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Chitosan Nanoparticles: A Promising Stimulant for Augmenting Cold Stress Resistance in Safed Velchi Cultivars of Banana Plants

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ABSTRACT

Banana plants lack effective defense mechanisms against cold weather. Low temperatures cause severe effects on their productivity. The objective of this research was to improve the growth, development and resistance of safed velchi cultivars of banana plants against cold stress by administering Chitosan-Nanoparticles using foliar treatments. In this study four different concentration levels of Chitosan Nanoparticles (0, 200, 400 and 800 mg $L^{-1)}$ in demineralized water were used to treat banana plants. A control group was considered as a reference and were not subjected to cold stress or Chitosan Nanoparticles treatment. The Safed Velchi variety were cultivated within a controlled environment having the temperature of 4˚C for a duration of 72 hours in a growth chamber. Giving foliar application at 800 mg L^{-1}, showing a substantial improvement in the fresh and dry weights by 16 and 38%. Various growth factors and nutrient levels showed significant improvements, with some enhancements reaching a p-value below 0.05. Decreased levels of Malonic aldehyde and reactive oxygen species i.e., 30 and 35 respectively were shown. Foliar application of chitosan nanoparticles may be a great and economical approach to prevent cold stress resistance. These findings suggest that *CH-NPs* can effectively mitigate the adverse effects of cold stress on Safed Velchi banana plants, holding promise for future applications in enhancing crop resilience to abiotic stressors.

Keywords: - *Chitosan nanoparticles, Cold Stress, Safed velchi, Stimulator*

Introduction

A sustainable and resilient crop production system is essential to meet the global food demands. Traditional chemical-based farming practices have become ineffective due to increased population pressures and extreme climate variations. Recently, nano biotechnology is considered to be a promising approach for sustainable crop production by improving the targeted nutrient delivery, pest management efficacy, genome editing efficiency, and smart plant sensor implications (Ijaz *et al.,* 2023). A major cash crop, the banana (*Musa spp. L.*) helps ensure global food security (Justine *et al.*, 2022). Cold stress resistance strategies are needed to sustain and improve banana production in low-temperature regions. Biopolymers made by living organisms resist abiotic stresses. Chitosan, the second most abundant polymer after cellulose, is cheap and eco-friendly, drawing attention. Biopolymers like chitosan reduce biotic and abiotic stress tolerance (Muthuramalingam *et al.,* 2023). Biocompatibility, biodegradability, and plant growth and stress tolerance have made chitosan nanoparticles popular. Chitosan nanoparticles improve plant drought, salinity, and heavy metal resistance, according to several studies (Faizan *et al.,*2021). Little is known about bananas and chitosan nanoparticles for cold stress. Bio stimulant can help plants fight climate change and grow more, so scientists are interested in it (Matthews *et al.,* 2022). Nanotechnology promises interdisciplinary research. It has pest control, pharmaceutical, electronics, and parasitology opportunities (Suresh *et al.,* 2018). First, nanoparticle size determines plant cell penetration. Pores in plant cells are 5-50 nm "Bio-stimulant based on nanoparticles can penetrate leaves and roots by various pathways through the cuticle, trichomes, stomata, hydathodes, root tip, lateral roots, ruptures, rhizosphere wounds and root junctions" (Matthews *et al.,*2022). Chitin, a nitrogen-rich polymer, is found in arthropod exoskeletons, fungi, green algae, microorganisms, mollusc and cephalopod radulae and beaks Chitosan's macromolecular structure, solubility, biocompatibility, biodegradability, and reactivity make it a promising candidate for many applications (Thambiliyagodage *et al.,*2023). In saline conditions, 600 mg/L SiO2 NPs improved photosynthetic rate, K+, and K+/N+ to preserve Cavendish banana. We reduced MDH formation and electrolyte leakage (Xiao-min *et al.,* 2020). Foliar spraying Si-NP increased PS-II photochemical efficiency (Fv/Fm), maximum photo-oxidable PS-I (Pm), photosynthesis gas exchange, chlorophyll, and carotenoid content in sugar cane, reducing chilling. SA-CS NPs improve maize plant defense and growth. SA-CS NPs were characterized for colloidal size distribution, functional group, surface chemistry, chemical composition, crystal structure, and morphology. Research found SA-CS NPs' synthesis, SA release profile, antifungal, and seedling growth-promoting properties (Kumarasamy *et al.,* 2019). Chitosan nanoparticles improved banana growth and cold stress resistance in this study. Banana plants are sensitive to cold and lack a chilling defense system, which reduces their productivity. Nanotechnology-produced *CH-NPs* have been used to improve plant tolerance and growth under abiotic stresses like salinity and drought, but their effects on banana plants under cold stress are unknown. The main objective of this paper is to evaluate the effectiveness of Chitosan Nanoparticles (CH-NPs) in enhancing the cold stress resistance of banana plants. Therefore, the main hypotheses are that banana plants treated with CH-NPs will exhibit significantly improved growth parameters, nutrient content, photosynthesis pigment levels, antioxidant enzyme activities, and stress indicators compared to the control group when exposed to cold stress conditions. For this purpose, in this study, banana plants were sprayed with 0, 200, 400, and 800 mg L-1 *CH-NPs*. *CH-NPs* are agriculturally versatile. Their fertilizer release controls ensure plants get nutrients slowly and efficiently. *CH-NPs* help plants access water in various environments. These nanoparticles protect plants from drought and heat. *CH-NPs* protect plants from non-biological stressors, ensuring crop sustainability. This study examined Safed Velchi banana cultivars' growth, development, nutrient levels, biochemical characteristics, stress indicators, and cold stress resistance in response to Chitosan Nanoparticles *(CH-NPs).* The study investigated how these nanoparticles can reduce cold stress and sustain banana cultivation in temperature-sensitive areas. The study examined banana plant responses to cold stress and *CH-NP* treatment at different concentrations and a control group.

Materials and Methods

Chitosan Nanoparticles and the Safed Velchi variety of banana were required for the study as shown in (Fig. 1.) The present study employed tissue culture methods to cultivate the Safed Velchi variety of banana plants. The plants were purchased from Dr. Rajendra Prasad Central Agricultural University of Samastipur, Bihar. The five months old tree of Safed Velchi variety of banana were taken with a height of 45 cm. The five months old tree of Safed Velchi consists 15 leaves. A growing medium composed of compost and perlite in a weight-to-weight ratio of 3:1. The ongoing experiment involved selecting thirty plants that were in good health and had consistent characteristics. Chitosan, which was obtained from Suvidhinath Laboratories in Vadodara, was converted into nanoparticles for use in the experiment. The purity of the chitosan nanoparticles was found to be 99%, while their specific surface area fell in 50-80 square meters per gram. The particle size of these nanoparticles was below 100 nm. Banana plants were subjected to the application of *CH-NPs* in varying concentrations, namely 0, 200, 400, and 800 mg L^{-1} dissolved in demineralized water.

Chilling Treatments

Plant Growth Walk-In Large chambers provide ample space for banana plants. Its capacities range from a few hundred to several thousand liters. Chilling was done with a Conviron Walk-in Plant Growth Chamber (MTPS144). Automatically controlled plant growth. The growth chamber maintained optimal plant growth. This included maintaining a temperature range of 25 ± 1ºC, a relative humidity of 70 ± 5%, and providing a 12-hour light period with the light intensity for photosynthesis is 250 µmol/ (m^2.s). They then placed selected plant samples inside the chamber. Water and 60% modified Hoagland Solution were given to the plants. The experiment used 1 g/L Chitosan Nanoparticle stock solutions. The stock solution of Chitosan Nanoparticles was mixed with 5% acetic acid to make 1% (w/v) solution. Demineralized water was added to dilute the solution one part solution to 10 parts water after mixing. The stock solution was diluted with demineralized water to 0, 200, 400, 800 mg/L. Each treatment involved spraying the plants with 250 mL of Chitosan nanoparticle solution containing 40 mL per liter polysorbate-80. Chitosan nanoparticles were applied to banana seedlings at concentrations from 0 to 800 mg/L. The treatment involved spraying Chitosan nanoparticles. The plants received 300 mL of *CH-NP* solution daily for three days. The incubator's internal temperature was then lowered to 4°C and maintained for 72 hours to maintain growth conditions. No Chitosan nanoparticles or cold stress were applied to a control group.

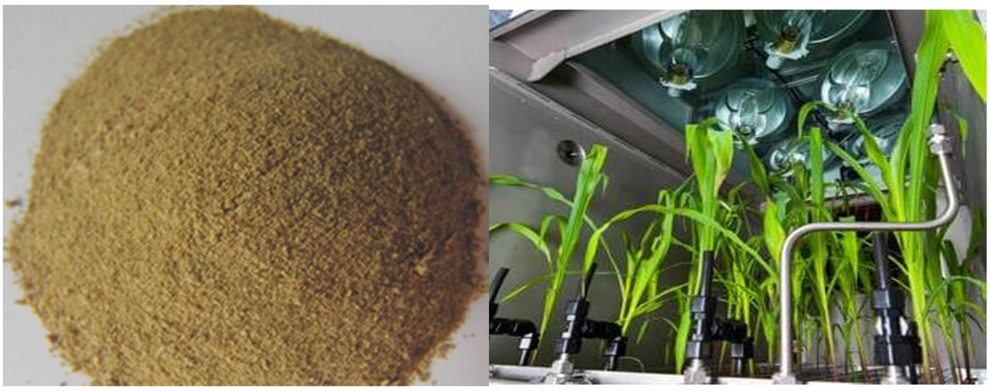

Fig.1. Safed Velchi Banana plant in Growth Chamber & Treatment with *CH-NP*

Examination and Analysis of Plant Samples

A modified technique based on Lichtenthaler and Buschmann's method (2001) was employed to quantify the levels of pigments responsible for photosynthesis in fresh leaves. These pigments were extracted using 85% acetone. Proline concentrations in freshly harvested banana plant leaves were evaluated employing the approach described by (Bates *et al.,* 1973). Extraction of the proline content from a mature leaf weighing 0.1 grams was carried out using a solution containing 4% sulfosalicylic acid. A modified approach, based on research conducted by (Ainsworth and Gillespie 2007), was employed to quantify the overall content of phenolic compounds. Banana leaves were subjected to phenolic compound extraction using 85% methyl alcohol. The anthrone reagent method was employed to quantify total soluble carbohydrates after extracting them with 80% ethanol. To initiate digestion, two grams of dried plant samples are mixed with a solution consisting of H_2O_2, Se, Li_2SO_4, and concentrated H_2SO_4. By the Kjeldahl distillation method 1883 was utilized to measure the nitrogen content in the extracts obtained from plant samples (Kirk 1950). A treatment involving digestion was applied to the plant samples, and subsequently, An ICP-OES instrument was employed to assess the levels of phosphorus, potassium, calcium, iron, manganese, zinc, and copper. The investigative strategy described by (Velikova *et al.,* 1999 and Kubis, 2008) was utilized to assess the concentrations of reactive oxygen species (ROS) in their research. The primary objective was to quantify the levels of hydrogen peroxide (H_2O_2), hydroxyl radicals, and superoxide anions. The process involved the extraction of hydrogen peroxide (H_2O_2) from recently harvested banana leaves using trichloroacetic acid (5%). A spectrophotometer was utilized to determine the concentration of hydrogen peroxide by analyzing the absorption of light at a predetermined wavelength of 390 nm. The K-phosphate buffer technique was applied to detect the occurrence of superoxide anions in the leaf samples, enabling their quantification based on the absorbance values obtained.

Statistical examination

The statistical analysis in this study utilized Tukey's HSD test as part of a one-way ANOVA to assess significant differences among the various treatments. Comparisons between treatment means were conducted at a 5% level of significance using Tukey's test. The results, as depicted in the figures and tables, are reported as means accompanied by their corresponding standard deviations (±SD), with each treatment group comprising 5 replicates (n = 5). All statistical analyses were carried out using the SPSS software, ensuring robust and reliable data interpretation.

Results and Discussion

Investigating the Impact of Chitosan Nanoparticles on Safed Velchi: Analyzing Growth Patterns and Nutritional Composition.

Banana is an excellent fruit full of micronutrients, especially vitamin A, iron, potassium, and magnesium, and is a source of energy for millions of inhabitants of tropical and subtropical regions. *Banana* can produce edible vaccines, paving the way for future syringe-less vaccine development. (Tripathi *et al.,* 2023).The group of banana plants that did not receive any treatment showed significantly superior growth when compared to the group that was exposed to cold stress, resulting in a significant hindrance to their overall growth (Fig. 2). Chitosan nanoparticle treatment administered to banana plant leaves yielded significant enhancements and the obtained data reached statistical significance with a p-value of less than 0.05 ($P < 0.05$). Weight of plants in their fresh state and weight of plants after drying, indicating a substantial improvement in plant development compared to untreated plants. These improvements were particularly notable under cold stress conditions, underscoring the potential benefits of Chitosan nanoparticles in protecting plants from adverse environmental factors. Nanoparticles enhance the germination of seed and maintain plant growth by promoting the production of enzymes in scavenging oxygen radicals, phytohormone balancing, nutrient metabolisms and expression of amino acid biosynthetic genes and photosystem (Hassim *et al.,*2021). The application of Chitosan nanoparticles at a concentration of 200 milligrams per liter resulted in a significant 6% augmentation in the fresh weight of banana plants, Furthermore, the dry weight of plants increased by 35%. The untreated group displayed a noticeable increase in plant weight when the concentration was increased to 400 milligrams per liter. The fresh weight of plants increased by 14%, while the dry weight experienced a remarkable boost of 60%. By employing Chitosan nanoparticles at their highest concentration (800 mg L^{-1}), notable improvements were observed in the weights of fresh and dried plant material in banana plants under the influence of cold stress. The plants that received treatment showed a 28% rise in their weight when measured fresh and the 82% increase in weight when measured dry, in comparison to the plants that were not treated. These results suggest that the application of *CH-NPs*, especially at higher concentrations (e.g., 800 mg L^{-1}), had a significant positive impact on the fresh and dry weights of banana plants under cold stress conditions, leading to substantial increases in plant growth compared to untreated plants. The differences between treatments were statistically significant ($p < 0.05$) based on Tukey's test. When chitosan nanoparticles were applied to banana plants under cold stress, there was a notable enhancement in the nutrient levels found in the leaves, as demonstrated in Table 1. The table presents the results of an experiment involving chitosan nanoparticles with varying concentrations and their effects on nutrient. The experiment was conducted under controlled conditions, and the following observations were recorded. The Control (C) group had the highest nutrient concentrations, with 45±5 mg/L chitosan nanoparticles. Among the elements, calcium (Ca) had the highest concentration at 220±15 mg/kg, followed by iron (Fe) at 155±6 mg/kg. The group with no chitosan nanoparticles added showed lower nutrient concentrations compared to the control group. Among the elements, copper (Cu) had the same concentration as in the control group 58±4 mg/kg, while other nutrients were generally lower. The group with 200 mg/L of chitosan nanoparticles showed slightly improved nutrient concentrations compared to the group with no nanoparticles. Potassium (K) and manganese (Mn) had higher concentrations than in the control group. When the concentration of chitosan nanoparticles was increased to 400 mg/L, nutrient concentrations further improved compared to the 0 mg/L group. Potassium (K) and calcium (Ca) levels increased significantly. The group with 800 mg/L of chitosan nanoparticles had similar nutrient concentrations to the 400 mg/L group, with only minor variations in some elements. In summary, the addition of chitosan nanoparticles influenced the nutrient concentrations in the sample. Generally, higher concentrations of chitosan nanoparticles tended to increase nutrient levels, with calcium (Ca) and potassium (K) showing the most significant improvements. Despite varying concentrations of chitosan nanoparticles, the impact on nutrient levels was not consistently proportional. This was evident in the case of copper (Cu), where its concentration remained constant across all experimental groups, irrespective of the chitosan concentration.

Table 1. The impact of Chilling stress (5°C for 72 hours) on the elemental composition of banana plant leaves and the role of chitosan nanoparticles

Chitosan nanopar-ticles mgL^{-1}	**N** g kg^{-1}	**P** g kg^{-1}	**K** g kg^{-1}	**Ca** g kg^{-1}	**Fe** mg kg^{-1}	**Mn** mg kg^{-1}	**Zn** mg kg^{-1}	**Cu** mg kg^{-1}
Control group	45± 5a	2.6 ±0.3a	30 ± 2.5a	25 ± 2.5a	220 ± 15a	155 ± 6a	135 ± 7a	58 ± 4a
0	35± 3d	2.6± 0.2b	16±2d	16± 2c	250± 5c	155± 8a	130± 6a	58 ± 4a
200	38±4c	2.9±0.3a	25±3c	28±3.5b	251± 6b	150±5a	130±3a	58 ± 4a
400	40±3b	2.8±0.4a	25±3c	21±4b	208 ±7b	155±7a	130±3a	55 ± 4a
800	40±3b	2.9±0.4a	30±4b	20±3b	215±9a	160±4a	136±7a	59 ± 3a

(mean ± SD, *n* = 5).

C = Control treatment was maintained without exposure to cold stress and was not supplemented with CH-NP amendment. HSD test at a significance level of p < 0.05 revealed significant differences among treatments, depicted by different letters in the same column. The reported values represent the mean ± standard deviation (SD) of five replicates (n = 5).

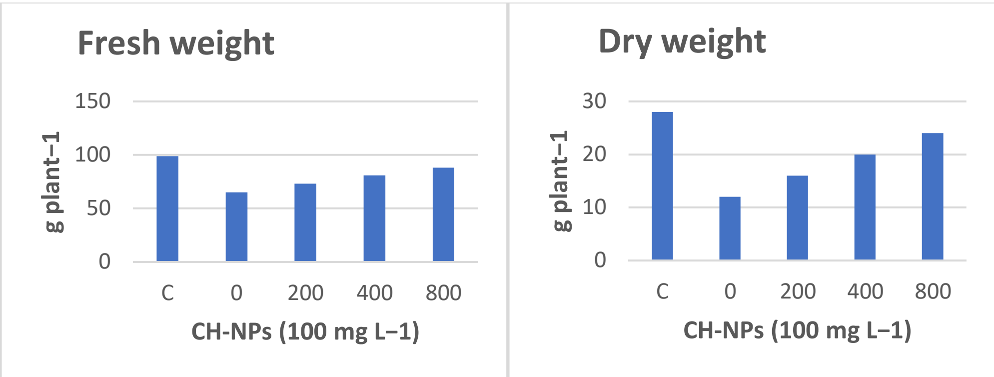

Fig. 2. Effect of chitosan nanoparticles (CH-NPs) on the fresh and dry weights of banana plants exposed to cold stress (4^0 C for 72 h). C = control treatment without cold stress and without CH-NP amendment Different letters refer to significant differences between treatments according to Tukey's test at *p* < 0.05. Each value is the mean of five replicates

Understanding the Interplay between Chitosan nanoparticles and Secondary Metabolites As well As Osmo-Metabolic Compounds

Nowadays, global climate change with inappropriate agricultural practices produces the severity and frequency of environmental worries which affect almost all living organisms including plants (Abeed *et al.,* 2023). The data presented in Table 2 provides compelling evidence of the marked advantageous effect that the inclusion of foliar chitosan nanoparticles has on the biochemical characteristics of banana plants when confronted with cold stress conditions. Under conditions of cold stress, there was an observed rise in the analyzed biochemical characteristics as compared to plants cultivated in normal environmental conditions. The application of chitosan nanoparticles led to substantial enhancements in the biochemical properties of banana plant leaf tissue under cold stress, resulting in a remarkable disparity when contrasted with plants that haven't received any treatment, the treated plants exhibit notable differences. Chitosan nanoparticles is known as a natural elicitor that triggers various physiological and biochemical responses in plants. It also has a growth-promoting effect on the plant (Matthews *et al.,*2022). The use of different concentrations of chitosan nanoparticles for treating banana plants via foliar application brought about a significant augmentation with regard to the buildup of total phenolic compounds, soluble carbohydrates, proline, and amino acids within the leaf tissue. The observed effect was particularly striking when the plants encountered cold stress. Increasing the concentration of chitosan nanoparticles generally led to an increase in phenolic compounds, soluble carbohydrates, proline, and amino acids. The highest concentrations of phenolic compounds, soluble carbohydrates, proline, and amino acids were observed at 800 mg/L of chitosan nanoparticles. The lowest concentrations were observed at 0 mg/L of chitosan nanoparticles. Phenolic compounds and soluble carbohydrates showed a significant increase with increasing chitosan nanoparticle concentrations, while proline and amino acids also increased but to a lesser extent. The data indicated that chitosan nanoparticles have an impact on the studied biochemical parameters, suggesting their potential influence on plant physiology or other relevant applications. Under the influence of cold stress, specifically at a temperature of 4°C endured for three consecutive days, banana plants displayed a significant upsurge in the presence of secondary and osmo-metabolic compounds within their leaves when treated with varying concentrations of *CH NPs.*

Table 2. Analyzing the effect of chitosan nanoparticles on various biochemical parameters in the leaves of banana plants when exposed to a prolonged period of Chilling stress (5°C for 72 hours).

Chitosan nanoparticles mgL-1	Phenolic Compounds	Soluble Carbohydrates	Proline	Amino Acids
C	3.02±0.9d	25.55± 2.25c	4±0.08e	25±3e
0	2.50±0.07	30.58± 2.54b	5.14±0.08d	28±3d
200	4.45±0.06b	35.68± 3.95a	4.90 ± 0.15c	38±4c
400	5.18±0.08a	38.25±3.50a	5.20 ± 0.15	45±4b
800	4.40±0.05a	40.15±4.60a	5.70±0.15a	56±5a

Analyzing the relationship between CH-NPs and photosynthesis-related pigments, as well as the activities of antioxidant enzymes.

Drought tolerance is boosted by nanoparticles, which increase root hydraulic conductance and water uptake while also showing a differential assortment of proteins involved in oxidation-reduction, ROS(Reactive oxygen sp.) removal, stress signaling, and hormonal mechanisms. Since nanoparticles, in their nature, are highly mobile, the nutrients can be easily transported to all the plant parts (Kumari *et al.,* 2022). By employing *CH-NPs* on banana plants, a substantial enhancement (The observed effects were statistically important, meeting the criterion of $p<0.05$.) was observed in the concentrations of photosynthesis pigments and the effectiveness of antioxidant enzymes (Fig. 3). Under cold stress conditions, the treatment of banana plant leaves with chitosan nanoparticles demonstrated significant benefits, Resulting in heightened levels of carotenoids and total chlorophyll (a + b) in the leaf tissue. By subjecting banana plants to chitosan particles at a concentration of 200 mg L^{-1}, a substantial elevation in carotenoid content was observed. The treated plants displayed an impressive 20% increment in carotenoid levels, underscoring a remarkable improvement compared to the untreated plants. Additionally, the chlorophyll (a + b) levels exhibited a substantial boost, showing a remarkable 56% increase in the treated plants. Banana plants treated with a solution containing 400 mg per liter of chitosan particles demonstrated a noticeable enhancement in carotenoid and chl (a + b) levels. Carotenoids demonstrated a notable expansion of 52%, whereas chl (a + b) experienced a significantly more substantial rise of 72% in comparison to the plants that were not subjected to any treatment. The introduction of 800 mg L^{-1} of chitosan nanoparticles to banana plants resulted in a remarkable elevation in carotenoid and chlorophyll (a + b) concentrations, signifying a substantial improvement. Applying chitosan nanoparticles to banana plant leaves that experienced cold stress resulted in a noteworthy boost in the levels of Peroxidative enzymes and superoxide dismutase (SOD) enzymes within the leaf tissue. The world depends on emerging technologies to improve crop productivity and overcome various abiotic and biotic stresses to meet the increasing food demand (Eevera *et al.,* 2023). The application of chitosan particles at a concentration of 200 mg L^{-1} on banana plants resulted in a 16% increase in Peroxidative enzymes activity and a 34% increase in superoxide dismutase (SOD) activity when compared to plants that were not treated with the spray. The application of 400 mg L^{-1} of chitosan nanoparticles on banana plants resulted in a 26% increase in peroxidase (POD) activity and a 48% increase in superoxide dismutase (SOD) activity, as compared to the plants that were not treated. Chitosan nanoparticles when applied at a concentration of 800 mg L^{-1} to banana plants, demonstrated a remarkable capacity to enhance enzymatic activity. Comparison to the untreated control group, peroxidase activity demonstrated a notable increment of 76%, while superoxide dismutase activity exhibited a considerable increase of 62%. Employing chitosan nanoparticles at different concentrations resulted in a marked enhancement ($p < 0.05$) in the quantities of photosynthetic pigments and the levels of antioxidant enzyme activities in banana plant leaves subjected to 72 hours of cold stress at a temperature of 4°C.

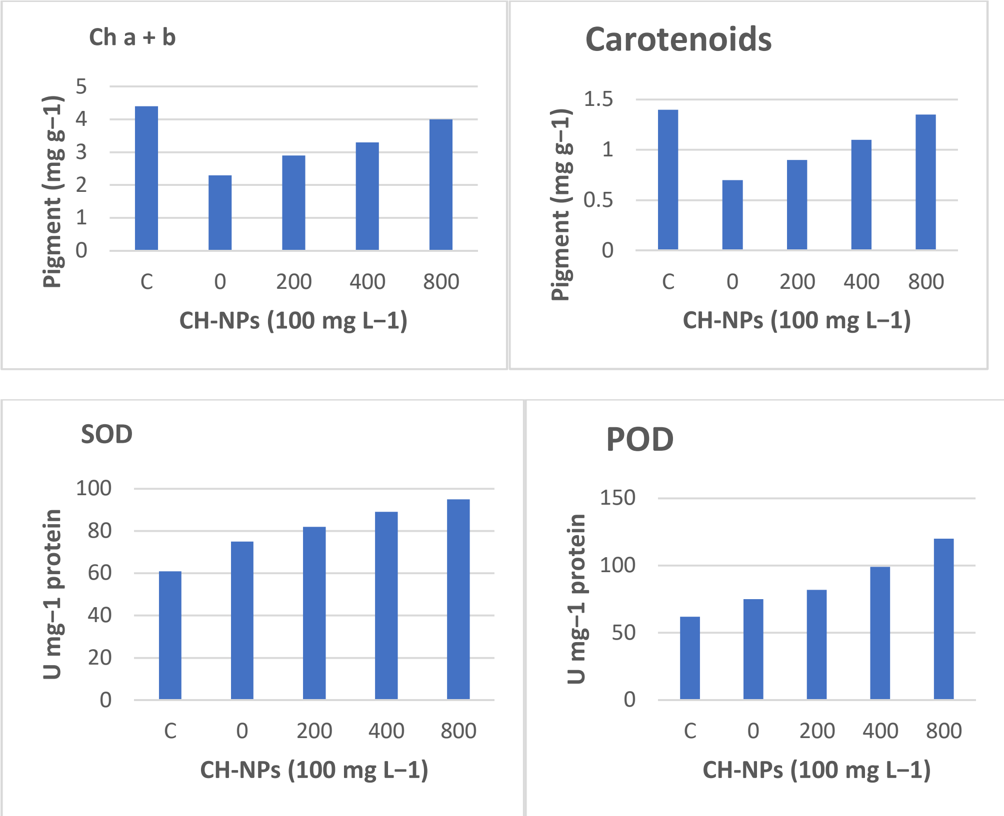

Fig. 3. Effect of chitosan nanoparticles *(CH-NPs)* on photosynthesis pigments (Chl (a + b) and carotenoids) and antioxidant enzymes superoxide dismutase (SOD) and peroxidase (POD) of banana plants exposed to cold stress (5 ◦C for 72 h). C = control treatment without cold stress and without *CH-NPs* amendment. Different letters refer to significant differences between treatments according to Tukey test at $p < 0.05$. Each value is the mean of five replicates (mean ± SD, $n = 5$).

Investigating the influence of CH-NPs on stress indicators and the subsequent impact on cell membrane integrity.

When subjected to a prolonged cold stress condition of 4°C for 72 hours, the banana plants demonstrated highly significant outcomes ($p < 0.05$) in terms of elevated MDA and ROS levels. Interestingly, these effects were observed in the absence of chitosan particles. The application of chitosan to banana plant leaves under cold stress conditions led to a notable reduction in malondialdehyde and reactive oxygen species levels within the leaf tissue. Drought stress impacts the photosynthesis pigment-protein complex, reducing PSII efficiency and the quantum yield of PSII electron transport chain. As a result, drought primarily hinders the photosynthetic process, reducing plant height, biomass and early senescence. Moreover, drought stress upregulates the alternative oxidase pathway and triggers the activity of nicotinamide adenine dinucleotide-malate dehydrogenase and nicotinamide adenine dinucleotide phosphate-malate dehydrogenase. Furthermore, drought stress frequently disrupts RWC in plants leading to oxidative and osmotic stress resulting in ionic inequity and severe injury to cell membranes and other in planta cellular functions. Gradual exposure to drought stress triggers ROS generation, including hydroxyl radical, singlet oxygen, superoxide anion free radical and H_2O_2, which increase lipid peroxidation, interfering with cellular metabolism by being detrimental to the functionality of proteins, lipids and macromolecules (Raza *et al.,*2023). Applying chitosan particles at a dosage of 200 milligrams per liter to banana plants resulted in a considerable alleviation of oxidative stress. The treated plants displayed a reduction of 40% in MDA levels, 64% in H_2O_2 levels, 78% in OH levels, and 92% in O_2 levels, when examining the treated plants and comparing them to their untreated plants, distinct disparities were evident. Banana plants treated with Chitosan nanoparticles at a concentration of 400 mg per liter experienced significant changes in their oxidative stress levels. The concentrations of MDA, H_2O_2, OH, and O_2 were notably decreased by approximately 54%, 58%, 72%, and 90%, respectively, in comparison to the plants that did not undergo this treatment. The presence of 800 mg L-1 chitosan in banana plants exhibited a considerable mitigation effect of oxidative stress. The treated plants demonstrated a substantial reduction of 66% in MDA, 67% in H_2O_2, 40% in OH, and 96% in O_2 levels contrasting to the untreated banana plants. The application of different concentrations of *CH-NP* through foliar spraying leads to a reduction in free radicals levels and minimizes membrane damage. Banana plants subjected to a cold stress environment (5°C for 72 hours) without the presence of chitosan particles exhibited the highest statistically important values ($p < 0.05$) for 3,4-Methylenedioxyamphetamine and free radicals. Upon treating banana plants experiencing cold stress with chitosan particles on their leaves, a striking decline in the amounts of malondialdehyde and reactive oxygen species was detected within the plant's leaf tissue. Under arid conditions, phenolic, flavonoids and antioxidant enzymes are affected to a large extent. Root-sourced signals are transported via the xylem to leaves, thus, affecting the cellular status in drought-stressed plants (El-Saadoni *et al.,* 2022). Banana plants exposed to chitosan particles with a dosage of 100 mg/L, exhibited a notable reduction in MDA levels (20%), H_2O_2 levels (32%), OH levels (39%), and O_2 levels (46%) in comparison to the untreated plants. Applying a solution of chitosan particles at a concentration of 200 mg L−1 to banana plants resulted in a reduction of 27% in MDA levels, 29% in H_2O_2 levels, 36% in OH levels, and 45% in O_2 levels when compared to plants that were not treated with the solution. Chitosan nanoparticles were applied to Safed velchi at a dose of 400 milligrams per liter exhibited a marked decrease in levels of MDA, H_2O_2, OH, and O_2, in stark contrast to untreated plants. The decrease observed was approximately 33% for MDA and H_2O_2, 40% for OH, and 48% for O_2 (Fig. 4). Nanoparticles are one of the most widely studied materials in this century, and they have important uses in various fields, including agriculture. The biologically synthesized nanoparticles provide promising solutions against biotic (insect pests, plant diseases) and abiotic (drought, salinity, thermal stresses, toxic metals, organic pollutants) stress factors (Nawaz, Ahmad, *et al.,*2023). Bio-stimulants play a vital role in the sustainable development of horticultural crops (Hassanein *et al.,* 2021). Many environmental factors, such as extreme heat, foggy weather, high moisture, heavy rainfall, and so on, can also impose adverse impacts on seed emergence uniformity, vigour, and thereby yield of crops (Suresh *et al.,* 2018). Exposure of plants to these stresses results in periodic adjustments and modification of the plant defense apparatus, as well as reorganization of the plant metabolism (Chakraborti *et al.,* 2022). Foliar spraying of nano-chitosan (5 mL/L) on mango trees enhanced the growth and fruit quality and also showed more resistance to malformation (Muthuramalingam *et al.,* 2023). The growing demand for food due to climate change has led to an increased recognition of the significance of the chilling problem. The recent research clearly indicated that exposure to cold temperatures hampers plant growth, leads to greater damage to cell membranes, and results in the accumulation of elevated levels of reactive oxygen species within leaf tissue. Applying chitosan nanoparticles *(CH-NPs)* to banana plants using water-based solutions, at concentrations ranging from 100 to 400 mg L−1, mitigated the negative impacts caused by chilling. *CH-NPs* exhibit a beneficial impact on improving cold tolerance due to their ability to alleviate Free radical damage by augmenting the functioning of antioxidant enzymes and elevating the concentrations of osmoprotectant compounds that govern cellular osmosis. One potential method for attaining resilience against the detrimental consequences of cold stress in a manner that is both sustainable and economically feasible could involve the application of *CH-NPs*. Through the utilization of *CH-NPs*, it might be feasible to reduce the negative effects caused by exposure to cold stress. Utilizing *CH-NPs* shows promise in addressing the challenges of cultivating bananas in cold-prone regions by mitigating the adverse impacts of cold stress. This is particularly important given the growing frequency of climate fluctuations in many agricultural regions around the world. Chitosan nanoparticles are nanoscale particles made from chitosan and have gained attention in various fields, including agriculture, due to their unique properties. So, the use of chitosan nanoparticles in enhancing cold stress resistance in banana plants are very much beneficial. As banana plants are sensitive to cold temperatures, which can lead to various physiological and biochemical changes, ultimately affecting plant growth and fruit production. Therefore, finding strategies to mitigate the negative effects of cold stress is crucial, especially for cultivars like Safed Velchi. In the present study, the banana plants exhibited the least noticeable growth when they were subjected to cold stress conditions (specifically, exposure to 4°C for a duration of 72 hours) without the incorporation of chitosan nanoparticles *(CH-NPs).* Cold stress is observed in banana plants when the temperature falls below a critical range, typically between 4 to 10°C. This temperature drop can trigger a range of physiological issues that have detrimental effects on the overall growth of the banana plants. It is essential to safeguard fruit trees from chilling injury, and achieving this goal involves the development of effective and feasible methods to alleviate cold stress, especially for banana plants. The utilization of chitosan nanoparticles *(CH-NPs)* demonstrates a positive impact on banana plant growth, as indicated by the observed rise in both fresh and dry weights. After *CH-NPs* penetrate the leaf tissue, they have the capacity to move and be transported to reach the centers of vital processes in the plant cell where vital processes occur, thereby enhancing their effectiveness in promoting the plant's stress resistance. The results of the present study validate that chitosan nanoparticles *(CH-NPs)* serve as an efficient method for mitigating the detrimental impacts of cold stress on banana plants.

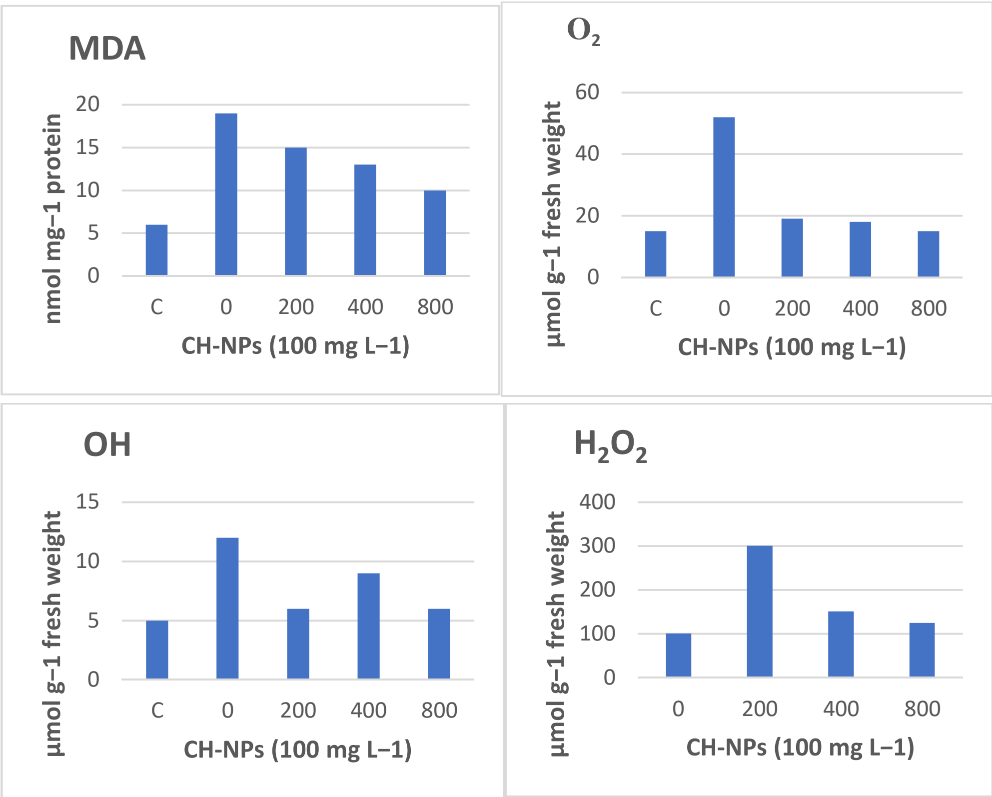

Fig. 4. Effects of chitosan nanoparticles (CH-NPs) on malondialdehyde (MDA) and reactive oxygen species (superoxide anions (O_2•-), hydroxyl radicals (•OH), and hydrogen peroxide (H_2O_2)). C = control treatment without cold stress and without CH-NP amendment. Different letters refer to significant differences between treatments according to Tukey's test at $p < 0.05$. Each value is the mean of five replicates (mean ± SD, $n = 5$)

The statistical analysis presented in this study provides robust evidence supporting the beneficial impact of Chitosan nanoparticles *(CH-NPs)* on Safed Velchi banana plants exposed to cold stress conditions. Abiotic stresses disturb the plant metabolism lead causing a significant reduction in plant growth, development, and yield formation. In addition to other effects, the over production of reactive oxygen species (ROS) is one of the major reasons for crop losses caused by abiotic stresses (Nawaz, Ahmad, *et al.,* 2023). The observed significant enhancements in plant growth, nutritional composition, biochemical characteristics, and stress indicators following the application of *CH-NPs* at various concentrations underscore their potential as a valuable tool for mitigating cold stress in banana cultivation. The findings of this study hold promise for future agricultural practices seeking to enhance cold tolerance in crops, potentially leading to increased yield and resilience in adverse environmental conditions. *Banana* cultivation largely regulates the agri-based global bio economy. Hence, understanding the associated challenges in its production and developing appropriate strategies for addressing these concerns are of paramount importance (Justin *et al.,* 2022). However, it is crucial to acknowledge and address potential limitations before considering the broader implementation of *CH-NPs.* One notable limitation is the variability observed in the control group's response, suggesting the need for a more homogeneous control group or increased replicates to mitigate confounding factors. While this study provides valuable empirical data, there is a notable absence of exploration sinto the molecular or physiological mechanisms underlying the observed effects of *CH-NPs.* Future research endeavors should prioritize elucidating the precise interactions between *CH-NPs* and plant physiology to enhance stress tolerance, thereby strengthening the scientific foundation of the findings. The study's focus on controlled conditions presents another limitation, as real-world environments exhibit greater variability. Future research initiatives should aim to validate the effectiveness of *CH-NPs* in open-field conditions, providing insights into their performance in more realistic scenarios. Furthermore, the study predominantly concentrates on the short-term effects of *CH-NP* application, emphasizing the necessity to investigate the long-term impacts on plant health, fruit quality, and soil health. This comprehensive understanding is essential to evaluate the practicality and sustainability of CH-NP application over extended periods. While attributing improvements to *CH-NPs,* it is essential to consider other potential contributing factors, such as changes in nutrient availability, soil composition, or microbial interactions. Future research should strive to rule out these factors, ensuring a clearer understanding of the specific contributions of *CH-NPs* to observed outcomes. Additionally, the study focuses specifically on Safed Velchi banana plants, and the efficacy of *CH-NPs* may vary across different plant species. To assess the generalizability of *CH-NPs,* future research should explore their applicability to a broader range of crops. Lastly, the promising prospect of *CH-NPs* in enhancing stress tolerance necessitates a comprehensive evaluation of their environmental impact, including persistence in the ecosystem and potential effects on non-target organisms. Future studies should address these environmental considerations to ensure the responsible application of *CH-NPs* in agriculture. In conclusion, while this study provides compelling evidence of *CH-NPs'* efficacy in mitigating cold stress in banana plants, acknowledging and addressing potential limitations is crucial. Further research addressing these limitations can refine our understanding and contribute to the practical application of *CH-NPs* in agriculture, promoting sustainable and resilient crop production systems.

Conclusion

In conclusion, this study explored the impact of Chitosan Nanoparticles *CH-NPs* on Safed Velchi banana plants facing cold stress, with a focus on enhancing various aspects of plant physiology. The experiments, incorporating diverse *CH-NP* concentrations and exposure to cold stress, successfully achieved the research objectives. The findings present a promising avenue for agricultural innovation. Specifically, *CH-NPs,* particularly at a concentration of 800 mg L^{-1}, significantly improved banana plant growth and development under cold stress conditions. This discovery is particularly relevant in regions prone to temperature fluctuations, offering a practical strategy for farmers to bolster banana plants against cold stress, thereby promoting more reliable and sustainable crop production. While the study showcases the positive impact of *CH-NPs*, further research is necessary for a deeper understanding of the underlying mechanisms. Additionally, considering the emphasis on Safed

Velchi banana cultivars, the potential variability in the effectiveness of *CH-NPs* across different banana varieties merits consideration. The study highlights the importance of investigating the long-term effects of *CH-NP* application and its potential ecological consequences. Comprehensive exploration in these areas is essential for ensuring the sustainable integration of *CH-NPs* into agricultural practices. In summary, this research provides valuable insights into the application of *CH-NPs* as a practical solution to mitigate the adverse effects of cold stress on banana plants. It not only addresses immediate challenges but also sets the stage for future research to uncover the intricacies of *CH-NP* mechanisms and explore broader applications in agriculture. Ultimately, this study contributes to sustainable farming practices by offering a viable approach to fortify crop resilience and enhance productivity in the face of environmental challenges.

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References

Ainsworth, E.A. and Gillespie, K.M. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature protocols*, **2**(4):875-877.

Abeed, A.H., AL-Huqail, A.A., Albalawi, S., Alghamdi, S.A., Ali, B., Alghanem, S.M. and El-Mahdy, M. T. 2023. Calcium nanoparticles mitigate severe salt stress in Solanum lycopersicon by instigating the antioxidant defense system and renovating the protein profile. *South African Journal of Botany,* **161**: 36-52.

Bates, L.S., Waldren, R.A. and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. Plant and soil, 39:205-207.

Chakraborti, S., Bera, K., Sadhukhan, S. and Dutta, P. 2022. Bio-priming of seeds: Plant stress management and its underlying cellular, biochemical and molecular mechanisms. Plant Stress, 3:1-16.

Eevera, T., Kumaran, S., Djanaguiraman, M., Thirumaran, T., Le, Q.H. and Pugazhendhi, A.2023. Unleashing the potential of nanoparticles on seed treatment and enhancement for sustainable farming. Environmental Research, 236:116849.

El-Saadony, M.T., Saad, A.M., Soliman, S.M., Salem, H. M., Desoky, E.S. M., Babalghith, A.O. and Abu Qamar, S.F. 2022. Role of nanoparticles in enhancing crop tolerance to abiotic stress: A comprehensive review. *Frontiers in plant science*, **13**:946717.

Faizan, M., Rajput, V.D., Al-Khuraif, A.A., Arshad, M., Minkina, T., Sushkova, S. and Yu, F. 2021. Effect of foliar fertigation of chitosan nanoparticles on cadmium accumulation and toxicity in Solanum lycopersicum. *Biology,* **10**(7): 1-14.

Hassanein, Y.Z., Abdel-Rahman, S.S.A., Soliman, W.S. and Salaheldin, S. 2021. Growth, yield, and quality of roselle (*Hibiscus sabdariffa* L.) plants as affected by nano zinc and bio-stimulant treatments. Horticulture, Environment, and Biotechnology, **62**:1-483.

Hassim, M. F. N., Chuen, N.L.,Ghazali, M. S. M., and Ahmad, A. 2021. Nanotechnology: Recent Progress in Agriculture and Effects on Physiology of Plants. *Journal of Tropical Plant Physiology,* **13**(2):1-37.

Ijaz, M., Khan, F., Ahmed, T., Noman, M., Zulfiqar, F., Rizwan, M. and Li, B. 2023. Nanobiotechnology to advance stress resilience in plants: Current opportunities and challenges. *Materials Today Bio,* **22**:1-17.

Justine, A.K., Kaur, N. and Pati, P. K. 2022. Biotechnological interventions in banana: current knowledge and future prospects. Heliyon, **8**:1-14.

Kirk, P.L. 1950. Kjeldahl method for total nitrogen. *Analytical chemistry,* **22**(2): 354-358.

Kumaraswamy, R.V., Kumari, S., Choudhary, R.C., Sharma, S.S., Pal, A., Raliya, R. and Saharan, V. 2019. Salicylic acid functionalized chitosan nanoparticle: a sustainable bio stimulant for plant. International *Journal of Biological Macromolecules,* **123**:59-69.

Kumari, A., Bhinda, M.S., Sharma, B. and Parihar, M. 2022. Climate change mitigation and nanotechnology: An overview. Sustainable Agriculture Reviews 53: Nanoparticles: A New Tool to Enhance Stress Tolerance, **28**:33-60.

Lichtenthaler, H.K., and Buschmann, C. 2001. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Current protocols in food analytical chemistry,* **1**(1):1-10.

Matthews, S., Ali, A., Siddiqui, Y., and Supramaniam, C.V. 2022. Plant bio-stimulant: Prospective, safe and natural resources. *Journal of Soil Science and Plant Nutrition,* **22**(2):1-15

Muthuramalingam, P., Muthamil, S., Shilpha, J., Venkatramanan, V., Priya, A., Kim, J. and Shin, H. 2023. Molecular Insights into Abiotic Stresses in Mango. Plants, **12**(10):1-20.

Nawaz, A., Rehman, H.U., Usman, M., Wakeel, A., Shahid, M.S., Alam, S. and Farooq, M. 2023. Nano biotechnology in crop stress management: an overview of novel applications. *Discover Nano,* **18**(1): 1-40.

Rajput, V. D., Minkina, T., Kumari, A., Harish, Singh, V. K., Verma, K. K. and Keswani, C. 2021. Coping with the challenges of abiotic stress in plants: New dimensions in the field application of nanoparticles. *Plants,* **10**(6):1-25.

Raza, A., Charagh, S., Salehi, H., Abbas, S., Saeed, F., Poinern, G.E. and Varshney, R.K. 2023. Nano-enabled stress-smart agriculture: Can nanotechnology deliver drought and salinity-smart crops? Journal of Sustainable Agriculture and Environment, **2**(3):189-214.

Suresh, U., Murugan, K., Panneerselvam, C., Rajaganesh, R., Roni, M., Al-Aoh, H.A.N. and Benelli, G. 2018. Suaeda maritima-based herbal coils and green nanoparticles as potential biopesticides against the dengue vector Aedes aegypti and the tobacco cutworm *Spodoptera litura*. *Physiological and molecular plant pathology,* **101**:1-11.

Thambiliyagodage, C., Jayanetti, M., Mendis, A., Ekanayake, G., Liyanaarachchi, H. and Vigneswaran, S. 2023. Recent Advances in Chitosan-Based Applications—A Review. Msaterials, **16**(5):1-58.

Tripathi, J.N., Ntui, V.O., Malarvizhi, M., Muiruri, S., Ravishankar, K.V. and Tripathi, L. 2023. Improvement of nutraceutical traits of banana: new breeding techniques. In Compendium of Crop Genome Designing for Nutraceuticals. Singapore: Springer Nature Singapore: 1-33.

Liu, X.M., GAO, T.T., Zhang, Z.J., Tan, K.X., Jin, Y B., Zhao, Y.J. and Chao, L.I. 2020. The mitigation effects of exogenous dopamine on low nitrogen stress in Malus hupehensis. *Journal of Integrative Agriculture,* **19**(11): 2709-2724.

Kubiś, J. 2008. Exogenous spermidine differentially alters activities of some scavenging system enzymes, H_2O_2 and superoxide radical levels in water-stressed cucumber leaves. *Journal of Plant Physiology,* **165**(4):397-406.

Velikova, G., Stark, D. and Selby, P.1999. Quality of life instruments in oncology. European *Journal of Cancer,* **35**(11):1571-1580.