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Study and selection of Selenium-tolerant genotypes of the economically important plants *Hordeum vulgare* and *Triticum aestivum*

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ABSTRACT

The aim of the study was to select selenium-tolerant genotypes of two economically important crops Hordeum vulgare (barley) and wheat Triticum aestivum (wheat). Selenium dioxide (SeO_2) was used as source of selenium. One hundred and sixty three accessions of barley and 175 accessions of wheat were used in this study. Concentration of Se 10^{-3} M was selected for treating the different accessions of barley and wheat. Accessions of barley and wheat were found nontolerant 52% and 93% respectively. Root and shoot length inhibition was found in all the accessions. Mitotic anomalies were present in the selenium treated seedlings of accessions. Frequencies of epidermal cells and stomata, structural anomalies in stomata and selenium accumulation were observed in root and shoot of selenium treated accessions of barley and wheat.

Keywords: Selenium, Accessions, nontolertant, *Triticum aestivu*m, *Hordeum vulgare,* anomalies.

INTRODUCTION

Increasing environmental pollution has become today a cause of global concern. Years of mining, industrial processing, use in agriculture, etc., has lead to regional and global redistribution of metals with consequent environmental pollution. Pollution of the biosphere with toxic metals has accelerated dramatically since the beginning of the industrial revolution. It is generally accepted that soil and other environmental heavy metal concentrations are substantially greater today than they were hundreds of years ago. There are various parts of the world where the natural soils are phytotoxic due to the presence of excess metal ions. The primary sources of this pollution are the burning of fossil fuels, mining and smelting of metalliferous ores, pesticides and sewage, etc. Toxic metal contamination of soil, aqueous waste streams and ground water poses a major environmental and human health problem. Accumulation of large number of heavy metals polluting ecosystem were reported by Brown [1].

According to Blum heavy metals are usually with densities greater than 5 gm/cm^3. However, in general, heavy metals include elements having atomic number ranging from 23 to 92, in group 2 to 7 of the periodic table [2]. Some chemists qualified heavy metals as metal ions that are not essential for life. On the contrary, there are essential metals that are also known to be toxic at raised concentration [3, 4, 5]. Biologists customarily use the term heavy metal ions for transition metal ions. Moreover, Woolhouse argues that the term “heavy metal” should be abandoned in favour of a classification based on ligands forming properties [6].

A current technology to apply Se fertilizer as a foliar spray or base fertilizer has been used to increase the Se content in the edible portion of crops and often to simultaneously counteract the injuries generated by different environmental stresses. It is generally accepted that soil and other environmental heavy metal concentrations are substantially greater today than they were hundreds of years [7, 8]. The present piece of work aimed towards analyzing the amount of genetic variability and inheritance for selenium tolerance using selenium-dioxide as a source in barley (*H. vulgare*) and wheat (*T. aestivum*).

MATERIALS AND METHODS

Presently the amount of genetic variability and pattern of inheritance for tolerance to heavy metal selenium was worked out in two economically important monocotyledonous crops, barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*). The source for selenium was selenium dioxide (SeO_2). One hundred and sixty three accessions of barley, procured from Directorate of Wheat Research, Karnal (70 accessions), National Bureau of Plant Genetic Resources, New Delhi (73 accessions) and Department of Agriculture, Canada (20 accessions). One hundred and seventy five accessions of wheat, procured by courtesy of Department of Genetics and Plant Breeding, C. C. S. University, Meerut.

For selecting the appropriate concentration of SeO_2 to be used for evaluating the genetic variability for Se-tolerance in the accessions of barley and wheat, various experiments were designed. In these experiments, tolerance and toxic limits for SeO_2 were estimated using thirteen molar concentrations (10^{-13}M-10^{-1}M) of these compounds and five accessions of barley and wheat as test systems. Length of radicle seven days old seedlings was used as a parameter for quantifying the toxic and tolerance limits of this heavy metal. At least fifty seeds each were grown in sterilized Petri plates lined with cotton pads, sandwiched between filter papers. Control sets were raised in Hoagland's solution lacking SeO_2 solutions prepared in Hoagland's solution. Both control and treated sets were raised in Calton's Seed Germinator in total darkness at 20^{o}C. A molar concentration, 10^{-3}M, was selected for SeO_2, for analyzing the genetic variability for tolerance.

All the accessions of barley and wheat were first multiplied after growing in the field. Multiplication was done in subsequent years also for fulfilling the need of seeds for other experimental works. Several morphological parameters Radicle length, Shoot length, weight of root and shoot and cytological parameters of the seven days old seedlings were used for estimating the relative importance of these parameters in screening for SeO_2 tolerance.

For cytological analysis, root tip samples were collected from both treatment sets and control sets after 48 hours of germination of seeds. These root tips were fixed in acetic alcohol (3 parts absolute ethanol + 1 part glacial acetic acid) for at least 48 hours. Fixed root tip samples were stored in 70% ethanol in refrigerator. The tips were smeared and squashed in 1.5% acetocarmine. For estimating the toxic effects of SeO_2 on the cytology of root meristem cells, the following parameters were analyzed:

(a) Mitotic index (MI), (b) Active mitotic index (AMI), and (c) Type and frequency of mitotic anomalies
These parameters were calculated using the below listed formulae:

$$MI = \frac{\text{Number of cells destined to devide x 100}}{\text{Total number of cells}}$$

$$AMI = \frac{\text{Number of actively dividing cells (Cells at metaphase and anaphase) x 100}}{\text{Total number of cells}}$$

$$\text{Mitotic anomalies} = \frac{\text{Number of cells showing anomalies x 100}}{\text{Number of cells in active division}}$$

A parameter called Response Coefficient (RC) was calculated, using the following formula for estimating the toxicity imposed by SeO_2 treatment.

$$RC = \frac{VT-VC}{VC}$$

(VT = value of the treated set; VC = value of the control set).

The negative values of RCs indicated inhibition while positive values indicated stimulation.

On the basis of RCs of above mentioned parameters, accessions were categorized into five category A-E (**A = > -0.20; B = -0.20 to -0.39; C = -0.40 to -0.59; D = -0.60 to -0.79; E = < -0.80**). Category A comprised of tolerant (T), category E of non-tolerant (NT) and categories B-D of partially tolerant (PT) accessions. Classification of the accessions on the basis of RCs for radicle length shall be considered primary classification in the subsequent text. Control sets were always raised side by side with every treated set. Various parameters in seven days old seedlings were estimated for calculating the corresponding response coefficients (RCs). The ranges of RCs for radicle lengths were divided into fourteen classes by using a class interval of -0.05 and class values were accordingly calculated.

RESULTS AND DISCUSSION

Selection of suitable concentration for estimating the tolerance and toxic level of selenium

Concentration of selenium dioxide, ranging from 10^{-13}M to 10^{-1}M was used for treating the seedlings. Seven days old seedlings of the five accessions of *H. vulgare* and *T. aestivum* were analyzed in detail using radicle length as a parameter for estimating the tolerance and toxic limits of selenium. In general, solutions ranging from 10^{-13} M to 10^{-4} M were having no general effect on the root lengths of the seedlings. The concentration, 10^{-4} M of the heavy metal responded as borderline solution between tolerance and toxic levels for seedlings. Molar concentrations, 10^{-2}M and 10^{-1}M were lethal for seedlings and concentration, 10^{-3} M was selected for treating the different accessions of barley and wheat and to study the genetic variability in them in response to selenium treatment.

Screening of accessions of *H. vulgare* and *T. aestivum*

Screening of 163 accessions of *H. vulgare* and 175 accessions of *T. aestivum* for distinguishing tolerant (T), partially tolerant (PT) and non-tolerant (NT) ones was done against Se using 10^{-3}M of selenium dioxide (SeO_2). RCs for length of radicle, shoot and whole seedling, RCs for fresh weight and dry weight of radicle, shoot and whole seedling and RCs for active mitotic index were analyzed for tolerant to selenium. About 52% accessions of barley and 93% accessions of wheat were non-tolerant to Se. The classification of the accessions of barley based on the RC values for radicle fresh weights and radicle dry weights in response to Se indicated that majority of the accessions were non-tolerant except for AMI. The RCs for the above parameters behaved similarly in case of wheat against Se.

Most of the accessions of barley were present in the partially tolerant class, based on the RC values for shoot lengths, shoot fresh weights and shoot dry weights where as in case of wheat most of the accessions belonged to the non-tolerant class against Se for the above parameters (Table 1). Classification of the accessions, based on the RCs for the total lengths and fresh weights of the seedlings showed that majority of the accessions were partially tolerant but on the basis of RCs for seedling dry weights majority were found to be tolerant, in case of barley. Similar results were observed in case of wheat for above parameters except seedling lengths where majority belonged to non-tolerant class (Table 2).

Table 1: Response coefficient of root and shoot of the Se treated seedlings of barley

Accession No.	RC Root length	RC Root - fresh weight	RC Root - dry weight	RC Shoot length	RC Shoot - fresh weight	RC Shoot dry weight
B-156	-0.09	-0.04	-0.37	0.35	0.51	0.17
B-14	-0.54	-0.82	-0.75	-0.61	-0.7	-0.39
B-20	-0.55	-0.75	-0.77	-0.56	-0.6	-0.37
B-26	-0.6	-0.81	-0.65	-0.58	-0.64	-0.4
B-28	-0.58	-0.84	-0.78	-0.61	-0.7	-0.42
B-113	-0.59	-0.92	-0.81	-0.85	0.06	-0.64
B-10	-0.97	-1	-0.98	-0.93	-0.93	-0.82
B-15	-0.95	-0.99	-0.98	-0.95	-0.94	-0.89
B-34	-0.99	-1	-1	-0.92	-0.89	-0.83
B-36	-0.99	-1	-1	-0.93	-0.94	-0.86
B-136	-0.96	-0.99	-0.98	-0.92	-0.94	-0.88

Table 2: Response coefficient of root and shoot of the Se treated seedlings of wheat

Accession No.	RC Root length	RC Root - fresh weight	RC Root - dry weight	RC Shoot length	RC Shoot - fresh weight	RC Shoot dry weight
W-3	-0.97	-0.99	-0.64	-0.97	-0.97	-0.9
W-6	-0.97	-1	-1	-0.97	-0.97	-0.91
W-19	-0.97	-0.99	-0.07	-0.97	-0.98	-0.95
W-39	-0.79	-0.91	-0.74	-0.94	-0.94	-0.83
W-55	-0.78	-0.92	-0.76	-0.94	-0.95	-0.87
W-97	-0.97	-0.99	-0.95	-0.93	-0.91	-0.73
W-138	-0.79	-0.97	-0.79	-0.89	-0.87	-0.65
W-149	-0.79	-0.93	-0.73	-0.9	-0.86	-0.66
W-172	-0.78	-0.83	-0.69	-0.8	-0.76	-0.55
W-173	-0.97	-1	-0.99	-0.97	-0.96	-0.98

Table 3: Response coefficient of root and shoot of the Se treated seedlings of barley

Acc. No.	Cytological response	Control	Treated	RC	Acc. No.	Control	Treated	RC
B-10	MI	99.63	99.89	0	B-34	99.7	99.89	0
	AMI	7.45	1.43	-0.81		7.25	1.63	-0.78
	TMA	2.26	28			2.28	71.67	
B-14	MI	99.58	99.86	0	B-36	99.85	99.67	0
	AMI	5.67	1.65	-0.71		8.38	1.95	-0.77
	TMA	0	39.24			2.64	63.64	-
B-15	MI	99.56	99.41	0	B-113	99.78	99.27	-0.01
	AMI	6.11	2.11	-0.65		7.3	3.76	-0.49
	TMA	2.97	67.67	-		1.14	15.59	
B-20	MI	100	99.2	-0.01	B-136	100	100	0
	AMI	5.39	2.73	-0.49		7.04	2.56	-0.64
	TMA	0	15.71	-		0	0	-
B-26	MI	99.69	98.75	-0.01	B-156	100	98.98	-0.01
	AMI	5.03	1.96	-0.61		8.91	1.62	-0.82
	TMA	2.07	23.21	-		1.81	23.57	-
B-28	MI	99.96	99.33	-0.01				
	AMI	4.98	2.7	-0.46				
	TMA	0	18.67	-				

Comparative analysis of the seedlings

Comparative analysis was carried out by studying the lengths of radicle, shoot and whole seedling, fresh and dry weights of radicle, shoot and whole seedling, Mitotic index (MI) and Active mitotic index (AMI) and types and total mitotic anomaly (TMA.) The phytotoxicity of Se (IV) was determined through root and shoot growth inhibition, biomass (dry (DM), fresh (FM)) production. The sensitivities of monocotyledonae (*Hordeum vulgare*, *Triticum aestivum*) and dicotyledonae plants (*Sinapis alba*, *Brassica napus*) were also compared. Except for *H. vulgare*, Se (IV) inhibited root growth more than shoot growth [10].

Table 4: Response coefficient of root and shoot of the Se treated seedlings of Wheat

Acc. No.	Cytological response	Control	Treated	RC	Acc. No.	Control	Treated	RC
W-3	MI	99.66	99.08	-0.01	W-97	100	100	0
	AMI	14.29	5.68	-0.6		9.65	3.28	-0.66
	TMA	0	13.89	-		0	0	-
W-6	MI	100	99.28	-0.01	W-138	100	98.53	-0.01
	AMI	9.07	4.34	-0.52		10.81	3.84	-0.64
	TMA	0	61.1	-		0	12	-
W-19	MI	100	99.45	-0.01	W-149	99.81	99.18	-0.01
	AMI	7.66	4.19	-0.45		10.5	7.49	-0.29
	TMA	0	8.29	-		3.51	51.78	-
W-39	MI	100	100	0	W-172	100	100	0
	AMI	8.15	3.06	-0.62		10.69	2.4	-0.78
	TMA	0	0	-		0	0	-
W-55	MI	100	100	0	W-173	99.04	98.94	0
	AMI	8.14	2.62	-0.68		9.2	2.98	-0.68
	TMA	0	0	-		0	41.67	-

Effect of Se on Seedling elongation, Fresh and dry weights

After the treatment with Se, root and shoot length inhibition was observed in all the treated sets of accessions. Selenium inhibited fresh weights and dry weights of roots and shoots in all the treated sets of both the crops except BS-113 which showed little stimulation in shoot fresh weights in case of barley (Table 3 and Table 4). Se reduced both FM and DM of all studied plants' roots. Although in shoots FM was decreased with increased Se concentration, DM was reduced only in monocotyledonae plants (*H. vulgare*, *T. aestivum*). No significant differences between roots and shoots were confirmed for the DM/FM relationship, except for *S. alba* seedlings [10].

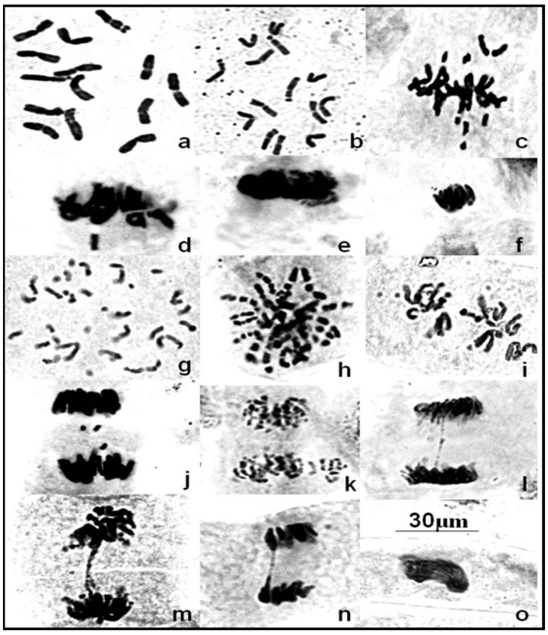
Root meristem cytology of the seedlings

Selenium treated barley seedlings, AMIs decreased in majority of the partially tolerant and non-tolerant accessions with some exceptions and in case of wheat, all the accessions showed inhibitive effect on AMI.

Mitotic Anomalies

Different types of mitotic anomalies were present in the selenium treated seedlings of accessions C-metaphase, late movement of chromosomes for metaphase alignment, clumped metaphase, chromosome fragmentation, chromosome erosion at metaphase, grouping of chromosomes at metaphase, lagging of chromosomes, formation of chromatin bridges during anaphase and formation of restitution nucleus (Figure 1 and Figure 2).

Figure 1: Photomicrographs of induced mitotic anomalies in *H. vulgare*



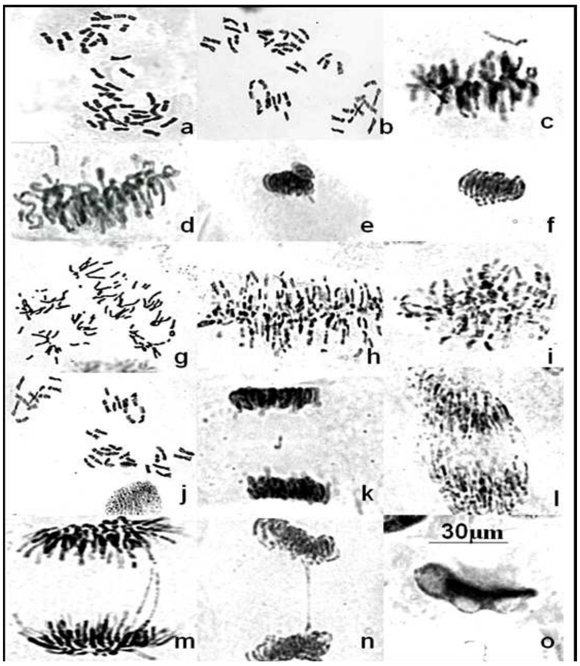
a, b- C-metaphase, c, d- late movement of chromosomes for metaphase alignment, e, f - clumped metaphase, g- chromosome fragmentation, h- Chromosome erosion at metaphase, i- Grouping of chromosomes at metaphase, j- Lagging of chromosomes, k- Chromosome erosion at anaphase, l, m, n- formation of chromatin bridges during anaphase, o- Formation of restitution nucleus

Study of foliar epidermis

In Se treatment, the frequency of epidermal cells, stomata and the values of guard cell indices were higher for abaxial sides in relation to those for adaxial sides. Se treated sets of accessions of barley and wheat the frequencies of epidermal cells and stomata, decreased as compared to corresponding controls. The same results were also available for guard cell indices, pore area, pore area indices and total pore area with rare exception. The proportions between frequency of epidermal cells, stomata and values of guard cell indices for abaxial epidermis with those for adaxial side were altered in relation to corresponding control sets.

In the leaves of treated accessions structural anomalies of stomata were noticed with unequally enlarged subsidiary cell, contiguous stomata with common subsidiary cell, stomata with one subsidiary cell, stomata with more than two subsidiary cells, stomata with obliquely placed subsidiary cells and contiguous stomata with unequal subsidiary cells.

Figure 2: Photomicrographs of induced mitotic anomalies in *T. aestivum*



a, b- C-metaphase, c, d- late movement of chromosomes for metaphase alignment, e, f - clumped metaphase, g- chromosome fragmentation, h, i- chromosome erosion at metaphase, j- grouping of chromosomes at metaphase, k- lagging of chromosomes, l- chromosome erosion at anaphase , l, m, n- formation of chromatin bridges during anaphase, o- formation of restitution nucleus

Accumulation of selenium

Selenium accumulation was also analyzed in the accessions of *H. vulgare* and *T. aestivum*. The accessions of barley (one tolerant, B-156; five partially tolerant, B-14, B-20, B-26, B-28, B-113; five non-tolerant, B-10, B-15, B-34, B-36, B-136) were accumulated Se in the roots and shoots. Accumulation of Se was higher in the roots than in the shoots of all studied plants. Selenium concentration in the roots was 3-times higher than that in controls. Se (IV) accumulation in the shoots was confirmed in *B. napus* 87 mg Se (IV) and *T. aestivum* 36 mg Se (IV) [10]. The concentration of selenium in barley was higher in the shoots as compared to the roots. Selenium treated accessions of wheat (five partially tolerant, W-39, W-55, W-138, W-149, W-172; five non-tolerant, W-3, W-6, W-19, W-97, W-173) also exhibited similar results.

CONCLUSION

Selected concentration (10^{-3} M) of selenium was tolerance limit of the accessions of *H. vulgare* and *T. aestivum*. However, this was important for anticipating the future increase level of SeO_2 in the environment and the tolerant gene(s) express better at tolerance levels. We conclude that Se tolerance in wheat and barley was observed at the range of Se application that would be used to increase grain Se for human consumption.

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