HEAVY METAL INHIBITION OF *IN VITRO* POLLEN GERMINATION AND POLLEN TUBE GROWTH IN *AMARYLLIS VITTATA* (AIT)

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HEAVY metal toxicity of plants is important because plants act as primary producers in all the food chains. It is only recently that different aspects of heavy metal toxicity in various plant physiological processes have been investigated[1]. There is very little information on the effects of heavy metals on pollen germination and pollen tube growth[2]. Co^{2+} and Zn^{2+} have been reported along with Ca^{2+} and Mg^{2+}, as stimulatory for pollen tube growth[3]. Recently Cd^{2+} (0.5–15 μM) and Pb^{2+} (5–500 μM) were reported to be stimulatory for pollen germination and tube growth at lower concentrations while at higher concentrations they were inhibitory[4]. It is interesting to note that the metal tolerance character exhibited in the sporophytic tissues of *Silene dioica* and *S. alba*, was also expressed in the pollen[5]. Pollen, therefore, is a suitable system for studying the mechanism of heavy metal toxicity and tolerance in plants.

Amaryllis vittata Ait (Amaryllidaceae) pollen was collected from freshly dehisced anthers and stored in air-tight glass vials in a deep freezer till further use. Pollen was cultured in a medium containing 3% sucrose and 10 $\mu g\,ml^{-1}$ boric acid at $28 \pm 2°C$. Heavy metal salts, $Cd(NO_3)_2$, $NiCl_2$, $CuSO_4$, $Pb(NO_3)_2$, $Co(NO_3)_2$ and $Zn(NO_3)_2$, were added to the basal medium so as to obtain concentrations of 150, 100, 150, 250, 200 and 200 μM respectively. Each treatment carried three replicates. Pollen germination and tube growth were scored by counting about 100 pollen grains in 5 different microscopic fields. To study the effect of heavy metal toxicity on pollen tube growth, pollen was cultured for 60 min in the basal medium followed by the addition of heavy metal salts to obtain the required concentration. Another experiment involved pollen culturing in the medium containing heavy metal salt for 60 min followed by washing (twice) and subsequent incubation in the basal medium.

To assess the enzyme activities, pollen was cultured for 60 min, separated through centrifugation (1000 *g* for 15 min at room temperature) and homogenized in cold 0.1 M tris-HCl buffer (pH 7) in a chilled pestle and mortar. The homogenate was centrifuged at 5000 *g* for 15 min at 4°C and the supernatant was used for enzyme assay. Activities of peroxidase and acid invertase were assayed by the method of Shannon *et al*[6] and Singh *et al*[7], respectively. Total soluble proteins were estimated according to Lowry *et al*[8]. Pollen was first cultured for 30 min and then used for the assay of trinitrophenyl tetrazolium chloride (TTC) reduction[9].

Complete inhibition of pollen germination was observed at different concentrations in most of the heavy metal salts. Co^{2+} treatment, however, did not cause complete inhibition; there was 51% pollen germination at 200 μM, which declined to about 25% at 500 μM. Pollen tube growth was also sensitive to heavy metal toxicity (table 1).

The inhibitory effect of different heavy metal salts except Cu^{2+} was partially reversible on pollen germination and tube growth (table 1). The response was not reversible with Cu^{2+}. Pollen cultured in Cu^{2+} containing medium had only negligible TTC reduction activity compared with the control pollen

Table 1 *Effect of pre- and post-treatment with heavy metal salts on pollen germination and tube growth. (Data were recorded after 2 h of the experiment)*

	Pre-treatment		Post-treatment
Metal salt	% germination*	% recovery in pollen tube growth	% pollen tube growth inhibition
$Cd(NO_3)_2$	26	14.2	84
$NiCl_2$	25	54	71
$CuSO_4$	—	—	88
$Pb(NO_3)_2$	54	27.4	79
$Co(NO_3)_2$	51	53.4	77
$Zn(NO_3)_2$	45	32.6	81

*Per cent germination in control was 93%.

Table 2 *Effect of heavy metal salts on trinitrophenyl tetrazolium chloride reduction, invertase and peroxidase activity. The activities were expressed as O D. 485 10 mg^{-1} pollen, µg glucose released mg^{-1} protein h^{-1} and ΔO D. 430 min^{-1} mg^{-1} protein respectively*

Heavy metal	TTC reduction	Invertase	Peroxidase
Control	0 17	270	0.038
$Cd(NO_3)_2$	0.07*	125*	0 018*
$NiCl_2$	0 09*	215*	0.89*
$CuSO_4$	0.02*	164*	0.10*
$Pb(NO_3)_2$	0.07*	117*	0.22*
$Co(NO_3)_2$	0.07*	60*	0.07*
$Zn(NO_3)_2$	0.08*	75*	0.122*

*Significantly different from control at 5% level.

(table 2). These features indicate loss of pollen viability with 150 µM Cu^{2+} treatment. Inhibition of TTC reduction in the case of Cd^{2+} and other heavy metal salts may reflect inhibition in pollen respiration (table 2). Bittell *et al*[10] reported inhibition of electron and energy transfer reactions in isolated corn mitochondria treated with Pb^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+} and Ni^{2+}. Inhibition of respiration, therefore, may be one of the factors leading to the inhibition of pollen germination and tube growth.

Acid invertase activity, which is a measure of the sucrose utilizing capacity of the system, was reduced by heavy metals (table 2). However, it is not clear whether the reduction of the enzyme activity was due to a direct interaction of metal ions with the enzyme or whether it is merely a consequence of inhibition of pollen germination and consequent reduction of carbon requirement for metabolism. Stimulation of the peroxidase activity by heavy metals indicates enhanced peroxidative metabolism (table 2). Inhibition of respiration and enhanced peroxidative activity appear to be the causative factors for heavy metal toxicity during pollen germination and tube growth.

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POLYPLOIDY AND RADIOSENSITIVITY IN *SOLANUM AMERICANUM* MILL. AND *SOLANUM VILLOSUM* MILL.

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THE chromosome number and ploidy are the decisive factors influencing radiosensitivity of any given species[1-3]. *Solanum americanum* Mill. and *Solanum villosum* Mill. are respectively the diploid and tetraploid species which belong to *Solanum* L. section *Solanum*[4]. Although considerable data are available on the cytogenetics of these two plants[5-9], studies on their radiobiological aspects are inadequate[10]. The present study was aimed at evaluating the radioresponse of these two species in regard to morphological parameters through R_1 and R_2 generations.

Dry seeds of *S. americanum* Mill. and *S. villosum* Mill. with uniform size and moisture content were irradiated with different doses of ^{60}Co gamma rays. The treated seeds were sown in the field following randomized block design with three replications and the R_1 generation was raised. The plants of R_1 generation were carefully screened and the data pertaining to the effect of gamma rays on morphological parameters such as plant height, the number of branches and the number of leaves were gathered. From the seed progeny of R_1, the R_2 generation was grown and the different parameters were studied.

The results (table 1) indicate a marked stimulatory effect at most of the doses of gamma rays in regard to