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Screening of pollen grains vis-à-vis whole plants of oilseed brassicas for tolerance to salt

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Abstract. Pollen grains and whole plants of 11 cultivars of oilseed brassicas (*B. juncea*, *B. campestris*, *B. carinata*) were screened for salt tolerance. Whereas pollen germination percentage in sitting drop cultures served as a reliable index of pollen tolerance to NaCl, pollen-tube growth did not. Seed yield in plants of the same 11 cultivars raised in artificially salinized soils also proved to be a good index of whole plant tolerance to soil salinity. A close correspondence between pollen (gametophyte) and whole plant (sporophyte) responses to salinity was discovered. Our studies show that tolerance to salt is yet another trait expressed in both the sporophyte and gametophyte.

Key words: Oilseed brassicas – Tolerance to salt – Pollen germination as index – Comparison with seed yield

Introduction

The use of pollen grains in place of whole plants as a screening system is based on reports that more than 60% of the genes expressed in an individual of a species are also expressed in its pollen grains (Tanksley et al. 1981; Willing and Mascarenhas 1984). The screening of whole plants of a species for desirable traits would demand a greater infrastructural input (space, time and labor) than the screening of pollen grains. Also, as individual plants produce a large quantity of pollen grains, a variety of factors can be tested on pollen produced by a given genotype. Pollen grains of certain taxa have been used in studies on tolerance to ozone, patho- and phyto-toxins, herbicides, heavy metals, salinity, and low and high temperatures (Ottaviano and Mulcahy 1989; Searcy and Mulcahy 1990; Hormaza and Herrero 1992; Shivanna and Sawhney 1993). Some of the tolerance traits are expressed in both the gametophyte and sporophyte.

We present results from the screening of pollen grains of 11 cultivars of oilseed brassicas for tolerance to NaCl and report the comparative response of whole plants grown in artificially salinized soils.

Materials and methods

Three cultivars of *Brassica juncea* Coss. and Czern. ('Krishna', 'Pusa Barani', and 'Pusa Bold'), 2 each of *B. campestris* L. var 'brown sarson' ('DBS-1' and 'Pusa Kalyani') and var 'yellow sarson' ('DYS-3' and 'YST-151'), and 4 of *B. carinata* A. Braun ('BICRIDA-169', 'BICRIDA-172', 'Carinata-337', and Carinata-435') were studied. Plants of all 11 cultivars were raised in the field from seeds procured from the Division of Genetics, Indian Agricultural Research Institute, New Delhi.

Tolerance of pollen to NaCl

Pollen grains from several anthers (that had been allowed to dehisce in the laboratory under a 40-W incandescent lamp) were cultured in vitro in basal medium of Roberts et al. (1983). Sitting drop cultures (Shivanna and Rangaswamy 1992) were raised; three concentrations of NaCl (4, 6, 8 dS/m[1]) were tested. For each treatment either four or six cultures of each cultivar were raised on different occasions. The cultures were maintained at 20° ± 2 °C under continuous laboratory light for 4 h, at the end of which the cultures were terminated by adding a drop of 1%

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[1] dS/m = m mhos/cm; 1 dS/m NaCl ≃ 0.05% NaCl

acetocarmine. From each culture up to eight microscopic fields were scored for pollen germination and pollen-tube growth. Only those pollen grains with a pollen-tube that measured at least twice as long as the grain diameter were considered to be germinated. The average percentage of pollen germination was calculated on the basis of 2000–3900 pollen grains. For determining the average pollen-tube length, 150–180 pollen-tubes were measured at random from all of the cultures in a treatment. Only those pollen-tubes that were intact at both ends (pollen grain and growing tip) and straight were measured; occasionally a tube with one or two coils was considered. Percentage pollen germination and average pollen-tube length over the control were used as indices of tolerance to NaCl.

Tolerance to salinity at the plant level

Plants were raised from seeds in a randomized block design (RBD) in soils salinized with a five-salt solution of different concentrations (Kumar 1984); the saline solutions were also used to irrigate the plants. The solutions were prepared in tube well water, which also served as the control. Four levels of saline solutions were used for irrigation (Table 1).

Table 1. Solutions used for salinizing the soil and for irrigation

Salinity of solution (dS/m)	Soil salinity[a] (dS/m)	Soil pH[a]
2.6 (control)	2.21	6.70
4.09	3.9	7.31
7.8	8.5	7.46
11.8	10.1	7.63
16.0	14.0	7.76

[a] At a depth of 30 cm at harvest

Soil salinity (i.e., electrical conductivity of the soil saturation extract, ECe) was estimated according to the method of Jackson (1967) by using a Conductivity Bridge (Type CL 01/01 S.V.M. 135) and was monitored through four irrigations given at different intervals up to seed harvest. The concentrations of the salts required in the irrigation solutions were determined on the basis of moisture content and salinity of the soil prevalent 1 day prior to irrigation; 10 days after each irrigation soil salinity and soil pH were re-measured. The RBD comprised 21–24 plants of each cultivar grown in three rows for each treatment; the treatments were replicated 3 times.

Seed yield (average weight of all seeds obtained from 15 to 18 individuals of a cultivar in each treatment) was determined. The seed yield (g) was considered to be an index of the tolerance of whole plants to soil salinity.

Regression analyses between pollen germination (independent variable) and seed yield (dependent variable) at all four levels of soil salinity were considered to infer whether or not a given cultivar is tolerant to salinity at both the pollen grain and whole plant levels.

Results and discussion

Pollen of all 11 cultivars germinated in the control medium; the gemination percentage ranged from 36% to approximately 86%. In culture medium supplemented with 8 dS/m NaCl, pollen grains of only 4 cultivars germinated, whereas in a medium supplemented with 4 dS/m or 6 dS/m NaCl, pollen grains of all 11 cultivars germinated. The maximal pollen germination percentage over the control in 6 dS/m NaCl medium was 67.8% (Table 2). We therefore consider *Brassica* pollen grains to be tolerant to 6 dS/m NaCl. ANOVA showed that the differences in pollen germination among the cultivars ($F = 105.4$, $df = 10$, $P \leq 0.01$) and among the treatments ($F = 804.4$, $df = 3$, $P \leq 0.01$), and the interactions between the cultivars and the treatments ($F = 14.6$, $df = 30$, $P \leq 0.01$) are highly significant.

Our study corroborates work carried out on tomato pollen (Sacher et al. 1983); the germination percentage of pollen from F_1 plants of tomato (grown on control soil) was nearly 50% lower in saline culture medium than in the control medium. Also, pollen from tomato plants grown on saline soil showed a higher germination percentage on saline medium than did the pollen derived from plants grown on control soil (Sacher et al. 1983). Both soil salinity and salinity of the pollen culture medium affect pollen performance. For example, pollen grains from individuals of *Armeria maritima* (Plumbaginaceae) grown in a maritime environment showed greater tolerance to saline water than the pollen grains from individuals grown inland (Eisikowitch and Woodell 1975).

In the treatment with 6 dS/m the average pollen-tube length over that in the control medium ranged from 18.1 μm to 71.6 μm (Table 2). Unlike the data on pollen germination, our data on pollen-tube growth did not show any meaningful trend. Similarly, in studies on tomato pollen response to aluminum treatments, pollen-tube length did not appear to be as sensitive a parameter as pollen germination (Searcy and Mulcahy 1990). Furthermore, in cultures of pollen grains from *Petunia hybrida* plants cultivated under NaCl and $MgCl_2$ treatments, pollen germination decreased in pollen from both sources, whereas pollen-tube growth was enhanced in cultures of pollen grains from $MgCl_2$-treated plants (Reddy and Goss 1971). Likewise, working with pollen cultures of tomato, McLeod (1975) observed that most of the growth substances tested inhibited pollen germination but promoted pollen-tube growth. Obviously, pollen germination and pollen-tube growth, which are distinct phenomena, are affected differently by the same treatment. Pollen-tube growth (length) may not therefore serve as a true index of salt tolerance.

If one considers the pollen germination percentage values we have obtained (Table 2), there arises a question: does as low a value as 2.6% truly reflect pollen tolerance to NaCl? To answer this we sought correspondence with the data on seed yield. ANOVA showed that the differences in seed yield among the cultivars ($F = 14.3$, $df = 10$, $P \leq 0.01$) and among the treatments ($F = 72.6$, $df = 4$, $P \leq 0.01$), and the inter-

Table 2. Seed yield of oilseed brassicas in saline soils, and in vitro pollen germination and pollen-tube growth in NaCl media

Species and cultivar	Seed yield under different levels of soil salinity (dS/m)					NaCl concentration in pollen culture medium (dS/m)[b]					
	2.2 (control)[a]	3.9	8.5	10.1	14.0	0.53 (control)[a]	4.0	6.0	8.0	0.53 (control)[a]	6.0
						Pollen germination percentage[c]				Pollen-tube length (μm)[c,d]	
B. juncea											
Krishna	25.8	99.1	54.6	45.6	38.2	53.9	68.1	14.7	0.0	121.6	31.7
Pusa Barani	20.0	108.5	100.5	64.5	61.5	57.2	65.6	18.2	0.0	113.8	33.5
Pusa Bold	26.7	98.1	57.7	38.6	37.0	36.4	59.4	4.7[e]	0.0	123.3	18.1
B. campestris											
DBS-1	10.5	98.1	65.6	36.1	33.3	56.0	47.2	15.4	4.1[e]	83.5	41.4
Pusa Kalyani	31.8	98.4	48.8	31.3	25.3	68.2	63.4	11.3	1.9[e]	89.3	50.1
DYS-3	24.4	98.3	31.6	12.7	11.9	52.6	38.0	7.4	0.0	68.9	44.0
YST-151	18.7	98.2	24.9	19.1	11.7	72.9	17.6	2.6[e]	0.0	76.6	52.2
B. carinata											
BICRIDA-169	18.4	100.0	95.7	90.8	57.8	85.5	77.0	65.1	0.0	157.6	45.6
BICRIDA-172	16.4	104.3	87.2	83.6	51.2	74.6	51.9	13.9	0.0	83.1	48.1
Carinata-337	20.2	105.0	91.0	87.0	68.5	85.4	80.9	67.8	22.0	205.7	42.1
Carinata-435	16.6	105.1	97.0	91.2	61.0	82.2	83.0	65.0	19.5	98.5	71.6
	$SE_d \pm 3.13$					± 6.53				± 6.53	

[a] Data are absolute values; SE_d (standard error of difference) is given; values under other salinity levels are percentages over the control
[b] dS/m = m mhos/cm; 1 dS/m NaCl ≃ 0.05% NaCl
[c] At the end of 4 h
[d] Data from two other NaCl treatments have been deliberately omitted
[e] As good as no germination

Table 3. Regression coefficients between seed yield (dependent variable) and pollen germination (independent variable) for cultivars of oilseed brassicas

Salinity level (dS/m) for estimating Seed yield[a]	Pollen germination[b]	'b'	R^2
3.9	4	0.095 ± 0.055	0.248
	6	0.065 ± 0.042	0.215
8.5	4	1.071 ± 0.292	0.597
	6	0.744 ± 0.244	0.507
10.1	4	1.156 ± 0.325	0.583
	6	0.934 ± 0.218	0.669
14.0	4	0.834 ± 0.188	0.689
	6	0.581 ± 0.166	0.576

[a] Seed yield as given in Table 2
[b] Pollen germination as given in Table 2

actions between the cultivars and the treatments ($F = 2.9$, $df = 40$, $P \leq 0.01$) are significant.

For many crop species a 50% seed set over the control is an accepted norm to qualify the species as being salt tolerant (Frenkel 1984). On this basis, only 5 of the 11 cultivars we studied were tolerant to 14 dS/m, the highest level of soil salinity tested by us (Table 2). We therefore deem the pollen germination percentage values obtained for these 5 cultivars to be a true reflection of pollen tolerance to NaCl. The cultivars that registered less than 50% seed yield in 14 dS/m were usually poor performers at the level of the pollen grain as well; rarely did these cultivars give a better seed yield even in soils of salinity less than 14 dS/m. The regressions between pollen germination and seed yield at soil salinity levels 8.5, 10.1, and 14.0 dS/m are more significant than those at 3.9 dS/m. Thus, the response of pollen grains of the 11 cultivars of oilseed brassicas to salinity and the ability of the same cultivars to produce seeds under salinized soil conditions show a close parallel.

Salt tolerance in *B. juncea* cvs 'Pusa Barani' and 'Pusa Bold' has been assessed using seed germination (Sarla and Chandrashekharan 1990) and seedling growth (Chandrashekharan and Sarla 1990) as parameters. 'Pusa Barani' was found to be more tolerant than 'Pusa Bold'. Our results on these cultivars confirm the earlier findings.

At the whole plant level allopolyploids are reported to tolerate generally higher levels of soil salinity than diploids (Sayed 1985; Rana 1986). *B. juncea* and *B. carinata* are both allopolyploids (Prakash and Chopra 1991). Ashraf and McNeilly (1990) reported that when grown in NaCl-treated sand *B. carinata* plants gave significantly higher seed yield than *B. juncea* and *B. campestris*. Our data on seed yield also clearly show that all 7 cultivars of the two allopolyploid species

(*B. juncea* and *B. carinata*) are more tolerant to all of the 4 levels of soil salinity tested; in fact 5 of these cultivars were found to be the highest seed yielders at 14 dS/m soil salinity (Table 2). On a seed yield basis cv 'Pusa Bold' of *B. juncea* has been reported to be non-tolerant to soil salinity (Kumar 1984); our data on seed yield as well as pollen germination vindicate Kumar's report. The absolute value for pollen germination in the control medium is, in fact, the lowest for cv 'Pusa Bold'. Relatively, therefore, the percentage pollen germination in 6 dS/m is lower for this cv 'Pusa Bold' (although an allopolyploid) than what it is for most of the diploid cultivars; a pollen germination of 4.7% of the control being indeed as good as no germination. Thus, salt tolerance seems to be a genotype-dependent phenomenon. Analyses of our data on 11 other agrobotanical traits such as the number of branches/plant, 1000 seed weight, oil content, and protein content in oil cake (not discussed here) have generally indicated a close correlation between pollen response to NaCl and most of the characters studied.

Briefly then, our studies show a close correspondence between pollen and whole plants of the same cultivars in their responses to salinity. Such an observation points to the great advantage which the screening of pollen grains, instead of whole plants, could offer in species for which yield data on saline soils are unknown or less known.

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