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MUTATION BREEDING STRATEGIES FOR GLUCOSINOLATE CONTENTS IN *Brassica juncea* (L.) COSS. & CZERN. Cv. Varuna

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ABSTRACT:

Low glucosinolate mustard is one of the major goals for quality oil and meal. To achieve this goal seeds of *Brassica juncea* (L.) COSS & CZERN CV. Varuna were treated with different concentrations of ethyl methane sulfonate and sodium azide. The mutagenized seeds were sown in the field to raise M_1 generation. Seeds of M_1 generation were harvested plant – wise and sown in the field to raise M_2 generation. M_2 seeds were harvested plant – wise and analyzed by Tes-tape for glucosinolate content. M_2 seeds with Westar level (<20 µmole/gm of deoiled meal) glucosinolate were selected. Glucosinolate levels were divided into three different categories depending upon the glucosinolate content. The Westar seeds contain <20 µmole of glucosinolate while control seeds of Varuna has about >155 µmole of glucosinolate. Hence, Westar level selected varuna seeds were sown in the field to raise M_3 generation. All plants were selfed and on maturity, harvested plant – wise. Upon screening for Westar level glucosinolate, the M_3 seeds showed variable levels of glucosinolate. M_3 seeds were observed to segregate in medium and high level of glucosinolate. The data obtained indicate positive possibilities of induced low glucosinolate mutation, however, the following generation need careful handling, selfing, crossing, screening and large population size.

Keywords: - *Brassia juncea*, Glucosinolate, Mutation breeding strategies, EMS, Sodium azide.

INTRODUCTION: -

Brassica juncea Cv. Varuna is a major oil yielding crop in India. It is commonly known as Indian mustard. After extraction of oil the remaining meal of mustard could become an important animal feed. The mustard meal has about 35 to 40% of crude protein content. This highly proteinaceous fraction increases the dietary value of the meal for animals. However, due to the presence of Glucosinolate the Indian mustard meal has limited usage. Indian mustard *B. juncea* Cv. Varuna has about >155 µmole of Glucosinolate/gm of deoiled meal. Hence, investigations on induced mutation for glucosinolate

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content in *B. juncea* Cv. Varuna were undertaken. Mutagens exposed populations have been screened for three generations. Results obtained have indicated certain problems which are discussed in this presentation.

MATERIAL AND METHODS:

Genetically pure and physiologically uniform *B. juncea* Cv. Varuna seeds were exposed to different doses of ethyl methane sulfonate (EMS) and sodium azide (SA). All concentration of EMS and SA were prepared on V/V and V/W basis, respectively. The mutagen treated seeds were utilized to raise M1 generation. They were harvested plant-wise and M_2 population raised. M_2 seeds were screened for glucosinolate content by Tes-tape method /1,3,5/. Seeds of Westar (*B. napus*) were used as low glucosinolate standard whereas non treated seeds of Varuna used as control. Upon selection, the M_2 low glucosinolate seeds were sown to raise the M_3 generation. M_3 seeds were screened for low glucosinolate. The levels of glucosinolate were categorized into 3 different types (-), (±) and (+) on the basis of presence or absence of glucosinolate.

RESULTS AND DISCUSSION:

In 1960's, Bronoski, identified a plant of *B. napus* Cv. Polish with low glucosinolate (about 10 to 12 µmole/gm of deoiled meal). Since-then the efforts to produce low glucosinolate *Brassica* species have caught speed. Westar (*B. napus*) was the first '00' produced in Canada.. Low glucosinolate lines of *B. napus* cannot grow in Indian agro -climate due to its long day dependency. Hence, efforts were made in our laboratory to induce mutation for low glucosinolate in *B. juncea* Cv. Varuna.

In the present investigation, the data obtained on glucosinolate level of M_2 and M_3 generations are recorded in table 1 and 2, respectively. The data obtained in M_2 show that Westar level of glucosinolate was recorded in 12 hPsw + 3h SA 0.03%; 12 h SA 0.008% and 18h SA 0.008% treatments. While (±) level of glucosinolate was observed in 12 h Psw + 3h SA 0.03%. Remaining treatment had plants with (±) level of glucosinolate. However, 12 h SA 0.008%

did not have any low glucosinolate plants. Plants with (±) level of glucosinolate were more (5) in 18h EMS 0.01% whereas in 12h Psw + 3h 0.03% and 12h EMS 0.01%, the (+) level of glucosinolate was not observed. 12h Psw + 3h SA 0.03%, 12h SA 0.008% and 18h SA 0.008% treatments had two plants with (+) level. The M3 data for glucosinolate content are surprisingly different than M_2 (Table 2), the Westar level of glucosinolate plant was not recorded in M3 however, 12h SA 0.008%, 18h SA 0.008% and 12h Psw + 3h SA 0.03% showed 4,2 and 1 plants with (±) level of glucosinolate, respectively. The (+) level of glucosinolate was apparent in all treatments in M_3 except 12h Psw + 3h EMS 0.03%.

These results clearly indicate that the breeding for glucosinolate is intricate. The glucosinolate level in *Brassica* is controlled by more than one locus with multiple alleles having additive effects/4. The (-) level glucosinolate plants were identified in M_2 generation but it segregated in the next generation. Such precious alterations can always get lost by improper handling of population. Therefore to breed low glucosinolate *B. juncea* the breeder must have well planned strategies. Some of the suggestions which have emerged out of our work are:

1. It is necessary to grow large population of M_3, M_4 and M_5 generations. Undertake screening of low glucosinolate character in M_5 generation and grow isolated Westar level lows in M_6 and self, screen and grow M_7, M_8, and M9 generations and evolve low glucosinolate lines.
2. By making crosses between (-) level glucosinolate plants or with either of (±) and (+) plants and grow F_1, F_2 and screen F_2 for low glucosinolate and then advance the material with desirable character.
3. Another approach is, microspore culture, the microspores of (-), (±) and (+) levels glucosinolate plants could be cultured and diplodized. Selection for low glucosinolate could be practiced in diploids which should be homozygous for low glucosinolate character.

4. Effective selfing of each inflorescence, plant-wise harvesting, correct way of analyzing the seeds and huge population size could increase the chance of getting low glucosinolate plants.

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TABLE: - 1 FREQUENCY OF LOW GLUCOSINOLATE PLANTS IN M_2

Treatments	Total No. of Plants studied	Number of Plants with different glucosinolate levels			Frequency per 100 M_2 plants		
		-	+ -	+	-	+ -	+
Control (*B. juncea* Cv. Varuna	17	-	-	-	-	-	-
Westar (*B. napus*)	17	17	-	-	100	-	-
12h Psw + 3h EMS 0.03%	411	-	1	-	-	0.24	-
12h Psw + 3h SA 0.03%	227	1	4	2	0.44	1.76	0.88
12h EMS 0.01%	290	-	1	-	-	0.34	-
12h SA 0.008%	244	1	-	2	0.44	-	0.89
18h EMS 0.01%	262	-	1	5	-	0.38	1.90
18h SA 0.008%	245	1	-	2	0.40	-	0.81

TABLE:- 2 FREQUENCY OF LOW GLUCOSINOLATE PLANTS IN M_3

Treatments	Total No. of Plants studied	Number of Plants with different glucosinolate levels			Frequency per 100 M_3 plants		
		-	+ -	+	-	+ -	+
Control (*B. juncea* Cv. Varuna	**5**	-	-	-	-	-	-
Westar (*B. napus*)	**5**	**5**	-	-	**100**	-	-

12h Psw + 3h EMS 0.03%	-	-	-	-	-	-	-
12h Psw + 3h SA 0.03%	96	-	1	6	-	1.04	6.2
12h EMS 0.01%	49	-	-	4	-	-	8.16
12h SA 0.008%	63	-	4	19	-	6.3	30.1
18h EMS 0.01%	49	-	-	4	-	-	8.16
18h SA 0.008%	19	-	2	11	-	10.5	57.8