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Variation in glucosinolate of seed and leaves through induction of sulfur in cotyledon and hypocotyls regenerates of mustard (*Brassica Juncea* L.)

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ABSTRACT

The present study were conducted in the crop breeding division, nuclear institute of food and agriculture (NIFA) to evaluate the glucosinolate contents in *Brassica Juncea* L. (Mustard). Therefore, the effect of different concentrations of $MgSO_4$ (Basal Salt) were studied on the total glucosinolate content in seed and leaves of hypocotyls and cotedonary regenerated plant of *B. juncea* L. For the callus induction and plant regeneration, the explants were harvested on MS synthetic media, containing modified two and four folds of the basal salt of $MgSO_4$ concentrations. The total glucosinolate contents in both the seed and leaves of mature regenerated plant were determined using UV-Visible spectroscopy. The higher concentrations of $MgSO_4$ were observed for the increase of glucosinolate content in the seed as well in the leaves of *B. juncea* L. Therefore, we concluded that the soil with higher concentration of sulfur in any form specifically in their salts like $MgSO_4$ or other will increase the glucosinolate contents in the *B. juncea* L.

KEY WORDS: Mustard,*Brassica juncea* L., Glucosinolate, Sulfur, Basal Salt.,

1. INTRODUCTION

Brassica juncea L. (Mustard) belongs to the families of *brassicaceae* that is a famous crops for the production of vegetable oil and meal (protein-rich)throughout the world [1]. Glucosinolate and erucic acid are the two important secondary metabolites of all the species of *Brassica*. Glucosinolate in the plants are responsible for the pungent flavor and the high concentration of erucic acid in oil may increase health risks [2].

Glucosinolate is a group of sulfur containing compounds [3]which occurs in all parts of the plant including root, stem, leaf and seeds. However its highest concentration is present in seeds [4-5], which may also act to attract some of the insects or repel the others [6]. *Brassica* family, if used as vegetables can inhibit the cancer growth [7-8]. The initial content of the GSL in the seed and vegetative parts can be influenced and modified by a number of factors such is moisture [9], temperature [10], air composition [11], and light [12] also in different growing seasons [13]. The content of GSL in *Brassica* plant is also be affected by the use of fertilizer and pesticides [12, 14].

The emergence of the advance biotechnological techniques, for example gene cloning, genetic engineering, molecular breeding and tissue culture have improved the quality of the seed of edible oil [15]. However the achievements will depend careful application and combination of techniques for the production of good quality and yielding genotype for sustained production[16]. Tissue culture technique has provided a new pathway for inducing variation and therefore, significant improvements have been achieved [17-18]. Therefore, in the present study, the concentration of sulfur was changed in the MS medium [19] to evaluate its effect on the total GSL content in the regenerates derived from canola or rapeseed (*Brassica juncea* L.) plant.

The aim of the preset study was to develop an approach for the reduction of GSL content in oil seed *Brassica* for its utilization as rich source of nutrition for animal, whereas; to increase of glucosinolate content in the leaves and stem as insect and pest repellent and the effect of different concentrations of sulfur in MS medium on total glucosinolate content.

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2. MATERIAL AND METHODS

The healthy and mature seed of three genotypes viz. DJL, BM-1 and JO-07 of *Brassica juncea* L. species were selected for the present study. The sterilized seed of all the genotype were germinated on half strength hormone-free MS medium with 1% sucrose and 0.7% plant agar of on pH of 5.8. Twenty (20) seed were placed on the germination medium in each petri dish. This allowed the germinating seed to grow taller and straighter which made it easier to harvest the cotyledon and hypocotyls explants. The plated seed were kept at 25 ^{o}C in the growth chamber. Three types of media i.e. MI, MII and MIII were prepared by using MS medium with 3% sucrose, 4.5 mg/L benzyl adenine (BA) and solidified with 7% agar. MII was prepared from MS medium with two times the concentration of $MgSO_4$ and MIII was prepared from MS medium with four times the concentration of $MgSO_4$, while the pH was maintained at 5.8. The cotyledon and hypocotyls were cut from the developed seedling within one week time from the 20 healthy and fully unfold cotyledon (from 10 seedling) were dropped on the surface of a plate of each medium, however the similar procedure was followed for the hypocotyls. Callus induction occurred within 2-3 week in both the explants. Once a good root system was developed, the plantlets were transferred to moist potting soil for the growth and further analysis. The total GSLs content were determined in the leaves and seeds of regenerated plants using UV-Vissible spectrophotometer. The study was conducted in randomized complex Block (RCB) design with split plot arrangement using media with different sulfur concentration as main plot and genotype as sub plot with four replications. The data were analyzed with Mstat-C statistical package [23].

3. RESULTS

3.1. Effect of Sulfur on GSL for Cotyledon

Three genotype of (*Brassica juncea* L.)i.e., DJL, BM-1 and JO-07 were culture on different types of media for cotyledon callus induction to characterize their GSL contents, the results have shown in (**Figure 1**). The mean value for the GSL content of the seeds of all the three genotypes were 63.82, 65.97 and 65.33µmol/g in the three media MI, MII and MIII respectively. While the mean of each individual genotype were also determined and their mean of total GSL content in the seeds of each genotypes were 15.05, 74.22 and 105.87 µmol/g of DJL, BM-1 and Jo-7 respectively. No significant variations were observed in the GSL concentration in cotyledon callus of the seeds of each individual genotype, (**Table 1**).Whereas, the mean value for the GSL content of the leaves of all the three genotypes were 14.95, 14.09 and 14.39 µmol/g in the three media MI, MII and MIII respectively. While the mean of each individual genotype were also determined and the mean of total GSL content in the leaves of each genotypes were 6.37, 19.63 and 18.40 µmol/g of DJL, BM-1 and Jo-7 respectively. No significant variations were observed in the GSL concentration in cotyledon callus of their leaves of each individual genotype, (**Table 1**).

Table 1.Effect of different concentration of sulfur on GSLs in the seed and leaves of plants regenerated from cotyledon in different genotype of (*Brassica juncea* L.).

Genotype	Seeds				Leaves			
	MI µmol/g	MII µmol/g	MIII µmol/g	Mean	MI µmol/g	MII µmol/g	MIII µmol/g	Mean
DJL	15.1	15.025	15.025	15.05	6.645	5.05	6.425	6.373
BM-1	71.925	75.875	74.85	74.22	19.6	19.5	19.5	19.63
JO-07	104.45	107.025	106.125	105.87	18.625	17.725	17.25	18.40
Mean	63.825	65.975	65.33333		14.95667	14.09167	14.39167	

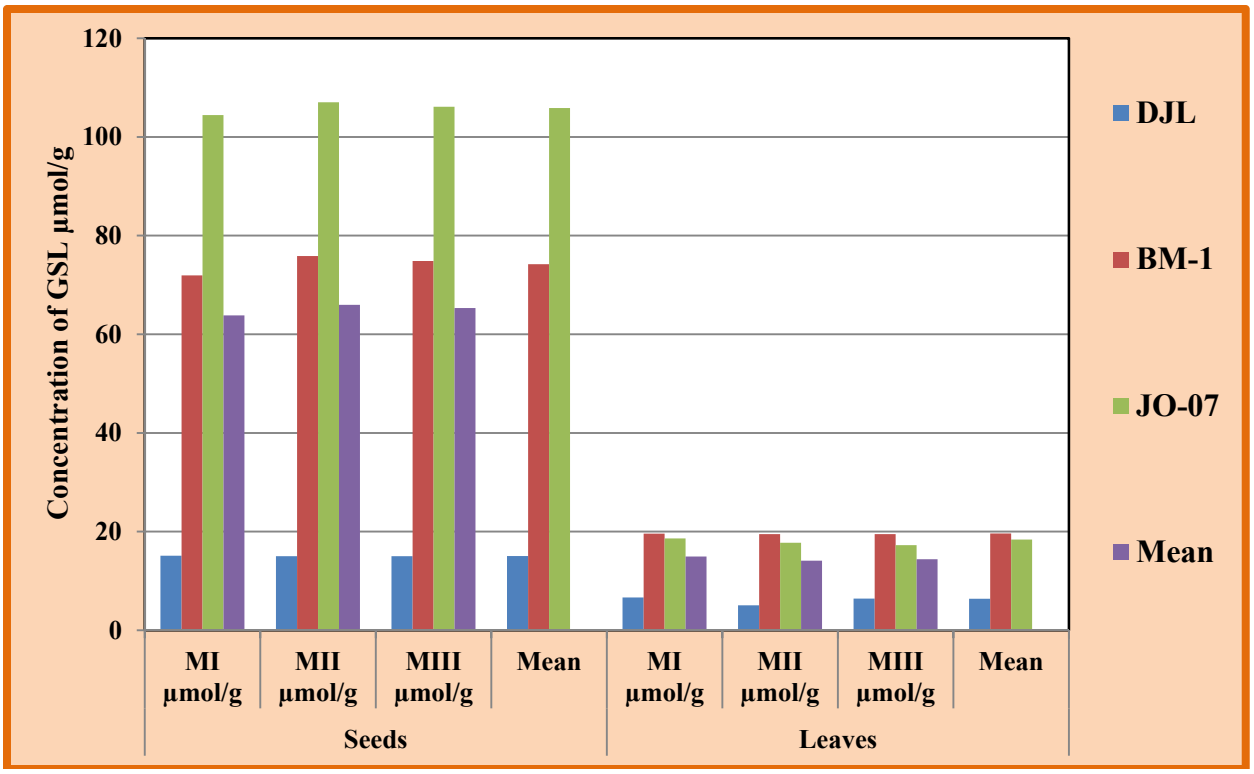

Figure1: Shows the total and mean of the GSL contents of the three genotypes

3.2. Effect of Sulfur on GSL for Hypocotyls

Three genotype of (*Brassica juncea* L.) i.e., DJL, BM-1 and JO-07 were also culture on different types of media for hypocotyl callus induction to characterize their GSL contents, the results have shown in (**Figure 2**). The mean value for the GSL content of the seeds of all the three genotypes were 63.07, 65.22 and 64.58 µmol/g in the three media MI, MII and MIII respectively. While the mean of each individual genotype were also determined and hence, the mean of total GSL content in the seeds of each genotypes were 14.30, 73.46 and 105.11 µmol/g of DJL, BM-1 and JO-07 respectively. No significant variations were observed in the GSL concentration in hypocotyl callus of their seeds of each individual genotype, (**Table 2**). Whereas, the mean value for the GSL content of the leaves of all the three genotypes were 14.75, 14.12 and 14.62 µmol/g in the three media MI, MII and MIII respectively. While the mean of each individual genotype were also determined and hence, the mean of total GSL content in the leaves of each genotypes were 6.07, 19.33 and 18.10 µmol/g of DJL, BM-1 and JO-07 respectively. No significant variations were observed in the GSL concentration in hypocotyl callus of their leaves of each individual genotype, (**Table 2**).

Table 2.Effect of different concentration of sulfur on GSLs in the seeds and leaves of plants regenerated from hypocotyls in different genotype of (*Brassica juncea*L.)

Genotype	Seeds				Leaves			
	MI µmol/g	MII µmol/g	MIII µmol/g	Mean	MI µmol/g	MII µmol/g	MIII µmol/g	Mean
DJL	14.35	14.275	14.275	14.30	6.345	5.75	6.125	6.0733
BM-1	71.175	75.125	74.1	73.4667	19.6	19.2	19.2	19.333
JO-07	103.7	106.275	105.375	105.117	18.325	17.425	18.55	18.1
Mean	63.075	65.225	64.5833		14.7567	14.125	14.625	

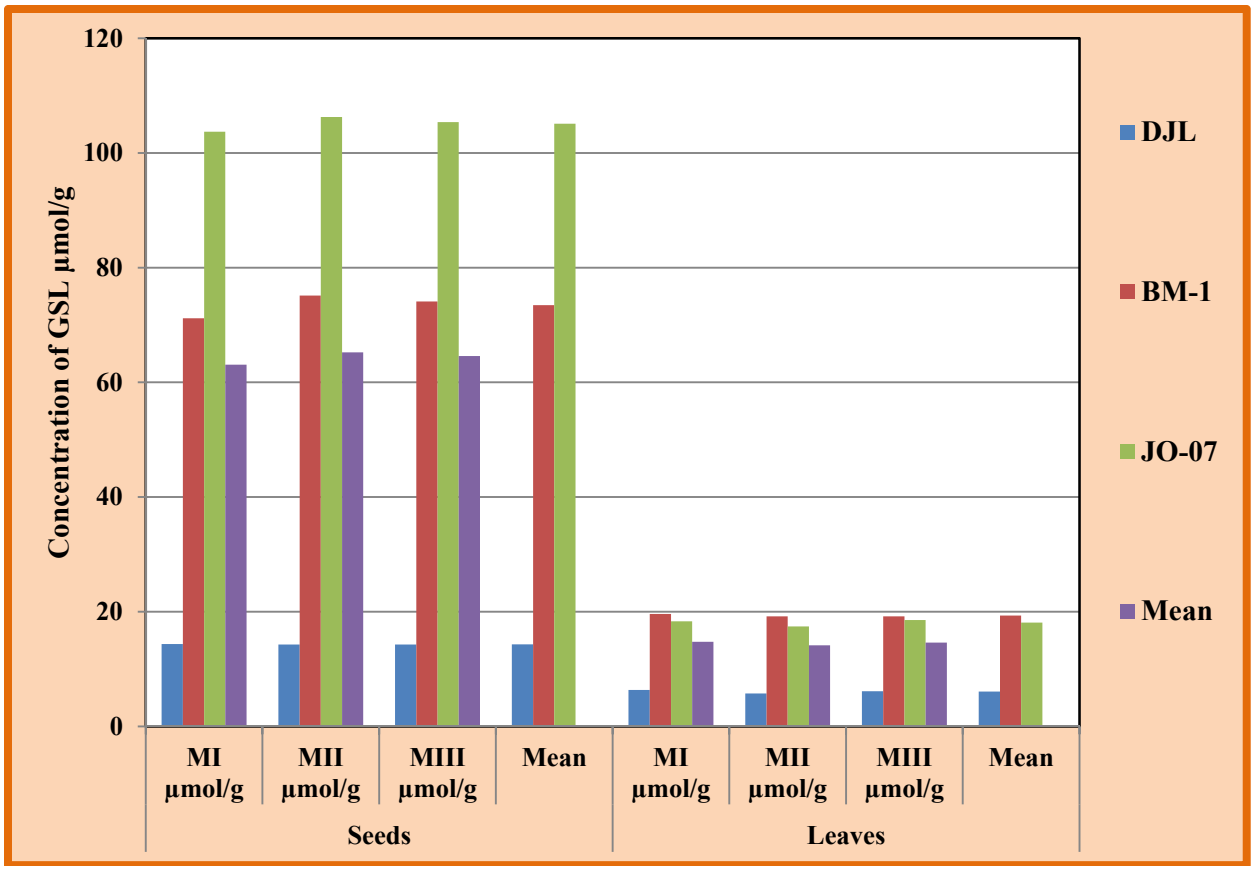

Figure2: Shows the total and mean of the GSL contents of the three genotypes

3. DISCUSSION

Literature survey reveal that 1708 entries of the 20 *Brassica* species were evaluated for seed glucosinolates (GS) in 2000 by using a new technique of near-infrared reflectance spectroscopy, in which 150 entries were observed to have high GS contents which was further confirmed by high performance liquid chromatography (HPLC)[20]. However in our case there is potential lower value of GS contents observed. Another study was conducted in 2004, by analyzing six cultivars of Canola (*Brassica. napus*) for total glucosinolates and fatty acids contents using near infrared reflection spectroscopy. Significant variations were observed in all the chemical constituents. However, Glocosinlates content was less than 30 uM/g [21], keeping in view these results, our results are quite complementary to those observed for the six canola type, and further suggested to be improved to lower the GS contents.

Further, studies were conducted in 2008 to evaluate the biochemical parameters of five F2 lines along with their nine parent lines, and they observed high GS contents in both set of population [22]. These findings again support our work for low GS contents. Khan et al., simultaneously evaluated six F3:4 derived interspecific Brassica population in 2008 to evaluate glucosinolates and some other biochemical's and hence they observed to have highly significant genetic variation for GS contents except protein. Glucosinolates, contents had high heritability values while protein content was observed to be low heritable trait [22].

4. Conclusion

In the present study we determined the effect of sulfur on the total GSL contents of the three genotypes of (*Brassica juncea* L.). The overall result of the present study showed that increase in the sulphate content in the MS Medium during in vitro studies has increased the GSL content in the regenerates derived from cotyledon and hypocotyls explants. While two fold increase in the sulphate content in the MS medium enhanced the callus induction from the cotyledon and hypocotyl explants of all the three genotypes used for this research study. However the four times increase in the sulphate content in MS medium have no effect on the growth and callus of cotyledons and hypocotyls in (*Brassica juncea* L.). The plant regeneration ability from cotyledon and hypocotyls

explants was not influenced by the modified concentration of sulfur in the MS medium. GSL contents are irrespective to the sulphate contents in MS media.

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