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Screening Sunflower Genotypes for Salt Tolerance using a Stress Response Index

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ABSTRACT. *Correlation between physiological and biochemical parameters with field endurance of genotypes towards salt stress may facilitate easy screening of genotypes towards sodicity/salinity. This experiment aimed at testing the validity of a stress response index and its association with morphological and physiological traits in sodic condition. The study was conducted with 25 different sunflower inbreds under both glass house and field condition. The soil from sodic tract and normal field soil were transported and utilized for glass house study. Similarly for field condition, the inbreds were raised on appropriate sodic tracts and normal soils in different locations. The role of proline, nitrate reductase and total soluble protein towards salt tolerance was proved. The utilization of stress response index to identify the stable genotypes across salt environments is discussed. The traits nitrate reductase and head diameter had positive correlation with stress response index and can be used as an indicator for improved yield and phenology under stress. The inbred SF 54 was identified as salt tolerant through stress response index, morpho-physiological/biochemical parameters are elucidated with yield data in stress condition.*

INTRODUCTION

Sunflower occupies next to soybean among edible oil crops in the world owing to its oil quality. The world now produces 21.6 million tons of seed in 15.7 million ha with a mean yield of 1300 kg ha^{-1}. Sunflower is also grown on saline soils, particularly under irrigated conditions in arid climates (Korell *et al.*, 1996). The homeostasis towards excess salt is conferred by adaptive traits that are complex in nature and involves various cellular reactions. Breeding for salt tolerance requires the identification of simple morphological or physiological traits that bestow an advantage under one or more specific stress conditions (Blum, 1979). The role of proline, nitrate reductase and total soluble protein and their correlated response to stabilize the phenology under stress in sunflower is well established (Heuer, 1999; Durgaprasad *et al.*, 1996; Khan, 1996). However breeding for salt tolerance should emphasize both enhanced yield potential and phenology related traits under stress and non stress environments. An index has to be identified which indicates the variation in yield prospective of genotypes and the relative contribution of physiological and morphological traits towards yield stability in stress. An index developed by Bidinger *et al.* (1987a) for assessing drought tolerance was utilized in this experiment to identify the traits that contribute to yield stability and the resistance of genotypes for salt stress.

MATERIALS AND METHODS

Plant materials

Twenty five sunflower inbreds comprising six high yielding varieties, five maintainer lines and 14 Germplasm accessions of different origin were subjected to screening for salt stress tolerance (Table 1).

Table 1. List of sunflower inbreds taken for study and their yield (g) in stress environments.

Genotype	Description		E1	E2	E3
CO 2	Derivative of seven single cross hybrids of Russian origin		30.02	19.58	19.75
CO 3	Mutant from CO 2 (5kR of gamma rays)		27.10	20.15	19.00
CO 4	Dwarf x Surya derivative		30.66	18.54	22.50
Surya	From Maharastra		26.01	19.92	25.75
Morden	EC 101495 – Cernianka 66 (Introduction)		31.51	19.59	21.68
Maintainer lines obtained from Department of Oil seeds, TNAU, Coimbatore					
5B	Seeds maintained at Department of Oil		23.56	13.59	16.75
6B	seeds, Tamil Nadu Agricultural University,		24.28	19.59	18.75
302B	Coimbatore		25.75	15.46	18.17
336B			25.46	12.21	16.07
400B			22.34	18.95	18.75
86B3			21.92	14.98	20.75
Germplasm accessions from Department of Oil seeds, TNAU, Coimbatore					
GP 161	Seeds maintained at Department of Oil seeds, Tamil Nadu Agricultural University, Coimbatore		28.59	19.12	15.87
GP 255			35.40	23.41	22.12
GP 86			35.04	21.17	23.25
GP 336			33.57	19.89	23.50
GP324			39.79	23.35	25.40
GP 93			25.40	15.00	18.29
Core collections received from Ames, IOWA					
SF 91	Acc. No. PI 433377	Origin Egypt	17.91	15.30	20.29
SF 54	Acc. No. PI 289626	Origin France	26.46	19.61	27.13
SF 45	Acc. No. PI 243074	Origin Jordan	24.84	16.74	20.69
SF 34	Acc. No. PI 243074	Origin Uruguay	24.64	18.56	17.62
SF 83	Acc. No. PI 431516	Origin Romania	29.91	14.44	29.59
SF 7	Acc. No. Ames 3300	Origin Germany	32.56	17.07	20.62
SF 30	Acc. No. Ames 20080	Origin Bulgaria	28.16	19.64	20.57
SF 60	Acc. No. PI 331176	Origin Argentina	26.89	21.49	23.63
SF 91	Acc. No. PI 433377	Origin Egypt	17.91	15.30	20.29

[Note: E1 - pH. 7.18, E2 – pH. 8.89, E3 – pH. 8.95 (E1, E2 &E3 refers to soil types in different field conditions)]

Lab experiments

The experiment was conducted at the cytogenetics glasshouse maintained in Tamil Nadu Agricultural University, Coimbatore, India. Earthen pots with a capacity of

8 kg of soil were used. For stress treatment, the soil from the sodic tracts was transported and utilized as such (EC: 1.76 dS m^{-1} and pH: 8.80). The normal sandy loam field soil was used for control (EC: 1.59 dS m^{-1} and pH: 7.48). No additional fertilizers were applied to either control or stress condition.

Five seeds of each genotype were sown for each treatment. On the tenth day after sowing, only three plants were maintained in each pot. The experiment was replicated twice and the genotypes were randomized within replication and treatment. The water with an EC of 1.43 dS m^{-1} was irrigated to the soil field capacity without any leakage.

The leaves were sampled for analysis at star bud stage to anthesis, as it is considered to be critical for stress condition (Prabudeva *et al.*, 1998). Proline content, total soluble protein content and nitrate reductase activity were estimated by the method described by Bates *et al.* (1973), Lowry *et al.* (1951) and Nicholas *et al.* (1976), respectively.

Field experiments

The seeds of 25 experimental genotypes that are endowed to screen for their salt tolerance via the biochemical and morphological characters along with consistent yield were raised in eight environments. Of these, five composed of sodic soil and three sandy loam soils. The genotypes were raised with a spacing of 60 x 45 cm in five-meter length ridges. Data on days to flowering and single plant yield on randomly selected plants in each genotype of each environment were documented and the data were utilized for estimating stress response index.

Stress Response Index

According to Bidinger *et al.* (1987 a), grain yield in a specific stress condition (Y_s) is a function of Yield potential (Y_p), time of flowering (FL) and stress response (SR); *i.e.*

$$Ysi = a + bY_{pi} + c\,FL_i + SR_i + E$$

Where, E is the random error with zero mean and unit variance.

Consistent with Bidinger *et al.* (1987 b), if the parameters a, b and c of the above equation are estimated by minimizing residuals (E + SR), then the estimated stress yield (Y_{si}) will be,

$$\overline{Y}_{si} = a + b\,\underline{Y}_{pi} + c\,FL_i$$

The difference between the actual yield ($\overline{Y}si$) and the estimated yield (Y_{si}) under stress is then the measure of the residuals; *i.e* ($Y_{si} - Y_{si}$) = SR_i + E. Hence from this, the test for the significance of stress response can be derived as;

$$Z = | Y_{si} - \overline{Y}_{si} | / \sigma \qquad :$$

Where σ is the standard error Y_{si}

In practice, Z is $\leq$ 1.3 (it selects those genotypes in the upper and lower 10% of the normal distribution of actual yield (Ysi) the stress response (SR) is considered to

be zero; i.e. if the absolute value of the difference between measured yield in the stress (Ysi) and the yield predicted (Ysi) from the time of flowering and yield potential was less than 1.3 times of the σ, then the genotypes is considered to have no specific response to salt stress (Bidinger, 1987 b).

Correlation with SRI

The SRI calculated for each genotype under stress was correlated to yield component and physiological data to identify traits related to positive SRI values which might be used as selection criteria (Fischer and Wood, 1979).

RESULTS AND DISCUSSION

The stress inducible biochemical parameters *viz.*, proline, nitrate reductase and total soluble protein were considered for screening the salt tolerance of 25 sunflower genotypes because of their vital role in improving the phenology of stressed plants. The primary effect of salt stress is the loss of turgor that triggers proline accumulation in plants for osmotic adjustment (Pessarakkali, 1999). In this study, proline content had increased 1.5 fold in stressed plants as compared to the normal conditions (Table 2) and the result is in accordance with the reports of Santos *et al.* (1999) and Kogan *et al.* (2000). Genotypes SF45, GP161 and Morden displayed insignificant level of proline accretion, whilst exorbitant increase (more than 100%) was witnessed in the genotypes SF7, SF34, SF60, SF83 and GP336. The increase could be attributed to the stimulation of proline synthesis from glutamate by the loss of feed back inhibition, decline in proline oxidation or decrease of its incorporation into protein (Kramer, 1983). Genotypes with high proline accumulation capability effectively combat salt stress through maintenance of high osmoticum than genotypes with lesser proline synthesis capacity (Sing and Sing, 1999). Accordingly SF7, SF34, SF83, SF60, GP336, GP324 and 400B were considered as tolerant and Morden, 302B, GP161, SF45 and 336B as susceptible.

A 1.5 fold decrement in total soluble protein and nitrate reductase activity in stressed plants was observed as compared to the unstressed plants (Table 2). The decrease in protein could be ascribed to salt enhanced proteolysis resulting in increased amino acid accumulation (Gururaja Rao *et al.*, 1999). The variation in nitrate reductase decrease among the genotypes ranged from 6.99 to 69.09% and 21.0 to 53.51% reduction in total soluble protein (Table 2). However in certain genotypes, the difference in protein content was insignificant between stressed and normal plants probably due to the synthesis of stress or shock proteins under stress (Diaz De Leon, 1980). Salt induced reduction in nitrate reductase activity might be credited to the alteration of function such as enzyme synthesis (Srivastava, 1980) and reduced nitrate ion uptake (Klobus *et al.*, 1988). Such decline was also connoted by Khan (1996), Lal and Bharadwaj (1987) and Rao and Gnanam (1990) in soybean, peas and sorghum respectively. Since maintenance of high levels of total soluble protein (Diaz De Leon *et al.*, 1980) and nitrate reductase activity (Gulati and Jaiwal, 1996) contributes to tolerance, the genotypes GP336, GP324, Sf54, SF38, SF30, CO3 and SF54, GP255, SF30, SF45, Sf60, GP93, GP324 and CO3 were grouped as relatively tolerant based on the above parameters.

The screening based on biochemical parameters have not yielded a common set of genotypes as tolerant and the genotypes examined varied with the parameters

Table 2. Effect of salt stress on proline content, nitrate reductase enzyme activity and total soluble protein contents of 25sunflower Genotypes.

Geno types	Proline content (µg/g dry weight)			Nitrate reductase enzyme activity (µ moles of No^{-2}/g/hr)			Total soluble protein (mg/g of dry weight)		
	Control	Stress	% increase	Control	Stress	% decrease	Control	Stress	% decrease
CO 2	263.50	338.40	28.43	363.36	151.35	58.35	27.10	17.05	37.08
Morden	389.90	429.90	10.26	746.33	437.59	41.37	27.64	12.85	53.51
CO 4	179.40	266.50	48.55	389.64	207.53	46.74	26.02	16.58	36.28
Surya	110.50	195.63	77.04	452.76	180.65	60.10	28.14	16.74	40.51
CO 3	317.20	416.98	31.46	255.40	187.76	26.48	26.30	18.09	31.22
302B	162.20	200.45	23.62	486.37	286.39	41.12	26.01	15.78	39.33
6B	252.10	345.00	36.85	362.74	123.82	65.87	24.10	15.32	36.43
400B	105.96	198.25	87.10	579.27	376.73	34.96	25.90	15.98	38.30
86B3	130.83	195.10	49.12	420.59	252.63	39.93	25.95	16.03	38.23
5B	130.65	174.95	33.91	389.13	226.39	41.82	25.53	13.67	46.46
336B	247.10	315.65	27.74	265.84	132.47	50.17	25.98	12.77	50.85
GP324	131.95	246.29	86.65	616.94	456.15	26.06	32.31	24.52	24.11
GP 93	230.63	349.21	51.42	237.47	175.89	25.93	25.54	17.15	32.85
GP 86	241.15	286.59	128.84	595.46	386.92	35.02	30.58	16.69	45.42
GP 336	119.60	231.95	93.94	422.36	235.72	44.19	22.32	17.61	21.10
GP 161	186.85	198.51	6.24	281.96	154.79	45.10	29.93	20.74	30.70
GP 255	150.15	261.30	74.03	312.89	289.69	7.40	26.72	16.00	40.12
SF 83	130.65	313.30	139.80	341.65	105.62	69.09	23.56	17.24	26.83
SF 45	254.15	285.13	12.19	415.65	343.16	17.44	36.62	19.14	47.73
SF 34	104.00	251.12	141.46	330.80	164.32	50.33	33.33	19.85	40.44
SF 30	111.80	148.85	33.14	338.65	289.08	14.64	31.18	22.74	27.07
SF 60	105.95	252.85	138.65	285.16	221.56	22.30	20.71	10.29	50.31
SF 7	97.50	251.65	158.10	560.63	385.42	31.25	27.32	14.11	48.35
SF 91	142.35	185.62	30.40	435.64	250.96	42.39	19.72	13.00	34.08
SF 54	102.35	136.16	33.03	350.69	326.19	6.99	20.72	15.65	24.46
Mean	175.93	259.01	58.88	409.50	253.95	37.80	26.77	16.62	37.67
	SEd	CD (0.05)	CD (0.01)	SEd	CD (0.05)	CD (0.01)	SEd	CD (0.05)	CD (0.01)
G	6.4	12.86	17.152	3.881	7.797	10.401	1.42	2.853	3.806
T	1.81	3.634	4.851	1.098	2.205	2.942	0.402	0.807	1.076
G x T	9.05	18.183	24.256	5.489	11.027	14.709	2.008	4.035	5.382

used. However screening of salt tolerance through biochemical methods could be used to identify parameters or plant characteristics that confer advantages in stress conditions (Fischer and Wood, 1979). If stress resistance would be a consequence of advantages conferred by one or more biochemical or morphological characteristics (Turner, 1982), it is to be manipulated in a breeding program as an independent genetic character, as well the assessment of salt tolerance should be free from the confounding effects of yield potential and phenology. Moreover cultivars can not be bred for salt tolerance alone but aiming to breed a cultivar with high yield potential along with phenology related characteristics under stress conditions would be optimum choice. Therefore, the properties mentioned before would not be sufficient to identify the salt tolerant genotypes.

Table. 3. Estimation of salt response index under stress condition (SRI).

Genotype	Potential yield	Stress yield	Days to 50 per cent flowering	Estimated $\overline{\text{yield}}$ (Ysi)	SRI
CO 2	21.14	18.52	57.50	15.53	3.59
Morden	26.63	18.65	57.50	22.35	5.65
CO 4	32.82	25.27	54.50	23.24	2.44
Surya	39.49	29.29	59.50	27.82	1.77
CO 3	42.08	22.65	50.50	23.01	**0.49**
302B	34.11	24.38	58.50	28.07	4.36
6B	31.82	23.52	60.50	23.12	**0.48**
400B	20.49	12.65	55.00	14.51	2.23
86B3	20.15	11.89	60.00	12.15	**0.31**
5B	34.30	22.24	57.50	24.00	2.11
336B	23.86	14.58	56.50	17.04	2.96
GP324	35.16	20.19	60.00	25.15	5.96
GP 93	30.14	23.69	52.50	20.13	4.28
GP 86	32.00	23.66	58.00	22.64	**1.23**
GP 336	32.73	26.40	56.00	25.99	4.52
GP 161	38.26	30.89	60.00	27.15	4.49
GP 255	33.48	24.63	56.00	23.12	1.81
SF 83	28.02	20.26	57.00	19.84	**0.50**
SF 45	26.72	18.62	52.50	17.93	**0.83**
SF 34	27.62	15.27	51.00	18.15	3.46
SF 30	20.06	15.95	49.00	12.81	3.77
SF 60	30.47	18.92	54.00	20.70	2.50
SF 7	25.70	16.62	51.50	20.79	4.52
SF 91	27.13	17.63	52.50	18.19	**0.67**
SF 54	35.25	23.59	54.00	23.78	**0.23**
Mean	29.99	20.79	55.66	20.82	-0.04

$\overline{\text{Ysi}} = -11.787 + 0.6442\text{Yp} + 0.23817\text{FL}$ SE = 0.354

Indexing yield to some quantifiable measures of stress severity which is independent of yield potential and phenology effects would therefore be the only mean of quantitatively evaluating relative stress resistance in a large collection of cultivars (Robin, 1997). In the present study, the stress indices established to quantify drought stress had been utilized for measuring the relative tolerance among cultivars to salt

stress as the latter chiefly imposes osmotic stress, which is a characteristic feature of drought. Nevertheless the formulae adopted for drought quantification involve the yield and yield components only without the putative traits specific to drought.

In this study, Stress Response Index (SRI) for genotypes was found to be of different magnitude (Table 3). For eight in-breds *viz.*, CO3, 6B, 86B3, GP86, SF83, SF45, SF91 and SF54, the SRI was less than 1.3 (Table 3) (10% upper and lower distribution of the stress yield), *i.e.* SR = 0, indicating that within the limits of experimental error, they had no specific response to stress (Bidinger *et al.*, 1987a) or average responsiveness across a range of salt environments. The remaining genotypes were found to respond to the sodic soil with a varying degree and the inbreds Morden, 302B, GP324, GP93, GP161 and SF7 had relatively high interaction to the sodic stress environments.

Table 4. Association of characters in sunflower with salt response index (SRI)

Characters	Control	Stress
Days 50 per cent flowering	-0.041	-0.027
Plant height	-0.308	-0.144
Number of leaves	-0.568**	-0.256
Stem girth	-0.174	-0.190
Head diameter	-0.074	0.103
100 seed weight	0.024	-0.420*
Per plant yield	0.227	-0.001
Proline content	0.002	-0.071
Nitrate reductase	0.206	0.505**
Total soluble protein	0.206	-0.130

* Significance 5% = 0.381 ** Significance 1% = 0.481

The absence of significant positive correlations between SRI and yield components measured in the normal soil (Table 4) indicated no consistent, a priori, advantages exist in one yield structure over another in stress treatment. The lack of such correlations in the data indicates that it may be possible to select sunflower for any desired combination of yield components in the absence of stress. In contrast, the existence of significant positive association between SRI and nitrate reductase enzyme activity and non significant but positive trend with head diameter (Table 4) suggests a better ability of certain genotypes to maintain phenology related traits and bigger sized capitulam under stress (Bidinger *et al.*, 1987b). From the foregoing analysis, one can conclude the maximum progress in developing varieties with better yield in sodic soils should be made by combining yield potential and the characteristics associated with a high positive SRI, *i.e.* nitrate reductase activity and head diameter. By adopting such criteria, the inbred SF54 was identified as salt resistant genotype and the validity was confirmed through replicated trial (Table 1) across the salt environments.

CONCLUSION

The biochemical parameters that were utilized for screening sunflower inbreds for sodicity have indicated that tolerant genotypes manifests exorbitant increase in proline content with insignificant reduction in protein content and nitrate reductase

activity and *vice versa* in susceptible genotypes. The inbreds CO3, 6B, 86B3, GP86, SF83, SF45, SF91 were identified as tolerant and the genotypes Morden, 302B, GP324, GP93, GP161 and SF7 as susceptible. The association pattern of SRI with yield components and biochemical parameters revealed that the traits head diameter and nitrate reductase activity can be used as a putative trait while screening for sodicity in sunflower.

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