Bot. Bull. Acad. Sin. (2005) **46:** 301-305

Antagonistic effect of selenium against aflatoxin G_1 toxicity induced chromosomal aberrations and metabolic activities of two crop plants

Güleray AGAR* and Lokman ALPSOY

Department of Biology, Faculty of Science and Arts, Atatürk University, 25240/Erzurum, TURKEY

(Received November 15, 2004; Accepted April 15, 2005)

Abstract. The antagonistic effect of selenium (Se^{4+}) against aflatoxin G_1 (AFG_1) toxicity induced chromosomal aberrations and metabolic activities in plants was studied. The results showed that 0.1, 0.2, 0.4 ppm concentrations of aflatoxin G_1 increased chromosomal aberration and mitotic index on total protein, and chlorophyll content decreased. When 0.08, 8, 800 ppm concentrations of Se^{4+} were added to AFG_1, the frequencies of chromosomal aberrations decreased and mitotic index, total protein and chloropyll content increased. Also, results suggested that Se^{4+} has an antagonistic effect against AFG_1, and that the degree of antagonistism of Se^{4+} against AFG_1 is probably related to its concentration ratio.

Keyword: Aflatoxin G_1; Genotoxicity; Selenium; *Vicia faba*; *Zea mays*.

Introduction

Aflatoxins, the most toxic of all the mycotoxins, have been described as natural contaminants in many consumables. These have been reported to affect the physiological processes of some crops. Most studies done have focused on the effect of aflatoxin B_1 (AFB_1). Other aflatoxins have received less attention, and very few studies have been carried out with AFG_1.

Previous papers on the effect of AFG_1 in higher plants indicate that it has an effect on total lipid, protein, and carbohydrate content, chlorophyll synthesis, inhibition of seed germination and growth, and chromosomal aberration (Asahi et al., 1969; Crisian, 1973; Abdou et al., 1984; Abdou et al., 1989; El-Zawahri et al., 1990; El-Naghy et al., 1999).

Recently, several studies have demonstrated that certain dietary substances (such as selenium, β carotene and vitamins) provide a protective effect against AFB_1 cytotoxicity in several animal species. The anticarcinogenic and antimutagenic effects of Se^{4+} against AFB_1 have been found in several animal studies (Chen, 1982; Chen et al., 1982; Bhattacharya et al.,1987; Gregory et al., 1984; Bronzetti et al., 2001). However, so far no report has shown a protective effect of Se^{4+} against AFG_1 toxicity. In the present study, we describe the effect of AFG_1 on total protein, chlorophyll content, and chromosomal aberration and discuss whether Se^{4+} has protective effects on these parameters.

*Corresponding author. E-mail: gagar@atauni.edu.tr; agar252001@yahoo.com; Tel: +90-442-2314341; Fax: +90-442-2360948.

Materials and Methods

AFG_1 and sodium selenite were obtained from Sigma Chemical Company, USA. *Vicia faba* seeds were obtained from the Department of Field Crops, Faculty of Agriculture, Atatürk University (Turkey). *Zea mays* seeds were obtained from the Department of Field Crops, Faculty of Agriculture, Karadeniz Technical University (Turkey). *Vicia faba* and *Zea mays* seeds of equal size were chosen and surface sterilized with 2.5 %/w/v of NaOCl for 3 min. After this treatment the seeds were rinsed with four changes of top water, and dried using sterile filter paper. The seeds were soaked in sterile distilled water for 1 h, and then fifteen seeds were germinated in 15 cm diameter petri dishes on four layers of sterile Whatman number 1 filter paper. Solution containing zero (control), 0.1, 0.2, 0.4 ppm of AFG_1 and the joint concentration of AFG_1 together with 0.8, 8, 800 ppm of Se^{4+} were added to each plate as seen Tables 1-4. The dishes were allowed to germinate in the dark at 25°C. When the roots reached 1.5-3.0 cm in length, they were collected and fixed in acetic acid: alcohol (1:3).

Cytological preparations were carried out using Feulgen's squash technique. The percentage of mitotic activity (mitotic index-MI.) was calculated by counting the total number of dividing cells to the total number of cells examined. Chromosomal aberrations (CA) were scored at anaphase and telophase, and the mutagenic effect was estimated as the percentage of cells with bridges and fragments. In addition to bridges and fragments, other forms of anomalies were identified in all mitotic stages.

For physiological studies, after 7 days, the total chlorophyll content of 20 g fresh tissue from shoot systems

or leaves of corn and broad bean seedling was estimated spectrophotometrically according to Arnon (1949). Quantitative estimation of total protein in the ground tissue of roots, shoot, and leaves systems of both corn and broad bean seedling were estimated according to Lowry et al. (1951).

Each experiment was performed at least three times. Analysis of variance was conducted using a one-way ANOVA test using SPSS 12.0 for Microsoft Windows, and means were compared by Duncan's multiple range test at the 0.001 level at confidence.

Results and Discussion

AFG_1 decreased significantly the mitotic index of *Vicia faba* and *Zea mays* root meristems when compared with control as seen in Table 1, and the mitotic index decreased progressively with increased AFG_1 concentration. Such a decrease was found to be statistically significant ($P<0.01$). All concentrations of AFG_1 used in this study increased the frequencies of abnormalities such as C-mitosis, chromosomal stickiness, and anaphase and telophase bridges involving one or more chromosome micronuclei and fragments (Table 2). Total frequencies of increasing chromosomal abnormalities were found statistically significant. The effects of AFG_1 on the frequencies of total chromosomal aberration and the mitotic index have been earlier reported in animals and plants (Lilly, 1965; Abdou et al., 1984; Abdou et al., 1989; El-Zawahri et al., 1990). The inhibition of mitotic activity and production of several aberrations caused by some fungal metabolites have been explained as being the effect of these compounds on DNA synthesis and their acting as an enzymatic inhibitor of the enzyme system required for the chain reaction of DNA synthesis (Smith and Sullivan, 1960; Taylor et al., 1962; Abdou et al., 1989).

Total chlorophyll and total protein of both plants decreased with increasing concentration of AFG_1 (Tables 3, 4). The decrease was more obvious with increased AFG_1 concentration. Aflatoxins inhibit chlorophyll synthesis, resulting in virescence or albinism in the affected plant (Schoental and White, 1965; Reiss, 1971; Sinha and Kumari, 1990; El-Naghy et al., 1999). Electron microscopic studies revealed the inhibition of grana formation in chloroplast of maize and oat leaves treated with aflatoxin (Slowatizky et al., 1969; El-Naghy et al., 1999).

AFG_1 affected the total proteins, and the protein content decreased with increased of AFG_1 concentration (Table 4). Little information is available on the effect AFG_1 on total protein concentration. Suppression of protein levels by aflatoxins was observed in germinating maize and other crop plants (El-Naghy et al., 1999). Singh et al. (1974) attributed the inhibition of protein synthesis to the non availability of m-RNA, which is necessary for the synthesis of proteins in the cell.

Table 1. Comparisons of the effects different concentrations Se^{4+} together with AFG_1 on mitotic index in *Vicia faba* and *Zea mays*.

		AFG_1		
		0.1 ppm (x±SD)	0.2 ppm (x±SD)	0.4 ppm (x±SD)
Vicia faba	AFG_1 (A)	6.8±1.05*	5.90±1.34*	5.50±2.35*
Control: 9.24	A+0.08 ppm Se^{4+}	7.13±1.92**	7.13±1.59**	6.80±2.55**
	A+8 ppm Se^{4+}	8.93±4.64**	8.40±2.19**	8.90±1.23**
	A+800 ppm Se^{4+}	7.57±1.90**	5.32±1.02**	5.53±3.31**
Zea mays	AFG_1	9.00±1.24**	7.70 ±1.92**	6.77±3.06**
Control: 20.64	A+0.08 ppm Se^{4+}	9.27±1.23**	9.30±2.6**	9.70±1.34**
	A+8 ppm Se^{4+}	11.43±1.43**	12.80±0.84**	10.27±0.83**
	A+800 ppm Se^{4+}	8.33±1.25**	6.67 ±2.01**	7.83±1.76**

Duncan's multiple range test *P<0.05; **P<0.01.

Table 2. Comparisons of the effects different concentrations Se^{4+} together with AFG_1 on chromosoma aberration in *Vicia faba* and *Zea mays*.

		AFG_1		
		0.1 ppm (x±SD)	0.2 ppm (x±SD)	0.4 ppm (x±SD)
Vicia faba	AFG_1 (A)	41.05±1.36*	50.55±1.79*	60.00±2.92*
Control: 13.29	A+0.08 ppm Se^{4+}	28.82±1.65**	30.48±4.86**	30.67±1.43**
	A+8 ppm Se^{4+}	18.48±1.65**	19.00±1.42**	19.26±1.44**
	A+800 ppm Se^{4+}	39.79±2.97**	47.53±2.73**	58.63±2.97**
Zea mays	AFG_1 (A)	44.92±1.94**	49.92±1.77**	55.12±1.20**
Control: 5.37	A+0.08 ppm Se^{4+}	34.24±1.65**	36.68±1.24**	42.45±2.13**
	A+8 ppm Se^{4+}	30.45±1.95**	30.89±1.42**	31.32±1.35**
	A+800 ppm Se^{4+}	45.42±1.72**	43.90±3.72**	49.09±1.13**

Duncan's multiple range test *P<0.05; **P<0.01.

Table 3. Comparisons of the effects different concentrations Se^{4+} together with AFG_1 on total chlorophyll content in *Vicia faba* and *Zea mays*.

		AFG_1		
		0.1 ppm (x±SD)	0.2 ppm (x±SD)	0.4 ppm (x±SD)
Vicia faba	AFG_1 (A)	7825±1.32**	4771±1.50**	3226±0.89**
Control: 9623	A+0.08 ppm Se^{4+}	8068±1.62**	5107±1.24**	4887±1.03**
	A+8 ppm Se^{4+}	8360±1.98**	5224±1.72**	4983±1.14**
	A+800 ppm Se^{4+}	7550±1.74**	4663±1.94**	3111±1.27**
Zea mays	AFG_1	6125±2.4**	3610±1.87**	3213±1.33**
Control: 8236	A+0.08 ppm Se^{4+}	6637±1.52**	4685±1.48**	4035±1.45**
	A+8 ppm Se^{4+}	7160±2.33**	4938±1.79**	4743±3.0**
	A+800 ppm Se^{4+}	6141±1.04**	3047±1.12**	3599±1.66**

Results given in mg/ml.
Duncan's multiple range test *P<0.05; **P<0.01.

Table 4. Comparisons of the effects different concentrations Se^{4+} together with AFG_1 on total protein content in *Vicia faba* and *Zea mays*.

		AFG_1		
		0.1 ppm (x±SD)	0.2 ppm (x±SD)	0.4 ppm (x±SD)
Vicia faba	AFG_1 (A)	273.42±1.93**	239.65±2.34**	201.53±1.64**
Control: 339.8	A+0.08 ppm Se^{4+}	287.58±1.02**	239.65±3.04**	199.35±1.59**
	A+8 ppm Se^{4+}	302.84±1.25**	250.55±1.89**	209.15±1.68**
	A+800 ppm Se^{4+}	264.71±1.38**	230.94±1.57**	194.99±1.95**
Zea mays	AFG_1	130.9±1.76**	124.66±1.33**	82.86±2.05*
Control: 151.3	A+0.08 ppm Se^{4+}	132.9±1.32**	128.90±2.55**	87.98±1.47*
	A+8 ppm Se^{4+}	137.60±1.83**	132.99±1.61**	100.77±1.03*
	A+800 ppm Se^{4+}	126.85±1.44**	121.74±2.28**	84.91±2.08*

Results given in mg/g dry.
Duncan's multiple range test *P<0.05; **P<0.01.

It is apparent that the effects exerted by AFG_1 are similar to those caused by AFB_1 and both are capable of inhibiting the synthesis of chlorophyll, protein, and mitotic division of *Vicia faba* and *Zea mays.*

However, these effects of AFG_1 seen at higher levels decreased after treatment with different concentrations of Se^{4+}. Among all the treatment groups with Se^{4+} together with AFG_1, especially the 8 ppm concentration of Se^{4+}, chromosomal aberrations decreased significantly, and mitotic index, total protein, and total chlorophyll concentration increased (Tables 1-4). Se^{4+} has been found to inhibit the mutagenic of effects AFB_1 in several animal studies (Chen, 1982; Chen et al., 1982; Francis et al., 1988; Shi et al., 1994; Shi and Hew, 1995). It is recognized as an antimutagenic and anticarcinogenic substance against the cytotoxicity and genotoxicity of AFB_1. The protective effects of Se^{4+} have been reported to be primarily associated with its presence in glutathione peroxidases, which are known to protect DNA and other cellular components from damage by oxygen radicals (Gregory and Edds, 1984; Nakae, 1987; Kodama et al., 1990).

Although the selenium has an antimutagenic and anticarcinogenic effect, its optimal concentration is not known. At high concentrations it is toxic, mutagenic, and carcinogenic while at low concentrations it is antimutagenic and anticarcinogenic (Oldifield, 1987; Biswas et al., 1996; Schrauzer, 2000). 10 µM of selenium concentration was observed to be cytotoxic to human blood lymphocytes (Abul Hassan et al., 2004). Moreover, the toxicity of elemental selenium was also obserbed when used in anticancer studies. Even though the recommended daily allowance (RDA) of selenium by the U.S. Food and Drug Administration is 50 µg/day, cancer preventive use of selenium is typically 200 µg daily. It is generally accepted that daily intake should not exceed 600 µg (Bahr et al., 1999).

The protective role of Se^{4+} against AFG_1 has not been reported. The antagonistic effect of selenium against cadmium induced chromosomal aberrations and micronuclei on higher plant has been studied. The 8-ppm concentration of Se^{4+}, in particular, has been shown to reduce the micronuclei and chromosomal aberrations effectively. This protective role of Se^{4+} has been attributed to glutathione peroxides (Zhang and Xiao, 1998; Ağar and Taşplnar, 2003).

In summary, the 8-ppm Se^{4+} concentration has been found to be an active inhibitor of the mutagenicity of AFG_1 as observed with AFB_1 and its effect is probably related to its action on the enzymatic activation system since selenium is the essential component of glutathione peroxides. Cleary, information on the biochemistry of an-

tioxidant protection against aflatoxin toxicity in plants is needed.

Literature Cited

Abdou, R.F., S.E. Megalla, A.M. Moharram, K.M. Abdal, T.H. I. Sherif, A.L. El-Syed-Mahmood, and A.E. Lottfy. 1989. Cytological effects of fungal metabolites produced by fungi isolated from Egyptian poultry feedstuffs. J. Basic. Microbiol. **29:** 131-139.

Abdou, R.F., S.E. Megalla, and S.G. Azab. 1984. Mutagenic effect of aflatoxin B_1 and G_1 on the Egyptian cotton leafworm, *Spodoptera littoralis*. Mycopathologia **88:** 23-26.

Abul-Hassan, K.S, B.E. Lehnert, L. Guant, and R. Walmsley. 2004. Abnormal DNA repair in selenium-treated human cells. Mutation Res. **565:** 45-51.

Ağar, G. and M.S. Taşplnar. 2003. Effects of calcium, selenium and zinc on cadmium induced chromosomal aberration in root of *Secale cereale*. Fresenius Environ. Bull. **12:** 1471-1475.

Arnon, D.L. 1949. Copper enzymes in isolated chroplasts PPO in *Beta vulgaris.* Plant Physiol. **24:** 1-15.

Asahi, T., Z. Mori, R. Majima, and I. Uritani. 1969. The effects of aflatoxins on metabolic changes in plant tissue in response to injury. J. Stored Prod. Res. **5:** 219.

Bahr, K., I. Dfreher, and J. Kohrle. 1999. Selenium supplementation by selenium yeast sodium selenite:analysis of the selenium status as well as risk deficiency and intoxication. J. Lab. Med. **23:** 594-599

Bhattacharya, R.K., P.F. Firozi, and V.S.S. Aboobaker. 1987. Modifying role of dietary factors on the mutagenicity of aflatoxin B_1: in vitro effect of vitamins. Mutat. Res. **188:** 121-128.

Biswas, S., G. Talukder, and A. Sharma. 1996. Selenium salts and chromosome damage. Mutation Res. **307:** 201-205.

Bronzetti, G., M. Cini, E. Andreoli, L. Caltavuturo, M. Panunzio, and C.D. Croce. 2001. Protective effects of vitamins and selenium compounds in yeast. Mutation Res. **496:** 105-115.

Chen, J. 1982. Effects of dietary selenium and vitamin E on hepatic mixed-function oxidase activities and in vivo covalent binding of aflatoxin B_1 in rats. J. Nutr. **112:** 324-331.

Chen, J., M.P. Goetchius, G.F. Combs, and T.C. Campbell. 1982. Effects of dietary selenium and vitamin E on covalent binding of aflatoxin to chick liver cell macromolecules. J. Nutr. **112:** 350-357.

Crisian, E.V. 1973. Effects of aflatoxin on seedling growth and ultrastructure in plants. Appl. Microbiol. **12:** 991-1000.

El-Naghy, M.A., E.M. Fadl-Allah, and M. Samhan. 1999. Effect of aflatoxin G_1 on germination growth and metabolic activities of some crop plants. Cytobios **97:** 87-93.

El-Zawahri, M., M. Morad, and A.F. Khishin, 1990. Mutagenic effect of aflatoxin G_1 in comparison with B_1. J. Environ Pathol. Toxicol. Oncol. **10:** 45-51.

Francis, A.R., T.K. Shetty, and R.K. Bhattacharya. 1988. Modifying role of dietary factors on mutagenenicity of aflatoxin B_1:In vitro effect of trace elements. Mutation Res. **199:** 85-93.

Gregory, J.F. and G.T. Edds. 1984. Effect of dietery selenium on the metabolism of aflatoxin B_1 in turkeys. Food Chem. Toxicol. **22:** 637-642.

Kodama, M., F. Inoue, and M. Akao. 1990. Enzymatic and non enzymatic formation of free radicals from aflatoxin B_1. Free Rad. Res. Comm. **10:** 137-142.

Lilly, L.J. 1965. Induction of chromosome aberrations by aflatoxin. Nature (London). **207:** 433-440.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Chem. **193:** 265-275.

Nakae, D., Y. Konishi, and J.L. Farbert. 1987. A role for oygen radicals in the hepatotoxicity of aflatoxin B_1 and dimethynitrosoamine. Proc. Jpn. Cancer. Assoc., pp. 38-43.

Oldifield, J.E. 1987. The two faces of selenium. J. Nutr. **117:** 2002-2008.

Reiss, J. 1971. Inhibition of germination of *Lepidium sativum* by aflatoxin B_1 and rubratoxin B. Biochem. Physiol. Pflanzen. **162:** 263-270.

Schoental, R. and A.F. White. 1965. Aflatoxins and albinism in plants. Nature **205:** 57-65.

Schrauzer, G.N. 2000. Anticarcinogenic effects of selenium. Cell. Mol. Life Sci. **57:** 1864-1873.

Shi, C.Y. and Y.C. Hew. 1995. Inhibition of aflatoxin B_1-induced cell injury by selenium; an in vitro study. Human Exper. Toxicol. **14:** 55-60.

Shi, C.Y., S.C. Chua, H.P. Lee, and C.N. Ong. 1994. Inhibition of aflatoxin B_1-DNA binding and adduct formation by selenium in rat. Cancer Letters. **82:** 203-208.

Singh, R., A. Singh, S. Vadhera, and I.S. Bhutia. 1974. Effect of aflatoxins on the changes in fats and carbohydrates during germinations and on the symbiotic of nitrogen in peanuts (*Arachis hypogea*). Physiol. Plant. **32:** 359-364.

Sinha, K.K. and P. Kumari. 1990. Some physiological abnormalities induced by aflatoxin B_1 in mung seeds *Vigna radiate* (variety Pusa Baishakhi). Mycopathologia **110:** 77-86

Slowatizky, I., A.M. Mayer, and A. Mayber. 1969. The effect of aflatoxin on greening of aetiolated leaves. Isr. J. Bot. **18:** 31-37.

Smith, L.H. and M. Sullivan. 1960. Feedback inhibition by fluorinated pyrimidines. Biochem. Biophys. Acta. **39:** 554-560.

Taylor, J.H., W.F. Hant, and J. Tung. 1962 Effects of fluorodeoxyuridine on DNA replication, chromosome breakage and reunion. Proc. Nat. Acad. Sci. USA **48:** 190-198.

Zhang, Y. and H. Xiao. 1998. Antagonistic effect of calcium, zinc and selenium against cadmium induced chromosomal aberrations and micronuclei in root cells of *Hordeum vulgare.* Mutation Res. **420:** 1-6.

硒可對抗黃麴毒素 G_1 對兩種作物引起之染色體不正常及代謝活性影響

Güleray AGAR and Lokman ALPSOY

Department of Biology, Faculty of Science and Arts, Atatürk University, 25240/Erzurum, TURKEY

本文研討硒對黃麴毒素 G_1 對兩種作物引起之染色體不正常及代謝活性影響的對抗作用。結果顯示 0.1, 0.2 及 0.4 ppm 濃度之黃麴毒素 G_1 增加染色體不正常，且細胞分裂指數、總蛋白質及葉綠素含量下降。當 0.08, 8 及 800 ppm 濃度之硒$^{4+}$ 和黃麴毒素 G_1 同時加入時，染色體不正常減緩，且細胞分裂指數、總蛋白質及葉綠素含量增加。同時，結果顯示硒$^{4+}$ 對黃麴毒素有拮抗作用，而此拮抗作用依二者之相對濃度而定。

關鍵詞：黃麴毒素 G_1 ；對基因之毒性；硒；*Vicia faba*；*Zea mays*。