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ESTIMATION OF QUALITY TRAITS IN INDIAN MUSTARD AFTER MUTATION

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ABSTRACT

Indian mustard PM21 and PM30, were pre-treated with gamma rays and EMS (Ethyl methyl sulphate) at varying concentrate with increasing exposure time. When compared to the control, the antioxidant capacity has increased while phytic acid concentration decreased. EMS treatment and gamma rays enhances the free radical scavenging activity. Our results shows increased nutritional traits after treatment and reduction of antinutritional parameters, because it provides increased nutritional benefits. Furthermore, Phytic acid and glucosinolates were also effectively reduced by mutation.

Keywords - Gamma radiation, Indian mustard, and EMS, Glucosinolates, Phytic acid antioxidants**.**

[1] INTRODUCTION

Mustard production ranks third in terms of behind China and Canada. Because of its exceptional edible oil, which has a 2:1 ratio of omega-6 to omega-3 fatty acids, and its protein-rich seed meal for animal feed, this crop has been known for being nutritionally rich (Jesch and Carr 2017). Its seed contains oil content, good nutritionally rich fatty acid composition, and a few nutritional and anti-nutritional elements including phytic acid and glucosinolates these quality traits are recognized as its nutritional value. The presence of natural antioxidants, primarily phenolics, tocopherols, phytosterols, and b-carotene, is crucial for the quality mustard oil. These elements fundamentally shield polyunsaturated fats from oxidation (Sharma et al.,2016). Consuming naturally high antioxidant oils has been linked to a lower incidence of obesity, cancer, and a number of heart problems, according to studies (Sharma et al.,2018). Plant thioglucosides, or glucosinolates, are mostly found in the Brassicaceae family. It is the cause of Indian mustard oil distinctive pungency. While some of the glucosinolate hydrolysis's cleavage products are known to be harmful to animals' health, they also have chemo preventive, antifungal, and antibacterial qualities (Sharma et al., 2016). Other anti-nutritional substances included in mustard oil and seed meal include erucic acid and phytic acid (Sharafi et al., 2015). It has been already reported that physical and chemical treatment of seeds increase the biochemical reactions in the seeds.This leads to the increase of nutritional parameters (Jambhulkar et al.,2022). As a result of its ease of use and minimal processing requirements, microwave radiation pre-treatment of seeds for (Gaikwad et al., 2017) There is an increasing interest in oil production. Because permanent pores in the cell wall allow oil to pass through them quickly, mutated seeds have demonstrated greater oil output (Abbey et al. 2017). The current study's goal is to find out effect of gamma radiation on the the nutritional and anti-nutritional traits in Indian mustard.

[2] MATERIAL AND METHOD

Sample collection

Seed material of PM21 and PM30 Indian mustard seeds were obtained from ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan, India and were used in the analysis.

Phytic Acid

Haug and Lantzsch's (1983) approach was applied in order to estimate the phytate content. Freshly ground seed samples (0.5 g) of all treatments were extracted using 25 ml of 0.2 N HCl in a shaking water bath set at 40 °C for three hours. Samples were filtered using Whattman No. 1 filter paper after extraction, and a 0.5 ml aliquot was used for the test. A boiling water bath was water and 1 ml of ferric ammonium sulphate to the extract. Once cooled, one mililitter of extract then add 1.5 ml of bipyridine solution was added after the supernatant was transferred to a new test tube. The absorbance was taken at 519 nm, and the findings were represented as mg g^{-1}.

Glucosinolate

Using methanolic extract made from the same genotypes, 0.2 g of defatted seed meal was homogenised with 80% methanol to perform spectrophotometric measurement (Kumar et al., 2010). Seed meal was incubated in n-Hexane to produce defatted seed meal. Following a night of incubation, the seed meal was allowed to dry naturally and then reconstituted in an 80% methanol solution. The homogenate was subsequently centrifuged for 4 minutes at room temperature at 3000 rpm. After centrifugation, the supernatant was collected. sodium tetrachloropalladate, concentrated HCl, and with 0.3 ml of double distilled water and 3 ml of 2 mM sodium tetrachloropalladate was incorporated into it. The absorbance was measured at 425 nm.

Total antioxidant activity

Using the (Prieto et al.,1999) method, the total antioxidant activity was measured in the defatted and methanolic extract of the samples. 2.5 ml of the reagent solution was added to the sample methanol extract. The reaction mixture was then incubated in a boiling water bath for half and hour. The absorbance was measured at 695 nm after cooling, and the results were expressed.

DPPH radical scavenging

The reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, is the foundation of the DPPH radical scavenging assay technique (Mensor et al., 2001). Every sample underwent defatting and processing in accordance with the protocol for estimating glucosinolate. Samples and standard compounds were taken, and 80% methanol was used to uniformly dilute the volume to 1 ml. After further diluting each sample with methanol to a volume of 5 ml, DPPH was added to each 5 ml after dark incubation, absorbance was measured at 517 nm using methanol as a blank on a spectrophotometer. Each compound's IC_{50} values and the standard preparation were computed. Using the following formula, the DPPH free radical scavenging activity was determined.

βeta-carotene

In order to determine the beta-carotene concentration, 1 g of from each sample was placed into screw-capped glass vials after the seeds of both Indian mustard kinds were completely ground to (Sathya et al., 2014). 5 ml of water-saturated n-butanol was added to each vial, and it was violently shaken for one minute. To extract all of the beta-carotene, the vials were left overnight at room temperature and in the dark. The following day, material was filtrate and absorbance was measured at 440 nm using a blank of pure water-saturated n-butanol.

Total tocopherol

Tocopherol was extracted from seeds using the method from Backer et al. (1980). In this, 0.2 ml of 0.06% ferric chloride reagent in absolute ethanol was added, along with 1 ml of 2, 20-dipyridyl reagent and 1 ml of the extracted sample. The mixture was thoroughly mixed for 10 seconds. Here, 0.2% ferric chloride reagent had a slight alteration. Before measuring absorbance, ethanol was added to each tube. This was another alteration. A minute was all it took to measure the mixture absorbance was taken at 522 nm.

Statistical analysis

The experiment was conducted in three replications for each parameter. The statistical analysis were performed using two way ANOVA. Different Treatments and analytical parameters were compared by DMRT (Duncan multiple range test).Values at p level <0.05 were considered as statistically significant. The analysis of glucosinolate content, phytic acid, β carotene, total antioxidant and tocopherol of each brassica genotypes was analyzed in triplicates and the results are expressed as mean value± standard error (SE).

[3] RESULT AND DISCUSSION

The mean values for total glucosinolate content were found to vary from 22.2µmole/g in genotype T1 to 63.2 µmole/g in genotype T6. Glucosinolate content of T6 was slighty higher than the mean value of control 60.6 µmole/g . Lower glucosinolate content (<30 µmole/g) were found in the genotypes of T1.As per the statical analysis Genotype T7 is better then other genotype which also showed similarity towards control TC1- TC2. The results were in accordance with the results reported by Kumar et al. (2010) and Niu et al. (2015). Phytic acid analysis also showed variation from 1.0 %-2.0 % where genotype T1 has 1.0% phytic acid and T5 has 2.2% phytic acid. From the data it is evident that genotype T8 is better than others genotypes that shows resemblance with control TC1-TC2. Sharma et al. (2019)studied phytic acid in mutant lines and also mentioned the higher phytic acid as compared to control.

In this study The mean values for Total antioxidant (TAA) were found from 20.1 µg/g (genotype T1) and 40.1µg/g (genotype T4) as compared to mean value of both control TC1 & TC2 17.5 µg/g and 17.1 µg/g. In this work out of seven genotype, T4 is found better then other genotype as it having highest TAA. The high antioxidant capacity in turn provides high protection against oxidative stress, and thus increases potential uses of mustard seed oil in food, pharmaceutical and cosmetic industries (Szydłowska-Czerniak et al., 2015). However, results for β carotene and tocopherol demonstrate there experiment were non significant as the variation in the values was not marked. β carotene ranges between (0.8 to 1.8ppm) and tocopherol ranges between (29.4 to 38.3µg/g).

Table 1 The effect of biochemical parameters of mustard seed (*Brassica juncea*) after mutation.

Genotypes	**phytic**	**GSL**	**β Carotene**	**TAA**	**Tocopherol**
T1	1.0^{a} ±0.00	22.2^{a} ±2.01	1.4±0.34	20.1^{a} ± 3.55	36.7±2.04
T2	1.4^{b} ±0.15	51.3^{c} ±2.31	1.2±0.14	29.5^{bc} ±4.87	31.0±1.70
T3	1.0^{a} ±0.02	41.6^{b} ±2.00	1.3±0.20	36.6^{cd} ±1.02	36.1±0.99

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T4	1.0 [a] ±0.03	40.5 [b] ±5.46	1.6±0.17	40.2 [d] ±1.19	29.4±1.21
T5	2.2[c] ±0.20	37.4[b] ±2.33	1.1±0.15	15.0 [a] ±0.51	33.5±5.66
T6	1.5 [b] ±0.05	63.2[d] ±3.53	1.2±0.15	37.7 [cd] ±0.93	35.1±2.54
T7	1.5 [b] ±0.07	58.7 [cd] ±2.88	1.3±0.17	34.7 [cd] ±0.60	30.6±3.23
T8	2.0 [c] ±0.07	51.3 [c] ±0.96	0.8±0.23	21.5 [ab] ±5.23	31.2±0.91
Tc1	2.2 [c] ±0.06	55.4 [cd] ±1.83	1.8±0.05	17.5 [a] ±2.06	38.3±2.10
Tc2	1.6 [b] ±0.08	60.6 [cd] ±1.60	1.4±0.10	17.1 [a] ±0.23	30.3±1.37
Total	1.5±0.10	48.2±2.84	1.3±0.07	38.7±4.97	33.2±0.90

The results are represented as mean±SD (n=3). a, b, c and d indicate significant differences between treated and control plants at $p<0.05$

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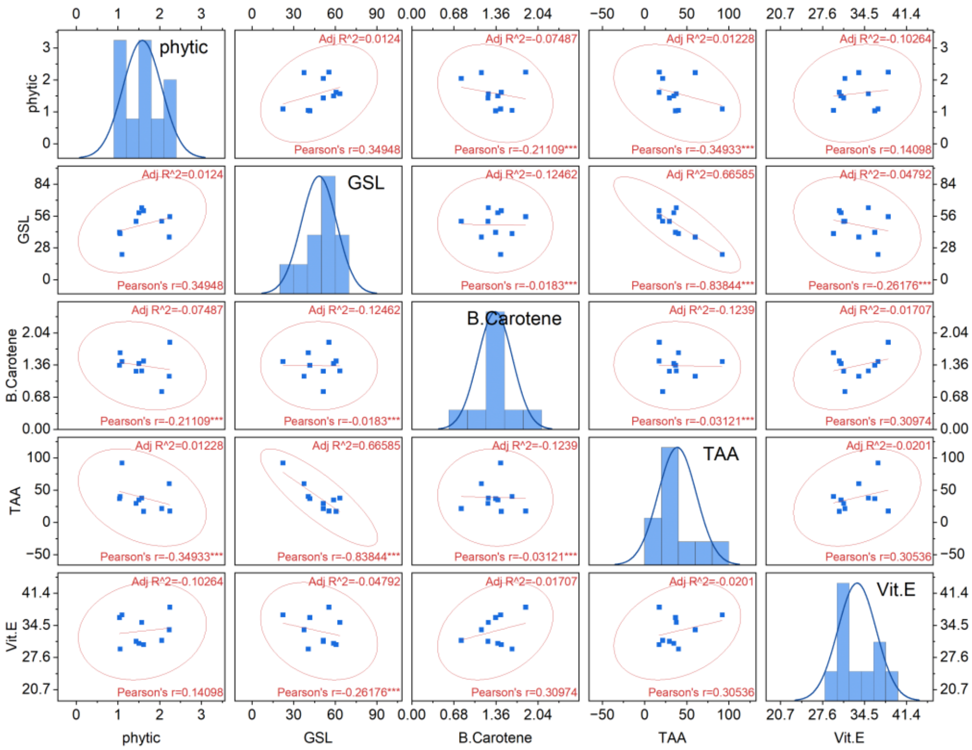

Figure:1 Correlation matrix showing correlation coefficients (r) of five parameters where, *p< 0.05; **p <0.01.

Correlation analysis

Fig. 1 shows the values of correlation analysis among the varieties, and analytical parameters. This kind of analysis helps in establishing relationship between studied parameters for better understanding of the impact of treatments on varietal performance. In this study, phytic acid showed maximum positive correlation with tocopherol content (r =-0.141) and negative correlation with TAA and β-carotene (r = -0.349 and -0.211). A significant, negative correlation of glucosinolates with tocopherol content (Verma et al.,2019). It means that mutated treatment has resulted in increase in antioxidant activity with concomitant decrease. These results an in agreement with (Kumar et al.,2017).

[4] CONCLUSION

Quality traits in indian mustard shows a broad range bioactive substance with different bioactivities. In this studies it was found that a treatments improves nutritional traits and lowers

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the antinutritional elements showing that these physical and chemical treatments are important to study the plant therapeutic qualities in brassica juncea. Further work is under way.

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