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Impact of seed nanopriming on biochemical properties of chilli (*Capsicum annuum* L.) cultivar GCh-1

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Abstract
Maintaining optimum plant population is an important factor in maximizing crop production and productivity. Our agriculture is threatened by climate change and the depletion of resources and biodiversity. A new agriculture revolution is needed in order to increase the production of crops and ensure the quality and safety of food, in a sustainable way. The present investigation was undertaken to find out the effect of seed nano priming on biochemical properties of chilli seeds. Chilli (GCh-1) seeds primed with eight different priming treatments (T_1: AgNPs @ 25 ppm, T_2: AgNPs @ 50 ppm, T_3: AgNPs @ 100 ppm, T_4: $AgNO_3$ @ 0.5%, T_5: $AgNO_3$ @ 1.0%, T_6: $AgNO_3$ @ 2.0%, T_7: GA_3 @ 100 ppm and T_8: H_2O) with soaking duration 24 hrs. Dray seeds were considered as control treatment. Priming treatments with silver nano particles significantly increased biochemical components such as α-amylase enzyme, chlorophyll, ascorbic acid, total phenol and total carbohydrates in GCh-1 as compared to control. Primed seeds showed better performance of chilli than control treatment in aspects of studied criteria.

Keywords: Nanopriming, biochemical and GCh-1

Introduction
As per Indian Minimum Seed Certification Standards, chilli has average 60 per cent germination, so it's important to increase the germination above 60 per cent, which will be an incremental achievement in seed quality attributes. As poor, delayed and erratic germination of chilli seeds are one of the reasons for the low yield of chilli. The germination period is the most critical period in a plant's life cycle because during this stage those plants have a high vulnerability to injury, environmental stress and disease, which leads to seed deterioration. To increase seed vigour and crop production, different chemical-based fertilizers and pesticides are used extensively in agriculture. In light of the leaching, degradation, hydrolysis and pollution associated with conventional chemical based practices, they are being discouraged. There is an urgent need to develop a sustainable technology that can contribute to the green revolution to address these growing concerns and to restore the damage caused to the ecosystem.

Seed priming is an unique and new method to improve the seed vigour, crop establishment and enhance the crop yield along with biochemical parameters without harming the ecosystem. Seed priming involves hydration of the seed in different ways, thus improving germination rate, uniformity in emergence and germination under a wide range of environmental and climatic conditions and also increasing seedling vigour and growth (Subramanian and Umarani, 2010) [10].

Biochemical and physiological deterioration during seed ageing has been mostly under accelerated ageing conditions using high temperature and high seed water content. Some studies indicate that the lipid peroxidation and the degradation of membrane phospholipids are major causes of seed ageing under accelerated ageing conditions. Significant changes in enzyme activities were noticed in primed seeds compared to un- primed seeds, desiccation and storage of seeds has been suggested to result in progressive loss of integrity of the membrane components of the seeds, which in turn contribute to seed deterioration as measured by loss of seed vigor and viability.

Materials and Methods

Experimental details

The research was conducted at the Department of Biochemistry, College of Basic Science and Humanities, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, during the years 2021-23. The seeds of Gujarat Chilli Hybrid-1 (GCh-1) cultivar of chilli, was used for this study. This research was arranged in complete randomized design with nine treatments and three replications.

Preparation of priming solution

Preparation of nanoparticles

The green synthesis of nanoparticles becomes imperative (Parashar *et al*., 2009) [6]. The *Ocimum basilicum* leaf extract was prepared by taking 10 g of thoroughly washed and finely cut fresh leaves in an Erlenmeyer flask in 100 ml of sterile deionized water, the mixture was boiled for 10 min. and then cooled followed by the filtration using the filter paper. Briefly, 20 ml of the leaf extract was added drop wise to 80 ml of 1.0 mM of $AgNO_3$ solution and vigorously stirred on the magnetic stirrer, until the colour change of the solution (brown solution) is observed which indicates the reduction of Ag^+ ions after a few minutes. The reactions were carried out in darkness (to avoid photo activation of $AgNO_3$) at room temperature. Complete reduction of $AgNO_3$ to Ag^+ ions was confirmed by the change in colour from colourless to colloidal brown. The formation of AgNPs was periodically monitored for 24 hrs. using the UV-Visible spectrophotometer. The solution was centrifuged at 11000 rpm for 30 min. and the dark brown solid product was collected and dried at room temperature. The resulting dried sample was crushed into powder and stored in an air tight container for further analysis.

Preparation of silver nitrate solution

Three different concentration of silver nitrate solution were used for the experiment. For making 0.5% solution of $AgNO_3$, taken 0.5 g of silver nitrate and dissolves in 100 ml of distilled water and mix it. While, 1.0% solution of $AgNO_3$, taken 1.0 g of silver nitrate and dissolves in 100 ml of distilled water and mix it. For making 2.0% solution of $AgNO_3$, taken 2.0 g of silver nitrate and dissolve in 100 ml distilled water and mix it.

Preparation of Gibberellic acid solution

Preparation of 100 ppm GA_3 solution taken 100, mg of Gibberellic acid and it dissolved in 100 ml of distilled water.

Seed surface sterilization

Seeds of chilli cultivar GCh-1 were 3-4 times washes with double distilled water after that seeds were deep in 0.1% mercury chloride solution for 1 min. Then washed thrice with distilled water and blotted on sterile blotting paper for drying.

Seed priming procedure

The sterilized seeds were treated with different priming and different concentration for 24.00 hrs duration under the aseptic condition mentioned in table 1. The 10 g seeds of each cultivar were soaked in 100 ml of solution for each treatment. The seeds were fully immersed in the solution at a room temperature in the dark condition. After that treated seeds were thoroughly rinsed with distilled water for 2 minutes. Then, it was labeled and keep in air dried on blotting paper at room temperature overnight. Than primed seeds of GCh-1 were sown in plug tray of each treatment in three replications.

Table 1: Treatment Details

Treatments	Soaking Periods (hrs)
T_1: AgNPs @ 25 ppm	24
T_2: AgNPs @ 50 ppm	24
T_3: AgNPs @ 100 ppm	24
T_4: $AgNO_3$ @ 0.5%	24
T_5: $AgNO_3$ @ 1.0%	24
T_6: $AgNO_3$ @ 2.0%	24
T_7: GA_3 @ 100 ppm	24
T_8: H_2O	24
T_9: Control	-

Extraction and estimation of biochemical parameters

The effect of different seed priming treatments on biochemical parameter *viz.*, α-amylase enzyme, chlorophyll, ascorbic acid, total phenol and total carbohydrates were studied in GCh-1 cultivar of chilli during present investigation.

Alpha amylase enzyme

Amylase activity was estimated according to the method suggested by Sadasivam and Manickam (2008) [9]. Six days old seedlings were taken from each treatment of both cultivars and washed thoroughly and excess moisture was removed by blotting with tissue paper. Then, one gram of seedlings were frozen in liquid nitrogen and ground with the help of pestle and mortar by using 1.0 ml of 0.1 M phosphate buffer (pH 7.0) was added and the ground material was transferred to Eppendorf tubes and kept at 4°C overnight and then transferred to -20°C. The slurry was centrifuged at 10,000 rpm for 15 minutes at 4°C and the supernatant was used for α-amylase assay.

Chlorophyll content

Estimation of chlorophyll was done by methods given by Arnon (1949) [2]. Fresh healthy leaves were collected from each treatment of GCh-1 and Bhut Jolokia cultivars of Chilli. One gram of each sample was taken and grounded with pestle and mortar with 10 ml of 80% acetone. The homogenate was centrifuged. The procedure was repeated till the residue was completely devoid of chlorophyll; the extract was made up to 50 ml. The absorbance of the solution was recorded at 645 nm and 663 nm against the solvent (acetone) blank.

Ascorbic acid

The ascorbic acid content will be estimated by using 2,4-dinitrophenylhydrazine reagent in conjunction with spectrophotometer at 540 nm described by Sadasivam and Manickam (1992) [8]. The unripped (65 days after transplanting) and ripped (85 days after transplanting) chilli fruits were collected from treated plants of both cultivars used for assay.

Total carbohydrates

Total carbohydrates were estimated according to the Anthrone method given by Yoshiba *et al*. (1997) [12]. The unripped (65 days after transplanting) and ripped (85 days after transplanting) chilli fruits were collected from treated plants of both cultivars for analysis.

Total phenol

Phenol level was estimated by Folin-Ciocalteau reagent method given by Roginsky and Lissi (2005) [7]. The unripped (65 days after transplanting) and ripped (85 days after transplanting) chilli fruits were collected from treated plants of both cultivars for analysis. A quantity of 0.5 g of fruit of each treatment was homogenized in 5 ml of 80% methanol and stirred continuously for 24 hrs. at room temperature. The mixture was then separated by centrifuging at 5000 rpm for 15 min. The supernatant was taken and filtered through the filter membrane with a size of 0.45 µm. The pellet was extracted twice and the solutions were mixed. The total extracts were dried using a rotary evaporator at 30 o C. Methanol was used to dissolve the dried extracts. Total phenolic content was estimated based on the Gallic acid standard curve and expressed as mg per gram.

Results and discussion

The biochemical data obtained are summarized in the following sessions.

α-amylase enzyme

The seed priming treatments had significant influenced on α-amylase content in 6 days old seedlings with respect to the non- primed seedlings (Table 2). When seed primed with GA 3 @ 100 ppm, the maximum α-amylase enzyme (63.28 absorption unit) was observed. It was statistically comparable to AgNPs @ 100 ppm and AgNPs @ 50 ppm, with 61.73 and 59.75 absorption units, respectively. In contrast to other treatments, the control group exhibited the lowest level of α-amylase enzyme (50.43 absorption unit) in the GCh-1 cultivar.

Effectiveness of priming on amylase enzyme activity of seedlings were recorded by many workers *viz*., Mahakham *et al*. (2017) [5] revealed that nanopriming treatments significantly enhanced α-amylase activity in rice seedlings. Similar trend was observed by Ghalavand *et al*. (2022) [3] in Lallemantia priming with hydropriming (for 24 hours) and Gibberellic acid (100 mg/l for 8 hours).

Table 2: Effect of priming on α-amylase enzyme

Treatments	α-amylase enzyme (absorption units)
T_1 : AgNPs @ 25 ppm	57.72^{bc}
T_2 : AgNPs @ 50 ppm	59.75^{ab}
T_3 : AgNPs @ 100 ppm	61.73^{ab}
T_4 : $AgNO_3$ @ 0.5%	52.03^{d}
T_5 : $AgNO_3$ @ 1.0%	53.21^{d}
T_6 : $AgNO_3$ @ 2.0%	58.91^{b}
T_7 : GA_3 @ 100 ppm	63.28^{a}
T_8 : H_2O	54.68^{cd}
T_9 : Control	50.43^{d}
S. Em. (±)	1.31
C. D. @ 5%	3.89
C.V. (%)	3.99

Treatment means with the common letters(s) are non-significant by Duncan's New Multiple Range Test at 5% level of significance.

Chlorophyll content

The data pertaining to chlorophyll content in leaves of GCh-1 seedlings as influenced by different priming treatments are presented in Table 3. It was clear from results that Chlorophyll-a, Chlorophyll-b and total chlorophyll content were significantly influenced by different seed priming. The maximum levels of chlorophyll-a (0.2147 mg/g), chlorophyll-b (0.2141 mg/g) and total chlorophyll (0.4288 mg/g) in the GCh-1 cultivar were observed under AgNPs @ 100 ppm, among other priming treatments. This was statistically equivalent to the levels of chlorophyll-a (0.2073 mg/g), chlorophyll-b (0.2016 mg/g), and total chlorophyll (0.4089 mg/g) recorded in GA 3 @ 100 ppm. Nonetheless, in the GCh-1 control group, the lowest concentration of chlorophyll-a (0.1808 mg/g), chlorophyll-b (0.1675 mg/g), and total chlorophyll (0.3483 mg/g) were noted.

Iqbal *et al*. (2019) [4] noted a similar tendency in wheat, where plants treated with AgNPs showed increases in chlorophyll-a of 10%, chlorophyll-b of 16.4% and total chlorophyll of 19.00%.

Table 3: Effect of priming on chlorophyll content

Treatments	Chlorophyll (mg/g)		
	Chlorophyll-a	Chlorophyll-b	Total Chlorophyll
T_1 : AgNPs @ 25 ppm	0.2001^{abc}	0.1992^{abc}	0.3993^{bc}
T_2 : AgNPs @ 50 ppm	0.2036^{abc}	0.1871^{bcd}	0.3907^{bcd}
T_3 : AgNPs @ 100 ppm	0.2147^{a}	0.2141^{a}	0.4288^{a}
T_4 : $AgNO_3$ @ 0.5%	0.1885^{bc}	0.1744^{d}	0.3629^{de}
T_5 : $AgNO_3$ @ 1.0%	0.1924^{abc}	0.1792^{d}	0.3716^{cde}
T_6 : $AgNO_3$ @ 2.0%	0.1932^{abc}	0.1816^{cd}	0.3748^{cde}
T_7 : GA_3 @ 100 ppm	0.2073^{ab}	0.2016^{ab}	0.4089^{ab}
T_8 : H_2O	0.1890^{bc}	0.1755^{d}	0.3645^{de}
T_9 : Control	0.1808^{c}	0.1675^{d}	0.3483^{e}
S. Em. (±)	0.007	0.006	0.009
C.D. @ 5%	0.02	0.02	0.03
C.V. (%)	6.17	5.57	4.07

Treatment means with the common letters(s) are non-significant by Duncan's New Multiple Range Test at 5% level of significance.

Ascorbic acid content

The data pertaining to ascorbic acid of unripe and ripe fruits of GCh-1 as influenced by different seed priming treatments are presented in Table 4. The seeds primed under AgNPs @ 50 ppm had a greater ascorbic acid content (1.0127 mg/g), which was shown to be statistically comparable to GA_3 @ 100 ppm and AgNPs @ 100 ppm with (1.0052 mg/g and 0.9746 mg/g), respectively. On the other hand, $AgNO_3$ @ 0.5% had the lowest ascorbic acid concentration (0.7891 mg/g) as compared to the control (0.8257 mg/g). In comparison to the control, AgNPs, GA_3, H_2O and $AgNO_3$ @ 1.0% caused a modest rise in ascorbic acid content. Additionally, ascorbic acid level in unripe chili fruit was

decreased by seed priming with a greater concentration of $AgNO_3$.

The significantly highest ascorbic acid content (2.2024 mg/g) was noted under seeds primed with AgNPs @ 100 ppm followed by GA_3 @ 100 ppm, $AgNO_3$ @ 0.5% and AgNPs @ 50 ppm with (2.0037 mg/g, 1.9734 mg/g and 1.9701 mg/g), respectively. Whereas, lowest ascorbic acid content (1.7037 mg/g) was found in seed primed with hydropriming as compared to control (1.7269 mg/g). Hydropriming treatment induced lower ascorbic acid content compared to other seed priming treatments in ripe fruit.

In present study, seed primed with AgNPs @ 50 ppm and 100 ppm showed the maximum ascorbic acid content in both unripe and ripe fruits of GCh-1 and Bhut Jolokia cultivar, respectively. Same trend was observed by Acharya *et al*. (2020) [1] that silver Nanoparticle-mediated seed priming improves ascorbic acid content in watermelons at multi locations in Texas.

Table 4: Effect of priming treatments on ascorbic acid content

Treatments	Ascorbic acid (mg/g)	
	Unripe	Ripe
T_1 : AgNPs @ 25 ppm	0.8632^{bc}	1.8327^{c}
T_2 : AgNPs @ 50 ppm	1.0127^{a}	1.9701^{b}
T_3 : AgNPs @ 100 ppm	0.9746^{a}	2.2024^{a}
T_4 : $AgNO_3$ @ 0.5%	0.7891^{c}	1.9734^{b}
T_5 : $AgNO_3$ @ 1.0%	0.8973^{b}	1.8129^{c}
T_6 : $AgNO_3$ @ 2.0%	0.8001^{c}	1.7669^{c}
T_7 : GA_3 @ 100 ppm	1.0052^{a}	2.0037^{b}
T_8 : H_2O	0.8685^{bc}	1.7037^{c}
T_9 : Control	0.8257^{bc}	1.7269^{c}
S. Em. (±)	0.024	0.040
C.D. @ 5%	0.07	0.12
C.V. (%)	4.66	3.67

Treatment means with the common letters(s) are non-significant by Duncan's New Multiple Range Test at 5% level of significance.

Total carbohydrates

The maximum total carbohydrates (7.05%) in unripe fruit of GCh-1 were recorded in seeds primed with GA_3 @ 100 ppm which was statistically at par with AgNPs @ 100 ppm with (6.79%). Whereas, lowest total carbohydrates content (5.87%) was found in control and it was statistically at par with seed priming treated through AgNPs @ 25 ppm, AgNPs @ 50 ppm, $AgNO_3$ @ 0.5%, $AgNO_3$ @ 1.0%, $AgNO_3$ @ 2.0% and H_2O (Table 5).

However, the seeds primed with AgNPs @ 100 ppm showed the highest percentage of total carbs (11.87%) of all the treatments. AgNPs at 25 ppm (11.21%), GA_3 @ 100 ppm (11.28%) and AgNPs at 50 ppm (11.06%) were shown to be statistically comparable. In contrast, in the case of ripe fruit, the smallest total carbohydrate amount (9.31%) was noted in the control treatment.

Present study, revealed that maximum increasing total carbohydrates content through seed priming with GA_3 in ripe and ripe fruits of both cultivars. The same result was found by Varier *et al.* (2010) [11].

Table 5: Effect of priming treatments on total carbohydrates content

Treatments	Total carbohydrates (%)	
	Unripe	Ripe
T_1 : AgNPs @ 25 ppm	5.91^{b}	11.21^{abc}
T_2 : AgNPs @ 50 ppm	5.97^{b}	11.06^{abc}
T_3 : AgNPs @ 100 ppm	6.69^{a}	11.87^{a}
T_4 : $AgNO_3$ @ 0.5%	6.02^{b}	10.68^{bc}
T_5 : $AgNO_3$ @ 1.0%	6.00^{b}	10.94^{bc}
T_6 : $AgNO_3$ @ 2.0%	5.92^{b}	10.36^{c}
T_7 : GA_3 @ 100 ppm	7.05^{a}	11.28^{ab}
T_8 : H_2O	5.89^{b}	9.38^{d}
T_9 : Control	5.87^{b}	9.31^{d}
S. Em. (±)	0.190	0.270
C.D. @ 5%	0.56	0.80
C.V. (%)	5.35	4.38

Treatment means with the common letters(s) are non-significant by Duncan's New Multiple Range Test at 5% level of significance.

Total phenol content

The data obtained regarding total phenol in unripe (65 days after transplanting) and ripe (85 days after transplanting) fruit were significantly influenced by different seed treatments with varying levels of concentration in both cultivars are presented in Table 6.

All the priming treatments were significantly increased total phenol content in unripe fruit as compared to control. The maximum (20.38 mg/g of GAE) total phenol was recorded in seeds primed with AgNPs @ 100 ppm and it was found statistically at par with GA_3 @ 100 ppm with (19.49 mg/g of GAE). Whereas, lowest total phenol content (10.23 mg/g of GAE) was noted in control as compared to other treatments.

Whereas, in ripe fruit, the data obtained regarding total phenol was significantly influenced by different seed treatments with varying levels of concentration. Out of all the treatments, AgNPs @ 50 ppm recoded highest (13.47 mg/g of GAE) total phenol content followed by AgNPs @

100ppm, GA_3 @ 100 ppm, $AgNO_3$ @ 0.5%, AgNPs @ 25 ppm, $AgNO_3$ @ 1.0% and $AgNO_3$ @ 2.0% with 11.98, 11.55, 10.97, 10.85 and 10.80 mg/g of GAE total phenol content, respectively. Whereas, lowest total phenol content (7.28 mg/g of GAE) was recorded in control as compared to other treatments.

Overall the seed primed with AgNPs @ 100 ppm observed maximum increasing the total phenol content in unripe as well as ripe fruit of both cultivars. Same research done by Ahmed and Dutta, (2019) [13] showed that AgNPs at 100 ppm can induce the plants in increasing total protein and biochemical parameters like phenol content of treated plants without any harmful effect on the tea plant.

Table 6: Effect of priming treatments on total phenol content

Treatments	Total phenol (mg/g of GAE)	
	Unripe	Ripe
T_1 : AgNPs @ 25 ppm	15.48^{c}	10.97^{b}
T_2 : AgNPs @ 50 ppm	17.09^{b}	13.47^{a}
T_3 : AgNPs @ 100 ppm	20.38^{a}	12.11^{b}
T_4 : $AgNO_3$ @ 0.5%	12.12^{de}	11.55^{b}
T_5 : $AgNO_3$ @ 1.0%	11.95^{de}	10.85^{b}
T_6 : $AgNO_3$ @ 2.0%	11.05^{ef}	10.80^{b}
T_7 : GA_3 @ 100 ppm	19.49^{a}	11.98^{b}
T_8 : H_2O	12.56^{d}	8.86^{c}
T_9 : Control	10.23^{f}	7.28^{d}
S. Em. (±)	0.440	0.410
C.D. @ 5%	1.31	1.22
C.V. (%)	5.26	6.53

Treatment means with the common letters(s) are non-significant by Duncan's New Multiple Range Test at 5% level of significance.

Conclusion

Nanotechnology is an emerging discipline with novel applications in agriculture. The most commonly used and widely applied types of nano particles are Silver nano particle. It has been concluded from the research work that seed priming treatments resulted in increased biochemical parameters of chilli cultivar GCh-1 than un-primed seed. In case of α-amylase enzyme maximum increasing was recorded in seed primed with GA_3 @ 100 ppm. While other biochemical parameters were maximum increase was observed with AgNPs @ 100 ppm, AgNPs @ 50 ppm and AgNPs @ 25 ppm than control treatment.

References

1. Acharya P, Jayaprakasha GK, Crosby KM, Jifon JL, Patil BS. Nanoparticle-mediated seed priming improves germination, growth, yield and quality of watermelons (*Citrullus lanatus*) at multi-locations in Texas. Scientific reports. 2020;10(1):1-16.
2. Arnon D. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *beta vulgaris*. Plant Physiology. 1949;24:1-15.
3. Ghalavand F, Jalal AL, Mashhadi AA, Siadat SA, Kochekzadeh A. The effect of different priming methods on seeds and seedlings of Lallemantia under salt stress. Journal of Plant Process and Function. 2022;10:46.
4. Iqbal M, Raja NI, Mashwani ZUR, Hussain M, Saira H. Assessment of AgNPs exposure on physiological and biochemical changes and antioxidative defense system in wheat (*Triticum aestivum* L.) under heat stress. IET nanobiotechnology. 2019;13(2):230-236.
5. Mahakham W, Sarmah AK, Maensiri S, Theerakulpisut P. Nanopriming technology for enhancing germination and starch metabolism of aged rice seeds using phytosynthesized silver nanoparticles. Scientific reports. 2017;7(1):1-21.
6. Parashar UK, Saxena PS, Srivastava A. Bio-inspired synthesis of silver nanoparticles. Digest Journal of Nanomaterials and Biostructures. 2009;4(1):212-225.
7. Roginsky V, Lissi EA. Review of methods to determine chain-breaking antioxidant activity in food. Food chemistry. 2005;92(2):235-254.
8. Sadasivam L, Manickam A. Biochemical methods for agricultural sciences. Wiley Eastern Ltd., Madras; c1992. p. 246.
9. Sadasivam S, Manickam A. Biochemical Methods. 3rd Edn., New Age International Publishers, New Delhi, India; c2008. p. 245-49.
10. Subramanian A, Umarani R. Storability of primed seeds of tomato (*Lycopersicon esculentum*), eggplant (*Solanum melongena*) and chili (*Capsicum annum*), Madras Agriculture Journal. 2010;97(4-6):117-124.
11. Varier A, Vari AK, Dadlani M. The sub-cellular basis of seed priming. Current Science. 2010;99:450-456.
12. Yoshiba Y, Kiyosue T, Nakashima K, Yamaguchi SK, Shinozaki K. Regulation of levels of proline as an osmolyte in plants under water stress. Plant Cell Physiology. 1997;38:1095-1102.
13. Dutta M, Ahmed MA. Calibration of VISSIM models at three-legged unsignalized intersections under mixed traffic conditions. Advances in transportation studies. 2019 Jul 1;48.