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Erucic acid and glucosinolate variability in *Brassica juncea* L

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Abstract
An experiment was conducted during 2016-2017 to study the most important anti-nutritional compounds i.e. erucic acid and glucosinolates in fifteen genotypes of *Brassica juncea* L. Significant differences ($p \leq 0.05$) were observed among the *Brassica juncea* L. genotypes for the undesirable characters of edible oil, viz. erucic acid and glucosinolates. The erucic acid content ranged from from 0.90 to 59.83 %. Maximum erucic acid content i.e. 51.83% was estimated in SKJM-5 closely followed by Pusa Tarak (50.74%) and Pusa Bold (49.57%), whereas maximum erucic acid content was noted in Pusa Karisma (0.9%). The glucosinolate content varied from 58.83-132.26 µmol/g defatted seed meal. RSPR-01 genotype showed minimum glucosinolate content, whereas maximum was observed in Pusa Tarak. The significant variability of the erucic acid and glucosinolate content in the various *Brassica juncea* L. genotypes observed in the present study can be utilized in the breeding programmes to develop genotypes with higher qualitative potential.

Keywords: *Brassica juncea* L, erucic acid and glucosinolate

Introduction
Mustard is a main source of comestible oil in Indian diet especially in North India. The major fatty acids of mustard oil are oleic, linoleic, linolenic, eicosanoic and erucic acid. Erucic acid in oil of mustard genotypes is relatively high (Chauhan *et al.*, 2007) [5]. High erucic acid oilseed rape cultivars has high amount of erucic acid in edible oils which may increase health risks (Khan *et al.*, 1985) [10]. Earliar studies reported impaired myocardial conductance that causes lipidosis in children and increases blood cholestrol (Ackman *et al.*, 1977) [1]. Rapeseed-mustard cultivars grown in India also have high level of glucosinolate content (Chauhan *et al.*, 2007) [5]. Glucosinolates are another most important anti-nutritional compounds. The glucosinolates are nitrogen and sulphur containing natural plant products and a group of plant thioglucosides, found principally among members of family *Brassicaceae* are responsible for the characteristic pungency of rapeseed-mustard oil. The glucosinolates are broken down by the enzyme thioglucoside glucohydrolase commonly known as myrosinase to yield sulphate, glucose and other aglucon products. Cleavage products from hydrolysis are detrimental to animal health as they reduce the feed palatability and affect the iodine uptake by the thyroid glands thus reducing feed efficiency and weight gains (Bell, 1984) [3] especially in non-ruminants. Because of the adverse effects of high erucic acid in oil (35.7–51.4%) and glucosinolates in seed meal (49.9-120.3 µmol/g defatted seed meal) of Indian mustard varieties (Chauhan *et al.*, 2007 and Rai *et al.*, 2018) [5, 14], rapeseed-mustard varietal improvement programme in India aims at reducing erucic acid level up to 2% and glucosinolate content up to 30 µmole/g defatted seed meal as per Internationally accepted norms. Breeders need large variability to initiate selection programs (Choudhary *et al.*, 2015) [7]. Therefore, study of genetic diversity of erucic acid and glucosinolates in Indian mustard collection would help breeders in genitors screening in order to develop *Brassica* germplasm having low undesirable fatty acids and glucosinolates. Keeping this in view, the present study was carried out with the aim of evaluating various *Brassica juncea* L. genotypes for their erucic acid content and glucosinolates content as well as assessing the variation for these traits. The variability of erucic acid content and glucosinolates content in the *Brassica juncea* L. genotypes/cultivars observed in the present study can be utilized in crop improvement programme to develop *Brassica juncea* L genotype with quality oil potential.

Materials and Methods

Plant materials

The seeds of 15 *Brassica juncea* L. were procured from Division of Plant Breeding and Genetics, SKUAST-Jammu. The procured seed material was cultivated at the experimental field of SKUAST- Jammu. Three rows of each genotype were planted and the recommended package practices and plant protection measures were followed. Seeds were harvested when the plants attained complete maturity. Harvested seeds were used for oil content and erucic acid analysis.

Determination of oil content

The oil content from the matured seeds was extracted in the soxhlet apparatus using petroleum ether as solvent for 6 h according to the AOCS method (AOCS, 1993) [2]. Seeds of different mustard genotypes were dried at 40 °C for 4 h in a hot air oven to reduce the moisture level to 4- 5%. The dried seeds were then thoroughly ground and mixed with ether for extracting the total oil content. Subsequently, ether was removed from the oil by rotary evaporator under pressure and the oil was collected in glass vials for further erucic acid analysis.

Fatty acid profiling

The erucic acid profiling of the *Brassica juncea* L genotypes was carried out using Gas- Liquid Chromatography (GLC). Fatty acid methyl esters (FAME) of oil sample from each genotypes were developed according to the method sugested by Goli *et al.,* (2008) [21]. Agilent 6890N gas chromatography equipped with a Flame Ionization Detector (FID) was used for FAME analysis. The oven injector and detector temperatures were regulated at 230 and 250^{0}C, respectively. Ultra-pure nitrogen gas was used as carrier. The peaks of FAME were analysed by comparing their retention time to that of the known erucic acid standard which has been subjected to similar separation conditions. The amount of erucic acid was expressed as % of the total fatty acids.

Estimation of Glucosinolates

Spectrophotometric estimation was done using methanolic extract prepared from the same genotypes by homogenizing 0.1 g defatted seed meal in a 2 ml vial with 80% methanol. This homogenate was centrifuged at 3000 rpm for 4 min after keeping overnight at room temperature. The supernatant was collected after centrifugation and made up to 2 ml with 80% methanol. 100 µl of this extract was used for estimation. 0.3 ml double distilled water and 3 ml of 2 mM sodium tetrachloropalladate (58.8 mg Sodium tetrachloropalladate + 170 µl concentrated HCl +100 ml double distilled water) were added to it. After incubation at room temperature for 1 hour, absorbance was measured at 425 nm using a spectrophotometer (Labomed UV-VIS Double beam UVD-3500). A blank was set following the same procedure without the extract. Total glucosinolates was calculated by putting the OD of each sample taken at 425 nm into the predicted formula y = 1.40 + 118.86 × A425 (Mawlong *et al.,* 2017) [22].

Statistical analysis

All tests were conducted in triplicate. Data are reported as means standard deviation (SD). Analysis of variance and significant differences among means were tested by one-way ANOVA using the SPSS computer package (version 10.1). The differences were considered to be significant difference (LSD) at $P < 0.05$.

Results and Discussion

Analysis of variance (ANOVA) revealed significant in the erucic acid and glucosinolates content of 15 *Brassica juncea* L. genotypes. Erucic acid is one of the most important undesirable fatty acids in found mustard oil. This 22-carbon fatty acid is detrimental for human consumption when present in higher amounts in edible oil. Higher erucic acid in cooking oil hampers the myocardial conductance in humans and leads to increased blood cholesterol levels (Bozzini *et al.,* 2007; Sinha *et al.,* 2007; Rai *et al.,* 2018) [4, 18, 14]. The erucic acid content in *Brassica juncea* L. genotypes varied from 0.90 to 51.83 % (Figure 1). Minimum erucic acid content (as per international oil quality norms ≥2.0%) was observed in PM-24 (1.56%), PM-21 (0.96%) and Pusa Karishma (0.90 %). However, highest erucic acid content was noted in SKJM-5 (51.83%). Similar finding were also reported by Rai *et al.,* (2018) [14], Singh (2018) [17], Sharafi *et al*., (2015) [15], Singh *et al.,* (2014) [16], Chhokar *et al.,* (2008) [6], Kumar *et al.,* (2002) [11], Kaushik and Agnihotri (2000) [9], Choudhary and Rai (2013) [20] and Rai *et al.,* (2018) [14]. The presence of high erucic acid in oil is considered anti-nutritional, as it has been reported to cause lipidosis in children and myocardial fibrosis in monkeys (Ackman *et al.,* 1977) [1]. At present, when the *Brassica* breeding programmes are focused towards the development of zero erucic lines for nutritional purpose the genotypes Pusa Karishma, PM-21 and PM-24 with low erucic acid will be of huge importance. Maximum genotypes in the present study which showed higher levels of erucic acid will be of utmost importance for various industries.

The glucosinolates are broken down by the myrosinase enzyme (thioglucoside glucohydrolase) to sulphate, glucose and other aglucon products. Hydrolysed products from hydrolysis of glucosinolates are detrimental to health as they reduce the feed palatability and affect the iodine uptake by the thyroid glands thus reducing feed efficiency and weight gains (Bille *et al.* 1983) [19] The glucosinolate content was estimated in all fifteen *Brassica juncea* L. genotypes. Significant variation in the glucosinolate content was observed in the test genotypes. The mean values for glucosinolate content varied from 58.83-132.26 µmol/g defatted seed meal (Figure 2). Minimum and maximum were observed in RSPR 01 and Pusa Tarak, respectively. The values of glucosinolate content are in close proximity to the published data on different varieties from India (Kumar *et al.,* 2010) [12] and to those of Beltagi and Mohamed (2010) [8] who reported that the 4.4 to 5.97 µmol /g dw in *Brassica napus*. These findings were confirmed in 4MTB-ITC analyses using 38 cultivars, mainly including Japanese and Chinese cultivars (Okano *et al.* 1990) [13]. The *Brassica species* genotypes grown in India have high amount of nutrionally and undesired components "Glucosinolates". The maximum research report were reported high glucosinolate range (80-160 µ mg^{-1}) in cultivars.The high glucosinolates content oil is not fit for human and animal consumption due to health issues (Agnihotri, 1999, Chaudhary and Rai, 2013) [20]. The information related to the significant variability of the erucic acid and glucosinolates content in the various *Brassica juncea* L. genotypes observed in the present study can be utilized in the breeding programmes to develop genotypes with higher qualitative potential.

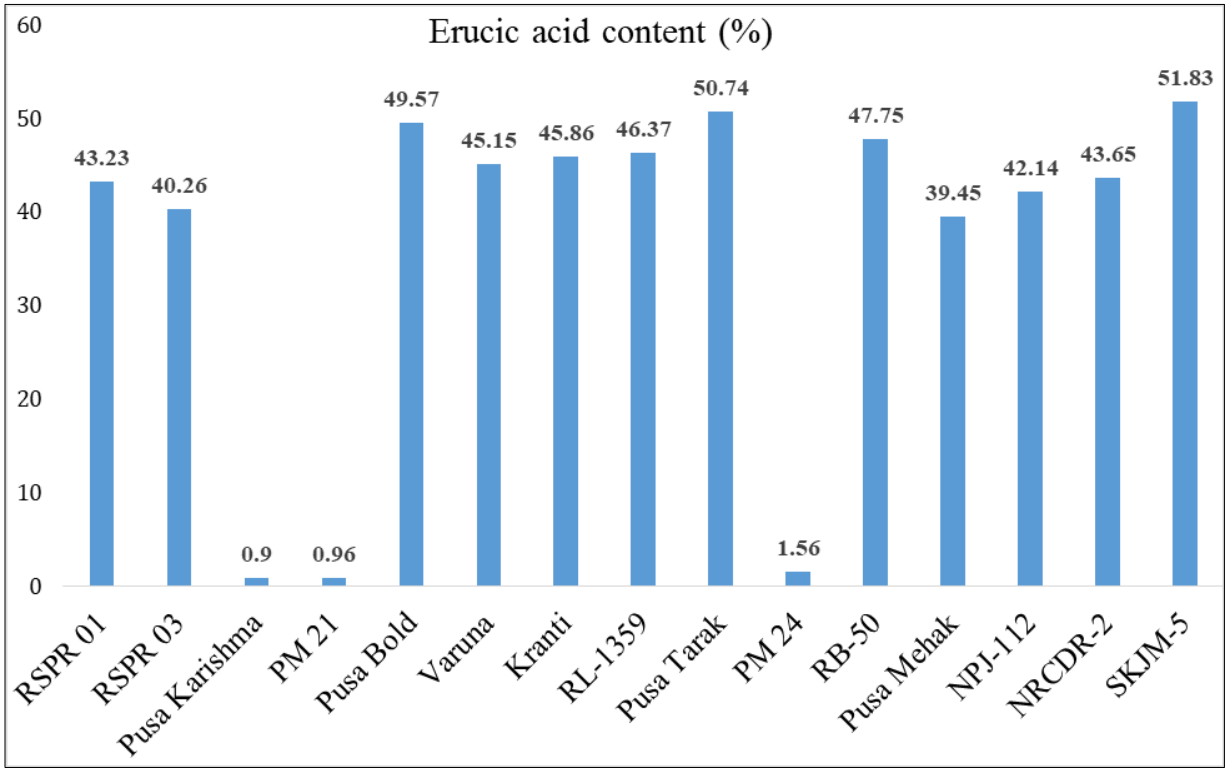

Fig 1: Erucic acid content in *Brassica juncea* L genotypes

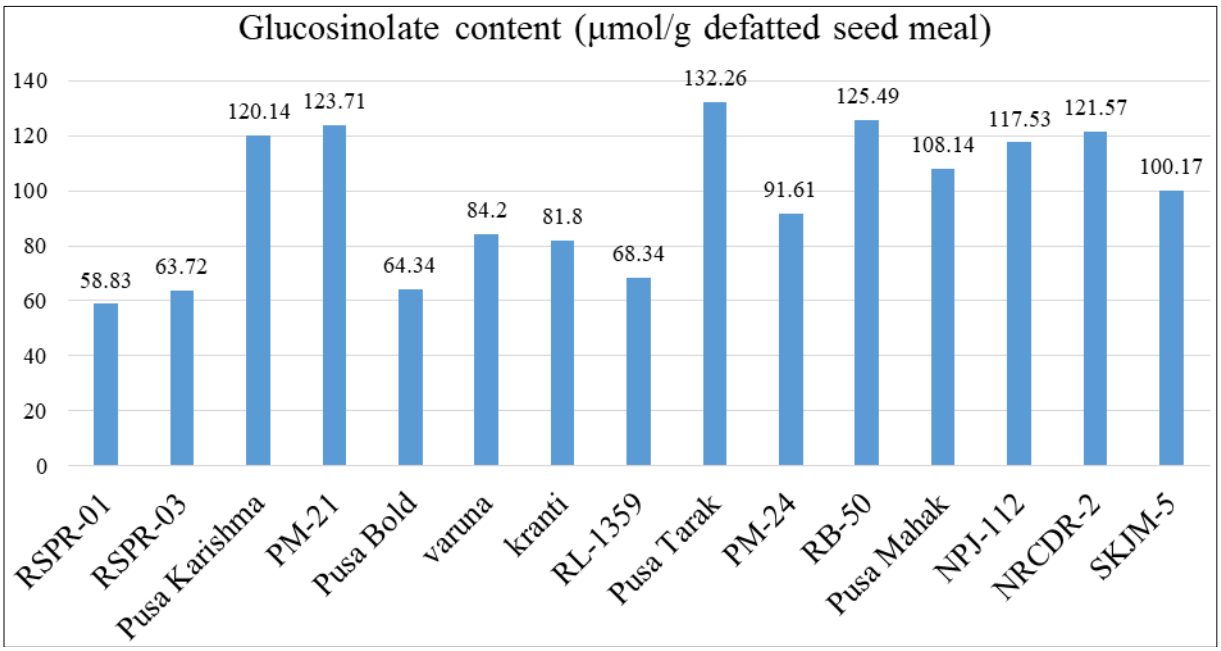

Fig 2: Glucosinolate content (µmol/g defatted seed meal) in *Brassica juncea* L. genotypes

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