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Rapid Screening of Tomato Varieties for Heavy Metal Stress Resistance Using Aseptic Seed Culture

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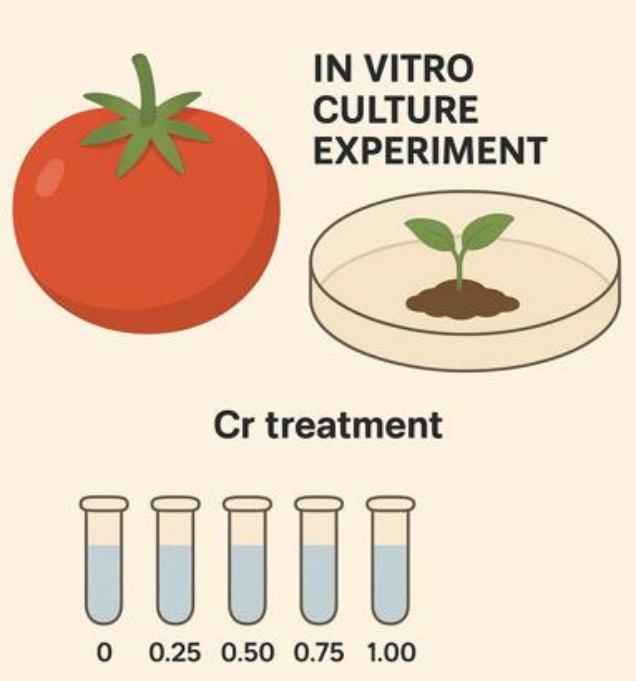
Graphical abstract



Abstract

Tomato (*Solanum lycopersicum*) considered an important vegetable throughout the world. It is also used as heavy metal tolerant crop. Local tomato varieties can perform better for Cr stress and remediate. Based on this hypothesis, the study was carried out to determine the tolerance and resistance of Cr. In vitro culture experiment was carried out on five Cr treatment 0, 0.25, 0.50, 0.75, and 1.00 mM/L along with three replications. Seed germination, mean germination time, shoot length, Dry and fresh weight of shoot and root, shoot and root tolerant index were examined. Results showed that, significant decreased was observed in seed germination percentage in response of Cr treatment compare with control. Whereas, the mean germination time of all cultivars increase as the treatment increases. Shoot height of all tomato cultivars significantly increases as Cr concentration increases, and the inhibition rate at the highest Cr concentration (1 mM) was more than 70%. However, the reducing in root length of all tomato cultivars were recorded. The sterile cultivation experiments exhibited a significant decrease in shoot and root fresh weight in response to Cr treatment compared to the control, in which the highest Cr concentration (1 mM) having a significant impact on shoot and fresh weight. Similarly, the Shoot tolerant index (STI) was observed higher (87.0) at Purple Beauty, followed by Milky Yellow (85.1) a similar trend was also observed in Root tolerant index (RTI) of all the tomato cultivars at different Cr concentrations. It is concluded that as the Cr concentration increases some tomato cultivars performed better for the tolerant of and resistance.

Keywords: Germination; Hexavalent Cr; Solanum lycopersicum; Seedling growth; sterile culture; tolerance

1. Introduction

Chromium (Cr) is one of the most toxic heavy metals found abundantly in the earth's crust, which attenuates the environment (Pokrovsky and Viers 2014). Comparing with the other toxic metals like cadmium, lead, mercury, and aluminum, Cr has received little attention from plant scientists (Shanker *et al.* 2005). After the revaluation industry and agricultural waste discharge from mining, electroplating, leather industry, paint industry, wood processing companies (Hafiz and Ma 2021; Ishchenko

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2018), municipal wastewater, sewage sludge and application of huge number of chemical fertilizers and compost in agriculture are the most significant sources of Cr contamination in soil (Drangert *et al.* 2018). The effects of Cr depend upon its speciation (Trivalent and hexavalent forms), and the mechanism involved in its transportation and distribution is still need to be uncovered. In soil Cr exists in two oxidation states Cr (VI) oxidized hexavalent and Cr (III) less oxidized trivalent. Cr (III) is less mobile, less toxic than Cr (VI) (Patil and Kaushik 2016). However, in the presence of manganese Cr (III) can be oxidized into Cr (VI) in soil. The total chromium (Cr) or hexavalent chromium Cr (VI) in soils can exert significant phytotoxic effects on plants and result in chromium accumulation in crops and vegetables, posing substantial risks to human health through its transfer in the food chain (Hafiz and Ma 2021).

Removal of heavy metal pollutants from wastewater and groundwater through the adsorption treatment shown the advantages of high efficiency (Bai *et al.* 2024). Similarly, no secondary poluution, simple operation and low energy consumption, and it has shown favorable application prospects (Bai *et al.* 2022). The experimental methods used for rapid screening of heavy metal tolerant plants usually include greenhouse soil pot experiments, sand culture, hydroponic methods, field experiments, and tissue culture methods. Plant tissue culture is a valuable tool to develop stress tolerance, screen stress tolerance plants or cultivars including drought tolerance, salinity tolerance, disease resistance, metal hyper-accumulators, and elucidate physiological and biochemical changes during stress. The methods of tissue culture or in vitro culture have the advantages of easy and precise control of conditions, short time required, and simple operation process (Elazab *et al.* 2023), especially for the study of heavy metal tolerance in woody plants, which has more obvious advantages because it reduces the time required for growth and treatment and the amount of space necessary for the tests (Confalonieri *et al.* 2003). However, due to the fact that the results of in vitro culture studies cannot fully reflect the combined effects of various biotic and abiotic factors under soil conditions, it can only serve as a fundamental screening and evaluation method.

In vitro culture methods for screening heavy metal tolerant plants or plant varieties include seed aseptic culture (Peralta *et al.* 2001; Gardea-Torresdey *et al.* 2004; Kumar *et al.* 2009; Buendía-González *et al.* 2010; Ozdener *et al.* 2011; Kundu *et al.* 2018) hypocotyl culture, shoot culture (Bojarczuk 2004; Gatti 2008; Vinterhalter *et al.* 2008; Adki *et al* 2013;Woo *et al.* 2014; Chaitanya *et al.* 2023), root culture (Santos-Díaz and Barrón-Cruz 2011; Ali *et al.* 2014), callus culture and embryo regeneration, (Wu *et al.* 2001). In vitro culture screening technology can establish effective connections with potted, hydroponic, and field experiments of whole plants. In vitro plant tissue cultures can provide a useful experimental system for the study of the mechanisms involved in the detoxification of heavy metal pollutants (Goldhirsh *et al.* 2004). Several studies have conducted in vitro culture for screening Cd or Cr tolerance and accumulation on multiple varieties of the same plant such as tomato (Hafiz and Ma. 2021), flax/linseed (Linum usitatissimum) (Smykalova *et al.* 2010), green gram (Varaprasad *et al.* 2014) and poplar (Di Santo *et al.* 2018), achieving significant results. Plant tissue and cell culture techniques have been used for plant breeding of heavy metal resistance (Ashrafzadeh and Leung 2015).

Usually, researchers prefer to choose wild plants grown under natural conditions especially those grow in pollution sites or heavy metal mine such as weeds (Samantaray *et al.* 2001; Wu *et al.* 2001), aquatic plants (Santos-Díaz and Barrón-Cruz 2011; Alfaro-Saldana *et al.* 2016), and small shrubs (Smantaray *et al.* 1999; Vinterhalter *et al.* 2008, Kumar *et al.* 2009; Waoo *et al.* 2015) for heavy metal tolerance and hyper-accumulation. This is because these plants can grow in nutrient poor environments and have relatively strong. Screening, identification and evaluation of chromium tolerance tomato cultivars were also carried out during the early growth stage of seedlings, which involves transplanting in sand or soil culture pot experiment and hydroponics culture after the seeds germination and seedlings grow normally.

Tomatoes are mostly utilized during in-laboratory experiments due to their high agricultural and nutritional value, together with the high degree of characterization of their genetic and physiological features. The scientific research has proved that tomato acts like a perfect model organism in explaining plant responses against environmental stresses such as heat, drought and salinity stress, in respect to improvement in crop resilience within variable climates (Solankey *et al.* 2015; Chaudhary *et al.* 2019). Moreover, owing to their relatively short growth cycle and high yield, they are ideal for setting up experiments to understand the processes of plant development and metabolic pathways. The genetic variation in the tomato species is also a useful resource in breeding programs aimed at increasing biotic and abiotic stress resistance and improving fruit quality. Finally, much of the background knowledge on tomato genetics and growing conditions helps to place an experiment within context, so that its results may be better understood, hence maximizing the overall impact of the findings.

The aim of this study is to evaluate Cr tolerance and Cr accumulation in shoots and roots of different cultivars of tomato treated with different concentration Cr (VI) by tomato seeds aseptic culture. Seed germination rate, mean germination time (MGT), shoot height and root length, fresh and dry weight of shoot and root, tolerance index of shoot and root of seedling were measured for Cr tolerance evaluation.

2. Materials and Methods

2.1. Plant material

Fourteen tomato varieties (*Solanum lycopersicum L.*) including 10 cherry tomatoes and 4 common tomatoes free from insects and diseases were examined in this study (**Table 1**). The seeds of these tomato varieties were

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purchased from the local market in Longmen Town, Fucheng District, Mianyang City, Sichuan province, China. The purchased seeds were sealed and kept in room temperature until the experiment conducted. The seed viability under heavy metal stress of all 14 tomato varieties was tested with potassium dichromate ($K_2Cr_2O_7$), which was obtained from Chengdu Jinshan, and the chemical reagent and medium not containing sucrose and agar were purchased from Shanghai Bio-way Technology (Sootahar *et al.* 2024).

Table 1. Fourteen Tomato varieties used in this experiment

Number	English name	Type
1	Black Currant	cherry tomato
2	Pink Jade	cherry tomato
3	Purple Beauty	cherry tomato
4	Red Jade	cherry tomato
5	Red Pearl	cherry tomato
6	Saint	cherry tomato
7	Taiwan Red Saint	cherry tomato
8	Taiwan Yellow Saint	cherry tomato
9	Yellow Milk	cherry tomato
10	Yellow Pearl	cherry tomato
11	Pink Cooperative 906	common tomato
12	Pink Cooperative 908	common tomato
13	Qinzu Shanghai 903	common tomato
14	Scarlet	common tomato

2.2. Experiment design and treatments

This study was performed at Plant Tissue Culture Laboratory of School of Life science and Engineering, Southwest University of Science and Technology, China. The experiment was lied on sterile culture media with four Cr (VI) concentrations CK/0, 0.25, 0.50, 0.75- and 1.00-mM L^{-1} prepared from 2mM stock solution using potassium dichromate ($K_2Cr_2O_7$). This screening was performed over four-week duration, the medium contained nutrition and Cr was loaded into transparent plastic tissue culture bottles with a diameter of 9.5 cm and a height of 13.8 cm respectively. A completely randomized block design was followed in this experiment. A total of 15 tissue culture bottles were used to test each tomato cultivar, with each bottle inoculating five seeds.

2.3. Preparation of culture medium

MS media (Murashige and Skoog. 1962), containing macro and micronutrients along with organic substances, was used to assess the seed germination percentage and early seedling growth of the 14 tomato cultivars. Initially, 4.74 g of MS media was accurately weighed, and then supplemented with 30 g of sucrose and 8 g of agar and then 700 ml of distilled water was added into enamel vessel and placed into a thermal furnace to melt the agar. Subsequently, the media was added into the working solution of Cr and distilled water to achieve the desired Cr concentration in total volume 1 liter, and the pH of the freshly prepared media was adjusted to 5.8~6.0 with HCl and NaOH. The prepared media was poured into plastic culture bottles (9.5 cm × 13.8 cm) with 50 mL in each bottle. The culture bottles with media were then sterilized and autoclaved at 121°C for 20 minutes.

2.4. Sterilization of tomato seed

The above mentioned (**Table 1**) fourteen tomato varieties seeds were surface sterilized with 70% ethanol for 1 minute, followed by 0.1% mercuric chloride ($HgCl_2$) solution for 10 minutes and washed thoroughly with distilled water, at least five times. To avoid further contamination, UV light of vertical flow clean bench was used for 20 minutes to completely sterilize the interior and contents before seed inoculation. Each culture bottle containing 50 mL solid medium was inoculated with 5 seeds, with each cultivar inoculated in 15 bottles, and each replicate inoculated in three bottles. This experimental process was repeated three times to avoid any ambiguity. All cultures were maintained at 25±2°C under white fluorescent light at an irradiance of 125 or 114 µmol m^{-2} s^{-1}, with a photoperiod of 16 hours of light and 8 hours of dark.

2.5. Growth parameter determination

The germination percentage of seeds was determined following the seedling evaluation procedure. Seeds were considered germinated if the radicle length reached 2 mm. Germination was defined as the point at which coleoptiles were visible and the plumules grew more than 5 mm in length. The germination percentage was assessed 20 days post-inoculation. Similarly, mean germination time (MGT) was calculated from the start of in vitro culture using the formula described by (Pradhan and Badola. 2012; Sootahar *et al.* 2024). After four weeks, the shoot and root lengths were measured following the removal of excess water by placing them on dry paper. The fresh weight of the shoots was recorded. To determine the dry weight of the shoots and roots, the tomato seedlings were placed in Petri dishes and dried in an oven at 65°C for 24 hours. The tolerance index (TI) to Cr of tomato was determined using the following formula of (El Rasafi *et al*. 2021).

$$\mathrm{STI}=(\text{Shoot length in treatment}/\text{Shoot length in control})\times 100$$

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$$RTI=(\text{Root length in treatment}/\text{Root length in control})\times 100$$

2.6. Statistical analysis

Data were analyzed using SPSS version 23 and GraphPad Prism version 9.0. Means ± standard error (SD) was calculated. One-way ANOVA was used to compare multiple treatment groups, with Tukey's LSD test for post-hoc analysis. Student's t-test was applied for comparisons between control and treatment groups, with significance set at $p< 0.05$.

3. Results and discussion

3.1. Influence of Cr on Seed Germination rate of Fourteen Tomato Cultivars

Sterile culture experiments demonstrated a significant decrease in seed germination percentages in response to Cr treatment concentrations compared with the control, in which the highest Cr concentration (1 mM) severely affected seed germination (**Figure 1**). Compared with the control, the germination rate significantly decreased under all four Cr treatments ($p<0.05$). The Seed germination rate at 1 mM Cr treatment of Purple Beauty, Pink Cooperative 908, Yellow Pearl and Milk Yellow showed the highest inhibition ranged 47.1%~54.1%, as shown in (**Figure 1A**). Furthermore, the tomato cultivars Pink Jade, Scarlet, Qinzu Shanghai 903 and Taiwan Yellow Saint showed similar germination inhibition at Cr stress and the seed germination rate at 1 mM Cr treatment was 42.7%, 40.0%, 39.4% and 39.2% respectively, as shown in (**Figure 1B**). Three tomato cultivars, Black Current, Saint and Pink Cooperative 906, showed germination inhibition from 36.25% to 37.14% in 1 mM Cr treatment compared to the control **(Figure 1C**). Red Jade, Taiwan Red Saint and Red Pearl were relatively less affected by Cr stress with inhibition percentage 35.7%, 33.7% and 31.4% at 1 mM Cr treatment (**Figure 1D**).

Seed germination is the first physiological indicator affected by metal stress. Therefore, the ability of seed germinate in medium containing Cr compared with control would be indicative of its tolerance level to Cr (Gardea-Torresdey *et al.* 2004). A significant decrease of seed germination rate at higher Cr concentration treatments have also found by other researchers. Kumar *et al.* (2009) reported that the seed germination rate of *Pongamia pinnata* decreased from 71% in control to 62%~69% at 200~800 µM Cr (VI) treatments under in vitro culture study, but with promotion effect with 87% germination rate at low Cr concentration (100 µM). Similarly, Babu *et al.* (2014) found a reduction in percentage of seed germination of green gram (*Vigna radiata*) with increasing concentrations of Cr, in which significant reduction in medium supplemented with 50 and 100 ppm Cr but without any nutrients and plant growth regulators was observed when compared to control, only 5% of seed germination at 100 ppm Cr treatment (Varaprasad *et al.* 2014). Seed germination in a dose-dependent manner under in vitro culture was also observed in *Plantago ovata* and *Brassica oleracea*. Kundu *et al.* (2018) found Cr (VI) had no significant effect on germination of *P. ovata* seeds at least up to 1 mM, but reduced to 86% and 59% in 1.5 mM and 1.8 mM doses, respectively, compared to 95% in seeds grown without Cr stress. Ozdener *et al.* (2011) studied the effect of Cr stress at 0.01~1.00 mM concentration on seed germination of *B. oleracea* and observed the significant inhibition when Cr concentration reached to 0.15 mM (Ozdener *et al.* 2011). However, Buendía-González *et al.* (2010) reported that *Prosopis laevigata* seeds could germinate 100% irrespective of the concentrations of Cd(II) (0~2.2 mM), Cr(VI) (0~3.4 mM)), Pb(II) (0~3.0 mM) and Ni(II) (0~4.2 mM) used in tomato Cr tolerance screening of seed sterile culture. Hafiz and Ma, (2021) investigated the response of 45 genotypes on 1.5 mM Cr stress and found four genotypes germinated 100%, while other four genotypes had higher germination percentage in Cr treatment (85%~92%) than in the control and no seed germination was observed in three genotypes. Statistical analysis showed that seed germination of 16 tomato cultivars was inhibited significantly by 1.5 mM Cr treatment (Hafiz and Ma 2021).

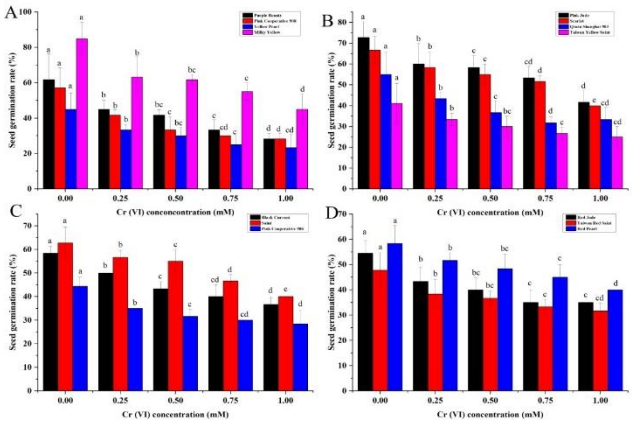

Figure 1. Seed germination rate of fourteen tomato cultivars under Cr concentrations. Different alphabets among the mean Values with different letters showing Signiant difference (LSD test, $p<0.05$). different varieties presented in (A) Purple Beauty, Pink Cooperative 908, Yellow Pearl and Milk Yellow, (B) Pink Jade, Scarlet, Qinzu Shanghai 903 and Taiwan Yellow Saint, (C) Black Current, Saint and Pink Cooperative 906, and (D) Red Jade, Taiwan Red Saint and Red Pearl (D) bar at each value represents the standard deviation of measured values (n=3).

3.2. Effect of Cr on mean germination time (MGT) of Fourteen Tomato Cultivars

All the cultivars showed an increase of MGT following exposure to different concentrations of Cr compared to the control (**Figure 2**). At low Cr concentration treatment (0.25 mM), MGT in Qinzu Shanghai 903, Milk Yellow, Taiwan Yellow Saint, Black Currant, and Scarlet were found no significant compared to the control ($p<0.05$), while the significant increasement of MGT was observed in all 14 tomato cultivars at high Cr concentration (0.5~1.0 mM). At 1 mM Cr treatment, the MGT of Red Pearl, Qinzu Shanghai 903 and Purple Beauty in increased 1.72, 1.77 and 1.77 days respectively than the control (**Figure 2A**), while in Scarlet, Black Currant and Taiwan Red Saint only increased in 1.16, 1.17 and 1.23 day respectively than the control (**Figure 2D**). Other eight tomato cultivars showed

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MGT increasement ranged from 1.31 to 1.66 days than that without Cr treatment (**Figure 2B, C**). MGT is an index of germination speed at time it takes for the seed to germinate. This study found that high concentrations of Cr treatment resulted in prolonged germination time of 14 tomato seeds. Similar studies have found that high concentrations of Cr treatment have a significant impact on seed germination time. Hafiz and Ma, (2021) found through in vitro culture experiments 42 out of 45 tomato genotypes exhibited prolonged MGT under 1.5mM Cr concentration treatment. However, some studies have found that even at a Cr concentration of 3.4 mM in culture medium, the germination time of *Prosopis laevigata* seeds was not affected (Buendía-González *et al.* 2010). Although the germination conditions of seeds in sterile culture medium are better and more consistent than in soil environment, the germination time of seeds is still influenced by various factors such as seed size, seed coat thickness, seed absorption of water and nutrients.

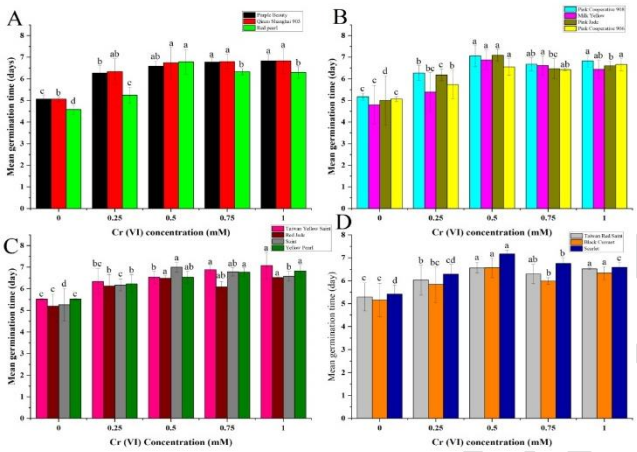

Figure 2. Effect of different Cr concentration on mean germination time of fourteen tomato cultivars under sterile culture Different alphabets among the mean Values with different letters showing Signiant difference (LSD test, p<0.05). Different cultivars show **(**A) Purple Beauty, Qinzu Shanghai 903, and Red Pearl, (B) Pink Cooperative 908, Milk Yellow, Pink Jade and Pink Cooperative 906, (C) Taiwan Yellow Saint, Red Jade, Saint and Yellow Pearl and (D) Taiwan Red Saint, Black Currant, and scarlet bar at each value represents the standard deviation of measured values (n=3).

3.3. Effect of Cr Shoot height and root length of Fourteen Tomato Cultivars

All tomato cultivars significantly decreased shoot height when increasing Cr concentration and the inhibition rate at the highest Cr concentration (1 mM) was more than 70% (**Figure 3**). Compared to the control, the shoot height significantly decreased under all four Cr concentrations (p<0.05). Pink Cooperative 908, Scarlet, Taiwan Yellow Saint and Saint had the highest inhibition rate from 82.9% to 84.6% (**Figure 3A**). Similarly shoot height inhibition in Pink Jade, Red Pearl, Yellow Pearl and Pink Cooperative 906 was found at Cr concentration of 1mM with 82.8%, 81.3%, 80.3% and 79.9% reduction respectively as showed in **Figures 2-3B**. The shoot height of the three tomato cultivars, Purple Beauty, Qinzu shanghai 903 and Red Jade, showed inhibition from 77.3%~77.6% at 1 mM Cr treatment compared to control (**Figure 3C**). Black Currant, Taiwan Red Saint and Milk Yellow were relatively less affected by Cr stress with inhibition percentage 77.1%, 74.5% and 74.1% at 1 mM Cr treatment (**Figure 3D**).

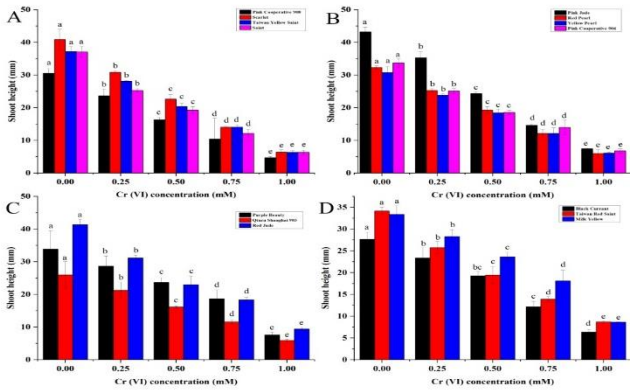

Figure 3. Effect of Cr concentration on shoot height of fourteen tomato cultivars under sterile culture. Different alphabets among the mean Values with different letters showing Signiant difference (LSD test, p<0.05). Different cultivars present in (A) Pink Cooperative 908, Scarlet, Taiwan Yellow Saint and Saint, (B) Pink Jade, Red Pearl, Yellow Pearl and Pink Cooperative 906, (C) Purple Beauty, Qinzu Shanghai 903 and Red Jade and (D) Black Currant, Taiwan Red Saint and Milk Yellow bar at each value represents the standard deviation of measured values (n=3).

3.4. Effect of Cr on tomato on Root length

The significant reduced effect of Cr on root length in all tomato cultivars was also recorded with a concentration dependent trend (**Figure 4**). The root length significantly decreased under all four concentrations as compared to the control (p<0.05). The root length at 1 mM Cr treatment of Taiwan Yellow Saint, Scarlet, Pink Cooperative 906 and Black Current showed the highest inhibition rate of 85.69~86.39% (**Figure 4A**), followed by Yellow Pearl, Saint, Purple Beauty and Taiwan Red Saint with inhibition percentage 83.17%, 83.13%, 82.71 and 81.23% respectively (**Figure 4B**). The root length of the three tomato cultivars, Red Jade, Pink Jade, Qinzu Shanghai 903, showed inhibition from 78.01%~81% at 1 mM Cr treatment compared to control (**Figure 4C**) and Red Pearl, Milk Yellow and Pink Cooperative 908 were relatively less affected by Cr stress, with inhibition percentages of 76.66%, 76.2%, and 75.43% (**Figure 4D**).

This study found that even under low concentration treatment (0.25 mM), Cr stress had a significant impact on the shoot and root growth of tomato seedlings. Several studies have been showed that Cr has significant negative effects on shoot height and root length in in-vitro cultures. The 1.5 mM Cr treatments resulted in significant decreased in shoot height across all tested 45 tomato genotypes and root growth inhibited with visual short taproot and less branch roots after 4 weeks (Hafiz and Ma 2021). In *Plantago ovata*, Cr treatment resulted to a decreased shoot length below one fourth and root length below one twenty fifth compared to control at higher doses (1 mM, 1.5 mM, 1.8 mM) (Kundu *et al.* 2018). Waoo *et al*. (2014) cultured *Lantana camara* plantlets in vitro for 20~25 days and transferred them to Cr containing

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medium (0.5~50 mg/L), who found that the shoot length was not significantly affected at concentrations of 35 mg/L and below, but completely inhibited at concentrations of 40~50 mg/L (Waoo *et al.* 2015). Significant decrease of shoot length and root length was also found in *Convolvulus arvensis* when the Cr level in agar-based medium was 20, 40, and 80 mg/L (Gardea-Torresdey *et al.* 2004). Seed in vitro culture under high Cr concentrations showed significant inhibition effect on seedling growth in shoot and root length, which has been observed in other plants such as alfalfa (*Medicago sativa*) (Gardea-Torresdey *et al.* 2001), *Prosopis laevigata* (Buendía-González *et al.* 2010), *Brassica oleracea* (Ozdener *et al.* 2011) and green gram (*Vigna radiata*) (Babu *et al.* 2014). However, it is interesting that after inoculating *Pongamia pinnata* seeds into Cr containing medium (0~800 µM) for 6 weeks, the shoot height and root length were not affected by Cr stress (Kumar *et al.* 2009). Similarly, after sterile germination of seeds in media containing other heavy metals such as Pb and Ni, significant inhibition of shoot height and root length was observed (Buendía-González *et al.* 2010). High concentrations of heavy metals in the culture medium have also been found to significantly reduce shoot height and/or root length in seedlings cultured in vitro using other explants (Bojarczuk. 2004; Gatti. 2008; Vinterhalter *et al.* 2008; Adki *et al.* 2013; Chaudhry *et al.* 2014; Waoo *et al.* 2015; Dogan 2019; Baktemur. 2023). However, the report by Waoo *et al.* (2014) was significantly different from most research results, as they found that lead concentrations ranged 0.1-45 mg/L had no inhibitory effect on shoot length.

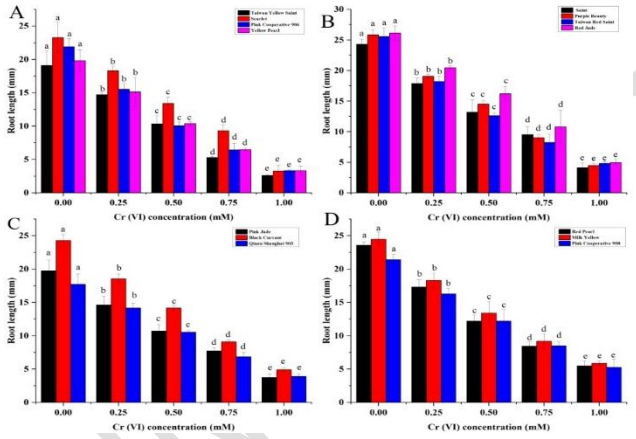

Figure 4. Effect of Cr root length of fourteen tomato cultivars under sterile culture Different alphabets among the mean Values with different letters showing Signiant difference (LSD test, p<0.05). Different cultivars present in (A) Taiwan Yellow Saint, Scarlet, Pink Cooperative 906 and Black Currant, (B)Yellow Pearl, Saint, Purple Beauty and Taiwan Red Saint, (C) Red Jade, Pink Jade and Qinzu Shanghai 903 and (D) Red Pearl, Milk Yellow and Pink Cooperative 908 bar at each value represents the standard deviation of measured values (n=3).

3.5. Influence of Cr on shoot and root fresh weight of Fourteen Tomato Cultivars

The sterile cultivation experiments exhibited a significant decrease in shoot and root fresh weight in response to Cr treatment quantities compared to the control, in which the highest Cr concentration (1 mM) having a significant impact on shoot and fresh weight (**Figure 5, 6**). All four concentration treatments significantly reduced shoot and root fresh weight compared to the control ($p<0.05$). Taiwan Yellow Saint, Yellow Pearl, Pink Cooperative 906 and Purple Beauty showed the highest reduction in shoot fresh weight at 1 mM Cr treatment ranged 79.59%~87.26% (**Figure 5A**), while Yellow Pearl, Purple Beauty, Taiwan Yellow Saint and Pink Jade were found the highest reduction in root fresh weight from 81.3% to 85.4% (**Figure 6A**). Shoot fresh weight of Red Jade, Milk Yellow and Red Pearl were less affected by Cr stress, with inhibition percentage of 64.84%~55.20% at 1 mM Cr treatment (**Figure 5D**) and root fresh weight of Red Jade, Qinzu Shanghai 903 and Taiwan Red Saint showed the lowest reduction at 1 mM Cr treatment compared to control among 14 tomato cultivars (**Figure 6D**). Under sterile in vitro culture conditions, the fresh weight of plantlets that could also show seedlings growth was influenced differently by Cr stress. Kumar *et al.* found that the alterations in fresh weight in the leaves, stems, cotyledons, roots and seed coat of *Pongamia pinnata* seedlings were not significant at five tested Cr concentration (100, 200, 400, 600 and 800 µM) (Kumar *et al.* 2009). However, even at a Cr concentration of 0.1 mM, the fresh weight of *Plantago ovata* was decreased very significantly ($p<0.001$) (Kundu *et al.* 2018). The harmful impact of Cr on the fresh weight of cotyledonary leaf and root in eight-day old seedlings of *Brassica oleracea* was also reported in the growth medium contained Cr at 0.1 mM by Ozdener *et al.* (2011). In another study with in vitro cultivation, 100 ppm Cr ($K_2Cr_2O_7$) applied to potatoes (*Solanum tuberosum*) seedlings induced and developed from nodal explants, the root fresh weight decreased by 72.95% compared to control, showing Cr inhibitory effects on root growth (Chaudhry *et al.* 2014).

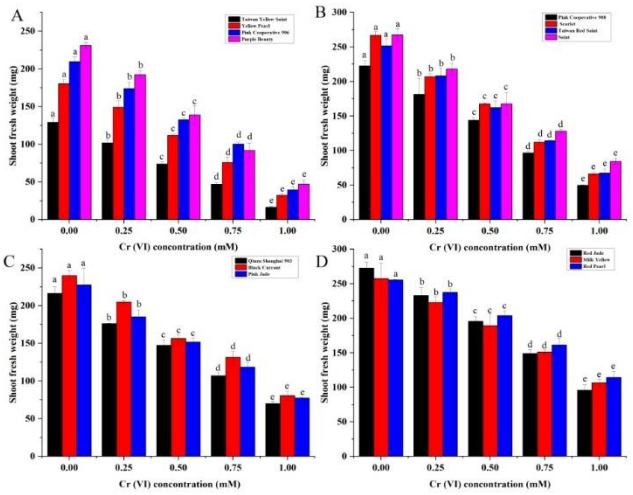

Figure 5. Effect of Cr concentration on shoot fresh weight of fourteen tomato cultivars under sterile culture Different alphabets among the mean Values with different letters showing Signiant difference (LSD test, p<0.05). Different cultivars present in (A) Taiwan Yellow Saint, Yellow Pearl, Pink Cooperative 906 and Purple Beauty, (B) Pink Cooperative 908, Scarlet, Taiwan Red Saint and Saint, (C) Qinzu Shanghai 903, Black Currant and Pink Jade and (D) Red Jade, Milk Yellow and Red Pearl bar at each value represents the standard deviation of measured values (n=3).

3.6. Effect of Cr on shoot and root Dry Weight of Fourteen Tomato Cultivars

Similar to the results of fresh weight determination, the shoot and root dry weight of 14 tomato varieties also showed a significant decrease at different concentrations, and had a concentration dependent effect with a greater decrease as the Cr concentration increased (**Figures 7-8**). At the highest Cr concentration (1 mM), the maximum reduction of shoot dry weight from 85.7% to 90.7% was found in Pink Cooperative 908, Purple Beauty, Scarlet and Pink Cooperative 906 (**Figure 7A**), while the maximum reduction of root dry weight from 78.4% to 82.5% was found in Saint, Pink Cooperative 908, Purple Beauty and Red Jade (**Figure 8A**). Saint, Red Pearl and Qinzu Shanghai 903 showed the lower reduction of shoot dry weight ranged at 69.0%~81.3% than other tomato cultivars (**Figure 7D**), while Red Pearl, Yellow Pearl and Pink Jade showed the low reduction of root dry weight ranged at 57.7%~67.8% (**Figure 8D**). From the overall results of dry weight measurement, Red Pearl was more tolerant to Cr stress, while Pink Cooperative 908 and Purple Beauty were more sensitive to Cr treatment than other tomato cultivars.

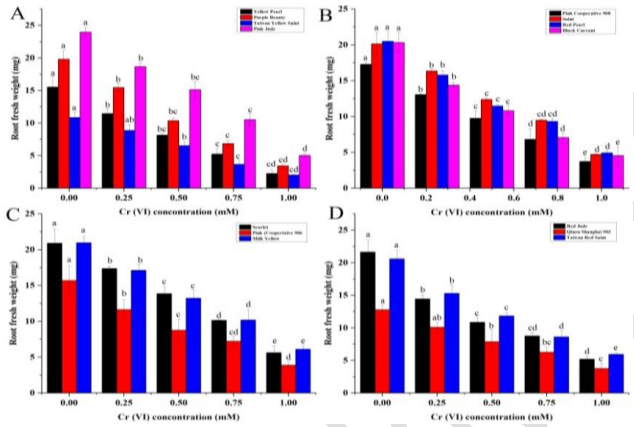

Figure 6. Effect of Cr concentration on Root fresh weight of fourteen tomato cultivars under sterile culture. Different alphabets among the mean Values with different letters showing Signiant difference (LSD test, p<0.05). Different cultivars present in (A) Yellow Pearl, Purple Beauty, Taiwan Yellow Saint and Pink Jade, (B) Pink Cooperative 908, Saint, Red Pearl and Black Currant, (C) Scarlet, Pink Cooperative 906 and Milk Yellow and (D) Red Jade, Qinzu Shanghai 903 and Taiwan Red Saint bar at each value represents the standard deviation of measured values (n=3).

Compared to fresh weight, the dry weight of plant growth can more accurately reflect the absorption and accumulation of nutrients under heavy metal stress, including the content of absorbed heavy metals. Fresh weight contains the moisture content in the plant tissue, and a higher fresh weight value does not necessarily indicate more dry matter. Multiple studies have shown that Cr adversely affected the shoot and root dry weight in vitro cultures. In 41 out 45 tomato cultivars, the Cr treatment at 1.5 mM significantly reduced shoot dry weight, showing that Cr inhibited biomass growth (Hafiz and Ma 2021). Shoot and leaf dry mass of *Convolvulus arvensis* in vitro culture were significant decreased at 80 mg/L Cr concentration but no significant difference at 20 and 40 mg/L Cr concentration compared to control, while the significant decrease in root dry mass under low concentration Cr treatment (20 and 40 mg/L) indicated that roots were more sensitive to heavy metal stress compared to aboveground parts (Gardea-Torresdey *et al.* 2004). The difference in dry weight of different parts of plant can indirectly reflect their capacity on heavy metal accumulation. For example, the DW^{-g} FW of roots, stems and cotyledons of *Pongamia pinnata* remained unaltered indicating non accumulation of Cr and Cu in these organs and a dramatic increase in DW^{-g} FW in the leaves and seed coat indicating possible accumulation of Cr in these two organs (Kumar *et al.* 2009). Ozdener *et al.* (2011) found that the roots and seedlings growth were completely inhibited in 0.25, 0.5 and 1 mM Cr treatments and the dry weight of roots and cotyledon were significantly decreased at 0.1 and 0.15 mM Cr treatments (Ozdener *et al.* 2011). A study found that the root dry weight decreased 84.74% when treated with 100 ppm Cr(VI) (Chaudhry *et al.* 2014). The dry weight of plantlets regenerated from node explants of *Momordica cymbalaria* was also significantly inhibited by cadmium, copper, and zinc, even at lower concentrations (50 µM) (Chaitanya *et al.* 2023).

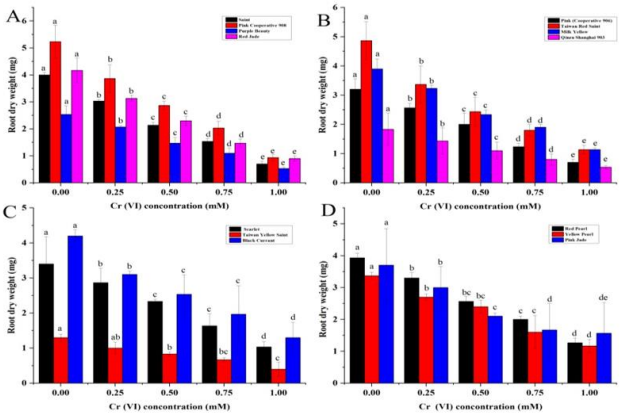

Figure 7. Effect of Cr concentrations on Shoot Dry Weight of fourteen Tomato cultivars under sterile culture. Different alphabets among the mean Values with different letters showing Signiant difference (LSD test, p<0.05). Different cultivars present in (A) Pink Cooperative 908, Purple Beauty, Scarlet and Pink Cooperative 906, (B) Taiwan Red Saint, Taiwan Yellow Saint, Red Jade and Pink Jade, (C) Black Currant, Yellow Pearl and Milk Yellow and (D) Saint, Red Pearl and Qinzu Shanghai 903 bar at each value represents the standard deviation of measured values (n=3).

However, the sensitivity of different plants to Cr stress varies significantly. The effect of Cr on *Plantago ovata* dry weight was drastic decreased at 0.3 mM dose compared to the control seedlings, but at the higher Cr (VI) concentrations (0.5~1.8 mM), the dry weight of seedlings was reduced only to about 63% of the control (Kundu *et al.* 2018). Similar to this, almost no obvious impact of Cd concentration at 5~250 µM and Cu concentration at 5~500 µM in medium on shoot dry mass and root dry mass of *Populus alba* clone have been reported from micro shoot cultures by (Marzilli *et al.* 2018).

3.7. Effect of Cr stress on Shoot and Root tolerance index of tomatoes

The shoot tolerance index (STI) under four Cr concentration treatments is shown in (**Figure 9**). At 0.25 mM Cr concentration treatment, STI of 14 tomato cultivars almost had no significant differences. Only STI of Saint (68.3) was significantly lower than Purple Beauty (87.0), Milky Yellow (85.1), Black Currant (84.6) and Qinzu Shanghai 903 (83.2). At 0.50 mM and 0.75 mM Cr concentration treatments, different tomato cultivars showed greater differences of STI than that at 0.25 mM Cr treatment, but overall, the STI were basically similar. The higher STI of Milky Yellow and Purple Beauty was recorded as 70.9 and 70.8 at 0.50 mM Cr treatment and as 54.1 and 55.6 at 0.75 mM Cr treatment. Saint was found samely the lowest STI with 51.8 at 0.50 mM and 32.8 at 0.75 mM Cr treatment. Under 1.00 mM Cr stress, STI of Milky Yellow and Taiwan Red Saint was significantly higher than eight tomato cultivars but had no significant difference when compared with Qinzu Shanghai 903, Purple Beauty, Black Currant and Red Jade. Although STI of Pink Cooperative 908 was recorded the lowest with 15.4, it did not show significant differences among the last seven tomato cultivars.

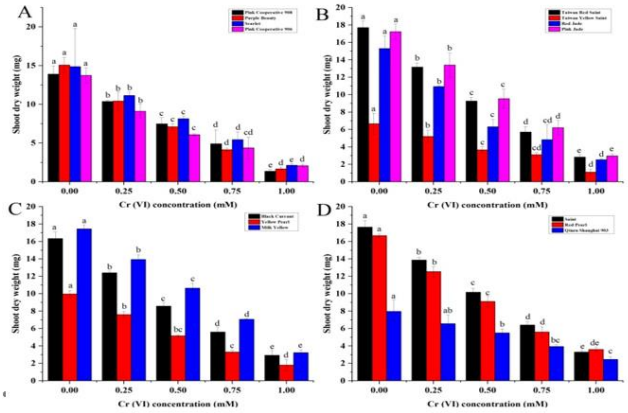

Figure 8. Effect of Cr concentrations on Root Dry Weight of fourteen Tomato cultivars under sterile culture. Different alphabets among the mean Values with different letters showing Signiant difference (LSD test, p<0.05). Different cultivars present in (A) Saint, Pink Cooperative 908, Purple Beauty and Red Jade, (B) Pink Cooperative 906, Taiwan Red Saint, Milk Yellow and Qinzu Shanghai 903, (C) Scarlet, Taiwan Yellow Saint and Black Currant and (D) Red Pearl, Yellow Pearl and Pink bar at each value represents the standard deviation of measured values (n=3).

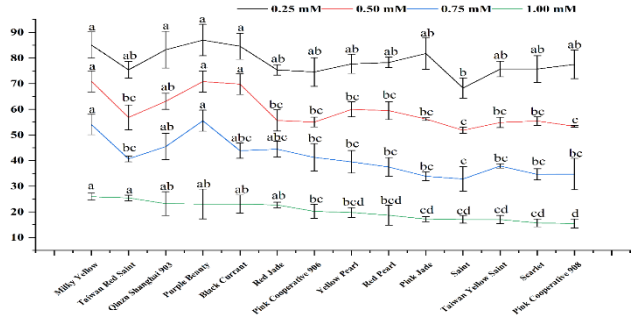

Figure 9. Shoot tolerance index (STI) of fourteen tomato cultivars, different alphabets among the mean Values with different letters showing Signiant difference (LSD test, p<0.05). Bars at each value represents the standard deviation of measured values (n=3).

The root tolerance index (RTI) under four Cr concentration treatments is shown in (**Figure 10**). At 0.25 mM Cr concentration treatment, RTIs had no significant differences among 14 tomato cultivars (p<0.05). At 0.50 mM, the highest RTI was found in Red Jade as 62.0 while the lowest RTI was recorded in Pink Cooperative 906 as 46.1. The RTI of Red Jade was significantly higher than RTI of Red Pearl, Taiwan Red Saint and Pink Cooperative 906. The RTI of Pink Cooperative 906 was also significantly lower than RTI of five tomato cultivars (Qinzu Shanghai 903, Black Currant, Scarlet, Pink Cooperative 908, Purple Beauty). Red Jade also showed the highest STI (41.6) at 0.75 mM Cr treatment and was significantly higher than Yellow Pearl (33.0), Taiwan Red Saint (32.3), Pink Cooperative 906 (29.5) and Taiwan Yellow Saint (28.0), but there were no significant differences among Red Jade and other nine tomato cultivars. However, RTI under the treatment of 1mM Cr concentration showed obvious change that was different from the results obtained at low Cr concentration. RTI of Red Jade was only an intermediate value (19.0) and was significantly lower than RTI of Pink Cooperative 908 (24.6), Milky Yellow (24.0) and Red Pearl (23.4) but was still higher than Scarlet (13.9) and Taiwan Yellow Saint (13.8) significantly (p<0.05). From the RTI presented in (Figure 10) alone, Pink Cooperative 908, Milky Yellow, Red Pearl and Qinzu Shanghai 903 showed relatively higher Cr tolerance, while Purple Beauty, Saint, Yellow Pearl, Pink Cooperative 906, Scarlet and Taiwan Yellow Saint had poorer tolerance.

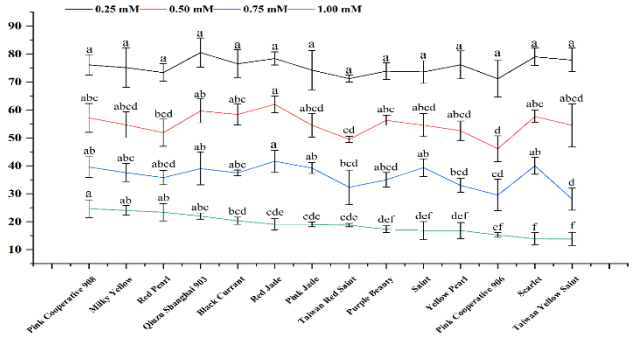

Figure 10. Root tolerance index (RTI) of fourteen tomato cultivars, different alphabets among the mean Values with different letters showing Signiant difference (LSD test, p<0.05). Bars at each value represents the standard deviation of measured values (n=3).

This study found significant differences in STI and RTI among different tomato varieties under higher concentration Cr treatment. Milky Yellow and Taiwan Red Saint exhibited better shoot tolerance while Pink Cooperative 908, Milky Yellow and Red Pearl displayed better root tolerance. Comparatively, no particular variety showed significant sensitivity in terms of STI and RTI, and there was no significant difference among six or seven cultivars with lower tolerance indices. Some researchers evaluated plant heavy metal tolerance through in vitro culture have used the shoot or root tolerance index evaluation method (Buendía-González *et al.* 2010). In a study on in vitro culture screening callus of *Echinochloa colona* tolerant to Cr and Ni, Samanta ray *et al.* intuitively evaluated tolerant and non-tolerant callus using the

growth tolerance index (Samantaray *et al.* 2001). Adki *et al*. (2013) used the formula as 100−[root length (Cr exposed)/root length (control)] for evaluating the RTI of in vitro culture seedlings of *Nopalea cochenillifera* after induction of rooting (Adki *et al.* 2013). In addition, the dry biomass of harvested cells from suspension cell culture of *Jatropha curcas* was also used to evaluate the tolerance index of cell lines (Bernabé-Antonio *et al.* 2015). Similarly, Marzilli *et al.* (2018) studied micro shoot cultures of *Populus alba* and used the dry weight tolerance index of shoots and roots to evaluate plant tolerance to Cd and Cu that could measure the plant ability to grow on metal-polluted medium (Marzilli *et al*. 2018). Except for STI and RTI, seeds vigor index and root toxicity index were used for evaluating the tolerance to Cu, Ni and as in vitro culture of fenugreek (*Trigonella foenum-gracium*) (El-Rasafi *et al.* 2021). However, relying solely on one or two indicators of tolerance cannot fully reflect the tolerance of in vitro cultured plantlets to heavy metals. Therefore, Hafiz and Ma (2021) used the average of four tolerance index i.e. seed germination, seedling survival rate, shoot height and shoot dry weight to comprehensively evaluate the growth tolerance index and found the shoot dry weight was the most sensitive parameter in four tested parameters (Hafiz and Ma 2021).

4. Conclusion

In this study, germination and growth parameters of all tested tomato cultivars were severely affected by 1.0 mM Cr under sterile in vitro culture. Seed germination rate significantly decreased in all cultivars, with Purple Beauty being the most affected and Red Pearl the least. Mean Germination time significantly increased only in Purple Beauty and Qinzu Shanghai 903. Shoot Length decreased in all cultivars, but Milky Yellow, Taiwan Red Saint, and Black Currant were less affected, while Pink Cooperative 908, Scarlet, and Taiwan Yellow Saint showed >83% inhibition. Root length of Pink Cooperative 908, Milky Yellow, and Red Pearl was less affected, whereas Taiwan Yellow Saint and Scarlet showed >86% inhibition. Taiwan Saint had the highest shoot fresh weight, while Qinzu Shanghai 903 had the lowest root fresh weight. Dry weight followed a similar trend. Qinzu Shanghai 903 and Red Pearl were most tolerant in shoot dry weight, while Pink Cooperative 908 showed 90.2% inhibition. For root dry weight, Pink Jade, Yellow Pearl, and Red Pearl were most tolerant, whereas Saint and Pink Cooperative 908 were most inhibited (>82%). Overall, Cr (VI) significantly inhibited germination and growth across all cultivars.

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Conflict of interest

The authors declare no conflicts of interest to report regarding the present study.

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