Variability of glucosinolates in *Brassica* germplasm collections

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Abstract

Glucosinolates are the secondary metabolites produced by the crucifer plants. In order to search for a new source of low glucosinolate more than 1200 Indian and exotic accessions of *Brassica* and its wide relatives obtained from National Bureau of Plant Genetic Resources, India were analyzed by HPLC comprising of 651 *B. juncea*, 461 *B. campestris/B. rapa,* 35 *B. napus*, 10 *B. nigra*, 18 *B. carinata*, 6 *B. chinensis*, 63 *Eruca sativa*, 5 *Sinapis alba*, 3 *Raphanus* sp., 5 *B. tourniforti*, and 1 *Crambe asyssinica, B. juncea* accessions from sub-species ragosa, characteristically yielded high allyl glucosinolate as compared to Indian *B. juncea* accessions, which registered medium allyl and high butenyl glucosinolate. Exotic *B. napus* recorded low hydroxy-butenyl glucosinolate except for some. Among Indian *B.napus* GSL-1-SEL recorded medium glucosinolate while others had high hydroxy-butenyl. The exotic accessions of *B. nigra* showed high allyl glucosinolate while Indian accessions recorded medium allyl and high butenyl, although a few accessions showed hydroxyl-butenyl in place of allyl glucosinolate. There were no significant differences among *B. campestris* sub-types toria, yellow sarson and brown sarson. They recorded predominantly high butenyl glucosinolate, with allyl and hydroxy-butenyl being either absent or present in negligible amounts. *B. chinensis* showed glucosinolate profile similar to that of *B. campestris/B.rapa*. Among the widely related species, *B. tournefortii* accessions ranged from high allyl to medium butenyl to high hydroxy-butenyl, thus representing a wide variability. *Raphanus* species recorded high allyl, high butenyl and low hydroxyl-butenyl. *B. carinata* germplasms recorded high hydroxy-butenyl and very low to medium butenyl. *Eruca sativa* accessions recorded varied glucosinolate profiles. All *S. alba* accessions were exotic collections and had butenyl as predominant glucosinolate with low to medium amount of allyl. A total of twenty six exotic accessions; twenty B. *napus,* two *B. juncea*, one *B. carinata*, one *Crambe asyssinica,* and two *E.sativa* have been identified having ⩽30 μmoles glucosinolate. This variability in glucosinolates can be further utilized for crop improvement and to study the inheritance pattern of various glucosinolates.

Key words: *Brassica* species, glucosinolate, HPLC

Introduction

The members of family Cruciferae includes several *Brassica* species such as *B. juncea*, B. napus, *B. rapa*, *B. nigra*, B. oleracea, *B. carinata* and related species such as *Raphanus* sativus, *Sinapis alba*, *Eruca sativa*, Diplotaxis species, etc. Glucosinolate are the secondary metabolites produced by the plants of the crucifers. So far, more than 90 different glucosinolates belonging to different classes have been reported for this family. Structurally they can be grouped into three different classes' viz. aliphatic, indolyl and alkyl glucosinolate. Among these, aliphatic glucosinolate are the most abundant and responsible for pungent flavour due to liberation of thio-and isothiocyanates after their hydrolysis. Presence of high amounts of aliphatic glucosinolates in feeds (>30μmole/g defatted meal) have been found to be nutritionally toxic. Allyl, butenyl and hydroxy butenyl are the main aliphatic glucosinolates present in different species of the *Brassica*. In order to make the *brassica* oil and meal nutritionally safe for food and feed purposes, efforts initiated in Canada and Europe have lead to the development of 'Canola' quality varieties having low erucic acid (⩽2% in seed oil) and low glucosinolate content (⩽ 30μm/g oil free meal) commonly known as double low varieties ('O-O'; Downey, 1990).

Bronowski, a Polish cultivar, was the first *B. napus* germplasm identified in 1967 to have low glucosinolate content (≈ 12 μ moles/g oil free meal; Finlayson et al., 1973). Incidentally it also had low erucic acid (≈ 7%). Discovery of Bronowski led to the development of many double low varieties of *B. napus* in Canada, Europe and Australia. So far, Bronowski is the only naturally occurring gene pool identified for low glucosinolate content and utilized globally as a donor source. India has rich stocks of oilseed crops, which can serve as a valuable resource for utilization under breeding program. Therefore, realizing an imperative need for systematic characterization of Indian rapeseed mustard germplasm, to search for a suitable low glucosinolate donor source in the Indian gene-pool, the work was initiated for the characterization of rapeseed-mustard varieties under the project NATP on Sustainable Management of Plant Biodiversity:

Materials and Method

With this objective more than twelve hundred *Brassica* germplasm comprising of 651 *B. juncea*, 443 *B. campestris*, 35 B. napus, 10 *B. nigra*, 18 *B. carinata*, 6 *B. chinensis*, 63 Eruca sativus, 5 *Sinapis alba*, 3 *Raphanus* sp., 5 B. tourniforti, 1 Crambe asyssinica, 18 *B. rapa* and 3 miscellaneous species, obtained from National Bureau of Plant Genetic Resources, India were analyzed by HPLC as per the method standardized in our laboratory (Kaushik and Agnihotri, 1999) to assess the glucosinolate profile.

Results

The glucosinolate content in the *Brassica* germplasm collection analyzed is summarized in Table 1 and the variation in the profile of glucosinolates in the *B. juncea* germplams is elucidated in Figure 1. The highlights of glucosinolate content in *Brassica* species are summarized below:

B. nigra

EC accessions of *B. nigra* are characterized by high allyl glucosinolate while Indian accessions recorded medium allyl and high butenyl. Total alkyl glucosinolate ranged from 51-210 μmole/g defatted meal. Moderately low glucosinolate (51μ mole) was recorded in Indian accession IC 253036.

B. campestris/ *B. rapa*

These accessions recorded predominantly high butenyl glucosinolate (>100μmole). There were no significant differences in the three varieties of *B. campestris* viz. toria, yellow sarson and brown sarson in terms of glucosinolate profile. Allyl and hydroxy-butenyl were either absent or present in negligible amounts (⩽5μ moles). The total glucosinolate ranged from 115–407 μ moles/g, none of the accessions recorded low glucosinolate.

B. chinensis

B. chinensis which is also referred as sub-species of *B. campestris,* showed glucosinolate profile similar to that of *B. campestris* having high butenyl and low amounts of allyl glucosinolate. The total glucosinolate ranged from 64-256 μ moles/ g. All the collections analyzed were exotic collections.

B. juncea

Predominantly high butenyl glucosinolate was recorded for the *B. juncea* accessions. Among the *B. juncea* samples analyzed, nearly 20 belonged to the sub-species ragosa, which characteristically yielded high allyl glucosinolate as compared to Indian *B. juncea* germplasm, which registered medium allyl and high butenyl glucosinolate. Although *B. juncea* accessions predominantly recorded high butenyl, medium allyl content, however, there were quite a few accessions which recorded high allyl and low butenyl combinations. Accession No. IC401769 recorded both high allyl (194μ moles) as well as high butenyl (162μ moles). Only a few recorded medium butenyl and medium hydroxy butenyl. Two of the EC accessions i.e. EC 367885 (22μ moles) and EC 394357 (11μ moles) recorded less than 30μ moles glucosinolate content. Therefore, these two exotic germplasm collections are valuable for transfer of low glucosinolate content in Indian *B. juncea* varieties. Overall, if we exclude these two varieties the total glucosinolate content varied from 64-373 μ moles.

B. napus

Most of the *B. napus* germplasm analysed were exotic collections and recorded characteristically high hydroxy butenyl glucosinolate, having low allyl glucosinolate. One of the Indian selection GSL-1-SEL recorded medium glucosinolate (40 μ moles).

B. nigra

B. nigra showed high butenyl, medium allyl type of glucosinolate profile. A few accessions showed hydroxy butenyl in place of allyl glucosinolate. This could be due to cross pollination. The total glucosinolate ranged from 51-210 μ moles. 51μmoles was recorded in Indian collection IC 253036.

B. tourniforttii

B. tourniforttii accessions recorded varied glucosinolate profiles, ranging from high allyl, medium butenyl and high hydroxy butenyl, representing wide variability in terms of glucosinolate content.

Raphanus species

These species recorded high allyl, high butenyl and low hydroxy butenyl. The total glucosinolate was more than 150 μmole/g.

B. carinata

B.carinata germplasm recorded high hydroxy butenyl and very low to medium butenyl. One of the accessions i.e. EC 223405 recorded low glucosinolate i.e 23μ moles/ g. The total glucosinolate of the rest of the accessions varied from 47 to 151μmoles/g except for EC 400044 which recorded very high glucosinolate i.e 292μ moles/g.

Eruca sativa

Eruca sativa accessions recorded varied glucosinolate profiles having predominantly high butenyl glucosinolate. Accessions EC 400096 and EC 400072 recorded less than 19-28μ moles/g glucosinolate respectively. None of the Indian accessions recorded low glucosinolate and it varied from 52-129 μ moles/g.

Sinapis alba

All the *S. alba* accessions were exotic collections and had butenyl as predominant glucosinolate with low to medium amount of allyl. The total glucosinolate ranged from 204-288μ moles.

Discussions

The Indian *B. juncea* accessions have allyl and butenyl glucosinolates while the exotic collections have the hydroxy-butenyl and butenyl glucosinolate. However, some of the IC numbers also showed this trend, and a few *B. juncea* accessions have very low allyl glucosinolate content. The *B. napus* has hydroxy-butenyl and butenyl as the predominant glucosinolate type. The accessions of *B. campestris* var. sarson, yellow sarson and toria have butenyl and allyl or hydroxy-butenyl glucosinolates, which may be contributed to cross pollination in these species due to self incompatibility. *B. carinata* has allyl

as the predominant glucosinolate. Allyl is also found in significantly high amounts in *B. juncea* var. ragosa and *B. nigra* while *B. tournifortii* is characterized by mediium amounts of allyl glucosinolate. Low amount of allyl is synthesized in *B. campestris* varieties, *B. chinensis, B. rapa, E. sativa* and *S. alba.* Twenty germplasm lines of *B. napus,* two *B. juncea*, one *B. carinata*, one *Crambe asyssinica,* two *E. sativa* and one miscellaneous species have been identified having ⩽30µ moles glucosinolate. However, all of these are exotic collections (EC). All Indian accessions of *B. juncea* and *B. campestris*, the most important and widely grown oilseed *brassica*s analyzed so far, have high glucosinolate (65 to 363µ mole/g) and there is no Indian source of *B. juncea* or *B. campestris* having ⩽ 30µ mole/g glucosinolate. Therefore, there is a need for further rigorous systematic characterization of Indian accessions for their quality to identify any potential donor source for low glucosinolate gene.

Table 1: Summary of Glucosinolate Characterization in *Brassica* species

Species	No. of Sample	Glucosinolate (µ mole/g oil free meal)			Significant Germplasm
		Range	⩽30µmole/g	30-60µmoles/g	
B. juncea	651	11 to 389	2	4	EC367885 -22 µmoles/g, EC394357 - 11 µmoles/g
B. campestris / *B. rapa*	461	44 to 480	-	12	None
B. napus	35	3 to 159	20	4	EC 400802, EC 400803 etc. – 3 to 26µmoles/g
B. nigra	10	52 to 211	-	1	None
B. carinata	18	23 to 292	1	3	EC223405 - 23 µmoles/g
B. chinensis	6	64 to 256	-	-	None
Eruca sativa	63	28 to 129	2	25	EC400072 - 28 µmoles/g, EC400096 - 19 µmoles/g
Sinapis alba	5	204 to 288	-	-	None
B. tournifortii	5	50 to 144	-	2	None
Raphanus sp.	3	111 to 163	-	-	None
Crambe asyssinica	1	25	1	-	EC400058 - 25 µmoles/g,
Misc. (SPP)	3	27-76	1	-	EC361801 - 28 µmoles/g
Total	1261		27	51	

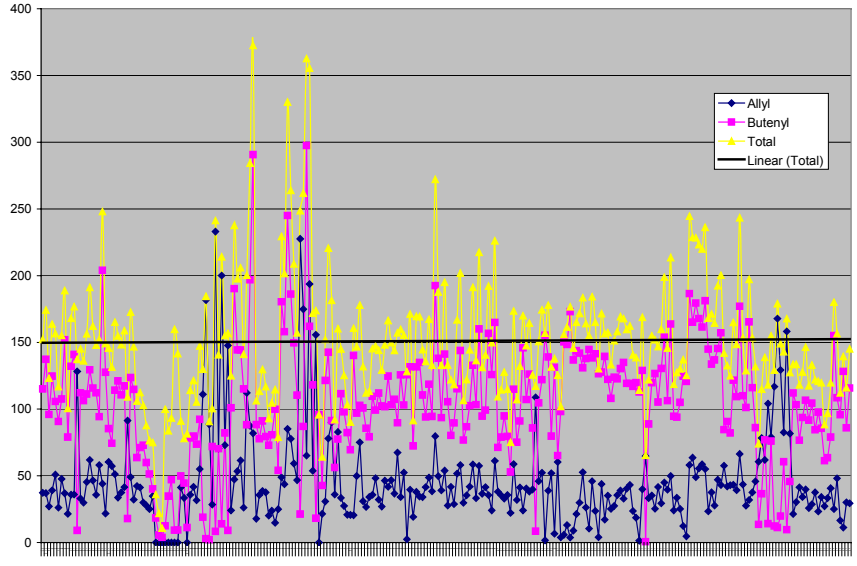

Figure 1: Glucosinolate pattern of *B. juncea* germplasm

Conclusion

The search for new low glucosinolate donors for Indian cultivars of rapeseed-mustard has provided useful insight into the rich diversity for this secondary metabolite. The present work showcases the diversity for glucosinolate profile and total content in various *Brassica* and related species. This cataloguing of variation is essential from the point of view of maintaining germplasm collections as well as identifying potential donors.

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