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# Potential effect of phyto-synthesized silver nanoparticles using *Cotula cinerea* Del Raw extract on salt tolerance of wheat seeds (*Triticum durum* desf, Boussellam variety) germination

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This study uncovers a novel and environmentally sustainable method for synthesizing silver nanoparticles (AgNPs) utilizing *Cotula cinerea* extract, a plant that has not been previously explored for nanoparticle (NP) fabrication. The study is to evaluate the efficacy of a nano primer in mitigating salt stress in durum wheat (*Triticum durum* Desf Boussaleme variety). AgNPs extract spectra showed a sharp peak at 445.91 nm in UV-vis while X-ray diffraction (XRD) reflected the patterns of the face-centred cubic (fcc) and crystalline structure and the scanning electron microscopy (SEM) study revealed that the NPs have an almost spherical and somewhat cuboidal morphology, with dimensions under 20 nm. The transmission electron microscopy (TEM) study revealed an average particle size distribution of 15.128 nm, largely exhibiting a spherical morphology. The hydrodynamic diameter measured (DLS) indicated a particle size of 351.6 nm, which is much larger. Seeds were treated with 0, 20, 40, and 80 mg L<sup>-1</sup> of AgNPs and exposed to 0 and 150 mM NaCl. At a concentration of 40 mg/L of AgNPs, germinability attained 90% under saline conditions, in contrast to 70% for the control group, while root length (RL) exhibited an 86% increase, measuring 7.28 cm compared to 3.9 cm in the control group. At a concentration of 20 mg/L of AgNPs, root fresh weight (RFW) increases from 0.04 to 0.06 g in saline conditions and from 0.06 to 0.09 g in the absence of salt. The control group exhibited a root number (RN) of 3.67, while plants treated with 20 mg/L demonstrated a significant increase to 5.54. Additionally, shoot length (SL) under saline stress reached 11.12 cm, compared to 8.26 cm in saline conditions. These findings underscore the potential of green synthesised AgNPs as an effective approach to enhance the salt tolerance of durum wheat and promote sustainable agriculture in saline conditions.

**Keywords** Wheat, Silver nanoparticle, Green synthesis, Salt stress, Germplasm, Food security

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The global world today faces two major challenges: the rising need for a sufficient and secure food supply, and the persistent environmental deterioration resulting from traditional farming methods<sup>1</sup>. Wheat, a globally ubiquitous crop, currently supports a substantial segment of the world's population. It is the third most produced cereal crop worldwide. It is responsible for generating 35% of the worldwide production of food grains<sup>2</sup>. Wheat crops, which sustain a significant segment of the global population, are susceptible to market volatility, geopolitical conflicts, and severe meteorological occurrences such as temperature variations, altered precipitation patterns, and other natural catastrophes. This dependence undermines the resilience of the food system, rendering it susceptible to shocks such as crop failures, price surges, and supply chain disruptions. The future of wheat production is increasingly uncertain, necessitating innovative strategies to guarantee food security<sup>4</sup>. Abiotic stressors, such as drought and salt, may significantly reduce agricultural output, leading to a potential loss of over 50%<sup>2</sup>.

Salinity in a particular agroclimatic zone may be ascribed to both natural and human-induced variables. Thus, the timing, length of exposure, and severity of salinity may vary, influencing the multi-trait responses of genotypes with diverse genetic origins, which eventually results in changes to the final grain production<sup>3</sup>. Soil salt stress is well recognized to adversely affect microbial biomass and enzyme activity, resulting in significant decreases in nutrient availability<sup>4</sup>.

In regions characterized by arid and semiarid climates, salinity significantly impact on agricultural productivity. This environmental factor inhibits plant growth and threatens the long-term viability of farming practices<sup>5</sup>. One major consequence of salt stress is a reduction in biomass accumulation. As plants experience water scarcity, their growth and biomass production decline, potentially impacting agricultural yields and ecological stability in the long-term<sup>6</sup>.

The influence of salt stress on the yield of salt-sensitive crops is a notable issue among environmental stressors, since it leads to a reduction in seed germination, attributable to the increase in osmotic pressure within the soil solution<sup>7</sup>. Elevated salinity levels in soil can induce a range of morphological changes in plants. A notable consequence is the decrease in plant biomass, which encompasses the total weight and dimensions of the plant<sup>8</sup>. Additionally, plants experiencing salt stress encounter difficulties in ion absorption, leading to an imbalance in the uptake of critical ions such as potassium, calcium, and magnesium. This disruption adversely affects various physiological functions in plants, including nutrient uptake, enzyme activity, and overall growth and development<sup>9</sup>. The impact of salinity stress can be effectively evaluated through the assessment of seedling growth, which provides insights into both the intensity and uniformity of the stress experienced. The speed and consistency of seedling emergence serve as reliable indicators of the degree of salinity stress<sup>10</sup>.

Salt, drought, waterlogging, and hypoxia may influence healthy seedling development<sup>11</sup>. Salinity stress impairs gene expression, thereby influencing plant development via modifying the expression of several genes associated with diverse cellular components and their derivatives<sup>12</sup>. The germination process was slowed down because salt greatly diminished the seeds' absorption and germination capabilities<sup>13</sup>. Wheat durum as *Triticum durum* Des, it's a member of the Poaceae family holds a significant position as a staple food crop in numerous regions across the globe thus holding significant economic importance and by the year 2050, it's imperative to increase wheat production two-fold in order to guarantee food security recognized<sup>10,14,15</sup>.

Many fields of science, engineering, and technology are collaborating to better life<sup>16</sup>. Nanotechnology provides significant benefits in many fields and seems to offer potential solutions<sup>5,17</sup>. Thus, nanotechnology may relieve stress<sup>2</sup> and solve complicated issues including global warming, sustainability, agricultural yields, and food insecurity<sup>5</sup>. The current innovation has the potential to substantially improve the sustainability of agricultural methods, the unique characteristics of nanomaterials have garnered increasing interest from researchers, scholars, and scientists alike<sup>16,18</sup>.

Despite the growing interest in nanotechnology in agriculture, a considerable gap persists in our comprehension of how NPs produced by plants might mitigate abiotic challenges, especially salinity stress. Physical procedures include disadvantages like inefficient energy use, irregular product distribution, and the incorporation of solvents in the final product<sup>19</sup>.

The use of physical and chemical methods for the synthesis of AgNPs is both inefficient and detrimental to the environment. Consequently, it is imperative to establish a system that is both economically viable and environmentally sustainable, one that eschews hazardous chemicals and mitigates the risks associated with chemical and physical production processes. Biological techniques can address these shortcomings<sup>19</sup>.

Plant components, including roots, bark, leaves, and fruits, have been recognized since antiquity as safe nutritional and therapeutic agents due to their great efficacy and little adverse effects<sup>20</sup>.

AgNPs serve as biocompatible precursors that induce particular features essential for the overall growth of plants, recognized for their non-toxicity and chemical stability under ambient circumstances, this makes AgNPs a valuable tool in enhancing plant productivity and ensuring sustainable agricultural practices<sup>21</sup>.

AgNPs function as seed priming agents that influence germination, with their effects largely dependent on the NPs concentration and the duration of the priming process. This duration varies across plant species, emphasizing the need for tailored seed priming protocols<sup>22</sup>. A comprehensive investigation into the biosynthetic pathways and regulatory mechanisms involved in the production of these NPs is warranted.

The utilization of green synthesis techniques for the production of NPs has been found to effectively induce physiological changes in plants, enabling them to combat the adverse effects of salinity stress<sup>23</sup>. The agricultural sector is increasingly adopting the use of AgNPs owing to the many fold benefits they offer in terms of crop improvement<sup>12</sup>. Additionally, when NPs are employed in the agricultural sector, they can have both advantageous and harmful effects on plant, they have the potential to induce phytotoxicity and exert a negative influence on both seeds germination and growth<sup>18</sup>. The appropriate nanomaterial concentration may increase plants' stress tolerance by changing stress response genes and microRNAs, according to Guleria et al.<sup>24</sup>. Nanoprimering represents an economically viable physio-biochemical method for the pre-treatment of seeds, distinguishing itself from traditional drying techniques<sup>18</sup>. This innovative approach has garnered attention for its potential to

enhance seed performance and resilience. *C. cinerea* Del is one of three species classified under the genus *Cotula* within the Asteraceae family that can be found in Algeria, is prevalent in Sahara-Arabic regions, particularly thriving in the arid conditions of the Sahara Desert and sandy terrains<sup>25</sup>. The phytochemical composition of *C. cinerea* encompasses a diverse array of chemicals, including saponins, essential oils, tannins, flavonoids, steroids, and terpenoids<sup>26</sup>. Guaouguaou et al.<sup>27</sup> highlighted the potential application of *C. cinerea* in both clinical and traditional medicine as a treatment for cancer. The biogenic of the production of Ag NPs employs plant extracts derived from various parts of the plant, including roots, leaves, flowers, fruits, seeds, latex, and bark<sup>28,29</sup>.

UV-Vis spectrophotometric analysis is a prevalent method for the primary research of AgNPs synthesis.

An analysis of the phytochemicals in the aqueous extract sourced from the aerial parts of *C. cinerea* demonstrated the existence of polyphenols and flavonoids, which are recognized as some of the most prevalent secondary metabolites in a variety of plants<sup>30</sup>. This study emphasizes the synthesis of AgNPs using extracts from *C. cinerea* and examines their potential to bolster the physiological and biochemical resilience of durum wheat when exposed to salinity levels. We want to improve the plant's resilience to salt stress by using the distinctive characteristics of these NPs. This method aims to enhance agricultural yield while adhering to sustainable agriculture principles that emphasize eco-friendly techniques.

## Materials and methods

Physical synthesis comprising plasma chemistry, vapor deposition, microwave irradiation, pulsed laser, sonochemical reduction, and gamma radiation is often chosen for its purity, controllability, and yield. Another common technique utilizes reducing agents in polyol, microemulsions, thermal decomposition, and electrochemical synthesis<sup>31</sup>. The use of physical, chemical and photochemical procedures methods for the synthesis of AgNPs are together inefficient and detrimental to the environment<sup>19</sup>, however, come with challenges, such as the necessity for high-quality materials, stringent practices, expensive budgets, and the potential for biological toxicity due to hazardous residues. The process of green synthesis, which is biological in nature, is recognized as the most environmentally sustainable, economically feasible, and health-conserving approach<sup>32</sup>. AgNPs have minimal inclination for resistance formation, many modes of action, and broad-spectrum activities<sup>33</sup>. Their efficacy as nano-stimulants for mitigating salt stress remains little researched. The non-toxic, eco-friendly, and easy-to-understand green nanotechnology (which uses microbes, plant/plant extract, and enzymes) is a good alternative to pure nanotechnology<sup>34</sup>. The idea of organic synthesis of metal NPs, which is also known as “green synthesis,” has become popular in both science and business in recent years<sup>35</sup>. This paper presents the use of *C. cinerea*, an indigenous plant from the dry zones of the Algerian Sahara, for the environmentally friendly production of bioactive AgNPs. Unlike conventional approaches, our approach leverages the plant's inherent stress tolerance systems while sustaining salt stress. The phytochemical examination of *C. cinerea* revealed the presence of flavonoids, tannins, alkaloids, saponins, terpenoids, steroids, and cardenolides<sup>30</sup>. These chemicals are acknowledged for their varied biological impacts, including antibacterial and antioxidant characteristics. This study provides novel evidence that biogenic NPs enhance salt tolerance in wheat via the combined manipulation of ionic homeostasis and antioxidant systems, offering a sustainable alternative to traditional stress mitigation methods.

## Product and reagent

The substances methanol, sodium hypochlorite, and silver nitrate (Ag NO<sub>3</sub>, 99.99%) were acquired from Sigma Aldrich, Germany. Double-distilled water was provided by the University of Ghardaïa in Algeria, while Hydroponic Nutrients A & B were obtained from the Green World Store in El-Hamiz, Algeria.

## Plant material and extraction AgNPs biosynthesis

The plant material, obtained from the Guerarra area in the Algerian Sahara. The identification of plant specimens was based on the morphological traits outlined by Quézel and Santa in their comprehensive study of Algerian flora<sup>36</sup>, further validated by Chehma Adelmadjid in his catalogue of spontaneous flora from northern Saharan Algeria<sup>37</sup>. These publications offered crucial diagnostic keys and thorough descriptions essential for precise species identification, as conducted by the Department of Methods Engineering at Ghardaïa University, without the inclusion of deposited voucher specimens, then was meticulously cleaned with tap water and then sliced into little pieces. To make the aqueous extract of *C. cinerea*, 5 g of dried, chopped plant material was combined with 50 ml of distilled water in a glass beaker. The mixture was heated to 60 ± 5 °C for 30 min and then cooled to ambient temperature. The resultant solution was filtered using Whatman no.1 filter paper to eliminate suspended particles and kept in an airtight container at 4 °C for future use as green reducing and capping agents in the production of AgNPs.

## Bio-fabrication of AgNPs

AgNPs were synthesized using a 1 mM L<sup>-1</sup> of silver nitrate solution, 10 ml of the plant extract was added to 90 ml of silver nitrate solution and stirred for 1 h at a temperature of 60 ± 5 °C. The colour changes from yellow to brown indicated the formation of AgNPs. The NPs solution was centrifuged at 4500 rpm for 10 min, and the resulting dark brown precipitate was washed distilled water and methanol, the dried precipitate was stored in an airtight container for further use<sup>38</sup>.

## The physical and optical properties of ag NPs

Two primary methods were used to characterize the synthesized Ag NPs: microscopic and spectroscopic techniques<sup>39</sup>. Keerawelle et al.<sup>40</sup> state that this is necessary to guarantee the production and stability of AgNPs and to provide a first description of the synthesized NPs.

The optical properties of the biosynthesised NPs were analysed using a UV-Visible spectroscopy on a SECAMAM UVILINE 9400 C Spectrophotometer (Loughborough, UK). The morphology and size of the NPs were examined using TEM on a Tecnai G2 at 200 kV with an EDX Bruker (Hillsboro, USA) attachment. And SEM from a Hituchi SU 5000. XRD analysis was performed. Using a Bruker D8 Advance ditratometer to determine the crystalline structure. Finally, DLS using a Dyna Pro Nanostar Whyatt (Santa Barbara, USA) was used to measure the particles size distribution n and the thickness of the capping agent.

Germination and seedling study: wheat seeds (*Triticum durum* desf, Bousselam variety)

Seeds material

All tests were Conducted in mid-2023 at the Faculty of Sciences and Technology, Ghardaia University (Ghardaia, Algeria). After evaluating four distinct varieties (Boutaleb, Bousselam, Megress, and Mohamed Ben Bachir), Bousselam was selected from the *Triticum durum* Desf variety as the most sensitive to salt. All seeds of *Triticum durum* Desf cultivars used in this research were obtained from the Technical Institute of Field Crops in El Khroub Province (Constantine, Algeria). analysis of dry weight revealed that the average weight per 100 seeds for the Bousselam variety was 6.19 g. Prior to the start of the tests, independent assessment indicated that the seeds had a germination rate of 100 ± 4%. Before the germination experiments, the seeds were surface sterilized with 70% ethanol for 5 min and 5% sodium hypochlorite for 10 min. Subsequently, they were rinsed five times with distilled water.

The trials were conducted in a single period to assess multiple germination indices of the seeds, facilitating the acquisition of preliminary data on the plant's response to various concentrations of silver nano-priming, in comparison to an untreated seed control and other AgNPs priming solutions, when subjected to the recommended salt levels. The experiments enabled the discovery and selection of the appropriate concentration. To nanoprime the seeds, they were submerged in solutions with AgNPs concentrations of 0, 20, 40, and 80 mg L<sup>-1</sup> for a length of 8 h at 25 ± 2 °C. Following nanoprimering, the seeds were subjected to an extensive washing procedure using distilled water. In contrast to traditional pre-sowing seed methods, the nanoprimered technology does not require a drying step. This differentiates it from previous seed preparation procedures (Shukla et al., 2019), especially at approximately 25 ± 2 °C, with the application of two distinct salinity levels. 0 mM of distilled water (DW) and 150 mM.

Germination test

Approximately 240 seeds of the Bousselam variety were used in this investigation, with each treatment consisting of three replicates of 10 seeds each. The seeds were placed in covered Petri dishes with a diameter of 9 cm. Each Petri dishes contained two filter sheets (N°1) that were saturated with an appropriate quantity of either distilled water or saline solution, depending on the treatment. The experimental setup was located in a greenhouse and maintained in darkness at a controlled temperature of 25 ± 2 °C. Seed germination was observed every day for seven days, with successful germination defined as the emergence of a 2 mm radicle. Upon conclusion of the experiment, the germination rate was determined:  $(GRP = \frac{\sum_{i=1}^k n_i}{N} 100)^{41}$ , GRP is a commonly used statistical measure, represents the total proportion of seeds that have germinated by the end of the experiment<sup>42</sup>.

Growth seedling response

After a seven-day germination period, five plantlets were transferred to into the hydroponic solution, a disposable plastic cups measuring 10 cm in height and 6 cm in top diameter, each with a volume of 250 mL were offered. To keep the seedlings in place above the liquid medium, a round sponge disc was inserted inside each cup. Because the bottom part of the sponge was in touch with the solution, capillary action enabled the absorption of nutrients. The seedlings were carefully placed within the sponge, making sure that the roots were immersed in the solution and that the shoots were left open to the air and light. Eleven days are needed for the seedling phase to attain three leaves. As a result, the hydroponic solution was replaced every two days.

The solution of Hydro A and B supply nitrate nitrogen (NO<sub>3</sub><sup>-</sup>), potassium oxide (K<sub>2</sub>O), calcium (Ca), magnesium (Mg), and a balanced NPK formulation (2-5-9), along with phosphorus pentoxide (P<sub>2</sub>O<sub>5</sub>), enriched with essential micronutrients such as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), and molybdenum (Mo) in chelated form (DTPA).

Multiple growth metrics were evaluated, including root length, shoot length, root count, leaf count, and the fresh and dry weights of both shoots and roots.

All necessary macro and micronutrients were provided. The methodical application of a nutrient-rich hydroponic solution, coupled with the careful transfer of seedlings to individual containers, created an optimal environment for plant growth. The frequent replenishment of the nutrient solution further facilitated the observed development, highlighting the advantages of hydroponic gardening methods Table 1.

In the context of experimental setups, monitoring TDS are essential for assessing both control and treatment solutions.

Solution	A	B	A + B	DW	AgNPs 20 mg L <sup>-1</sup>	AgNPs 40 mg L <sup>-1</sup>	AgNPs 80 mg L <sup>-1</sup>
TDS (ppm)	333	108	199	15	17	21	27

**Table 1.** Total dissolved solids (TDS) for control and treatments solution. A hydro a, B hydro b, DW distiller water, mg L<sup>-1</sup> milligrams per liter, ppm particle per million, AgNPs silver nanoparticles.

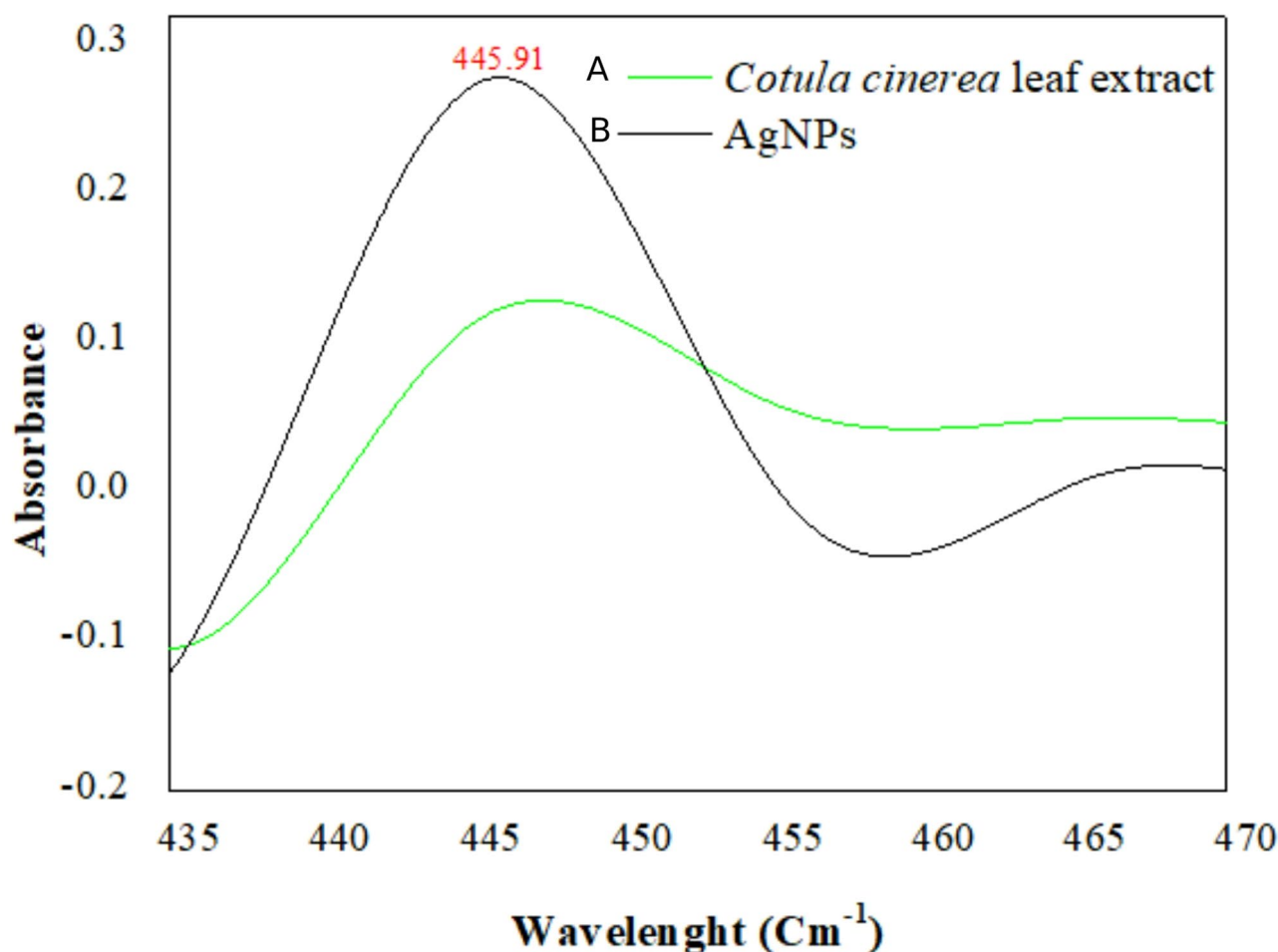
### Statistical analysis

Data pertaining graphs, tables, descriptive statistics, and the calculation of germination indices for the registered seeds and seedlings were processed, the mean values and standard deviation of the traits were computed using the interactive web applications “GerminaQuant for R”<sup>41</sup> and “Yupana”<sup>43</sup>. The Pearson correlation approach was employed to determine the relationships among the different parameters; correlation coefficients were considered statistically significant at the 5% level ( $P < 0.05$ ) using the Tukey test. A multivariate principal component analysis (PCA) graph was generated to assess the eight morphological characteristics. The same interface was used to generate a dendrogram based on Euclidean distances.

## Results and discussion

### Characterization by UV–Vis spectrophotometer

The incorporation of raw extracts into an aqueous silver nitrate solution resulted in a notable alteration of the solution's hue. The colour transitioned from pale yellow to yellowish-brown after 1.5 h following the addition of aqueous leaf extract of *C. cinerea* to silver nitrate solution for the synthesis of AgNPs. The solution first exhibited a subtle solution that gradually transformed into a yellowish-brown. Subsequently, the colour deepened to a reddish-brown, ultimately yielding a colloidal brown solution, indicating the presence of AgNPs<sup>44</sup>. This change resulted from the excitation of free electrons in the metal<sup>45</sup>, indicating that the optical properties of the silver nitrate solution shifted as silver ions reduced to elemental silver and ultimately to AgNPs upon interaction with the bioactive constituents of the plant extract<sup>46</sup>, proving that *C. cinerea*-AgNPs were being synthesised. Upon comparing the absorbance of the AgNPs and the aqueous leaf extract simultaneously, we observed that the UV-vis spectra of the AgNPs exhibit a pronounced peak at 445.91 nm (Fig. 1), which stays consistent over many days, with no rise in absorption seen. This signal unequivocally signifies the creation of AgNPs in the solution, attributable to the surface plasmon resonance (SPR) electrons on the NPs surface<sup>47</sup>. The dimensions and morphology of the metal particles, together with the dielectric characteristics of the synthesis medium and the interactions between NPs, collectively influence the surface plasmon resonance pattern. Throughout the procedure, the SPR band amplified, confirming the synthesis of the AgNPs<sup>48</sup>.



**Fig. 1.** Biosynthesis of AgNPs: colour change of the extract from pale yellow to dark brown after addition of  $\text{AgNO}_3$ ; UV spectra of the *C. cinerea* Del aqueous leaf extract and AgNPs synthesized; Cm = centimetre; AgNPs = silver nanoparticles.



XRD spectroscopy characterization

The size and shape of the metal particles, together with the dielectric properties of the synthesis medium and the interactions among NPs, collectively affect the surface plasmon resonance pattern. During the operation, the SPR band intensified, validating the synthesis of the AgNPs<sup>49</sup>. However, this secondary phase is usually neglected in published reports. Here, the XRD pattern shows that synthesis method and conditions highly influence Ag/AgCl secondary phase ratio.

The size of the NPs was calculated based on the Debye–Scherrer equation: ( $D = k\lambda/\beta\cos\theta$ ). In the above equation: D represents particle diameter size, K: a constant with a value of 0.9,  $\lambda$ : X-ray source wavelength (0.1541 nm),  $\beta$  and  $\theta$  represent the FWHM (full width at half maximum), respectively. The average crystalline size was found to be approximately 15.26 nm. Table 2.

The size of the NPs will significantly influence the XRD peak patterns<sup>50</sup>. The presence of various reducing agents in the extract is responsible for the stabilization of AgNPs and, thus, for providing the crystalline structure of AgNPs, Fig. 2. which was well studied in various biosynthesized NPs<sup>51</sup>.

SEM characterization

SEM analysis was conducted to determine the morphology of AgNPs that were synthesized using the raw extract of *C. cinerea* for the purpose of reduction and capping. The results, as depicted in (Fig. 3.a, b, c and d), clearly indicate that the NPs possess an almost spherical and sort of cuboidal large shape and have a size of less than 20 nm. The dissimilarity in the amount and type of capping agents detected in distinct raw extract may contribute to this phenomenon.

TEM characterization

The examination of the synthesized NPs’ size and morphology is conducted using TEM (Fig. 4.a, b,c and d). The measured average particle size distribution at 15.128 nm was determined by TEM micrographs (Fig. 4.e), falling within the reported size range of 1.42–47.62 nm by TEM analysis. The shape of the AgNPs is predominantly spherical in nature and the polydispersity of NPs and the presence of individual AgNPs are both evident in this TEM image.

The clear visualization of NPs aggregates in the test suspension provided further insight into the larger hydrodynamic diameter determined by DLS. In contrast to the sizes reported by TEM and XRD, the hydrodynamic diameter calculated using DLS is notably larger, measuring 351.6 nm Table 3, and (Fig. 5). The variation observed in the AgNPs can be explained by their polydisperse nature due to the interference of the dispersant into the hydrodynamic diameter, as evidenced by the polydispersity index value of 0.5662<sup>52,53</sup>.

DSL characterization (Fig. 5)

Effect of AgNPs on salt tolerance of wheat (*Triticum durum* Desf) Bousselam

To elucidate the function of AgNPs in plant germination, growth, and salt stress response, durum wheat plants underwent germination and growth studies.

Plants were treated with AgNPs throughout cultivation under both salt and standard environmental conditions. The addition of NaCl resulted a reduction in the average seed germinability during early stages of emergence in wheat plants compared to the control groups. This research aimed to investigate the effects of biosynthesized AgNPs on salt stress concerning seed germination and seedling development of the Bousselam variety of Wheat (*Triticum durum* Desf).

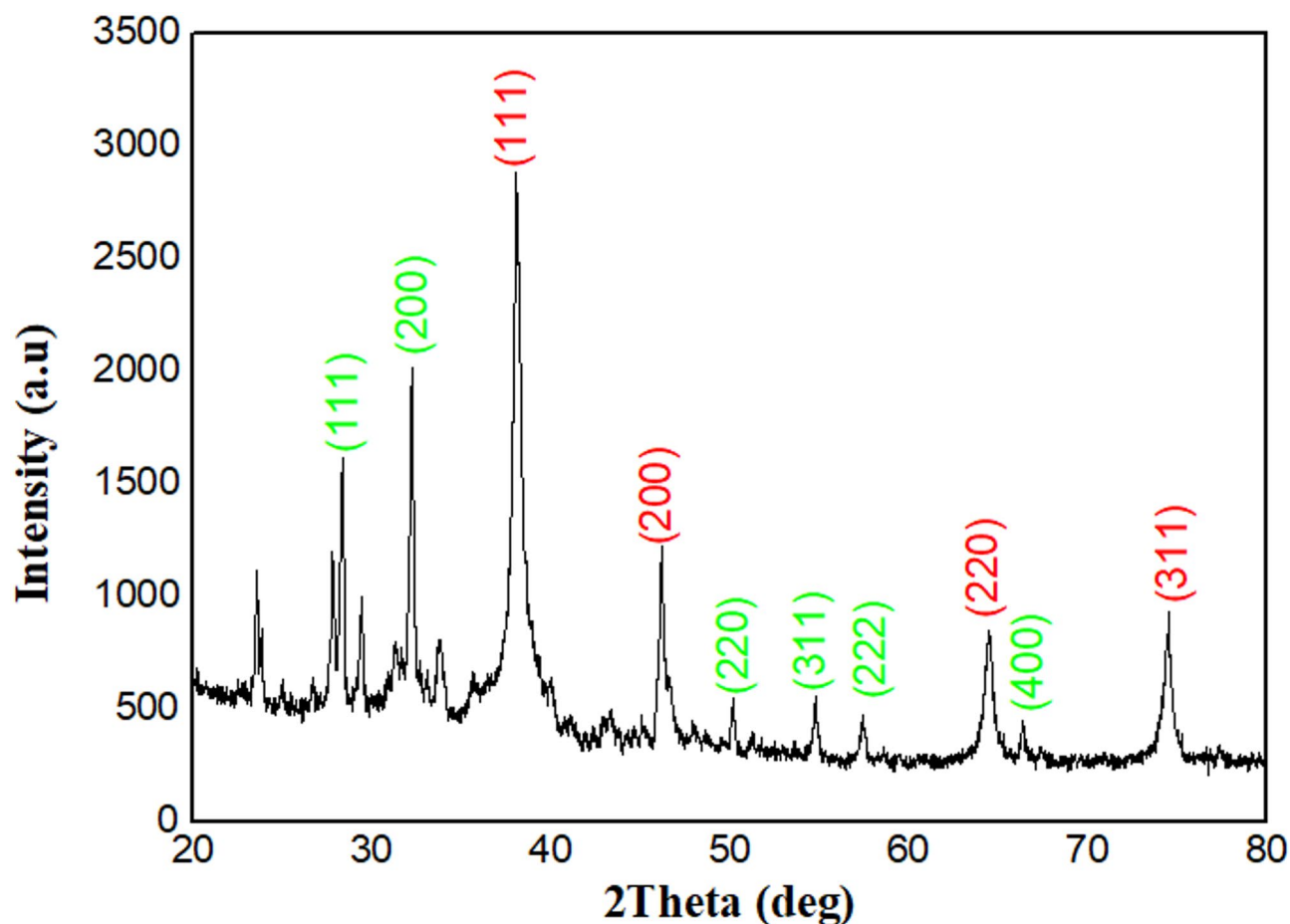
This study clarifies the impact of salt stress on seed germination and seedling development, providing valuable insights into adaptive processes.

Germination test

Physiologically, seed germination is the stage most sensitive to the effects of abiotic stress<sup>54</sup>. The primary germination index considered in this study was based on Bousselam variety of wheat seeds, with germinability serving as the key metric for evaluating seed germination. On the seventh day of the germination study, the control group untreated with salt, exhibited a germinability rate of 90% (Fig. 6). Seeds treated with AgNPs showed germinability rates of 93.33%, 96.66%, and 93.33% at concentrations of 20, 40, and 80 mg L<sup>-1</sup>, respectively. In contrast, under salt stress, the control group demonstrated a germinability of 70%, while seeds treated with AgNPs recorded rates of 66.66%, 90%, and 76.66% at the same concentrations. The efficacy of 40 mg L<sup>-1</sup> AgNPs in mitigating stress highlights its potential as a valuable tool for enhancing crop tolerance and ensuring sustainable agricultural productivity in saline environments<sup>55</sup>. Figure 6 illustrates the comparative

K	$\lambda$ (Å)	Peak position $2\theta$ (°)	FWHM $\beta$ (°)		D (nm)	Average D (nm)
0.94	1.5406	38.121	0.993	2.359	8.83	15.26
		46.231	0.571	1.962	15.78	
		64.467	0.558	1.444	17.57	
		74.468	0.552	1.273	18.87	

**Table 2.** XRD analysis results of the green synthesized AgNPs using *C. cinerea* extract. K Scherrer constant,  $\lambda$  Wavelength of the X-rays, Å angstrom, ° degree, FWHM  $\beta$  Full width at half maximum of the diffraction peak, D Crystallite size, nm nano meter.



**Fig. 2.** XRD pattern of the green synthesized AgNPs using *C. cinerea* extract. The diffraction peaks observed at  $2\theta$  values of approximately  $38.12^\circ$ ,  $46.23^\circ$ ,  $64.47^\circ$ , and  $74.47^\circ$  correspond to the (111), (200), (220), and (311) lattice planes of face-centered cubic (FCC) silver. An average crystallite size of 15.26 nm obtained from the Scherrer equation confirms nanocrystalline development. The strong peaks point to great purity, therefore verifying effective biosynthesis; a.u = arbitrary units; deg = degree.

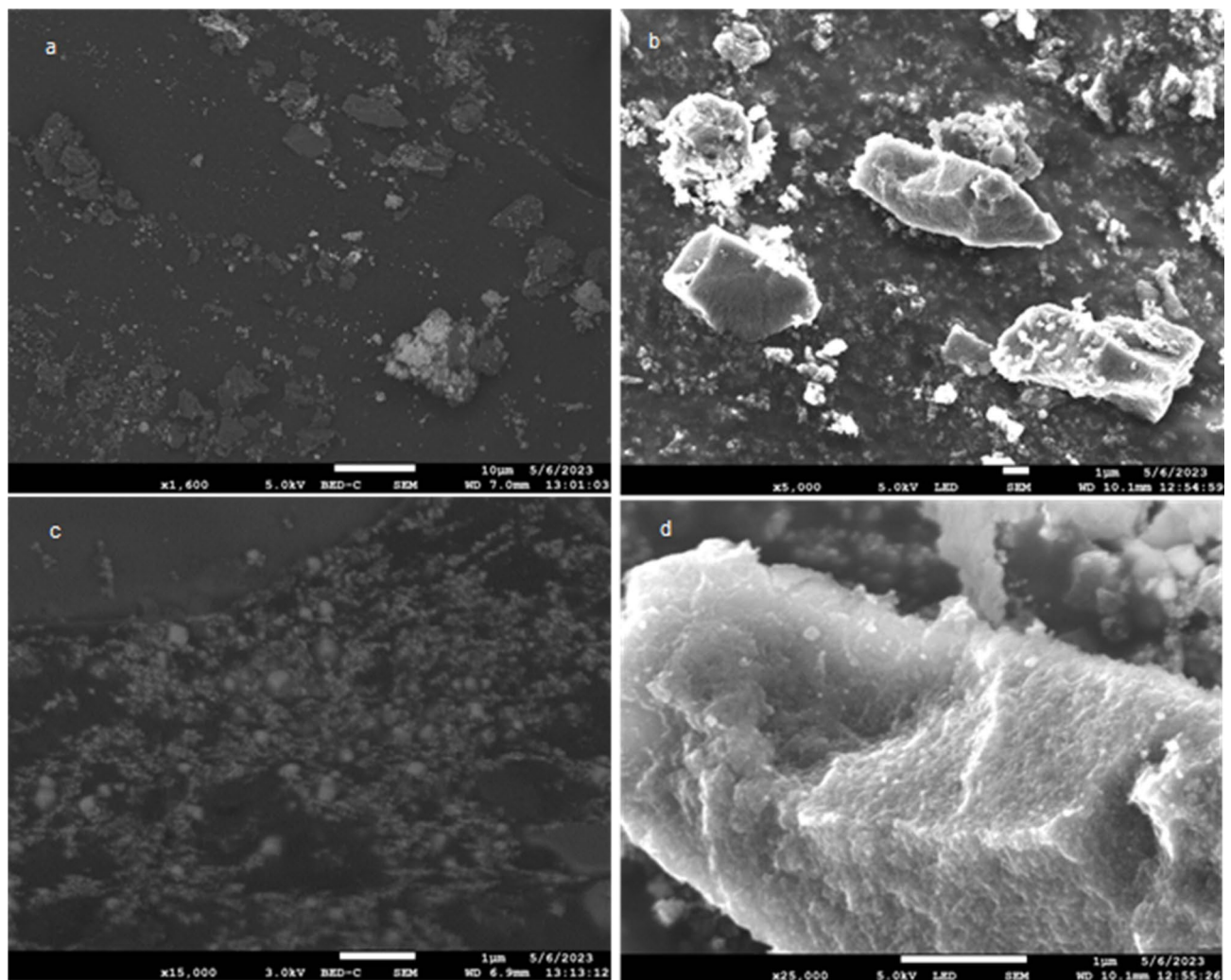
effects of AgNPs, indicating their potential as beneficial agents for improving seed germination. Some research indicated that the impact of nanoparticles (NPs) on plant growth is contingent upon their concentration; elevated concentrations may be detrimental to plant development<sup>1–3</sup>, whereas appropriate quantities can be advantageous<sup>4,5,56–59</sup>.

AgNPs applied showed a concentration-dependent influence on plant responses under salt stress. Among the investigated concentrations,  $40 \text{ mg L}^{-1}$  AgNPs always showed the most notable protective properties. This enhancement can be attributed to increased AgNPs penetration into seed pores<sup>60</sup> and their uptake by seeds, which can induce modifications in the cell membrane, various structural elements, the plant's defense system, and the cellular division mechanisms. AgNPs improved the capacity of the plants to fight salt stress, lower damage from oxidation, and modify the function of the antioxidant enzymes<sup>61</sup>.

#### Seedling growth parameters of durum wheat

This research aimed to analyse the effects of varying concentrations of biosynthesized AgNPs on the Boussalem variety of durum wheat, both under saline stress conditions and in a non-stressed control group. A comprehensive investigation was performed to assess the impact of AgNPs on several morphological characteristics of wheat plants. This research examined the following parameters: root length (RL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW), root number (RN), leaf number (LN), root dry weight (RDW), and shoot dry weight (SDW).

Figure 7 impact of biogenic NPs priming on wheat seed development metrics over an eleven-day during seedling period, considering different salinity levels and NPs concentrations. The data presented herein are the mean derived from three distinct replicates of 5 seeds. Under ideal conditions and with salt treatment, RL raised dramatically and RFW was positively correlated with root development, underlines the part played by non-enzymatic and plant enzymatic antioxidant systems in lowering reactive oxygen species. While AgNPs promote root and leaf growth, salt stress seriously compromises plant health. Treatment encourages ionic equilibrium, so lowering the salt accumulation in plant tissues, particularly in wheat leaves. Salinity levels likewise produced



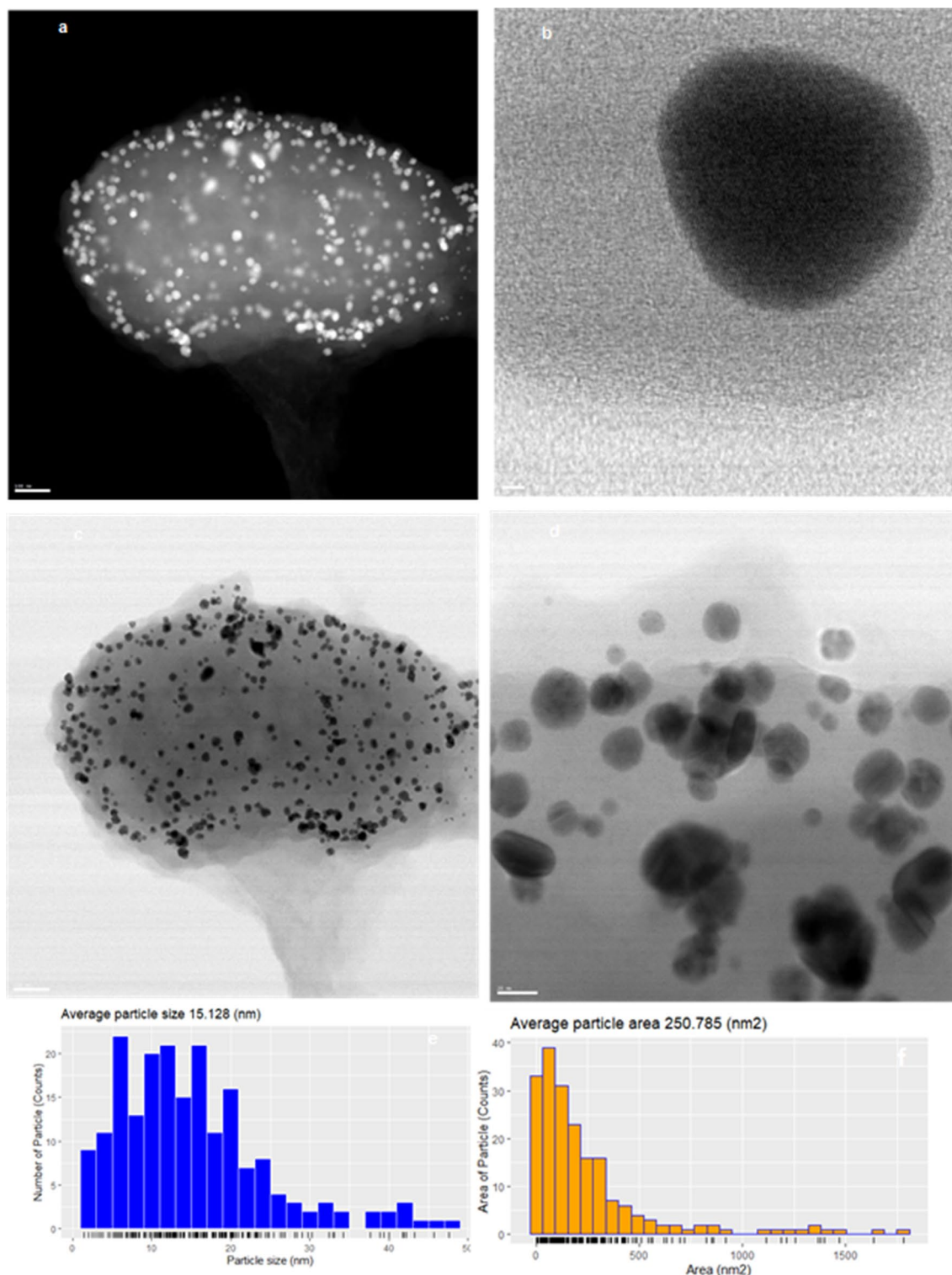
**Fig. 3.** SEM image shows the morphology and shape AgNPs green synthesized using *C.cinerea* at (a) 1600x magnified image of AgNPs, (b) 5000x magnified image of AgNPs (c) 15000x magnified image of AgNPs (d) 25000x magnified image of AgNPs. Exhibiting spherical and significantly cuboidal large morphological particles with an average size of 20 nm. Aggregates exhibit random dispersion. The surface has a faceted texture and lacks smoothness.

significant variations in shoot development. Under saline stress, AgNPs at  $20 \text{ mg L}^{-1}$  promoted shoot development; and under non-saline conditions, they reduced it. SFW data reveal context-dependent reactions. In non-saline conditions, the SFW of the control group stayed constant at 0.27 g, proving strong development free from AgNPs. But under saline stress,  $80 \text{ mg L}^{-1}$  AgNPs dropped SFW to 0.11 g, suggesting that higher concentrations might induce stress or toxicity in already stressed plants; mM = millimole; g = gram; cm = centimetre; Ag NPs = silver nanoparticles; mg = milligram.

#### *Effect of AgNPs on the root system of durum wheat Boussalem variety*

The nineteen-day old seedlings were chosen for further investigation into the effects of AgNPs on root development under both optimal and saline conditions. Salinity induced growth decline is often attributed to reduce in the plants' ability to uptake nutrients uptake and increased sodium translocation from the roots to the shoots. Our findings demonstrate that Seed priming with green AgNPs synthesized from *C.cinerea* extract, significantly improved growth of wheat, whether in the absence of stress or under saline conditions, relative to the control. While AgNPs synthesized from *Salvia officinalis* L they can promote germination through antioxidative pathways with their potential to generate free radicals and disrupt photosynthesis necessitates a careful evaluation of their application in agricultural practices<sup>62</sup>. The results demonstrate the effect of AgNPs on all morphological attributes (Fig. 7). The differences in RL observed in the non-salt treatment group underscore the role of AgNPs in influencing plant growth under optimal conditions. Control seedlings exhibited the longest RL at 15.76 cm, whereas those exposed to  $20 \text{ mg L}^{-1}$  AgNPs experienced a slight reduction in RL to 14.56 cm. This indicates that, in the absence of stress, the NPs may have a marginally inhibitory effect on root elongation, possibly due to their concentration-dependent influence on nutrient absorption or cellular mechanisms. For



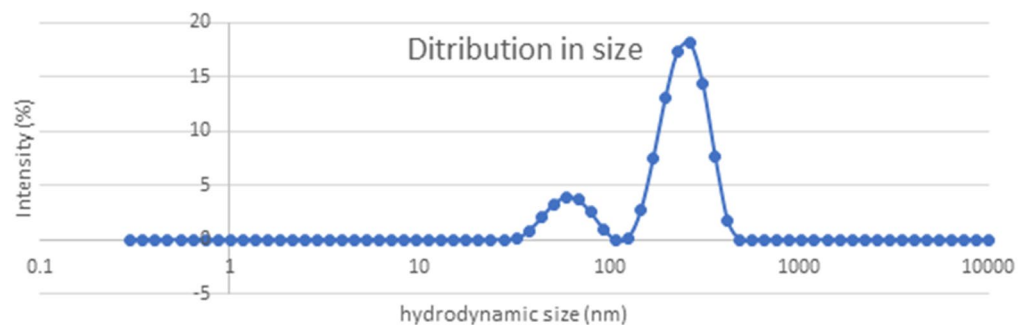


**Fig. 4.** Green synthesized AgNPs TEM image showing spherical particles (a) STEM HAADF at 100 nm (b) STEM bright field at 2 nm (c) STEM bright field at 100 nm (d) STEM bright field at 20 nm (e, f) particle size and area histogram. The narrow size distribution is 1.42–47.62 nm and the picture shows NP polydispersity; nm = nanometre.

roots to grow normally, it is essential that key elements like calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn) are available, as their availability is compromised under salt stress conditions, in a concentration-dependent way, as in roots, it should be hypothesised that nanoparticle transfer from the roots to the coleoptile should likewise slow down its development<sup>63</sup>. Therefore, a reduction in root growth could lead to diminished

Z-Average (nm)	351.6
Polydispersity Index (PI)	0.5662
Peak 2 Mean by Intensity ordered by area (nm)	61.56
Peak 2 Area by Intensity ordered by area (%)	17.28
Peak 1 Mean by Intensity ordered by area (nm)	254.8
Peak 1 Area by Intensity ordered by area (%)	82.72

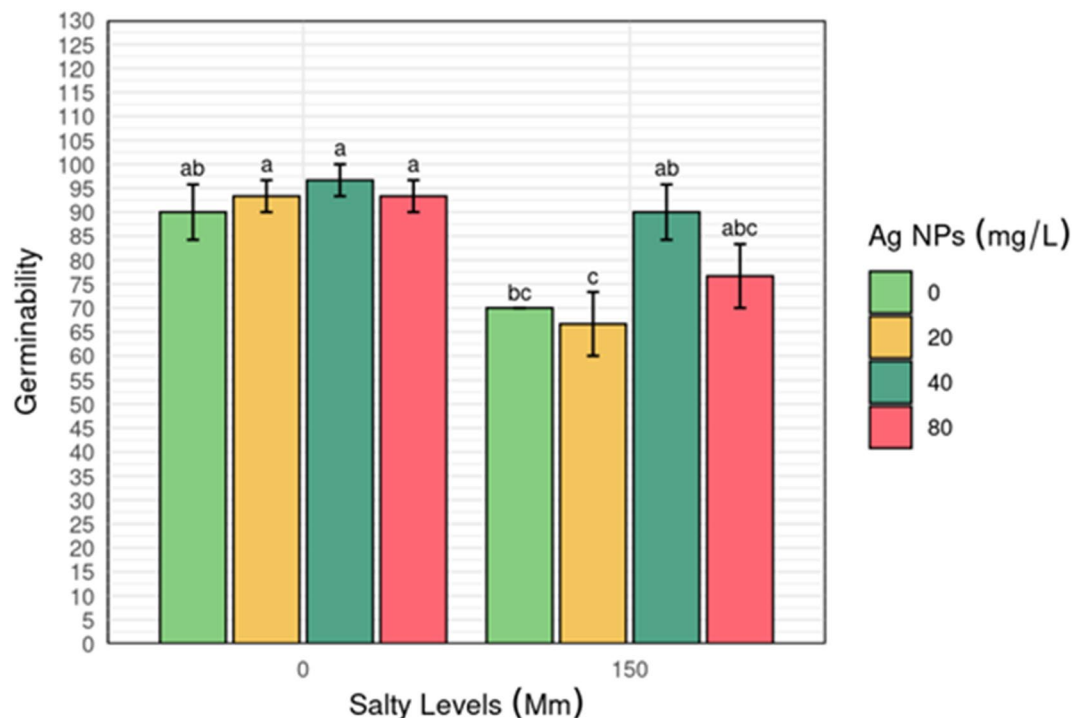
**Table 3.** DLS distribution particle size results of biosynthesized agnps; Z-Average = intensity-weighted mean hydrodynamic size of the particles.



**Fig. 5.** DLS distribution particle size of biosynthesized AgNPs. Showing a hydrodynamic diameter of 351.6 nm, which differs from the dimensions seen in TEM and XRD, DLS gave a unique view of AgNPs. One explains this disparity by the polydisperse character of the particles; nm = nanometre.

uptake of these vital nutrients<sup>64</sup>. Nevertheless, this minor decrease does not diminish the potential advantages of NPs in stress conditions. In the context of salt treatment, the substantial increase in RL observed with 40 mg L<sup>-1</sup> AgNPs treatment (7.28 cm) compared to the control group (3.9 cm) signifies an 86% enhancement in root growth. This remarkable improvement highlights the efficacy of AgNPs in counteracting salt-induced growth restrictions. The assessment of correlation coefficients among various traits indicated that RL exhibited a positive correlation with all measured traits, with the exception of RFW. According to the results of Salama<sup>65</sup> higher concentrations slowed down it. Increasing concentrations from 20 to 60 ppm, however, raised shoot and root lengths, leaf surface area, chlorophyll, carbohydrate, and protein contents. Control plants had the lowest range of these values, but the increasing concentration of silver nanoparticles decreased these chemicals, the correlation coefficient between RL and RFW was notably the lowest, recorded at  $r=0.72$ , suggesting a static relationship between root growth and fresh weight. This finding is illustrated in Fig. 9, which highlights the distinct nature of this correlation. The findings from the study underscore the significant role of AgNPs in enhancing RFW in plants, demonstrating their potential as effective agents in promoting plant growth. AgNPs clearly affected the features of wheat plant production in AgNPs at 40 ppm registered maximum yields. Applying a greater concentration (80 ppm) of AgNPs occupied the growth and adversely impacted the weight of 1000-grain and the straw and grain yield<sup>65</sup>.

The data reveals a marked improvement in RFW under both non-saline and saline conditions, where the application of AgNPs resulted in RFW increases from 0.06 g to 0.09 g in the absence of salt and 0.04 g to 0.06 g in saline conditions. The application of seed priming with AgNPs markedly enhanced plant height, relative water content, proline concentrations, and both fresh and dry biomass, especially at a concentration of 20 mg L<sup>-1</sup> AgNPs, regardless of whether it was administered alone or under conditions of salt stress<sup>66</sup>. By facilitating better absorption, AgNPs may assist in bolstering plant resilience, a crucial factor in maintaining productivity in challenging agricultural settings. The consistent RDW values, particularly the control group's RDW reaching 0 g under saline conditions, indicate complex interactions between RFW and overall biomass accumulation. While RFW increased significantly with AgNPs treatment, the relatively stagnant RDW suggests that plants may prioritize root expansion over dry weight accumulation, particularly under stress conditions. This phenomenon points to possible metabolic resource allocation strategies that enhance root systems capable of tapping into available nutrients and water. Zahra et al.<sup>67</sup> indicate a significant increase in the growth of *B. juncea* seedlings was seen with 25 and 50 ppm silver nanoparticle treatment and proved that the growth profile was assessed based on shoot fresh weight, shoot and root length, and vigour index. AgNPs treatment over 50 ppm was damaging to seedling development. In non-salt conditions, the RN for the control group was recorded at 3.67, while a notable increase to 5.54 was observed in plants treated with a concentration of 20 mg L<sup>-1</sup> of AgNPs. This trend extends to saline conditions as well, where RN values for the control and 40 mg L<sup>-1</sup> AgNPs groups were measured at 5.89

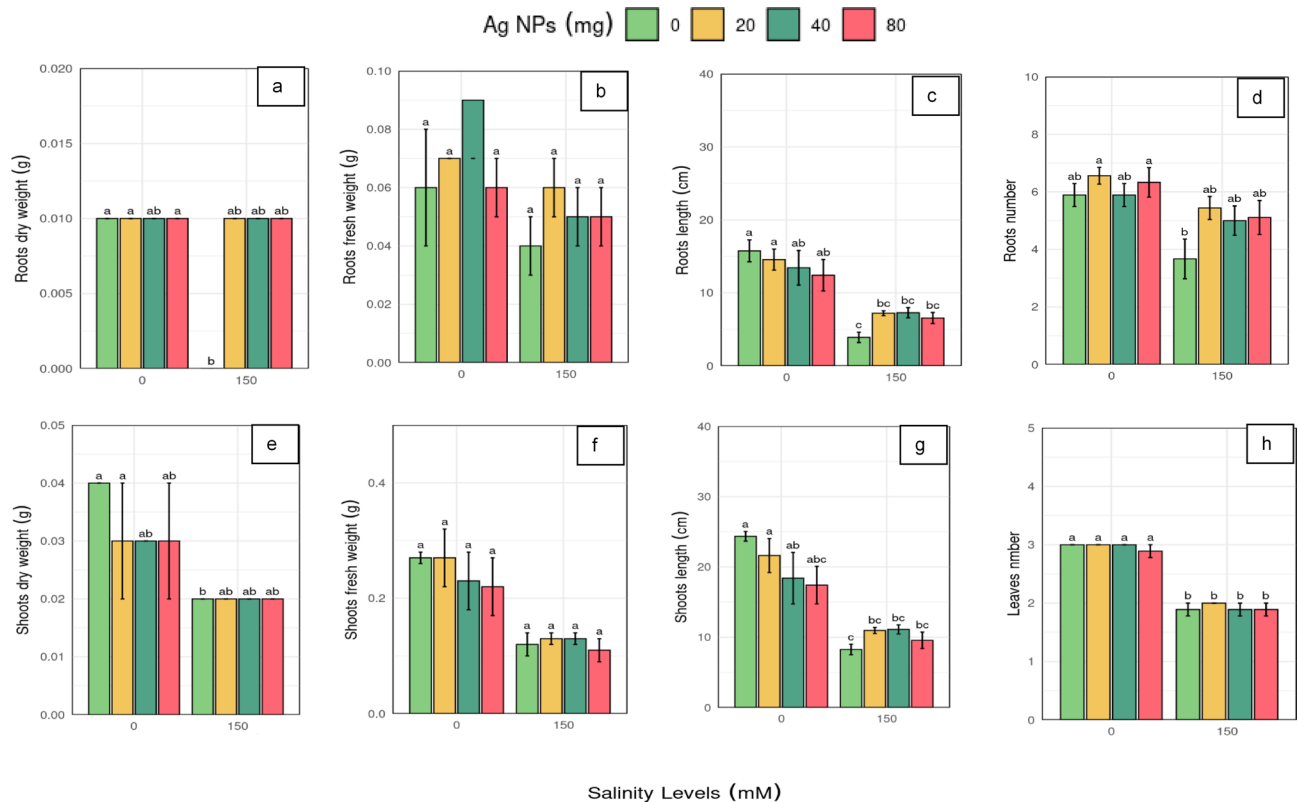


**Fig. 6.** Germinability (%), analysis of wheat seeds Boousselam variety after 7th day of germination Under salt stress, AgNPs greatly raised germinability on wheat seeds when treated with 40 mg L<sup>-1</sup>. AgNPs act as seed priming agents, so influencing germination, structural elements, cell membrane, and cellular division processes. AgNPs' ability to reduce stress, however, emphasizes their possibility to improve crop tolerance and sustainable agricultural output in saline conditions.; Mm = millimole; mg / L = milligram per liter; AgNP = silver nanoparticles.

and 6.56, respectively. By comparing with Soaking corms at 40 or 80 ppm AgNPs concentration but in flooding stress, an increased root number was observed. And a stressed roots grew longer with 40 ppm nano silver<sup>68</sup>. The correlation between RN and other attributes was strong, with correlation coefficients (*r* values) ranging between 0.67 and 0.90 (Fig. 9), indicating a robust relationship between root development and overall plant health. The reduction in auxin concentration under salt stress conditions indicates a potential disruption in the normal hormonal balance within the plant. Auxins are essential in several growth processes, such as cell elongation and differentiation<sup>69</sup>. AgNPs effectively stimulate root growth, likely due to their influence on hormone signalling pathways, notably auxins, which play a vital role in root development. The substantial increase in RN not only enhances anchorage but also improves the plant's ability to absorb water and nutrients, which is critical under both regular and saline conditions.

Salinity tolerance in plants is a multifaceted attribute, relying on a synthesis of physiological mechanisms including osmotic tolerance, the exclusion of toxic ions, and tissue tolerance. Plants adapt to saline environments through mechanisms such as the synthesis of osmo-protectants like proline and efficient absorption of K<sup>+</sup> ions. Under salinity stress, salt-tolerant plants critically depend on maintaining adequate K<sup>+</sup> ion levels. Wheat, for instance, typically experiences a deleterious rise in Na<sup>+</sup> accumulation alongside a reduction in K<sup>+</sup> levels, as the absorption of vital K<sup>+</sup> ions is inversely correlated with the higher concentrations of antagonistic Na<sup>+</sup> and Cl<sup>-</sup> ions. Our study demonstrates that AgNPs treatment significantly ameliorated this ion imbalance, leading to lowered Na<sup>+</sup> and Cl<sup>-</sup> ion accumulation and elevated K<sup>+</sup> ion concentrations in both leaf and root tissues. This crucial effect explains the observed increase in the K<sup>+</sup>/Na<sup>+</sup> ratio in plant tissues and the concurrent decrease in Na<sup>+</sup>-induced K<sup>+</sup> efflux, consistent with findings by Wahid et al. (2020)<sup>64</sup>, which highlighted AgNPs' role in regulating ion homeostasis under salt stress.

Beyond ion balance, changes in endogenous phytohormone levels represent another crucial plant mechanism for overcoming the impacts of salt and drought, influencing numerous reactions throughout the plant's life cycle under both stressful and non-stressful situations<sup>64</sup>. It is evident that AgNPs treatment robustly improved growth features. This enhancement can be partly explained by the accumulation of soluble carbohydrates resulting from seed priming activities, given that soluble sugar concentrations in germinating seeds are reliable indicators of seedling emergence and development<sup>65</sup>. Furthermore, AgNPs have been shown to inhibit ethylene sensing and potentially suppress ethylene production<sup>64</sup>, a key stress hormone, thereby contributing to stress mitigation. The biological activity of plant-synthesized NPs is intricately linked to their surface charge, natural chemical content, and physical characteristics. Moreover, the host organism's metabolism and reaction to these NPs are heavily influenced by the metallic core and the phytochemical capping agents used in AgNPs biosynthesis, which



**Fig. 7.** Impact of biogenic NP.

collectively have a major effect on seed germination, protein and carbohydrate synthesis, and overall growth profiles in various crops like *Bacopa monnieri*, *Brassica juncea*, common bean, and maize<sup>66</sup>.

#### *Effect of AgNPs on the shoot system of durum wheat Boussalem variety*

Regarding LN, our results revealed that while there were minor fluctuations across various concentrations of AgNPs, a beneficial effect was noted at 20 mg L<sup>-1</sup>, where plants exhibited enhanced leaf development. Under salt concentrations of 40 to 80 mg L<sup>-1</sup> of AgNPs, as well as in the control group, LN displayed a degree of preservation or marginal enhancement. However, it is essential to note that a higher concentration of 80 mg L<sup>-1</sup> in non-saline conditions resulted in a decrease in LN, with some experimental groups recording LN values as low as 3. The relationships with other attributes related to LN showed correlation coefficients ranging from 0.70 (Fig. 9), further emphasizing the interconnectedness of leaf development with root health and overall plant vigour. The data indicate that the application of AgNPs contributes to improved salt tolerance in plants. Particularly, the treatment seems to support ionic balance, helping mitigate the negative effects of sodium accumulation in plant tissues. This aligns with the established understanding that maintaining potassium levels relative to sodium is critical for cellular function in saline conditions. In wheat leaves, the presence of AgNPs led to an enhancement in the activities of key antioxidant enzymes and a reduction in the levels of malondialdehyde and hydrogen peroxide, particularly when compared to plants that were subjected to salt stress<sup>70</sup>.

Our findings illustrate the potential of AgNPs as a bio-stimulant to enhance leaf development, particularly at moderate concentrations such as 20 mg L<sup>-1</sup>. The significant increases in RN and the careful modulation of LN across various conditions highlight the applicability of AgNPs in agricultural practices, especially in regions impacted by soil salinity. The investigation into the effects of AgNPs on plant development reveals significant disparities based on salinity conditions, particularly concerning SL. At a concentration of 20 mg L<sup>-1</sup>, AgNPs improved SL under saline stress, recording values of 11.12 cm and 8.26 cm, which implies a notable enhancement in shoot development during adverse conditions. Similarly, Sadak<sup>71</sup> highlight the use of AgNPs via foliar application at different concentrations of 20, 40, and 60 mg L<sup>-1</sup> has been found to significantly boost the growth traits of fenugreek plants. Significant enhancements were noted in various parameters, including SL, LN, and SDW. In contrast, the presence of AgNPs in non-saline environments led to a reduction in SL, with control measurements of 24.36 cm dropping to 21.63 cm. This decline indicates that the stimulatory effects of AgNPs are context-dependent, providing less benefit in optimal conditions and emphasizing the need to consider environmental factors when evaluating NPs applications in agriculture.

#### *Effect of AgNPs on the biomass of durum wheat Boussalem variety*

The findings regarding SFW further support the notion of context-dependent responses. In non-saline conditions, the control group's SFW remained stable at 0.27 g, indicating healthy growth without the need



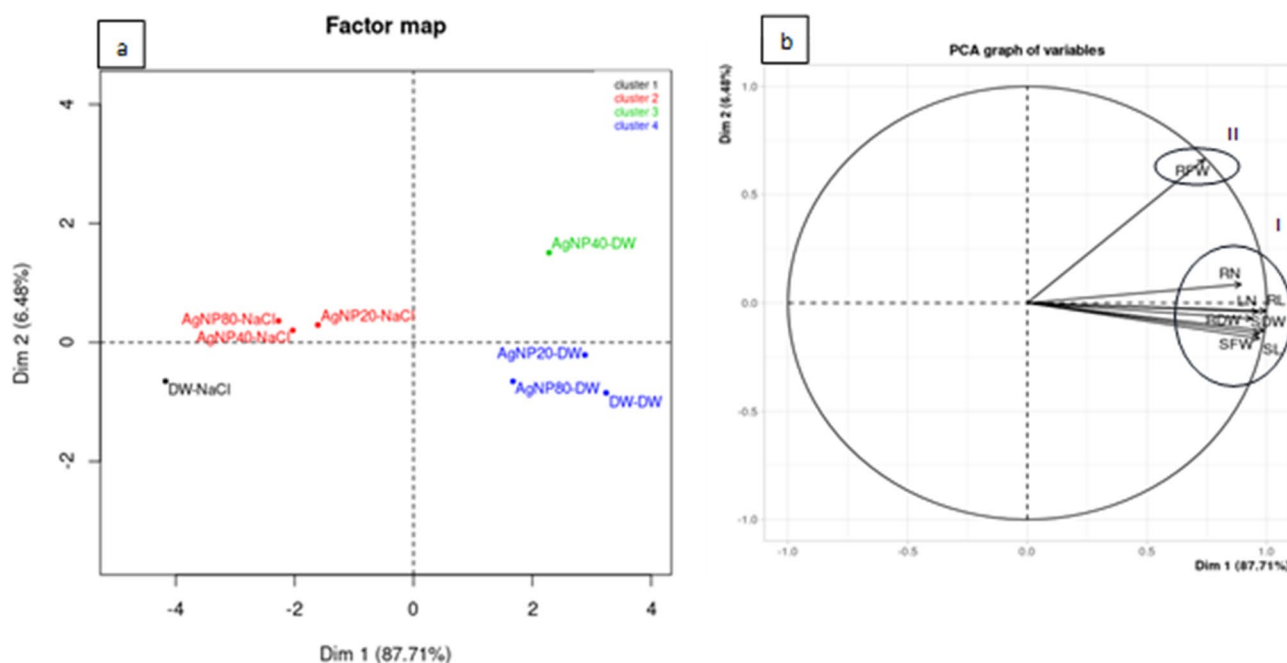
for AgNPs intervention. However, under saline stress, the elevated concentration of 80 mg L<sup>-1</sup> resulted in a substantial decrease in SFW to 0.11 g, suggesting that higher levels of AgNPs may induce toxicity or exacerbate stress in already challenged plants. Conversely, lower concentrations of 20 and 40 mg L<sup>-1</sup> moderately enhanced SFW, reaching 0.13 g. This indicates a nuanced interaction where AgNPs can be beneficial at specific concentrations, but excessive amounts may pose risks, aligning with findings in other studies that show varying optimal concentrations for different plant responses, and the low concentrations of AgNPs promoted plantlet development, but higher quantities resulted in an inhibitory effect<sup>72</sup>. The mechanisms of action of green AgNPs include improving nutrient uptake, which directly supports biomass growth, and stimulating phytochelatin induction, which helps sequester toxins and maintain metabolic efficiency, indirectly supporting biomass retention.

### Durum wheat variety Boussalem seedling trait assessment

#### Factor map

Factor Map (Fig. 8.a) depicts the distribution and grouping of treatment groups according to two major components: Dimension 1 (87.71% of variance): This axis denotes the main source of variation across treatments, indicating it encompasses the major variables affected by saline and AgNPs treatments. It probably encompasses extensive growth responses concerning biomass, vigour, and tolerance under simultaneous stress situations. Dimension 2 (6.49% of variance): Although it represents a little fraction of the overall variance, this component emphasizes secondary distinction within the dataset. It may indicate more nuanced physiological or morphological changes among treatments that are less significant than those in Dimension 1.

The treatment groups are arranged along these axes, demonstrating clustering patterns that signify unique physiological responses: DW-DW (control under non-saline circumstances) and DW-NaCl (control under saline conditions) function as reference points, delineating the impact of salinity alone. AgNP20-DW, AgNP40-DW, and AgNP80-DW denote non-saline settings with increasing Ag NPs concentrations of 20, 40, and 80 mg



**Fig. 8.** Principal component analysis (PCA) and Factor Map to better understand the process of durum wheat Boussalem variety with its seedling attributes in non-saline and saline stress, and AgNPs treatments with different concentration. Dim 1 represents the major variables affected by saline and AgNPs treatments, such as biomass, vigour, and tolerance under simultaneous stress situations. Dim 2 emphasizes secondary distinction within the dataset, suggesting more nuanced physiological or morphological changes among treatments. Variables such as RFW and SFW have elevated loading values along Dim 1, suggesting they are very sensitive to the applied treatments. Secondary contributors, such as RN, RL, and SL, exhibit modest contributions to both. Traits situated near the plot's beginning have low influence on total treatment variance, indicating that these characteristics are largely stable or less impacted by the examined stressors. Features further from the origin indicate more sensitivity and variability, acting as differentiators of treatment effects; Dim 1 = dimension 1; Dim 2 = dimension 2; DW = distilled waters; RDW = root dry weight; RFW = root fresh weight; RN = root number; RL = root length; SDW = shoot dry weight; SFW = shoot fresh weight; SN = shoot number; LN = leaves number; AgNP = silver nanoparticles.

$L^{-1}$ , respectively, while AgNP20-NaCl, AgNP40-NaCl, and AgNP80-NaCl signify analogous treatments under saline circumstances.

The aggregation of various treatments indicates that AgNPs concentration, together saline stress, provokes unique phenotypic responses, resulting in the formation of discrete clusters for each treatment. This suggests that the simultaneous application of AgNPs and salt may elicit distinct tolerance mechanisms or stress responses, contingent upon the concentration of NPs.

#### Principal component analysis (PCA)

The use of PCA is essential for uncovering hidden patterns, reducing duplication, and enhancing the clarity of data representation. This approach allows a comprehensive examination of the interconnections among many growth parameters and their reciprocal impacts, yielding insights into the processes that govern seedling development. The PCA identified two different sets of features associated with elevated seedling parameters when analysing both Dimension 1 and Dimension 2 simultaneously (Fig. 8.b). Group I included the qualities RN, RL, LN, SDW, RDW, SFW, and SL, whereas the trait RFW was included in group II.

PCA Variable elucidates the contributions of certain seedling qualities to the principal components, highlighting the traits most significantly correlated with treatment-induced variance: Dimension 1: Variables such as RFW and SFW have elevated loading values along Dim 1, suggesting that these qualities are very sensitive to the applied treatments. Their significant impact on Dim 1 indicates they function as principal measures of growth performance and stress resilience across treatments. Secondary contributors, such as RN, RL, and SL, exhibit modest contributions to both Dimension 1 and Dimension 2. Although these qualities are affected by treatments, their variability is less significant than that of RFW and SFW, indicating that root and shoot morphological parameters are altered by stress but may not be the principal determinants of treatment responses. Absciscic acid applied in conjunction with lower amounts of growth stimulators more especially, auxins and cytokinins in the shoots and roots of wheat resulted in changes in hormone levels within the wheat plant might be the first mechanism controlling the salinity related decrease of growth<sup>73</sup>.

Traits situated near the plot's beginning have low influence on total treatment variance, indicating that these characteristics are largely stable or less impacted by the examined stressors. In contrast, features positioned further from the origin indicate more sensitivity and variability, therefore acting as differentiators of treatment effects.

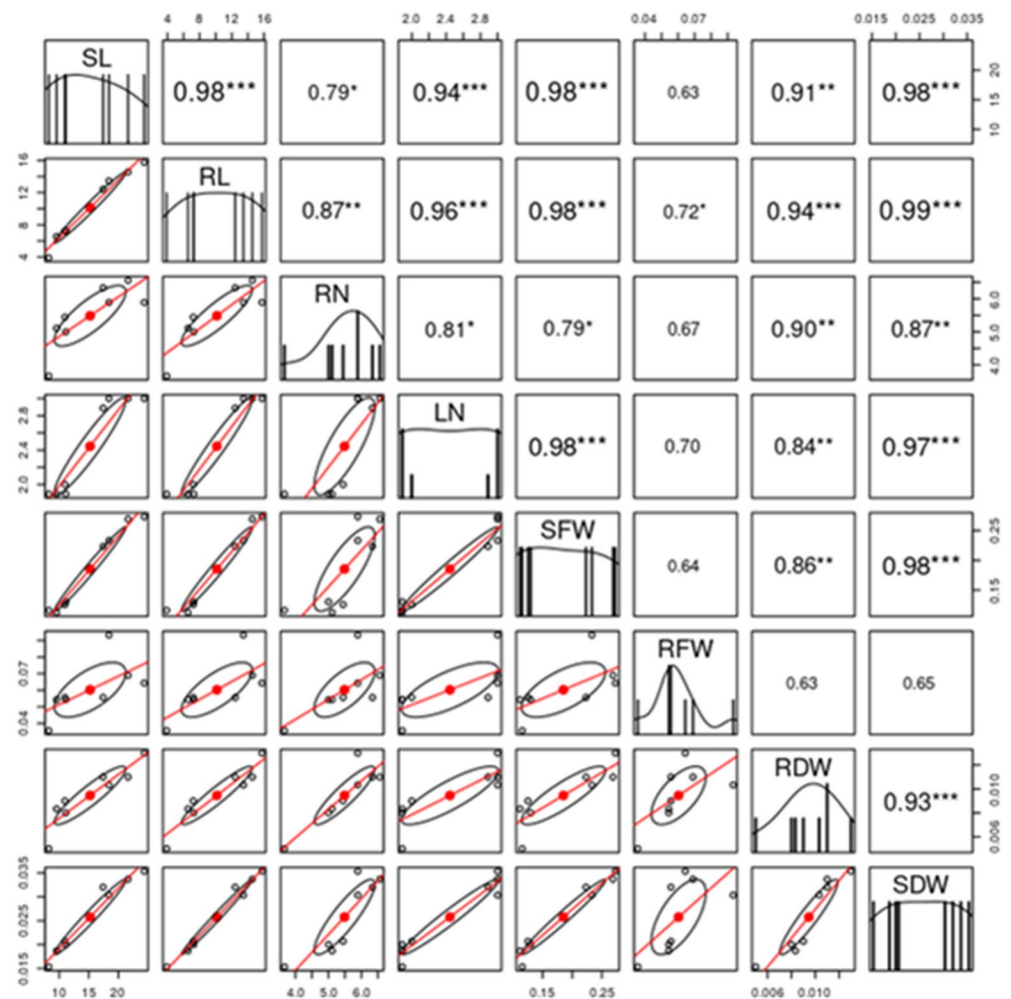
#### Pearson correlation test

The correlation analysis (Fig. 9) provides additional insight into the dynamics of growth metrics. While SFW displayed a moderate correlation with RFW at  $r=0.64$ , it showed a strong correlation with SDW at  $r=0.98$ . This suggests that factors influencing SFW are closely linked to overall biomass accumulation in terms of shoots but less so with root development. The lower correlation with RFW might indicate that while shoots can reflect immediate growth responses to AgNPs, root development may not be as influenced or could be responding to other environmental and physiological factors. Collectively, these findings highlight the complex interplay between AgNPs, salinity, and plant growth, underscoring the importance of targeted research to optimize NPs use in enhancing agricultural resilience under various environmental constraints.

Overall, the positive correlation between RFW and other growth traits, despite the lower correlation with SL, highlights the nuanced dynamics of plant growth influenced by AgNPs. The findings advocate for the inclusion of nanotechnology in agricultural practice, particularly in saline-prone areas. Future studies should delve deeper into the mechanisms driving these changes and assess the long-term effects and environmental implications of AgNPs in agriculture, aiming to optimize their application and enhance crop resilience sustainably. The mechanisms by which plants reduce reactive oxygen species largely include both enzymatic and non-enzymatic antioxidant systems. The enzymatic system comprises essential components like superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and glutathione peroxidase (GPX). Conversely, the non-enzymatic system consists of substances such as ascorbic acid, glutathione, vitamin E, carotenoids, and mannitol, as highlighted by<sup>74</sup>.

#### Strong positive correlations between growth traits

SL shows a very high correlation with multiple traits, including LN ( $r=0.98^*$ ), RDW ( $r=0.98^{***}$ ), and RL ( $r=0.96^{***}$ ). This suggests that an increase in SL is closely associated with an increase in LN, RDW, and RL, indicating a coordinated growth pattern between the above-ground and below-ground parts of the plant. SFW and SDW have a very strong correlation ( $r=0.98^{***}$ ), as do RFW and RDW ( $r=0.86^{**}$ ), indicating a strong association between fresh and dry biomass in both shoots and roots. The high correlations among biomass traits imply a balanced allocation of resources between shoot and root growth, crucial for durum wheat's adaptation to various environmental conditions. RN exhibits moderate correlations with SL ( $r=0.79^*$ ) and RL ( $r=0.81^*$ ), suggesting a role in structural complexity rather than direct biomass increase. LN shows strong correlations with SL ( $r=0.98^{***}$ ) and RL ( $r=0.96^{***}$ ), reflecting coordinated resource allocation and supporting greater photosynthetic activity. The strong associations between these traits suggest they could serve as indicators of general plant vigour and resilience, with potential for indirect selection in breeding programs. Insights derived from the Pearson correlation analysis shed light on the growth interrelations of durum wheat seedlings facing potential salt stress, emphasizing the significant correlations among SL, RL, LN, SDW, and RDW, which imply a unified physiological strategy for resource allocation. Previous research has highlighted that while elevated concentrations of NPs can be detrimental to plant health, lower concentrations may actually promote positive growth effects. This duality suggests a complex interaction between NPs concentration and plant physiological responses, warranting further investigation into the underlying mechanisms<sup>75</sup>.



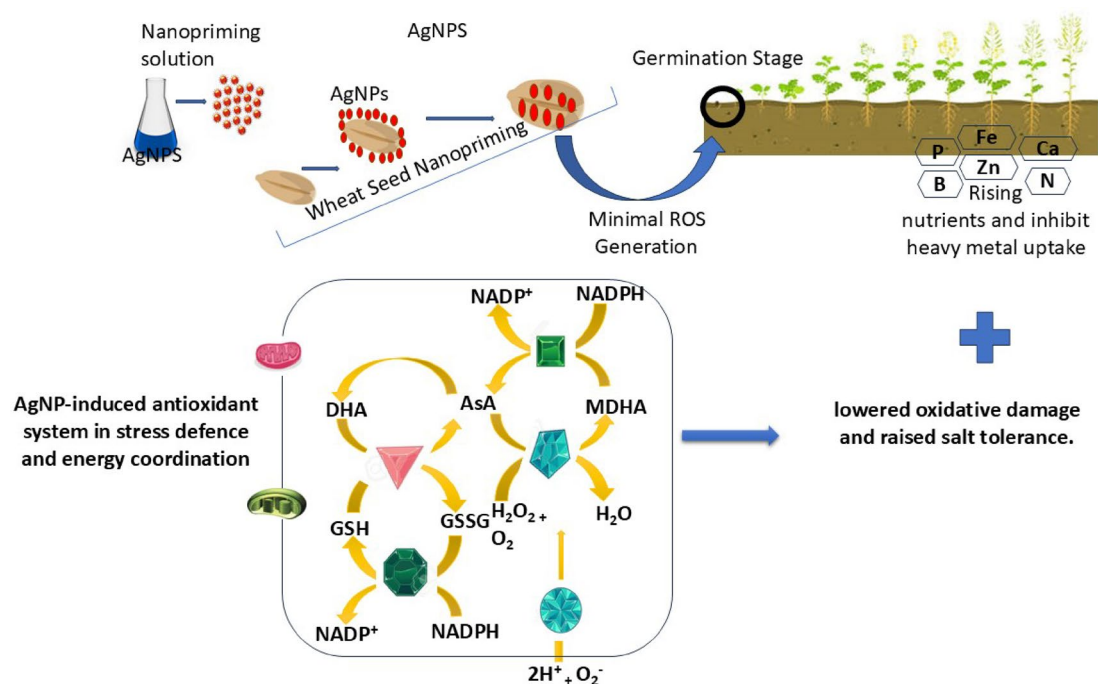
**Fig. 9.** The Pearson correlations matrix (R-value) was computed to examine the relationships among eight critical growth and physiological traits of *Triticum durum* Desf (durum wheat) Bousselam variety. The matrix displays pairwise correlation coefficients that represent recognizing traits which are closely linked and may exhibit similar responses to experimental factors, such as salinity stress or AgNPs treatments. Statistical significance is marked by asterisks, with the following criteria: \*\*\* $p < 0.001$ \*\*, \*\* $p < 0.01$ \*, and \* $p < 0.05$ \*: RDW = root dry weight; RFW = root fresh weight; RN = root number; RL = root length; SDW = shoot dry weight; SFW = shoot fresh weight; SN = shot number; LN = leaves number.

Numerous researches have evidenced the beneficial impacts of AgNPs in alleviating salt stress in plants, and various hypotheses have emerged from investigations aimed at elucidating the mechanics of AgNPs activity (Fig. 10).

Abiotic stressors are recognized for eliciting several responses that adversely affect plant cellular processes and induce oxidative and osmotic effects, hence limiting plant growth and productivity<sup>76</sup>. Given this condition, the application of AgNPs, due to their capability to enhance antioxidant activity and mitigate oxidative stress, has been identified as a viable technique to combat abiotic stress in several plant species, demonstrating significant potential to resolve this issue<sup>77</sup>.

AgNP treatments can initiate oxidative signaling and enhance the antioxidant capacity of treated plants, hence mitigating oxidative damage when subjected to biotic stressors<sup>78</sup>.

Kumar et al.<sup>79</sup> proposed that the utilization of AgNPs on tomato plants subjected to saline stress enhanced their growth, photosynthetic efficiency, and antioxidant activity. The AgNPs served as a source of silver ions, which activated the plants' defense mechanisms against salt-induced oxidative stress. Javadi et al.<sup>80</sup> similarly showed that AgNP treatment in basil plants in saline circumstances improved biomass production, chlorophyll content, and antioxidant enzyme activity. While the exact processes by which AgNPs alleviate salt stress remain under investigation, increasing evidence indicates their multifaceted role in promoting plant development and strengthening stress resilience.



**Fig. 10.** Proposal mechanisms of AgNPs on salt tolerance of wheat seeds.

A hypothesized mechanism suggests that AgNPs enhance nutrient intake by modifying root membrane permeability or promoting the expression of ion transporters, thus ensuring superior acquisition of critical minerals despite ionic imbalance induced by salt stress. AgNPs may enhance aquaporin activity, hence improving water absorption and balance during osmotic stress.

Moreover, numerous studies indicate that AgNPs markedly improve the antioxidant defense mechanisms in plants. Moghaddam et al.<sup>81</sup> noted elevated activity of essential antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), in plants treated with AgNP, which assist in neutralizing reactive oxygen species (ROS) and mitigating cellular oxidative damage. This enzymatic enhancement is crucial for preserving cellular redox equilibrium during stressful circumstances.

Furthermore, AgNPs can alter soil physicochemical characteristics, enhancing water-holding capacity and cation exchange capacity, hence improving water retention and nutrient bioavailability in saline soils<sup>82</sup>. This enhanced microenvironment not only promotes plant hydration but also enables prolonged metabolic activity under abiotic stress.

These data collectively indicate that AgNPs aid in stress reduction via physiological, biochemical, and soil-mediated mechanisms, establishing them as prospective agents for improving plant tolerance to salt and potentially other abiotic stresses.

## Conclusion

The findings indicate that AgNPs, synthesized from *C. cinerea* Del. raw extract, significantly improve wheat seed germination and growth in saline stress conditions, with optimal concentrations ranging from 20 to 40 mg L<sup>-1</sup>. The application of AgNPs resulted in significant enhancements in critical morphological parameters, including RL, RN, SFW, and SL, while also markedly mitigating the negative impacts of salinity. A concentration of 40 mg L<sup>-1</sup> demonstrated the most significant overall benefit, enhancing RL by 86% and RN by 11%. In contrast, 20 mg L<sup>-1</sup> was notably effective in increasing RFW by 50% and SL by 34%.

The dry weight of the roots and shoots showed minimal change, suggesting that the main advantages of AgNPs lie in enhancing early-stage growth and development rather than in biomass accumulation. The results indicate that AgNPs significantly enhance wheat salt tolerance by alleviating osmotic and ionic stresses related to salinity, positioning them as a promising nanoprimering agent for agricultural use in saline soils.

This study demonstrates the potential of green-synthesized AgNPs as a sustainable and eco-friendly method for enhancing plant resilience to salinity, especially in neglected or salt-affected regions. The findings offer insights into the interaction between saline stress and NPs application, facilitating further investigations into the mechanisms underlying these responses and their broader applications in agriculture.

## Data availability

Data Availability The data that support the findings of this study are available upon reasonable request for Brahim Kesbi: Kesbi.brahim@univ-ouargla.dz.

Received: 30 March 2025; Accepted: 25 July 2025



Published online: 01 August 2025

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## Acknowledgements

This work has been partially cofounded by The Crops For Change: Tackling the global warming effects in crops (C4C) project (part of the ERA-NET Cofund FOSC within the European Union's Horizon 2020 research and innovation programme. (<https://www.c4c-cropsforchange.net/>)

## Author contributions

B.K., N.S., and Y.Kh conceived and designed the experiments. S.A. and A.A. performed bioinformatics data analysis. B.K., N.S., Z.A., H.A., and F.A. wrote the manuscript. All authors contributed substantially and approved the final submission.

## Funding

This research received no external funding.

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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