Australian Journal of Crop Science AJCS
AJCS 6(4):641-648 (2012) ISSN:1835-2707

Tolerance for cadmium pollution in a core-collection of the model legume, *Medicago truncatula* L. at seedling stage

Ghodratollah Saeidi[1,2*], Martina Rickauer[2,3], Laurent Gentzbittel[2,3]

[1]Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran
[2]Université de Toulouse, INP, UPS, EcoLab (Laboratoire Ecologie Fonctionnelle et Environnement), ENSAT, 18 chemin de Borderouge, 31326 Castanet Tolosan, France
[3]CNRS, EcoLab, 31326 Castanet Tolosan, France

***Corresponding author: gsaeidi@cc.iut.ac.ir**

Abstract

Development of cadmium (Cd) tolerant cultivars in legumes is important for crop production in contaminated soils. This research was carried out to quantify Cd-tolerance of 27 accessions from a core-collection of the model legume, *Medicago truncatula*. Root growth, relative root growth (Cd-treated /untreated seedlings) and tolerance index (difference of root growth between Cd-treated and untreated seedlings) were considered as the indices of tolerance to this toxic metal. Cadmium significantly and considerably decreased root growth, root fresh weight, root and shoot dry weight, but increased shoot water content and root diameter. Root growth was most affected by Cd and decreased to 59, 57 and 60% of control treatment after 2, 4 and 6 days, respectively. The accessions significantly differed for seedling root growth in Cd-stress condition, tolerance index and relative root growth. These indices varied from 4.33 to 12.51 mm, 3.58 to 18.92 mm and 0.25 to 0.64 with broad-sense heritabilities of 61, 73 and 57%, respectively. Based upon the results of root growth in Cd-stress condition, accessions L144 (originated from Jordan) and L648 (originated from France) were recognized as the most tolerant and susceptible genotypes, respectively. In conclusion, the results indicated the existence of genetic variation for Cd- tolerance at seedling stage and root growth can be rapidly and simply used as a good index of tolerance in breeding programs.

Keywords: Genetic variation; Germplasm; Heavy metal; *Medicago truncatula* L.; Seedling growth.
Abbreviations: Cd cadmium; DM dry mass; FM fresh mass; GCV genetic coefficient of variation; LSD least significant difference; PCV phenotypic coefficient of variation; WC water content.

Introduction

Legumes are among the most economically important crop families, due to their high protein content. The annual legume *Medicago truncatula* L., commonly known as barrel medic is considered both as a model plant and a forage crop. Its diploid structure and comparatively small genome size (2n = 16 with about 550 Mbp), self-fertility nature to produce fixed genotypes, short life cycle, prolific seed production and its close relation to many economically important legumes (Barker et al., 1990; Colditz and Braun, 2010) has made this model plant suitable to study biology, development during microbial interactions and other aspects of legumes molecular genetics and genomics (Cook, 1999; Ronfort et al., 2006). Furthermore, the close genetic relationship and high genome similarity of *M. truncatula* with *M. sativa* (Choi et al., 2004) make it possible to use this species as a source of genes in alfalfa breeding (Sledge et al., 2005). *M. truncatula* is also grown as an important forage species, for example in Australia (Crawford et al., 1989) and its forage quality (Derkaoui et al., 1993) and dry matter yield (Zhu et al., 1998) is comparable to cultivated alfalfa. Cadmium (Cd) is an extremely dangerous heavy metal pollutant of the environment due to high solubility in water (Lockwood, 1976), its prompt uptake by plants, bioaccumulation through the food chain causing serious problems to human health and its long half-life which make it enormously persistent in the environment (Salt et al., 1995; DalCorso et al., 2010;). Although Cd is naturally a trace element in the soil, its concentration is increasing due to various agricultural, mining and industrial activities and also from the exhaust gases of automobiles (Foy et al., 1978) that caused Cd contamination becoming a serious environmental problem in the world (Davis, 1984; Nouairi et al., 2006). Cadmium has no known nutrient function and small quantities of this metal can negatively affect plant growth (Hall, 2000) and crop production and quality (Prasad, 1995; Wu et al., 2007). Cadmium strongly inhibits enzyme activities (Lockwood, 1976) and cell division (Rosas et al., 1984), causes chlorosis due to suppression of iron uptake (Das et al., 1997) and denatures proteins and creates oxidative stress (Prasad 1995; Benavides et al., 2005). Generally, germination and the early seedling growth stages are more sensitive to Cd toxicity, due to lack of some defence mechanisms at this stage (Prasad 1995; Das et al., 1997; Wahid et al., 2007). The problem of cadmium toxicity might be ameliorated through better management practices to reduce Cd input in the soil and through introduction of tolerant cultivars in contaminated areas. It has been described that growing tolerant cultivars in

polluted soil can be a way to reduce the harmful effects of excessive exposure to heavy metal ions in plants (Tyler et al., 1989). Finding of genetic variation for tolerance to different heavy metal toxicity has been emphasized in various crop species such as Cd in *Thlaspi caerulescens* (Roosens et al., 2003) and *Triticum aestivum* L. (Ci et al., 2010), Pb in *Triticum aestivum* L. (Awaad et al., 2010), Cr in *Oryza sativa* L. (Gyawali and Lekhak, 2006) and Al in *Medicago truncatula* (Sledge et al., 2005). It was demonstrated that Cd-tolerance is widely varied among plant species and the genotypes within the same species (Li et al., 1997; Verma et al., 2007; Wu et al., 2007). For example, legume crops are less tolerant to Cd toxicity than cereals and grasses and encounter strong inhibition of biomass production due to this toxicity (Inouhe et al., 1994). In addition, different degrees of tolerance have been found among different genotypes of the same species (Li et al., 1997; Roosens et al., 2003; Ci et al.,2010). Germplasm sources of *M. truncatula* with a high degree of tolerance to Cd can increase the chance of improving Cd-tolerance in alfalfa or possibly other legumes. Molecular tools make the possibility of using the identified genes in *M. truncatula* to improve tolerance of cultivated alfalfa either by genetic transformation or by high throughput DNA homology searches for the same or similar genes in alfalfa germplasm (Sledge et al., 2005). The objectives of this study were to study the effect of Cd on seedling growth, quantify the extent of genetic variation for Cd- tolerance toxicity within a new core-collection of the model legume, *M. truncatula* at seedling stage and to identify Cd-tolerant genotypes for the future use in genetic studies and breeding programs.

Results and Discussion

Cadmium effects on seedling growth traits

In order to study the response of different accessions of *M. truncatula* to Cd toxicity, various seedling growth parameters were assessed. Our results showed that Cd-stress had significant effect on all growth parameters, except shoot fresh weight, root water content, dry shoot/root ratio and fresh biomass (Table 2). Compared with non-stress conditions, Cd-stress considerably reduced seedling root growth, root fresh weight, root and shoot dry weight and dry biomass, but increased shoot water content, fresh shoot/root ratio and root diameter (Table 3). Significant inhibition of root and shoot growth due to Cd treatment implied that the seedlings were subjected to severe stress. These results also revealed that *Medicago truncatula* is a Cd-sensitive plant and were in agreement with other reports indicating a strong inhibitory effect of Cd on root length and the fresh and dry biomass of *M. truncatula* (Aloui et al., 2009; Xu et al., 2010), *Medicago sativa* (Peralta et al., 2001; Ortega-Villasante et al., 2005; Dražić et al., 2006) and other plant species (Poschenrieder et al., 1989; Roosens et al., 2003; Metwally et al., 2005; Wójcik et al., 2005; Nada et al., 2007; Groppa et al., 2008; Finger-Teixeira et al., 2010). In the present study, root diameter significantly increased due to Cd treatment which seems not to be associated with swelling of the root cells due to more water absorption, since Cd treatment showed no significant effect on root water content (Tables 2 and 3). In this study, seedling growth was strongly negatively affected by Cd treatment (Table 3). Plants are highly susceptible to Cd toxicity in early seedling growth that can be due to rapid uptake and accumulation of Cd in the root (Chen et al., 2003), high metabolic activity and root lignification (Donaldson, 2001; Finger-Teixeira et al., 2010), loss of cell viability (Finger-Teixeira et al., 2010), disturbance of cell division (Liu et al., 2003) and its effect on metabolism of growth regulators (Prasad, 1995). It was mentioned that exposure of seedlings to Cd caused important physiological changes such as cell death in *Medicago sativa* (Dražić et al., 2006), oxidative damage and disruption of auxin equilibrium in *Medicago truncatula* (Xu et al., 2010). In spite of decreasing shoot dry weight and dry biomass due to Cd-stress, there was no significant effect of Cd on shoot fresh weight and fresh biomass that could be due to increased shoot water content by Cd-stress (Table 3). Higher shoot water content due to Cd toxicity also increased fresh shoot/root ratio, but had no significant effect on dry shoot/root ratio (Table 3). Cadmium stress can affect on plant tissue water content (Prasad, 1995), however, our results were not in agreement with those reported in *Phaseolus vulgaris* L. where Cd-treated plants had lower water content than non-treated (Poschenrieder et al., 1989). Root growth linearly increased with time up to the end of the experiment (6 days after treatment) and all of its variation (99.9%) was significantly described by linear regression (Table 4). However, Cd treatment markedly decreased root growth to 59, 57 and 60% of control seedlings after 2, 4 and 6 days of growing, respectively (Table 3). These results are in agreement with other reports indicating that after 48h of exposure to 50µM Cd ($CdSO_4$), root growth in *Medicago truncatula* seedlings decreased to 57% of untreated control plants (Xu et al., 2010). These results indicated that root growth in *Medicago truncatula* is sensitive and considerably affected by Cd. Root growth is characterized by high metabolic activity and more affected than shoot growth by Cd-stress (Belimov et al., 2003; Tiryakioglua et al., 2006). More sensitivity of roots might be related to the fact that they are the first organs to be in contact with Cd and are the primary site of its accumulation (Kevrešan et al., 2003; Nada et al., 2007), which can cause excessive production of monolignols forming lignin to solidify the cell wall and restricts seedling root growth (Finger-Teixeira et al., 2010).

Genetic variability for Cd-tolerance

The results of analysis of variance showed the significant effect of the accessions on all of the studied traits (Table 2). These results along with the coefficients of variation (Table 5) indicated the existence of genetic variability for those seedling growth parameters among the accessions. However, significant interaction between the accessions and Cd treatments for root growth (Table 2) implied that the accessions had different response of root growth to Cd-stress and generally the accessions with higher root growth were more affected (Fig. 1). This was also in agreement with the high positive correlation ($r = 0.95^{**}$) which was found between tolerance index and root growth in control condition. The sensitivity of root growth to Cd-stress suggests that this trait can be used as a good indicator of Cd toxicity in *Medicago truncatula*. Inhibition of root elongation is among the most sensitive responses to Cd exposure and occurs more rapidly than most of other physiological reaction (Schutzendubel et al., 2001). Therefore, supplying the Cd ions with simple Cd salt solutions and quantification of its inhibitory effects on root elongation is the most commonly used method for monitoring Cd toxicity (Prasad, 1995). Root growth and/or indices constructed by root growth have been used as the indicators of toxicity to Cd or other heavy metals in different plant species (Metwally et al., 2005; Ortega-Villasante et al., 2005; Sledge et al., 2005; Dražić et al., 2006; Groppa et al., 2008). The accessions highly differed for

Table 1. Country of origin for the accessions used in this study.

Accession	Origin	Accession	Origin
L 144	Jordan	L 542	Algeria
L 154	Italy	L 543	Algeria
L 163	Syria	L 544	Spain
L 174	Cyprus	L 545	Spain
L 198	Libya	L 549	France
L 213	Morocco	L 552	France
L 239	Morocco	L 554	France
L 245	Jordan	L 557	Greece
L 263	Israel	L 648	France
L 290	Turkey	L 679	France
L 321	Italy	L 734	Algeria
L 368	Algeria	L 736	Algeria
L 369	Pottugal	L 738	Australia
L 530	France		

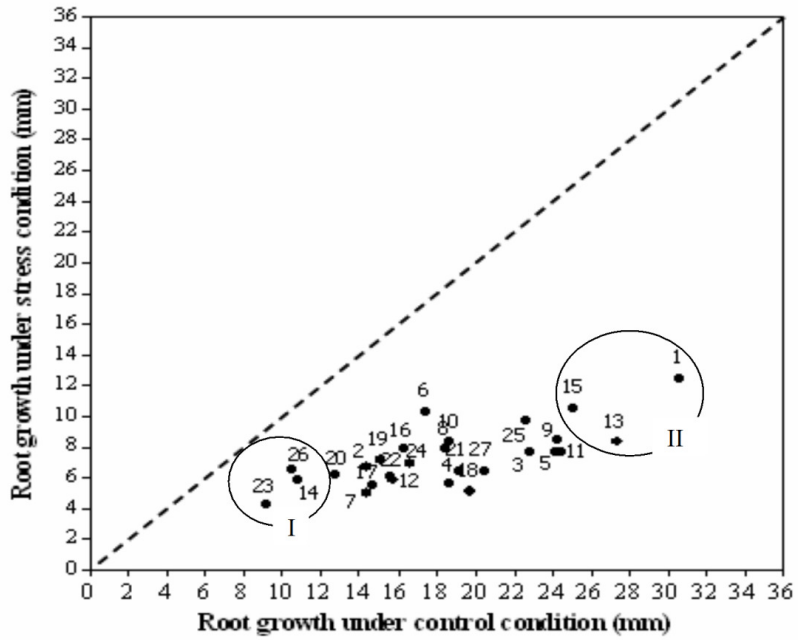

Fig 1. Different responses of the accessions for root growth to Cd-stress condition. I: more susceptible accessions 23 (L648), 14 (L530), 26 (L736); II: more tolerant accessions 1 (L144), 13 (L369), 15 (L542).

root growth in stress condition, tolerance index and relative root growth which varied from 4.33 to 12.51mm, 3.58 to 18.92mm and 0.25 to 0.64, respectively (Table 6). These results indicate the occurrence of variability for Cd-tolerance in this collection in which most of the observed variation for these tolerance indices was due to genetic factors, since they showed relatively high genetic coefficients of variation and broad-sense heritabilities (Table 6). Although genetic differences for Cd-tolerance have been reported in other plant species such as *Thlaspi caerulescens* (Roosens et al., 2003; Wójcik et al., 2005), *Pisum sativum* L. (Belimov et al., 2003; Metwally et al., 2005) and *Hordeum vulgare* (Tiryakioglua et al., 2006), this has not been reported in *M. truncatula*. Knowledge about the genetic variability for Cd-tolerance in germplasm of a plant species is important not only for studying of mechanisms in plant-metal interactions, but also in genetic studies and providing genetic material for developing tolerant cultivars. Both relative root growth and tolerance index indicated that the accessions L736 and L648 were more tolerant than the others and L545 and L369 were more susceptible (Table 7). These results and significant correlation coefficient between the results of relative root growth and tolerance index ($r = -0.75^{**}$) indicated that these two indices were comparable in terms of discriminating susceptible and tolerant genotypes; however, there was no agreement between the results of these two indices with absolute root growth in stress condition (Table 7). Accession L144 which had the highest root growth in Cd-stress condition was categorized as susceptible genotype based upon the relative root growth and tolerance index; however, accession L648 with the lowest root growth in Cd treatment, was recognized as a tolerant genotype based on these two indices (Table 7). Conversely, the recognized tolerant accessions based upon relative root growth and tolerance index, had very low root growth in both stress and non-stress conditions (Table 7). These results were also in agreement with the correlation coefficients found between root growth in stress condition with the relative root growth ($r = 0.07$) and tolerance index ($r = 0.49^{*}$). Relative root growth has been previously used as an indicator of Al tolerance for evaluation of *M. truncatula* germplasm (Sledge et al., 2005) and quantifying toxicity to Cd (Xu et al., 2010), and absolute root

Table 2. Results of analysis of variance for different traits.

Source of variation	DF	*F*- Values													
		Root growth after 2d	Root growth after 4d	Final root growth	Root fresh weight	Shoot fresh weight	Root dry weight	Shoot dry weight	Root water content	Shoot water content	Shoot/ Root ratio (fresh)	Shoot/ Root ratio (dry)	Fresh biomass	Dry biomass	Root diameter
Replication	2	10**	5.7**	1.6ns	4.6*	26.0**	6.7**	20.2**	9.7**	5.1**	41.1**	0.9ns	11.7**	19.0**	38.3**
Treatment (Tr)	1	287**	316**	438**	4.3*	0.1ns	5.5*	8.6**	0.1ns	19.8**	14.6**	0.6ns	0.3ns	8.3**	7.2**
Line (L)	26	3.2**	4.2**	6.1**	5.0**	14.0**	4.8**	12.2**	2.1**	1.8*	7.7**	3.8**	11.2**	11.5**	3.5**
Tr*L	26	1.4ns	1.8*	2.4**	0.6ns	0.9ns	1.5ns	0.9ns	1.5ns	1.2ns	1.1ns	1.3ns	0.8ns	0.9ns	0.6ns
Covariate [a]	1	0.1ns	0.1ns	0.2ns	11.9**	12.0**	0.1ns	13.5**	0.3ns	0.4ns	2.4ns	0.3ns	10.8**	9.1**	5.6*
Covariate [b]	1	3.5ns	3.7ns	1.7ns	39 **	1.8ns	16.4**	0.1ns	1.8ns	0.8ns	42.3**	24.3**	10.2**	1.2ns	0.8ns
Error	104	7.7†	9.6	11.5	2.6	13.9	0.01	0.1	1.1	0.7	0.2	1.9	22.4	0.2	0.01

*, ** Significant at 0.05 and 0.01 level of probability, respectively; ns, not significant. [a] Seed weight. [b] Initial root length.
† The numerical values for source of variation of error are mean squares.

Table 3. Means (± SE) of different traits under both control and Cd-stress conditions.

Condition	Traits													
	Root growth after 2d (mm)	Root growth after 4d (mm)	Final root growth (mm)	Root fresh weight (mg) †	Shoot fresh weight (mg) †	Root dry weight (mg) †	Shoot dry weight (mg) †	Root water content (%)	Shoot water content (%)	Shoot/Root ratio (fresh)	Shoot/Root ratio (dry)	Fresh biomass (mg) †	Dry biomass (mg) †	Root diameter (mm)
Control	12.48a	15.12a	18.47a	10.08a	29.51	0.41a	2.32a	95.89	92.18b	3.02b	6.09	39.60	2.74a	1.09b
	±0.46	±0.55	±0.70	±0.26	±0.74	±0.02	±0.07	±0.14	±0.09	±0.08	±0.20	±0.87	±0.07	±0.01
Cd - Stress	5.09b	6.46b	7.33b	9.56b	29.62	0.39b	2.16b	95.90	92.76a	3.19a	5.93	39.19	2.55b	1.13a
	±0.29	±0.31	±0.28	±0.23	±0.79	±0.01	±0.06	±0.13	±0.11	±0.09	±0.21	±0.91	±0.08	±0.01

In each column, means followed by different letters are significantly different at the 0.05 or 0.01 level of probability using the *F* –test. † For eight seedlings.

Table 4. Results of analysis of variance for root growth over different times.

Source of variation	DF	Mean squares
Replication (R)	2	135.80**
Treatment (Tr)	1	9980.00**
Line (L)	26	123.50**
Tr*L	26	45.90*
Error 1	105	25.28
Time (T)	2	685.60**
Linear	1	1370.90**
Quadratic	1	0.25ns
T*Tr	2	146.30**
T*L	52	5. 35**
T*Tr*L	52	4.00**
R*T	4	8.16**
Error 2	212	1.87
Covariate †	1	1.98ns

*, ** Significant at 0.05 and 0.01 level of probability, respectively; ns, not significant. † Seed weight.

growth has been used as an index of different heavy metal toxicity including Cd in *Medicago sativa* (Peralta et al., 2001; Dražić et al., 2006). Generally, selection of those genotypes with desired root growth in both stress and non-stress conditions are preferred to develop genetically tolerant cultivars to Cd toxicity. In our study, accessions L648 and L144 had the lowest and highest root growth, respectively in both stress and non-stress conditions. Furthermore, significant and positive correlation coefficient for root growth between Cd-stress and control ($r = 0.71^{**}$) indicated that genotypes with good root growth in Cd-stress also had a good performance in non-stress condition. Significant correlation was found between final root growth (after 6 days) with those obtained after 2 days ($r = 0.90^{**}$) and 4 days ($r = 0.98^{**}$) of Cd treatment. These correlations showed that the results of root growth after 2, 4 and 6 days of Cd treatment were comparable and furthermore indicate that root growth was rapidly affected by Cd toxicity. These results suggest the possibility of quantifying tolerance to Cd-stress after a short-time treatment which was in agreement with previous reports indicating very early phytotoxic effects of Cd on *Medicago sativa* (Ortega-Villasante et al., 2005; Dražić et al., 2006). Therefore, it seems that seedling root growth can be used as a good index of Cd-tolerance in breeding programs of *Medicago truncatula* and offers the possibility of low-cost screening of a large number of genotypes, since this method is simple, rapid and easy to perform (Prasad, 1995).

Materials and methods

Plant materials

Twenty seven accessions from a 32-entry nested core-collection of *M. truncatula* were used in this study. This core-collection has been built up based on the patterns of microsatellite molecular markers diversity in a collection of 346 inbred lines with a broad scale of geographical origin (Ronfort et al., 2006). The accessions used in our study (Table 1) were originated from different countries (http://www1.montpellier.inra.fr/BRC-MTR/accueil.php).

Experimental procedure

Seeds of the accessions were scarified by fine sand paper and then cold treated by placing on moist filter paper in a Petri dish and incubated at 4°C for 4 days. After cold treatment, seeds were germinated in the dark at room temperature for 12h. Eight uniformly germinated seedlings of each accession were placed on filter paper in a Petri dish (12 × 12cm) and considered as an experimental unit. A factorial experiment with two factors of Cd treatment (control and Cd-stress conditions) and genotypes (27 accessions) in a randomized complete block design with three biological replications was used in this study. In control and Cd-stress conditions, 6ml of distilled water and a solution of 100µM $CdCl_2$ was added to each Petri dish, respectively. Petri's were then sealed with a strip of Parafilm to prevent evaporation and wrapped with Aluminum paper to provide dark conditions for the roots of seedlings. Seedlings were grown in a growth room at 25°C /20°C (day/night) with 18h/6h (day/night) photoperiod and 75% relative humidity. Seed weight and root length of the germinated seedlings at the start of experiment were recorded to use as covariates in statistical analysis. During the experiment, root length of the seedlings was measured three times at 48h intervals. The data for root diameter of the seedlings were collected at the end of the experiment (after 6 days). Root length and diameter of the seedlings were measured by photographing and image processing program (Image J). After 6 days of growing in growth room, seedlings were washed with distilled water and dried on tissue paper to measure fresh and dry weight of roots and shoots. Roots and shoots dry weights were determined after oven-drying at constant temperature of 74°C for 4 days. Shoot and root water content (WC) were calculated base on the formula $WC = \frac{FM - DM}{FM} \times 100$ and FM and DM designate for fresh mass and dry mass, respectively.

Relative root growth was calculated by dividing the root growth under Cd treatment by the one under the control condition (Sledge et al., 2005). The tolerance index was calculated as ($Y_c - Y_s$) in which Y_c and Y_s denote for the root growth under control and stress conditions, respectively (Rosielle and Hamblin, 1981).

Table 5. Range, accession with minimum and maximum amount of the trait, LSD value, coefficient of variation (CV) and population mean for different traits over two conditions.

Trait	Range	Accession with minimum amount	Accession with maximum amount	LSD (0.05)	CV (%)	Population mean
Root growth after 2d (mm)	5.17-13.51	L648	L144	3.18	52.90	8.78
Root growth after 4d (mm)	6.11-16.20	L648	L144	3.54	53.20	10.79
Final root growth (mm)	6.71-21.53	L648	L144	3.87	54.00	12.90
Root fresh weight (mg) †	7.32-13.33	L530	L545	1.84	51.40	9.80
Shoot fresh weight (mg) †	20.63-41.73	L738	L545	4.27	51.20	29.57
Root dry weight (mg) †	0.23- 0.57	L554	L263	0.12	53.00	0.40
Shoot dry weight (mg) †	1.43-3.16	L738	L545	0.40	22.20	2.24
Root water content (%)	94.95 – 96.99	L239	L738	1.21	50.40	95.90
Shoot water content (%)	91.57 – 93.18	L154	L734	0.94	50.40	92.50
Shoot/Root ratio (fresh)	2.24-4.06	L738	L154	0.54	52.00	3.10
Shoot/Root ratio (dry)	4.13-7.98	L552	L245	1.57	52.10	6.00
Fresh biomass (mg) †	28.66-55.06	L554	L545	5.42	51.70	39.39
Dry biomass (mg) †	1.70-3.67	L738	L545	0.46	52.70	2.64
Root diameter (mm)	1.02-1.25	L738	L213	0.08	50.70	1.11

† For eight seedlings.

Table 6. Range, accession with minimum (Min) and maximum (Max) amount, LSD value, mean squares for accessions, estimated variance components, phenotypic coefficient of variation (PCV), genetic coefficient of variation (GCV) and broad-sense heritability (H_b^2) for root growth at different times and Cd-tolerance indices.

Conditions	Trait	Range	Accession with Min	Accession with Max	LSD (0.05)	Mean squares for accessions	Variance Components			GCV (%)	PCV (%)	H_b^2 (%)
							$V_g^{\dagger}$	V_e	V_{ph}			
Control	Root growth after 2d (mm)	7.62 - 17.86	L648	L321	5.45	25.20**	4.70	3.70	8.41	17	23	56
Control	Root growth after 4d (mm)	8.62 – 20.98	L648	L144	6.20	46.07**	10.57	4.78	15.35	23	27	69
Control	Final root growth (mm)	9.08 - 30.55	L648	L144	6.81	86.06**	22.93	5.76	28.69	26	29	80
Cd - stress	Root growth after 2d (mm)	2.32 -10.24	L174	L144	3.29	9.94**	1.97	1.35	3.32	28	35.8	59
Cd - stress	Root growth after 4d (cm)	3.59 - 11.72	L648	L144	3.35	10.34**	2.07	1.39	3.46	22	28.8	61
Cd - stress	Final root growth (mm)	4.33 -12.51	L648	L144	3.33	10.56**	2.14	1.38	3.52	20	25.6	61
-	Tolerance index	3.58 - 18. 92	L736	L369	6.19	52.41**	12.72	4.75	17.47	32	38	73
-	Relative root growth	0.25 - 0.64	L545	L736	0.18	0.025**	0.005	0.004	0.008	17	23	57

** Significant at 0.01 level of probability. † V_g, V_e and V_{ph} stand for genetic, environmental and phenotypic variance, respectively.

Table 7. The accessions with maximum (Max) and minimum (Min) root growth, relative root growth and tolerance index.

Accession	Final root growth in control condition (mm)	Final root growth in stress condition (mm)	Relative root growth	Tolerance index (mm)
L736	10.83c	6.55c	0.64a (Max)	3.58b (Min)
L545	19.73b	5.04c	0.25b (Min)	14.69a
L144	30.55a (Max)	12.51a (Max)	0.41b	18.04a
L648	9.08c (Min)	4.33c (Min)	0.52a	4.75b
L369	27.32a	8.40b	0.34b	18.92a (Max)
Population mean	18.47	7.33	0.40	11.13

In each column, means followed by the same letter are not significantly different at the 0.05 level of probability using the LSD test.

Statistical analysis

The data were subjected to analysis of variance based on a factorial combination in a randomized complete block design model, using PROC GLM in SAS (2002). However, for the root growth where data were collected over three times, the data were analysed based on the model of a factorial-split in time and time was considered as sub factor. Seed weight and root length of germinated seedling at the start of experiment were used as covariates in analysis of variance of the data. The least significant difference test (LSD) was applied to determine the statistically differences between those means with significant *F*-value. Phenotypic coefficient of variation (PCV), genetic coefficient of variation (GCV), and broad-sense heritability (H_b^2) were estimated for root growth and tolerance indices based upon their variance components (Burton and DeVane, 1953; Miller et al., 1958). To estimate the variance components, accessions were considered random.

Conclusion

Generally, the results of this study revealed that *M. truncatula* is a Cd-sensitive plant and its seedlings growth is rapidly and severely affected by Cd toxicity. Inhibition of root growth was a highly sensitive response to this stress and can be used as a good indicator of Cd toxicity and tolerance for screening of breeding materials. Also, there is considerable genetic variability for Cd-tolerance at seedling stage among the accessions of this core-collection which can provide the opportunity of selection for more tolerance to Cd toxicity-in *M. truncatula*. Genetic improvement of Cd-toleranc in germplasm resources of this plant species may increase the chance of developing tolerant cultivars in other related crop species such as alfalfa or other legumes for a safe production in contaminated soils. For breeding purposes, root growth in Cd-stress conditions might be more suitable and simple to use as an index of tolerance for screening genetic materials which usually are large in number. Correlation coefficients showed that selection for Cd-tolerance based on absolute root growth can genetically improve this trait for both stress and non-stress environment, but this may not be relevant to selection based upon the relative root growth or tolerance index. However, for genetic or physiological studies, using the relative root growth or tolerance index might be more favored for characterizing tolerant and susceptible genotypes.

Acknowledgements

The first author gratefully acknowledges Isfahan University of Technology, Iran for the financial support of his sabbatical leave in EcoLab, ENSAT-INP. France. The authors also appreciate the practical helps of Reza Estiri, Anastasia Shushova and Jun Xie.

References

Aloui A, Recorbet G, Gollotte1 A, Robert F, Valot B, Gianinazzi-Pearson V, Aschi-Smiti S, Dumas-Gaudot E (2009) On the mechanisms of cadmium stress alleviation in *Medicago truncatula* by arbuscular mycorrhizal symbiosis: A root proteomic study. Proteomics 9:420–433

Awaad HA, Youssef MAH, Moustafa ESA (2010) Identification of genetic variation among bread wheat genotypes for lead tolerance using morpho- physiological and molecular markers. J Am Sci. 6 1142-1153

Barker DG, Bianch S, Blondon F, Dattée Y, Duc G, Essad S, Flament P, Gallusci P, Génier G, Guy P, Muel X, Tourneur J, Dénarié J, Huguet T (1990) Medicago truncatula, a model plant for studying the molecular genetics of the Rhizobium-legume symbiosis. Plant Mol Biol Rep. 8:40–49

Belimov AA, Safronova VI, Tsyganov VE, Borisov AY, Kozhemyakov AP, Stepanok VV, Martenson AM, Gianinazzi-Pearson V, Tikhonovich IA (2003) Genetic variability in tolerance to cadmium and accumulation of heavy metals in pea (*Pisum sativum* L.). Euphytica 131:25-35

Benavides MP, Gallego SM, Tomaro ML (2005) Cadmium toxicity in plants. Braz J Plant Physiol. 17:21-34

Burton G, DeVane EH (1953) Estimating heritability in tall fescue (*Festuca arundinacea*) from replicated clonal material. Agron J. 45 478-481

Chen YX, He YF, Yang Y, Yu YL, Zheng SJ, Tian GM, Luo YM, Wong MH (2003) Effect of cadmium on nodulation and N2-fixation of soybean in contaminated soils. Chemosphere 50 781–787

Choi HK, Kim DJ, Uhm T, Limpens E, Lim H, Mun JH, Kalo P, Penmetsa RV, Seres A, Kulikova O, Roe BA, Bisseling T, G.B. Kiss, Cook DR (2004) A sequence-based genetic map of *Medicago truncatula* and comparison of marker colinearity with *M. sativa*. Genetics 166 1463–1502

Ci D, Jiang D, Dai T, Jing Q, Cao W **(2011)** Variation in cadmium tolerance and accumulation and their relationship in wheat recombinant inbred lines at seedling stage. Biol Trace Elem Res. 142 807-818

Colditz F, Braun HP (2010) Medicago truncatula proteomics. J Proteomics 73 1974-1985

Cook DR (1999) Medicago truncatula-a model in the making. Curr Opin Plant Biol. 2 301-304

Crawford EJ, Lake AWH, Boyce KG (1989) Breeding annual *Medicago* species for semiarid conditions in southern Australia. Adv Agron. 42:399–437

DalCorso G, Farinati S, Furini A (2010) Regulatory networks of cadmium stress in plants. Plant Signal Behav. 5 663-667

Das P, Samantaray S, Rout GR (1997) Studies on cadmium toxicity in plants: a review. Environ Pollut. 98 29–36

Davis RD (1984) Cadmium-A complex environmental problem. Part II, Cell Mol Life Sci. 40 117–126

Derkaoui M, Caddel JL, Rommann LL (1993) Forage quality in annual *Medicago spp*. Agric Mediterranea 123 86–91
Donaldson LA (2001) Lignification and lignin topochemistry-an ultrastructural view. Phytochemistry 57 859–876
Dražić G, Mihailović N, Lojić M (2006) Cadmium accumulation in *Medicago sativa* seedlings treated with salicylic acid. Biol Plantarum 50 239-244
Finger-Teixeira A, Ferrarese MLL, Soares AR, Silva D, Ferrarese-Filhon O (2010) Cadmium-induced lignification restricts soybean root growth. Ecotox Environ Safe. 73 1959–1964
Foy CD, Chaney RL, White MC (1978) The physiology of metal toxicity in plants. Ann Rev Plant Physio. 29 511-566
Groppa MD, Zawoznik MS, Tomaro ML, Benavides MP (2008) Inhibition of root growth and polyamine metabolism in sunflower (*Helianthus annuus*) seedlings under cadmium and copper stress. Biol Trace Elem Res. 126 246–256
Gyawali R, Lekhak HD (2006) Chromium tolerance of rice (*Oryza sativa* L.) cultivars from Kathmandu valley. Nepal, Scientific World 4 102-108
Hall JL (2000) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot. 53 1–11
Inouhe M, Ninomiya S, Tohoyama H, Joho M, Murayama T (1994) Different characteristics of roots in the cadmium-tolerance and Cd-binding complex formation between mono- and dicotyledonous plants. J Plant Res. 107 201–207
Kevrešan S, Kiršek S, Kandrač J, Petrovič N, Kelemen D (2003) Dynamics of cadmium distribution in the intercellular space and inside cells in soybean roots, stems and leaves. Biol Plantarum 46 85–88
Li YM, Chaney RL, Schneiter AA, J.F. M, Elias M, Elias EM, Hammond JJ (1997) Screening for low grain cadmium phenotypes in sunflower, durum wheat and flax. Euphytica 94 23–30
Liu D, Jiang W, Gao X (2003) Effects of cadmium on root growth, cell division and nucleoli in root tip cells of garlic. Biol Plantarum 47 79-83
Lockwood MP (1976) Effects of Pollutants on Aquatic Organisms. Cambridge University Press, New York
Metwally A, Safronova VI, Belimov AA, Dietz KJ (2005) Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. J Exp Bot. 56 167-178
Miller PA, Williams JC, Robinson HF, Comstock RE (1958) Estimates of geotypic and environmental variances and covariances in upland cotton and their implications in selection. Agron J. 50 126-131
Nada E, Ferjani BA, Rhouma A, Bechir BR, Imed M, Makki B (2007) Cadmium-induced growth inhibition and alteration of biochemical parameters in almond seedlings grown in solution culture. Acta Physiol Plant. 29 57–62
Nouairi I, Ammar WB, Youssef NB, Daoud DBM, Ghorbel MH, Zarrouk M (2006) Comparative study of cadmium effects on membrane lipid composition of *Brassica juncea* and *Brassica napus* ‘‘leaves’’. Plant Sci. 170 511–519
Ortega-Villasante C, Rellan-Alvarez R, Campo FFD, Carpena-Ruiz RO, Hernandez LE (2005) Cellular damage induced by cadmium and mercury in *Medicago sativa*. J Exp Bot. 56 2239-2251
Peralta JR, Gardea-Torresdey JL, Tiemann KJ, Gomez E, Arteaga S, Rascon E, Parsons JG (2001) Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (*Medicago sativa* L.). Bull Environ Contam Toxicol. 66 727–734
Poschenrieder C, Gunse B, Barcelo J (1989) Influence of cadmium on water relations, stomatal resistance, and abscisic acid content in expanding bean leaves. Plant Physiol. 90 1365-1371
Prasad MNV (1995) Cadmium toxicity and tolerance in vascular plants. Environ Exp Bot. 35:525-545
Ronfort J, Bataillon T, Santoni S, Delalande M, David JL, Prosperi JM (2006) Microsatellite diversity and broad scale geographic structure in a model legume: building a set of nested core collection for studying naturally occurring variation in *Medicago truncatula*. BMC Plant Biol. 6 28
Roosens N, Verbruggen N, Meerts P, Ximénez-Embún P, Smith JAC (2003) Natural variation in cadmium tolerance and its relationship to metal hyperaccumulation for seven populations of *Thlaspi caerulescens* from western Europe. Plant Cell Environ. 26 1657–1672
Rosas I, Carbajal ME, Gomez-Arroyo S, Belmont R, Villalogos-Pietrini R (1984) Cytogenetic effects on cadmium accumulation on water hyacinth (*Eichornia crassipes*). Environ Res. 33 386-395
Rosielle AA, Hamblin J (1981) Theoretical aspects of selection for yield in stress and non-stress environments. Crop Sci. 21 943-946
Salt DE, Blaylock M, Kumar NPBA, Dushenkov V, Ensley BD, Chet I, Raskin I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Nat Biotechnol. 13 468–474
SAS, Institute (2002) SAS Users Guide: Statistics. Version 9. SAS Inc, Cary, North Carolina, USA.
Schutzendubel A, Schwanz P, Teichmann T, Gross K, Langenfeld-Heyser R, Godbold DL, Polle A (2001) Cadmium-induced changes in antioxidative systems, hydrogen peroxide content, and differentiation in Scots Pine Roots. Plant Physiol. 127:887–898
Sledge MK, Pechter P, Payton ME (2005) Aluminum tolerance in *Medicago truncatula* germplasm. Crop Sci. 45 2001-2004
Tiryakioglua M, Ekerb M, Ozkutluc F, Hustedd S, Cakmaka I (2006) Antioxidant defence system and cadmium uptake in barley genotypes differing in cadmium tolerance. J Trace Elem Med Bio. 20 181–189
Tyler G, Pahlsson AMB, Bengtsson G, Baath E, Tranvik L (1989) Heavy metal ecology and terrestrial plants, micro-organisms and invertebrates: a review. Water Air Soil Poll. 47 189-215
Verma P, George KV, Singh HV (2007) Modeling cadmium accumulation in radish, carrot, spinach and cabbage. Appl Math Model. 31 1652–1661
Wahid A, Ghani A, Ali I, Ashraf MY (2007) Effects of cadmium on carbon and nitrogen assimilation in shoots of mungbean [*Vigna radiata* (L.) Wilczek] seedlings. J Agron Crop Sci. 193 357–365
Wójcik M, Vangronsveld J, Tukiendorf A (2005) Cadmium tolerance in *Thlaspi caerulescens* I. Growth parameters, metal accumulation and phytochelatin synthesis in response to cadmium. Environ Exp Bot. 53 151–161
Wu F, Zhang G, Dominy P, Wu H, Bachir DM (2007) Differences in yield components and kernel Cd accumulation in response to Cd toxicity in four barley genotypes. Chemosphere 70 83–92
Xu J, Wang W, Yin H, Liu X, Sun H, Mi Q (2010) Exogenous nitric oxide improves antioxidative capacity and reduces auxin degradation in roots of *Medicago truncatula* seedlings under cadmium stress. Plant Soil 326 321–330
Zhu YP, Sheaffer CC, Russelle MP, Vance CP (1998) Dry matter accumulation and dinitrogen fixation of annual *Medicago* species. Agron J. 90 103–108