non-antioxidative enzymes are involved in removing ROS to protect plants from oxidative damage under various stressful conditions upon Cs and CsNPs treatment (Geng et al., 2020; Hassan et al., 2021; Turk, 2019).

### 8.1. Effect of Cs/CsNPs on antioxidant activity

Cs and CsNPs application improved plant productivity under different stressed conditions by boosting plant immune responses and by the activation of various defense related enzymes and decreasing reactive oxygen species (ROS) of plants (E. F. Ali et al., 2021; Attia et al., 2021; Picchi, Gobbi, Fattizzo, Zefelippo, & Faoro, 2021; Quitadamo, De Simone, Beleggia, & Trono, 2021).

#### 8.1.1. Antioxidant activity under Cs treatment

Foliar application of 1  $\text{mg L}^{-1}$  Cs significantly induced antioxidant activities of SOD, CAT and APX. Interestingly, 5844  $\text{mg L}^{-1}$  salt stress combined with 1  $\text{mg L}^{-1}$  Cs by foliar means activated defense antioxidant enzymes such as SOD, CAT and APX by 35%, 45.1% and 119.8% and reduced GPX levels by 20.1% compared to control plants. The decrease in the MDA content due to the antioxidant system and the stimulating effects on alternative respiration was induced by Cs treatment under salt stress (Turk, 2019). Thus, proving the role of Cs to improve salt stress tolerance by activating various antioxidant enzymes.

The application of 15  $\text{mg L}^{-1}$  Cs immobilized with *Methylobacterium oryzae* CBMB20 in tomato seeds resulted in activation of defense mechanism against ROS by enhancing GR, APX and CAT, SOD and decrease in the MDA and  $\text{H}_2\text{O}_2$  level under 5844  $\text{g L}^{-1}$  salt stress treatment (Chanratana, Joe, Anandham, et al., 2019). Similarly, foliar application of 10 and 20  $\text{mg L}^{-1}$  Cs or 25 and 50  $\text{mg L}^{-1}$  SA to wheat cultivars improved antioxidant activities such as SOD, CAT and peroxidase thus eliminating ROS compared to corresponding control. 10  $\text{mg L}^{-1}$  Cs were more effective in enhancing POX and CAT activities than any other treatment in Sakha 94 whereas, SA 50  $\text{mg L}^{-1}$  was more effective than other treatments in Gemmeiza 9. The maximum increase in SOD activity was noted either at 25  $\text{mg L}^{-1}$  of SA or 20  $\text{mg L}^{-1}$  of Cs

treatments (Abdallah et al., 2020). The maize and wheat seeds soaked in Cs resulted in an increase in the activity of POD and CAT at 250 mg L<sup>-1</sup> of Cs, and a decrease in activity was seen at a higher dose, which was 750 mg L<sup>-1</sup> of Cs, under 11,688 mg L<sup>-1</sup> NaCl treatment (Peykani & Sepehr, 2019) indicating that Cs concentration affects the accumulation of antioxidant enzymes response under salt stress. During salt stress, antioxidant enzymes such as SOD, APX, CAT were significantly increased but decreased POD activity was observed in the leaves of creeping grass without Cs application. However, when creeping grass is pre-treated with Cs exhibited 17.24%, 18.8% or 18.62% increase in SOD, POD or CAT activity, respectively, compared to untreated plants under salt stress. Surprisingly, exogenous Cs didn't show significant effects on POD activity under normal and stress conditions indicating that pre-treatment of seeds is advantageous over exogenous application of Cs with respect to POD activity (Geng et al., 2020). Cs application by mixing with soil in milk thistle enhanced the activity of POD in the leaves under 2560, 6400, 9600 mg L<sup>-1</sup> salinity stress. When 0.1 mg L<sup>-1</sup> and 0.5 mg L<sup>-1</sup> Cs were applied under 9600 mg L<sup>-1</sup> salt stress increased POD activity and maximum POD activity was noticed under 9600 mg L<sup>-1</sup> and 0.1 mg L<sup>-1</sup> Cs treatment in milk thistle plants (Safikhani et al., 2018). *In vitro* culture of callus under 15 mg L<sup>-1</sup> of salinity stress and 50 mg L<sup>-1</sup> Cs elicitation showed no significant effect on DPPH activity compared to Cs application of control callus indicating that Cs didn't increase the DPPH activity of callus under salinity stress (Golkar et al., 2019). CAT activity increased with increased salt concentration (2240, 4880, 6480 and 8640 mg L<sup>-1</sup>) in sunflower and safflower seedlings. During maximum salinity stress ie 8640 mg L<sup>-1</sup>, CAT activity was increased by 62.27% in safflower and 60.83% in sunflower compared to control which is 8640 mg L<sup>-1</sup> salt treated. Low concentration 2.5 mg L<sup>-1</sup> Cs treatment without salt stress increased CAT by 53.95% and 52.16% in safflower and sunflower compared to control seedlings without salt treatment. However, Cs treatment at 2.5, 5.0 and 7.5 mg L<sup>-1</sup> under salinity stress decreased CAT activity compared to control seedlings (Jabeen & Ahmad, 2013). This might be due to inability of Cs to activate CAT under salt stress. However, the effect of Cs was not studied for other antioxidant enzymes under salinity stress. Stevia plants treated with 2922, 5844 and 8766 mg L<sup>-1</sup> levels of salinity stress showed a significant difference in CAT activity. Plants treated with 2922 and 8766 mg L<sup>-1</sup> raised CAT levels to 327% and 303.3%. Whereas Cs and salt stress combined treatment showed induced CAT activity in the following attributes: 200 mg L<sup>-1</sup> Cs treatment under 2922 mg L<sup>-1</sup> NaCl, 200 mg L<sup>-1</sup> Cs treatment under 5844 mg L<sup>-1</sup> NaCl, 600 mg L<sup>-1</sup> Cs treatment under 8766 mg L<sup>-1</sup> NaCl indicating that low concentration of Cs and salt stress induced CAT activity.

On the other hand, POD was also affected by 2922, 5844 and 8766 mg L<sup>-1</sup> of salinity stress in stevia plants. Compared to the control plants, the increasing salt concentration increased POD activity up to 61%, 79%, and 71%, respectively, to each salinity stress concentration. Among different Cs treatment, 200 mg L<sup>-1</sup> Cs showed increased CAT activity at 2922 and 5844 mg L<sup>-1</sup> NaCl treatment compared to control plants whereas, other two treatments, which was 400 and 600 mg L<sup>-1</sup> of Cs, showed reduced POD activity up to 21% and 16%, under 2922 and 5844 mg L<sup>-1</sup> NaCl treatments, respectively. Interestingly, when 400 mg L<sup>-1</sup> Cs application under 8766 mg L<sup>-1</sup> salt stress showed increased POD activity and decreased MDA content at 200 mg L<sup>-1</sup> and 400 mg L<sup>-1</sup> Cs treatment at 5844 mg L<sup>-1</sup> salt stress treatments resulting in increased salt tolerance by increase of CAT and POD and decrease of MDA in stevia plants (Gerami et al., 2020). Overall, Cs treated to leaves or seeds showed tremendous increase in antioxidative enzymes. Therefore, choosing the plant organs is crucial for the antioxidant related defense response under salt stress of plants.

### 8.1.2. Antioxidant activity under CsNPs treatment

During vegetative and flowering stage, *Catharanthus roseus* (L) subjected to salt stress (8766 mg L<sup>-1</sup>) caused a significant increase in CAT, APX, and GR enzymes compared to the control groups. Further, foliar

application of 10 mg L<sup>-1</sup> CsNPs enhanced antioxidant enzymes (CAT, APX, and GR) compared to salt and control plants. The significant upregulation of CAT, APX, GR activities and the reduction in MDA content and H<sub>2</sub>O<sub>2</sub> were recorded when 10 mg L<sup>-1</sup> Cs-NPs treated to 8766 mg L<sup>-1</sup> salt stressed *Catharanthus roseus* (L), proving enhanced tolerance against salt stress and preserved membrane function (Hassan et al., 2021).

High salt exposure as much as 5844 mg L<sup>-1</sup> NaCl significantly increased POD, SOD, APX, and CAT by 29.19%, 67.03%, 36.79% and 70.58% compared to control bitter melon plants under normal conditions. 20 mg L<sup>-1</sup> Cs-SeNPs treatment significantly increased CAT, POD and APX by 18.83%, 42.49% and 16.84%. When 20 mg L<sup>-1</sup> Cs-SeNPs and salt stress combined (2922 and 5844 mg L<sup>-1</sup>), CAT increased by 14.97% and 16.5%; POD increased by 63.33% and 36.66%; APX increased by 24.29% and 23.25%. However, SOD activity was increased only at 5844 mg L<sup>-1</sup> NaCl treatment under 20 mg L<sup>-1</sup> Cs treatment (Sheikhalipour et al., 2021). Similarly, priming seeds of *Silybum marianum* (L) Gaertn using Cs-NPs showed significant increase in SOD activity (54.63%) at 8766 mg L<sup>-1</sup> of NaCl post treatment. However, seeds only treated with CsNPs did not show any significant effect on CAT and SOD enzymes in milk thistle plants (Mosavikia et al., 2020). These results confirm that the role of Cs/Cs-NPs as an activator of antioxidant enzyme activities against ROS generation was higher during salinity stress. Solid matrix priming is an efficient and advantageous method of priming in comparison with liquid priming. The solid matrix priming with CsNPs reduced MDA and H<sub>2</sub>O<sub>2</sub> concentration in salt stressed (2560 and 6400 mg L<sup>-1</sup>) mung bean which eventually reduced the accumulation of lipid peroxidation and oxidative stress. Priming with CsNPs also increased the activity of SOD, CAT and PPO at higher salinity stress. The maximum POD activity was observed in CsNPs compared to Cs alone treatment. The APX activity showed maximum activity in CsNPs treated seedlings under high salinity indicating that APX is crucial member in scavenging ROS that detoxify H<sub>2</sub>O<sub>2</sub> in plant tissues by maintaining homeostasis of ascorbate and thus improves salt tolerance (Sen et al., 2020). *Phaseolus vulgaris* exposed to CsNPs (1, 2 and 3 mg L<sup>-1</sup>) significantly affected the antioxidant enzymes under salt stress (2922, 5844 and 8766 mg L<sup>-1</sup>). The maximum increase of CAT up to 427.77% was observed at 1 mg L<sup>-1</sup> CsNPs treatment under 5844 mg L<sup>-1</sup> of salt stress while 3 mg L<sup>-1</sup> Cs on 5844 mg L<sup>-1</sup> salt stress showed a maximum increase of PPO up to 224.83% and increased POD until 218.94% (Zayed et al., 2017).

These reports suggested that the application of CsNPs effectively combating ROS production by activating the defense antioxidant system in salt stressed plants. However, CsNPs treatment of plants like milk thistle did not affect the expression of antioxidant system but when it is combined with salt stress, there was a significant increase in antioxidant enzyme activities indicating that CsNPs and salt stress works synergistically works to activate defense response for improved salt tolerance. However, it should be noted that different plants elicited different types of response based on the plant species and concentration of Cs used. Therefore, identifying optimum concentration of Cs/Cs-NPs with respect to plant species is crucial to alleviate salt stress conditions.

## 8.2. Effect of Cs/CsNPs on secondary metabolites under salt stress

### 8.2.1. Effect of Cs treatment on polyphenol content

The total phenol content showed significant changes in Cs and non-Cs treated plants, however, phenol content increased with increased salinity stress (1461, 2922, 5844 and 8766 mg L<sup>-1</sup>) and reached the highest at 8766 mg L<sup>-1</sup> NaCl (Rashidi et al., 2020). Co-application of Cs and SA as the elicitors alleviated salinity stress partly by increasing polyphenols as the defensive component, elevated hormones, PAL synthesis, SA signal transduction pathways, anthocyanins and antioxidant defensive mechanisms (Golkar et al., 2019; Govindaraju & Indra Arulselvi, 2018; Rivas-San Vicente & Plasencia, 2011; Salimgandomi & Shabrangi, 2016). Thus, Cs is proposed as a possible tool for inducing systemic acquired resistance (SAR) and induced systemic resistance

(ISR). However, detailed research needs to be conducted in this area to investigate the molecular mechanisms involved.

### 8.2.2. Effect of CsNPs treatment on polyphenol content

Plant adapts to salt stress by maintaining homeostasis between ROS and phytochemicals such as polyphenols (Kiani, Arzani, & Mirmohammady Maibody, 2021). Foliar application of 20 mg L<sup>-1</sup> Cs-SeNPs under 2922 and 5844 mg L<sup>-1</sup> NaCl increased phenol accumulation by 9.55% and 19.71%, respectively. Increased flavonoid by 10.25% and 25.6% was observed when bitter melon plants treated under 2922 and 5844 mg L<sup>-1</sup> NaCl, indicating that higher the salt stress, higher the content of phenol and flavonoid (Sheikhalipour et al., 2021).

### 8.2.3. Effect of modified Cs/CsNPs on lycopene content

Ascorbic acid/Vitamin C is well-known non-enzymatic antioxidants that play crucial role in scavenging ROS and modulating various functions in both abiotic and biotic stresses (Akram, Shafiq, & Ashraf, 2017; Wang & Huang, 2019). CuNPs absorbed on Cs-PVA increased lycopene and vitamin C content in tomatoes by 63.4% compared to non-stressed conditions. Under 5844 mg L<sup>-1</sup> salt stress and Cs-PVA treatment, slight increase in lycopene content was observed compared to salt stressed and control plants (without salt stress). Interestingly, compared to control, salt stress alone and CuNPs treated plants, Cs treatment under salinity stress significantly increased lycopene content indicating that Cs play crucial role under salinity condition to improve salt tolerance in tomato plants (Hernández-Hernández, González-Morales, et al., 2018). The decreased content of vitamin C under 2922 and 5844 mg L<sup>-1</sup> salinity stress was alleviated by the foliar application of 10 and 20 mg L<sup>-1</sup> of Cs-SeNPs in bitter melon. With no exposure to salt or 2922 mg L<sup>-1</sup> NaCl, foliar treatment with 20 mg L<sup>-1</sup> Cs-SeNPs increased vitamin C by 28.30% and 25.71%, respectively. Meanwhile, plant exposed to 10 mg L<sup>-1</sup> Cs-SeNPs and 5844 mg L<sup>-1</sup> NaCl significantly elevated vitamin C content by 18.53%. The positive effect of Cs-SeNPs on salt stressed plants was shown by decreased cellular damage caused by ROS (Sheikhalipour et al., 2021). Higher the concentration of Cs-SeNPs, better the lycopene accumulation, increased vitamin C content without salt stress; whereas increase of lycopene and vitamin C was observed in lower concentration of Cs-SeNPs under salt stress indicating that different environment needs different concentration of Cs-SeNPs to show improved plant tolerance in tomato plants.

### 8.2.4. Effect of modified CsNPs on anthocyanin content

Anthocyanins are involved in multiple stress responses, acts as antioxidants, and reduce oxidative damage to plants by increased accumulation of polyphenols, scavenge free radicals and reduced lipid peroxidation (Sakamoto & Suzuki, 2019). Salt treatments on 2922 and 5844 mg L<sup>-1</sup> of NaCl with 20 mg L<sup>-1</sup> or even Cs-SeNPs itself could induce anthocyanin production by 35.21%, 21.73% and 48.93% in leaves of bitter melon plants (Sheikhalipour et al., 2021). There is only few evidence on the effect of Cs/Cs-NPs on anthocyanins under salt stress and therefore future research needs to be focused in this area.

### 8.2.5. Effect of CsNPs on alkaloids and diterpene glycosides

Several secondary metabolites including alkaloids and terpenes are involved in various stress responses in plants (Heydarian et al., 2018). When *Catharanthus roseus* (L.) was irrigated with CsNPs, increased alkaloid production was recorded in flowering stage than at vegetative stage in both roots and leaves, and even higher in roots than in leaves to resist 8766 mg L<sup>-1</sup> salinity stress. Furthermore, expression of a terpene indole alkaloid ORAC3 transcription factor, octadecanoid-derivative responsive AP2-domain, key gene involved in terpenoid indole alkaloid pathway, is responsible for the enhanced salt tolerance (Hassan et al., 2021).

The interaction of CsNPs and 8766 mg L<sup>-1</sup> salt stress modulated the biosynthesis of steviol glycosides derivatives by encouraging the conversion of stevioside to rebaudioside A indicating that CsNPs is

specifically affecting special pathways in alkaloid biosynthesis in *C. roseus*. Geissoschizine synthase (GS) gene is involved in the biosynthesis of stemmadenine, a terpene indole alkaloid induced transcription of GS and ORAC3 ultimately helps to improve salt stress tolerance in *Catharanthus roseus* (L.) G.Don (Hassan et al., 2021). Further research in other plant species is essential to unravel the role of alkaloids or terpenes in Cs/Cs-NPs treated salt stressed plants.

### 8.2.6. Effect of modified CsNPs on essential oils

Phytochemicals are secondary metabolites present in essential oils affected by several environmental factors especially osmotic stress that cause reduced absorption and transfer of nutrients in plants (Fernández-Sestelo & Carrillo, 2020). Foliar application of Cs-SeNPs under saline (2922 and 5844 mg L<sup>-1</sup>) and non-saline conditions significantly enhanced essential oil content. Without saline treatment, only 20 mg L<sup>-1</sup> Cs-SeNPs application increased essential oil content by 54.38%. 10 mg L<sup>-1</sup> Cs-SeNPs increased the content of essential oil up to 17.74% and 15.44%, in the fruits of bitter melon and thus increased the quality of fruits even under 2922 and 5844 mg L<sup>-1</sup> of salinity stress (Sheikhalipour et al., 2021), indicating that low concentration of CsNPs is sufficient to recover essential oil content in bitter melon fruits under salt stress.

### 8.3. Effect of Cs/CsNPs on membrane permeability

Plasma membrane is the primary part of plant affected by salinity stress, and therefore it is considered crucial when studying salt tolerance in plants (Muhammad Jamil, 2012). The treatment of 20 mg L<sup>-1</sup> CsNPs improved the membrane stability index, and decreased lipid peroxidation caused by ROS under 5844 mg L<sup>-1</sup> salt stress in *Phaseolus vulgaris* (Zayed et al., 2017). When periwinkle plants exposed to CsNPs showed low ion leakage and better membrane function under 8766 mg L<sup>-1</sup> of salt stress (Hassan et al., 2021; Xie, Xu, & Liu, 2001). Thus, the application of CsNPs could be as an effective tool to improve membrane stability and function under salinity stress.

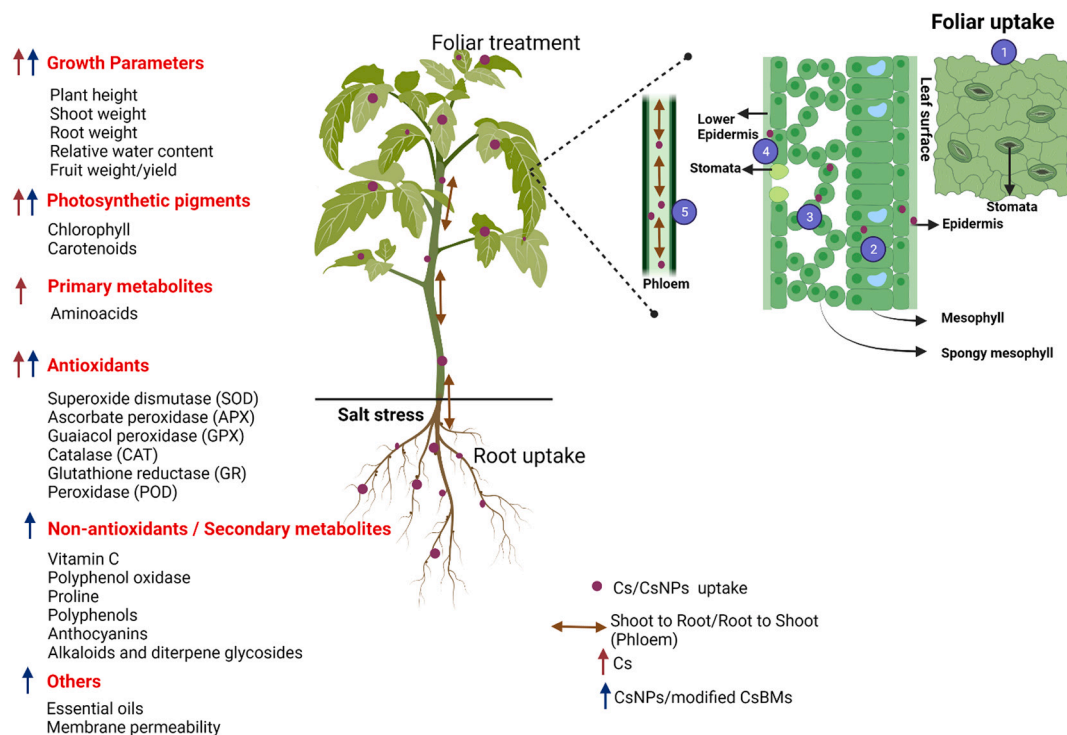
## 9. Molecular mechanism adapted by plants under Cs/CsNPs/modified CsBMs treatments during salt stress

### 9.1. Molecular mechanisms under Cs/modified Cs treatment

Cs can induce important plant physiological activities in cells and tissues through regulation of biochemical processes at the molecular level to improve plant growth and development (Bakhroum et al., 2020). Cs can also be used to efficiently mitigate salt stress by expressing various genes through activation of antioxidative enzymes and JA signaling pathway. The application of 1 g Cs-PVA and 10 mg CuNPs in tomato increased expression of SOD and decreased expression of GPX and CAT due to the higher production of anion superoxide radicals and lower production of H<sub>2</sub>O<sub>2</sub> compared to control plants. Cs activated octadecanoid pathway of JA by regulating the concentration of ions under salt stressed condition resulting in the downregulation of *PR1* and upregulation of JA biosynthetic transcripts (Hernández-Hernández, González-Morales, et al., 2018). Considering the downregulation of *PR1* and accumulation of JA transcripts upon CsBM treatment under salt stress, it is expected that the respective condition can result in induced systemic resistance (ISR) against pathogens, that is JA-dependent. Cs induces defense response genes through JA pathway under abiotic stress and SA pathway under biotic stress. The JA pathway is one of the key hormonal signaling pathways to mitigate salt stress in the plants (Zhao et al., 2014).

There are three types of alternate oxidase (AOX) genes, where only AOX1 is responsive to abiotic stress. Cs directly affects the expression of AOX1 in biochemical level possibly by mitochondrial respiration via AOX enzyme or by stimulating AOX gene expression. Application of Cs significantly up regulated the expression of AOX1 gene by 154% in maize seedlings under salt stress. Cs modulates alternative respiration by





**Fig. 3.** Cs/CsNPs/modified CsBMs uptake and transport through leaves and their physiological and biochemical response under salt stress. Cs/CsNPs/ modified CsBMs possibly enter plants through leaf surface and reach phloem vessels (1–5) to initiate various physiological responses, including plant growth, increasing photosynthetic pigments, primary and secondary metabolites, antioxidant, non-antioxidative enzymes, and other defense related genes *etc.*, as an adaptive mechanism toward salt stress.

regulating the activity and by regulating transcription factor of the AOX gene under normal and salinity conditions (Turk, 2019).

The primary factor of salt toxicity is the accumulation of high level of sodium in plants. Therefore, in a saline environment plant tends to isolate the excess sodium from roots and inhibits the transfer of sodium from roots to the shoots as a survival strategy (Chanratana, Joe, Anandham, et al., 2019). High affinity  $K^+$  transporter (HKT) belongs to the HKT/Trk/Ktr-type, exhibit species-specific function due to its variable selectivity for  $Na^+$  and  $K^+$  (Z. Ali et al., 2012; Rubio, Gassmann, & Schroeder, 1995). The exogenous application of  $500 \text{ mg L}^{-1}$  Cs to creeping bentgrass showed enhanced expression of *AsHKT1* in leaves and roots under salt stressed condition representing the involvement of Cs to induce *AsHKT1* gene to recruit sodium in roots and inhibit the transfer of sodium from roots to shoots under salt stress treated with  $5844 \text{ mg L}^{-1}/4$  days,  $8766 \text{ mg L}^{-1}/4$  days,  $11,688 \text{ mg L}^{-1}/16$  days (Geng et al., 2020). Sodium compartmentalization (separation of sodium into vacuoles) can also provide osmotic adjustment for water maintenance under saline stress.  $500 \text{ mg L}^{-1}$  Cs enhanced the expression of sodium hydrogen exchanger (*AsNHX4*, *AsNHX5*, and *AsNHX6*) genes under salt stress, i.e., on  $5844 \text{ mg L}^{-1}/4$  days,  $8766 \text{ mg L}^{-1}/4$  days,  $11,688 \text{ mg L}^{-1}/16$  days, all of them increased the capacity of sodium compartmentalization (Geng et al., 2020).  $500 \text{ mg L}^{-1}$  Cs is also able to increase SOS pathway associated with the excretion of  $Na^+$  from the cytosol to the rhizosphere, increase the expression of *AsHKT1* to inhibit  $Na^+$  transport to photosynthetic tissues, and upregulate the expression of *AsNHX4*, *AsNHX5*, and *AsNHX6* to increase the compartmentalization capacity of  $Na^+$  in roots and leaves. These findings reveal the involvement of Cs in regulating  $Na^+$  transport, increasing sugar and amino acid metabolism for osmotic adjustment and energy supply, and increasing plant polyamine (PA) accumulation in response to salt stress (Geng et al., 2020). A foliar spraying method of Cs caused increase in  $K^+/Na^+$  ratio possibly by acting as a biostimulators under salinity stress (Ashour et al., 2020). Cs-immobilized aggregated *M. oryzae* CBMB20 decreased  $Na^+$  influx into the plant cells and subsequently decreased  $Na^+/K^+$  ratio

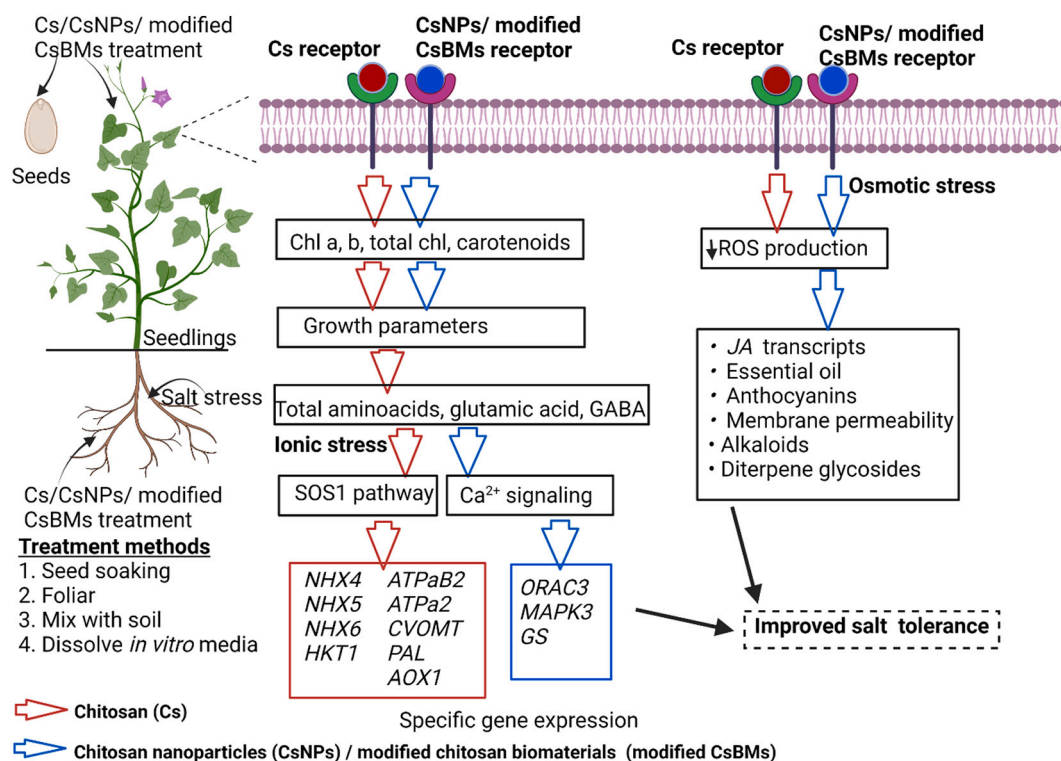
under salt stress conditions (Chanratana, Joe, Anandham, et al., 2019).

Additionally, under salt stress, Cs also enhanced the expression of *AsATPaB2* a transcription factor involved in light-controlled synthesis of photosystem proteins in roots, and *ATPa2-* sodium/potassium-transporting ATPase subunit alpha in leaves of creeping bentgrass to enhance proton motive force in the plant (Geng et al., 2020). A sweet basil showed enhanced expression of *phenylalanine ammonia lyase (PAL)* and *chavicol O-methyltransferase (CVOMT)* when  $2 \text{ mg L}^{-1}$  Cs (foliar application) was treated under salt stressed condition namely 1461, 2922, 5844, and  $8766 \text{ mg L}^{-1}$ . *PAL* is one of the most important enzymes in the biosynthesis of phenolic compounds and *CVOMT* gene belongs to the family of *O*-methyltransferase enzymes, and it performs the methylation of chavicol (enzyme family of *O*-methyltransferase). Enhanced expression of *PAL* and *CVOMT* genes at  $2 \text{ mg L}^{-1}$  Cs and  $5844 \text{ mg L}^{-1}$  NaCl leads to the increased production of phenolic compounds, which helps mitigate salt stress in plants (Rashidi et al., 2020).

## 9.2. Molecular defense related mechanisms under CsNPs treatment

There are few reports on the effect of CsNPs and defense related genes response under salt stress.  $10 \text{ mg L}^{-1}$  CsNPs was applied to *Catharanthus roseus* (L.) G.Don. challenged with salt stress to study the expression profile of *mitogen-activated protein kinase 3 (MAPK3)*, *GS* and *ORAC3*. Compared to control plants, salt stress slightly decreased, but no significant difference in *ORAC3* and *MAPK3* and *GS* expression was observed.  $10 \text{ mg L}^{-1}$  CsNPs treatment to *C. roseus* plants caused significant upregulation in all three genes such as *GS*, *MAPK3*, and *ORAC3* compared to control and salt stressed plants. Interestingly, the expression of all three genes increased further by  $10 \text{ mg L}^{-1}$  CsNPs and salt stress (Hassan et al., 2021) indicating the synergistic role of CsNPs and salt to improve growth and developmental process of plants under salinity conditions.





**Fig. 4.** Molecular signaling in plants after Cs/CsNPs/ modified CsBMs treatment under salt stress. Various plants parts were used for the treatment of Cs/CsNPs/ modified CsBMs. Cs/ CsNPs/ modified CsBMs attached to the receptor of plant cells and activated chlorophyll contents, carotenoids, growth parameters in plants under salinity stress. Cs and CsNPs/ modifiedCsBMs activated both ionic, and osmotic stress upon salinity stress increased specific gene expression and metabolites to improve salt tolerance in various plants. *NHX4*, *NHX5* and *NHX6*- sodium hydrogen exchanger 4; *HKT1*- sodium, transporter; *ATPaB2*- a transcription factor involved in light-controlled synthesis of photosystem proteins; *ATPa2*- sodium/potassium-transporting ATPase subunit alpha; *CVOMT*-chavicol O-methyltransferase; *PAL*-phenylalanine ammonia lyase; *AOX1*- aldehyde oxidase-1; *ORAC3*-terpene indole alkaloid transcription factor; *MAPK3*- mitogen activated protein kinase; *GS*-geissoschizine synthase.

## 10. Conclusion and future prospects

Cs are non-toxic in nature and hence they are widely used as bioactive materials in the field of agriculture. The salt tolerance effect was majorly dependent on the size and molecular weight of the CsBMs treated irrespective of plants class types (monocot or dicot). However, still numerous studies should be carried out in this area to further validate this conclusion. Compared to all other nanoparticles treatment methods, seed priming is considered as an optimum method for treating Cs/CsNPs/modified CsBMs in plants, as seed priming requires a low concentration of Cs/CsNPs for exhibiting significant physiological functions compared to other treatment methods. The reason for that may be due to the direct and constant contact of plants to Cs/CsNPs solution allowed to break the dormancy period faster and thus improved the seed and germination performance. Cs/CsNPs/ modified CsBMs uptake by plants activates several metabolites including primary, secondary, and defense related genes as the adaptive mechanism toward salt tolerance (Fig. 3, Table 4). Moreover, the foliar spray is advantageous over mixing Cs/CsNPs/ modified CsBMs with soil, as soil micro-organisms may regulate Cs/CsNPs/ modified CsBMs, thereby causing decreased effect. Overall, we conclude that foliar or seed priming methods are suggested as appropriate methods for applying Cs/CsBM/ modified CsBMs in agriculture.

The chlorophyll content is affected by Cs/CsNPs/modified CsBMs depending on the plant species and salt concentration, therefore the concentration of Cs/CsBMs must be optimized before the treatment. Depending on the salt concentration, various defense mechanisms can be affected by modulating various signaling pathways. We also found that CsNPs/ modified CsBMs increased certain parameters including anthocyanins, alkaloids, diterpene glycosides, essential oil content, and

membrane stability. Therefore, evaluating these parameters can be considered when developing a similar type of biomaterials (Fig. 4). From this review, we clearly understand that modified CsBMs and CsNPs is better than Cs to regulate proline and improve plant performance under salinity stress. Furthermore, Cs combined with SA under salt stress activated SA signaling pathway and thus possibly induced SAR and ISR in plants under salt stress. However, this is a preliminary study and therefore, more research should focus in this area to explore the potential role of Cs/CsNPs in inducing SAR and ISR.

The use of Cs/CsBMs/modified CsBMs to alleviate salt stress is a new trend and emerging area thus the application of these materials needs further investigation. Although bulk Cs is non-toxic in nature, use of CsBMs/modified CsBMs needs extensive bench and field trials to use them safely in agriculture. Further, selection of active component that works synergistically with CsBMs to perform selective functions, and optimization of the dosage of an active component according to plant species and salt concentrations can make the research expensive, time consuming, and result in delay of the use of CsBMs in agriculture. Further, the addition of hazardous active components during CsBMs synthesis can pose potential environmental, health risks and therefore should be chosen cautiously. When plants are treated with formulated CsBMs alone or in modified form, dose-dependant issues can arise and therefore should be carefully analyzed, deeply scaled-up before application in field as the higher concentration can negatively affect the plant growth, and has high chance to accumulate in ecosystem. Eventhough nano-Cs and modified CsBMs showed promising results in combating salt stress compared to bulk Cs, more research should be done to explore its molecular mechanism of action, for the safety use of the material without causing major damage to the environment, plant growth and profiting the farmers by providing sustainable agriculture.

## Declaration of competing interest

The authors declare they do not have any conflict of interest.

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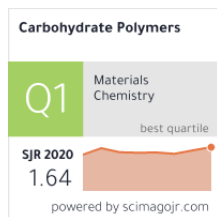
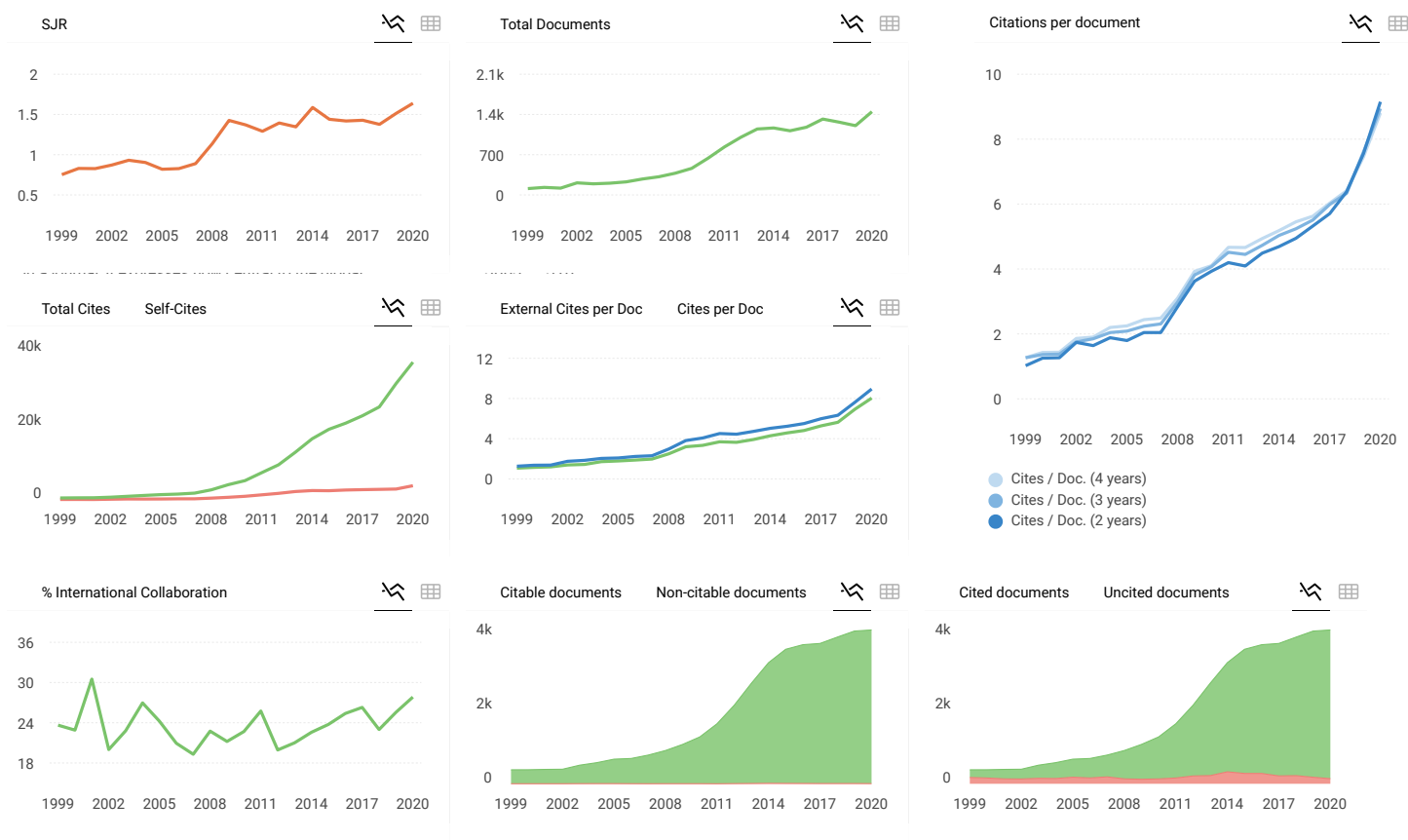
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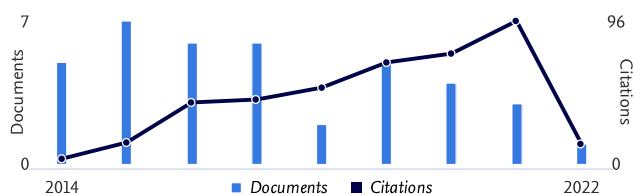
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