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

J. S. Chauhan, Manju Singh, V. P. S. Bhadauria, Arvind Kumar

National Research Centre on Rapeseed-Mustard, Bharatpur 3231 303 (Rajasthan) Email: js_chau@yahoo.com

Abstract

Genetics of glucosinolate content was investigated using six generations (P_1, P_2, F_1, F_2, B_1 and B_2) of three crosses, viz., NUDHYJ-3×Varuna, NUDHYJ –3×RL 1359 and NUDHYJ-3×PCR 7. NUDHYJ-3 is a double low strain and Varuna, RL 1359 and PCR 7 are the important varieties of Indian mustard. High glucosinolate content was partially dominant over low glucosinolate content in all the three crosses as revealed by F_1 means. The parents NUDHYJ-3, source of low glucosinolate and three high glucosinolate varieties differed by at least 4-5 pairs of major genes for glucosinolate content. Non-allelic interactions were predominant in the genetic control of this trait in all the three crosses as simple additive-dominance model was inadequate to explain total genetic variability for this trait in different generations of the crosses. Although both additive and dominant effects were significant but prevalence of dominant effects along with their interactions suggested that early generation selection for low glucosinolate would not be quite effective. 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.

Key words : Indian mustard, *Brassica juncea*, glucosinolate content, genetics

Introduction

India contributes 25.2 and 13.8% to the world's rapeseed-mustard hectarage and production, respectively. In India, these crops account for 21.6 and 23.2% of the total oilseed cropped area and production, respectively. Indian mustard (*B. juncea* L.) is the predominant crop among rapeseed-mustard, occupying nearly 80% of the total cropped area under these crops in the country. Indian cultivars have high glucosinolate content (80-125 μ moles/g defatted seed meal). Glucosinolates is a 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 (Fenwick *et a*l. 1983) Glucosinolates co-exist with an enzyme called myrosinase which mediates their breakdown to a range of active compounds, isothiocyanates, nitriles, oxazolidimethiones which rendered the seed meal unsuitable for use as animal feed, especially for non-ruminants. The breakdown products of glucosinolates are goitrogenic (Bell, 1995). 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 in seed meal of Indian mustard cultivars is a strong non - tariff barrier in international market and fetches low prices. In the breeding programme in the country efforts are underway to reduce the level of the 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 (Sodhi *et al*. 2002). Therefore, the present investigation attempts to study the genetics of glucosinolate content in Indian mustard

Materials and methods

The material for the present investigation consisted of high glucosinolate parent (Varuna, RL 1359 and PCR-7) and a low glucosinolate parent (NUDHYJ-3), F_1, F_2, B_1 and B_2 generations of three crosses, NUDHYJ-3×Varuna, NUDHYJ-3×RL-1359 and NUDHYJ-3×PCR-7 (Table 1). These 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 a single row each of P_1, P_2, B_1, B_2 and F_1 and five rows for F_2 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 ranged from 8 for P_1, 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* (2004). The seeds were dried overnight in an oven at 50°C. The dried seeds (200 mg) were crushed with a mortar and pestle and transferred to screw capped tubes, 70% methanol (300 ml) was added and kept in a water bath (80°C) for 5 minutes. After cooling at room temperature, double distilled water (2 ml) was added to the tubes and heated in a water bath (80°C) for 15 minutes. Subsequently, tubes were left to cool at room temperature and centrifuged at 5,000 rpm for 5 minutes. The upper layer containing intact glucosinolate (5 ml) was then transferred to an ELISA plate and 0.002 M sodium tetrachloropalladate solution (300 μl) was added to each hole of ELISA plate. The plate was heated in an oven at 70°C for 30 minutes. The intensity of colour was measured at 405 nm using microscan ELISA reader based on complex formation between glucosinolate and tetrachloropalladate (II).

Mean and variances were calculated for each generation separately and used for statistical analysis. Adequacy of additive – dominance model was tested using scales given by Hayman and Mather (1955) and Cavalli (1952). Gene effects for glucosinolate content were estimated following Hayman (1958) 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 (1985). The minimum number of effective factor pairs was calculated by the method of Burton (1951); Castle and Wright (1921) and Weber (1950).

Results and discussion

Analysis of variance indicated significant differences for glucosinolate content among different generations. The mean glucosinolate content of NUDHYJ-3 (26.5 µmoles) was significantly lower than that of Varuna (116.3 µmoles), RL 1359 (109.8 µmoles) and PCR- 7 (117.4 µmoles). The mean glucosinolate content of F_1 of the three crosses did not differ significantly from the F_2 means (Table 1). However, F_2 and backcross generation means were significantly different from each other. The mean glucosinolate contents of the parents were significantly different from the means of F_1, F_2 and backcross generations. The means of B_1 and B_2 were significantly different from each other in the three crosses. The means of B_1 and B_2 were towards the recurrent parent suggesting the important role of additive effects in the genetics of trait. The glucosinolate content of the F_1 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. The F_2 segregants fell within the parental range with no transgressive segregants toward high glucosinolate parent. Nevertheless, a low transgressive segregant (1.1-2.5%) surpassing the low glucosinolate parent were screened in the crosses NUDHYJ-3 × Varuna and NUDHYJ-3 × 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×Varuna, NUDHYJ-3×RL 1359 and NUDHYJ-3×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 χ^2 values for the adequacy of 3- parameter model were highly significant indicated 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×dominant [l] and dominant×additive [j] interaction effects were significant in the crosses NUDHYJ-3×RL 1359 and NUDHYJ-3×PCR-7. In the cross, NUDHYJ-3×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 inheritance of glucosinolate content in these crosses as the magnitude of non-additive gene effects higher than the flexible component (additive effects). The opposite sign of both [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×Varuna, from 5.2 to 5.6 in the cross NUDHYJ-3×RL 1359 and from 4.1 to 4.4 in the cross NUDHYJ-3×PCR-7. 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*. (1993) who reported that five unlinked loci controlling this trait in *Brassica napus.* However, Sodhi *et al*. (2002) and Thiagarajah (1995) 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 of [i] made it obvious selection should be made in advance generations. The selection to be useful should be deferred to advanced generations when dominance effects are substantial 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

Bell, J M., 1995. Meal and by- product utilization in animal nutrition. In : D. Kimber and D.I. Mc Gregor (eds), *Brassica* Oilseed Production and Utilization, 301-337. CAB Int., Wallingford.

Burton, G.W. 1951. Quantitative inheritance in pearl millet (*Pennisetum glaucum*). *Agron. J*. 43 : 409-417

Castle, W.E. and S. Wright. 1921. An improved method of estimating the number of genetic factors concerned in cases of blending inheritance. *Science*, 54 : 223

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

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

Hayman, B. I. 1958. The separation of epistatic from additive and dominance variation in generation means. *Heredity*. 12 : 371-390

Hayman, B. I., and K. Mather. 1955. The description of genetic interactions in continuous variation. *Biomatrics*. 11 : 69-82

Kumar, Satyanshu., S.K. Yadav, J.S. Chauhan, A. K. Singh, N. A. Khan and P. R. Kumar. 2004. Total glucosinolate estimation by complex formation between glucosinolate and tetrachloropalladate (II) using ELISA reader. *J. Food Sci. Technol*., 41 : 63-65

Magrath, R., C. Herron, A. Giamoustaris, and R. Mithen, 1993. The inheritance of Aliphatic glucosinolates in *Brassica napus*. *Plant Breeding* 111, 55-72

Singh, R.K. and B.D. Chaudhary. 1985. In : *Biometrical Methods in Quantitative Genetic Analysis*. R.K. Prinetrs, Delhi, India. Pp. 284

Sodhi, Y.S., A.K. Mukhopadhyay, N Arumugam, J.K. Verma, V. Gupta, D. Pental and A.K. Pradhan. 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

Stringam, G.R., and M.R. Thiagarajah. 1995. Inheritance of alkenyl glucosinolates in traditional and microspore-derived doubled haploid populations of *Brassica juncea* L. Czern and Coss. *Proc. 9 th Int. Rapeseed Congr*. Cambridge, 3, 804-806.

Weber, C. R. 1950. Inheritance and inter-relationship of some agronomic and chemical characteristics in an inter-specific cross in soybean. *Res. Bul*l. *Iowa. Agrc'l. St*. 374:816.

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

Population	NUDHYJ-3 × Varuna		NUDHYJ-3 × RL 1359		NUDHYJ-3 × PCR-7	
	Range	Mean ± Sem	Range	Mean ± Sem	Range	Mean ± SEm
P_1	23.2-29.1	26.5[c]* ± 0.4	23.2-29.1	26.4[c] ± 0.4	23.2-29.1	26.5[c] ± 0.4
P_2	105.7-122.9	116.3[a] ± 1.4	102.8-116.6	109.8[a] ±1.2	106.7-130.4	117.4[a] ± 2.4
F_1	40.9-57.8	49.4[c] ± 2.4	49.0-58.3	52.1[c] ± 2.0	48.6-61.5	55.5[c] ± 1.2
F_2	22.7-96.3	57.1[c] ± 0.9	31.4-92.7	56.0[c] ± 0.8	21.8-125.7	61.8[c] ± 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[b] ± 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 (µmoles/g defatted seed meal) in the three crosses of mustard

Parameter	Estimate		
	NUDHYJ-3 × Varuna	NUDHYJ-3×RL 1359	NUDHYJ-3 × PCR-7
A	1.8 ± 4.6	-4.9 ± 4.5	-4.4 ± 3.5
B	-36.9** ± 7.8	-32.9** ± 10.2	-21.5** ± 7.0
C	-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
χ^2 joint scaling 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
j	19.4** ± 4.2	14.0** ± 5.4	8.5* ± 3.8
l	57.3** ± 17.7	59.4** ± 22.0	39.5* ±15.4

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