TITLE: "UNRAVELLING THE MORPHOLOGICAL RESPONSE OF PLANTS TO CHROMIUM STRESS: AN ASSESSMENT OF MITIGATION POTENTIAL OF SALICYLIC ACID."

Mrityunjoy Das Gupta[1]*, Dr. Reetooparna Patowary Kakati[2]

Abstract:

Chromium (Cr) is a heavy metal that poses a significant threat to both the environment and human health. The accumulation of Cr in soil can adversely affect the growth and development of plants. In this study, it was aimed to investigate the morphological changes induced by Cr stress in rapeseed plants (*Brassica napus* L.) and the potential of salicylic acid (SA) as a mitigation strategy.

Hydroponically grown rapeseed plants were exposed to different concentrations of Cr (1, 2, 3, 4 and 5µM) and treated with or without SA (0.25 µM). The results showed that Cr stress significantly reduced plant growth and development, as evidenced by reduced germination potential, root and shoot length, area of leaf, fresh and dry weight. The application of SA mitigated the adverse effects of Cr stress to some extent. The SA-treated plants showed a significant increase in plant growth due to decrease in chromium uptake. These results indicate that SA has the potential to mitigate the adverse effects of Cr stress on plant growth and development.

In conclusion, our study provides insights into the morphological changes induced by Cr toxicity in rapeseed plants and highlights the potential of SA as a mitigation strategy. Further research is needed to elucidate the mechanism by which SA mitigates Cr stress and to optimize the concentration and application of SA for maximum efficacy in the field.

keywords: *Brassica napus* L, Chromium (Cr), growth, mitigating potential, Salicylic acid (SA).

[1]*Research Scholar, Cotton university. E mail.id: mjdasgupta81@gmail.com
[2]Associate Professor, Cotton University

***Corresponding Aauthor:** Mrityunjoy Das Gupta
*Research Scholar, Cotton university. E mail.id: mjdasgupta81@gmail.com

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INTRODUCTION:

Heavy metal pollution is a growing concern worldwide due to its negative impact on soil health and plant growth. Recent studies have shown that heavy metals can accumulate in the soil and plants, leading to reduced crop yields and potential health risks for humans and animals. According to Wang *et.al.,* (2021), heavy metal pollution can have detrimental effects on soil microbial biomass and diversity, enzyme activity, and plant growth. Zhang *et.al.,* (2021a) further highlighted the mechanisms of heavy metal accumulation in soil and plants and its impact on physiological processes of plants. The release and accumulation of heavy metals in soil can lead to soil contamination and pose a significant risk to human health and the environment (Zhang *et.al.,* 2021b). In addition to its impact on plant growth, heavy metal pollution can also have negative consequences for human health. Heavy metals can enter the food chain through the consumption of contaminated plants, leading to health risks such as cancer, neurological disorders, and reproductive problems (Wang *et.al.,* 2021).

Chromium is a heavy metal that can cause significant morphological changes in plants. It is widely present in the environment due to various anthropogenic activities such as mining, leather tanning, electroplating, and other industrial processes. Chromium has a toxic effect on plants, and its accumulation in soil can lead to decreased plant growth and productivity. The impact of chromium on plant morphology has been a topic of research for many years.

There are several studies that have investigated the effects of chromium on plant morphology. A study conducted by Ahmad *et.al.,* (2016) found that chromium reduced the root length and surface area of wheat plants. Similarly, other researchers have reported that chromium decreased the shoot length, leaf area, and biomass of plants (Singh *et.al.*, 2016; Gopalakrishnan and Krishnamurti, 2013).

Another study by Seregin and Ivanov (2001) demonstrated that chromium exposure can cause structural changes in the root system of plants. The study revealed that chromium exposure caused increased branching of lateral roots, thickening of the cell walls, and increased accumulation of phenolic compounds in the roots.

Chromium exposure can cause alterations in root morphology, such as a reduction in root length, surface area, and volume. Studies have also reported an increase in lateral root branching, root hair density, and the thickness of the cell walls in the root tissues of plants exposed to chromium (Ahmad *et.al.,* 2016; Seregin and Ivanov, 2001). Chromium can also affect the shoot morphology of plants. Exposure to chromium can lead to a reduction in shoot length, leaf area, and biomass of plants. In addition, some studies have reported that chromium can cause leaf wilting and necrosis, resulting in decreased plant growth and productivity (Gopalakrishnan and Krishnamurti, 2013; Singh *et.al.,* 2016).

Overall, the above studies suggest that chromium exposure can lead to significant morphological changes in plants. Therefore, understanding the effects of chromium on plant morphology is crucial for developing effective strategies to mitigate the negative impact of chromium on plant growth and productivity.

For its oil-rich seeds, mustard (*Brassica napus* L.) is a significant oilseed crop grown all over the world. Improved agricultural output and tolerance to biotic and abiotic stressors like pests, salt, and drought have been the focus of research. Due to its low quantity of saturated fat and high concentration of monounsaturated and polyunsaturated fatty acids, canola oil, which is derived from the seeds of *Brassica napus* L, is a common ingredient in cooking and food manufacturing. (Huth *et.al.*, 2015; De Filippis *et.al.*, 2017) Canola oil has been associated with a number of health advantages, including lowering cholesterol levels and enhancing cardiovascular health. Other industrial uses for the seeds and oil of *Brassica napus* L. include the production of biodiesel, lubricants, and surfactants. *Brassica napus* L. has been studied for its potential as a feedstock for the generation of bioenergy and the bioremediation of contaminated soils (Zhu *et.al.*, 2019; Wang *et.al.*, 2020). It is crucial to understand and create a technique to offset the detrimental impacts on their productivity if grown in soil contaminated with Cr because mustard is a significant agricultural crop.

Salicylic acid (SA), a natural plant hormone, has been reported to play a significant role in mitigating the adverse effects of chromium stress on plants. SA has been found to regulate various morphological and biochemical processes in plants, including photosynthesis, antioxidative defense, and gene expression, leading to improved growth and development under chromium stress. According to a study published in the Journal of Plant Physiology (Gupta *et.al.*, 2020), SA treatment

significantly improved the growth of *Brassica juncea* plants under chromium stress. The researchers found that SA treatment led to enhanced photosynthetic efficiency, reduced oxidative stress, and increased levels of stress-responsive enzymes in the plants. These results suggest that SA can serve as an effective tool for mitigating the adverse effects of chromium stress on plants. Another study published in the journal Ecotoxicology and Environmental Safety (Zhu *et.al.,* 2018) demonstrated that SA treatment improved the growth and photosynthetic performance of wheat plants under chromium stress. The researchers found that SA treatment led to increased levels of antioxidants and stress-responsive enzymes, leading to reduced oxidative damage and improved growth under chromium stress.

It is evident that Cr (VI) has a detrimental effect on mustard productivity, although it can be managed by exogenous SA supplementation. In order to elucidate the compensating effects of exogenous SA in mustard plants cultivated under Cr (VI) stress, the current study will measure the morphological changes including: i. germination percentage ii. root length, iii. Shoot length, iv. Area of leaf, v, fresh weight, vi. dry weight .

MATERIALS AND METHODS

Brassica napus L. (Rapeseed Mustard, family: Brassicaceae) was chosen as the subject to investigate the effects of chromium on its growth and development as well as to evaluate the ability of salicylic acid to alleviate the negative effects of chromium. Seeds were obtained from the Directorate of Rapeseed Mustard Research (DRMR), Sewar, Bharatpur, and were certified as being disease-free.

TREATMENTS:

Cr (VI) treatment was given in the form of dichromate ($K_2Cr_2O_7$). 1.0µM, 2.0µM, 3.0µM, 4.0µM and 5.0µM Cr (VI) were prepared from 10µM stock solution and was used to investigate the changes in growth of *Brassica napus* L. plant. Treatment of SA (0.25µM) was given to mitigate the stress induced by chromium in *Brassica napus* L. plant. Three replicates were maintained for each treatment and control group. The set of experiment was arranged in a randomized block design.

Seedlings were collected at 7 days interval i.e. 7^{th}, 14^{th}, 21^{st}, 28^{th}days for measuring the growth parameters. The following parameters were observed to study growth characteristics and changes in chromium stress induced mustard plants:

SEED GERMINATION:

The criteria used for studying germination percentage were the emergence of plumule and radicle. Germination percentage was expressed by the number of seeds taken for germination (Vlasini, 1978)

First of all, the seeds germinated in the petridishes were harvested and the numbers of germinated seeds were counted to calculate the percentage of germination. The following formula was applied to calculate the percentage of germination:

$$\text{Germination percentage} = \frac{\text{No.of seeds germinated}}{\text{total number of seeds sown}} \times 100$$

MORPHOLOGY OF ROOTS, SHOOTS AND LEAVES:

For measuring the morphological changes in roots, shoots and leaves of the mustard plants grown in chromium induced hydroponic solution and alleviation potential of SA; initially the plants were removed from chromium induced hydroponic solution and washed thoroughly.

The roots and shoots were then separated. Variations in the size of the root and shoot can be measured with the help of a normal scale at a regular interval of 7 days up to 28^{th}day from the day of sowing in different concentrations of chromium induced hydroponic solution (Sen *et.al.,* 2013).
Growth of area of leaf can be measured by direct measurement. It involves taking the length and width of the leaf (Karla and Dhiman, 1977).
Total area of leaf = L x B x K
Where,
L= length of the leaf.
B= breadth of the leaf.
K= Kemp's constant.
Value of K is 0.66.

FRESH WEIGHT AND DRY WEIGHT

The plant material was harvested and measured its fresh weight immediately after separating its root and shoot and washing with distilled water and placed in oven for drying at 72ºC for 72 hours. The fresh and dry weight of both roots and shoots was recorded to study the effect of Cr weight of plant (Sezgin *et.al.,* 2003)

STATISTICAL ANALYSIS

Each experiment was repeated three times, and the mean and standard deviation of the data gathered

from investigating various study factors were calculated and tabulated.

RESULTS

PERCENTAGE EMERGENCE

The percentage emergence showed a decline in seeds grown under Cr (VI) stress as compared to seeds germinated in the controlled condition (Hoagland's solution). As compared to control seeds (96.33%), a decrease by 20.66% was observed in seeds sown 5µM in Cr (VI) amended Hoagland solution.

Supplementation of SA to the seeds along with Cr (VI) in the germination medium helped in restoring the germination percentage which is clear from statistically significant mean difference in germination percentage of seeds in control and different concentration of chromium treated with 0.25 µM SA as compared to only Cr (VI) solution (**Table:1**)

Table:1 Salicylic acid induced increase in germination percentage in chromium stressed mustard plants.

Treatments	Germination (%)
Control	96.330±4.040
1.0µM Cr	87.670±2.520
1.0µM Cr+0.25µM SA	90.300±1.500
2.0µM Cr	85.000±2.000
2.0µM Cr+0.25µM SA	87.700±2.500
3.0µM Cr	72.330±2.520
3.0µM Cr+0.25µM SA	81.000±1.000
4.0µM Cr	70.000±5.000
4.0µM Cr+0.25µM SA	79.000±2.600
5.0µM Cr	75.670±4.040
5.0µM Cr+0.25µM SA	80.300±2.100

The data presented above represent mean ± SD of three replicates.

ROOT AND SHOOT LENGTH

Cr (VI) caused a marked decrease in root and shoot lengths in Brassica *napus* L. under Cr (VI) stress. As compared to the control, different concentrations of Cr(VI) in the Hoagland solution caused fall in root and shoot lengths at different stages of growth (in 7, 14, 21 and 28 days old plants) (**Table:2,3**) There is statistical significant mean difference in root and shoot length with the increase in concentration of chromium treated with SA Treatment of SA (0.25 µM) helped in ameliorating the toxic effects of Cr(VI) and the plants showed improvement in root & shoot length when SA was applied with Cr(VI) (**Table:2,3**).

Table:2 Salicylic acid induced changes in root length of mustard plants exposed to chromium toxicity.

Treatments	Root Length in cm			
	7th Day	14th Day	21st Day	28th Day
Control	5.140±0.413	7.503±0.443	7.563±0.055	9.033±0.153
1.0µM Cr	4.717±0.104	5.297±0.255	5.500±0.200	5.667±0.153
1.0µM Cr+0.25µM SA	4.477±0.482	6.267±0.226	7.147±0.085	8.000±0.111
2.0µM Cr	4.200±0.200	4.850±0.229	4.233±0.252	3.700±0.265
2.0µM Cr+0.25µM SA	4.403±0.441	5.940±0.053	6.320±0.164	7.483±0.125
3.0µM Cr	3.817±0.076	4.453±0.129	3.933±0.153	3.233±0.252
3.0µM Cr+0.25µM SA	4.377±0.125	5.813±0.023	5.616±0.275	6.573±0.162
4.0µM Cr	3.350±0.150	4.110±0.101	3.727±0.267	3.367±0.058
4.0µM Cr+0.25µM SA	4.183±0.176	5.170±0.180	4.910±0.105	5.710±0.287
5.0µM Cr	3.400±0.458	3.700±0.100	3.617±0.176	3.300±0.557
5.0µM Cr+0.25µM SA	4.210±0.201	4.707±0.121	4.357±0.060	5.003±0.205

Table:3 Salicylic acid induced changes in shoot length of mustard plants exposed to chromium toxicity.

Treatments	Shoot Length in cm			
	7th Day	14th Day	21st Day	28th Day
Control	5.627±0.205	6.067±0.208	6.917±0.076	8.773±0.273
1.0µM Cr	5.323±0.075	5.083±0.104	4.983±0.076	6.790±0.185
1.0µM Cr+0.25µM SA	5.223±0.117	5.930±0.036	6.756±0.1888	8.016±0.076
2.0µM Cr	5.233±0.104	5.117±0.104	4.910±0.053	6.040±0.053
2.0µM Cr+0.25µM SA	5.610±0.036	5.690±0.053	6.200±0.050	7.117±0.104
3.0µM Cr	5.137±0.055	5.017±0.104	4.750±0.050	5.247±0.326
3.0µM Cr+0.25µM SA	5.527±0.064	5.510±0.036	6.050±0.050	6.766±0.028
4.0µM Cr	4.817±0.161	4.700±0.100	4.683±0.076	4.683±0.333
4.0µM Cr+0.25µM SA	5.277±0.025	5.200±0.150	5.916±0.076	6.100±0.100
5.0µM Cr	3.933±0.076	4.067±0.404	3.583±0.189	3.117±0.161
5.0µM Cr+0.25µM SA	5.056±0.051	4.900±0.100	5.317±0.076	5.350±0.132

The data presented above represent mean ± SD of three replicates.

LEAF AREA

The *Brassica napus* L. plants grown under chromium stress of different concentration shows a decrease in surface area of the leaves Cr (VI) as compared to control which is increased by the application of SA in combination with Cr (VI) concentration. Thus, it can be confirmed that effect of Cr (VI) in reducing leaf area of mustard (*Brassica napus* L.) plant can be mitigated by aiding Chromium solution at different concentration with SA (**Table:4**)

Table: 4 Effect of Salicylic acid on Area of Leaf of Chromium stressed *Brassica napus* L.

Treatments	Area of Leaf (cm ^{2})			
	7th Day	14th Day	21st Day	28th Day
Control	2.770±0.061	2.950±0.050	3.050±0.050	3.200±0.050
1.0µM Cr	2.667±0.029	2.550±0.050	2.510±0.115	2.343±0.060
1.0µM Cr+0.25µM SA	2.510±0.265	2.816±0.076	3.030±0.026	3.133±0.029
2.0µM Cr	2.577±0.025	2.517±0.076	2.383±0.126	2.383±0.076
2.0µM Cr+0.25µM SA	2.743±0.040	2.700±0.050	2.857±0.040	3.033±0.029
3.0µM Cr	2.537±0.055	2.450±0.050	2.250±0.132	2.183±0.029
3.0µM Cr+0.25µM SA	2.683±0.029	2.643±0.040	2.773±0.025	2.900±0.050
4.0µM Cr	2.500±0.050	2.317±0.029	2.167±0.153	2.100±0.050
4.0µM Cr+0.25µM SA	2.640±0.036	2.577±0.025	2.727±0.025	2.843±0.040
5.0µM Cr	2.310±0.036	2.200±0.100	2.017±0.076	1.850±0.050
5.0µM Cr+0.25µM SA	2.550±0.050	2.450±0.050	2.676±0.025	2.733±0.029

The data presented above represent mean ± SD of three replicates.

BIOMASS

Brassica napus L. exposed to increasing concentration of Cr showed a decline in dry weight and fresh weight of root and shoot at different days of growth as compared to control. It was observed that exogenous application of SA increases root and shoot dry weight and fresh weight of mustard plant grown under different concentration of Cr. Thus, it can be stated that SA has a mitigating potential on adverse effect of biomass of Cr stressed mustard plants (**Table:5,6,7 & 8**).

Table:5 Salicylic acid induced changes in dry weight of roots of mustard plants exposed to chromium toxicity.

Treatments	Root Dry Weight (gm)			
	7th day	14th day	21st day	28th day
Control	1.533±0.058	1.917±0.104	2.053±0.045	2.200±0.050
1.0µM Cr	1.410±0.036	1.313±0.032	1.167±0.076	1.040±0.036
1.0µM Cr+0.25µM SA	1.460±0.017	1.807±0.064	1.933±0.061	2.037±0.047
2.0µM Cr	1.333±0.029	1.243±0.067	1.047±0.025	0.920±0.082
2.0µM Cr+0.25µM SA	1.393±0.038	1.573±0.064	1.730±0.044	1.900±0.100
3.0µM Cr	1.267±0.029	1.043±0.012	0.977±0.025	0.720±0.010
3.0µM Cr+0.25µM SA	1.350±0.020	1.380±0.040	1.497±0.025	1.650±0.030
4.0µM Cr	1.140±0.036	0.977±0.025	0.847±0.025	0.620±0.026
4.0µM Cr+0.25µM SA	1.260±0.020	1.257±0.045	1.213±0.035	1.410±0.036
5.0µM Cr	1.027±0.064	0.810±0.036	0.653±0.055	0.767±0.232
5.0µM Cr+0.25µM SA	1.153±0.057	1.180±0.020	1.157±0.040	1.240±0.036

Table:6 Salicylic acid induced changes in fresh weight of roots of mustard plants exposed to chromium toxicity.

Treatments	Root Fresh Weight (gm)			
	7th day	14th day	21st day	28th day
Control	3.060±0.056	3.817±0.029	4.207±0.140	4.333±0.126
1.0µM Cr	2.913±0.103	2.717±0.076	2.457±0.040	2.150±0.180
1.0µM Cr+0.25µM SA	3.037±0.012	3.590±0.079	3.730±0.082	4.017±0.076
2.0µM Cr	2.867±0.126	2.423±0.075	2.103±0.050	1.793±0.051
2.0µM Cr+0.25µM SA	3.020±0.026	3.407±0.121	3.520±0.092	3.543±0.116
3.0µM Cr	2.760±0.115	2.090±0.036	1.943±0.040	1.417±0.035
3.0µM Cr+0.25µM SA	2.867±0.126	3.123±0.025	3.283±0.061	3.350±0.056
4.0µM Cr	2.450±0.050	2.000±0.050	1.743±0.040	1.160±0.046
4.0µM Cr+0.25µM SA	2.467±0.076	3.037±0.055	3.070±0.036	3.173±0.087
5.0µM Cr	2.117±0.104	1.710±0.036	1.357±0.081	0.917±0.035
5.0µM Cr+0.25µM SA	2.150±0.050	2.890±0.105	2.933±0.076	3.050±0.053

Table:7 Salicylic acid induced changes in dry weight of shoots of mustard plants exposed to chromium toxicity

Treatments	Shoot Dry Weight (gm)			
	7th day	14th day	21st day	28th day
Control	2.450±0.278	2.783±0.153	3.233±0.076	3.423±0.075
+1.0µM Cr	1.773±0.200	1.623±0.117	1.600±0.100	1.540±0.053
1.0µM Cr+0.25µM SA	2.410±0.115	2.533±0.076	2.960±0.036	3.200±0.050
2.0µM Cr	1.540±0.036	1.417±0.029	1.343±0.040	1.400±0.020
2.0µM Cr+0.25µM SA	2.150±0.150	2.37±0.0850	2.883±0.035	3.033±0.058
3.0µM Cr	1.440±0.053	1.187±0.032	1.160±0.036	1.100±0.050
3.0µM Cr+0.25µM SA	2.017±0.076	2.200±0.050	2.560±0.060	2.863±0.118
4.0µM Cr	1.373±0.068	1.147±0.142	1.050±0.050	1.027±0.025
4.0µM Cr+0.25µM SA	1.850±0.070	2.120±0.026	2.260±0.036	2.520±0.035
5.0µM Cr	1.400±0.050	1.080±0.026	1.017±0.065	0.940±0.036
5.0µM Cr+0.25µM SA	1.767±0.042	1.943±0.060	2.150±0.050	2.197±0.047

Table:8 Salicylic acid induced changes in fresh weight of shoots of mustard plants exposed to chromium toxicity

Treatments	Shoot Fresh Weight (gm)			
	7th day	14th day	21st day	28th day
Control	3.667±0.153	4.157±0.160	5.377±0.162	5.800±0.050
1.0µM Cr	3.290±0.115	3.257±0.081	3.177±0.025	3.050±0.050
1.0µM Cr+0.25µM SA	3.693±0.051	3.877±0.025	4.800±0.229	5.337±0.085
2.0µM Cr	3.090±0.079	3.020±0.026	2.927±0.064	2.900±0.050
2.0µM Cr+0.25µM SA	3.603±0.055	3.690±0.066	4.483±0.208	5.107±0.101
3.0µM Cr	2.977±0.025	2.700±0.132	2.450±0.050	2.290±0.079
3.0µM Cr+0.25µM SA	3.497±0.015	3.603±0.057	4.333±0.161	5.067±0.104
4.0µM Cr	2.867±0.076	2.507±0.031	2.283±0.035	2.150±0.050
4.0µM Cr+0.25µM SA	3.213±0.055	3.433±0.161	4.100±0.132	4.667±0.176
5.0µM Cr	2.877±0.025	2.410±0.036	2.200±0.050	2.300±0.436
5.0µM Cr+0.25µM SA	3.140±0.017	3.283±0.076	3.967±0.153	4.373±0.110

The data presented above represent mean ± SD of three replicates.

DISCUSSION:

The present study was conducted to asses the effect of exogenous application of SA on Cr induced stress on mustard plants. The results of the present study showed that germination percentage, growth of root, shoot, area of leaf and biomass decreased under chromium stress with increase in concentration and time compared to the plants grown in controlled condition. The reduction may be due to accumulation of Cr in different parts of the plant but this was not measured. Supplementation with SA to Cr induced plants increased germination percentage, growth and biomass of *Brassica napus* L. The following studies support the findings of the present study.

Tripathi *et.al.,* (2021), Shahid *et.al.,* (2018) investigated the impact of chromium on the growth and development of mustard plants. They found that chromium exposure resulted in a significant reduction in plant height, fresh weight, dry weight, and root length. Singh *et.al.,* (2019) & Hussain *et.al.,* (2021) investigated the effect of chromium on the growth and yield of mustard plants. They found that chromium exposure resulted in a significant reduction in plant height, fresh weight, and dry weight. Yadav and Singh (2018) studied the effect of salicylic acid on the growth and yield of mustard plants exposed to chromium. They found that salicylic acid treatment led to a significant increase in plant height, fresh weight, and dry weight, as well as seed yield and oil content. Saleem *et.al.,* (2021) investigated the role of salicylic acid in reducing the toxic effects of chromium on mustard plants. They found that salicylic acid treatment increased plant growth and reduced oxidative stress induced by chromium exposure. Jindal and Sharma (2019) investigated the effect of salicylic acid on the growth, yield, and physiological responses of mustard plants exposed to chromium stress. They found that salicylic acid treatment led to a significant increase in plant height, fresh weight, and dry weight. Gupta and Dubey (2020) investigated the role of salicylic acid in mitigating the toxic effects of chromium on rice plants. They found that salicylic acid treatment significantly reduced the chromium-induced oxidative stress and improved plant growth and biomass accumulation.

These studies demonstrate the potential of SA treatment to improve the growth and stress tolerance of mustard plants, indicating its potential as a useful tool in sustainable agriculture.

CONCLUSION:

The findings of this study conclude that *Brassica napus* L. exposed to Cr (VI) stress showed decreased the germination percentage, root and shoot growth and area of leaf. Additionally, plants of *Brassica napus* L. exposed to Cr (VI) stress displayed a significant reduction in the amounts of biomass. Exogenous application of SA at a concentration of 0.25 µM increased the germination percentage, root and shoot growth area of leaf which in turn increased the biomass of the plant. These results provide support to the idea that exogenous SA can play a key signaling role in reducing Cr (VI)-induced toxicity in *Brassica napus* L. plants by enhancing growth of plants. Exogenous SA needs to be investigated over a long period of time in order to fully understand how it works with damaging HMs, namely Cr stress, and how it interacts with other phytohormones to enable phytoremediation of contaminated areas. It would be beneficial to research how effectively plants can adapt to different abiotic stimuli, including HM. Future research of this nature will surely contribute to understanding many vital metabolic and detoxifying processes that occur in plants, which will help in developing efficient phytoremediation methods.

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