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Original article

Investigation of frost stress on yield, yield components and a number of biochemical traits in rapeseed genotypes

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Extended abstract

Introduction

Rapeseed is one of the most important oilseeds in the world. Rapeseed with the scientific name of Brassica napus L. is ranked third in the world in terms of grain production after soybean and in terms of oil production after soybean and oil palm. Plants are always exposed to a wide range of environmental stresses, both biological and non-biological, that strongly affect their growth and production. Cold stress and frost are one of the environmental factors that affect crop production and other important agronomic traits in many plant species. Frost in the early stages of germination causes the destruction of the whole plant. The amount of frost damage depends on many important factors such as the duration and severity of frost stress, different stages of plant growth period and the amount of air humidity. Rapeseed is sensitive to frost stress and shows a yield reduction of up to 70%.

Identifying cultivars that tolerate low temperatures can help researchers improve new cultivars and increase the flexibility of choosing the right planting date for farmers. Various studies have been performed to evaluate canola genotypes against cold stress in terms of morphological or physiological traits, but few studies have been conducted to study the combined traits. This study was designed to identify frost stress resistant genotypes, evaluate the effects of frost stress on yield and yield components as well as physiological traits to identify and modify the traits affected by the stress.

Materials and methods

In this experiment, 24 autumn Rapeseed genotypes were obtained from the oilseeds section of Karaj Seed and Plant Breeding Research Institute. Selected genotypes included a number of promising lines and free pollen and rapeseed hybrid cultivars, including: SLM046, Opera, L963, Okapi, L62, Nima, KH4, Talayeh, L957, Ahmadi, KR18, L1009, Zarfam, Nafis, HW101, Licord, KS7, L14, SW101, L1008, L83, L120, Natali and Hydromel. Experiment with two different planting dates as normal sowing on 10/6/1397 and delayed sowing on 6/25/1397 in the form of split plot design based on randomized complete blocks with three replications in 1397-98 crop year It was implemented in the agricultural lands of Tikmeh Dash city of East Azerbaijan province. Morphological traits including number of pods per plant, number of seeds per pod, 1000-seed weight, yield per hectare and physiological traits including leaf chlorophill, proline, malondialdehyde, soluble sugars, electrolyte leakage percentage and superoxide dismutase were measured.

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Results and discussion

The effects of frost stress were significant for three traits: number of pods per plant, 1000-seed weight and yield at 1% probability level, and this indicates that delay in planting all cultivars tested caused a decrease in yield and its components. The mean squares of genotypes were significant for two traits: number of seeds per pod and yield at 1% probability level. Mean stress squares were significant in terms of chlorophill b, proline, soluble sugars, electrolyte leakage, malondialdehyde and superoxide dismutase at the level of 1% probability. There was also a significant difference between the genotypes in terms of the above traits as well as chlorophill a and total chlorophill at the level of one percent probability. The results of comparing the mean of genotypes together for stress-free and frost stress conditions showed that KS7 line with 1699.9 kg ha^{-1} had the highest average yield and the lowest was related to L62 line with 107 kg ha^{-1}. According to this study, the decrease in yield is due to the decrease in the number of pods per plant as components of grain yield. Both under stress and non-stress conditions, the total yield showed a positive and significant correlation with the number of pods per plant and the number of seeds per pod. In non-stress conditions, no correlation was observed between yield and physiological traits, while in frost stress conditions, there was a positive and significant correlation between yield with proline, soluble sugars, malondialdehyde and superoxide dismutase, so this can be Introduced traits as positive traits for selecting frost-resistant cultivars. Based on the results of factor analysis, 4 factors were identified for each of the conditions without stress and frost stress, which in the non-stress state was 77.52% of the total variance and in the frost stress state was 81.06% of the total variance.

Conclusions

According to the results of the correlation between the studied traits, it was found that in non-stress conditions, the number of pods per plant and the number of seeds per pod can be used to improve grain yield. In terms of frost stress, in addition to the number of pods per plant and the number of seeds per pod, the amount of proline, soluble sugars, malondialdehyde and superoxide dismutase had the greatest contribution in justifying the diversity of studied genotypes. Due to the low heritability of the yield, it is usually difficult to improve. As a result, other aspects of frost stress resistance, such as physiological parameters, should be considered. In general, physiological traits provide more complete information and are a good alternative to functional traits.

***Keywords*:** Analysis of variance, Correlation, Factor analysis, Morphological and physiological traits

Table 1. Charaectristics of cultivars and promising lines in canola

No.	Genotypes	Genotype type	No.	Genotypes	Genotype type
1	SLM046	Open Pollinated	**13**	Zarfam	Open Pollinated
2	Opera	Open Pollinated	**14**	Nafis	Open Pollinated
3	L963	Inbred Line	**15**	HW101	Inbred Line
4	Okapi	Open Pollinated	**16**	Licord	Open Pollinated
5	L62	Inbred Line	**17**	KS7	Inbred Line
6	Nima	Open Pollinated	**18**	L14	Inbred Line
7	KH4	Inbred Line	**19**	SW101	Inbred Line
8	Talayeh	Open Pollinated	**20**	L1008	Inbred Line
9	L957	Inbred Line	**21**	L83	Inbred Line
10	Ahmadi	Open Pollinated	**22**	L120	Inbred Line
11	KR18	Inbred Line	**23**	Natali	Hybrid
12	L1009	Inbred Line	**24**	Hydromel	Hybrid

Table 2. Monthly mean values of precipitation, minimum and maximum temprature in Bostanabad station in 2018-2019

Month	Min temp (^{0}C)	Max temp (^{0}C)	Precipitation (mm)
Sep.	10.4	28.4	1
Oct.	16.7	20.8	0.6
Nov.	1.1	11.3	0.6
Dec.	-0.1	7.4	1.3
Jan.	-5.5	2.8	1.4
Feb.	-6.3	3.8	2.3
Mar.	2.3	6	2.5
Apr.	1.9	11.1	2.5
May.	3.9	17.9	1
Jun.	10.3	27	0.9
Jul	13.2	31.2	0

Table 3. Analysis of variance of yield and yield components of the canola genotypes in non-stress and frost stress conditions

S.O.V	d.f	Mean of squares			
		NPP	NSP	TSW	Yield
Replication	2	13006.9 ^{ns}	62.01**	4.69 $^{n.s}$	7602025*
Stress (S)	1	16325.38 $^{n.s}$	3.66^{ns}	11.49 $^{n.s}$	7591026 $^{n.s}$
Error a	2	1538.81	2.12	1.32	451635.74
Genotype (G)	23	840.27*	13.85**	0.87*	980975.9**
G * S	23	381.08$^{n.s}$	3.15$^{n.s}$	0.65$^{n.s}$	344842.8**
Error b	92	500.88	6.55	0.45	102334.94
CV (%)	-	*27.27*	*11.08*	*20.26*	*41.16*

NPP: Number of pods per plant; NSP: Number seed per pod; TSW: Thousand seed weight
*, ** and ^{ns} significant 5% and 1% probability and non-significant respectively

Table 4. Analysis of variance of physiological and biochemical traits of the canola genotypes in non-stress and frost stress conditions

S.O.V	d.f	Chl. a	Chl. b	Chl. t	Pro.
Replication	2	9.4**	0.31$^{n.s}$	13.11**	1.6$^{n.s}$
Stress	1	0.08$^{n.s}$	2.7$^{n.s}$	3.67$^{n.s}$	462.46**
Error a	2	0.23	0.52	0.24	0.04
Genotype	23	10.76**	2.31**	20.38**	88.27**
Genotype * stress	23	13.86**	2.49**	22.80**	59.2**
Error b	92	0.9	0.31	1.04	2.98
CV (%)	-	*10.61*	*10.25*	*7.1*	*7.8*

Table 4. Continued

S.O.V	d.f	SS	EL	MDA	SOD
Replication	2	6.37$^{n.s}$	6.72$^{n.s}$	3.21$^{n.s}$	0.14$^{n.s}$
Stress	1	899.05**	1005.78**	558.53**	30.17**
Error a	2	13.15	2.09	4.37	0.33
Genotype	23	176.78**	1272**	106.47**	7.04**
Genotype * stress	23	116.82**	1188.72**	71.79**	4.38**
Error b	92	2.86	10.08	2.62	0.43
CV (%)	-	*7.6*	*5.04*	*12.09*	*14.77*

Chla: Chlorophill a; Chlb: Chlorophill b; Chlt: Chlorophill total; Pro: Proline; SS: Soluble Sugars; EL: Electrolyte Leakage; MDA: Malondialdehyde; SOD: Superoxide dismutase
** and ^{ns} significant 1% probability and non-significant respectively

Table 5. Mean comparison of traits by LSD method in 24 rapeseed genotypes

Genotypes	NPP	NSP	TSW (g)	Yield
SLM046	71.23^{a-c}	23.97^{a-d}	3.49^{a-e}	687.9^{e-k}
Opera	93.57^{a}	21.95^{b-f}	3.94^{a}	1383^{a-c}
L963	78.70^{a-c}	25.17^{a}	3.25^{a-e}	835.3^{d-j}
Okapi	8.23^{a-c}	25.5^{a}	3.1^{b-e}	668.4^{e-k}
L62	58.72^{c}	22.63^{a-f}	3.22^{a-e}	107^{l}
Nima	75.23^{a-c}	23.83^{a-d}	2.75^{e}	1240.4^{a-d}
KH4	80.6^{a-c}	25.06^{a-c}	3.48^{a-e}	504.3^{g-l}
Talaye	89^{ab}	23.12^{a-e}	3.48^{a-e}	948.1^{b-h}
L957	90.93^{a}	25.13^{a}	3.28^{a-e}	1439.8^{ab}
Ahmadi	81.23^{a-c}	25.08^{a-c}	3.04^{b-e}	556.7^{f-l}
KR18	86.83^{ab}	19.93^{f}	3.03^{b-e}	375.7^{j-l}
L1009	87.6^{ab}	25.04^{a}	3^{b-e}	902.8c-i
Zarfam	61.18^{bc}	25.59^{ab}	3.3^{a-e}	784.1^{d-j}
Nafis	76.83^{a-c}	22.70^{a-f}	3.88^{a}	962.4^{b-g}
HW101	73.4^{a-c}	23.16^{a-d}	3.96^{a}	426.1^{i-l}
Licord	80.67^{a-c}	21.93^{b-f}	2.93^{c-e}	1068.7^{b-e}
KS7	87.47^{ab}	23.06^{a-e}	3.19^{b-e}	1699.9^{a}
L14	97.1^{a}	22.99^{a-e}	3.26^{a-e}	532.2^{f-l}
SW101	89.27^{ab}	23.22^{a-d}	3.72^{ab}	451.1^{h-l}
L1008	96.23^{a}	22.63^{a-f}	3.56^{a-d}	1011.9^{b-f}
L83	96.47^{a}	22.94^{a-e}	3.28^{a-e}	227.9^{kl}
L120	54.26^{c}	20.25^{ef}	3.29^{a-e}	264.8^{kl}
Natatli	87.2^{ab}	21.26^{c-f}	3.64^{a-c}	617.4^{e-k}
Hydromel	93.47^{a}	21.06^{d-f}	2.83^{de}	958.4^{b-g}

NPP: Number of pods per plant; NSP: Number seed per pod; TSW: Thousand seed weight
Means followed by similar letters in each column are not significant at %5 probability level

Table 6. Mean comparison for interaction effect of stress × genotype on yield and some of physiologic traits for 24 canola cultivars under non stress and frost stress using

	Yield		Chla		Chlb		Chlt		Pro	
Genotypes	**Normal**	**Stress**	**Normal**	**Stress**	**Normal**	**Stress**	**Normal**	**Stress**	**Normal**	**Stress**
	------ kg ha^{-1} ------		---------- mg ----------						------ µmol ------	
SLM046	854.9	520.7	9.56	5.18	5.02	4.28	14.58	9.46	15.72	26.94
Opera	1521.4	1244	10.26	5.67	5.49	2.75	15.75	8.42	17.87	28.66
L963	888.8	781.7	11.44	8.82	6.65	6.09	18.09	14.91	25.09	27.30
Okapi	705.4	631.3	10.64	7.43	6.57	5.78	17.21	13.21	18.67	27.19
L62	144.7	69.23	8.7	8.24	4.71	4.67	13.41	12.91	14.81	17.49
Nima	2000.3	480.5	10.83	9.77	5.31	6.17	16.14	15.94	15.47	21.40
KH4	791.1	217.4	8.91	8.27	4.62	4.80	13.53	13.07	20.21	21.06
Talaye	1206.8	689.3	9.7	9.34	5.40	5.05	15.10	14.39	15.47	27.44
L957	2043.7	835.7	10.57	10.33	5.91	5.47	16.24	16.04	19.43	27.36
Ahmadi	811.7	301.6	10.63	8.14	5.94	5.66	16.57	13.8	14.28	23.20
KR18	543.7	207.7	9.75	8.22	5.99	5.63	15.74	13.85	16.43	20.23
L1009	1353.6	451.9	9.61	8.09	6.35	5.34	15.96	13.43	16.69	20.43
Zarfam	1033.2	534.9	8.23	6.47	4.47	5.31	12.70	11.78	14.63	17.35
Nafis	1392.5	532.2	9.56	6.21	5.73	4.48	15.29	11.10	15.13	21.77
HW101	593.5	258.8	10.36	8.66	5.90	5.66	16.26	14.32	14.74	19.67
Licord	1442.2	695.2	9.98	6.87	5.13	4.19	15.11	11.06	19.17	35.77
KS7	2450.2	949.6	10.55	10.13	6.02	5.46	16.57	15.59	20.40	26.35
L14	647.1	417.4	11.82	10.35	6.45	4.84	18.27	15.19	18.05	30.73
SW101	568.7	333.4	10.50	10.28	5.84	5.63	16.34	15.91	25.98	26.10
L1008	1330.5	693.4	11.30	6.93	6.81	5.75	18.11	12.68	19.13	28.88
L83	258.5	197.2	8.57	6.21	5.33	3.62	13.9	9.83	25.80	26.52
L120	457.8	71.81	9.03	6.33	5.74	4.75	14.77	11.08	16.94	19.94
Natatli	649.8	584.8	10.24	5.32	4.23	3.52	14.47	8.84	26.69	30.85
Hydromel	991.7	925.0	12.01	10.43	7.21	5.17	19.22	15.6	24.46	26.14
LSD 0.05	*711*		*1.537*		*0.903*		*1.654*		*2.799*	

Table 6. Continued

Genotypes	SS		EL		MDA		SOD	
	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
	------- mg/g -------		--------- % ---------		---------- µmol/g ----------			
SLM046	13.43	29.18	26.73	69.55	6.51	19.95	2.74	5.99
Opera	17.14	30.67	29.46	82.39	9.38	19.88	3.85	6.32
L963	26.55	29.42	63.47	73.78	16.68	18.90	5.22	5.92
Okapi	17.54	29.54	51.29	84.20	9.70	19.00	3.75	6.13
L62	12.24	15.76	44.22	51.13	5.46	8.31	2.33	3.64
Nima	12.90	21.31	34.56	40.67	6.09	12.61	2.89	4.11
KH4	19.52	20.83	84.36	86.08	11.29	12.24	3.77	4.06
Talaye	25.18	30.07	41.20	80.16	15.62	19.41	5.05	6.08
L957	18.56	29.77	49.59	93.38	10.54	19.18	3.95	6.11
Ahmadi	11.36	23.87	88.29	90.96	4.82	14.60	1.87	4.33
KR18	14.27	19.66	69.09	91.64	7.16	11.37	3.11	4.02
L1009	14.63	19.94	38.72	52.16	7.43	11.55	3.15	4.13
Zarfam	11.70	15.51	61.58	82.87	5.17	8.13	2.31	3.47
Nafis	12.21	21.84	56.99	73.55	5.58	13.03	2.49	4.19
HW101	12.07	18.86	59.03	87.36	5.40	10.72	2.36	3.18
Licord	18.11	41.70	35.52	43.83	10.16	28.53	3.58	7.52
KS7	19.75	28.34	32.68	69.79	11.40	18.07	4.08	5.98
L14	16.51	34.55	61.07	74.36	8.91	22.88	3.39	7.02
SW101	27.81	27.99	28.85	51.34	17.66	17.68	5.11	5.52
L1008	18.07	31.93	39.80	74.41	10.12	20.85	3.69	6.26
L83	27.63	28.58	74.39	76.54	17.52	18.26	5.39	5.55
L120	14.98	19.29	45.27	94.21	7.71	11.04	3.11	3.68
Natatli	28.86	34.73	69.83	77.08	18.44	23.02	5.72	7.33
Hydromel	25.96	27.99	47.07	86.40	16.22	17.79	4.96	5.77
LSD 0.05	*2.742*		*5.149*		*2.623*		*1.068*	

Chla: Chlorophill a; Chlb: Chlorophill b; Chlt: Chlorophill total; Pro: Proline; SS: Soluble Sugars; EL: Electrolyte Leakage; MDA: Malondialdehyde; SOD: Superoxide dismutase

Table 7. Correlation coefficients between physiological and morphological traits under the non-stress (Down) and frost stress (Top) conditions

Traits	Chla	Chlb	Chlt	Pro	SS	EL
	----------mg----------			µmol	mg	%
Chla		0.543**	0.960**	0.049	0.060	-0.233*
Chlb	0.439**		0.757**	-0.202	-0.194	-0.250*
Chlt	0.937**	0.725**		-0.029	-0.018	-0.265*
Pro	0.209	0.264*	0.263*		0.937**	-0.021
SS	0.203	0.280*	0.264*	0.950**		-0.018
EL	0.447**	0.198	0.420**	0.085	0.078	
MDA	0.236*	0.285*	0.291*	0.950**	0.942**	0.078
SOD	0.115	0.197	0.165	0.909**	0.858**	0.069
NPP	0.059	0.131	0.096	0.061	0.093	0.159
NSP	0.001	0.054	0.022	-0.168	-0.138	-0.011
TSW	-0.258*	-0.292*	-0.311**	-0.099	-0.129	-0.042
Yield	0.088	0.020	0.075	-0.073	-0.029	-0.056

Table 7. Continued

Traits	MDA	SOD	NPP	NSP	TSW	Yield
	---------- µmol ----------				g	kg/ha
Chla	0.021	0.027	0.122	0.180	-0.022	0.229
Chlb	-0.110	-0.140	-0.026	-0.087	-0.039	0.076
Chlt	-0.020	-0.026	0.086	0.111	-0.030	0.204
Pro	0.933**	0.901**	0.325**	0.175	0.130	0.295*
SS	0.944**	0.889**	0.370**	0.170	0.082	0.342**
EL	-0.038	-0.017	0.063	0.036	0.067	-0.058
MDA		0.905**	0.369**	0.109	0.109	0.269*
SOD	0.908**		0.293*	0.148	0.117	0.258*
NPP	0.067	0.054		0.361**	0.156	0.601**
NSP	-0.177	-0.147	0.226		0.097	0.324**
TSW	-0.097	-0.118	-0.067	-0.079		0.005
Yield	0.025	-0.009	0.502**	0.325**	0.082	

Chla: Chlorophill a; Chlb: Chlorophill b; Chlt: Chlorophill total; Pro: Proline; SS: Soluble Sugars; EL: Electrolyte Leakage; MDA: Malondialdehyde; SOD: Superoxide dismutase; NPP: Number of pods per plant; NSP: Number seed per pod; TSW: Thousand seed weight; Yield

*, ** significant 5% and 1% probability respectively

Table 8. Rotated factor coefficients, the ratio of variance by each factor, the cumulative proportion of variance in 24 rapeseed genotypes under the non–stress and frost stress conditions

	Factor Coefficients of Non-frost stress				Factor Coefficients of Frost Stress			
Traits	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Chla	0.051	**0.779**	0.453	0.090	**0.774**	0.092	0.155	0.063
Chlb	0.193	**0.788**	0.054	0.058	0.139	0.140	0.049	**0.880**
Chlt	0.112	**0.890**	0.363	0.091	0.198	0.063	**0.660**	-0.065
Pro.	**0.977**	0.180	0.070	-0.027	**0.597**	0.396	-0.122	-0.191
SS	**0.981**	0.159	0.067	-0.030	0.069	**0.928**	-0.065	0.201
EL	-0.031	0.230	**0.754**	0.058	-0.143	**0.922**	-0.052	-0.111
MDA	**0.982**	0.157	0.067	-0.027	0.009	**0.977**	-0.064	0.119
SOD	**0.987**	0.080	0.070	0.017	**0.974**	-0.075	0.010	0.123
NPP	0.148	0.087	0.402	**0.635**	-0.063	-0.293	**0.745**	0.118
NSP	-0.268	0.171	-0.304	**0.619**	**0.969**	-0.081	0.007	0.130
TSW	-0.143	**-0.692**	0.065	0.118	0.974	-0.041	0.007	0.108
Yield	0.015	-0.126	0.037	**0.832**	-0.233	0.001	0.587	**-0.594**
The ratio of variance	31.15	20.79	13.89	11.68	37.52	22.77	10.67	10.09
The cumulative variance	31.15	51.94	65.83	77.52	37.52	60.29	70.97	81.06

Chla: Chlorophill a; Chlb: Chlorophill b; Chlt: Chlorophill total; Pro: Proline; SS: Soluble Sugars; EL: Electrolyte Leakage; MDA: Malondialdehyde; SOD: Superoxide dismutase; NPP: Number of pods per plant; NSP: Number seed per pod; TSW: Thousand seed weight; Yield