HELIA, **16**, Nr. 18, p.p. 71-76 (1993)

EFFECT OF SALT STRESS ON CALLUS DEVELOPMENT FROM HYPOCOTYL SEGMENTS OF SUNFLOWER (*Helianthus annuus* L.) GENOTYPES

A.H. Prakash, S.N. Vajranabhaiah and P.C. Reddy

University of Agricultural Sciences, Bangalore 560 065, India.

SUMMARY

Effect of sodium chloride on callus development in hypocotyl explants and seed germnation in two c.v. Morden and EC 68415 of sunflower was studied. Better callus development and germination were noticed in the former. Pattern of changes in water relations and accumulation of reducing sugars, soluble amino acids and proline were also studied in germinated seeds and calli. These studies indicated that turgor can not be related to salt tolerance. Proline accumulation seems more due to the effect of salinity. In the light of inconsistencies noticed in water relations and solute contents with respect to germination and seedling vigour, role of any one of these in greater salt tolerance of c.v. EC 68415 is not beyond doubt. Since better callus development and seed germination and vigour are associated, the former could be a more reliable index of salt tolerance in the two cultivars.

Key words: Sunflower, callus, salt stress, proline accumulation.

INTRODUCTION

Effect of salt on the survival and growth of callus is used as a method to develop salt resistant somaclones (Dix and Street, 1975; McCoy, 1987; Warne and Hickok, 1987). Growth of salt tolerant callus under osmotic stress (Fitch and Moore, 1981; Liu and Yeh, 1982; Binzel et al., 1985; and Paek et al., 1988) is a good indication of tolerance. Water relations (Binzel et al., 1987, 1985; Hasegava et al., 1986) and solute contents (Binzel et al., 1987, 1985; Hasegava et al., 1986), including ion accumulation patterns (Binzel et al., 1987, 1985; Watad et al., 1983) under stress are subject to intensive investigations. Most of the attempts in these lines are to relate the maintenance of turgor (Binzel et al., 1987, 1985; Hasegava et al., 1986), accumulation of solutes like proline (Paleg and Aspinall, 1981; Pandey and Ganapathi, 1985; Steward and Lee, 1974), carbohydrates (Steward and Lee, 1974; Mousseau, 1970; Popchristov and Zaganska, 1977), and ionic contents (Binzel et al., 1987 and 1985; Watad et al., 1983) with salt tolerance. However, there are no reports on effects of salinity on callus development and changes in water relations and solute contents in sunflower explants. Hence, the present report deals with the effect of salinity on callus development from hypocotyl explants of two sunflower genotypes.

MATERIALS AND METHODS

Hypocotyl segments from three-day old seedlings of EC–68415 and Morden sunflower genotypes were inoculated on Murashige and Skoog medium (1962) supplemented with sucrose (30g/l), NAA(2mg/l) and kinetin (0.25mg/l). The experimental

Table 1: Effect of sodium chloride on callus development, water relations and solute contents of hypocotyl explants

Treat-ment/ cultivar	Growth/ (in DAI)						Water relations (MPa)			Reducing sugars (mM) (DAI)			Amino acids (mM) (DAI)			Proline (mM) (DAI)		
	10		20		30		(30th day after inoculation)			10	20	30	10	20	30	10	20	30
Stress level (–MPa)	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	$-\Psi_w$	$-\Psi_s$	$-\Psi_p$									
EC–68415																		
Control	297.0	25.25	583.0	49.56	760.0	64.6	0.2	0.632	0.432	61.26	59.5	38.85	119.28	76.66	78.79	2.701	7.66	13.51
100	324.0	27.54	583.0	48.75	700.0	59.5	0.3	0.513	0.213	93.24	83.69	60.83	184.55	119.25	127.76	8.92	25.28	44.60
200	145.0	12.33	301.0	25.59	427.0	40.57	0.7	0.72	0.02	125.87	101.23	47.73	168.22	95.04	75.95	6.09	30.45	30.47
300	40.0	3.4	98.0	8.33	154.0	28.64	1.1	0.745	–0.355	37.35	26.42	43.51	119.25	46.85	53.24	2.07	20.04	25.29
Morden																		
Control	321.0	27.29	642.0	54.57	810.0	68.85	0.2	0.642	0.442	77.03	68.82	36.63	114.28	114.33	92.98	2.7	10.02	11.15
100	217.0	18.45	555.0	47.18	642.0	54.57	0.4	0.76	0.36	93.24	74.81	37.86	132.73	123.51	108.65	3.74	11.84	22.46
200	97.0	8.25	198.0	16.83	221.0	18.79	1.0	0.7	–0.3	57.74	63.05	29.08	114.28	57.55	36.91	4.11	13.95	29.37
300	40.0	3.10	92.0	7.82	105.0	13.09	1.1	0.763	–0.37	41.51	38.63	35.08	104.32	39.75	45.43	1.60	6.75	9.58
CD (P=0.01)	10.97	1.26	17.16	2.99	14.62	5.19	0.153	0.049	0.077	9.13	11.47	9.54	10.42	25.42	13.74	0.326	2.71	3.32
Interaction																		
CD (P=0.01)	21.52	2.47	33.67	5.87	28.69	10.13	0.13	0.096	0.114	17.90	22.51	18.72	20.44	49.87	26.95	0.64	5.319	11.13
CV(%)	2.39	3.20	1.83	3.67	1.25	4.74	9.02	2.88	7.44	4.76	6.85	8.60	3.04	11.33	6.74	3.22	6.70	1.40

Inoculum size = 50 mg per culture
Fresh weight expressed in mg
Dry weight expressed in mg

media contained 100, 200, and 300mM sodium chloride. Growth, by fresh and dry weights, was determined after thirty days. Water ($-\Psi_w$) and solute ($-\Psi_s$) potentials were determined by using water potential data system (Wescor–HP 115) and vapour pressure osmometer (Wescor 5100C) in the dew point mode. Turgor ($+\Psi_p$) potential was calculated as $+\Psi_p=(-\Psi_w--\Psi_s)$. Total alcohol soluble amino acids were determined as described earlier (Spiess, 1957). Proline contents were assayed by adopting the method reported by Bates et al., (1973). Reducing sugars were estimated by the method previously reported (Nelson–Somogyi, 1944).

Seeds of the two genotypes were also germinated for five days in Petri plates containing sodium chloride in water at 100, 200 and 300mM concentrations. Germination percent and seedling vigour were calculated. Water relations and solute contents were determined as described above.

RESULTS AND DISCUSSION

There was poor callus development from hypocotyl segments of the two genotypes incubated in the presence of salt (Table 1). This reduction in callus development was more with increasing NaCl. The differences were more conspicuous in Morden than in EC–68415, at 200 and 300 mM salinity levels. However, callus development was better in EC 68415 at the highest salt concentration in the medium. Studies on water relations, which did not vary much during the course of culture, revealed greater reduction in water potential in Morden, except at 300mM salinity, where it was equal to that of EC–68415. Turgor potential of Morden tissues at 200 and 300mM salinity was more negative. At 100mM it is more positive than the corresponding treatment in EC–68415. This suggests that turgor, which is often linked to stress tolerance (Hsiao et al., 1985), does not indicate its role in the present study. In spite of the maintenance of almost equal turgor at 300mM salinity, the two genotypes differed in callus development.

Accumulation of reducing sugars and alcohol soluble amino acids seems to be higher in EC–68415 when compared with Morden tissues. However, a decreasing trend of these solutes with increase in salinity was noticed. This suggests relatively higher rate of uptake and turnover of carbohydrates and nitrogen in EC–68415. Proline which is often implicated in salt tolerance (Paleg and Aspinall, 1981; Pandey and Ganapathi, 1985; Steward and Lee, 1974) is also higher in EC–68415 under salinity. However, there is a decline in the accumulation of proline with increase in salinity level. This decline is more conspicous at the highest salinity.

The reductions in germination percent and seedling vigour due to salt treatment was greater in Morden (Table 2). There was a failure of germination at 200 and 300mM concentrations. Complete inhibition of radicle elongation was noticed only at 300mM salinity in EC 68415. Reduction in seedling vigour was due to reduced radicle and plumule growth in Morden. But in EC–68415, it was due to the greater reduction in radicle length. Yet, seedling vigour was greater in EC–68415 at all salinity levels.

Water and solute potentials were more negative in seedlings germinated under salinity. This was more conspicuous at 300mM salinity in Morden. Negative turgor potentials were noticed in Morden at 200mM and EC 68415 at 300mM salt concentrations. This was due to more negative water potentials and less negative solute potentials.

Table 2. Effect of sodium chloride stress on germination, water relations and solute contents

Treatment/ Cultivar	Germination (% of control)	Root length (% of control)	Shoot length (% of control)	Seedling vigour	Water relations (MPa)		
					$-\Psi_w$	$-\Psi_s$	$+\Psi_p$
Stress levels (mM)							
EC–68415							
Control	*90.0 (100)**	90.0 (100)	90.0(100)	720(100)***	0.4	0.41	0.01
100	68.8 (87)	40.2 (41.7)	40.2 (41.7)	270(37.5)	0.6	0.84	0.24
200	58.7 (73)	13.4 (5.5)	40.2 (41.7)	136(18.8)	0.9	1.12	0.22
300	40.9 (43)	0.0 (0.0)	21.9 (13.9)	28.3(3.9)	1.1	1.00	–0.10
Morden							
Control	90.0 (100)	90.0 (100)	90.0(100)	480(100)***	0.4	0.41	0.01
100	71.6 (90)	39.2 (40)	56.5 (60.6)	1040(21.6)	0.7	0.91	0.21
200	21.9 (14)	0.0 (0.0)	6.3 (1.2)	12(2.5)	0.9	0.89	–0.01
300	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0(0.0)	1.7	1.71	0.01
CD (P=0.0)	5.82	0.55	3.61	24.47	0.09	0.12	0.09
CD interaction	11.41	1.08	7.08	48.01	0.42	0.28	0.08
(P=0.01),							
CV(%)	4.30	0.67	3.42	4.49	11.89	5.84	15.54

* Angular transformed values
** Per cent over control
*** Seedling vigour

Better germination and seedling vigour in EC–68415 at 300mM salinity, in spite of negative turgor, suggest doubts on the role of turgor in promoting germination and growth. Generally, higher reducing sugars may indicate higher rate of carbohydrate metabolism in EC–68415. The increased amino acids and proline maintained in Morden at 200mM salinity and no germination at the highest salt level indicate that accumulation of proline may be a response to stress and not a tolerance mechanism as reported earlier (Lawlor and Leach, 1985).

CONCLUSION

Better callus development and germination were not always associated with positive turgor or accumulation of proline, amino acids, or solutes. Therefore, the role of turgor in salt tolerance is to be doubted. Simultaneously, accumulation of solutes like proline may be due to salt stress, rather than it being a mechanism of salt tolerance.

REFERENCES

Binzel, M.L., Hasegawa, P.M., and A.K., and Bressan, R.A. (1985). Adaptation of tobacco cells to NaCl. *Plant Physiol.*, 79.

Binzel, M.L., Hasegawa, P.M., Rhodes, D., Handa, S., Handa, A.K. and Bressan, R.A. (1987). Solute accumulation in tobacco cells adapted to NaCl. *Plant Physiol.*, 84, 1408–1415.

Dix, P.J. and Street, H.E. (1975). Sodium chloride resistant cultured cell lines from *Nicotiana sylvestris* and *Capsicum annuum. Plant Science Letters*, 5, 231–237.

Hasegawa, P.M., Bressan, R.A. and Handa, A.K. (1986). Cellular mechanisms of salinity tolerance. *Hort. Sci.*, 21, 1317–1324.

Hsiao, T.C., Silk, K.W. and Jing, J. (1985). Leaf growth and water deficits: biophysical effects. *Control of leaf growth:* Society for Experimental Biology. Seminar series 27. Edited by N.R. Baker, W.J. Davies and C.K. Ong. pp239–267.

Lawlor, D.W. and Leach, J.E. (1985). Leaf growth and water deficits: Biochemistry in relation to biophysics. In: *Control of leaf growth.* SEB seminar series 27. Ed., N.R.Beker, W.J.Davies and C.K. Ong. Pub. Cambridge Univ. Press, U.K.

McCoy, J.J. (1987). Characterization of alfalfa plants regenerated from selected NaCl tolerant cell lines. *Plant Cell Reports*, 6, 417–422.

Mousseau, J. (1970). Fluctuation indvites par la neoformation debourgeons in vitro. In: Cultures des Tissues Reductase. *Mol. Gen. Genet.*, 161, 67–76.

Paleg, L.G. and Aspinal, G. (1981). Physiology and biochemistry of drought resistance in plants. Australia: Academic Press.

Pandey, R. and Ganapathy, P.S. (1985). The proline enigma: NaCl tolerant and NaCl sensitive callus lines of *Cicer arietinum. Plant Science*, 40, 13–17.

Popchristov, V.D. and Zaganska, N.A. (1977). Study of the seed progeny of regenerated plants obtained by the tissue culture of tobacco. In: Uses of Plant Tissue Culture in Breeding. *Experimental Botanikustav* CSAV.

Steward, G.R. and Lee, J.A. (1974). Effect of proline and carbohydrates on the metabolism of exogenous proline by excised bean leaves in the dark. *Plant Physiol.*, 50, 551–555.

Warne, T.R. and Hickok, L.G. (1987). Single gene mutants tolerant to NaCl in the fern *Ceratopteris*: Characterization and genetic analysis. *Plant Science*, 52, 49–55.

Watad, A., Reinhold, L. and Learner, H.R. (1983). Comparison between a stable sodium chloride resistant *Nicotiana* cell line and wild type: K and Na and proline pools as a function of salinity. *Plant physiol.*, 73, 624–629.

EFECTO DEL ESTRES SALINO OBRE EL DESARROLLO DE CALLO A PARTIR DE SEGMENTOS DE HIPOCOTILOS DE GIRASOL (*Helianthus annuus* L.)

RESUMEN

El efecto de cloruro sódico sobre el desarrollo de callo en explantes de hipocotilos y germinación de semilla en dos cultivares, Morden y EC 68415 de girasol. Se encontró mejor desarrollo do calle y garminación en el primero. El tipo de cambios en relaciones hídricas y acumulación de azúcares reductores, aminoácidos solubles y prolina fueron también estudiados en semillas germinados y callo. Estos estudios indicaron que la turgencia no puede ser relacionada a la tolerancia a la sal. A la luz de las inconsistencias encontradas en relaciones hídricas y contenido de solutos con respecto a germinación y vigor de plántulas, el papel de estos en mayor tolerancia a la salinidad del cultivar EC 68415 esta fuera de duda. Dado que mejor desarrollo de callo y germinación y vigor de la semilla están asociados, el primero puede ser un indice mas real de tolerancia a la salinidad en los dos cultivares.

EFFETS DU SEL SUR LE DÉVELOPPEMENT DE CALS ISSUS DE SEGMENTS D'HYPOCOTYLES DE DIFFÉRENTS GÉNOTYPES DE TURNESOL

RÉSUMÉ

L'effet du chlorure de sodium sur le developpement de cals à partir d' expiants d'hypocotyles et sur la germination de graines a été etudié sur deux cultivars de tournesol Morden et EC68415. Sur ce demier, on a observé un meilleur developpment de clas et une meilleure germination. Les profils de modification dans les relations hydriques et dans les accumulations de sucres réducteurs, d'acides aminés solubles et de proline ont été egalement étudiés dans les semnces en germination et sur les cals. Ces études indiquent que la turgescence ne peut être mise en relation avec la tolérance au sel. L'accumulation de proline semble être davantage due aux effets de la salinité. A la lumière des résultats irréguliers enregistrés sur les relations hydriques et les compositions des ces paramèters sur une meilleure tolérance au sel n'est hors de doute. Puisqu' une association entre un meilleur developpment de cals et la germination et la vigueur a été trouuvée, le premier critère pourrait constituer un index valable de tolérance au sel shez les deux cultivars.