

## Variability for Leaf and Seed Glucosinolate Contents and Profiles in a Germplasm Collection of the *Brassica juncea*

Shilpa Gupta<sup>1\*</sup>, Manjeet K Sangha<sup>1</sup>, Gurpreet Kaur<sup>2</sup>, Amarjeet K Atwal<sup>2</sup>, Shashi Banga<sup>2</sup> and Surinder S. Banga<sup>2</sup>

<sup>1</sup>Department of Biochemistry, Punjab Agricultural University, Ludhiana, Punjab- 141004, India

<sup>2</sup>Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab- 141004, India

### Abstract

**Aim:** A renewed interest in glucosinolates (GSLs) as compounds with biocidal and anticarcinogenic activity demands evaluation of the available variability in germplasm collections. The objective of the present study was to evaluate a germplasm collection of the *Brassica juncea* for total content and profile of leaf and seed GSLs.

**Methods:** A total of 366 entries of a RIL population derived from cross of NUDH-YJ-04 and RL-1359, were nondestructively analyzed by near-infrared reflectance spectroscopy by means of previously developed calibration equations. Out of 366 lines, 97 lines were selected on the basis of glucosinolate range and further analyzed by ultra performance liquid chromatography for total GSL content and the concentrations of individual components i.e. sinigrin, glucoiberin, epiprogoitrin, gluconapin, gluconasturtiin and gluconeobrassicin.

**Results and conclusion:** The collection contained great variability for GSL content and profile. Remarkable variation in glucosinolate content and profiles from different tissues within one plant may reflect different control mechanism operating on the glucosinolate biosynthetic pathway in different tissues. In the present study no correlation has been observed in leaf and seed glucosinolates. NIRS screening followed by further HPLC analyses on preselected entries led to a fast and comprehensive evaluation of variability for total content and profile of seed GSLs, which represents an important advance in the evaluation of GSLs in *Brassica* germplasm.

**Keywords:** *Brassica juncea*; Glucosinolate; Recombinant inbred lines; NIRS; UPLC

### Introduction

Modern cultivars and breeding lines of Indian mustard (*Brassica juncea* (L.) Czern. + Coss.) yield high-quality oil, high-protein seed meal, and are relatively resistant to heat stress, water stress, pod-shattering and fungal diseases [1], and could be used as an oilseed crop in the Mediterranean area [2]. Glucosinolates (GSLs) are a family of secondary plant metabolites particularly abundant in seeds and green tissues of the family *Brassicaceae*. They consist of a glycone moiety and a variable aglycone side chain, which derives from the amino acid pathway. More than 140 different GSLs, showing different side chain structure, have been identified in the plant kingdom, mainly in the *Brassicaceae* [3-5]. Both the GSLs and their degradation products are associated with antinutritive and toxic effects, limiting the usefulness of seeds and seed meals for human and animal feed [6]. Because of their detrimental effects, plant breeding has been focused on a drastic reduction of seed GSL content in the major oil crops of the family, i.e. *Brassica napus*, *B. rapa*, and *B. juncea* [7] with a view to using the defatted meal for animal feeds on a large scale. On the other hand, GSLs and their hydrolysis products possess beneficial properties as well. They show broad biocidal activity which can be used as an alternative to synthetic pesticides for pest and disease control [8,9]. Furthermore, GSLs exert a positive influence against biological processes associated with cellular damage and cancer development [10-12]. The biological activity of the GSLs is determined by both the GSL concentration and composition [8,9]. Therefore, the identification of sources of variability for individual GSLs is important for the development of cultivars with increased levels of specific GSLs. Large-scale germplasm evaluation for GSL profiles with traditional chromatographic methods is expensive, time consuming, and requires the destruction of valuable germplasm materials. Alternatively, total GSL content and the concentration of several individual GSLs can be nondestructively analysed by near infrared reflectance spectroscopy (NIRS) [13]. NIRS analyses are faster

but also less accurate than the standard method of High Performance Liquid Chromatography (HPLC) [14,15]. However, previous studies on evaluation of *Brassica* germplasm for seed quality traits revealed that a combination of NIRS screening followed by analysis of the preselected accessions by a reference method leads to an efficient identification of variants with a simultaneous minimization of time, costs, and destruction of material [16]. The objective of the present study was to evaluate a germplasm collection of the genus *Brassica* for total seed and leaf GSL content and profile.

### Materials and Methods

The original germplasm collection included a RIL population comprising 366 entries from cross of European *juncea* NUDH-YJ-04 and Indian *juncea* RL-1359, which were analysed for GSL content and composition by NIRS (Near infrared reflectance spectrometry) and UPLC (ultra performance liquid chromatography). The nomenclature used and the delimitation of the species is based on Gladis [17]. The germplasm accessions were collected from Oilseeds section, department of plant Breeding and Genetics, Punjab agricultural University, Ludhiana, Punjab.

Near-infrared reflectance spectroscopy and HPLC analyses were performed as described by Font et al. [18]. Intact-seed samples of

\*Corresponding author: Shilpa Gupta, Department of Biochemistry, Punjab agricultural University, Ludhiana, Punjab-141004, India, E-mail: [shilpagupta09@gmail.com](mailto:shilpagupta09@gmail.com)

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the 366 entries were scanned by NIRS. Calibration equations for total GSL content were used for estimation (see systematic names of glucosinolates in table 1). A selection based on NIRS estimations was performed and 97 selected samples were further analyzed by UPLC. The selection was made to include entries which covered the complete range of glucosinolates in RIL population i.e. entries with very high, high, intermediate, low and very low concentration of GSL.

Seed and leaf glucosinolates profiles were estimated by ultra performance liquid chromatography (UPLC) (Waters UPLC) following the method of Sorenson et al. [19]. Solvents used were acetonitrile and water. The gradient used for separation is mentioned in table 2

The percentages of aliphatic, aromatic and aryl glucosinolates were calculated following the software “Empower”.

$$\text{Conc. of individual glucosinolate component} = \frac{\text{Conc. of internal std}}{\text{Area of internal std}} \times \text{Area of unknown} \times \text{Response factor for each glucosinolate component}$$

## Results and Discussion

Initially, total seed glucosinolate content has been estimated in 366 lines via NIRS. Out of these 366, 97 RILs were selected on the basis of glucosinolate range observed in whole RIL population. These 97 lines were distributed in seven categories covering complete range (Table 3). Seed and leaves of these 97 lines were further screened for total GSL content and composition via NIRS and UPLC (Table 4 and 5).

### Variation for total seed glucosinolate content in seeds

Glucosinolates are antinutritional components and the international standards for canola varieties require glucosinolate level to be < 30 μmol/g seed. With NIRS, in selected accessions, total GSL ranged from 28.85 to 115.88 μmol/g tissue, with mean value of 69.39 ± 0.26. The frequency distribution of glucosinolates in these 97 lines also showed a near normal distribution (Figure 1). In this case also,

Systematic name	Trivial name	Abbreviation
2-Propenyl	Sinigrin	SIN
3-Methylsulphinylpropyl	Glucoiberin	IBE
3-Butenyl	Gluconapin	GNA
(2S)2-Hydroxy-3-butenyl	Epiprogoitrin	EPI
N-Methoxy-3-indolmethyl	Neoglucobrassicin	NEO
2-Phenylethyl	Gluconasturtiin	NAS

Table 1: systematic names of glucosinolates.

Time (min)	Acetonitrile (%)	Water (%)	Flow rate (ml/min.)
0-3.64	1	99	0.4
3.64-4.04	19	81	0.4
4.04-4.93	1	99	0.4
4.93-5.00	1	99	0.4

Table 2: gradient used for separation in UPLC (Ultra Performance Liquid Chromatography).

Range	No. of genotypes
0-20	0
21-40	8
41-60	21
61-80	46
81-100	15
101-120	7

Table 3: Selected 97 individuals in different ranges of glucosinolate content in seeds (μmole/g seed) of RIL population.

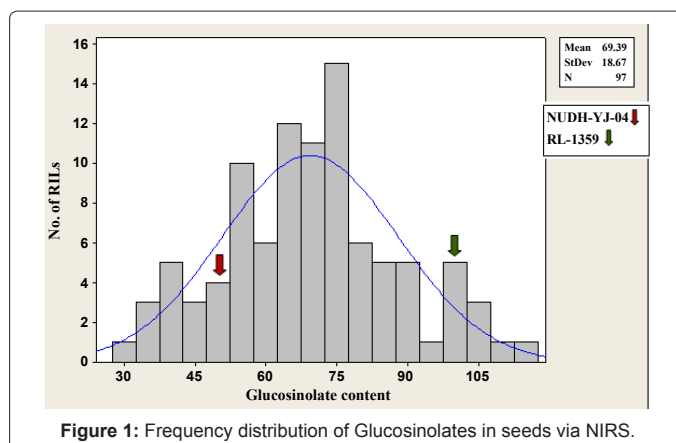


Figure 1: Frequency distribution of Glucosinolates in seeds via NIRS.

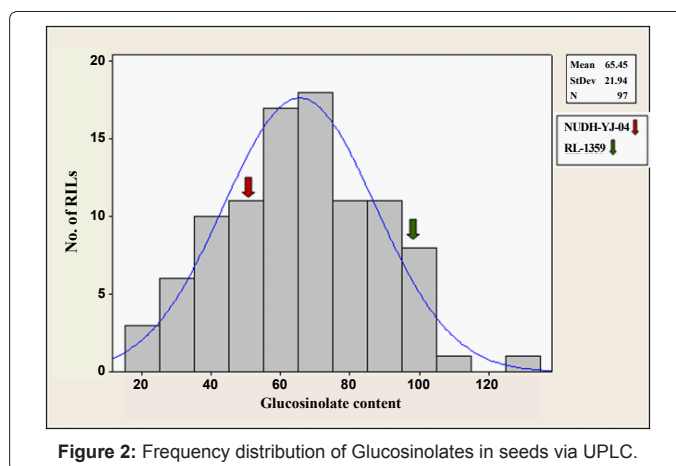


Figure 2: Frequency distribution of Glucosinolates in seeds via UPLC.

most of the inbred lines were in the range of 41 to 60 μmol/g seed glucosinolate content (21), 46 had occurred in a range of 61-80 μmol/g seed and 15 had in range of 81-100 μmol/g seed while range of 21-40 and 101-120 showed lesser number of lines i.e. 8 and 7 respectively and it was found that there was no line in 0-20 μmol/g seed. With UPLC, a little bit variation has been observed as compared to NIRS. The selected 97 lines harboured the range of 15.73-127.61 μmol/g DW seed with mean value of 65.45 ± 0.33. RL-1359 and NUDH-YJ-04 showed glucosinolate content of 97.51 and 52.54 μmol/g DW seed respectively. The frequency distribution of glucosinolates again showed a normal distribution (Figure 2), indicating a quantitative inheritance. Most of the recombinant inbred lines fell in the range of 41 to 60 μmol/g DW seed glucosinolate content (32), 30 had in range of 61-80 μmol/g DW seed and 17 had in range of 81-100 μmol/g DW seed. 21-40 μmol/g DW seed had 11 lines and 101-120 μmol/g DW seed had 5 lines whereas only a single line was there in 0-20 μmol/g DW seed range and 121-140 μmol/g DW seed range (Table 4). Variation in seed glucosinolate concentrations of Indian mustard (*Brassica juncea* L.) Czern and Coss has also earlier been reported by many authors [1,20,21].

### Variation for total glucosinolate content in leaves

In leaves of selected 97 lines, NIRS data showed a range of 0.82-102.30 with mean ± CV value of 44.18 ± 0.64. RL-1359 had a high glucosinolate content of 86.92 μmol/g DW leaf and NUDH-YJ-04 had a low glucosinolate content of 10.17 μmol/g DW leaf. The frequency distribution of glucosinolate content in these 97 lines showed a discrete distribution (Figure 3) with 29 lines in range of 0-25 μmol/g DW leaf

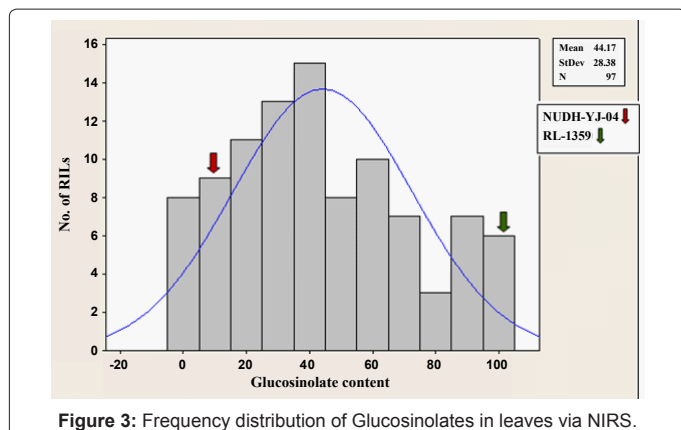


Figure 3: Frequency distribution of Glucosinolates in leaves via NIRS.

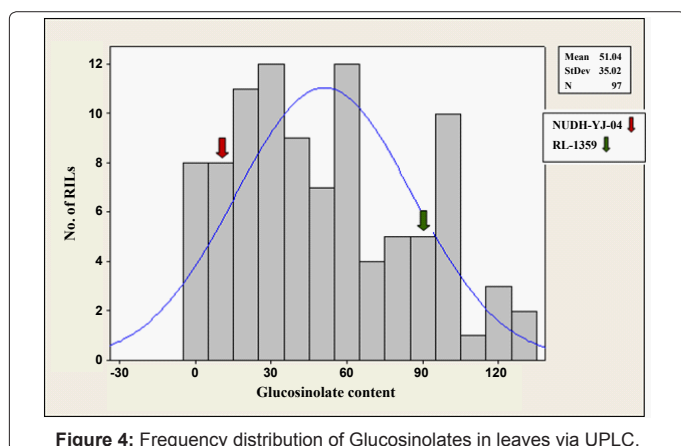


Figure 4: Frequency distribution of Glucosinolates in leaves via UPLC.

Tissue		RL-1359	NUDH-YJ-04	Range	Mean ± CV
Seeds	97 (NIRS)	102.15	53.50	28.85-115.88	69.39 ± 0.26
	97 (UPLC)	97.51	52.54	15.73-127.61	66.28 ± 0.33
Leaves	97 (UPLC)	86.92	10.17	0.82-102.30	44.18 ± 0.64
	97 (UPLC)	102.25	14.19	4.33-129.93	51.04 ± 0.68

Table 4: Distribution of glucosinolate content in seeds ( $\mu\text{mole/g}$  seed) and leaves ( $\mu\text{mole/g}$  DW leaf) of *B. juncea* in selected 97 individuals.

tissue, 31 lines had 26-50  $\mu\text{mole/g}$  DW leaf range, 21 genotypes had 51-75  $\mu\text{mole/g}$  DW leaf range, 15 lines had glucosinolates in range of 76-100  $\mu\text{mole/g}$  DW leaf with only single individual in 101-125  $\mu\text{mole/g}$  DW leaf range. In UPLC analysis, these 97 lines showed variation of 4.33-129.93 with RL-1359 having 102.25  $\mu\text{mole/g}$  DW leaf and NUDH-YJ-04 was having 14.19  $\mu\text{mole/g}$  DW leaf. The mean  $\pm$  CV value is 51.04  $\pm$  0.68. The frequency distribution (Figure 4) showed a decreasing trend of glucosinolate content with range, as 0-25  $\mu\text{mole/g}$  DW leaf glucosinolate content had 30 lines, 26-50  $\mu\text{mole/g}$  DW leaf range had 23 lines, 51-75  $\mu\text{mole/g}$  DW leaf range had 19 lines, 76-100  $\mu\text{mole/g}$  DW leaf range had 15 lines, 101-125  $\mu\text{mole/g}$  DW leaf range had 9 lines and 126-150  $\mu\text{mole/g}$  DW leaf had a single genotype i.e. with increase in range of glucosinolate content, there was decrease in number of genotypes, thus suggesting lower glucosinolate content predominated in leaves (Table 4).

Similar results have also been observed by many authors [22-26]. As there is very little variation in total GSL content via NIRS and UPLC, so it can be suggested that non-destructive and cost effective NIRS may be an alternative to laborious UPLC. Similar studies have also been done by Velasco and Becker [13].

### Profiling of glucosinolates

The individual glucosinolates identified in extracts from the seed and leaf tissues of *B. juncea* lines studied are listed in the Table 5. In seeds and leaves, 5 and 3 different individual components were identified by comparison with the purified standard (Sinigrin) and on the basis of the use of internal standards (Glucotropaeolin). When glucosinolate profiles of leaf and seed tissue were compared, trend of concentration of individual components was found to be similar. In both leaves and seeds, the dominant glucosinolate was gluconapin (GNA), with sinigrin (SIN) as the next most abundant component. These results are in agreement with previous studies on this [27-30,7,13]. Vaughan and Gordon [28] and Röbbelen and Thies [29] found a relationship between geographical origin and GSL profile of *B. juncea*; whereas the accessions from India and Pakistan contained mainly GNA, the accessions from other origins contained mainly SIN. Another main component in seeds was found to be glucoiberin (IBE) which was not reported in leaves and epiprogoitrin (EPI) showed its presence in leaf tissue only, not in seeds.

In seeds, GNA component ranged from 2.36-90.87  $\mu\text{mole/g}$  DW seed with mean  $\pm$  CV value of 48.01  $\pm$  0.00. The parent, RL-1359 showed high GNA content (75.12  $\mu\text{mole/g}$  DW seed tissue) and NUDH-YJ-04 had showed low GNA content (37.51  $\mu\text{mole/g}$  DW seed tissue) as expected. The lowest concentration of SIN component was found to be 0.194  $\mu\text{mole/g}$  DW seed, highest was the 35.16  $\mu\text{mole/g}$  DW seed, and the mean  $\pm$  CV value of 11.78  $\pm$  0.00 in the RIL. RL-1359 had 19.73  $\mu\text{mole/g}$  DW seed SIN and NUDH-YJ-04 had 11.09  $\mu\text{mole/g}$  DW seed SIN. IBE showed lesser concentration. In this case, high glucosinolate parent i.e. RL-1359 had lesser concentration of IBE (2.12  $\mu\text{mole/g}$  DW seed tissue) whereas low glucosinolate parent i.e. NUDH-YJ-04 had higher concentration of IBE (3.16  $\mu\text{mole/g}$  DW seed). Range observed was 0.117-10.93  $\mu\text{mole/g}$  DW seed with mean  $\pm$  CV value of 2.08  $\pm$  0.00. Traces of two individual components NAS and NEO have also showed their presence. NAS was observed in RL-1359 only (0.547  $\mu\text{mole/g}$  DW seed) and it ranged from 0.0-6.10  $\mu\text{mole/g}$  DW seed with mean  $\pm$  CV value of 0.706  $\pm$  0.00. Again in case of NEO, low glucosinolate parent had high concentration (0.728  $\mu\text{mole/g}$  DW seed) as compared to high glucosinolate parent (0.0027  $\mu\text{mole/g}$  DW seed). NEO concentration ranged from 0.0027-0.99  $\mu\text{mole/g}$  DW seed in selected 97 individuals with mean  $\pm$  CV value of 0.1446  $\pm$  0.00. Bellostas et al. [5] also observed that GNA concentration was highest in seeds of red cabbage (0.2  $\mu\text{mole/g}$  DW seed in cauliflower to 7.3  $\mu\text{mole/g}$  DW seed in red cabbage). Also, SIN has already been reported as the quantitatively dominating glucosinolate in edible cabbage and cauliflower heads [25,31-34] with values ranging from 6-125  $\mu\text{mole/g}$  DW seed [35], depending upon the cultivar and the tissue assessed. Palmer et al. [36] reported SIN as the major glucosinolate in *B. carinata*, *B. nigra* and *B. juncea*, whereas other glucosinolates such as GNA appeared in lower concentration. But in

Tissue		RL-1359	NUDH-YJ-04	Range	Mean $\pm$ CV
Seeds	IBE	2.12	3.16	0.117-10.93	2.08 $\pm$ 0.00
	SIN	19.73	11.09	0.194-35.16	11.78 $\pm$ 0.00
	GNA	75.12	37.51	2.36-90.87	48.01 $\pm$ 0.00
	NAS	0.547	-	0.0-6.10	0.706 $\pm$ 0.00
	NEO	0.0027	0.728	0.0027-0.99	0.1446 $\pm$ 0.00
Leaves	EPI	10.61	2.04	0.65-16.37	3.98 $\pm$ 0.08
	SIN	18.60	1.09	0.089-64.85	14.10 $\pm$ 0.11
	GNA	73.04	11.06	0.653-70.12	12.46 $\pm$ 0.00

IBE- glucoiberin, SIN- sinigrin, GNA- gluconapin, NAS- gluconasturtiin, NEO- gluconeobrassicin, EPIPRO- epiprogoitrin

Table 5: Distribution of individual glucosinolate components in seeds ( $\mu\text{mole/g}$  DW seed) and leaves ( $\mu\text{mole/g}$  DW leaf) of *B. juncea* in selected 97 individuals.

our study, GNA was found to be maximum. Bellostas et al. [5] also reported that some *B. juncea* cultivars have SIN as the main component while GNA as the dominant compound in others. Variability among previous studies is not surprising and can be explained considering first of all the different *B. juncea* varieties used in those studies which also interplay with other factors, such as growing conditions and agronomic practices that can affect plant glucosinolate profiles [37].

In leaves also, GNA concentration showed a diverse range from 0.653-70.12  $\mu\text{mol/g}$  DW leaf with mean  $\pm$  CV value of  $12.46 \pm 0.00$ . RL-1359 had 73.04  $\mu\text{mol/g}$  DW leaf GNA and NUDH-YJ-04 had concentration of 11.06  $\mu\text{mol/g}$  DW leaf tissue. SIN concentration was very low in NUDH-YJ-04 (1.09  $\mu\text{mol/g}$  DW leaf tissue) as compared to high glucosinolate parent (18.60  $\mu\text{mol/g}$  DW leaf tissue). SIN component ranged from 0.089-64.85  $\mu\text{mol/g}$  DW leaf with mean  $\pm$  CV value of  $14.10 \pm 0.11$ . In EPI, range observed was 0.65-16.37  $\mu\text{mol/g}$  DW leaf and mean  $\pm$  CV value was  $3.983 \pm 0.08$ . The parents, RL-1359 showed 10.61  $\mu\text{mol/g}$  DW leaf EPI and NUDH-YJ-04 showed 2.04  $\mu\text{mol/g}$  DW leaf EPI.

Remarkable variation in glucosinolate content and profiles from different tissues within one plant may reflect different control mechanism operating on the glucosinolate biosynthetic pathway in different tissues or it may simply result from alterations in substrate availability [31]. Since, glucosinolates are known to arise from amino acid precursors [38], a comparative study of glucosinolate and amino acid profiles in different tissues should prove informative. Dynamic changes of glucosinolate levels in any particular tissue also depend upon regulation of de novo biosynthesis, degradation and mobilization of glucosinolates [39], with the capacity for the de novo synthesis varying according to the type of tissue concerned. Moreover, the chemical structure and glucosinolate concentrations in plants vary considerably, depending on the stage of development, tissue type and environmental conditions [37,40], suggesting a storage function for these compounds which are required at specific stages of development [41]. Wide variation in glucosinolate content among genotypes, also suggest differences in their health promoting properties and the opportunity for enhancement of their levels through genetic manipulation [33].

Reports available so far on the correlation between total seed GSL content and GSL content in other plant tissues appear to be contradictory. Some authors have reported high positive correlations [42,43], but others did not find any in other materials [44,45,32] as in present study. Schilling and Friedt [43] suggested that weak correlations between seed and leaf GSL content might be caused by the dependence of leaf GSL content on environmental effects and growing stage. Other authors have indicated that intra-plant variations for GSLs are quantitative rather than qualitative [46,29]. Therefore, further evaluation of the glucosinolate content and composition in different plant tissues will have to elucidate how the variability for seed GSLs identified in the present study is reflected in other plant organs.

In conclusion, a typical GSL profile of selected accessions was detected after NIRS analyses and confirmed with HPLC. Additionally to the inherent interest of identifying variability for seed quality traits in *Brassica*, the results of the present study reveal that a fast NIRS screening including the evaluation of individual GSLs may also be useful to identify wrong taxonomic assignments in this genus. The application of an approach consisting of nondestructive NIRS screening followed by further HPLC analyses on preselected entries led to a fast and comprehensive evaluation of variability for total content and profile of seed GSLs in a germplasm collection of *Brassica*. This approach allowed us to complete the evaluation without destroying any

seed in most of the accessions, which represents an important advance in the evaluation of GSLs in *Brassica* germplasm. \*Details of individual components has been given in online resources (I-II)

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