

SCREENING FOR FROST TOLERANCE IN INDIAN MUSTARD  
/BRASSICA JUNCEA/L/ COSS/

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Abstract

Forty three genotypes of Indian mustard, consisting of six recommended varieties, 36 advanced generation progenies of the cross B.junceae cv. RLM 198 x B.napus cv. Oro and a frost tolerant variety RW 175 as a check, were screened.

Initial screening based on an extent of stem bending was done in the morning after a frosty night. Genotype with maximum stem bending was assigned a score of 1 and those with no bending a score of 4. Based on this, genotypes were grouped in four categories i.e. susceptible, moderately resistant, resistant and highly resistant. Two representative genotypes of each group plus the check RW 175 were assessed for damage to reproductive organs. The siliqua at intermediate stage of development were most susceptible followed by immature stage.

Entries, FS Sel 3 and IS 99 were most resistant. Most apparent damage was the bud abortion, after repeated frost exposure female fertility was adversely affected when frost exposure occurred 2-4 days prior to anthesis. The estimate of frost injury to plant tissue by electrical conductivity method indicated FS sel. 7 and FS Sel.3 had lowest electrolyte leakage while IS 10 and RLM 619 the maximum. Two varieties IS 99 and FS Sel 3 had maximum yield stability ratio /85 per cent/ as against minimum ratio recorded in a most susceptible genotype IS 10. Rank correlations between electrical conductivity of leaf tissue, visual score and aborted seeds were significant. Yield stability index was highly correlated with visual

score.

### Introduction

Ground frost is one of the most common environmental factor limiting mustard productivity in India. Frost is most damaging when it occurs at pod filling stage. Up to 70 per cent of yield losses have been reported from a single exposure at this stage /Yadava and Bhola, 1977/. In view of severe economic losses it has become necessary to breed for frost resistant/tolerant varieties for frost prone areas. Many physiological and biochemical procedures have been developed for screening for frost resistance. These include seedling growth /Dhillon and Larsson, 1985/, yield losses /Dhawan et.al., 1983/, electrolyte leakage /Acikgoz, 1982/ and many other colorimetric methods /Burke et.al., 1976/. In this communication we present the results of a frost screening trial based on both field and laboratory techniques.

### Material and method

In all, 43 genotypes of Indian mustard /Brassica juncea /L./ Coss,  $2n=36$ / were screened of these, six were recommended varieties of the area, 33 were advanced generation juncea progenies / $2n=36$ / of B.juncea cv. RLM 198 x B.napus cv. ORC cross 3 were C-genome chromosome substitution lines and one was frost tolerant variety RW 175 which was used as a check. The material was sown in a randomized complete block design with three replications. Each replication consisted of three lines spaced 30 cm apart with a plant to plant distance of 15 cm. Initial visual scoring was done in the morning after the frosty night. It consisted of arbitrary scores assigned depending upon the degree of stem bending /e.g. 1=maximum bending; 4 = no bending/. Based on the visual scoring the genotypes were divided into four groups: namely highly susceptible, moderately susceptible, resistant and highly resistant. From each group, two genotypes plus the check RW 175 were assessed for damage to the reproductive organs. Percentage of aborted seeds per genotype was used to estimate the damage to

reproductive organs. For this hundred pods per replication in each selected genotypes were sampled at three pod developing stages i.e. immature pod stage, intermediate stage, and final stage. Effect on female fertility was estimated on the basis of seed setting obtained after frost attack. Buds of three different stages were tagged one day after the frost attack. These buds were hand pollinated and number of seeds present in these pods at maturity was compared to ovule number. Seed setting obtained in frost free buds was used as control.

### Results and discussion

A correct estimate of abiotic stress is invariably obscured by a number of factors influencing organisms response to external stimuli. These include plant's growth stage, its nutritional status and disease-pest syndrome. The genotype may respond differentially to frost, whose impact, however, is most critical during reproductive phase. Any damage during this phase will be definitely reflected in the reduced yield level. Results of various experiments related to frost tolerance are presented below:

#### Stem bending

One of the most apparent visual symptom observed in Indian mustard after a frosty night is stem bending resulting from a temporary loss of turgor in the stem and leaves. This bending is more conspicuous at flower initiation stage and is attributed to the extracellular ice formation and frost plasmolysis /Levitt, 1972/. In the present study, the cultivars differed for this external symptom while variety x year interaction was non-significant. Six entries out of the total viz. F.S. 3, F.S. Sel. 6, IS 98, IS 86, IS 13 and IS 36 were found to be almost resistant to stem bending. Nine were tolerant, seven were moderately susceptible and remaining 21 were highly susceptible. Previously recorded frost tolerant variety RW 175 /Ohlsson 1983/ figured in susceptible group. Local commercial varieties RLM 619 and Kranti were observed to be highly

susceptible. Frost damage was more severe during 1985-86, season with 15 frosty nights as compared to 1984-85 season with eight frosty nights.

#### Damage to reproductive structures

From an agronomist's point of view, the active seed filling period is the most critical frost sensitive stage as frost at this junction will invariably result in yield losses. In the present study, siliquae at intermediate stage of development were most susceptible followed by immature stage /Table 1 and 2/. The differences, however, were not very much evident at final stage of siliqua development. Similar observation has been reported by Dhawan /1985/. The entries FS Sel-3 and IS-59 had least number of aborted seeds at intermediate stage. The entries Kranti and IS-26 which appeared highly susceptible at immature and intermediate stages were statistically at par with FS-Sel.3 and IS-99 at maturity. This could be due to greater pod shell thickness in Kranti and IS-26 at maturity.

#### Effect on female fertility

Visually one can assess the damage to female fertility by mortality of immature buds after repeated frost exposure. Such buds first show reduced growth, then turn yellow, and finally abscise. Such a damage cannot be used as a true estimate as bud mortality is irregular and random even amongst buds of same developmental stage. The crucifers in general are also much prone to bud abscission /Banga, 1986 unpublished/. This damage is maximum when frost exposure coincides with flowering initiation than at late flowering stages. The female fertility as studied in the present study was significantly reduced when frost exposure occurred 2 or 4 days prior to bud opening but was at par to control at 1 day prior to flower opening.

#### Electrical conductivity method

Assessment of the damage to the reproductive structure, besides being labour intensive, is time specific and is localised in the apparently more exposed parts of the plant

canopy. The estimate of frost injury to plant tissue by electrical conductivity method may provide a rapid screening technique if it is correlated with the field observations. At cellular level, the frost injury results in increased permeability of the electrolyte, especially  $K^+$  in the cell sap. The conductivity value of tissue or tissue extract is a direct measure of frost injury /Levitt,1972/.

The data for electrical conductivity for stem and leaf portion are presented in Table 4. FS-Sel-7 and FS-Sel-3 had lowest electrolyte leakage while IS-10 and RLM 619 had the maximum for stem and leaf tissue respectively.

#### Yield stability index

Stability index was estimated to determine the relationship between the yield and relative severity of natural frost over 1984-85 and 1985-86 seasons. Of the various entries evaluated, IS 99 and FS-Sel-3, both frost tolerant varieties had maximum yield stability ratio i.e. 85 per cent as against minimum of 59 per cent in susceptible genotype IS-10. Highest yielding Kranti and RLM 619 had stability ratios of 72 and 71 per cent respectively. Though every effort was taken to keep similar cultural treatments during both years the yield stability might not be a true reflection of frost damage as yield is a complex response of environmental conditions, of which frost is but one component.

#### Correlation studies

Rank correlation /Table 6/ between electrical conductivity, visual score and aborted seeds was significant when conductance of leaf tissue was considered, on the other hand yield stability ratio was correlated with conductance of the stem tissue. Per cent aborted seeds were correlated with both visual score and yield stability index. Yield stability was highly correlated /.80/ with the visual score. This study demonstrated that the initial screening of a large germplasm collection could be undertaken by the visual assessment of loss in turgor of upper parts of plant canopy and electrical conductance. The final selec-

tion may, however, be based on damage to reproductive structures as reflected in final yield loss.

### References

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Table 1. Effect of frost on reproductive phase

Variety	Stages of silique development					
	Immature (25 per cent growth)		Intermediate (50 per cent growth)		Mature (75 per cent growth)	
	N	A	N	A	N	A
FS Sel 3	11.2±0.34	1.6±0.21	10.7±0.42	2.1±0.33	11.9±0.62	1.3±0.20
IS 99	10.9±0.57	1.5±0.29	9.1±0.41	2.2±0.25	11.8±0.39	1.6±0.33
FS Sel 7	8.3±0.28	3.5±0.32	7.0±0.51	3.7±0.51	11.0±0.34	1.7±0.20
IS 26	9.6±0.77	2.5±0.32	8.1±0.52	3.8±0.44	10.7±0.74	1.5±0.25
RW 175	9.7±0.64	2.4±0.43	8.8±0.65	3.2±0.18	10.3±0.42	2.1±0.40
RLM 514	12.8±0.51	1.8±0.31	10.1±0.36	3.6±0.29	10.6±0.29	1.9±0.39
IS 10	9.3±0.67	2.5±0.38	7.4±0.70	3.8±0.31	9.7±0.45	2.1±0.23
Krant1	11.0±0.42	2.7±0.20	9.3±0.29	4.4±0.21	12.8±0.33	1.9±0.29
RLM 619	10.3±0.62	2.2±0.31	8.1±0.48	4.3±0.68	10.8±0.37	2.1±0.32

N = Normal; A = Aborted

Table 2. Per cent aborted seeds at various stages of silique development

Variety*	Stage of silique development		
	Immature (25% growth)	Intermediate (50% growth)	Before maturity (75% growth)
FS-Sel-3	12.15 <sup>a</sup>	16.4 <sup>a</sup>	9.85 <sup>a</sup>
IS-99	12.09 <sup>a</sup>	10.46 <sup>ab</sup>	11.94 <sup>ab</sup>
FS-Sel-6	29.66 <sup>c</sup>	34.57 <sup>c</sup>	13.38 <sup>b</sup>
IS-26	20.66 <sup>b</sup>	31.93 <sup>c</sup>	12.29 <sup>ab</sup>
RLM-514	12.32 <sup>a</sup>	26.27 <sup>bc</sup>	15.20 <sup>bc</sup>
IS-10	21.18 <sup>b</sup>	33.92 <sup>c</sup>	17.8 <sup>c</sup>
Kranti	19.70 <sup>b</sup>	32.11 <sup>c</sup>	12.92 <sup>ab</sup>
RLM 619	17.60 <sup>b</sup>	34.67 <sup>c</sup>	16.27 <sup>bc</sup>
RW-175(check)	19.83 <sup>b</sup>	26.67 <sup>bc</sup>	16.93 <sup>c</sup>

Table 3. Effect of frost on female fertility

Variety	Days prior to flower opening			Control
	4	2	1	
FS-Sel-3	83.5	82.0	84.3	85.8
IS-99	88.7	87.7	89.5	91.3
FS-Sel-6	84.2	88.6	87.7	87.5
IS-26	88.0	86.5	87.8	89.4
RLM-514	76.5	76.0	78.9	80.3
IS-10	83.0	80.1	79.5	84.3
Kranti	83.6	82.1	89.4	86.7
RLM 619	83.1	85.5	84.2	83.8
RW-175	84.1	84.5	86.2	85.5
	*	*	ns	

\*Significantly different from control



Table 4. Biochemical analysis for frost tolerance in Indian mustard

Variety	Visual rating	Electrical conductivity (Mm/hos)		Per cent aborted seeds
		Stem	Leaf	
FS Sel 3	3.7	84	52	12.8
IS 99	4.0	82	100	14.5
FS Sel 7	3.4	69	95	25.8
IS 26	2.9	80	96	21.6
RW 175	1.9	105	86	21.1
RLM 514	1.8	112	65	17.9
IS 10	1.6	118	140	24.3
Kranti	1.1	108	115	21.5
RLM 619	1.0	95	165	22.8
C.D.	0.88	55	6.71	-

Table 5. Yield and stability ratio of some mustard strains over two years

Variety	Yield (gm)		Stability ratio (%)
	1984-85	1985-86	
FS Sel. 3	130.2	110.8	85
IS 99	102.1	86.7	85
FS Sel 7	149.2	116.5	78
IS 26	158.4	120.4	76
RW 175	113.2	74.5	65
RLM 514	176.5	135.2	76
IS 10	118.0	70.4	59
Kranti	211.0	152.1	72
RLM 619	230.5	163.0	71
C.D. (5%)	-	-	11

Table 6. Rank correlations amongst electrolyte leakage with field traits

Variant	Visual frost index	Aborted seeds	Yield stability index
Electrolyte leakage			
(a) Stem	0.52	0.02	0.63*
(b) Leaf	0.57*	0.60*	0.48
Aborted seeds	0.58*	-	0.51

\* Significant at 5 per cent level of significance