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Environmental effects on oil, fatty acid profile, protein and glucosinolate content in Indian mustard (*Brassica juncea*)

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ABSTRACT

Environmental influence over oil, fatty acid profile, protein, glucosinolates, oil stability index, monounsaturated fatty acids to polyunsaturated fatty acid, polyunsaturated fatty acid to saturated fatty acids and ω-6 to ω-3 fatty acid ratio was investigated by growing 25 Indian mustard *(Brassica juncea* L. Czern & coss) varieties during winter season (*rabi*) (October–April) 2003–06. The expression of all the parameters studied was significantly affected by genotypes, environment and genotype × environment interactions. Oil and protein content were relatively consistent over the seasons as indicated by low coefficients of variability (1.7–4.8%). Similarly, glucosinolates (CV 7.0–7.5%) and erucic acid (CV 4.0–8.0%) content were less affected by the cropping seasons. The oil stability index, ω-6 to ω-3 fatty acid and polyunsaturated fatty acid to saturated fatty acid ratio were more prone to environmental conditions than the fatty acids *per se.* Of all the nutritional and shelf-life indices of oil, monounsaturated fatty acid to polyunsaturated fatty acid ratio had the least variability across the years. The pattern of correlations was also affected by the prevailing environmental conditions during the cropping season. The relationship of maximum temperature up to 42 days after flowering and oil content was negative and significant. The negative correlation coefficients of erucic acid with mean temperature were significant only during (2004–05 and 2005–06) and 43–56 days after flowering in 2004–05 cropping season. In the 2005–06 cropping season, mean relative humidity during 15–28 days after flowering had negative and significant relationship with linoleic acid but significantly positive with linolenic acid.

Keywords: *Brassica juncea* oil, Environmental factors, Glucosinolate content, Oil stability index, ω-6 / ω-3 fatty acid ratio, Protein

Fatty acid profile and ratio of different fatty acids such as saturated fatty acids (SFA) comprising palmitic and stearic, monounsaturated fatty acid (MUFA) comprising oleic, eicosenoic and erucic and poly-unsaturated fatty acid (PUFA) consisting of linoleic and linolenic acid, determines the shelf-life and nutritional quality of mustard oil, whereas glucosinolate content judges the quality of seed meal. Climatic variables such as temperature and relative humidity have differential effects on the expression of different characters depending upon their genetic architecture. In rapeseed *(Brassica napus*) environmental conditions during crop growth have been reported to alter fatty acid composition of oil and glucosinolate content in meal (Tribi-Blondel and Renard 1999, Pritchard *et al.* 2000, Scarth and Tang 2006) as well as oil and protein content (Pritchard *et al.* 2000, Si and Walton 2004). However, such studies are lacking in Indian mustard. Therefore, in the present investigation the effects of different weather variables on oil, protein and glucosinolate content, fatty acid profile, oil stability index, PUFA to SFA, MUFA to PUFA and ω-6 to ω-3 fatty acid ratios were analyzed in Indian mustard.

MATERIALS AND METHODS

The experimental materials comprising breeder seed of 25 varieties of Indian mustard were grown in randomized complete block design with three replications during *rabi* (October-April) season of 2003–06. The experiment was sown on 4 November 2003; 26 October 2004 and 24 October 2005. There were five rows of 5 m length in a plot with 45 cm row-to-row and 15 cm plant-to-plant spacing. The experiment was conducted under 80: 40: 40 kg / ha of N: P_2O_5: K_2O of fertilizer with two irrigations at 30 and 60 days after sowing. The observations were recorded on composite sample from the central three rows. The method for the analysis of oil, protein, glucosinolate content and fatty acid profile were essentially the same as described earlier

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(Chauhan *et al*. 2008). Palmitic and stearic acid, oleic, eicosenoic and erucic acid were put together as SFA and MUFA, respectively, whereas; linoleic and linolenic acid were pooled together as PUFA to work out the ratio of MUFA to PUFA and PUFA to SFA. The oil stability index was determined following Carpenter *et al.* (1976) and ω-6 / ω-3 fatty acid ratio was also computed.

The range, mean and coefficients of variability for each character were worked out. The duration between 50% flowering and physiological maturity was divided into 4 phases, each of two meteorological weeks, viz 1–14, 15–28, 29–42 and 43–56 days after 50% flowering. Means of various climatic variables were computed for each phase separately and simple correlation coefficients of quality characters with minimum, maximum and mean temperature, relative humidity, sunshine duration, wind speed and evepo-transpiration were also worked out following standard statistical methods (Gomez and Gomez 1984) to elucidate the role of environmental factors.

RESULTS AND DISCUSSION

Weather variables

The three cropping seasons showed wide variation in temperature, humidity, sunshine duration, wind speed and evaporation during the seed development of the investigated varieties. The 2003–04 cropping season was characterized by low humidity, relatively bright sunny days and consequently high evaporation. The 2004–05 cropping season had the lowest mean maximum temperature, highest humidity and reduced sunshine duration. Because of high mean humidity (65.4–96.0%), the evaporation was also the lowest. A high temperature, high morning and low after noon relative humidity with bright sunny days characterized the 2005–06 cropping season (Table 1). The crops in the 2004–05 and 2005–06 cropping season also experienced a rainfall of 24.2 mm in 8 days and 29.1 mm in two days, respectively, between 50% flowering and physiological maturity.

Analysis of variance

The mean sum of squares due to genotypes, environments and genotype × environment interactions for oil, fatty acid profile, protein and glucosinolates were highly significant, indicating thereby, presence of genetic variability in the varieties investigated. Further, environmental factors and genotype × environment interactions had high influence on the expression of all the characters and genotypes had differential performance in the three cropping seasons. Therefore, pattern of variation in quality characters was discussed separately under the three environments.

Oil and protein content

The mean oil content showed the highest range of 36.3–42.1% during 2004–05 but the highest mean and variability were recorded during 2005–06 and 2003–04 cropping seasons, respectively. The coefficients of variability among the three cropping seasons ranged from 1.7 to 3.5%. Variety ‘Rohini’ had the highest oil content (42.4%), followed by ‘GM 1’ (42.3%) during 2003–04 cropping season. Protein content was more variable during 2004–05 as compared to other seasons as seen by relatively high coefficients of variability (CV 4.8%). The highest protein content (21.5%) was recorded for variety ‘Rajat’ during 2004–05 cropping season.

Fatty acid and glucosinolates

Environmental factors had large effects over the saturated fatty acid (SFA), as coefficients of variance across the cropping seasons were high. The maximum mean SFA was recorded during 2005–06 but the variability for this character was high during 2004–05 cropping season. The range for SFA in different cropping seasons was 2.2% for ‘Basanti’ (2003–04) to 6.2% for ‘Vardan’ (2005–06). The varieties had appreciable variation for oleic acid in different cropping seasons. It varied from 8.0% in ‘Krishna’ to 18.5% in ‘GM 2’ during 2003–04. This character showed moderate to high variation over the cropping seasons (CV 8.7–17.7%).

Linoleic acid, an essential fatty acid ranged from 10.9% in ‘Krishna’ and 24.1% in ‘RCC 4’ during 2004–05. The mean linoleic acid was maximum during 2003–04. In general, this character had moderate variability during different cropping

Table 1 Weather conditions from 50% flowering to physiological maturity of Indian mustard during different cropping seasons

Parameter	Cropping season					
	2003–04		2004–05		2005–06	
	Range	Mean±SEM	Range	Mean±SEM	Range	Mean±SEM
Maximum temperature (°C)	9.1–42.0	25.4±0.9	1.8–31.8	21.7±0.6	17.6–35.0	27.1±0.6
Minimum temperature (°C)	1.2–18.0	8.2±0.4	1.7–17.1	7.3.0±0.5	–0.5–14.5	7.5±0.5
Maximum relative humidity (%)	40.0–100.0	87.0±1.3	72.0–100	96.0±0.6	80.0–100.0	94.9±0.6
Minimum relative humidity (%)	3.0–93.0	41.0±2.7	21.0–95.0	65.4±2.1	25.0–90.0	49. 0±1.9
Sunshine duration (hr / day)	0.0–10.7	7.6±0.4	0.0–10.5	5..5±0.4	0.0–9.6	7.1±0.4
Evaporation (mm)	0.0–9.8	3.4±0.2	0.1–6.0	1.7±0.2	0.0–6.0	1.7±0.2
Wind speed (km/hr)	1.0–8.0	3.5±0.2			1.0–6.9	3.3±0.2

seasons, the highest CV being 16% during 2004–05. The variability in linolenic acid was also moderate with CVs varying from 10.3 (2005–06) to 15.4% (2003–04). 'Kranti' and 'RH 781' had the minimum (7.4%) and maximum (19.4%) linolenic acid during 2003–04 and 2004–05 cropping season, respectively. In general mean linolenic acid showed a narrow range of 12.7–15.8% during all cropping seasons.

Eicosenoic acid was largely influenced by the environmental factors as suggested by higher CVs over the cropping seasons, 13.1 (2005–06) –28.2% (2004–05)). The mean eicosenoic acid among the varieties did not vary much among the cropping seasons (6.1–8.4%). It ranged from 3.7% in 'RH 781' (2004–05) to 10.7% for 'CS 52' (2005–06). Erucic acid showed low variation over the years (CV 4.0–8.0%) with least environmental effects. The lowest (37.2%) and highest erucic acid (54.3%) was recorded in 'Basanti' and 'Krishna', respectively, during the 2004–05 cropping season.

Glucosinolate content also showed low variability across different cropping seasons as for erucic acid and was less affected by the environmental factors (CV 7.0–7.6%). The mean gucosinolates content showed a narrow range of 96.9–100.2 moles / g defatted seed meal.

Oil quality indices

Since oil quality indices, like oil stability index (OSI), ω-6 to ω-3 fatty acid, MUFA to PUFA and PUFA to SFA were based on the ratio of different fatty acids, analysis of variance was not conducted for these parameters assuming that they were also affected by the environmental × genotype interactions similar to that of the constituent fatty acid. But the trend of variability in different cropping seasons was analyzed for these indices. The mean OSI ranged from 0.65 (2004–05) to 0.82 (2005–06) with large variability across the three cropping seasons (CV: 15.2–22.1%). 'RCC 4' had the lowest OSI (0.37) in 2004–05 while 'GM 2' had the highest OSI (1.27) during 2005–06 cropping season. The highest mean ω-6 to ω-3 fatty acid (1.71±0.09) and PUFA to SFA ratio (10.88±0.44) was observed during 2003–04 cropping season. Variety 'Krishna' and 'Kranti' recorded the lowest and highest ω-6 to ω-3 fatty acid ratio of 0.71 and 3.02 in 2004–05 and 2003–04, respectively. The PUFA to SFA ratio was the highest (15.20) for the variety 'Basanti' in the 2003–04. In the cropping season of 2005–06 variety 'Vardan' showed the lowest value (4.44). The mean MUFA to PUFA ratio was highest during 2005–06 cropping season and the highest variability (CV 15.4%) was observed during 2004–05. The MUFA to PUFA ratio was the highest (2.67) in 'Krishna' during 2004–05. The ω-6 to ω-3 fatty acid ratio across the three seasons was the most variable, whereas, MUFA to PUFA ratio, which indicates the shelf-life of the oil, was relatively consistent across the years as compared to other indices showing the moderate to low coefficient of variation (6.3–15.4%). In general, all the characters except linoleic acid and glucosinolate content showed lower CVs during 2005–06 seasons than those of others. The results of the present investigation suggested that the OSI, ω-6 to ω-3 fatty acid, MUFA to PUFA and PUFA to SFA were more prone to environmental conditions than the fatty acids *per se*.

Correlation coefficients of oil and oil quality parameters with weather variables

The pattern of correlations was also affected by the prevailing environmental conditions during the cropping seasons. None of the characters expressed consistent nature of association with the weather variables studied. Since the genotype × environment interactions were significant in the expression of all the quality characters, therefore, correlations were worked out and discussed separately for the three cropping seasons.

Temperature

The relationship of maximum temperature (up to 42°C) and oil content was negative and significant. The mean minimum temperature during 1–14 and 29–42 days after flowering also had negative and significant correlations with oil content (Table 2). Oil content had significantly negative association with mean temperature during 1–14 (r = –0.580**) and 29–42 DFA (r = –0.518**) in the 2005–06 cropping season. The results suggested that the high temperature during seed development would reduce oil content and were in agreement with the earlier studies (Pritchard *et al.* 2000; Si and Walton 2004; Aslam *et al.* 2009). The minimum temperature during 1–14 days after flowering had negative and significant association with protein contents while minimum temperature during 15–28 days after flowering was positively correlated with protein content in the 2003–04 cropping season. The inverse relationship of oil and protein content with temperature was expected as these two characters were reported to be negatively correlated (Pritchard *et al.* 2000; Chauhan *et al.* 2007; Aslam *et al.* 2009). Further, low minimum temperature in the initial stage of seed development would be detrimental for protein accumulation. The maximum temperature in the 2004–05 and 2005–06 cropping season during 43–56 DAF and eicosenoic acid exhibited positive and significant association (r =0.522**, 0.412*). The similar pattern was observed between minimum temperature (43–56 DAF) and eicosenoic acid in the 2005–06 cropping season (r = 0.431*). Erucic acid, in general, recorded negative association with mean temperature during seed development. Nevertheless, the negative correlation coefficients were significant only during 15–28 (2004–05 and 2005–06) and 43–56 DAF in 2004–05 cropping season. The results revealed that low temperature during seed development would increases erucic acid. Deng and Scarth (1998) reported that seeds produced under high temperature conditions had increased saturated and mono-saturated fatty acids.

Table 2 Correlation coefficients of weather parameters with quality components of Indian mustard

Character	Crop stage	Cropping season	Correlation coefficient (r)								
			Oil content	Protein	Saturated fatty acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid	Glucosin-olates
Mean temperature	I	2003–04	–0.103	–0.216	0.272	0.235	0.043	0.179	–0.246	–0.341	0.266
		2004–05	–0.081	–0.01	0.221	0.148	0.167	–0.102	0.063	–0.127	–0.082
		2005–06	–0.580**	00.175	0.183	0.006	0.314	–0.253	–0.024	–0.300	–0.067
	II	2003–04	0.170	0.348	0.004	0.082	–0.176	0.193	–0.115	–0.004	0.065
		2004–05	–0.057	–0.026	0.013	0.321	0.349	–0.104	0.325	–0.479*	0.085
		2005–06	–0.282	–0.002	0.095	0.258	0.130	–0.222	0.114	–0.399*	–0.089
	III	2003–04	–0.016	0.058	0.120	0.153	–0.232	0.118	0.009	–0.084	0.088
		2004–05	–0.098	0.081	0.190	0.088	0.195	–0.172	0.300	–0.158	0.073
		2005–06	0.518**	0.239	0.219	0.102	0.333	–0.346	–0.095	–0.319	–0.032
	IV	2003–04	0.128	0.174	–0.205	0.246	–0.359	–0.023	0.085	0.068	–0.011
		2004–05	–0.142	–0.077	–0.266	0.235	0.171	–0.136	0.503*	–0.410*	0.342
		2005–06	–0.014	–0.286	–0.154	0.019	–0.044	0.211	0.427*	–0.278	–0.128
Mean humidity	I	2003–04	–0.214	–0.397*	0.068	–0.111	0.238	–0.139	0.124	–0.074	–0.107
		2004–05	–0.066	–0.014	–0.059	0.256	0.376	–0.083	0.398*	–0.505**	0.191
		2005–06	0.526**	–0.136	–0.174	–0.112	–0.269	0.274	–0.010	0.361	0.089
	II	2003–04	0.121	0.115	–0.234	–0.171	0.165	–0.144	–0.014	0.212	–0.083
		2004–05	–0.118	–0.058	0.216	–0.054	–0.177	–0.075	–0.198	0.294	–0.153
		2005–06	0.432*	–0.418*	–0.263	–0.076	0.400*	0.396*	0.257	0.211	–0.016
	III	2003–04	–0.079	–0.220	–0.013	–0.152	0.315–	0.069	–0.013	–0.032	–0.075
		2004–05	0.170	0.113	0.171	–0.297	0.277	0.144	–0.438*	0.440*	–0.262
		2005–06	0.049	–0.102	–0.059	0.120	–0.135	0.101	0.131	–0.079	–0.171
	IV	2003–04	–0.118	–0.141	0.161	–0.281	0.260	0.002	–07.01	0.018	0.004
		2004–05	0.097	0.043	–0.246	–0.019	0.092	0.086	0.157	–0.192	0.175
		2005–06	–0.445**	0.000	0.097	0.125	0.243	–0.193	0.180	–0.452*	–0.080
Sunshine duration	I	2003–04	0.210	–0.418*	–0.075	0.043	–0.239	0.163	–0.111	0.102	0.074
		2004–05	–0.159	0.007	0.206	–0.041	–0.068	–0.158	0.070	0.165	–0.001
		2005–06	0.307	0.368	0.097	0.018	0.105	–0.162	–0.396*	0.261	0.180
	II	2003–04	–0.167	–0.263	0.273	0.244	–0.216	0.055	0.090	–0.233	0.124
		2004–05	0.134	0.105	–0.126	0.087	0.296	0.057	0.250	–0.382	0.131
		2005–06	0.591**	0.084	0.149	–0.042	0.253	–0.192	0.024	–0.266	–0.068
	III	2003–04	0.050	0.102	0.014	0.204	–0.353	0.084	–0.001	–0.010	0.105
		2004–05	–0.201	–0.103	–0.075	0.297	0.143	–0.207	0.410*	–0.325	0.198
		2005–06	0.489*	–0.087	–0.150	–0.141	–0.227	0.246	–0.053	0.377	0.109
	IV	2003–04	–0.356	–0.575**	0.245	0.136	0.098	–0.156	0.148–	0.248	0.032
		2004–05	–0.063	0.049	0.284	0.006	0.097	–0.069	–0.003	0.058	–0.107
		2005–06	–0.276	0.382	0.249	0.019	0.186	–0.342	–0.440*	0.113	0.060
Evaporation	I	2003–04	0.283	0.544**	0.186	0.063	0.128	0.093	–0.290	0.121	–0.049
		2004–05	0.028–	–0.026	–0.231	0.246	0.287	–0.053	0.426*	–0.500*	0.260
		2005–06									
	II	2003–04	0.107	0.248	0.082	0.104	–0.162	0.148	–0.026	–0.056	0.075
		2004–05	–0.139	0.024	0.201	0.080	0.083	–0.179	0.196	–0.039	0.023
		2005–06	–0.497*	0.093	0.153	0.133	0.252	–0.256	0.058	–0.392	–0.093
	III	2003–04	0.145	0.237	0.016	0.141	–0.256	0.167	–0.056	–0.022	0.109
		2004–05	–0.083	0.006	–0.086	0.280	0.338	–0.143	0.506**	–0.507**	0.239
		2005–06	–0.507**	0.276	0.223	0.049	0.376	–0.345	–0.121	–0.287	0.008
	IV	2003–04	0.129	0.128	–0.183	0.265	–0.329	–0.005	0.105	0.017	0.030
		2004–05	–0.130	0.035	0.237	–0.029	–0.026	–0.154	0.076	0.133	–0.024
		2005–06	–0.247	–0.195	–0.072	–0.009	0.094	0.103	0.383	–0.361	–0.129
Wind speed	I	2003–04	–0.091	–0.224	0.095–	0.277	–0.132	0.101	–0.067	–0.202	0.243
		2004–05									
		2005–06	0.337	0.133	0.110	0.184	0.215	0.122	0.110	0.002	0.083
	II	2003–04	–0.010	–0.145	0.316	–0.014	0.082	–0.109	0.348	–0.158	–0.315
		2004–05									
		2005–06	–0.519**	0.085	0.127	–0.064	0.308	–0.184	0.062	–0.294	–0.004
	III	2003–04	0.385	0.629**	–0.272	–0.048	–0.128	0.182	–0.160	0.187	0.009
		2004–05									
		2005–06	–0.519**	0.085	0.127	–0.064	0.308	–0.184	0.062	–0.294	–0.004
	IV	2003–04	–0.070	–0.054	0.074	0.252	–0.025	0.052	0.108	–0.171	0.059
		2004–05									
		2005–06	–0.364	–0.101	0.030	0.112	0.196	–0.102	0.293	–0.474*	0.084

[a] I, 1–14 days after 50% flowering; II, 15– 28 days after 50% flowering; III, 29– 42 days after 50% flowering and 43–56 days after 50% flowering; * and **, Significant at $P = 0.05$ and $P = 0.01$, respectively.

Relative humidity

Positive and significant relationship of oil content was observed with maximum humidity (morning relative humidity) during 1–14 (r = 0.408*) and 15–28 DAF (r = 0.455*) in 2005–06 cropping season. The afternoon relative humidity during early seed development (1–14 DAF) was positively and significantly correlated with oil content (r = 0.516**) but during later stage (43–56 DAF) it showed negative association (r = –0.474*). Mean humidity during 1–14 and 15–28 DAF in 2003–04 and 2004–05 cropping season, respectively, was correlated negatively (r = –0.397* and r = –0. 418*) with protein content.The association of eicosenoic acid was positive and significant with mean relative humidity during 1–14 DAF in the 2004–05 cropping season. But during 29–42 DAF had negative association (r = –0.438*) with eicosenoic acid. Nevertheless, afternoon relative humidity during 43–56 DAF in the 2005–06 cropping season revealed positive association with erucic acid (r =0.497*). Erucic acid had positive and significant correlation with morning relative humidity during 29–42 in 2004–05 and 15–28 DAF in 2005–06. In the 2005–06 cropping season, the morning relative humidity near physiological maturity (43–56 DAF) and erucic acid were negatively correlated (r =0.427*). Variable trend of relationship was observed between mean relative humidity and erucic acid. The ω-6 to ω-3 fatty acid ratio showed negative relationship with morning and mean relative humidity during 15–28 DAF in the 2005–06 cropping season. In 2005–06 cropping season, mean relative humidity during 15–28 DAF had negative and significant relationship with linoleic acid but significantly positive with linolenic acid. Pritchard *et al.* (2000) also observed that linolenic acid in canola was positively correlated with higher cumulative spring rainfall.

Sunshine duration

The relationship between oil content and sunshine during 15–28 DAF was negative and significant while it was positive and significant during 29–42 DAF (Table 2). The longer duration of sunshine near maturity 43–56 DAF might reduce protein content as suggested by their negative association. Sunshine during 29–42 DAF recorded positive relationship with eicosenoic acid in 2004–05. In the 2005–06 cropping season, sunshine during 1–14 DAF and 43–56 DAF exhibited negative and significant relationship with eicosenoic acid.

Wind speed

Wind speed during 29–42 DAF influenced protein content positively and significantly (r = 0. 629**) in the 2003–04 cropping season. Wind speed near maturity (43–56 DAF) exerted negative effect on erucic acid as revealed by their negative relationship (r = –0.474*).

Evaporation

The evaporation during 15–28 and 29–42 DAF had negative and significant correlation with oil content and exhibited positive association with protein content during 1–14 DAF in the 2003–04 cropping season. Evaporation during 29–42 DAF recorded positive relationship with eicosenoic acid. Erucic also exhibited negative and significant relationship with evaporation during 1–14 and 29–42 DAF in the 2004–05 cropping season.

Because all the varieties investigated in the present study had high erucic and glucosinolate content, therefore, weather variables investigated did not have substantial influence on fatty acid profile and glucosinolates even though open pollinated seeds were analyzed. Further, the results of the present investigation suggested that the OSI, ω-6 to ω-3 fatty acid, MUFA to PUFA and PUFA to SFA ratios were more prone to environmental conditions than the fatty acids *per se* as higher CVs were invariably observed over different growing seasons. Further investigations with low erucic and/ or glucosinolate genotypes grown over different seasons and analyzing open-pollinated seeds should be undertaken to precisely know the effect of growing seasons and also define the role of weather variables. However, our earlier studies (Chauhan *et al.* 2002) revealed that if the low erucic lines were properly maintained through selfing and selfed seeds analyzed, there was very little effect of environmental conditions on erucic acid.

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