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IMPACT OF NANO-PRIMED GREEN-SYNTHESIZED POTASSIUM NANOPARTICLES (GSKNPs) ON BIOCHEMICAL PARAMETERS UNDER DIFFERENT TEMPERATURE REGIMES IN CHICKPEA

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ABSTRACT

High temperatures due to climate change constrain chickpea (*Cicer arietinum* L.) productivity worldwide. The present study investigated the effect of nano seed priming of green-synthesized potassium nanoparticles (GS-KNPs), synthesized from onion dry scale extract using potassium sulphate (1.0 M) as a precursor, in chickpea *cv.* JG-11. Seeds were primed with GS-KNPs using three concentrations (N1: 10 mg/l, N2: 20 mg/l, N3: 30 mg/l) and evaluated at 25°C and 35°C. The total phenol content (mg GAE/g), and ascorbate peroxidase (µmol/min) were estimated and the treatment N2 (20 mg/l KNPs) recorded highest phenol accumulation (1.446 mg GAE/g), while the treatment N3 (30 mg/l KNPs) exhibited increased ascorbate peroxidase activity (0.814 µmol/min/g) confirming robust activation of antioxidant defense mechanisms at 35°C. These findings demonstrate that GS-KNP nano seed priming with 20/30 mg/l effectively enhances high temperature stress tolerance in chickpea by improving biochemical parameters by activating secondary metabolite-based antioxidant defence mechanisms, offering a sustainable agronomic strategy for heat-prone environments.

***Keywords* :** Green synthesis; Nano seed priming; Potassium nanoparticles, antioxidant enzymes, ascorbate peroxidase activity, phenol and alkaloid content.

Introduction

Chickpea (*Cicer arietinum* L.), the world's third most important pulse crop (Jukanti *et al*., 2012), is highly vulnerable to heat stress during germination and reproductive stages (Devasirvatham *et al*., 2012). Temperatures above 35°C impair germination, seedling establishment, flowering and seed setting which in turn cause huge yield loss (Kumar *et al*., 2013). Climate change-induced temperature fluctuations pose a major concern for chickpea yield reduction, wherein emerging nanotechnology-based approaches offer promising strategies to overcome these adverse effects on crop productivity.

Nanotechnology has become one of the most transformative disciplines exhibiting unique physiochemical properties distinct from their bulk counterparts (Ahire *et al*., 2022). In agriculture, nanotechnology offers a precision tool for enhancing stress resilience (Azameti and Imoro, 2023). Seed technology interventions, particularly nano-based seed priming utilizing engineered nanoparticles, enhance germination efficiency, seedling vigor, stress tolerance and nutrient uptake, representing a transformative strategy for sustainable agriculture. Nanoparticle-mediated seed priming offers a targeted, low-dose alternative to conventional techniques (Acharya *et al*., 2020).

Environmental friendly approaches in nanotechnology using plant extracts, microorganisms and agricultural by-products as reducing and capping agents (Alabdallah and Hasan, 2021) is gaining importance nowadays which generally termed as green synthesis of nanoparticles. Green-synthesized nanoparticles (GSNPs) will show low toxicity and strong biocompatibility, making them well suited to agricultural applications. Abiotic stresses such as heat, drought and salinity account for over 50% of global crop yield losses (Elsakhawy *et al*., 2018). Among these, heat stress is the major one that cause damage to crops by disrupting cellular homeostasis through protein denaturation and excess reactive oxygen species (Wahid *et al*., 2007). GSNPs alleviate stress by enhancing antioxidant enzyme activity, upregulating heat shock protein expression and improving osmotic adjustment (Alabdallah and Hasan, 2021; Nair *et al*., 2022).

Potassium (K) plays an important role in plant nutrition, governing enzyme activation, osmotic regulation and stomatal functioning (Sardans and Penuelas, 2015). Green-synthesized potassium nanoparticles (GS-KNPs) helps in slow-release of bioavailable potassium with nanoscale delivery. Nano seed priming is a technique that hydrates seeds with nanoparticle solutions to trigger metabolic activation without radicle protrusion (Rahman *et al*., 2022; Sharma *et al*., 2021). Potassium GSNPs activates H^+-ATPase pumps and regulates the turgor pressure critical for seedling establishment. (Shabala and Cuin, 2008).GS-KNP priming is hypothesized to enhance antioxidant defences and osmoprotection (Nair *et al*., 2022; Guleria *et al*., 2023). In this context, an attempt has been made to evaluate GSKNPs on defence mechanisms under different temperature regimes in chickpea.

Material and Methods

Methodology

The onion dry scale was used as green extract to synthesize potassium nanoparticles (GS KNPs) with 1.0 M potassium sulphate as its precursor. The synthesized nanoparticles were used for nano priming of chickpea seeds (*var* JG-11) with various concentrations of 10 (N1), 20 (N2), 30 (N3) mg/l GSKNPs and the control seeds (N4) were hydroprimed. The primed seeds were subjected to different temperature regimes *viz*., for 35°C/25°C (Day/Night) by keeping them in plant growth chamber and for 25°C/18°C (Day/Night) in walk-in seed germinator for 30 days and the biochemical observations were recorded by taking the seedlings.

Total Phenol content (mg GAE/g)

The total phenol content was measured as per the procedure given by Bray and Thrope (1954). 0.5g of seedling was grinded using pestle and mortar by adding 10 ml of 80% ethanol, and the homogenate was centrifuged at 10,000 rpm for 20 min. Supernatant was discarded and the pellet was dissolved in 5 ml of distilled water. 2 ml of aliquots were pipetted out into test tubes and volume was make up to 3ml with distilled water. Later, 0.5ml of Folin-Ciocalteu reagent was added to the above test tubes and kept for 3 mins. 2ml of 20% Na_2CO_3 solution was added to each tube and mixed thoroughly. The tubes were placed in boiling water for 1 min, then cooled and the absorbance was recorded at 650nm against a blank reagent. Different concentrations of catechol were used to prepare a standard curve.

Alkaloid content

The alkaloid content was determined using the procedure given by Ushie and Adamu (2013). 0.5g of seedling was grinded with 10 ml of 98% ethanol and centrifuged at 10,000 rpm for 10 min at 4°C. 2 ml of plant extract was taken and 2 drops of Wagner's reagent (1.27g of iodine and 2g of Potassium iodide (KI) and make up 100ml with distilled water) was added resulting in the formation of brown/reddish precipitate which indicates the presence of alkaloids.

Ascorbate peroxidase (μmol/min.)

Ascorbate peroxidase enzyme activity was assayed as per the protocol described by Nakano and Asada *et al*. (1981). Extract was prepared by grinding 1g of seedling using extraction buffer (0.1 M phosphate buffer (pH 7.5), containing 0.5mM ethylene diamine tetra acetic acid and 1mM ascorbic acid) and centrifuged for 20 min at 15000rpm and supernatant was taken as enzyme extract. In a test tube, 3 ml reaction mixture (50mM potassium phosphate buffer, 0.5Mm ascorbic acid, 0.1 mM ethylene diamine tetra acetic acid (EDTA), 0.2 ml enzyme extract and 0.5ml distilled water were added and the reaction was initiated by adding 0.1 ml of 0.1 M H_2O_2. For every 30 seconds up to 2 minutes, absorbance was measured at 290 nm using spectrophotometer.

Statistical analysis

The experiment was laid out in Factorial Completely Randomized Design (FCRD). Data was statistically analysed (ANOVA) as per the procedure given by Panse and Sukhatame (1985).

Results and Discussion

Analysis of variance

The chickpea (JG-11) seed was primed with the green-synthesized potassium nanoparticles (GSKNPS) and were subjected to different temperatures *viz.*, 35°C and 25°C for 30 days and the seedlings were used for biochemical tests. The results pertaining to analyses of variance (ANOVA) were presented in Table 1. The treatment means sum of squares for nano priming treatments (N), Temperature conditions (C) and their interaction (N x C) revealed significant variation.

Total phenol content (mg GAE/g)

Nano-primed GSKNPS significantly influenced the total phenol content (mg GAE/g) of chickpea seedlings kept at 35°C and 25°C (Table 2 and Figure 1). At 35°C, total phenol content was increased across all treatments compared to chickpea seedlings kept at 25°C, highlighting the role of phenols as stress-responsive secondary metabolites. At 25°C, N2 (20 mg/l KNPs) recorded the highest phenol content (0.456 mg GAE/g) and the same treatment kept at 35°C recorded phenol content of 1.446 mg GAE/g, revealing higher phenol accumulation at 35°C in GSKNP-primed seeds and this may be due to enhanced activation of phenylpropanoid biosynthesis pathways, potentially triggered by potassium-mediated signalling. High per cent change over control was recorded in N2 (20mg/l KNPs) (+741.59%), followed by N1 (10mg/l KNPs) (+451.33%) and N3 (30mg/l KNPs) (+179.65%). Similar observations were reported by Etesami *et al*. (2021) who stated that nanoparticle-plant interactions under abiotic stress activate both enzymatic and non-enzymatic antioxidant pathways, including phenolic compound synthesis, as a coordinated defence response. Guleria *et al*. (2023) also affirmed that appropriate nanomaterial concentrations alter stress response gene expression and microRNA activity, leading to enhanced secondary metabolite production, including phenolics.

Alkaloid content

Nano-primed GSKNPS significantly influenced the Alkaloid content in chickpea seedlings kept at 35°C and 25°C (Table 3). At 25°C, N1 (10 mg/l K NPs) exhibited light red color, while N2 (20mg/l KNPs), N3 (30mg/l KNPs) and N4 (control) displayed dark red color, indicating that alkaloid levels were broadly elevated by moderate-to-high GSKNP concentrations. At 35°C, all treatments exhibited dark red color, suggesting upregulation of alkaloid biosynthesis. The shift from light red to dark red in N1 (10mg/l KNPs) at 35°C is particularly notable, as it implies that even the lowest GSKNP concentration (10 mg/l KNPs) stimulates alkaloid accumulation at high temperatures. Alkaloids are nitrogen-containing secondary metabolites with established roles in plant defence against biotic and abiotic stresses through their involvement in osmotic adjustment and ROS scavenging. Adhikary *et al*. (2022) nano-primed rice seed with ZnO-NP and selenium nanoparticles (SeNPs) and observed significant increase in soluble proteins, alkaloid content in primed seedlings. Khan *et al*. (2023) similarly demonstrated that silver nanoparticle (AgNPs) priming elicited secondary metabolite biosynthesis including alkaloids in wheat seeds, a phenomenon attributed to nanoparticle-mediated modulation of metabolic enzyme activity and gene regulatory networks. In the present study, consistent dark red intensity across GSKNP treatments at 35°C supports that GSKNP priming broadly sustains and enhances alkaloid biosynthesis as part of a stress defence response in chickpea.

Ascorbate peroxidase (APX) (µmol/min/g)

Nano-primed GSKNPs significantly influenced the Ascorbate peroxidase content in chickpea seedlings kept at 35°C and 25°C (Table 4 and figure 2). At 35°C, substantially elevated APX activity was observed across all treatments, with the overall mean of 0.678 µmol/min/g, reflecting a marked upregulation of the ascorbate-glutathione pathway in response to stress-induced ROS accumulation. Among treatments at 35°C, N3 (30 mg/l KNPs) recorded the highest APX activity (0.814 µmol/min/g) with the highest per cent change over control (+59.70%), followed by N1 (10 mg/l KNPs) (0.74 µmol/min/g; +44.17%) and N2 (20 mg/l KNPs) (0.658 µmol/min/g; +37.61%), while the N4 (control) recorded the lowest (0.501 µmol/min/g). The per cent change of 35°C over 25°C was most pronounced in N1 (+228.88%), followed by N3 (+219.21%) and N2 (+145.31%), confirming that nano-priming with GS-KNPs, particularly at 30 mg/l, most effectively enhanced APX-mediated antioxidant defense under heat stress conditions in chickpea seedlings

The stimulation of APX activity by GS KNPs in the present study aligns with the view that nanoparticles will provide essential micronutrients and trigger stress-memory responses, prepare the antioxidant system ahead of stress exposure (Ahmad *et al*., 2020). Proline and soluble protein accumulation, which is also enhanced by nano-priming (El-Badri *et al*., 2021), may further complement APX-mediated H_2O_2 detoxification by stabilising enzyme conformation at 35°C. Rizwan *et al*. (2019) showed that zinc and iron oxide nanoparticles improved APX and CAT activities in wheat under cadmium stress conditions, reducing oxidative damage. Haripriya *et al*. (2018) similarly reported elevated APX activity in finger millet treated with foliar ZnO nanoparticles under salt stress conditions.

Table 1: Analysis of variance of biochemical parameters at different temperature regimes under *in vitro* conditions

	Phenol content	Ascorbate peroxidase
Nano primed Treatments (N)	1.073***	0.057***
Temperature conditions	1.884***	1.608***
Nano primed treatments (N) x Temperature conditions (C)	0.346***	0.023**
Error	0.003	0.005

NOTE: *, **, *** denote significance at the 5 %, 1%, and 0.1% probability levels, respectively.

Table 2: Effect of nano seed priming with green synthesized Potassium nanoparticles (GS KNPs) on total phenol content (mg GAE/g) in chickpea at 25° C and 35° C

Nano primed Treatments (N)	Total phenol content (mg GAE/g)		Mean	35° C (C2) Percent change over 25°C (C1)	Mean percent Change over Control
	25° C (C1)	35° C (C2)			
N1 (10mg/l K NPs)	0.309 ± 0.041	0.936 ± 0.025	0.623[b]	202.912	451.327
N2 (20mg/l K NPs)	0.456 ± 0.08	1.446 ± 0.091	0.951[a]	217.105	741.59
N3 (30mg/l K NPs)	0.167 ± 0.012	0.465 ± 0.05	0.316[c]	178.443	179.646
N4 (Control)	0.099 ± 0.008	0.126 ± 0.007	0.113[d]	27.272	0
Mean	0.258	0.743			
CD (α≤0.05)	**N=0.051, C= 0.036, NxC= 0.073**				
SE(m)	0.025				
CV	9.935				

Table 3: Effect of nano seed priming with green synthesized Potassium nanoparticles (GS KNPs) on total alkaloid content in chickpea at 25° C and 35° C

Nano primed Treatments (N)	Alkaloid content	
	25° C (C1)	35° C (C2)
N1 (10mg/l K NPs)	Light red	Dark red
N2 (20mg/l K NPs)	Dark red	Dark red
N3 (30mg/l K NPs)	Dark red	Dark red
N4 (Control)	Dark red	Dark red

Table 4: Effect of nano seed priming with green synthesized Potassium nanoparticles (GNPs) on Ascorbate peroxidase (μmol/min/g) in chickpea at 25° C and 35° C

Nano primed Treatments (N)	Ascorbate peroxidase (n moles/ min)		Mean	Percent change over C1	Percent Change over Control
	25° C (C1)	35° C (C2)			
N1 (10mg/l K NPs)	0.225 ± 0.07	0.740 ± 0.07	0.483	228.88	44.17
N2 (20mg/l K NPs)	0.267 ± 0.04	0.655 ± 0.154	0.461	145.31	37.61
N3 (30mg/l K NPs)	0.255 ± 0.01	0.814 ± 0.06	0.535	219.21	59.70
N4 (Control)	0.170 ± 0.013	0.501 ± 0.05	0.335	194.70	-
Mean	0.229	0.678			
CD (α≤0.05)	**N= 0.076, C= 0.053, NxC= 0.107**				
SE(m)	0.037				
CV	16.158				

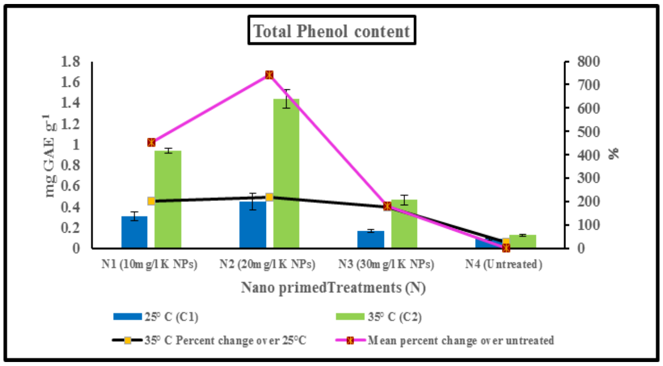

Fig. 1: Effect of nano seed priming with green synthesized Potassium nanoparticles (GNPs) on total phenol content (mg GAE/g) in chickpea at 25° C and 35° C

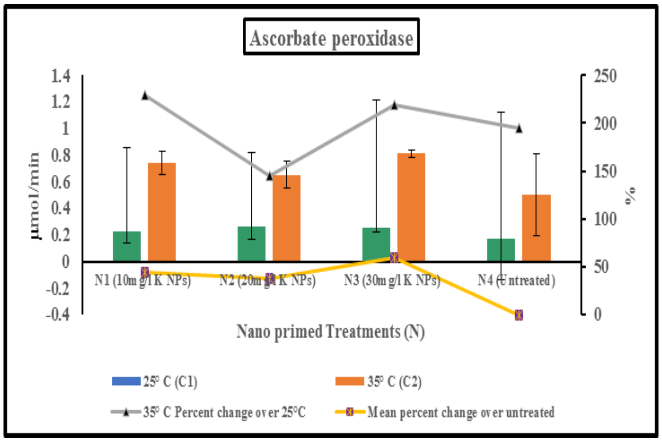

Fig. 2: Effect of nano seed priming with green synthesized Potassium nanoparticles (GNPs) on Ascorbate peroxidase content (µmol/min) in chickpea at 25° C and 35° C

Conclusion

Nano seed priming with green-synthesized potassium nanoparticles (GS-KNPs) derived from onion dry scale extract significantly enhanced heat stress tolerance in chickpea *cv.* JG-11. N2 (20 mg/L) optimally improved phenolic accumulation, while N3 (30 mg/L) recorded elevated ascorbate peroxidase activity at 35°C. High temperatures upregulated total phenolics, ascorbate peroxidase and alkaloid biosynthesis across all GS-KNP treatments, reflecting activated antioxidant defense mechanisms of green synthesized potassium nanoparticles. The optimal concentration range of 20-30 mg/L GS-KNPs is recommended as a sustainable, eco-friendly nano-agronomic strategy to improve chickpea establishment and productivity in high temperature conditions.

Author Contribution

Conceptualization, R.K., B.Y., and S.C. Ch, J.M.R.P.; methodology R.K., B.Y., and S.C. Ch.; software R.K.; validation, B.Y., J.M.R. P.; formal analysis R.K., data curation R.K.; writing original draft preparation, R.K., Y. B., writing-review and editing, R.K., Y.B. S.P., P. SNCVL.; visualization & supervision B.Y., J.M.R.P., S. C. Ch.

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Conflict of Interest

There was no conflict of interest among the authors.

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