Physiological and biochemical basis of salinity tolerance in Indian mustard (*B. juncea*)

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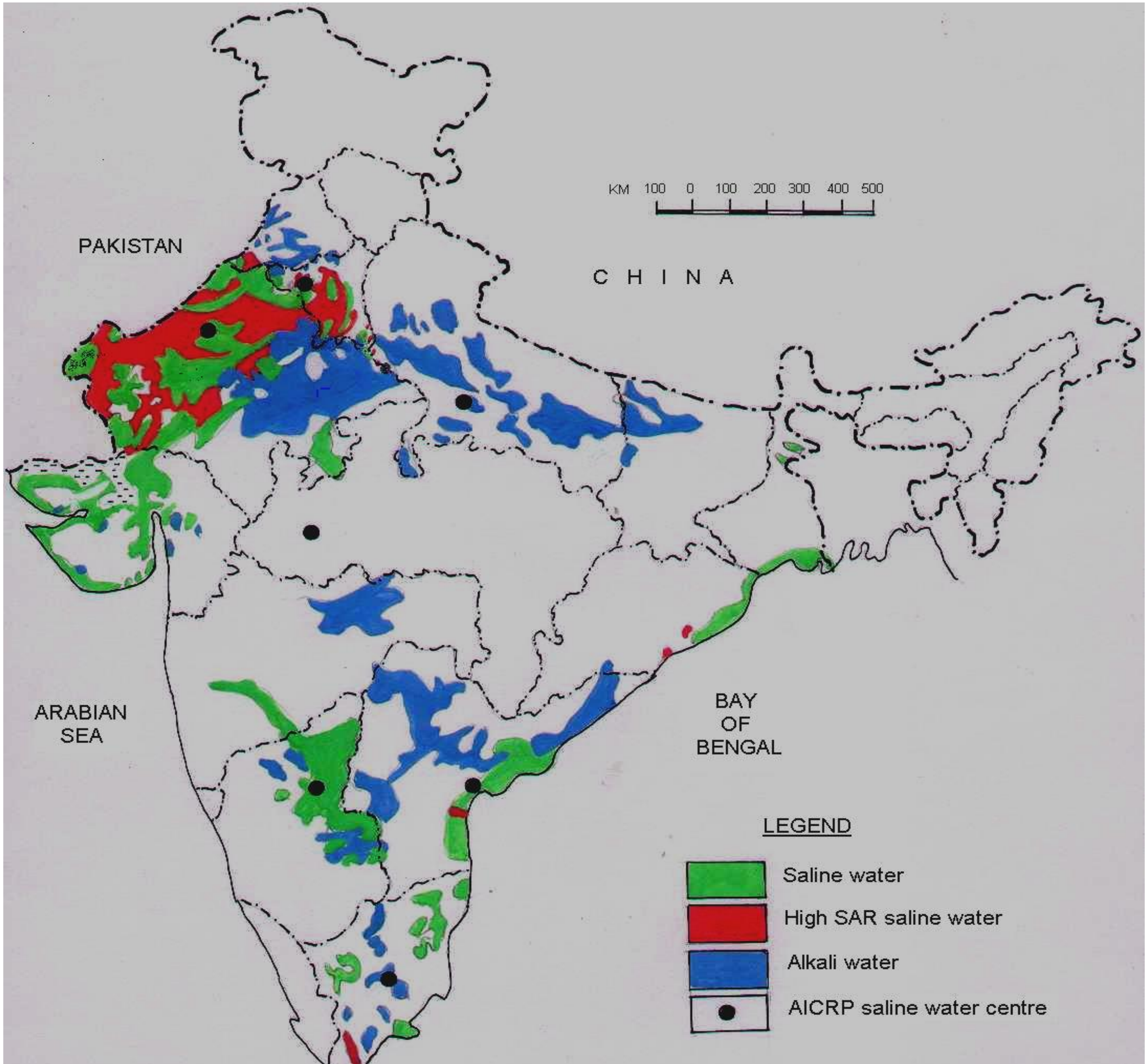
Introduction

- India with share of 17.2% in area and 8.5% in production of rapeseed-mustard ranks third in the world. Rapeseed-mustard is the second most important oilseed crop after soybean in India.

- Good quality water resources are becoming increasingly scarce for use in agriculture and are allocated with priority to domestic and industrial users. Consequently, marginal quality waters are being used for irrigation with little or no consideration of their long-term impact on soils especially in the arid and semi-arid regions

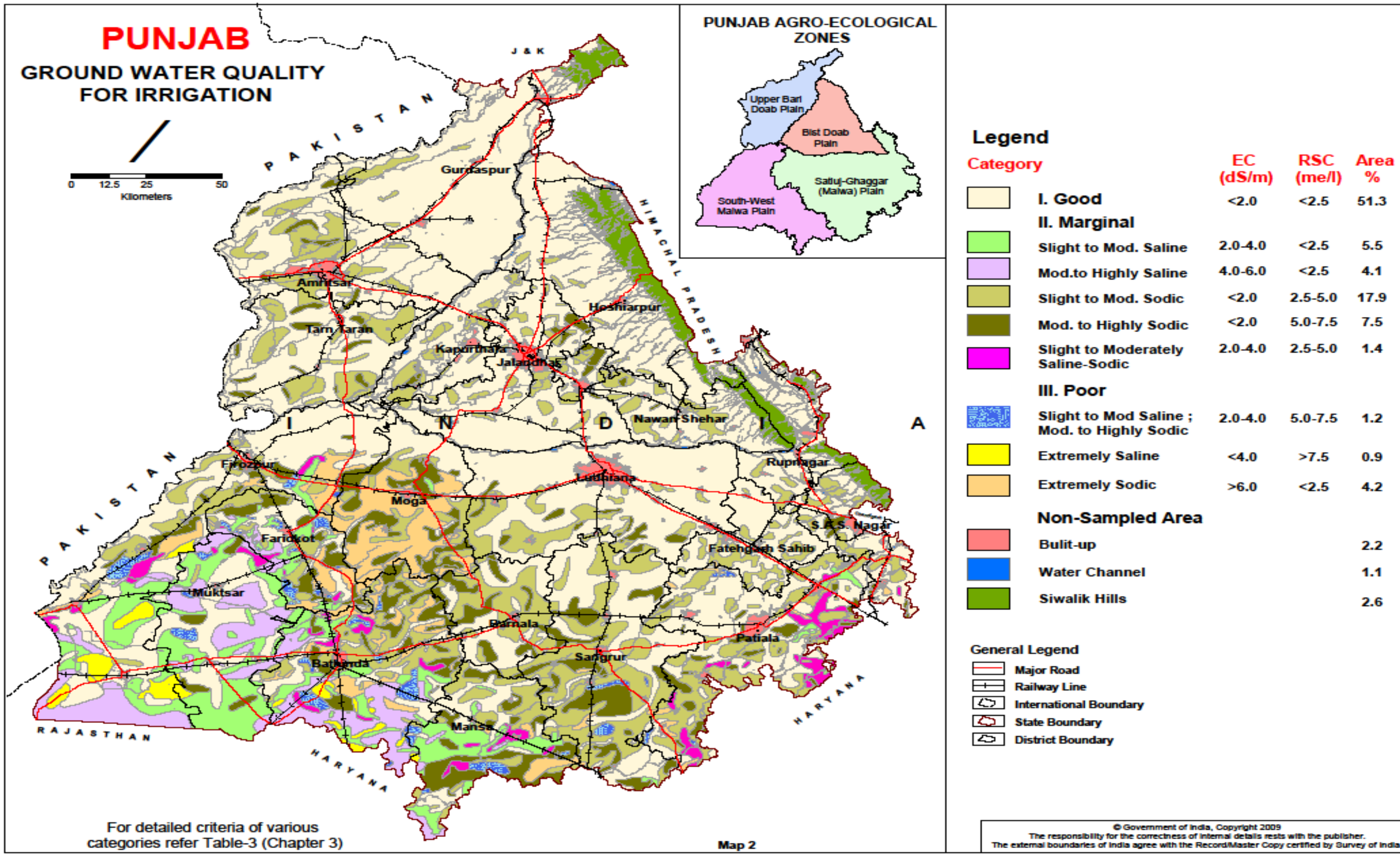
- **WORLD** : About 10 m ha of irrigated land suffers from secondary salinization and sodification

Groundwater Quality for Irrigation in India



40-60% of the groundwater in northwestern parts of India have higher (30-50 %) residual alkalinity.

Many more areas with good quality aquifers are endangered with contamination a consequence of excessive withdrawals of ground water and subsequent flows from poor quality aquifers.



Out of 42 % poor quality ground waters, 25 % are saline, 69 % are sodic and 6 % are saline-sodic in nature.

Irrigation water induced salinity and sodicity are becoming serious threat to mustard cultivation.

Phenotyping genotypes for morpho-physiological and biochemical traits conferring salinity tolerance will aid to develop salt/drought tolerant mustard cultivars.

Objectives

- To study the effect of different salt stress regimes on morpho-physiological and biochemical traits of *Brassica juncea* genotypes.
- To identify the traits responsible for salinity tolerance.
- To characterize genotypes for physiological responses under different salinity regimes.
- To explore the selection possibility of stress indices on the physiological responses.

Material and Methods

Permanent plots irrigated with different levels of saline and sodic water are maintained at the research farm of the Department of Soil Science, Punjab Agricultural University,Ludhiana

Soil type : sandy loam soil (*Typic Ustochrept*)

Irrigation induced salinization		Levels	2015-16	2016-17
A	**Irrigation with sodic water (sodium bicarbonate) Sodicity/alkalinity**	**RSC (4) : 0, 3, 6.5 and 10 me/l**	**36 advanced introgressed lines (ILs) along with wild donor species (*B. fruticulosa*, *Erucastrum abysinnicum*, *Diplotaxis tennisiliqua* , UP *B.nigra*)**	**Four ILs (JA106, JA108, JT 163 & JT498) along with the released variety (PBR357) and national salinity check, CS 52**
B	**Irrigation with saline water (sodium chloride) Salinity**	**EC (5) : 0, 3, 6, 9 and 12ds/m**	**36 advanced introgressed lines (ILs) along with wild donor species (*B. fruticulosa*, *Erucastrum abysinnicum*, *Diplotaxis tennisiliqua* , UP *B.nigra*)**	**Four ILs (JA106, JA108, JT 163 & JT498) along with the released variety (PBR357) and national salinity check, CS 52**

CS 52 : Developed by ICAR-Central Soil Salinity Research Institute ,Karnal INDIA

Observations		Instrument/Method
SPAD values Leaf area and Photosynthetic active radiations		SPAD 502 Plus, Minolta Digital Canopy Imager CL-110
❖Extinctions coefficient (k)		-In (PAR b/PAR a) /LAI
❖Chlorophyll content		Hiscox and Israelstam 1979
Soluble Na and K		Sodium and potassium measured in the 1:2 soil extract using Flame-photometer (Richards 1954)
Soluble Ca+ Mg		Versenate method (Richards 1954)
Soluble HCO_3^- and Cl-	:	Richards 1954
Total soluble sugars		Dubois *et al.* (1956)
Proline		Bates (1973)
Ascorbate		Roe and Kuether (1943)
ἀ tocopherol		Asthir (2009)
❖ Growth, yield and yield attributes at maturity		
❖Oil content NMR (Newport Analyzer (Model MKIIIA) ❖N content and glucosinolates NIRS (Model Foss 6500)		
❖Tolerance parameters (Fisher and Maurer (1978) Salinity susceptibity index (SSI): (1-(Ys/Yp))/(1-Ys / Yp), where Ys and Yp yield under stress and non-stress for each cultivar Ys and Yp yield mean in stress and conditions for all cultivars		
Salinity tolerance index (STI): Ypi x Ysi/Ypi^2 Ypi: seed yield under non stressed/irrigated conditions Ysi: seed yield under stress conditions		

Results

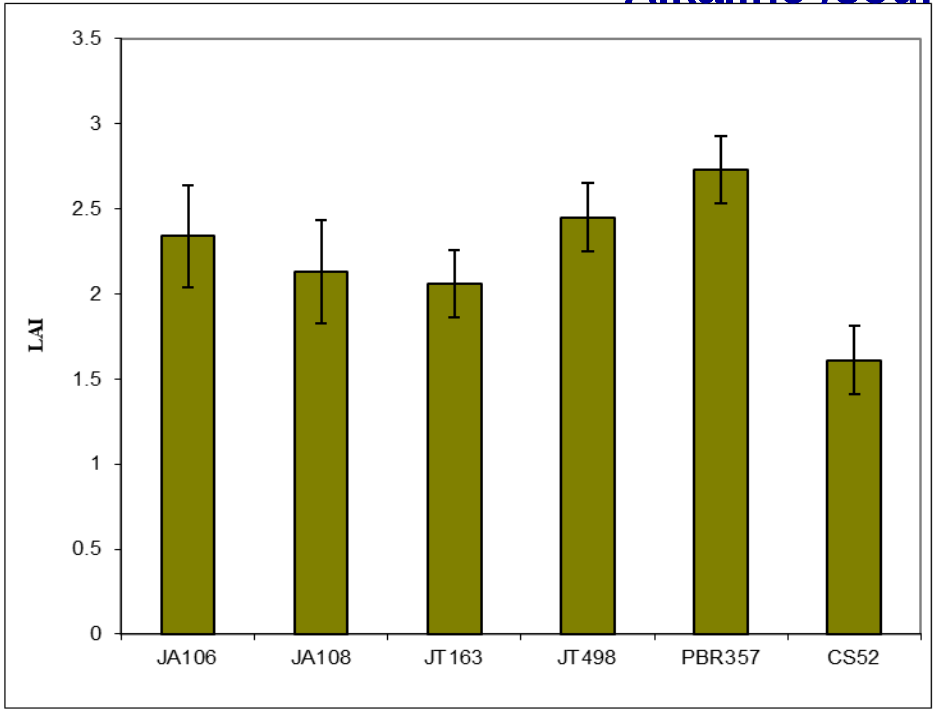
Reproductive phase was longer by 4-5 days in ILs and PBR357 with alkalinity and only in JA108 and PBR357 by 5 days with salinity

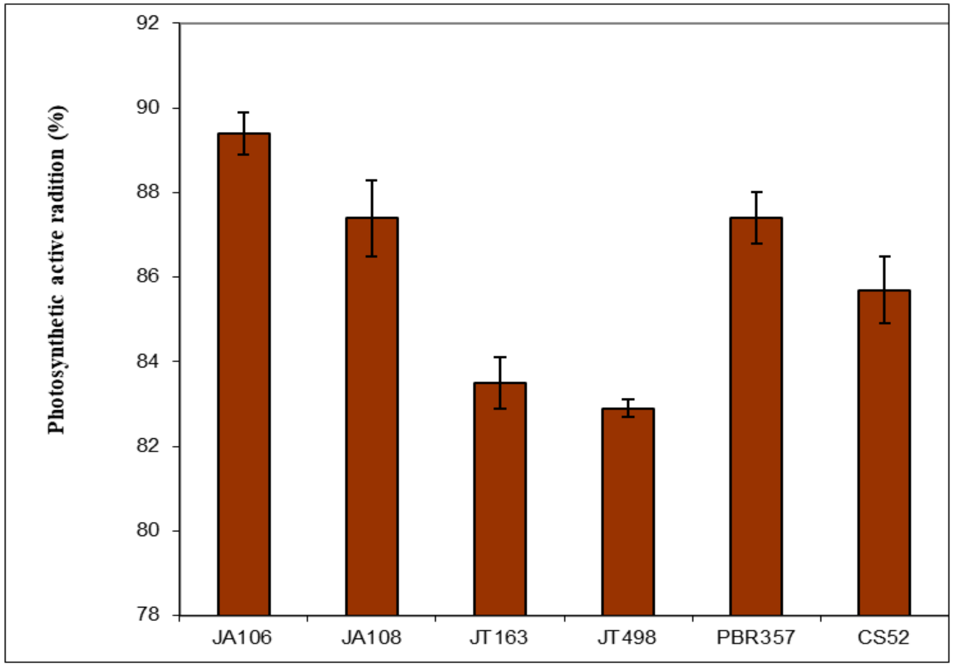
Results

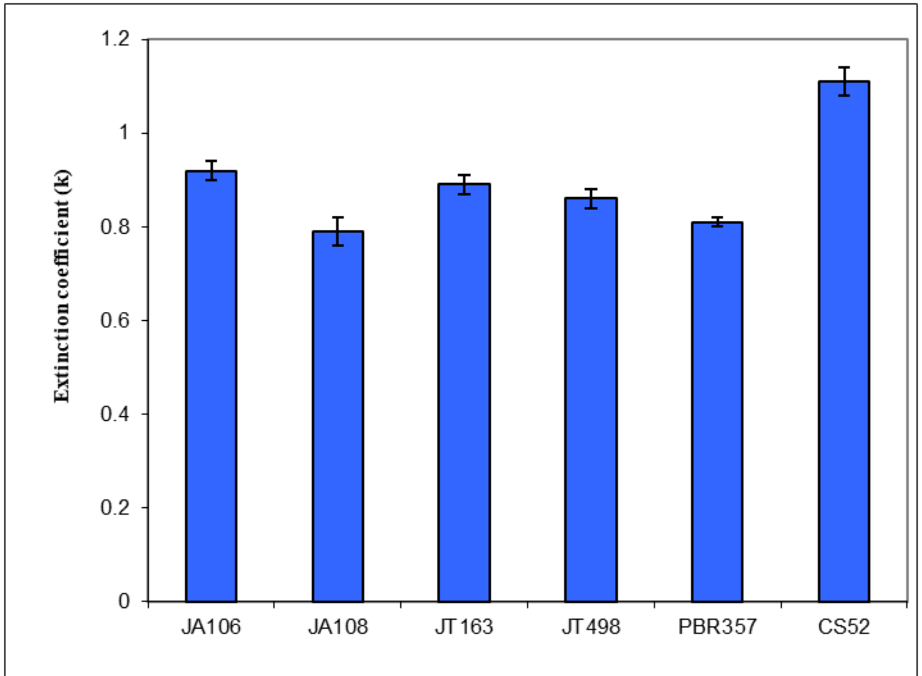
With the increase in alkaline and salinity levels ,leaf area index (LAI), photosynthetic active radiations (PAR) and extinction coefficient (k) decreased.

PAR and k showed inverse relationship .

Alkaline /sodic condition



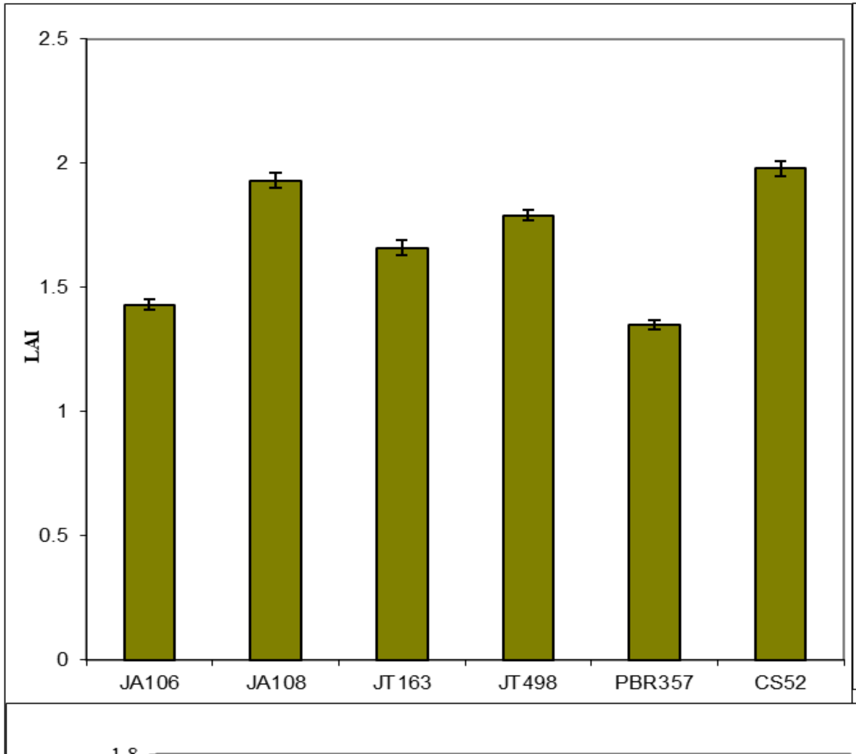


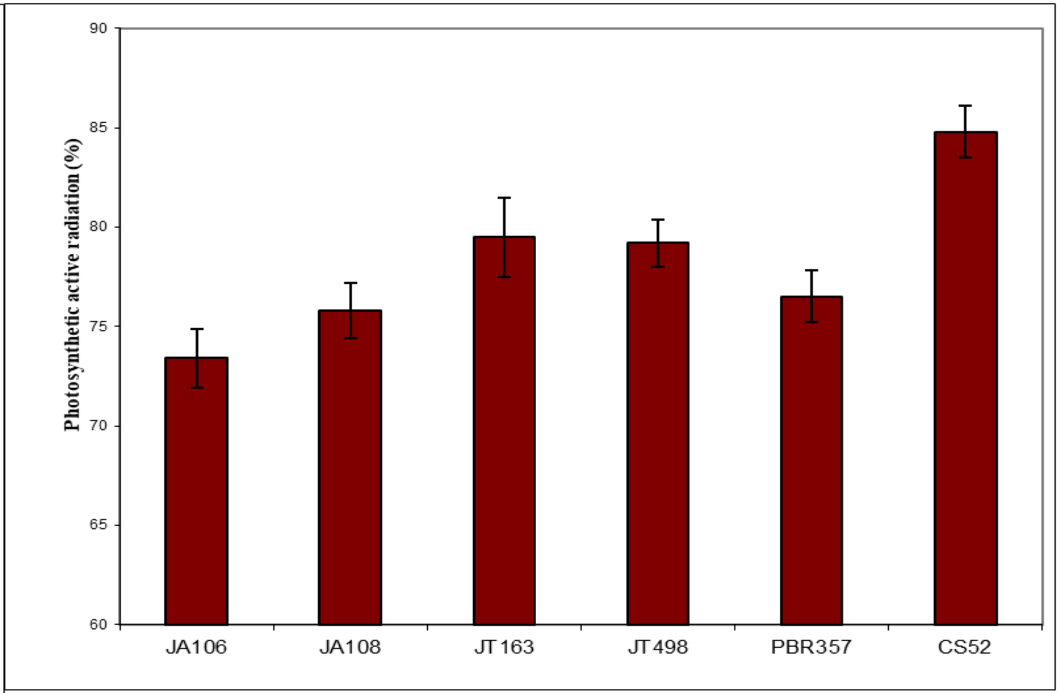


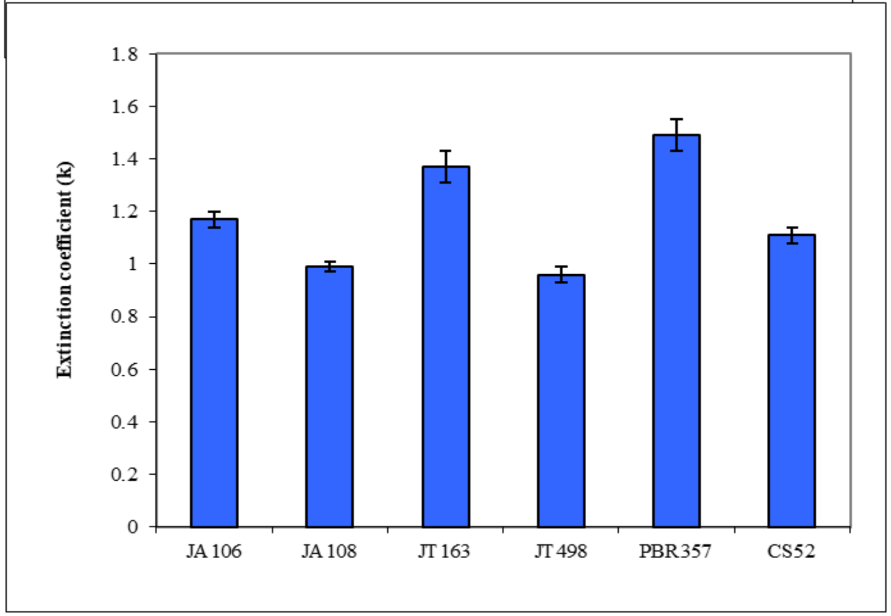
Variation existed for LAI ,

PAR interception and extinction coefficient at flowering stage under sodic /alkaline condition

Saline condition







Differences existed for LAI,PAR interception and extinction coefficient under saline condition

Effect of alkalinity levels on photosynthetic pigments (mg/g FW)

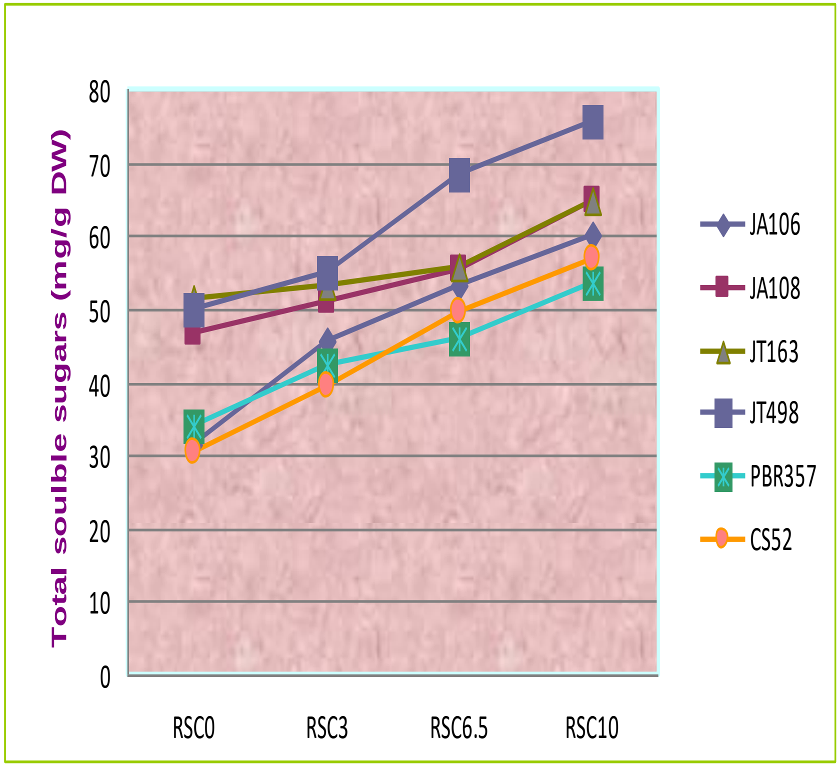
Pigments	Alkaline levels			
	RSC0	RSC3	RSC6.5	RSC10
	Chl a			
Range	1.57-1.66	1.45-1.60	1.29-1.57	1.16-1.38
Mean	1.65	1.52	1.44	1.3
	Chl b			
Range	0.35-0.50	0.27-0.40	0.24-0.34	0.21-0.29
Mean	0.42	0.34	0.30	0.25
	Total Chlorophyll			
Range	1.89-1.96	1.79-1.88	1.63-1.86	1.54-1.84
Mean	1.93	1.85	1.73	1.65
	Carotenoids			
Range	0.46-0.59	0.44-0.49	0.42-0.46	0.36-0.42
Mean	0.52	0.47	0.44	0.40
	SPAD			
Range	43.9-47.8	42.8-44.8	41.8-43.8	39.9-42.8
Mean	45.4	44.1	42.8	41.7

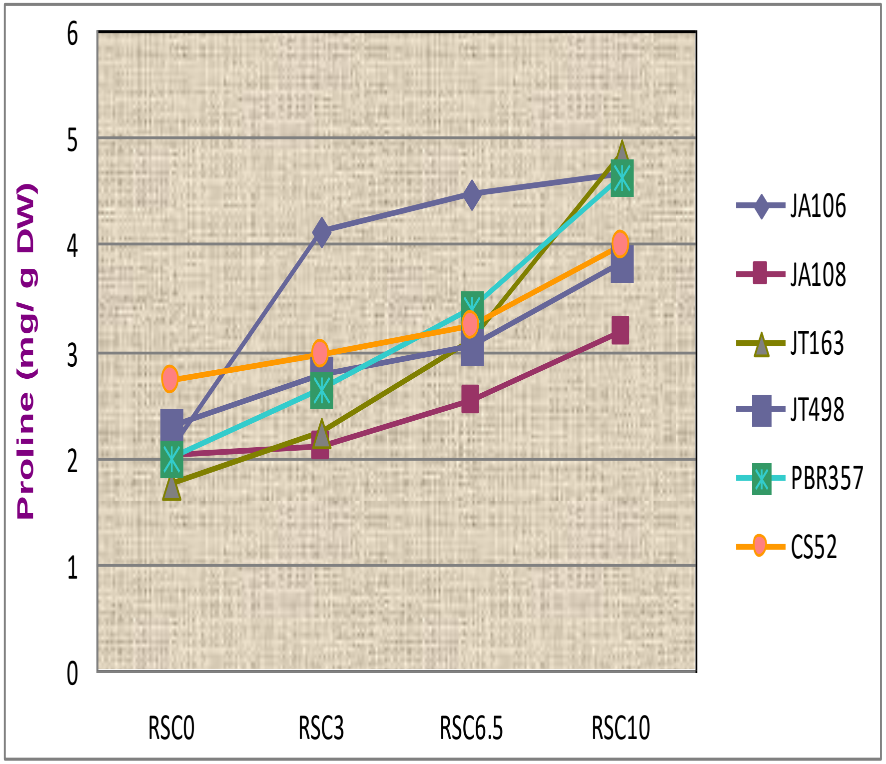
Effect of salinity levels on photosynthetic pigments

Pigments	Saline levels				
	EC0	EC3	EC6	EC9	EC12
	Chl a				
Range	1.52-1.84	1.47-1.72	1.39-1.64	1.34-1.6	1.15-1.57
Mean	1.67	1.59	1.48	1.42	1.33
	Chl b				
Range	0.35-0.48	0.31-0.36	0.29-0.33	0.18-0.32	0.18-0.29
Mean	0.43	0.34	0.32	0.27	0.25
	Total Chlorophyll				
Range	1.86-2.27	1.75-2.08	1.75-1.96	1.64-1.87	1.34-1.87
Mean	2.04	1.91	1.84	1.73	1.65
	Carotenoids				
Range	0.48-0.61	0.47-0.54	0.43-0.51	0.41-0.46	0.35-0.45
Mean	0.55	0.51	0.46	0.44	0.42
	SPAD				
Range	43.55-46.1	41.05-44.2	40.85-42.8	40.2-41.4	37.25-40.95
Mean	44.6	43.16	41.82	40.83	39.59

Compatible solutes or osmolytes

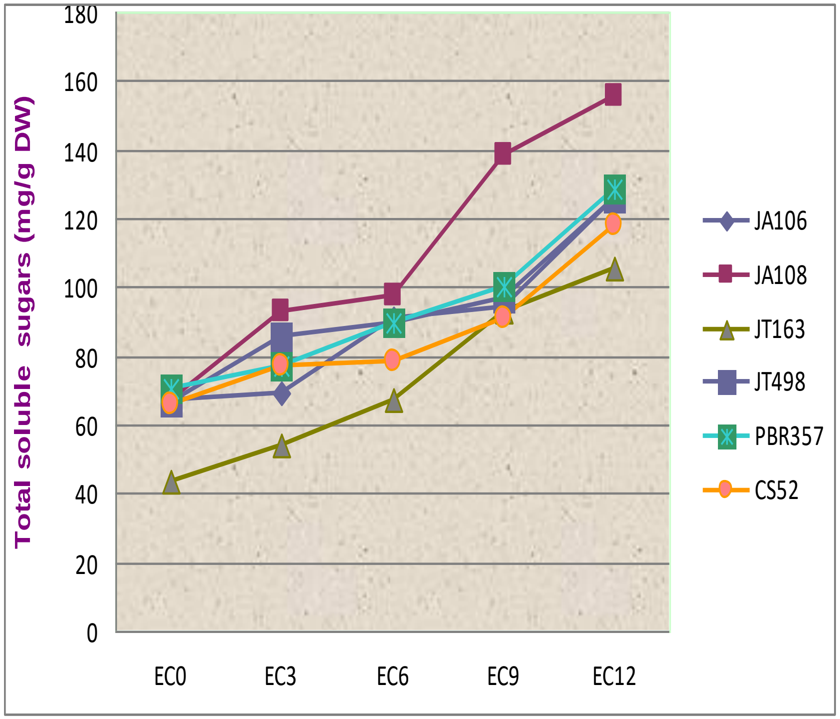
Effect of alkalinity

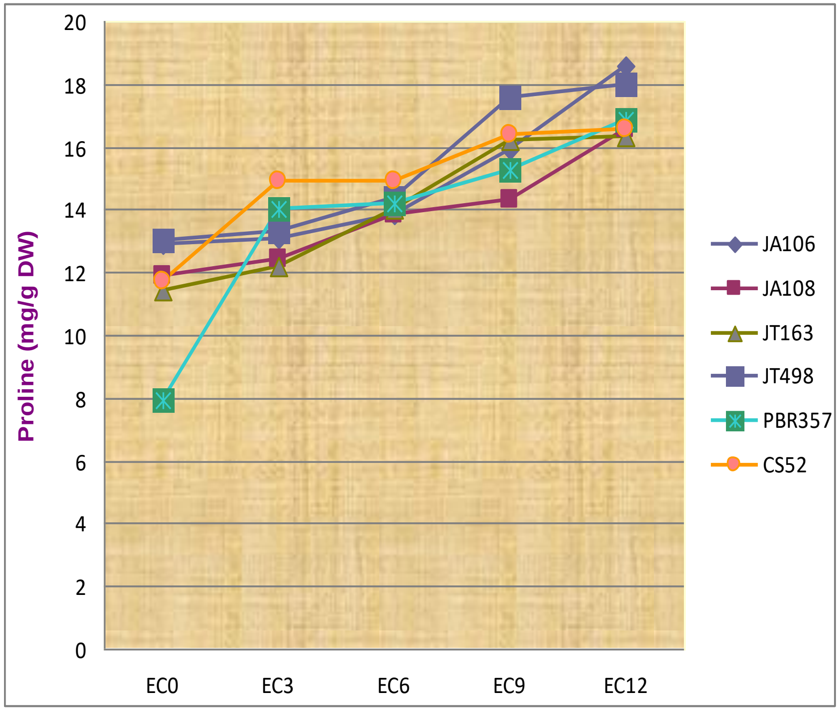




Total soluble sugars and proline content increased with increase in alkalinity levels

Effect of salinity



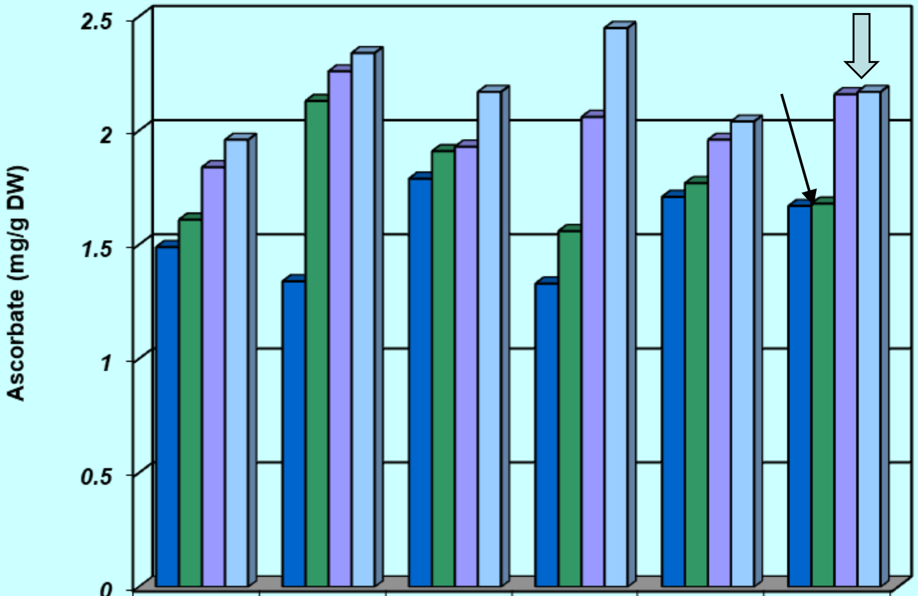


Genetic variations for osmoprotectants

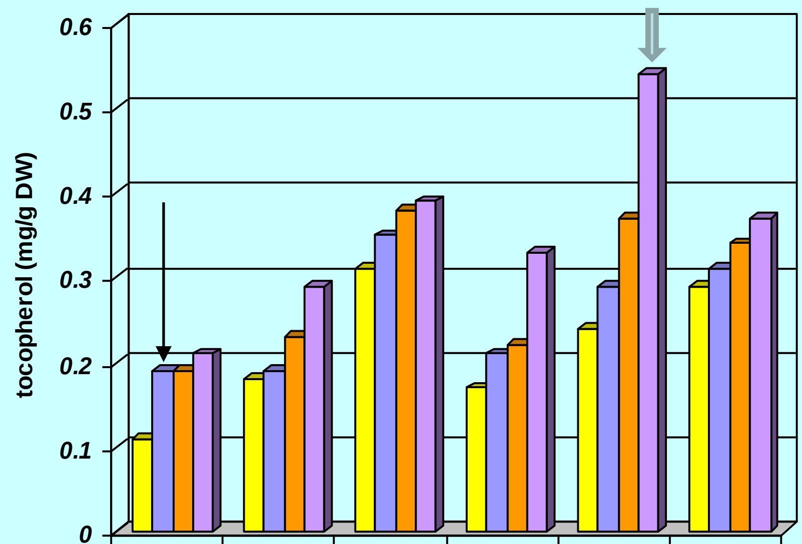
Total soluble sugars and proline content increased with increase in salinity levels

Antioxidative molecules

Effect of alkalinity

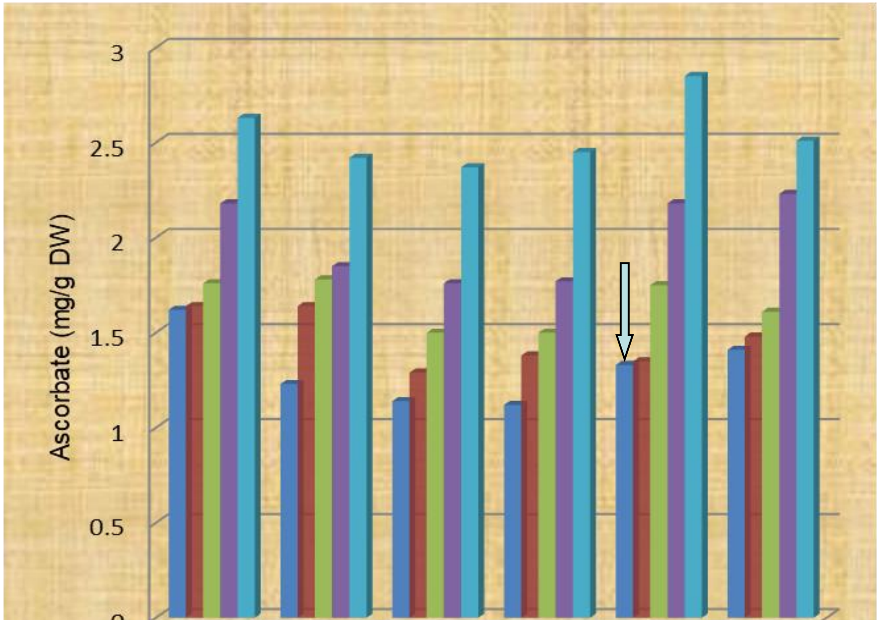


	JA106	JA108	JT163	JT498	PBR357	CS52
RSC0	1.49	1.34	1.79	1.33	1.71	1.67
RSC3	1.61	2.13	1.91	1.56	1.77	1.68
RSC6.5	1.84	2.26	1.93	2.06	1.96	2.16
RSC10	1.96	2.34	2.17	2.45	2.04	2.17

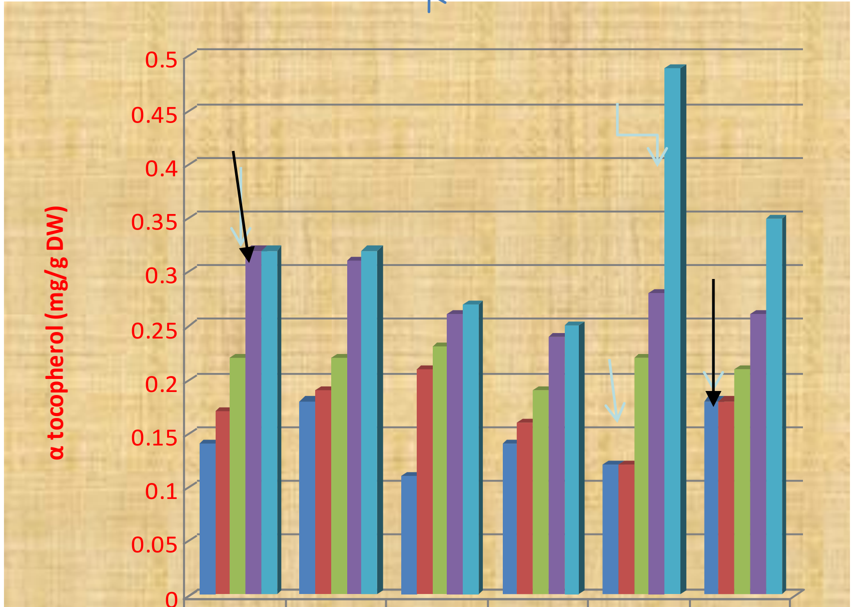


	JA106	JA108	JT163	JT498	PBR357	CS52
RSC0	0.11	0.18	0.31	0.17	0.24	0.29
RSC3	0.19	0.19	0.35	0.21	0.29	0.31
RSC6.5	0.19	0.23	0.38	0.22	0.37	0.34
RSC10	0.21	0.29	0.39	0.33	0.54	0.37

Effect of salinity



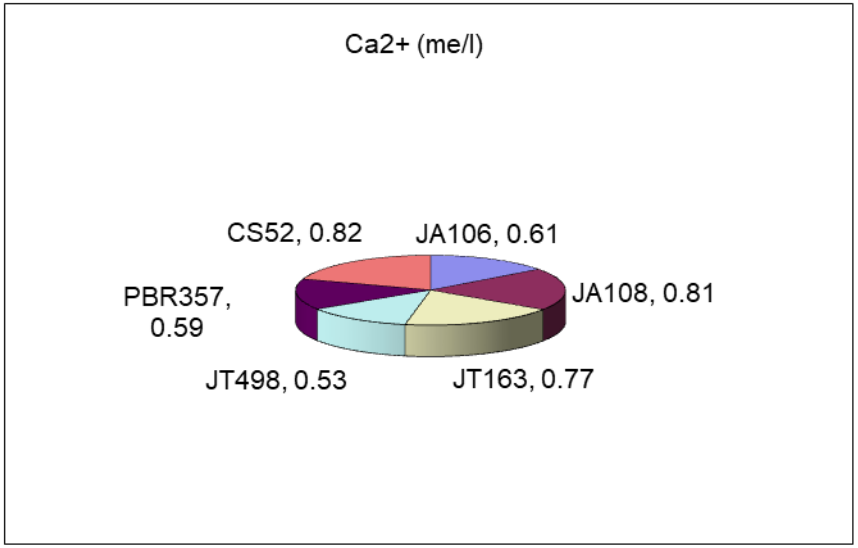
	JA106	JA108	JT163	JT498	PBR357	CS52
EC0	1.62	1.23	1.14	1.12	1.33	1.41
EC3	1.64	1.64	1.29	1.38	1.35	1.48
EC6	1.76	1.78	1.5	1.5	1.75	1.61
EC9	2.18	1.85	1.76	1.77	2.18	2.23
EC12	2.63	2.42	2.37	2.45	2.85	2.51

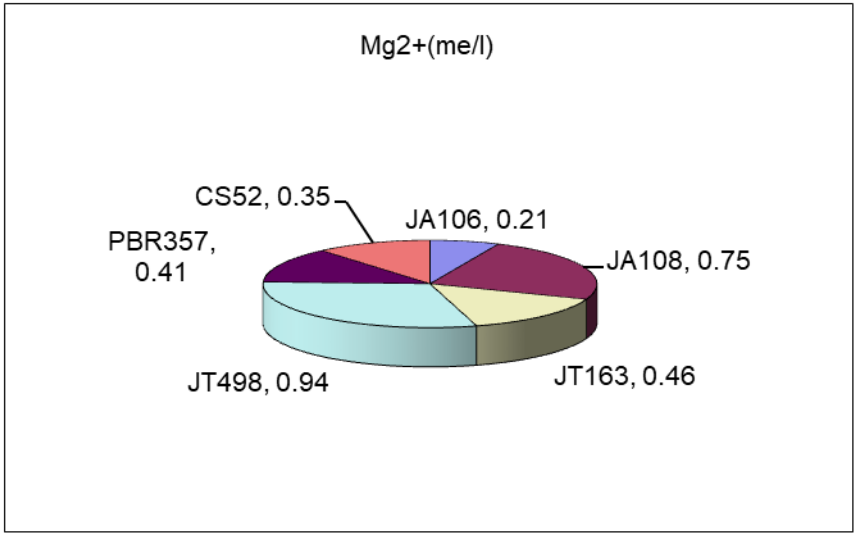


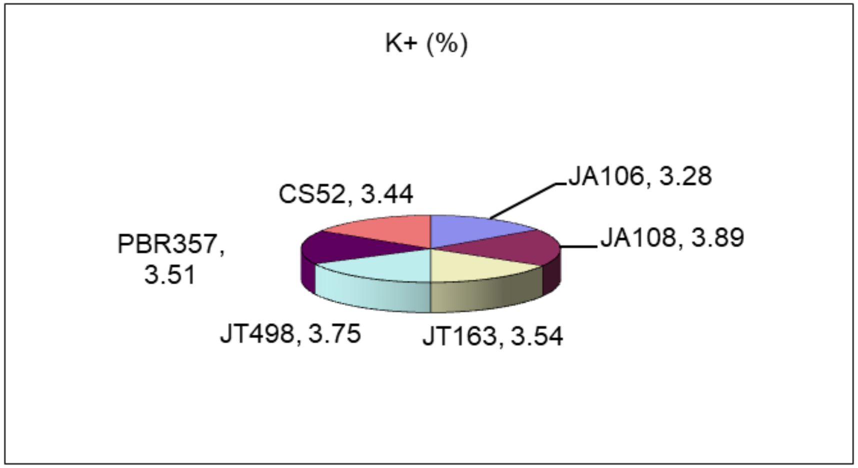
	JA106	JA108	JT163	JT498	PBR357	CS52
EC0	0.14	0.18	0.11	0.14	0.12	0.18
EC3	0.17	0.19	0.21	0.16	0.12	0.18
EC6	0.22	0.22	0.23	0.19	0.22	0.21
EC9	0.32	0.31	0.26	0.24	0.28	0.26
EC12	0.32	0.32	0.27	0.25	0.49	0.35

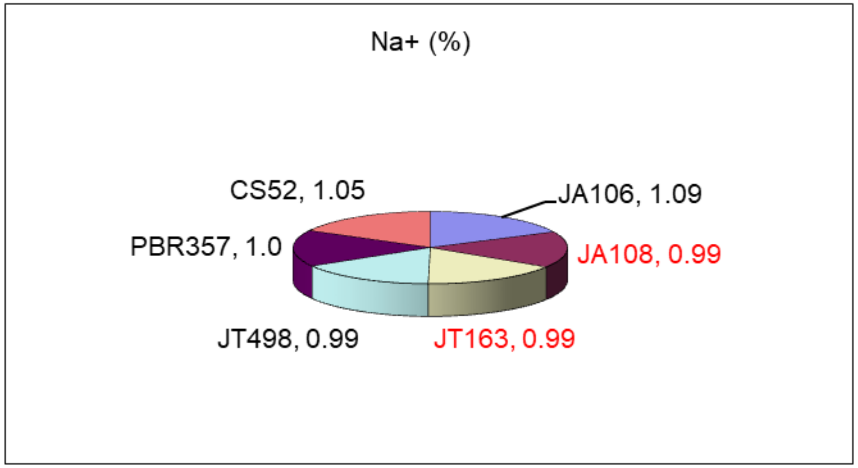
Up regulation of ascorbic acid and ά tocopherols with increased saline and alkaline levels

Variation in ionic content under alkaline condition in the leaves at flowering stage

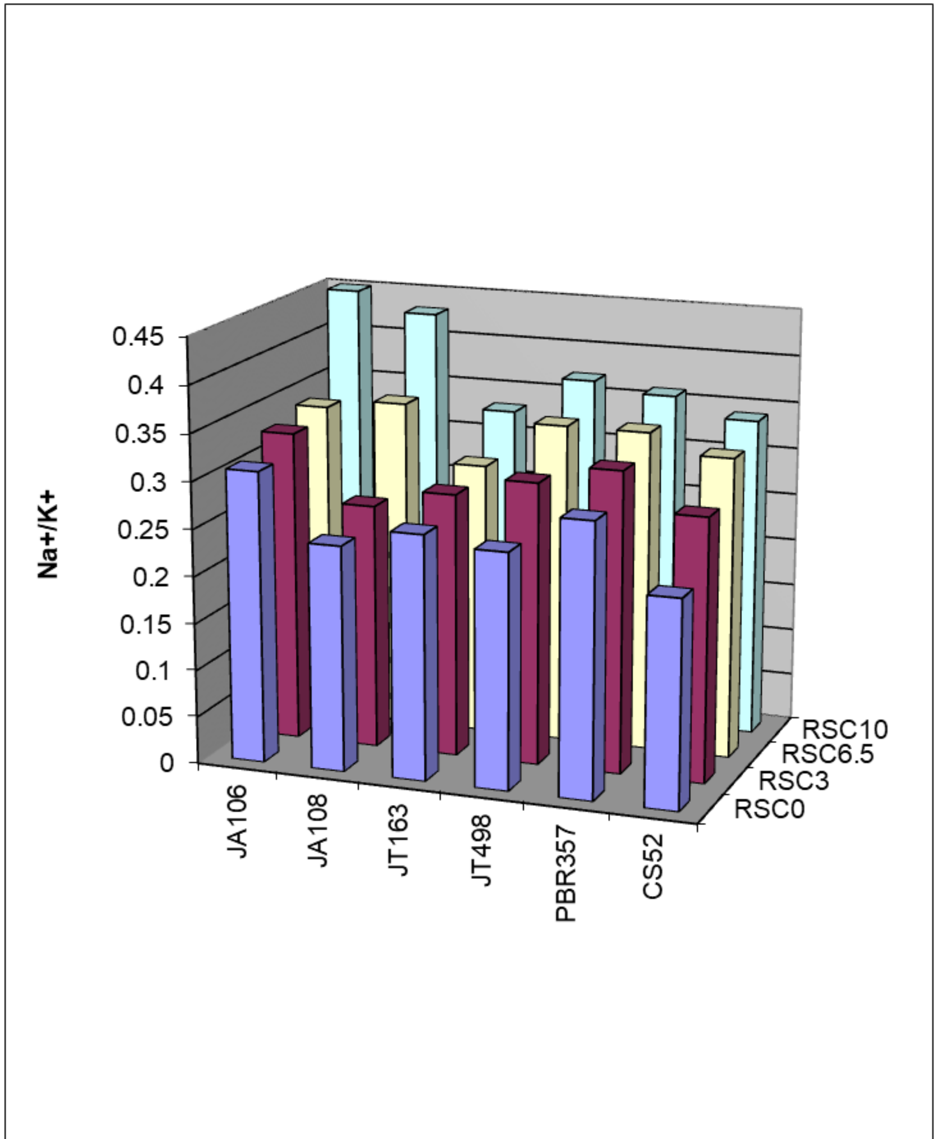


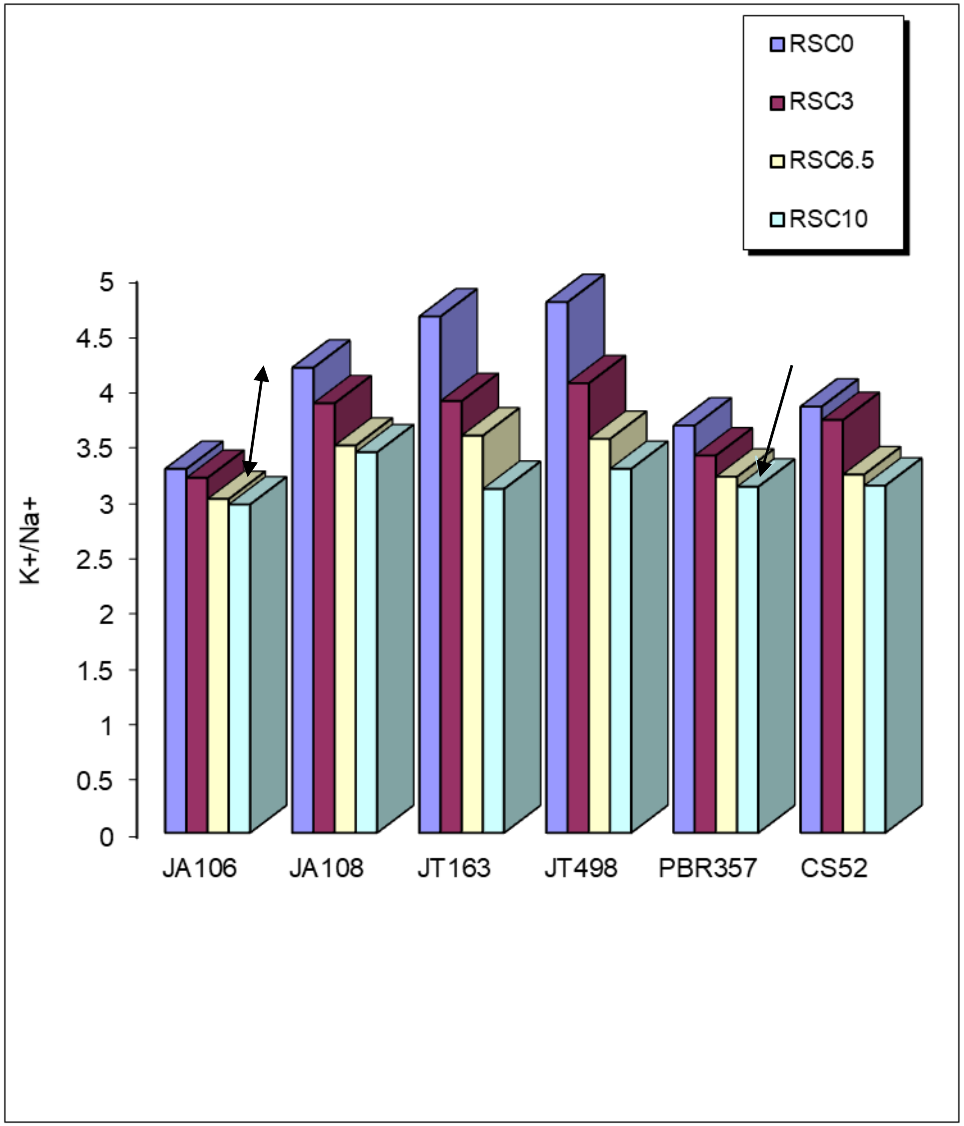


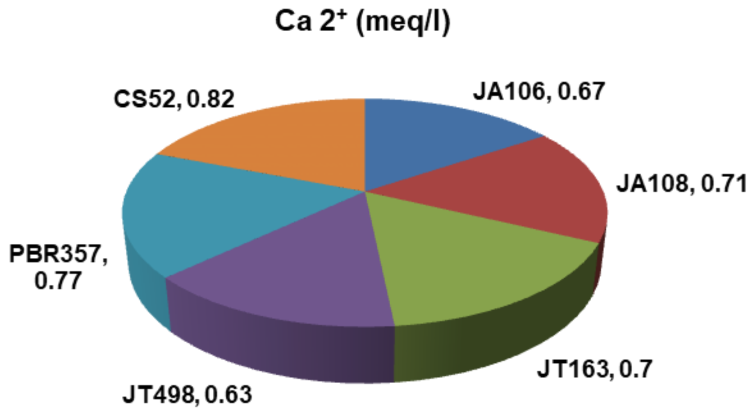


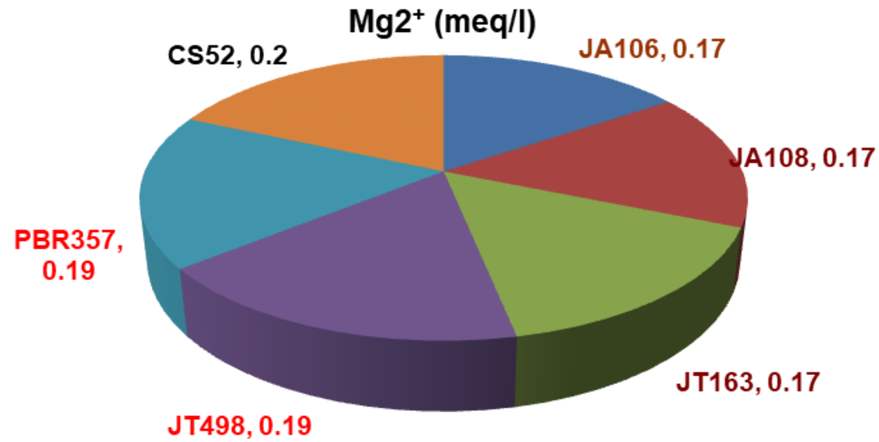


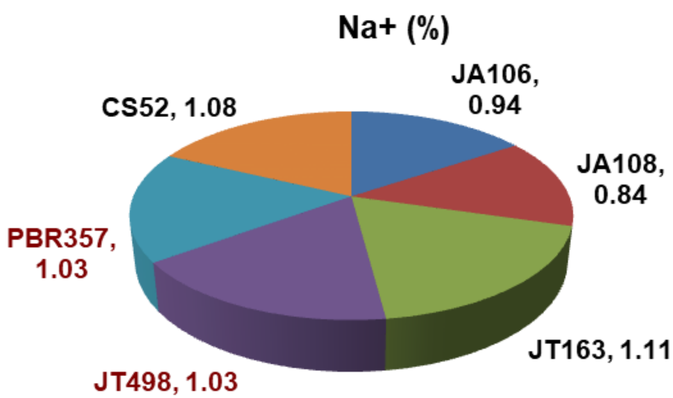
Effect of alkalinity levels on Na^+/K^+ and K^+/Na^+ ratio

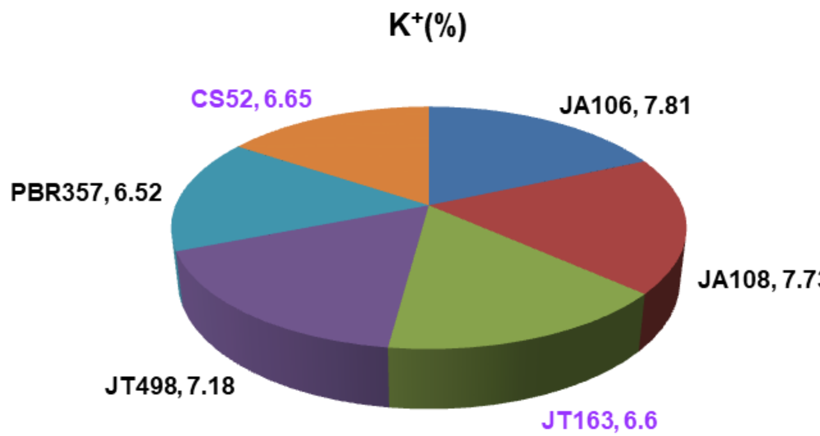


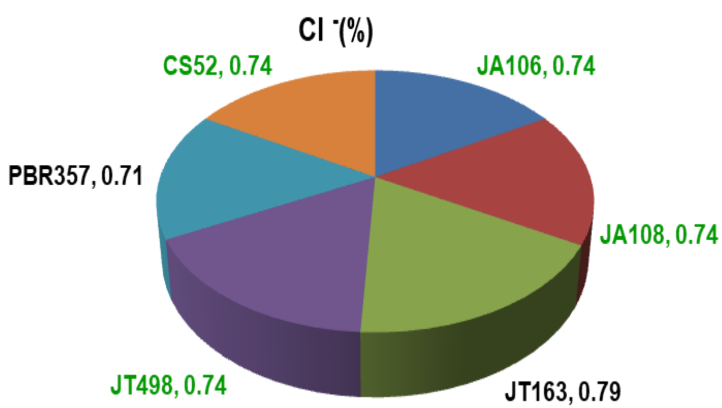




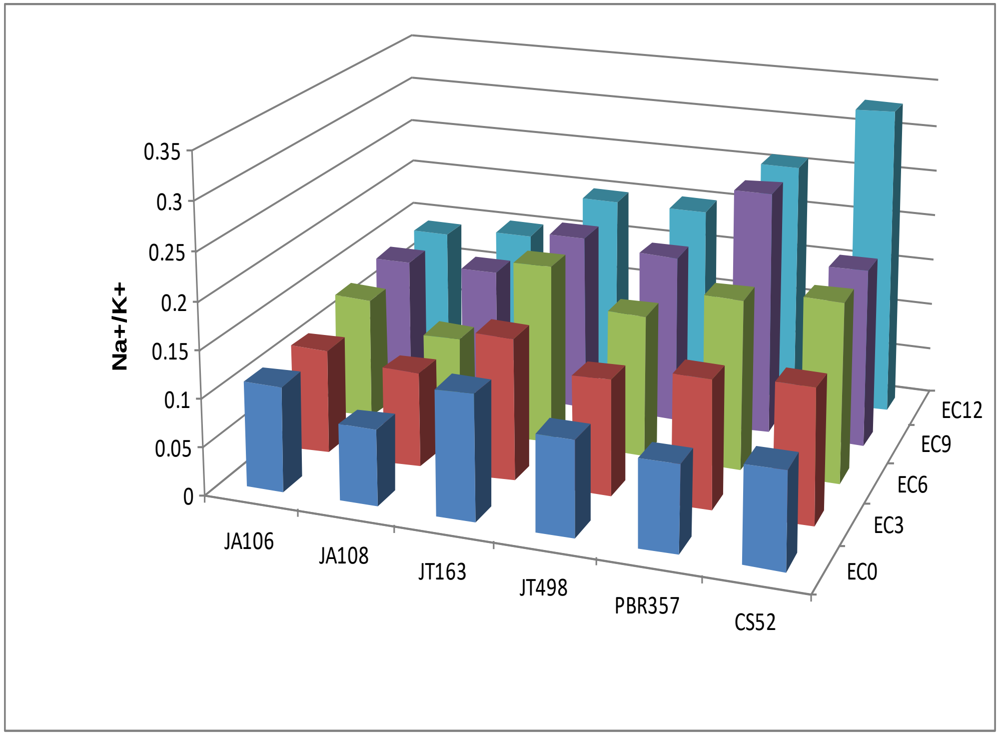


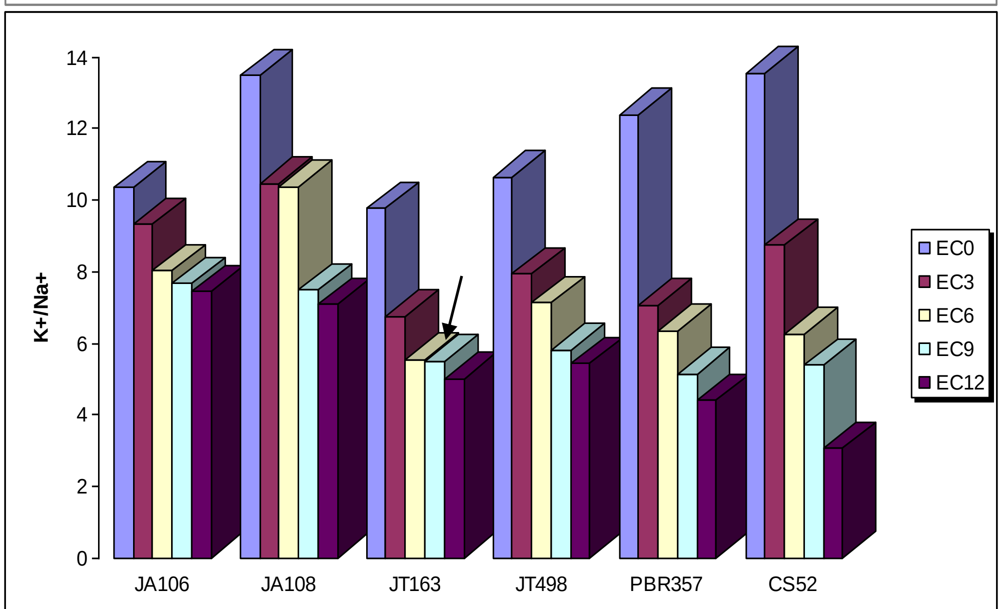






Variation in ionic content under saline condition

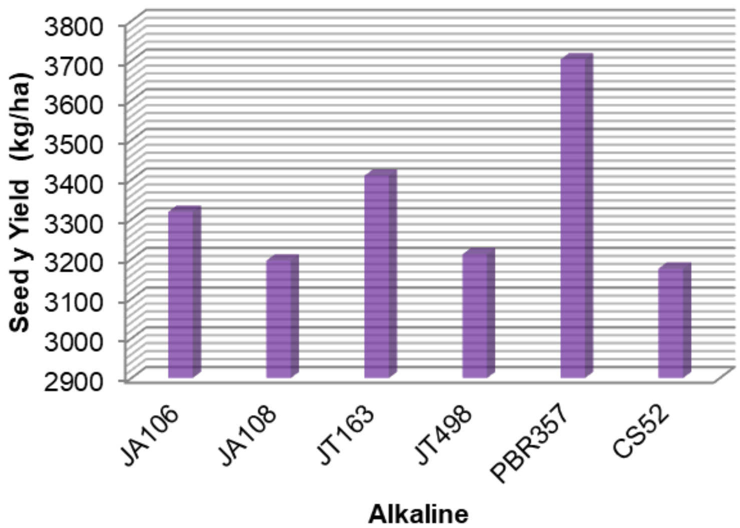


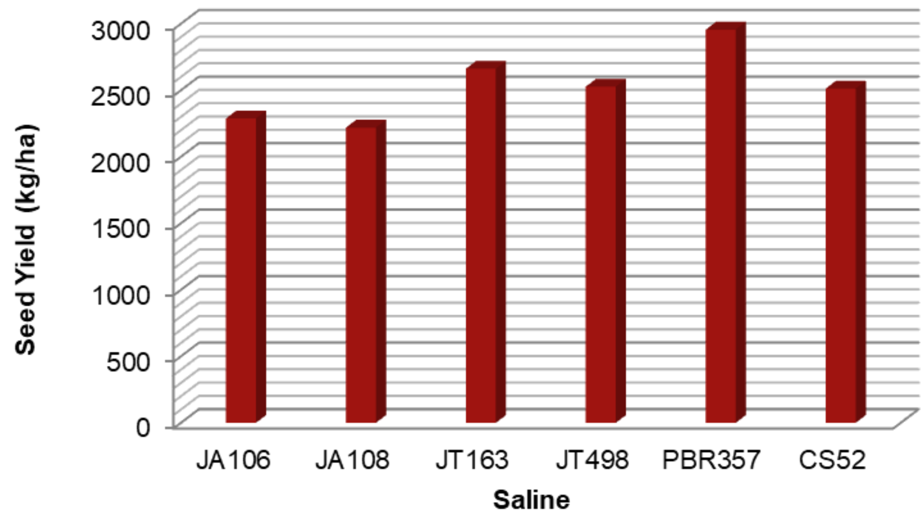


- ✓ Increased salinity levels increased Na^+/K^+ but decreased K^+/Na^+
- ✓ Differential responses to salinity and magnitude of variation exists

Alkaline levels/ Traits	RSC0		RSC3		RSC6.5		RSC10	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Growth parameters								
Plant height (cm)	192.1-203	195.4	187.4-195.1	187.9	177.8-187.3	184.3	154.0-167.4	168
Main shoot length (cm	67.2-86.1	74.1	60.3-78.5	68.6	57.9-78.3	66.4	54.4-76.7	63.4
Primary branches	5.6-7.8	6.3	5.1-7.2	5.8	5.0-6.6	5.6	4.6-6.1	5.3
Secondary branches	12.8-16.7	15.1	11.9-16.3	14.1	10.7-15.5	12.9	9.2-14.0	11.2
Yield components								
Siliquae on main shoot	39.9-47.9	44.4	39.5-46.1	42.9	37.5-44.1	41.3	34.0-42.2	39.6
Total Siliquae /plant	272.6-379.6	341.4	269.9-372	319.4	253.1-336.9	291.1	204.7-325.2	253.3
Seed weight (g)	3.6-5.7	4.6	3.4-5.6	4.2	3.2-5.1	4.0	3.0-4.9	3.7
Siliqua length (cm)	4.4-4.8	4.6	4.1-4.8	4.5	4.1-4.7	4.4	3.7-4.3	4.1
Seeds/siliqua	14.2-15.4	14.8	12.7-14.6	13.7	12.4-14.5	13.2	12.0-14.4	12.8
Yield								
Biological yield (q/ha)	16.3-21.1	17.6	14.4-20.3	15.9	11.3-17.5	13.5	8.8-11.9	9.8
Seed yield (kg/ha)	3880-4283	4080	3194-3969	3514	2869-3550	3092	2372-3013	2638
oil content (%)	40.2-42.2	41	39.6-41.8	40.6	39.6-41.6	40.4	39.0-40.9	39.9
Crude protein (%) in seeds	29.2-30.4	29.6	28.1-29.3	28.8	28.1-28.9	28.5	27.3-28.7	28.1
Nitrogen (%) in seeds	4.7-4.9	4.7	4.5-4.7	4.6	4.5-4.6	4.6	4.4-4.6	4.5
Glucosinolates (µmole /g defatted meal)	77.7-83.9	81.1	75.8-83.1	79.3	71.4-82.4	76.8	67.2-81.3	73.4

Salinity levels / Traits	EC0		EC3		EC6		EC9		EC12	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Growth parameters										
Plant height (cm)	168.4-183.7	172.9	163.5-176.9	168.6	141.2-161.9	154	135.4-152.8	143.9	122.6-136.4	127.6
Main shoot length (cm)	59.9-75.5	67.4	55.7-67.6	61.6	50.8-60.9	56.7	46.1-56.1	49.4	48.1-49.7	45.8
Primary branches	6.4-6.8	6.2	4.5-6.5	5.5	4.4-6.2	5.2	3.7-5.4	4.7	3.7-4.6	4.4
Secondary branches	8.9-12.6	11.1	7.8-10.6	9.1	6.2-10.1	8.2	5.6-8.6	7.0	3.9-8.1	5.6
Yield components										
Siliquae on main shoot	39.2-43.0	40.9	34.7-40.2	38.5	33.2-38.3	36.2	32.7-36.9	35.3	29.5-34.5	32.6
Total Siliquae /plant	144-247.9	181.4	140.5-197.4	155.5	102-179.0	136.3	94.5-172.9	123.9	90.1-127	98.5
Seed weight (g)	4.0-6.7	5.5	3.2-5.8	4.7	2.9-5.3	4.3	2.9-4.8	3.8	2.7-4.8	3.6
Siliqua length (cm)	4.7-5.5	5	4.4-5.2	4.8	4.2-5.1	4.6	4.1-4.7	4.4	3.8-4.5	4.2
Seeds/siliqua	11.3-13.3	12.4	11.4-13.1	12.1	10.6-12.3	11.8	10.1-11.6	10.7	9.1-11.0	9.8
Yield										
Biological yield (q/ha)	9.4-14.7	12	9.1-13.6	11.2	7.5-12.2	9.1	5.2-8.3	7.2	4.7-8.0	6.4
Seed yield (Kg/ha)	2061-2966	2619	2219-2955	2460	1538-2736	2257	1497-2408	1950	1011-2038	1651
oil content (%)	40.6-42.7	41.4	40.2-40.9	40.9	38.5-41.3	39.9	38.4-40.5	39.1	37.6-38.9	38.3
Crude protein(%) in seeds	31.2-32.0	31.6	30.2-30.6	38.4	28.2-30.4	29.2	27.7-28.9	28.1	26.4-28.2	27.5
Nitrogen (%) in seeds	5-5.1	5.1	4.8-4.9	4.8	4.5-4.9	4.7	4.4-4.6	4.5	4.2-4.5	4.4
Glucosinolates (µmole /g defatted meal)	73.3-86.9	82.6	70.6-85.6	80.9	70.5-85.0	78.2	65.2-80.7	74.7	63.8-80.5	73.2





Stress Indices	Sodic/ alkaline levels		
	JA163	PBR357	CS52
Salinity tolerance index (STI)	0.82	0.82	0.68
Salinity susceptibility index (SSI)	0.6	0.7	1.2
	Saline levels		
Salinity tolerance index (STI)	JA163	PBR357	CS52
	0.74	0.84	0.84
Salinity susceptibility index (SSI)	0.64	0.62	0.69

Over the years

- Decrease in Ca^{2+} and Mg^{2+} ions resulted in decline in chlorophyll.
- JT 163 and PBR 357 possessed higher total soluble sugars, proline, ascorbate and tocopherols as compared to check, CS 52 .
- JT 163 registered more number of branches and length of main raceme while PBR 357 had higher seed weight, biomass and seed nitrogen.
- PBR 357 and JT 163 holds promise under saline conditions based on higher seed yield and tolerance index (STI≥0.62).
- Seed yield was positively correlated with tolerance index (0.892, 0.955**) but negatively associated with susceptibility index (-0.896, -0.955**).

Conclusions

- Salt stress reduced productivity but there existed genotypic variation for salinity tolerance.

- Accumulation of osmolytes and antioxidative molecules imparts tolerance.

- Lower Na^+ and Na^+/K^+ ratio but higher K^+ and K^+/Na^+ associated with tolerance.

- Higher tolerance index (STI) and lower susceptibility index (SSI) indicate salinity tolerance.

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