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Somdutt
Research Scholar, Department of Agronomy, Rajasthan College of Agriculture, MPUAT- Udaipur, India.

Himangini Joshi
Research Scholar, Department of Agronomy, Rajasthan College of Agriculture, MPUAT- Udaipur, India.

K.S. Rajawat
Asst. Professor, Department of Horticulture, Bhupal Nobels' University- Udaipur, India.

J Choudhary
Asst. Professor, Department of Agronomy, Rajasthan College of Agriculture, MPUAT- Udaipur, India

Corresponding Author:
Somdutt
Research Scholar, Department of Agronomy, Rajasthan College of Agriculture, MPUAT- Udaipur, India.

Controlled release action of chitosan nanoparticles to improve nutrient use efficiency

Somdutt, Himangini Joshi, K.S. Rajawat and J Choudhary

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Abstract
Chitosan is an effective organic molecule that improve productivity by supplying valuable nutrients to plant and due to site specific action of this nanoparticle it also enhance activity of protective enzymes that enables for biotic and abiotic stress resistance in plants. It acts as a carrier of major and micro nutrients, pesticide, herbicide and increase crop yield in a sustainable manner.

Keywords: Chitosan nanoparticles (CNP), target action, stress resistance, sustainable manner

Introduction
Chitosan is the most lavish carbohydrate biopolymer in the earth. It is obtained from marine crustaceans as functioned biopolymers (chitin and chitosan) **(**Kurita *et al.,* 2006) [43]. It is the linear polysaccharide which is made up of deacetylated unit (β-1-4-linked D-glucosamine) obtained from either partially or fully deacetylated chitin (Calvo *et al.,* 1997) [16]. Chitosan nanoparticles (CNP) showed their ability as a nano carrier for nutrient delivery systems. It has good bonding and loading efficiency values of a model substance. It gave more advantages like biocompatibility, biodegradability and low toxicity with high cationic potential (Kim *et al.,* 2007) [37]. Now a day's Farmers must move toward more efficient and sustainable practices of agriculture to face the increasing cost of fertilizer and wish to environmentally-friendly agriculture. In this context, chitosan-based polymers may be the solution (Davidson *et al.,* 2012) [20]. Materials which are used for encourage plant growth controlled release of nutrients and molecules were efficient. When chitosan used in farming (without chemical fertilizer) increase microbial population and improve mineralization rate of nutrients and nutrients easily absorbed by plant root. To accelerate crop productivity, stress avoidance and disease suppression natural products and favorable microbes was used which is based on alternative approaches (Chenguang *et al.,* 2007) [10].

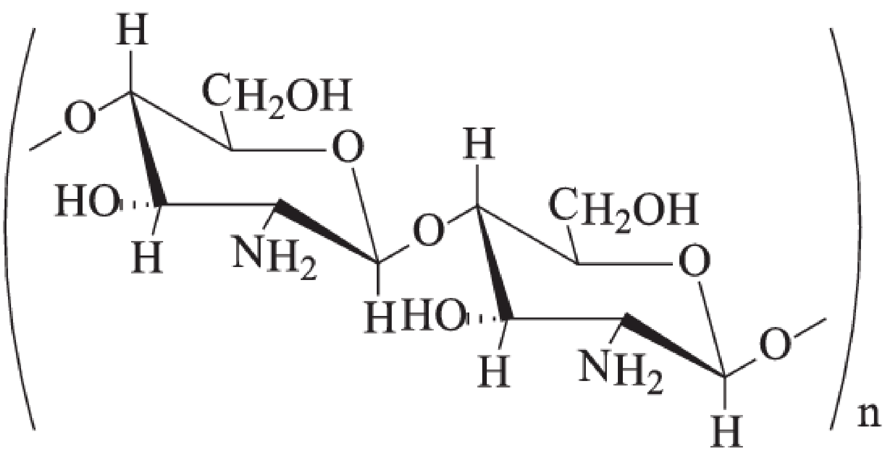

Fig 1: Molecular structure of chitosan

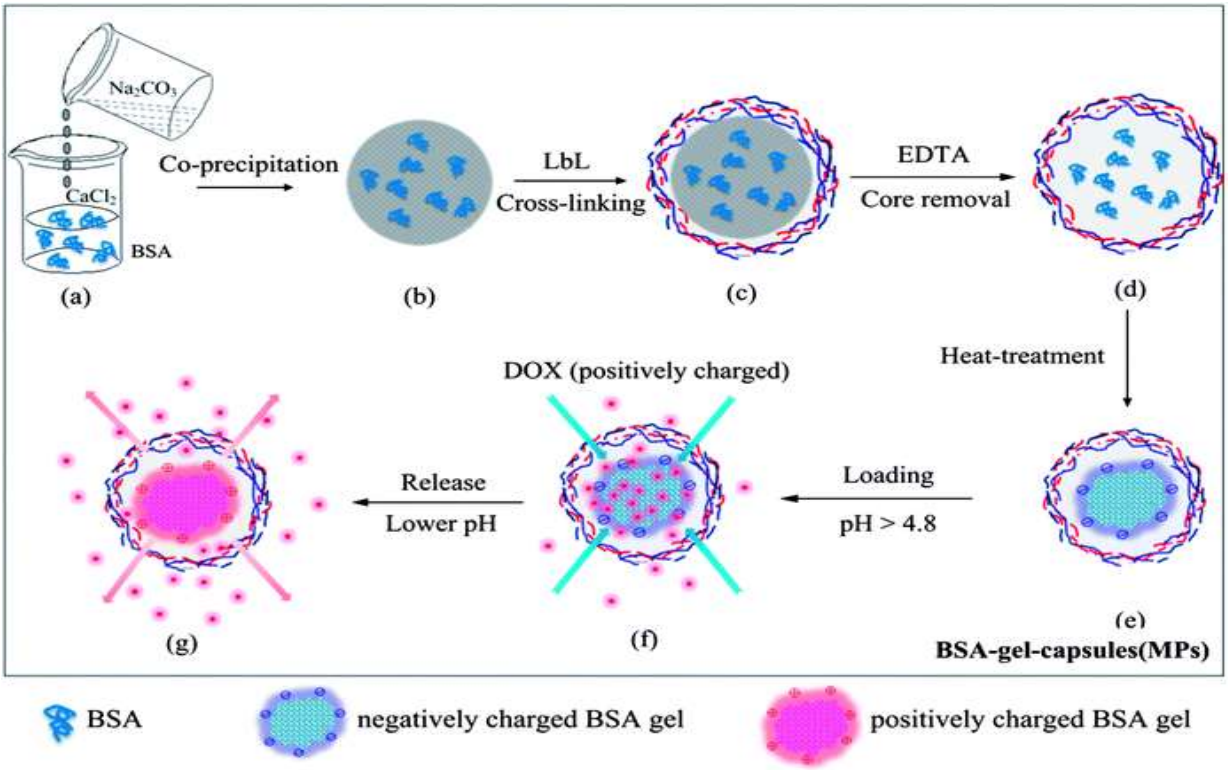

Fig 2: Biodegradable chitosan BSA-gel-capsules for pH-controlled loading

CNP on seed germination

Cho *et al.* (2008) [15] reported that with increase in concentration of chitosan nanoparticles significant effect observed on morphological and vigor index of sunflower seedling. Similar results were observed by Shao *et al.* (2005) [58] in maize plant. Germination percentage, hypocotyl length, radical length, hypocotyl dry weight and radical dry weight were recorded maximum when lentil seed was stratified with 3 g/l chitosan (DeRosa *et al.*, 2010) [21]. Seed treatment with chitosan biopolymer (100 mg/L) induced salinity tolerance by increasing concentration of proline catalase and peroxidase enzymes activities and decreasing malwondialdehyde level on rice (*Oryza sativa L.*) seeds variety INCA LP-5 (Boonlertnirun *et al.* (2013) Agbodjato *et al.* (2016 [9, 2]) examined the joint effects of plant growth promoting rhizobacteria (PGPR) and chitosan on germination, growth and nutrient uptake of maize seeds. The combination of chitosan-*A. lipoferum-P fluorescens* resulted in maximum heights of plant (17.66%), increased the leaves per plant (50.09%), aerial (84.66%) and underground biomass (108.77%) production by maximum. *A. lipoferum* inoculated plants had large leaf areas 54.08%, whereas combination of *A. lipoferum-P. fluorescens-P putida* and chitosan-*A lipoferum* increased the aerial as well as underground dry matter of plant by 26.35% and 18.18% respectively. So, the combination and plant growth promoting rhizobacteria may be used as bio fertilizers to boost maize production.

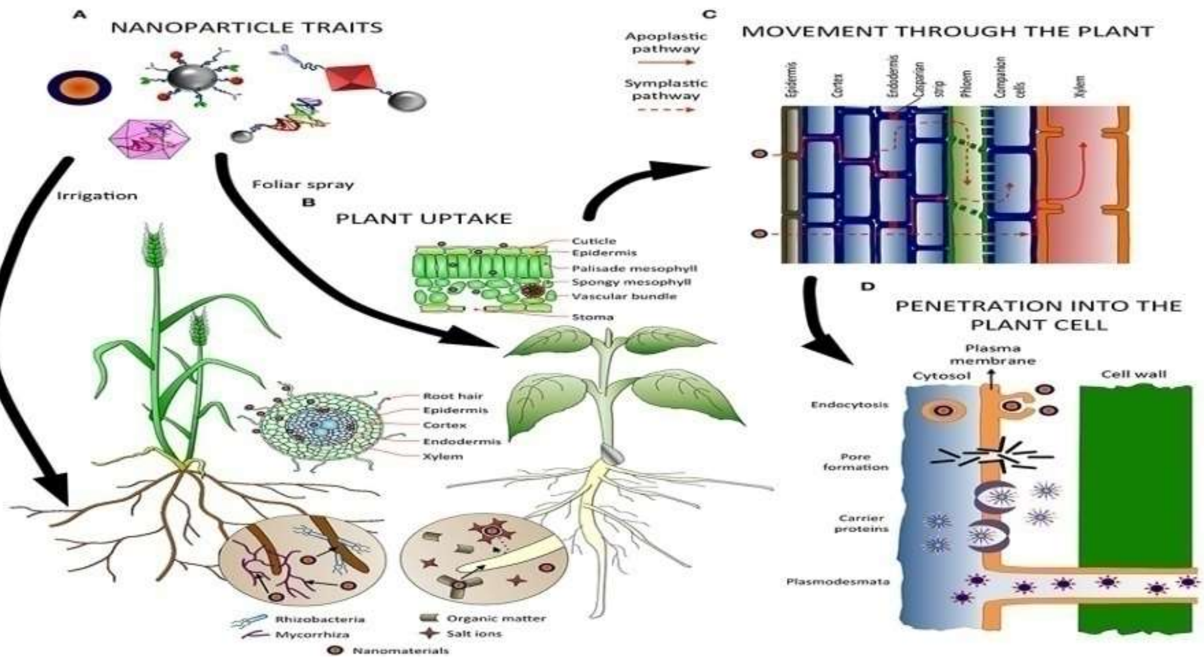

Fig 3: Target site action of chitosan nanoparticles

CNP on growth and activities of protective enzymes

Chitosan coated seeds significantly improved the growth indices of germination rate, fresh weight, root length, root active, and also influenced physiological index such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)), the content of malwondialdehyde (MDA) and chlorophyll compared with control under drought stress condition (Zeng *et al.,* 2012) [71]. Ohta *et al.* (2016) [52] reported that the number, weight and quality of *Eustoma grandiflorum* (Raf.) Were higher in chitosan-treated seed compared to control plants. Perera *et al*. (2017) in an experimental study suggested that bioactivity assays using *Phaseolus vulgaris* showed chitosan nanoparticles effectively increased leaf area, chlorophyll content, carotenoids and gibbrellic acid concentration. The developed systems prove noble potential with more stability and application efficiency of this phytohormone in agricultural practices (Biagio *et al.,* 2013) [7]. Chinese cabbage seeds amalgamated with chitosan @ 0.4-0.6 mg/g of seed followed by foliar spraying @ 20-40 mg/l countered with increased height of plants and also leaf area (Wang *et al.,* 2012). Chitosan was extensively used in agriculture to boost plant secondary metabolite. Different forms of chitosan had been used as a chelated nutrient and minerals (Aziz *et al.,* 2004) [6]. Chitosan has been effective in improving harvested yield of several ornamental and horticultural commodities (Wang *et al.*, 2015) [61]. The changing regulation of relative greenness of leaves correlated with the transformation in the concentration of chitosan (Xie *et al.*, 2001) [66]. Furthermore, Chibu *et al.* (2001) [11] and Ghadi *et al.* (2014) [25] investigated leaves of tomato and lettuce turned to darker green colour, responding with concentration of chitosan. A similar result was obtained by in rice seeds.

Additionally, chitosan had positive ionic charges that bind it with plant nutrients chemically, resulted in a slowly released action in rice plants which contributed to increased yield. There were many reports supporting this result such as the report of who established in rice, panicle numbers and yield were amplified by application of polymeric or oligomeric chitosan. Compared to other methods, application of chitosan by seed priming in chitosan solution before sowing, produce more number of panicle and seeds in rice plant. Mondal *et al.* (2012) [50] revealed that most of the morphological indices such as plant height, leaf number/plant, total dry mass/plant, absolute growth rate, relative growth rate as well as biochemical parameters (nitrate reductase and photosynthesis) and yield attributes (number of fruits/plant and fruit size) were improved with increasing concentration of chitosan up to 25 ppm, which gave maximum fruit yield of okra (27.9% yield increased over control). Chitosan was mainly recognized due to stimulation of plants (Dzung *et al.,* 2011) [24]. Gornik *et al.* (2008) [26] demonstrated that chitosan improved the growth and development of plant. They found that application of chitosan enhanced the activity of enzymes such as nitrate reductase, glutamine synthetase and protease that involved in the nitrogen metabolism and also increased the translocation of nitrogen in the functional leaves which in turned improved the performance of plant and over all development. Results revealed that application of chitosan in okra at early growth stages had a very enormous effect on its growth and development. The results were consistent with Liu *et al*. (2001) [66], described that growth and development of tomato plants were improved after chitosan application. Besides, Zhou *et al.* (2002) [72] reported the application of carboxymethyl chitosan the activities of crucial enzymes of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease) increased, which also boosted the growth and development in rice. The results were reliable with Khan *et al.* (2002) [35] who reported chitosan act as an elicitor. Chitosan was shown to play role on the activation of plant defensive genes through octadecanoid pathway. Chitosan application increased photosynthetic activity in leaves of maize and soybean. Shi *et al.* (2006) [59] reported the photosynthetic pigment increased by chitosan application which in turn increase photosynthesis phenomenon of the plant. Chitosan increased fiber percentage in rice plant by seed coating before planting followed by four times of soil applications (Doares *et al.,* 1995) [23].

Recently chitosan nanoparticles used for crop production by a sustained nutrition. These nanoparticles act as a valuable delivery system for fertilizers, herbicides, pesticides, and micronutrients for enhancing agricultural productivity per unit area per unit time. Pichyangkuraa *et al.* (2015) [55] revealed that chitosan worked as an expedient tool for agricultural livability. Chitosan led to the induction of secondary metabolite such as polyphenolics, lignin, flavonoids, and phytoalexin. Biochemical and molecular transformations observed in plant fed with chitosan in callose apposition (Ali *et al*., 2011; Kohli *et al.*, 1985 and Faoro *et al.*, 2007). It enhanced cytosolic $Ca^{2+,}$ MAP-kinases activation (Yin *et al.,* 2010) [68], H^+-ATPase embarrassment in plasma membrane, synthesis of alkaloids i.e. tannin, saponins, phenolic compounds etc. (Orlita *et al.,* 2008) [53], growth regulators such as jasmonic acid and abscisic acid (Doares *et al.*, 1995) [23]. Złotek *et al.* (2014) [75] proved chitosan mediated signalling pathway to recognize the chitosan by a specific cellular receptor, secondary messenger, must transduce the signal to induce the physiological responses and the involvement of reactive oxygen species, $Ca^{2+,}$ nitric oxide, phytohormones. H_2O_2 regulates numerous responses (mainly reactive oxygen species) persuaded by chitosan in many plant species. Chitosan based materials used to produce nanoparticles efficacy supply plant to minerals and nutrients. Chitosan easily absorbed to leaves epidermis and stems extend the contact time and promote the uptake of the bioactive molecules (Kah *et al.,* 2013). Chitosan materials were used to enclosed synthetic brassinosteroids and diosgenin derivatives that performed as a slow release fertilizer (Hazara *et al.,* 2014). Chitosan nanoparticles could be used for plant growth and development because it has ability to bind with negative charged bio-molecules. It can act as a good carrier for nucleic acid and functional for delivery of genetic materials in biological system. (Hadwiger *et al.,* 2013 [29]; Meng *et al.*, 2010 [49] and Kurosaki *et al.,* 1988) [44]. Chitosan induced as well as enhanced defense responses like reactive oxygen species, membrane depolarization, biosynthesis of abscisic, salicylic acid, jasmonic acid, hypersensitive responses, production of phytoalexins, callose formation, programmed cell death and expression of defense related genes in plants when pathogen invaded (Jayalakshmi *et al.,* 2010) [33]. Seed priming with different concentrations of chitosan solutions on the growth and physiological variations of two inbred lines of maize *(Zea mays* L.) (Guan *et al.,* 2009). Various crops like peanut, soybean, maize, wheat cabbage, rape etc. were primed with chitosan and increased the germination, growth and yield (Zhou *et al.,* 2011 [74] and Yin *et al.,* 2012) [69]. Chitosan performed better under stress conditions because it released antioxidant that scavenging the hydroxylated radicles, hydrogen peroxidase etc. through drought avoidance mechanism (Chen *et al.,* 1994) [14]. In pepper plants reported that chitosan reduced the transpiration rate by 26-43% through closing stomata without much effect on yield. Rice plant treated with chitosan showed higher production than control group (Bittelli *et al.*, 2001) [8]. When grapevine leaves treated with chitosan led to induction of lipoxygenase, phenylalanine ammonia-lyase and chitinase activities occurs (Kim *et al.*, 2005) [36]. Shi *et al.* (2003) Chitosan chelated metal ions and also cause enzyme immobilization for matrix. Cross-linked Chitosan (suberoyl chloride particles) increased delivery of microelements such as Zn^{2+} and Cu^{2+}. Tao *et al.* (2012) [63] reported that the chitosan used for the controlled delivery of agricultural important molecules (1-naphthylacetic acid) which was beneficial for plant growth due to slow release action.

Sahu *et al.* (2017) [57] described the effect of chitosan derivatives on growth enhancement, elicitation of secondary metabolites for conserving yield and quality of crops. Zeng and Shi (2009) [70] developed a new type of organic rice seed coating agent using Chitosan and found to be effective on enhancing the growth and productivity of rice. Ruan and Xue (2002) [56] revealed the rice seed coating with Chitosan may accelerate germination of it and improved their resistance to stress conditions. The field data of Toan *et al.* (2013) [62] showed the yields of rice significantly increased (almost 31%) after spread over solution of chitosan. In general, applying chitosan increased rice production and significantly reduced cost of production. Chitosan improved the self defense mechanism of plants and work as a growth promoter and increased successful agricultural production reported by Thai farmers (Chamnamanoonthom *et al.*, 2015). Chitosan accelerated growth indices of treated plants (Dutta *et al.*, 2004) [19]. The soluble chitosan can be successfully encapsulated into seed of potato *(solanum tuberosum)* from *invitro* plantlets (Zakaria *et al.*, 2009). Chitosan boosted plant growth and development. Zhao *et al.* (2011) [73] suggested that chitosan originated self defense system in standing plant by various physicochemical changes include lignifications, ion flux variations, cytoplasmic acidification, membrane depolarization protein phosphorylation, chitinase and glucanase activation, phytoalexin biosynthesis in addition to generation of reactive oxygen species (Kaku *et al.*, 1997). Kurita *et al.* (1998) [42] proved chitosan has ability to attract tremendous attention as an efficient biological source because of biocompatibility, non-toxicity and biodegradability characteristics. Kmanjulaand *et al.*, (2006) [41] concluded the role of chitosan in stimulation of microbiological action in the soil increased the prospective for the chitosan production for agriculture resolutions. Booth *et al.* (1965) [10] reported that chitosan improved crop yield, seed germination, biotic and abiotic resistance. The Positive effect was observed in seeds treated with chitosan or stressed with acidic pH, in rice and maize plants (Malerba *et al.*, 2011) [46]. Chitosan is extensively used for enzyme immobilization, as a carrier for controlled drug delivery and as a growth enhancer in agriculture (Divya *et al.*, 2017) [22]. Yien *et al.* (2012) [67] evaluated the joint effect of chitosan and H_2O_2, was improved the protein content in two dissimilar maize varieties. Therefore, chitosan and H_2O_2 developed certain phenological and biochemical features of maize but it depended on their method of application. Consequently, seed priming with chitosan at low concentration boost the production of anise *(Pimpinella anisum* L.) (Mahdavi *et al.*, 2013) [48]. Chitosan could be a better option for sustainable organic cultivation (Chandra *et al.*, 2015) [13]. Hadwiger *et al.* (2002) [28] applied commercially produced chitosan to cereal crop seeds @ 60 µg to 1000 pg per gram of seed that boosted root development, crown diameter, mature straw strength and crop yield. Foliar application (01-1.5% weight) of water-Soluble Salt of chitosan (514/55) to the improved the overall performance of the vegetables, tubers, cereal grains, fruits and blossoms (Hasaneen *et al.*, 2014) [30]. The stimulating effect of different enzyme activities to detoxify reactive oxygen species recommended the involvement of hydrogen peroxide and nitric oxide in chitosan signaling (Malerba *et al.*, 2016) [47]. Martin *et al.* (2009) demonstrated the effects of soluble chitosan on plantlets growth *invitro* condition and increased the yield of potato by micropropagation technique. However, the soluble chitosan can successfully integrate or encapsulated into plantlets for vigor seed production (Silva *et al.*, 2011) [61]. Applied chitosan improve the quality of plantlets *invitro* and facilitating the subsequent acclimatization of plantlets to *exvitro* conditions (Nge *et al*., 2006) [51]. Kashyap *et al.* (2015) [40] reported that the seed quality of minitubers derived from chitosan treatments *invitro* was improved the tuber number and yield.

CNP-NPK fertilizer effect on growth and productivity

The study of Aziz *et al.* (2016) [1] reported that Nano chitosan integrated with NPK fertilizer encouraged harvest index, crop index and mobilization index of the wheat yield variables significantly than control group (normal non-fertilized and normal fertilized NPK both). Thus, the application of nanofertilizers can open a new perspective for encouragement of agricultural productivity. However, the nanofertilizers responses towards plants differ with crop to crop or even variety to variety. In the work of Coradini *et al.* (2010) the interaction and stability of chitosan nanoparticle suspensions containing nitrogen (N), phosphorus (P) and potassium (K) were determined by FTIR spectroscopy, particle size analysis and zeta-potential. The stability of the Chitosan nanoparticle colloidal suspension was advanced with the addition of nitrogen and potassium as compared with the addition of phosphorus, this effect was due to the higher anion charge from the calcium phosphate than the anion charges from the potassium chloride (KCl) and urea (NH_2-CO-NH_2). Wu *et al.* (2008) [65] proposed that chitosan nanoparticles coated with cross-linked poly (acrylic acid)/diatomite that containing urea and a core of water-soluble granular nitrogen (N), phosphorus (P), and potassium (K). (NPK) fertilizer act as a slow and controlled release of the nutrients without any injurious influence on the soil health. Hussain *et al.* (2012) [32] reported that encapsulation of urea with chitosan microspheres attained a controlled release of the nutrient.

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