Enhancement of growth and physiological traits under drought stress in Faba bean (*Vicia faba* L.) using nanocomposite

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**ABSTRACT**

Scarcity of water is a substantial impediment to the growth and yield of crop species. In this study, free-radical copolymerization was used to tailor novel formulations of superabsorbent nanocomposites (SANCs). The prepared SANCs were characterized using FT-IR spectra, SEM micrograph, TGA and X-Ray diffraction. Following SANC preparation, the swelling behavior was examined. Also, SANC’s soil burial degradation and water retention were investigated. A pot trial was performed to examine the growth performance and physio-biochemical traits of Faba bean in the presence and absence of SANC under water-stress (40% FC). Water stress decreased chlorophyll and sugars contents, CAT, APX, SOD, and PPO activities. Nonetheless, water stress raised POD and GR activities, and AsA, DPPH, PMA, H$_2$O$_2$, MDA, and soluble proteins levels. SANC alleviated water stress by boosting Faba bean growth and physiological characteristics. SANC enhanced Chl b, carotenoids, Fv/Fm, CAT, GR and SOD, contributing to better growth of stressed Faba bean.

**1. Introduction**

Water shortage is one of nature’s most destructive calamities that can threaten the economic, social, and environmental status of any country. Inadequate water resources, poor irrigation practices, soil degradation, and soil’s inadequate retention capacity aggravate its consequences. Drought has an impact on the soil ecosystem, perhaps leading to erosion and soil degradation, so effective land management is the most feasible solution during drought (Saha et al. 2020). Being the basic axis of the development, water resources and how they are managed provide benefits and services including poverty reduction, economic growth and environmental sustainability. The restrictions imposed by finite or insecure water resources management constrain the progress of sustainable development. Water contributes to advances in social well-being and inclusive growth. Hence, creatures’ life, environmental health, food security and energy demand are affected, and there can be no sustainable development unless the demand-supply balance is restored (UN 2018).

Freshwater resources distribution and availability, as a result of precipitation and runoff, can be irregular, with different parts of the world receiving varying amounts of water at different times of the year (Abd Ellah 2020). There is a lot of difference between arid and humid regions, as well as between wet and dry seasons. Compounded yearly averages reveal large differences in per capita water availability between countries. Egypt is among the countries experiencing significant water issues as a result of its limited resources, which are primarily reflected by its fixed share of Nile water, and the country’s aridity (Metwally et al. 2019).

Egypt’s agriculture sector is the largest user of water, accounting for more than 80% of overall demand. Given the anticipated growth in water demand from other sectors, such as industrial and municipal water supplies, Egypt’s economic growth is heavily reliant on its ability to conserve and manage its water resources (Omar and Moussa 2016).

Composites have a wide range of uses in everyday life and technology. These composites are dispersions of a filler or reinforcement in a matrix, which is often polymeric. Nanocomposites, a novel class of composites, are reshaping science and engineering around the world. A nanocomposite is indeed a heterogeneous system made up of two or more constituents remaining in a homogeneous distribution in a polymeric matrix, that has at least one constituent having a nanoscale dimension (recognized as nanofiller) disseminated in a first phase to obtain a combination of the constituents’ individual properties (Velasco-Soto et al. 2016). Nanofillers have an advantage over traditional micrometer-sized fillers in that they usually give superior performance enhancement for the same concentration (Bokobza 2017). Fillers in nanocomposites include nanoscale clays, nanoscale metal oxides, and carbonaceous nanomaterials (Velasco-Soto et al. 2016).

As a result, several studies on novel fillers have been managed to fill in the superabsorbent polymer composites (SPCs). These SPCs are inexpensive, biodegradable, and eco-friendly (Wan et al. 2008). Starch, guar gum, alginate, cellulose, wheat bran, wheat straw, chitosan, and other natural polymers are widely employed as polymers utilized in superabsorbent composites. Polymer/clay superabsorbent composites have recently attracted researchers interest due to their inexpensive manufacturing costs, excellent water...
retention, and a broad range of applications in agriculture and horticulture (Sikder et al. 2021).

Faba bean (Vicia faba L.) is a prominent legume crop in the Fabaceae family that thrives in many agroecosystems, although its performance is severely hampered by many environmental challenges, including salinity and water stress (Abdel Latef et al. 2021). It is a commonly grown crop for human consumption and livestock feed owing to its great content of protein, fibers, minerals, lecithin and sugars (Etemadi et al. 2018). Water shortage is among the greatest stressful aspects for plants, as it has a significant impact on their growth and development, as well as a significant impact on their production. Faba bean consumes more water than other legumes and is more drought susceptible (Martínez et al. 2018). Water shortage is among the greatest stressful aspects for plants, as it has a significant impact on their growth and development, as well as a significant impact on their production. Faba bean consumes more water than other legumes and is more drought susceptible (Martínez et al. 2007). In the current work, we attempted to determine whether soil amendment with the superabsorbent nanocomposite (SANC) endows defense to Faba bean against water stress by evaluating the effect of this SANC on growth, antioxidant status, oxidative stress markers, sugar and protein contents at the seedling stage.

2. Materials and methods

2.1. Chemicals

Acrylic acid (AA), cellulose, polyethylene glycol (PEG 6000), \(N,N\)’ methylene bisacrylamide (MBA), Gelatin, cetyl tri-methyl ammonium bromide (CTAB), \(N,N,N',N'\) Tetramethylene diamine (TEMED) and Potassium persulfate (KPS) were donated from ACROS Organics™ (Germany). Acrylamide (AM), bentonite and Potato starch were purchased from Sigma Aldrich (Germany). Activated carbon (AC), sodium hydroxide, potassium hydroxide, sodium chloride, calcium chloride, ferric chloride and ethanol were purchased from El-Nasr pharmaceutical chemicals Co., Egypt.

2.2. Preparation of organo-modified nano clay (bentonite)

The method of Zawrah et al. (2014) was applied to synthesize the organo-modified nano clay (OMNC). The powdered clay was spread in distilled water at 600 rpm for 24 h, then the surfactant (CTAB) was gradually added and agitated at 80°C for an additional 12 h. The product was washed many times with a 50/50 (v/v) mixture of ethanol and water until the filtrate was devoid of bromide anions. Filtration was used to recover the OMNC, which was subsequently dried at 40°C for 12 h. The OMNC was then milled for 15 h with a planetary ball miller to produce a nanoscale powder, which was then stored in plastic bottles. The same method was used to mill the other fillers (cellulose and activated carbon).

2.3. Preparation of superabsorbent nanocomposite (SANC)

The SANC was prepared based on preliminary experiments for determining the maximal swelling ratio (1646.8 g water/g SANC). The technique of free-radical copolymerization in an aqueous medium of nanocellulose (8.2%), \(N,N\)’ methylene bisacrylamide (0.033%), potassium persulphate (0.5%), gelatin (8.2%), acrylamide (8.2%), starch (8.2%), activated carbon nanoparticles (4.1%), organo-modified nano-clay (4.1%), polyethylene glycol (8.2%) and acrylic acid (49.7%) was used in SANC preparation. The produced composite was purified by washing with ethanol and then dried at 70°C using a vacuum oven until constant weight. The prepared SANC was then scissored, milled, and sieved through an 80 mesh screen.

2.4. SANC swelling in saline solutions and different pH values

To determine the swelling ratio, 0.1 g SANC was placed in distilled water or saline solutions (NaCl, CaCl₂, or FeCl₃ at 25-200 mmol/L) and held at room temperature until the swelling equilibrium was reached (24 h). In addition, aqueous solutions with varying pH (2-12) were employed to see how the medium pH affects the swelling ratio. Swollen samples were separated from the various examined solutions by filtering them through a 60-mesh screen (for 40 min) without pressing. The separated SANC was then weighted and swelling ratio was calculated.

2.5. Water retention in the soil

In a 500 mL glass beaker, 0.5 g of well-powder SANC was mixed thoroughly with 100 g of air-dried sandy loam soil, then 100 g of tap water was gently added to the beaker. The beaker’s weight was determined and recorded as W₀. A control experiment was likewise carried out without the SANC. The beakers were incubated at RT and weighed daily (Wᵢ) for 30 days then soil water retention (WR) over time was calculated.

2.6. Soil burial degradation

The SANC’s biodegradability was assessed by tracking samples weight loss in the soil over time, as reported by Wang et al. (2008). 1 g of SANC was buried in the soil for 150 days at 15 cm depth, with the soil kept at 20% moisture. The buried SANC was dug out at constant time bands, washed with deionized water, dried for 24 h in a vacuum at 60°C, equilibrated in a desiccator for at least 24 h and the weight loss was determined.

2.7. Characterization

IR spectra of SANC samples (KBr discs) were recorded with a Jasco-4100 LE spectrophotometer operating in the region from 400 to 4000 cm⁻¹. The SANC morphology was detected using the JSM IT-100 SEM instrument. TGA (thermogravimetric analysis) was achieved using the TGA-4000 instrument with a scanning rate of 10°C/min in a range from 25-800°C. The X-ray diffractometer GNR-APD 2000 PRO was used to identify the crystalline phases of the SANC at room temperature. The data collection was over the 2-theta range of 4-80 in steps of 0.030/s.

2.8. Pot experiment and experimental design

A pot investigation was led in a controlled growth chamber at the Faculty of Science, Tanta, Egypt, from 10th November to 21st March (2020) to evaluate the water-stressed Vicia faba L. cv. Giza 3 performance in the existence and absence...
of the superabsorbent nanocomposite [Gelatin/Starch-g-p (AA-co-Am-co-PEG)/NC/bentonite/ACNPs]. The soil employed in this experiment has a loamy texture with a pH of 7.02, EC of 3.1 mS cm⁻¹ and a bulk density of 1.43 g cm⁻³. The mineral analyses of the study soil showed that it contained total nitrogen (0.76), phosphorus (1.82), potassium (18.76), magnesium (36.88), sodium (5.79), chloride (2.48), total carbonate (11.61) and active carbonate (3.14) mg kg⁻¹ soil. The soil (10 kg) was filled in plastic pots of 40 cm width and 25 cm depth.

Pots were allocated into two main clusters; the first cluster comprised 6 pots and received SANC-free soil and was considered as control. The second group comprised 6 pots occupied with the soil-SANC mixture (30 kg ha⁻¹) and was considered as test. The soil-SANC mixture divided into two subgroups (3 each); the first subgroup received water of 70% FC (well-irrigated) and the second one received 40% FC (water-stressed). Also, the second group pots were divided into well-irrigated and water-stressed subgroups on the 8th day of seed sowing. After complete seedling establishment, 4 uniform seedlings were maintained in each pot. Until the end of the growing season, the plants were irrigated once weekly with the prescribed field capacity. Chemical fertilizers (NPK) were added as endorsed by the Egyptian Ministry of Agriculture (kg ha⁻¹). The pots were allowed to grow in controlled growth conditions (28/16°C ± 3 d/night, 72–76% relative humidity and 12 h photoperiod) in the growth chamber.

### 2.8.1. Assessment of growth attributes

Faba bean plants were harvested during pre-flowering stage (60–days old), washed thoroughly with tap water then with deionized water. A measuring tape was employed to determine the shoot height and root depth. Shoots and roots were weighed as fresh and dried at 60°C to constant weight for the determination of shoot and root dry masses (g/plant).

### 2.8.2. Determination of chlorophyl, carotenoids and photosynthetic performance (Fv/Fm)

Fresh leaf samples were homogenized in 85% acetone then centrifuged at 7000 rpm and the color intensities of these extracts were measured at three wavelengths (663, 644 and 452.5 nm). Pigment content in leaf extracts was expressed as mg/g FM (Metzner et al. 1965).

The photosynthetic performance of photosystem II as a measure of PSII photochemical efficiency (Fv/Fm) was measured by digital fluorometer (OS-30 P, Hudson, USA) as prescribed by Saad-Allah and Ragab (2020) at the vegetative stage.

### 2.8.3. Estimation of antioxidant enzymes

Leaf samples of Faba bean were extracted in 0.05 M cold phosphate buffer containing 50 mg PVP, 10 mL DDT and 0.1 mM EDTA (pH 7.0) (Beauchamp and Fridovich 1971). The extracts were then centrifuged at 7000 rpm using a cooling centrifuge and the supernatant was used for the enzymatic assay.

The activity of catalase (CAT, EC 1.11.1.6) was appraised using Patterson et al. (1984) method. The decomposition of H₂O₂ due to CAT activity was calculated at 240 nm with 43.6 mM⁻¹ cm⁻¹ as extinction coefficient.

Peroxidase (POD, EC 1.11.1.7) colorimetric activity was estimated spectrophotometrically by monitoring guaiacol oxidation into tetraguaiacol at 470 nm based on the extinction coefficient (26.6 mM⁻¹ cm⁻¹) (Zaharieva et al. 1999).

Glutathione reductase (GR, EC 1.6.4.2) activity was tested by measuring the rate of NADPH oxidation as a decline in light absorbance at 340 nm based on the extinction coefficient (6.2 mM⁻¹ cm⁻¹) (Halliwell and Foyer 1978).

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined based on the absorbance decrease at 290 nm (as a result of ascorbate oxidation) using 2.8 mM⁻¹ cm⁻¹ as an extinction coefficient (Nakano and Asada 1981).

Superoxide dismutase (SOD, EC 1.15.1.1) activity assessment was based upon nitroblue tetrazolium photochemical reduction to formazan according to the procedures of Beyer and Fridovich (1987). After measuring the mixture absorbance (at 560 nm), 21.1 mM⁻¹ cm⁻¹ was used as extinction coefficient to calculate SOD activity.

Polyphenol oxidase (PPO, EC 1.10.3.1) activity was determined based on monitoring pyrogallol rate of oxidation into purpurogallin at 495 nm. PPO activity was calculated using 26.40 M⁻¹ cm⁻¹ as an extinction coefficient (Kumar and Khan 1982).

#### 2.8.4. Ascorbic acid content

Ascorbic acid (AsA) content of Faba bean fresh leaf extracts in aqueous sulfosalicylic acid was estimated in consonance with Oser (1979). The reaction mixture comprised Na-molybdate (2%), H₂SO₄ (0.15 N), Na₂HPO₄ (1.5 mM) and the tissue extract. The absorbance was then measured (at 660 nm), and AsA content (µmol/g FM) was evaluated using a previously prepared calibration curve by ascorbic acid.

#### 2.8.5. DPPH scavenging activity

The leaf ethanol extracts were mixed with the free radical DPPH⁺ (2,2 Diphenyl -1- picrylhydrazyl) and the absorbance decrease was observed at 517 nm after 30 min. The percentage of scavenged DPPH was calculated as proposed by Bondet et al. (1997).

#### 2.8.6. Phosphomolybdate assay (PMA)

The total antioxidant activity of leaf extracts was measured using the method reported by Ahmed et al. (2012). The phosphomolybdate reagent was well-mixed with the leaf extracts and incubated at 95°C for 90 min. After absorbance measurement (at 765 nm), the antioxidant activity was calculated as µg ascorbic acid equivalent (AAE)/g DM.

#### 2.8.7. Hydrogen peroxide content

The content of H₂O₂ was determined using Velikova et al. (2000) approach. Fresh leaf tissues were extracted with trichloroacetic acid then centrifuged at 8000 rpm. A mixture of the supernatant, K-phosphate buffer and potassium iodide (1 M) was measured at 390 nm. The extinction coefficient (0.28 M⁻¹ cm⁻¹) was employed to determine the quantity of H₂O₂ (µmol/g FM).

#### 2.8.8. Assessment of lipid peroxidation

Malondialdehyde (MDA) as a criterion of lipid peroxidation was measured in Faba bean leaves by Heath and Packer (1968) method. Leaves were extracted with TCA (5%), centrifuged at 4000 rpm then the supernatant was mingled...
with 0.67% (w/v) thiobarbituric acid and incubated at 100°C. The absorbance was recorded at two wavelengths (532 and 600 nm) and the MDA level was calculated (nmol/g FM) employing the extinction coefficient (155 mM−1 cm−1).

2.8.9. Estimation of total soluble proteins and sugars

Borate buffer (pH 8) was used to extract soluble proteins and sugars from leaf powders. The total soluble proteins were estimated quantitatively (mg/g DM) in the borate extract employing the extinction coefficient of mean ± standard deviation (SD) of three different replica. The absorption at 280 nm was assessed (mg/g DM) through the extinction coefficient (155 mM−1 cm−1).

2.9. Statistical analysis

The findings of the pot experiment were expressed in terms of mean ± standard deviation (SD) of three different replications. One-way analysis of variance (ANOVA) was applied to the results using SPSS software (V 20). The differences among means were compared with Tukey’s HSD test at the level of 5%.

3. Results and discussion

3.1. FT-IR spectroscopy

According to the FT-IR spectra of NC, the absorption was observed at 3377.88 cm−1 (hydroxyl stretch), 2986.55 cm−1 (methylene) and 1033.65 cm−1 (β1, 4-glycosidic bond). There was also a peak at 1423.46 cm−1 that belonged to the C−O−H group of cellulose (Grande et al. 2009) which were characteristic absorptions in NC structure. The distinctive absorption bands of bentonite at 3632.26 cm−1 and 3445.20 cm−1 (stretching vibration of Al−OH and Mg−OH) and the bands at 1024.98 cm−1 (stretching vibration of Si−O) and 454.15 cm−1 (bending vibration of Si−O−Si) (Figure 1A).

Figure 1(A) shows the surface acidity and basicity of ACNPs. The surface acidity is apparently caused by the occurrence of carboxyl, lactones, and phenols at 3445.20 cm−1 for O−H groups, and the peak at 1642.08 cm−1 corresponds to C = O stretching of carbonates and lactic acid groups of ACNPs (Fuente et al. 2003). Whereas, 1375.75 cm−1 peak represents C = C ring stretching for all aromatic groups in ACNPs. The peak at 1078.97 cm−1 could be due to aliphatic ether C−O or alcohol C−O stretching (Özçimen and Ersoy-Meriçboyu 2010), whereas the presence of carbonates may have contributed to the surface alkalinity peak at 819.59 cm−1 (Fuente et al. 2003; Yadav et al. 2016).

Comparison of absorption peaks of NC, bentonite and the AC-SANC revealed some change of absorption, which suggested that the serial compositions of NC had changed during the reaction. The absorption peak at 1033.65 cm−1 indicated the existence of C−O−C bond, which was intense in NC spectrum but decreased in the AC-SANC spectrum. It suggested that the C−O−C bond in NC was fractured. The absorption peak of 3454.85 cm−1, which came from the hydroxyl stretch, was high in the prepared composite spectrum but low in NC. It can be deduced that after the reaction more −OH were grafted on the carbon chain, which resulted mostly from the increase of −COOH. Also, it became clear that the specific absorption bands of bentonite at 3632.26 cm−1 and 3445.20 cm−1 disappeared and that the bands at 1024.98 and 454.15 cm−1 weakened, as seen in Figure 1(A), suggesting that bentonite −OH groups reacted with AA and chemically bonded with the polymer chains (Wen et al. 2016). In the prepared nanocomposite spectrum, the band at 3464.49 cm−1 is coming from the −NH (in AM unit) stretching vibration, that has overlapping pattern with −OH groups. The peaks at about 2933.19 cm−1 were owing to the absorption band of C−H that may present in methyl and methylene groups. A band 1638.23 cm−1 is analogous to the shift of C = O stretching vibration of carboxylate, caused by the superposition of the amide group (−CONH2), which could be noticed in the spectra. The peak at 1570.73 cm−1 corresponded to the asymmetric −COO− stretching that is corroborated by peaks at 1391.38 cm−1 which is attributable to the carboxylate anion’s symmetric stretching mode, which indicated the introduction of p (AA-co-AM) into the graft-copolymer composite. The absorption at 1035.8 cm−1 was due to β 1,4-glycosidic bond, and the peak near 1391.38 cm−1 was distinctive to skeletal C = C stretching vibrations in the aromatic rings. Both these peaks manifested the existence of NC in the composite. In addition, the bands at 1391.38 and 1185.73 cm−1 were attributed to the −C−O− and −OH coupling interactions of the −COOH and C−N stretching vibrations. The absorption peak at 1638.23 cm−1 is attributed to carboxamide. It indicated that these three functional groups were grafted in the NC after graft copolymerization. The characteristic peaks of starch, gelatin and PEG were overlapped with the other peaks of the similar functional groups present in the other components. All these FT-IR information verified that the P (AA-co-AM) chains were successfully grafted onto NC, ACNPs and bentonite. The obtained FT-IR results are consent with some previous reports (Chaudhuri et al. 2020; Kassem et al. 2020).

3.2. Morphologies of superabsorbent composite

The water absorbency and retention rate of hydrogels are affected by their porosity and an average size of hydrogel pores. Hydrogel microstructure morphologies are thus among the most noteworthy aspects to address. Figures 1 (B-E) show the SEM images of the ACNPs, NC, bentonite and different superabsorbent nanocomposites. SEM images of ACNPs, NC and bentonite indicated close-packed tiny spherical nano-particles, which appeared as bright dots (Salimi et al. 2020).

The superabsorbent nanocomposites surface was coarse, fluffy, undulant, interlaced network with highly porous morphology, with size of pores in the range of μm and open-pore geometry. This indicates that the hydrophilic groups in the SANCs caused electrostatic repulsion to increase the space of the cross-linked gel network (Alam and Christopher 2018) and structurally increased the surface area of the superabsorbent nanocomposites that facilitates and accelerates the diffusion of liquids into the matrix (Liang et al. 2009). Therefore, the nanocomposites were able to rapidly absorb water and form a swollen hydrogel when immersed in water. The inner portion of the samples had a porous structure, where many small, uniform, and
interconnected pores were observed. These pores were the regions of water permeation, where water easily diffused inside. This porous microstructure brings about an increased surface area and capillary effect which makes it possible to transport the water into and through the superabsorbent nanocomposites and is likely responsible for their rapid swelling (Capezza et al. 2020).

The main cause of porosity in the produced nanocomposites was dehydration with ethanol and the drying procedure. Large pores also contributed to the superabsorbent composite's high water absorbency and swelling rate (Kabiri and Zohuriaan-Mehr 2004). Furthermore, the ACNPs-NC-bentonite-SANC network architecture is less compressed, and the high porosity allows water molecules to efficiently permeate and be sustained. The porosity and network assembly of NC-bentonite SANC and ACNPs-NC-bentonite-SANC are more than NC-SANC; therefore, higher porosity makes it easier for water molecules to permeate and stay in place. The results demonstrated that the water-absorbing properties of NC-bentonite SANC and ACNPs-NC-bentonite-SANC are better than those of NC-SANC.
3.3. Thermo-gravimetric analysis

Figure 1(F) shows the thermo-gravimetric analysis (TGA) curves of gelatin/starch/PEG-g-P(AA-co-AM)/NC, gelatin/starch/PEG-g-P(AA-co-AM)/NC/bentonite and gelatin/starch/PEG-g-P(AA-co-AM)/NC/bentonite/ACNPs superabsorbent nanocomposites. The thermo-gram of these superabsorbent materials could be categorized into three steps. At the initial stage, in the range of 50–150°C (around 15%) weight loss is due to a loss of moisture present in the samples. Following, the minor weight loss (about 10%) from 150 to 310°C is ascribed to the dehydration of saccharide rings and the breaking of C–O–C bonds in the chain of cellulose of both treated and untreated rice husk (Yang et al. 2009). Within the temperature of 310–620°C, the weight loss for the studied nanocomposites revealed 67% loss for NC-SANC, 63% for NC-bentonite-SANC and 60% for NC-ACNPs-bentonite-SANC. This loss could be related to carboxylate and amide side-groups of the copolymers thermal decomposition, as well as MBA moieties in the network, resulting in the formation of ammonia and other gases as the elimination of CO₂ molecule from the polymeric backbone (Sand et al. 2010). It is also related to protein chain degradation (Barreto et al. 2003). The temperature at which maximum degradation occurs, i.e. T_{max} has been found at 422°C. The weight loss is about 50%. This T_{max} can be explained by the withdrawal of a water molecule from two adjacent carboxylic groups in the polymer chains due to anhydride production, main-chain scission, and the breakdown of cross-linked network structure (Huang et al. 2007).

The third stage was linked to the breaking of copolymer chains, in which higher temperatures ranging from 500 to 800°C were detected as a displacement. As a result of the TGA data, the temperature in which ACNPs have an influence on the corresponding superabsorbent composites thermal stability is within the range 500-650°C. The last stage at 620°C to 800°C might be due to SO₂ molecules elimination from the pendant chain attached to the polymeric backbone. The higher temperature step (T > 600°C) explain the decomposition of more thermally stable structures due to the crosslinking reactions produced during heating (Barreto et al. 2003). Cross-linking reactions may occur between hydroxyl groups and carboxylic acid formed by the chain scission, as in the case of polyamides (Peebles and Huffman 1971). The properties of ACNPs in the superabsorbent composite polymeric network may be the main reasons for TGA results difference in this system as it may operate as a mass transport barrier to the volatiles created during polymer decomposition, hence improving SANC’s overall thermal stability (Hosseinzadeh and Ramin 2018).

The presence of bentonite nanoparticles incorporated in SANC is observed to increase the overall resistance against thermal degradation of the polymer backbone because bentonite resisted polymer matrix’s shrinkage as it dries, and so more water is held by meniscus forces. The bentonite gives rigidity against the shrinkage forces which otherwise would collapse the matrix during drying and so more water would be lost without this shrinkage resistance. Additionally, the bentonite participated in char formation also slowing the...
degradation of the SPA matrix. The TGA results demonstrated that the bentonite nanoparticles in the network might operate as a heat barrier, improving the nanocomposite’s overall thermal stability (Tao et al. 2006). Therefore, it might be concluded that bentonite nanoparticles improve the stability against the thermal degradation of the polymer matrix.

3.4. X-Ray diffractograms

The XRD patterns of ACNPs-bentonite-SANC, bentonite and bentonite-SANC in the range 2θ = (4-35°) are plotted in Figure 1(G). Scherrer’s law predicted a crystal size of 16.023 nm for bentonite, demonstrating nanocrystal size. Bentonite XRD pattern showed a strong peak at 2θ = 6.17° which corresponds to a basal spacing of 14.32 Å. The lack of diffractions peaks for ACNPs-bentonite-SANC suggests that the bentonite clay has been exfoliated and disseminated in the matrix of the polymer developing a nanocomposite structure and implying that the nanocomposite is amorphous. Because the superabsorbent with the amorphous structure has an uneven composition, the solution will be easier to get into and bind with the superabsorbent, which will boost the swelling capacity (Abbas et al. 2017). For bentonite-SANC a peak appeared at approximately 2θ = 11.35°, as in bentonite pattern, implying that bentonite layers were primarily exfoliated in the matrix of the polymer producing composite structure.

The original bentonite exhibits a peak associated with a spacing of 14.32 nm at 6.17°, whereas the absence of this basal peak suggests clay platelets high exfoliation in the nanocomposite materials. In the nanocomposites, it is supposed that the bentonite gallery structure breaks through pressure applied by the inserted co-monomers, causing co-polymerization exfoliation of nanocomposite material. By combining FTIR spectroscopy and XRD patterns results, it could be realized that co-monomers grafting on the bentonite was carried out in an aqueous solution. There are two methods for adsorbing co-monomers onto the surface of bentonite galleries: (i) via creating H-bonds between their acid and amide groups and the molecules of water encircling the exchangeable cations and (ii) by developing ion-dipole through the interaction of the interlayer exchangeable cations and acrylate groups. These interactions, relative to bentonite, lead to basal spacing increase causing exfoliation (Solhi et al. 2012). Experimentally, co-monomers and bentonite interactions lead to the exfoliation by thickening of the solution after vigorous stirring. On the other hand, clay can adsorb co-monomers either by hydrogen bonding between amide and acrylic acid groups and the OH groups on the boundaries of bentonite platelets or by esterification (Santiago et al. 2007). Hence, the ACNPs-bentonite-SANC forms a completely exfoliated nanocomposite while the bentonite-SANC produces a comparatively exfoliated composite.

3.5. Effect of salt solution on the water uptake

The superabsorbent’s swelling capacity in a salt solution has many important practical applications, especially in agriculture and horticulture. The swelling ratio is determined by the external solution’s characteristics as well as the polymer’s composition. The interaction of various saline solutions (NaCl, CaCl2, and FeCl3) with various concentrations and the superabsorbent composite is represented in Figure 2(A).

The type of salt supplied to the composite swelling medium has a significant impact on the composite swelling. According to the study results, as cation charges increase, the crosslinking degree increases and swelling decreases. Therefore, the uptake for the prepared nanocomposite in the inspected saline solutions is in the following order: mono > di > trivalent cations. Increasing ionic strength decreases movable ionic concentration difference between the nanocomposite matrix and the outside solution (osmotic swelling pressure) causing an instant contraction of gel, so the water absorbency decreases with the increase of the concentration of all the three salt solutions. The absorbency dropped with the increased metal cation activity (from Na+ to Ca2+ and Fe3+), so equilibrium water absorbencies in saline solutions for the prepared superabsorbent composite decrease in order of NaCl > CaCl2 > FeCl3 (Zhao et al. 2005).

The carboxylate anions can be shielded from the hydrogel matrix by monovalent cations (e.g. Na+). Because of the minimal repulsion forces between the charges embedded in the hydrogel matrix, a compact tridimensional structure is constructed. As a result, the osmotic pressure decreases, and the capacity of water uptake decreases as well. Also, due to the physical interaction of multivalent cations (e.g. Ca2+ and Al3+) in the swelling media and the carboxylate groups present in the hydrogel matrix, crosslinking sites are formed (Mahdavinia et al. 2004). The increased crosslinking sites prevent the hydrogel structure from expanding, making it stiffer and lowering the water uptake capacity.

3.6. Effect of pH on water absorbency

Figure 2(B) illustrates the swelling behavior of the composite at different pH values at room temperature. The capacity of the superabsorbent composites to absorb water was tested in different solutions with varying pH values (2-12). The swelling of the prepared nanocomposite grew as the pH values increased from 2 to 10, then decreased at pHs between 10 and 12, though it was kept roughly constant at a pH range from 6 to 8. At very acidic conditions (pH < 4.0), most of the (−COO−) groups were protonated into −COO− (Zohourian and Kabiri 2008). So, the gel is neutral at acidic pHs and the polymeric chain flexibility is relatively low because the main anion–anion repulsive forces were eliminated, and as a result, equilibrium water absorbency remarkably decreased.

At a relatively higher pH (pH > 4.0), the polymeric network carboxylic acid’s groups ionize to −COO−, attracting cations into the gel to interchange H+ ions. As a result, the proportion of free ions within the composite effectively raises. Therefore, because of the ionic swelling pressure increase, the nanocomposite tends to expand and thereby maximizes the repulsion between the ionized polycarboxylate groups. At pH 10, water absorbency maximum limit of the prepared SANC has been achieved. At this point, totally −COOH groups are converted to −COO−. Increasing the anion density in the prepared nanocomposite results in high swelling capacity. The raise of ionic strength and shielding effect may be responsible for the decreased swelling capacity beyond pH 10 (Flory 1953). The −COO− groups are shielded by the Na+ cations from NaOH at high pH, preventing perfect anion–anion repulsion (Lee and Wu 1996).
3.7. Water retention in soil

In agricultural and horticultural applications, superabsorbent has been successfully utilized as a soil supplement to improve the physical attributes of soils to increase the water-holding capacity (Zohuriaan-Mehr et al. 2010). It can increase seedling survival rates and accelerate plant growth. The water retention capacity of the prepared superabsorbent composite in the soil at different dosages is manifested in Figure 2(C).

The rate of water reduction in soil containing the superabsorbent is slower than in soil that does not contain the superabsorbent. The rate of water retention in 0.5% superabsorbent-dosed soil was prolonged to 37 days, but without the superabsorbent, the soil lost all of the water it had absorbed within 14 days. After 37 days, 6.5% of the initial absorbed water can be found when 0.5% superabsorbent was used. Therefore, the use of ACNPs-NC-bentonite-SANC in the soil improves its water retention capability.

3.8. Soil burial degradation

Figure 2(D) illustrates the biodegradability of the produced superabsorbent composite over time. As can be observed, the weight loss of the sample rose as the degradation time grew, and after 154 days, the weight loss of the sample in soil was 50%. As a result, the biodegradability of the superabsorbent composite prepared in this study was found to be satisfactory.

3.9. Growth attributes of Faba bean

Data shown in Figure 3 represent the effect of soil amendment with 0.5% (w/w) superabsorbent nano-composite (SANC) on growth attributes of water-stressed Faba bean. The exposure of Faba bean plants to water stress significantly caused a pronounced reduction in the growth attributes. Whereas water stress (40% FC) had resulted in 5.56, 35.15 and 43.01% decreases in shoot height, fresh mass and dry...
mass, respectively as compared with the non-stressed control (70% FC). In the meantime, 40% FC drastically affected root attributes, as it resulted in 29.06, 36.26 and 38.33% reductions in root depth, fresh mass, and dry mass, respectively. Nonetheless, soil amendment with SANC positively enhanced the growth attributes of both water treatments; non-stressed and stressed, particularly the shoot and root dry masses as compared to SANC non-amended treatments.

The reduction in the growth rate due to water stress in the shoot and the root of Faba bean could be ascertained to the loss of turgidity, decrease in relative water content, reduction in cell division and elongation, the decline of net CO₂ assimilation rate, stomatal conductance and transpiration rate (Abid et al. 2017). Furthermore, it has been documented that prolonged water stress deleteriously influences nutrient uptake and metabolism, partitioning of photoassimilates in addition to limiting energy availability for nitrogen assimilation (Saud et al. 2017). Faba bean shoot and root growth improvement by the application of SANC could be due to its role in improving water holding capacity and available soil water (Abdallah 2019), in addition to the role of SANC in uptake then gradually release of water and nutrients, resulting in increased production of biomass and decreased ROS accumulation (Kargar et al. 2017).

3.10. Photosynthetic pigments and photosynthetic performance

From the data expressed in Figure 4, it is clear that the exposure of Faba bean to water stress negatively affected chlorophyl content. The exposure to water stress (40% FC) had resulted in 12.31 and 25.31% decreases in Chl a and Chl b, respectively. Meanwhile, carotenoids non-significantly increased (2.96%) with the exposure to water stress compared to the fully irrigated control. Soil amendment with SANC non-significantly affected photosynthetic pigments (chlorophyl and carotenoids) content, except Chl b which showed a 29.16% increase due to the treatment with SANC.

The decreased content of chlorophyl in Faba bean leaves under water stress could be attributed to the excessive production of ROS, which in turn destroys the photosynthetic apparatus, inactivates chlorophyl biosynthesizing enzymes and catalyzes the activation of the chlorophyllase enzyme. Further, the increased carotenoids biosynthesis in response to water deficit suggested that Faba bean plants have a good photoprotective defense mechanism against water stress. It is essential for plants to suppress the development of reactive oxygen species. By quenching triplet chlorophyl, carotenoids help to minimize singlet oxygen production in photosynthetic tissues (Farooq et al. 2009). The presence of SANC in the water-stressed soil slightly affected photosynthetic pigments. SAC had previously been shown to have a non-significant effect on leaf chlorophyl content by Tongo et al. (2014) in Acacia victoriae.

Regarding photosynthetic performance (Fv/Fm), water stress non-significantly decreased Fv/Fm compared to the fully irrigated control. However, SANC treatment, either with normal or water-stressed Faba bean, had resulted in a significant increase in Fv/Fm, compared to the corresponding controls. This could be attributed to that application of SANC resulted in better and more effective water and nutrients use efficiencies, increasing the available water and light-harvesting complex in Faba bean leaves. A similar result was obtained in water-stressed maize, where the application of SAP effectively maintained a high net photosynthetic rate and decreased leaf stomatal transpiration under drought.
conditions (Yang et al. 2019). Another reason for increased photosynthetic performance following soil amendment with SANC, even in the case of water scarcity, may be the defensive role of SANC on chloroplast membranes through continuous moistening of the cellular organelles.

3.11. Enzymatic antioxidant activity

The effect of soil amendment with SANC on the activity of six antioxidant enzymes; catalase (CAT), peroxidase (POD), glutathione reductase (GR), ascorbate peroxidase (APX), superoxide dismutase (SOD) and polyphenol oxidase (PPO), of water-stressed (40% FC) Faba bean is represented in Figure 5. As shown from data, water stress had resulted in 14.28, 24.59, 54.29 and 40.59% decreases in CAT, APX, SOD and PPO activities, as compared to the non-stressed control. Meanwhile, this treatment had resulted in 58.08 and 60.56% increases in POD and GR activities, respectively. The pre-sowing addition of SANC to the soil did not affect CAT and APX activities of Faba bean, but significantly increased POD, GR, SOD and PPO activities relative to the control activities. In the case of water-stressed treatment, SANC application effectively increased the activities of CAT, GR, SOD and PPO, but decreased those of POD and APX.

Similar to our findings, Kabiri et al. (2018) showed that drought stress (40% FC) profoundly raised the activities of SOD, CAT and APX in Moldavian balm leaves. Li et al. (2019) showed that the increased activities of these antioxidant enzymes were triggered by the increased oxidative stress attained by water stress, consequently the increased overexpression of the genes encoding these enzymes. Furthermore, Farooq et al. (2009) stated that drought tolerance is conferred by the plants ability to produce various types of antioxidant enzymes to mitigate the oxidative damage. Thereby, the increased CAT, APX, SOD and PPO activities could be used as a judge on the capacity of Faba bean to resist the adverse effects of drought stress, as the balance between the antioxidant

![Figure 5. Impact of soil amendment with SANC on enzymatic antioxidant activity of water-stressed faba bean. Similar letters indicate non-significant variations at 0.05 level.](image-url)
enzymes and ROS production determines whether oxidative signaling and/or damage will occur (Møller et al. 2007). On the other hand, the decreased POD activity under various stress conditions has been described in many crop species like sorghum and sunflower (Zhang and Kirkham 1996), alfalfa (Wang et al. 2009) and common bean (Türkan et al. 2005). This result could be considered as an indicator that Faba bean relies on CAT more than POD in the elimination of excessive H$_2$O$_2$. GR plays a fundamental role in restoring the reduced glutathione (GSH) pool occurring in the antioxidant response depending on the NADPH pool of the stressed plant. Hence, the reduction in GR activity in water-stressed Faba bean could be attributed to the lower usage of NADPH, which is mainly hindered by plants’ exposure to water stress (Hajiboland 2014).

The increased antioxidant enzymes activity by adding the SANC to the stressed plants had been explained by Islam et al. (2011), who reported that when used under drought stress, SAC ensures more available water for the plant and reduces oxidative stress at the phyto-physiological level, resulting in improved growth and redox homeostasis. In harmony with our results, Nazarli et al. (2011) reported that the application of SAC to the water-stressed sunflower had reduced the activity of some antioxidant enzymes (APX and POD). Eneji et al. (2013) concluded that plants subjected to water deficit and treated with SAC showed reduced stress symptoms (decreased enzymatic activities) based on SOD, CAT, POD, APX and GR activities in maize leaves.

3.12. Ascorbic acid, DPPH activity and PMA of Faba bean

The effect of soil conditioning with SANC on the content of ascorbic acid (AsA), free radical scavenging activity (DPPH$^*$), and the total antioxidant capacity (PMA) of the water-stressed Faba bean is illustrated in Figure 6. The exposure of Faba bean to water stress (40% FC) significantly increased AsA content (65.96%), DPPH activity (5.58%), and PMA (12.17%) compared to the normally grown plants. The soil-added SANC slightly decreased AsA and PMA, but merely increased DPPH of normally grown Faba bean plants. Nevertheless, the pre-mixing of SANC with the soil before drought exposure resulted in the decline in AsA, DPPH and PMA as compared to the SANC non-supplemented plants.

Plants benefit from ascorbic acid as an antioxidant because it supports cell growth, division, differentiation, and metabolism (Athar et al. 2009). Furthermore, AsA has been accounted to upregulate the enzymatic antioxidant system (CAT, POD and SOD), sustain nutrients and water homeostasis and protect photosynthetic machinery against the imposed oxidative stress (Athar et al. 2009). Ascorbic acid has also been linked to the regulation of plant defense genes and the modulation of plant growth through phytohormone signaling (Pastori et al. 2003). In several crop species, higher levels of DPPH activity have been linked to improved water stress tolerance (Kang and Saltveit 2002). Water stress potently increased the DPPH activity of Thymus vulgaris, according to Khalil et al. (2018), and this result has been accredited to the induction of polyphenols, especially flavonoids. Furthermore, plant resistance to various stresses has been related to antioxidant capacity and increased antioxidant levels, which protect plants from stress-induced damage. Furthermore, total antioxidant capacity (PMA) increased by water stress could help the plant cope with oxidative stress and sustain normal growth under stressful conditions.

According to the findings of our study, the application of SANC to well-watered or water-stressed Faba bean plants decreased AsA, DPPH, and PMA. This suggests that SANC has a beneficial impact on Faba bean development, as well as making plants more tolerant to water stress conditions that can occur during vegetation growth, by providing additional protection against water stress through lowering ROS production and increasing the antioxidative capacity of stressed plants, hence reducing oxidative damage.
3.13. H$_2$O$_2$ content and lipid peroxidation level of Faba bean

The impact of pre-sowing soil conditioning with SANC on H$_2$O$_2$ content as an oxidative marker and malondialdehyde (MDA) as a product of lipid peroxidation of cellular membranes in water-stressed Faba bean is epitomized in Figure 7. As evident from the results, water stress (40% FC) significantly increased the accumulation of H$_2$O$_2$ and MDA in Faba bean leaves. This treatment had resulted in 131.17 and 29.95% increases in H$_2$O$_2$ and MDA contents, respectively. On the other hand, soil amendment with SANC resulted in a pronounced decline in H$_2$O$_2$ and MDA contents of both normal and stressed Faba bean plants.

Under water stress, ROS-induced oxidative damage occurs frequently, resulting in membrane lipid peroxidation at the cellular and subcellular levels, lowering growth and biomass production as well as seed yield (Habib et al. 2020). In the present investigation, increased MDA and H$_2$O$_2$ concentrations in Faba bean under water stress were reported in other plant species including wheat (Habib et al. 2020), mung bean (Ali et al. 2018) and sugar beet (Ghaffari et al. 2019). Accordingly, the elevated lipid peroxidation and H$_2$O$_2$ levels arise from higher oxidation stress due to the disturbance in the enzymatic response in growing plants undergoing water stress (Ghaffari et al. 2019). In contrast, the reduced MDA and H$_2$O$_2$ concentrations in water-stressed plants treated with SANC are due to the positive effects of SANC in offering good hydraulic status in the rhizosphere of the stressed plants. As a result, the antioxidative defense system activities of APX, SOD, and PPO are thought to be responsible for the decreased levels of H$_2$O$_2$ and MDA in water-stressed plants. Our results suggested that SANC-treated Faba bean plants expressed enhanced APX, SOD and PPO production with the concomitant down-regulation of H$_2$O$_2$ and MDA production.

3.14. Soluble proteins and sugars contents of Faba bean

The data shown in Figure 8 represent the effect of the SANC on the level of soluble proteins and sugars in water-stressed Faba bean leaves. Water stress treatment oppositely affected soluble proteins and sugars contents; it resulted in a 71.62% increase in soluble proteins, but slightly decreased soluble sugars (6.87%) compared to the normally grown control. In the meantime, SANC significantly decreased soluble proteins in Faba bean irrigated with 70 or 40% FC. In the case of soluble sugars, SANC treatment slightly increased its level compared to both the normal and the stressed controls.

Many plant species have experienced changes in protein expression, transcription, and accumulation as a result of water stress since proteins play critical physiological roles in the early stages of growth. Liu et al. (2011) reported that plants enhance their stress resistance by accumulating large quantities of soluble protein and other metabolites to improve cell sap concentration, which can preserve cell turgidity and prevent excessive plasma dehydration. This improvement in soluble protein under water stress was
correlated to the expression of specific protein types called adverse environment or stress protein (Guo et al. 2018). Furthermore, Zhong et al. (2017) postulated that soluble protein increment as induced by water stress could partially result from the decline in glutamate dehydrogenase activity. The slight decline in soluble sugars level by water stress could be elucidated by the disturbance in net photosynthetic assimilation rate, partial inhibition of Rubisco activity, drought-induced stomatal closure, dehydration of the protoplasm and the decrease in leaf area of water-stressed plants.

SANC treatment significantly reduced soluble proteins while increased soluble sugars as compared to water deficit treatment. The incorporation of proteins in structural roles such as protoplasm, cellular membranes, and enzyme formation, as well as functional roles such as cell elongation, active transport, and photosynthates assimilation, could account for the decrease in soluble proteins level. In contrast, the increased soluble sugars in Faba bean leaves due to the addition of SANC could be ascertained to the improved hydraulic status of the stressed plants, which could be reflected in sustaining the activity of photosynthesizing enzymes, increased photosynthetic carbon fixation and eliminating the oxidative injury to the photosynthetic apparatus.

4. Conclusion

We found that co-mixing SANC before exposing Faba bean plants to water stress resulted in substantial changes in growth characteristics, pigmentation, antioxidant status, stress markers, as well as nitrogen and carbon metabolism of water-stressed Faba bean plants in this study. Our findings propose that the biodegradable and environmentally friendly SANC has the potential to imbibe water in its matrix and subsequently release it in the rhizosphere according to the plant requirements, enabling prolonged periods of root moistening and promoting the plant growth. Furthermore, through promoting growth and antioxidant homeostasis of water-stressed plants, the application of SANC effectively reduced drought consequences on Faba bean. So, while this study can be viewed as an extension of existing concepts regarding the role of SACs in drought mitigation, additional large-scale research is needed to better comprehend the influence of these polymers on soil structure and microbial community.

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Author contribution statement

E-RK involved in conceptualization, methodology, software, data curation, original draft preparation, software and validation. MR involved in conceptualization, visualization, supervision and reviewing. AH involved in conceptualization, methodology, software, data curation, original draft preparation, software and validation. SS involved in conceptualization, visualization, software, data curation, original draft preparation and investigation. DG involved in conceptualization, visualization, methodology, investigation validation and original draft preparation. KS involved in conceptualization, visualization, methodology, investigation validation, software, original draft preparation, reviewing and editing.

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**References**
Abbas GH, Kurniawan S, et al. 2017. Superabsorbent nanocomposite synthesis of cellulose from rice husk grafted poly (acrylate acid-co-acrylamide)/Bentonite. Mater Sci Eng. 188:12024.
Abdallah AM. 2019. The effect of hydrogel particle size on water retention properties and availability under water stress. Int Soil Water Conserv Res. 7:275–285.
Abd Ellah RG. 2020. Water resources in Egypt and their challenges, Lake Nasser case study. Egypt J Aquat Res. 46:1–12.
Abdel Latief AA, Hanansuzumam M, Tahjib-Ul-Arif M. 2021. Mitigation of salinity stress by exogenous application of cytokinin in faba bean (Vicia faba L.). Not Bot Horti Agrobot Cluj-Napoca. 49:1–22.
Abid G, Hessini K, Aouida M, et al. 2017. Agro-physiological and biochemical responses of faba bean (Vicia faba L. var. ‘minor’) genotypes to water deficit stress. Biotechnol Agron Soc Environ. 21:146–159.
Ahmed D, Baig H, Zara S. 2012. Seasonal variation of phenolics, flavonoids, antioxidant and lipid peroxidation inhibitory activity of methanolic extract of Eryngium maritimum. J Food Sci Technol. 30:609–617.
Ahmad D, Baig H, Zara S. 2012. Seasonal variation of phenolics, flavonoids, antioxidant and lipid peroxidation inhibitory activity of methanolic extract of melilotus indicus and its sub-fractions in different solvents. Int J Phytomedicine. 4:326–332.
Ahmed MR, Amin SM, Rahman S. 2019. Natural cellulose-chitosan cross-linked superabsorbent hydrogels with superior swelling properties. ACS Sustain Chem Eng. 7:8736–8742.
Ali Q, Jawed MT, Noman A, et al. 2018. Assessment of drought tolerance in mung bean cultivars/lines as depicted by the activities of germination enzymes, seedling’s antioxidative potential and nutrient acquisition. Arch Agron Soil Sci. 64:84–102.
Athar HuR, Khan A, Ashraf M. 2009. Inducing salt tolerance in wheat by exogenously applied ascorbic acid through different modes. J Plant Nutr. 32:1799–1817.
Barreto PLM, Pires ATN, Soldi V. 2003. Thermal degradation of edible films based on milk proteins and gelatin in inert atmosphere. Polym Degrad Stab. 79:147–152.
Beauchamp C, Fridovich I. 1971. Superoxide dismutase: improved assay and an assay applicable to acrylamide gels. Anal Biochem. 44:276–287.
Beyer WE, Fridovich I. 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem. 161:539–566.
Bokobza L. 2017. Mechanical and electrical properties of elastomer derived from plants, algae, and fungi. J Geosci Environ Prot. 5:27–39.
Bokobza L. 2017. Mechanical and electrical properties of elastomer derived from plants, algae, and fungi. J Geosci Environ Prot. 5:27–39.
Bonet V, Brand-Williams W, Besert C. 1997. Kinetics and mechanisms of antioxidative activity using the DPPH free radical method. LWT - Food Sci Technol. 30:609–615.
Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72:248–254.
Bruzzone DA, Landman M, Olsson RT, et al. 2020. Carboxylated wheat gluten proteins: a Green solution for production of sustainable superabsorbent materials. Biomacromolecules. 21:1709–1719.
Chaudhuri SD, Mandal A, Dey A, Chakraborty D. 2020. Tuning the swelling and rheological attributes of bentonite clay modified starch grafted polyacrylic acid based hydrogel. Appl Clay Sci. 185:105405.
Dubois M, Gilles K, Hamilton J, et al. 1965. Colorimetric method for determination of sugars and related substances. Anal Chem. 28:350–356.
Ennej AF, Islam R, An P, Amalu UC. 2013. Nitrate retention and physiological adjustment of maize to soil amendment with superabsorbent polymers. J Clean Prod. 52:474–480.
Etemadi F, Baker A V, Hashemi M, et al. 2018. Nutrient accumulation in faba bean varieties. Commun Soil Sci Plant Anal. 49:2064–2073.
Farooq M, Wahid A, Kobayashi N, et al. 2009. Plant drought stress: effects, mechanisms and management. Agron Sustain Dev. 29:185–212.
Flory PJ. 1953. Principles of polymer chemistry, first edit. Ithaca: Cornell University Press.
Fuente E, Menéndez JA, Diez MA, et al. 2003. Infrared spectroscopy of carbon materials: a quantum chemical study of model compounds. J Phys Chem B. 107:6530–6539.
Ghaffari H, Tadayon MR, Nadeem M, et al. 2019. Proline-mediated changes in antioxidant enzymatic activities and the physiology of sugar beet under drought stress. Acta Physiol Plant. 41:1–13.
Grande CJ, Torres FG, Gomez CM, et al. 2009. Development of self-assembled bacterial cellulose-starch nanocomposites. Mater Sci Eng C. 29:1098–1104.
Guo YY, Yu HY, Yang MM, et al. 2018. Effect of drought stress on lipid peroxidation, osmotic adjustment and antioxidant enzyme activity of leaves and roots of lycium ruthenicum murr. seedling. Russ J Plant Physiol. 65:244–250.
Habib N, Ali Q, Ali S, et al. 2020. Use of nitric oxide and hydrogen peroxide for better yield of wheat (triticum aestivum L.) under water deficit conditions: growth, osmeregulation, and antioxidative defense mechanism. Plants. 9:285–308.
Hajboland R. 2014. Reactive oxygen species and photosynthesis. In: Ahmad P. editor. Oxidative damage to plants. New York: Elsevier; p. 1–63.
Halliwell B, Foyer CH. 1978. Properties and physiological function of a glutathione reductase purified from spinach leaves by affinity chromatography. Planta. 139:9–17.
Heath RLR, Packer L. 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys. 125:189–198.
Hosseinzadeh H, Ramin S. 2018. Fabrication of starch-graft-poly (acrylamide)/graphene oxide/hydroxyapatite nanocomposite hydrogel adsorbent for removal of malachite Green dye from aqueous solution. Int J Biol Macromol. 106:101–115.
Huang Y, Lu J, Xiao C. 2007. Thermal and mechanical properties of cat-ionic guar gum/poly (acrylic acid) hydrogel membranes. Polym Degrad Stab. 92:1072–1081.
Islam MR, Xue X, Mao S, et al. 2011. Effects of water-saving superabsorbent polymer on antioxidant enzyme activities and lipid peroxidation in oat (Avena sativa L.) under drought stress. J Sci Food Agric. 91:680–686.
Kabiri K, Zohuriaan-Mehr MJ. 2004. Porous superabsorbent hydrogel composites: synthesis, morphology and swelling rate. Macromol Mater Eng. 289:653–661.
Kabiri R, Hatami A, Oloumi H, et al. 2018. Foliar application of melatonin induces tolerance to drought stress in Moldavian balm plants (Dracocephalum moldavica) through regulating the antioxidant system. Folia Hortic. 30:155–167.
Kang HM, Saltveit ME. 2002. Antioxidant enzymes and DPPH-radical scavenging activity in chilled and heat-shocked rice (Oryza sativa L.) seedlings radicles. J Agric Food Chem. 50:513–518.
Kargar M, Suresh R, Legrand M, et al. 2017. Reduction in water stress for tree saplings using hydrogels in soil. J Geosci Environ Prot. 05:27–39.
Kassem I, Kassab Z, Khouloud M, et al. 2020. Phosphoric acid-mediated green preparation of regenerated cellulose spheres and their use for all-cellulose cross-linked superabsorbent hydrogels. Int J Biol Macromol. 162:136–149.
Khalil N, Fekry M, Bishr M, et al. 2018. Foliar spraying of salicylic acid induced accumulation of phenolics, increased radical scavenging activity and modified the composition of the essential oil of water stressed Thymus vulgaris L. Plant Physiol Biochem. 123:65–74.
Kumar KB, Khan PA. 1982. Peroxidase and polyphenol oxidase in Thymus vulgaris L. Plant Physiol Biochem. 20:412–416.
Lee W-F, Wu R-J. 1996. Superabsorbent polymeric materials. I. swelling behaviors of crosslinked poly(sodium acrylate-co-hydroxyethyl methacrylate) in aqueous salt solution. J Appl Polym Sci. 62:1099–1114.
Li J, Yang Y, Sun K, et al. 2019. Exogenous melatonin enhances cold, salt and drought stress tolerance by improving antioxidant defense in tea plant (Camellia sinensis (L.) O. Kuntze). Molecules. 24:1826–1840.
Grande CJ, Torres FG, Gomez CM, et al. 2009. Development of self-
