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# GENETIC EVALUATION OF LEGUME SPECIES UNDER HEAVY METAL AND BIOGAS WASTEWATER TREATMENTS

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**Abstract:** *The legumes are very important food crops, called pulses grown throughout the world for their grain which contains essential vitamins, carbohydrates, protein, fat, minerals, and dietary fiber. Chickpea (Cicer arietinum L.), pea (Pisum sativum L.), green mung bean (Vigna radiate L.), and black mung bean (Vigna mungo L.) is important pulse crops that belong to the family Fabaceae or Leguminosae. The present experiment was conducted at the greenhouse of Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore during the season of July to August of 2020 to study the effects of different treatments of* $ZnSO_4$ *and biogas wastewater on the growth of chickpea, pea, green bean, and black bean. The experiment consisted of two treatment combinations comprising of two levels of* $ZnSO_4$ *at 0.5M and 0.25M along with the two levels of biogas wastewater at 500ml and 250ml along with including the control group. It was observed from results that the performance of Chickpea, pea, green mung bean, and black mung bean genotypes were variable under heavy metal zinc sulfate treatment. The results suggested that the treatment of a higher concentration of 0.5M* $ZnSO_4$ *was toxic as compared with 0.25M* $ZnSO_4$*. The application of biogas wastewater was found relatively fit for the seedling growth of all of four pulse crop species. The results showed that there was a significant correlation among root length, shoot length, and leaf length under the application of different treatments. A higher genetic advance was reported for shoot length and root length which revealed that the selection of legumes may be fruitful to improve yield under stress conditions.*

**Keywords:** chickpea, pea, green mung bean, black mung bean, stress, $ZnSO_4$

## Introduction

Chickpea (*Cicer arietinum* L.), pea (*Pisum sativum* L.), green mung bean (*Vigna radiate* L.) and black mung bean (*Vigna mungo* L.) are an important pulse crops belong to family Fabaceae or Leguminosae. Chickpea has been cultivated in the Middle East while green bean and black bean have been cultivated in East Asia and Southeast Asia from ancient times of old civilizations. Chickpea and pea are sensitive to abiotic stress such as heat, drought, cold and heavy metals. The introduction and use of high input fertilizer varieties has caused micronutrient depletion in soils, mainly zinc (Zn). About 60 % of the world's soil is deemed insufficient for crops production of some essential nutrient elements due to mineral stress deficiency, lack of availability or toxicity (Pathak *et al*., 2012; Moller, 2009). The legumes are important to boost soil fertility and to increase crop productivity in developing countries' cultivation systems of arid and semi-arid areas. Furthermore, Bio fertilizers are products made of living microorganisms that can increase crop production in an environmentally friendly, sustainable way, through a variety of direct and indirect mechanisms (Amjad, 2002). Chickpea constitutes a significant source of human dietary protein, particularly for a large vegetarian population. It produces an average of 126 kg of protein per hectare and is potentially the top protein producing vegetables alongside soybean. Peas have been cultivated for many centuries as a significant source of animal feed and human food Cousin, 1997). Field pea is a cool legume crop cultivated worldwide in the cool season. 20% of the available protein is provided by beans in many developing countries. Beans are also a key component of dietary protein in 50% of the world's population (Deshpande *et al.,* 1984; Sai et al., 2017).

Stress is any external elements that affect physiology, development, productivity, metabolism and plant survival. Stress has been divided into two categories: abiotic and biotic (Mahajan *et al.,* 2018). Abiotic stress is a stress mainly caused by environmental changes such as drought , salinity, heat, colds, water recesses, extreme soil pH changes, mechanical stress (for example wind, hail, wounds, etc.) and effects of herbicides and weedicides and exposure to other heavy metals. Although, the biotic stress is often pathogenic stress which is caused by living organisms like bacteria, fungi, viruses, nematodes, etc., insects and weeds, which caused higher loss of crop productivity (Chen, 2006; Sangolli *et al.,* 2018). Across different terrestrial habitats around the world, heavy metal toxicity has become a key concern. The damage to soil texture i.e. pH of soil, the presence of various elements, heavy metal causes a direct and indirect decrease in plant growth by adversely affecting diverse physiological and molecular activity of plants. Heavy metals, including Hg, Co, Cd, Fe, Ni, Al, Cr, Ar, Zn, Cu, Mo and Mn, etc., today extensively used in industries and imparts damage to soil as well as to crop productivity (Mahanty *et al.*, 2017; Ranpariya *et al.,* 2017; Zubair *et al*., 2016).

**Materials and methods**

Chickpea (*Cicer arietinum* L.), pea (*Pisum sativum* L.), green mung bean (*Vigna radiate* L.) and black mung bean (*Vigna mungo* L.) seeds were collected from local market of Lahore. The present experiment was conducted at the greenhouse of Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore during the season of July to August of 2020 to study the effects of different treatments of $ZnSO_4$ and biogas wastewater on the growth of chickpea, pea, green bean and black bean. The experiment consisted of two treatment combinations comprising of two levels of $ZnSO_4$ at 0.5M and 0.25M along with the two levels of biogas wastewater at 500ml and 250ml along with including control group. Seeds were sown and after complete germination five plants from each pot of chickpea, pea, green mung bean and black mung bean were taken to collect data and after removing the plants from each pot, treatments of biogas wastewater and $ZnSO_4$ was applied. Five groups were made; the first group was given no treatment because it was kept as control group. The next four groups were included; second group was treated with 0.5M $ZnSO_4$, third group with 0.25M $ZnSO_4$, fourth group with 500ml of biogas wastewater and fifth group was 250 ml of biogas wastewater. After a period of one week of first treatment, five plants were again removed from each pot to record data. After that second treatment was given to each pot and after period of one week again plants were removed to collect the data. The application of treatments and data recording was carried out five times. After collection of data, pooled analysis of variance, correlation and regression analysis was carried out for traits including root length, roots per plant, shoot length and leaf length.

**Results and Discussions**

It was persuaded from results given in table 1 that significant differences were among treatments, genotypes and genotypes × treatment. It was found that the average leaf length (5.524±0.0035cm), number of roots per plant (5.125±0.0044cm), root length (5.1045±0.0011cm) and shoot length (3.5±0.0023cm) were found under combined effects of all of the treatments. The genetic advance was found higher for all of the studied traits of legume species. The coefficient of variation was found lower for all of the studied traits which indicated that the results for root length, shoot length, leaf length and number of roots per plants were highly consistent and reliable for making selection under heavy metal stress conditions. The results from table 2 indicated that survival of legume genotypes was higher under control conditions as compared with $ZnSO_4$ and biogas wastewater treatment. The lowest survival of black mung bean was found under the treatment of 0.5M $ZnSO_4$ followed by 0.25 M $ZnSO_4$. The application of $ZnSO_4$ showed adverse effects on black mung bean only to reduce the survival. The lower survival percentage indicated that the genotype was sensitive to application of $ZnSO_4$. The genotypes which showed higher survival percentage under applications of $ZnSO_4$ and biogas wastewater indicated that the legume genotype may be used as heavy metal tolerance genotype (Akhtar *et al.,* 2017; Bhardwaj *et al*., 2014; Garci-Gomez *et al*., 2017).

**Table 1 Pooled analysis of variance**

| **Source** | DF | LL | NRP | RL | SL |
|---|---|---|---|---|---|
| Replication | 1 | 3.23761 | 2.025 | 0.03721 | 0.121 |
| Genotypes | 3 | 0.04902* | 0.825* | 0.32551* | 0.15* |
| Treatment | 4 | 0.67771* | 1* | 0.14857* | 0.00437* |

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| | | | | | |
|---|---|---|---|---|---|
| Genotypes × Treatment | 12 | 0.84646* | 1.53333* | 0.28637* | 0.10104* |
| Error | 19 | 0.35954 | 0.28816 | 0.35686 | 0.03468 |
| Grand Mean | | 5.524 | 5.125 | 5.1045 | 3.5 |
| Standard error | | 0.0035 | 0.0044 | 0.0011 | 0.0023 |
| Coefficient of variance | | 10.85 | 10.47 | 11.7 | 5.32 |
| Genetic Advance | | 13.237 | 15.654 | 16.342 | 17.346 |

* = Significant at 5% probability level, LL = Leaf length, RL = Root length, SL = Shoot length, NPR = Number of roots per plant

**Table 2 Survival percentage of seedlings under different treatments**

| Treatments | Chickpea | Pea | Green mung bean | Black mung bean |
|---|---|---|---|---|
| Control | 94.94 | 97.14 | 94.94 | 92.50 |
| 0.5 M $ZnSO_4$ | 84.33 | 78.75 | 84.33 | 70.25 |
| 0.25 M $ZnSO_4$ | 89.59 | 80.13 | 84.61 | 72.67 |
| 500 ml Biogas wastewater | 89.82 | 92.86 | 89.82 | 92.50 |
| 250 ml Biogas wastewater | 89.41 | 87.14 | 89.41 | 85.34 |

The results from table 3 for chickpea indicated that the higher number of roots per plant was found higher under control condition (9.10) while lowest was found under 250ml biogas wastewater. The shoot length was also found under control condition (18.19cm) while lowest for 0.5M $ZnSO_4$ (13.18cm). The higher leaf length was found under control conditions (5.26cm) while lowest for 0.5M $ZnSO_4$ (4.15cm) while root length was found higher under control condition (19.91cm) while lowest under 0.25M $ZnSO_4$ (16.88cm). The higher shoot length and root length under applications of $ZnSO_4$ indicated that the genotype has tolerance against heavy metals applications and selection may be helpful to improve chickpea yield under stressful environment (Akhtar *et al.*, 2017; Bhardwaj *et al*., 2014; Garci-Gomez *et al*., 2017; Moller and Stinner 2010; Verma *et al*., 2017). The results from table 3 for pea indicated that the higher number of roots per plant was found higher under 250 ml Biogas wastewater condition (8.1) while lowest was found under 0.5M $ZnSO_4$ (6.2). The shoot length was also found under control condition (15.28cm) while lowest for 0.5M $ZnSO_4$ (11.22cm). The higher leaf length was found under 250 ml Biogas wastewater conditions (7.16cm) while lowest for 0.5M $ZnSO_4$ (6.17cm) while root length was found higher under 250 ml Biogas wastewater condition (17.91cm) while lowest under 0.5M $ZnSO_4$ (15.89cm). The higher shoot length and root length under applications of $ZnSO_4$ indicated that the genotype has tolerance against heavy metals applications and selection may be helpful to improve pea yield under stressful environment (Akhtar *et al.*, 2017; Bhardwaj *et al*., 2014; Garci-Gomez et al., 2017; Usman *et al*., 2014).

**Table 3 Mean comparison among legumes for different traits of seedlings**

| Genotypes | Treatment | NRP | SL | LL | RL |
|---|---|---|---|---|---|
| **Chickpea** | Control | 9.1a | 18.19a | 5.26a | 19.91a |
| | 0.5 M $ZnSO_4$ | 7.3b | 13.18d | 4.15b | 16.89c |
| | 0.25 M $ZnSO_4$ | 6.4c | 14.23c | 4.19b | 16.88c |
| | 500 ml Biogas wastewater | 6.5c | 13.27d | 5.11a | 18.87b |
| | 250 ml Biogas wastewater | 5.1d | 15.20 b | 5.19a | 18.92b |
| **Pea** | Control | 7.6b | 15.28a | 7.14a | 16.87b |
| | 0.5 M $ZnSO_4$ | 6.2c | 11.22d | 6.17b | 15.89c |
| | 0.25 M $ZnSO_4$ | 7.3b | 12.26c | 6.19b | 16.78b |
| | 500 ml Biogas wastewater | 7.7b | 14.19b | 7.14a | 16.78b |
| | 250 ml Biogas wastewater | 8.1a | 14.25b | 7.16a | 17.91a |
| **Green mung bean** | Control | 8.3a | 17.24a | 8.19a | 15.86 b |
| | 0.5 M $ZnSO_4$ | 7.2b | 13.23c | 6.18c | 14.87 c |

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| | | | | | |
|---|---|---|---|---|---|
| | 0.25 M $ZnSO_4$ | 6.3c | 14.27b | 6.17c | 13.88d |
| | 500 ml Biogas wastewater | 8.1a | 13.21c | 7.19b | 16.89a |
| | 250 ml Biogas wastewater | 8.4a | 12.26d | 7.13b | 16.79a |
| **Black mung bean** | Control | 8.5a | 16.29a | 8.14a | 17.91a |
| | 0.5 M $ZnSO_4$ | 5.9c | 13.21d | 6.17c | 15.85c |
| | 0.25 M $ZnSO_4$ | 5.8c | 12.24e | 6.12c | 14.87d |
| | 500 ml Biogas wastewater | 7.1b | 15.19b | 7.18b | 16.84 b |
| | 250 ml Biogas wastewater | 7.4b | 14.27c | 7.16b | 17.82a |

LL = Leaf length, RL = Root length, SL = Shoot length, NPR = Number of roots per plant

The results from table 3 for green mung bean indicated that the higher number of roots per plant was found higher under 250 ml Biogas wastewater condition (8.4) while lowest was found under 0.25M $ZnSO_4$ (6.3). The root length was also found under 250 ml Biogas wastewater condition (16.79cm) while lowest for 0.25M $ZnSO_4$ (13.88cm). The higher shoot length was found under control conditions (17.24cm) while lowest for 250 ml Biogas wastewater (12.26cm) while leaf length was found higher under control condition (8.19cm) while lowest under 0.25M $ZnSO_4$ (6.17cm). The higher shoot length and root length under applications of $ZnSO_4$ indicated that the genotype has tolerance against heavy metals applications and selection may be helpful to improve green mung bean yield under stressful environment. The results from table 3 for black mung bean indicated that the higher number of roots per plant was found higher under control condition (8.5) while lowest was found under 0.25M $ZnSO_4$ (5.8). The root length was also found under 250 ml Biogas wastewater condition (17.82cm) and control conditions (17.91cm) while lowest for 0.25M $ZnSO_4$ (14.87cm). The higher shoot length was found under control conditions (16.29cm) while lowest for 0.25M $ZnSO_4$ (12.24cm) while leaf length was found higher under control condition (8.14cm) while lowest under 0.25M $ZnSO_4$ (6.12cm). The higher shoot length and root length under applications of $ZnSO_4$ indicated that the genotype has tolerance against heavy metals applications and selection may be helpful to improve black mung bean yield under stressful environment (Ali et al., 2012; Akhtar *et al.*, 2017; Bhardwaj *et al.*, 2014; Garci-Gomez et al., 2017; Hussain *et al.*, 2015; Usman *et al.*, 2014).

The results from table 4 about pooled correlation analysis of studied traits of legume crop species indicated that there was significant and positive correlation among all of the studied traits viz. root length, shoot length, number of roots per plant and leaf length. The significant correlation indicated that the selection may be helpful to improve legume seed yield under various stress conditions (Ali and Ahsan 2012; Ali *et al.*, 2010ab; Ali *et al.*, 2013; Ali *et al.*, 2014; Ali *et al.*, 216).

**Table 4. Pooled correlation among morphological traits of legumes under different treatments**

| **Source** | LL | NRP | RL |
|---|---|---|---|
| NRP | 0.4627* | | |
| RL | 0.3832* | 0.6312* | |
| SL | 0.4972* | 0.5823* | 0.6759* |

* = Significant at 5% probability level, LL = Leaf length, RL = Root length, SL = Shoot length, NPR = Number of roots per plant

**Conflict of interest**

The authors declared absence of any type of conflict of interest.

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