TOXICOLOGICAL STUDY OF CHROMIUM AND ITS INTERACTION WITH GA_3 ON GERMINATION OF DIFFERENT CULTIVARS OF PADDY (*ORYZA SATIVA* L.)

Rajendra Gyawali and **H.D. Lekhak***
Department of Food and Nutrition
Chosun University, Dong-gu, Gwangju 501-759, Korea
Email: sagarmathap@yahoo.com
*Central Department of Botany
Tribhuvan University, Kathmandu, Nepal
Email: hdlekhak@wlink.com.np

ABSTRACT

The study was carried out to find the toxic effect of chromium and the role of GA_3 to overcome the toxic effect imposed by chromium on five different cultivars of paddy (*Oryza sativa* L.) *viz.* cv Pokhareli Masino, cv Khumal-2, cv Jorpati Local, cv Khumal-6 and cv Taichung Local from Kathmandu Valley. Germination experiments were carried out in petridishes for seven days at 26±2 °C temperature and potassium dichromate ($K_2Cr_2O_7$) was used as a source of chromium. The degree of inhibition in germination was variable with type of the cultivars and range of chromium concentration. Increase in concentration led to inhibition in germination. Tolerance of the five different cultivars of paddy was: Taichung Local < Khumal-6 < Mansuli Local < Khumal-2 < Pokhareli Masino. The value of effective concentration (EC_{50}) that inhibited the 50 percent germination was found at 287.5, 177.5, 147.5, 87.5 and 32.5 ppm for Pokhareli Masino, Khumal-2, Mansuli Local, Khumal-6 and Taichung Local, respectively. Exogenously applied plant hormone GA_3 in combination with chromium was found partly able to overcome the chromium toxicity but was unable to fully supress the toxicity imposed by chromium on seed germination of paddy.

Key words: Paddy, germination, chromium, EC_{50}, GA_3 and toxicity.

INTRODUCTION

Metals are ubiquitous in the modern industrialized environment. Some metals have no beneficial effects for humans but some others are essential for man. However, the essential trace elements can also be dangerous at high levels. When the concentration of the metal in the soil reaches a threshold level, the ability of the plant to hold the metal breaks down and thus metals may exert their toxic effect on any system of cell metabolism. At that condition, sensitive species serve as an indicator and tolerant species as accumulators that collect the large amount of metals in their cell wall without damaging them (Bradshaw *et al.* 1965). The heavy metal pollution not only decreases agricultural production, they also accumulate in crops, transferred to higher trophic levels in the soil-plant-animal/human system. Therefore, use of the new species or varieties of crop plants at potential toxic level of some elements in agricultural land is likely to become more prominent in commercial agriculture.

Phytohormones might have designated functions in the control of germination and dormancy, with gibberellins assuming the primary role (Bajracharya and Gupta 1978). Lower

concentration of hormones stimulate the growth upto certain limit but at higher concentration the growth was rather suppressed (Silwal 1999). In seeds, substantial amount of gibberellic acid (GA) have been found during seed enlargement and germination (Osada *et al.* 1973) and this hormone is involved in the regulation of some growth processes in young seedling (Mishra *et al.* 1994). GA_3 in paddy has frequently been reported and such increase of GA_3 level may be due to its liberation from bounded forms or due to synthesis. GA_3 treatment in seed germination induced at least four enzymes *viz.* α-amaylase, protease, ribonuclease and β1,3 glucanase. Thus the GA_3 act for α-amylase synthesis and release enzyme from the aleurone layer into the endosperm which support germination. Exogenous application of the GA_3 overcome the germination inhibition of paddy seedlings which is caused by low oxygen concentration due to water logging (Paul and Mukherjee 1977). In previous report dormancy was completely removed in paddy by subjecting the seeds to GA_3 after dehulling (Seshu and Dadlani 1991).

Although, there are many reports on phytotoxicity of chromium metal, to date no data has been found about its toxicity on paddy cultivars from Nepal. The purpose of this study was to examine the phytotoxicity of chromium on germination of paddy (*Oryza sativa* L.) seeds and role of exogenously applied GA_3 to overcome its toxicity.

MATERIAL AND METHODS

Seeds of different paddy cultivars, *viz.*, Pokhrali Masino, Khumal-2 and Khumal-6 were collected from National Agricultural Research Council (NARC), Khumaltar, Lalitpur, Nepal and other cultivars like Taichung Local was collected from Kirtipur area and Mansuli Local was collected from Jorpati area of Kathmandu valley. Seeds were selected on the basis of their wide distribution in the irrigated land around Bagmati river. The seeds were kept at room temperature in airtight packets. Chromium VI was obtained form potassium dichromate ($K_2Cr_2O_7$) and GA_3 was obtained in pure form. The stock solution of 1000 ppm was prepared by dissolving 2.8282 g of $K_2Cr_2O_7$ and 1 g of GA_3 in 1000 ml of distilled water. The concentrations 10, 50, 100, 200, 400, 800 ppm of the Cr and GA_3 solutions were prepared from stock solutions and distilled water was considered as control. The seeds were sterilized with 0.1 percent of mercuric chloride for 2 minutes and were then washed with the fresh water and followed by distilled water. Fifty healthy and uniform sized seeds were selected and shown at equidistance in sterilized petridishes ranged with filter paper. Five ml of test solution was added to each petridishes and kept inside the incubator maintained at 26±2°C. The petridishes were regularly maintained moist by adding the test solutions. The rate of seed germination was recorded for every 24 h up to five days till it becomes constant. The seeds were considered germinated once the radicle emerged 2 mm, out of the seed. Seven days old seedlings were used to determine the germination parameters. Experiments were carried out three times each with three replicates.

Relative toxicity

Degree of relative toxicity of chromium on seed germination of cultivars was calculated. The calculated value gives the percentage of inhibition or enhancement over control. The relative degree of toxicity was calculated by the following formula. Relative toxicity (%) = X-Y / X

Where, X= Germination percentage in control at particular time interval.

Y= Germination percentage in treatment at same time interval.

The tolerance of the five different varieties of paddy was calculated by evaluation of relative degree of germination inhibition and relative degree of toxicity.

Interaction experiment

Highly resistant and highly susceptible cultivars were selected for the interaction experiment after toxicity experiment. The seeds were treated with 0.1, 1, 10 and 100 ppm concentrations of GA_3 for 24 h and were allowed to germinate with EC_{50} value of chromium solution of respective samples. Where, the value of EC_{50} represents a 50 percent germination of seed either effect may be promontory or inhibitory in the respective solutions.

Statistical Analysis

Results from the above study were statistically analyzed following Bailey (1959) and Gupta and Kapoor (1984). Analysis of variance was applied for testing the statistical significance of variations of means at 0.05 levels, which was followed by another test Least Significance Difference (LSD) and Standard Deviation (SD).

RESULTS

Effect of Chromium on seed germination

The rate of seed germination of different paddy cultivars in different concentrations of chromium was found to exhibit inhibitory effect. The Taichung Local showed poor germination percentage as compared to others. All the cultivars germinated up to 400 ppm concentration of chromium except Khumal-6, but none of them were able to germinate at 800 ppm (Table 2). The relative degree of toxicity on seed germination of five different cultivars is shown in Table 1. All the cultivars showed greater degree of toxicity even at lower concentration except the Pokhareli Masino on seed germination. The average relative degree of toxicity was lowest in the Pokhareli Masino and was highest in Taichung Local.

Evaluation of EC_{50} on Germination

From the results obtained above, the value of EC_{50} for different paddy cultivars was calculated for 7 days of germinated seedlings. Two parameters were taken for toxicity evaluation whereas the mean value of EC_{50} was calculated (Table 3).

Table 1. Average relative degree of toxicity (%) of chromium on seed germination of paddy.

Concentration (ppm)	Pokhareli Masino	Khumal-2	Taichuing Local	Mansuli Local	Khumal-6
Control	0	0	0	0	0
10	0.86	8.39	44.04	20.80	23.50
50	1.19	19.14	73.94	26.40	38.86
100	6.26	36.99	82.47	44.58	65.12
200	31.22	74.30	98.65	83.33	89.91
400	89.30	95.03	96.97	95.43	100
800	100	100	100	100	100

Table 2. Percentage of germination inhibition of chromium on seed germination of paddy.

Concentration (ppm)	Pokhareli Masino	Khumal-2	Taichuing Local	Mansuli Local	Khumal-6
Control	0	0	0	0	0
10	0	2.72	3.01	0.79	14.58
50	1	3.40	54.54	8.16	16.66
100	1.34	14.67	65.15	14.28	50.12
200	12.56	46.13	74.24	59.18	79.52
400	85	93.85	87.87	82.77	100
800	100	100	100	100	100

Table 3. Evaluation of average EC50 value of chromium during seed germination of paddy cultivars.

SN	Cultivar	EC50 from germination inhibition (%)	EC50 from average relative degree of toxicity	Mean value
1	Pokhareli Masino	305	270	287.5
2	Khumal-2	220	135	177.5
3	Taichung Local	45	20	32.5
4	Mansuli Local	180	115	147.5
5	Khumal-6	100	75	87.5

Table 4. Germination percentage of Pokhareli Masino after interaction experiment.

Treatment	24 h	48 h	72 h	96 h	120 h
Control	0	93±0.69	100±0	100±0	100±0
EC50 (Cr)	0	10±1	48±3	50±4	50±3
EC50+0.1 GA3	0	16±2	70±2	82±5	84±8
EC50+1 GA3	0	18±5	70±7	82±6	90±4
EC50+10 GA3	0	20±3	80±5	90±4	95±2
EC50+100 GA3	0	18±2	78±8	96±5	98±5

Table 5. Germination percentage of Mansuli Local after interaction experiment.

Treatment	24 h	48 h	72 h	96 h	120 h
Control	0	26±2	98±5	89±0	98±0
EC50 (Cr)	0	6±2	40±5	50±3	50±6
EC50+0.1 GA3	0	8±3	60±4	76±2	90±8
EC50+1 GA3	0	12±2	66±4	84±5	94±10
EC50+10 GA3	0	12±4	80±4	94±5	96±7
EC50+100 GA3	0	10±2	88±4	96±6	96±3

The tolerance index of five different cultivars of paddy was determined in the following order, where higher index value suggested higher tolerance and lower value less tolerance for germination: Taichung Local < Khumal–6 < Mansuli Local < Khumal-2 < Pokhareli Masino. Thus, from the above experiment it was seen that Pokhareli Masino was the most tolerant cultivars that showed the germination capacity at higher concentration of chromium and Taichung Local was the most sensitive cultivar.

Interaction experiment

Interaction experiment was carried out by treatment between culture solution of chromium and exogenous GA_3 on two cultivars of paddy.

These cultivars were selected as one is most tolerant, i.e., Pokhareli Masino and another is most susceptible, i.e., Mansuli Local with the help of above data. Though the Taichung Local was the most susceptible cultivar, it was not taken for interaction experiment due to its poor germination

performance and same case was with the Khumal – 6 also. Seed of highly resistant and highly susceptible on paddy cultivar were treated with a range of GA_3 solutions for 24 h and then allowed for germination experiment with EC_{50} of chromium solution.

Tables 4 and 5 show the role of GA_3 on seed germination at EC_{50} of chromium toxicity of paddy cultivars. The toxicity was found to overcome even at 0.1 ppm of GA_3 treatment but the degree of alleviation of the toxicity was comparatively less. Treatment with 100 ppm concentration of GA_3 was found most effective for both the cultivars. Though germination inhibition mostly overcame by 100 ppm of GA_3, their germination speed was found delayed than control.

DISCUSSION

Though the essentiality of the Cr to the plants is still equivocal, this careful study with five different paddy cultivars reveals that there is no benefit from exogenous chromium even at 10 ppm. Huffman and Allaway (1973) obtained similar result with several crop species in nutrient medium as low as 20 ppm of Cr. This type of toxicity may be due to the various external and internal factors and influenced on hydrolytic enzyme activity like α-amylase, protenase, ribonuclease, β-glucanase, phosphotase. All these are synthesized on the aleurone cells of seed in cereal crops. In present study chromium was observed to delay the initiation of germination of paddy seeds. Therefore, the study indicated that higher concentration of the chromium in water will cause the depression on germination percentage of different cultivars of paddy from Kathmandu valley. Roots were found highly affected by chromium. Absorption study of chromium and cadmium on *Limnanthemum cristatum* Griseb showed that highest accumulation of chromium was found in root than leaf and shoot (Chandra and Garg 1992). Generally the germination was found more enhanced during early hours, i.e., before 72 h after showing and totally inhibited at 800 ppm. The germination percentage has been observed to be decreased at 10 ppm for all cultivars except Pokhareli Masino which exhibited 100 percent germination at that treatment. The result was found just opposite to that obtained by Upadhaya (1999) who had found 100 percent germination of paddy upto the 100 ppm on Cr metal solution. This type of variation in result may be due to the genetic variation of cultivar of paddy. Reddy and Prasad (1992) compared 40 cultivars of paddy under cadmium chloride and categorized them into three different groups on the basis of tolerance. This type of toxicity may be due to the depression on oxygen uptake and physiological disturbance in mobilization of reserve food materials of seeds (Agrawal *et al.* 1961). According to Roberts and Smith (1977) oxygen is required in germination as a terminal acceptor for electrons in respiration as well as other oxidative process of regulatory nature.

The term metal tolerance generally refers to any individual which is able to withstand greater amounts of toxicity than their immediate relatives of normal conditions (Antonovics *et al.* 1971). Plant species or cultivars within species differ widely in tolerance to metal. The metal contents in paddy were related to paddy variety, locality, and soil type (Schuhmacher *et al.* 1994). In the present work Taichung Local was found more susceptible and Pokhareli Masino was comparatively more tolerant than other four cultivars. It is because of the appreciable genotypic differences towards the uptake of heavy metals in the crops. Bradshaw *et al.* (1965) advocated the genetic factors to be responsible for the tolerance capacity of the metal in the plants and such tolerance of chemical pollutants may be due to certain inherited potentiality of the seed (Baker 1981). The extent of

the metal binding by the cell wall was related to the degree of tolerance to a specific metal (Foy *et al.* 1978). Although the binding of metals on the cell wall of root may contribute to metal tolerance, it is not sufficient for plants to prevent metal transport in their leaves.

From the Tables 4 and 5 two different value of EC_{50} were calculated for two separate parameters. The variation in the value of EC_{50} might depend upon different ecological and physiological factors. This type of study for EC_{50} on toxicity of Cr was made by Mangi *et al.* (1978) and Wang (1986) in *Spirodela* species. Nepal (1999) reported EC_{50} at 7.52 ppm for *Spirodela polyrhiza* L. from 14 days of culture in chromium (VI) solution. But here in this case only 7 days germinated seedling was considered to evaluate the EC_{50} value. EC_{50} varied with test cultivars where Taiching Local (32.5ppm) showed lowest value and Pokhareli Masino showed highest (287.5ppm) value.

In interaction experiment against EC_{50} value of chromium concentration, about 97% of the seeds were found germinated upto 100 ppm GA_3 treatment. These results are in agreement with Silwal (1999) who concluded that, germination of paddy seeds was not inhibited even at 1000 ppm solution of GA_3. It indicated that treatment of 100 ppm GA_3 was most effective to overcome the toxicity of chromium but was not a fair difference between the treatment 100 ppm and 10 ppm of GA_3. Shakya (1998) concluded that 10 ppm concentration of GA_3 was found most effective to overcome the toxicity due to industrial effluent. But in our study, for the chromium 100 ppm of GA_3 was required for such type of result. This may be due to the strong pollutant media like chromium (EC_{50}) for the germination than that of the effluent. In fact the interaction of the chromium with GA_3 can progressively decrease the toxicity but is not able to fully overcome the toxicity imposed by chromium during seed germination of paddy cultivars.

ACKNOWLEDGEMENTS

Authors express thanks to Prof. Dr. S.D. Joshi, former Head and to Prof. Dr. P.K. Jha, Head of the Central Department of Botany, Tribhuvan University for laboratory facilities and encouragement.

REFERENCES

Agrawal, S.C., A. Kumar and C.P. Sharma. 1961. Effect of excess supply of heavy metals on barley during germination with special reference to Catalase and Roxidase. *Nature* **191:**726-727.

Antonovics, J., A.D. Bradshaw and R.G. Turner. 1971. Heavy metal tolerance in plants. In: *Advances in Ecological Research.* (ed.) J.B. Cragg. Academic Press, London **7:**85.

Bailey, N.T.J. 1959. *Statical Methods in Biology.* ELBS London.

Bajracharya, D. and V.N. Gupta. 1978. The effect of growth hormones on germination and dormancy behaviour of developing paddy seed. *J. Ins. Sc.* **1:**14-21.

Baker, A.T.M. 1981. Accumulation and exclusion strategies of plants in response to heavy metals. *Journal of Plant Nutrition* **3:**643-654.

Bradshaw, D., R.S. McNeilly and R.P.G. Gregory. 1965. Industrialization evaluation and the development of heavy metal tolerance in plants. *Symp. Brit. Ecol. Soc.* **5:**327-343.

Chandra, P. and P. Garg. 1992. Absorption and toxicity of chromium and cadmium in *Limnanthemum cristatum* Griseb. *(The) Sci. Total Env.* **125:**175-183.

Foy, C.D., R.L. Chaney and M.C. White. 1978. The physiology of metal toxicity in plants. *Ann. Rev. Plant. Physiol.* **29:**511-566.

Gupta, S.C. and V.K. Kapoor. 1984. *Fundamentals of Applied Statistics.* S. Chand and Sons, New Delhi.

Huffman, E.N.D and W.H. Allaway. 1973. Growth of plants in solution culture containing low level of chromium. *Plant Physiol* **52:**72-75.

Mangi, J.J., L. Pankow and P. Turner. 1978. Effect of chromium on some aquatic plants. *Env. Poll.* **16:**285-291.

Mishra J., V. Poudeyl and N. Singh. 1994. Effect of some heavy metals on root growth and germinating seeds of *Vicia faba. Journal of Env. & Health* Part A, **29:**2229-2234.

Nepal, B. 1999. Evaluation of chromium toxicity and its accumulation on *Spirodela polyrihza* L. Schleden. M.Sc. thesis submitted to Central Department of Botany, Tribhuvan University, Kathmandu.

Osada, A.H., S.S. Shibhukawa and I. Noguchi. 1973. Difference in endogenous gibberllins in paddy plant as affected by the growth stage and different growth conditions. *Proc. Crop. Sci.* Japan **42:**41-45.

Paul, A.K. and S. Mukharjee. 1977. Germination behaviour and metabolism of paddy seed under water logged condition. *Zeitshrift fiir pflanzephysiologic* **82:**117-124.

Reddy, C.N. and M.V. Prasad. 1992. Peroxidase (POD) activity increased in *Oryza sativa* treated with different concentration of cadmium chloride and cadmiyum induced additional peroxidase enzymes on roots and leaves. *Biochem. Arch.* **8:**101-106.

Roberts, E.H. and R. Smith. 1977. Dormancy and the pentose phosphate pathway. In: *The Physiology and Biochemistry of Seed Dormancy and Germination.* (ed.) Khan A.A. Elsevier, Amsterdam, pp. 385-411.

Schuhmacher, M., J.M. Llobet, J.L. Domingo and J. Corbella. 1994. *Cadmium, chromium, copper, and zinc in paddy and paddy field soil from southern Catalonia,* Spain, pp. 54-60.

Seshu, D.V. and M. Dadlani. 1991. Mechanism of seed dormancy in paddy. *Seed Science Research* **1(3):**187-194.

Shakya, J.D. 1998. Effect of industrial effluents on seed germination and seedling growth with reference to *Helianthus annus* L. cultivar "Modem Dwarf", M.Sc. thesis submitted to Central Department of Botany, Tribhuvan University, Kathmandu.

Silwal, U. 1999. Effect of heavy metals (Fe, Zn, Pb, Al) and hormone (GA_3) on seed germination and seedling growth of paddy *(Oryza sativa* L. var. Khumal-3). M.Sc. thesis submitted to Central Department of Botany, Tribhuvan University, Kathmandu.

Upadhaya, G.R. 1999. Effect of heavy metals (Al, Cr and Co) on seed germination, water uptake, seedling growth, fresh weight and dry weight of paddy *(Oryza sativa* L. CV Khumal-4). M.Sc. thesis submitted to Central Department of Botany Tribhuvan University, Kathmandu.

Wang, W. 1986. The effect of river water on phytotoxicity of Barium, Cadmium and Chromium ions. *Env. Poll.* (Ser-B):11-193.