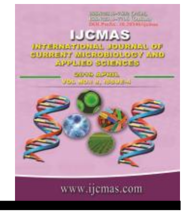
Int.J.Curr.Microbiol.App.Sci (2019) *8*(4): 1338-1349

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 8 Number 04 (2019)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2019.804.156

Effect of Zn Application on Root Growth Parameters and Shoot Dry Matter Content of Some Cowpea Genotypes

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Keywords

Zinc, Cowpea genotypes, Root and shoot characteristics

Article Info

Accepted:
12 March 2019
Available Online:
10 April 2019

A B S T R A C T

A Pot experiment was conducted with nine cowpea genotypes and two levels of zinc in sand culture medium to study the response of different cowpea genotypes to zinc fertilization in root characteristics. The highest average total root length (944.9 cm), surface area (227.4 cm^2), diameter (0.75 mm) and root volume (0.71 cm^3) were recorded in V1.The highest average number of root tips was observed in V11 (1676.1). The highest average number of forks (7085.0) and number of crossings (1194.8) was noted in V10. The highest average cation exchange capacity of roots (0.398 meq g^{-1}) was recorded in V5.

Introduction

Zinc is essential for the normal healthy growth and reproduction of plants. Plants absorb Zn as zinc ions (Zn^{+2}). Zinc sufficient plants contain 27 to 150 ppm Zn in mature tissues. Zinc plays a key role as a structural constituent or regulatory co-factor of a wide range of different enzymes and proteins in many important biochemical pathways and these are mainly concerned with: carbohydrate metabolism, both in photosynthesis and in the conversion of sugars to starch, protein metabolism, auxin (growth regulator) metabolism, pollen formation, the maintenance of the integrity of biological membranes, the resistance to infection by certain pathogens. Alloway (2004) reported that zinc is one of the trace elements which are essential for the normal healthy growth and reproduction of crop plants.

Differential responses of plants to Zn deficiency indicate the existence of genotypic variation for efficient utilization of native soil zinc. Genotypic variations in Zn efficiency have been associated with different mechanisms operating within the plant and in the rhizosphere. Some plant genotypes possess mechanisms for efficient acquisition of Zn from soils low in Zn. These mechanisms include: increased Zn bioavailability in the rhizosphere due to release of root exudates, higher Zn uptake by roots, and efficient utilization and (re)-translocation of Zn (Hart *et al.,* 1998). In the present study, we attempted to identify genotypic variability in terms of accumulation and utilization of zinc for plant growth by studying the root parameters of some cowpea genotypes.

Materials and Methods

A bulk sample of quartz sand was thoroughly screened and washed several times in tap water to remove dirt. Finally, the quartz sand was soaked in dilute HCl and repeatedly washed by deionized water till the effluent water reached a pH around 6.0 and finally kept for air-drying. After air-drying, sand was filled in ½ kg plastic pots. The experiment was laid in a completely randomized design replicated twice with two level of Zn (i) + Zn: 0.05 mg L^{-1}, ii) –Zn: 0.0 mg L^{-1}).

Seeds of nine contrasting cowpea genotypes (V1, V2, V3, V5, V6, V7, V9, V10 and V11) were pre-germinated in towel paper in a seed germinator. Three pre-germinated seedlings (4 days old) of each genotype were transplanted to the pots in duplicate under green house conditions. For the next few days, the pots were watered with distilled water to keep them moistened. The details of genotypes selected for study were V1= Pant Lobia-1 (IT 205-1), V2= Pant Lobia-2 (IT1042-3), V3 = Pant Lobia-3 (IT889-1), V5 = PGCP 12 (IT 82E-18), V6 = PGCP 15(PL-10 K1-1-4-1-3), V7 = PGCP 16 (PGCP-5 × PGCP-1), V9= PGCP-32(PGCP-3 × PGCP-6 13), V10 = PGCP-33 (PGCP-8 × PGCP-22) and V11= PGCP-34 (PGCP-12 × PGCP-4-17). Prior to preparation of Hoagland solution, the stock solutions of NH_4NO_3, $CaCl_2.2H_2O$, KNO_3, KH_2PO_4, $MgSO_4.7H_2O$ were made by taking 80, 147.1, 101.1, 136.1 and 246.5 g L^{-1} respectively and stock solution of tracer elements H_3BO_3, $MnCl_2.4H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$ and $NaMoO_4$ were prepared by taking 2.8, 1.8, 0.05, 0.1 and 0.025 g L^{-1} respectively. To prepare Fe-EDTA solution, the pH of KOH solution (56.1 g L^{-1}) was adjusted to 5.5 using H_2SO_4 and then EDTA.2Na (10.4 g) and FeSO4.7H2O (7.8 g) were added to it and diluted to 1 L. This solution was considered as Fe-EDTA. The following amounts of stock solutions were added in 1 L volumetric flask and pH was adjusted to 7.0 using $Ca(OH)_2$ and then diluted to 1 L with distilled water to get the nutrient solution (Hoagland solution). NH_4NO_3 = 6mL, $CaCl_2.2H_2O$ = 7 mL, KNO_3 = 5 mL, KH_2PO_4 = 2 mL, $MgSO_4.7H_2O$ = 2 mL, Trace elements = 1 mL and Fe-EDTA = 1 mL. This solution was designated as '+ Zn' and when this solution prepared without $ZnSO_4.7H_2O$ it was designated as '- Zn'.

A 40 mL of '+Zn' and '–Zn' Hoagland solution was added to the pots on 5 days after transplanting (DAT). The application of Hoagland solution was practiced three times a week and continued till the crop attained physiological maturity and then at this growth stage plants were uprooted for chemical analysis in roots and shoots. Uprooted plants were thoroughly and sequentially washed, first with tap water then in dilute HCl (0.1 N) and finally in deionized water. The roots were separated from shoots. Roots and shoots were soaked between bloating paper to remove moisture and their fresh weights were recorded. Washed plant roots were stored in refrigerator until scanned by scanner and one root sample of each cowpea genotype was stored in deep freezer for the estimation of root cation exchange capacity. Plant shoots and remaining roots samples were kept for oven drying at 60˚C for 48 h. The oven dry weight of shoots and roots were recorded for each pot. The oven dried root and shoot samples were finally crushed with the help of pestle and mortar and stored in paper-bags for chemical analysis. The details of chemical analysis performed are given below. The oven dry weight of shoots and roots were recorded for each pot. The oven dried root and shoot samples were finally crushed with the help of pestle and mortar and stored in paper-bags for chemical analysis.

Results and Discussion

Root length

The data on total root length (cm) of all nine cowpea genotypes both under application of Zn (+Zn) and no application of Zn (-Zn) are presented in Table 1.

It is clearly seen from the data that the highest average total root length (944.9 cm) was recorded in V1 while it was lowest in V9 (475cm). The average total root length observed in V9 was at par with that of V2, V6 and V11.The main effect of Zn application indicated that application of Zn increased the average total root length significantly by 22.5 percent over no application of Zn. The interaction effect of genotypes and Zn level (V × Zn) had no statistically significant effect on total root length.

Surface area

The data on surface area of root (cm^2) of all nine cowpea genotypes under application of Zn (+Zn) and no application of Zn (-Zn) are presented in Table 2.

The data contained in the Table 4 clearly indicate that the highest average surface area of root was recorded in V1 with a value of 227.4 cm^2 while it was the lowest (64.8 cm^2) in V9. The average surface area of root noted in V9 was at par with that of V2, V6, V7, V10 and V11. The main effect of Zn level indicated that application of Zn significantly increased the mean surface area of roots in all cowpea genotypes by 43.8 percent over no application of Zn. The interaction effect of genotypes and Zn levels (V × Zn) had no statistically significant effect on root surface area.

Root diameter

The data pertaining to effect of Zn application on average diameter of root (mm) of all nine cowpea genotypes are presented in Table 3.

It is evident from the data that the highest average root diameter was recorded in V1 (0.75 mm) while it was the lowest (0.41 mm) in V11.

The average root diameter noted in V11 was at par with V2, V5, V6, V7, V9 and V10. The main effect of Zn levels on the average root diameter was statistically non-significant. The interaction effect of genotypes and Zn levels (V × Zn) also had no statistically significant effect on the average root diameter.

Root volume

The data pertaining to effect of Zn application on root volume of all nine cowpea genotypes are presented in Table 4.

The data presented in Table 6 clearly indicate that the highest average root volume (4.66 cm^3) was recorded in V1 while it was lowest inV9 (0.71 cm^3). The average root volume observed in V9 was at par with V2, V5, V6, V7, V10 and V11. As regard the main effect of Zn levels, application of Zn increased the average root volume significantly by 72.1 percent over no application of Zn.

The interaction effect of genotypes and Zn levels (V × Zn) had no statistically significant effect on root volume.

Root tips

The data on effect of Zn application on the number of root tips in all cowpea genotypes are presented in Table 5.

It is evident from the data that the highest average number of root tips was recorded in V11 (1676.1) while it was lowest (983.5) in V9. The main effect of zinc levels had no statistically significant effect on number of root tips in all cowpea genotypes. The interaction effect of zinc levels and genotypes (V × Zn) had no statistically significant influence on number of root tips in cowpea genotypes.

Number of forks

The data on effect of Zn application on the number of forks in roots of all nine cowpea genotypes are presented in Table 6.

It is evident from the data that the highest average number of forks (7085.0) was observed in V10 while it was the lowest in V9 (4297.1). The average number of forks recorded in roots of V9 was at par with V2, V3, V5, V6 and V11. The main effect of zinc levels indicated that Zn application increased the average number of forks in roots of cowpea genotypes significantly by 16.2 percent over no application of zinc. The interaction effect of zinc levels and genotypes (V × Zn) had no statistically significant effect on number of forks in roots of cowpea genotypes.

Number of crossings

The data on numbers of crossings in roots of all nine cowpea genotypes under the application of Zn (+Zn) and no application of Zn (-Zn) are presented in Table 7. The data contained in Table 9 clearly indicated that the highest average number of crossings (1194.8) was recorded in V10 while it was the lowest (716.4) in V9. The number of crossing noted in V9 was at par with V1, V2, V3, V5, V6 and V7. The main effect of Zn levels on the average number of crossings in roots of cowpea genotypes was statistically not significant. The interaction effect of zinc levels and genotypes (V × Zn) had no statistically significant influence on number of crossings in roots of cowpea genotypes

Cation exchange capacity

The data on root cation exchange capacity of all nine cowpea genotypes under the application of Zn (+Zn) and no application of Zn (-Zn) are presented in Table 8.

The data clearly indicated that the highest average cation exchange capacity of roots (0.398 meq g^{-1}) was recorded in V5 while it was lowest (0.317 meq g^{-1}) noted in V2. The average root cation exchange capacity noted in V2 was at par with V1, V3, V6 and V10. The average root cation exchange capacity values observed for V1 and V10 were numerically similar. As regard the main effect of zinc levels, Zn application decreased the average root cation exchange capacity significantly by 8.2 percent over no application of zinc. The interaction effect of genotypes and zinc levels (V × Zn) had statistically significant effect on root cation exchange capacity of cowpea genotypes. In the case of V9, the application of zinc brought a significant increase in root cation exchange capacity while in case of genotypes V5, V6 and V7, application of zinc significantly decreased the root exchange capacity in comparison to no application of zinc.

Root weight per plant

The data on root weight per plant (g) of all nine cowpea genotypes under the application of Zn (+Zn) and no application of Zn (-Zn) are presented in Table 9. It is evident from the data that the highest mean root weight per plant (0.190 g) was recorded in V10 while it was the lowest (0.105 g) in V2. The main effect of zinc levels had no statistically significant effect on the average root weight per plant. The interaction of genotypes and zinc levels (V × Zn) had statistically significant effect on root weight per plant. In the case of V6 and V9, Zn application increased the root weight per plant by 14.1 and 26.1 percent over no application of zinc, respectively. On the other hand, in the case of V1, Zn application decreased the root weight per plant by 21.3 percent in comparison to no application of zinc.

Shoot weight per plant

The data on shoot weight per plant (g) of nine cowpea genotypes under different Zn levels are presented in Table 10. It is evident from the data that the highest average shoot weight per plant (0.92 g) was recorded in V6 while the lowest average shoot weight per plant (0.49 g) was in V2. The average shoot weight per plant recorded in V2 was at par with V11. As regard the main effect of zinc levels, Zn application increased the average shoot weight per plant significantly by 11.6 percent over no application of zinc. The interaction effect of genotypes and zinc levels (V × Zn) had statistically significant effect on shoot weight per plant. A close perusal of data revealed that in case of genotypes V3, V6 and V9 the application of zinc significantly increased the shoot weight per plant while in rest of genotypes (V1, V2, V5, V7, V10 and V11) the shoot weight per plant was not significantly influenced by the application of zinc in comparison to no application of zinc.

Dry weight ratio in shoot and root

The data on dry weight ratio in shoot and root (g) of all nine cowpea genotypes under different Zn levels are presented in Table 11. It clearly apparent from the data that the highest average dry weight ratio in shoot and root (5.50 g) was recorded in V6 while it was the lowest in V11 (4.02 g). The average dry weight ratio in shoot and root noted in V11 was at par with V5. As regard the main effect of zinc levels, application of Zn increased the average dry weight ratio in shoot and root significantly by 10.9 percent in comparison to no application of zinc. The interaction effect of zinc levels and genotypes had statistically significant influence on dry weight ratio in shoot and root of cowpea genotypes. A close perusal of data revealed that application of zinc increased the dry weight ratio in shoot and root significantly in genotypes V1, V3 and V10 in comparison to no application of zinc while genotype V7 showed a slight decrease in the dry weight ratio in shoot and root with the application of Zn in comparison to no application of zinc.

As a rule under nutrient efficiency, the acquisition of nutrients by the roots plays the most important role (Gutschick, 1993). Efficiency in acquisition largely depends on root size and morphology. A large surface area (fine roots, long root hairs) is either an inherent property (e.g., grasses vs. legumes) or deficiency-induced trait (e.g., by P or N, but not K or Mg deficiency). It is of key importance for acquisition particularly of P, and most likely also ammonium, in upland soils (Marschner, 1998).

A significant effect of Zn application on average root length of cowpea with the application of Zn over no application of Zn showed that zinc is required for the synthesis of tryptophan, which is most likely precursor for the biosynthesis of IAA and responsible for growth parameters. Impairment in auxin synthesis in plants might be either due to decreased synthesis of IAA or enhanced oxidative degradation of IAA by reactive oxygen species produced under Zn-deficient conditions in the plants (Robson, 1994 and Cakmak, 2011). Singh and Bhatt (2013) also reported that Zn application increased the root length. They observed 53.2 percent increment in root length with the foliar application of 0.08 percent Zn over no application of Zn. Chen *et al.,* (2009) reported from their study that Zn efficiency was closely associated with a larger surface area (longer fine root and larger root surface). Further, they concluded that under moderate Zn deficient stress, fine root development of the efficient genotype was enhanced, and the greater surface area could help an increase the plant's ability to acquire Zn from soil.

Table.1 Effect of Zn application on total root length (cm) of cowpea genotypes

Genotypes	**Average total root length (cm)**		
	+Zn	**-Zn**	**Mean**
V1	1101.2	788.6	944.9
V2	726.1	562.7	644.4
V3	773.1	633.9	703.5
V5	813.3	610.2	711.7
V6	623.7	504.8	564.2
V7	724.4	672.4	698.4
V9	604.0	347.5	475.7
V10	729.2	828.6	778.9
V11	686.2	593.1	639.7
Mean	753.4	615.8	684.6
Effect	**V**	**Zn levels**	**V × Zn levels**
S.Em. ±	65.9	31.1	93.2
C.D. ($p \leq 0.05$)	186.8	88.1	NS

Table.2 Effect of Zn application on surface area (cm2) of cowpea genotypes

Genotypes	**Surface area of root (cm^2)**		
	+Zn	**-Zn**	**Mean**
V1	297.1	157.7	227.4
V2	116.3	82.5	99.4
V3	160.3	110.1	135.2
V5	156.1	94.6	125.4
V6	85.6	102.1	93.9
V7	133.5	88.8	111.2
V9	85.6	43.9	64.8
V10	108.4	115.5	111.9
V11	95.3	72.6	83.9
Mean	137.6	96.4	117.0
Effect	**V**	**Zn levels**	**V × Zn levels**
S.Em. ±	18.5	8.7	26.2
C.D. ($p \leq 0.05$)	52.6	24.8	NS

Table.3 Effect of Zn application on average root diameter (mm) of cowpea genotypes

Genotypes	Average root diameter (mm)		
	+Zn	**-Zn**	**Mean**
V1	0.86	0.63	0.75
V2	0.51	0.46	0.49
V3	0.63	0.56	0.59
V5	0.60	0.49	0.54
V6	0.43	0.61	0.52
V7	0.54	0.42	0.48
V9	0.44	0.41	0.42
V10	0.45	0.44	0.45
V11	0.42	0.39	0.41
Mean	0.54	0.49	0.52
Effect	**V**	**Zn levels**	**V × Zn levels**
S.Em. ±	0.05	0.02	0.07
C.D. (p≤0.05)	0.14	NS	NS

Table.4 Effect of Zn application on root volume (cm3) of cowpea genotypes

Genotypes	Root volume (cm^3)		
	+Zn	**-Zn**	**Mean**
V1	6.70	2.62	4.66
V2	1.51	0.97	1.24
V3	2.89	1.55	2.22
V5	2.44	1.19	1.82
V6	0.94	2.00	1.47
V7	2.35	0.93	1.64
V9	0.98	0.44	0.71
V10	1.30	1.29	1.30
V11	1.06	0.71	0.89
Mean	2.24	1.30	1.77
Effect	**V**	**Zn levels**	**V × Zn levels**
S.Em. ±	0.52	0.24	0.73
C.D. (p≤0.05)	1.47	0.69	NS

Table.5 Effect of zinc application on number of root tips in cowpea genotypes

Genotypes	Number of root tips		
	+Zn	**-Zn**	**Mean**
V1	1134.0	1445.0	1289.5
V2	1516.5	1283.0	1399.8
V3	1378.8	1111.3	1245.0
V5	1093.5	1132.3	1112.9
V6	1752.3	1145.8	1449.0
V7	1510.8	1537.0	1523.9
V9	1083.8	883.3	983.5
V10	1342.0	1984.5	1663.3
V11	1648.0	1704.3	1676.1
Mean	1384.4	1358.5	1371.4
Effect	**V**	**Zn levels**	**V × Zn levels**
S.Em. ±	179.7	84.7	254.1
C.D. (p≤0.05)	NS	NS	NS

Table.6 Effect of zinc application on number of forks in roots of cowpea genotypes

Genotypes	Number of forks		
	+Zn	**-Zn**	**Mean**
V1	7586.0	6108.3	6847.1
V2	6319.3	4718.3	5518.8
V3	5759.0	5037.8	5398.4
V5	6337.8	5290.3	5814.0
V6	5457.3	3903.0	4680.1
V7	5783.0	6220.0	6001.5
V9	5435.5	3158.8	4297.1
V10	6478.8	7691.3	7085.0
V11	6208.5	5514.0	5861.3
Mean	6151.7	5293.5	5722.6
Effect	**V**	**Zn levels**	**V × Zn levels**
S.Em. ±	573.8	270.5	811.5
C.D. (p≤0.05)	1627.1	767.0	NS

Table.7 Effect of zinc application on number of crossings in roots of cowpea genotypes

Genotypes	Number of crossings		
	+Zn	**-Zn**	**Mean**
V1	1067.0	899.8	983.4
V2	1089.8	771.3	930.5
V3	815.0	624.3	719.6
V5	819.8	893.8	856.8
V6	949.8	653.3	801.5
V7	893.3	1074.3	983.8
V9	890.0	542.8	716.4
V10	1035.5	1354.0	1194.8
V11	1055.3	1046.3	1050.8
Mean	957.3	873.3	915.3
Effect	**V**	**Zn levels**	**V × Zn levels**
S.Em. ±	104.3	49.1	147.4
C.D. (p≤0.05)	295.6	NS	NS

Table.8 Effect of zinc application on cation exchange capacity (meq. g-1) in roots cowpea genotypes

Genotypes	Root cation exchange capacity (meq. g^{-1})		
	+Zn	**-Zn**	**Mean**
V1	0.327	0.360	0.343
V2	0.321	0.314	0.317
V3	0.332	0.359	0.345
V5	0.373	0.424	0.398
V6	0.268	0.397	0.333
V7	0.314	0.418	0.366
V9	0.421	0.333	0.377
V10	0.330	0.356	0.343
V11	0.359	0.356	0.357
Mean	0.338	0.368	0.353
Effect	**V**	**Zn levels**	**V × Zn levels**
S.Em. ±	0.010	0.049	0.015
C.D. (p≤0.05)	0.031	0.015	0.044

Table.9 Effect of zinc application on root weight per plant (g) in cowpea genotypes

Genotypes	Root weight per plant (g)		
	+Zn	**-Zn**	**Mean**
V1	0.132	0.167	0.149
V2	0.105	0.105	0.105
V3	0.128	0.154	0.141
V5	0.164	0.157	0.160
V6	0.182	0.152	0.167
V7	0.190	0.167	0.178
V9	0.177	0.140	0.158
V10	0.177	0.202	0.190
V11	0.139	0.150	0.144
Mean	0.155	0.155	0.155
Effect	**V**	**Zn levels**	**V × Zn levels**
S.Em. ±	0.0064	0.0030	0.0091
C.D. (p≤0.05)	0.0190	NS	0.027

Table.10 Effect of zinc application on shoot weight per plant (g) in cowpea genotypes

Genotypes	Shoot weight per plant (g)		
	+Zn	**-Zn**	**Mean**
V1	0.72	0.76	0.74
V2	0.48	0.50	0.49
V3	0.81	0.64	0.73
V5	0.76	0.65	0.70
V6	1.04	0.80	0.92
V7	0.83	0.85	0.84
V9	0.90	0.67	0.78
V10	0.92	0.83	0.88
V11	0.56	0.59	0.58
Mean	0.78	0.70	0.74
Effect	**V**	**Zn levels**	**V × Zn levels**
S.Em. ±	0.03	0.01	0.04
C.D. (p≤0.05)	0.09	0.04	0.13

Table.11 Effect of zinc application on ratio of dry weight of shoot and root in cowpea genotypes

Genotypes	Ratio of dry weight of shoot and root		
	+Zn	**-Zn**	**Mean**
V1	5.49	4.57	5.03
V2	4.55	4.76	4.65
V3	6.37	4.16	5.27
V5	4.64	4.14	4.39
V6	5.70	5.30	5.50
V7	4.34	5.18	4.76
V9	5.08	4.78	4.93
V10	5.21	4.13	4.67
V11	4.07	3.96	4.02
Mean	5.05	4.55	4.80
Effect	**V**	**Zn levels**	**V × Zn levels**
S.Em. ±	0.20	0.09	0.28
C.D. ($p \leq 0.05$)	0.59	0.28	0.83

Longer and thinner roots and a greater proportion of thinner roots were associated with Zn efficiency in wheat (Dong *et al.*, 1995). The Zn deficit stress also greatly influenced the activity of root tip cells in cowpea genotypes. Similar to root length; surface area, root volume, number of forks, shoot-dry matter production and dry weight ratio in shoot and root were significantly increased with the application of Zn over no application of Zn while average diameter of root, number of root tips, number of crossings in root and root weight was not altered.

Significant shoot-dry matter production was observed in the all nine cowpea genotypes. Genotypes V3, V6 and V9 were most efficient in extracting Zn from low-Zn growth medium, possibly due to an efficient ion-transport system (Grewal *et al.*, 1997 and Khan *et al.*, 1998). The average dry weight ratio in shoot and root was significantly increased in the V1, V3 and V10 genotypes with the application of Zn over no application of Zn may which might be due to efficient absorption of Zn by these genotypes while genotype V7 showed a significant decrease in dry weight ratio of shoot and root. Similarly, for a range of plant species under Zn deficiency, the root: shoot ratio has been found to increase (Cumbus 1985; Loneragan *et al.*, 1987; Khan *et al.*, 1998). The average root cation exchange capacity of cowpea genotype decreased significantly with the application of Zn while a close perusal of data regarding the interaction effect of genotypes and Zn levels revealed that the root cation exchange capacity increased in the V9 genotype while decreased in V5, V6 and V7. Crooke and Knight (1962) made an evaluation of the data of different workers.

They drew an inference that the CEC was positively correlated with the content of the tops of (a) total cations, (b) the ash, (c) the excess base, and (d) the total trace elements. Williams and Coleman (1950) reported that plant root surfaces possessed cation exchange capacities which may be measured by the adsorption and release of various cations. They added that the CEC was the same on live or killed roots which indicated that the CEC on the surface of the root was metabolically inactive.

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How to cite this article:

Santosh Chandra Bhatt, Deepa Rawat and Prakash Chandra Srivastava. 2019. Effect of Zn Application on Root Growth Parameters and Shoot Dry Matter Content of Some Cowpea Genotypes. *Int.J.Curr.Microbiol.App.Sci*. 8(04): 1338-1349.
doi: https://doi.org/10.20546/ijcmas.2019.804.156