Indian J.Sci.Res. 16 (2): 19-26, 2017

ISSN: 0976-2876 (Print)
ISSN: 2250-0138(Online)

EFFECT OF ALUMINIUM ON THE SURVIVAL OF *Rhizobium* ISOLATES UNDER STRESS CONDITION

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ABSTRACT

The present experiments were carried out during 2004 - 2007 at Dept. of Agrl. Microbiology, Tamil Nadu Agricultural University, Coimbatore. The best performing nitrogen fixing *Rhizobium* strains *viz*., CO 5, COG 15, TNAU 14, COS 1 and CRR 6 were selected for the development of temperature and acid tolerance study in which the effect of aluminium on the temperature and acid tolerant rhizobia isolates of (Bcp1, Bcp2, Scp1, Scp2, Gcp1, Gcp2, Gncp1, Gncp2, Ccp1 and Ccp2) Blackgram, Soybean, Groundnut, Green gram and Cowpea under acid stress conditions were carried out and recorded the maximum growth (9.00 log $_{10}$ cfu ml^{-1}) in Bcp1 isolates at '0' µM concentration of aluminium after 15 days period of incubation.

Keywords: *Rhizobium* strains, Aluminium, Temperature tolerance, Acid tolerance

Wood *et al.* (1988) reported that the fast growing lotus rhizobia (*Rhizobium loti*) were tolerant of acidity and aluminium (at least 50 µM Al at pH4.5). Slow growing lotus rhizobia (*Bradyrhizobium* sp.) were less tolerant to acidity but equally tolerant to aluminium. Both *Rhizobium loti* and *Bradyrhizobium* sp. were able to nodulate*Lotus pedunculatus* in an acid soil (pH 4.1 in 0.01 M $CaCl_2$) and the slow growing strains were not effective than the fast growing strains in this soil over 30 days. Martin (1988) reported that aluminium is the third most abundant element in the earth crust after oxygen and silicon. It is found in soils predominantly as insoluble alumino silicates or oxides.

Johnson and wood (1990) reported that the aluminium was taken up and bound to the DNA of both sensitive and tolerant strains but that DNA synthesis by the tolerant strains of *Rhizobium loti* was not affected. However, Richardson *et al*. (1988) found that 7.5 µM concentration of aluminium depressed nod gene expression at low pH 4.8.

Strains of *Rhizobium* and *Bradyrhizobium* (Graham, 1992) were resistant to aluminium 50 µM) at low pH less than 5.0 were identified. *Rhizobia* showed varied responses to aluminium toxicity in acidic soils and cultures. Ryan *et al*. (1993) observed that soluble aluminium reduces plant growth because its targeted action at the root apex inhibits plant growth. Flis *et al*. (1993) suggested that the symbiosis between *Rhizobium-Bradyrhizobium* and legumes, aluminium has been shown to adversely affect the process of nodulation through inhibition of root hair formation and nodule initiation.

In acidic soils with pH of more than 5.0, where heavy metal activity is relevant, the presence of available aluminium inhibits nodulation (Bell *et al*., 1989; Bordeleau and Prevost, 1994). Kochian (1995) suggested that in acid soils, aluminium primarily in the form of Al^{3+} is mobilized into soil solution impairing the growth of most plant species. Von Uexkull and Mutert (1995) reported that acid soils occupy approximately 30 per cent of the world's ice free land area and occur mainly in two global belts: one in the humid northern temperate zone that is covered predominantly by coniferous forests; another in the humid tropics covered by savanna and tropical rain forests. The poor fertility of the acid soils is due in part to high H^+ concentrations and especially below pH 5.0, to Al, Mn and Fe toxicity and limited availability of Ca, Mg, K and P.

Grabski and Schindler (1995) reported that aluminium can lower phosphorus availability and block the normal uptake of Ca^{2+} and Mg^{2+} causing an imbalance in plant mineral nutrition; aluminium produces rigidity in the actin cytoskeleton. Ryan *et al.* (1995) reported that in the process of exclusion, aluminium is immobilized outside the plant by

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complexation with organic acids such as malic acid and citric acid, released from roots. In plants aluminium cation is tolerated within the symplasm. Matsumoto *et al.* (1977) observed that the aluminium binding to nucleic acids and it ihibits cell division.

RESULT AND DISCUSSION

Effect Of Aluminum on the Survival of Temperature and Acid Tolerant Rhizobial Isolates Under Acid Stress Conditions

The effect of aluminium on the survival of ten rhizobial isolates (Scp1, Scp 2, Gcp1, Gcp 2, Bcp1, Bcp 2, Gncp1, Gncp 2, Ccp1 and Ccp2) under acid (pH 5.5 and 6.0) stress conditions was studied. The culture samples were taken at three different concentrations of aluminium (0, 25 and 50 μM) and recorded the population for a period of 15 days incubation.

***Rhizobium* Sp. (Blackgram)**

Bcp1 isolates grown at pH 5.5 had maximum growth (9.00 $\log_{10}$cfu ml^{-1}) at '0'μM aluminium concentration significantly after 15 days of incubation(Table1;Fig 1). When the aluminium concentration was increased from 0 to 50 μM, the population decreased. Bcp2 isolates grown at pH 6.0 showed the maximum growth (9.15 $\log_{10}$cfu ml^{-1}) at '0'μM aluminium concentration, compared to Bcp1 isolates (Fig.1a).

***Rhizobium* Sp. (Greengram)**

At '0'μM aluminium concentration, the Gcp1 isolates grown at pH 5.5 recorded the maximum growth (7.50 $\log_{10}$cfu ml^{-1}) and it was reduced to 7.41 $\log_{10}$cfu ml^{-1} at 25 μM and 7.35 $\log_{10}$cfu ml^{-1} at 50 μM aluminium concentration.

Gcp2 isolates grown at pH 6.0 showed the maximum growth (9.20 $\log_{10}$cfu ml^{-1}) at '0'μM aluminium concentration. Then it was reduced to 9.10 $\log_{10}$cfu ml^{-1} at 25 μM and 9.07 $\log_{10}$cfu ml^{-1} at 50 μM aluminiumconcentration(Table 2).

***Rhizobium* Sp. (Groundnut)**

At '0'μM aluminium concentration, the Gncp1 isolates grown at pH 5.5 recorded the maximum growth (7.45 $\log_{10}$cfu ml^{-1}) and it was found reduced to 7.37 $\log_{10}$cfu ml^{-1}at 25 μM and 7.20 $\log_{10}$cfu ml^{-1} at 50μM aluminium concentration.

Gncp2 isolates grown at pH 6.0 showed the maximum growth (9.14 $\log_{10}$cfu ml^{-1}) at '0'μM aluminium concentration. Then it was reduced to 9.05 $\log_{10}$cfu ml^{-1} at 25 μM and 8.85 $\log_{10}$cfu ml^{-1} at 50 μM aluminiumconcentration(Table 3).

***Rhizobium* Sp. (Soybean)**

At '0'μM aluminium concentration, the Scp1 isolates grown at pH 5.5 recorded the maximum growth (7.42 $\log_{10}$cfu ml^{-1}) and it was reduced to the level of 7.31 $\log_{10}$cfu ml^{-1} at 25 μM and 7.17 $\log_{10}$cfu ml^{-1} at 50 μM aluminium concentration.

The maximum growth (9.10 $\log_{10}$cfu ml^{-1}) inScp2 isolates was recorded at '0'μM aluminium concentration whereas it was minimum (8.90 $\log_{10}$cfu ml-1) at 25 μM and 8.75 $\log_{10}$cfu ml^{-1} at 50 μM aluminium concentration (Table 4).

***Rhizobium* Sp. (Cowpea)**

At '0'μM aluminium concentration, Ccp1isolates grown at pH 5.5 recorded the maximum growth (7.35 $\log_{10}$cfu ml^{-1}) and it was reduced to the level of 7.27 $\log_{10}$cfu ml^{-1} at 25μM and 7.14 $\log_{10}$cfu ml^{-1} at 50μM aluminium concentration.

The maximum growth (9.00 $\log_{10}$cfu ml^{-1}) recorded at '0'μM aluminium concentration in Ccp2 isolates. Then it was reduced to the level of 8.75 $\log_{10}$cfu ml^{-1} at 25 μM and 8.68 $\log_{10}$cfu ml^{-1} at 50 μM aluminiumconcentration(Table 5).

The interaction between the three factors (day, concentration and pH) did not show any significant effect of aluminium on the rhizobial growth but individually they were found significantly higher. The interaction between the two factors (days and concentration) showed significant variation on the rhizobial growth.

Screening procedures based on the ability of *Rhizobium* strains to multiply in laboratory media at low pH values with aluminium have successfully identified strains with improved survival and nodulating ability in acid soil (Keyser *et al.*, 1979; Graham *et al.*, 1982; Hartel *et al.*, 1983; Lowendorf and Alexander, 1983 and Thornton and Davey, 1983). Such tests are useful if multiplication in the rhizosphere is limiting nodulation in soil. If later stages of the nodulation process are also limiting the development of the symbiosis, they can only be identified by using *Rhizobium* strains tolerant to stresses at earlier stages.

The present study reveals that Bcp1 isolates at pH 5.5 had significantly higher growth (9.00 log_{10}cfu ml^{-1}) at the concentration of '0'µM aluminium after 15 days of incubation. When the concentration of aluminium increased from 0 to 50 µM, the population decreased. Bcp2 isolates at pH 6.0 showed the maximum growth (9.15 log_{10}cfu ml^{-1}) at '0'µM concentration of aluminium, compared to Bcp1 isolates.These results disagree with the earlier findings of Wood and Cooper (1985), who reported that the aluminium had no additional effect on Lotus rhizobia at pH 4.5; aluminium had no effect on multiplication of strain CC 814S at pH 5.5. Also suggested by the same author that the fast growing Lotus rhizobia (*R. loti*) were generally tolerant to acidity and aluminium at least to 50 µM Al at pH 4.5. Slow growing Lotus rhizobia (*Bradyrhizobium* sp.) are less tolerant to acidity but equally tolerant to aluminium. The multiplication in liquid culture is not an indicator of nodulating ability under acid conditions when a strain is presented to the host as a single culture. Multiplication may, however, improve a strains competitive ability under acid conditions.

CONCLUSION

The ability of *Rhizobium* strains to multiply in laboratory media at low pH values with aluminium have successfully identified strains with improved survival and nodulating ability in acid soil.*Bradyrhizobium* sp. are less tolerant to acidity but equally tolerant to aluminium. Multiplication may, however, improve a strains competitive ability under acid conditions.

Table :. Effect of aluminium on the survival of temperature and acid tolerant rhizobial (Bcp1; Bcp2) isolates

S.No	Days	Population (log $_{10}$cfu ml^{-1})					
		Aluminium rate (µM)					
		Bcp1			Bcp2		
		0µM	25µM	50µM	0µM	25µM	50µM

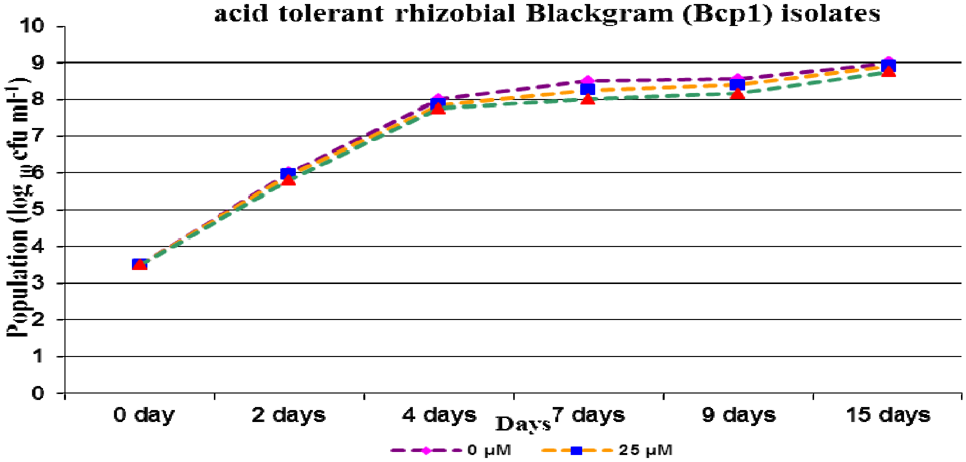
S.No	Days	Population (log $_{10}$cfu ml^{-1})					
		Aluminium rate (µM)					
		Gncp1			Gncp2		
		0µM	25µM	50µM	0µM	25µM	50µM
1.	0	3.18 x10^3 (3.50)	3.18 x10^3 (3.50)	3.18 x10^3 (3.50)	3.18 x10^3 (3.50)	3.18 x10^3 (3.50)	3.18 x10^3 (3.50)
2.	2	8.95 x10^3 (3.95)	6.35 x10^3 (3.80)	4.98 x10^3 (3.70)	2.84 x10^4 (4.45)	2.45 x10^4 (4.39)	1.77 x10^4 (4.25)
3.	4	1.6 x10^6 (6.20)	1.25 x10^6 (6.10)	1.00 x10^6 (6.00)	6.39 x10^5 (5.81)	5.05 x10^5 (5.70)	4.95 x10^5 (5.65)
4.	7	8.95 x10^6 (6.95)	7.15 x10^6 (6.85)	4.45 x10^6 (6.65)	8.0 x10^7 (7.90)	4.95 x10^7 (7.70)	4.00 x10^7 (7.60)
5.	9	19.5 x10^6 (7.29)	15 x10^6 (7.18)	10 x10^6 (7.00)	30.0 x10^7 (8.48)	20.0 x10^7 (8.30)	16.2 x10^7 (8.20)
6.	15	28.5 x10^6 (7.45)	23.5 x10^6 (7.37)	16 x10^6 (7.20)	13.9 x10^8 (9.14)	11.4 x10^8 (9.05)	7.00 x10^8 (8.85)

1.	0	3.18 x10^3 (3.50)	3.18 x10^3 (3.50)	3.18 x10^3 (3.50)	3.18 x10^3 (3.50)	3.18 x10^3 (3.50)	3.18 x10^3 (3.50)
2.	2	9.95 x10^5 (6.00)	8.85 x10^5 (5.95)	6.35 x10^5 (5.80)	8.85 x10^5 (5.95)	6.35 x10^5 (5.80)	4.45 x10^5 (5.65)
3.	4	9.05 x10^7 (8.00)	7.15 x10^7 (7.85)	5.75 x10^7 (7.75)	1.12 x10^8 (8.05)	0.8 x10^8 (7.90)	5.75 x10^7 (7.75)
4.	7	3.18 x10^8 (8.50)	1.78 x10^8 (8.25)	0.9 x10^8 (8.00)	4.00 x10^8 (8.60)	2.25 x10^8 (8.35)	1.78 x10^8 (8.25)
5.	9	3.58 x10^8 (8.55)	2.49 x10^8 (8.40)	1.40 x10^8 (8.15)	5.65 x10^8 (8.75)	3.58 x10^8 (8.55)	2.49 x10^8 (8.40)
6.	15	9.75 x10^8 (9.00)	7.78 x10^8 (8.90)	5.65 x10^8 (8.75)	14 x10^8 (9.15)	9.75 x10^8 (9.00)	6.75 x10^8 (8.83)

Particulars	SEd	CD (0.05%)
Days	0.222	0.443
Concentration	0.157	0.313
pH	0.128	0.256
Days x Concentration	0.385	0.768
Concentration x pH	0.222	NS
Days x pH	0.315	NS
Days x Concentration x pH	0.545	NS

Log values are represented in paranthesis

Fig 1. Effect of aluminium on the survival of temperature and acid tolerant rhizobial Blackgram (Bcp1) isolates



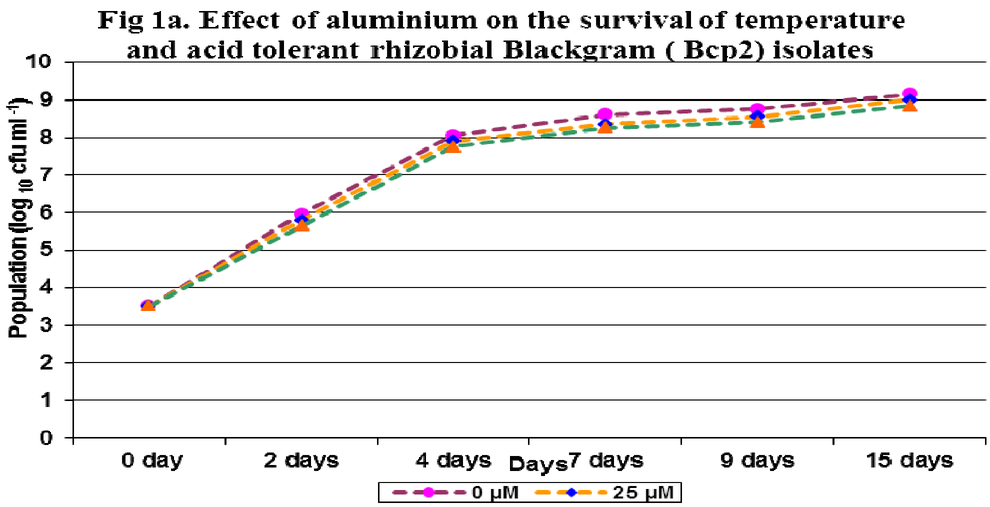


Table 2: Effect of aluminium on the survival of temperature and acid tolerant rhizobial (Gcp1; Gcp2) isolates

Particulars	SEd	CD (0.05%)
Days	0.194	0.386
Concentration	0.137	0.273
pH	0.112	0.223
Days x Concentration	0.335	0.669
Concentration x pH	0.194	NS
Days x pH	0.274	NS
Days x Concentration x pH	0.474	NS

Log values are represented in paranthesis

Table 3: Effect of aluminium on the survival of temperature and acid tolerant rhizobial (Gncp1; Gncp2) isolates

Particulars	SEd	CD (0.05%)
Days	0.192	0.382
Concentration	0.136	0.270
pH	0.111	0.221
Days x Concentration	0.332	0.662
Concentration x pH	0.192	NS
Days x pH	0.271	NS
Days x Concentration x pH	0.470	NS

Log values are represented in parenthesis

S.No	**Days**	**Population (log_{10}cfu ml^{-1})**					
		Aluminium rate (µM)					
		Gcp1			**Gcp2**		
		0µM	**25µM**	**50µM**	**0µM**	**25µM**	**50µM**
1.	0	3.18×10^3 (3.50)	3.18×10^3 (3.50)	3.18×10^3 (3.50)	3.18×10^3 (3.50)	3.18×10^3 (3.50)	3.18×10^3 (3.50)
2.	2	3.18×10^4 (4.50)	7.9×10^3 (3.90)	3.95×10^3 (3.60)	3.13×10^4 (4.49)	2.25×10^4 (4.35)	1.95×10^4 (4.29)
3.	4	2.25×10^6 (6.35)	1.6×10^6 (6.20)	1.22×10^6 (5.09)	7.01×10^5 (5.85)	5.70×10^5 (5.76)	4.45×10^5 (5.65)
4.	7	1.0×10^7 (7.00)	8.0×10^7 (6.90)	5.6×10^7 (6.75)	8.85×10^7 (7.95)	7.15×10^7 (7.85)	5.72×10^7 (7.76)
5.	9	2.25×10^7 (7.35)	1.95×10^7 (7.29)	1.73×10^7 (7.24)	3.33×10^7 (8.52)	3.12×10^7 (8.49)	2.45×10^7 (8.39)
6.	15	3.15×10^7 (7.50)	2.6×10^7 (7.41)	2.25×10^7 (7.35)	16.0×10^8 (9.20)	12.65×10^8 (9.10)	11.65×10^8 (9.07)

Table 4:Effect of aluminium on the survival of temperature and acid tolerant rhizobial (Scp1; Scp2) isolates

Particulars	SEd	CD (0.05%)
Days	0.190	0.379
Concentration	0.134	0.268
pH	0.110	0.219
Days x Concentration	0.329	0.656
Concentration x pH	0.190	NS
Days x pH	0.269	NS
Days x Concentration x pH	0.466	NS

Log values are represented in paranthesis

Table 5. Effect of aluminium on the survival of temperature and acid tolerant rhizobial (Ccp1; Ccp2) isolates

S.No	Days	Population ($\log_{10}$cfu ml^{-1})					
		Aluminium rate (µM)					
		Ccp1			Ccp2		
		0µM	25µM	50µM	0µM	25µM	50µM
1.	0	3.18 x10^3 (3.50)	3.18 x10^3 (3.50)	3.18 x10^3 (3.50)	3.18 x10^3 (3.50)	3.18 x10^3 (3.50)	3.18 x10^3 (3.50)
2.	2	6.75 x10^3 (3.83)	4.98 x10^3 (3.70)	3.40 x10^3 (3.53)	2.35 x10^7 (4.37)	1.8 x10^4 (4.25)	1.465x10^4 (4.16)
3.	4	4.95 x10^5 (6.17)	8.25 x10^5 (5.92)	6.23 x10^5 (5.79)	4.75 x10^5 (5.68)	3.45 x10^5 (5.54)	3.18 x10^3 (5.45)
4.	7	6.95 x10^6 (6.83)	5.40 x10^6 (6.73)	4.00 x10^6 (6.51)	5.22 x10^7 (7.72)	3.75 x10^7 (7.57)	2.85 x10^7 (7.45)
5.	9	18 x10^6 (7.19)	14 x10^6 (7.0)	8.0 x10^7 (6.79)	21.5 x10^7 (8.33)	15.5 x10^7 (8.19)	12.4 x10^7 (8.09)
6.	15	2.25 x10^7 (7.35)	18.5 x10^6 (7.27)	13.8 x10^6 (7.14)	97.5 x10^7 (9.00)	14.75 x10^6 (8.75)	47.6 x10^7 (8.68)

Particulars	SEd	CD (0.05%)
Days	0.188	0.375
Concentration	0.133	0.265
pH	0.109	0.216
Days x Concentration	0.326	0.649
Concentration x pH	0.188	NS
Days x pH	0.266	NS
Days x Concentration x pH	0.461	NS

Log values are represented in paranthesis

ACKNOWLEDGEMENT

I expressed my indebt thanks to Dr.K.Ramasamy,Vice Chancellor of TamilNadu Agricultural University,Coimbatore and Dr.K.Ilamurugu, Professsor, Department of Agricultural Microbiology,Coimbatore for their encouraging words and suggestions given in completion of research article.

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