

DIFFERENCES IN GROWTH, WATER RELATIONS AND SOLUTE ACCUMULATION IN THE SELECTED CALLUSES OF SUNFLOWER (*Helianthus annuus* L.) UNDER SODIUM CHLORIDE STRESS

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SUMMARY

Recurrent selection of sunflower hypocotyl calluses maintained at different levels of salinity improved the tolerance capacity. At 10th sub-culture the selected tissues of cvs - EC 68415 and Morden, showed better growth at the highest (-1.368 MPa) salinity. Growth, turgor and accumulation of solutes and ions were greater in these tissues. However, the involvement of turgor and proline in salt tolerance seem to be doubtful. The increase in sodium chloride in the medium enhanced the uptake of sodium and potassium leading to maintenance of a greater K/Na ratio in selected tissues, thereby suggesting a role for ionic equilibrium between potassium and sodium.

Key words: Recurrent selection, sunflower, salt stress, water relation, hypocotyl callus.

INTRODUCTION

Salinity affects growth and development of crops *in vivo*. *In vitro* selection, a quick alternative method in crop improvement, has led to the development of salt tolerant tissues (Vinod Kumar and Sharma, 1989) and plants (McCoy, 1987). However, in sunflower, there are no reports of the development of salt tolerant somaclones. Hence, the somaclonal selection technique was applied for crop improvement, and salt tolerant clones were obtained.

Patterns of changes in growth, water relations and solute accumulation associated with salt tolerance in selected tissues are reported in the present paper.

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MATERIALS AND METHODS

Hypocotyl calluses of two sunflower cultivars, EC-68415 and Morden, initiated (Prakash et al., 1993) on MS medium containing NAA (2 mg/l) and kinetin (0.25 mg/l), were transferred to the same medium containing 0.0, 100, 200 and 300 mM sodium chloride, corresponding to 0.0, -0.462, -0.915 and -1.368 MPa of stress, respectively.

Table 1: Growth (g/culture) in culture of selected and non-selected tissues of two sunflower cultivars under sodium chloride stress.

Treatment/ cultivar stress (mM)	Growth (g/days after inoculation)								
	10			20			30		
	Fresh weight	Dry weight	FW/DW weight	Fresh weight	Dry weight	FW/DW weight	Fresh weight	Dry weight	FW/DW weight
Control									
EC-68415	0.503	0.051	9.86	3.72	0.247	15.06	4.4	0.291	15.12
Morden	0.520	0.034	15.29	3.463	0.229	15.12	4.100	0.270	15.19
SELECTED TISSUES									
EC-68415									
100	0.460	0.031	14.80	2.400	0.160	15.00	3.960	0.267	14.83
200	0.420	0.029	14.48	0.840	0.057	14.76	1.550	0.140	14.90
300	0.230	0.075	3.10	0.340	0.095	3.74	0.55	0.137	4.02
Morden									
100	0.360	0.024	15.00	1.102	0.074	14.89	3.100	0.209	14.83
200	0.360	0.024	15.00	0.864	0.058	14.89	1.450	0.098	14.80
300	0.225	0.068	3.11	0.310	0.350	5.85	0.053	0.350	0.090
CP (P=0.01)	0.127	0.027	-	0.109	0.077	-	0.307	0.070	-
Interaction									
CD (P=0.01)	0.048	0.016	-	0.184	0.058	-	0.604	0.032	-
CV (%)	2.580	11.18	-	2.290	10.00	-	4.98	3.860	-
NON-SELECTED TISSUES									
EC-68415									
100	0.410	0.025	15.19	1.600	0.070	20.77	2.600	0.106	24.52
200	0.275	0.018	15.27	0.764	0.015	14.98	0.900	0.060	15.00
300	0.245	0.016	15.31	Dead	Dead	Dead	Dead	Dead	Dead
Morden									
100	0.320	0.017	18.80	1.200	0.079	15.19	1.450	0.096	15.11
200	0.250	0.019	13.16	0.660	0.044	15.00	0.800	0.053	15.00
300	0.240	0.016	15.00	Dead	Dead	Dead	Dead	Dead	Dead
Interaction	NS	0.008	-	0.344	0.021	-	0.443	NS	-
CD (P=0.01)									
CV (%)	5.96	6.41	-	4.69	4.86	-	5.03	78.85	-

After 9 subcultures by recurrent selection in the corresponding media, surviving tissues were subjected to stress treatments in the 10th subculture along with non-selected tissues (Table 1). Growth of fresh and dry callus weights were recorded at 10-day intervals. Water (Ψ_w) and solute ($-\Psi_s$) potentials were deter-

mined by water potential data system (Wescor-HP 115) and vapour pressure osmometer (Wescor 5100C) in the dew point mode. Turgor ($\Psi_p = \Psi_w - \Psi_s$) potential was calculated as $\Psi_p = (-\Psi_w - \Psi_s)$. Reducing sugars, alcohol soluble amino acids and proline (Bates et al., 1973) were determined periodically. Sodium and potassium levels in the tissues were also examined during the period of culture, as described earlier (Prakash et al., 1993).

Table 2: Effect of sodium chloride stress on water relations (Ψ_w , Ψ_s and Ψ_p) in culture of selected and non-selected tissues of two sunflower cultivars

Treatment/ cultivar stress (mM)	Water Relations											
	Water potential (MPa)				Solute potential (MPa)				Pressure potential (MPa)			
	(DAI)				(DAI)				(DAI)			
1	0	10	20	30	0	10	20	30	0	10	20	30
Control												
EC-68415	0.5	0.5	0.8	1.4	1.1	1.18	1.52	1.83	0.6	0.68	0.72	0.43
Morden	0.4	0.5	1.2	1.4	0.9	1.10	1.60	1.97	0.5	0.60	0.40	0.57
SELECTED TISSUES												
EC-68415												
100	1.4	1.4	1.6	1.8	2.07	1.66	1.80	2.01	0.67	0.26	0.21	0.21
200	1.8	2.0	2.2	2.4	2.27	2.23	2.43	2.95	0.47	0.23	0.23	0.55
300	2.2	2.2	3.0	2.8	2.43	2.43	3.59	3.55	0.23	0.23	0.60	0.75
Morden												
100	1.2	1.4	1.8	2.0	2.00	1.84	2.43	2.73	0.80	0.44	0.63	0.73
200	1.8	2.2	2.8	3.0	2.47	2.43	3.55	3.55	0.67	0.23	0.75	0.55
300	2.0	2.2	2.8	3.1	2.47	2.73	3.60	3.85	0.47	0.53	0.80	0.77
CD (P=0.01)	-	0.2	0.41	0.34	-	0.11	1.54	0.13	-	0.13	0.02	0.11
Interaction												
CD (P=0.01)	-	NS	NS	0.13	-	NS	NS	NS	-	0.12	0.20	0.31
CV (%)	-	7.1	8.1	6.04	-	2.16	22.92	1.92	-	13.36	4.49	6.83
NON-SELECTED TISSUES												
EC-68415												
100	0.5	2.0	2.0	2.8	1.1	2.20	2.55	3.40	0.6	0.21	0.55	0.65
200	0.5	2.5	2.8	3.2	1.1	2.55	2.90	3.35	0.6	0.05	0.10	0.15
300	0.5	4.0	-	-	1.1	4.01	-	-	0.6	0.01	-	-
Morden												
100	0.4	2.0	2.6	3.2	0.9	2.20	2.90	1.97	0.5	0.2	0.30	0.30
200	0.4	3.0	3.4	3.2	0.9	3.12	4.01	3.50	0.5	0.12	0.61	0.30
300	0.4	3.5	-	-	0.9	3.53	-	-	0.5	0.03	-	-
CD (P=0.01)	-	0.53	0.75	NS	-	0.32	0.54	0.31	-	0.07	0.23	0.12
Interaction												
CD (P=0.01)	-	NS	NS	NS	-	0.33	0.72	0.83	-	NS	0.32	0.27
CV (%)	-	1.50	17.2	3.20	-	21.05	17.5	18.9	-	3.1	5.1	14.3

RESULTS AND DISCUSSION

The growth of selected and non-selected sunflower hypocotyl tissues under all treatments was conspicuous. The differences in weights of the two fresh and dry tissues, were affected to a greater extent with increase in salinity (Table 1). However, the selected tissues performed better under all conditions than the non-selected ones. There was even senescence of non-selected tissues at the highest level of salinity. Selected hypocotyl tissues of EC-68415 were significantly superior in their performance at all stress levels. This shows that *in vitro* selection increased salt tolerance in EC 68415 and Morden, to a greater extent in the former.

Table 3: Effect of sodium chloride on solute accumulation during growth of selected and non-selected tissues of two sunflower cultivars

Treatment/ cultivar stress (mM)	Reducing sugars (mM)				Amino acids (mM)				Proline (mM)			
	(DAI)				(DAI)				(DAI)			
	0	10	20	30	0	10	20	30	0	10	20	30
Control												
EC-68415	83.00	59.94	77.70	55.50	63.88	48.59	92.59	63.88	16.90	25.32	48.37	57.18
Morden	90.80	139.86	64.38	66.60	70.98	63.88	81.31	69.18	15.35	21.53	26.69	37.69
SELECTED TISSUES												
EC-68415												
100	90.47	74.26	122.54	133.64	125.00	113.57	191.65	291.01	22.50	36.08	53.38	60.77
200	81.51	90.80	133.64	115.99	132.50	212.94	220.04	326.51	29.10	24.81	61.56	65.21
300	79.25	55.94	116.94	104.62	130.75	205.84	212.94	255.83	30.27	20.30	68.79	73.66
Morden												
100	85.50	50.84	105.89	126.87	127.52	129.95	191.10	193.83	19.54	30.45	40.69	51.51
200	89.68	74.81	97.01	133.64	147.22	321.59	255.53	397.49	25.40	29.58	68.75	64.23
300	75.12	49.28	102.56	125.72	149.75	156.17	191.65	227.14	32.18	36.09	51.87	82.14
CD (P=0.01)	-	10.14	11.75	12.27	-	35.94	23.75	23.07	-	8.60	18.68	7.80
Interaction												
CD (P=0.01)	-	19.89	23.05	24.07	-	70.56	46.61	45.27	-	16.08	36.64	15.49
CV (%)	-	5.87	3.93	4.11	-	8.90	5.27	3.85	-	11.63	12.29	4.89
NON-SELECTED TISSUES												
EC-68415												
100	83.00	146.52	79.92	93.24	63.88	127.76	240.50	227.14	16.90	13.82	45.86	45.23
200	83.00	99.90	86.58	124.32	63.88	604.96	326.51	242.10	16.90	14.76	52.77	61.56
300	83.00	77.90	-	-	63.88	113.57	-	-	16.90	14.45	-	-
Morden												
100	90.80	64.38	75.48	99.98	70.98	28.39	191.65	217.31	15.35	19.56	41.46	43.97
200	90.80	53.28	31.08	89.91	70.98	156.17	397.49	224.61	15.35	50.27	55.91	72.01
300	90.80	86.58	-	-	70.98	20.03	-	-	15.35	56.85	-	-
CD (P=0.01)	-	24.74	10.45	20.03	-	36.91	72.44	NS	-	2.32	5.10	7.40
Interaction												
CD (P=0.01)	-	7.22	5.10	NS	-	10.23	17.10	3.70	-	6.40	5.51	3.74
CV (%)	-	1.65	2.05	18.79	-	1.39	2.10	NS	-	4.10	3.52	6.30

There seems to be no definite trend in water and solute potential parameters (Table 2), among the treatments and tissues. The general fall noticed in water and solute potentials seems to be greater in non-selected tissues with increasing salinity. However, pressure potential was generally more positive in the selected tissues during the course of observation, especially at higher levels of salt. This gives some credence to the concept of turgor-related salt tolerance (Hasegawa et al., 1986). However, the selected tissues of Morden exhibiting greater turgor showed lesser tissue weights and those of EC-68415 which produced greater fresh and dry weights had lower pressure potential (Ψ_p) values, show the inconsistent relationship between growth and water relations at present. This leads to doubt the role of pressure potential in salt tolerance which was expressed earlier also (Binzel et al., 1985 and Prakash et al., 1993).

Table 4: Pattern of ion accumulation in selected and non-selected tissues of two sunflower genotypes grown under sodium chloride stress.

Treatment/ cultivar stress (mM)	Ion accumulation (mg/g dry wt.)											
	Sodium (DAI)				Potassium (DAI)				Potassium/sodium ratio (DAI)			
	0	10	20	30	0	10	20	30	0	10	20	30
Control												
EC-68415	6.25	10.00	12.50	6.25	7.50	10.00	17.50	15.00	2.31	1.00	1.40	2.40
Morden	3.75	5.00	6.25	12.50	10.00	27.50	11.25	19.25	2.67	3.50	1.80	1.54
SELECTED TISSUES												
EC-68415												
100	100	145.00	145.00	200.00	50.00	60.00	82.50	65.00	0.50	0.41	0.57	0.33
200	117	307.50	280.00	215.00	45.00	100.00	125.00	92.50	0.38	0.33	0.45	0.43
300	147	200.00	285.00	235.00	57.50	75.00	125.00	107.00	0.39	0.38	0.44	0.46
Morden												
100	77.50	115.00	290.00	340.00	40.00	47.50	120.00	47.50	0.52	0.41	0.41	0.14
200	131.00	370.00	292.50	292.50	60.00	87.50	100.00	100.00	0.46	0.24	0.34	0.34
300	157.00	215.00	365.00	495.00	43.00	65.00	167.50	100.00	0.27	0.30	0.45	0.20
CD (P=0.01)	-	15.71	3.17	15.01	-	15.03	8.36	9.75	-	0.005	0.001	0.065
Interaction												
CD (P=0.01)	-	47.00	52.17	78.05	-	NS	32.70	NS	-	0.032	0.12	0.18
CV (%)	-	2.92	14.42	2.61	-	6.16	2.51	4.22	-	9.36	1.65	6.12
NON-SELECTED TISSUES												
EC-68415												
100	6.25	32.50	119.00	142.50	7.50	37.50	30.00	32.00	0.94	0.27	0.21	-
200	6.25	60.00	197.00	145.00	7.50	15.00	52.50	35.75	0.25	0.36	0.25	-
300	6.25	42.50	-	-	7.50	7.50	-	-	0.18	-	-	-
Morden												
100	6.25	110.00	155.00	165.00	10.00	12.50	27.50	20.00	0.11	0.18	0.12	-
200	6.25	121.25	185.00	172.50	10.00	16.50	42.50	25.00	0.13	0.23	0.15	-
300	6.25	143.75	-	-	10.00	27.00	-	-	0.12	-	-	-
CD (P=0.01)	-	21.32	37.50	NS	-	12.50	NS	NS	0.09	NS	0.07	-
Interaction												
CD(P=0.01)	-	53.21	27.10	NS	-	17.32	NS	NS	0.15	NS	NS	-
CV (%)	-	6.53	13.13	7.27	-	1.80	20.13	14.73	4.31	9.10	12.50	-

Greater accumulation of reducing sugars, amino acids and proline noticed in the selected tissues of the two cultivars did not show a definite trend in between the two cultivars (Table 2). Though reducing sugars were more in EC-68415 on some days, the accumulation of amino acids and proline was generally higher in Morden on another day, specifically at 300 mM salinity (Table 2). The previous reports ascribe a role of carbohydrate (Steward and Lee, 1974; Popchristov and Zaganska, 1977), and proline (Pandey and Ganapathi, 1985) in salt tolerance. However, the present observations on pattern of growth and accumulation of reducing sugars and proline and earlier reports (Ben-Hayyim and Kochba, 1983) lead us to doubt the role of these in salt tolerance, at least in the present situation.

The greater accumulation of sodium ions with increasing levels of sodium chloride was very high in the selected tissues, which also showed better growth unlike non-selected tissues, which became senesced. However, there was almost parallel increase in the potassium ion content. This suggests that better salt tolerance was not due to the inhibition in the uptake of sodium. However, the greater uptake of potassium associated with the accumulation of sodium by the selected tissues seems to help maintain a greater K/Na ratio (Table 4), thereby reducing the deleterious effects of sodium ion on membrane and salt-sensitive systems, as reported earlier (Binzel et al., 1988 and Watad et al., 1983). This is also supported by the maintenance of balance between sodium and potassium ions in the non-selected tissues on control medium.

CONCLUSION

In vitro selection has improved the salt tolerance of the sunflower cultivars EC-68415 and Morden calluses. The per cent improvement in growth was better in the latter. Apparently, turgor, solute and ion accumulations seem to play some role in salt tolerance at present. However, absence of a definite pattern in changes among different parameters and smaller differences among them leads to doubt the role of these parameters in salt tolerance.

However, the somaclonal selection technique has definitely improved salt tolerance capacity of the tissues and the attempts for regeneration of plants from selected somaclone tissues are under way.

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**DIFERENCIAS EN CRECIMIENTO, RELACIONES
HÍDRICAS Y ACUMULACIÓN DE SOLUTO EN CALLOS
SELECCIONADOS DE GIRASOL (*Helianthus annuus* L.)
BAJO DIFERENTES NIVELES DE CLORURO SÓDICO**

RESUMEN

La selección recurrente de callos de hipocotilos mantenidos a niveles diferentes de salinidad mejoraron la capacidad de tolerancia. Después de 10 días de cultivo los tejidos seleccionados se cultivaron EC-G8415 y Morden mostraron mejor crecimiento a los niveles de salinidad (-1.68 MPa) más altos. Crecimiento, turgencia, acumulación de solutos e iones fueron mayores en estos tejidos. Sin embargo, la implicación de la turgencia y prolina en la tolerancia a salinidad parece ser dudosa. El incremento en cloruro sódico en el medio, incrementó la absorción de sodio y potasio, dando lugar al mantenimiento de una mayor proporción K/Na en los tejidos relacionados, sugiriendo por tanto un papel para el equilibrio iónico entre potasio y sodio.

**DIFFÉRENCES DE CROISSANCE, DE RELATIONS
HYDRIQUES ET D'ACCUMULATION DE SOLUTÉS DANS
DES CALS DE TOURNESOL (*Helianthus annuus* L.)
SÉLECTIONNÉS SUR CHLORURE DE SODIUM**

RÉSUMÉ

Une sélection récurrente de cals d'hypocotyles maintenus à divers niveaux de salinité a amélioré le potentiel de tolérance. A la 10^e génération de sous-culture les tissus sélectionnés de cvs EC-68415 et Morden, ont montré une meilleure croissance à la salinité la plus élevée (-1.368 MPa). La croissance, la turgescence et l'accumulation de solutés et d'ions était plus forte dans ces tissus. Cependant, l'implication de la turgescence et de la prolina dans la tolérance à la salinité apparaît incertaine. L'augmentation du chlorure de sodium dans le milieu, accroît le prélèvement du sodium et du potassium, conduisant au maintien d'un ratio K/Na plus élevé dans les tissus sélectionnés, suggérant ainsi un rôle de l'équilibre ionique entre le potassium et le sodium.