Res. on Crops **22** (1) : 18-23 (2021)
With seven figures
Printed in India
DOI : 10.31830/2348-7542.2021.031

Impact assessment of zinc stress on pigmented rice (*Oryza sativa*) cultivars in Northeast India

SAGOLSHEM PRIYOKUMAR SINGH AND Y. TUNGINBA SINGH*

Department of Botany
Mizoram University, Aizawl-796004, Mizoram, India
**(e-mail : tunginba@mzu.edu.in)*

(Received : January 27, 2021/Accepted : March 08, 2021)

ABSTRACT

Heavy metal is a serious abiotic factor that affects the growth and production of rice. In view of important properties such as anti-oxidant, anti-diabetic, anti-cancer activities and anti-cancer activities of pigmented rice, two important local pigmented rice varieties - Chakhou (Manipur) and Kawnglawng (Mizoram) were selected for this study. This study was conducted during the month of March to October (2018 to 2020) at the Department of Botany, Mizoram University, Mizoram, India to investigate the effects of zinc stress on two pigmented and aromatic local rice cultivars Chakhou and Kawnglawng. The pigmented rice cultivars were analyzed for germination percentage, protein contents, chlorophyll a and chlorophyll b, antioxidant enzymes- catalase (CAT) and ascorbate peroxidase (APX) and genome template stability (GTS) under different zinc stress conditions. Zinc stress affected chlorophyll and protein contents while CAT and APX activities were increased significantly. PCR-based Random Amplified Polymorphic DNA (RAPD) technique was employed to investigate the genome stability of the rice cultivars against zinc stress. Genome template stability (GTS) was found to be high (>94%) in both the cultivars. Hence, these cultivars can be suggested as a source for future breeding programs against zinc stress.

Key Words : Antioxidant enzyme, genome template stability, pigmented rice, zinc stress

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple foods and feeds about half of the world's population. It is mainly grown in tropical and temperate regions of the world and serves as sole source of calories for many people. Abiotic and biotic stresses are major problems that affect crop production while abiotic factors alone account for about 70% reductions in crop yield (Almeida *et al.,* 2016; Jagtap *et al.*, 2018; Mani and Sankaranarayanan, 2018). Heavy metal contamination and accumulation in soil and water is again an important abiotic constraint that reduces crop production. Due to the increase of heavy metal accumulation in the soil, there is an increased risk of food safety and health, which is another important concern to the general public as well as government agencies (Kandel and Shrestha, 2018; Mohammadi *et al.,* 2019). Heavy metals enter an agroecosystem through natural and anthropogenic processes. Anthropogenically heavy metals contamination occurs through excessive use of fertilizers, organic manures and industrial and municipal wastes (Madhusudanan *et al.*, 2019; Zulkafflee *et al.*, 2019).

Zinc is required for normal growth and development of plants and acts as an important elements for the synthesis of chlorophyll, carotenoid and cofactor for many enzymes when they are present in an appropriate concentration (Umair Hassan *et al.,* 2020). However, excessive accumulation of zinc leads to a reduction in photosynthetic rate, chlorophyll content and then induces production of reactive oxygen species (ROS) and creates oxidative stress, which causes damage to protein, lipids, etc. (Subba *et al.*, 2014; da Silva *et al.,* 2017; Sudhagar Rao *et al.*, 2020).

Many earlier studies have supported the significant properties of pigmented rice grain like anti-oxidant, anti-diabetic, anti-hyperlipidemic and anti-cancer activities (Baek *et al.,* 2015; Boue *et al.,* 2016). Therefore, a comparative analysis for two pigmented and aromatic local zinc tolerant rice cultivars is of prime importance for future plant breeding programs.

MATERIALS AND METHODS

The experiment was conducted during the month of March to October (2018 to 2020) at the Department of Botany, Mizoram University, Mizoram to study the level of phytotoxicity, genotoxicity and identify zinc tolerant rice cultivars Chakhou (Manipur) and Kawnglawng (Mizoram) in Northeast India.

Plant Material and Treatment Conditions

Seeds of two local rice varieties (Chakhou and Kawnglawng) were collected from local rice farmers. Seeds were surface sterilized in 10% sodium hypochlorite solution and washed with distilled water. Then two experimental sets were undertaken :

(A) Germination percentage: Seeds were exposed to different concentration of zinc sulphate solution (5, 10 and 15 mM). Germination percentage was calculated after 14 days.

(B) Fourteen days old germinated seeds were taken and exposed to different concentrations of zinc sulphate solution (5, 10 and 15mM) for 48 hrs and further analysis was done from these plants.

Isolation and Quantification of Chlorophyll and Total Protein

The amounts of photosynthetic pigments in terms of chlorophyll a, chlorophyll b was determined according to the method of Hartmut *et al.* (1983). Total protein estimation was performed using standard Lowry's method, with bovine serum albumin (BSA) as a standard.

Enzymatic antioxidants

Plant leaves (0.5 g) were taken per treatment and homogenized in 8 mL of 50 mM potassium phosphate buffer (pH 7.8) under ice cold conditions. Homogenate was centrifuged at 10,000 g for 20 min at 4°C and the supernatant was used for the determination of enzyme activity. Catalase (CAT) activity was measured by reduction in absorbance at 240nm due to the decline of extinction H_2O_2 (Aebi, 1983). Ascorbate peroxidase (APX) activity was measured according to Nakano and Asada (1981). The assay depended on the decrease in absorbance at 290 nm as ascorbate was oxidized.

DNA Isolation and RAPD-PCR

DNA was isolated from the leaf following Edwards *et al.* (1991). Briefly, 100 mg of the leaf was macerated in Eppendorf tubes for 10-30 sec and 400 µL of extraction buffer (200 mm Tris-HCl, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) is added. The sample was vortex for 1 min and centrifuged at 13,000 rpm for 5 min and the supernatant was collected and mixed with an equal amount of cold isopropanol and kept at room temperature for 2 mins. Then the mixture was centrifuged at 13,000 rpm for 5 mins and the dried pellet was dissolved in 100 µL TE buffer. The RAPD assays were performed using 5 different RAPD primers (OPA1, OPA2, OPA3, OPA4, and OPA5) (Liu *et al.*, 2007).

Estimation of Genomic Template Stability (GTS)

Changes in the RAPD profiles were expressed as GTS, a qualitative measure showing the obvious changes in the number of RAPD profiles. The GTS was calculated using the formula:

$$GTS = (1 - \frac{a}{n}) \times 100$$

Where, *a* is the average number of changes in DNA profiles and n is the number of bands selected in control DNA profiles.

RESULTS AND DISCUSSION

Zinc treatment did not have a significant impact on germination in the studied rice varieties. Germination percentage was found to be 100% however, zinc treatment did reduce the growth of shoot and root lengths. When 14 days old rice seedlings were treated with different concentrations of $ZnSO_4$ for 48h, both chlorophyll and protein significantly decreased with increasing $ZnSO_2$ concentration (Figs. 1, 2 and 3). Heavy metal stress interferes the morphological, physiological and developmental process. Zinc is essential micronutrient for plants but at high concentration it retards the growth and development and disturbs important metabolic processes (Cherif *et al.*, 2011). High level of zinc concentration has severe phytotoxic effect on rice and inhibits growth. Several studies have shown that zinc stress had significant effect on growth and chlorophyll contents. They also opined that reduction in chlorophyll content might be due to the increased production and accumulation of ROS in the cell and damage of chloroplastic pigments. Heavy metal stress is known to alter reactions that increase production of ROS, OH and H_2O_2. Increasing production of ROS, superoxide radicals, hydroxyl radicals, and hydrogen peroxides inhibits vital cellular processes and causes oxidative damage on plant (Fahad *et al.*, 2019). Increase in antioxidant enzymes such as catalase (CAT), peroxidase (POD) and superoxides dismutase (SOD) are considered to be important defense mechanism in response to excess production of ROS due to heavy metal stress (Arif *et al.*, 2016).

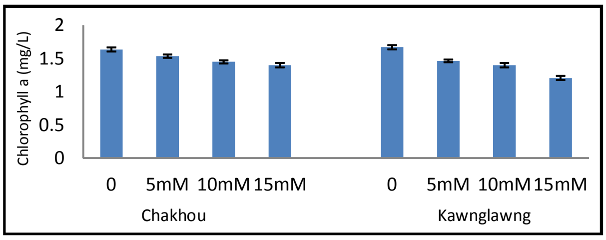

Fig. 1. Changes in chlorophyll 'a' content due to zinc stress.

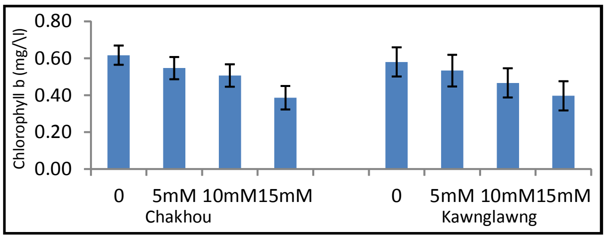

Fig. 2. Changes in chlorophyll 'b' content due to zinc stress.

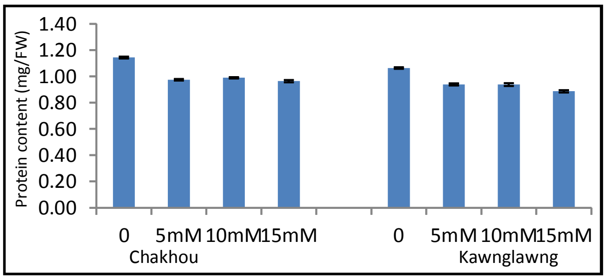

Fig. 3. Changes in protein content due to zinc stress.

Catalase plays an important role in preventing accumulation of H_2O_2 in plant cell. In our present study, due to zinc stress, catalase (CAT) activity significantly increased in both varieties of rice with increasing concentrations of zinc. CAT activity was found to be the highest in rice seedling treated with 15mM $ZnSO_2$ in both the rice varieties (Fig. 4). Earlier studies also concluded similar results in other plants (Gupta *et al.*, 2009). Generally, increase in CAT activity indicates the response of plant under stress. Increase in CAT activity could be due to increase in formation of CAT substrate or inactivation by peroxisomal protease (Cakmak, 2000). Under many biotic and abiotic stress condtions, ascorbate peroxidase (APX) activity also increases to detoxify H_2O_2 in different plant species (Asada, 1999). The present study shows that in response to the increasing zinc concentration, there is an increase in APX activity in both the varieties (Fig. 5). Earlier studies reported that CAT and APX represent the important enzyme in degradation of H_2O_2 (Anjum *et al.*, 2016).

Use of PCR-based RAPD technique to detect DNA damage and mutations in plants due to toxic chemicals has been a successful method. Earlier studies also reported that metal can enhance DNA damages such as

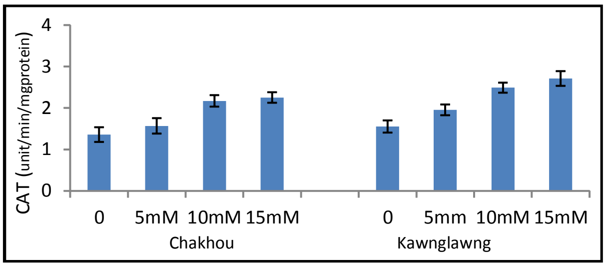

Fig. 4. Effects of zinc stress on Catalase (CAT) activity.

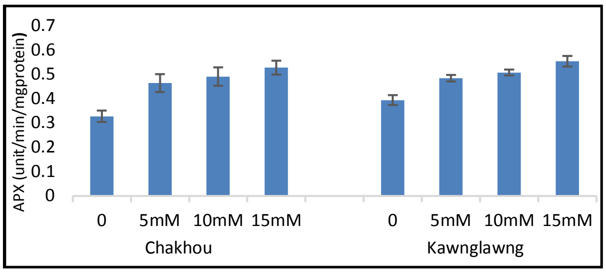

Fig. 5. Effects of zinc stress on Ascorbate peroxidase (APX) activity.

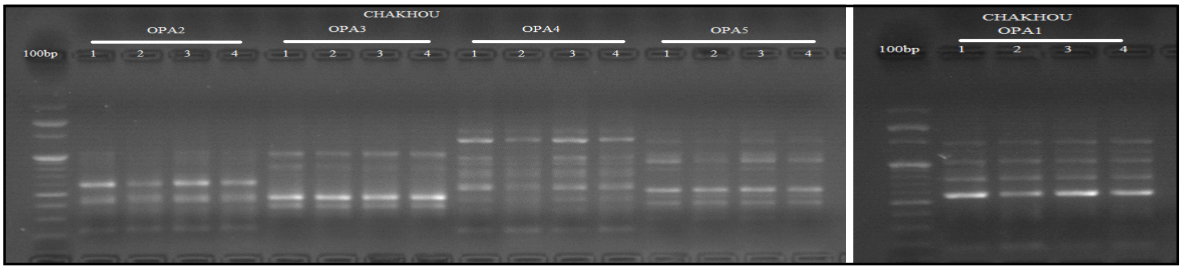

Fig. 6a. Change in banding pattern of Chakhou under zinc stress. 100bp represents 100bp DNA ladder.

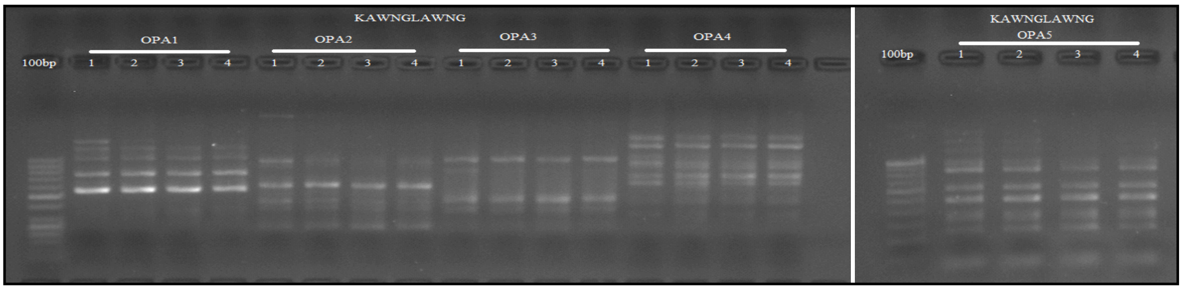

Fig. 6b. Change in banding pattern of Kawnglawng under zinc stress. 100bp represents 100bp DNA ladder.

single and double strand breaks, modified bases, point and deletion mutations. Changes in RAPD profiles like disappearance and appearance of new bands may be due to DNA damage, point mutations or complex chromosomal rearrangements induced by genotoxins (Atienzar *et al.*, 1999). To evaluate the genetic effects of zinc stress, the RAPD-PCR was performed with DNA extracted from both the varieties. Five RAPD primers were employed to screen the rice genomes for genotoxicity. The RAPD profile showed 29 bands in Chakhou and 28 in Kawnglawng (Figs. 6a and 6b). The changes in RAPD profiles generated due to zinc stress in both varieties such as appearance/disappearance of new bands and variation in band intensity were found when compared with control experiments (Tables 1 and 2).

The genomic template stability (GTS) value, a qualitative measure based on change in RAPD profile was calculated for each of the five primers in both the rice cultivar (Table 3). It was observed that Chakhou showed 100% GTS under studied conditions indicating zinc stress had no significant effect on the genome stability other than increase and/or decrease in band intensity. Furthermore, Kawnglawng also showed 94.2% GTS under zinc stress. Our finding on GTS of Chakhou and Kawnglawng cultivars indicate that zinc stress has very little effect on the genomes of the studied cultivars suggesting good candidates as source for future rice breeding programs against zinc stress.

CONCLUSIONS

The present study showed that zinc

Table 1. Changes of total bands in control and polymorphic bands (Chakhou)

Primer	No. of bands	5 mM				10 mM				15 mM			
		a	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*
OPA1	5	0	0	0	0	0	0	0	0	0	0	0	0
OPA2	4	0	0	2	0	0	0	0	1	0	0	1	0
OPA3	4	0	0	1	0	0	0	1	0	0	0	1	0
OPA4	8	0	0	3	1	0	0	0	0	0	0	1	0
OPA5	8	0	0	2	0	0	0	0	0	0	0	1	0
Total bands	29	0	0	7	1	0	0	1	1	0	0	4	0
a+b	0					0				0			

a indicates appearance of new bands, *b* disappearance of normal bands, *c* decreases in band intensities, *d* increase in band intensities, *a+b* polymorphic bands.

Table 2. Changes of total bands in control and polymorphic bands (Kawnglawng)

Primer	No. of bands	5 mM				10 mM				15 mM			
		a	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*
OPA1	5	0	0	0	0	0	0	1	0	0	1	0	0
OPA2	5	0	0	2	0	0	0	0	0	0	1	1	0
OPA3	4	0	0	0	0	0	0	0	0	0	0	0	2
OPA4	8	0	0	0	0	0	0	0	0	1	0	0	0
OPA5	6	0	0	0	0	0	0	1	0	0	0	1	2
Total bands	28	0	0	2	0	0	0	2	0	1	2	2	4
a+b	0					0				3			

a indicates appearance of new bands, *b* disappearance of normal bands, *c* decreases in band intensities, *d* increase in band intensities, *a+b* polymorphic band.

Table 3. Changes of GTS for all primer

Primer	CHAKHOU				KAWNGLAWNG			
	0	5 mM	10 mM	15 mM	0	5 mM	10 mM	15 mM
OPA1	100	100	100	100	100	100	100	94.2
OPA2	100	100	100	100	100	100	100	94.2
OPA3	100	100	100	100	100	100	100	100
OPA4	100	100	100	100	100	100	100	94.2
OPA5	100	100	100	100	100	100	100	100

stress has effect on growth, chlorophyll content, protein content and enzyme activity, which are earlier, reported as an indicator of metal stress in rice. Detection of changes in RAPD profiles due to heavy metal stress using PCR-based RAPD technique can be a powerful and reliable tool for studying genotoxicity in rice. Our results suggest that molecular, physiological and enzyme assay could be used together as a reliable and powerful biomarker to detect the genotoxic effect of heavy metal in different varieties of plants.

ACKNOWLEDGMENTS

Sagolshem Priyokumar Singh thanks University Grant Commission, Govt. of India for UGC-MZU Fellowship.

REFERENCES

Aebi, H. E. (1983). Catalase. In: Bergmeyer, H. U., Ed., Methods of Enzymatic Analysis, Verlag Chemie, Weinhem. Pp. 273-86.

Almeida, D. M., Almadanim, M. C., Lourenço, T., Abreu, I. A., Saibo, N. J. and Oliveira, M. M. (2016). Screening for abiotic stress tolerance in rice: salt, cold, and drought. In Environmental Responses in Plants. Humana Press, New York, NY. pp. 155-82.

Anjum, N. A., Sharma, P., Gill, S. S., Hasanuzzaman, M., Khan, E. A., Kachhap, K., Mohamed, A. A., Thangavel, P., Devi, G. D., Vasudhevan, P. and Sofo, A. (2016). Catalase and ascorbate peroxidase - representative H_2O_2 - detoxifying heme enzymes in plants. *Environ. Sci. Pollut. Res.* **23** :19002-029.

Arif, N., Yadav, V., Singh, S., Kushwaha, B. K., Singh, S., Tripathi, D. K., Vishwakarma, K., Sharma, S., Dubey, N. K. and Chauhan, D. K. (2016b). Assessment of antioxidant potential of plants in response to heavy metals. In Plant Responses to Xenobiotics. Springer, Singapore. Pp. 97-125.

Asada, K. (1999). The water-water cycle in chloroplast: scavenging of active oxygens and dissipation of excess photons. *Annu. Rev. Plant Biol.* **50** : 601-39.

Atienzar, F. A., Conradi, M., Evenden, A. J., Jha, A. N. and Depledge, M. H. (1999). Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in *Daphnia magna* to benzo [a] pyrene. *Environ. Toxicol. Chem.* **18** : 2275-282.

Baek, J. A., Chung, N. J., Choi, K. C., Hwang, J. M. and Lee, J. C. (2015). Hull extracts from pigmented rice exert antioxidant effects associated with total flavonoid contents and induce apoptosis in human cancer cells. *Food Sci. Biotechnol.* **24** : 241-47.

Boue, S. M., Daigle, K. W., Chen, M. H., Cao, H. and Heiman, M. L. (2016). Antidiabetic potential of purple and red rice (*Oryza sativa* L.) bran extracts. *J. Agric. Food Chem.* **64** : 5345-353.

Cakmak, I. (2000). Tansely Review No. 111: possible role of zinc in protecting plant cells from damage by reactive oxygen species. *New Phytol.* **146** : 185-05.

Cherif, J., Mediouni, C., Amma, W. B. and Jemal, F. (2011). Interactions of zinc and cadmium toxicity in their effects on growth and in antioxidative systems in tomato plants (*Solarium lycopersicum*). *J. Environ. Sci.* **23** : 837-44.

da Silva, D. O. M. and Santos, C. A. F. (2017). Adaptability and stability parameters of iron and zinc concentrations and grain yield in cowpea lines in the Brazilian semiarid region. *Crop Sci.* **57** : 2922-931.

Edwards, K., Johnstone, C. and Thompson, C. (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res. 19 : Article No. 1349. doi: 10.1093/nar/19.6.1349.

Fahad, S., Rehman, A., Shahzad, B., Tanveer, M., Saud, S., Kamran, M., Ihtisham, M., Khan, S.U., Turan, V. and Ur Rahman, M.H. (2019). Rice responses and tolerance to metal/metalloid toxicity. In Advances in rice research for abiotic stress tolerance. Woodhead Publishing. pp. 299-12.

Gupta, M., Sharma, P., Sarin, N. B. and Sinha, A. K. (2009). Differential response of arsenic stress in two varieties of *Brassica juncea* L. *Chemosphere* **74** : 1201-208.

Hartmut, K., Lichtenthaler, A. and Welburn, R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **11** : 591-92.

Jagtap, D. N., Pawar, P. B., Sutar, M. W., Jadhav, M. S. and Pinjari, S. S. (20218). Response of rice to different fertilizer sources : A mini review. *Farm. Manage.* **3** : 146-52.

Kandel, B. P. and Shrestha, J. (2018). Characterization of rice (Oryza sativa L.) germplasm in Nepal : A mini review. *Farm. Manage.* **3** : 153-59.

Liu, W., Yang, Y. S., Zhou, Q., Xie, L., Li, P. A. and Sun, T. (2007). Impact assessment of cadmium contamination on rice (*Oryza sativa* L.) seedlings at molecular and population levels using multiple biomarkers. *Chemosphere* **67** :1155-163.

Madhusudanan, M., Singh, K. and Ramawat, N. (2019). Evaluation of the effectiveness of zinc oxide nano particles on growth and yield of rice (*Oryza sativa* L.). *Res. Crops* **20** : 468-76.

Mani, A. and Sankaranarayanan, K. (2018). Heavy metal and mineral element-induced abiotic stress in rice plant. In Rice crop Current Developments. IntechOpen.

Mohammadi, A. A., Zarei, A., Esmaeilzadeh, M., Taghavi, M., Yousefi, M., Yousefi, Z., Sedighi, F. and Javan, S. (2019). Assessment of heavy metal pollution and human health risks assessment in soils around an industrial zone in Neyshabur, Iran. *Biol. Trace Element Res.* **195** : 343-52.

Nakano, Y. and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplast. *Plant Cell Physiol.* **22** : 867-80.

Subba, P., Mukhopadhyay, M., Mahato, S. K., Bhutia, K. D., Mondal, T. K. and Ghosh, S. K. (2014). Zinc stress induces physiological, ultrastructural and biochemical changes in mandarian orange (*Citrus reticulata* Blanco) seedlings. *Physiol. Mol. Biol. Plants* **20** :461-73.

Sudhagar Rao, G. B., Balachandrakaumar, V., Immanuel, R. R., Nambi, J. and Raj, T. S. (2020). Influence of zinc and iron fortified micronutrients on the growth, yield and economics of rice (*Oryza sativa* L.). *Crop Res.* **55** : 202-07.

Umair Hassan, M., Aamer, M., Umer Chattha, M., Haiying, T., Shahzad, B., Barbanti, L., Nawaz, M., Rasheed, A., Afzal, A., Liu, Y. and Guoqin, H. (2020). The critical role of zinc in plants facing the drought stress. Agriculture 10 : doi : 10.3390/ agriculture10090396.

Zulkafflee, N. S., Mohd Redzuan, N. A., Hanafi, Z., Selamat, J., Ismail, M. R., Praveena, S. M., and Abdull Razis, A. F. (2019). Heavy metal in paddy soil and its bioavailability in rice using in vitro digestion model for health risk assessment. Int. J. Environ. Res. Public Health. **16** : doi : 10.3390/ ijerph16234769.