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ANALYSES OF GENETIC VARIABILITY FOR LEAD AND ARSENIC TOLERANCE IN OIL CROP *CARTHAMUS TINCTORIUS* L.

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ABSTRACT:
The present piece of work aimed towards analyzing the amount of genetic variability and the mode of inheritance for tolerance to lead (Pb) and arsenic (As) using lead acetate and sodium arsenate as a source for lead and arsenic in two hundred and thirty four accessions of safflower (*Carthamus tinctorius* L.). For testing variability of Pb and As 10^{-3} M solution was used. The variability was quantified using seedling's growth and cytological parameters and categorized in five categories A-E, using the parameter Response Coefficient (RC). The accessions belonging to category A were fully tolerant, belonging to category E were non tolerant and those belonging to category B-D were partially tolerant. The accessions displayed differential tolerance behavior.

INTRODUCTION:

The excessive and unplanned use of biological resources for the betterment of human race jeopardizes the maintenance of the natural ecosystem. The rapid growth of industries, fast urbanization and injudicious planning, are speedily leading to fatal environmental pollution. Presence of a very large number of chemical toxicants is progressively being identified in smaller or larger quantities in air, soil and water. Among the various kinds of chemicals which are continuously being pumped out in to biosphere, the 'heavy' metals are one of the biologically highly reactive ones. They can neither be destroyed and nor can be transformed into another. While working out the genetics of tolerance for several heavy metals in different crops, safflower tolerance for lead and arsenic was analyzed using seedling growth and cytological parameters.

MATERIALS AND METHODS:

For selecting the appropriate concentration of lead acetate and sodium arsenate to be used for evaluating the genetic variability for Pb-tolerance and As-tolerance in the accessions of safflower, experiments were designed. In these experiments, tolerance and toxic limits for Pb and As were estimated using thirteen molar concentrations (10^{-13}M-10^{-1}M) of these compounds prepared in Hoagland's solution and five accessions of safflower as test systems. Control sets were raised in Hoagland's solution lacking Pb and As. Both control and treated sets were raised in Calton's Seed Germinator in total darkness at 25^0C-27^0C. Radicle lengths of seven days old seedlings were used as a parameter for quantifying the toxic and tolerance limits of these heavy metals. At least fifty seeds of each accession were grown in sterilized petriplates lined with cotton pads, sandwiched between filter papers. On the basis of these experiments molar concentration, 10^{-3}M, was selected for both Pb and As, for analyzing the genetic variability for tolerance. The following parameters were analyzed:

1. Radicle length,
2. Cotyledon length,
3. Hypocotyl length,
4. Fresh and dry weight of roots,
5. Fresh and dry weight of cotyledon,
6. Fresh and dry weight of hypocotyl, and
7. Cytological parameters.

For cytological analysis, root tip samples were collected after 48 hours of the start of experiment. 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 root tips smeared and squashed in 1% aceto-orcein. For estimating the effects of Pb and As 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 (TMA).

These parameters were calculated using the following formulae:

$$\mathbf{MI} = \frac{\text{Number of cells destined to divide}}{\text{Total number of cells}} \mathbf{\times} 100$$

Number of actively dividing cells

$$\mathbf{AMI} = \frac{\text{Cellsat metaphase and anaphase}}{\text{Total number of cells}} \mathbf{\times} 100$$

$$\mathbf{TMA} = \frac{\text{Number of cells showing anomalies}}{\text{Number of cells in active division}} \mathbf{\times 100}$$

A parameter called Response Coefficient (RC) was calculated, using the following formula for estimating the toxicity imposed by Pb and As treatments.

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

(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.

OBSERVATIONS AND DISCUSSIONS:

The frequency distribution of the accessions showing response to both heavy metals for differential growth parameters and AMI are presented in table 1 and table 2.

Table 1. Overall assortment (%) of the accessions to different classes of RC's for Pb treatment.

Parameters	A	B	C	D	E
RL	7.69	20.15	67.95	3.42	0.43
RFW	63.68	22.22	8.97	5.13	0
RDW	74.79	23.08	1.71	0.43	0
CL	89.32	8.55	2.14	0	0
CFW	82.05	16.67	1.25	0	0
CDW	90.6	8.12	0.85	0	0.43
HL	61.11	34.19	3.85	0.85	0
HFW	55.13	36.75	5.98	1.28	0.85
HDW	86.75	12.82	0.43	0	0
SeL	20.94	65.38	13.25	0.43	0
SeFW	71.37	23.5	5.13	0	0
SeDW	98.29	1.71	0	0	0
AMI	50	44.02	5.56	0	0.43

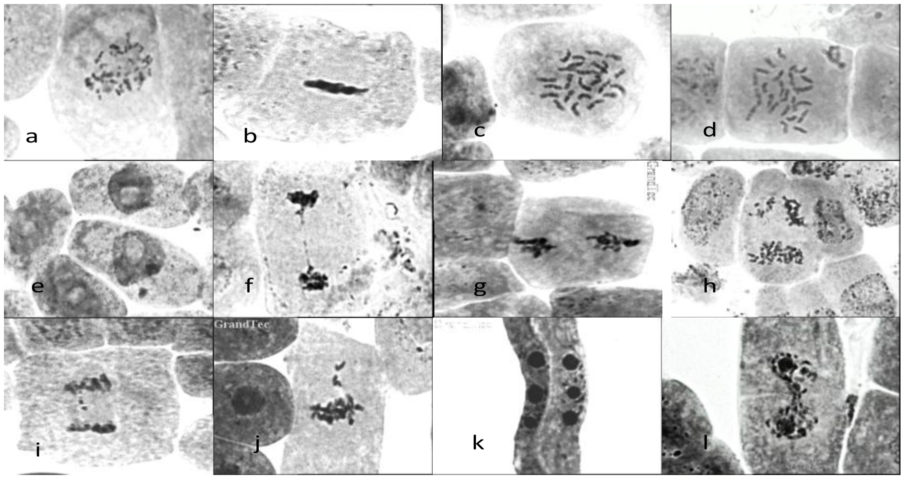
Table 2. Overall assortment (%) of the accessions to different classes of RC's for As treatment.

Parameters	A	B	C	D	E
RL	0.85	0.85	18	74	4.7
RFW	11	11	36	36	4.7
RDW	16	40	40	2.5	0
CL	59	35	4.2	0.42	0
CFW	65	31	2.9	0	0
CDW	97	1.2	0.85	0	0
HL	7	24	54	13	0
HFW	7.6	21	48	22	0.85
HDW	63	32	2.9	0.42	0
SeL	3.4	6.4	67	22	0
SeFW	14	41	43	1.3	0
SeDW	96	3.8	0	0	0
AMI	55	34	52	0	0

The frequencies of accessions showing response for the length, fresh weight and dry weight of total seedling and its part, against treatment with these heavy metals, varied significantly evidencing the variability of the safflower accessions towards both the heavy metals. On the basis of RCs about 8% accessions of safflower were tolerant to lead but only 1% accessions were tolerant to arsenic. The classification of the accessions based on the RC values for radicle lengths did not agree with the classifications based on the RC values for other parameters. Classification of the accessions based on the RC values for radicle fresh weight, radicle dry weight cotyledon length cotyledon fresh weight and dry weight hypocotyls length, fresh weight, and dry weight and active mitotic indices in response to lead indicated that majority of the accessions were tolerant to this heavy metal. The RCs for the above parameters behaved in a different way for arsenic, showing that majority of the accessions were either partially tolerant or non-tolerant.

The behavior of the accessions for the effect of both the heavy metals on AMIs and TMAs was significantly different these heavy metals could not change AMIs in more than 50% accessions. More than 70%

accessions of safflower were not showing any mitotic anomaly against the treatment of these heavy metals. Rests of the accessions were showing following types of anomalies.



a. Chromosome erosion,
b. Clumped metaphase,
c,d. C-metaphase,
e. Enlarged nucleolus,
f. Formation of chromatin bridges during anaphase,
f. Grouping of chromosomes at metaphase,
h. Grouping of chromosomes at anaphase,
i. Lagging of chromosomes,
j. Late movement of chromosomes for metaphase alignment,
k. Presence of multi-nucleate cells, and
l. Formation of restitution nucleus.

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