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Trait modelling for stress tolerance in Indian mustard : Evidenced from seedling stage

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Abstract

The current scanty knowledge about the physiological mechanisms underlying plants' ability to tolerate salt stress that hinders potential production of numerous crops, including mustard. To explore the traits and mechanism for salt tolerance in mustard, we used 250 stabilized $F_{7:8}$ recombinant inbred lines (RILs) mapping population developed by crossing indigenous contrasting genotypes CS 614-1-1-100-13 (salt sensitive) × CS 56 (salt tolerant) and evaluated them under control and irrigated water salinity of EC_{iw} 12 dS/m to characterize for growth, photosynthetic and ionic traits. Step wise regression revealed, instantaneous water use efficiency, transpiration rate and fresh weight of root together accounted for more than 93% of the overall variation in photosynthesis rate under salt stress condition, indicating their critical contribution to reducing salt stress. The salt tolerance index (STI) categorized 23 RILs as salt tolerant, 99 RILs as moderately tolerant and remaining 128 RILs were sorted as salt sensitive RILs. These identified salt tolerant RILs can be exploited for QTLs and gene discovery and serve as potential doners/future ready lines to combat abiotic stress and development of salt tolerant varieties of mustard.

***Keywords:** Mustard, RILs, photosynthetic traits, STI*

Introduction

Salinity and sodicity stress damage an area of 932.2 mha worldwide, of which 6.73 mha are affected by these stresses in India (Metternicht and Zinck, 2003; Singh *et al.*, 2014) however, this will be increased up to 16.25 mha by 2050 (Kumar *et al.*, 2022b). Groundwater utilized for irrigation contains 32-84 percent salty or brackish water and the soil salinity build due to this irrigation results in an annual loss of 10 million ha of land, consequently adverse effect on the food basket. High salt stress leads to cellular osmotic stress, ion-specific toxicity, reduced plant growth and photosynthetic traits which ultimately lead to a minimal yield of the crop (Singh and Sharma, 2016). By improving crop tolerance to salt or by draining salt from the soil, this low yield in saline places can be combated (Kumar, 2014; Singh and Sharma, 2016). This tolerance is achieved in crop plants *via* different mechanisms such as maintenance of cell turgidity through the accumulation of osmolytes, ion exclusion from the root, ion compartmentation in vacuole, tissue tolerance and ion-independent tolerance.

Indian mustard, *Brassica juncea* (L.) Czern & Coss (AABB, 2n=36, Genome size: 1068 Mb) is a significant oilseed crop with wide adaptability (Kang *et al.*, 2021). Globally, India ranked second in rapeseed-mustard cultivation after only China and third in production behind Canada and China (Kumari *et al.*, 2019). It ranks as India's third-largest edible oilseed crop after peanut and soybean, which accounts for 24.36% of the country's oilseed market among nine edible oilseed crop (Kumar *et al.*, 2022a). The amphidiploid species (*B. carinata, B. juncea and B. napus*) appear to be superior to the diploid species (*B. rapa*, *B. nigra* and *B. oleracea*) in terms of saline tolerance, as per several studies (Ashraf *et al*., 2001; Ashraf and Mehmood, 1990).

Salt stress causes Na^+ and K^+ ion imbalance, by disrupting the Na^+/K^+ ratio in leaves, salinity during the seedling stage has a deleterious impact on photosynthesis. The transfer of carbohydrates from source to sink is slowed down by this aberrant Na^+/K^+ ratio, which also affects mustard growth. Due to restrictions on growth, seed yield is reduced by up to 60% in mustard (Singh *et al*., 2019). The lowered rate of carbon absorption, assimilation and partitioning to seedlings during the initial stage of salt stress makes it more detrimental (Singh *et al*., 2019). Ultimately, plant growth, photosynthesis rate and yield is reduced due to toxic salt stress levels (Pant *et al*., 2022).

It's well-recognized that *Brassica* an acceptable reclamation crop but introduction of salt mitigation or salt tolerance mechanisms into the Indian mustard crop proceeds slowly due to a lack of genetic variability, research or inadequate information in these areas. One of the main initiatives in *Brassica* development is cultivar improvement for saline tolerance. Therefore, the creation of salinity-tolerant Indian mustard cultivars with higher yields in the salt-affected semi-arid tropics will be more effective and efficient by integrating breeding, physiological, biochemical, novel omics as well as bioinformatics approaches studies (Pant *et al*., 2022). Our study included 250 RILs mapping population which were produced by crossing indigenous contrasting genotypes CS 614-1-1-100-13 (salt sensitive) × CS 56 (salt tolerant) of *B. juncea.* These RIL mapping population serve as mustard genetic resource to identify salt tolerance genotypes to combat salt stress for salt prone areas.

Materials and Methods

Study site

The experimental materials consisted of 250 stabilized $F_{7:8}$ recombinant inbred lines (RILs) mapping population of Indian mustard developed by crossing indigenous sources of mustard; CS 614-1-1-100-13 (salt sensitive) an advanced breeding line developed at ICAR-CSSRI, Karnal with gamma-ray irradiation treatment and stabilized for M_6 generation (Sharma *et al*., 2008) and CS 56, a national released high yielding salt tolerant variety. These 250 RILs along with parents grown during consecutive *Rabi* seasons 2020-21 and 2021-22 under control and irrigation water salinity ECiw 12 dS/m in the pots with three replications at ICAR-CSSRI, Karnal (29°43'N, 76°58'E; 245 m above the average sea level) (Singh *et al*., 2020) (Fig. 1).

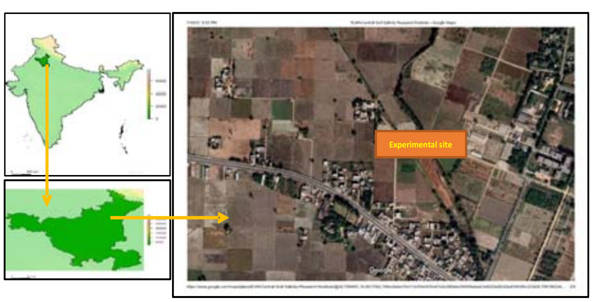

Fig. 1: Location of experimental site

Experimental details

The RIL mapping population was cultivated in pots of 20 kg capacity in sand culture. For the salinity environment, irrigated with saline water of EC_{iw} 12 dS/m throughout the experiment. The chloride and sulphate salts of Na^+, Ca^{2+} and Mg^{2+} to keep the SAR (Sodium absorption ratio) within the permissible limits used for the preparation of EC_{iw} 12 dS/m saline irrigation water. Prior to planting, seeds were surface sterilised for 5 minutes in a solution of 10% sodium hypochlorite before being rinsed with distilled water. Twenty seeds of each RIL were planted in a plastic pot filled with properly washed river sand at a depth of one centimetre. Each pot's bottom was dug out to allow any extra water to drain. The pots were set up in a factorial experiment using a completely random block design. The pots were watered with Hoagland's solution, a nutrient solution and kept at maximum field capacity until the seedling stage. Throughout the experiment, salinity levels were kept constant by draining the salt out of the pots every day (Singh *et al*., 2019).

Data collection

Growth attributing traits at the seedling stage

Initially, randomly ten seedlings (15 days old) from each RIL under control and salinity EC_{iw} 12 dS/m conditions were uprooted and washed with distilled water to record the fresh weight (mg) of leaves, stems and roots. The oven-dried plant samples at 55-65 °C for 5-6 days were used for reading of dry weight (g) of the leaf, stem and root.

Photosynthetic traits

Randomly selected three plants from each genotype under control and EC_{iw} 12 dS/m salinity regime were used for photosynthetic data at the seedling stage *i.e.*, Photosynthesis rate (Pn; µmol m^2 s^1), transpiration rate (E; mol m^2 s^1), stomatal conductance (gS; mol m^2 s^1), intracellular CO_2 assimilation (Ci/Ca), Instantaneous water use efficiency (*i* WUE; µ mol CO_2/m mol H_2O) and intrinsic water use efficiency (*in* WUE). Pn and other gas exchange parameters were measured on the fully expanded leaf of three representative plants per RILs using a portable photosynthetic system: infrared gas analyzer LI-6800XT [Li-COR, USA) (Singh *et al*., 2019). All the above traits were measured between 10:00 and 12:00 AM in sunlight under these weather conditions; PAR ~700 µmol $m^{-2}s^{-1}$, temperature ~ 25±1°C relative humidity ~ 70% and air CO_2 355 µmol mol^{-1}. The K^+ and Na^+ concentration of plant sample measured with Inductively coupled plasma-optical emission spectrometry ICPE-9800 (Shimadzu, Japan) after checking the standards (Piper, 2019).

Statistical analysis

The statistical analyses regression and STI was carried out for all the studied seedling stage traits using the *STAR 2.0.1* (IRRI, 2014) and MS Excel.

Stress tolerance index (Fernandez, 1992) = $\frac{Ys.Yp}{Yp^2}$

Where, Ys and Yp are the mean yield of genotypes under stress and non-stress conditions, respectively. The RILs with high STI values will be salt stress tolerance Result and discussion

Twenty-one traits were further sub-categorized into six growth attributing traits [fresh weight (root, stem and leaves), dry weight (root, stem and leaves)], six photosynthetic traits and nine ionic traits (Na^+, K^+ and Na^+/K^+ of root, stem and leaves) were used for trait modeling and characterized salt tolerant RILs at the seedling stage.

Results and Discussion

Mustard traits prioritization under salinity

All feasible and stepwise regression analyses were carried out to ascertain the impact of component variables on Pn (dependent variable) (Shannon *et al.*, 2000; Sharma and Sinha, 2012). All conceivable regression analyses revealed that gS, E, *i*WUE, *in*WUE, RFW and LFW significantly influenced the Pn of mustard leaves under salt stress, while the remaining traits did not (Table 1). Therefore, during the stepwise regression method, these remaining non-significant traits were eliminated. According to the results, *i*WUE, E and RFW together accounted for more than 93% of the overall variation in Pn under salt stress conditions. Additionally, Pn variation was substantially influenced by *i*WUE, E, RFW, *in*WUE, gS and LFW with cumulative R^2 = 94.36, which could be best fitted since it reflected the least Mallow's Cp criteria. The following equation was created to estimate the projected Pn under saline conditions based on regression coefficients of the relevant traits (Table 2):

Predicted photosynthetic rate = -17.05 + (3.8 × E) + (gS × 13.13) + (2.44 × *i*WUE) + (0.08 × *in*WUE) + (-0.02 × RFW) + (-0.01 × SFW) + (0.23 × LFW) + (0.01 × LK15).

Hence, these traits play major in contribution to enhance photosynthetic rate under saline condition. By targeting these traits, further research will be extending to vegetative and harvesting stage to combat salt stress.

Characterization of RILs based on salt tolerance index

To characterized tolerant and sensitive RILs over the environment, the salt tolerance index (STI) was calculated. Based on STI, RILs were characterized into three groups *i.e.*, highly tolerant (STI e"1), tolerant (STI = 1-0.75), moderately tolerant (STI = 0.75-0.50) and sensitive RILs (STI d"0.50). Total 24 RILs were selected as highly salt tolerant (RIL24, RIL32, RIL74, RIL87 and RIL73), 99 RILs were sorted as tolerant (RIL14, RIL31, RIL13, RIL95 and RIL142), 109 RILs as moderately tolerant while remaining 18 RILs were characterized as sensitive which score STI <0.50 (RIL170, RIL247, RIL225, RIL228 and RIL223) over the environment (Table 3 and Fig. 2). The salt-tolerant

Table 1: Salinity stress tolerance's regression coefficient, standard error and significance of the prioritized attributes

Dependable variable	Variable	Estimate	Standard Error (SE)		t value	Pr(>|t|)
Pn	Intercept	-17.05	0.71	-23.97	0.000	
	E	3.80	0.19	19.60	0.000	
	gS	13.13	1.95	6.72	0.000	
	*i*WUE	2.44	0.13	18.93	0.000	
	*in*WUE	0.08	0.01	6.66	0.000	
	RFW	-0.02	0.01	-3.85	0.000	
	SFW	-0.01	0.00	-1.53	0.127	
	LFW	0.23	0.14	1.72	0.087	
	LK15	0.01	0.00	1.78	0.077	

Table 2: Traits modelling for salinity tolerance through multiple linear regressions approach

Variables	C(p)	R-square	Adj R-sq
*i*WUE	2845.40	29.19	28.90
E + *i*WUE	56.81	93.07	93.01
E + *i*WUE + RFW15DAS	47.63	93.32	93.24
GSW + E + *i*WUE + *in*WUE	19.57	94.01	93.92
GSW + E + *i*WUE + *in*WUE + RFW15DAS	8.49	94.31	94.20
GSW + E + *i*WUE + *in*WUE + RFW15DAS + LFW15DAS	8.40	94.36	94.22

Table 3: Grouping of mustard RILs based on salt tolerance index (STI) of photosynthetic rate

Highly tolerant RILs (STI = >1)			Tolerant RILs (STI = 1-0.75)						Moderately tolerant RILs (STI = 0.75-0.50)						Sensitive RILs (STI = <0.50)		
RILs	STI	Rank	RILs	STI	Rank	RILs	STI	Rank	RILs	STI	Rank	RILs	STI	Rank	RILs	STI	Rank
RIL24	1.26	1	RIL14	0.99	25	RIL121	0.85	75	RIL128	0.74	124	RIL169	0.64	179	RIL201	0.49	233
RIL32	1.22	2	RIL31	0.99	26	RIL141	0.85	76	RIL8	0.74	125	RIL187	0.64	180	RIL222	0.49	234
RIL74	1.18	3	RIL13	0.99	27	RIL111	0.84	77	RIL166	0.74	126	RIL65	0.64	181	RIL180	0.48	235
RIL87	1.17	4	RIL95	0.97	28	RIL97	0.84	78	RIL184	0.74	127	RIL154	0.64	182	RIL175	0.48	236
RIL73	1.15	5	RIL142	0.97	29	RIL34	0.84	79	RIL70	0.74	128	RIL61	0.64	183	RIL155	0.47	237
RIL77	1.15	6	RIL78	0.97	30	RIL96	0.84	80	RIL118	0.74	129	RIL148	0.63	184	RIL183	0.46	238
RIL25	1.13	7	RIL56	0.97	31	RIL174	0.84	81	RIL105	0.73	130	RIL113	0.63	185	RIL182	0.46	239
RIL82	1.12	8	RIL161	0.96	32	RIL88	0.84	82	RIL43	0.73	131	RIL221	0.63	186	RIL185	0.45	240
RIL30	1.12	9	RIL102	0.96	33	RIL147	0.84	83	RIL204	0.73	132	RIL133	0.63	187	RIL249	0.44	241
RIL79	1.1	10	RIL51	0.96	34	RIL29	0.84	84	RIL233	0.73	133	RIL237	0.63	188	RIL179	0.43	242
RIL22	1.1	11	RIL49	0.95	35	RIL17	0.83	85	RIL54	0.73	134	RIL136	0.62	189	RIL167	0.43	243
RIL37	1.07	12	RIL41	0.95	36	RIL149	0.83	86	RIL189	0.73	135	RIL240	0.62	190	RIL246	0.38	244
RIL83	1.06	13	RIL108	0.95	37	RIL19	0.83	87	RIL1	0.72	136	RIL235	0.62	191	RIL186	0.36	245
RIL117	1.04	14	RIL26	0.94	38	RIL110	0.83	88	RIL120	0.72	137	RIL239	0.62	192	RIL170	0.36	246
RIL3	1.03	15	RIL57	0.94	39	RIL6	0.83	89	RIL217	0.72	138	RIL168	0.62	193	RIL247	0.34	247
RIL46	1.03	16	RIL242	0.94	40	RIL5	0.83	90	RIL135	0.72	139	RIL158	0.62	194	RIL225	0.34	248
RIL99	1.02	17	RIL129	0.94	41	RIL47	0.83	91	RIL230	0.71	140	RIL81	0.61	195	RIL228	0.34	249
RIL36	1.02	18	RIL93	0.93	42	RIL241	0.82	92	RIL112	0.71	141	RIL216	0.61	196	RIL223	0.33	250
RIL94	1.01	19	RIL85	0.93	43	RIL220	0.82	93	RIL143	0.71	142	RIL11	0.61	197			
RIL109	1.01	20	RIL91	0.93	44	RIL90	0.82	94	RIL4	0.71	143	RIL206	0.61	198			
RIL72	1.01	21	RIL100	0.93	45	RIL209	0.81	95	RIL231	0.71	144	RIL153	0.61	199			
RIL107	1.01	22	RIL131	0.92	46	RIL101	0.81	96	RIL211	0.71	145	RIL28	0.6	200			
RIL76	1.00	23	RIL27	0.92	47	RIL39	0.81	97	RIL194	0.71	146	RIL214	0.6	201			
RIL33	1.00	24	RIL68	0.92	48	RIL16	0.81	98	RIL172	0.71	147	RIL62	0.59	202			
			RIL197	0.91	49	RIL115	0.81	99	RIL114	0.71	148	RIL190	0.59	203			
			RIL145	0.91	50	RIL42	0.8	100	RIL157	0.71	149	RIL137	0.59	204			
			RIL69	0.91	51	RIL159	0.8	101	RIL21	0.7	150	RIL156	0.59	205			
			RIL71	0.91	52	RIL84	0.8	102	RIL152	0.7	151	RIL162	0.59	206			
			RIL132	0.91	53	RIL196	0.8	103	RIL52	0.7	152	RIL163	0.59	207			
			RIL160	0.91	54	RIL125	0.8	104	RIL35	0.7	153	RIL165	0.58	208			
			RIL23	0.91	55	RIL218	0.8	105	RIL205	0.69	154	RIL238	0.56	209			
			RIL53	0.9	56	RIL127	0.8	106	RIL151	0.69	155	RIL213	0.56	210			
			RIL12	0.9	57	RIL59	0.8	107	RIL124	0.69	156	RIL224	0.56	211			
			RIL140	0.9	58	RIL130	0.8	108	RIL10	0.69	157	RIL236	0.55	212			

RIL86	0.9	59	RIL191	0.79	109	RIL188	0.69	158	RIL229	0.55	213
RIL55	0.9	60	RIL146	0.79	110	RIL171	0.69	159	RIL244	0.54	214
RIL48	0.89	61	RIL150	0.79	111	RIL234	0.69	160	RIL202	0.54	215
RIL63	0.89	62	RIL50	0.79	112	RIL44	0.69	161	RIL164	0.54	216
RIL126	0.89	63	RIL2	0.78	113	RIL192	0.68	162	RIL176	0.53	217
RIL104	0.89	64	RIL98	0.78	114	RIL20	0.68	163	RIL138	0.53	218
RIL139	0.89	65	RIL60	0.77	115	RIL144	0.68	164	RIL195	0.52	219
RIL103	0.87	66	RIL7	0.77	116	RIL9	0.67	165	RIL210	0.52	220
RIL122	0.87	67	RIL18	0.77	117	RIL215	0.67	166	RIL181	0.52	221
RIL106	0.87	68	RIL45	0.76	118	RIL198	0.67	167	RIL177	0.52	222
RIL116	0.86	69	RIL40	0.76	119	RIL66	0.67	168	RIL227	0.52	223
RIL208	0.86	70	RIL89	0.76	120	RIL64	0.66	169	RIL226	0.52	224
RIL15	0.86	71	RIL119	0.76	121	RIL134	0.66	170	RIL245	0.52	225
RIL75	0.85	72	RIL38	0.75	122	RIL193	0.66	171	RIL203	0.52	226
RIL58	0.85	73	RIL123	0.75	123	RIL219	0.65	172	RIL200	0.51	227
RIL80	0.85	74				RIL67	0.65	173	RIL173	0.51	228
						RIL232	0.65	174	RIL250	0.5	229
						RIL248	0.65	175	RIL243	0.5	230
						RIL212	0.65	176	RIL178	0.5	231
						RIL92	0.65	177	RIL199	0.5	232
						RIL207	0.65	178			

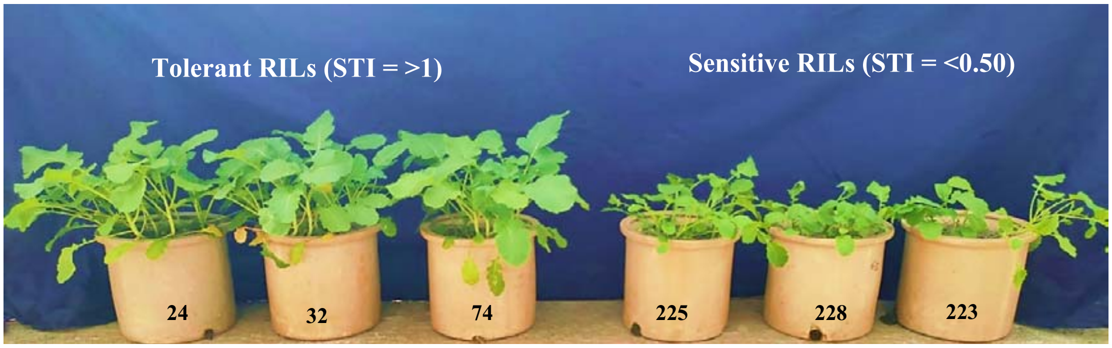

Fig. 2: Salinity tolerant and sensitive RILs of mustard under salinity environment ECiw 12dS/m at seedling stage

RILs under salt stress circumstances due to the preservation of high photosynthetic activity, high K^+ concentration, low Na^+ and Na^+/K^+ ratio (Keisham *et al*., 2018). These research' conclusions agreed with our experimental results.

A multiple regression model's fit can be evaluated using Mallows' Cp Criterion; smaller Cp values are preferable because they signify lower levels of unexplained error.

Conclusion

The Indian mustard RILs evaluated in our study have a significant variation in measured growth, photosynthetic and ionic traits. The stepwise regression approach *i*WUE, E, RFW, *in*WUE, gS and LFW as defining traits for Pn, indicating their critical contribution to reducing salt stress. Based on our study on RIL24, RIL32, RIL74 and RIL 87 were identified as potential resource or donor for salt-tolerance and may be used for cultivation under salinity stress. Further these lines may be employed in hybridization programs to create future ready new high-yielding, salt-tolerant breeding lines to combat salt stress. Additionally, these genotypes might be used to comprehend the genetic and molecular mechanism of Indian mustard salt tolerance.

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