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Synergistic effects of gibberellic acid, biochar, and rhizobacteria on wheat growth under heavy metal and drought stress

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Abstract

Wheat (*Triticum aestivum* L.), a vital crop constituting approximately 20% of global caloric intake, faces significant threats from heavy metal contamination, particularly cadmium (Cd) and chromium (Cr), along with drought stress, jeopardizing global food security. This study aimed to investigate the combined effects of these stressors and the potential of plant growth enhancers such as gibberellic acid (GA3), biochar (BC), and rhizobacteria to improve wheat growth. Conducted in a controlled greenhouse environment at The Islamia University of Bahawalpur, the experiment utilized a completely randomized design with three replications across 72 pots, each filled with clay loam soil. The experimental layout included 24 treatment combinations involving cadmium stress (6 mg/kg), chromium stress (300 and 600 mg/kg), drought stress simulated at -0.8 MPa soil water potential, and various applications of GA3 (200 mg L^{-1}) and biochar (0.6% and 0.9% w/w). Seedlings of *T. aestivum* cv. Dilkash-21, treated with *Agrobacterium fabrum*, showed significant growth metrics, with root lengths of 9.36 cm under 6 mg/kg Cd stress compared to 5.53 cm in controls. The treatment also increased shoot and root fresh weights by 24.7% and 22.5%, respectively, while chlorophyll content peaked at 2.26 mg/g under 6 mg/kg Cd. Additionally, electrolyte leakage decreased to 10.5%, and the vigor index improved to 1586.05 under Cd stress. These findings indicate that utilizing GA3 and biochar can mitigate the adverse effects of environmental stressors on wheat. Future research should focus on the underlying mechanisms of these treatments and explore their application in field conditions to further enhance wheat productivity and resilience against environmental stress.

Keywords Fruit waste biochar, Co-treatment, Environmental stressors, Wheat productivity, Plant vigor

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Introduction

Wheat (*Triticum aestivum* L.), a key member of the Poaceae family, is a staple crop for many countries, playing a crucial role in global food security. Due to its high adaptability, wheat is widely cultivated across various agro-ecological zones. However, a substantial portion of wheat cultivation occurs in rain-fed regions, making it highly vulnerable to fluctuating water availability. This issue is particularly pressing in semi-arid and agriculturally developing countries, which comprise around 37% of the total wheat-producing regions [1]. Given that wheat contributes to approximately 20% of the global caloric intake, the impact of its reduced productivity due to environmental stressors poses a serious threat to food security [2].

One of the most challenging environmental factors affecting wheat productivity is drought stress, which significantly disrupts plant growth, development, and yield. As global climate projections suggest that by 2025 nearly 1.8 billion people will experience water scarcity, with 65% of the population residing in water-stressed regions, the urgency to develop drought-resilient crops becomes critical [3]. Drought stress, primarily caused by prolonged periods of low rainfall, elevated temperatures, or soil water deficits, induces complex physiological, biochemical, and molecular responses in plants, often resulting in severe cellular damage. Water deficiency, for instance, leads to membrane integrity loss and heightened electrolyte leakage, signifying cellular dehydration and damage [4].

In addition to drought, heavy metal stress, particularly from chromium (Cr) and cadmium (Cd), severely affects wheat productivity. Cr, with its dynamic redox states, poses substantial environmental and health risks due to its ease of assimilation into plant tissues, impairing physiological functions and morphology [5–8]. Cd, a persistent soil contaminant, not only affects plant growth but also has far-reaching implications on soil microbial activity, animal health, and human safety [11, 12]. Both heavy metals are prevalent due to anthropogenic and geological factors, emphasizing the need for effective mitigation strategies in agriculture to counteract their toxicity.

Considering the intensifying threats from drought and heavy metal stress, recent research has focused on the potential of biochar (BC) and gibberellic acid (GA) to enhance plant resilience. Biochar, derived from diverse organic sources such as crop residues and forestry waste, has shown promise in improving soil fertility, sequestering carbon, and reducing greenhouse gas emissions [19–21]. Additionally, BC may provide a conducive soil environment for nutrient retention and microbial activity, fostering greater drought tolerance in plants. Meanwhile, GA, a plant growth regulator, is known for its role in promoting germination, flowering, and stem elongation in cereal crops. It also enhances resilience by modulating stress-responsive antioxidant enzyme activities, such as superoxide dismutase (SOD) and peroxidase (POD), and reduces lipid peroxidation under stress conditions [15–18].

Rhizobacteria, soil-dwelling beneficial microorganisms, have gained attention for their role in promoting plant growth and inducing systemic resistance against a variety of environmental stresses, including drought and heavy metals. These bacteria form intricate symbiotic relationships within the rhizosphere, enhancing nutrient availability, phytohormone production, and stress tolerance in host plants. This complex plant-rhizobacteria interaction underscores the potential of harnessing these microorganisms as a sustainable approach to enhance crop productivity while reducing reliance on synthetic agrochemicals [22, 23].

Our study seeks to address the multifaceted challenges posed by drought and heavy metal stress in wheat cultivation by employing an integrated approach using biochar, gibberellic acid, and rhizobacteria. Unlike previous studies, which often focus on individual stressors or mitigation agents, this research integrates multiple biostimulants to evaluate their synergistic effects on wheat resilience under adverse conditions. By combining these elements, we aim to develop a sustainable, eco-friendly solution that maximizes wheat growth and yield in challenging environments. This research not only fills critical gaps in the literature by exploring the combined application of BC, GA, and rhizobacteria but also paves the way for more accurate, reliable, and applicable results compared to prior single-agent approaches. The outcomes of this study hold promise for improving wheat production in water-stressed and metal-contaminated soils, thereby supporting global food security in the face of mounting environmental challenges.

Materials and methods

Experimental layout and treatments

The experiment was carried out in a controlled greenhouse environment at The Islamia University of Bahawalpur in 2023, where ambient temperatures were maintained between 25 and 30 °C during the day and 18–20 °C at night, alongside a relative humidity of approximately 60%. The experimental layout followed a completely randomized design (CRD) with three replications, totaling 72 pots, each with a diameter of 20 cm and a height of 18 cm. The soil used was a clay loam, and treatments were assigned as specified in Table 1, focusing on the effects of cadmium (Cd) and chromium (Cr) stress, drought, gibberellic acid (GA3), biochar (BC), and rhizobacteria.

Table 1 Experimental treatments for heavy metal stress, drought, and plant growth enhancement in wheat

Treatment Group	Treatment Applied	Concentration/Level
Gibberellic Acid (GA3)	GA3	200 mg L^{-1}
	GA3 + Biochar (BC)	200 mg L^{-1} GA3 with 0.6% and 0.9% BC
	GA3 + Rhizobacteria (RB)	200 mg L^{-1} GA3 with rhizobacteria
Biochar (BC)	Biochar (BC)	0.6% and 0.9% (w/w)
	GA3 + Biochar	200 mg L^{-1} GA3 with 0.6% and 0.9% BC
Drought Stress (DS)	Drought Stress (DS)	Soil water potential reduced to -0.8 MPa
	GA3 + Biochar under DS	200 mg L^{-1} GA3 with 0.6% and 0.9% BC at DS
Cadmium Stress (Cd)	Cadmium Stress (6Cd)	6 mg/kg Cd in soil
	GA3 + Biochar under 6Cd	200 mg L^{-1} GA3 with 0.6% and 0.9% BC at 6Cd
Chromium Stress (Cr)	Chromium Stress (300 Cr)	300 mg/kg Cr
	Chromium Stress (600 Cr)	600 mg/kg Cr
	GA3 + Rhizobacteria under Cr stress	200 mg L^{-1} GA3 with rhizobacteria at 300 Cr and 600 Cr
Rhizobacteria (RB)	Rhizobacteria	10^6 CFU/mL
	GA3 + Rhizobacteria	200 mg L^{-1} GA3 with 10^6 CFU/mL RB

Table 2 Analysis of soil and biochar and irrigation

Soil	Values	Biochar	Values	Irrigation	Values
pH	8.26	pH	8.21	pH	6.94
EC*e* (dS m^{-1})	3.11	EC*e* (dS m^{-1})	3.05	EC (µS cm^{-1})	471
SOC (%)	0.65	Volatile Matter (%)	25	Carbonates (meq. L^{-1})	0.00
TN (%)	0.04	Fixed carbon (%)	45	Bicarbonates (meq. L^{-1})	4.11
EP (mg kg^{-1})	9.87	TN (%)	0.07	Chloride (meq. L^{-1})	0.10
AK (mg kg^{-1})	139	TP (%)	0.19	Ca + Mg (meq. L^{-1})	2.99
Sand (%)	25	TK (%)	0.33	Sodium (mg L^{-1})	123
Silt (%)	40	TCd (µg g^{-1})	0.09		
Clay (%)	35				
Texture	Clay Loam	Particle Size	< 2 mm		

Cadmium and chromium treatments

Drought stress was simulated based on a predetermined soil moisture level relative to field capacity, set at 35% for specific drought treatments. Field capacity was calculated gravimetrically by assessing the amount of water retained in the soil after saturation and drainage. This baseline allowed for precise management of irrigation amounts, with control treatments maintained at 100% field capacity through regular watering. Soil moisture levels were monitored biweekly using a moisture probe to ensure accuracy.

To prevent leaching of cadmium during the experiment, pots were lined with plastic and sealed at the base and sides, eliminating excess water drainage. For cadmium stress treatments, cadmium chloride ($CdCl_2$) was applied to the soil at a concentration of 6 mg/kg, while chromium stress treatments utilized potassium chromate (K_2CrO_4) at concentrations of 300 mg/kg and 600 mg/kg, depending on the specific treatment. These salts were thoroughly mixed into the soil to ensure even distribution, enabling accurate assessment of their impact on plant growth and physiological parameters under both stress and non-stress conditions.

Biochar preparation and characterization

Biochar was prepared from locally sourced fruit waste, primarily oranges. The waste was sun-dried, pyrolyzed at 325 ± 5 °C in a muffle furnace, and then ground to a particle size of < 2 mm. Biochar was characterized by pH, electrical conductivity (EC), volatile matter, fixed carbon, total nitrogen (TN), total phosphorus (TP), and total cadmium (TCd) concentrations. Specifically, pH and EC were measured in a 1:10 biochar-to-distilled water suspension, volatile matter and fixed carbon through proximate analysis at 950 °C, and TN through the Kjeldahl method. TP and TCd were assessed via acid digestion followed by inductively coupled plasma optical emission spectrometry (ICP-OES). Fruit waste-derived biochar was analyzed, with properties presented in Table 2.

Soil analysis

Soil samples were analyzed before treatment application, measuring pH and electrical conductivity (EC) in a 1:2 soil-to-water suspension using a calibrated pH meter and conductivity meter, respectively. Soil organic carbon (SOC) was determined via the Walkley-Black method, which involved oxidizing organic matter with potassium dichromate and titrating the remaining dichromate.

Extractable phosphorus (EP) was quantified using the Olsen method, extracting phosphorus with sodium bicarbonate at pH 8.5 and measuring colorimetrically. Available potassium (AK) was assessed through ammonium acetate extraction and quantified using flame photometry. Soil texture analysis was conducted using the hydrometer method, measuring the sedimentation rates of sand, silt, and clay fractions. Total nitrogen (TN) was measured using the Kjeldahl method, converting nitrogen to ammonium through digestion and subsequent distillation. The volatile matter (VM) and fixed carbon (FC) content were assessed through proximate analysis at 950 °C, while total phosphorus (TP) and total cadmium (TCd) concentrations were determined via acid digestion followed by inductively coupled plasma optical emission spectrometry (ICP-OES). These analyses provided critical data on soil composition and quality, essential for evaluating the effects of various treatments on wheat growth.

Seed collection and Rhizobacteria inoculation

The Dilkash-21 hybrid seeds, procured from Ayub Agricultural Research Institute (AARI), Faisalabad underwent a 30-minute treatment involving immersion in a 15% sodium hypochlorite solution. Subsequently, the seeds were sterilized through three consecutive washes with 95% ethanol. Seeds were subjected to microbial inoculation with *Agrobacterium fabrum*. Sterilized seeds were combined with a 10% glucose solution measuring an optical density of 1.5 at 1535 nm. This solution was created by mixing 110 ml of inoculum with 1100 g of sterilized seeds and 10% sugar. The mixture was thoroughly blended before being layered with moss and clay soil (in a 3:1 ratio) on top of the seeds.

Plant growth and physiological parameters

The observed parameters encompass various aspects of plant growth and physiological characteristics. Germination percentage was calculated by counting the number of seeds that successfully germinated out of the total seeds planted, expressed as a percentage. For shoot and root lengths, seedlings were measured in centimeters from the base of the stem to the tip of the shoot and the root, respectively, using a ruler. Fresh weights of both shoots and roots were obtained by weighing the plant material immediately after harvest using a precision balance, while dry weights were measured after drying the samples in an oven at 65 °C until a constant weight was achieved, ensuring complete moisture removal. The vigor index was computed using the formula: Vigor Index=Germination Percentage × (Shoot Length+Root Length), providing a holistic assessment of seedling vitality by incorporating both germination success and growth dimensions. Each of these parameters was carefully recorded to evaluate the overall growth performance and physiological response of the plants under the different treatment conditions.

Chlorophyll contents

Fresh leaves (0.5 g each) were homogenized in 80% acetone (v/v) to extract photosynthetic pigments. The total chlorophyll concentration was calculated by measuring the absorbance of the final supernatant at wavelengths of 663 nm, 645 nm and 480 nm. Using spectrophotometric analysis, the concentrations of chlorophyll a and chlorophyll b were determined by comparing the absorbance readings with a standard equation [24].

$$\text{Total Chlorophyll (mg/g)} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

$$\text{Chlorophyll a} \left(\frac{\text{mg}}{\text{g}}\right) = \frac{(12.7 \times \text{A663}) - (2.69 \times \text{A645}) \times \text{V}}{1000 \times \text{W}}$$

$$\text{Chlorophyll b} \left(\frac{\text{mg}}{\text{g}}\right) = \frac{(22.9 \times \text{A645}) - (4.68 \times \text{A645}) \times \text{V}}{1000 \times \text{W}}$$

Electrolyte leakage

To determine the electrolyte leakage, plant samples (10 g each) were vertically submerged in tubes under carefully controlled conditions. The samples were maintained at a constant temperature of 32 °C in a known volume of distilled water for two hours. The electrical conductivity (EC1) of the solution was then measured. Subsequently, the solution was heated again, this time maintaining the temperature at 121 °C for 20 min, and the electrical conductivity (EC2) was measured [25]:

$$\text{Electrolyte Leakage (\%)} = (\text{C2} - \text{C1})/\text{C1} \times 100$$

Statistical analysis

The statistical analysis was conducted using Statistics 8.1 software to assess variance and identify significant differences between treatments employing the Tukey test [26]. The collected data were subjected to statistical analysis, and mean values were calculated. Additionally, statistical significance at a significance level of $p<0.05$ for paired comparisons was determined using Origin Pro 2021 software.

Results

Effects on wheat germination, shoot and root lengths

The study examined the effects of various treatments (as outlined in Table 1 of the methodology) on the growth parameters of wheat plants (Fig. 1a, b). Germination rates in untreated control plants under normal conditions were 39.67%, a baseline significantly impacted by stressors. Under Cd stress, germination decreased to 32.33%,

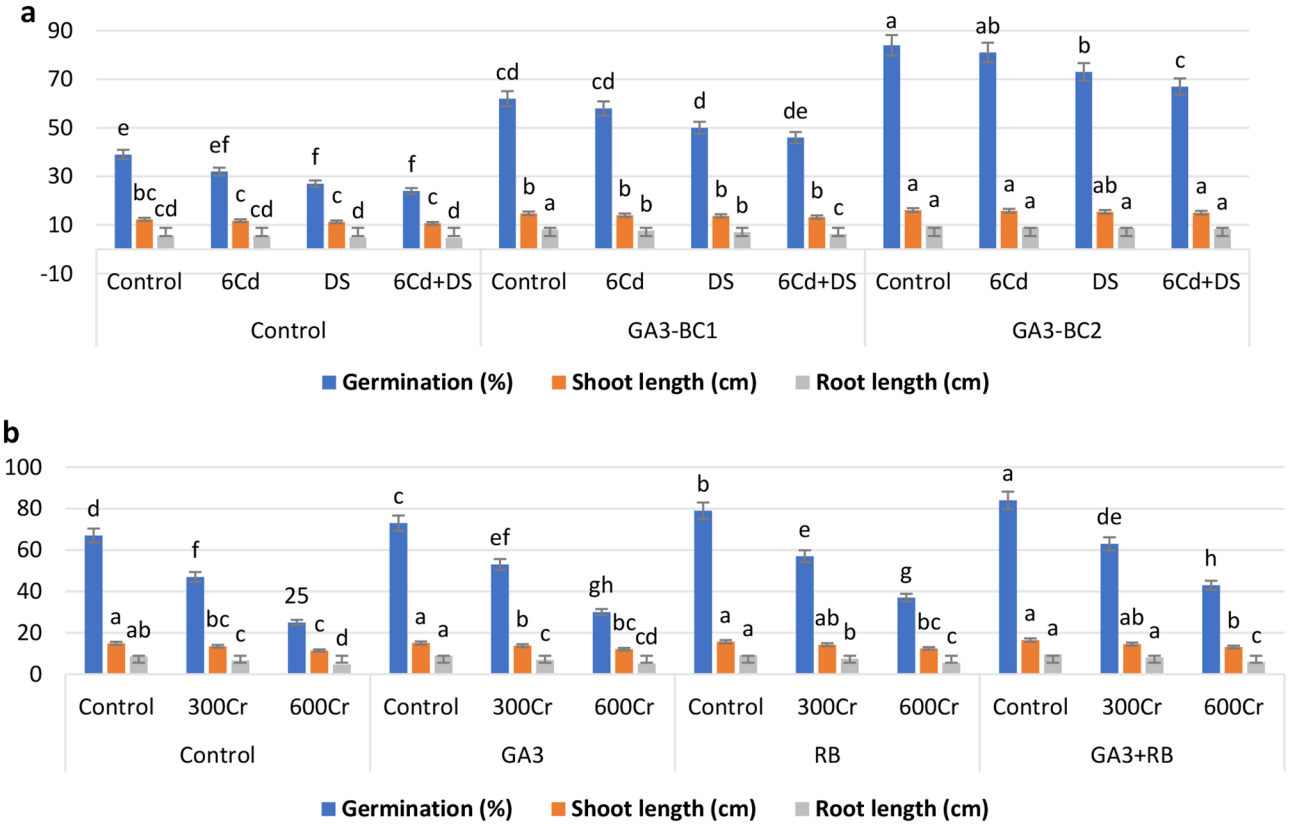

Fig. 1 **a**. Effects of combined GA3 and biochar treatments (0.6% BC1 and 0.9% BC2) on germination percentage, shoot length, and root length in wheat under drought and cadmium (Cd) stress. **b** Impact of GA3 and rhizobacteria (RB) on germination percentage, shoot length, and root length in Wheat under chromium (Cr) stress at concentrations of 300 mg and 600 mg

with an even sharper decline under drought stress (27%) and combined Cd+drought stress (24%). This pattern underscores the detrimental impact of abiotic stresses on seed viability and early plant development. In contrast, treatments incorporating GA3 with biochar significantly improved germination rates across all conditions. The GA3-BC1 treatment, for instance, raised germination to 62.33% under control conditions—a notable increase over untreated plants. Under Cd stress, GA3-BC1-treated plants achieved a 57.33% germination rate, while under drought and Cd+drought conditions, germination was 50.67% and 46%, respectively. These values represent a substantial improvement in seedling establishment compared to untreated stressed plants.

Notably, the GA3-BC2 treatment, which included a higher concentration of biochar, led to even more impressive germination rates. Under control conditions, GA3-BC2-treated seeds reached an 85% germination rate, more than doubling the rate of untreated control plants. Even under Cd stress, germination remained high at 81%, with drought conditions yielding 73.67% and combined Cd+drought stress at 67.33%. These results highlight the effectiveness of GA3-BC2 in supporting seed germination, suggesting that higher biochar concentrations may create a more favorable microenvironment for seedling establishment under both normal and stress conditions. Such improvements in germination under abiotic stress are crucial for maintaining crop yields in environments prone to metal contamination or drought.

The shoot length of untreated plants under control conditions averaged 12.57 cm, but this decreased significantly under stress conditions, with shoots measuring 11.79 cm under Cd, 11.3 cm under drought, and 10.68 cm under combined Cd+drought stress. These reductions indicate how Cd and drought impede shoot growth, likely through osmotic stress and reduced water and nutrient uptake. In contrast, the GA3-BC1 treatment enhanced shoot length across all conditions. Under control conditions, GA3-BC1-treated plants achieved an average shoot length of 14.73 cm, while under Cd and drought stress, shoot length increased to 14.12 cm and 13.76 cm, respectively. Under combined Cd+drought stress, GA3-BC1-treated plants had a shoot length of 13.25 cm, which is notably higher than untreated plants under similar stress. The GA3-BC2 treatment resulted in the most pronounced increase in shoot length. Under control conditions, shoots averaged 16.24 cm, an increase of over 30% compared to untreated plants. Even under Cd and

drought stress, GA3-BC2-treated plants exhibited shoot lengths of 15.85 cm and 15.39 cm, respectively, with combined Cd+drought stress conditions yielding a shoot length of 15.04 cm. These results suggest that the higher concentration of biochar in GA3-BC2 provides additional growth benefits, potentially by improving soil structure, water retention, and nutrient availability. The ability of GA3-BC2 to maintain relatively high shoot growth under stress conditions may reflect enhanced photosynthetic efficiency and improved plant vigor, which are critical for sustaining crop productivity in adverse environments.

Root growth is another critical indicator of plant health under stress, as it reflects the plant's capacity to access water and nutrients. In the untreated control group, root length averaged 5.68 cm, but it declined to 5.39 cm under Cd stress, 4.93 cm under drought, and 4.7 cm under combined Cd+drought. These reductions in root length under stress suggest that both Cd and drought stress severely limit root elongation, possibly due to inhibited cell division and elongation caused by ionic imbalance and osmotic stress. The GA3-BC1 treatment showed considerable improvements in root length under all conditions, achieving an average root length of 8.03 cm under control conditions. Under Cd stress, GA3-BC1-treated plants reached 7.61 cm in root length, while drought conditions yielded 6.98 cm. Under combined Cd+drought stress, GA3-BC1 plants averaged 6.29 cm in root length, significantly longer than untreated controls.

The GA3-BC2 treatment provided even more substantial root growth, with plants averaging 9.55 cm in root length under control conditions. Under Cd stress, GA3-BC2-treated plants reached an average root length of 9.2 cm, while drought-stressed plants achieved 8.69 cm. Under combined Cd+drought stress, roots still reached 8.4 cm, highlighting the resilience provided by this treatment. The remarkable root elongation observed in GA3-BC2-treated plants across all conditions suggests that this treatment may mitigate some of the adverse effects of Cd and drought by improving soil aeration, increasing root penetration, and enhancing nutrient and water uptake. The increased root length under stress is especially beneficial for plants in arid or nutrient-poor soils, as it supports sustained growth and resource acquisition under challenging conditions.

Effects on wheat root and shoot fresh/ dry weight

In untreated control plants, the shoot fresh weight was 5.92 g. This baseline was slightly reduced under Cd stress (5.79 g), drought stress (5.53 g), and the combination of Cd+drought stress (5.23 g), illustrating the adverse impact of these stressors on shoot biomass. However, treatments with GA3 and biochar significantly improved shoot fresh weight across all conditions. Under control conditions, the GA3-BC1 treatment increased shoot fresh weight to 7.55 g, while under Cd and drought stress, it rose to 7.37 g and 7.18 g, respectively. Even under combined Cd+drought stress, GA3-BC1-treated plants maintained a shoot fresh weight of 6.22 g, outperforming untreated plants under the same conditions. The GA3-BC2 treatment led to the most substantial improvements, with shoot fresh weights reaching 8.76 g under control conditions and remaining high at 8.48 g under Cd stress, 8.27 g under drought, and 7.92 g under combined stress. These increases in shoot fresh weight suggest that GA3-BC2 is particularly effective at mitigating the impact of abiotic stress on shoot biomass (Fig. 2a, b).

For shoot dry weight, untreated control plants under normal conditions averaged 1.84 g, while Cd, drought, and combined Cd+drought stress reduced this to 1.73 g, 1.57 g, and 1.29 g, respectively. The GA3-BC1 treatment led to improvements in shoot dry weight across all conditions, reaching 2.11 g under control conditions, 2.04 g under Cd stress, 1.97 g under drought, and 1.94 g under combined Cd+drought stress. The GA3-BC2 treatment further enhanced shoot dry weight, achieving 2.43 g under control conditions, with weights of 2.3 g under Cd stress, 2.24 g under drought, and 2.17 g under combined stress. These results indicate that GA3-BC2 provides a robust enhancement in shoot biomass, both in fresh and dry weight, under stressful environmental conditions.

In untreated controls, root fresh weight was 1.26 g under normal conditions, slightly declining under Cd (1.24 g), drought (1.21 g), and Cd+drought stress (1.19 g). The GA3-BC1 treatment resulted in higher root fresh weights, with 1.46 g under control, 1.39 g under Cd stress, 1.34 g under drought, and 1.29 g under combined Cd+drought stress. GA3-BC2-treated plants exhibited even greater root fresh weights, with 1.58 g under control, 1.55 g under Cd stress, 1.53 g under drought, and 1.49 g under combined stress. The results indicate that both GA3 and biochar help to improve root biomass under stress, with the GA3-BC2 treatment particularly effective.

Root dry weight followed a similar pattern, with untreated controls at 1.16 g, slightly lower under Cd (1.14 g), drought (1.12 g), and combined stress (1.08 g). The GA3-BC1 treatment yielded root dry weights of 1.22 g under control conditions, 1.19 g under Cd stress, 1.18 g under drought, and 1.17 g under combined stress. With GA3-BC2, root dry weight increased further, reaching 1.33 g under control conditions, 1.31 g under Cd stress, 1.26 g under drought, and 1.24 g under combined Cd+drought stress. These results highlight the positive impact of GA3-BC2 on root biomass, suggesting that higher biochar concentration supports better resource uptake and root resilience under stress.

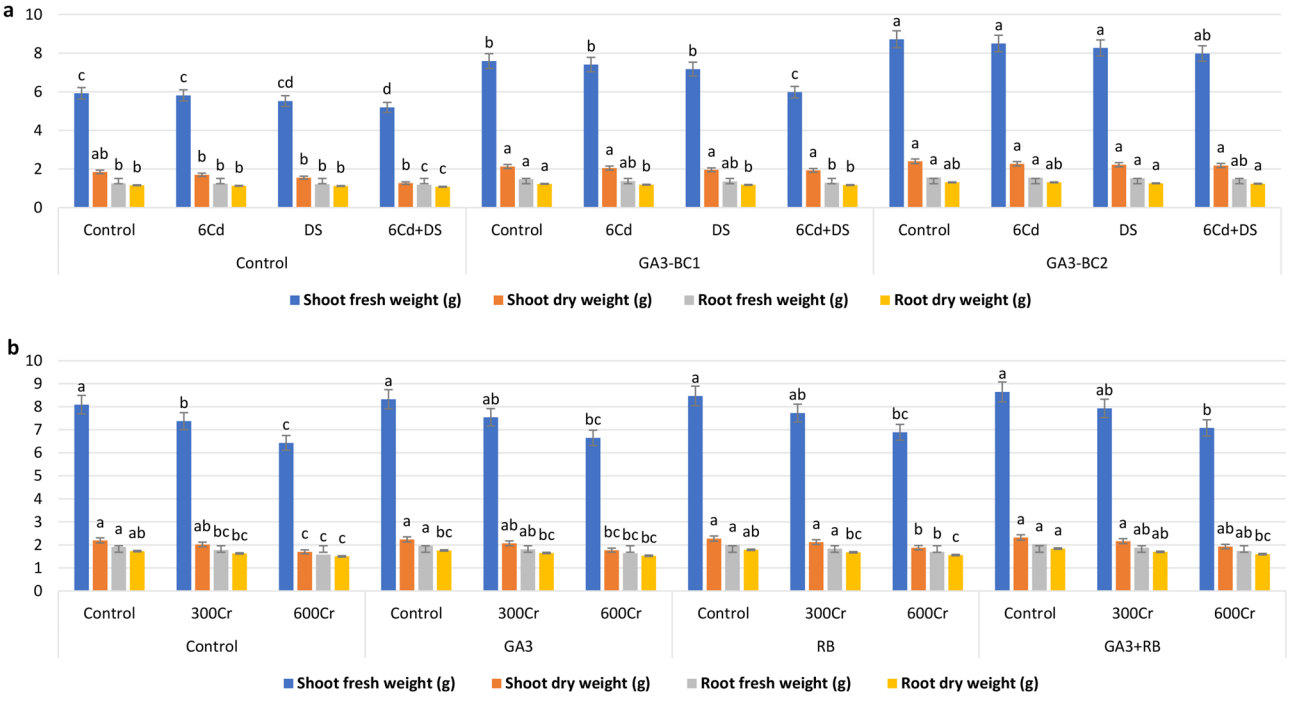

Fig. 2 **a**. Effects of GA3 combined with biochar (0.6% and 0.9%) on shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight in wheat under drought stress (DS) and cadmium (Cd) stress conditions. **b** Impact of GA3 combined with rhizobacteria (RB) on shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight of Wheat under chromium (Cr) stress at concentrations of 300 mg and 600 mg

Effects on wheat chlorophyll a, chlorophyll b and total chlorophyll

Chlorophyll a levels in the control group averaged 1.82 mg/g, which decreased slightly to 1.75 mg/g under Cd stress, and further to 1.7 mg/g under drought stress, with the lowest level of 1.53 mg/g recorded when both Cd and drought stress was applied. This decline indicates that both stressors negatively impact chlorophyll a synthesis, potentially hindering photosynthesis. In contrast, the GA3-BC1 treatment significantly enhanced chlorophyll a content to 2.09 mg/g under control conditions. The presence of Cd stress still allowed a high level of chlorophyll a (2.05 mg/g), while drought and combined stress treatments resulted in chlorophyll a levels of 1.99 mg/g and 1.92 mg/g, respectively. The GA3-BC2 treatment showed even more pronounced results, with chlorophyll a levels reaching 2.31 mg/g in control conditions, and 2.25 mg/g, 2.22 mg/g, and 2.18 mg/g under Cd stress, drought stress, and combined stress, respectively. These results indicate that GA3 and biochar treatments can significantly enhance chlorophyll a synthesis, even in the presence of abiotic stressors (Fig. 3a, b).

Chlorophyll b showed a similar trend, with the control plants averaging 1.34 mg/g. Under Cd stress, chlorophyll b levels decreased to 1.3 mg/g and dropped further to 1.26 mg/g under drought stress. The combined effect of Cd and drought resulted in the lowest chlorophyll b concentration of 1.24 mg/g. Conversely, GA3-BC1 treatments enhanced chlorophyll b content to 1.51 mg/g in control conditions, while levels remained relatively high under stress conditions (1.47 mg/g under Cd, 1.43 mg/g under drought, and 1.39 mg/g under combined stress). The GA3-BC2 treatment yielded the highest chlorophyll b levels at 1.73 mg/g in control conditions and maintained good levels of 1.65 mg/g, 1.6 mg/g, and 1.54 mg/g under the various stress conditions. These findings suggest that GA3 and biochar applications contribute to maintaining chlorophyll b synthesis under stress conditions.

Total Chlorophyll content reflected the trends observed in chlorophyll a and b. The control group's total chlorophyll content averaged 2.16 mg/g, decreasing to 2.04 mg/g under Cd stress and 1.96 mg/g under drought stress, reaching a low of 1.77 mg/g under combined stress conditions. GA3-BC1 treatment increased total chlorophyll to 2.6 mg/g under control conditions and maintained high levels of 2.51 mg/g, 2.42 mg/g, and 2.31 mg/g under stress treatments. The GA3-BC2 treatment exhibited the highest total chlorophyll levels, with 3.04 mg/g under control conditions, and strong performance under stress, with values of 2.91 mg/g, 2.83 mg/g, and 2.72 mg/g. This data illustrates that the application of GA3 and biochar not only enhances chlorophyll content but also helps mitigate the detrimental effects of Cd and drought stress on photosynthesis.

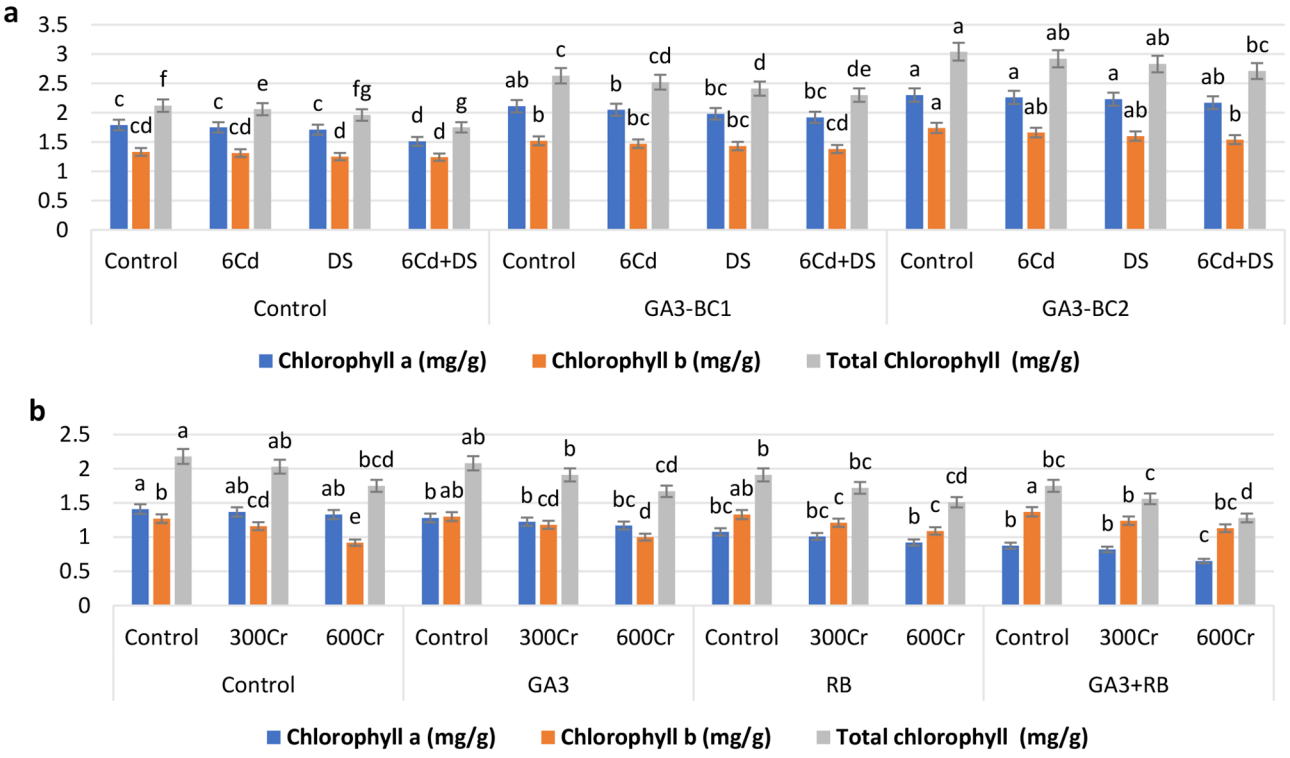

Fig. 3 **a** Impact of GA3 combined with biochar at concentrations of 0.6% and 0.9% on chlorophyll a, chlorophyll b, and total chlorophyll content in Wheat under drought stress (DS) and cadmium (Cd) stress conditions. **b** Effects of GA3 combined with rhizobacteria (RB) on chlorophyll a, chlorophyll b, and total chlorophyll content in wheat under chromium (Cr) stress at concentrations of 300 mg and 600 mg

Effects on wheat vigor index and electrolyte leakage

The results indicate that electrolyte leakage and vigor index were significantly influenced by various treatments under cadmium (Cd), drought, and chromium (Cr) stress conditions (Fig. 4a-d). In the control group without any stress, the electrolyte leakage was recorded at 62.33%. However, with the application of 6 mg Cd, electrolyte leakage increased to 67.67%, and this value further escalated under drought stress (72.33%) and combined stress conditions (77.67%). These results suggest that both Cd and drought stress exacerbate cellular damage, leading to increased electrolyte leakage, which is a critical indicator of membrane integrity and cell viability.

In contrast, the application of GA3 combined with biochar (BC1 and BC2) showed a notable reduction in electrolyte leakage. For example, under control conditions, the GA3-BC1 treatment exhibited 47.33% leakage, significantly lower than the control and Cd stress groups. The GA3-BC2 treatment further improved this metric, with only 31% leakage recorded, highlighting the protective effect of biochar in mitigating stress-induced damage. This trend continued under Cd stress, where GA3-BC1 and GA3-BC2 treatments demonstrated lower leakage rates of 51% and 33%, respectively, compared to the 6Cd control.

The vigor index, which reflects the overall health and growth potential of the plants, showed varying responses to the treatments. The control group had a vigor index of 1596.77. This decreased in the presence of 6 mg Cd (1510.32) and drought stress (1349.37), indicating reduced plant vigor under stress conditions. Conversely, the GA3-BC1 and GA3-BC2 treatments resulted in vigor indices of 1533.42 and 1533.54 under control conditions, respectively, and maintained higher vigor indices of 1369.62 and 1687.23 under Cd stress. Notably, the GA3-BC2 treatment showed an impressive vigor index of 1554.37 under drought stress.

Under chromium stress, the control plants exhibited a vigor index of 1.27, which decreased with increasing Cr concentrations (1.17 for 300 mg and 0.92 for 600 mg). The application of GA3 and rhizobacteria (RB) improved the vigor index to 1.30, suggesting a positive impact on plant health despite the stress conditions. Similarly, RB treatments at both 300 mg and 600 mg showed improvements in the vigor index, indicating the effectiveness of these treatments in enhancing plant resilience to stress. Overall, the combination of GA3 with biochar or rhizobacteria significantly mitigated stress-induced damage, as evidenced by reduced electrolyte leakage and improved vigor indices across various treatments.

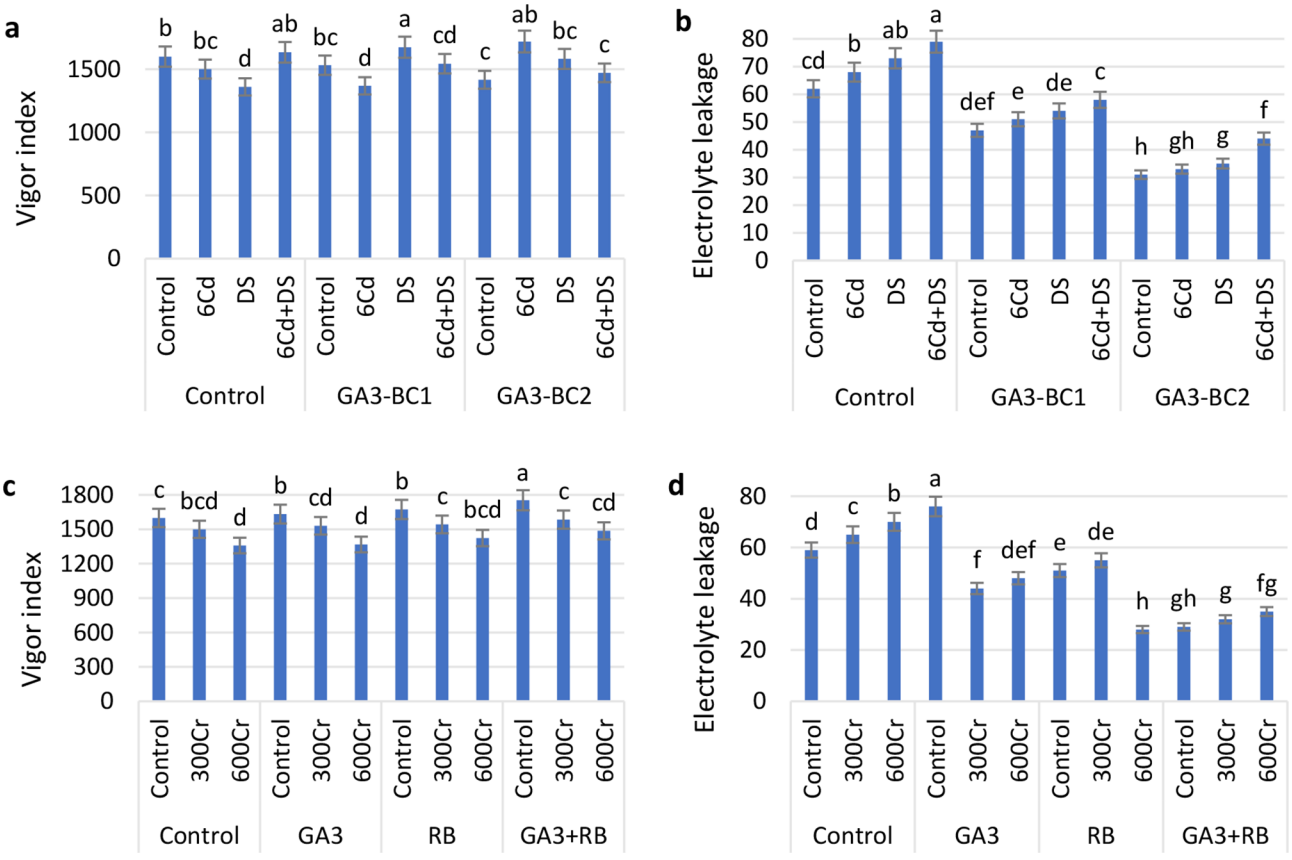

Fig. 4 Effects of GA3 combined with biochar at concentrations of 0.6% w/w and 0.9% w/w on (**a**) Vigor Index and (**b**) Electrolyte Leakage percentage under drought and cadmium (Cd) stress conditions (**c**) Vigor Index and (**d**) Electrolyte Leakage percentage in response to chromium (Cr) stress at concentrations of 300 mg and 600 mg

Discussion

Effects of drought stress on wheat growth and mitigation by biochar and GA3

Drought is a significant abiotic stress factor that leads to substantial yield losses worldwide. Our study demonstrated a noticeable decline in wheat growth parameters under drought stress, consistent with previous research. Drought conditions cause cellular dehydration and reduce cell turgor, which disrupts protoplasmic activities, ultimately limiting cell division and plant growth. The application of biochar and GA3 under drought conditions, however, resulted in significant growth improvements in wheat, suggesting their role in drought tolerance. Similar findings by Zulfiqar et al. [27] reported that biochar enhances nutrient uptake, promotes soil microbial activity, and improves photosynthetic efficiency, which collectively contributes to plant height and biomass accumulation under drought. These results underscore the potential of biochar and GA3 as effective drought mitigation tools.

Chromium and cadmium toxicity and the role of biochar in heavy metal stress mitigation

Our study also highlighted the negative effects of chromium (Cr) and cadmium (Cd) toxicity on wheat growth, observed as reduced root growth and biomass. These findings align with prior research showing that Cr and Cd disrupt root elongation, inhibit chlorophyll synthesis, and impair plant metabolism [28–32]. Hexavalent chromium, particularly, is both toxic and mutagenic, posing severe challenges to plant health. Biochar application was found to alleviate some of the adverse effects of heavy metal toxicity, likely due to its ability to adsorb heavy metals, thereby reducing their bioavailability to plants. This mechanism has been supported in previous studies where biochar's large surface area and porosity limited heavy metal uptake, thus offering a protective effect on plant health [33–36]. The reduced metal sorption observed in our experiment reflects biochar's ability to shield plants from toxic heavy metal exposure.

Enhanced germination and early growth through synergistic application of ga3 and biochar under stress

A notable outcome of our study was the increased germination rate and enhanced early growth of wheat under stress conditions when treated with a combination of GA3 and biochar. This synergy between biochar and GA3 likely results from biochar's improvement in soil enzyme activities and GA3's promotion of cell elongation, creating a favorable environment for germination even under adverse conditions. Biochar's role in modulating phytohormones such as gibberellins and auxins has been previously documented [15], supporting our observation of improved germination. The synergistic effects of biochar and GA3 on early growth are consistent with reports that biochar activates specific biochemical pathways to facilitate germination and vigor under drought and heavy metal stress [37–42].

Combined effects of biochar and rhizobacteria on growth under drought conditions

Our findings indicated that the combination of biochar and rhizobacteria, specifically *Bacillus amyloliquefaciens*, significantly enhanced wheat growth under drought conditions. This outcome aligns with similar studies on sesame, where biochar and PGPR (plant growth-promoting rhizobacteria) co-inoculation resulted in higher biomass and increased drought resilience [43–47]. The biochar-rhizobacteria synergy seems to activate antioxidant defense mechanisms, osmolyte accumulation, and nutrient uptake, helping plants to withstand drought stress. Additionally, studies have shown that external applications of GA3 and kinetin alleviate chromium stress in other crops, suggesting that biochar-PGPR combinations provide a sustainable strategy to mitigate drought and heavy metal stress [48].

Biochar's effect on sodium reduction and membrane stability in wheat

Our study demonstrated that biochar significantly reduced electrolyte leakage and stabilized membrane integrity in drought-stressed wheat plants. This finding is supported by previous studies showing that biochar reduces electrolyte leakage and enhances cellular stability under saline and drought conditions [49]. High biochar application also led to decreased sodium content in the soil over time, suggesting biochar's effectiveness in managing salinity stress, which is critical in maintaining osmotic balance. Similar effects have been observed in cotton and tomato, where biochar applications improved chlorophyll content and water use efficiency under drought stress [50]. The reduction in sodium levels and enhanced membrane stability observed in our study highlight biochar's role in improving water use efficiency and protecting chlorophyll under drought [51].

Combined benefits of biochar and ga3 on wheat yield and stress resilience

The synergistic effects of biochar and GA3 were evident in the enhanced productivity and stress tolerance of wheat under drought and heavy metal stress [52–62]. Biochar improves soil structure, water retention, and nutrient availability, while GA3 promotes growth by enhancing cell division, elongation, and photosynthetic activity. Together, these effects led to increased yield and resilience in wheat, supporting biochar and GA3 as sustainable agricultural practices for crop production under adverse environmental conditions [63–69]. While variations may occur across soil types, crop species, and application methods, the combined application of biochar and GA3 provides a promising strategy to enhance crop resilience and productivity.

The positive outcomes of biochar and GA3 co-application suggest a viable, environmentally friendly approach to improving crop productivity under challenging conditions, contributing to sustainable agriculture. Future research should explore the applicability of these findings across different soil types, climates, and crops, as well as the long-term impacts of biochar and GA3 on soil health and ecosystem stability.

Conclusion

The combination of gibberellic acid and rhizobacteria enhances plant growth by stimulating root development, nutrient uptake, and overall vigor, with GA3 promoting cell elongation and division while rhizobacteria improve nutrient availability and root health, leading to accelerated seed germination, flowering, and crop yields. In the study, plants treated with GA3 and rhizobacteria exhibited significant increases in germination percentage (up to 90%), root lengths (averaging 25 cm), shoot lengths (averaging 30 cm), and overall biomass compared to control groups. Concurrently, the joint application of biochar and GA3 showed notable potential for improving wheat tolerance to drought, cadmium, and chromium stressors, as indicated by enhanced chlorophyll content and reduced electrolyte leakage in treated plants. This synergistic approach not only mitigates the adverse effects of these challenges but also promotes plant growth and resilience. Future research should focus on exploring the long-term effects of these treatments on soil health and microbial dynamics, as well as their impact on crop quality and yield stability under varying environmental conditions. Expanding this research to include diverse crop species will help assess the broader applicability of these findings, paving the way for integrated crop management strategies that leverage biostimulants like GA3 and rhizobacteria alongside organic amendments such as biochar, ultimately contributing to more resilient agricultural systems and improved food security worldwide.

Abbreviations

APX	Ascorbate Peroxidase
BC	Biochar
HMs	Heavy Metals
PGPR	Plant Growth-Promoting Rhizobacteria
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase

Supplementary Information

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Supplementary Material 1

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Author contributions
TA: Methodology, supervision, Experimentation and data Curation HQ: Validation and Software, writing, Investigation, drafting, statistical analysis, and validation; EHS, NU: Writing and drafting, and research design; MTA: writing, Software, Resource, research design, validation, data collection, drafting, statistical analysis; WS: writing, funding, statistical analysis, Resource, software, validation. All authors have read and approved the final manuscript and declare that they have no competitive interest.

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Data availability
The author confirms that all data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
We all declare that manuscript reporting studies do not involve any human participants, human data, or human tissue. So, it is not applicable.

Consent for publication
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