Genetic analysis of glucosinolate content in Indian mustard (*Brassica juncea* L.)

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Glucosinolates are group of plant thioglucosides found principally among the members of family Brassicaceae. The vegetative tissue and seed of Cruciferous contain one or more of the 120 known glucosinolates [1]. Glucosinolates co-exist with an enzyme called myrosinase which mediates their breakdown to a range of active compounds, isothiocyanates, nitrites, oxazolidimethiones which rendered the seed meal unsuitable for use as animal feed, especially for nonruminants. The breakdown products of glucosinolates are goitrogenic [2]. The toxicity manifestation of these products is goiter, as a result of iodine uptake impairment, liver damage, increased liver weight, reduced body weight and food intake in farm animals. The presence of high glucosinolates (80-125 µ moles/g defatted seed meal) in seed meal of cultivars of Indian mustard (B. juncea L.), the predominant crop among rapeseed-mustard, occupying nearly 80% of the total cropped area under these crops in the country, is a strong non -tariff barrier in international market and fetches low prices. Reducing glucosinolate content in seed meal would also improve the feeding value of mustard meal for livestock especially for non-ruminants. In the breeding programme d-efforts are underway to reduce the level of glucosinolate content up to the internationally acceptable norms (< 30 µ moles/g defatted seed meal). Knowledge of genetic architecture of a character is imperative for the success of the breeding programme. Information on this aspect for glucosinolate content in Indian mustard is meager [3]. Therefore, the present investigation attempts to study the genetics of glucosinolate content in Indian mustard.

The materials for the present investigation consisted of high glucosinolate parent (Varuna, RL 1359 and PCR-7) and a low glucosinolate parent (NUDHYJ

3), F₁, F₂, B₁ and B₂ generations of three crosses, NUDHYJ 3 x Varuna, NUDHYJ 3 x RL 1359 and NUDHYJ 3 x PCR 7 were grown in a randomized complete block design with two replications during rabi (Oct.-April) 2004-05. The rows were 5 m long and spaced 30 cm apart and spacing between plants was maintained at 10 cm with-in-a row. There were single row each of P1, P2, B1, B2 and F1 and five rows for F2 generations in each replication. Standard agronomic practices were followed to raise a good crop. The plants were selfed and selfed seeds were harvested separately. The number of plants taken randomly from each replication was from 8 for P_2 , F_1 140 for F_2 , 8 for B_1 and 9 for B_2 generations of each cross. Total glucosinolate content was estimated by using the method based on complex formation between glucosinolate and tetrachloropalladate (II) as described by Kumar et al. [4].

Mean and variances were calculated for each generation separately and used for statistical analysis. Adequacy of additive - dominance model was tested using scale given by Hayman and Mather [5] and Cavalli [6]. Gene effects for glucosinolate content were estimated following Hayman [7] using a six-parameter model. The significance of gene effects was tested by calculating variances, standard errors and "t" values separately for each effect as discussed by Singh and Chaudhary [8]. The minimum number of effective factor pairs was calculated by the method of Burton [9]; Castle and Wright [10] and Weber [11].

Analysis of variance indicated significant differences for glucosinolate content among different generations. The mean glucosinolate content of NUDHYJ 3 (26.5 μ moles) was significantly lower than

that of Varuna (116.3 µ moles), RL 1359 (109.8 µ moles) and PCR 7 (117.4 µ moles). The mean glucosinolate content of F, of the three crosses did not differ significantly from the F2 means (Table 1). However, F2 and backcross generation means were significantly different from each other. The mean glucosinolate contents of the parents were significantly different from the means of F₁, F₂ and backcross generations. Similarly means of B₁ and B₂ were significantly different from each other in the three crosses. The means of B, and B, were towards the recurrent parent suggesting the role of additive effects in the genetics of this trait. The glucosinolate content of the F, in all the crosses were towards the low glucosinolate parent and close to the mid-parental-value (Table 1) suggesting that genes displaying partial dominance for high glucosinolate content might be controlling synthesis of glucosinolate in these crosses. In all the three crosses, the F, frequency distribution showed distinct peaks suggesting that major genes controlled the glucosinolate in these crosses. The F, segregants fell within the parental range with no transgressive segregants towards the high glucosinolate parent. Nevertheless, a low transgressive segregant (1.1-2.5%) surpassing the low glucosinolate parent were recovered in the crosses NUDHYJ 3 x Varuna and NUDHYJ 3 x PCR 7.

The simple additive dominance model was inadequate, as revealed by different scaling tests, to explain the total genetic variability for glucosinolate content in different generations of the crosses, NUDHYJ 3 x Varuna, NUDHYJ 3 x RL 1359 and NUDHYJ 3 x PCR 7 (Table 2), suggesting the presence of non-allelic interactions in the genetic control of glucosinolate. The results were also supported by the joint scaling test as $\rm c^2$ values for the adequacy of 3-parameter model were

highly significant indicating involvement of digenic or multigenic interactions in the genetic control of glucosinolate content In these crosses, both additive [d] and dominance [h] gene effects were significant but dominance effects were larger than additive effects. Further, dominant x dominant [1] and dominant x additive [j] interaction effects were significant in the crosses NUDHYJ 3 x RL 1359 and NUDHYJ 3 x PCR 7. In the cross, NUDHYJ 3 x Varuna, all the three interactions, [i], [j] and [l] were significant (Table 2). Although both additive and dominant effects were significant but dominant effects and their interactions were predominant in the inheritance of glucosinolate content in these crosses as the magnitude of non-additive gene effects was higher than the fixable component (additive effects). The opposite sign of [h] and [l] suggested duplicate type of gene action in the genetics of this trait.

The minimum number of effective factor pairs for glucosinolate content as estimated by different methods ranged from 4.8 to 5.5 in the cross NUDHYJ-3 x Varuna, from 5.2 to 5.6 in the cross NUDHYJ-3 x RL 1359 and from 4.1 to 4.4 in the cross NUDHYJ-3 x PCR-7 (Table 3). The results indicated that the parents utilized in these crosses differed by at least 4-5 pairs of major genes for glucosinolate content. These results were in agreement with Magrath *et al.* [12] who reported that five unlinked loci controlling this trait in *Brassica napus.* However, Sodhi *et al.* [3] and Stringam and Thiagarajah [13] also reported 6-7 genes controlling inheritance of glucosinolate content in *Brassica juncea.*

The study suggested that early generation selection for low glucosinolate content would not be quite effective owing to prevalence of non-additive gene effects and negative sign of [i] made it obvious that selection

Table 1. Range and mean (± standard error) for glucosinolate content (μ moles/g defatted seed meal) of parental and segregating generation in the three crosses of Indian mustard

Population	NUDHYJ-3 x Varuna		NUDHYJ-3 x RL1359		NUDHYJ-3 x PCR-7	
	Range	Mean ± Sem	Range	Mean ± Sem	Range	Mean ± SEm
P ₁	23.2-29.1	26.5°*±0.4	23.2-29.1	26.4°±0.4	23.2-29.1	26.5°±0.4
P_2	105.7-122.9	116.3°±1.4	102.8-116.6	109.8°±1.2	106.7-130.4	117.4°±2.4
F ₁	40.9-57.8	49.4°±2.4	49.0-58.3	52.1°±2.0	48.6-61.5	55.5°±1.2
F_2	22.7-96.3	57.1°±0.9	31.4-92.7	56.0°±0.8	21.8-125.7	61 .8°±1.8
B ₁	22.3-49.7	38.9 ^d ±1.9	31.7-47.2	36.8 ^d ±1.9	33.1-53.3	38.8 ^d ±1.6
B ₂	56.6-94.2	70.3 ^b ±3.6	58.9-99.4	64.5 ^d ±4.9	57.7-90.2	75.7 ^b ±3.2

^{*}In a column, means followed by different letters are significantly different from each other,

Table 2. Scaling tests and estimates of gene effects for glucosinolate content (μ moles/g defatted seed meal) in the three crosses of mustard

Parameter		Estimate				
	NUDHYJ-3 x Varuna	NUDHYJ-3x RL 1359	NUDHYJ-3 x PCR-7			
A	1.8±4.6	-4.9±4.5	-4.4±3.5			
В	-36.9**±7.8	-32.9**±10.2	-21. 5**±7.0			
С	-13.0*±6.2	-16.3**±5.4	-12.3**±5.2			
D	11.1*±4.5	10.8±5.6	6.7±4.1			
X² joint scali	ng test 25.7**	16.7**	12.9**			
[m]	93.5**±9.1	89.7**±11. 2	85.5**±8.3			
[d]	-44.9**±0.7	-41.7**±0.6	-45.5**±1.2			
[h]	-101. 4**±26.1	-97.0*±32.8 -	69.5**±23.4			
[i]	-22.1*±9.0	-21.6±11. 2	-13.5±8.3			
[i]	19.4**±4.2	14.0**±5.4	8.5*±3.8			
[1]	57.3**±17.7	59.4**±22.0	39.5*±15.4			

and **: Significant at 5% and 1% probability level.

Table 3. Minimum number of effective factor pairs for glucosinolate content (μ moles/g defatted seed meal) in the three crosses of Indian mustard

Method	No. of effective factor pairs					
	NUDHYJ-3 x Varuna	NUDHYJ-3x RL 1359	NUDHYJ-3 x PCR-7			
Castle-Wright(1	1921) 5.3	5.4	4.1			
Weber (1950)	4.8	5.2	4.1			
Burton (1951)	5.5	5.6	4.4			
Mean	5.2	5.4	4.2			

should be made in advance generations. The selection to be useful should be deferred to advanced generations when dominance effects are substantially reduced. The bi-parental mating followed by pedigree selection in F_3 / F_4 generation may a suitable approach to select for low glucosinolate content.

References

- Fenwick G. R., Heaney R.K. and Mullin W. J. 1983. Glucosinolate and their break down products in food and food plants. CRC Critical Reviews of Food Nutrition, 18: 123-201.
- Bell J. M. 1995. Meal and by-product utilization in animal nutrition. In: D. Kimber and D.I. Me Gregor (eds.), Brassica Oilseed Production and Utilization, 301-337. CAB Int., Wallingford.
- Sodhi Y, S., A. K. Mukhopadhyay N., Arumugam J. K. Verma V., Gupta D. Rental and Pradhan A. K. 2002. Genetic analysis of total glucosinolate in crosses involving a high glucosinolate Indian variety and a low glucosinolate line of *Brassica juncea*. Plant Breeding, 121: 508-511.
- Kumar, Satyanshu, Yadav S.K., Chauhan J.S., Singh A. K., Khan N. A. and Kumar P. R. 2004. Total glucosinolate estimation by complex formation between glucosinolate and tetrachloropalladate (II) using ELISA reader. J. Food Sci. Technol., 41: 63-65.
- 5. **Hayman B. I. and Mather K.** 1955. The description of genetic interactions in continuous variation. Biometrics, **11**: 69-82.
- 6. **Cavalli L. L.** 1952. An analysis of linkage in quantitative inheritance. *In*: E.C.R. Reeve and C.H. Waddeington, *Quantitative Inheritance*. London: HMSO. pp. 135-144.
- 7. **Hayman B. I.** 1958. The separation of epistatic from additive and dominance variation in generation means. Heredity, **12**: 371-390.
- 8. Singh R. K. and Chaudhary B. D. 1985. *In*:
 Biometrical Methods in Quantitative Genetic Analysis.
 R.K. Printers, Delhi, India, Pp. 284
- Burton G. W. 1951. Quantitative inheritance in pearl millet (*Pennisetum glaucum*). Agron. J., 43: 409-417.
- Cattle W. E. and Wright S. 1921. An improved method of estimating the number of genetic factors concerned in cases of blending inheritance. Science, 54: 223.
- Weber C. R. 1950. Inheritance and inter-relationship of some agronomic and chemical characteristics in an inter-specific cross in soybean. Res. Bull. Iowa. Agrc'l. St., 374: 816.
- 12. **Magrath R., Herron C., Giamoustaris A. and Mithen R.** 1993. The inheritance of "Aliphatic glucosinolates in *Brassica napus*. Plant Breeding, **111**: 55-72.
- Stringam G. R. and Thiagarajah M. R. 1995. Inheritance of alkenyl glucosinolates in traditional and microspore-derived doubled haploid populations of *Brassica juncea* L. Czern and Coss. Proc. 9th Int. Rapeseed Congr. Cambridge, 3: 804-806.