

Supplementary Information

The application of melatonin and organic waste derived from vitamin C industry effectively promotes seed germination and seedling growth of cotton in saline-alkali soil.

Methods

Determination of seedling physiological parameters

Crude enzyme: (A) Weigh 0.2 g of fresh leaf with primary veins from one or two young leaves, grind them with a mortar and pestle in liquid nitrogen. (B) Homogenize the leaf powder by adding 3 ml of 100 mM PBS buffer (pH 7.8). (C) Transfer the homogenate to two 1.5 ml centrifuge tubes and centrifuge at 10,000×g for 20 min at 4 °C. (D) Transfer the supernatant to new centrifuge tubes for further analysis. (E) Measure the concentration of crude protein (mg/ml) in the supernatant with Analytik Jena ScanDrop 250 by a spectrophotometric method with Formula Warburg-Christian (protein).

Crude enzyme concentration (mg/ml) = $1.55 \times A_{280} - 0.76 \times A_{260}$.

A₂₈₀: the absorbance at 240 nm.

A₂₆₀: the absorbance at 240 nm.

CAT activity: The activity of CAT was measured by monitoring the decomposition of hydrogen peroxide (H₂O₂) at 240 nm. The reaction mixture, prepared with 77.5 µL of 30% H₂O₂ in 50 mL of 100 mM PBS (pH 7.0), was combined with 50 µL of crude enzyme in a cuvette. The light absorption value was recorded every 15 seconds for one minute using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Inc., Waltham, MA, USA).

CAT activity (u/mg protein) = $A_{240} \times (V / V_t) (0.1 \times t) / C_p$.

A₂₄₀: the change of absorbance at 240 nm during every 15 sec

V: total volume of crude enzyme solution

V_t: volume of crude enzyme used in the testing tube

t: reaction time (min)

C_p: crude protein concentration (mg/ml)

0.1: One unit of CAT is defined as the amount of enzyme that decreases 0.1 of absorbance at 240 nm per minute.

POD activity: The activity of POD was determined by measuring the absorbance at 470 nm due to the oxidation of guaiacol. The reaction solution, prepared by mixing 28 μ L of 0.2% guaiacol in 50 mL of 100 mM PBS (pH 7.0) and 19 μ L of 30% H₂O₂, was combined with 50 μ L of crude enzyme. The light absorption value was recorded every 15 seconds for one minute using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Inc., Waltham, MA, USA).

POD activity (u/mg protein) = $A_{470} \times (V / V_t) (0.01 \times t) / C_p$.

A₄₇₀: the change of absorbance at 470 nm during every 15 sec

V: total volume of crude enzyme solution

V_t: volume of crude enzyme used in the testing tube

t: reaction time (min)

C_p: crude protein concentration (mg/ml)

0.01: One unit of POD is defined as the amount of enzyme that increases 0.01 of absorbance at 470 nm per minute.

SOD activity: The activity of SOD was determined by measuring the rate of nitrite production. The reaction mixture, containing 50 μ L of crude enzyme solution in 1 mL of reaction solution, was exposed to light at an intensity of 4,000 lux for 10-15 minutes, with a dark control. The light absorption value at 560 nm was measured using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Inc., Waltham, MA, USA).

SOD activity (u/mg protein) = $[(A_{ck} - A_s) \times V] (0.5 \times A_{ck} \times V_t) / C_p$

A_{ck}: the absorbance at 560 nm of Control II (exposed to light with no crude enzyme)

A_s: the absorbance at 560 nm of candidate sample tube

V: total volume of crude enzyme solution

V_t: volume of crude enzyme used in the testing tube

C_p: crude protein concentration (mg/ml)

0.5: One unit of SOD is defined as the amount of enzyme that inhibits 50% nitroblue tetrazolium photoreduction.

ASA content: ASA content was measured by high-performance liquid

chromatography (HPLC). A fresh leaf sample weighing 0.2 g was immersed in 2.0 mL of a 1.0% (w/v) metaphosphoric acid solution, protected from light, and ground on ice until no obvious tissue remained. The resulting homogenate was then centrifuged at 6000 r/min for 5.0 minutes, and the supernatant was collected and filtered through a filter membrane with a pore size of 0.22 μ m to obtain the test solution. The HPLC (YL9100, Young In Chromass, Anyang-si, Republic of Korea) analysis was performed under the following conditions: mobile phase consisting of NaH₂PO₄ solution (20 mM, pH = 2.8 \pm 0.05) / acetonitrile = 95:5; flow rate was set at 1.0 mL/min; UV detector wavelength was at 243 nm; column (ZORBAX Eclipse Plus C18, Agilent Technologies, Santa Clara, CA, USA) temperature was maintained at 40°C. The content was calculated by a standard curve of 0–50 (0, 5, 10, 25, 50) μ g/mL ASA.

MDA content: MDA content was measured using the TBA (thiobarbituric acid) method. Samples were fully ground in pH 7.8 phosphate buffer, followed by centrifugation at 6000 rpm for 10 minutes. Two milliliters of supernatant was added to the scale test tube, to which 1 mL of 0.5% thiobarbituric acid and 3 mL of 5% trichloroacetic acid solution were added. The solution was heated in a boiling water bath for 10 min and then cooled rapidly. After centrifugation at 6000 rpm for 10 min, the light absorption value was measured at 532 nm and 600 nm using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Inc., Waltham, MA, USA). With distilled water as the blank and 100% light transmittance.

Soluble sugar: The content of soluble sugar was measured by the phenol-sulfuric acid method. Fresh leaf samples (0.5 g) were homogenized in a centrifuge tube through a homogenizer until complete tissue disruption was achieved. Then, 5.0 mL of desalted water was added and the mixture was subjected to boiling in a water bath for 30 minutes, followed by cooling to room temperature with tap water and centrifugation at 6000 r/min for 10 minutes. Subsequently, 1.0 mL of the supernatant was transferred into a stopper test tube. To this, 0.5 mL of a 9.0% (w/v) phenol solution and 2.5 mL of concentrated sulfuric acid were added, thoroughly mixed by shaking, and then boiled again in a water bath for an additional period of 15 minutes. After cooling to room temperature with tap water, the absorbance was detected at 490 nm using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The content was calculated by a standard curve of 0–80 (0, 20, 40, 60, 80) μ g/mL glucose.

Soluble protein: The content of soluble protein was measured by the Coomassie brilliant blue method. After thoroughly grinding 0.5 g of fresh leaf sample using a homogenizer until no visible tissue remained, 5.0 mL of desalted water was added to

the tube. Following centrifugation at 6000 r/min for 5.0 minutes, the supernatant was collected for further analysis. Then, 1.0 mL of the test solution and 5.0 mL of Coomassie brilliant blue solution were mixed thoroughly and the absorbance value was measured at a wavelength of 595 nm using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Waltham, MA, USA). The content was calculated by a standard curve of 0-100 (0, 20, 40, 60, 80, 100) $\mu\text{g/mL}$ bovine serum albumin.

Proline content: The proline content is determined by the following methods. First, 0.3-g samples were cut into pieces and added to a mortar, to which an appropriate amount of 80% (v/v) ethanol and a small amount of quartz were added prior to grinding the tissue into a homogenate. Then, the volume was filled with 80% ethanol to 25 mL and incubated in an 80°C watered bath for 20 minutes, after which 0.4 g of artificial zeolite and 0.2 g of activated carbon were added. Samples were subsequently oscillated and filtered, and 2 mL of the above extraction solution was transferred into a test tube, to which 2 mL of glacial acetic acid and 2 mL of indanone were added before being heated in boiling water for 15 min. After cooling, the light absorption values of the samples were measured at 520 nm using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Determination of soil characteristics

Soil catalase activity: 5.0 g air-dried soil after passing through a 1 mm sieve was weighed into a stoppered conical flask (using a flask without soil as a blank control). and then 0.5 mL of toluene was added, mixed well, and the flask was placed in a 4°C refrigerator for 30 minutes. 25 mL of 3% hydrogen peroxide solution, pre-chilled in the refrigerator, was then rapidly added and mixed thoroughly. The mixture was returned to the refrigerator for 1 hour, after that, 25 mL of 2 M H_2SO_4 solution, also pre-chilled, was swiftly added, mixed well, and filtered through neutral filter paper. 1 mL of the filtrate was added in a conical flask, and 5 mL of distilled water and 5 mL of 2 M H_2SO_4 solution were added, and then was titrated with 0.02 M potassium permanganate solution. The catalase activity was determined by the difference in titration between the control and the sample, representing the amount of decomposed H_2O_2 and the corresponding consumption of KMnO_4 . The activity was expressed as the volume of 0.1 M KMnO_4 consumed per gram of dry soil per hour (in mL).
$$\text{Enzyme Activity (mL 0.1 M KMnO}_4) / (\text{h} \cdot \text{g}) = (\text{Blank sample residual hydrogen peroxide titration volume} - \text{Soil sample residual hydrogen peroxide titration volume}) / \text{Soil sample mass}.$$

Soil protease activity: 4.0 g air-dried soil after passing through a 1 mm sieve was

weighed into a stoppered conical flask (using a flask without soil as a blank control). Then 20 mL of 1% (w/v) casein solution and 1 mL of toluene were added, carefully mixed, then tightly sealed with a wooden stopper and incubated at 30°C for 24 hours. After incubation, 2 mL of 0.1N sulfuric acid and 12 mL of 20% (w/v) sodium sulfate solution were added to the mixture to precipitate proteins, followed by centrifugation at 6000 rpm for 15 minutes. 2 mL of the supernatant was transferred to a 50 mL volumetric flask. The absorbance was measured at 500 nm using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The content was determined using a standard curve of 0–0.52 µg/mL amino nitrogen solution. The activity of protease was represented by the milligrams of amino nitrogen in 1 gram of soil after 24 hours. Protease activity (mg NH₃-N) = $a \times 5$, where 'a' is the amino nitrogen milligrams obtained from the standard curve, and 5 is the coefficient for converting to 1 g of soil.

Soil sucrase activity: Soil sucrase activity was determined by the absorbance of 3-amino-5-nitro salicylic acid, which is produced by the reaction of reducing sugars with 3, 5-dinitrosalicylic acid. 5.0 g air-dried soil after passing through a 1 mm sieve was weighed into a stoppered conical flask (using a flask without soil as a blank control). Then 15 mL of 8% sucrose solution, 5 mL of pH 5.5 phosphate buffer, and 5 drops of toluene were added, mixed well, and incubated at 37°C for 24 hours. After incubation, the mixture was quickly filtered, and 1 mL of the filtrate was added to a 50 mL volumetric flask, followed by the addition of 3 mL of DNS reagent and heated in a boiling water bath for 5 minutes. The flask was then cooled under tap water for 3 minutes. The solution turned orange-yellow due to the formation of 3-amino-5-nitro salicylic acid and was diluted to 50 mL with distilled water. The absorbance was measured at 508 nm using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Inc., Waltham, MA, USA). Sucrase Activity = $(a \text{ sample} - a \text{ blank}) \times n / m$, where 'a sample' and 'a blank' represent the glucose milligrams obtained from the standard curve, 'n' is the dilution factor, and 'm' is the weight of the air-dried soil.

Soil phosphomonoesterase activity: 1.0 g air-dried soil after passing through a 2 mm sieve was weighed into a stoppered conical flask (using a flask without soil as a blank control). Then 0.2 mL of toluene, 4 mL of pH 6.5 phosphate buffer, and 1 mL of 0.05 M disodium p-nitrophenyl phosphate solution were added. The mixture was gently shaken, sealed, and incubated at 37°C for 1 hour. After incubation, the flask was uncapped, and 1 mL of 0.5 M CaCl₂ solution and 4 mL of 0.5 M NaOH solution were added, gently mixed for a few seconds, and then filtered. The absorbance was measured at 410 nm using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The content was calculated using a standard

curve of 0 – 0.005 mg/ mL p-aminophenol solution (Shanghai Yuanye Co., Ltd., Shanghai, China). The activity of phosphomonoesterase was represented by the absorbance of p-nitrophenol produced per unit time. Phosphomonoesterase activity = m_1 / m_2 , where m_1 is the mass of p-nitrophenol in the test solution (mg), and m_2 is the mass of the soil sample (g).

Soil urease activity: 5.0 g air-dried soil after passing through a 1 mm sieve was weighed into a stoppered conical flask (using a flask without soil as a blank control). Then 1 mL of toluene was added and left for 15 minutes, followed by the addition of 10 mL of 10% (w/v) urea solution and 20 mL of citrate buffer solution (pH 6.7), mixed thoroughly. The flask was then incubated at 37°C for 24 hours. After incubation, the mixture was diluted with water heated to 38°C to the mark (100 mL), shaken well, and filtered through a dense filter paper into a conical flask. 3 mL of the filtrate was added to a 50 mL volumetric flask, mixed with 10 mL of distilled water, then added 4 mL of 1.35 M sodium phenolate solution, mixed thoroughly, followed by the addition of 3 mL of 0.9% (w/v) Sodium hypochlorite solution, shaken well, and left for 20 minutes. The solution was diluted to the mark with water, turning a phenolphthalein blue color. The absorbance was measured at 578 nm using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Inc., Waltham, MA, USA) within one hour. The content was calculated using a standard curve of 0 – 0.0026 mg/mL nitrogen standard solution. Urease activity was expressed as the milligrams of $\text{NH}_3\text{-N}$ in 1 g of soil after 24 hours. Urease activity ($\text{mg NH}_3\text{-N}$) = $a \times 2$, where 'a' is the $\text{NH}_3\text{-N}$ milligrams obtained from the standard curve, and 2 is the coefficient for converting to 1 g of soil.

Ammonium nitrogen content: 2.0 mL of soil combined extractant was aspirated into a glass vial as a blank. Another 2.0 mL of soil mixed standard solution was aspirated into a separate glass vial, and 2.0 mL of soil extract filtrate was aspirated into a third glass vial. Sequentially, 6 drops of soil ammonium nitrogen mask agent, 3 drops of soil ammonium nitrogen coloring agent, and 4 drops of soil ammonium nitrogen developer were added. After shaking well and standing for 10 minutes, the absorbance was measured at 420 nm using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Inc., Waltham, MA, USA). Ammonium nitrogen content (mg/kg) = $A_2 / A_1 \times 48.0$, where A_1 is the absorbance of the soil mixed standard solution, and A_2 is the absorbance of the soil extract filtrate.

Nitrate nitrogen content: 5.0 g of air-dried soil was weighed and added to a container with 50 mL of 2 M KCl solution. After shaking for 1 hour, the suspension was allowed to stand for 3 to 5 minutes before filtration. The absorbance was measured at 220 nm and 275 nm using a Multiskan Spectrum Photometer (1500, Thermo Fisher

Scientific, Inc., Waltham, MA, USA). The corrected absorbance A was calculated as $A_{220} - A_{275}$. A standard curve was prepared with nitrate nitrogen standard solutions ranging from 0 to 1.4 mg/L in 50 mL volumetric flasks, diluted with redistilled water, and the absorbance A was determined at 220 nm and 275 nm. Nitrogen content (mg/kg) = $C \times V / m$, where C is the amount of $\text{NH}_3\text{-N}$ obtained from the standard curve, V is the volume of the extractant (mL), and m is the mass of the soil sample (g).

Available phosphorus content: 2.0 mL of the combined extractant was aspirated into a glass vial as a blank. Another 2.0 mL of soil mixed standard solution was aspirated into a separate glass vial, and 2.0 mL of soil extract filtrate was aspirated into a third glass vial. Then, 4 drops of soil available phosphorus mask agent were added, shaken well until bubble-free, followed by the addition of 5 drops of soil available phosphorus coloring agent and 1 drop of soil available phosphorus reducer. After shaking well and standing for 10 minutes, the absorbance of the soil mixed standard solution and soil extract filtrate was measured at 685 nm using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Inc., Waltham, MA, USA). Available phosphorus (P_2O_5) content (mg/kg) = $A_2 / A_1 \times 48.0$, where A_1 is the absorbance of the soil mixed standard solution, and A_2 is the absorbance of the soil extract filtrate.

Available potassium content: 2.0 mL of the combined extractant was aspirated into a glass vial as a blank. Another 2.0 mL of soil mixed standard solution was aspirated into a separate glass vial, and 2.0 mL of soil extract filtrate was aspirated into a third glass vial. Then, 2 drops of soil available potassium mask agent, 6 drops of soil available potassium assistant, and 4 drops of soil available potassium turbidity agent were added. After shaking well, the absorbance was immediately measured at 685 nm using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Inc., Waltham, MA, USA). Available potassium (K_2O) content (mg/kg) = $A_2 / A_1 \times 200.0$, where A_1 is the absorbance of the soil mixed standard solution, and A_2 is the absorbance of the soil extract filtrate.

Soil organic matter content: The low-temperature external heat dichromate oxidation-colorimetric method was used. 1 g air-dried soil after passing through a 0.149 mm sieve was weighed and placed into a 50 mL standard tube. Then 5 mL of 0.8 M potassium dichromate solution and 5 mL of concentrated H_2SO_4 were added, mixed well, and then placed in a 100°C constant temperature oven for 90 minutes. After cooling in a water bath, water was added in two portions to reach 50 mL using a syringe, mixed well, and left overnight. The supernatant was taken up to a 10 mM cuvette for colorimetry, and the absorbance was measured at 685 nm using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Inc., Waltham, MA,

USA). The content was calculated using a standard curve of organic carbon standard solution concentrations from 0 to 0.3 mg/mL. Organic matter (% OM) = $(m_1 \times 1.724 \times 1.08 / m \times 103) \times 100$, where m_1 is the carbon content (mg) determined from the standard curve, 1.724 is the coefficient for converting organic carbon to organic matter, and m is the mass of the soil sample (g).

m_1 : the carbon content (mg) of the soil sample determined by the standard curve.

1.7240: the coefficient for converting organic carbon to organic matter.

m : the mass (g) of the soil sample

Soil cation exchange capacity (CEC): The trichloro hexaammine cobalt extraction-colorimetric method was used. 2.5 g air-dried soil after passing through a 2 mm sieve was weighed and placed into a 100 mL centrifuge tube, then 50.0 mL of 0.0167 M trichloro hexaammine cobalt extractant was added. The tube was sealed and shaken at $20 \pm 2^\circ\text{C}$ for 1 hour, followed by centrifugation at 4000 r/min for 10 minutes to collect the supernatant. The supernatant was taken up to a 10 mM cuvette for colorimetry, and the absorbance was measured at 475 nm using a Multiskan Spectrum Photometer (1500, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The content was calculated using a standard curve of trichloro hexaammine cobalt extractant concentrations from 0 to 4.458 mg/mL. The effective cation exchange capacity (CEC) (cmol+/kg) = $[300 \times (C_0 - C) \times V] / m$, where CEC is the effective cation exchange capacity of the soil sample (cmol+/kg).

C_0 : the blank test concentration (M),

C : the sample concentration (M),

V : the volume of the extractant (mL)

M : the mass of the soil sample (2.50 g).

Soil pH: 10.0 g air-dried soil after passing through a 2 mm sieve was weighted and placed into a 50 mL beaker. 25 mL of carbon dioxide-free water was added, and the mixture was vigorously stirred with a glass rod for 1 to 2 minutes, then allowed to stand for 30 minutes. The pH was then measured using a pH meter (PHS-3, Lei Ci, Shanghai, CN).

Ammonia nitrogen standard solution: Dissolved 0.1 g glycine ($\text{C}_2\text{H}_5\text{NO}_2$) in reverse osmosis water (RO), adjusted the volume to 1L, resulting in a standard solution containing 0.02 mg of ammonia nitrogen per 1 mL. Further diluted this solution 10 times to prepare the standard solution.

Nitrogen standard solution: Weighed 0.4717 g ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$, dissolved it in reverse osmosis water (RO), and dilute to 1000 mL, that was the standard solution containing 0.1mg of nitrogen per 1 mL.

Nitrate nitrogen standard solution: Weighed 0.722 g potassium nitrate (KNO_3) that had been dried and cooled in a 105°C oven, place it in a small beaker, added distilled water to dissolve, transfer quantitatively to a 1 L volumetric flask, adjusted the volume and mixed well to obtain a nitrate nitrogen standard solution of 100 mg/L. Take 10 mL of this solution and dilute to 100 mL in a 100 mL volumetric flask, adjusted the volume and mixed well to obtain a nitrate nitrogen standard solution of 10 mg/L.

Soil combined extractant: Weighed 53.12 g anhydrous sodium sulfate (Na_2SO_4) and 37.80 g sodium bicarbonate (NaHCO_3), dissolved them in approximately 800 mL of reverse osmosis water (RO), adjusted the pH to 8.5 with concentrated sulfuric acid solution or sodium hydroxide solution, and adjusted the volume to 1 L with RO.

Soil mixed standard stock solution: Weighed 0.4602 g potassium dihydrogen phosphate (K_2HPO_4), 1.1319 g ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$, 1.7323 g potassium nitrate (KNO_3), and 0.0611 g potassium sulfate (K_2SO_4), dissolved them in approximately 800 mL of reverse osmosis water (RO), added 10.0 mL of concentrated sulfuric acid (H_2SO_4), making them completely dissolved, transferred the solution to a volumetric flask, and adjust the volume to 1 L with RO.

Soil mixed standard solution: 1.0 mL of the soil mixed standard stock solution was diluted to 100.0 mL with the soil combined extractant, mixed well.

Soil extraction filtrate: Weighed 2.5 g air-dried soil sample that had passed through a 2 mm sieve, placed it in a 100 mL conical flask, added approximately 0.5 g of phosphorus-free activated carbon and 50.0 mL of soil combined extractant, tighten the stopper, maintain the temperature at $25^\circ\text{C} \pm 2^\circ\text{C}$, oscillate at a frequency of 220 r/min for 10 minutes, and filtered through neutral filter paper. The filtrate can be used for the rapid determination of soil ammonium nitrogen, available phosphorus, and available potassium.

Soil ammonium nitrogen masking agent: Weighed 400.0 g potassium sodium tartrate ($\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$), dissolved it in approximately 700 mL of reverse osmosis water (RO) (can be heated to assist dissolution); separately weighed 20.0 g sodium hydroxide (NaOH), dissolve it in approximately 100 mL of RO, cooled slightly and added to the potassium sodium tartrate solution, transferred the new solution to a volumetric flask, and adjust the volume to 1 L with reverse osmosis water (RO)

Soil ammonium nitrogen coloring agent: Weighed 50.0 g arabic gum powder, dissolved it in approximately 30 mL of boiling reverse osmosis water (RO); additionally weighed 30.0 g sodium fluoride (NaF), dissolve it in approximately 100 mL of water; mixed the above two solutions, transferred to a volumetric flask, and adjusted the volume to 1 L with decarbonated water. Let it stand overnight, and kept the upper clear liquid for use.

Soil ammonium nitrogen color developer: Weighed 50.0 g potassium iodide (KI), dissolved it in approximately 50 mL of reverse osmosis water (RO), added saturated mercuric chloride (HgCl) solution while stirring, until a small amount of purple-red precipitate appeared and did not dissolve even after thorough stirring. Slowly added 150.0 g potassium hydroxide (KOH), stirred until dissolved, transferred to a 1L volumetric flask while hot, cooled, adjusted the volume, and transferred to a large beaker, let it stand overnight, and kept the upper clear liquid for use.

Soil ammonium nitrogen intensifier: Weighed 300.0 g sodium hydroxide (NaOH), dissolved in approximately 800 mL of reverse osmosis water (RO), cooled to room temperature, transferred to a volumetric flask, and adjusted the volume to 1 L with RO.

Soil available phosphorus masking agent: Measured 305 mL of concentrated sulfuric acid and slowly poured it into a beaker containing approximately 500 mL of reverse osmosis water (RO), let it cool, added 10.0 g sodium tartrate ($\text{C}_4\text{H}_4\text{Na}_2\text{O}_6 \cdot 2\text{H}_2\text{O}$), stirred until dissolved, transferred to a volumetric flask, and adjusted the volume to 1L with RO.

Available phosphorus color developer: Measured 146 mL of concentrated sulfuric acid and dissolved it in approximately 500 mL of reverse osmosis water (RO), let it cool; separately weighed 35.0 g ammonium molybdate $[(\text{NH})_4\text{Mo}_2\text{O}_7 \cdot 4\text{H}_2\text{O}]$ and dissolved it in approximately 200 mL of water; slowly poured the sulfuric acid solution into the ammonium molybdate solution, stirring while adding, mixed well, transferred to a volumetric flask, and adjusted the volume to 1 L with RO.

Available phosphorus reductant: Weighed 20.0 g tin (II) chloride (SnCl_2), dissolved it in 100.0 mL of hydrochloric acid (heat slightly to assist dissolution, shake as little as possible, perform this operation in a fume hood), fully dissolved, transferred to a 1L volumetric flask, and adjusted the volume with glycerin.

Soil Available potassium masking agent: Weighed 5.0 g copper (II) sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and 12.0 g tartaric acid ($\text{C}_4\text{H}_6\text{O}_6$), dissolved in approximately 500 mL of reverse osmosis water (RO), added 200.0 mL of concentrated sulfuric acid, cooled to room temperature, transferred to a volumetric flask, and adjusted the volume to 1

L with RO.

Soil available potassium auxiliary masking agent: Weighed 75.0 g disodium EDTA ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_8\text{Na}_2 \cdot 2\text{H}_2\text{O}$) and 130.0 g sodium hydroxide (NaOH), dissolved in an appropriate amount of reverse osmosis water (RO), cooled, transferred to a volumetric flask, and adjusted the volume to 1 L with RO.

Soil available potassium turbidity agent: Weighed 8.0 g sodium hydroxide (NaOH) and dissolved in approximately 80 mL of reverse osmosis water (RO), cooled and adjusted the volume to 100 mL to obtain a 2 M sodium hydroxide solution for standby; separately weighed 62.5 g sodium tetraphenylborate [$\text{NaB}(\text{C}_6\text{H}_5)_4$], dissolved in approximately 900 mL of RO, added 0.5 mL of the 2 M sodium hydroxide solution, mixed well, transferred to a volumetric flask, adjusted the volume to 1 L with RO, and filtered until the solution was clear.

Organic carbon standard solution: Weighed 1.375 g glucose and dissolved it in reverse osmosis water (RO), adjusted the volume to 100 mL.

Hexaammine cobalt(III) chloride extractant: Weighed 4.458 g hexaammine cobalt (III) chloride [$\text{Co}(\text{NH}_3)_6\text{Cl}_3$], dissolved it in reverse osmosis water (RO), and diluted to 1000 mL, mixed well.