African Journal of Biotechnology Vol. 9(50), pp. 8608-8612, 13 December, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.890
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Effects of lead ($PbCl_2$) stress on germination of lentil (*Lens culinaris* Medic.) lines

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Accepted 13 October, 2010

The presence of heavy metals in solid and liquid wastes is an important issue that is related to the pollution of the environment. They are one of the most important environmental pollutants and reaching dangerous amount requires more research. This study was conducted to investigate the effects of lead ($PbCl_2$) stress on germination of different lentil lines. Seeds of four different lentil (*Lens culinaris* Medic.) lines (BM601-46, BM706-8, BM449-13 and BM152-33) were placed on temperature adjustable germination cabinet at 15°C with 10 different lead solutions (Control, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 mM) and germination percentage, mean germination time, vigour index, plumule length and radicle length were investigated. According to result gotten from the study, considerably decrease was observed in all parameters except mean germination time, depending on increasing lead concentration in all lines which had been used. On the other hand, effect of the concentrations of $PbCl_2$ on germination was observed to be statistically important in all parameters. The results also revealed that significant variation is found among lines. BM449-13 and BM706-8 lines showed better performance for all parameters.

Key words: Lentil (*Lens culinaris* L.), germination, vigour index, plumule length, radicle length.

INTRODUCTION

Heavy metals are one of the most important environmental pollutants (Kirbag and Munzuroglu, 2003). It is generally accepted that the solubility, bioavailability and toxicity of heavy metals are dependent on various physicochemical parameters such as pH, hardness, interactive effects and presence of natural organic matter (Janssen et al., 2003; Heijerick et al., 2003; Hadjispyrou et al., 2001; Peijnenburg and Jager, 2003; Manusadzianas et al., 2003). pH affects the solubility, speciation and transportation of metals from solid to liquid phase. Additionally, heavy metals are often present in the environment in mixtures, making the assessment of environmental hazards even more difficult due to the antagonistic or synergistic actions that may occur. The investigation of the joint toxic effects of chemicals in a mixture is generally based on comparison of the actual toxic effect of the mixture with the theoretically expected toxic effect deduced by a statistical model, using the toxic effects of the individual chemicals (Aoyama et al., 1987; Kungolos et al., 1999; Mowat and Bundy, 2002).

Due to the rapid development of industry, the quantity of hazardous materials is also increasing rapidly and the environmental and economic problems posed by this factor are becoming more and more serious. These pollutants may cause environmental damage to soil, plants, groundwater and air. Owing to industrial development and population expansion, heavy metal pollution is becoming increasingly serious worldwide.

Heavy metals may come from many different sources in urbanized areas. One of the most important heavy metal sources is vehicle emission (Harrison et al., 1981; Gibson

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Abbreviations: GP, Germination percentage; **MGT,** mean germination time; **VI,** vigour index; **PL,** plumule length; **RL,** radicle length.

and Farmer, 1986). Atmospheric pollution is a major contributor to heavy metal contamination. Heavy metals can accumulate on topsoil from atmospheric deposition by sedimentation, impaction and interception. The persistence of heavy metals in soil is a long process (Kelly et al., 1996). Top soils and roadside dusts in urban area are indicators of heavy metal contamination from atmospheric deposition. It has been noted that roadside soils near heavy traffic are polluted by Pb and other metals (Culbard et al., 1988; Wong and Mak, 1997). The large particles of lead from vehicle emissions deposit close to the road (>90% within 1.5 m when the size is >5 Am) (Hamamci et al., 1997).

Lead is found in the environment in various forms. Today, most large-scale heavy metal are the common types (Nriagu, 1992). Lead contamination is an important problem, especially in agriculturally developed zones. The contamination of agricultural lands caused by heavy metals in and around industrial areas is a serious problem. Such contamination is due largely to injudicious anthropogenic activities such as indiscriminate use of pesticides containing heavy metals in agriculture, discharge of untreated industrial wastes and effluents, faulty waste disposal, high rate of burning of fossil fuels, mining, etc (Mehera and Farago, 1994). As an indicator of metal phytotoxicity, various authors have reported from time to time, different biomonitoring indices based on germination and seedling growth to indicate metal stress effects using different plant systems (Baki and Anderson, 1973; Mhatre and Chaphekar, 1982).

This study was carried out in order to define the effect of lead which has a very wide space of use such as battery factories, metal industries, petrol refineries, paint industry, vehicle accumulators and explosive industry; however, it also has a very important role in environment pollution upon the germination of various lentil lines.

MATERIALS AND METHODS

Lentil lines

This study was conducted at the Faculty of Agriculture, Kahramanmaras Sutcu Imam University and Kahta Vocational School, Adiyaman University in 2010. Four lentil lines (BM601-46, BM706-8, BM449-13 and BM152-33) were used and their seeds were obtained from Field Crops Department, Faculty of Agriculture, Dicle University.

$PbCl_2$ solutions

$PbCl_2$ (lead II chloride) whose molecular weight is 278.116 g was obtained from Sigma-Aldrich and prepared by using distilled water (Kiran and Sahin, 2005). Four different lentil (*Lens culinaris* Medic.) lines (BM601-46, BM706-8, BM449-13 and BM152-33) were placed on temperature adjustable plant growth cabinet at 15°C with 10 different lead solutions [0.0 (Control), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 mM] for 24 h. Having 25 seeds in each petri dish, 4 replications were used. Then, seeds were washed and placed in germination cabin at a temperature of 15 ± 0.5°C for 10 days.

During this period, the petri dishes were observed daily (Tanveer et al., 2010).

Germination percentage (%)

Germinated seeds were counted daily according to the seedling evaluation procedure in the Handbook of Association of Official Seed Analysts. The number of germinated seeds was recorded every 24 h (AOSA, 1990). Ten days after germination, the germination percentage (GP) was calculated using the formula below for each replication of the treatment (Tanveer et al., 2010):

$$GP = \frac{Germinated \quad Seed}{Total \quad Seed} x100$$

Mean germination time (MGT)

MGT was calculated in days according to the equation of Ellis and Roberts (1981):

$$\mathrm{MGT} = \frac{\sum [\mathrm{D.n}]}{\sum \mathrm{n}}$$

Where, n is the number of seeds that germinated on day D, and D is the number of days counted from the beginning of germination.

Vigour index

Vigour index (VI) was calculated by using the formula of Baki and Anderson (1973):

$$VI = [MPL + MRL] x GP$$

Where, MPL is mean plumule length, MRL is mean radicle length and GP is germination percentage (%).

Plumule and radicle length (cm)

Plumule and radicle parts of the seeds in petri dishes were seperated after germination, and measured in centimetres from the point where the radicle and plumule joins together at the end of the radicle and to the top of the plumule (Tanveer et al., 2010).

Data collection

A completely randomized experimental design (CRD) with four replicates was involved. Data were subjected to analysis of variance using the Statistical Analysis System (SAS) software (SAS, 1997) and mean separation was performed by Fisher's least significant difference (LSD) test when F test was significant at $P < 0.01$.

RESULTS AND DISCUSSION

Significant differences were determined between lines and $PbCl_2$ treatments. Lead affected germination

Table 1. Analysis of variance for germination percentage (GP), mean germination time (MGT), vigour index (VI), plumule length (PL) and radicle length (RL) of lentil.

Source	Degree of freedom	Mean square				
		GP	MGT	VI	PL	RL
Lines	3	274.77**	0.625**	45842**	0.350**	0.406**
$PbCl_2$ concentration	9	7420.10**	1.119**	16629**	11.727**	13.295**
Lines x $PbCl_2$ concentration	27	127.06**	0.231**	1305217**	0.071	0.119
Error	117	26.02	0.066	2650	0.056	0.100
Total	159	-	-	-	-	-
CV (%)		8.359	5.278	10.122	5.786	8.626
Mean		61.025	4.849	508.568	4.100	3.671

** Significant at 0.01.

Table 2. Means of $PbCl_2$ concentrations for all parameters.

$PbCl_2$ conc. (mM)	Germination percentage (%)	Mean germination time (day)	Vigour index	Plumule length (cm)	Radicle length (cm)
0.0 (Control)	98.5 a	4.51 e	1027.4 a	5.30 a	5.13 a
0.5	86.5 b	4.52 e	860.9 b	5.08 b	4.85 b
1.0	78.0 c	4.62 de	720.6 c	4.83 c	4.39 c
1.5	66.0 d	4.78 cd	570.6 d	4.59 d	4.04 d
2.0	64.0 d	4.95 bc	510.1 e	4.28 e	3.70 e
2.5	54.0 e	4.82 bc	399.0 f	4.06 f	3.37 f
3.0	47.0 f	4.81 c	317.5 g	3.59 g	3.16 f
3.5	44.0 f	4.99 b	271.5 h	3.31 h	2.86 g
4.0	39.5 g	5.23 a	231.8 i	3.10 i	2.74 g
4.5	32.8 h	5.27 a	175.4 j	2.87 j	2.47 h
LSD	3.571	0.179	36.045	0.166	0.221

Different letters shows significant differences at 0.01 level.

percentage, mean germination time, vigour index, plumule and radicle length (PL and RL). All these parameters decreased with increasing lead (II) chloride level, except MGT. On the other hand, lines x $PbCl_2$ concentration interactions were found statistically significant for germination percentage, mean germination time and vigour index parameters (Table 1).

Germination percentage

The results indicated significant differences among treatments and lines (Tables 2 and 3). According to result from the study, considerably decrease was observed in germination percentage, depending on level of lead concentration in all lines which used.

The highest germination percentage was obtained from control (98.5%), whereas the lowest was obtained from 4.5 mM $PbCl_2$ solution (32.8%). The germination percentage was decrease when the lead concentration increased, which shows that higher lead concentrations inhibit germination (Fargasova, 1994; Kiran and Munzuroglu, 2004; Wierzbicka and Obidzinska, 1998). This can also be due to the toxic effects of ions on the germination process (Khajeh-Hosseini et al., 2003).

Lines BM449-13 and BM706-8 (respectively, 63.4 and 62.9%) showed higher germination percentage, while BM601-46 and BM152-33 (respectively, 60.0 and 57.8%) were lower statistically. Germination patterns could be different between species and different varieties (McWilliam and Phillips, 1971; Therios, 1982).

Mean germination time

Values determined in terms of mean germination time feature contrasted with germination percentage. That is, seeds of which germination percentage was high displayed germination in a shorter time. Germination in short time was determined in the control.

The highest mean germination time was observed in 4.0 and 4.5 mM $PbCl_2$ solutions (5.23 and 5.27 days, respectively). It showed that average velocity of germination in this treatment is the slowest. The shortest

Table 3. Means of lentil lines for all parameters.

Line	Germination percentage (%)	Mean germination time (days)	Vigour index	Plumule length (cm)	Radicle length (cm)
BM601-46	60.0 b	4.84 b	491.1 c	4.03 b	3.58 b
BM706-8	62.9 a	5.04 a	521.8 b	4.17 a	3.70 ab
BM449-13	63.4 a	4.80 b	549.1 a	4.19 a	3.80 a
BM152-33	57.8 b	4.74 b	472.3 c	4.01 b	3.60 b
LSD	2.259	0.113	22.797	0.105	0.140

Different letters shows significant differences at 0.01 level.

mean day germination was observed in control and 0.5 mM $PbCl_2$ solution with 4.51 and 4.52 days (Table 2). The highest mean germination time increases when the lead concentration increased which showed that increased lead concentration caused a decrease in germination velocity. Inhibition of germination and increase in germination time of lentil are supported by Stavrianakou et al. (2004), Dongre and Yadav (2005), Kadioglu et al. (2005) and Tanveer et al. (2010).

Significant variation on mean germination time was found between lentil lines. The highest mean of day germination belonged to BM706-8 lines (5.04 days). It showed that mean velocity of germination in this line are very slow. The shortest mean day germination was observed in other lines, indicating their high germination velocity (Table 3). Lentil lines gave various reactions to lead (II) chloride because of their genetic potentials. Same experiment was conducted by Tanveer et al. (2010).

Vigour index

There was a direct relation between lead (II) concentration and reduction in vigour index because, as the $PbCl_2$ concentration level increased, the vigour index decreased (Table 2). The highest vigour index was obtained from the control (1027.4), whereas the lowest was obtained from 4.5 mM $PbCl_2$ solution (175.4). Similar results were observed by Channappagoudar et al. (2005).

There were significant differences among lentil lines in terms of vigour index. The highest belonged to BM449-13 line (549.1) and the lowest to BM601-46 and BM152-33 lines (491.1 and 472.3, respectively). These rates resulted from their genetic potentials which support the report of Ganesh et al. (2009).

Plumule length

Plumule length decreased when the lead concentration increased. Highest plumule length was observed in control treatment of lentil (5.30 cm). Shortest plumule was observed under 4.5 mM lead concentration (2.87 cm). The control lead treatment and the other treatments were compared, particularly with the high lead concentrations which showed significant difference (Table 2). The presence of inhibitory chemicals in higher concentrations might be the reason for differential behaviour of the extracts and maximum reduction in seedling growth (Fargasova, 1994; Kumar and Singh 1991; Shukla et al. 2003).

Substantial variation on plumule was found between lentil lines (Table 3). Among lines, the highest plumule length belonged to BM449-13 and BM706-8 lines (4.19 and 4.17 cm, respectively) but least plumule length belonged to other lines. Similar results were observed by Ganesh et al. (2009). Germination patterns could be different between species and between different varieties (McWilliam and Phillips, 1971; Therios, 1982).

Radicle length

There was a direct relation between lead (II) concentration and reduction in radicle length because as the $PbCl_2$ concentration level increased, radicle length decreased. The highest radicle length was observed in control with 5.13, whereas the lowest radicle length was obtained with 2.47 cm of 4.5 mM. Its shows that lead (II) concentrations inhibit radicle length (Table 2). Our findings are similar to Kiran and Munzuroglu (2004).

The tallest radical belonged to BM449-13 line with 3.80 cm and the shortest radical belonged to other lines (Table 3). But with BM449-13 and BM706-8 lines, radicle length difference is not statistically significant. Plants give various reactions to lead (II) chloride because of their genetic potentials. The result was considered in parallel to the findings of Bybordi and Tabatabaei (2009).

In conclusion, lead (II) chloride has a very negative effect on germination in lentil. Especially, the considerably negative effect of lead (II) chloride at high concentration appeared evidently. The use of the $PbCl_2$ caused significant decrease in germination percentage, vigour index, plumule length and radicle length, while mean germination time increased. The negative effect of high concentration of the $PbCl_2$ was clearly observed.

The results also revealed that there was significant variation between lines in all parameters. On the other hand, BM449-13 and BM706-8 lines showed better performance for all parameters.

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