Effect of heavy metal Stress on seed Germination and Relative Water Content in Tomato Genotypes

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ABSTRACT

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| Heavy metal contamination is a serious problem because it harms soil health, reduces crop yield, and affects food safety. Among these metals, lead (Pb) is especially harmful due to its high toxicity, long persistence in the environment, and tendency to accumulate in food crops like tomato. Six tomato genotypes were treated with four levels of lead heavy metal stress (50, 100, 200, and 300 ppm of lead nitrate) along with normal water as control. The results clearly showed that lead stress caused changes in physiological parameters, especially germination percentage and relative water content (RWC). Germination percentage decreased with the increase in lead concentration. The lowest germination percentage (29.3 %) was recorded in JTL-2105 at 300 ppm, while the highest mean germination percentage (68.7%) was maintained by GT-1. Similarly, RWC also declined as the stress level increased. The lowest RWC (69.24%) was observed in JTL-2105 at 300 ppm, whereas the highest RWC (87.33%) was found in GT-1 across all stress levels. These results suggest that GT-1 is more tolerant to lead stress, while JTL-2105 is the most sensitive genotype.Tolerant genotypes like GT-1 could be used to breed stronger tomato varieties, |

*Keywords:* *Tomato genotypes, Heavy metal stress, Lead stress, Seed germination, Relative Water Content (RWC),*

1. INTRODUCTION

Tomato (*Solanum lycopersicum*) is a member of the Solanaceae family, with a diploid chromosome number of 2n = 24, with a modest diploid genome size of 950 Mb. The worldwide production of tomato is reported to be 186.82 million tonnes from an area of 5 million hectares, with a productivity of 36.97 tonnes/hectare. India is second leads in tomato production by contributing 20.57 million tonnes which is approximately 11.01 % of the total global production (Lata *et al.* 2024). Tamil Nadu, Andhra Pradesh, Karnataka, Madhya Pradesh, Gujarat, Odisha, West Bengal, Bihar, Telangana, Uttar Pradesh, Maharashtra, Chhattisgarh, Haryana, and Himachal Pradesh are the major tomato producing states in India. In Gujarat, during the same period, the area under tomato cultivation was around 52,000 hectares, yielding approximately 1.44 million tonnes (yasangi-pre-harvest-tomato-2023.pdf [ND]).

Tomato grown either in an open field or in controlled environments like greenhouse and tunnels. It requires sufficient water especially at critical stages, such as immediately after sowing and [transplanting](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/transplanting), and irrigation is often needed to ensure sufficient [moisture](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/moisture)  during these delicate growth stages namely; [vegetative growth](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/vegetative-growth) stage, [flowering](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/anthesis) and fruit set (Nuruddin *et al.* 2003). Irrigation often becomes necessary to maintain optimal soil moisture during these critical phases. However, when irrigation water is contaminated with heavy metals (HMs), it can significantly impair plant growth. Common heavy metals found in agricultural water include zinc (Zn), cadmium (Cd), lead (Pb), chromium (Cr), nickel (Ni), and copper (Cu). Although certain heavy metals are essential micronutrients that contribute to key metabolic functions, excessive accumulation beyond safe thresholds turns them toxic, leading to reduced growth, poor yield, and overall plant stress (Ogugua *et al.* 2022).

Heavy metal contamination arises from natural and human-induced sources, posing risks to soil, water, air, crops, and human health. Naturally, metals like Cr, Pb, As, and Hg exist in mineral ores and are released during geological processes (Cairns *et al.* 2023). Ground water pollution occurs due to fertilizers, pesticides, and industrial waste seepage, with high Cd, Pb, and Hg levels detected in Eastern India and Punjab. Air pollution from vehicles and industries releases Pb, Zn, Mn, and Cd, with significant levels found in urban corridors (Shreya Singh and Devi 2023). Agricultural activities contribute through phosphate fertilizers and manure, increasing Cd, As, and Pb in soil and crops. Industrial emissions, such as from power plants, further contaminate surrounding areas. Effective monitoring and management are essential to mitigate these environmental and health risks(Moghimi Dehkordi *et al.* 2024).

Tomatoes cultivated in contaminated soils tend to accumulate elevated concentrations of heavy metals, resulting in adverse physiological effects. Toxic metals such as lead (Pb) and aluminum (Al) are known to inhibit ATP synthesis, enhance reactive oxygen species (ROS) generation, and induce DNA damage. Consequently, these metals significantly suppress seed germination, root elongation, vegetative growth, and relative water content (RWC) (Sharma and Dubey 2005). Heavy metals in the environment can significantly impact seed germination and seedling growth, particularly in tomato plants. These metals, even at low concentrations, can disrupt various physiological and biochemical processes within the plant, leading to reduced germination rates, stunted growth, and even cell death. High concentrations can be toxic causing significant damage and potentially leading to plant death.

2. material and METHODS:

**Plant Material**

Six tomato (*Solanum lycopersicum* L.) genotypes, namely GT6, GT1, JT3, JTL2105, JTL1908, and JTL2103 seed were collected from Vegetable Research Station, Junagadh Agricultural University, Junagadh and selected for the screening experiment. In order to screen tomato genotypes for tolerance or sensitivity to heavy metal stress, graded concentrations of lead (Pb) were applied through lead nitrate [Pb(NO₃)₂] as the source. The treatments consisted of control, 50 ppm, 100 ppm, 200 ppm, and 300 ppm Pb, which were thoroughly mixed into the soil to achieve uniform distribution. The experiment was conducted under greenhouse conditions following a completely randomized design (CRD) with three replications. Observations on various growth and physiological parameters were recorded 21 days after germination.

Germination percentage

Nearly twenty-five healthy tomato seeds, devoid of any damage or disease, were carefully selected. Each petri dish was prepared with a base layer of moist filter paper. These seeds were distributed evenly among the petri dishes, each subjected to varying heavy metal treatments. The petri dishes were then positioned in incubator with controlled environment with appropriate temperature and light conditions, ideally maintained at 25-30 °C, optimal for tomato germination. Monitoring commenced, with the number of germinated seeds recorded 6 days post sowing (DAS). The germination percentage (%) was subsequently calculated following the formula outlined by I.S.T.A. (1976)(International Seed Testing Association (1976) International rules for seed testing. Seed Science and Technology, 4, 51-177. - References - Scientific Research Publishing [ND]).

Germination Percentage (GP %) = Ng/Nt \*100

Where, Ng = Total number of germinated seeds;

Nt = Total number of seeds sown

Relative Water Content(RWC)

To determine the relative water content (RWC) of tomato leaves, fresh leaf samples were collected at **20 days after germination (DAG)**. A known fresh weight (FW) of leaves was immediately recorded and transferred into Petri dishes containing **25 ml of distilled water**. The samples were allowed to hydrate for **four hours** at room temperature. After hydration, the leaves were gently blotted dry using tissue paper, and their **turgid weight (TW)** was measured. The samples were then oven-dried at **84 °C for 5 hours**, until a constant weight was obtained, to determine the **dry weight (DW)**(Weatherley 1951).

  **Relative Water Content (RWC %) = TW−DW**

 **FW−DW**

$$×100$$

3. results and discussion

The germination percentage data was recorded based on the seedling growth of tomato over a period of six days after sowing (DAS) in petri dishes treated with five different concentrations of lead heavy metal containing water along with control. This data was collected for six different genotypes. The impact of various lead heavy metal treatments on the germination percentage of tomato varieties is presented in Table 1

An examination of the data in table 1 indicates that various genotypes of tomato seedlings exhibited significant differences in germination percentage at 6 days after sowing (DAS). The significantly higher (68.77 %) mean value of germination percentage was recorded for the genotype GT1. The significantly lower germination percentage (29.3%) was found in GT (V4).These variations in germination percentages can be attributed to genetic differences among the genotypes, as well as potential variations in their tolerance to Lead heavy metal tolerant levels in , impacting seed germination rates.

Mean effect of treatments on germination percentage was significantly varied between 100% and 7.83 % as given in table 1, and treatment irrigated with distilled water showed highest mean value of germination percentage (90 %) while the irrigated with 300 ppm showed lowest mean value of germination percentage (7.83 %) after 6 DAS. This variation in germination percentages can be attributed to the osmotic stress induced by different levels of lead heavy metal. High heavy metal levels, such as those in treatment at 300 ppm, can lead to higher osmotic stress compared to other treatments, may be due to hindering water uptake by seeds and thus reducing germination rates.

**Table 1 : Effect of lead heavy metal stress on germination percentage of tomato genotypes at 6 DAS**

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| --- |
| **Relative Water Content** |
|  **Genotypes\* (V)** | **Treatment** | **Mean V** |
|  |  |  |  |  |
| **control** | **50 ppm** | **100 ppm** | **200 ppm** | **300 ppm** |
| **GT6** | 90 | 64.34 | 36.87 | 11.54 | 0 | 40.15 |
| **GT1** | 90 | 82.34 | 66.21 | 69.89 | 35.42 | 68.77 |
| **JT3** | 90 | 63.44 | 27.63 | 0 | 0 | 36.61 |
| **JTL2105** | 90 | 39.02 | 17.46 | 0 | 0 | 29.3 |
| **JTL1908** | 90 | 68.24 | 75.95 | 71.56 | 20.94 | 65.74 |
| **JTL2103** | 90 | 61.29 | 39.02 | 14.32 | 0 | 40.93 |
| **Mean T** | **90** | **63.78** | **43.19** | **27.88** | **9.73** |  |
|   | **SE.m** **±** | **C.D.** | C.V. % 2.62 |
| V | 0.398 | 0.799 |
| T | 0.363 | 0.729 |
| (V X T) | 1.786 | 0.89 |

The interaction effect of genotype and treatment was found to be significant for germination percentage in tomato seedling. All the genotypes showed 90% germination under control condition, which decreased with an increase in heavy metal stress concentration. The lower (29.3 %) germination percentage was found in genotype JTL 2105 at 300 ppm heavy metal stress concentrations. The germination percentage was found significantly higher in GT-1 genotype compared to other genotype under the influence of different level of lead heavy metal (50 ppm to 300 ppm). The rate of decreasing germination percentage was found lowest in GT1 compared to other genotypes, when heavy metal stress level increased up to 300 ppm.

Several studies have reported that heavy metals adversely affect tomato seed germination in a concentration-dependent manner. (Karthika *et al.* 2017) observed that chromium stress (0–250 µg g⁻¹) significantly reduced germination percentage from **95% (control) to 55%** at the highest concentration. Similarly, (Shekar *et al.* 2024) reported a progressive decline in germination under mercury stress (1–20 mg/kg), with germination decreasing from **95% in control to 40%** at 20 mg/kg. In line with these findings,(SANTOSH Singh *et al.* 2012) demonstrated that cadmium exposure (1–500 ppm) markedly suppressed germination of tomato seeds, with the highest concentration causing nearly a **52% reduction** compared to the control.

Heavy metals, such as lead (Pb), adversely affect seed germination primarily by interfering with water uptake, enzyme activity, and energy metabolism in seeds. The process of germination requires water absorption (imbibition) to activate metabolic pathways that drive radicle emergence. Heavy metals can create osmotic stress by reducing the water potential around seeds, making water uptake more difficult. Additionally, toxic ions like Pb can disrupt the activity of key hydrolytic enzymes, such as amylases and proteases, which are essential for mobilizing stored nutrients required for early seedling growth (Sharma and Dubey, 2005).

Moreover, heavy metal exposure induces oxidative stress through the overproduction of reactive oxygen species (ROS), which can damage cellular membranes, proteins, and DNA in the embryonic tissues. This oxidative damage further suppresses germination and slows seedling development (Patra and Sharma, 2000). The combined osmotic and oxidative stresses result in delayed or reduced germination rates, as observed in tomato genotypes under increasing Pb concentrations in the present study.

# **Relative Water Content (RWC)**

An examination of the data in table 2 indicates that various genotypes of tomato leaves exhibited significant differences in relative water content at 20 days after gemination. The significantly higher mean value (87.33%) of relative water content was recorded for the genotype GT-1. The significantly lower (77.713%) relative water content was found in JTL 2105. GT-1 might possess efficient stress tolerance mechanisms, enabling them to maintain higher water content even under suboptimal conditions, while genotypes JTL 2105 may be more susceptible to water stress. Genotypes exhibiting higher relative water content likely possess mechanisms that minimize water loss via transpiration and optimize water absorption, while genotypes with lower relative water content may have less effective water uptake mechanisms or may undergo increased rates of transpiration.

Mean effect of treatments on relative water content was significantly varied between 94.39 % and 69.24 % as given in table 2. Control showed highest (94.39 %) mean value of relative water content, while the tomato plant irrigated with 300 ppm heavy metal showed lowest (69.24 %) mean value of relative water content at 20 days after germination. Similar results also observed by, they observed lower mean relative water content was probably caused by the plants absorbing less water as a result of increased osmotic stress. Relative water content can also be impacted by heavy metal stress. Plants may display modified leaf shape and decreased stomatal conductance in high salinity environments, both of which can affect relative water content.

**Table 2 Effect of heavy metal stress on Relative Water Content (RWC) in tomato leaves at 20 days after germination.**

|  |  |
| --- | --- |
| **Genotypes\* (V)** | **Relative Water Content (RWC)** |
| **Treatment** | Mean(V) |
| **Control** | **50 ppm** | **100 ppm** | **200 ppm** | **300 ppm**  |
| **GT6** | 90.22 | 83.38 | 79.37 | 78.08 | 77.89 | 79.89 |
| **GT1** | 96.58 | 89.56 | 85.66 | 83.27 | 81.63 | 87.34 |
| **JT3** | 96.31 | 83.78 | 80.97 | 75.28 | 73.95 | 82.06 |
| **JTL2105** | 92.83 | 84.27 | 80.80 | 72.01 | 58.66 | 77.71 |
| **JTL1908** | 96.08 | 83.90 | 80.30 | 77.49 | 64.44 | 80.44 |
| **JTL2103** | 94.36 | 83.93 | 80.69 | 77.65 | 64.37 | 80.20 |
| **Mean(T)** | 94.40 | 84.80 | 81.13 | 76.80 | 69.24 |   |
|   | **S.Em.±** | **C.D. at 5 %** | **C.V. %**  | 1.72 |
| **V** | 0.36 | 1.02 |
| **T** | 0.33 | 0.93 |
| **V X T** | 0.80 | 2.28 |

The interaction effect of genotype and treatment was found statistically significant for relative water content in tomato leaves (Table 2). The lower (69.24%) relative water content was found in genotype JTL-2105 at 300 ppm lead heavy metal concentrations. The relative water content was found significantly higher (87.33%) in GT-1 genotype compared to other genotype under the influence of heavy metal at all level (50 ppm to 300 ppm). The rate of decreasing relative water content was found lowest in GT1 compared to other genotypes, when heavy metal level increased up to 300 PPM. Genotype GT 1 retains highest relative water content all the heavy metal stress treatments as compare to other genotypes.

Heavy metals have been shown to significantly influence the relative water content (RWC) of plants, either by reducing or enhancing it depending on the metal and concentration. (Emamverdian *et al.* 2023) reported that in *Pleioblastus pygmaeus*, RWC decreased by **26% under 150 µM Mn** and by **31% under 150 µM Cr** compared to control. Similarly, (Alyemeni *et al.* 2018)found that cadmium exposure (150 mg/L) in *Solanum lycopersicon* cv. K-21 reduced RWC by **31.57%**. In contrast, (Kohli *et al.* 2018) observed that lead treatments (0.25–0.75 mM) in *Brassica juncea* significantly increased RWC, with the highest Pb concentration (0.75 mM) enhancing RWC by **20.45%** over the control. These findings suggest that while Cd, Mn, and Cr generally impair water status in plants, Pb may exert a stimulatory effect on RWC under certain conditions.

Heavy metals, such as lead (Pb), can significantly reduce the relative water content (RWC) of plant tissues by disrupting both water uptake and retention mechanisms. The reduction in RWC under heavy metal stress primarily results from osmotic imbalance and impaired root water absorption. Heavy metals often accumulate in root tissues, causing structural and functional damage to root cells, including disruption of plasma membranes and xylem vessels, which limits water transport from the soil to the shoot

CONCLUSION

The present study demonstrated that **lead (Pb) stress exerts a dose-dependent inhibitory effect on tomato seed germination and relative water content (RWC)**. All genotypes exhibited reduced germination percentages and lower RWC values with increasing Pb concentrations, though the extent of reduction varied across genotypes. Among the tested varieties, **GT-1** consistently maintained the highest germination percentage and RWC across treatments, indicating a comparatively greater tolerance to Pb stress, while **JTL-2105**  was the most sensitive genotype. The observed decline in germination and RWC under higher Pb concentrations can be attributed to osmotic stress, impaired water uptake, and toxic effects of Pb on seed metabolism and cell function.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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