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Establishment of an in vitro Protocol for Chromium Toxicity Tolerance Screening in Banana

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Abstract

Several factors negatively affecting plant growth and production. Abiotic stresses play a vital role against many plant species. Among various abiotic stresses, heavy metals provide one of the most harmful effects on plant performance. Thus, this study was designed to establish an efficient *in vitro* protocol for chromium (Cr) toxicity tolerance screening in banana. One of the most important commercial banana cultivars (i.e., Grand Nain) was used. Five different concentrations of potassium dichromate (i.e., 50, 100, 200, 400 and 600 ppm) were tested along with free chromium medium as a control. Analysis of variance showed highly significant differences among Cr concentrations, affecting shoot length and number of shoots per explants, compared to control. In addition, results clearly showed the harmful effects of chromium on both shoot length and number of shoots per explant. In this regard, the percentage of reduction in shoot length due to Cr treatment ranged from 5.77 to 84.09% regarding treatment with 50 and 600ppm, respectively. Moreover, the number of shoots per explant was decreased by a range of 6.00 to 86.48% caused by treatment of 50 and 600ppm, respectively. According to these results and the analysis of variance, it is recommended to use the concentration of 400ppm potassium dichromate for in vitro screening of chromium tolerance in banana. The established protocol is efficient and of great importance and could be used for selecting chromium-tolerant genotypes in banana and other species.

Keywords:
Musa, Potassium dichromate, in vitro selection, protocol

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. 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 (Hasan et al., 2020). 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). Several abiotic stresses stand against banana production, among which toxicity of heavy metals plays vital role in reducing banana growth and production. 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. Banana plants are commercially propagated through the tissue culture technique (Dagnew, 2012) which can provide mass propagation, rejuvenation of older varieties, disease elimination, conservation of genetic resources, and the management of abiotic and biotic stresses (Mahmoud et al, 2020). The objectives of the present study were to establish an *in vitro* screening protocol for chromium tolerance in banana and to evaluate the performance banana commercial cultivar under chromium *in vitro* stress condition based on morphological assessment.

MATERIALS AND METHODS

Plant materials

In vitro regenerated plantlets of banana commercial cultivar (i.e., Grand Nain, Musa acuminata Colla, subgroup Cavendish, AAA) was obtained from the private Zamzam Tissue Culture Laboratory, Cairo, Egypt.

Culture media preparation

In vitro regenerated shoots of 'Grand Nain' banana cultivar 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 1.5 IP/b2 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**

In order to determine the optimum concentration of chromium (Cr) for banana tolerance screening, an 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.

***In vitro* evaluation of chromium tolerance**

After 5 weeks, number of shoots per explant (SN) and shoot length (SL, cm) were recorded.

Experimental design and data analysis

A complete randomized design was used to perform the experiment. Three replicates with 6 jars each were used per treatment, six concentrations of Cr were used in the preliminary experiment. A total of 108 experimental unit were used, (1 cultivar × 6 concentrations × 3 replicates × 6 jars). Analysis of variance was performed utilizing MSTAT-C significant program (**Nissen, 1984**). Means were compared using Duncan's multiple range test at 1% probability level.

RESULTS AND DISCUSSION

In the present study, the toxic effect of chromium (Cr) in a form of chromium bisulfate was evaluated on *in vitro* performance of a commercial banana cultivar (i.e., 'Grand Nain', Cavendish group "AAA"). The experiment was done to establish an efficient protocol for chromium tolerance screening in banana. Banana proliferation medium supplemented with six different concentrations of potassium dichromate was used for tolerance screening.

Shoot length (cm)

Chromium treatments showed harmful effects on shoot length reflected by significant reduction, which was parallelly matched with chromium concentrations. In this regard, the average of shoot length in untreated plants was 10.58 cm, while it was significantly reduced in the treated plants to the range of 9.97 to 6.19 cm belong to 50 and 400 mg/l potassium dichromate, respectively. Moreover, shoot length reached 1.68 cm when plants treated with 600 mg/l potassium dichromate. Table (1) and Figure (1) showed the averaged shoot length in control and treated plants of Grand Nain banana cultivar. In the same context, the percentage of reduction in the shoot length ranged from 5.77 to 41.47% due to treatment with 50 and 400 mg/l potassium dichromate, respectively (Table 1). Meanwhile, the highest concentration (600 mg/l) of potassium dichromate was enough to reduce shoot length by 84.09% compared with control. Furthermore, analysis of variance showed highly significant ($p < 0.01$) differences among potassium dichromate concentrations affecting shoot length (Table 2).

Number of shoots per explant

The effect of chromium treatment on number of shoots per explant was slightly different of that on shoot length (Table 1, Fig. 2). In this regard, the lowest concentration of potassium dichromate gave almost the same number of shoots per explants compared with the control (4.33 and 4.61, respectively). However, the higher concentrations of potassium dichromate reduced the number of shoots per explant significantly, compared to untreated plants. Thus, SN ranged from 2.11 to 1.44 by treatment with 100 and 400 mg/l, while it was minimized to 0.62 due to 600 mg/l treatment. These numbers were reflected in the percentage of reduction due to potassium dichromate treatment. The initial concentration (50 mg/l) of potassium dichromate caused only 6.00% reduction, compared to control. However, the higher concentrations were harmful enough to give reduction in number of shoots per explant ranged from 54.22 to 68.67% due to treatment with 100 and 400 mg/l potassium dichromate. Furthermore, the highest concentration of potassium dichromate (600 mg/l) extremely affected the number of shoots per explants and showed 86.48% of reduction, compared to untreated plants of Grand Nain cultivar (Table 1). Analysis of variance showed highly significant ($p < 0.01$) differences among potassium dichromate concentrations affecting number of shoots per explant (Table 2). The results clearly showed the harmful effects of chromium on banana *in vitro* growth at morphological level. In this respect, all morphological traits were affected and decreased significantly due to Cr treatment especially at its higher concentrations. The results are matched with the previous findings of Amalia et al., (2016). They reported that, the growth rate of banana plantlets decreased with increasing Cr concentrations in the growth media, especially at 200 and 400 ppm. Also, they found that, the level of CAT and APX gene expression in plantlets under Cr stress condition were higher than the

control. Gene expression level in the roots is higher than the shoots. Chromium uptake into the plant body can interfere with important metabolic processes, such as photosynthesis and respiration (Amalia et al., 2016).

Results gave helpful information about the harmful effect of potassium dichromate on vegetative traits of Grand Nain banana cultivar. Based on the above-mentioned results, the concentrations of 50, 100 and 200 mg/l showed low to moderate reduction on the studied traits. While, the concentration 600 mg/l was extremely harmful on banana plants, which sometimes caused complete plant death. Therefore, the concentration 400 mg/l potassium dichromate was chosen as a selectable level for chromium tolerance screening. Figure (3) shows the percentage of reduction occurred in shoot length and number of shoots per explant due to treatment of Cr different concentrations. The performance of Grand Nain cultivar under different concentrations of Cr is shown in Figure (4).

Chromium (Cr) is one of the top seven toxic heavy metals. It is ranked 17th among the abundantly found metals in the earth's crust. A huge amount of Cr releases from various industries and Cr mines, 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 chromium on banana *in vitro* performance. According to the available literature, there is only one previous study focused on banana in vitro growth under chromium stress (Amalia et al., 2016).

Exposure of plants to different levels of heavy metals toxicity caused a wide range of physiological and metabolic alterations (Villiers, 2011). The most widespread visual evidence of heavy metal toxicity is a reduction in plant growth (Sharma and Dubey, 2007; Pisam et al., 2020; El Mahdy et al., 2021) including leaf chlorosis, necrosis, turgor loss, a decrease in the rate of seed germination, and a crippled photosynthetic apparatus, often correlated with progressing senescence processes or with plant death (Carrier, 2003). All these effects are related to ultrastructural, biochemical, and molecular changes in plant tissues and cells brought about by the presence of heavy metals (Gamalero, 2009; El-Mahdy et al., 2022).

Table (1): Effect of different concentrations of potassium chromate on shoot length and number of shoots per explant of Grand Nain banana cultivar

Cr levels (mg/l)	Shoot length (cm)		Number of shoots per explant	
	Trait	% Reduction	Trait	% Reduction
Control	10.58±0.05^{A}	-	4.61±0.45^{A}	-
50	9.97±0.07^{B}	5.77	4.33±0.51^{A}	6.00
100	9.14±0.15^{C}	13.65	2.11±0.36^{B}	54.22
200	7.39±0.07^{D}	30.18	1.5±0.42^{BC}	67.47
400	6.19±0.10^{E}	41.47	1.44±0.29^{BC}	68.67
600	1.68±0.09^{F}	84.09	0.62±0.02^{C}	86.48

Values represent means, SE, different letters within the same trait indicate significant differences (Duncan's multiple range test, n=3, □ = 0.01)

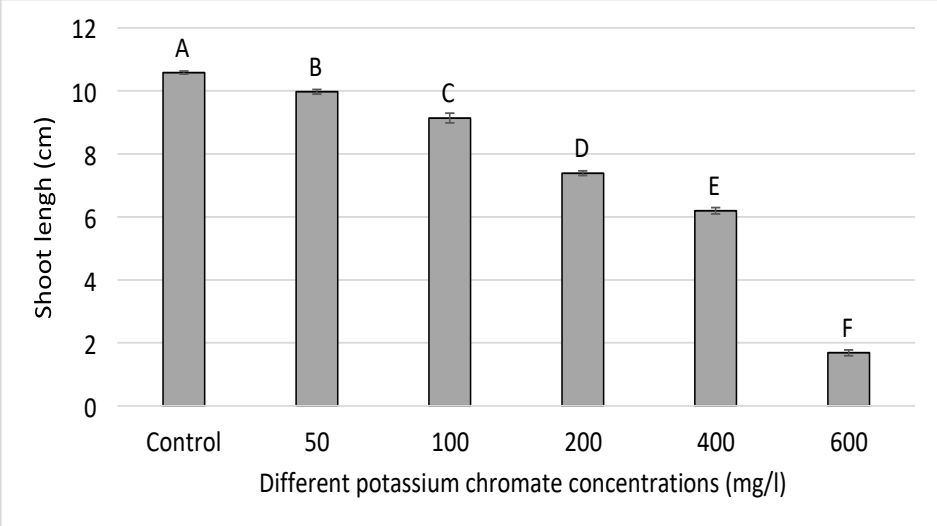

Figure (1): Effect of different concentrations of potassium chromate on shoot length of Grand Nain banana cultivar. Different letters indicate significant differences (Duncan's multiple range test, α = 0.01, bars indicate standard error, n=3)

Table (2): Analysis of variance of shoot length (SL) and number of shoots per explant (SN) in Grand Nain banana cultivar under different concentrations of potassium dichromate.

Trait	Source of variance	df	SS	MS	F	Prob.
SL	Cr concentrations	5	161.52	32.30	1157.604	0.0000
	Error	12	0.34	0.03		
	Total	17	161.85			
SN	Cr concentrations	5	40.739	8.148	18.92	0.0000
	Error	12	5.17	0.43		
	Total	17	45.91			

SL: shoot length, SN: number of shoots per explant, df: degrees of freedom, SS: sum of squares, MS: mean sum of squares, F: Fisher's value and Prob.: probability.

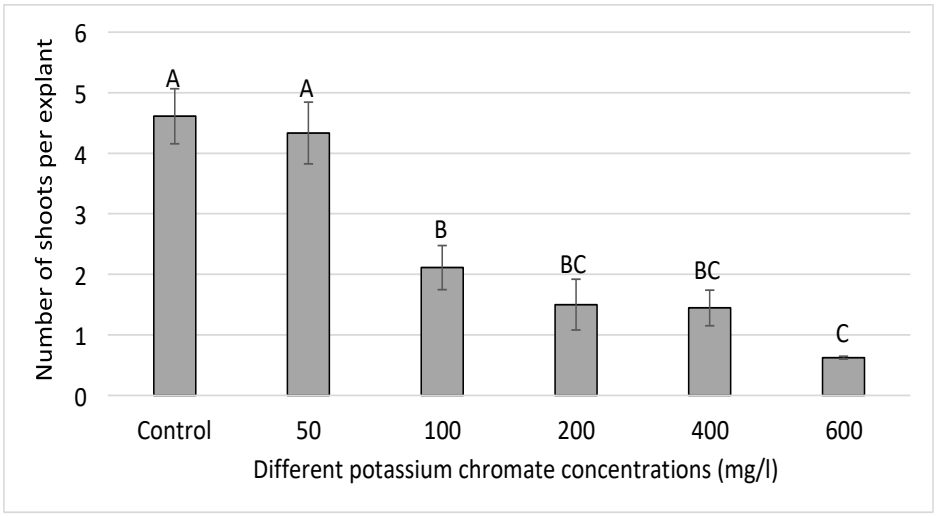

Figure (2): Effect of different concentrations of potassium chromate on number of shoots per explant of Grand Nain banana cultivar. Different letters indicate significant differences (Duncan's multiple range test, α = 0.01, bars indicate standard error, n=3)

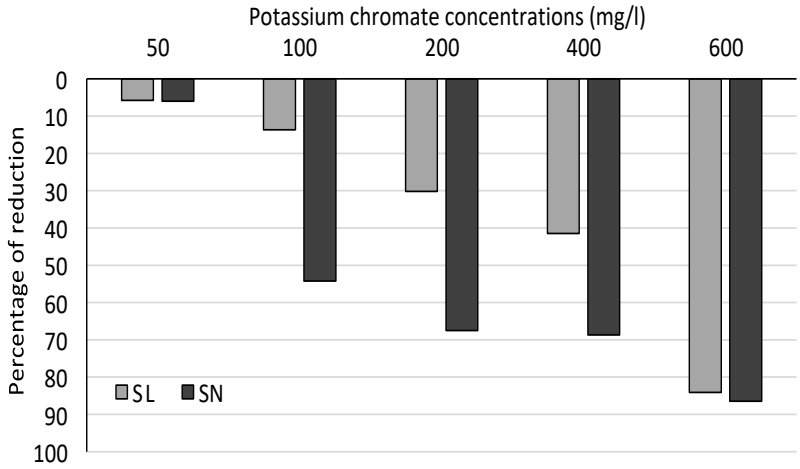

Figure (3): Percentage of reduction due to different concentrations of potassium chromate on shoot length and number of shoots per explant of Grand Nain banana cultivar

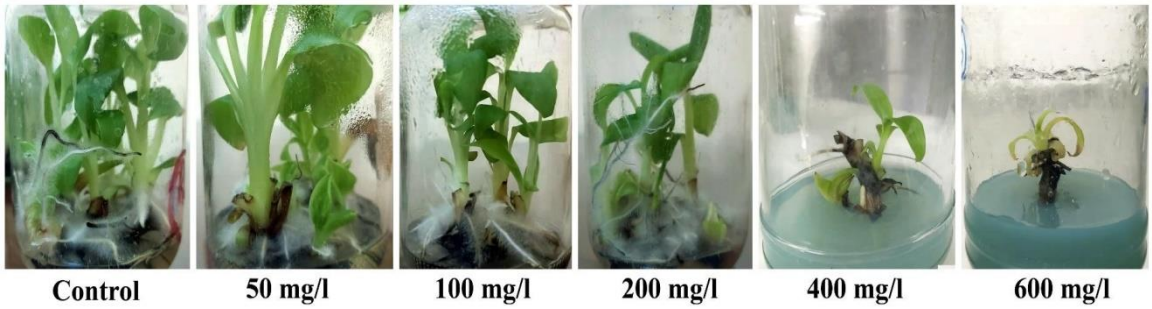

Figure (4): Growth performance of Grand Nain plant under different Concentrations of chromium.

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تأسيس بروتوكول لكشف تحمل الموز لسمية الكروم تحت ظروف زراعة الأنسجة

هناك العديد من العوامل التي تؤثر سلباً على نمو وإنتاجية النباتات. وللاجهاد البيئي دوراً أساسياً يؤثر سلباً لمعظم النباتات. ومن بين الاجهادات البيئية العديدة، تعتبر العناصر الثقيلة مصدراً هاماً للتأثيرات السلبية على آداء النباتات. للك، كان الهدف من هذه الدراسة هو تأسيس بروتوكول فعال معتمداً على زراعة الأنسجة لبيان سمية الكروم على الموز. وتم استخدام أحد أصناف الموز التجارية الهامة (جراند ناين) في هذه الدراسة. تم اختبار خمسة تركيزات من ثنائي كرومات البوتاسيوم (50 و 100 و 200 و 400 و 600 جزء في المليون) بالاضافة الى بيئة غذائية خالية من الكروم ككنترول. أوضح تحليل التباين وجود فروق معنوية جداً بين تركيزات الكروم وتأثيرها على صفتي طول الفرع وعدد الأفرع لكل جزء نباتي، مقارنة بالكنترول. كما أوضحت النتائج جلياً التأثيرات الضارة للكروم على كلتا الصفتين محل الدراسة. وتراوحت نسبة الاختزال في صفة طول الفرع من 5.77 إلى 84.09% نتيجة المعاملة بـ 50 و 600 جزء في المليون من ثنائي كرومات البوتاسيوم، على التوالي. بالاضافة لذلك، حدث نقص في عدد الأفرع لكل جزء نباتي بمدى يتراوح من 6.00 إلى 86.48% بسبب المعاملة بـ 50 و 600 جزء في المليون من ثنائي كرومات البوتاسيوم، على التوالي. وبناء على هذه النتائج وتحليل التباين، يوصى باستخدام التركيز 400 جزء في المليون لفحص النباتات وبيان درجة التحمل للكروم في الموز. يعتبر البروتوكول الذي تم تأسيسه في هذه الدراسة، فعال وذو أهمية عالية، حيث يمكن تطبيقه في الانتخاب للتراكيب الوراثية المتحملة للكروم في الموز وبعض الأنواع النباتية الأخرى.