Rapid hydroponic screening for molybdenum tolerance in rice through morphological and biochemical analysis

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ABSTRACT

High yielding varieties of rice (*Oryza sativa*) cultivars were tested for their tolerance to different levels of molybdenum (Mo) (0.1 µM – control, 0.2, 0.4, 0.8 and 1.6 µM) in nutrient solution at pH 6.8. Seeds of rice were germinated and grown in presence of molybdenum under controlled environmental conditions. Standard growth parameters such as root length, shoot length, root/shoot dry biomass and root/shoot tolerance index were tested as markers of molybdenum toxicity. Measurements as early as 48 hours after the germination did not yield consistent results. However, root measurement on 3rd, 6th and 9th day after root emergence showed significant differences among cultivars of rice. Rice cultivars Annapurna, Kusuma, Deepa and Vaghari developed better root system while, Paridhan-1, Pusa-2-21 and Ratna showed poor growth of the roots in presence (0.8 µM) of molybdenum. The root tolerance index (RTI) and the shoot tolerance index (STI) in Annapurna, Kusuma and Deepa in rice were high indicating their tolerance to molybdenum; Paridhan-1 and Ratna, however, showed low RTI and STI. Based on the growth parameters, twenty cultivars of rice were ranked in respect of their tolerance to molybdenum: Annapurrna > Deepa > Kusuma > Vaghari > Hamsa > Vikram > Bharati > Paridhan-2 > Aswathi > Subhadra > Sankar > Sakti > Nilgiri > Rudra > Hema > Pragati > Pusa-2-21 > Ratna > Paridhan-1, respectively. Molybdenum toxicity was correlated with increased peroxidase and catalase activity in different cultivars of rice. This method can be employed for quick screening of rice cultivars for molybdenum tolerance in breeding programmes.

Keywords: molybdenum toxicity; catalase; hydroponic; peroxidase; rice; screening

Molybdenum plays an important role in plant metabolism mainly for its role in nitrogen metabolism and protein synthesis (Hewitt 1984, Marschner 1986, Welch 1995). Molybdenum (Mo) acts as an essential element for the development of reproductive parts of the plant (Shkolnik 1984); uptake of molybdenum at high concentrations, however, induces physiological disorders and changes in metabolic pathways in plants (Nicholas and Egan 1975, Hewitt 1983, Kabata-Pendias and Pendias 1992, Warner and Kleinhofs 1992). Molybdenum interacts with other mineral elements in plant nutrition (Aparicio et al. 1971, Zumft 1978); under certain conditions, the addition of these elements (B, Cu, Zn and Mn) causes molybdenum deficiency (Possingham 1954, 1957, Tiffin 1972). The presence of waste materials within the vicinity of mines and chemical industries contributes to chemical pollution of the soil, possibly resulting in the development of phytotoxic phenomena causing severe alterations in the plant system (Bradshaw 1983). Increasing interest is addressed to the combined effects of heavy metals and other pollutants, like acidity, as they often occur in the same site, and it is well known that besides causing nutritional deficiency, soil acidification also increases the availability of toxic metals to plants (Ericsson 1995). Molybdenum toxicity is a major factor for crop growth on acidic poorly drained soils. Toxic soil factors may mould the plants through natural selection to adapt to such conditions (Foy et al. 1992). Since there are good evidences that metal tolerance is under genetic control, breeding crop plants for cultivation in poor drained soils may be useful. To facilitate breeding programmes, a rapid, non-destructive, inexpensive and repeatable seedling-based bioassay is required for selection of tolerant genotypes from early segregating generations (Devine 1982). The use of hydroponic culture has been suggested as a means of assessing the plant tolerance to the toxic elements or the efficiency in mineral utilization (Foy et al 1978, Barcelo and Poschenrieder 1990). Hydroponic cultures allow easy observation, making quick screening on the basis of relative growth rate and toxicity (Carver et al. 1988). The present study was intended to identify the Mo-tolerant cultivars based on growth rate, tolerance index, biomass production and enzyme activity in rice in hydroponic cultures for breeding programmes.

MATERIAL AND METHODS

Plant material and environmental conditions

High yielding varieties of twenty rice cultivars (Annapurna, Kusuma, Deepa, Nilgiri, Subhadra, Vaghari, Khandagiri, Aswathi, Sankar, Vikram, Hamsa, Bharati, Sakti, Paridhan-1, Paridhan-2, Ratna, Pusa-2-21, Pragati, Hema and Rudra) were collected from the Department of Plant Breeding and Genetics, Orissa University of Agriculture and Technology, Orissa, India. Seeds were treated with detergent solution Teepol (Glaxo, India) for 10 min and washed with running tap water for 15 min. Further,

the seeds were sterilized with 0.1% aqueous mercuric chloride solution for 20 min and sown over plastic nets on glass trays (12 × 15 × 7 cm) (Borosil, India) containing the nutrient solution. The ratio of the seeds and the solution used were 1:30 (m/v). The trays were kept in a growth room at 25 ± 2°C lighted with cool, white fluorescent lamp (55 µmol.m^{-2}.s^{-1}) under a 16 h photoperiod. The modified Hoagland nutrient solution consisted of 4.0mM $CaNO_3$, 2.0mM $MgSO_4$, 4.0mM KNO_3, 0.4mM $(NH_4)_2SO_4$, 2µM $MnSO_4$, 0.3µM $CuSO_4$, 0.8µM $ZnSO_4$, 30µM NaCl, 0.1µM Na_2MoO_4, 1.43mM KH_2PO_4, 10µM H_3BO_3 and 20µM Fe-Na-EDTA (Hoagland and Arnon 1950). The pH of the nutrient solution was adjusted to 6.8 using 0.1N HCl or 0.1N KOH; the solution was changed regularly at 3-d-intervals to maintain the desired level of nutrients and the pH. Molybdenum was used in the form of Sodium molybdate ($Na_2MoO_4.2H_2O$) at 0.1µM (control), 0.2, 0.4, 0.8 and 1.6µM. The experiment was laid in a Completely Randomized Block Design (CRBD) with six replications. The experiments were repeated three times. The length of the primary root, shoot and number of lateral roots/plant were measured at 3-d-intervals from the date of root emergence up to the 9th day. The rate of root elongation in each experiment was determined by subtracting the length of the root recorded on the day of germination from that noted on the 9th day. Tolerance index (TI) for the tested plants was calculated using the formula: TI (%) = (mean root or shoot elongation in solution with Mo/mean root or shoot elongation in solution without Mo).100.

Biomass analysis

For biomass analysis, plants were harvested after 9th days of root emergence in the nutrient solution with Mo treatment. Shoot and root were separated and measured the initial weight and kept at 70°C for 48 h, and the dry matter measured until the constant weight.

Molybdenum analysis

Nine days after root emergence, plants were harvested and separated into leaves, roots and stems. Tissues were dried at 70°C, weighed, and ground to pass a 40 µm mesh sieve. One mg powdered shoot (stem + leaf) and root samples were predigested in 10 ml concentrated HNO_3 for 12 h followed by digestion with 5 ml diacid mixture, i.e. nitric acid (HNO_3):perchloric acid ($HClO_4$) in the ratio of 3:2. Distilled water was added to the digested samples which were then filtered by Whatman-42 filter paper and, after suitable dilution, the samples were ready for Mo analysis by ICP 8410 Plasmascan (Australia) by using wave length 203.844 nm.

Table 1. Shoot length, root length and number of lateral roots/plant of twenty rice cultivars in nutrient solutions (0.1µM – control) and with 0.8µM molybdenum after 9th day of root emergence; values are mean of 20 replicates; repeated three times

Oryza sativa cvs.	Root length (cm)		Shoot length (cm)		Number of lateral roots/plant	
	molybdenum level (µM)					
	0.1	0.8	0.1	0.8	0.1	0.8
Annapurna	5.09 ± 0.8q	6.25 ± 0.6n	7.25 ± 0.8q	8.13 ± 0.7g	4.01 ± 0.6	4.13 ± 0.7
Subhadra	3.82 ± 0.8m	4.02 ± 0.7k	6.72 ± 0.8n	6.98 ± 0.9n	3.15 ± 0.5	3.65 ± 0.8
Nilgiri	3.91 ± 0.7n	4.05 ± 0.8k	6.84 ± 0.7o	7.03 ± 0.8n,o	3.60 ± 0.8	3.75 ± 0.5
Deepa	4.22 ± 0.6o	5.15 ± 0.7l	6.95 ± 0.8p	7.22 ± 0.6p	3.25 ± 0.7	3.89 ± 0.8
Hamsa	2.75 ± 0.7f	3.00 ± 0.8f	5.15 ± 0.7i	5.35 ± 0.9h	1.85 ± 0.5	3.20 ± 0.7
Paridhan-1	2.21 ± 0.7a	2.00 ± 0.8a	3.12 ± 0.4a	2.42 ± 0.5a	1.32 ± 0.4	1.02 ± 0.5
Paridhan-2	2.50 ± 0.8d	2.65 ± 0.8d	5.25 ± 0.7j	5.45 ± 0.5i	1.62 ± 0.8	1.98 ± 0.6
Kusuma	4.35 ± 0.7p	5.24 ± 0.7m	7.01 ± 0.8p	8.05 ± 0.6q	3.54 ± 0.5	3.94 ± 0.6
Vaghari	3.54 ± 0.6l	3.99 ± 0.7k	6.73 ± 0.8n	6.95 ± 0.4n	2.10 ± 0.6	2.62 ± 0.4
Khandagiri	3.25 ± 0.5k	3.55 ± 0.6j	6.86 ± 0.7o	6.75 ± 0.5m	2.05 ± 0.5	2.59 ± 0.5
Aswathi	3.15 ± 0.6j	3.33 ± 0.8i	6.47 ± 0.5m	6.65 ± 0.6l	2.01 ± 0.4	2.54 ± 0.7
Vikram	2.84 ± 0.5h	3.05 ± 0.6g	4.20 ± 0.6b	4.45 ± 0.7e	1.92 ± 0.5	2.25 ± 0.6
Sankar	2.98 ± 0.7i	3.13 ± 0.7h	5.35 ± 0.8k	5.55 ± 0.6j	1.98 ± 0.4	2.50 ± 0.5
Bharati	2.80 ± 0.6g	2.98 ± 0.6f	4.95 ± 0.6h	5.01 ± 0.8h	1.89 ± 0.5	2.12 ± 0.6
Shakti	2.66 ± 0.7e	2.77 ± 0.8e	5.64 ± 0.6l	5.88 ± 0.7k	1.75 ± 0.6	1.92 ± 0.4
Ratna	2.45 ± 0.6c	2.40 ± 0.7c	4.60 ± 0.5f	4.20 ± 0.8d	1.34 ± 0.7	1.04 ± 0.6
Rudra	2.69 ± 0.8e	2.64 ± 0.6d	4.85 ± 0.8g	4.64 ± 0.6f	1.75 ± 0.7	2.05 ± 0.8
Hema	2.25 ± 0.5b	2.12 ± 0.6b	4.34 ± 0.7c	4.00 ± 0.7b	1.39 ± 0.8	1.06 ± 0.7
Pragati	2.20 ± 0.9a	2.10 ± 0.8b	4.55 ± 0.8e	4.20 ± 0.6d	1.77 ± 0.5	1.53 ± 0.6
Pusa-2-21	2.45 ± 0.8c	2.35 ± 0.6c	4.45 ± 0.6d	4.09 ± 0.7c	1.52 ± 0.4	1.29 ± 0.8

Within a column means having a letter in common of rice cultivars are not significantly different at $p \leq 0.05$ level by Duncan's multiple range test

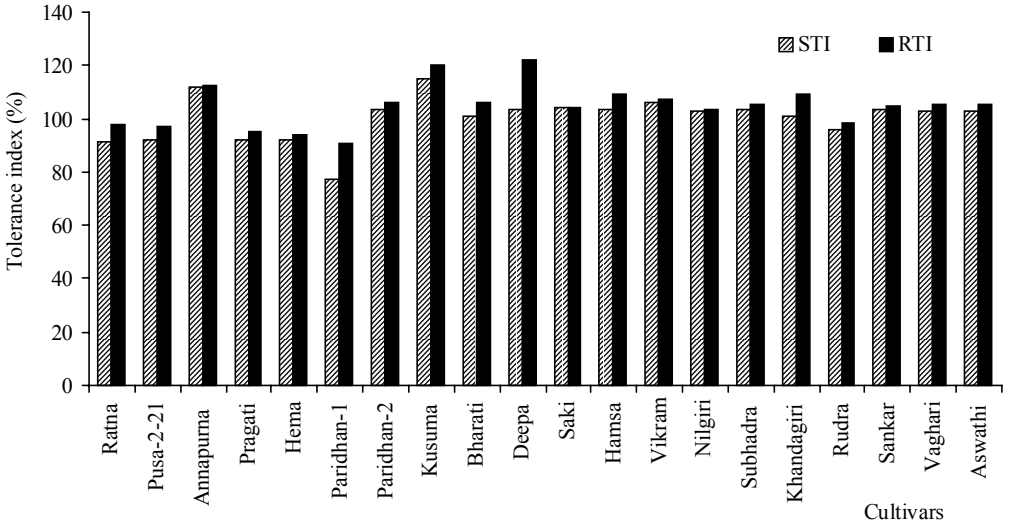

Figure 1. Tolerance index of shoot (STI) and root (RTI) of twenty rice (*Oryza sativa* L.) cultivars in 0.8μM molybdenum; values are mean of twenty samples

Enzyme assay

Peroxidase. Fresh shoots (100 mg) were collected after 9th days of root emergence from plants grown in Hoagland solution supplemented with 80.0 μg/l Mo and without addition of extra Mo (0.1μM – control) and homogenized with mortar and pestle in 4.0 ml of cold 0.1M phosphate buffer (pH 6.1) containing 30 mg of insoluble polyvinylpyrrolidone (PVP) and 15 mg sodium ascorbate. The homogenate was filtered through four layers of miracloth and centrifuged at 12 000 g for 10 min at 4°C. The supernatant was used for the peroxidase assay. The assay mixture contained 0.1M phosphate buffer (pH 6.1), 4mM guaiacol, 3mM H_2O_2 and 0.4 ml of crude enzyme extract. The total reaction volume was 1.2 ml. The rate of change in absorbance (OD) at 420 nm was measured using a double beam UV-Spectrophotometer (Jasco, UVIDEC-650, Japan). The levels of enzyme activity were expressed as μmoles H_2O_2 destroyed/min/mg protein (Bergmeyer et al. 1974).

Catalase. Fresh shoots (100 mg) were collected after 9th days of root emergence from plants grown in Hoagland solution containing 0.8μM Mo and without extra addition of Mo (0.1μM – control) and homogenised in 0.1M sodium phosphate buffer (pH 7.0) and centrifuged at 1000 g for 10 min at 4°C. The supernatant was used as the enzyme extract for catalase activity (Maehly and Chance 1967). For the catalase assay, 1 ml of the enzyme extract was added to the reaction mixture containing 1 ml of 0.1M H_2O_2 and 3 ml of 0.1M sodium phosphate buffer (pH 7.0). The reaction was stopped by adding 10 ml of 2% H_2SO_4 after 1 min of incubation at 20°C. The

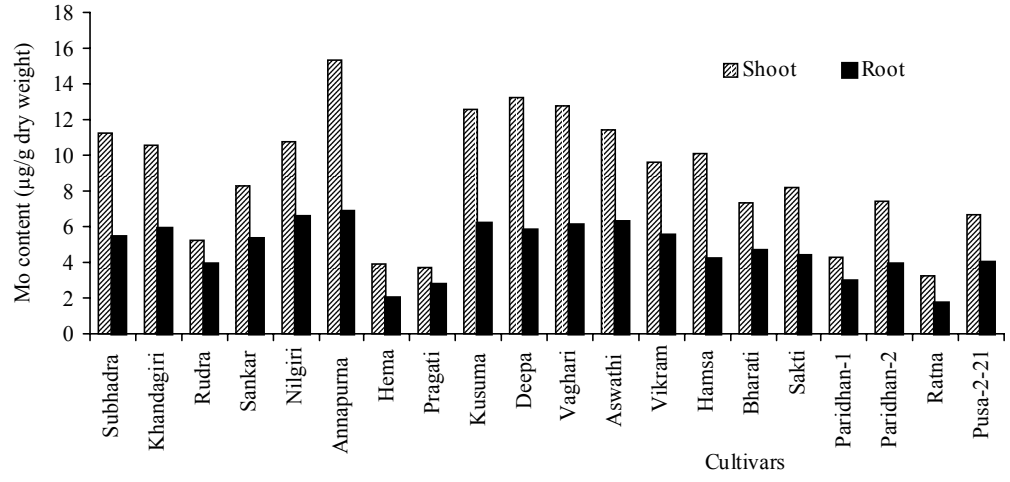

Figure 2. Molybdenum content (μg/g dry weight) in twenty rice cultivars in presence of 0.8μM molybdenum after 9 days of culture; values are means of ten replicates; repeated thrice

Table 2. Biomass yield of twenty rice cultivars in absence (0.1µM – control) and presence (0.8µM) of molybdenum after 9th day of root emergence; values are mean of 20 replicates; repeated three times

Oryza sativa cvs.	Biomass yield (mg/plant)					
	root		shoot		shoot/root	
	0.1µM	0.8µM	0.1µM	0.8µM	0.1µM	0.8µM
Subhadra	30.7m	39.2k	46.2m	57.2n	1.50	1.41
Khandagiri	24.1h	30.1i	45.0l	56.0m	1.86	1.86
Rudra	15.0d	10.0d	29.2f	26.0e	1.94	2.60
Sankar	20.1f	28.7g	43.5j	52.5k	2.16	1.82
Nilgiri	30.8m	39.7k	47.5n	50.3i	1.54	1.46
Annapurna	35.7p	44.1m	50.2p	60.3q	1.42	1.36
Hema	13.3b	9.25c	20.4b	15.5b	1.54	1.67
Pragati	14.8c	9.34c	25.3d	17.2c	1.70	1.84
Kusuma	33.8o	42.0l	48.2o	59.5p	1.42	1.41
Deepa	31.7n	34.3j	48.0o	59.0p	1.51	1.72
Vaghari	29.4l	34.2j	45.3l	54.2l	1.54	1.58
Aswathi	22.3g	29.3h	44.3k	50.0i	1.98	1.70
Vikram	22.1g	26.3e	42.9j	51.9j	1.93	1.97
Hamsa	24.7i	27.3f	40.3i	50.2i	1.63	1.83
Bharati	28.3k	34.2j	38.7h	40.5g	1.36	1.40
Paridhan-1	9.35a	5.71a	11.2a	5.45a	1.20	0.97
Paridhan-2	26.0j	28.3g	37.8g	39.5f	1.45	1.39
Ratna	13.7b	8.0b	26.0e	22.4d	1.89	2.78
Pusa-2-21	17.0e	10.2d	24.5c	22.6d	1.44	2.20

Within a column means having a letter in common of rice cultivars are not significantly different at $p \leq 0.05$ level by Duncan's multiple range test

acidified reaction mixture with or without enzyme extract was titrated against 0.01N $KMnO_4$ to determine the quantity of H_2O_2 utilized by the enzyme. The catalase activity was expressed as µmoles H_2O_2 destroyed/min/mg protein (Bergmeyer et al. 1974).

Soluble proteins in the supernatant were determined according to Bradford (1976) using bovine serum albumin as standard.

Statistics

In order to ascertain the significant differences of growth among various cultivars of rice, an ANOVA test was performed (Sokal and Rohlf 1973). Regression analyses were performed to assess the response of root length of different cultivars of rice to molybdenum over the time of exposure. Effects of molybdenum on growth variables at each level were noted with the separation of mean using the Waller-Duncan multiple range test (Harter 1960).

RESULTS AND DISCUSSION

Twenty cultivars of rice (*Oryza sativa*) treated with five levels of molybdenum showed significant variations in respect of seed germination, elongation of root and shoot and the total biomass production. Our results show that the seed germination was not affected at 0.1–0.8µM of molybdenum. Whereas at higher concentration of molybdenum (1.6µM), germination rate declined; however, the percentage of germination was the maximum in the control nutrient solution without addition of extra molybdenum (data not shown). A good degree of variation in germination and growth response was observed at 0.8µM molybdenum; therefore, this concentration was chosen to compare the performance of different cultivars. The results indicated that the root and shoot growth of rice varied in presence of molybdenum (Table 1). Root length in rice (*Oryza sativa* cvs. Paridhan-1 and Ratna) decreased by 9.50 and 2.04%, respectively, in presence of molybdenum (0.8µM) as compared to their respective controls, while the root length was enhanced by 22.78, 22.03, 20.45 and 12.71%, respectively, in Annapurna, Deepa, Kusuma and Vaghari rice. In rest of the cultivars, the effects on root length were intermediate. Shoot growth also varied in different cultivars in presence of molybdenum (0.8µM). The root elongation method developed by Wilkins (1978) to quantify the inhibitory effect of metal ions on root growth was used widely in ecological studies for testing of tolerance of plants to metals. Toylor and Foy (1985) suggested that the root tolerance index (RTI) is one of the most important markers to screen genotypes and varieties for metal tolerance. Tolerance index (TI) derived from ratios between the data

Table 3. Correlation between different levels of molybdenum (0.1μM – control, 0.2, 0.4, 0.8 and 1.6μM) and root length at three time intervals for 20 cultivars of rice in nutrient solutions

Oryza sativa cvs.	Days	Correlation coefficient (r^2)	Equation	*Oryza sativa* cvs.	Days	Correlation coefficient (r^2)	Equation
Subhadra	3	0.689	$Y = 2.83 + 0.83x$	Hamasa	3	0.836	$Y = 2.07 + 0.46x$
	6	0.903	$Y = 3.05 + 0.97x$		6	0.940	$Y = 2.38 + 0.71x$
	9	0.928	$Y = 3.83 + 0.36x$		9	0.933	$Y = 2.77 + 0.53x$
Vaghari	3	0.719	$Y = 2.55 + 1.09x$	Bharti	3	0.802	$Y = 2.08 + 0.48x$
	6	0.811	$Y = 3.14 + 1.01x$		6	0.894	$Y = 2.57 + 0.54x$
	9	0.872	$Y = 3.61 + 0.71x$		9	0.971	$Y = 2.80 + 0.39x$
Khandagiri	3	0.873	$Y = 2.11 + 0.96x$	Sakti	3	0.693	$Y = 1.82 + 0.79x$
	6	0.707	$Y = 2.51 + 0.87x$		6	0.888	$Y = 2.27 + 0.47x$
	9	0.970	$Y = 3.26 + 0.65x$		9	0.908	$Y = 2.66 + 0.20x$
Aswasthi	3	0.624	$Y = 2.35 + 0.71x$	Paridhan-2	3	0.751	$Y = 1.85 + 0.70x$
	6	0.686	$Y = 2.97 + 0.45x$		6	0.676	$Y = 2.21 + 0.36x$
	9	0.870	$Y = 4.21 + 0.31x$		9	0.931	$Y = 2.51 + 0.28x$
Annapurna	3	0.445	$Y = 4.78 + 1.68x$	Paridhan-1	3	0.974	$Y = 1.97 - 1.06x$
	6	0.613	$Y = 5.47 + 1.26x$		6	0.817	$Y = 2.25 - 1.00x$
	9	0.615	$Y = 3.61 + 0.71x$		9	0.868	$Y = 2.76 - 0.62x$
Kusuma	3	0.726	$Y = 3.51 + 1.70x$	Ratna	3	0.965	$Y = 1.94 - 0.91x$
	6	0.730	$Y = 4.08 + 1.35x$		6	0.917	$Y = 2.04 - 0.74x$
	9	0.727	$Y = 4.55 + 1.10x$		9	0.880	$Y = 2.52 - 0.56x$
Deepa	3	0.844	$Y = 3.26 + 2.05x$	Pusa-2-21	3	0.692	$Y = 1.97 - 0.43x$
	6	0.913	$Y = 4.00 + 1.45x$		6	0.860	$Y = 2.25 - 0.51x$
	9	0.850	$Y = 4.30 + 1.45x$		9	0.818	$Y = 2.50 - 0.53x$
Nilgiri	3	0.716	$Y = 2.73 + 0.99x$	Pragati	3	0.971	$Y = 1.71 - 0.43x$
	6	0.767	$Y = 3.17 + 1.19x$		6	0.895	$Y = 1.97 - 0.34x$
	9	0.915	$Y = 3.93 + 0.25x$		9	0.974	$Y = 2.22 - 0.38x$
Sankar	3	0.682	$Y = 2.20 + 0.77x$	Hema	3	0.927	$Y = 1.50 - 0.66x$
	6	0.746	$Y = 2.54 + 0.58x$		6	0.993	$Y = 1.98 - 0.96x$
	9	0.847	$Y = 3.00 + 0.25x$		9	0.920	$Y = 2.32 - 0.82x$
Vikram	3	0.850	$Y = 1.86 + 0.91x$	Rudra	3	0.975	$Y = 1.76 - 0.57x$
	6	0.948	$Y = 2.28 + 0.70x$		6	0.870	$Y = 1.97 - 0.56x$
	9	0.895	$Y = 2.86 + 0.34x$		9	0.881	$Y = 2.28 - 0.74x$

of different treatments and the control solutions have been useful to characterize individual populations for metal tolerance. Our observations therefore provide further evidences that Annapurna, Deepa, Kusuma and Vaghari rice were tolerant to molybdenum having RTI values as 122.7, 122.0, 120.4 and 105.2, respectively (Figure 1). The cultivars of Paridhan-1, Ratna and Pusa-2-21 rice showed some disorders such as chlorosis, dark brown speckles, leaf yellowish green and inter veinal spots at the leaf due to molybdenum toxicity. Similar observations were made in different plant species at higher concentrations of manganese either in solution or in soil (Nason and McElroy 1963, Tiffin 1972, Moore and Patrick 1991). Root and shoot biomass production were in accordance with root and shoot length and metal accumulation; Annapurna, Deepa, Kusuma and Vaghari had 23.5, 8.20, 24.3 and 16.3% increase in root biomass as compared to the control. The results presented in Table 2 indicated that Paridhan-1, Ratna and Pusa-2-21 were sensitive to molybdenum toxicity showing 38.9, 41.6 and 40.0% reductions in the root biomass when compared to the respective controls. The shoot/root biomass also varied in different cultivars of rice as compared to control (Table 2). The above ground (shoot) biomass was more than the underground (root) biomass. The increase of above ground (shoot) biomass and decrease of underground (root) biomass were due to the fact that molybdenum accumulated principally in the shoot at higher concentrations (Gupta 1991). The greater movement of molybdenum was also due to the interaction with K, Fe and P moving through the roots (Nason and McElroy 1963, Burkin 1968, Xia and Xiong 1991). Root growth declined at higher concentrations of molybdenum, which may be due to the reduction in the protein content of roots by the metal ions (Welch 1995).

The accumulation of molybdenum in root and shoot varied in different cultivars of rice (Figure 2). The accumulation of molybdenum in shoot was higher than in root. The Mo content in the control plant was very low (1.4–2.4 μg/g) (data not shown). Lower accumulation was observed in Paridhan-1, Ratna, and Pusa-2-21. The uptake of Mo was more in the tolerant genotypes than non-tolerant one. Our results confirmed earlier findings in various crops (Possingham 1957, Moore and Patrick 1991, Ervio and Sippola 1993, Welch 1995). One of the basic strategies of metal tolerance is metal accumulation

Table 4. Effect of molybdenum (0.8μM) on enzyme activity of different cultivars of rice (*Oryza sativa* L.); activities are expressed as μmoles of H_2O_2 destroyed per min per mg of protein; parenthesis indicates percent as compared to the control; values given are the averages of three assays

Oryza sativa cvs.	Enzyme activity (mean ± *SE*) peroxidase	catalase
Subhadra	37.8 ± 1.1h (+ 19.8)	38.9 ± 0.8i (+ 11.4)
Khandagiri	38.8 ± 1.0i (+ 26.7)	39.8 ± 1.1j (+ 27.5)
Rudra	20.6 ± 0.9b (+ 4.62)	16.2 ± 1.2a (+ 5.04)
Nilgiri	35.2 ± 0.8g (+ 14.8)	40.7 ± 1.0j (+ 11.7)
Sankar	34.4 ± 1.1g (+ 17.5)	34.2 ± 1.3g (+ 12.1)
Annapurna	49.2 ± 0.9k (+ 52.2)	44.2 ± 0.8k (+ 37.2)
Kusuma	36.6 ± 0.7h (+ 28.9)	46.9 ± 0.9l (+ 31.6)
Deepa	38.2 ± 0.9i (+ 33.9)	48.2 ± 1.0m (+ 36.9)
Vaghari	39.2 ± 1.0j (+ 18.0)	40.2 ± 1.1j (+ 10.1)
Aswathi	36.6 ± 0.8h (+ 16.2)	32.7 ± 0.8f (+ 11.4)
Vikram	32.7 ± 1.0f (+ 22.7)	36.4 ± 1.0h (+ 20.4)
Hema	14.3 ± 0.9a (+ 5.99)	22.4 ± 1.1b (+ 7.51)
Pragati	23.2 ± 1.1c (+ 9.42)	24.7 ± 0.9c (+ 8.82)
Pusa-2-21	21.3 ± 0.8b (+ 12.9)	23.7 ± 0.8b (+ 10.9)
Hamsa	30.7 ± 1.2e (+ 26.1)	30.6 ± 0.7e (+ 19.5)
Bharati	24.7 ± 0.9d (+ 22.6)	36.2 ± 0.6h (+ 18.1)
Sakti	23.0 ± 0.8c (+ 16.9)	33.4 ± 1.1f (+ 10.5)
Paridhan-2	24.5 ± 0.6d (+ 19.3)	31.8 ± 0.8e (+ 12.3)
Paridhan-1	22.2 ± 1.2c (+ 16.3)	27.8 ± 1.0d (+ 10.5)
Ratna	20.9 ± 0.9b (+ 15.2)	25.2 ± 0.9c (+ 11.4)

Within a column means having a letter in common of rice cultivars are not significantly different at $p \leq 0.05$ level by Duncan's multiple range test

where there is no such restriction and metals are accumulated in a detoxified form. Detoxification may result from cell wall binding, active pumping of ions into vacuoles, complexing by organic acids and possibly by specific metal-binding proteins, and alteration of membrane structures (Verkleij and Schat 1990).

The relationship between molybdenum concentration and root length varied with the cultivars (Table 3). The effect of molybdenum became accentuated over time, increasing the slope and this trend was stronger in Paridhan-1, Ratna and Pusa-2-21 of rice being susceptible to molybdenum. Cultivars like Annapurna, Deepa, Kusuma and Vaghari were tolerant, other cultivars were in intermediate position. Acceleration in the enzyme activities such as catalase and peroxidase are believed to play a metabolic role under conditions of metal stress (Van Assche and Clijsters 1990) and therefore may have a subtle role in metal tolerance. Both catalase and peroxidase activity varied in different cultivars (Table 4). The catalase activity was higher in case of tolerant than the non-tolerant cultivars. The activity increased from 4.62 to 52.2% in case of peroxidase and 5.04 to 37.2% in case of catalase, respectively, as compared to control. Greater activity of catalase and peroxidase in tolerant cultivars indicate that the tolerant plants were under stress, a feature often associated with tolerance (DeVos and Schat 1991). Nashikhar and Chakrabarti (1994) reported that catalase and peroxidase activity were indicators of heavy metal toxicity and subsequent stress situation in plants. Several suggestions on the mechanism of metal tolerance in plants were proposed which include production of intracellular metal binding compounds, alterations in metal compartmentation patterns, alteration of cellular metabolism and membrane structure (Verkleij and Schat 1990); once absorbed, toxic metals were not completely inert, but stimulated the activity of certain enzymes (Van Assche and Clijsters 1990, Van Gronsfeld and Clijsters 1994). Based on growth parameters, regressions of root growth versus different concentrations of Mo at 3, 6 and 9th day of exposure and accumulation, the cultivars were categorised with regard to Mo tolerance as: Annapurrna > Deepa > Kusuma > Vaghari > Hamsa > Vikram > Bharati > Paridhan-2 > Aswathi > Subhadra > Sankar > Sakti > Nilgiri > Rudra > Hema > Pragati > Pusa-2-21 > Ratna > Paridhan-1. Nutrient culture is an efficient method for screening metal-tolerant plants of rice for breeding programmes. Further studies are necessary to unravel the hidden facts on the mechanisms of Mo tolerance in rice, a starch rich plant.

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ABSTRAKT

Rychlý hydroponický test tolerance rýže k molybdenu pomocí morfologické a biochemické analýzy

Toleranci k různým hladinám molybdenu (Mo) (0,1µM – kontrola, 0,2, 0,4, 0,8 a 1,6µM) jsme ověřovali u vysoce produktivních kultivarů rýže (*Oryza sativa*) v živném roztoku s hodnotou pH 6,8. Obilky rýže klíčily a rostly za přítomnosti molybdenu v řízených podmínkách prostředí. Standardní růstové charakteristiky, jako je délka kořenů, délka nadzemních částí, hmotnost sušiny kořenů a nadzemních částí a index tolerance kořenů a nadzemních částí, jsme ověřovali jako markery toxicity molybdenu. Měření provedená už za 48 h po vzejití nepřinesla odpovídající výsledky. Avšak měření kořenů 3., 6.

a 9. den po vytvoření kořenů prokázala významné rozdíly mezi jednotlivými kultivary rýže. Kořenový systém kultivarů rýže Annapurna, Kusuma, Deepa a Vaghari se vyvíjel lépe, zatímco kultivary Paridhan-1, Pusa-2-21 a Ratna vykazovaly v přítomnosti molybdenu (0,8µM) slabý růst kořenů. Indexy tolerance kořenů (RTI) a tolerance nadzemních částí (STI) byly u kultivarů rýže Annapurna, Kusuma, Deepa vysoké, což naznačovalo jejich toleranci k molybdenu; naopak hodnoty indexů RTI a STI u kultivarů Paridhan-1 a Ratna byly nízké. Na základě růstových charakteristik jsme určili pořadí tolerance k molybdenu u dvaceti kultivarů rýže: Annapurna > Deepa > Kusuma > Vaghari > Hamsa > Vikram > Bharati > Paridhan-2 > Aswathi > Subhadra > Sankar > Sakti > Nilgiri > Rudra > Hema > Pragati > Pusa-2-21 > Ratna > Paridhan-1. Toxicita molybdenu vykazovala u jednotlivých kultivarů rýže korelaci se zvýšenou aktivitou peroxidázy a katalázy. Tuto metodu lze použít ve šlechtitelských programech k rychlému testu kultivarů rýže na toleranci k molybdenu.

Klíčová slova: toxicita molybdenu; kataláza; hydroponie; peroxidáza; rýže; skrínink

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