Assiut Journal of Agriculture Science 53 (2) 2022 (14-28) *Website:* http://ajas.journals.ekb.eg/
ISSN: 1110-0486 / E-ISSN: 2356-9840 *E-mail:* ajas@aun.edu.eg

Evaluation of Chromium Harmful Effects on *In Vitro* Banana Performance

Karema E.A. Morsy[1]; Galal A.R. Elsherbini[1] and Muhammad Youssef [2]*

[1]Genetics Department, Faculty of Agriculture, Sohag University, Egypt
[2]Genetics Department, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt

*Corresponding author email: mkhirshy@aun.edu.eg

DOI: 10.21608/ajas.2022.126810.1112

Abstract

Abiotic stresses have a significant negative impact on plant growth and production. Among which, toxicity of heavy metals is considered one of the most factors affecting plant growth and agriculture on new reclaimed lands. In this study, the harmful effect of chromium was evaluated on *in vitro* banana plants of Grand Nain and Williams-Zeef cultivars based on morphological, physiological, and molecular assessments. Results showed that chromium significantly decreased all studied traits, including plant fresh weight, plant length, number of shoots per explant, and photosynthesis related pigments (chlorophyll and carotenoid content). The percentage of reduction due to chromium treatment ranged from 36.48 to 79.69% in shoot length of Grand Nain and chlorophyll-*b* in Williams-Zeef, respectively. Both banana cultivars were negatively affected by chromium treatment. However, Williams-Zeef showed higher reduction than that of Grand Nain in all studied traits. On the other hand, molecular analysis was performed to detect any variation between chromium-treated and untreated plants using inter simple sequence repeats (ISSR) and start codon targeted (SCoT) polymorphism. Results of molecular analysis confirmed the morphophysiological findings, by detection some polymorphic bands due to chromium treatment. In this regard, a total of six polymorphic bands were detected in the two banana cultivars, discriminating treated and control plants. In agreement with morphophysiological results, Williams-Zeef showed more polymorphism (five bands) due to chromium treatment than Grand Nain (one band). The screening protocol used in this study was efficient and helpful and could be used in successive studies to evaluate other toxicants and with other plant species as well.

Keywords: *Heavy metals, In vitro screening, Musa, Chromium, Molecular markers*

Introduction

Banana belongs to the genus *Musa*, family *Musaceae,* is considered one of the most common fresh fruits worldwide. The important cultivars of banana are derived from the two valuable species, i.e., *Musa acuminata* Colla and *M. balbisiana* Colla (Hasan *et al*., 2020). Banana is grown in several countries, mainly in tropical and subtropical regions of the world with abundant rainfall, including Africa, Latin America, Caribbean, Asia, and Pacific (Nansamba *et al*., 2020). The

Received: 12 March 2022 / Accepted: 10 May 2022 / Published online: 1 June 2022

global production of banana is about 114 million tons (FAOSTAT, 2019). It provides 25% of dietary energy for over 70 million people in Africa (Edward and Fredy, 2012). Their fruits are rich in vitamin B_6, carbohydrates, proteins, minerals such as calcium, sodium, potassium, and magnesium along with low levels of zinc, iron, and carotenoids (Fineli, 2007).

A complex of biotic and abiotic stresses is being affecting plant production in the developing world. Abiotic stresses including drought, heat, cold, salinity and toxicity of heavy metals, are important factors affecting the growth and productivity of plant species, resulting in up to 70% yield losses (Rai *et al*., 2019; Roorkiwal *et al*., 2020; Raza *et al*., 2021; Varshney *et al*., 2021). Banana production is restricted by a wide range of abiotic stresses which affects fruit productivity and quality (El-Mahdy and Youssef, 2019). Major plant physiological and metabolic processes can be altered by the presence of toxic compounds, such as heavy metals, that can cause damage to plants (Hossain *et al*., 2009). Heavy metals include a vast range of metals and metalloids which are toxic to plants such as copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), nickel (Ni), cobalt (Co), cadmium (Cd), arsenic (As) and chromium (Cr) etc.

Chromium (Cr) is a naturally occurring heavy metal and the 17th most abundant element in the earth's mantle (Bhalerao *et al*., 2015). The contaminated soil with Cr reaches an average of 200 ppm, or even more, with significant difference to the soil quality standard for the content of Cr, amounting to 76 ppm (Diwan *et al*., 2010). The phytotoxicity of Cr can be mediated either by direct interaction with different plant parts and metabolic pathways or it generates internal stress by inducing the accumulation of reactive oxygen species (ROS) (Abdul Wakeel *et al*., 2020). The effect of Cr on banana growth has been evaluated Amalia *et al*. (2016). The higher concentration of Cr in the medium decreased plan weight drastically. Also, root-shoot ratio and Cr content in root and shoot were increased by increasing Cr concentration in the medium.

To assess genetic variability caused by toxicants such Cr, various methods can be used to detect and monitor *in vitro* raised plants under stress. These methods include morphological and physiological evaluation as well as biochemical and molecular markers approaches. The molecular marker technologies have become a powerful tool in crop improvement through their use in germplasm characterization and fingerprinting, genetic analysis, linkage mapping, and molecular breeding (Gaafar and Sakerm, 2006). Identification of possible variation at molecular level is very useful for abiotic stress screening in plant tissue culture (Soniya *et al*., 2001). Simple and less laborious molecular markers like inter simple sequence repeats (ISSR) and start codon targeted (SCoT) polymorphism can be used for genetic stability against abiotic stress in micro-propagated plants. Therefore, the objective of the present study was to evaluate the performance of *in vitro* banana plants under chromium stress condition in two commercial cultivars using morphological, physiological, and molecular analyses.

Materials and Methods

Banana plant materials

In vitro regenerated plantlets of two banana commercial cultivars (i.e., Grand Nain and Williams-Zeef, *Musa acuminata* Colla, subgroup Cavendish, AAA) were obtained from the private Zamzam Tissue Culture Laboratory, Cairo, Egypt.

Culture media preparation

In vitro regenerated shoots of 'Grand Nain' and 'Williams-Zeef' banana cultivars were sub-cultured three times on proliferation medium at an interval of 30 days. The proliferation culture media consisted of the full strength MS (Murashige and Skoog, 1962) medium with vitamins, supplemented with 22 µM 6-benzyleaminopurine (BAP), 30.0 g/L sucrose and solidified with 8.0 g/L agar. The pH of the medium was adjusted to 5.7±0.1 before sterilization using 0.1 or 1M of sodium hydroxide. Fifty ml of the medium was poured into 500 ml glass jars. All media were autoclaved under 1.2 IP/b^2 at 121°C for 20 min and then kept overnight at room temperature before culture. The explants were incubated in a growth chamber for three weeks at 26±2°C under 16 hours of cool white fluorescent light (21 umol/s/m^2) and 8 hours of darkness.

In vitro screening of banana cultivars for chromium toxicity tolerance

To determine the optimum concentration of chromium (Cr) for banana tolerance screening, a preliminary experiment was conducted. Proliferation medium was supplemented with six different concentrations of potassium dichromate ($K_2Cr_2O_7$), i.e., 50, 100, 200, 400 and 600 ppm, along with a Cr-free medium as a control. The preliminary experiment was done on Grand 'Nain cultivar' only. After determination of the optimum concentration of chromium, the main experiment was conducted on the two banana cultivars. Proliferation medium was supplemented with the optimum concentration of potassium dichromate (400 ppm) along with a free Cr medium as a control.

In vitro evaluation of chromium tolerance

After 5 weeks on proliferation medium, rooted plantlets were collected and some morphological (plantlets fresh weight (FW, g), number of shoots per explant (SN) and shoot length (SL, cm)) and physiological measurements (chlorophyll a, b, total and carotenoid) were recorded on proliferated plantlets (Lichtenthaler, 1987).

Experimental design and data analysis

Both preliminary and main experiments were designed using complete randomized design (CRD). For the preliminary experiment, 3 replicates with 6 jars each were used per treatment along with six concentrations of Cr on Grand Nain cultivar. While the main experiment was designed by 3 replicates with 6 jars each were used per treatment along with two concentrations of Cr on the two banana cultivars. Analysis of variance was performed utilizing MSTAT-C significant program (Nissen, 1984). Duncan's multiple range test was used for means comparison using at 1% probability level.

Molecular evaluation

This part of study was performed and analyzed partially at Genetics Department, Faculty of Agriculture, Sohag University and the molecular biology laboratory, Central laboratories, Faculty of Agriculture, Assiut University.

DNA extraction

Total genomic DNA was extracted from banana plantlets of both cultivars under control and the optimum concentration of potassium chromate, following the protocol of Youssef *et al*., (2015). DNA concentration was determined using the spectrophotometer and electrophoresis.

Molecular markers

Ten primers of each ISSR and SCoT markers were tested, out of which seven and eight primers were selected and used for the analysis, respectively (Table 1) according to band clearness. PCR program and conditions of ISSR and SCoT was done according to Gupta *et al*. (1994) and Collard and Mackill (2009), respectively. PCR products of both ISSR and SCoT were separated on 1.5% agarose gel, respectively and visualized by staining with ethidium bromide.

Table 1. Codes and sequences of ISSR and SCoT primers used in molecular analysis

ISSR		SCoT	
Code	**Sequence (5` - 3`)**	**Code**	**Sequence (5` - 3`)**
UBC-807	$(AG)_8T$	SCoT-01	CAACAATGGCTACCACCA
UBC-808	$(AG)_8C$	SCoT-02	CAACAATGGCTACCACCC
UBC-810	$(GA)_8T$	SCoT-16	ACCATGGCTACCACCGAC
UBC-811	$(GA)_8C$	SCoT-18	ACCATGGCTACCACCGCC
UBC-812	$(GA)_8A$	SCoT-22	AACCATGGCTACCACCAC
UBC-815	$(CT)_8G$	SCoT-28	CCATGGCTACCACCGCCA
UBC-826	$(AC)_8C$	SCoT-32	CCATGGCTACCACCGCAC
UBC-834	$(GA)_{10}T$	SCoT-34	ACCATGGCTACCACCGCA
UBC-840	$(GA)_8TT$	SCoT-35	CATGGCTACCACCGGCCC
UBC-846	$(CA)_8AT$	SCoT-36	GCAACAATGGCTACCACC

ISSR primers were based on The University of British Columbia and SCoT primers were based on Collard and Mackill (2009)

Analysis of Molecular Marker Data

Binary matrices were made of presence “1” and absence “0” of bands for ISSR and SCoT profiles. Only strong and clear bands were considered and were applied in the analysis. Polymorphic bands due to Cr treatment were scored in a comparison with the control in the two banana cultivars.

Results

To figure out the harmful effect of chromium on banana, *in vitro* screening experiment was performed. Proliferation medium supplemented with selective concentration (400 mg/l) of potassium chromate, along with free chromium medium as a control was used for screening. Two banana commercial cultivars i.e., Grand Nain and Williams-Zeef were used. The effect of chromium on both

banana cultivars was evaluated by measuring some morphological and physiological traits.

Morphological traits

Chromium treatment negatively influenced all morphological traits under study. Analysis of variance showed highly significant differences in all morphological traits between the two cultivars except for number of shoots per explant, and between control and treatment, as well as the interaction between cultivar and chromium concentrations except shoot length. The averaged fresh weight of Williams-Zeef under control condition was higher than that of Grand Nain. However, the fresh weight of Williams-Zeef was highly affected by chromium stress than that of Grand Nain. In this context, the percentage of reduction in fresh weight of Grand Nain was less (44.81%) than that of Williams-Zeef (78.32%), as shown in Table (2) and Fig. (1). Similar to fresh weight, the reduction in shoot length was higher in Williams-Zeef than that of Grand Nain. In this respect, percentage of reduction in shoot length was 36.48 and 53.13% in Grand Nain and Williams-Zeef, respectively (Table 2, Fig. 1).

Table 2. Effect of potassium chromate on morphological traits of two banana cultivars

Cultivar	Condition	Morphological traits		
		FW	SL	SN
Grand Nain	Control	6.36 ± 0.06^{B}	10.5 ± 0.25^{A}	6.00 ± 0.29^{A}
	Stress	3.51 ± 0.12^{C}	6.67 ± 0.17^{C}	1.83 ± 0.17^{C}
	% Reduction	44.81	36.48	69.50
Williams-Zeef	Control	10.61 ± 0.101^{A}	8.00 ± 0.14^{B}	5.00 ± 0.00^{B}
	Stress	2.30 ± 0.16^{D}	3.75 ± 0.14^{D}	2.67 ± 0.17^{D}
	% Reduction	78.32	53.13	46.60

Values represent mean ± SE, FW: fresh weight (g), SL: shoot length (cm), SN: number of shoots per explant. Different letters indicate significance within the same trait (Duncan's multiple range test, n=3 α=0.01)

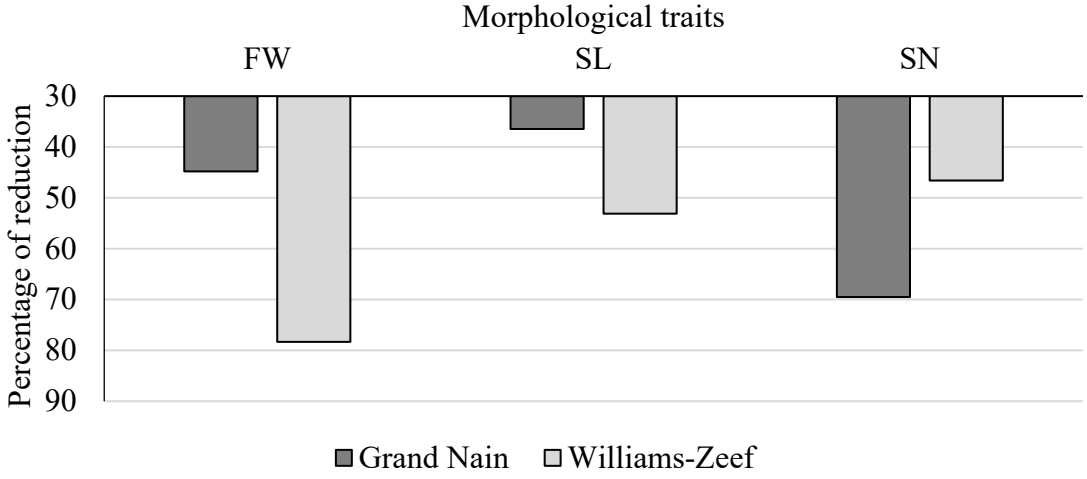

Fig. 1. Percentage of reduction due to different potassium chromate stress on morphological traits of two banana cultivars. FW: fresh weight (g), SL: shoot length (cm) and SN: number of shoots per explant

Both banana cultivars exposed significant reduction in the number of shoots per explant under chromium stress. However, unlike fresh weight and shoot length, the reduction in number of shoots per explant was higher in Grand Nain than that of Williams-Zeef. Moreover, the percentage of reduction in number of shoots per explant was 69.50 and 46.60% in Grand Nain and Williams-Zeef, respectively (Table 2, Fig. 1).

Physiological traits

The harmful effect of chromium was able to reduce the chlorophyll content in both banana cultivars in the present study. In this regard, total chlorophyll content under normal conditions was 1.70 and 2.06 mg/g FW in Grand Nain and Williams-Zeef, respectively. Meanwhile, under chromium stress the total chlorophyll content was minimized to 0.79 and 0.42 mg/g FW in both cultivars, with a reduction of 53.53 and 79.61%, respectively. Moreover, the chlorophyll components (*a* and *b*) were reduced unequally in the tested cultivars. Thus, the chlorophyll *a* reduction in Grand Nain (55.00%) was higher than that of chlorophyll *b* (48.98%), however both pigments were diminished equally in Williams-Zeef (79.43 and 79.69%, respectively), and in a higher percentage than that of the other cultivar. Chlorophyll contents and its reduction under chromium stress are displayed in Table (3) and Fig. (2).

Table 3. Effect of potassium chromate on physiological traits of two banana cultivars

Cultivar	Condition	Physiological traits			
		Ch*t*	Ch*a*	Ch*b*	Car
Grand Nain	Control	1.70±0.18^{A}	1.2±0.14^{A}	0.49±0.05^{A}	0.67±0.06^{A}
	Stress	0.79±0.12^{B}	0.54±0.10^{B}	0.25±0.02^{B}	0.31±0.06^{B}
	% Reduction	53.53	55.00	48.98	53.73
Williams-Zeef	Control	2.06±0.18^{A}	1.41±0.14^{A}	0.64±0.04^{A}	0.78±0.07^{A}
	Stress	0.42±0.09^{B}	0.29±0.06^{B}	0.13±0.03^{B}	0.18±0.05^{B}
	% Reduction	79.61	79.43	79.69	76.92

Values represent mean ± SE, Ch*t*: total chlorophyll, Ch*a*: chlorophyll *a*, Ch*b*: chlorophyll *b*, Car: carotenoids. Different letters indicate significance within the same trait (Duncan's multiple range test, n=3 α=0.01)

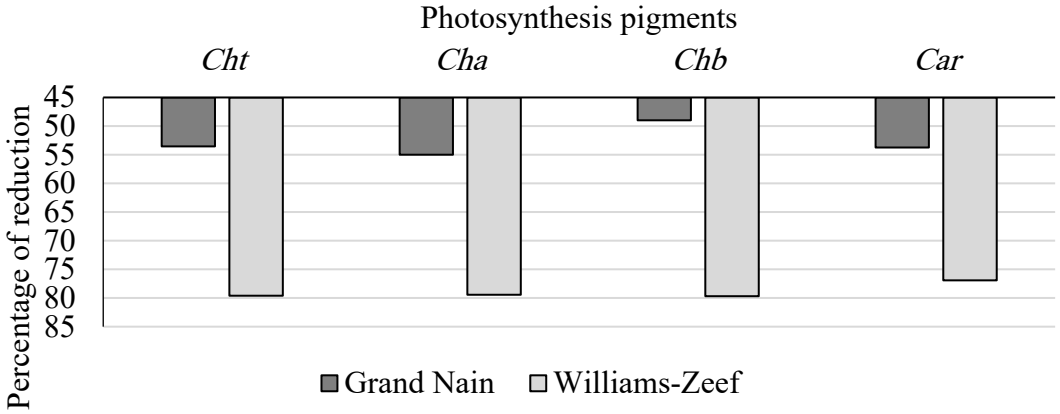

Fig. 2. Percentage of reduction due to different potassium chromate stress on physiological traits of two banana cultivars. Ch*t*: total chlorophyll content, Ch*a*: chlorophyll *a* content, Ch*b*: chlorophyll *b* content and Car: carotenoids content

Similar to chlorophyll, the carotenoid content was decreased in both cultivars due to the chromium effect, compared to control. Under untreated conditions, carotenoid contents were 0.67 and 0.78 mg/g FW. Treatment with potassium dichromate reduced the carotenoid content to 0.31 and 0.18 mg/g FW in both cultivars, by a 53.73 and 76.92% reduction, respectively (Table 3 and Fig. 2). Fig. (3) shows *in vitro* response of the two banana cultivars to 400 ppm chromium.

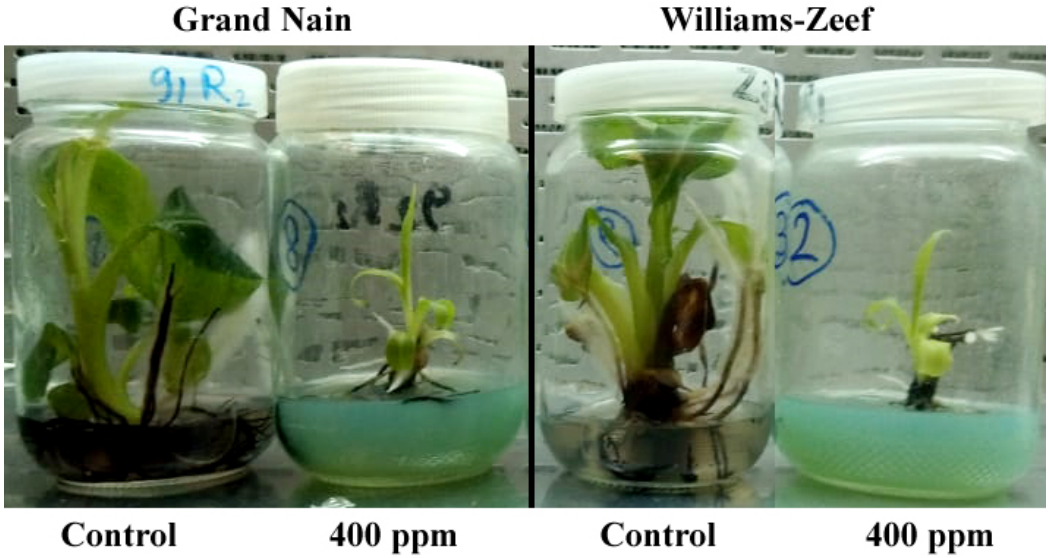

Fig. 3. *In vitro* performance of two banana cultivars, i.e., Grand Nain and Williams-Zeef under chromium treatment (400 ppm), compared with control.

Assessment of genetic variability under chromium stress by molecular Analysis

Two molecular markers were used in the current study to detect genetic variation – if any – due to the treatment of potassium dichromate. Both markers gave good patterns and exposed some polymorphic bands when DNA form treated, and untreated plants were compared.

ISSR marker

Out of ten tested primers of ISSR marker, seven primers were used for molecular analysis, based on band clearness. A total of 40 and 38 bands were generated by 7 ISR primers in Grand Nain and Williams-Zeef, respectively. The number of bands per primer was ranged from three to 7 bands. The two banana cultivars showed different ISSR pattern and different levels of polymorphism due to chromium treatment. In this regard, in Grand Nain cultivar and among 40 bands, only one band with a size of 1146bp was polymorphic. The specific band was generated by UBC-815 which was present only in plants treated with chromium. On the other hand, Williams-Zeef was more influenced by chromium treatment, resulted by showing three polymorphic bands. The three bands were generated by UBC-807, UBC-815 and UBC-826, of size 575, 1273 and 980 bp, respectively. The first band was present only in untreated sample while it was absent in the chromium-treated sample. The second and third bands (1273 and 980 bp) were

absent in untreated plant while they were present in treated plants (Table 4 and Figs. 4 and 5).

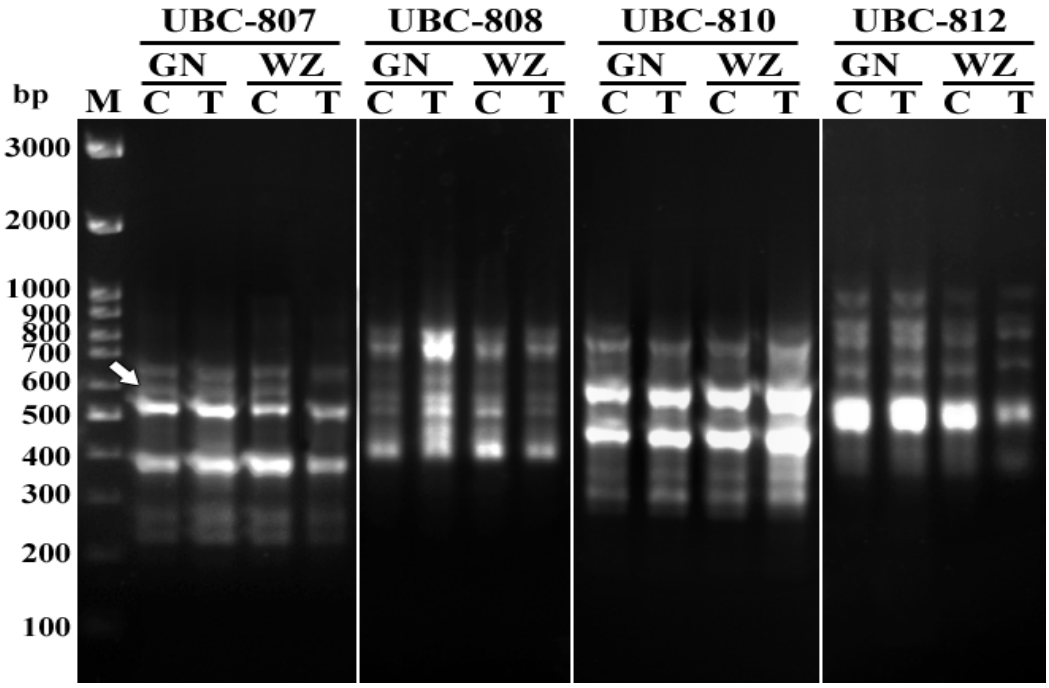

Fig. 4. ISSR profiles of two banana cultivars (GN: Grand Nain and WZ: Williams-Zeef) under control (C) and chromium treatment (T). Arrows indicate polymorphic bands.

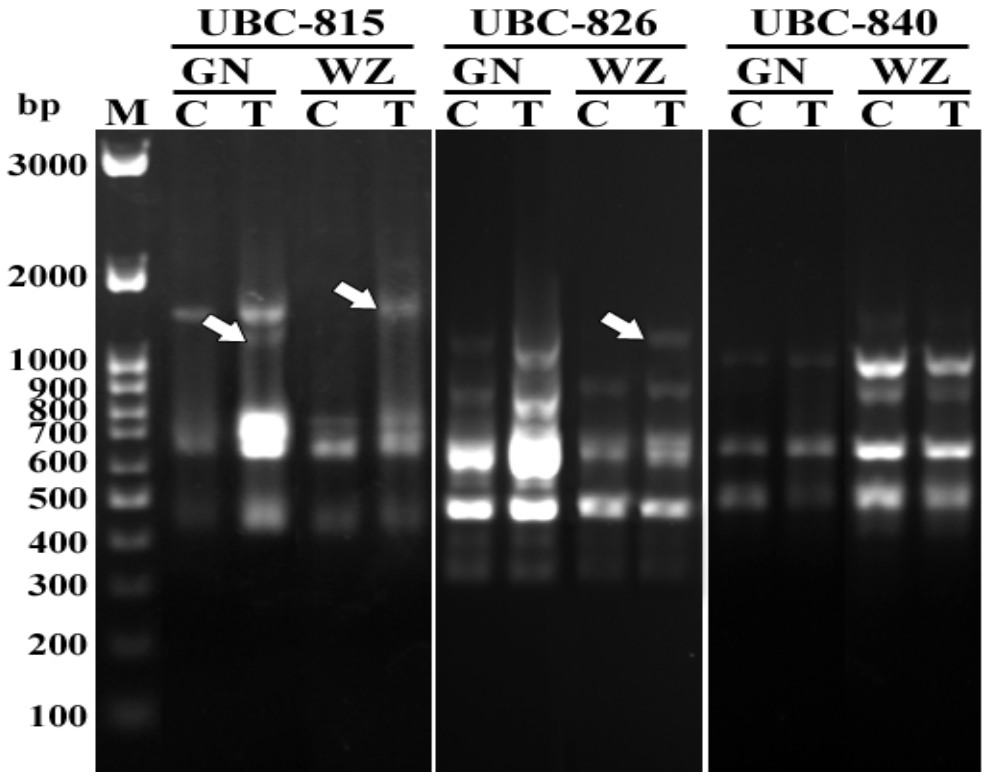

Fig. 5. ISSR profiles of two banana cultivars (GN: Grand Nain and WZ: Williams-Zeef) under control (C) and chromium treatment (T). Arrows indicate polymorphic bands.

Table 4. A survey of total number of bands, number of polymorphic bands and their size generated by ISSR primers in two banana cultivars treated with chromium stress

Primer	Grand Nain			Williams-Zeef		
	TNB	NPB	bp	TNB	NPB	bp
UBC-807	6	0	-	6	1	575
UBC-808	6	0	-	6	0	-
UBC-810	5	0	-	5	0	-
UBC-812	7	0	-	7	0	-
UBC-815	5	1	1146	4	1	1273
UBC-826	8	0	-	5	1	980
UBC-840	3	0	-	5	0	-
Total	40	1	-	38	3	-

Primer codes are related to Table (1), TNB: total number of bands, NPB: number of polymorphic bands

SCoT marker

Ten primers of SCoT marker were tested, out of them eight primers were used for molecular analysis, based on band clearness. The total number of bands generated by eight SCoT primers was 49 and 45 in Grand Nain and Williams-Zeef, respectively. The effeceincy of SCoT primers in detecting polymorphism was less than that shown by ISSR. In this regard, no polymorphic bands were detected in Grand Nain, while two polymorphic bands (502 and 830 bp) were detected in Williams-Zeef, generated by primer SCT-36. These bands were presented only in chromium treated plant (Table 5 and Figs. 6 and 7).

Table 5. A survey of total number of bands, number of polymorphic bands and their size generated by SCoT primers in two banana cultivars treated with chromium stress

Primer	Grand Nain			Williams-Zeef		
	TNB	NPB	Size (bp)	TNB	NPB	Size (bp)
SCT-01	3	0	-	3	0	-
SCT-16	5	0	-	5	0	-
SCT-18	6	0	-	6	0	-
SCT-22	5	0	-	5	0	-
SCT-28	5	0	-	5	0	-
SCT-34	11	0	-	7	0	-
SCT-35	7	0	-	7	0	-
SCT-36	7	0	-	7	2	830, 502
Total	49	0	-	45	2	-

Primer codes are related to Table (1), TNB: total number of bands, NPB: number of polymorphic bands

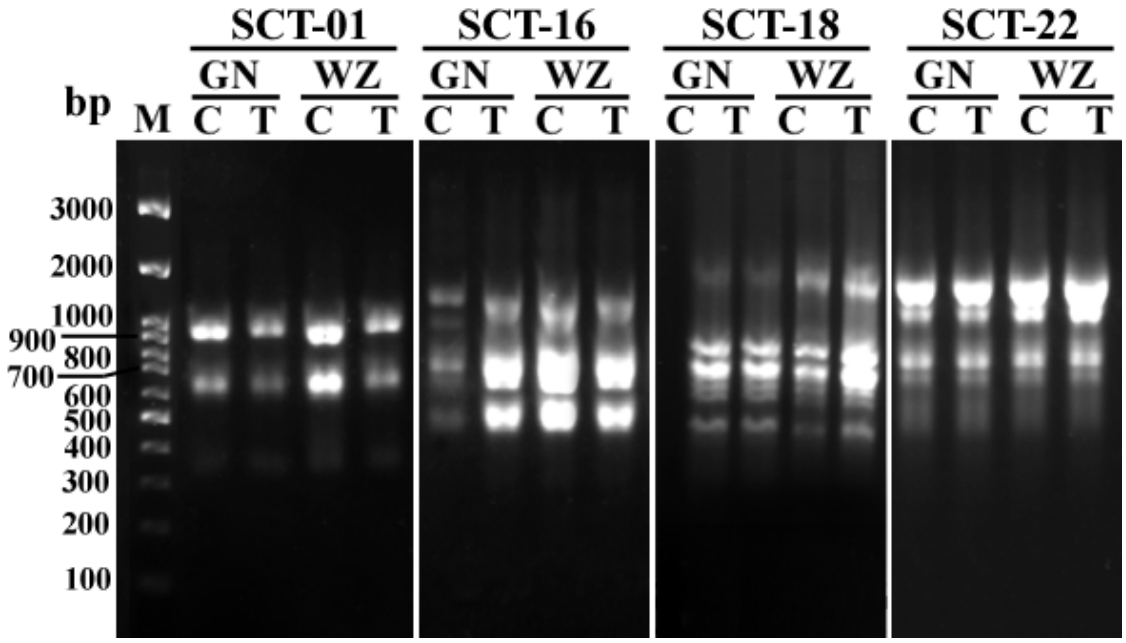

Fig. 6. SCoT profiles of two banana cultivars (GN: Grand Nain and WZ: Williams-Zeef) under control (C) and chromium treatment (T).

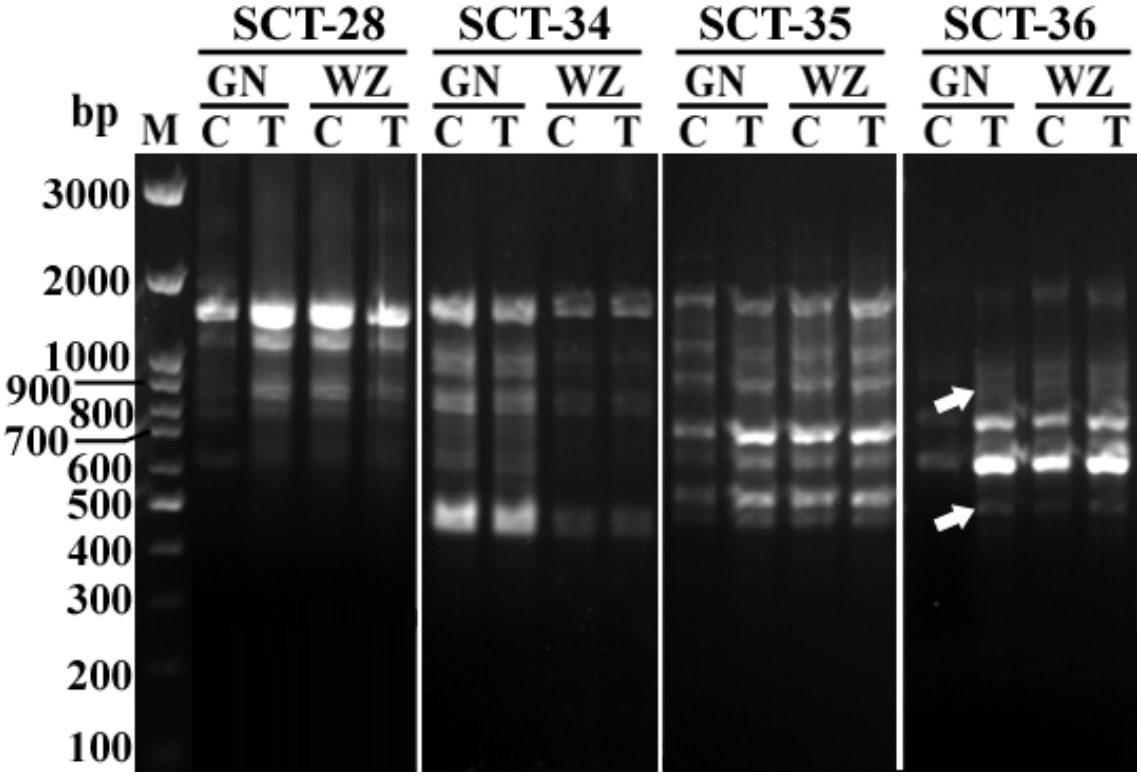

Fig. 7. SCoT profiles of two banana cultivars (GN: Grand Nain and WZ: Williams-Zeef) under control (C) and chromium treatment (T). Arrows indicate polymorphic bands.

Discussion

Several abiotic stresses faced banana production, such as drought, salinity, and toxicant metals. In this respect, heavy metal pollution is one of the most worrying environmental challenges faced by plant species. In the present study,

the toxic effect of chromium (Cr) in a form of chromium bisulfate was evaluated on *in vitro* performance of two commercial banana cultivars (i.e., 'Grand Nain' and 'Williams-Zeef', Cavendish group "AAA").

Chromium is a very toxic heavy metal and is placed as one of the top seven toxicants. Various industries and Cr mines are source of a huge amount of Cr, which is accumulating in the agricultural land, is significantly reducing the crop development, growth, and yield (Abdul Wakeel *et al*., 2020). There is a lack of research work on the effect of Cr on banana *in vitro* performance. According to the available literature, there is only one previous study focused on banana *in vitro* growth under Cr stress (Amalia *et al*., 2016). Increasing Cr concentration in plant cells caused the production of Reactive Oxygen Species (ROS) that would successively increase oxidative stress. Subsequently, the damage in cell membrane' structure and function, DNA damage, gene mutations, protein oxidation, lipid peroxidation and cell death can occur (Panda and Choudhury, 2005; Hossain *et al*., 2012).

In the present study, the results clearly showed the harmful effects of chromium on banana *in vitro* growth at morphological, physiological, and molecular levels. The results are matched with the previous findings of Amalia *et al*., (2016). They reported that, the growth rate of banana plantlets decreased by higher concentrations (200 and 400 ppm) of Cr. Also, they found that the Cr treatment increased the level of CAT and APX gene expression, compared with the control. Chromium uptake can be inhibited with important metabolic processes, such as photosynthesis and respiration (Amalia *et al*., 2016). In this regard, the photosynthesis related pigments (chlorophyll and carotenoid) were decreased significantly in the present study due to *in vitro* Cr treatment.

Results of the current study showed that Grand Nain cultivar was more tolerant than Williams-Zeef when they *in vitro* treated with Cr. However, contrary findings have been reported for the two cultivars in their tolerance to copper and lead toxicity (Pisam *et al*., 2020). This finding demonstrates that the same genotype may differ in its response according to the stress it faces.

Molecular analysis using simple and efficient tools such as ISSR and SCoT offers a good understanding and explaining for morphological and physiological observations. The two markers utilized in the current study (ISSR and/or SCoT) have been used previously in several studies, e.g., evaluation of genetic diversity (Youssef and Ibrahim, 2015; Khalil *et al*., 2020), abiotic stress screening (Abouzaid *et al*., 2016; El-Mahdy *et al*., 2021; El-Mahdy *et al*., 2022), mutation detection (Al-Qurainy, 2010) and assessment of heavy metals toxicity (Pisam *et al*., 2020). In the present study, ISSR and SCoT exposed their efficiency by confirming the observations at morphology and physiology levels. Polymorphism detected by ISSR/SCoT due to chromium treatment in this study proved the toxicity and mutagenicity of the chromium on both banana cultivars. However, ISSR was more powerful than SCoT in exposing variability between treated and untreated plants by showing more polymorphic bands. This difference between the two molecular markers might be due to the difference in their basis and regions they target in the genome. The results of the current study agreed with the previous

researches who reported the efficiency of ISSR in detecting DNA damages due to toxic metals and other mutagenic causes in many plant species e.g., , *Eruca sativa* (Al-Qurainy, 2010), *Viola tricolor* (Słomka *et al*., 2011), *Solanum nigrum* (Al Khateeb and Al-Qwasemeh, 2014), *Sphagnum palustre* (Sorrentino *et al*., 2017), *Musa acuminata* (Pisam *et al*., 2020; El-Mahdy *et al*., 2021) and *Punica granatum* (El-Mahdy *et al*., 2022).

In conclusion, the protocol of chromium tolerance screening used in the present study was efficient to discriminate between the two banana cultivars. Grand Nain showed more tolerance than Williams-Zeef by showing less reduction in the evaluated traits under Cr treatment. Both morphological and physiological traits evaluated in this study were significantly affected by higher concentrations of Cr. Additionally, molecular analysis using ISSR, and SCoT confirmed the morphophysiological findings. Moreover, ISSR was more powerful than SCoT in differentiation between treated plants and those under control. Results of this study are valuable and could be help in successive screening studies in banana and other species.

References

Abdul Wakeel, Xu M., and Gan Y. (2020). Chromium- Induced Reactive Oxygen Species Accumulation by Altering the Enzymatic Antioxidant System and Associated Cytotoxic, Genotoxic, Ultrastructural, and Photosynthetic Changes in Plants. Int. J. Mol. Sci. 21, 728.

Abouzaid E., El-Sayed E.N., Mohamed E.A., Youssef M. (2016). Molecular analysis of drought tolerance in guava based on *in vitro* PEG evaluation. Trop Plant Biol 9:73–81. https://doi.org/10.1007/s12042- 016- 9164-5

Al Khateeb, W., and Al-Qwasemeh, H. (2014). Cadmium, copper and zinc toxicity effects on growth, proline content and genetic stability of *Solanum nigrum* L., a crop wild relative for tomato; comparative study. Physiology and Molecular Biology of Plants, 20(1): 31-39.

Al-Qurainy F. (2010). Application of inter simple sequence repeat (ISSR marker) to detect genotoxic effect of heavy metals on *Eruca sativa* (L.) Afr J Biotechnol 9(4): 467–474. https:// doi. org/ 10. 4314/ AJB.V9I4

Amalia, L., and Widiyanto, S.N.B. (2016). Catalase (CAT) and ascorbate peroxidase (APX) genes expression level in growth of banana plantlets (*Musa acuminata*) cv. ambon lumut under chromium stress condition. Journal of Plant Sciences, 11(4): 69-74.

Bhalerao, S.A. and Sharma, A.S. (2015). Chromium: As an environmental pollutant. Int. J. Curr. Microbiol. Appl. Sci., 4: 732–746.

Collard, B.C., and Mackill, D.J. (2009). Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant molecular biology reporter, 27(1): 86-93.

Diwan, H., Khan, I., Ahmed, A. and Iqbal, M. (2010). Induction of phytochelations and Antioxidant Defense System in *Brassica iuncea* and *Vigna radiate* in Response to Chromium Treatment. Plant Growth Regul. 61: 97- 107.

Edward, E. and Fredy B. (2012). Banana Market. Florida: University of Florida/IFAS extension.

El-Mahdy, M.T., and Youssef, M. (2019). Genetic homogeneity and high shoot proliferation in banana (*Musa acuminata* Colla) by altering medium thiamine level

and sugar type. *In Vitro* Cellular and Developmental Biology-Plant, 55(6): 668-677.

El-Mahdy, M.T., Youssef, M., and Elazab, D.S. (2022). *In vitro* screening for salinity tolerance in pomegranate (*Punica granatum* L.) by morphological and molecular characterization. Acta Physiologiae Plantarum, 44(2): 1-11.

El-Mahdy, M.T., D.A. Abdel-Wahab and M. Youssef (2021). *In vitro* morpho-physiological performance and DNA stability of banana under cadmium and drought stresses, *In Vitro* Cellular and Developmental Biology - Plant (2021) 57:460–469.

FAOSTAT Statistical Database (2019). Food and Agriculture Organization of the United Nations [FAO]. 2019. Retrieved from http://www.fao.org/faostat/en/

Fineli, K. (2007). Banana without peel, (Finnish National Public Health Institute) Available online. http://www.fineli.fi/food.php?foodid=11049andlang=en

Gaafar, R.M. and Saker, M.M. (2006). Monitoring of cultivars identity and genetic stability in strawberry varieties grown in Egypt. World Journal Agricultural Science, 2 (1): 29-36.

Gupta M., Chyi Y-S., Romero-Severson J., Owen J.L. (1994). Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. Theor Appl Genet 89: 998—1006

Hasan, A.S., Khasim, S.M. and Ramudu, J. (2020). Development of standard protocols for *in vitro* regeneration of some selected banana cultivars (*Musa* spp.) from India. In Medicinal Plants: Biodiversity, Sustainable Utilization and Conservation; Khasim, S.M., Long, C., Thammasiri, K., Lutken, H., Eds.; Springer Nature: Singapore, Volume 45, pp. 743–759.

Hossain, M.A., Piyatida, P., da Silva, J.A.T. and Fujita, M. (2012). Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. Journal of Botany. 2012.

Hossain, M.A., Hossain, M.Z. and Fujita, M. (2009). Stress-induced changes of methylglyoxal level and glyoxalase I activity in pumpkin seedlings and cDNA cloning of glyoxalase I gene, Australian Journal of Crop Science. 3 (2) 53–64.

Khalil O.A., Ibrahim R.A., Youssef M. (2020). A comparative assessment of phenotypic and molecular diversity in Doum (*Hyphaene thebaica* L.) Mol Biol Rep. 47:275–284. https:// doi. org/ 10. 1007/s11033- 019- 05130-w

Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In Methods in enzymology (Vol. 148, pp. 350-382) Academic Press.

Murashige T. and Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473–497

Nansamba, M., Sibiya, J., Tumuhimbise, R., Karamura, D., Kubiriba, J., and Karamura, E. (2020). Breeding banana (*Musa* spp.) for drought tolerance: A review. Plant Breeding, 139(4): 685-696.

Nissen, O. (1984). MSTAT. A microcomputer program for statistical analyses of experiments and surveys. In 10. General Meeting of the European Grassland Federation, Aas (Norway), 26-30 Jun 1984. Norwegian State Agricultural Research Stations.

Panda, S.K. and Choudhury, S. (2005). Chromium stress in plants. Brazil J. Plant Physiol., 17: 95-102.

Pisam, W.M., Abdel Aal, A.H., Khodair, O.A., and Youssef, M. (2020). *In vitro* performance of two banana cultivars under copper stress. Assiut J. Agric. Sci, 51: 143-153.

Rai, P. K., Lee, S.S., Zhang, M., Tsang, Y.F. and Kim, K.H. (2019). Heavy metals in food crops: health risks, fate, mechanisms, and management. Environ. Int. 125: 365–385.

Raza, A., Habib, M., Charagh, S. and Kakavand, S.N. (2021). Genetic engineering of plants to tolerate toxic metals and metalloids. in Handbook of Bioremediation, eds M. Hasanuzzaman and M. N. V. Prasad (Cambridge, MA: Academic Press), 411–436.

Roorkiwal, M., Bharadwaj, C., Barmukh, R., Dixit, G.P., Thudi, M. and Gaur, P.M. (2020). Integrating genomics for chickpea improvement: achievements and opportunities. Theor. Appl. Genet. 133: 1703–1720.

Słomka, A., Sutkowska, A., Szczepaniak, M., Malec, P., Mitka, J., and Kuta, E. (2011). Increased genetic diversity of *Viola tricolor* L. (Violaceae) in metal-polluted environments. Chemosphere, 83(4): 435-442.

Soniya, E.V., Banerjee, N.S. and Das, M.R. (2001). Genetic analysis of somaclonal variation among callus-derived plants of tomato. Current Science, 80: 1213-1215.

Sorrentino, M.C., Capozzi, F., Giordano, S., and Spagnuolo, V. (2017). Genotoxic effect of Pb and Cd on *in vitro* cultures of Sphagnum palustre: An evaluation by ISSR markers. Chemosphere, 181: 208-215.

Varshney, R. K., Bohra A., Yu J., Graner A., Zhang Q. and Sorrells M.E. (2021). Designing future crops: genomics-assisted breeding comes of age. Trends Plant Sci. 26: 631–649.

Youssef M., Ibrahim R.A. (2015). Molecular markers associated with high vitamin-c content in guava. J. Agric. Chem. and Biotechn., Mansoura Univ. 7(3): 49 – 55.

Youssef M., Valdez-Ojeda R., Ku-Cauich J.R., Escobedo-Gracia Medrano R.M. (2015). Enhanced protocol for isolation of plant genomic DNA. J Agri Environ Sci 4(2):172–180.

تقييم التأثيرات الضارة للكروميوم على أداء نباتات الموز معملياً

كريمة السيد عبد الحفيظ مرسي[1]، جلال أحمد رزق الشربينى[1]، محمد أحمد الملقب بالخرشي محمد يوسف[2]

[1] قسم الوراثة – كلية الزراعة – جامعة سوهاج

[2] قسم الوراثة – كلية الزراعة – جامعة أسيوط

الملخص

الاجهادات البيئية لها تأثير سلبي كبير على نمو النبات وإنتاجيته. ومن بين هذه الاجهادات البيئية، تُعتبر سمية المعادن الثقيلة من أكثر العوامل التي تؤثر على نمو النبات واستصلاح الأراضي. في هذه الدراسة، تم تقييم التأثير الضار للكروم على نباتات الموز تحت ظروف زراعة الأنسجة في صنفين تجاريين (جراند ناين وويليامز-زيف) بناءً على التقييمات المورفولوجية والفسيولوجية والجزيئية. أظهرت النتائج أن الكروم قلل معنوياً جميع الصفات المدروسة، بما في ذلك الوزن الطازج للنبات، وطول النبات، وعدد الأفرع لكل نبات، والأصباغ المتعلقة بالتمثيل الضوئي (محتوى الكلوروفيل والكاروتينويد). حيث تراوحت نسبة الاختزال بسبب معاملة الكروم من 36.48 إلى 79.69٪ لصفة عدد الأفرع لكل نبات لصنف جراند اين وصفة الكلوروفيل ب في ويليامز-زيف، على التوالي. كما تأثر كلا الصنفين سلبيا بمعاملة الكروم. حيث أظهر الصنف ويليامز-زيف انخفاضًا أعلى من جراند ناين في جميع الصفات المدروسة. ومن ناحية أخرى، تم إجراء التحليل الجزيئي لاكتشاف أي تباين بين النباتات المعاملة بالكروم والنباتات غير المعاملة باستخدام الواسمات الجزيئية (ISSR) و (SCoT). أكدت نتائج التحليل الجزيئي النتائج المتحصل عليها على المستوى المورفولجي والفسيولوجي، وذلك من خلال الكشف عن بعض الحزم متعددة الأشكال الناتجة عن معاملة الكروم. في هذا الصدد، تم اكتشاف عدد ستة حزم متعددة الأشكال في صنفي الموز، مما يميز النباتات المعاملة عن الكنترول. بالاتفاق مع النتائج المورفوفسيولوجية، أظهر الصنف ويليامز-زيف تعدد أشكال (خمسة حزم) بسبب معاملة الكروم أكثر من جراند ناين (حزمة واحدة). كان بروتوكول الفحص المستخدم في هذه الدراسة فعالًا ومفيدًا ويمكن استخدامه في دراسات متتالية لتقييم المواد السامة الأخرى مع الموز ومع الأنواع النباتية الأخرى أيضًا.