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Mutagenic impact of picric acid on chloroplast genome and a selection of biological attributes of *Brassica napus* L. (Brassicaceae)

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Abstract

The present study was conducted to investigate the possible mutagenic effects of picric acid on the chloroplast genome and certain biological properties of *Brassica napus* L. under field conditions. The experimental design for this study was a randomized complete block trial, based on 3 factors (dose, duration of priming, and three oilseed rape (cultivars). The seeds of oilseed rape cultivars (*Abasin- 95*, *Dur e Nifa*, and *Nifa Gold*) were exposed to five doses (0 mM, 5 mM, 10 mM, 15 mM, and 20 mM) of picric acid for different soaking times (3 h, 6 h, and 9 h) and the data on agro-morphological characteristics were recorded. Control and test samples were collected and compared for genomic chloroplast studies. The results confirmed the inhibitory effect of picric acid on days to emergence, completion of germination, and percent emergence. Seeds treated with a dose of 20 mM delayed seedling emergence (6.15) compared to other treatments. In addition, the differences between seeds primed for 3 h (6.07), and 6 h (5.89) were also highly significant. Similarly, the effects of 0 mM (7.52), 5 mM (8.26) and 10 mM (8.11) doses on germination time were not significant. Comparison of the mean values with respect to germination showed that picric acid led to a reduction in the percentage emergence of the oilseed rape seedlings. A significant increase in leaf size was observed in the *Nifa Gold* cultivar (10.27). On the other hand, the differences in leaf size between the cultivars *Dur e Nifa* (9.51) and *Abasin*-95 (9.20) were not significant. The inhibitory effect of picric acid on the number of leaves could be due to the fact that the meristematic cells are damaged by the mutagen, leading to a reduction in the number of leaves. The *Nifa Gold* cultivar showed the highest value for plant height (17.53), and the highest number of siliqua (14.69) compared to other genotypes. The number of siliqua confirms that *Nifa Gold* is best adapted to picric acid in contrast to the other genotypes. However, the differences between the *Abasin*-95 cultivar (0.068) and the *Nifa Gold* cultivar (0.062) in terms of seed weight were not significant. The dose of 15 mM (37.72) showed the highest moisture content, followed by 10 mM (37.08), 0 mM (33.87), 5 mM (33.11) and 20 mM (32.73). The chloroplast genome of plants grown from picric acid-treated seeds was mutated. Therefore, annotation of the genome was not possible. Numerous single nucleotide substitutions (187) were detected between the control and test samples. In addition, 43 additions/

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deletions were observed. The present study suggests that picric acid has a mutagenic effect, which is confirmed by numerous mutations in chloroplast samples. Subsequently, the doses have stimulating effects on plant height and seed weight. Furthermore, priming can improve the emergence and certain biological characteristics of *B. napus*. In the present study, all parameters were investigated and the significant response of the selected genotypes was confirmed.

Keywords Oilseed rape cultivars, Chloroplast genome, Mutagenic impact, Picric acid

Introduction

B. napus is a bright-yellow flowering member of the Brassicaceae family with genus Brassica and includes 40 species [1–5]. Rapeseed is the third largest source of vegetable oil and biofuel and the third largest oil-producing crop in the world after oil palm (*Elaeis guineensis* Jacq.) and soybean (*Glycine max* (L.) Merr.) [6]. It is the second largest source of protein food in the world. Rapeseed is better suited to places with heavy rainfall, and its yield suffers dramatically when drought strikes. In these places, water scarcity is prevalent throughout critical periods of plant growth, resulting in decreased photosynthetic capacity and leaf chlorophyll levels [1, 7]. *B. napus* reaches a height of about 100 cm. The lower leaves are petiolated, hairless, fleshy and pinnatifid. The upper leaves have no petioles. The leaf color is a darker, bluish-green. A single leaf is attached to the stem at each node, with about 15 to 20 internodes per plant, 5 to 10 mm apart. The flowers of *B. napus* are bisexual and develop in terminal racemes. The flowers are regular with 4 sepals and 4 petals. The diagonally opposite, yellow petals narrow at the basal end and form a cross, hence the original name of the family Cruciferae. The flowers also contain 6 stamens (2 of which are shorter and lower than the others), a pistil with 2 carpels and superior ovary. The seeds develop in an elongated, two-celled capsule called a siliqua, which has a pronounced midrib [8]. The production and quality of rapeseed oil depends primarily on the growth of the siliques, i.e. the number, size and volume of the fully matured siliques. This is due to the crucial function of siliques within the rapeseed plant. It is not only the carrier of the oilseeds but also contributes to photosynthesis and transmits developmental cues to the seeds during maturation [9–12].

Mutation breeding, a type of plant breeding, has been used to improve rapeseed (*B. napus*) varieties by creating genetic mutations through physical or chemical means. This has led to genetic diversity and enabled the development of desired traits [13]. Mutation breeding oilseed rape began in the mid-twentieth century when it was realized that induced mutations could be used to improve crops. Scientists exposed oilseed rape seeds to X-rays or gamma rays to induce random mutations in the plant's DNA [14]. These mutations can affect numerous traits, including oil content, seed yield, disease resistance, and plant shape. Mutant lines exhibiting the desired traits were selected for further analysis and inbreeding. Mutation breeding was used to develop improved agronomic traits in oilseed rape varieties. To combine the desired traits and improve overall performance, better varieties were often crossed with mutation lines created by mutation breeding. Through hybridization and rigorous selection, breeders succeeded in breeding rapeseed varieties with higher yield, better oil quality, and resistance to biotic and abiotic stress factors [15]. Thanks to the commercial release of oilseed rape varieties created through mutation breeding, farmers now have more choices for oilseed cultivation. The improved resistance to pests and diseases, better oil composition, and higher yield potential of these varieties have helped to promote oilseed rape cultivation worldwide [16].

Fluctuations in climatic conditions have a negative effect on the productivity of crops and oil plants [17]. The vulnerability of oilseed rape (*B. napus*) yields to increased temperatures is a major challenge in the quest for global food security. Yields have been reported to decrease by up to 40% under elevated temperatures [18, 19]. This problem emphasizes the critical impact of climatic variability on the growth stages, seedling emergence, and reproductive performance of *B. napus*, leading to a significant decline in productivity [20–22]. These findings have far-reaching implications as the global agricultural sector is faced with the need to significantly increase food production. More than 2 billion tons of cereals are produced annually worldwide, which are important for both human consumption and livestock feed, accounting for about two-thirds of total direct and indirect protein consumption. Interestingly, only about 10% of this immense production, i.e. 200 million tons, is available for international trade [23]. Given the expected population increase to 9.3 billion people in 2050 [24] and to 12.3 billion people in 2100 [25, 26], there is an urgent need to double current food production, particularly grain production, from 2 billion to over 4 billion tons annually [23]. Although this ambitious target is challenging, it is considered achievable if crop yields are further increased without an equal increase in the exploitation of arable land [27,

28]. In order to meet the demand caused by population growth and changing eating habits, the actual food supply must be increased by 70% [29]. Although the focus is on reducing the distribution gap, the pressure on existing agricultural frameworks to meet this demand indicates that either a sustained increase in crop yields, an expansion of agricultural land, or a synergistic approach combining both is required [30]. The introduction of new crops and oil plants is therefore of immense importance. Genotype changes induced by nature or selection are a slow process. Therefore, techniques to produce new varieties need to be explored. The only solution in this scenario seems to be mutation breeding [31]. Charles Darwin highlighted the importance of genetic variation for evolutionary adaptation and stressed that diversity is crucial not only for the survival of plant species but also for improving the quality of different crops [32]. This genetic diversity plays a central role in breeding programs as it enables plants to adapt effectively to environmental changes [33]. Therefore numerous methods have been introduced in recent years to increase genetic diversity in fruit trees [34]. Mutation, an important source of diversity, refers to unexpected changes in DNA sequences that occur without segregation or recombination. These changes are not limited to nucleotide substitutions but also include deletions/insertions, inversions, and translocations [35]. The concept of plant mutation can be traced back to 300 BC, but it was Hugo de Vries in the late nineteenth century who introduced the term"mutation"in 1903 in his work “The Mutation Theory”, and explained it as the cause of phenotypic changes in *Oenothera lamarckiana* Ser. [36]. Mutation breeding as a field for the creation of new cultivars was first recognized by Stadler [37] in his work on maize, barley and wheat. In the early 1930 s, the first commercial tobacco variety produced by mutation breeding was released. To date, more than 3088 mutagenic varieties of different fruit trees (e.g. apples, citrus, fruits and peaches), food crops (e.g. rice, barley, wheat, maize, and peas), oil crops (e.g. soybeans, rapeseed, sunflowers) and ornamental plants (e.g. chrysanthemums, dahlias, and poinsettias) have been released [38]. In addition, mutations are considered the basis of modern plant breeding techniques [34]. These mutations occur naturally in organisms to repair genetic material, including DNA and RNA, that has been damaged by physical, chemical, or biological factors [33, 39]. Hugo De Vries coined the term and described mutations as sudden changes in the DNA or RNA of a plant organ without recombination or segregation [36]. The resulting genetic variation, referred to as a"mutant", is a cornerstone of evolutionary progress [38, 40]. These mutations cause heritable changes in DNA and RNA that lead to phenotypic changes [38]. The induction of genetic variability by mutations, either by irradiation or by chemical mutagens, differs from genetic modification in that it does not involve the insertion of genes, which distinguishes it from genetically modified organisms (GMOs) [41]. Since 1925, when it was discovered that mutations in plants can be induced by irradiation or chemical treatments, mutation breeding has become a standard procedure for plant breeders. This approach is favored in regions where transgenic plants are banned and offers a non-transgenic alternative for plant development [42]. While mutations certainly occur in nature, but their impulsive frequency is not sufficient to achieve the rapid progress required in plant breeding. Nonetheless, mutations can be deliberately induced in various plant and animal species using physical and chemical means. These induced mutations range from extensive DNA deletions to minor point mutations. Physical methods of mutation induction, such as irradiation with non- ionising radiation (e.g. UV light) or ionising radiation (including X-rays, gamma rays and fast and slow neutrons), often lead to significant DNA deletions and changes in chromosome structure. In contrast, chemical mutagens usually attack individual nucleotide pairs. Chemical mutagens frequently used in plant breeding include ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), hydrogen fluoride (HF), sodium azide, N-methyl-N-nitrosourea (MNU), and hydroxylamine. The extent of the mutation depends on the exposed tissue and the level of exposure (taking into account dose and duration). Mutations of single nucleotide pairs are usually the focus of interest for breeders, as major chromosomal changes usually have adverse effects. However, the use of mutagens that alter chromosome structure can help to increase the number of recombination events and break unwanted genetic linkages, which is of considerable value for genetic improvement [43]. The present study aimed to investigate the efficacy of a little- studied mutagen called picric acid on an important oilseed crop. Picric acid is an organic compound with the formula $(O_2N)_3\ C_6H_2OH$. Its IUPAC name is 2, 4, 6-trinitrophenol (TNP). The name"picric acid"comes from the Greek (pikros) and means"bitter", which refers its bitter taste. It is one of the most acidic phenols [44]. Among the nitroaromatic compounds, picric acid is a very stable molecule [44]. The mutagenic effect of nitroaromatic compounds begins with their entry into cellular systems, the resulting interactions and binding to specific enzymes, the formation of important complexes (e.g. hydroxylamine and electrophilic intermediates), which in turn react with nucleophiles such as proteins or DNA and form an

addition product that makes them a potent DNA-altering substance [45]. The mutagenic tendencies of picric acid have already been studied by several authors [44, 45]. Thus, the current scenario of genetic diversity in oilseed rape is influenced by conservation measures, breeding programmes, genomic research, genetic engineering, and the use of wild forms. To ensure the resilience, sustainability, and long-term viability of oilseed rape cultivation in the face of changing agricultural and environmental challenges, it is essential to maintain and improve genetic diversity. The goal of the study is presumably to better understand how picric acid exposure affects the chloroplast genome and associated biological traits of *B. napus*. For this reason, variability was created by mutation with the following objectives: 1) evaluation of mutagenic effects, 2) investigation of chloroplast function, 3) identification of stress responses, and 4) characterization of genetic diversity.

Materials and Methods

Research site details and experimental layout

The study was conducted in the Department of Botany, Islamia College Peshawar, Khyber Pakhtunkhwa, Pakistan. The trial was designed in a randomized complete block design based on 3 factors (1 st factor: doses, 2nd factor: priming duration, 3rd factor: canola cultivars). Each treatment was replicated three times. The canola genotypes (*Abasin- 95*, *Dur e Nifa*, and *Nifa Gold*) were obtained from Nuclear Institute for Agriculture, Peshawar (NIFA), Khyber Pakhtunkhwa, Pakistan. The seeds were soaked in beakers of distilled water and allowed to stand at room temperature for 6 h. Aeration was encouraged by occasional shaking. The seeds were removed from the water and the excess moisture was removed by pressing in folded filter paper. They were treated with the required concentrations (0 mM, 5 mM, 10 mM, 15, and 20 mM) of picric acid solution for three time durations (3 h, 6 h, and 9 h). After each treatment, the seeds were washed under cold running tap water for about 20 min to remove excess picric acid. Excess moisture was removed by spreading the seeds on blotting paper. Ten seeds were sown in pots filled with soil. Each pot contained about 7 kg of soil. The soil was prepared by mixing clay and loam in almost equal proportions. The soil sample was well-aerated, drained and sterilized. Data on the number of leaves were recorded by counting the leaves of all plants in each pot. Similarly, the length of the 4th leaf from the base of each plant was recorded. In the same way, the height of the plants (in inches) was determined using a common scale. The siliques were collected at the time of plant maturity and data regarding the number of siliqua/plant, average siliqua weight/replicate, seed weight/siliqua, fresh/dry biomass and moisture content were recorded.

Molecular study

The plants were grown in pots to study the chloroplast genome. Plants grown from untreated seeds served as controls. The plants treated with picric acid were the treated samples. Plant tissue was taken from both the control and test samples and subjected to DNA extraction.

DNA extraction

Leaf samples of each genotype were collected, and DNA was extracted from these samples using a standard protocol. The samples were ground in liquid nitrogen, and the warmed (65 ∘C) CTAB buffer (800 µL) was added to each tube, and the resulting mixture was vortexed thoroughly to homogenize. Tubes were then incubated in a water bath for 1 h at 65 ∘C. Chloroform and phenol-isoamyl-alcohol (800 µL) at a ratio of 24:1 were added to each tube, and the solution was mixed gently by inverting tubes for 2 min. The samples were centrifuged at 14,000 rpm for 15 min. The supernatant was transferred to new 1.5 µL Eppendorf tubes. Cold isopropanol (2/3rd volume of supernatant) was added to each tube and mixed gently. Samples were again centrifuged at 14,000 rpm for 10 min to pellet DNA. The supernatant was discarded, and the sample was washed with the help of ethanol (70%). Ethanol was discarded, and the DNA pellets were allowed to dry at 27 ∘C for 10 min. The DNA samples were dissolved in 100 µL TE (pH 8.0) and stored at − 20 ∘C [46].

Study of the chloroplast genome

The approach of Ahmed et al. 2009 [47], was used to extract the whole genome DNA. Hydrogenated ether is then used to remove polysaccharides and other impurities from the DNA. The extracted whole genome DNA was checked for quality and quantity using a 1% agarose gel and Nanodrop (Thermo Scientific). The high-quality DNA was sent to Novogene, Hong Kong. The control sample was assembled using NovoPlasty [48] and annotated with GeSeq [49]. The reads of treated samples were mapped through Bowtie 2 [50] to the assembled genome, and the number of indels and substitutions were counted by inspecting the alignment manually with care. The number of chloroplast genomes is higher in the leaf than in the nuclear genome, which provides high coverage for the chloroplast genome even from low-depth sequencing [51]. Here, despite the conventional approach in which isolation or enrichment of the chloroplast genome by amplification with long-range PCR is done [46, 52, 53], followed by DNA extraction [54, 55], we used the whole genome sequencing approach and assembled the genome

Table 1 Mean-square values and significance tests for emergence (days), completion of germination (days), number of leaves, leaf size (cm), plant height (inches), number of siliqua, siliqua weight (g), seed weight (g), fresh biomass (g), dry biomass (g) and moisture content (%) of three oilseed rape cultivars evaluated with different doses treated with picric acid

Source	Degree of Freedom	Emergence	Germination completion	% emergence	No. of leaves	Leaf size	Plant height	No. of siliqua	Weight of siliqua	Seed weight	Fresh biomass	Dry biomass	Moisture contents
Replication	2	1.126	0.274	276.296	4.484	4.402	7.648	19.860	0.000	0.000	148.208	9.968	396.482
Doses (A)	4	9.437^{S}	14.141^{S}	1482.593^{S}	3.597^{S}	4.708^{S}	19.969^{S}	17.419^{S}	0.001^{S}	0.000^{NS}	47.532^{NS}	5.682^{S}	146.534^{S}
Priming durations (B)	2	14.948^{S}	0.363^{NS}	76.296^{NS}	5.964^{S}	31.907^{S}	44.222^{S}	12.985^{S}	0.001^{S}	0.001^{NS}	141.903^{S}	14.896^{NS}	103.606^{NS}
Cultivars (C)	2	3.126^{S}	7.674^{S}	571.852^{S}	95.675^{S}	13.644^{S}	952.062^{S}	130.904^{S}	0.001^{NS}	0.001^{S}	85.843^{S}	146.639^{S}	307.488^{S}
A × B	8	2.385^{NS}	3.974 N^{S}	233.704^{NS}	0.486^{NS}	2.055^{S}	5.456^{NS}	21.370^{S}	0.000^{NS}	0.000^{NS}	28.221^{NS}	3.415^{NS}	115.683^{S}
B × C	4	1.096^{NS}	3.085^{NS}	409.630^{S}	10.057^{S}	26.001^{S}	15.099^{NS}	7.543^{NS}	0.001^{NS}	0.000^{NS}	70.246^{S}	9.098^{NS}	214.634^{S}
A × C	8	3.319^{NS}	3.535^{NS}	323.704^{S}	0.313^{NS}	2.307^{NS}	3.737^{NS}	25.570^{NS}	0.000^{NS}	0.000^{NS}	42.765^{NS}	5.675^{NS}	93.514^{S}
A × B × C	16	6.459^{S}	7.752^{S}	169.815^{S}	1.147^{S}	2.811^{NS}	4.911^{NS}	21.134^{NS}	0.001^{NS}	0.000^{S}	40.759^{S}	6.445^{NS}	57.792^{NS}
Error	88	15.541	2.380	120.993	1.017	2.074	7.725	18.471	0.001	0.001	24.000	4.834	34.208

* Significant (S), Non-significant (NS), (A × B) = 1 st order interaction, (B × C) = 2nd order interaction, (A × C) = 3rd order interaction, (A × B × C) = 4 th order interaction

successfully. The same has been reported for several plant lineages [47, 56–60].

Statistical analysis

The experimental data were analysed using MSTAT-C software to establish statistical significance between treatments. An analysis of variance (ANOVA) was used to discover variations in the measured parameters. The Least Significant Difference (LSD) test was used at a 5% probability level ($p \leq 0.05$) to compare mean values and assess the significance of detected differences. To facilitate interpretation, results were provided as mean ± standard error [61].

Results and discussion

Statistical interpretation

The effect of doses on seedling emergence, completion of germination, percentage emergence, number of leaves, leaf size, plant height, number of siliqua, weight of siliqua and moisture content of oilseed rape was highly significant. Seed weight and fresh/dry biomass showed no-significant responses to the doses. In contrast, priming resulted in significant changes in seedling emergence, number of leaves, leaf size, plant height, siliqua weight and fresh biomass of oilseed rape. However, the priming techniques were not able to produce significant changes in percentage emergence, number of siliquae, seed weight, dry biomass and seedling moisture content. Significant differences were observed between the selected varieties for The traits such as seedling emergence, germination completion, percentage emergence, fresh/dry biomass and moisture content, number of leaves, leaf size, plant height, number of siliqua and seed weight. The effect of cultivar differences on siliqua weight was not significant. The 1 st order interaction resulted in significant variations in leaf size, number of siliqua and moisture content. However, attributes such as seedling emergence, germination, number of leaves, plant height, siliqua weight, seed weight and fresh/dry biomass did not vary-significantly under the influence of 1 st order interaction. The 2nd order interaction was found to be significant for percentage emergence, number of leaves, leaf size, fresh biomass and moisture content. On the other hand, the 3rd order interaction did not result in significant changes in germination, number of leaves, leaf size, plant height, number of siliqua, fresh seed weight, and dry biomass. Similarly, the studied attributes (except germination, sprouting, number of leaves, seeds, and weight) showed no-significant changes under the 4 th order interaction (Table 1).

Impact on germination traits

Effect on days to seedling emergence

Seeds treated with a dose of 20 mM delayed seedling emergence (6.15) compared to other treatments. However, the effects of 15 mM (5.96) and 20 mM (6.15) were statistically similar. The doses of 0 mM (5.63), 5 mM (5.56) and 10 mM (5.44) also did not result in significant seedling emergence. The data on days to emergence confirmed two statistical groups at the applied doses. The control, 5 mM and 10 mM belong to the first group and 15 mM and 20 mM to the 2nd group.

Priming the rapeseed for 9 h resulted in significantly higher seedling emergence (5.29) compared to the other priming durations. In addition, the differences between seeds primed for 3 h (6.07), and 6 h (5.89) were also highly significant. Seeds of *Dur e Nifa* cultivar required the least time for emergence (5.53) compared to *Abasin-95* (5.87) and *Nifa Gold* (5.84). However, the response of *Abasin- 95* cultivar (5.87) and *Nifa Gold* cultivar (5.84) was statistically similar.

The 3rd order interaction (A × C) delayed the emergence (6.33) of *Nifa Gold* cultivar seeds treated with 20 mM picric acid. In contrast, seeds of *Dur e Nifa* cultivar treated with 0 mM and 10 mM took least time to emerge (5.11). The effect of 4 th order interaction (A × B × C) was found to be inhibitory for the seeds of *Abasin- 95* cultivar (6.67) treated with 0 mM picric acid for 6 h. In contrast, the cultivar *Dur e Nifa* showed the fastest emergence (4.33) under the influence of a 0 mM dose and a soaking time of 9 h (Table 2).

The results on germination characteristics showed that higher doses of picric acid delayed the emergence of the oilseed rape varieties. In contrast, pre-soaking of the seed accelerated seedling emergence. Among the oilseed rape genotypes, cultivar *Dur e Nifa* showed rapid seedling emergence and proved to be more adaptable to picric acid than the other cultivars. The delayed emergence of the oilseed rape seedlings can be attributed to damage to the embryo by picric acid [62]. It is also possible that the mutagen caused some physiological changes (disruption of enzymes) in the oilseed rape seeds that slowed down seedling emergence [63–65]. Mutations in genes involved in metabolic pathways responsible for the conversion of fatty acid to glucose could be another reason for delayed seedling emergence [66, 67]. In addition, there is a possibility that treatment with picric acid creates stress conditions for the embryos that lead to dormancy, which in turn results in delayed seedling emergence [47]. Many researchers have reported negative effects of mutagens on the germination of different plants, which are in complete agreement with the results of the present study [65, 68–71]. Some researchers reported rapid growth of various plants grown from

Table 2 Effect of various doses of picric acid (0, 5 mM, 10 mM, 15 mM, and 20 mM) and different priming durations (3 h, 6 h, and 9 h) on the days to seedling emergence of three oilseed rape cultivars

Treatment Levels × Priming Durations × Oilseed rape Cultivars Interaction						
		Abasin-95		*Dur e Nifa*		*Nifa Gold*
Control	3 h	6.00^{abc}		6.00^{abc}		5.67^{bcd}
5 mM		6.00^{abc}		6.00^{abc}		6.00^{abc}
10 mM		6.00^{abc}		5.67^{bcd}		6.00^{abc}
15 mM		6.00^{abc}		6.33^{ab}		6.33^{ab}
20 mM		6.33^{ab}		6.00^{abc}		6.67^{a}
Control	6 h	6.67^{a}		5.00^{def}		6.00^{abc}
5 mM		6.00^{abc}		5.33^{cde}		5.67^{bcd}
10 mM		6.00^{abc}		5.00^{def}		6.00^{abc}
15 mM		6.00^{abc}		6.33^{ab}		6.00^{abc}
20 mM		6.00^{abc}		6.33^{ab}		6.00^{abc}
Control	9 h	5.33^{cde}		4.33^{f}		5.67^{bcd}
5 mM		5.00^{def}		5.33^{cde}		4.67^{ef}
10 mM		5.00^{def}		4.67^{ef}		4.67^{ef}
15 mM		5.67^{bcd}		5.00^{def}		6.00^{abc}
20 mM		6.00^{abc}		6.00^{abc}		6.00^{abc}
Treatment Levels × Priming Durations Interaction						
	3 h	6 h		9 h		Treatment means
Control	5.89	5.89		5.11		5.63^{b}
5 mM	6.00	5.67		5.00		5.56^{b}
10 mM	5.89	5.67		4.78		5.44^{b}
15 mM	6.22	6.11		5.56		5.96^{a}
20 mM	6.33	6.11		6.00		6.15^{a}
Priming Durations × Oilseed rape **Cultivars Interaction**						
	Abasin-95	*Dur e Nifa*		*Nifa Gold*		Priming means
3 h	6.07	6.00		6.13		6.07^{a}
6 h	6.13	5.53		6.00		5.89^{b}
9 h	5.40	5.07		5.40		5.29^{c}
Treatments Levels × Oilseed rape **Cultivars Interaction**						
	Control	5 mM	10 mM	15 mM	20 mM	Cultivars means
Abasin-95	6.00^{abc}	5.67^{cde}	5.67^{cde}	5.89^{bcd}	6.11^{ab}	5.87^{a}
Dur e Nifa	5.11^{f}	5.57^{de}	5.11^{f}	5.89^{bcd}	6.00^{abc}	5.53^{b}
Nifa Gold	5.78^{bcde}	5.44^{ef}	5.56^{de}	6.11^{ab}	6.33^{a}	5.84^{a}

* LSD value at the 5% significance level for treatment level = 0.2365, priming duration = 0.1763, oilseed rape cultivars = 0.1763, treatment levels × oilseed rape cultivars = 0.3941 and treatment levels × priming duration × oilseed rape cultivars = 0.6827. Values with the same bearing similar letters in rows and columns are not statistically significant

seeds treated with many other mutagens, which is in contradiction with our results [67, 72–78].

Effect on days taken to germination completion

The untreated rapeseed (7.52) took the fewest days to germinate compared to the other doses. However, the effects of the doses of 0 mM (7.52), 5 mM (8.26) and 10 mM (8.11) on germination time were not significant. The seeds treated with a dose of 20 mM took the longest time to germinate (9.41). However, the doses of 15 mM and 20 mM had a statistically similar effect on germination. In contrast to the other cultivars, rapid germination was observed in the *Abasin*− 95 cultivar. In contrast, the cultivar *Nifa Gold* (8.89) took the longest time to complete germination. However, the differences between the *Dur e Nifa* cultivar (8.31) and the *Nifa Gold* cultivar (8.89) were highly insignificant. Conversely, the responses of *Abasin*− 95 cultivar and *Nifa Gold* cultivar were highly significant in terms of germination rate. The 4th order interaction increased the germination rate (6.33) of *Abasin- 95* cultivar under the influence of 0 mM dose and soaking time. However, the maximum time to complete

Table 3 Effect of various doses (0, 5 mM, 10 mM, 15 mM, and 20 mM) and different priming durations (3 h, 6 h, and 9 h) of picric acid on days taken to germination completion of three oilseed rape cultivars

Treatment Levels × Priming Durations × Oilseed rape Cultivars Interaction						
		Abasin-95		*Dur e Nifa*		*Nifa Gold*
Control	3 h	7.00^{bc}		8.00$^{b–f}$		8.33$^{b–f}$
5 mM		9.00$^{b–e}$		7.33^{def}		7.67$^{c–f}$
10 mM		7.67$^{c–f}$		8.33$^{b–f}$		8.00$^{b–f}$
15 mM		7.67$^{c–f}$		8.00$^{b–f}$		9.67^{bcd}
20 mM		8.67$^{b–f}$		12.33^{a}		10.00^{abc}
Control	6 h	8.00$^{b–f}$		7.33$^{c–f}$		7.67$^{c–f}$
5 mM		8.00$^{b–f}$		7.00^{ef}		8.67$^{b–f}$
10 mM		8.67$^{b–f}$		7.00^{ef}		8.00$^{b–f}$
15 mM		8.00$^{b–f}$		12.33^{a}		8.67$^{b–f}$
20 mM		8.33$^{b–f}$		7.67$^{c–f}$		9.67^{bcd}
Control	9 h	6.33^{f}		8.00$^{b–f}$		7.00^{ef}
5 mM		7.00^{ef}		7.33^{def}		12.33^{a}
10 mM		7.00^{ef}		8.00$^{b–f}$		10.33^{ab}
15 mM		9.67^{bcd}		8.33$^{b–f}$		7.33^{def}
20 mM		10.33^{ab}		7.67$^{c–f}$		10.00^{abc}
Treatment Levels × Priming Durations Interaction						
	3 h	6 h		9 h		Treatment means
Control	7.78	7.67		7.11		7.52^{c}
5 mM	8.00	7.89		8.89		8.26^{bc}
10 mM	8.00	7.89		8.44		8.11^{bc}
15 mM	8.44	9.67		8.44		8.85^{ab}
20 mM	10.33	8.56		9.33		9.41^{a}
Priming Durations × Oilseed rape Cultivars Interaction						
	Abasin-95	*Dur e Nifa*		*Nifa Gold*		Priming means
3 h	8.00	8.80		8.73		8.51
6 h	8.20	8.27		8.53		8.33
9 h	8.07	7.87		9.40		8.44
Treatments Levels × Oilseed rape Cultivars Interaction						
	Control	5 mM	10 mM	15 mM	20 mM	Cultivars means
Abasin-95	7.11	8.00	7.78	8.44	9.11	8.09^{b}
Dur e Nifa	7.78	7.22	7.78	9.56	9.22	8.31^{ab}
Nifa Gold	7.67	9.56	8.78	8.56	9.89	8.89^{a}

* LSD value at the 5% significance level for treatment levels = 0.8672, oilseed rape cultivars = 0.6463 and treatment levels × priming duration × oilseed rape cultivars = 2.503. Values with the same bearing similar letters in rows and columns are not statistically

germination was 12 (days), which could not be specifically assigned to any cultivar or dose (Table 3).

The results confirmed the inhibitory effect of picric acid on the number of days to full germination. Among the three oilseed rape varieties *Abasin- 95* seed germinated the fastest and proved to be the most adaptable to picric acid for the same parameter. According to reports by Roychowdhury & Tah [65], interference with the formation of enzymes involved in the germination process may be one of the physiological effects of mutagens. Secondly, picric acid could physically damage the cells of seed embryos, and disrupt many physiological processes required for rapid germination [62] [62–65]. There is also the possibility that picric acid affects some genes necessary for fatty acid metabolism, leading to slow completion of germination [66, 79]. In [65, 68–70], negative effects of various other mutagens on the germination behavior of different plants were reported, which is fully consistent with our results. In contrast to our results, some researchers have reported rapid germination of various plants grown from seeds treated with different other mutagens [66, 67, 72–76, 80].

Effect on germination percentage (%)

The seeds grown in the control group showed the highest germination (94.44) compared to the other doses. However, the dosages of 0 mM (94.44), 5 mM (92.59) and 10 mM (92.22) had a similar statistical effect. Similarly, the effect of 10 mM (92.22) and 15 mM (86.30) was not significant. The dose of 20 mM (76.30) caused a significant inhibition of percent incidence compared to the other doses. The lethal dose that caused 50% inhibition was 42.597 mM.

The cultivar *Dur e Nifa* (91.78) recorded the highest percentage of field emergence compared to the other genotypes. In addition, the *Dur e Nifa* cultivar (91.78) and the *Nifa Gold* cultivar (84.67) showed significant differences in percentage field emergence. However, the *Dur e Nifa* cultivar (91.78) and the *Abasin*-95 cultivar (88.67) did not show any significant variations. Similarly, the differences between the *Abasin*-95 cultivar (88.67) and the *Nifa Gold* cultivar (84.67) were not significant.

The 2nd order interaction (B ×C) increased the germination of the *Dur e Nifa* cultivar primed for a period of 3 h duration (95.33). However, the *Nifa Gold* cultivar, which was primed for 9 h recorded the lowest germination (79.33). The 3rd interaction stimulated the percent emergence (97.78) of the *Abasin*-95 cultivar and the *Dur e Nifa* cultivar treated with a dose of 5 mM and 15 mM doses of picric acid, respectively. In contrast, the interaction between dose and cultivar (A ×C) resulted in maximum inhibition of percent emergence (68.89) in *Nifa Gold* cultivar treated with a dose of 20 mM (Table 4).

Comparison of mean values with respect to germination showed that picric acid resulted in a reduction in percentage emergence of rapeseedlings. Among the three rapeseed varieties, *Dur e Nifa* cultivar showed the highest percentage of germination. The reduction in seed germination induced by mutagenic treatments may be the result of damage to cellular components at the molecular level or altered enzyme activity [63–65]. Micco et al. [79] have indicated abnormalities in the mitotic cycles and metabolic pathways of cells due to mutagens as the cause of low germination. Kovacs & Keresztes, [62] reported that the reduction in germination and survival could be due to the incorporation of mutagens into biological materials that act directly on critical targets in the cell. Most researchers reported a decrease in germination rate due to the application of mutagens in various plants [67, 69, 72, 74, 75, 78, 81–85]. Abdul Rahaman et al. [80] studied the effects of sodium azide and nitric acid on the growth parameters of *Citrullus* and *Moringa* and concluded that the mutagens have no effect on germination, while Roychowdhury & Tah [65] and El-Nashar & Asrar [70] reported stimulatory effects of various mutagens on germination of different plants in contrast to the present findings.

Response of growth and agronomic traits

Effect on number of leaves

Plants grown from seeds treated with a picric acid dose of 20 mM (11.37) produced most leaves compared to the control (11.06). However, the differences between the doses of 0 mM and 20 mM were not significant. At the same time, a dose of 10 mM (10.48) caused a reduction in the number of leaves compared to the control. Furthermore, the differences between the plants grown from seeds treated with 5 mM (10.67), 10 mM (10.48) and 15 mM (10.81) were not significant. Similarly, no significant differences were observed for the parameter in question in seeds treated with 0 mM (11.06), 5 mM (10.67) and 15 mM (10.81) picric acid.

Soaking the seeds for 6 h resulted in a maximum number of leaves (11.16) compared to other priming durations. At the same time, seeds primed for 3 h (11.06) and 6 h (11.16) resulted in non-significant variations in the number of leaves. In contrast, the differences between plants grown from seed with a soaking time of 9 h (10.49) and the other two soaking times were highly significant.

The *Dur e Nifa* cultivar (12.02) produced the highest number of leaves. It was also found that the *Abasin*-95 cultivar (9.25), *Dur e Nifa* cultivar (12.02), and *Nifa Gold* cultivar (11.43) showed highly significant variations for the parameter in question. The 2nd order interaction study (B ×C) showed that priming for 6 h is stimulated the number of leaves of *Dur e Nifa* (13.03). Subsequently, *Abasin*-95 cultivar primed for 3 h recorded the highest inhibition (8.90) for the attribute in question (Table 5).

The inhibitory effect of picric acid on the number of leaves could be due to the fact that the meristematic cells are damaged by the mutagen, leading to a reduction in the number of leaves. Since mutagens are known to induce physical, physiological and genetic changes in plants, the reduction in the number of leaves of oilseed rape plants could be related to the inhibition of polymerization of tubulin into microtubules or RNA synthesis, which affects the mitotic divisions of cells, and leads to a reduction in leaf production [67]. Soaking the seeds for 6 h was found to be favorable for improving leaf number. The *Dur e Nifa* cultivar showed sufficient adaptability to picric acid in terms of leaf number. Our findings on the decrease in leaf number of oilseed rape plants with picric acid are confirmed by [17, 74, 83] and Olawuyi & Okoli [69] when they studied the effects of different mutagens on horse gram, cowpea, tomato, and maize respectively. In contrast, Kumar et al. [84], Kumar et al. [86], Barman et al. [87] and El-Nashar & Asrar [70] reported positive

Table 4 Impact of various treatment levels (0, 5 mM, 10 mM, 15 mM, and 20 mM) and different priming durations (3 h, 6 h, and 9 h) on germination percentage (%) of three oilseed rape cultivars treated with picric acid

Treatment Levels × Priming Durations × Oilseed rape Cultivars Interaction						
		Abasin-95		*Dur e Nifa*		*Nifa Gold*
Control	3 h	83.33		96.67		100.00
5 mM		93.33		96.67		93.33
10 mM		93.33		100.00		90.00
15 mM		66.67		96.67		86.67
20 mM		76.67		86.67		73.33
Control	6 h	100.00		93.33		93.33
5 mM		100.00		93.33		100.00
10 mM		90.00		90.00		93.33
15 mM		83.33		96.67		66.67
20 mM		86.67		76.67		76.6
Control	9 h	90.00		96.67		96.67
5 mM		100.00		90.00		66.67
10 mM		100.00		93.33		80.00
15 mM		83.33		100.00		96.67
20 mM		83.33		70.00		56.67
Treatment Levels × Priming Durations Interaction						
	3 h	6 h		9 h		Treatment means
Control	93.33	95.56		94.44		94.44^{a}
5 mM	94.44	97.78		85.56		92.59^{a}
10 mM	94.44	91.11		91.11		92.22^{ab}
15 mM	83.33	82.22		93.33		86.30^{b}
20 mM	78.89	80.00		70.00		76.30^{c}
Priming durations × Oilseed rape cultivars interaction						
	Abasin-95	*Dur e Nifa*		*Nifa Gold*		Priming means
3 h	82.67^{cd}	95.33^{a}		88.67^{abc}		88.89
6 h	92.00^{ab}	90.00^{abc}		86.00^{bcd}		89.33
9 h	91.33^{ab}	90.00^{abc}		79.33^{d}		86.89
Treatment Levels × Oilseed rape Cultivars Interaction						
	Control	5 mM	10 mM	15 mM	20 mM	Cultivars means
Abasin-95	91.11$^{a–d}$	97.78^{a}	94.44^{ab}	77.78^{ef}	82.22^{de}	88.67^{ab}
Dur e Nifa	95.56^{ab}	93.33^{abc}	94.44^{ab}	97.78^{a}	77.78^{ef}	91.78^{a}
Nifa Gold	96.67^{ab}	86.67$^{b–e}$	87.78$^{a–e}$	83.33^{cde}	68.89^{f}	84.67^{b}

* LSD value at the 5% significance level for treatment levels = 6.183, priming duration = 0.2625, oilseed rape cultivars = 4.608, priming durations × oilseed rape cultivars = 7.982, treatment levels × oilseed rape cultivars = 10.30. Values with the same letters in rows and columns are statistically not significant. LD_{50} = 42.597 mM

effects of chemical mutagens on mulberry, brassica, jamun and calendula respectively.

Effect on leaf size (cm)

The effect of picric acid on the seeds to increase leaf size proved to be as effective as with untreated seeds. The plants with the highest leaf size (10.53) emerged from seed soaked for 3 h, leaf size followed by 6 h (9.60) and 9 h (8.85). A significant increase in leaf size was observed in the *Nifa Gold* cultivar (10.27). On the other hand, the differences in leaf size between the cultivars *Dur e Nifa* (9.51) and *Abasin*-95 (9.20) were not significant.

The 2nd order interaction (B × C) recorded the highest value for leaf size (11.93) in *Dur e Nifa* cultivar primed for 3 h. On the other hand, *Dur e Nifa* primed for 9 h recorded the lowest value for leaf size (7.82) (Table 6). The improvement in leaf size with priming could be due to the fact that the oilseed rape made the necessary adaptation to the mutagen before sowing [88]. The pre-exposure of the seeds stimulated the defense mechanisms of the oilseed rape plants against the mutagen, which led to an increase in leaf size. The increase in leaf size would maximize the plants'biomolecule synthesis apparatus of the plants needed to cope with the effects of the mutagen

Table 5 The number of leaves of oilseed rape cultivars under the influence of different doses (0, 5 mM, 10 mM, and 15 mM, and 20 mM) and different priming durations (3 h, 6 h, and 9 h) of picric acid

Treatment Levels × Priming Durations × Oilseed rape Cultivars Interaction						
		Abasin-95		*Dur e Nifa*		*Nifa Gold*
Control	3 h	8.60		12.73		12.60
5 mM		8.50		12.00		11.17
10 mM		8.80		12.40		11.83
15 mM		9.20		12.60		10.97
20 mM		9.47		11.93		13.03
Control	6 h	10.43		13.40		10.30
5 mM		8.73		13.00		11.10
10 mM		8.54		12.60		11.03
15 mM		9.67		12.37		11.37
20 mM		10.13		13.77		11.03
Control	9 h	9.50		11.03		12.00
5 mM		9.43		10.70		11.43
10 mM		8.87		10.00		10.27
15 mM		8.93		11.03		11.13
20 mM		10.00		10.77		12.20
Treatment Levels × Priming Durations Interaction						
	3 h	6 h		9 h		Treatment means
Control	11.31	11.38		10.84		11.18^{ab}
5 mM	10.56	10.94		10.52		10.67^{bc}
10 mM	11.01	10.72		9.71		10.48^{c}
15 mM	10.92	11.13		10.37		10.81^{bc}
20 mM	11.48	11.64		10.99		11.37^{a}
Priming Durations × Oilseed rape Cultivars Interaction						
	Abasin-95	*Dur e Nifa*		*Nifa Gold*		Priming means
3 h	8.90^{e}	12.33^{ab}		11.92^{bc}		11.06^{a}
6 h	9.50^{e}	13.03^{a}		10.97^{d}		11.16^{a}
9 h	9.35^{e}	10.71^{d}		11.41^{cd}		10.49^{b}
Treatments Levels × Oilseed rape Cultivars Interaction						
	Control	5 mM	10 mM	15 mM	20 mM	Cultivars means
Abasin-95	9.51	8.89	8.73	9.27	9.87	9.25^{c}
Dur e Nifa	12.39	11.90	11.67	12.00	12.16	12.02^{a}
Nifa Gold	11.63	11.23	11.04	11.16	12.09	11.43^{b}

* LSD value at the 0.05% significance level for treatment levels; 0.5454, priming duration; 0.4225, oilseed rape cultivars; 0.4225 and interaction of priming duration × oilseed rape cultivars; 0.7318. Values with same letters in rows and columns are not statistically significant

[47, 88]. Researchers such as [72], Mshembula et al. [83] and El-Nashar & Asrar [70] reported an increase in leaf growth of sesame, cowpea and calendula respectively, when chemical mutagens were used. On the other hand, Akhtar [17] and Olawuyi & Okoli [69] reported deleterious effects of chemical mutagens on leaf growth of tomato and maize plants.

Effect on plant height (inches)

The increase in seedling height was observed and the maximum increase over the control (12.85) was recorded at 15 mM (15.10) and it was not significantly different from the 5 mM (14.38), 10 mM (14.74) and 20 mM (14.50) doses. Soaking the seeds for 6 h (15.19) resulted in a significant increase in plant height compared to 3 h (13.24). Similarly, soaking the seeds for 3 h and 9 h resulted in significant variations in plant height. On the other hand, the effects of 6 h (15.19) and 9 h (14.51) duration on plant height were not significant.

The *Nifa Gold* cultivar showed the highest value for plant height (17.53), followed by the *Dur e Nifa* cultivar (16.36) and the *Abasin- 95* cultivar (9.05) (Table 7). The stimulatory effect of picric acid confirmed that it affects the plant growth hormone genes that enable plants to

Table 6 Leaf size (cm) of oilseed rape cultivars as a function of different doses (0, 5 mM, 10 mM, 15 mM, and 20 mM) and different priming durations (3 h, 6 h, and 9 h) of picric acid

Treatment Levels × Priming Durations × Oilseed rape Cultivars Interaction						
		Abasin-95		*Dur e Nifa*		*Nifa Gold*
Control	3 h	9.45		10.53		9.67
5 mM		9.95		12.65		9.54
10 mM		7.43		12.32		10.82
15 mM		9.90		12.17		10.01
20 mM		10.99		11.98		10.57
Control	6 h	9.75		8.61		8.13
5 mM		10.12		7.60		9.75
10 mM		8.62		8.69		10.17
15 mM		11.17		8.11		11.58
20 mM		10.00		10.88		10.88
Control	9 h	8.24		7.38		10.83
5 mM		8.45		9.54		10.33
10 mM		7.87		7.67		10.14
15 mM		7.75		7.81		10.33
20 mM		8.38		6.71		11.33
Treatment Levels × Priming Durations Interaction						
	3 h	6 h		9 h		Treatment means
Control	9.88	8.83		8.82		9.18
5 mM	10.72	9.16		9.44		9.77
10 mM	10.19	9.16		8.56		9.30
15 mM	10.69	10.28		8.63		9.87
20 mM	11.18	10.59		8.81		10.19
Priming Durations × Oilseed rape Cultivars Interaction						
	Abasin-95	*Dur e Nifa*		*Nifa Gold*		Priming means
3 h	9.54^{cd}	11.93^{a}		10.12^{bc}		10.53^{a}
6 h	9.93^{bc}	8.78^{de}		10.10^{bc}		9.60^{b}
9 h	8.14^{e}	7.82^{e}		10.60^{b}		8.85^{c}
Treatments Levels × Oilseed rape Cultivars Interaction						
	Control	5 mM	10 mM	15 mM	20 mM	Cultivars means
Abasin-95	9.15	9.51	7.97	9.60	9.79	9.20^{b}
Dur e Nifa	8.84	9.93	9.56	9.36	9.86	9.51^{b}
Nifa Gold	9.54	9.88	10.38	10.64	10.93	10.27^{a}

* LSD value at the 0.05% significance level for priming durations; 0.6034, oilseed rape cultivars; 0.6034 and interaction of priming durations × oilseed rape cultivars; 1.045. Values with the same letters in rows and columns are not statistically significant

reach maximum height [63, 64, 69]. Since many researchers have reported the effects of mutagens on cytological aspects of plants, a link between the growth of picric acid and the meristematic stem cells of oilseed rape plants could also be hypothesised in the present case [62]. It is likely that, picric acid-induced rapid mitosis in the meristematic tissue, which helped the plants to reach maximum height [69, 89]. It is also possible that picric acid increases the stomata density of plants [76]. As the number of stomata increases, so does the number of guard cells. We know that guard cells contain chloroplasts, which are the assimilation factories of plants. The more assimilation machines there are, the more carbon is fixed and the more raw materials are made available for the plants, which gain height as a result. Researchers such as Mshembula et al. [83], Birara et al. [76], Barman et al. [87], El-Nashar & Asrar [70], and Abdul Rahaman et al. [80] reported an increase in the height of plants grown from seeds treated with different chemical mutagens. In contrast, Obadoni et al. [72], Bolbhat et al. [74], Jagajanantham et al. [75], Arisha et al. [90], Akhtar [17], Olawuyi & Okoli [69] reported a decrease in plant height when different chemical mutagens were used, which contradicts the results of the present study.

Table 7 Plant height of oilseed rape cultivars as a function of different doses (0, 5 mM, 10 mM, 15 mM, and 20 mM) and different priming durations (3 h, 6 h, and 9 h) of picric acid

Treatment Levels × Priming Durations × Oilseed rape Cultivars Interaction						
		Abasin-95		*Dur e Nifa*		*Nifa Gold*
Control	3 h	7.66		11.65		12.04
5 mM		8.77		14.11		18.11
10 mM		9.95		15.34		16.94
15 mM		8.70		15.93		17.99
20 mM		7.77		13.89		19.73
Control	6 h	9.04		17.96		17.86
5 mM		8.89		17.62		19.02
10 mM		10.13		15.97		20.76
15 mM		9.01		17.89		19.25
20 mM		9.26		18.06		17.15
Control	9 h	8.63		14.65		16.19
5 mM		8.52		17.65		16.72
10 mM		10.05		17.58		15.92
15 mM		9.64		19.60		17.85
20 mM		9.67		17.53		17.43
Treatment Levels × Priming Durations Interaction						
	3 h	6 h		9 h		Treatment means
Control	10.45	14.95		13.16		12.85[b]
5 mM	13.63	15.18		14.30		14.38[a]
10 mM	14.08	15.62		14.52		14.74[a]
15 mM	14.21	15.38		15.70		15.10[a]
20 mM	13.79	14.82		14.88		14.50[a]
Priming Durations × Oilseed rape Cultivars Interaction						
	Abasin- 95	*Dur e Nifa*		*Nifa Gold*		Priming means
3 h	8.57	14.18		16.96		13.24[b]
6 h	9.27	17.50		18.81		15.19[a]
9 h	9.30	17.40		16.83		14.51[a]
Treatments Levels × Oilseed rape Cultivars Interaction						
	Control	5 mM	10 mM	15 mM	20 mM	Cultivars means
Abasin- 95	8.44	8.73	10.04	9.12	8.90	9.05[c]
Dur e Nifa	14.75	16.46	16.30	17.80	16.49	16.36[b]
Nifa Gold	15.36	17.95	17.84	18.37	18.11	17.53[a]

[*] LSD value at a significance level of 0.05% for treatment levels; 1.503, priming duration; 1.164 and oilseed rape cultivars; 1.164. Values with the same letters in rows and columns are not statistically significant

Effect on the number of siliqua/plant

The analysis of variance regarding the number of siliqua/plant confirmed the non-significant reaction of the plants to picric acid. Priming the seeds for 6 h did not significantly increase the number of siliqua compared to other durations. The *Nifa Gold* cultivar (14.69) produced the highest number of siliqua compared to the other genotypes. At the same time, the number of siliquas did not show-significant variations between the *Dur e Nifa* cultivar (14.36) and the *Nifa Gold* (14.69) cultivar. In contrast, the *Abasin- 95* cultivar (11.58) recorded a significant decrease in the number of siliqua observed in the Abasin- 95 cultivar (Table 8).

The number of siliqua confirms that *Nifa Gold* is best adapted to picric acid in contrast to the other genotypes. It appears that picric acid has the least effect on the genes responsible for the synthesis of siliqua in oilseed rape plants. It can also be concluded that oilseed rape plants are mature enough at the fruiting stage and less susceptible to the effect of mutagens, which makes them a good crop for cultivation under stress conditions [36]. A reduction in the number of fruits/plants was

Table 8 Number of siliqua in oilseed rape cultivars influenced by various doses of picric acid (0, 5 mM, 10 mM, 15 mM, and 20 mM) and different priming durations (3 h, 6 h, and 9 h)

Treatment Levels × Priming Durations × Oilseed rape Cultivars Interaction						
		Abasin-95		*Dur e Nifa*		*Nifa Gold*
Control	3 h	11.20		13.67		15.67
5 mM		8.07		15.33		16.33
10 mM		13.40		14.33		15.33
15 mM		12.63		13.00		14.33
20 mM		13.93		14.67		14.33
Control	6 h	9.43		18.00		18.67
5 mM		11.63		11.67		17.67
10 mM		11.30		13.00		8.33
15 mM		18.41		12.00		19.00
20 mM		12.41		17.00		10.67
Control	9 h	8.73		13.33		11.33
5 mM		9.87		18.00		12.67
10 mM		11.37		11.33		13.00
15 mM		10.35		14.33		16.67
20 mM		11.00		15.67		16.33
Treatment Levels × Priming Durations Interaction						
	3 h	6 h		9 h		Treatment means
Control	13.51	15.37		11.13		13.34
5 mM	13.24	13.66		13.51		13.47
10 mM	14.36	10.88		11.90		12.38
15 mM	13.32	16.47		13.78		14.53
20 mM	14.31	13.36		14.33		14.00
Priming Durations × Oilseed rape Cultivars Interaction						
	Abasin-95	*Dur e Nifa*		*Nifa Gold*		Priming means
3 h	11.85	14.20		15.20		13.75
6 h	12.64	14.33		14.87		13.95
9 h	10.26	14.53		14.00		12.93
Treatments Levels × Oilseed rape Cultivars Interaction						
	Control	5 mM	10 mM	15 mM	20 mM	Cultivars means
Abasin-95	9.79	9.86	12.02	13.80	12.45	11.58[b]
Dur e Nifa	15.00	15.00	12.89	13.11	15.78	14.36[a]
Nifa Gold	15.22	15.56	12.22	16.67	13.78	14.69[a]

[*] LSD value at 0.05% significance probability for oilseed rape cultivars; 1.801, treatment levels × priming duration; 0.5870, priming duration × oilseed rape cultivars; 0.4547 and treatment levels × oilseed rape cultivars; 0.7189. Values with the same letters in rows and columns are not statistically significant

observed by Bolbhat et al. [74], Mshembula et al. [83] and Jagajanantham et al. [75] using different chemical mutagens. In contrast, Birara et al. [76] reported an increase in the number of fruits/plants in sesame with chemical mutagens.

Effect on siliqua weight of the husk (g)

The dose of 20 mM (0.120) significantly increased the weight of rapeseed compared to the control (0.105). However, the changes induced by the doses of 5 mM (0.117), 10 mM (0.110), 15 mM (0.113) and 20 mM (0.120) were highly non-significant. Priming the priming of seeds for 9 h resulted in a significant increase in siliqua weight compared to 3 h duration (0.108). However, the differences between 6 h (0.114) and 9 h duration were not significant (Table 9).

It should be clear that both the weight of the seeds seed and that of the pods contribute to the total weight of the siliqua. And it was the weight of the husk of the siliqua, not that of the seeds, that was higher than that of the untreated seeds. The increase in husk mass of oilseed rape plants treated with picric acid could be due to the production of large protein molecules to cope with the altered conditions caused by the mutagen. Similar to the

Table 9 Siliqua weight (g) in oilseed rape cultivars influenced by various doses of picric acid (0, 5 mM, 10 mM, 15 mM, and 20 mM) and different priming durations (3 h, 6 h, and 9 h)

Treatment Levels × Priming Durations × Oilseed rape Cultivars Interaction						
		Abasin-95		*Dur e Nifa*		*Nifa Gold*
Control	3 h	0.100		0.090		0.087
5 mM		0.137		0.090		0.107
10 mM		0.113		0.100		0.110
15 mM		0.123		0.097		0.117
20 mM		0.123		0.117		0.103
Control	6 h	0.117		0.107		0.103
5 mM		0.123		0.120		0.107
10 mM		0.123		0.117		0.093
15 mM		0.093		0.123		0.120
20 mM		0.123		0.113		0.123
Control	9 h	0.120		0.107		0.113
5 mM		0.117		0.103		0.147
10 mM		0.100		0.120		0.117
15 mM		0.120		0.117		0.110
20 mM		0.137		0.127		0.117
Treatment Levels × Priming Durations Interaction						
	3 h	6 h		9 h		Treatment means
Control	0.092	0.109		0.113		0.105[b]
5 mM	0.111	0.117		0.122		0.117[a]
10 mM	0.108	0.111		0.112		0.110[a]
15 mM	0.112	0.112		0.116		0.113[a]
20 mM	0.114	0.120		0.127		0.120[a]
Priming Durations × Oilseed rape Cultivars Interaction						
	Abasin-95	*Dur e Nifa*		*Nifa Gold*		Priming means
3 h	0.119	0.099		0.105		0.108[b]
6 h	0.116	0.116		0.109		0.114[a]
9 h	0.119	0.115		0.121		0.118[a]
Treatments Levels × Oilseed rape Cultivars Interaction						
	Control	5 mM	10 mM	15 mM	20 mM	Cultivars Means
Abasin-95	0.112	0.126	0.112	0.112	0.128	0.118
Dur e Nifa	0.101	0.104	0.112	0.112	0.119	0.110
Nifa Gold	0.101	0.120	0.107	0.116	0.114	0.112

[*] LSD value at the significance level of 0.05% for the treatment levels; 0.01710 and the priming duration; 0.01325. Values with the same letters in rows and columns are not statistically significant

present results, Birara et al. [24] reported an increase in capsule weight in sesame using hydroxylamine. In contrast to the present study, Bolbhat et al. [74], Mshembula et al. [83], Jagajanantham et al. [75], Arisha et al. [90], and Olawuyi & Okoli [69] confirmed a decrease in pod weight in many plants due to chemical mutagens.

Effect on seed weight (g)

The *Abasin- 95* cultivar (0.068) had the highest seed weight compared to the other genotypes. However, the differences between the *Abasin*-95 cultivar (0.068) and the *Nifa Gold* cultivar (0.062) in terms of seed weight were highly non-significant. In contrast, the *Dur e Nifa* cultivar (0.059) recorded a significant decrease in seed weight. The *Nifa Gold* cultivar (0.100) showed the highest seed weight at a dose of 10 mM for 9 h. On the other hand, priming of the *Nifa Gold* cultivar (0.043) at a dose of 10 mM for 6 h had an inhibitory effect on seed weight (Table 10).

The results suggest that picric acid has the least effect on the genes responsible for seed production in oilseed rape plants [91]. It can also be deduced from this that genes responsible for seed production in oilseed rape plants counteract the mutagenic effects of

Table 10 Seed weight (g) of oilseed rape cultivars under the effect of different doses (0, 5 mM, 10 mM, 15 mM, and 20 mM) and various priming durations (3 h, 6 h, and 9 h) of picric acid

Treatment Levels × Priming Durations × Oilseed rape Cultivars Interaction						
		Abasin-95		*Dur e Nifa*		*Nifa Gold*
Control	3 h	0.060^{ab}		0.050^{ab}		0.047^{b}
5 mM		0.083^{ab}		0.047^{b}		0.057^{ab}
10 mM		0.063^{ab}		0.053^{ab}		0.060^{ab}
15 mM		0.070^{ab}		0.050^{ab}		0.063^{ab}
20 mM		0.067^{ab}		0.067^{ab}		0.063^{ab}
Control	6 h	0.070^{ab}		0.057^{ab}		0.053^{ab}
5 mM		0.060^{ab}		0.063^{ab}		0.053^{ab}
10 mM		0.080^{ab}		0.060^{ab}		0.043^{b}
15 mM		0.050^{ab}		0.070^{ab}		0.060^{ab}
20 mM		0.070^{ab}		0.060^{ab}		0.077^{ab}
Control	9 h	0.070^{ab}		0.057^{ab}		0.063^{ab}
5 mM		0.070^{ab}		0.053^{ab}		0.100^{a}
10 mM		0.050^{ab}		0.067^{ab}		0.070^{ab}
15 mM		0.070^{ab}		0.063^{ab}		0.057^{ab}
20 mM		0.080^{ab}		0.067^{ab}		0.067^{ab}
Treatment Levels × Priming Durations Interaction						
	3 h	6 h		9 h		Treatment means
Control	0.052	0.060		0.063		0.059
5 mM	0.062	0.059		0.074		0.065
10 mM	0.059	0.061		0.062		0.061
15 mM	0.061	0.060		0.063		0.061
20 mM	0.066	0.069		0.071		0.069
Priming Durations × Oilseed rape Cultivars Interaction						
	Abasin-95	*Dur e Nifa*		*Nifa Gold*		Priming means
3 h	0.069	0.053		0.058		0.060
6 h	0.066	0.062		0.057		0.062
9 h	0.068	0.061		0.071		0.067
Treatment Levels × Oilseed rape Cultivars Interaction						
	Control	5 mM	10 mM	15 mM	20 mM	Cultivars means
Abasin-95	0.067	0.071	0.064	0.063	0.072	0.068^{a}
Dur e Nifa	0.054	0.054	0.060	0.061	0.064	0.059^{b}
Nifa Gold	0.054	0.070	0.058	0.060	0.069	0.062^{a}

* LSD value at a significance level of 5% for oilseed rape cultivars; 0.01325 and treatment levels × priming duration × oilseed rape cultivars; 0.05131. Values with the same letters in rows and columns are not statistically significant

picric acid [47]. A similar result, namely a non-significant increase in seed weight in chickpea genotypes, was reported by Wani & Anis [92]. In contrast to our results, Bolbhat et al. [74], Mshembula et al. [83], Jagajanantham et al. [75], and Arisha et al. [90] reported a reduction in seed weight of horse gram, cowpea, lady finger and bell pepper by the application of chemical mutagens. In contrast, some researchers reported positive effects of chemical mutagens on the weight of sesame and hibiscus seeds [75].

Effect on fresh and dry weight (gm)

In contrast to the other soaking times, the seeds soaked for 6 h yielded the plants with the highest fresh weight (37.58) in contrast to other priming durations. At the same time, soaking times of 3 h (35.77) and 6 h (37.58) were found to have no significant effect on fresh biomass of oilseed rape. Likewise, pre-soaking the seeds for 3 h and 9 h did not result in significant changes in the fresh biomass of oilseed rape. Conversely, plants grown from seeds pre-soaked for 6 h (37.58 gm) and

9 h durations (34.03 g) showed significant changes in fresh weight.

The *Dur e Nifa* cultivar recorded the highest fresh/dry weight (36.91/24.31), followed by the *Nifa Gold* cultivar (36.22/23.71) and the *Abasin*-95 cultivar (34.25/20.93). At the same time, the *Dur e Nifa* cultivar and the *Nifa Gold* cultivar did not show significant differences in fresh/dry biomass. In contrast, the differences between the *Dur e Nifa* cultivar and the *Abasin*-95 cultivar were highly significant in fresh biomass. However, the *Abasin- 95* cultivar showed significant differences in dry biomass compared to the other genotypes. The 2nd order interaction (B × C) increased the fresh biomass (40.2) of the *Nifa Gold* cultivar, primed for 6 h. On the other hand, *Abasin- 95* cultivar, primed for 9 h recorded the lowest fresh biomass (31.73) (Table 11 & 12).

The compiled results on biomass attributes showed no-significant effects of picric acid on fresh/dry weight of oilseed rape plants. At the same time, priming the seeds for 6 h resulted in significant changes in the fresh weight of in the plants. There were also significant differences between the rapeseed varieties in the fresh and dry weights of the plants. An increase in fresh weight due to the application of various mutagens has already been

Table 11 Effect of various treatment levels of picric acid (0, 5 mM, 10 mM, 15 mM, and 20 mM) and different priming durations (3 h, 6 h, and 9 h) on the fresh weight (g) of three oilseed rape cultivars

Treatment Levels × Priming Durations Oilseed rape Cultivars Interaction						
		Abasin-95		*Dur e Nifa*		*Nifa Gold*
Control	3 h	37.11		35.01		32.46
5 mM		39.28		38.71		36.95
10 mM		36.21		34.50		34.11
15 mM		37.88		40.22		34.89
20 mM		31.98		32.82		34.40
Control	6 h	30.61		41.31		38.75
5 mM		35.45		41.73		33.24
10 mM		35.14		37.48		38.97
15 mM		39.38		33.80		47.30
20 mM		32.05		35.59		42.89
Control	9 h	25.31		32.68		35.95
5 mM		30.99		37.69		28.31
10 mM		38.04		36.27		35.44
15 mM		30.34		36.86		39.02
20 mM		33.97		39.01		30.55
Treatment Levels × Priming Durations Interaction						
	3 h		6 h		9 h	Treatment means
Control	34.86		36.89		31.33	34.36
5 mM	38.31		36.80		32.33	35.86
10 mM	34.94		37.20		36.58	36.24
15 mM	37.66		40.16		35.41	37.74
20 mM	33.07		36.84		34.51	34.81
Priming Durations × Oilseed rape Cultivars Interaction						
	Abasin-95		*Dur e Nifa*		*Nifa Gold*	Priming means
3 h	36.49^{bc}		36.25^{bc}		34.56^{bcd}	35.77^{ab}
6 h	34.53^{bcd}		37.98^{ab}		40.23^{a}	37.58^{a}
9 h	31.73^{d}		36.50^{bc}		33.86^{cd}	34.03^{b}
Treatment Levels × Oilseed rape Cultivars Interaction						
	Control	5 mM	10 mM	15 mM	20 mM	Cultivars Means
Abasin-95	31.01	35.24	36.46	35.86	32.67	34.25^{b}
Dur e Nifa	36.33	39.38	36.08	36.96	35.81	36.91^{a}
Nifa Gold	35.72	32.83	36.17	40.41	35.95	36.22^{ab}

* LSD value at the 5% significance level for priming duration = 2.052, oilseed rape cultivars = 2.052 and priming duration × oilseed rape cultivars = 3.555. Values with the same letters in rows and columns are not statistically significant

Table 12 Effect of various treatment levels of picric acid (0, 5 mM, 10 mM, 15 mM, and 20 mM) and different priming durations (3 h, 6 h, and 9 h) on the dry weight (g) of three oilseed rape cultivars

Treatment Levels × Priming Durations × Oilseed rape Cultivars Interaction						
		Abasin-95		*Dur e Nifa*		*Nifa Gold*
Control	3 h	19.00		22.94		23.90
5 mM		22.11		24.25		25.32
10 mM		21.93		25.92		23.61
15 mM		21.79		26.73		22.83
20 mM		19.96		25.24		23.18
Control	6 h	20.37		26.05		23.71
5 mM		23.55		23.03		24.30
10 mM		21.36		22.31		23.99
15 mM		21.94		23.36		26.45
20 mM		21.92		23.86		25.06
Control	9 h	18.27		23.29		24.41
5 mM		21.10		25.87		21.94
10 mM		21.38		22.03		21.50
15 mM		18.73		23.65		23.74
20 mM		21.24		26.14		21.91
Treatment Levels × Priming Durations Interaction						
	3 h	6 h		9 h		Treatment means
Control	21.95	23.38		21.99		22.44
5 mM	23.89	23.63		22.97		23.50
10 mM	23.57	22.55		21.55		22.56
15 mM	23.78	23.92		22.04		23.25
20 mM	22.79	23.61		23.10		23.17
Priming Durations × Oilseed rape Cultivars Interaction						
	Abasin-95	*Dur e Nifa*		*Nifa Gold*		Priming means
3 h	20.81	25.02		23.77		23.20
6 h	21.83	23.72		24.70		23.42
9 h	20.14	24.20		22.65		22.33
Treatment Levels × Oilseed rape Cultivars Interaction						
	Control	5 mM	10 mM	15 mM	20 mM	Cultivars means
Abasin-95	19.21	22.25	21.31	20.82	21.04	20.93[b]
Dur e Nifa	24.09	24.38	23.42	24.58	25.08	24.31[a]
Nifa Gold	24.01	23.85	22.95	24.34	23.38	23.71[a]

[*] LSD value at 5% probability of significance for oilseed rape cultivars = 0.9211. Values with the same letters in rows and columns are not statistically significant

observed in many plants such as mulberry [84], sesame [76], calendula [70] and kenaf [93]. In contrast, studies by Jagajanantham et al. [75] confirmed the inhibitory effect of mutagens on the fresh weight of plants and Mshembula et al. [83] and Jagajanantham et al. [75] also reported a decrease in dry weight in plants such as cowpea and *Hibiscus* sp. from seeds treated with some mutagens. On the other hand, [76, 84] reported an increase in dry weight of mulberry, sesame and hibiscus by mutagens.

Effect on moisture contents (%)

Moisture contents showed significant variations for the applied doses, the selected cultivars and the order 1st (A × B), 2nd (B × C) and 3rd (A × C) orders interactions. At the same time, priming and the 4th order interaction had no significant effects on the moisture content of rapeseed.

The dose of 15 mM (37.72) showed the highest moisture content, followed by 10 mM (37.08), 0 mM (33.87), 5 mM (33.11) and 20 mM (32.73). At the same time, the dosages of 10 mM and 15 mM did not result in significant changes in the moisture content compared to the other dosages. Likewise, the dosages of 0 mM and 10 mM did not show significant differences in moisture content. The same trend of non-significance in moisture content was observed at the 0 mM, 5 mM and 20 mM doses. In

contrast, a dose of 15 mM picric acid resulted in significant changes in moisture content in contrast to the 0 mM, 5 mM and 20 mM treatment levels. Similarly, a dose of 10 mM resulted in significant effects on moisture content compared to 5 mM and 20 mM.

The *Abasin*-95 cultivar (37.92) had the highest moisture content compared to the other genotypes. At the same time, the *Dur e Nifa* cultivars (33.24) and the *Nifa Gold* cultivar (33.55) showed highly non-significant variations in moisture content. The 1 st order interaction (A ×B) resulted in a significant increase in the moisture content of rapeseed (40.28) compared to the other treatments under the effect of the 15 mM dose and the 9 h presoaking. In contrast, the moisture content decreased to 28.04% when the seeds were soaked for 9 h with a 5 mM dose. The 2nd order interaction (B ×C) increased the elevated moisture content of the *Abasin- 95* cultivar (42.27) primed for 3 h in contrast to the other treatments. Priming the *Dur e Nifa* cultivar for 3 h decreased the moisture content (30.39) compared to the other treatments. The 3rd order interaction (A ×C), stimulated the moisture content (41.13) of the *Abasin- 95* cultivar

Table 13 Effect of various treatment levels of picric acid (0, 5 mM, 10 mM, 15 mM, and 20 mM) and different priming durations (3 h, 6 h, and 9 h) on the moisture content (%) of three oilseed rape cultivars

Treatment Levels × Priming Durations × Oilseed rape Cultivars Interaction						
		Abasin-95		*Dur e Nifa*		*Nifa Gold*
Control	3 h	48.28		34.45		26.04
5 mM		43.79		37.39		31.08
10 mM		40.98		24.60		30.60
15 mM		41.74		33.09		34.56
20 mM		36.56		22.42		32.60
Control	6 h	33.49		35.75		38.24
5 mM		32.33		42.37		26.88
10 mM		38.79		39.56		38.36
15 mM		43.34		30.73		43.77
20 mM		31.23		32.80		40.85
Control	9 h	27.77		28.80		32.04
5 mM		31.65		30.45		22.03
10 mM		43.61		37.70		39.54
15 mM		37.99		35.60		38.67
20 mM		37.17		32.93		27.98
Treatment Levels × Priming Durations Interaction						
	3 h	6 h		9 h		Treatment means
Control	36.26^{abc}	35.83$^{a–d}$		29.54^{ef}		33.87^{bc}
5 mM	37.42^{abc}	33.86$^{b–e}$		28.04^{f}		33.11^{c}
10 mM	32.06$^{c–f}$	38.90^{ab}		40.28^{a}		37.08^{ab}
15 mM	36.46^{abc}	39.28^{bc}		37.42^{abc}		37.72^{a}
20 mM	30.53^{def}	34.96$^{a–e}$		32.69$^{c–f}$		32.73^{c}
Priming Durations × Oilseed rape Cultivars Interaction						
	Abasin-95	*Dur e Nifa*		*Nifa Gold*		Priming means
3 h	42.27^{a}	30.39^{d}		30.98^{d}		34.55
6 h	35.84^{bc}	36.24^{bc}		37.62^{b}		36.57
9 h	35.64^{bc}	33.10^{cd}		32.05^{cd}		33.60
Treatment Levels × Oilseed rape Cultivars Interaction						
	Control	5 mM	10 mM	15 mM	20 mM	Cultivars means
Abasin-95	36.52^{abc}	35.92^{abc}	41.13^{a}	41.03^{a}	34.99^{bc}	37.92^{a}
Dur e Nifa	33.00^{cd}	36.74^{abc}	33.96^{bcd}	33.14^{cd}	29.38^{de}	33.24^{b}
Nifa Gold	32.10^{cde}	26.66^{e}	36.16^{abc}	39.00^{ab}	33.10^{bcd}	33.55^{b}

* LSD value at the 5% significance level for treatment levels = 3.288, priming durations = 0.2625, oilseed rape cultivars = 2.450, treatment levels × priming durations = 5.479, priming durations × oilseed rape cultivars = 4.244 and treatment levels × oilseed rape cultivars = 5.479.Values with the same letters in rows and columns are not statistically significant

exposed to a dose of 10 mM. In contrast, the *Nifa Gold* cultivar treated with a dose of 5 mM showed the lowest moisture content (26.66) (Table 13).

In contrast to traits such as fresh/dry weight, picric acid can increase or decrease the moisture content of oilseed rape plants. Moreover, oilseed rape cultivars showed significant variations between varieties in terms of moisture content. The increase in moisture content at some picric acid doses (10 mM and 15 Mm), indicates that some anatomical variations (increase in trichomes) are induced by the mutagens in oilseed rape plants. Trichomes and stomata play a very important role in the conservation of water in the plant [77]. Trichomes are the epidermal outgrowths of plants. Trichomes increase the reflection of sunlight which helps to lower the internal temperature and thus reduces transpiration in plants growing under water deficit conditions. This helped the plants to intercept the dew drops, reduce transpiration and conserve water [80]. Studies by Kumar et al. [84], Akhtar [17] and Birara et al. [76] confirm the possibility of increasing the moisture content of plants by using mutagens [94] also reported five genotypes of *B. napus* and their physiological attributes and growth characters under drought induced osmotic stress [95] examined the transgenic rape seed lines and their phenophysiological traits under glyphosate treatment.

Effect on chloroplast genome

Control sample

The chloroplast genome of the control sample (contig 1) consists of 153,452 base pairs including a total of 10 rRNA and 38 t -RNA genes. It has two inverted repeat regions (a,b), which can be clearly seen in Fig. 1.

Treated sample

The chloroplast genome of plants grown from picric acid-treated seeds was mutated. Therefore, annotation of the genome was not possible. Numerous single nucleotide substitutions (187) were detected between the control and test samples (Table 14). In addition, 43 additions/deletions were observed (Table 15).

Future research directions

Future research on the carcinogenic effects of picric acid on *Brassica napus* L. (Brassicaceae)'s chloroplast genome and biological characteristics may go in the following directions:

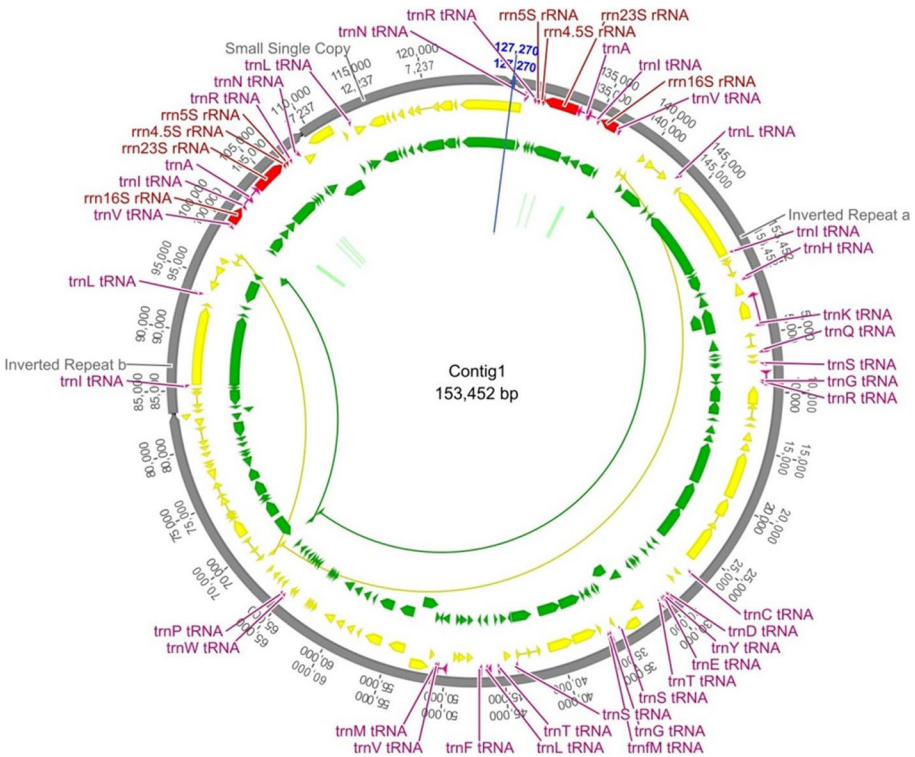

Fig. 1 Diagrammatical representation of the chloroplast genome (153, 452 bp), illustrating gene annotations i.e. green represented as protein-coding genes, purple represented as tRNA genes, red represented as rRNA genes, gray represented as inverted repeat regions. Arrows demonstrated the direction of gene transcription

Table 14 Comparison of control and treated samples based on single nucleotide polymorphism

Control	Test	No. of substitutions
Adenine	Cytosine	22
Adenine	Guanine	10
Adenine	Thymine	20
Thymine	Adenine	8
Thymine	Cytosine	18
Thymine	Guanine	22
Cytosine	Adenine	19
Cytosine	Guanine	3
Cytosine	Thymine	11
Guanine	Adenine	25
Guanine	Cytosine	3
Guanine	Thymine	26
Total		187

1. Examine whether these mutations enhance or decrease the plant's resistance to environmental stressors such as heat, salinity, or drought.
2. Examine the possibility of creating new Brassica cultivars with desired features by using the produced mutations.
3. Examine alterations in secondary metabolites that may affect therapeutic usage or pest resistance.
4. Research possible hazards related to picric acid emission into the environment and how it affects organisms that are not the intended targets.
5. Integrate research with CRISPR/Cas9 or other gene-editing tools to implement targeted modifications based on mutagenesis outcomes.

Conclusion

This study shows that picric acid has a strong mutagenesis effect on the chloroplast genome and biological properties of *Brassica napus* cultivars. Picric acid's mutagenic influence was obvious in delayed seedling emergence, lower percentage emergence, and significant genomic alterations, including 187 single nucleotide changes and 43 insertions/deletions. Despite its inhibitory effects on early growth metrics, specific doses and priming durations had stimulatory effects, particularly on plant height and seed weight, with the *Nifa Gold* cultivar surpassing others in terms of adaptation and productivity under mutagenic conditions. These findings highlight picric acid dual role as an inhibitor and potential promoter of agronomic traits, depending on the dosage and exposure period. The findings also point to its possible utility in mutation breeding programs targeted at boosting specific agricultural attributes. More research into optimising its use and understanding its broader effects on genomic stability and plant physiology is required. Moreover, priming can improve germination and certain

Table 15 Comparison of control and treated samples based on number of indels (insertions/deletions)

	Control	Treatment
1	CAGTATTGA	CAGTATTGAGTATTGA
2	TTATATATATAAATATATATATAAATATAT	TTATATATATATAAATATAT
3	GTGAAA	GTGAAATAATGAAA
4	TAAA	TAAAA
5	TA	TAA
6	TTCTTTATCTTTA	TTCTTTA
7	A	AG
8	TA	TAA
9	TA	TAA
10	CAGAAAAAA	CAGAAAAAAGAAAAA
11	GAAAAAA	GAAAAAATAAAAAA
12	TTTACTTTCTTTAACTTTCTATTACTT TCTTTAACTTT	TTTACTTTCTTTAACTTTCTAT
13	CTTCTTTCTTTC	CTTCTTTCTTTCTTTC
14	TC	TCTTAC
15	G	GT
16	CTTTTTTGTTTTTTG	CTTTTTTG
17	TTCATATCATA	TTCATA
18	TAAA	TAA
19	GAATTAAATTA	GAATTA
20	CTGAAATCAAATGAAATCAAAT	CTGAAATCAAAT
21	TTAATTCTA	TTAATTCTATAATTCTA
22	CTA	CTATAATTATA
23	TTTATCTTATCTTATC	TTTATCTTATC
24	G	GT
25	GC	G
26	TAGTGAAAGTG	TAGTG
27	TT	TTCTATAT
28	ATT	AT
29	CTATTTTATT	CTATTTTATTTTATT
30	TTATTA	TTATTATATTA
31	ATT	ATTTTGTT
32	CCCT	C
33	CTGAAC	CTGAACGATGAAC
34	CATTT	CATTTTATTT
35	AAAATCAGAA	AAAATCAGAAATCAGAA
36	CAGAA	CAGAAATAAGAA
37	CTTTTTTTTTT	CTTTTTTTTTTTT
38	TTAAGTCAAA	TTAAGTCAAATAAGTCAAA
39	GTCAAA	GTCAAATAATTCAAA
40	TATATTAG	TATATTAGATATTAG
41	ATCAA	ATCAACTCAA
42	CTTCTTTCTTTCTTT	CTTCTTTCTTT
43	ATCAAATTCAAATTC	ATCAAATTC

morpho-agronomic attributes of *B. napus*. The studied parameters confirmed the variations among the selected cultivars and can be used for further studies. The chloroplast genome studies categorically confirmed the mutagenic effect of picric acid. Taken together, these results provide new information to understand the mutagenic interactions on the chloroplast genome, while the application of picric acid, which can affect the growth and development of *B. napus*, has a positive morpho-agronomic effect.

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Authors' contributions
I.A.: Conceptualization, writing – original draft, data curation, methodology, software; B.U., Z.M.: Visualization, validation, project administration, software, supervision; M.N.K, A.K.: Methodology, formal analysis, investigation, validation; M.A.J.: Writing – review & editing, resources, software, formal analysis, visualization; D.D.O., M.I., U.B.A., S.A.R., M.A.A: Writing – review & editing, resources, data curation, funding acquisition; S.K.A., L.A.A.S & D.A. All authors contributed significantly, and have read and agreed to the published version of the manuscript."

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Data availability
Availability of chloroplast genome data and materials has been deposited in NCBI GenBank Database and under the accession number PV294938 (https://www.ncbi.nlm.nih.gov/nuccore/2930385756?log$ = activity). All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Oilseed rape genotypes (Abasin- 95, Dur e Nifa and Nifa Gold) were obtained from Nuclear Institute for Agriculture, Peshawar (NIFA), Pakistan. All the experiments were performed in accordance with relevant guidelines and regulations".

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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