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Induction of tolerance to mercury in barley (*Hordeum vulgare* L.)

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Abstract

Barley (*Hordeum vulgare* L.) plants, raised after soaking seeds for 24 hours in 10 ppm mercuric chloride, showed tolerance to effects of higher dose (100 ppm) in the second generation using different agronomic characters as endpoints.

Key words : Barley, mercuric chloride, tolerance

Introduction

Mercury, a rare element with ubiquitous distribution enters the ecosystem through seepage and leaching of its compounds and leads to severe health hazards through bio-magnification in ecosystem. Plants are both the first consumers and sufferers since heavy metals affect or block plant metabolism, growth and development and are accumulated in the plant body, later entering the biosystem. Though mercury pollution is principally due to the use of pesticides containing mercurials, yet increase in mercury load in soil and water through industries is also becoming a major factor. Mercury concentration in various soil samples and industrial effluents discharged in rivers from the eastern part of India is much higher than the level of 20-150 ppb prescribed by WHO [1]. Mercury poisoning has thus again become a problem of current interest as a result of environmental pollution on a global scale.

Mercury tolerance has been recorded in different plants, like *Chloris barbata, Cyperus rotundus* [2] and *Zostera marina* [3]. The present investigation was undertaken to find out whether exposure to low levels of mercury could lead to tolerance to the metal in subsequent generations.

Materials and methods

Inorganic salt of mercury, namely mercuric chloride [$HgCl_2$; CAS no. 7487-94-7], Loba Chemie, Mumbai, India was used for the experiment. 1.0 g of the salt was dissolved in 100 ml of distilled water to obtain a strength of 10^4 ppm and further diluted with distilled water to get strength of 10^3, 10^2 and 10 ppm concentrations. In order to study the induction of tolerance to mercury toxicity, seeds of *Hordeum vulgare* L. (Family Poaceae, 2n = 14), common barley, bought locally from Amtala Seed Stores, Calcutta, were used.

In the first year, healthy viable seeds were divided equally into separate sample lots and presoaked in sterile distilled water for six hours. Seeds were then soaked in 10 ppm (LECT, Lowest Effective Concentration Tested) of mercuric chloride for 24 hours and finally washed in running tap water for 12 hours. A control set of seeds was maintained by treatment with distilled water. Both treated and control seeds were sown in the field in three replications in a randomized block design. The plots were given the recommended basal dose of fertilizer during the soil preparation and the crop was irrigated regularly as required.

In the second year, seeds were treated with seven different combinations of mercuric chloride. Seeds harvested from the treated plants with 10 ppm of first year were divided into three sets and treated separately with 100ppm (E), 10ppm (D) and distilled water (F). Fresh seed samples were also treated separately with 1000 (A), 100 (B), 10 ppm (C) of $HgCl_2$. Following the treatment, treated and control seeds were sown in the field in a randomized block design with three replications. Application of fertilizer and irrigation was similar to the first year.

Seeds obtained in the second year from the plots C, D, E, F and G were again grown in the third year in a randomized block design with three replications along with control to find out whether the effects observed in second year were carried on to next year or not. Data was recorded for the characters studied earlier and statistically analyzed.

The morphological characters of *Hordeum vulgare* L. studied were plant height (cm), number of total tillers per plant, number of fertile tillers per plant, number of leaves per plant, length of flag leaf (cm), length of

panicle (cm), filled grain percentage, 1000-seed weight(g) and grain yield per plant (g). Student's t test was carried out in the first year to compare the treatment means of each character with control [4]. In the second year, analysis of variance (one way) was followed for each character studied and accordingly least significant difference test (LSD) [5], was carried out wherever needed.

Results and discussion

In the first year mean values of treated set for all characters (Table 1) did not differ appreciably much from control. No significant difference was recorded between the treated and the control set when 't' test was performed, which indicates that 10ppm of the salt, the threshold concentration, did not affect the plant characters studied.

Table 1. Comparison of the characters observed in treated and control plants of *Hordeum vulgare* L. in field experiment of year 1995-96

Characters Observed	Range		Mean ± SD		't' value
	Control	Treated	Control	Treated	
Plant height (cm)	52-105	50-88	74.108 ± 0.81	69.825 ± 0.5	1.155^{NS}
Number of tillers/plant	1-16	1-6	2.45 ± 1.249	1.7 ± 0.507	1.578^{NS}
Number of leaves/plant	4-61	3-34	12.35 ± 3.654	10.383 ± 3.268	3.4527^{NS}
Length of flag leaf (cm)/plant	3-17.5	5-19	7.916 ± 0.675	8.34 ± 1.425	0.88^{NS}
Length of panicle (cm)	1.5-9	1.5-8	5.033 ± 0.689	5.006 ± 1.538	0.054^{NS}
Percentage of filled grains	3.4-100	50-100	79.7997 ± 5.366	84.96 ± 4.711	0.935^{NS}

NS - Non-significant

Analysis of variance (ANOVA) for different characters of *Hordeum vulgare* L. in second generation after mercuric chloride treatment showed significant difference between the control and treated set with respect to plant height, number of total and fertile tillers, number of leaves, percentage of filled grain, 1000-seed weight and grain yield per plant (Table 2).

Statistical comparison of mean values with control for all the characters were carried out by LSD test and presented in Table 3. Mean values of plant height of all the treatments were higher than that of control but the sets pre-treated with 10 ppm (D, E and F) differed significantly. Number of total tillers, number of fertile tillers, number of leaves and percentage of filled grains of plants raised from seeds treated directly with 1000ppm, 100ppm and 10ppm $HgCl_2$ were significantly less than those of control. Pretreatment of seeds with 10ppm before exposure to 10, 100ppm and distilled water increased mean values appreciably. Grain yield per plant of treated plants previously exposed to 10 ppm showed no statistically significant reduction of mean values when compared to control. Comparison of the mean values of 1000-seed weight revealed significant reduction of all the treatment means compared to control, effects being most toxic with plants directly treated with 1000 and 100ppm.

Analysis of variance for different characters of *Hordeum vulgare* L. in third generation after mercuric chloride treatment showed than mean of any of the characters of treated sets did not vary from those of control (Table 4). No significant difference was recorded between the treated and the control sets. This may be attributed to the tolerance the species had acquired in course of cultivation after exposure to mercuric chloride for consecutive years.

Seeds of barley, with or without residual mercury, had been earlier exposed to ethyl methane sulphonate (EMS), maleic hydrazide (MH), methyl mercuric chloride (MMC) and mercury- contaminated soil [2, 6, 7]. Measurements of germination, seedling height, mitotic index, mitotic chromosome or spindle aberrations in embryonic shoot cells, and meiotic chromosome aberration showed that residual mercury in seed conferred protection against the genotoxicity of EMS, MH, MMC and mercury contaminated soil. Pre-exposing the Hg treated seeds to buthionine sulfoximine, an inhibitor of phytochelatin synthesis, significantly prevented the genotoxic adaptation to MH and MMC. As compared to normal seedlings, the seedlings grown from treated seeds exhibited a higher amount of

Table 2. ANOVA for different morphological characters of *Hordeum vulgare* L. in second generation after mercuric chloride treatment in 1996-97

Sources of Variation	d.f.	Mean Squares								
		Plant Height	No. of Tillers	No. of Fertile Tillers	No. of Leaves	Length of Flag Leaf	Length of Panicle	%-age of filled grains	1000-seed weight	Grain yield/ plant
Replication	2	79.352	0.016	0.006	3.103	0.481	17.141	4.554	0.057	0.0003
Treatment	6	256.852^{**}	6.237^{**}	1.371^{**}	92.922^{**}	2.256	7.688	187.043^{**}	46.760^{**}	0.193^{**}
Error	12	22.437	1.308	0.013	4.752	0.834	3.129	5.709	0.032	0.006

**significant at 1% level of significance; d.f. - degrees of freedom

Table 3. Response of *Hordeum vulgare* L. after exposure and re-exposure for consecutive years to different concentrations of $HgCl_2$

Treatments	Plant height	No. of total tillers	No. of fertile tillers	No. of leaves	%-age of filled grains	Grain yield/ plant (g)	1000-seed weight
A (1000ppm)	71.977^{BC}	2.600^{E}	1.867^{D}	23.667^{C}	58.273^{E}	3.167^{D}	28.745^{E}
B (100ppm)	73.783^{BC}	3.333^{D}	2.117^{CD}	25.567^{C}	71.797^{D}	3.377^{C}	30.373^{D}
C (10ppm)	73.992^{BC}	3.517^{CD}	2.933^{B}	35.550^{AB}	74.125^{CD}	3.753^{AB}	36.090^{C}
D (10 + 10 ppm)	92.058^{A}	3.983^{ABC}	3.050^{B}	34.550^{B}	77.238^{BC}	3.710^{AB}	37.223^{B}
E (10 + 100ppm)	91.417^{A}	3.633^{BCD}	2.317^{C}	35.217^{AB}	81.493^{A}	3.677^{B}	36.128^{C}
F (10ppm + water)	79.675^{B}	4.133^{AB}	3.133^{B}	33.250^{B}	79.723^{AB}	3.763^{AB}	37.286^{B}
G (0ppm)	69.675^{C}	4.367^{A}	3.800^{A}	38.700^{A}	78.934^{AB}	3.887^{A}	39.514^{A}

For each character, means followed by common letter(s) are not significantly different at $LSD_{.01}$

Table 4. ANOVA for different morphological characters of *Hordeum vulgare* L. in third generation after mercuric chloride treatment in 1997-98

		Mean Squares							
Sources of Variation	d.f	Plant Height	No. of Tillers	No. of Fertile Tillers	No. of Leaves	Length of Flag Leaf	Length of Panicle	Grain Yield/ plant	1000-seed weight
Replication	2	79.352	0.016	0.014	1.103	0.481	1.141	4.554	0.345
Treatment	4	101.86	2.32	0.936	9.922	1.054	7.688	12.043	1.556
Error	8	34.876	1.308	0.235	4.752	0.934	3.429	5.709	1.22
Total	14								

non-protein SH (thiol) group. The findings indicate a possible involvement of phytochelatins in the mercury-induced adaptive response. Exposure to Hg compounds for 2 hours induced genotoxic adaptation to later challenge exposure to mercurials.

The results of the present investigation showed that previous exposure of plants to 10ppm of Hg could protect the plants exposed to mercury up to 100ppm in the second year indicating induction of tolerance by pretreatment with Hg. In the third year field experiment mean of any of the characters of previously treated sets C (10ppm), D (10+10ppm), E (10+100ppm) and F (10ppm + water) did not vary from those of control (G) indicating possible tolerance induced by previous exposure to low concentration of $HgCl_2$.

Previous exposure to 10ppm of Hg (II) in the bacterium *Rhodococcus erythripolis* A_3 induced mercury resistance up to 50ppm [8]. The concentration 10ppm of Hg (II) appears to be optimal both for the bacterium and *Hordeum vulgare* as observed here.

In the experiments the uptake (µg/g) measured by Atomic Absorption Spectrophotometry following direct treatment with $HgCl_2$ is directly related to the concentrations of $HgCl_2$ (Table 5). The difference between 1000ppm and 100ppm is highly significant (1.382 and 0.436 respectively). In the seeds, which were exposed to 10ppm (M_1), harvested and then exposed to 100ppm the uptake of Hg (0.159) is less than the former. This may indicate that previous exposure to low doses of Hg may have induced alterations in susceptibility of the cell membrane to Hg uptake. These findings confirm the earlier report of site directed mutagenesis of many mercury sensitive aquaporins present in vacuolar membranes of cells of *Arabidopsis thaliana* [9, 10].

Production of phytochelatin and its complexation to mercury may be a possible explanation for development of tolerance through consecutive exposures. The genes for mercury resistance have been identified in several bacterial species [11] and have been successfully introduced through genetic engineering in higher plants [12-14]. However, development of tolerance to *mercury* in plants through repeated exposure as discussed here may be a first report. Such reduction in toxic effects of high concentrations of cadmium following pre-treatment with lower concentration (0.2 µg/ml) in *Holcus lanatus* has been previously reported as well [15].

Table 5. Measurement of Mercury (µg/g) in Plant Tissue Samples of *Hordeum vulgare* L. by Atomic Absorption Spectrophotometer

Concentrations (ppm)	Treated Seed	Harvested Seed
1000	1.382	ND
100	0.436	ND
10+100	0.159	ND

ND : Not detected

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