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In vitro screening for salinity stress in cotton genotypes

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Abstract: Effect of salinity stress on growth parameters in 27 cotton (*Gossypium hirsutum*) genotypes were studied under in vitro condition. The salinity stress was induced by incorporating NaCl to generate EC 12 ds/m. Reduction in seedlings fresh weight, shoot length, root weight, root length and root dry weight was significantly less in JK-4 (G_{11}), PH 1009 (G_{12}) and RDT-17 (G_{10}) under salt stress as compared to other genotypes while, high per cent reduction in all the traits were observed in CPD 464 (G_4), CPD 2007-4 (G_5) and HLS 321729 (G_{18}). Based on the results obtained, we conclude that JK-4 (G_{11}), PH 1009 (G_{12}) and RDT-17 (G_{10}) were more tolerant to salinity stress.

Key words: Cotton, Genotypes, Salinity stress

Introduction

Cotton is an important source of fibre, Cotton "the silver fibre" is an important commercial crop of India playing a significant role in Indian farming and industrial economy of the country, by providing 65-75 per cent of the raw material for the textile industry of our country. Salinity is one of the most serious factors that limit the crop production. Plants show variable capacity to salt tolerance that could range from negligible effect to plant death. Considerable differences are found between plant species, for example, after exposure to 200 mM NaCl, a salt-tolerant species such as sugar beet exhibited a reduction of only 20% in dry weight, whereas, a moderately tolerant species such as cotton exhibited a 60% reduction, and a sensitive species such as soybean might die (Saleh, 2011). Generally, salinity reduces the vegetative growth of cotton, but increase in growth with low concentration of salts has also been observed. Salinity deceases shoot/root ratio because shoots are sensitive than roots (Ahmad *et al.,* 1991).

Cotton appears to be more sensitive for salinity at germination stage. It can tolerate salinity level of 4 mmho/cm at 23^{o}C in saturation extracts during germination but can tolerate three times this salt level once the seedling were established (Ayears and Hayward, 1948). Cotton is considered a moderately salt tolerant crop, but its yield is markedly affected due to poor germination and subsequent abnormal plant development under severe saline conditions (Ashraf, 2002).

According to Munns (2006), salt stress decreases growth in most plants, including halophytes. Salinity can affect germination of seeds either by creating osmotic potential which prevent water uptake, or by toxic effects of ions on embryo viability and it is generally accepted that the germination and seedling stage of plant life cycle is more sensitive to salinity than adult stage (Lianes *et al.,* 2005).

Among several activities, the development of salt tolerance in crop plants and the identification of salt tolerant genetic resources through screening is most important. Screening for salt tolerance under natural condition is difficult because of non-availability of uniformly salt affected fields and also it is time consuming and labour intensive. The conventional breeding methods are therefore not useful. The conventional breeding methods including screening methods have also been slow in improving salt tolerance crop plants. Through *in vitro* technique it is possible to develop less labour intensive, short

Table 1. Effect of salt concentrations on seedling fresh weight (g)

Genotypes	Control	Treated	% Reduction
Gcot-16 surath (G_1)	0.134	0.081	39.55
HBS-128 (G_2)	0.140	0.134	4.29
Abhadita (G_3)	0.160	0.128	20.00
CPD 464 (G_4)	0.131	0.051	61.07
CPD 2007-4 (G_5)	0.138	0.062	55.07
AK 23 B (G_6)	0.154	0.114	25.97
RAJ-2 (G_7)	0.144	0.136	5.56
NH 615 (G_8)	0.153	0.117	23.53
543374 A 02 N68 (G_9)	0.125	0.120	4.00
RDT-17 (G_{10})	0.144	0.140	2.78
JK-4 (G_{11})	0.138	0.134	2.90
PH 1009 (G_{12})	0.133	0.131	1.50
CPD 446 (G_{13})	0.132	0.129	2.27
IC35701Coker 417-68 (G_{14})	0.133	0.127	4.51
CPD 433 (G_{15})	0.127	0.119	6.30
5433 A2 A03 N83 (G_{16})	0.140	0.132	5.71
HBS 123 (G_{17})	0.134	0.124	7.46
HLS 321729 (G_{18})	0.143	0.075	47.55
CCH 1831 (G_{19})	0.129	0.107	17.05
AKA 081 (G_{20})	0.125	0.099	20.80
IC 359963 (G_{21})	0.154	0.095	38.31
PS-20-2-1 (G_{22})	0.136	0.124	8.82
543403 A03 N106 (G_{23})	0.160	0.131	18.13
RHC 0811 (G_{24})	0.126	0.085	32.54
211412W247-85-D14-26 (G_{25})	0.137	0.102	25.55
JBWR 23 (NSP-18) (G_{26})	0.136	0.109	19.85
EC 560392 (G_{27})	0.127	0.091	28.35
Mean	0.138	0.111	
CV	0.8009		
	C.D.	S.Em±	
Genotype	0.003	0.001	
Treatment	0.001	0.001	
GxT	0.004	0.001	

duration oriented and more efficient screening method for abiotic stresses by manipulating salt concentrations to induce salt stress. The present investigation was carried out to identify salinity tolerant genotypes of cotton by *in vitro* screening.

Material and methods

A laboratory experiment was conducted in tissue culture laboratory at Agricultural Research Station, Dharwad farm, University of Agricultural Sciences, Dharwad during 2013-14. The details of the material used and the techniques adopted during the course of this investigation are as described below.

Genetically diverse 27 genotypes were used in the experiment. Based on the survey report of the AICCP, the genotypes CCH 1831, 5433 A2 A03 N83, PH 1009 and JK-4 which were identified as drought tolerant and genotypes G.cot-16 surath, RHC 0811, HLS 321729, CPD 2007-4 and CPD 464 which were identified as susceptible were included in 27 genotypes used in the experiment (Laddi, M. A., 2014). Delinted seeds were dipped in 0.2% mercuric chloride for 20 minutes with constant stirring followed by the repeated washes with sterile water under laminar airflow and kept overnight for germination. Shoot apical meristem along with radical was isolated from germinating seeds under aseptic conditions to use as explant.

Table 2. Effect of salt concentrations on shoot fresh weight (g)

Genotype	Control	Treated	% Reduction
G.cot-16 surath (G_1)	0.089	0.042	52.81
HBS-128 (G_2)	0.101	0.097	3.96
Abhadita (G_3)	0.096	0.085	11.46
CPD 464 (G_4)	0.086	0.021	75.58
CPD 2007-4 (G_5)	0.092	0.028	69.57
AK 23 B (G_6)	0.093	0.067	27.96
RAJ-2 (G_7)	0.092	0.086	6.52
NH 615 (G_8)	0.093	0.066	29.03
543374 A 02 N68 (G_9)	0.079	0.075	5.06
RDT-17 (G_{10})	0.063	0.061	3.17
JK-4 (G_{11})	0.092	0.089	3.26
PH 1009 (G_{12})	0.087	0.085	2.30
CPD 446 (G_{13})	0.083	0.068	18.07
IC35701Coker 417-68 (G_{14})	0.084	0.078	7.14
CPD 433 (G_{15})	0.079	0.074	6.33
5433 A2 A03 N83 (G_{16})	0.088	0.085	3.41
HBS 123 (G_{17})	0.086	0.079	8.14
HLS 321729 (G_{18})	0.081	0.039	51.85
CCH 1831 (G_{19})	0.079	0.05	36.71
AKA 081 (G_{20})	0.08	0.059	26.25
IC 359963 (G_{21})	0.094	0.044	53.19
PS-20-2-1 (G_{22})	0.088	0.078	11.36
543403 A03 N106 (G_{23})	0.096	0.072	25.00
RHC 0811 (G_{24})	0.079	0.039	50.63
211412W247-85-D14-26(G_{25})	0.076	0.06	21.05
JBWR 23 (NSP-18) (G_{26})	0.076	0.058	23.68
EC 560392 (G_{27})	0.085	0.046	45.88
Mean	0.086	0.064	
CV	0.72884		
	C.D.	S.Em±	
Genotype	0.003	0.001	
Treatment	0.001	0.001	
GxT	0.004	0.001	

Table 3. Effect of salt concentrations on shoot length (cm)

Genotype	Control	Treated	% Reduction
G.cot-16 surath (G_1)	3.933	1.533	61.02
HBS-128 (G_2)	4.633	3.433	25.90
Abhadita (G_3)	3.133	2.467	21.26
CPD 464 (G_4)	3.733	1.067	71.42
CPD 2007-4 (G_5)	4.733	1.867	60.55
AK 23 B (G_6)	3.333	2.133	36.00
RAJ-2 (G_7)	4.833	3.733	22.76
NH 615 (G_8)	3.233	1.067	67.00
543374 A 02 N68 (G_9)	2.533	1.933	23.69
RDT-17 (G_{10})	2.033	1.933	4.92
JK-4 (G_{11})	4.733	4.533	4.23
PH 1009 (G_{12})	3.533	3.167	10.36
CPD 446 (G_{13})	2.533	1.933	23.69
IC35701Coker 417-68 (G_{14})	2.433	2.167	10.93
CPD 433 (G_{15})	3.9	2.767	29.05
5433 A2 A03 N83 (G_{16})	3.433	3.033	11.65
HBS 123 (G_{17})	2.533	1.667	34.19
HLS 321729 (G_{18})	4.4	0.967	78.02
CCH 1831 (G_{19})	2.533	1.8	28.94
AKA 081 (G_{20})	3.367	2.833	15.86
IC 359963 (G_{21})	4.3	1.033	75.98
PS-20-2-1 (G_{22})	4.5	2.867	36.29
543403 A03 N106 (G_{23})	3.067	1.5	51.09
RHC 0811 (G_{24})	3.533	0.967	72.63
211412W247-85-D14-26 (G_{25})	2.533	1.067	57.88
JBWR 23 (NSP-18) (G_{26})	2.533	1	60.52
EC 560392 (G_{27})	3.633	1.133	68.81
Mean	3.468	2.059	
CV	0.957		
	C.D.	S.Em±	
Genotype	0.078	0.028	
Treatment	0.021	0.008	
GxT	0.110	0.039	

Murashige and Skoog medium (MS form Himedia make with vitamins; without $CaCl_2$, sucrose, IAA, Kinetin, dehydrated Agar) was supplemented with growth regulator Benzyl Adenine (2.0 mg/l), $CaCl_2$ (440 mg/l) and different salts at different concentrations were added to medium to generate required EC levels.

Freshly isolated explants were cultured and cultures were incubated at 26 ± 2°C temperature, 50-60 per cent relative humidity and 16 / 8 h light and dark spells. Light intensity of 1000 lux was maintained. Observations such as Seedling fresh weight, Shoot weight, Shoot length, Shoot dry weight, Root weight, Root length, Root dry weight were recorded on samples made at 8 days after culture.

Results and discussion

The growth parameters of cotton get affected under salinity stress. The root and shoot lengths are the most important parameters for salt stress because roots are in direct contact with soil and absorb water from the soil and shoot supplies it to the rest of the plant. For this reason, root and shoot length provides an important clue to the response of plant to salt stress (Jamil and Rha, 2004). Soil salinity is shown to increase P, Mn and Zn and decrease K and Fe concentration of plants.

Table 4. Effect of salt concentrations on shoot dry weight (mg)

Genotype	Control	Treated	% Reduction
G.cot-16 surath (G_1)	6.733	3.417	49.25
HBS-128 (G_2)	7.439	7.011	5.75
Abhadita (G_3)	7.634	6.338	16.98
CPD 464 (G_4)	6.778	1.927	71.57
CPD 2007-4 (G_5)	7.223	1.987	72.49
AK 23 B (G_6)	7.398	5.07	31.47
RAJ-2 (G_7)	7.332	6.12	16.53
NH 615 (G_8)	7.423	4.995	32.71
543374 A 02 N68 (G_9)	5.671	5.283	6.84
RDT-17 (G_{10})	4.723	4.68	0.91
JK-4 (G_{11})	7.356	6.937	5.70
PH 1009 (G_{12})	6.866	6.257	8.87
CPD 446 (G_{13})	6.11	5.138	15.91
IC35701Coker 417-68 (G_{14})	6.238	5.671	9.09
CPD 433 (G_{15})	5.734	5.426	5.37
5433 A2 A03 N83 (G_{16})	6.638	6.196	6.66
HBS 123 (G_{17})	6.287	5.576	11.31
HLS 321729 (G_{18})	6.196	2.851	53.99
CCH 1831 (G_{19})	5.708	3.926	31.22
AKA 081 (G_{20})	6.02	4.62	23.26
IC 359963 (G_{21})	7.672	3.465	54.84
PS-20-2-1 (G_{22})	6.731	5.522	17.96
543403 A03 N106 (G_{23})	7.47	5.019	32.81
RHC 0811 (G_{24})	5.677	2.976	47.58
211412W247-85-D14-26 (G_{25})	5.597	4.598	17.85
JBWR 23 (NSP-18) (G_{26})	5.626	4.531	19.46
EC 560392 (G_{27})	6.437	3.751	41.73
Mean	6.545	4.788	
CV	0.558		
	C.D.	SEm±	
Genotype	0.031	0.011	
Treatment	0.008	0.003	
GxT	0.043	0.015	

Table 5. Effect of salt concentrations on root fresh weight (g)

Genotype	Control	Treated	% Reduction
G.cot-16 surath (G_1)	0.045	0.039	13.33
HBS-128 (G_2)	0.039	0.037	5.13
Abhadita (G_3)	0.065	0.043	33.85
CPD 464 (G_4)	0.045	0.03	33.33
CPD 2007-4 (G_5)	0.046	0.034	26.09
AK 23 B (G_6)	0.061	0.047	22.95
RAJ-2 (G_7)	0.052	0.05	3.85
NH 615 (G_8)	0.06	0.051	15.00
543374 A 02 N68 (G_9)	0.046	0.045	2.17
RDT-17 (G_{10})	0.081	0.079	2.47
JK-4 (G_{11})	0.046	0.045	2.17
PH 1009 (G_{12})	0.046	0.045	2.17
CPD 446 (G_{13})	0.05	0.046	8.00
IC35701Coker 417-68 (G_{14})	0.049	0.048	2.04
CPD 433 (G_{15})	0.047	0.045	4.26
5433 A2 A03 N83 (G_{16})	0.051	0.047	7.84
HBS 123 (G_{17})	0.047	0.045	4.26
HLS 321729 (G_{18})	0.062	0.036	41.94
CCH 1831 (G_{19})	0.05	0.038	24.00
AKA 081 (G_{20})	0.045	0.04	11.11
IC 359963 (G_{21})	0.061	0.051	16.39
PS-20-2-1 (G_{22})	0.048	0.045	6.25
543403 A03 N106 (G_{23})	0.064	0.059	7.81
RHC 0811 (G_{24})	0.047	0.046	2.13
211412W247-85-D14-26 (G_{25})	0.061	0.042	31.15
JBWR 23 (NSP-18) (G_{26})	0.06	0.051	15.00
EC 560392 (G_{27})	0.042	0.034	19.05
Mean	0.052	0.047	
CV	0.6481		
	C.D.	S.Em±	
Genotype	0.003	0.001	
Treatment	0.001	0.001	
GxT	0.005	0.002	

Shoots are generally more sensitive to cation disturbances than roots and there are great differences among plant species in the ability to prevent or tolerate the excess salt concentrations (Turan *et al.,* 2010).

Significant reduction in all phenological parameter sunder salt treated condition compared to control condition in all the genotypes indicates effective induction of salt stress in in vitro. Less per cent reduction in seedling fresh weight in PH 1009 (G_{12}) (1.50%), followed by CPD 446 (G_{13}) (2.27%) and RDT-17 (G_{10}) (2.78%), indicates their higher ability of tolerance to salinity than other genotypes and higher per cent reduction in CPD 464 (G_4), (61.01%) followed by CPD 2007-4 (G_5) (55.07%) and HLS 321739 (G_{18}) (47.55%) indicates their susceptibility to salinity (Table 1). The present findings are in close agreement with Kaymakanova (2009), who reported that among three bean cultivars (Lody, Gyna, Tara), Tara cultivar showed highest seedling growth than Lody and Gyna. Decrease in seedling growth in these two varieties may be due to inability of the seedling to adjust osmotically or toxic effects of salts like Cl, SO_4^-, and Na^+.

The present findings revealed that in salt treated condition PH 1009 (G_{12}) (2.30%) recorded least shoot fresh weight per cent reduction followed by RDT-17 (G_{10}) (3.17%) and JK-4 (G_{11}) (3.26%), whereas in CPD 464 (G_4) (75.58%) the per cent reduction was highest, followed by CPD 2007-4 (G_5) (69.57%) and G.cot-16 surath (G_1) (52.81%) (Table 2). Less per cent reduction of shoot length was observed in JK-4 (G_{11}) (4.23%), followed by RDT-17 (G_{10}) (4.92%) and IC 35701 Coker 417-68 (G_{14}) (10.93%) (Table 3). HLS 321729 (G_{18}) (78.02%) showed highest per cent reduction, followed by IC 359963 (G_{21}) (75.98%) and RHC 0811 (G_{24}) (72.63%). Increasing NaCl concentration antagonistically affected shoot dry weight (Table 4). The present investigation revealed that, RDT-17 (G_{10}) (0.91%) showed less per cent reduction in shoot dry weight in salt treated condition, followed by CPD 433 (G_{15}) (5.37%) and JK-4 (G_{11}) (5.70%), indicating these genotypes as tolerant to salinity. CPD 2007-4 (G_5) (72.49%) showed highest per cent reduction, followed by CPD 464 (G_4) (71.57%) and IC 359963 (G_{21}) (54.84%). Turan et al. (2010) studied the effect of applied NaCl on shoot and root growth in maize plant. They reported that applied NaCl inhibited the growth of maize plant and caused to decrease both shoot and root dry weights. Shoot and root growth of maize were negatively correlated to the concentration of NaCl. Maize plants grown at the low levels of NaCl (0 and 25 mM) reached relatively higher dry weights and did not imply toxicity symptoms, however, the

Table 6. Effect of salt concentrations on root length (cm)

Genotype	Control	Treated	% Reduction
G.cot-16 surath (G_1)	3.167	2.600	17.90
HBS-128 (G_2)	3.167	2.767	12.63
Abhadita (G_3)	4.333	2.800	35.38
CPD 464 (G_4)	4.000	3.333	16.68
CPD 2007-4 (G_5)	4.533	3.800	16.17
AK 23 B (G_6)	3.767	2.867	23.89
RAJ-2 (G_7)	4.533	2.433	46.33
NH 615 (G_8)	3.033	2.867	5.47
543374 A 02 N68 (G_9)	3.400	3.100	8.82
RDT-17 (G_{10})	5.533	5.367	3.00
JK-4 (G_{11})	4.933	4.767	3.37
PH 1009 (G_{12})	5.100	4.867	4.57
CPD 446 (G_{13})	5.167	4.900	5.17
IC35701Coker 417-68 (G_{14})	4.467	4.100	8.22
CPD 433 (G_{15})	4.333	2.767	36.14
5433 A2 A03 N83 (G_{16})	4.233	4.000	5.50
HBS 123 (G_{17})	3.200	2.967	7.28
HLS 321729 (G_{18})	5.200	3.267	37.17
CCH 1831 (G_{19})	3.767	3	20.36
AKA 081 (G_{20})	3.467	2.800	19.24
IC 359963 (G_{21})	5.000	3.833	23.34
PS-20-2-1 (G_{22})	3.067	2.467	19.56
543403 A03 N106 (G_{23})	3.233	2.100	35.04
RHC 0811 (G_{24})	4.400	4.167	5.30
211412W247-85-D14-26 (G_{25})	3.133	2.433	22.34
JBWR 23 (NSP-18) (G_{26})	3.267	1.567	52.04
EC 560392 (G_{27})	2.567	2.167	15.58
Mean	4.000	3.310	
CV	0.8708		
	C.D.	S.Em±	
Genotype	0.120	0.043	
Treatment	0.033	0.012	
GxT	0.170	0.060	

Table 7. Effect of salt concentrations on root dry weight (mg)

Genotype	Control	Treated	% Reduction
G.cot-16 surath (G_1)	3.342	2.656	20.53
HBS-128 (G_2)	2.842	2.501	12.00
Abhadita (G_3)	4.739	2.967	37.39
CPD 464 (G_4)	3.011	1.984	34.11
CPD 2007-4 (G_5)	3.44	2.01	41.57
AK 23 B (G_6)	4.526	3.419	24.46
RAJ-2 (G_7)	3.715	3.676	1.05
NH 615 (G_8)	4.364	3.915	10.29
543374 A 02 N68 (G_9)	3.552	3.473	2.22
RDT-17 (G_{10})	6.446	5.971	7.37
JK-4 (G_{11})	3.338	3.045	8.78
PH 1009 (G_{12})	3.119	3.004	3.69
CPD 446 (G_{13})	3.596	3.32	7.68
IC35701Coker 417-68 (G_{14})	3.571	3.322	6.97
CPD 433 (G_{15})	3.518	3.385	3.78
5433 A2 A03 N83 (G_{16})	3.501	3.004	14.20
HBS 123 (G_{17})	3.359	3.137	6.61
HLS 321729 (G_{18})	4.644	2.556	44.96
CCH 1831 (G_{19})	3.711	3.23	12.96
AKA 081 (G_{20})	3.371	3.114	7.62
IC 359963 (G_{21})	4.44	3.553	19.98
PS-20-2-1 (G_{22})	3.479	3.198	8.08
543403 A03 N106 (G_{23})	4.885	4.133	15.39
RHC 0811 (G_{24})	3.565	3.168	11.14
211412W247-85-D14-26 (G_{25})	4.36	3.168	27.34
JBWR 23 (NSP-18) (G_{26})	4.527	4.156	8.20
EC 560392 (G_{27})	3.298	3.054	7.40
Mean	3.861	3.334	
CV	0.8876		
	C.D.	S.Em±	
Genotype	0.027	0.01	
Treatment	0.007	0.003	
GxT	0.038	0.013	

growth was significantly reduced at higher levels of salinity (50, 75 and 100 mM) indicating the symptoms of salt toxicity as growth depression.

Significant less per cent reduction of root fresh weight was observed in IC 35701 Coker 417-68 (G_{14}) (2.04%), followed by RHC 0811 (G_{24}) (2.13%) and 543374 A 02 N68 (G_9) (2.17%) (Table 5). HLS 321729 (G_{18}) (41.94%) recorded highest per cent reduction in root fresh weight, followed by Abhadita (G_3) (33.85%) and CPD 464 (G_4) (33.33%). Rauf *et al.* (2014), conducted an experiment to determine the effect of different levels of NaCl salinity on growth of Cotton varieties, by growing them under different salinity levels @ 0, 30, 60, 90, 120 and 150mM NaCl. They reported that salinity reduced the root and shoot growth significantly and root weight was the most limiting growth parameter.

Least per cent reduction of root length under salt treated condition as compared to normal condition was recorded in RDT-17 (G_{10}) (3.00%), followed by JK-4 (G_{11}) (3.37%) and PH 1009 (G_{12}) (4.57%). JBWR 23 (NSP-18) (G_{26}) (52.04%) showed highest per cent reduction, followed by RAJ-2 (G_7) (46.33%) and HLS 321729 (G_{18}) (37.17%) (Table 6). Hussain *et al.* (2009) reported that salinity caused significant reduction in root length in black seeds (*Nigella sativa* L.). The results indicated that lower reduction in shoot and root length is found to be tolerant at higher salinity levels. RAJ-2 (G_7) (1.07%) showed least per cent reduction in root dry weight under salt treated condition compared to normal condition, followed by 543374 A02 N68 (G_9) (2.22%) and PH 1009 (G_{12}) (3.69%)(Table 7). Highest per cent reduction was recorded in HLS 321729 (G_{18}) (44.96%), followed by CPD 2007-4 (G_5) (41.57%) and Abhadita (G_3) (37.39%). Similar results were observed by Mehmet Atak et al. (2006), among 3 Triticale varieties Presto showed highest root dry weight as the salt concentration increased, than Karma 2000 and Tathcak-97 varieties.

Percentage reduction in seedling fresh weight (g), shoot fresh weight, shoot length (cm), shoot dry weight (mg), root fresh weight (g), root length (cm) and root dry weight (mg) under salt stress condition, in comparison with normal, directly or in directly is due to higher tolerance of the genotype to salt tolerance. Therefore, in present study genotype 543374 A 02 N68 (G_9), RDT-17 (G_{10}), JK-4 (G_{11}) and PH 1009 (G_{12}) recorded lesser reduction for five and more traits. It indicates their tolerance to salt, on further validation they may become good genetic resource in development of salt tolerant cotton varieties.

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