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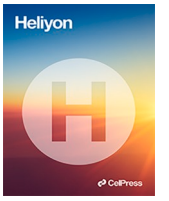

Research article

Ultraviolet-enhanced detoxification of chromate and protection of *Brassica napus* by *Aspergillus sojae* SH 20

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ABSTRACT

The pervasive issue of heavy metal contamination in agricultural lands poses significant concerns and has wide-ranging implications for ecosystems. However, an encouraging solution lies in exploiting the potential of fungal endophytes to alleviate these detrimental effects. This study emphasized on improving the growth-promoting and chromium-alleviating capabilities of fungal endophytes, particularly *Aspergillus sojae* strain SH20, through ultraviolet (UV) irradiation. Following UV treatment, SH20 exhibited significantly enhanced growth-promoting and chromium-alleviating capabilities in comparison to its non-irradiated counterpart. Distinctly, the UV-treated SH20 strain demonstrated an improved ability to accumulate and reduce toxic chromate in the soil, effectively addressing the growth constraints imposed by elevated chromium levels in *Brassica napus* L. The UV-irradiated SH20 variant boosted shoot length up to 3 times that of the control. Similarly, this fungal strain displayed a remarkable increase in the total fresh weight of the seedlings, recording nearly 17 times greater than the control. The isolate treated with UV light reduced the absorption of chromium by about 3 times in the roots, helping the young plants to grow well even when exposed to chromate stress. A drop in root colonization by the UV-treated strain further resulted in reduced chromate absorption by the roots. Also, the strain showed great skill in boosting the host's antioxidant defenses by reducing the buildup of harmful reactive oxygen species (ROS), increasing the removal of ROS, and improving the plant's antioxidant levels, including phenols and flavonoids. When the host plants were exposed to 25 ppm of Cr stress, the UV-irradiated variant SH 20 stimulated the production of flavonoids (246 μg/ml) and phenols (952 μg/ml) in comparison to the control (with 220 μg/ml of flavonoids and 919 μg/ml of phenols). In conclusion, this report highlights how exposing the *A. sojae* strain SH20 to UV light has the potential to enhance its abilities to promote growth and bioremediate. This suggests a promising solution for addressing heavy metal contamination in agricultural lands.

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1. Introduction

In the ecosystem, it's customary for a range of mutants to emerge as a result of accidental and random mutagenesis [1]. Induced mutagenesis is a labor-intensive and tedious trial-and-error procedure [2]. To induce random mutations in organisms, various modes and methods have been introduced, including exposure to UV radiation [3]. UV radiation is commonly used to boost the accumulation of secondary metabolites in various species to combat stresses [4,5]. UV light can redirect carbon flux, resulting in a beneficial change in various metabolites, such as phenols, carotenoids, and glucosinolates [6]. These changes can be useful in addressing stresses. Moreover, it is well known that some of the endophytes can help the host plants under severe stress conditions [7–11]. They are successful at keeping a good relationship with their host because they can release metabolites that are like a valuable treasure for the host plants [12]. Research has indicated that endophytes might be able to endure heavy metals and help their host plants survive in areas contaminated with heavy metals [13,14].

When plants are exposed to higher concentrations of these metals, it can result in decreased yield and have harmful effects on the overall health of the plants [15]. Amongst the numerous heavy metals, hexavalent chromium (Cr^{+6}) is noted for its reputation as an industrial contaminant. Crop species that cultivate in polluted soils often absorb high levels of Cr^{+6}, resulting in a decrease in both root and shoot biomass, crop yield, hindrance of seedling growth [14]. This features the detrimental effect of Cr^{+6} on plant growth and productivity [16]. However, endophytic fungi, which have similar secondary metabolic traits as their host plants, can produce secondary metabolites when exposed to UV radiation [5]. This capability can boost their effectiveness in promoting growth, especially in situations with heavy metal stress [17].

Given the detrimental effects of Cr^{+6} on crops, it is imperative to explore cost-effective and robust methods for their remediation [18]. Bioremediation stands as a contemporary practice for the elimination of Cr^{+6}, with endophytic fungi emerging as prime candidates due to their diverse mechanisms for detoxifying this metal [19]. Additionally, the UV treatment of promising strain might enhance its capability to improve its bioremediation property. Therefore, this study was designed to explore the impact of UV-C radiations on the growth-promoting and heavy metal-alleviating tendencies of plant growth promoting endophyte, *A. sojae*. The scarcity of studies in this specific domain highlights the unique contribution of our research. The induction of mutations via UV-C radiation is hypothesized to have the potential to enhance the growth-promoting capabilities and Cr^{+6} alleviation tendencies of endophytic fungi. The study aimed to explore various aspects, including the survival and growth-promoting abilities of *A. sojae*, the production of metabolites and phytohormones, the capacity of UV-C-irradiated strains to alleviate Cr^{+6} stress in *Brassica napus*, and the ability of UV-C-irradiated *A. sojae* to improve the host's antioxidant system and scavenge reactive oxygen species (ROS).

2. Materials and methods

Euphorbia helioscopia plants, found in the industrialized area of Mardan, were gathered and relocated to the Department of Botany at Abdul Wali Khan University Mardan. Roots, stem and leaves of the plants were subjected to a thorough cleaning process, involving rinsing with flowing tap water. Sterilization of the surface was accomplished by immersing the plant parts in 70 % ethanol for 1 min, followed by a 3-min immersion in a 2 % hypochlorite (NaOCl) solution. Subsequently, segments of roots, stem and leaves were carefully rinsed with distilled water, as described by Stone et al. [20]. Cleaned and surface-sterilized segments of the plant, measuring between 1 and 3 cm, were placed onto Hagum minimal media under sterile conditions within a fume hood. The composition of 250 ml of Hagum minimal media includes the following components: 1.25 gm of glucose, 0.125 gm of ammonium chloride, 0.125 gm of potassium phosphate, 0.125 gm of magnesium sulfate, 3.75 gm of agar, 0.25 gm of iron chloride, and 20 ppm of streptomycin. All these components were dissolved in 250 ml of distilled water. These plates were then incubated for a period of 7 days at a temperature of 30 °C. To obtain pure colonies, fungal strains isolated on Hagum media were sub-cultured onto Potato Dextrose Agar (PDA).

2.1. Cr resisting and plant growth promoting assay

A selection of isolated endophytic fungi was evaluated for their potential to promote growth and alleviate chromium (Cr) stress in *Brassica napus*. The choice of *Brassica napus* was made due to its status as the world's second-largest source of oil and its significance as a crucial protein source for dietary purposes. To conduct this assessment, uniformly germinated *B. napus* seedlings were transplanted into sterilized soil containing varying concentrations of Cr (specifically, 25 ppm and 50 ppm), with or without fungal biomass. The process involved adding 3 g of fresh fungal biomass to 300 g of autoclaved soil. As a point of comparison, seedlings were also grown in soil that was devoid of both metal and endophytic fungus. Following that, we permitted these seedlings to develop over the course of 14 days. Subsequently, the seedlings were carefully harvested, and several parameters were measured, including shoot and root lengths, fresh weight, as well as the levels of chlorophyll and carotenoids present in the plants. These measurements were taken to determine how endophytic fungi affect growth and the capacity to endure stress in *Brassica napus* under chromium exposure.

2.2. Morphology of the fungal strains

The morphology of both the endophytic fungi and their mutants was examined using the slide culturing technique [21]. Under sterile conditions, a piece of potato dextrose agar was affixed to a slide and inoculated with fungal spores from all four directions. To prevent surface growth, the agar block was shielded with a cover slip. These slides were then positioned within sterile Petri dishes and subjected to a three-day incubation period at 30 °C. After incubation, the developing endophytic fungi were stained using lactophenol cotton blue dye and observed at 40X magnification using a light microscope.

2.3. Molecular identification of fungal isolate SH 20

To isolate DNA for molecular identification, freeze-dried mycelia were employed trailing the methodology illustrated by Li et al. [22]. A fungal biomass weighing approximately 200 mg was ground in a buffer solution (composed of sodium dodecyl sulfate, 0.1 M NaCl, and 0.5 M Tris-HCl) with a pH of 8 and vortexed for 10–15 s. The mixture was then exposed to a 65 °C temperature for 25 min and subsequently centrifuged at 7500 rcf for 5 min. Added a phenol-chloroform-isoamyl alcohol blend (25:24:1) to the supernatant in a 1:1 ratio and centrifuged again at 7500 rcf for 5 min. The aqueous phase was blended with chloroform-isoamyl alcohol (24:1) and subjected to another 5-min centrifugation at 7500 rcf. After mixing with ethanol, the collected supernatant underwent incubation for 50 min at 4 °C. Following this; the mixture was centrifuged at 7500 rcf for 15 min to isolate the DNA. For DNA intensification, the ITS1 and ITS4 regions were directed exhausting PCR. The PCR reaction contained 15 μL of PCR master mix, 1.5 μL of DNA sample, 1.5 μL of each primer, and 10.5 μL of double-distilled water. For PCR intensification, we followed a protocol that began with an initial denaturation at 95 °C for 2 min, trailed by annealing at 55 °C for 1 min, and extension at 72 °C for 5 min. The intensified DNA fragment was attained after running a total of 35 cycles. The gel-purified PCR fragment then underwent a sequencing reaction, succeeding the protocol outlined by White et al. [23]. The resultant sequence reads were aligned, and the homology of the consensus sequence was ascertained by exploiting the nucleic acid blast tool available at http://www.ncbi.nlm.nih.gov/BLAST/. In order to pinpoint the exact nomenclature of the isolate, we retrieved sequences that displayed the highest similarity to it. The phylogenetic analysis of the sample was conducted using MEGA-7 [24,25].

2.4. UV mutagenesis and screening of Cr^{+6} resistant A. sojae

To facilitate UV mutagenesis, a spore suspension of the endophytic fungi was prepared by scraping spores from the surface of the colony and suspending them in sterilized distilled water. Spore concentration of 1×10^7 spores per ml was achieved by diluting the spore suspension in the subsequent stage, which was then verified through spore counting using a hemocytometer. This diluted spore suspension served as the inoculum for UV-C light exposure. For the selected endophytic fungus *A. sojae*, the prepared spore suspension was transferred into autoclaved Petri plates. These plates were then positioned beneath a UV-C light source at a distance of 12 cm for varying exposure times: 15, 30, and 45 min. The entire irradiation process was conducted in the absence of light exposure to avoid photo-reactivation effects. After each UV treatment period, 1.5 ml of the irradiated spore suspension was withdrawn and serially diluted up to 10 times using sterilized distilled water. A small portion of the diluted spore suspension was placed on Petri dishes covered with potato dextrose agar (PDA) medium. These plates were then kept in incubation at 28 °C for three days to allow the fungal culture to start sporulation. For screening the selected UV-C irradiated strains of endophytic fungi, Czapek broth media supplemented with different concentrations of Cr^{+6} (specifically, 300 ppm, 600 ppm, and 900 ppm) were employed. The growth of these endophytic fungi, including measurements of fresh and dry mycelial weight, was recorded after incubating the mutated fungal strains in a shaking incubator (model: LSI-1005R) for seven days. Additionally, the UV-irradiated strains were grown on potato dextrose agar (PDA) media and incubated for seven days at 30 °C to observe their colony morphology.

2.5. Determination of fungal (wild type and their mutants) metabolites

A calorimetric bioassay was conducted to measure the levels of indole-3-acetic acid (IAA), salicylic acid, flavonoids, and phenolic contents in the fungal culture filtrate (FCF) of both the wild-type and UV-C irradiated strains. The FCF was obtained by filtering the fungal culture grown in Czapek broth through Whatman No.1 filter paper. This allowed for the subsequent quantification of these bioactive compounds in the culture filtrate.

2.5.1. Indole acetic acid (IAA)

The strength of indole-3-acetic acid (IAA) in the FCF was assessed utilizing the Salkowski reagent, and measurements were acquired employing a spectrophotometer (PerkinElmer Lambda 25 double-beam spectrophotometer) at a wavelength of 540 nm [26].

2.5.2. Salicylic acid (SA)

The procedure outlined by Warrier et al. [27] was exercised to compute the concentration of salicylic acid (SA) in the FCF. Under this approach, 0.2 ml of FCF was blended with 5.8 ml of newly prepared 1 % ferric chloride solution. The absorbance of this mixture was then computed at a wavelength of 540 nm.

2.5.3. Flavonoids

Quantification of flavonoids was performed through $AlCl_3$ method, adhering to the methodology set forth by El Far et al. [28]. To be more precise, we incorporated 0.25 ml of FCF into 50 μL of 10 % $AlCl_3$, 50 μL of 10 % potassium acetate, and 2.4 ml of 80 % methanol. After the mixture had been well-mixed, it was placed in incubation at 25 °C for 25 min. The absorbance was then computed at 415 nm, exhausting methanol as a blank.

2.5.4. Phenolic content

The phenolic content in FCF was quantified utilizing a modified approach as outlined by Ainsworth and Gillespie [29]. In this method, 1.4 ml of double distilled water and 0.25 ml of Folin-ciocalteu reagent were added in a 1:1 ratio to 100 μL of FCF. Following a 4-min incubation at 26 °C, 1 ml of 20 % sodium carbonate was introduced. The resulting mixture was heated for 1 min, and after it

cooled down, the absorbance was measured at 650 nm.

2.6. Screening of wild type and UV-C irradiated A. sojae for growth promotion and Cr^{+6} alleviation in B. napus

B. napus seeds underwent sterilization for 6 min using a 0.1 % $HgCl_2$ solution, followed by three rinses with autoclaved distilled water. These disinfected seeds were then evenly distributed within pots each filled with 300 g of sterilized, autoclaved soil. The soil used in this study was obtained from the botanical garden of Abdul Wali Khan University. It underwent thorough cleaning and was then autoclaved to ensure its suitability for experimentation. A randomized complete block design (RCBD) was implemented for the pot experiment, with each treatment being replicated three times, featuring 10 sterilized seeds per treatment. After germination, any *B. napus* seedlings displaying non-uniform growth were removed, leaving only three uniform seedlings in each pot. The experimental setup was organized as follows:

Ctrl = endophyte free *B. napus* seedlings without undergoing Cr stress.
T1 = endophyte free *B. napus* seedlings exposed to 25 ppm Cr^{+6}
T2 = endophyte free *B. napus* seedlings exposed to 50 ppm Cr^{+6}
T3 = *A. sojae* SH 20 associated *B. napus* seedlings without undergoing Cr stress.
T4 = *A. sojae* SH 20 associated *B. napus* seedlings exposed to 25 ppm Cr^{+6}
T5 = *A. sojae* SH 20 associated *B. napus* seedlings exposed to 50 ppm Cr^{+6}
T6 = *A. sojae* SH 20 (45f) associated *B. napus* seedlings without undergoing Cr stress.
T7 = *A. sojae* SH 20 (45f) associated *B. napus* seedlings exposed to 25 ppm Cr^{+6}
T8 = *A. sojae* SH 20 (45f) associated *B. napus* seedlings exposed to 50 ppm Cr^{+6}

All the pots were situated outdoors in the field during the winter season, exposed to natural environmental conditions, and received a daily irrigation of 50 ml of water per pot. After 28 days from the initial germination, the growth parameters of the plants were systematically recorded.

2.7. Root colonization

The roots of *B. napus* seedlings were exposed to staining with lactophenol cotton blue to facilitate the examination of fungal mycelia and hyphae under a light microscope, following the method described by Larone and Larone [30]. In brief, a few drops of lactophenol cotton blue were applied to the root section positioned on a glass slide. After 3–4 min, any excess stain was removed by rinsing the root section with 70 % ethanol, followed by two rinses with distilled water. To visualize fungal hyphae, thin sections of the target roots were placed on glass slides and examined using a light microscope equipped with a 40X objective lens.

2.8. Total pigments analysis

In the presence of Cr^{+6} stress, the leaves of the plants were utilized for pigment analysis, employing a spectrophotometer following the method outlined by Sumanta et al. [31]. To perform this analysis, fresh leaves (0.5 gm) were crushed and combined with 5 ml of 80 % acetone. The supernatants were separated from the extract by centrifugation at 7500 rcf for approximately 15 min. Following centrifugation, absorbance of the resulting supernatants was measured at two wavelengths, namely 645 nm and 663 nm, utilizing a PerkinElmer Lambda 25 Double Beam Spectrophotometer. Furthermore, the carotenoid content in the samples was determined by recording absorbance at 480 nm [32].

2.9. Metabolites profile of the chromate stressed seedlings

2.9.1. Total flavonoids and indole acetic acid

To extract total flavonoids, 1 g of seedlings was crushed and mixed with 10 ml of 80 % ethanol. The mixture was left to sit overnight in a shaking incubator at 25 °C. Afterward, the supernatants were separated from the extract by centrifugation at 7500 rcf for approximately 15 min and were then stored in 25 ml Falcon tubes at 4 °C. The estimation of total flavonoids was conducted using the $AlCl_3$ method, as previously described [28]. To measure the total indole acetic acid in the leaf extract, the Salkowski reagent method was employed as discussed earlier.

2.9.2. Salicylic acid

The extraction of salicylic acid from the seedlings involved grinding 0.5 g of seedling material in 5 ml of 80 % ethanol. The ground sample was then allowed to stand overnight in a shaking incubator at 28 °C. Afterward, the supernatant from the extract was separated by centrifugation at 7500 rcf for approximately 15 min. The collected supernatant was carefully stored at 4 °C. The quantification of salicylic acid in the extract was carried out using the previously mentioned method [27].

2.9.3. Total phenol content

To estimate the phenolic contents, 1 g of seedlings was homogenized in 10 ml of ethanol. The homogenized sample was then incubated at 40 °C for approximately 3 h. Following incubation, the mixture underwent centrifugation at 7500 rcf for 15 min at 25 °C. After carefully isolating the supernatant, it was subsequently re-dissolved in 10 ml of distilled water and stored at 4 °C before analysis. The estimation of phenolic contents in the extract was conducted using the specified methodology [29] as discussed earlier.

2.10. Antioxidant enzyme activities in Brassica napus under Cr stress

2.10.1. Peroxidase activity

Peroxidase activity was determined using guaiacol as a substrate for the dehydrogenation reaction. To extract the enzyme, 0.2 g of plant sample were ground in 6 ml of 0.1 M phosphate buffer (pH 7.0) using a mortar and pestle. The resulting mixture was then subjected to centrifugation for approximately 15 min at 7500 rcf. The total reaction mixture consisted of 0.2 ml of the enzyme extract, 6 ml of 0.1 M phosphate buffer at pH 7, 0.06 ml of 12.3 mM hydrogen peroxide, and 0.1 ml of 20 mM guaiacol, following the protocol described by Pütter [33]. After vigorously shaking the reaction mixture in a cuvette, any change in optical density (ΔOD) of 0.1 was recorded at 436 nm. The POD activity was calculated by the following formula:

$$\text{Peroxidase enzymatic activity} = \left(\frac{500}{\Delta t}\right) \times \left(\frac{1}{1000}\right) \times \left(\frac{TV}{VU}\right) \times \left(\frac{1}{F\,wt}\right)$$

in the given formula;

Δt = time change (per minute),

TV = total volume of extract

VU = Volume used (Sousa et al., 2012).

F wt = fresh weight of leaf tissue in (g).

2.10.2. Catalase activity

Catalase activity in the leaves of *B. napus* was assessed following the method outlined by Huseynova [34]. To perform this assay, 2.6 ml of potassium phosphate buffer (50 mM) and 40 μL of enzyme extract were thoroughly mixed, as previously described. This mixture was then supplemented with 0.4 ml of H_2O_2 (15 mM), and the absorbance was measured at 240 nm against a blank reference.

2.10.3. Ascorbate peroxidase activity

Ascorbate peroxidase activity (APX) was determined using the method described by Asada [35]. In this procedure, 0.3 ml of enzyme extract was mixed with 900 μL of potassium phosphate buffer (50 mM, pH 7.0), 150 μL of ascorbic acid (0.5 mM), and 150 μL of H2O2 (0.1 mM). The absorbance was recorded at 290 nm at 30-s intervals for a total of 5 min, using a blank reference for comparison.

2.10.4. 1, 1-diphenylhydrazyl assay

The DPPH radical scavenging activity was assessed following the technique outlined Meng et al. [36]. Briefly, 0.2 g of plant leaves were homogenized in 2 ml of methanol and then centrifuged at 7500 rcf for 10 min. A DPPH solution was prepared by dissolving powdered DPPH in methanol (0.004 %). Next, 1 ml of the plant sample was mixed with 2 ml of the DPPH solution and incubated in the dark for approximately 30 min. The absorbance was subsequently measured spectrophotometrically at 517 nm against a blank reference.

2.11. Analysis of heavy metal Cr using atomic absorption spectroscopy

2.11.1. BCR sequential method

The quantification of two distinct species of the heavy metal Cr in plant tissues and fungal culture filtrates was conducted utilizing the methodology provided by the Community Bureau of References (BCR) [37]. The extraction was done in three key stages.

1) Acid soluble and exchangeable

A dried sample weighing 0.5 g was combined with 20 ml of 0.11 M acetic acid (Sigma Aldrich in Burlington, MA, USA). The resulting mixture was then placed in a shaking incubator and left for 24 h at a temperature range of 25–30 °C. Following this 24-h period, the residue and the supernatant were separated by subjecting the mixture to centrifugation at 675 rcf for 3 min at room temperature.

2) Reducible fraction

The residue obtained from the first step was further treated by mixing it with 20 ml of 0.5 M hydroxylamine hydrochloride at a pH of 1.5. This mixture was agitated for approximately 16 h at a temperature of 30 °C. After this incubation, the residue and the supernatant were once again separated using the same procedure as described in the first step.

3) Oxidizable fraction

The remaining residues from step 2 were mixed with 5 ml of 30 % hydrogen peroxide (H_2O_2) at a pH of 2. The mixture was continuously shaken for 1 h and then fully dehydrated through evaporation. To ensure precise results, the residues were washed and dried twice. After thorough washing, the dried residue was re-suspended in 25 ml of 1 M ammonium acetate (CH_3COONH_4) and left to

incubate for 16 h at 28 °C.

During each extraction step, the pellets were meticulously washed, shaken for 15 min, and subsequently subjected to centrifugation for 20 min at 504 rcf. This deliberate washing process was carried out to ensure accurate measurements and prevent any contamination from residues.

2.12. Atomic absorption spectroscopy (AAS)

The measurements were conducted using Flame Atomic Absorption Spectroscopy, specifically utilizing the PerkinElmer AAnalyst 700 instrument from the USA. This analysis was performed with an air/acetylene flame, following the standard operational procedure as recommended for this instrument.

2.12.1. Bio-concentration factor (BCF)

Using the given equation, heavy metal (Cr) accreted in the samples was measured.

$$BCF = \frac{Metals\ in\ biomass}{Metals\ added\ to\ media}$$

2.13. ROS visualization using DAB

To visualize the accumulation of total ROS in the leaves, the staining technique involving 3,3′-diaminobenzidine (DAB) was employed, following the methodology described by Jambunathan and protocols [38]. In summary, leaves were immersed in a DAB solution with continuous shaking at 100 rcf for approximately 3 h to allow the solution to infiltrate the leaves. Any excess dye was removed by washing with a bleaching solution made by mixing ethanol, acetic acid, and glycerol in a ratio of 3:1:1. Subsequently, the leaves were treated with ethanol in a water bath to remove the chlorophyll content. The stained leaves were then visualized using a light microscope.

2.14. Statistical analysis

To ensure the reliability and precision of the results, all experiments and assays were repeated three times while maintaining consistent and uniform environmental conditions and laboratory procedures. Statistical analysis was carried out using Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at a significance level of $P \leq 0.05$. This analysis was performed using SPSS software (IBM SPSS Statistics 21). Additionally, graphs were generated using GraphPad Prism (Version 5.03).

3. Results

3.1. Isolation of chromium resistant endophytic fungi

A diverse range of endophytic fungi were isolated from various parts of *E. helioscopia*. Among the assortment of isolated endophytic fungi, SH 20 was singled out due to its remarkable capacity to enhance the growth of *B. napus* and alleviate the effects of chromate stress. In the presence of both 25 ppm and 50 ppm chromate, seedlings associated with SH 20 displayed superior shoot and root growth compared to the control seedlings, as illustrated in Table 1.

Table 1
Influence of endophytic fungi (natural and uv-irradiated variants) on the growth parameters and pigment content of *Brassica napus* cultivated in Cr^{+6}-enriched or Cr^{+6}-free growth media.

Treatment	Shoot length (*C*m)	Root length (*C*m)	Fresh weight (gm)	Chlorophyll *a* (mg/gm)
Ctrl	4.0 ± 0.12^{c}	5.0 ± 0.11^{de}	0.15 ± 0.041^{b}	1.03 ± 0.002^{d}
T1	3.7 ± 0.0^{b}	4.8 ± 0.11^{d}	0.12 ± 0.012^{a}	0.96 ± 0.001^{c}
T2	3.2 ± 0.05^{a}	4.5 ± 0.06^{c}	0.11 ± 0.023^{a}	0.71 ± 0.002^{a}
T3	9.0 ± 0.08^{f}	5.6 ± 0.07^{g}	0.41 ± 0.011^{d}	1.56 ± 0.005^{g}
T4	7.10 ± 0.08^{e}	3.6 ± 0.05^{b}	0.52 ± 0.013^{e}	1.48 ± 0.005^{f}
T5	6.20 ± 0.05^{d}	3.2 ± 0.07^{a}	0.19 ± 0.013^{c}	1.01 ± 0.001^{d}
T6	16.5 ± 0.11^{i}	5.4 ± 0.05^{fg}	2.86 ± 0.016^{h}	0.86 ± 0.002^{b}
T7	13.5 ± 0.08^{h}	5.3 ± 0.04^{f}	1.80 ± 0.012^{g}	1.23 ± 0.004^{e}
T8	9.5 ± 0.05^{g}	5.0 ± 0.05^{e}	0.95 ± 0.009^{f}	1.70 ± 0.002^{h}

Ctrl = control; T1 = plants exposed to Cr at 25 ppm; T2 = plants exposed to Cr at 50 ppm; T3 = plants associated with SH 20 and without undergoing Cr stress; T4 = plants associated with SH 20 and exposed to Cr stress at 25 ppm; T5 = plants associated with SH 20 and exposed to Cr stress at 50 ppm; T6 = plants associated with SH 20 45f (UV irradiated) and without undergoing Cr stress; T7 = plants associated with SH 20 45f (UV irradiated) and exposed to Cr stress at 25 ppm; T8 = plants associated with SH 20 45f (UV irradiated) and exposed to Cr stress at 50 ppm. Values are means of 3 replicates with ±SE (Duncan test $P < 0.05$).

3.2. Morphological characteristics of the target strain

The morphological characteristics of the chosen endophyte were thoroughly examined. The fungal strain SH 20 displayed rapid growth and exhibited a filamentous structure with a brownish coloration (Fig. 1A &B). This strain demonstrated a remarkable ability to produce a significant quantity of conidia on its conidiophores. Notably, the conidia themselves were observed to be spherical to oval in shape, as depicted in Fig. 1B.

3.2.1. Identification on the basis of ITS sequencing and phylogenetic analysis

Fungal strain SH 20 was subjected to identification through ITS rDNA sequencing and subsequent phylogenetic analysis. In order to determine its genus or species, the obtained sequence was compared with existing data in the GenBank sequence database (GenBank Accession No. PP152229). The homology search on GenBank provided classification of the fungal strain SH 20 up to the genus level, revealing a strong similarity to *Aspergillus sojae*, as illustrated in Fig. 1C.

3.3. Screening of Aspergillus sojae and their mutants for Cr tolerance

Following UV exposure, all the obtained variants were subjected to various levels of Cr^{+6} exposures in Czapek Dox broth, as outlined in (Supplementary Table 1). Out of the 21 variants, SH 20 (45f) exhibited the highest growth in the presence of chromate stress. This particular strain, SH 20 (45f), demonstrated superior growth even when exposed to 900 ppm of chromate, outperforming the wild-type strain. Due to its remarkable resistance to increasing chromate stress, SH 20 (45f) was chosen for a comparative study alongside the wild-type strain. The morphology of the fungal colony on PDA and microscopic characteristics of SH 20 (45f) can be observed in Fig. S1.

3.4. Quantification of phytohormones and metabolites in fungal culture filtrate (FCF) under HM stress

In our current study, the fungal strain SH 20 displayed a notable capacity to produce a significant quantity of IAA, even in the presence of heavy metal (HM) stress, as depicted in Fig. 2A. IAA production increased in response to higher levels of heavy metal stress. In contrast, the wild-type strain exhibited considerably lower IAA production, while the UV-irradiated variant SH 20 (45f) consistently produced significantly higher amounts of IAA. Furthermore, IAA production by the mutant strain increased proportionally as the concentration of chromium in the media increased. Additionally, the UV-irradiated strain (SH 20 45f) produced substantially higher quantities of salicylic acid than the wild type. The highest amount of salicylic acid (237 μg/ml) was produced by the mutant strains exposed to 300 ppm of chromate. However, the production of salicylic acid tended to decrease at higher levels of chromate (Fig. 2B). Similarly, there was an increase in flavonoid production up to 600 ppm of chromate, followed by a slight decrease at 900 ppm, as indicated in the figure. Under normal conditions without chromate stress, the wild-type SH 20 produced more flavonoids in the Czapek Dox broth. However, this pattern changed when the strain was exposed to chromate stress. Under Cr stress, the UV-irradiated strain (SH 20 45f) produced a significant amount of flavonoids compared to the wild type (Fig. 2C). The pattern of total phenolics in the fungal strains differed from that of IAA and flavonoids. Under normal conditions, the UV-irradiated strain produced more phenolics. However, this pattern shifted when the strains were exposed to chromate stress. The wild-type strain SH 20 was capable of producing 637 μg/ml of phenolics, whereas the UV-irradiated strain produced 578 μg/ml in Czapek broth supplemented with 900 ppm of chromate (Fig. 2D).

3.5. Bio-reduction, biosorption and bio-concentration factor of Cr^{+6}

Microorganisms often employ a unique strategy called bio-reduction to mitigate the toxicity of hexavalent chromium (Cr^{+6}) by

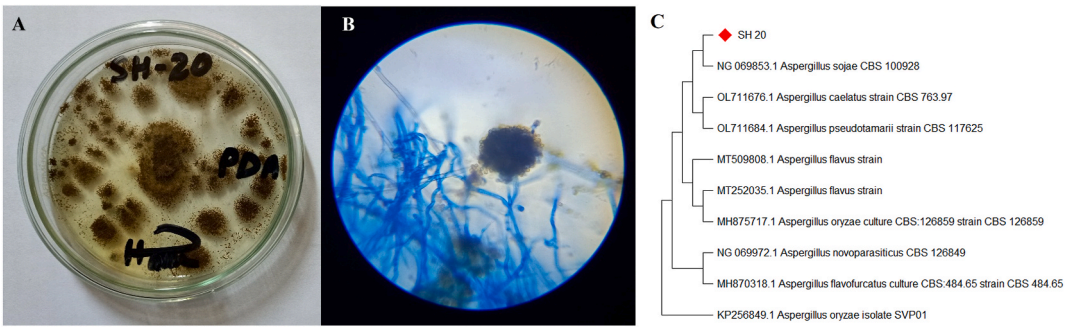

Fig. 1. Characterization of the chromate-resistant endophytic fungus *Aspergillus sojae* SH20 included the following aspects: (A) Examination of colony morphology on PDA, (B) Light microscopy analysis of hyphae and conidiophore stained with lactophenol cotton blue, and (C) Phylogenetic relationship illustrated through a neighbor-joining tree, showcasing the ITS rDNA sequence similarity between the isolate and closely related sequences obtained from the NCBI GenBank database.

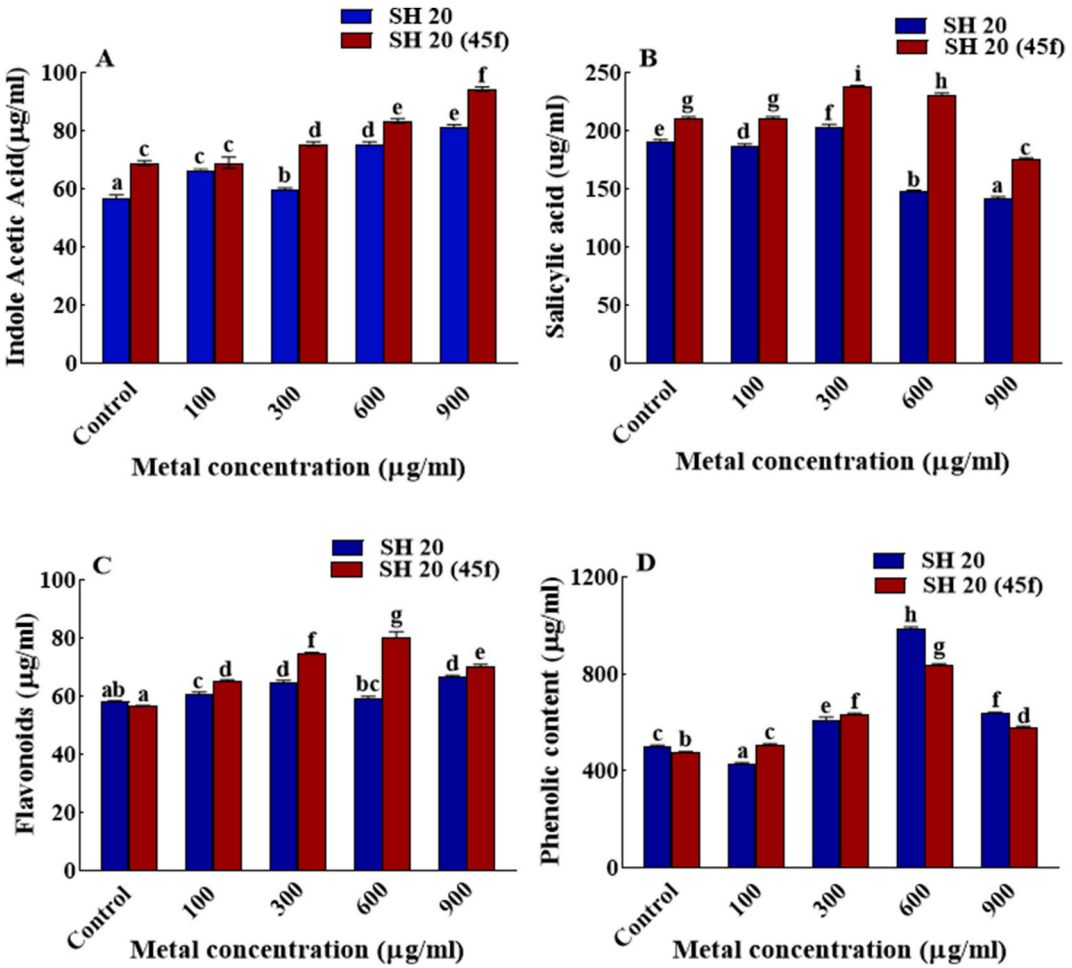

Fig. 2. Production of **A**) IAA, **B)** Salicylic acid, **C)** Flavonoids and **D)** Phenols by fungal strain SH 20 and its mutant (SH 20 45f) under varying concentration of chromate stress. Data shown are mean ± SE of 3 replicates and labels on bars denotes significance among treatments (Duncan test $P < 0.05$).

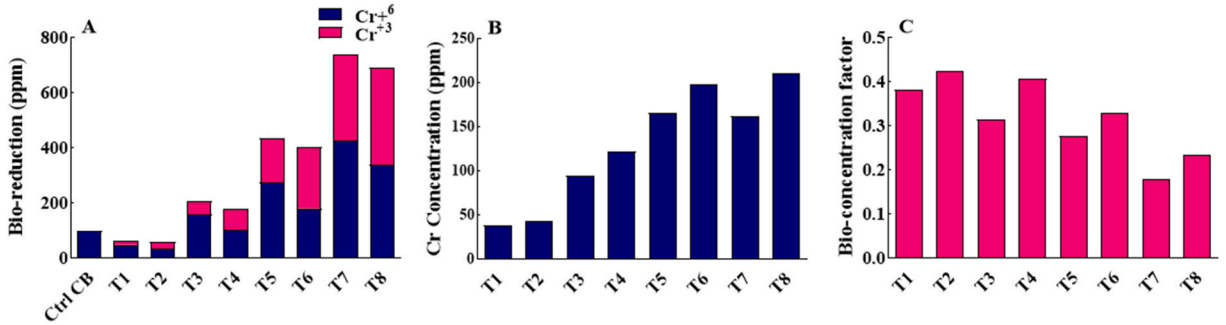

Fig. 3. Impact of *Aspergillus sojae* (SH 20) and its mutant SH 20 (45f) on **A)** Bio-reduction of Cr^{+6} to Cr^{+3} in Fungal cultural filtrate (FCF), **B)** Biosorption and **C)** Bio-concentration factor. Ctrl CB = Czapek broth spiked with 100 ppm of Cr without endophytes; T1 = Czapek broth spiked with 100 ppm of Cr and inoculated with SH 20; T2 = Czapek broth spiked with 100 ppm of Cr and inoculated with SH 20 (45f); T3 = Czapek broth spiked with 300 ppm of Cr and inoculated with SH 20; T4 = Czapek broth spiked with 300 ppm of Cr and inoculated with SH 20 (45f); T5 = Czapek broth spiked with 600 ppm of Cr and inoculated with SH 20; T6 = Czapek broth spiked with 600 ppm of Cr and inoculated with SH 20 (45f); T7 = Czapek broth spiked with 900 ppm of Cr and inoculated with SH 20; T8 = Czapek broth spiked with 900 ppm of Cr and inoculated with SH 20 (45f).

converting it into its less toxic trivalent form (Cr^{+3}). In our current study, we observed that the UV-C irradiated strain was significantly more effective in converting chromium from its hexavalent state to the trivalent form compared to the control fungal strain (non-irradiated). After a one-week incubation period in Czapek Dox broth, the UV-C irradiated strain successfully converted half of the total chromium present in FCF to its trivalent form (Fig. 3A). To further investigate the process, we conducted an assessment of abiotic chromate reduction by adding 100 ppm of hexavalent chromium to blank Czapek media, without the involvement of microorganisms. However, no reduction of chromium was observed in the absence of microorganisms. Additionally, we assessed the biosorption capacity of UV-C irradiated *A. sojae*, which was found to be significantly higher than that of the wild strain (Fig. 3B). Notably, UV-C irradiated fungal mycelia supplemented with 900 ppm of Cr^{+6} exhibited the highest level of chromium adsorption. This suggests that the UV-C irradiated strain has an enhanced ability to adsorb chromium from the environment (Fig. 3B & C).

3.6. Chromium stress mitigation in B. napus

The application of different levels of chromate stress had a significant impact on the growth attributes of *B. napus* seedlings, as summarized in Table 1 & Fig. S2. When exposed to chromate at 50 ppm, the root and shoot lengths of the seedlings were reduced to only 20 % and 10 % of the control seedlings, respectively. The fresh weight of the seedlings exposed to 25 ppm and 50 ppm chromate was reduced to 20 % and 26.7 % of the control, respectively. Additionally, a decline in chlorophyll *a* content was observed in the chromate-stressed seedlings compared to the control. Both the wild and mutant strains significantly promoted the growth attributes of the seedlings compared to the control without chromate stress. In particular, the shoots of SH 20 (45f) inoculated seedlings showed a remarkable increase, approximately 4 times greater than the control. However, it's worth noting that SH 20 (45f) inoculated seedlings had lower chlorophyll content than the control seedlings. When the fungi-associated seedlings were exposed to chromate stress, the shoot growth and fresh weight of the fungi-associated seedlings remained higher than those of the control. On the contrary, the root length was severely reduced to 28 % and 36 % of the control in SH 20-associated seedlings exposed to 25 ppm and 50 ppm of chromate, respectively. Interestingly, in chromate-stressed SH 20-associated seedlings, chlorophyll *a* concentration was higher than in the control seedlings. The chromate stress appeared to synergistically interact with the mutant strain, promoting an increase in chlorophyll *a* content in the seedlings (Table 1).

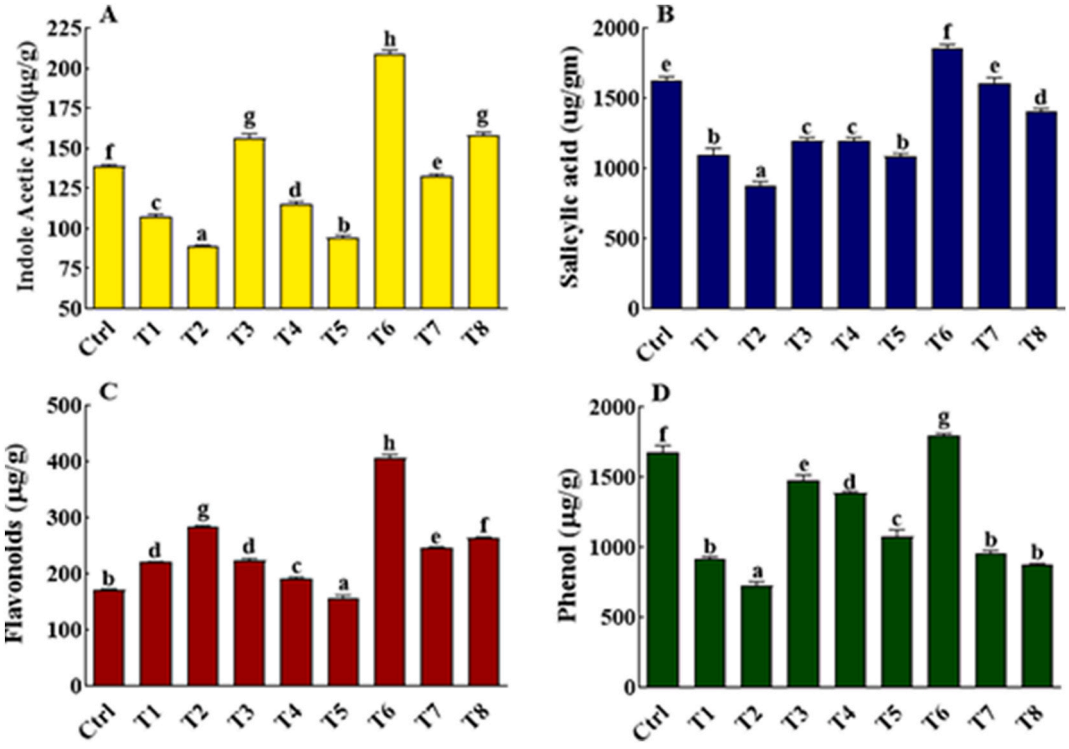

Fig. 4. Effect of heavy metal (Cr^{+6}) and *A.sojae* inoculation on **A)** IAA, **B)** Salicylic acid **C)** Flavonoids and **D)** Phenol contents in *Brassica napus* seedlings. Ctrl = endophyte free *B. napus* seedlings without undergoing Cr stress; T1 = endophyte free *B. napus* seedlings exposed to 25 ppm Cr^{+6}; T2 = endophyte free *B. napus* seedlings exposed to 50 ppm Cr^{+6}; T3 = *A. sojae* SH 20 associated *B. napus* seedlings without undergoing Cr stress; T4 = *A. sojae* SH 20 associated *B. napus* seedlings exposed to 25 ppm Cr^{+6}; T5 = *A. sojae* SH 20 associated *B. napus* seedlings exposed to 50 ppm Cr^{+6}; T6 = *A. sojae* SH 20 (45f) associated *B. napus* seedlings without undergoing Cr stress; T7 = *A. sojae* SH 20 (45f) associated *B. napus* seedlings exposed to 25 ppm Cr^{+6}; T8 = *A. sojae* SH 20 (45f) associated *B. napus* seedlings exposed to 50 ppm Cr^{+6}. Data shown are mean ± SE of 3 replicates and labels on bars denotes significance among treatments (Duncan test $P < 0.05$).

3.7. Effect of wild and UVC irradiated SH 20 on the plant metabolites under heavy metal stress

The levels of IAA in both shoot and root exudates of B. napus decreased as the Cr^{+6} stress increased, as shown in Fig. 4. The IAA content declined from 138.7 μg/gm to 88.5 μg/gm with increasing chromium stress from 0 to 50 ppm. However, inoculating the seedlings with the wild SH 20 and UV-C irradiated SH 20 strains benefited the plants by enhancing IAA production. The highest level of IAA was recorded in seedlings inoculated with UV-C irradiated SH 20 (45f). Seedlings inoculated with UV-C irradiated SH 20 under heavy metal stress were able to produce a significant amount of IAA compared to seedlings inoculated with the wild SH 20 strain under Cr stress (Fig. 4A). Seedlings inoculated with the wild SH 20 strain produced a similar amount of salicylic acid as the control. However, the production of salicylic acid was enhanced when the seedlings were inoculated with UV-C irradiated SH 20 (45f). UV-C irradiated SH 20 (45f) stimulated the seedlings to produce increasing amounts of salicylic acid, which promoted overall plant growth. Seedlings inoculated with UV-C irradiated SH 20 (45f) were able to produce 1851 μg/ml of salicylic acid, which was significantly higher than seedlings inoculated with wild SH 20 (1195 μg/ml of salicylic acid) (Fig. 4B). The production of flavonoids in the seedlings and root exudates increased in a dose-dependent manner with increasing chromium stress. The amount of flavonoids in seedlings inoculated with SH 20 and UV-C irradiated SH 20 (45f) showed significant variation. Seedlings inoculated with UV-C irradiated SH 20 produced greater quantities of flavonoids compared to seedlings supplemented with wild SH 20 (Fig. 4C). The levels of total phenolics were considerably enhanced in seedlings inoculated with UV-C irradiated SH 20 compared to the control and wild SH 20-inoculated seedlings (Fig. 4D). The total amounts of phenolics were significantly higher in UV-C irradiated SH 20 seedlings.

3.8. Chromium in soil and seedlings parts

The increase in soil Cr content led to higher levels of chromium in various plant parts (Fig. 5A). The bioaccumulation of Cr was most pronounced in seedlings supplemented with 50 ppm of Cr. However, the translocation of chromium from the soil to different parts of the plants was reduced when the seedlings were supplemented with the fungal strain SH 20. Interestingly, distinct results were observed when the seedlings were supplemented with UV-irradiated SH 20 (45f). Seedlings inoculated with wild SH 20 exhibited significant absorption of Cr, which accumulated in the roots (Fig. 5B) and leaves (Fig. 5C) of the seedlings. On the other hand, seedlings supplemented with UV-irradiated SH 20 (45f) displayed the lowest absorption of Cr from the soil, resulting in reduced translocation and bioaccumulation of Cr (Fig. 5A, B & 5C).

3.8.1. Bio-concentration factor

Seedlings of *B. napus* that were co-cultivated with or without UV-irradiated and wild SH 20 strains were assessed for the absorption

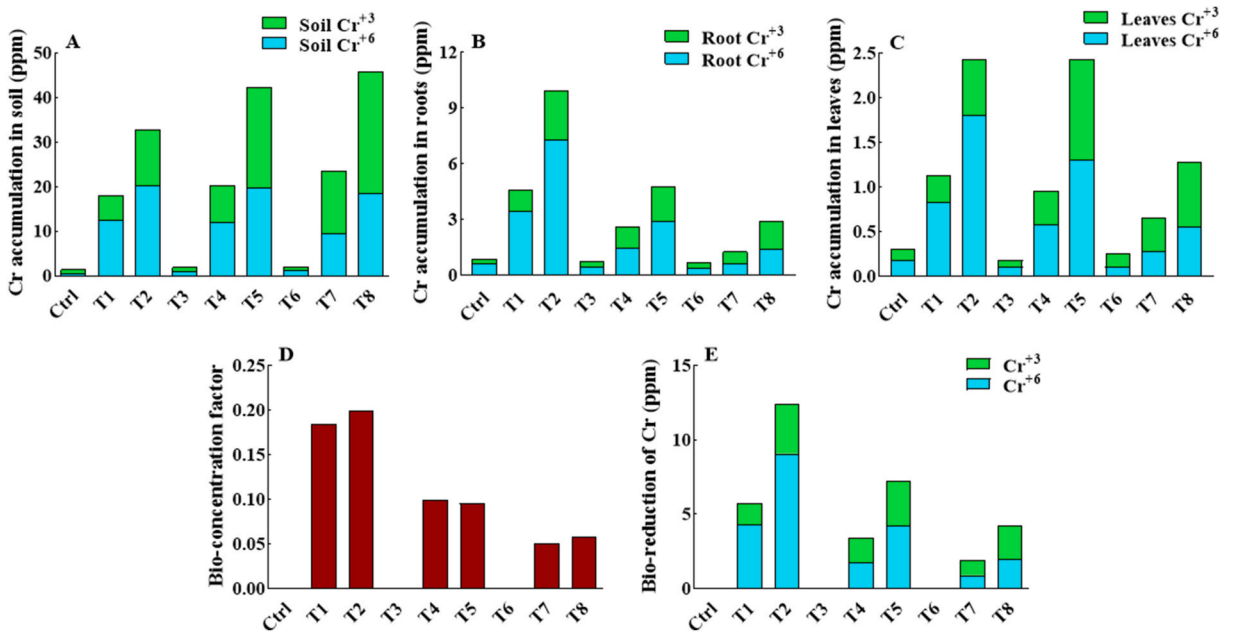

Fig. 5. Effect of *Aspergillus sojae* on **A)** Chromium in soil, **B)** Chromium translocated to roots, **C)** Chromium translocated to leaves, **D)** Bio-concentration factor and **E)** Bio-reduction of Cr. Ctrl = endophyte free *B. napus* seedlings without undergoing Cr stress; T1 = endophyte free *B. napus* seedlings exposed to 25 ppm Cr^{+6}; T2 = endophyte free *B. napus* seedlings exposed to 50 ppm Cr^{+6}; T3 = *A. sojae* SH 20 associated *B. napus* seedlings without undergoing Cr stress; T4 = *A. sojae* SH 20 associated *B. napus* seedlings exposed to 25 ppm Cr^{+6}; T5 = *A. sojae* SH 20 associated *B. napus* seedlings exposed to 50 ppm Cr^{+6}; T6 = *A. sojae* SH 20 (45f) associated *B. napus* seedlings without undergoing Cr stress; T7 = *A. sojae* SH 20 (45f) associated *B. napus* seedlings exposed to 25 ppm Cr^{+6}; T8 = *A. sojae* SH 20 (45f) associated *B. napus* seedlings exposed to 50 ppm Cr^{+6}.

and bioaccumulation of Cr. The bioaccumulation of Cr in the seedlings increased as the amount of Cr in the environment increased. Seedlings supplemented with wild SH 20 accumulated 59.87 % and 58.18 % of the Cr in comparison to control seedlings at 25 ppm and 50 ppm Cr levels. However, bioaccumulation was significantly reduced to 33.18 % and 33.93 % of the control in seedlings supplemented with UV-irradiated SH 20 (45f) (Fig. 5D).

3.8.2. Biotransformation of chromium from toxic to least toxic state

The biotransformation of chromium (Cr) from its toxic form (Cr^{+6}) to the less toxic form (Cr^{+3}) was identified using the BCR extraction method. The fungal strain SH 20 not only limited the absorption of heavy metals but also demonstrated significant abilities in transforming Cr from its highly toxic Cr^{+6} state to the less toxic Cr^{+3} state. Plants inoculated with the mutant SH 20 (45f) strain exhibited the highest biotransformation rates of 53.9 % and 53.57 % at Cr concentrations of 25 ppm and 50 ppm, respectively (Fig. 5E).

3.9. A. sojae regulates antioxidant enzyme system in B. napus

A gradual increase in the activities of catalase (CAT), ascorbate peroxidase (APX), and peroxidase enzymes was observed with increasing Cr stress, as depicted in Fig. 6A, B & 6C. The seedlings associated with both the wild-type and UV-irradiated strains exhibited significantly higher activities of these enzymes compared to the control seedlings. Exposure of these seedlings to heavy metal stress further enhanced the levels of catalase and APX. On the other hand, there was a gradual decrease in 1,1-diphenylhydrazyl (DPPH) free radical scavenging activity with increasing chromium stress. However, the ability of the leaf extract to scavenge DPPH free radicals under heavy metal stress was restored by inoculating the seedlings with UV-irradiated *A. sojae* (Fig. 6D). This suggests that the fungal treatment helped mitigate the oxidative stress caused by chromium, as indicated by the increased activity of antioxidant enzymes and the restoration of free radical scavenging capacity.

3.9.1. 3, 3-diaminobenzidine (DAB) stain assay

The production of hydrogen peroxide (H_2O_2) was directly correlated with the increasing concentration of Cr^{+6}. Control leaves showed no visible spots, whereas plants grown under higher concentrations of Cr^{+6} exhibited denser brown spots spread over a larger

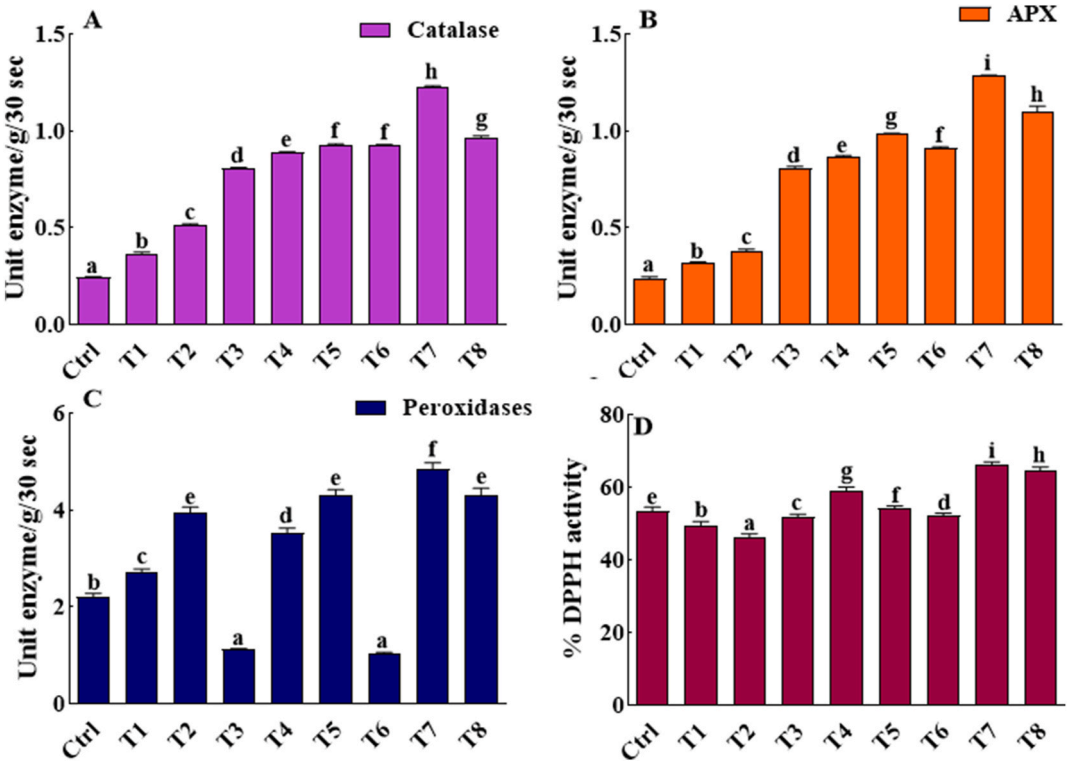

Fig. 6. Effect of Cr^{+6} stress and SH 20 inoculation on **A)** Catalase, **B)** Ascorbate peroxidase, **C)** Peroxidase and **D)** DPPH in host plants. Ctrl = endophyte free *B. napus* seedlings without undergoing Cr stress; T1 = endophyte free *B. napus* seedlings exposed to 25 ppm Cr^{+6}; T2 = endophyte free *B. napus* seedlings exposed to 50 ppm Cr^{+6}; T3 = *A. sojae* SH 20 associated *B. napus* seedlings without undergoing Cr stress; T4 = *A. sojae* SH 20 associated *B. napus* seedlings exposed to 25 ppm Cr^{+6}; T5 = *A. sojae* SH 20 associated *B. napus* seedlings exposed to 50 ppm Cr^{+6}; T6 = *A. sojae* SH 20 (45f) associated *B. napus* seedlings without undergoing Cr stress; T7 = *A. sojae* SH 20 (45f) associated *B. napus* seedlings exposed to 25 ppm Cr^{+6}; T8 = *A. sojae* SH 20 (45f) associated *B. napus* seedlings exposed to 50 ppm Cr^{+6}. Data shown are mean ± SE of 3 replicates and labels on bars denotes significance among treatments (Duncan test $P < 0.05$).

area, in comparison to plants supplemented with lower levels of chromate (Fig. 7A, B & 7C). Moreover, the production of H_2O_2 was significantly reduced upon inoculation with the SH 20 strain (Fig. 7D, E & 7F). Exposure of the plants to Cr^{+6} along with the UV-irradiated SH 20 strain resulted in spotless tissues when exposed to DAB stain. This indicates the least production of H_2O_2 in the leaf tissues, suggesting that the fungal treatment effectively reduced oxidative stress caused by Cr^{+6} exposures (Fig. 7G, H & 7I).

3.10. Root colonization

The root colonization potential of the endophytic fungi was assessed by examining lactophenol cotton blue-stained sections under a light microscope. It was observed that the endophytic fungi had successfully colonized the plant roots after the growth media were supplemented with endophytic fungal biomass (Fig. 8A, B & 8C). The level of colonization was the least in seedlings supplemented with the mutant SH 20 (45f) in comparison to seedlings supplemented with wild-type SH 20 strains (Fig. 8D, E & 8F).

4. Discussion

UV radiation, being a physical agent, is considered an effective method for generating mutant endophytes with improved capabilities [39]. In our study, UV-C radiation was used to induce mutagenesis in *A. sojae*, leading to the creation of a mutant strain (SH 20 45f) that can withstand the adverse effects of Cr^{+6} while promoting plant growth. Among the 21 variants obtained after UV-C irradiation, *A. sojae* (SH 20 45f) exhibited not only the highest resistance to increasing concentrations of Cr^{+6} but also displayed significant

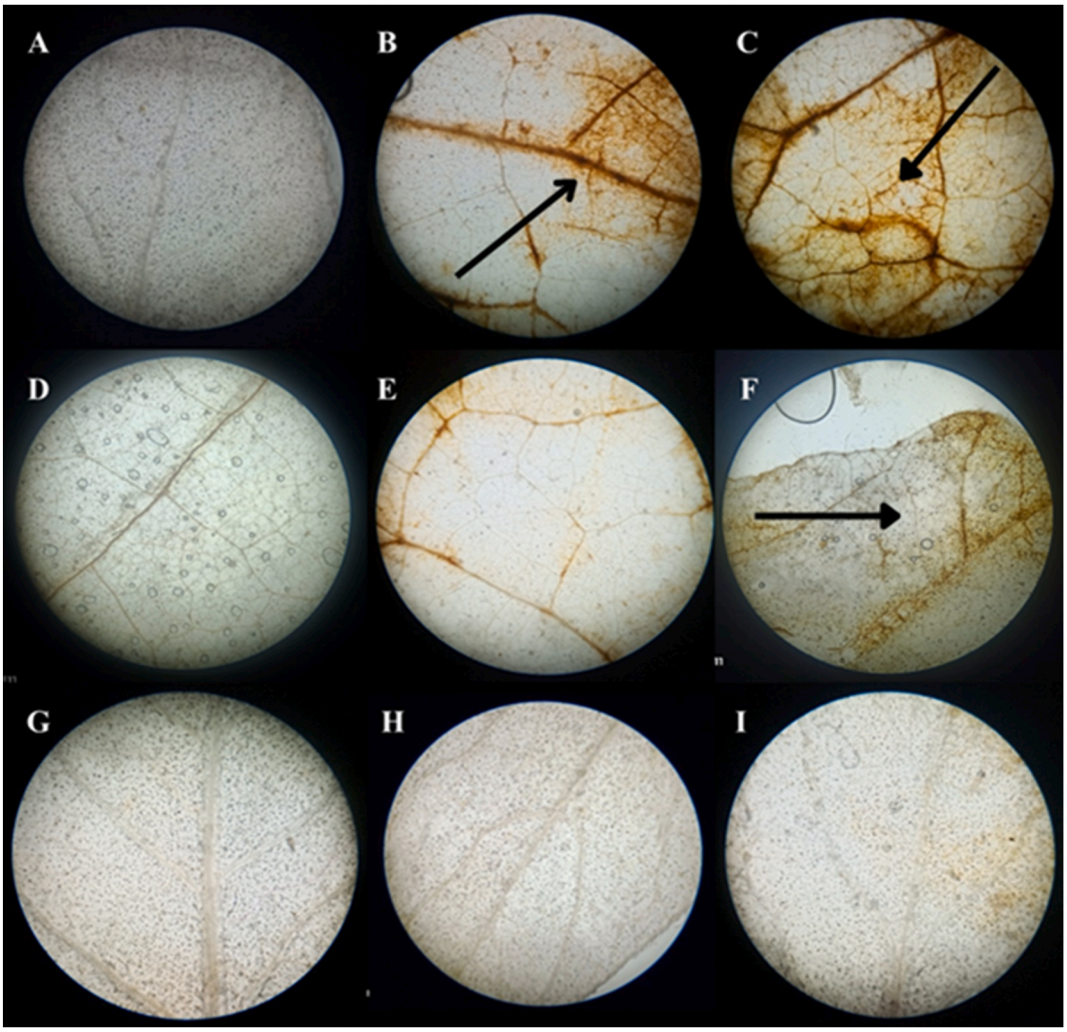

Fig. 7. ROS accumulation in SH 20 and UV irradiated SH 20 (45f) inoculated *Brassica napus* leaves in comparison to control under varying levels of Cr^{+6} stress. **A)** Control, **B)** 25 ppm chromate, **C)** 50 ppm chromate, **D)** SH 20, **E)** SH 20 + 25 ppm chromate, **F)** SH 20 + 50 ppm chromate, **G)** SH 20 (45 F), **H)** SH 20 (45 F) + 25 ppm chromate and **I)** SH 20 (45 F) + 50 ppm chromate. Fresh leaves of 16 days old seedlings were detached from the plants and were stained with DAB in order to visualize ROS.

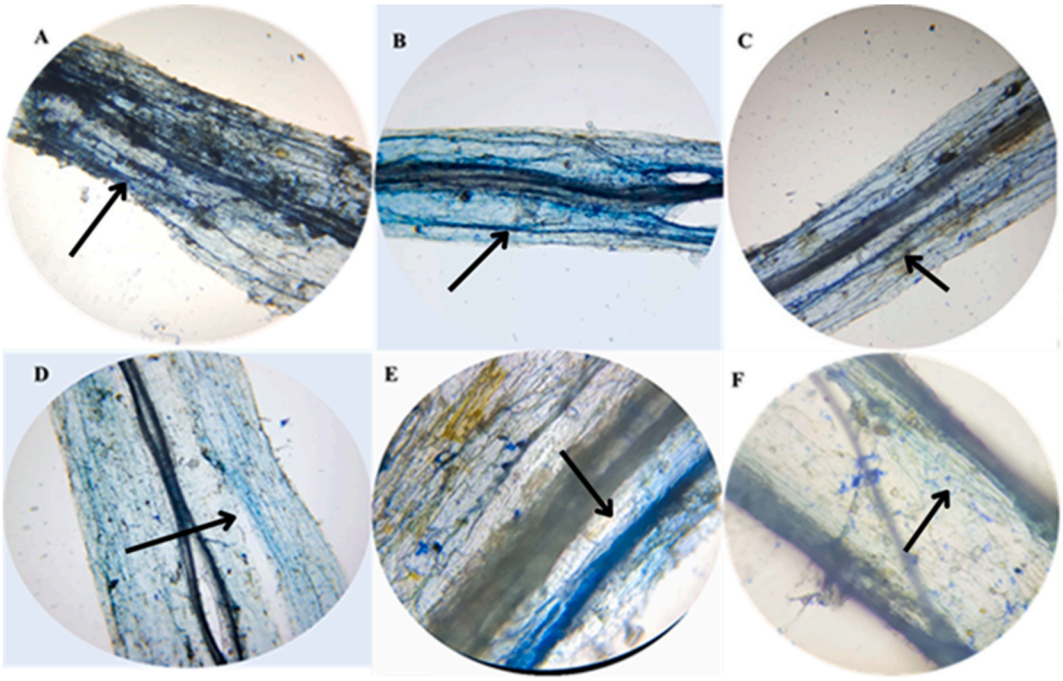

Fig. 8. Colonization of endophytic fungus *Aspergillus sojae* SH20 and UV irradiated SH 20 (45F) under varying Cr^{+6} stresses. **A)** SH 20, **B)** SH 20 + 25 ppm chromate, **C)** SH 20 + 50 ppm chromate, **D)** SH 20 (45 F), **E)** SH 20 (45 F) + 25 ppm chromate and **F)** SH 20 (45 F) + 50 ppm chromate in the cortical constituency of *Brassica napus* root stained with lactophenol cotton blue dye after 21 days of successful colonization.

abilities to enhance plant growth. A noteworthy observation was that UV-C irradiated *A. sojae* secreted elevated levels of various compounds such as indole acetic acid (IAA), salicylic acid (SA), flavonoids, sugar, and phenols, which played a crucial role in enabling the fungus to withstand stressful conditions. In comparison to the non-UV-C irradiated *A. sojae*, the mutant strain produced considerably higher quantities of phytohormones. Additionally, the fungal culture maintained a greater phytohormone-to-biomass ratio in the presence of Cr stress, indicating its adaptability to stressful and challenging environmental conditions [40]. Similarly, Hasanien et al. [41] observed enhanced bioremediation capabilities of heavy metals in *Alternaria alternata* and *Chaetomium globosum* that can play vital role in plant growth promotion. In case *of A. sojae*, the mutant phenotype exhibited superior growth accompanied by higher release of beneficial metabolites. The superior growth and greater availability of the stress mitigating substances made this strain ideal for heavy metal detoxification. This not only helped the mutant to cope better with excessive amount of Cr^{+6} but also promoted its benefit as plant symbiont. Previously, strains of *Trichoderma harzianum* were subjected to UV radiation to enhance the growth of their host species. Following UV exposure, these strains actively promoted the growth of their respective host plants [42]. One crucial strategy employed by endophytes to adapt to heavy metal (HM) stress is to reduce their surface area. UV-irradiated *A. sojae* exhibited impressive resistance to Cr by effectively decreasing its surface area. This reduction in surface area enables the fungus to divide and mitigate the stress. Moreover, it aids in efficiently bio-transforming the heavy metal into a less toxic state in the immediate vicinity of the fungus [43].

Brassica seedlings exposed to different concentrations of Cr stress experienced significant reductions in agronomic traits such as root and shoot lengths, fresh and dry weights, as well as chlorophyll and carotenoid contents. However, when the seedlings were co-cultivated with UV-irradiated *A. sojae*, positive changes were observed in the agronomic characteristics of the host plant. Moreover, there was a significant increase in the levels of plant hormones such as IAA (indole-3-acetic acid), flavonoids, and SA (salicylic acid). The observed hormonal changes in the plant indicate a potential solution for protecting plants against various stresses, including heavy metal stress [44]. In this study, UV-irradiated *A. sojae* demonstrated the ability to produce the highest amounts of IAA, SA, and flavonoids, which played a key role in mitigating heavy metal (HMs) stress. It also triggered a response in the host plant to produce larger quantities of these compounds as the levels of Cr stress increased. On the contrary, the non-irradiated strain of *A. sojae* produced lower amounts of IAA, SA, and flavonoids and exhibited a slower growth rate. This highlights the importance of UV exposure in enhancing the fungal strain's ability to generate these essential compounds and its overall stress-management capabilities [45].

Brassica plants, co-cultivated with UV-irradiated *A. sojae* under heavy metal stress, accumulated higher quantities of flavonoids to counteract the detrimental effects of HMs. The synthesis of phenolic compounds by Brassica plants increased as the levels of heavy metal stress escalated. The increased production of phenolics acts as a potential defense mechanism against metal and oxidative stress by scavenging ROS and chelating metal ions [46]. UV-irradiated *A. sojae* exhibited an exceptional capacity to reduce oxidative stress through increased enzymatic antioxidant synthesis like APX, CAT, peroxidase, and DPPH scavenging activity. This causes a rise in ROS elimination and condensed ROS accumulation in plants. As a result, this not only aids in preserving the typical growth traits of the plant

during abiotic stress but also leads to noticeable enhancements in growth [47].

The bio-reduction of HMs from a harmful to a less harmful state is considered an effective strategy for diminishing toxicity. According to AAS data, when UV-irradiated *A. sojae* was present, approximately half of the hexavalent Cr was transformed into trivalent Cr within one week of inoculation [48]. The process of bio-reduction of hexavalent Cr to trivalent Cr involves two basic mechanisms. First, Cr^{+6} may be reduced to Cr^{+3} by direct interaction with the biomass cell walls. Second, Cr^{+6} may bind to the positively charged amines present in the cell wall's chitin and chitosan, which have a lower reduction potential than Cr^{+6} [49,50]. Furthermore, in addition to the bio-reduction of heavy metals, the normal growth patterns of the seedlings were restored when they were supplemented with *A. sojae*. This growth stimulation, induced by the aforementioned endophytic fungi under both normal and heavy metal stress conditions, suggests that heavy metal reclamation might not be the sole mechanism of phyto-stimulation [51]. The current study indicates that UV exposure enhances the effectiveness of *A. sojae* in promoting the agronomic features of plants and alleviating heavy metal stress through biotransformation and bio-reduction, rendering the heavy metals less accessible to the roots [52].

5. Conclusion

A. sojae strain SH 20, especially the UV-irradiated variant SH 20 (45f), demonstrates significant potential for bioremediation and plant growth enhancement in the presence of chromium stress. This strain exhibits exceptional growth even under high chromium levels, produces essential phytohormones, improves the growth of *B. napus* seedlings, and efficiently adsorbs and transforms toxic chromium. Furthermore, it enhances antioxidant enzyme activities, reducing oxidative stress. Generally, these findings highlight the promising role of endophytic fungi, particularly *A. sojae* SH 20 (45f), in combating chromium toxicity and promoting plant growth under heavy metal stress conditions.

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CRediT authorship contribution statement

Hamza Bacha: Writing – original draft, Investigation, Formal analysis. **Anwar Hussain:** Supervision, Project administration, Methodology, Data curation, Conceptualization. **Waheed Murad:** Supervision, Project administration, Methodology, Conceptualization. **Muhammad Irshad:** Formal analysis, Data curation. **Muhammad Hamayun:** Supervision, Resources, Project administration. **Asma A. Al-Huqail:** Writing – review & editing, Resources, Funding acquisition. **Amjad Iqbal:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Sajid Ali:** Methodology, Investigation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35501.

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