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EFFECT OF NaCl STRESS AMELIORATED WITH $CaCl_2$ ON NUCLEOTIDES AND POLYPEPTIDES DURING GERMINATION IN GROUNDNUT (*ARACHIS HYPOGAEA* L.)

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ABSTRACT
The present research work was carried out to elucidate the adverse effect of NaCl salinity and its amelioration by $CaCl_2$ during early seedlings growth of two groundnut cultivars (cv, TPT-2 and cv. TCGS-29) $CaCl_2$ treated seedlings showed higher levels of DNA and RNA contents when compared to all other treatments during seedlings growth. However, the DNA content was more in the cv. TCGS-29 on all the days after sowing. NaCl treatment caused decrease in the levels of lipoxygenase (LOX) activity when compared to the seedlings treated with either $CaCl_2$ or its combination with NaCl. LOX showed a single peak with an UV absorption maximum at 235 nm on HPLC. Three major polypeptides (66kD, 47kD and 18.4 kD) appeared predominantly in control and treated seedlings of cotyledons in both varieties but they were absent in the embryonic axis. Cv. TPT-2 is stress tolerant than cv. TCG-29.30 mM and 15mM $Ca2^+$ treatment alleviated the stress effect in cv, TPT-2 and TCGS-29 respectively.

INTRODUCTION

Groundnut is one of the important oil seed crops grown in various parts of India, it is cultivated extensively in the districts of Rayalaseema area of Andhra Pradesh under rainfed conditions as karif and rabi. The crop is sensitive to salinity, it is a major and wide spread abiotic stress that contains crop productivity under rain fed as well as irrigated conditions (Agnihotri *et al.,* 2006). Salt tolerance and susceptibility do not reside in a single factor but result from the possession of a number of biochemical characteristics (Stewart and Larher, 1980). According to La Haye and Epstein (1969) $Ca2^+/Na^+$ interaction takes place at the plasmalemma and Na^+ acted by displacing $Ca2^+$ from membranes leading to increase membrane permeability and intercellular Na^+ concentration. $Ca2^+$ is involved in the regulation of plant responses to various environmental stresses (Nayyar, 2003). According to porta and Rocha Sosa (2002) plant LOX is of considerable interest. In germinating cucumber seeds, LOX associated with lipid bodies is capable of adding O_2 to the esterified fatty acids (Feussner *et al.,* 2001). Salinity decreased the utilization of reserve protein in the cotyledons of soyabean seedlings (Durgaprasad *et al.,* 1996). Hence the present study was aimed to evaluate the physiological and biochemical basis of tolerance on two groundnut cultivars.

MATERIALS AND METHODS

Plant growth and treatment

Seeds of two groundnut cultivates were obtained from the Regional Agricultural Research Station (Tirupati, Andhra Pradesh, India). The seeds were surface sterilized with 0.2 % $HgCl_2$ solution. The seeds were divided into four batches and germinated in bread boxes containing fluted filter paper towels. They were subjected to the following treatments: irrigation with (1). Distilled water (control) (2). 100 mM NaCl for cv.TPT-2 and NaCl 90 mM for cv. TCGS-29, (3). 30 mM $CaCl_2$ for cv. TPT-2, 15 mM $CaCl_2$ for TCGS-29, (4). 100 mM NaCl + 30 mM $CaCl_2$ for cv. TPT-2 and 90 mM NaCl + 15 mM $CaCl_2$ for cv. TCGS-29. Seedlings were illuminated continuously with fluorescent lamps in a growth room. The temperature was maintained at $25 \pm 2°C$. The seedlings were harvested randomly from the bread boxes on 3, 6, 9 and 12^{th} day. Measurements were carried out separately in the cotyledons and the embryonic axis.

Estimation of fresh and dry weight

Fresh and dry weight of the each cultivar from each treatment was determined separately in the cotyledons and embryonic axis. After taking fresh weight they were kept in a hot air oven at 80°C for 48 hrs. in a hot air oven and
weight of the oven-dried material was determined.

Extraction and estimation of DNA and RNA

Extraction and estimation of nucleotides was done by the method of Jayaraman (1981). 1g fresh weight of cotyledons and embryonic axis were homogenized with 5 mL of ice cold 10% TCA and centrifuged at 3,000 Xg for 10 min. The pellet was suspended in 5 mL of ethanolether mixture and centrifuged at 3,000 Xg for 10 min. The precipitate obtained was suspended in 5 mL of 0.5 N NaOH, kept for 18 hr. and

centrifuged at 3,000 Xg for 10 min. The supernatant contained RNA and the precipitate contained DNA along with proteins. 1 mL of perchloric acid was added to the DNA precipitate and centrifuged at 3,000 Xg for 10 min. Then DNA was released from supernatant RNA extract was added to 3 mL of orcinol reagent boiled for 5 min and measured at 665 nm. DNA extract was added to the 2 mL of diphenyl reagent, heated in boiling water bath and the solution was read at 595 nm.

Estimation of LOX activity and HPLC analysis

LOX activity was assayed by determining O_2 consumption using Clark's oxygen electrode (Model 5300). HPLC analysis was carriedout to obtain the LOX product on reverse phase column equipped with a pump model 6000A and an injector model U 6K. The reaction was initiated by the addition of arachidonic acid (133 mM) and incubated for 2 min at 30°C and the products were extracted immediately twice with hexane: ether (1:1v/v). The organic extracts were pooled, dried over anhydrous sodium sulphate and evaporated to dryness. The residue was reconstituted in the HPLC solvent system of hexane: 2-propanol: acetic acid (1000:4:1). The HPETE was determined on RP-8 column at a flow rate of 2 mL per min.

SDS -page analysis

Polypeptide analysis was done according to Laemmli (1970). 100 mg of the plant material was macerated in a mortar with 0.1 M Tris-HCl buffer pH 8.3 and 0.5 % (v/v) b-mercaptoehanol. Extract was centrifuged at 15,000 Xg for 30 min. The precipitate was collected by centrifuging at 10,000 Xg for 10 min and washes twice with 5 % TCA. The final protein pellet was lyophilized. Sample was dissolved in a small volume of 0.057 M Tris- HCl buffer pH 6.1 containing 4% SDS and undissolved material was discarded by centrifugation at 5000 Xg for 5 min. The protein concentration was determined by Lowry *et al.,* (1951).

Statistical analysis

Data were analyzed by ANOVA. The values are mean ± SE of five replications, each replication consists of 5 seedlings and means compared by the least significant difference test at the $p < 0.05$ level.

RESULTS AND DISCUSSION

Fresh weight of cotyledons decreased continuously from 3 day to 12 days after sowing in all treatments including control on the otherhand in embryonic axis it increases progressively. NaCl treatment caused considerable reduction in the fresh weight than the control, $CaCl_2$ or NaCl + $CaCl_2$ in both the varieties (Table 1). It has reported that the fresh weight of the embryonic axis have been shown to decreased with increasing concentration of NaCl (Sharma and Garg, 1985; Sarvesh *et al.,* 1996). Addition of $CaCl_2$ to the NaCl stressed seedlings caused decrease in the fresh weight of cotyledons and increase in embryonic axis. However there is a less significant difference between the $CaCl_2$ treated NaCl stressed and unstressed seedlings on the positive growth response to Ca^{2+} under saline conditions on sorghum (Grieve and Mass, 1988). NaCl treatment caused decrease in dry weight than the other treatment including control (Table 2). However the embryonic axis of the seedlings treated with the $CaCl_2$ showed greater dry weight than the other treatments during the progressive seedlings growth. Addition of $CaCl_2$ to NaCl caused increase in dry weight of the embryonic axis at all the stages of seedlings growth than the seedlings treated with NaCl in both the cultivars. In general the reduction in growth and dry matter in the NaCl stressed the seedlings have been attributed to the accumulation of Na^+ ions (Lauter and Munns, 1986).

The cotyledons treated with NaCl caused a lower level of DNA contents on the 3rd day and steadily declined upto 12th day after sowing (Table 3) ($p < 0.01$). Seedlings treated with $CaCl_2$ and in combination with NaCl caused a decline and it was in between the control and NaCl treatments in both the varieties of groundnut. In the cv. TCGS-29 the DNA content was lower than cv. TPT-2. The DNA content of the embryonic axis increased rapidly in control seedlings from 3rd to 12th day after sowing. However the DNA content was more in the cv.

Table 1: Effect of NaCl, $CaCl_2$ and their combinations on the changes in fresh weight of cotyledons (mg/cotyledons) and embryonic axis (mg/embryonic axis) of two groundnut cultivars

Days after treatment	Seedlings part	TPT-2				TCGS-29			
		T1	T2	T3	T4	T1	T2	T3	T4
3	COTY	0.780 (±0.008)	0.760 (±0.003)	0.675 (±0.006)	0.615 (±0.007)	0.820 (±0.009)	0.752 (±0.024)	0.722 (±0.004)	0.670 (±0.014)
	EA	0.181 (±0.001)	0.560 (±0.007)	1.490 (±0.040)	2.140 (±0.051)	0.136 (±0.007)	0.576 (±0.015)	1.540 (±0.040)	2.142 (±0.020)
6	COTY	0.705 (±0.003)	0.672 (±0.009)	0.560 (±0.020)	0.510 (±0.006)	0.705 (±0.004)	0.679 (±0.018)	0.639 (±0.009)	0.585 (±0.018)
	EA	0.096 (±0.004)	0.360 (±0.003)	0.736 (±0.056)	1.610 (±0.042)	0.091 (±0.003)	0.301 (±0.006)	0.676 (±0.029)	1.350 (±0.026)
9	COTY	0.760 (±0.002)	0.740 (±0.001)	0.640 (±0.010)	0.584 (±0.009)	0.770 (±0.012)	0.717 (±0.011)	0.676 (±0.007)	0.626 (±0.005)
	EA	0.164 (±0.009)	0.501 (±0.022)	1.405 (±0.006)	1.950 (±0.026)	0.126 (±0.004)	0.502 (±0.006)	1.411 (±0.056)	1.950 (±0.059)
12	COTY	0.725 (±0.004)	0.686 (±0.004)	0.623 (±0.004)	0.532 (±0.006)	0.735 (±0.010)	0.692 (±0.002)	0.659 (±0.008)	0.612 (±0.145)
	EA	0.129 (±0.005)	0.459 (±0.008)	1.121 (±0.019)	1.690 (±0.012)	0.101 (±0.004)	0.461 (±0.005)	1.156 (±0.004)	1.651 (±0.046)

(Values are mean ± SE of five replications); (T1 -control; T2-100 mM NaCl; T3- 30 mM $CaCl_2$; T4 -100 mM NaCl + 30 mM NaCl) TPT-2; (T1 -control; T2-90 mM NaCl; T3- 15 mM $CaCl_2$; T4 - 90 mM NaCl + 15 mM NaCl) TCGS-29.

Table 2: Effect of NaCl, $CaCl_2$ and their combinations on the changes in dry weight of cotyledons (mg/cotyledons) and embryonic axis (mg/ embryonic axis) of two groundnut cultivars

Days after treatment	Seedlings part	TPT-2				TCGS-29			
		T1	T2	T3	T4	T1	T2	T3	T4
3	COTY	0.456 (±0.008)	0.412 (±0.007)	0.362 (±0.003)	0.332 (±0.008)	0.459 (±0.006)	0.439 (±0.009)	0.381 (±0.008)	0.356 (±0.008)
	EA	0.381 (±0.008)	0.341 (±0.002)	0.301 (±0.018)	0.276 (±0.005)	0.038 (±0.006)	0.092 (±0.002)	0.201 (±0.002)	0.327 (±0.007)
6	COTY	0.436 (±0.010)	0.389 (±0.002)	0.345 (±0.012)	0.301 (±0.002)	0.401 (±0.002)	0.381 (±0.008)	0.337 (±0.012)	0.301 (±0.002)
	EA	0.301 (±0.005)	0.271 (±0.003)	0.251 (±0.050)	0.232 (±0.011)	0.030 (±0.004)	0.059 (±0.004)	0.119 (±0.009)	0.256 (±0.005)
9	COTY	0.421 (±0.005)	0.379 (±0.007)	0.321 (±0.010)	0.280 (±0.004)	0.439 (±0.007)	0.415 (±0.007)	0.361 (±0.003)	0.321 (±0.005)
	EA	0.341 (±0.005)	0.312 (±0.005)	0.281 (±0.003)	0.256 (±0.007)	0.034 (±0.003)	0.081 (±0.002)	0.161 (±0.004)	0.292 (±0.016)
12	COTY	0.409 (±0.005)	0.355 (±0.002)	0.301 (±0.002)	0.265 (±0.005)	0.421 (±0.010)	0.407 (±0.004)	0.347 (±0.009)	0.312 (±0.005)
	EA	0.312 (±0.005)	0.289 (±0.000)	0.262 (±0.009)	0.241 (±0.015)	0.032 (±0.003)	0.069 (±0.005)	0.151 (±0.014)	0.276 (±0.009)

(Values are mean ± SE of five replications); (T1 -control; T2-100 mM NaCl; T3- 30 mM $CaCl_2$; T4 -100 mM NaCl + 30 mM NaCl) TPT-2; (T1 -control; T2-90 mM NaCl; T3- 15 mM $CaCl_2$; T4 - 90 mM NaCl + 15 mM NaCl) TCGS-29.

Table 3: Effect of NaCl, $CaCl_2$ and their combinations on the changes in the DNA content (mg fw) of the cotyledons and embryonic axis of groundnut cultivars

Days after treatment	Seedlings part	TPT-2	TCGS-29						
		T1	T2	T3	T4	T1	T2	T3	T4
3	COTY	116.75 (±1.45)	107.81 (±1.19)	94.25 (±0.17)	78.95 (±0.99)	126.95 (±0.86)	117.81 (±0.89)	104.25 (±0.17)	88.95 (±0.78)
	EA	49.62 (±0.78)	69.41 (±0.38)	74.62 (±0.30)	89.69 (±0.52)	55.41 (±0.79)	71.59 (±0.58)	81.24 (±0.46)	92.85 (±0.53)
6	COTY	56.73 (±0.67)	42.79 (±0.89)	49.79 (±0.94)	27.25 (±0.17)	66.73 (±0.46)	52.79 (±0.63)	47.79 (±0.41)	37.25 (±0.21)
	EA	30.87 (±0.93)	45.57 (±0.43)	49.52 (±0.87)	56.69 (±0.66)	40.87 (±0.41)	55.57 (±0.40)	51.72 (±0.60)	71.95 (±0.50)
9	COTY	109.81 (±0.93)	89.54 (±0.24)	81.98 (±0.93)	72.91 (±1.80)	119.81 (±0.58)	99.54 (±0.71)	91.98 (±0.85)	81.96 (±0.80)
	EA	45.41 (±0.63)	61.59 (±0.62)	71.24 (±0.40)	82.35 (±1.06)	59.62 (±0.61)	77.41 (±0.49)	84.62 (±0.60)	99.69 (±0.45)
12	COTY	87.91 (±1.02)	71.83 (±0.941)	64.94 (±0.47)	51.92 (±0.93)	77.91 (±0.43)	81.83 (±0.57)	74.94 (±0.50)	61.92 (±0.50)
	EA	40.41 (±0.30)	54.65 (±0.66)	59.69 (±0.47)	64.95 (±0.99)	50.41 (±0.30)	64.65 (±0.43)	72.69 (±0.49)	84.95 (±0.40)

(Values are mean ± SE of five replications); (T1 -control; T2-100 mM NaCl; T3- 30 mM $CaCl_2$; T4 -100 mM NaCl + 30 mM NaCl) TPT-2; (T1 -control; T2-90 mM NaCl; T3- 15 mM $CaCl_2$; T4 - 90 mM NaCl + 15 mM NaCl) TCGS-29.

TCGS-29 on all the days after sowing. Ca^{2+} regulates various nuclear activities including DNA synthesis and nuclear fusion (Dauwalder, 1985).

In the cotyledons of the two cultivars the RNA content declined sharply in the controls and treated seedlings (Table 4) ($p<0.01$). The decline was at different levels based on the nature of treatments. NaCl treatment caused a slower decline than the other two treatments. RNA content of the embryonic axis increased steadily from 3rd to 12th day after steadily from 3rd day to 12th day after treatment in the control seedlings. While in the other treatments the level of increase was slower than the control and in both the varieties. NaCl treatment caused a lower level of increase in RNA content. The DNA and RNA contents increased in the embryo throughout its developing period (Bewley and Black, 1982).

The level of LOX activity of the cotyledons increased upto 6th day of sowing followed by continuously decline in all treatments, this trend was observed by Tappel *et al.,* (1963). NaCl treatment caused decrease in the level of LOX activity when compared to the seedlings treated with $CaCl_2$ and its combination with NaCl (Table 5). In $CaCl_2$ treated seedlings LOX activity was found to be higher compared to the NaCl and $CaCl_2$ with NaCl treatments in both the cultivars. The poly unsaturated fatty acids (PUFA) are catabolized by LOX to the hydroperoxidized products, these acts as specific endogenous Ca^{2+} ionophores enabling more Ca^{2+} to penetrate, induction and release of PUFA (Leshem, 1987). During the progressive germination of groundnut cotyledons $CaCl_2$ prevents the formation of linoleic and linolenic acid from Sn^{-2} acyl-side chain may be by maintaining cytosolic Ca^{2+} at homeostatic

Table 4: Effect of NaCl, $CaCl_2$ and their combinations on the changes in the RNA content (mg fw) of the cotyledons and embryonic axis of groundnu. cultivars

Days after treatment	Seedlings part	TPT-2				TCGS-29			
		T1	T2	T3	T4	T1	T2	T3	T4
3	COTY	162.92 (±0.49)	151.62 (±1.32)	142.31 (±0.53)	131.28 (±2.03)	166.21 (±0.82)	156.52 (±1.34)	146.31 (±2.37)	136.28 (±1.31)
	EA	67.52 (±0.88)	76.95 (±0.80)	85.95 (±0.97)	94.94 (±0.93)	77.52 (±0.85)	80.95 (±0.60)	91.95 (±2.66)	105.94 (±1.61)
6	COTY	79.52 (±0.41)	68.52 (±0.57)	55.69 (±0.74)	47.82 (±0.93)	81.52 (±0.77)	71.95 (±1.26)	64.52 (±0.63)	51.52 (±1.34)
	EA	56.84 (±0.42)	74.62 (±0.44)	69.99 (±1.37)	75.81 (±1.34)	59.91 (±1.66)	76.34 (±1.01)	78.21 (±1.70)	87.63 (±1.26)
9	COTY	136.82 (±0.89)	122.95 (±1.09)	111.95 (±0.694)	101.45 (±1.36)	142.82 (±0.51)	129.95 (±1.33)	116.95 (±0.86)	106.45 (±1.26)
	EA	71.52 (±0.71)	81.95 (±0.72)	89.95 (±1.33)	91.82 (±0.44)	79.62 (±1.15)	91.72 (±0.92)	99.94 (±1.24)	108.92 (±1.71)
12	COTY	107.52 (±0.94)	98.52 (±0.68)	81.69 (±0.29)	72.72 (±1.18)	109.52 (±1.40)	97.52 (±0.95)	86.69 (±1.08)	71.82 (±1.67)
	EA	61.54 (±0.80)	74.52 (±0.83)	79.69 (±1.57)	81.95 (±1.06)	67.34 (±0.93)	89.31 (±1.25)	89.74 (±1.56)	96.32 (±2.51)

(Values are mean ± SE of five replications); (T1 -control; T2-100 mM NaCl; T3- 30 mM $CaCl_2$; T4 -100 mM NaCl + 30 mM NaCl) TPT-2; (T1 -control; T2-90 mM NaCl; T3- 15 mM $CaCl_2$; T4 - 90 mM NaCl + 15 mM NaCl) TCGS-29.

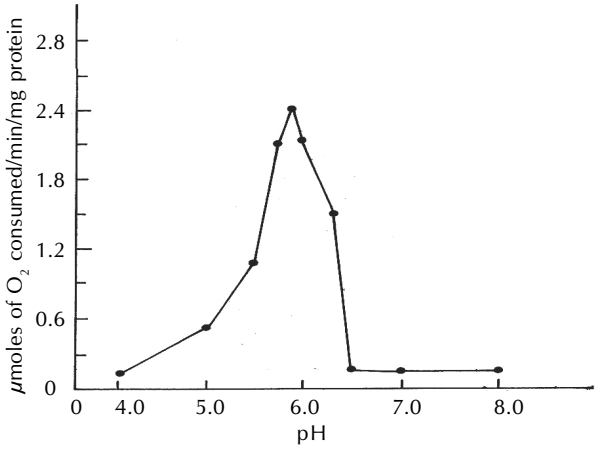

Figure 1a: pH optima of LOX activity of groundnut seedlings

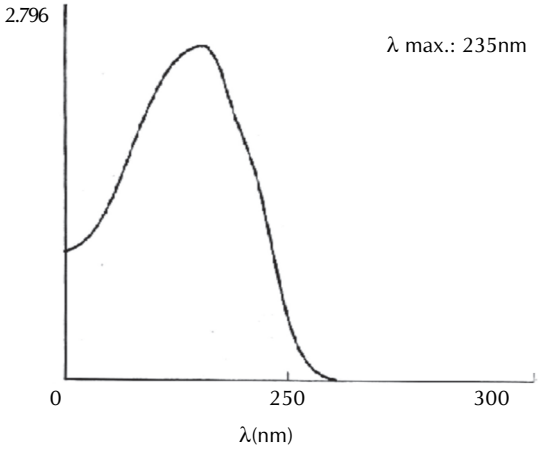

Figure 1b: UV absorption spectra of LOX product of groundnut seedlings

Table 5: Effect of NaCl,$CaCl_2$ and their interaction on changes in the LOX activity (μ moles of O_2 consumed min^{-1} mg^{-1} protein) of cotyledons and embryonic axis of groundnut cultivars

Days after treatment	Seedlings part	TPT-2				TCGS-29			
		T1	T2	T3	T4	T1	T2	T3	T4
3	COTY	7.26 (±0.05)	8.19 (±0.08)	6.91 (±0.05)	7.96 (±0.07)	8.86 (±0.272)	9.12 (±0.078)	7.01 (±0.20)	8.4 (±0.05)
	EA	7.92 (±0.13)	8.31 (±0.06)	7.16 (±0.122)	6.54 (±0.30)	8.61 (±0.156)	9.21 (±0.103)	7.6 (±0.06)	6.92 (±0.19)
6	COTY	5.1 (±0.04)	4.72 (±0.07)	3.4 (±0.07)	5.9 (±0.30)	5.96 (±0.132)	4.92 (±0.014)	3.81 (±0.02)	6.36 (±0.09)
	EA	8.7 (±0.28)	7.6 (±0.05)	6.4 (±0.09)	5.72 (±0.23)	9.24 (±0.04)	8.62 (±0.16)	7.11 (±0.24)	6.9 (±0.03)
9	COTY	7.0 (±0.19)	7.18 (±0.08)	6.11 (±0.08)	7.12 (±0.05)	7.92 (±0.28)	7.98 (±0.13)	6.76 (±0.21)	7.96 (±0.21)
	EA	6.91 (±0.03)	5.92 (±0.135)	4.62 (±0.03)	3.96 (±0.18)	7.92 (±0.28)	6.87 (±0.08)	5.82 (±0.24)	4.91 (±0.30)
12	COTY	6.14 (±0.02)	5.48 (±0.15)	4.72 (±0.25)	6.2 (±0.10)	6.94 (±0.05)	5.81 (±0.11)	4.92 (±0.02)	6.91 (±0.07)
	EA	7.6 (±0.12)	6.9 (±0.05)	5.8 (±0.25)	4.9 (±0.01)	8.1 (±0.04)	7.72 (±0.02)	6.92 (±0.13)	5.42 (±0.08)

(Values are mean ± SE of five replications); (T1 -control; T2-100 mM NaCl; T3- 30 mM $CaCl_2$; T4 -100 mM NaCl + 30 mM NaCl) TPT -2; (T1 -control; T2-90 mM NaCl; T3- 15 mM $CaCl_2$; T4 - 90 mM NaCl + 15 mM NaCl) TCGS-29.

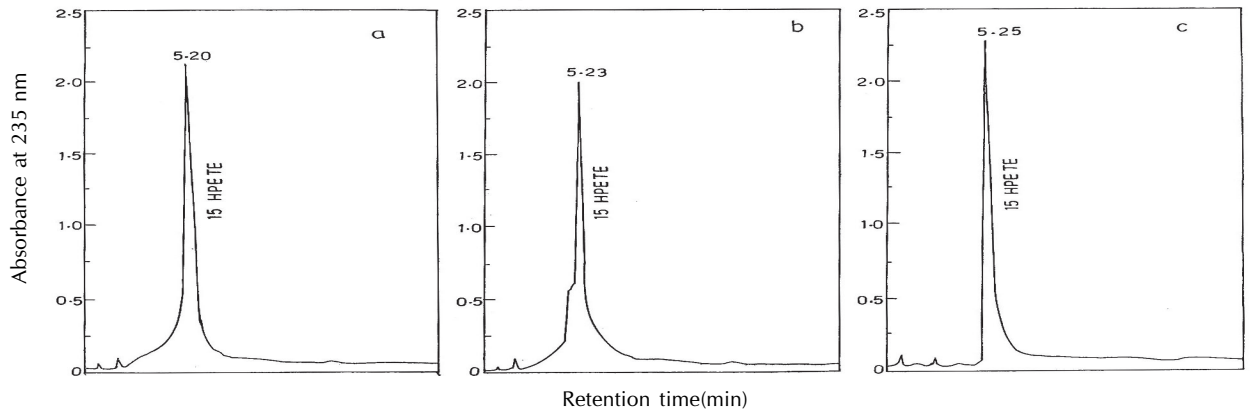

Figure 1c: HPLC profiles at 235 nm of a) standard 15-HPETE b) products generated after incubation of LOX with acid and c) mixture of 'a' and 'b'.

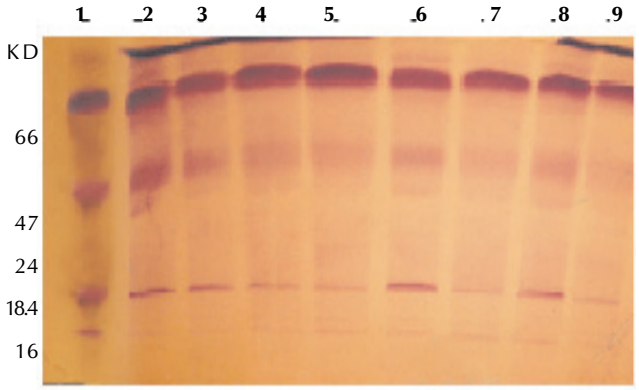

Figure 2: Effect of NaCl, $CaCl_2$ and their interaction on changes in polypeptides of the cotyledons of the two groundnut cultivars by SDS-PAGE analysis

[Lane 1: Marker proteins; Lane 2: Control cv. TPT-2; Lane 3: 100 mM NaCl cv. TPT-2; Lane 4: 30 mM $CaCl_2$ cv. TPT-2; Lane 5: 100 mM NaCl + 30 mM $CaCl_2$ cv.TPT-2; Lane 6: Control cv. TCGS-29; Lane 7: 90 mM NaCl cv. TCGS-29; Lane 8: 15 mM $CaCl_2$ cv. TCGS-29; Lane 9: 90 mM NaCl + 15 mM $CaCl_2$ cv. TCGS-29].

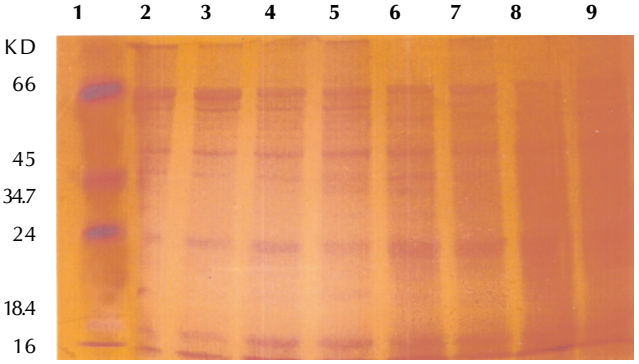

Figure 3: Effect of NaCl, $CaCl_2$ and their interaction on changes in polypeptides of the embryonic axis of the two groundnut cultivars by SDS-PAGE analysis. [Lane 1: Marker proteins; Lane 2: Control cv. TPT-2;

Lane 3: 100 mM NaCl cv. TPT-2; Lane 4: 30 mM $CaCl_2$ cv. TPT-2; Lane 5: 100 mM NaCl + 30 mM $CaCl_2$ cv.TPT-2; Lane 6: Control cv. TCGS-29; Lane 7: 90 mM NaCl cv. TCGS-29; Lane 8: 15 mM $CaCl_2$ cv. TCGS-29; Lane 9: 90 mM NaCl + 15 mM $CaCl_2$ cv. TCGS-29].

level, because of the decrease in substrate level LOX activity declines. This may be the reason for the decrease in LOX activity in the treatment of $CaCl_2$ and its combination with NaCl. The level of LOX activity of embryonic axis of the control seedlings showed a gradual decrease from 3rd day to 12th day after sowing. NaCl treatment caused a gradual decline in the LOX activity of the embryonic axis of the seedlings upto 9th day followed by a sudden decrease by 12th day after sowing. On the otherhand in $CaCl_2$ seedlings LOX activity increased steadily upto 9th day followed a decline. The LOX enzyme is widely distributed in plant tissue being particularly abundant in leguminous plants and its activity initiate lipidperoxidation (Funk *et al.,* 1986). The biological implication of the $CaCl_2$ in lowering endogenous activity of the LOX enzyme include production of the free radicals normally resulting in the biological damage and possibly by preventing oxidation of phospholipids (Grossman and Leshem, 1978). The pH optima of the groundnut LOX was found to be 6.0 (Fig. 1a). When arachidonic acid was incubated with the groundnut LOX, a single major peak with an UV absorption maximum at 235 nm was observed (Fig. 1b), Analysis of the product profile of the LOX with linolenic acid as the substrate of HPLC has shown a single peak co-migrating with standard 15-HPETE (Fig. 1c). This data suggest that groundnut LOX is rather specific for the insertion of O_2 and 15th carbon on amino acid molecule.

The SDS-PAGE polypeptide analysis of the cotelydons and embryonic axis of the 12th day after sowing with two groundnut cultivars showed that the treatments in both the cultivars did not show appreciable changes in quality of peptides (Fig. 2). Three major polypeptides (66 kD, 47 kD and 18.4 kD) were appeared predominantly in cotyledons of both control and treated seedlings in both the varieties, other polypeptides appeared heavily over the gel. In embryonic axis a large number of polypeptides appeared on the gel varying from high to low molecular weights (Fig. 3). The heavy molecular weight polypeptides (66 kD, 47 kD and 18.4 kD) were absent in the embryonic axis in all the treatment of the cultivars.

Thus our results concluded that the two cultivars varied in their sensitivity to NaCl stress and $CaCl_2$ treatment alleviated NaCl stress. The cV TPT-2 showed resistant to NaCl stress and the 30 mM of Ca^{2+} alleviated the stress condition.

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