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# Genetic Evaluation of Rice (*Oryza sativa* L.) Genotypes at Seedling Stage for Their Tolerance to Lead

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***Authors' contributions***

*All authors designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

Twelve Egyptian Rice (*Oryza sativa* L.) genotypes have been used in Randomized Complete Block Design (RCBD) experiment with three replicates to study their tolerance to lead at seedling stage Those genotypes were: Giza 177, Giza 178, Giza 179, Giza 181, Giza 182, Sakha 101, Sakha 102, Sakha 103, Sakha 104, Sakha 105, Sakha 106 and Egyptian Yasmin. Final Germination Percentage (FGP), Germination Index (GI), Root Length (RL), Shoot Length and Root/Shoot Ratio (R/S %) were estimated under different concentrations of lead. Results indicated that there were highly significant differences between studied genotypes and treatments. Mean performance for studied characters was discussed.

*Keywords: Rice; final germination percentage; germination index; root length; shoot length; root/shoot ratio; lead.*

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## 1. INTRODUCTION

Rice (*Oryza sativa* L.) is considered as one of the most important cereal crops not only in Egypt but also all over the world. In Egypt, the yearly area cultivated annually by rice is almost more than one-fifth of the total area. According to the statistics of the Ministry of Agriculture in 2003, the total cultivated area of rice was about 1,507,000 feddan, which produced 6,174,000 tons of paddy rice with an average of 4,095 t/fed. [1]. Rice is a cereal foodstuff that forms an important part of the diet of many people worldwide and as such, it is a staple food for many. It is also known that people, especially those who take rice as staple food for daily energy, are inevitably exposed to significant amounts of heavy metals because fertilizers that are used in farm, had amounts of heavy metals [2].

Heavy metals species are trace elements, because of their toxicity can have a serious impact if released into environment as a result of bioaccumulation and they may be extremely toxic even in trace quantities [3,4]. They enter the environment from natural and anthropogenic sources. The fate of heavy metals in soils would be controlled by a complex set of physical, chemical and biological reactions acting within the soil and also their mobility largely depend on the form of heavy metals and their sources [5].

Lead (Pb) naturally occurs in uncontaminated soils are generally in the range of 20 to 50 mg $kg^{-1}$ [6]. In industrialized areas, Pb up to 1000 mg $kg^{-1}$ or above has been recorded [7]. Although it is not an essential element for plants, it gets easily absorbed and accumulates in different parts of plants, causes anatomical changes by binding with essential enzymes and cellular components and inactivates them in primary leaves and decreases the number of epidermal cells/mm and growth parameters [8]. Toxicity of Pb in plants causes a number of toxicity symptoms as stunted growth, chlorosis, and blackening of root system and inhibits photosynthesis, upsets mineral nutrition and water balance, changes hormonal status and affects membrane structure and permeability [9].

Heavy metals can enter the food chain from aquatic and agricultural ecosystems and threaten human health indirectly. These metals contaminate food source and accumulate in both agricultural products and seafood through water, air and soil pollution. Some of these elements are toxic to humans even at considerably low concentrations. Especially, toxic trace heavy metals like lead are known to pose a variety of health risks such as cancer, mutations or miscarriages [10]. In humans, it has been implicated as the cause of renal disturbances, lung insufficiency, bone lesions, cancer and hypertension [11]. Farmers used some chemical fertilizer on rice farms to make rice plant grow better. Chemical fertilizers such as super phosphate have Pb and they can be the major source of lead uptake in rice [2]. There have not been many studies conducted to estimate the content of heavy metals in food such as rice. Therefore, the present study will help to understand sensitivities of various rice cultivars in Egypt under Pb stress conditions and help the grower to manage rice crop in Pb contaminated soils.

## 2. MATERIALS AND METHODS

A laboratory experiment was conducted at Rice Research and Training Center (RRTC), Sakha, Kafr EL-Sheikh, Egypt and laboratory of Genetics Department, Faculty of Agriculture, Alexandria University. During the growing season 2014 to study the response of rice (*Oryza sativa* L.) cultivars to germinate it under Lead (Pb) stress and to confirm the seedling growth performance to examine a range of genetic variability for heavy metals tolerance among twelve Egyptian rice cultivars.

### 2.1 Treatments and Experimental Design

The experiment was arranged in factorial design in Randomized Complete Block Design (RCBD) with three replicates. The experiment included 12 different rice cultivars which were provided by Initial Seed Increase of Rice Research and training center at Sakha, Kafr El Sheikh, Egypt. Studied rice cultivars were include 9 cultivars belong to Japonica type, 3 cultivars belong to Indica type and 2 cultivars belong to Indica-japonica type as showed in (Table 1). Four different concentrations of lead chloride ($Pb\ Cl_2$) *i.e*. 0, 100, 200 and 400 ppm were used. Fifty seeds of uniform size in each cultivar were allowed to germinate to primary roots of 2 mm length in a Petri dish containing a filter paper of 9 cm diameter; the Petri dishes were placed in a growth chamber for 7 days at 28±1℃, germinated seeds were selected and transferred to pots (diameter 10 cm) containing quartz sand. Then, they were transferred to the green house with day and night temperature of 25℃ and grown in plastic pots.

**Table 1. List of rice genotypes, pedigree and type of studied cultivars**

| No | Genotypes | Pedigree | Type |
|---|---|---|---|
| 1 | Giza 177 | Giza 171 / Yomji No.1 // Pi No.4 | Japonica |
| 2 | Giza 178 | Giza 175 / Milyang 49 | Indica-japonica |
| 3 | Giza 179 | IR 6269-12-1-2-1-1 / GZ 1368-5-5-4 | Indica-japonica |
| 4 | Giza 181 | IR 28 / IR 22 | Indica |
| 5 | Giza 182 | Giza 181/ IR 39422-163-1-2// Giza 181 | Indica |
| 6 | Sakha 101 | Giza 176 / Milyang 79 | Japonica |
| 7 | Sakha 102 | GZ 4096-7-1 / Giza 177 | Japonica |
| 8 | Sakha 103 | Giza177 / Suweon 349 | Japonica |
| 9 | Sakha 104 | GZ 4096-8-1 / GZ 4100-9-1 | Japonica |
| 10 | Sakha 105 | GZ 5581-46-3 / GZ 4316-7-1-1 | Japonica |
| 11 | Sakha 106 | Giza 177 / Hexi 30 | Japonica |
| 12 | Egyptian Yasmin | IR 262-43-8-1 / NAHNG SARN | Indica |

Seeds were germinated, in the presence of lead chloride. Each dish contained fifty seeds and a total of 3 dishes were used for each treatment. After One week from the initiation of germination, selected seedlings (stressed and non-stressed) were carefully transferred to suitable pots containing quartz sand to continue their growth in the greenhouse. After 21 days of sowing, plantlets were harvested and were homogenized in liquid nitrogen; the plant material was stored in liquid nitrogen until used.

## 2.2 Studied Characters

After 14 days seedlings were selected from each replicate and then were evaluated as follows:

A- Final Germination Percentage (FGP): FGP was calculated according to the following equation [12].

$$(FGP) = \frac{\text{Number of Germinated Seed}}{\text{Total Number of Seed Tested}} \times 100.$$

**B**- Germination Index (GI): according to the following equation described by [13] it was calculated according the following equation

$$(GI) = \frac{\text{\%Germination Each Treatment}}{\text{\%Germination in the Control}}$$

A- Root Length, RL (cm).
B- Shoot Length, SL (cm).
C- Root/Shoot Ratio, R/S (%).

## 2.3 Statistical Analysis

All obtained data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the split-plot design [14], by using means of “MSTAT-C” computer software package. Least Significant Difference (LSD) method was used to test the differences between treatment means at 5% level of probability [15]. The comparison of the means was done by Duncan test at a probability level of 5 percent.

# 3. RESULTS AND DISCUSSION

The results obtained as shown in (Table 2) revealed that highly significant differences in final germination percentage (FGP), germination index (GI), root length (RL), shoot length (SL) and root/shoot ratio (R/S) traits among genotypes and Lead application as well as their interaction.

With regard to final germination percentage, significant differences were detected among the twelve studied rice cultivars (Table 3), the cultivar Giza 181 gave highly significant mean value (91.00%), while, Sakha 103 variety provide decreased mean value (76.50%). These differences among rice genotypes might be attributed to their genetic background. Many investigators found differences among rice cultivars in germination characters and seedling parameters [16,17]. The analysis of variance indicates that final germination percentage was significantly influenced by Lead application levels. As shown in (Table 4), the treatment without Pb application (control) produced the highly mean of final germination percentage (91.88%), while the application Lead with 400 ppm gave the lowest mean value for final germination percentage (82.19%). Similar results were claimed by [18] in cereal crops and [19] for other plants. The interaction between rice cultivars and Pb doses affected significantly final germination percentage. The results obtained as shown in (Fig. 1A) clearly indicated that the untreated cultivar Giza 179 gave the highest mean (97.33%). Otherwise, the lowest value (74.33%) for germination percentage obtained from the cultivar Sakha 103 with 400 ppm Pb dosage. The effect of lead on seed germination and vigor of rice cultivars that performed differently under Pb stress may due to genetic diversity [20].

For germination index (GI), the rice cultivars found to be highly significant differences, the cultivar Sakha 105 and Sakha 104 produced superior mean (0.975 and 0.974, respectively), while, the lowest mean obtained from Giza 182 cultivar (0.866). The germination index was significantly influenced by Lead application levels, (Table 3). The untreated seedling (control) gave the highly mean value of germination index,

**Table 2. Analysis of variance for final germination percentage, germination index, root length, shoot length and root/shoot ratio traits affected by Pb treatments**

| S.O.V | df | Germination percentage (FGP) | Germination index (GI) | Root length (cm) | Shoot length (cm) | Root/ Shoot ratio |
|---|---|---|---|---|---|---|
| Replication | 2 | $3.22^{ns}$ | $0.0001^{ns}$ | $0.002^{ns}$ | $0.28^{ns}$ | $2.04^{ns}$ |
| Genotypes | 11 | $205.70^{**}$ | $0.017^{**}$ | $2.604^{**}$ | $7.89^{**}$ | $175.33^{**}$ |
| Treatments | 3 | $614.41^{**}$ | $0.070^{**}$ | $391.52^{**}$ | $195.90^{**}$ | $37990.94^{**}$ |
| G x T | 33 | $24.40^{**}$ | $0.003^{**}$ | $2.74^{**}$ | $3.20^{**}$ | $200.60^{**}$ |
| Error | 94 | 4.53 | 0.001 | 0.08 | 0.13 | 17.41 |

*ns: not significant;* ***** *,* ****** *: significant at 5% and 1%, respectively*

**Table 3. Mean performance for final germination percentage, germination index, root length, shoot length and root/shoot ratio of the twelve rice cultivars studied**

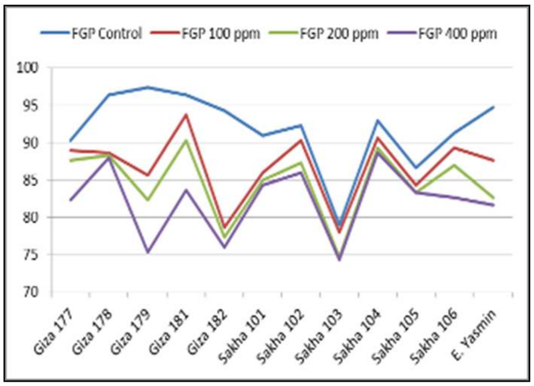
| Genotypes | Germination percentage (FGP) | Germination index (GI) | Root length (cm) | Shoot length (cm) | Root/Shoot ratio |
|---|---|---|---|---|---|
| Giza 177 | $87.333^{cd}$ | $0.968^{ab}$ | $2.933^{b}$ | $7.250^{c}$ | $33.481^{a}$ |
| Giza 178 | $90.333^{ab}$ | $0.940^{bc}$ | $2.117^{f}$ | $6.350^{d}$ | $29.456^{bc}$ |
| Giza 179 | $85.167^{ef}$ | $0.877^{d}$ | $2.450^{cd}$ | $6.525^{d}$ | $29.445^{bc}$ |
| Giza 181 | $91.000^{a}$ | $0.943^{ab}$ | $2.267^{def}$ | $5.900^{e}$ | $34.117^{a}$ |
| Giza 182 | $81.583^{g}$ | $0.866^{d}$ | $2.183^{ef}$ | $5.883^{e}$ | $30.904^{ab}$ |
| Sakha 101 | $86.583^{de}$ | $0.951^{ab}$ | $2.400^{cde}$ | $5.942^{e}$ | $29.190^{bc}$ |
| Sakha 102 | $89.000^{bc}$ | $0.964^{ab}$ | $3.217^{a}$ | $7.325^{c}$ | $32.840^{ab}$ |
| Sakha 103 | $76.500^{h}$ | $0.968^{ab}$ | $3.017^{ab}$ | $7.325^{c}$ | $32.252^{ab}$ |
| Sakha 104 | $90.417^{ab}$ | $0.974^{a}$ | $2.417^{cde}$ | $7.058^{c}$ | $25.832^{cd}$ |
| Sakha 105 | $84.417^{f}$ | $0.975^{a}$ | $3.075^{ab}$ | $7.817^{b}$ | $30.688^{ab}$ |
| Sakha 106 | $87.583^{cd}$ | $0.960^{ab}$ | $2.592^{c}$ | $8.125^{a}$ | $23.913^{de}$ |
| E. Yasmin | $86.667^{de}$ | $0.915^{c}$ | $1.625^{g}$ | $5.817^{e}$ | $21.972^{e}$ |

** Means followed by the same latter in each column or significantly different by the least significant at p 0.05 according to Duncan's test*

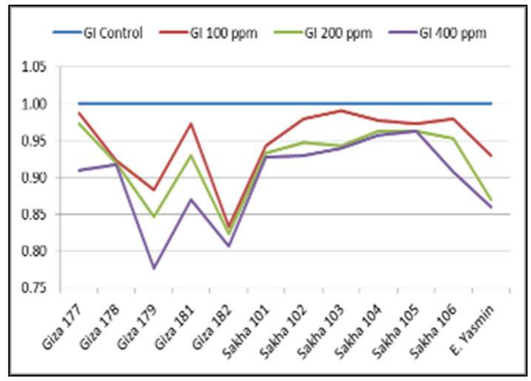
**Table 4. Mean performance for germination percentage, germination index, root length, shoot length and root/shoot ratio affected by Pb application levels**

| Treatment | Germination percentage (FGP) | Germination index | Root length (cm) | Shoot length (cm) | Root/Shoot ratio |
|---|---|---|---|---|---|
| Control | $91.889^{a}$ | $1.000^{a}$ | $7.297^{a}$ | $9.667^{a}$ | $75.449^{a}$ |
| 100 ppm | $86.833^{b}$ | $0.948^{b}$ | $2.158^{b}$ | $7.450^{b}$ | $29.499^{b}$ |
| 200 ppm | $84.611^{c}$ | $0.922^{c}$ | $0.350^{c}$ | $5.758^{c}$ | $6.145^{c}$ |
| 400 ppm | $82.194^{d}$ | $0.897^{d}$ | $0.292^{c}$ | $4.231^{d}$ | $6.937^{c}$ |

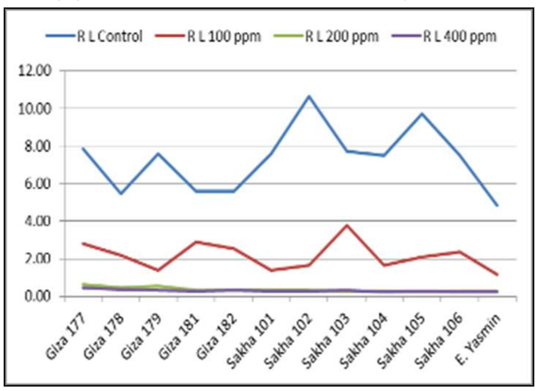
** Means followed by the same latter in each column or significantly different by the least significant at p0.05 according to Duncan's test* where the application Lead with 400 ppm gave the lowest one for germination index (0.897) as shown in (Table 4).The interaction between rice cultivars and Pb doses affected significantly germination index as shown in (Fig. 1B). The results obtained revealed that the maximum mean value with cultivar Sakha103 as (0.990) with 100 ppm lead dose in comparison with the control treatment. On other hand, the lowest mean (0.777) for germination index was obtained from the cultivar Giza179 treated with 400 ppm Pb dosage.



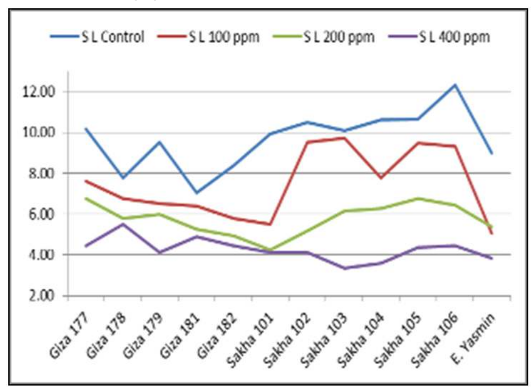

(A) Final Germination Percentage FGP



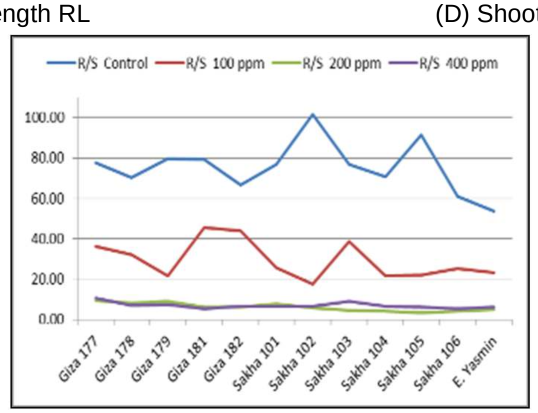

(B) Germination Index GI




(C) Root Length RL




(D) Shoot Length SL




(E) Root/Shoot ratio R/S

**Fig. 1. Interaction between cultivars genotypes and Pb concentrations on (A); final germination percentage FGP, (B); germination index GI, (C); root length RL, (D); shoot length SL and (E); root/shoot ratio R/S**

The results obtained also revealed that as shown in (Table 3), root length was varied significantly among cultivars and Pb application. The highest mean root length achieved from Sakha102 (3.217 cm), where, Egyptian Yasmin rice genotype gave the lowermost mean of root length (1.625 cm). Similar results were reported that significant inhibition of root length of seedlings at higher Pb [21]. The results obtained also demonstrated that (Table 4), Pb applications were high significant differences affected for root length, since the control treatment provided the longest mean (7.297 cm), whilst the shortest mean value was (0.292 cm) obtained from 400 ppm Lead dose. The rice genotypes and Lead doses interaction (Fig. 1C) was highly significant for root length. The uppermost root length mean value was realized from genotypes, Sakha 102 (10.63 cm) with control, on the other hand, Sakha 104, Sakha 106 and Egyptian Yasmin treated with 400 ppm Pb dose donated the lowest one (0.23 cm). A similar trend was found by Sakha 105 treated with 200 ppm. Similar results were reported by [22,23]. The root growth inhibition by Pb toxicity is most probably resulted from non-selective suppressive of both cell division and cell elongation of the seedlings [24].

Considering the shoot length it was affected significantly by cultivars and Lead application. The highest mean of shoot length gained from Sakha106 (8.13 cm). Where, Egyptian Yasmin rice genotype got the decreased mean of shoot length (5.82 cm) as present in (Table 3). The results in (Table 4) showed that there were highly significant differences for shoot length among treated cultivar with Pb, where the control treatment gave the longest mean (9.67 cm), while the shortest mean value was (4.23 cm) found from 400 ppm Pb dose. The rice genotypes and Lead doses interaction was highly significant for shoot length (Fig. 1D). The highest shoot length value was realized from genotypes Sakha106 with control (12.33 cm). On the other hand, the Sakha 103 treated with 400 ppm Pb dose donated the declined one (3.33 cm). Various degrees of permeability of seed coats to metals lead to a range of seed germination inhibitions [25]. A number of studies have reported that plant seedlings responded quickly to a higher concentration of metals in terrestrial ecosystems by changing in their growth rates and root branching patterns compared to shoot growth [21,26-28].

With respect to root/shoot ratio, significant differences were detected among the twelve studied rice cultivars (Table 3). The cultivar Giza 181 gave highly significant mean (34,12), while, Egyptian Yasmin variety provide decreased mean (21,97). These differences among rice genotypes might be attributed to their genetic background [29]. The analyses of variance indicated that root/shoot ratio was significantly influenced by Pb application levels. As shown in (Table 4), the treatment without Pb application produced the highly mean of root/shoot ratio (75,45), while the application Lead with 300 ppm gave the lowest mean for root/shoot ratio (6,15).The interaction between rice cultivars and Pb doses affected significantly root/shoot ratio. The results also showed that the highest mean value (101,5) with cultivar Sakha 102 and without Pb dose. Otherwise, the lowest value 5,25 for root/shoot ratio obtained from the cultivar Sakha 106 treated with 400 ppm Pb dosage (Fig. 1E). The change in root growth characteristics is probably due to the consequences of the direct exposure of the radical to metal toxicity and preferential accumulation of metals in the emerging roots followed by slow mobility to the plant shoots [30,31].

## 4. CONCLUSION

From the obtained results of this study, it could be concluded that great diversity was observed among rice genotypes for different concentrations of Pb at the seedling stage. The cultivars Giza 179, Sakha 103 and Sakha 106 showed the highest mean values for final germination percentage, germination index and shoot length traits, respectively. Meanwhile, the cultivar Sakha 102 recorded the highest mean values for root length and root/shoot ratio traits. The Pb treatments were significantly affected all studied rice genotypes and traits.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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