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Effect of genotypes, reproductive developmental stages, and environments on glucosinolates content in rapeseed mustard

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ABSTRACT

The current study reports changes in glucosinolate content in rapeseed mustard at different floral developmental stages. Variation in glucosinolate content seems to be primarily under control of reproductive developmental stages, which contribute to 47.18- 69.98% of total variance. Significant differences were found in glucosinolate content among 10 different genotypes of rapeseed mustard. Glucosinolate content was higher in protected than unprotected environment. Although significant stage x environment and stage x genotype effect was observed, these effects were small as compared influence due developmental stage and genotypes. Total glucosinolate content increases from flower initiation stage (FIS) to full bloom stage (FBS) while at pod maturity stage (PMS), it tends to decrease.

Key Words: *Brassica*; Genotypes; glucosinolate; reproductive developmental stages; FBS-flower bloom stage; FIS –Flower initiation stage; PMS-pod maturation stage, rapeseed-mustard

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INTRODUCTION

India is the second largest rapseed growing country in the world. Rapeseed -mustard are the third most important source of edible oil in the world after soyabean and and palm oil. *Brassica* oil especially that of oilseed rape (*Brassica napus*) is nutritionally superior to most of the other edible oils due to the lowest amounts of harmful saturated fatty acids (SFAs) and a good proportion of mono and poly-unsaturated fatty acids (FAs) and is also a source of the two essential FAs, linoleic and linolenic, that are not present in some of the other edible oils[1]. The meal is a rich source of good quality proteins. The functional and nutritional values of different vegetable oils are dependent on the nature of the different fatty acids, which are incorporated into the oil (triacylglycerols). High erucic acid in the oil may increase health risks. After oil extraction, the remaining meal contains different nutritional and anti-nutritional compounds (e.g. glucosinolates, sinapine and fiber). Among these, glucosinolates have a wide range of biological functions including anticarcinogenic properties in humans, anti-nutritional effects of seed meal in animals, insect pest repellent and fungal disease suppression [1-5] . Glucosinolates play important role in the nutritional qualities of *Brassica* products which are consumed as oil, meal and as vegetables[6-8]. On the other hand, glucosinolates are important for the resistance of the plant to pest insects. Upon insect feeding or mechanical disruption, glucosinolates are hydrolyzed by myrosinase to form a range of toxic products- isothiocyanates, nitriles, thiocyanates, and epithio-nitriles and other products [9] which are deterrent to generalist insects [10,11]. Due to manifold toxic effects of glucosinolate degradation product, these compounds have attracted interest in organic pest control. During the recent past, breeding objectives have been focused on the production of “double low” rape varieties. The introduction of the low glucosinolate genotypes of oilseed rape was accompanied by concerns about how this would affect the pest and disease status of the crops [12]. The differences in composition of glucosinolate compounds are possibly of critical importance when assessing plant susceptibility to infestation [13] . Variation in the amount and pattern of glucosinolates in Brassica plants has been attributed to genetic and environmental factors, including plant age, temperature, water stress, and soil type [14]. They are found in all plant

parts, but their quantities may vary considerably among organs [15]. The content of seed glucosinolate is controlled by multiple genes and is complexly regulated in the cell [16].

Not much information is available on glucosinolate profile of in different genotypes of Indian rapeseed-mustard and its consequence on upon insect herbivore. A little is known about their locations within individual leaves, stems, or other organs and the way these influence patterns of herbivory [17]. With the concern that aphids infestation occur during flowering and pod maturation period, we attempted to explore changes in total glucosinolate content during reproductive developmental stages (flowering and pod maturation stages) in rapeseed mustard. Also, the current research was designed to determine the effects of genotypes and environmental conditions (protected and unprotected) on total glucosinolate content in Rapeseed-Mustard.

MATERIALS AND METHODS

A field experiment was conducted during winter seasons of 2004-05. 2005-06 and 2006-07 during at the experimental farm of Directorate of Rapeseed-Mustard Research Bharatpur, Rajasthan aimed at evaluation of effect of genotypes, reproductive developmental stages, and environments on glucosinolates content in rapeseed –mustard. Five varieties of *Brassica juncea* (Raya)-RK-9501, RH- 7846, Purple Mutant, RH- 9501, and JMM-927; two of *Brassica napus* (Gobhi sarson)-NUDB-09, and Teri (OE)R-9903; one of *Brassica compestris* (Toria)-BSH-1; one each of *Brassica carinata* (Ethiopian mustard)- DLSC-2 and *Eruca sativa* (Taramira)-T-27, were taken in the study. The experiment was laid out in randomized block design (RBD) with three replications.. The treatments consisted of observations on glucosinolate contents on 10 genotypes grown under two environmental conditions (Protected and unprotected). The conditions referred here as protected environment (PR) is that, that was kept protected by spray of recommended pesticide (Metasystox 0.025%) at weekly intervals, and the one that referred as unprotected (UPR), where no pesticides have been used. There were a total of 60 plots, each plot area- 4 X 3 m, and the row to row distance was 30 cm, and plant to plant distance was 10 cm. Plant samples were taken for analysis of total glucosinolate content at three different stages of plant growth from protected and unprotected environment. The three different stages of plant growth: flower initiation stage (FIS), flower bloom stage (FBS) and pod maturation stage (PMS). Plant samples collected from top 10 cm central shoot/twig. The tender portion is area which is more prone to aphid infestation. A standardized growth stage scale developed by BASF (Bayer, Ciba-Geigy and Hoechst) called the BBCH decimal system [18,19] was adopted to collect data on growth stages, FIS on the BBCH decimal system scale is stage 3; FBS is stage 4, and PMS is stage 5 .

Plant material was oven dried in oven at 80 °C to prevent enzymatic degradation by myrosinase, and homogenized in an analytical grinder. The finely grounded material (0.1 g) plant material was extracted three times, each extraction cycle of 15 minutes with 45 ml deionized water at 50 °C. Glucosinolate content was estimated from samples from two environments, protected (PR) and unprotected (UPR) environment. In each environment Glucosinolate was estimated in the samples by complex formation between Glucosinolate and tetrachloropalladate using Microscan MS % 05 ELISA Reader [20].

Analysis of variance analysis (ANOVA) was performed using SAS 9.3 software. Genotypes, reproductive developmental stages, environmental conditions-protected (PR) and unprotected (UPR) were taken into consideration as variables to compare the variation among genotypes, plant developmental stages, and environmental effects. Significant differences were evaluated at $P<0.05$ error level. Data were presented as mean values, and the the means were compared using Duncan's multiple range test (DMRT). Means with the same letter are not significantly different at $P<0.05$.

RESULTS AND DISCUSSION

Damage due to insect pests at flowering and pod maturation stages may severely affect crop yield. In the present study, quantitative variation in glucosinolate content at three different stages of reproductive growth: bud stage (flower initiation stage, FIS), flower stage (full bloom stage, FBS) and ripening stage (pod maturation stage, PMS) was studied. All the plant samples used in the study were collected from top 10 cm central shoot/twig, which is the area more prone to most aphid infestation. The result of analysis of variance (ANOVA) given in Table 1 showed that genotypic differences, environments (protected and unprotected), reproductive developmental stages, and their interaction components were highly significant for glucosinolate under the study.

Significant differences between environmental (protected and unprotected) means indicated that there existed difference between the environments in which genotypes were tested (Table 2). The significant interaction components showed that growing conditions impact the stability of individual genotype for glucosinolate content. The mean performance of all genotypes (averaged over stages) in each condition (protected and unprotected) will

give an estimate that which condition results in highest glucosinolate. In our present studies, the protected environment showed the highest glucosinolate content ranging 92.86**,** 92.34 and 93.08 µmoles/g plant dry weight, in year 2004-05,2005-06, and 2006-07 respectively than the unprotected conditions, which showed the lowest mean glucosinolate content ranging from 88.67, 89.34 and 89.99 µmoles/g plant dry weight , in year 2004-05, 2005-06 and 2006-07 respectively (Table 2).

Genotypes, and stages and interaction variance components as a percentage of the total variance were estimated and presented in Table 3.The genetic and stage components for total glucosinolate content were 27.25, and 69.98 percent respectively in 1st year trial (2004-05); 41.16 and 47.18 in 2nd year trial (2005-06) ; and 35.037and 59.690 in 3rd year trial (2006-07). Our data indicates that significant variation in glucosinolate content, which is primarily under control of reproductive developmental stages existed among 10 rapeseed mustard cultivars: stages contributing to most of percent variance component (47.18-69.98) followed by genotypes (27.25-41.16). Although available reports on glucosinolate content in rapeseed -mustard suggest that the environment plays a major role in determining glucosinolate content. However, analysis of data in our study based on two environments (three years trials) indicated that environmental effects are not key in the regulation of glucosinolate content in rapeseed mustard, and most of the observed variation was described by reproductive developmental stages and genotypes (Table 3).There are reports of influence of environment on glucosinolate content [21]. Healthy growth of plant under protected environment may be responsible for higher glucosinolate content. The studies of Spak *et al*. [22] reported that infested plants have decreased glucosinolate content than healthy uninfested plants.

Comparison 10 different cultivars of rapeseed –mustard differing (averaged over two conditions-protected and unprotected) is given in Table 4 showed significant influence due to genotypes on glucosinolate content. The genotypes *B. compestris* cv. BSH-1 and *B. napus* cv. TERI (OE)R 9903 had lowest level of total glucosinolate ranging from 69.31-71.93 and 77.26-89.49 umoles/g plant dry weight respectively ; while in *B. juncea* cv. RK-9501 , *B. juncea* RH- 7846 , *B. juncea* RH-9501, Purple Mutant and JMM-927 had moderately high glucosinolate level varying from 85.01to 91.67 umoles/g plant dry weight; whereas the genotypes *B. carinata* cv. DLSC-2 , *E. sativa* cv. T-27 had the highest level of total glucosinolate, 106.05-109.52 and 110.55 to 133.47 umoles/g plant dry weight in three years trials (2004-05-2006-07). More recent work has shown that the glucosinolate content of horticultural *Brassica* such as broccoli, *Brassica oleracea* [23], and turnip greens, *Brassica rapa* subsp. *rapa* [24] are under genetic control, although strongly influenced by environmental factors.

The significant interaction component (genotype and Environment interaction) showed that the individual genotype behaved differentially in the different environments (Table 5). In general, all genotypes have shown higher glucosinolate content in protected environment than unprotected environment. Drought conditions induce glucosinolate accumulation in cultivated *Brassica* [25].

In our studies, reproductive developmental stages (FIS, FBS and PMS) had significant influence on total glucosinolate content of rapeseed (Table 6). There has been increase in total glucosinolate content from flower initiation stage (FIS) to full bloom stage (FBS). While growing towards full maturity (PMS stage) glucosinolate content tend to decrease to certain extent (Table 6). Although, there exists significant interactions for glucosinolate content (stage x environment, and stage x genotype) (Table 7 and Table 8).

Higher glucosinolate content in seeds is considered as a risk factor for health. Therefore, there is need to develop cultivars where higher glucosinolate content remain restricted to the part of plants other than seeds. The concentration glucosinolate varies widely among different developmental stages of the plant, and also among different organs [26] . Glucosinolate accumulation and myrosinase activity differ by plant age and tissue type and respond to environmental stimuli such as planting density and herbivory [27]. The pattern of change of the glucosinolate content in *Brassica* spp. along the growth cycle has been described by earlier investigators suggest that higher glucosinolate content was present in reproductive organs compared to the other vegetative parts [28]. The seed is the ultimate sink for glucosinolate, and the highest concentration of glucosinolate is found in seeds. There is a little de novo biosynthesis of glucosinolate in seeds, majority of glucosinolate is de novo biosynthesized in the silique wall and subsequently transferred to the seeds [29].

Table 1. Analysis of variance (ANOVA) of glucosinolate content from Rapeseed Mustard genotypes grown at three developmental stages under two environmental conditions

Sources	Degrees of Freedom	Sum of squares	Sum of squares	Sum of squares
		2004-05	2005-06	2006-07
Replications	2	3.42	38.81	15.38
Genotypes	9	20564.00**	52923.26**	33604.50**
Reproductive developmental Stages	2	52818.94**	60657.00**	57248.82**
Stages*Genotypes	18	624.04**	13054.67**	3437.20**
Environmental Conditions	1	790.15**	404.76**	430.41**
Genotypes X Environmental Conditions	9	172.94**	191.81**	152.30*
Reproductive developmental Stages X Environmental conditions	2	156.40**	145.08**	26.36
Reproductive developmental Stages Stages X Genotypes X Environmental conditions	18	188.63**	329.86**	167.88
Error	118	152.42	832.62	827.56
Corrected Total	179	75470.93	128577.88	95910.40

** Significant at P<0.05, ** Significant at P< 0.01*

Table 2. Comparison of Glucosinolate content in two environments (Protected and Unprotected environments) in genotypes of Rapeseed –Mustard

Sl. No.	Genotypes	Year 2004-05 Mean* Glucosinolate Content (µmoles/g plant dry weight)	Year 2005-06 Mean* Glucosinolate Content (µmoles/g plant dry weight)	Year 2006-07 Mean* Glucosinolate Content (µmoles/g plant dry weight)
1	Protected Environment	92.86[a]	92.34[a]	93.08[a]
2	Unprotected Environment	88.67[b]	89.34[b]	89.99[b]
	CD Values at 5%	**0.335**	**0.78**	**0.78**

**Means within a column with the same letters are not significantly different at P < 0.05.*

Table 3. Genotypes, and stages and interaction variance components as a percentage of the total variance

Components	df	2004-05	2005-06	2006-07
Replications	2	0.005	0.030	0.016
Genotypes	9	27.25	41.16	35.04
Reproductive developmental Stages	2	69.98	47.18	59.69
Reproductive developmental Stages X Genotypes	18	0.827	10.153	3.584
Environmental conditions	1	1.047	0.315	0.449
Genotypes X Environmental conditions	9	0.229	0.149	0.159
Reproductive developmental Stages X Environmental conditions	2	0.207	0.113	0.027
Reproductive developmental Stages X Genotypes X Environmental conditions	18	0.250	0.257	0.175
Error	118	0.202	0.648	0.863

Table 4. Comparison 10 different cultivars of rapeseed –mustard differing in Glucosinolate content

Sl No.	Brassica species	Genotypes	Year 2004-05 Mean* Glucosinolate Content (µmoles/g plant dry weight)	Year 2005-06 Mean* Glucosinolate Content (µmoles/g plant dry weight)	Year 2006-07 Mean* Glucosinolate Content (µmoles/g plant dry weight)
1	*B. juncea*	RK-9501	90.54d^{e}	86.59^{d}	89.31^{c}
2	*B. juncea*	RH- 7846	89.89^{ef}	86.27^{d}	88.84^{c}
3	*B. juncea*	Purple Mutant	91.67^{c}	85.01^{d}	89.62^{c}
4	*B. juncea*	RH- 9501	89.53^{f}	89.34^{c}	89.95^{c}
5	*B. juncea*	JMM-927	90.92^{cd}	86.41^{d}	89.53^{c}
6	*B. carinata*	DLSC-2	106.05^{b}	109.52^{b}	107.61^{b}
7	*B. napus*	NUDB-09	88.15^{g}	85.25^{d}	89.49^{c}
8	*B. napus*	Teri (OE)R-9903	78.41^{h}	77.26^{e}	89.49^{c}
9	*B. compestris*	BSH-1	71.93^{i}	69.31^{f}	71.21^{e}
10	*E. sativa*	T-27	110.55^{a}	133.47^{a}	122.57^{a}
CD Values at 5%			**0.750**	**1.75**	**1.75**

**Means within a column with the same letters are not significantly different at P < 0.05.*

Table 5. Effect of Environments on Genotypes (Genotype and Environment Interaction) glucosinolate content

Brassica species	Genotypes	Environment*	Year 2004-05 Mean** Glucosinolate Content (µmoles/g plant dry weight)	Year 2005-06 Mean** Gucosinolate Content (µmoles/g plant dry weight)	Year 2006-07 Mean** Gucosinolate Content (µmoles/g plant dry weight)
B. juncea	RK-9501	1	92.33^{f}	87.92^{def}	90.70^{def}
		2	88.75^{g}	85.27^{fg}	87.92^{fgh}
B. juncea	RH- 7846	1	92.44^{ef}	86.69^{def}	89.77^{defgh}
		2	87.34^{h}	85.85^{efg}	87.91^{fgh}
B. juncea	Purple Mutant	1	94.28^{d}	87.43^{def}	92.68^{d}
		2	89.06^{g}	82.59^{gh}	86.57^{h}
B. juncea	RH- 9501	1	92.27^{f}	89.75^{d}	91.05^{def}
		2	86.80^{h}	88.92^{de}	88.86^{efgh}
B. juncea	JMM-927	1	93.83^{de}	87.86^{def}	91.91^{de}
		2	88.01^{gh}	84.95^{fg}	87.16^{gh}
B. carinata	DLSC-2	1	107.53^{b}	111.22^{b}	110.75^{b}
		2	104.57^{c}	107.82^{c}	104.47^{c}
B. napus	NUDB-09	1	91.94^{f}	89.12^{de}	90.14^{defg}
		2	84.35^{i}	81.39^{hi}	88.84^{efgh}
B. napus	Teri (OE)R-9903	1	80.05^{j}	79.30^{i}	78.52^{i}

		2	76.77[k]	75.22[j]	75.86[i]
B. compestris	BSH-1	1	73.25[l]	70.30[k]	71.61[j]
		2	70.60[m]	68.33[k]	70.81[j]
E. sativa	T-27	1	110.66[a]	133.85[a]	123.67[a]
		2	110.43[a]	133.09[a]	121.46[a]
		CD Values at 5%	**1.060**	**2.48**	**2.47**

**=Environment-1 Protected Environment; 2 Unprotected Environment*
***Means within a column with the same letters are not significantly different at P < 0.05.*

Table 6. Effect of different reproductive developmental stages on glucosinolate content in Rapeseed –Mustard

Sl.No.	Stages	Year-2004-05 Mean* Glucosinolate Content (µmoles/g plant dry weight)	Year-2004-05 LS Mean* Glucosinolate Content (µmoles/g plant dry weight)	Year-2004-05 LS Mean* Glucosinolate Content (µmoles/g plant dry weight)
1	FIS	69.93[c]	70.93[c]	70.46[c]
2	FBS	111.88[a]	115.22[a]	114.07[a]
3	PMS	90.48[b]	86.38[b]	90.07[b]
CD Values at 5%		**0.411**	**0.96**	**0.96**

FIS=Flower Initiation Stage; FBS=Full Bloom Stage; and PMS= Pod Maturation Stage
**Means within a column with the same letters are not significantly different at P < 0.05.*

Table 7. Effect of environment on glucosinolate content at three different stages of Growth in Rapeseed-Mustard

Sl. No.	Stages*	Environment**	Year 2004-05 Mean*** Glucosinolate Content (µmoles/g plant dry weight)	Year 2005-06 Mean*** Glucosinolate Content (µmoles/g plant dry weight)	Year 2006-07 Mean*** Glucosinolate Content (µmoles/g plant dry weight)
1	FIS	PR	72.43[e]	71.16[e]	71.63[e]
2	FIS	UPR	67.42[f]	70.69[e]	69.29[f]
3	FBS	PR	112.69[a]	117.45[a]	115.47[a]
4	FBS	UPR	111.08[b]	112.99[b]	112.67[b]
5	PMS	PR	93.45[c]	88.41[c]	92.14[c]
6	PMS	UPR	87.51[d]	84.34[d]	88.00[d]
CD Values at 5%			**0.581**	**1.36**	**1.35**

**Stages: FIS=Flower Initiation Stage; FBS=Full Bloom Stage; and PMS= Pod Maturation Stage*
*** Environment : PR=Protected Environment; and UPR=Unprotected Environment*
****Means within a column with the same letters are not significantly different at P < 0.05.*

Table 8. Effect of Different Stages and Genotypes (Genotype and Stage Interaction)

Sl. No	Brassica species	Genotypes	Stages*	Year 2004-05 Mean ** Glucosinolate content (µmoles/g plant dry weight)	Year 2005-06 Mean** Glucosinolate content (µmoles/g plant dry weight)	Year 2006-07 Mean ** Glucosinolate content (µmoles/g plant dry weight)
1	*B. juncea*	RK-9501	FIS	70.83^{op}	73.27^{lmn}	72.05^{kl}
2	*B. juncea*	RH- 7846	FIS	70.44^{op}	71.92^{n}	71.44^{l}
3	*Brassica juncea*	Purple Mutant	FIS	71.68^{o}	76.01^{klm}	74.10^{jkl}
4	*B. juncea*	RH- 9501	FIS	70.54^{op}	72.06^{mn}	71.30^{l}
5	*B. juncea*	JMM-927	FIS	69.96^{p}	72.57^{lmn}	71.30^{l}
6	*B. carinata*	DLSC-2	FIS	86.17^{l}	88.19^{h}	88.01^{gh}
7	*B. napus*	NUDB-09	FIS	63.32^{q}	63.78^{p}	62.99m
8	*B. napus*	Teri (OE)R-9903	FIS	56.44^{r}	54.12^{q}	54.28^{n}
9	*B. compestris*	BSH-1	FIS	48.59^{s}	44.95^{r}	47.02^{o}
10	*E. sativa*	T-27	FIS	91.30^{ij}	92.42^{g}	92.12^{f}
11	*B. juncea*	RK-9501	FBS	110.38^{de}	108.60^{e}	110.27^{d}
12	*B. juncea*	RH- 7846	FBS	109.22^{ef}	107.84^{e}	109.25^{d}
13	*Brassica juncea*	Purple Mutant	FBS	111.02^{d}	106.76^{e}	110.09^{d}
14	*B. juncea*	RH- 9501	FBS	108.70^{ef}	114.14^{d}	111.99^{d}
15	*B. juncea*	JMM-927	FBS	111.65^{d}	106.27^{e}	109.56^{d}
16	*B. carinata*	DLSC-2	FBS	127.16^{b}	142.27^{b}	129.92^{b}
17	*B. napus*	NUDB-09	FBS	115.08^{c}	124.11^{c}	123.37^{c}
18	*B. napus*	Teri (OE)R-9903	FBS	101.42^{h}	101.31^{f}	101.58^{e}
19	*B. compestris*	BSH-1	FBS	92.31^{i}	86.57^{h}	89.81^{fg}
20	*E. sativa*	T-27	FBS	131.90^{a}	154.37^{a}	144.88^{a}
21	*B. juncea*	RK-9501	PMS	90.41^{jk}	77.92^{ijk}	85.61^{hi}
22	*B. juncea*	RH- 7846	PMS	90.01^{jk}	79.04^{ijk}	85.83^{ghi}
23	*Brassica juncea*	Purple Mutant	PMS	92.31^{i}	72.26^{mn}	84.69^{hi}
24	*B. juncea*	RH- 9501	PMS	89.36^{k}	81.81^{i}	86.58^{gh}
25	*B. juncea*	JMM-927	PMS	91.14^{ij}	80.38^{ij}	87.73^{gh}
26	*B. carinata*	DLSC-2	PMS	104.82^{g}	98.09^{f}	104.91^{e}
27	*B. napus*	NUDB-09	PMS	86.05^{l}	67.88^{o}	82.10^{i}
28	*B. napus*	Teri (OE)R-9903	PMS	77.36^{m}	76.35^{kl}	75.72^{jk}
29	*B. compestris*	BSH-1	PMS	74.89^{n}	76.42^{jkl}	76.80^{j}
30	*E. sativa*	T-27	PMS	108.44^{f}	153.62^{a}	130.71^{b}
		CD Values at 5%		**1.299**	**3.04**	**3.03**

**Stages: FIS=Flower Initiation Stage; FBS=Full Bloom Stage; and PMS= Pod Maturation Stage*
***Means within a column with the same letters are not significantly different at P < 0.05.*

CONCLUSION

Based on results of the studies on the total glucosinolate content of 10 Rapeseed-Mustard in a three years trial under two environments at different stages (FIS, FBS and PMS) during its life cycle, it was concluded that variation in glucosinolate content is primarily under control of reproductive developmental stages. The highest glucosinolate content was found at the pod maturation stage (PMS). Mean glucosinolate content was slightly lower in samples collected at flower initiation stage (FIS) and flower bloom stage (FBS) compared to the pod maturation stage (PMS).

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