

## Research Article

# Physiological response of wheat seeds grown under NaCl and HgCl<sub>2</sub> stress

Jibrin Ashiru Ibrahim, Aisha Auwal, Vimlendu Bhushan Sinha\*

Department of Biotechnology, School of Engineering and Technology, Sharda University, Greater Noida, Uttar Pradesh - 201306, India

**Received:** 10 May 2016

**Revised:** 14 May 2016

**Accepted:** 08 June 2016

### \*Correspondence:

Dr. Vimlendu Bhushan Sinha

E-mail: [vimlendusinha@gmail.com](mailto:vimlendusinha@gmail.com); [vb.sinha@sharda.ac.in](mailto:vb.sinha@sharda.ac.in)

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### ABSTRACT

**Background:** Plants are continuously exposed to various abiotic and biotic stresses like cold, high temperature, drought, salinity and exposure to heavy metals. However, it is also argued that the effect of these stresses on the germination of seeds represents the most critical aspect of plant survival. The aim of the study was to observe the differences in germination potential of seeds in the presence of salt and heavy metal stress.

**Methods:** The seeds were germinated under the effect of NaCl (0, 25, 50, 75, 100mM) and HgCl<sub>2</sub> (0.001, 0.01, 0.1 and 1 ppm), Timson's index was calculated as a growth identifier, average shoot length and RWC was calculated for surviving seedlings.

**Results:** Germination rate was observed to decrease with increasing concentrations of the NaCl and HgCl<sub>2</sub>. The maximum germination (100%) was recorded in NaCl (25 Mm,) treatment and the minimum percentage of germination was observed as 84% in HgCl<sub>2</sub> (1 mg/ml). The maximum shoot length was observed in HgCl<sub>2</sub> (0.001 mg/ml) with average shot length of 16.50 cm and 13.92 cm of NaCl (25 mM) respectively. It was also observed that the seedlings having high RWC were more resistant against salinity stress.

**Conclusions:** The use of both genetic modification as well as traditional breeding approaches are to be needed so as to unravel the mechanisms to salinity tolerance and at the same time to involve in the development of salt-tolerant cultivars that are better to adapt with any increase in soil salinity constraints.

**Keywords:** Wheat, RWC, Metal stress, Abiotic stress, Germination

## INTRODUCTION

Abiotic stresses, including drought, salinity, temperatures (low and high), chemical toxicity as well as oxidative stress etc are amongst some of the serious threats to agriculture. If the agricultural crops are affected by abiotic stresses, it thereby reduces the average yields of most of the major crops by about 50%.<sup>1</sup> Abiotic stress is nothing other than any environmental conditions or combination of them which affect the expression of potential for growth, development and reproduction of plant species.<sup>1</sup> The important method used to deal with environmental stress in the past was to limit the stress through soil irrigation, the type of fertilizer use, soil

reclamation, etc. Seeds germination depends on many factors like presence of water, optimum temperature, oxygen etc.

Water initiates the various metabolic processes required for the seed to germinate.<sup>2</sup> Whenever proper conditions exist, the seed usually imbibes water through its permeable seed coat. The plants show variations in their physiological characteristics, amongst which the prominent are decreased biomass, decreased leaf area, reduced cell growth, and decreased yield. The cells grow, become large and eventually the seed coat breaks to mark the start of germination stage. The root emerges first, followed by the shoot, which contains the stem and

leaves. It is always assumed that seeds being more sensitive to abiotic stresses as compared to the mature plant, the exposure affect the overall growth close to the soil surface.<sup>3</sup> Cold stress may cause injuries to seedling and reduction of average yield may be due to spikelet sterility<sup>4</sup> and chilling stress may result into reduced leaf expansion, wilting, chlorosis, necrosis and strongly disturbed reproductive development of plants.<sup>5</sup> Low temperatures and metal toxicity cause damage to wheat at all stages of crop development including germination, crop yields, soil biomass and fertility.<sup>5</sup> Presence of heavy metals, such as mercury, zinc, cadmium, lead, cobalt and nickel in air, soil and water are said to be the risk factors for the causation of bioaccumulation which significantly known to affect the whole ecosystem and pose the detrimental health consequences to the entire life forms. Among the biochemical changes known to occur when plants are subjected to the abiotic stress conditions is the production of ROS. Salt stress was known to play a significant role in the generation of oxidative stress which is the major component of abiotic stress. Salinity is regarded as one of the major environmental abiotic stress present in the arid, semi-arid as well as the coastal regions. The crops shows variable resistance to the salinity in accordance to the plant ontogeny, germination represents a critical stage for direct sowing as well as for the transplantation of crops production.<sup>6,7</sup> The serious damage arises as a result of salinity at seed germination level is said to be attributed to the various environmental factors such as water reduction, modifications in the mobilisation of most of the stored reserves as well as some of the various structural organisation of protein machinery at the plant level which is considered to be a remarkably constant characteristics. Previous reports have established the fact that the rate of germination decrease with increase in salt concentration.<sup>6,7</sup> Generally it has been noticed that during competition of the same resources larger plant seeds dominate the smaller ones irrespective of the growing conditions. However, researchers argue over the potency of larger seeds having advantages in abiotic stress conditions, the smaller seeds actually thrive well in abundant sun and water.<sup>8</sup> On the other hand, some groups came to the conclusion that the smaller seeds may germinate more rapidly than large seeds and thus, to gain a competitive advantages.<sup>9</sup> However, numerous studies have demonstrated that weight of the seeds of the same species may vary greatly and such variation in seed weight within a species possibly reflects germination variability and/or seedling characteristics and finally population recruitment.<sup>9</sup> The present study was undertaken to observe the physiological effect on the seed germination on the studied cultivar.

## METHODS

### *Procurement of seeds and verification of the viability of seeds/germination efficiency*

The seed of wheat cultivar (HALNA) was procured from pantanagar Tarai AGri Seeds, Uttarakhand, India. About

hundred seeds of the procured cultivars were germinated in the presence of controlled condition on wet blotting paper kept in petri-dish in which their viability of the procured seed material was verified.

### *Determination of seeds with high frequency of occurrence*

About three thousand seeds were randomly selected and weighed on weighing balance for determining the weight of the seeds having highest frequency of occurrence.

### *Effect of salt stress on wheat seeds*

The cultivars were washed with deionized water and dispersed in petri dishes (25 seeds per petri dish) on a wet filter paper. The solutions used to moisten the filter paper contained NaCl (0, 25, 50, 75, 100 mM) and HgCl<sub>2</sub> (0.001, 0.01, 0.1 and 1 ppm). Seeds were germinated at room temperature of 25±2 °C. Radicle emergence to a length of 2-5 mm was the criterion for seed germination. Germination was recorded everyday continuously for 15 days at every 24 h interval.

### *Calculation of Timson's index efficiency*

Germination efficiency and Timson's index was calculated using standard statistical procedures and method described by.<sup>10</sup>

### *Determination of relative water content in treated seedling*

To evaluate the relative water content (RWC). The fresh weight samples were weighed, then were submerged in distilled water and finally were dried at 48 °C for 72 h (after weighing) and weighed again. RWC was calculated according to with the help of the formulae  $RWC = ((FW - DW) / (TW - DW)) \times 100$ , where, FW is fresh weight, DW is dry weight and TW is turgor weight of plant sample.<sup>11</sup>

## RESULTS

### *Viability testing and determination of seed weight with highest frequency*

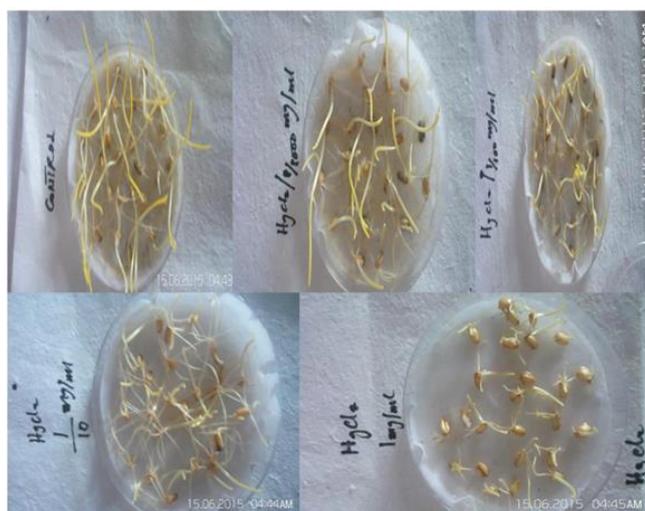
The seed having 38 mg was found to have the highest occurring in number and were used as sample seeds for experimental purpose. The viability was found to be close to 100 percent for the experimental set.

### *Determination of percent germination:*

Seed germination was checked at varying treatments levels of salt and heavy metal stress and a decrease in germination percentage with increasing chemical concentration in the solution was observed (Table 1; Figure 1 and 2).

**Table 1: Germination percentage of seedling.**

Treatment	No. of seed	No. of seeds germinated	Percentage germination
Control	25	25	100
25 mM NaCl	25	25	100
50 mM NaCl	25	24	96
75 mM NaCl	25	24	96
100 mM NaCl	25	24	96
0.001mg/ml HgCl <sub>2</sub>	25	25	100
0.01mg/ml HgCl <sub>2</sub>	25	25	100
0.1mg/ml HgCl <sub>2</sub>	25	23	92
1 mg/ml HgCl <sub>2</sub>	25	21	84



**Figure 1: Germination of wheat seeds at various concentrations of HgCl<sub>2</sub>.**



**Figure 2: Germinated seeds at various concentrations of NaCl.**

**Determination of Timson’s index**

Timson’s index is regarded as a benchmark to determine the germination efficiency. A better TI for the lower treatment range was obtained which signifies that higher the TI better is the rate of germination (Table 2).

**Table 2: Calculation of Timson’s index.**

Treatment	TI calculated
Control	24.50
25 mM NaCl	19.67
50 mM NaCl	15.00
75 mM NaCl	10.67
100 mM NaCl	9.00
0.001mg/ml HgCl <sub>2</sub>	24.17
0.01mg/ml HgCl <sub>2</sub>	23.67
0.1mg/ml HgCl <sub>2</sub>	21.33
1 mg/ml HgCl <sub>2</sub>	19.5

**Measurement of the average shoot length of plant samples after treatment**

The seedlings germinated modal seeds of the wheat cultivar being studied were treated with various concentrations of NaCl and HgCl<sub>2</sub> respectively under varying concentration. The surviving seedling when studied shows that the shoot length of wheat cultivars were decreased significantly with increasing concentration of NaCl and HgCl<sub>2</sub> as compared with the control (Table 3; Figure 3 and 4).

**Table 3: Measurement of shoot length.**

Treatment	Shoot length
Control	17.31±1.25
25 mM NaCl	13.92±1.87
50 mM NaCl	11.86±1.20
75 mM NaCl	10.60±1.53
100 mM NaCl	9.22±1.48
0.001mg/ml HgCl <sub>2</sub>	9.22±1.48
0.01mg/ml HgCl <sub>2</sub>	16.50±2.45
0.1mg/ml HgCl <sub>2</sub>	12.44±3.23
1 mg/ml HgCl <sub>2</sub>	7.96±1.01

**Fresh weight (FW) of wheat plants:**

The fresh weight of the plant showed variations and the increasing salt concentration reduced the value of the FW and thus, differences in fresh weight of the wheat plant in different treatments were observed. The results obtained depicts that the highest FW for the seedlings were observed for NaCl (25 mM) and that of HgCl<sub>2</sub> (1/1000 mg/l) (Table 4).

**Table 4: Average weight of the plant.**

Treatment	Average FW	Average DW
Control	0.112±0.012	0.084±0.010
25 mM NaCl	0.100±0.022	0.026±0.011
50 mM NaCl	0.089±0.019	0.027±0.032
75 mM NaCl	0.076±0.011	0.020±0.009
100 mM NaCl	0.057±0.018	0.016±0.006
0.001 mg/ml HgCl <sub>2</sub>	0.101±0.009	0.024±0.008
0.01 mg/ml HgCl <sub>2</sub>	0.059±0.015	0.028±0.023
0.1 mg/ml HgCl <sub>2</sub>	0.068±0.012	0.016±0.007
1 mg/ml HgCl <sub>2</sub>	0.046±0.00	0.019±0.00

**Figure 3: Wheat seedling at various concentrations of NaCl.****Figure 4: Wheat seedling at various concentrations of HgCl<sub>2</sub>.****Dry weight (DW) of wheat plants:**

These traits decreased with increasing in salinity level. There were significant differences among wheat cultivar at NaCl and HgCl<sub>2</sub> stress when compared with control.

The highest dry weight of plant wheat was observed for plants treated with 0.01 mg/ml HgCl<sub>2</sub> (Table 4).

**Evaluation of relative water content (RWC):**

The highest relative water content was observed for 25 mM NaCl and 1/1000 mg/ml HgCl<sub>2</sub> respectively (Table 5).

**Table 5: RWC calculation.**

Treatment	RWC
Control	5.13
25 mM NaCl	27.00
50 mM NaCl	23.50
75 mM NaCl	21.88
100 mM NaCl	17.22
0.001mg/ml HgCl <sub>2</sub>	27.57
0.01mg/ml HgCl <sub>2</sub>	13.42
0.1mg/ml HgCl <sub>2</sub>	20.45
1 mg/ml HgCl <sub>2</sub>	11.89

**DISCUSSION**

Wheat is the second most important cereals in India and grown worldwide ranging from temperate, dry and high rain fall areas, from warm/humid to dry and cold environments. However, investigation on wheat categorizes it into C3 plant and thus, it thrives well in cooler environment.<sup>12</sup> It particularly grows well on the loamy soils of Northern plains covering Punjab, Haryana, western Uttar Pradesh and black soils of Madhya Pradesh and about 35-40% of moisture content is required for seed germination in wheat.<sup>13</sup> Wheat yields are decreased by drought, heat, low temperatures, low fertility, especially nitrogen, and soil salinity. Interestingly, for breeding purposes, the resistance to various stresses may be combined and may create opportunities for better yielding wheat varieties.<sup>14,15</sup> Plant response to stress is a complex phenomenon and wheat has a moderate tolerance to salinity.<sup>14,16</sup> In the present study we selected modal weight as the seeds to be used for precisely studying the effect of stress. Seed germination is said to occur due to the water intake by the seed but it is also said to decrease due to the salt. Generally, it has been observed that the highest germination percentage can only occurs in non salty environment and it said to be decreases by ascending salt concentrations. Salt stress is said to affect seed germination by decreasing the capacity with which the seeds take up water because all the activities as well as the events that are associated with germination are said to be either getting delayed and/ or proceed at undetermined reduced rate.<sup>17</sup> Salinity (NaCl) is known to affect seed germination by facilitating the level of toxic ions intake which may alter the enzymatic as well as hormonal activities of the seed.<sup>18</sup> The results of our study also emphasized that by increasing NaCl concentrations, germination in the cultivar delayed and decreased which is in accordance with the previous

reports.<sup>18</sup> It may be deduced from the work that increasing salinity concentrations causes osmotic and/or specific toxicity reducing the germination percentage and higher concentration produces toxic effects on the embryo which leads to delay and reduced germination.<sup>19</sup> Since germination percentage or rate of germination of wheat cultivars was observed to decrease under salt stress condition, hence it can be concluded that salinity may be the causes of reduced seed vigour which may consequently be the risk factor that causes the delay of the emergence of seedlings. Heavy metal stress especially mercury (Hg) is a hazard to human health as well as detrimental to microorganisms, plants and animals.<sup>20</sup> In our experiment the seed germination gradually decreased with the increase in concentration of mercury chloride and it can be deduced that it happened due to the toxicity of HgCl<sub>2</sub> on root growth and shoot length. As far as the continued effect of salt and mercury stress on growth of plant is concerned, it was observed that shoot length decreased with increasing treatment (NaCl and HgCl<sub>2</sub>) concentration. Generally, low salt concentration is known to decrease the germination rate whereas high salt concentration is known to decrease germination percentage. All these results point towards the importance of seed germination in unfavourable conditions, where salinity is pronounced by scarce and erratic precipitation. Seeds that germinate without delay can make use of the limited soil water pool to establish seedlings before adverse climatic conditions prevail. Similar observation about germination speed was made by who found that wheat cultivars have relatively rapid germination at moderate salt concentrations.<sup>21</sup> These results further confirm the negative impact of salinity on cumulative germination percentage and that the responses are variable and cultivars specific. It may also be inferred that the decrease in seed germination percentage happened due to osmotic retention of water/toxicity to the embryo. The radicle and plumule growth are among the major parameters for salt and heavy metals stress, this is because roots that are involved in water absorption from the soil after which it distributed to the other part of the plant through the shoot are in direct contact to the soil. Because of this, radicle and plumule are said to be an important clue to the responding of the plants to salt and heavy metal stress.<sup>20</sup> The timson index obtained for the radicle and plumule of seedlings that grown in salty conditions have shown a decline, this is an indication that salt stress is not only affected the germination processes but also the mechanism of growth seedlings, which indicates that synthetic ability of seed, and supports the previous reports on a similar problem.<sup>17</sup> Reduced seedling growth has also been reported by previous workers for salt and heavy metal stress on different crops like barley, tomato and wheat.<sup>17</sup> Further, the reduction in radicle and plumule development, most especially length or weight may be due to the toxic effects of the NaCl used and unbalanced nutrient uptake by the seedling at different developmental stage. RWC was introduced as criteria for studying the plant water status and in our results the seedling grew better in higher treatments used

for the study and thus, indicates the balance between absorbed water by the plant and water consumed through transpiration.

## CONCLUSION

Salinity and heavy metal stresses are significant problems that are known to cause serious damage to agriculture worldwide and assured to become major problems in the coming decades. The lethality of high salinity stress on plants can be predicted at the whole-plant level in terms of plant death as well as decrease in plant's productivity. Some species of the plants are known to be more flexible than the other types in their requirements for tolerance and survival in salinity and heavy metal toxicated environments. Salt stress occurs from both osmotic stress pertaining to low water potentials/salt specific effect. Hence, salt-stressed plants develop adaptive mechanisms for better tolerance to the toxic effects resulting due to ion accumulation and affects plant metabolism. The use of both genetic modification as well as traditional breeding approaches are to be needed so as to unravel the mechanisms to salinity tolerance and at the same time to involve in the development of salt-tolerant cultivars that are better to adapt with any increase in soil salinity constraints.

## ACKNOWLEDGEMENTS

The authors thank Sharda University, Greater Noida, India for providing facilities for the work.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: Not required*

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**Cite this article as:** Ibrahim JA, Auwal A, Sinha VB. Physiological response of wheat seeds grown under NaCl and HgCl<sub>2</sub> stress. *Int J Sci Rep* 2016;2(6):130-5.