Stomatal characteristics, endogenous cytokinins, and abscisic acid-like inhibitors in pigeon pea and gingelley leaves under NaCl salinity.

Resumen. Se estudiaron los cambios en características estomatales, citocininas e inhibidores del tipo ABA, transpiración y cationes en gandul y ajonjolí, durante el desarrollo foliar, bajo la influencia de salinidad. La salinidad ocasiona una reducción en la frecuencia estomatal, en su abertura y en las fases de transpiración. Las hojas de las plántulas cultivadas en alta salinidad de NaCl presentan su máxima abertura estomatal durante las primeras horas del día.

El tratamiento salino provoca una disminución de citocininas y niveles superiores de inhibidores tipo ABA, tanto en gandul como en ajonjolí. Las hojas de las plántulas salinizadas presentan altas concentraciones de sodio, pero baja de potasio y calcio. Se discute la importancia de estos cambios en relación a la adaptabilidad de estas plantas a condiciones de salinidad.

Soil salinity is one of the frequent constraints in crop productivity. Salts in the root medium generally stunt plant growth, often without any other symptoms of damage. It was suggested that the stunted plant growth and other modifications of growth habit such as increased leafiness, indicated that growth hormones may be involved in response of plants to the salinity of the root medium (2). It has been reported that the hormone changes brought by salinity arise essentially due to water deficits (11, 12). Work on different physiological aspects of the same plant species under the influence of salinity seems to be meagre. A comprehensive study on the effect of salinity on different physiological aspects of the same species would provide a better picture of the physiological alterations induced by salinity. An attempt is made in the present study to elucidate the effects of salinity on changes in endogenous cytokinins, ABA-like inhibitors, stomatal characteristics, transpiration and cation content and their role in adaptation to salinity in pigeon pea and gingelley during leaf maturation.

Materials and methods

Pigeon pea (*Cajanus indicus* Spreng Var. LRG 30) and gingelley (*Sesamum indicum* L. Var. TMV 1) were screened for tolerance to varying levels of salinity ranging from 0.1 to 0.6% NaCl in the soil. A salinity level of 0.4% has been selected in the present study at which pigeon was tolerant and gingelley was susceptible. The growth of plants, salt treatment and sampling of leaves for various experiments were carried out as reported earlier by Gururaja Rao (3, 4).

Stomatal frequency and diurnal rhythms of stomatal opening were determined by the imprint method described by Sampson (15). Stomatal aperture width was measured using a pre-calibrated occular micrometer. Transpiration rates were determined by the pot-weight method as described by Sarada Devi (16).

Endogenous cytokinins (17) and ABA-like inhibitors (10) were extracted and separated by unidirectional paper chromatography using iso-propanol: ammonia:water (10:1:1, by volume). The chromatograms were dried after development and cut transversely into 10 equal strips and each strip was eluted in methanol. The eluates were evaporated to dryness and the residues were dissolved in 5 mM phosphate buffer, pH 5.8 and 0.5% sucrose for the bioassays of cytokinins and ABA-like inhibitors, respectively. The biological activity of cytokinins and ABA-like inhibitors was determined by the cucumber cotyledon greening bioassay (1) and wheat coleoptile bioassay (13), respectively. Cations, sodium, potassium and calcium were determined using a flame photometer.

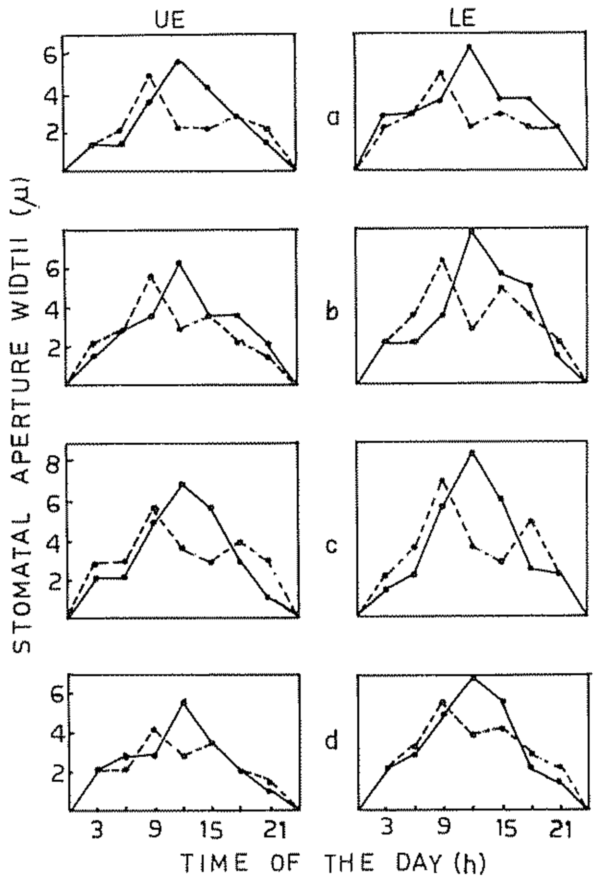

Fig. 1. Diurnal rhythms of stomatal opening in pigeon pea leaves under NaCl salinity.

● —— ● Control ● - - - - ● Salinized

Results and discussion

Stomatal frequency (Table 1) showed a decrease both in pigeon pea and gingelley under salinity; the decrease being more in gingelley. Stomatal opening (aperture width in microns) was found to be affected in the salt treated plants (Figures 1, 2). The stomates were found to be completely closed at mid-night in both the species under control and salinized conditions. Maximum stomatal opening was observed at 12:00 h and 9:00 h under control and salinized conditions respectively, in both the species on both abaxial and adaxial surfaces of the leaves.

Stomata control diffusion of water vapour and of CO_2. The stomata in pigeon pea and gingelley under salinity showed maximum opening in the morning (Figures 1, 2). The stomata of mangroves also remined open during the early hours of the day and closed in the after-noon suggesting the possibility of maximum CO_2 assimilation in the morning (9). From the results of the present study it is inferred that these alterations were considered to be a measure of physiological adaptability to the saline environment because the changes would reduce transpiration. Reduction in transpiration is evident under salinity in both pigeon pea and gingelley (Figure 3) and these observations are in conformity with those of Sarada Devi (16).

Cytokinin activity was substantially higher in the control plants than the salinized ones in pigeon pea (Figure 4) and was found to be almost absent in gingelley under salinity (Figure 5). But control gingelley plants showed high cytokinin activity at all stages of growth.

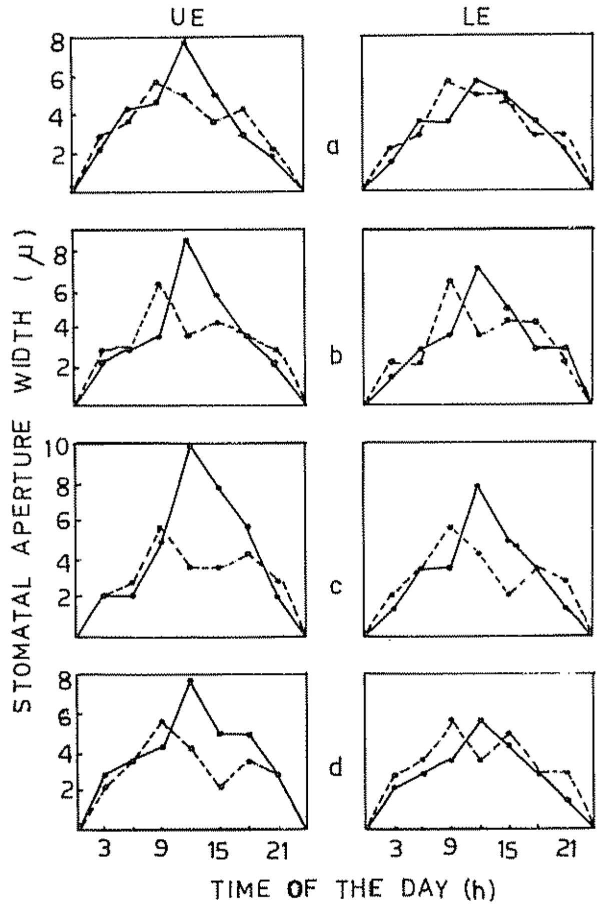

Fig. 2. Diurnal rhythms of stomatal opening in gingelley leaves under NaCl salinity.

●——● Control ●- - - -● Salinized

Table 1. Effect of NaCl salinity on stomatal frequency in pigeon pea and gingelley leaves. (Number of stomata/cm^2 leaf area).

Stage	Treatment	Pigeon pea Upper Epidermis	S.E. ±	Pigeon pea Lower Epidermis	S.E. ±	Gingelley Upper Epidermis	S.E. ±	Gingelley Lower Epidermis	S.E. ±
1	Control	85	2	537	5	495	3	693	2
	Salinized	57	2	382	6	283	3	311	3
2	Control	85	2	523	5	509	3	594	3
	Salinized	71	2	354	4	269	5	297	3
3	Control	113	3	417	4	453	3	679	2
	Salinized	71	2	410	3	240	2	255	3
4	Control	113	2	537	3	281	2	580	3
	Salinized	57	2	438	3	170	3	339	2

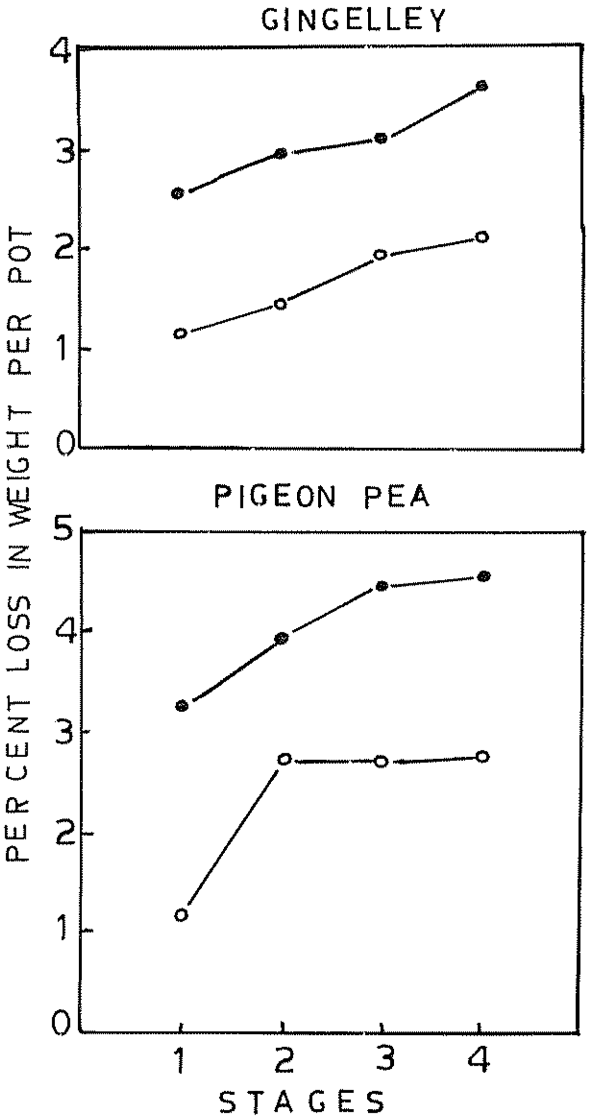

Fig. 3. Rates of transpiration in pigeon pea and gingelley under control and NaCl salinity.

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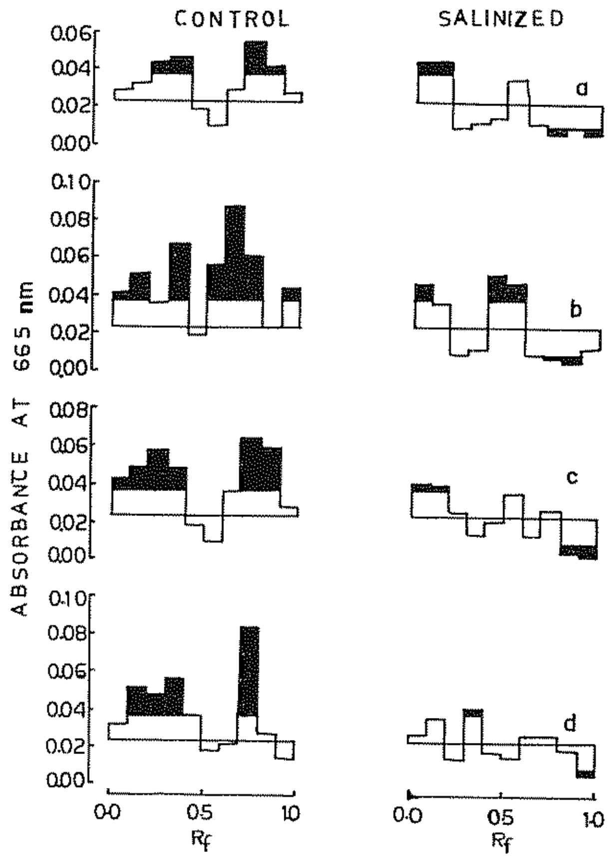

Fig. 4. Effect of NaCl salinity on changes in cytokinins in pigeon pea leaves as shown by cucumber cotyledon greening bioassay. Darkened areas represent regions significantly different from standard at 5% probability level. a, b, c and d indicate stages 1, 2, 3, and 4 respectively.

ABA-like inhibitors were found to be high in the salinized plants of pigeon pea and gingelley (Figures 6, 7) and almost absent in both pigeon pea and gingelley under control conditions.

Table 2. Effect of NaCl salinity on changes in sodium, potassium and calcium in pigeon pea and gingelley leaves (mg/g dry weight).

Stage	Treatment	Pigeon pea						Gingelley					
		Sodium	S.E. ±	Potassium	S.E. ±	Calcium	S.E. ±	Sodium	S.E. ±	Potassium	S.E. ±	Calcium	S.E. ±
1	Control	0.92	0.2	0.62	0.1	0.38	0.1	0.84	0.4	0.64	0.2	0.42	0.8
	Salinized	1.21	0.2	0.53	0.1	0.27	0.2	1.31	0.1	0.44	0.1	0.21	0.1
2	Control	0.95	0.3	0.64	0.1	0.38	0.1	0.86	0.1	0.66	0.2	0.42	0.1
	Salinized	1.29	0.3	0.48	0.1	0.30	0.1	1.37	0.1	0.38	0.1	0.23	0.02
3	Control	0.97	0.4	0.69	0.1	0.4	0.1	0.88	0.1	0.69	0.1	0.46	0.1
	Salinized	1.43	0.5	0.47	0.1	0.37	0.1	1.48	0.1	0.37	0.1	0.25	0.1
4	Control	0.94	0.1	0.69	0.2	0.43	0.1	0.92	0.1	0.71	0.04	0.44	0.1
	Salinized	1.35	0.1	0.47	0.2	0.35	0.1	1.39	0.1	0.37	0.1	0.21	0.1

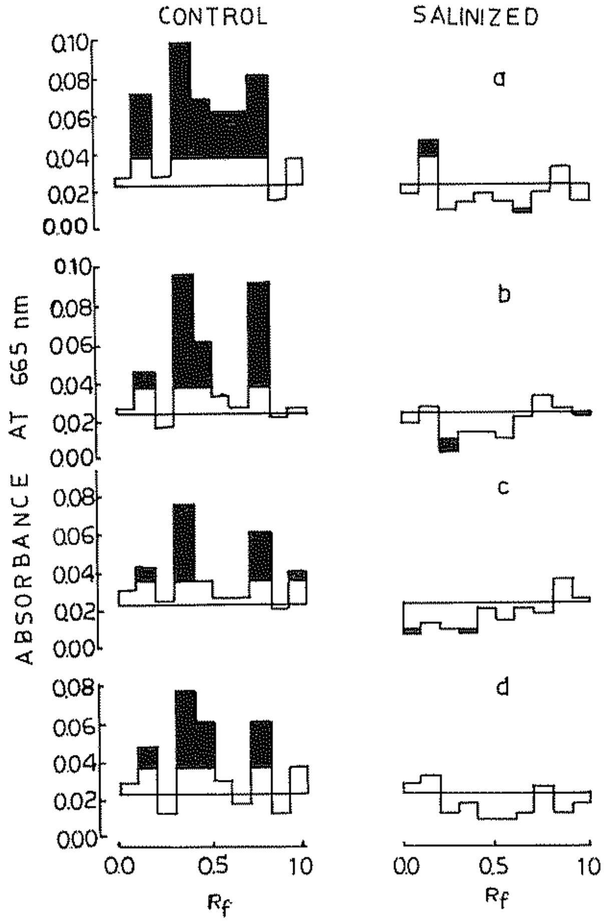

Fig. 5. Effect of NaCl salinity on changes in cytokinins in gingelley leaves as shown by cucumber cotyledon greening bioassay. Darkened areas represent regions significantly different from standards at 5% probability level. a, b, c and d indicate stages 1, 2, 3 and 4 respectively.

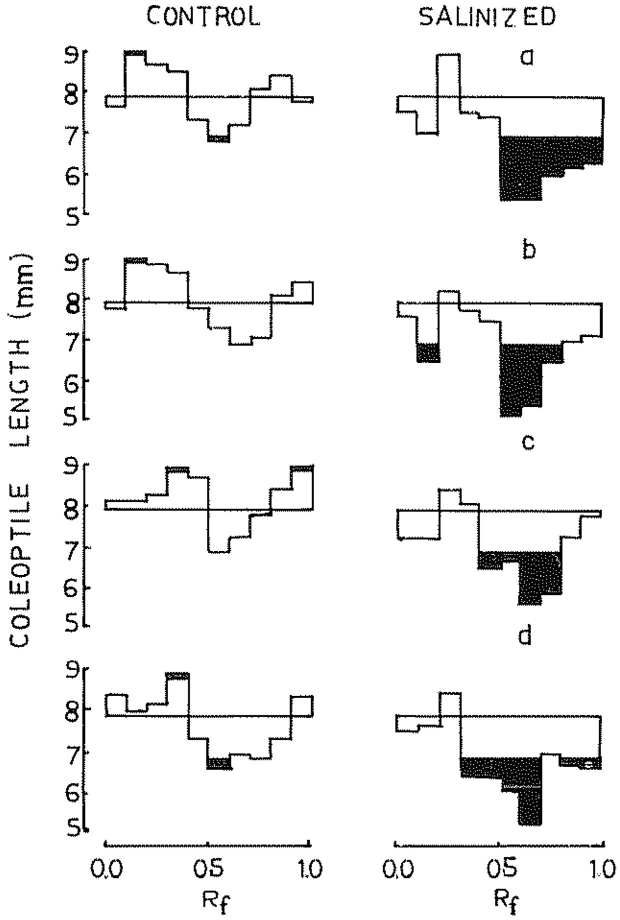

Fig 6. Effect of NaCl Salinity on changes in ABA-like inhibitors in pigeon pea leaves as shown by wheat coleoptile growth bioassay. Darkened areas represent areas significantly different from standards at 5% probability level. a, b, c and d indicate stages 1, 2, 3 and 4 respectively.

Increase in sodium and decrease in potassium and calcium were noticed in both the species under salinity (Table 2); the changes being more in gingelley.

The partial closure of stomata in pigeon pea and gingelley is associated with low amounts of endogenous cytokinins and high amounts of ABA-like inhibitors. Closure of stomata may even be tranced to hormone changes, which in turn will be influenced by reduced CO_2 fixation under salinity. Both pigeon pea and gingelley showed reduced $^{14}CO_2$ uptake (5), there by decreasing the rate of photosynthesis (3).

Plant hormones, especially cytokinins and ABA have been suggested to play an important role in plant water relations through their effects of stomata. Earlier studies (7, 8) showed that water deficit caused by drought or osmotic shock (short exposure of roots to NaCl or mannitol) decreased cytokinin levels in leaves and xylem exudates. It has been reported that salinity is also characterised by hormone changes and their influence on other symptoms are similar to those of water deficits. These hormonal responses may contribute to the adjustment of plants to salinity by reducing stomatal opening and water loss, hence retaining water potential and hence turgor at higher levels than when the stomates should be open.

Changes in hormonal levels may also influence ion uptake. Both pigeon pea and gingelley showed high levels of Na^+ and low levels of K^+ and Ca^{++} under salinity. These changes could perhaps be attributed to high ABA-like inhibitors and low cytokinins. The effect of cytokinins on Na^+/K^+ selectivity in leaves and cotyledons were already reported (6, 14, 18). All the above studies indicated that cytokinins cause changes in selectivity towards K^+ and Na^+ so that the

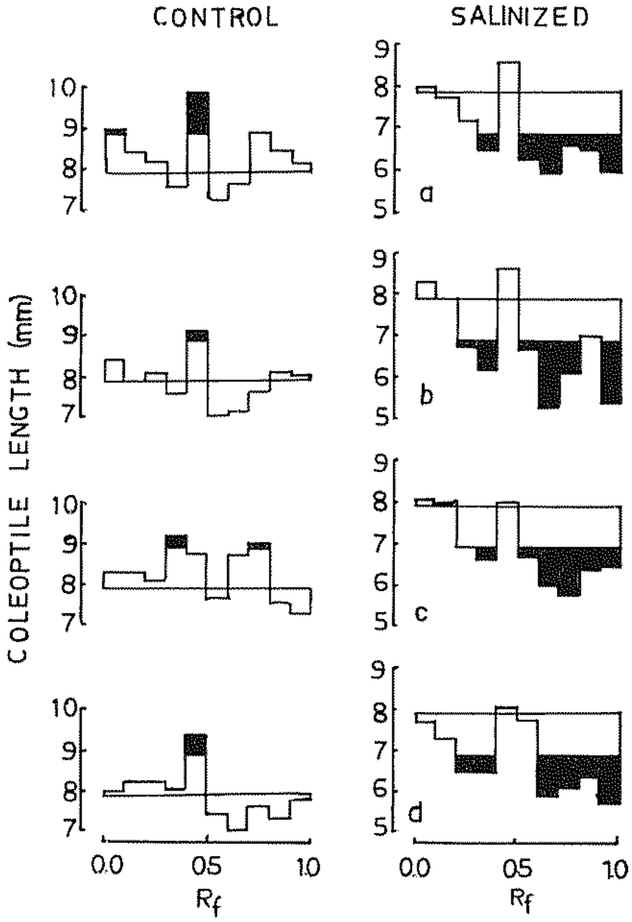

Fig 7 Effect of NaCl salinity on changes in ABA-like inhibitors in gingelley leaves as shown by wheat coleoptile growth bioassay. Darkened areas significantly different from standards at 5% probability level. ab, b, c and d indicate states 1, 2, 3 and 4 respectively.

affinity of cells of K^+ is increased while that for Na^+ is decreased. ABA on the other hand causes preference to Na^+ uptake and lowers K^+ uptake. Low amounts of potassium may even cause stomatal closure, and thus reducing water loss and CO_2 uptake

It is concluded that high levels of ABA-like inhibitors and low cytokinins play a pivotal role in adaptation to salinity in pigeon pea and gingelley by minimising water loss, thus adjusting to the adverse water relations which are inevitably associated with salinity.

Summary

Changes in stomatal characteristics, endogenous cytokinins and ABA-like inhibitors, transpiration and cations were studied in pigeon pea and gingelly during leaf development as influenced by salinity. Salinity resulted in reduced stomatal frequency, stomatal opening and transpiration rates. The leaves of plants grown at high NaCl salinity had their widest stomatal opening during the early hours of the day. Salt treatment resulted in lower cytokinins and higher levels of ABA-like inhibitors in both pigeon pea and gingelley. The leaves of salinized plants showed high sodium and low potassium and calcium. The relevance of these changes in relation to adaptability of plants to salinity is discussed.

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