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Evaluation of gamma-irradiated *Pisum sativum* germplasm for agronomic traits and tolerance to *Didymella pinodes*

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Abstract: Ascochyta blight, caused by a complex of pathogenic fungi including *Didymella pinodes*, *Ascochyta pisi*, and *Phoma pinodella*, is a major disease of field pea (*Pisum sativum*), causing severe losses through lesions on leaves, stems, and pods. Mutation breeding using gamma irradiation is a non-GMO strategy to induce genetic variation and accelerate the development of improved genotypes. In this study, the M_2 generation of the forage pea cultivar Dodoni (*Pisum sativum* L. var. *arvense*), derived from M_0 seeds irradiated with 100 Gy, was evaluated for tolerance to *D. pinodes* (CBS 251.47) using a detached-leaf assay under controlled greenhouse conditions. Disease progression was quantified via image-based analysis on the 3rd and 5th days post-infection, calculating diseased area and disease severity index. Extensive phenotypic evaluation was also conducted on 16 families in the greenhouse and 100 families under field conditions, using an augmented incomplete block design. Screening revealed several M_2 families with significantly improved tolerance compared to non-irradiated controls. Among these, some individuals combined enhanced resistance with improved yield-related traits, such as higher pod number and biomass, while others exhibited reduced agronomic performance. These findings highlight the phenotypic diversity induced by gamma irradiation and demonstrate the potential to generate dual-purpose pea genotypes with both disease resistance and enhanced productivity, providing valuable material for future breeding of resilient cultivars.

Keywords: Ascochyta blight; disease tolerance; field pea; mutagenesis; mutation breeding

Field pea (*Pisum sativum* L.) is one of the world's most important pulse crops, cultivated on over 7 million hectares globally and prized for nitrogen-fixing ability and role in sustainable cropping systems (FAOSTAT 2022; Brhane & Hammenhag 2024). Its contribution to soil fertility improvement and controlled

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use of fertilisers makes it a key component in crop rotation and climate-resilient agriculture strategies. One of the most valued attributes of field pea is its high seed protein content, typically ranging between 20–25%, making it an important source of plant-based protein in both human and animal diets (Tzitzikas et al. 2006). This nutritional profile positions pea as a promising crop in the context of rising global demand for sustainable protein sources. The protein in field pea is rich in essential amino acids such as lysine (Duranti & Gius 1997). Advances in breeding and genomics have enhanced further pea protein yield and quality, making field pea an attractive crop for addressing nutritional security in both developed and developing countries (Smýkal et al. 2012; Klein et al. 2020). However, pea production is increasingly constrained by Ascochyta blight, an aggressive disease that can cause yield losses ranging from 10% to 60% depending on environmental conditions and cultivar susceptibility (Martin-Sanz et al. 2011; Fonseka et al. 2023). Ascochyta blight is caused by a pathogen complex that includes *Didymella pinodes* (syn. *Mycosphaerella pinodes*, formerly *Ascochyta pinodes*), *Didymella pinodella* (syn. *Phoma medicaginis* var. *pinodella*), and *Ascochyta pisi* (*Didymella pisi*) (Tivoli & Banniza 2007; Barilli et al. 2016). Among these, *D. pinodes* is the most prevalent and virulent species worldwide. It infects seedlings and all aerial parts of the plant, such as leaves, stems, and pods, leading to necrotic lesions, defoliation, foot rot, and seed discolouration. Its survival and spread are facilitated by durable reproductive structures (pycnidia and pseudothecia) that persist on crop residues, enabling rapid epidemic development under humid conditions (Fonseka et al. 2023).

Controlling Ascochyta blight in pea, mainly caused by *Didymella pinodes*, is challenging due to the disease's complexity and limited efficacy of control measures (Fonseka et al. 2023). Cultural practices and rotation reduce inoculum but provide inconsistent protection, while fungicides raise sustainability and resistance concerns. Thus, genetic resistance is the most viable long-term option (Tivoli & Banniza 2007). However, durable resistance is difficult since most pea germplasm sources are partial, quantitative, and influenced by environment and pathogen variability (Fondevilla et al. 2011; Barilli et al. 2016). Genome wide association studies (GWAS) have identified loci linked to resistance in *Peyronellaea pinodes* and *Ascochyta koolunga* (Lee et al. 2023), and genomic/transcriptomic studies of *Ascochyta pisi* revealed pathogenicity factors and effector genes (Liu et al. 2023). Advanced strategies combining genomic selection, speed breeding, and molecular markers aim to accelerate resistance introgression (Timmerman-Vaughan et al. 2016; Jha et al. 2017; Carpenter et al. 2018; Parihar et al. 2022). Broadening pea's genetic base through diverse germplasm and wild relatives is also critical. Genomic prediction further supports breeding for resistance and other traits in intercropping systems (Annicchiarico et al. 2021). Overall, integrating multi-omics, functional genomics, and high-throughput phenotyping is essential, alongside deeper knowledge of pathogen biology and host defences, to achieve durable resistance in pea.

Pea resistance to *Mycosphaerella pinodes* has also been studied at the genomic and molecular level. Partial resistance was shown to be polygenic, a complex trait governed by multiple quantitative trait loci (QTLs) expressed at seedling and adult plant stages (Prioul et al. 2004). Several studies have identified key genomic regions and candidate genes associated with hypersensitive response (Carrillo et al. 2014; Boutet et al. 2023), some of which are also involved in response to abiotic stress such as frost, as well as in plant development and architecture such as plant height and flowering time (Prioul et al. 2004, 2007; Boutet et al. 2023). Furthermore, stress and metabolism-related proteins involved in early defence signalling and pathogen containment (Castillejo et al. 2010), and also genomic regions directly involved in cellular defence responses (Carrillo et al. 2014) were revealed by proteomic and histological phenotyping studies, respectively.

One promising strategy to introduce new allelic variation is mutation breeding, particularly using gamma irradiation. This non-transgenic approach induces a broad spectrum of heritable genetic changes, from point mutations to chromosomal rearrangements, thereby accelerating the development of improved phenotypes, including disease resistance (Ahloowalia et al. 2004; Shu et al. 2012). Gamma mutagenesis has proven effective in other legumes, including lentil, chickpea, and soybean, where it has been used to improve a range of traits such as seed yield, disease resistance, drought tolerance, early maturity, and seed quality. This approach is especially valuable for species like pea that exhibit limited natural diversity and are less amenable to genetic transformation (Shu et al. 2012). Importantly, because it does not involve transgenes, mutation breeding is generally well-accepted by both regulatory authorities and consumers. In our previous study, gamma irradia-

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tion of seeds from the forage pea cultivar Dodoni at 100 Gy produced M_1 plants exhibiting a wide range of agronomic phenotypes, indicating that the M_2 generation may harbour valuable allelic variants related to traits like disease tolerance and high yield (Sarri et al. 2024).

However, large-scale screening of irradiated populations requires precise, high-throughput phenotyping platforms. Traditional field evaluations are often hampered by environmental variability and inconsistent disease pressure. In contrast, ex-planta bioassays offer a controlled and scalable alternative for evaluating host–pathogen interactions (Annan et al. 2023). When combined with digital image analysis, this approach enables accurate quantification of lesion development and calculation of disease severity indices, facilitating early selection of resistant genotypes (Schneider et al. 2012; Liu et al. 2016). Detached-leaf assays have been successfully used in pea to evaluate responses to *D. pinodes*, and are well suited for screening mutagenized populations under uniform infection pressure. For example, Joshi et al. (2022) differentiated resistant and susceptible field pea genotypes using a *P. pinodes* and *D. pinodella* detached-leaf assay coupled with digital lesion quantification (r = 0.89 and 0.75, respectively). Barilli et al. (2016) used detached pea leaves inoculated with *D. pinodes* to study defence mechanisms, while Pandey et al. (2022) screened breeding lines against the blackspot complex under controlled detached-leaf conditions with reliable genotype discrimination. In this study, we assessed the M_2 irradiated forage pea cultivar Dodoni (*P. sativum* var. *arvense*) for tolerance to *Didymella pinodes* (strain CBS 251.47) using a detached-leaf bioassay conducted under controlled conditions. Evaluation of the M_2 families revealed considerable variation in resistance to *D. pinodes* and agronomic traits. Some individuals combined enhanced resistance with improved yield, while others showed reduced performance, highlighting the importance of individual-level selection for future pea breeding.

MATERIAL AND METHODS

Plant material and genetic background. The plant material used in this study consisted of M_2 generation individuals derived from the forage pea (*Pisum sativum* L. var. *arvense*) cultivar Dodoni. This Greek landrace, registered in the National Catalogue of Major Crops in 1985, is characterised by its adaptability to the country's agro-climatic conditions. Dodoni is a fall-sown, mid- to late-maturing variety with high tolerance to low temperatures and frost, strong lodging resistance, and partial resistance to several important pathogens, including *Erysiphe polygoni*, *Fusarium oxysporum*, *Septoria pisi*, and *Uromyces fabae*. Plants typically reach a length of 150 cm, produce numerous lateral shoots, and bear purple flowers. Seed production is robust, with a 1 000-seed weight ranging from 90 to 110 grams. M_0 seeds of Dodoni were exposed to 100 Gy of gamma irradiation at the laboratories of the International Atomic Energy Agency (IAEA) in Austria to induce genetic variation. The resulting M_1 generation was cultivated under field conditions during the and served as a first-level phenotypic screening. Agronomic traits such as plant height, biomass, and seed yield were evaluated. Based on this assessment, high-performing M_1 individuals were selected to move to the M_2 generation (Sarri et al. 2024). The M_2 population was subsequently evaluated both under field conditions and in a controlled laboratory inoculation assay with *Didymella pinodes*, aiming to assess phenotypic variation, particularly in relation to disease tolerance. Non-irradiated Dodoni plants were included as controls in both trials. The *Didymella pinodes* strain CBS 251.47 used for inoculations was obtained from the Westerdijk Fungal Biodiversity Institute.

Plant cultivation and experimental setup. Comparisons were made between 16 irradiated families (20 plants per family) and non-irradiated control plants (4 per family), summing 384 plants (320 irradiated and 64 controls). The experimental layout was according to completely randomized design (CRD) with seven replications.

Seeds from both irradiated and control plants were surface sterilised in a 10% sodium hypochlorite solution and germinated on sterile, moist filter paper within disinfected Petri dishes. Germination was conducted in the absence of light at ambient temperature. Seedlings exhibited radicle and plumule emergence within five days. Germinated seedlings were transplanted into 5 × 4-cell seedling trays filled with a sterilised soil-to-perlite mixture (3 : 1 v/v) and grown under controlled greenhouse conditions at the Agricultural University of Athens (37°59'9''N, 23°42'23''E). Each seedling was tagged with a unique identifier corresponding to its irradiated family. After approximately 30 days of growth, when seedlings had developed at least seven true leaves, leaves were excised (removed) from each plant using sterile

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scissors. The seedlings were then transplanted into 2-L pots and relocated to the greenhouse (37°58'58''N, 23°42'19''E). Plants were arranged in rows by family, with control plants systematically placed at the end of each row to ensure the comparative evaluation of the harvested M_3 seeds.

In vitro detached leaf bioassays. Detached leaf bioassays were performed in vitro using the *Didymella pinodes* (syn. *Ascochyta pinodes*) CBS 251.47 strain and excised pea leaflets from both gamma-irradiated and non- irradiated plants, following the protocol of Liu et al. (2016) with modifications. The fungal strain was maintained on potato dextrose agar (PDA) plates and incubated at 21 °C for 8–10 days until mycelial growth covered approximately three quarters of the agar surface, without entering the sporulation phase. Cylindrical agar plugs (5–6 mm diameter) were excised from the colony periphery using the wide end of sterilised pipette tips. For inoculation, each plug was placed with the mycelial side in direct contact with the adaxial leaflet surface, carefully avoiding the central vascular bundle tissues. Sterile PDA plugs, treated identically but without fungal inoculation, served as negative controls.

Detached pea leaflets were collected from the first, second, and third nodes of healthy composite leaves (up to six leaflets per composite leaf) from each family. Leaflets were surface-sterilised in 70% ethanol for 10 s, rinsed twice in sterile distilled water, and briefly dried on sterile Whatman paper. To prevent desiccation, leaflets were rehydrated and then transferred to sterile Petri dishes lined with moist filter paper. For each family, seven leaflets from independent plants (20 irradiated and 4 control plants per family) were randomly and evenly distributed across two Petri dishes (3 and 4 leaflets per dish), ensuring consistent replication. Inoculated Petri dishes were incubated in a growth chamber (Model GRW 1000SB CMP) under controlled conditions (22 °C temperature, 50% relative humidity, and 16 h light/8 h dark photoperiod) for five days.

Disease progress was assessed on the 3rd and 5th days post-inoculation. Digital images of inoculated leaflets were captured using a standardised photographic setup inside a dark photobox. Images were analysed with ImageJ software (National Institutes of Health, USA), and lesion area and the disease severity index (DSI) – lesion area relative to total leaflet area – were calculated. The pixel-to-area calibration was standardised at 245 pixels per cm^2.

Data acquisition and analysis. Digital images of the inoculated leaves were captured on the 3rd and 5th days post-inoculation using a standardised photographic setup within a dark photobox. Total leaf area and infected area (cm^2) were quantified, and per cent infection relative to total leaf area was calculated. Image processing and analysis were performed using a custom protocol in ImageJ (National Institutes of Health, USA), with lesion area calibrated based on 245 pixels per square centimetre. Pixel-based area values were exported to Excel for further calculations. Based on calibration using a known scale, 1 cm was estimated to correspond to approximately 240–250 pixels.

Field trial conditions. A parallel field trial was conducted to assess the agronomic performance and phenotypic variation among M_2 irradiated lines and non-irradiated control plants of the forage pea cultivar Dodoni. Seeds were pre-germinated in sterile Petri dishes, transferred to greenhouse trays for early growth, and later transplanted to the experimental field of the Agricultural University of Athens (37°59'07.8''N, 23°42'21.6''E). The experiment was conducted using an augmented block design, in which families were assigned as treatments. Standard spacing and labelling were applied to enable the tracking of individual plant performance. Key growth and reproductive traits – such as height, flowering time, pod number, and seed yield – were recorded under field conditions. No chemical inputs were used.

Statistical analysis. Data from both trials were analysed using the R programming language (R Core Team 2024) within the RStudio software environment (RStudio Team 2024). Prior to analysis, assumptions of normality and homogeneity of variances were verified using the stats package (R Core Team 2024) for the Shapiro–Wilk test and the car package (Fox & Weisberg 2019) for Levene's test, respectively. For the controlled trial under a completely randomised design (CRD) and for the field trial conducted under the augmented incomplete block design, analysis of variance (ANOVA) was applied. Mean comparisons were performed using the least significant difference (*LSD*) test from the Agricola package (De Mendiburu 2020) at a significance level of $\alpha = 0.05$.

RESULTS

Evaluation of pea leaf tolerance to *Didymella pinodes* infection. The study aimed at the evaluation of the *Pisum sativum* cv. Dodoni germplasm

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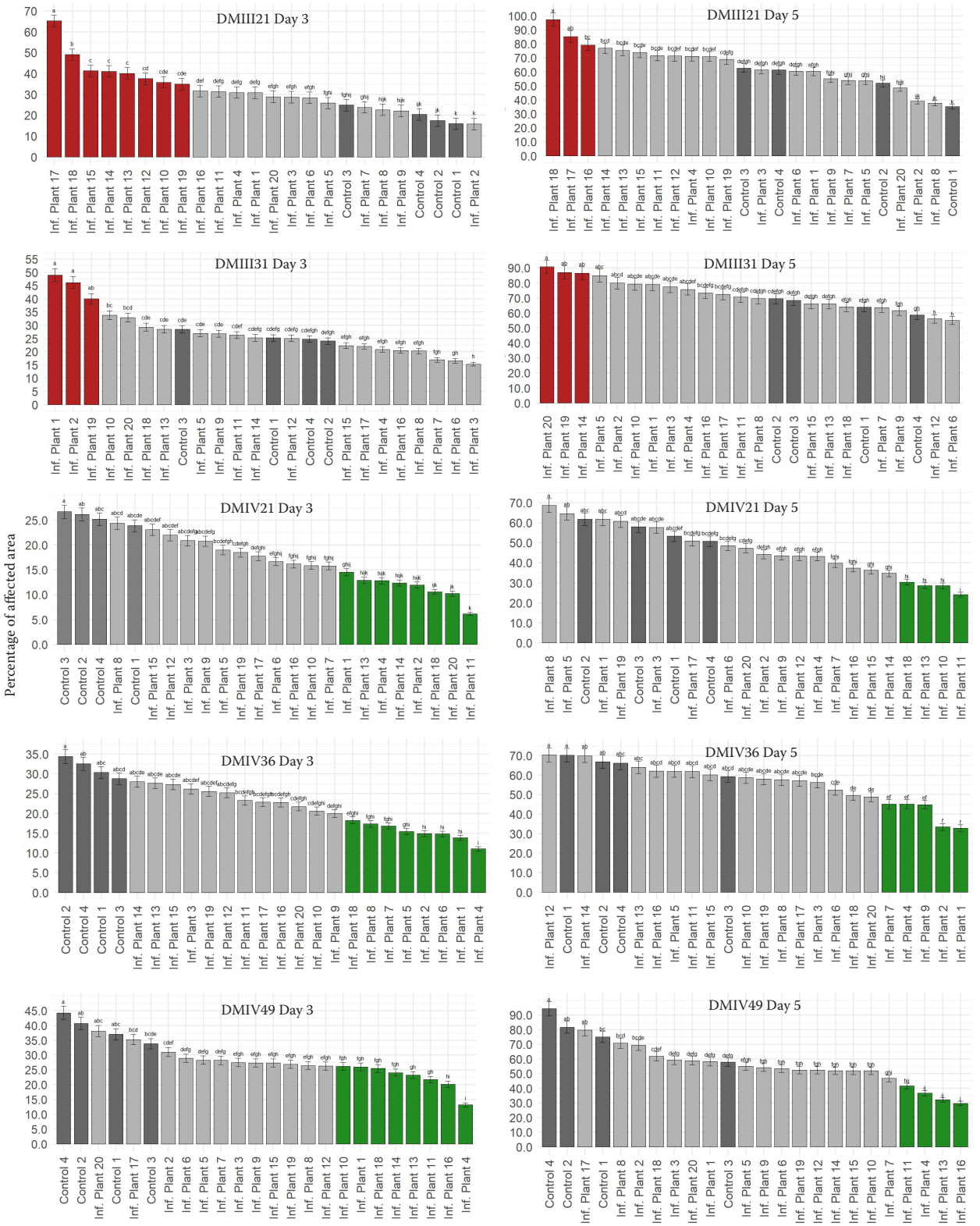

Figure 1. Mean disease severity index (DSI) values for five *Pisum sativum* families following inoculation at days 3 and 5 Dark-grey bars represent the non-irradiated control plants for each genotype; in families DMIV21, DMIV36, and DMIV49, green bars indicate irradiated plants that exhibited a statistically significant reduction in DSI compared to their respective controls; in contrast, in families DMIII21 and DMIII31, red bars represent irradiated plants with a significantly higher DSI than the controls; error bars represent standard error; statistically significant differences were determined using the least significant difference (*LSD*) test ($P < 0.05$)

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tolerance to infection by *Didymella pinodes*, using detached leaf bioassays. Calculation of the DSI was performed the 3rd and 5th days post artificial inoculation. Among the 16 different families derived from seeds exposed to gamma radiation, only five exhibited statistically significant differences compared to their corresponding non-irradiated controls.

In families DMIV21, DMIV36, and DMIV49, a significant reduction in the DSI was recorded in a portion of the irradiated plants, suggesting a potential emergence of tolerant mutations (Figure 1). On the 3rd day, approximately 40% of the plants in each of these families displayed markedly lower DSI values: DMIV21 ranged between 7–15%, compared to 25% in the controls; DMIV36 ranged between 12–19%, compared to 31%; and DMIV49 ranged between 12–26%, compared to 36%. On the 5th day, although a general increase in the DSI was observed in the control plants, reaching 44.5% for DMIV21, 49% for DMIV36, and 40.5% for DMIV49, tolerant individuals were still present in lower frequencies. Specifically, DSI values in the tolerant mutants ranged between 23–30% in DMIV21, 35–45% in DMIV36, and 30–42% in DMIV49.

In contrast, families DMIII21 and DMIII31 included irradiated plants with significantly higher DSI values, indicating increased susceptibility likely due to mutagenesis. On the 3rd day, 40% of the plants in DMIII21 showed DSI values ranging from 35–65%, compared to 20% in the control group, while 15% of the plants in DMIII31 had DSI values between 40–48%, compared to 25% in the controls. This trend became more pronounced by the 5th day, with 15% of DMIII21 plants reaching DSI values between 60–96%, compared to 56% in the controls, and 15% of DMIII31 plants showing values between 85–90%, compared to 65% in the controls. Notably, statistically significant differences were observed in a greater number of plants on the 3rd day, while by the 5th day, these differences remained significant in a smaller proportion of the population. This finding suggests that differences in tolerance are more evident at the early stages of evaluation and tend to diminish as the disease progresses.

In conclusion, families DMIV21, DMIV36, and DMIV49 constitute promising genetic resources for the selection of tolerant mutant lines, while DMIII21 and DMIII31 underline the importance of concurrently evaluating sensitising mutations within genetic improvement programs.

Associated agronomic traits performance. The gamma-irradiated pea germplasm was evaluated in field trials for differences in agronomic traits (Figure 2, Table 1). For plant height, notable increases were observed in plants of DMIV21 and DMIV49 compared to their respective controls, whereas DMIV36 showed a slight decrease. Similarly, for the number of stems, certain families such as DMIII21 and DMIII31 displayed an increased stem number, while other genotypes, including DMIV36 and DMIV49, exhibited either a mild reduction or no clear difference.

Gamma irradiation had a variable impact on seed productivity traits across the evaluated families. According to Figure 3 and Table 1, in terms of seeds per plant, DMIV49 showed a substantial increase under irradiation, with irradiated plants producing

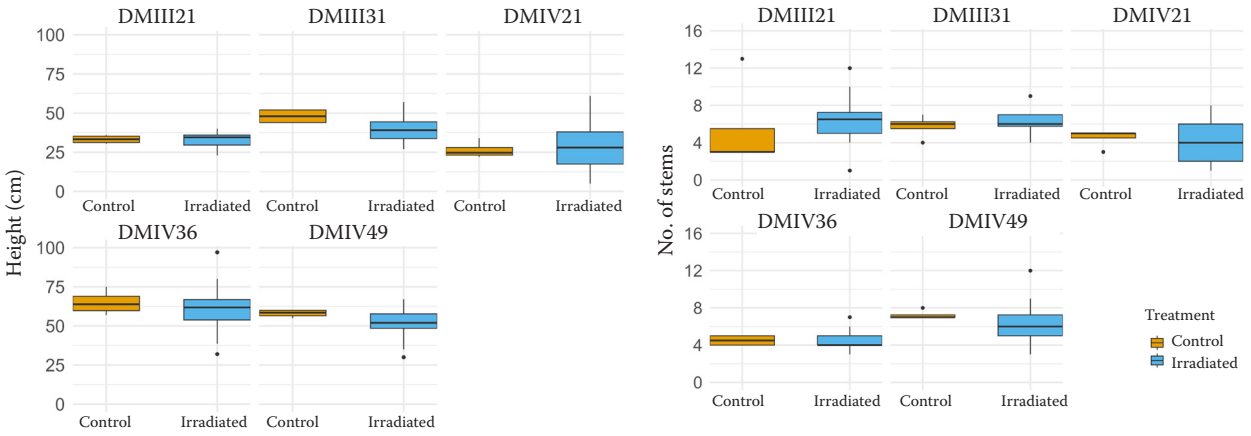

Figure 2. Boxplots showing the effect of gamma irradiation on plant height (left) and number of stems (right) in selected *Pisum sativum* families under control and irradiated conditions

Each family is presented separately to visualise treatment response; the median, interquartile range, and outliers are indicated

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Table 1. Mean values (± SD) of morphological and yield-related traits in irradiated and control *Pisum sativum* families (α = 0.05)

Families	Plant height (cm)	No. of stems	Plant weight wet (g)	Plant weight dry (g)	No. of pods per plant	Average pod length/plant	No. of seeds per pod	No. of seeds per plant	TWK (g)
DMIV4 9	54.42 ± 9.20	6.55 ± 2.21	14.49 ± 6.72	10.34 ± 4.41	4.33 ± 2.95	2.84 ± 0.51	2.88 ± 2.31	10.11 ± 9.76	95.17 ± 46. 72
Control	58.00 ± 2.45	7.52 ± 2.50	20.79 ± 7.34	14.29 ± 4.79	2.75 ± 0.50	2.98 ± 0.31	2.21 ± 0.85	6.00 ± 2.58	101.91 ± 32.39
P-value	ns	ns	ns	*	*	ns	ns	ns	ns
DMIV3 6	61.33 ± 14.01	4.35 ± 1.09	18.47 ± 12.19	15.81 ± 9.94	12.35 ± 9.14	3.51 ± 0.53	3.49 ± 1.69	32.21 ± 20.96	108.84 ± 82.72
Control	64.92 ± 7.88	4.50 ± 0.57	17.68 ± 7.57	16.18 ± 6.68	5.66 ± 6.43	3.21 ± 0.21	2.53 ± 0.61	10.00 ± 10.58	122.73 ± 11.08
P-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
DMIII3 1	39.57 ± 8.10	5.94 ± 1.34	19.96 ± 17.93	18.80 ± 12.47	11.92 ± 6.76	3.34 ± 0.58	3.5 ± 1.03	23.76 ± 18.58	70.32 ± 34.49
Control	44.66 ± 5.18	5.33 ± 0.94	6.62 ± 5.44	19.79 ± 6.10	15.00 ± 11.04	3.46 ± 0.05	4.6 ± 1.81	43.00 ± 8.69	86.47 ± 32. 12
P-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
DMIV2 1	29.02 ± 14.6	4.00 ± 2.39	6.24 ± 4.40	4.94 ± 3.18	–	–	–	–	–
Control	26.37 ± 5.34	4.50 ± 1.00	7.09 ± 4.33	4.78 ± 2.05	–	–	–	–	–
P-value	ns	ns	ns	ns					
DMIII2 1	32.87 ± 4.69	6.45 ± 2.85	–	–	–	–	–	–	–
Control	35.00 ± 9.26	5.50 ± 5.00	–	–	–	–	–	–	–
P-value	ns	ns							

SD – standard deviation; TWK – thousand kernel weight; ns – non-significant

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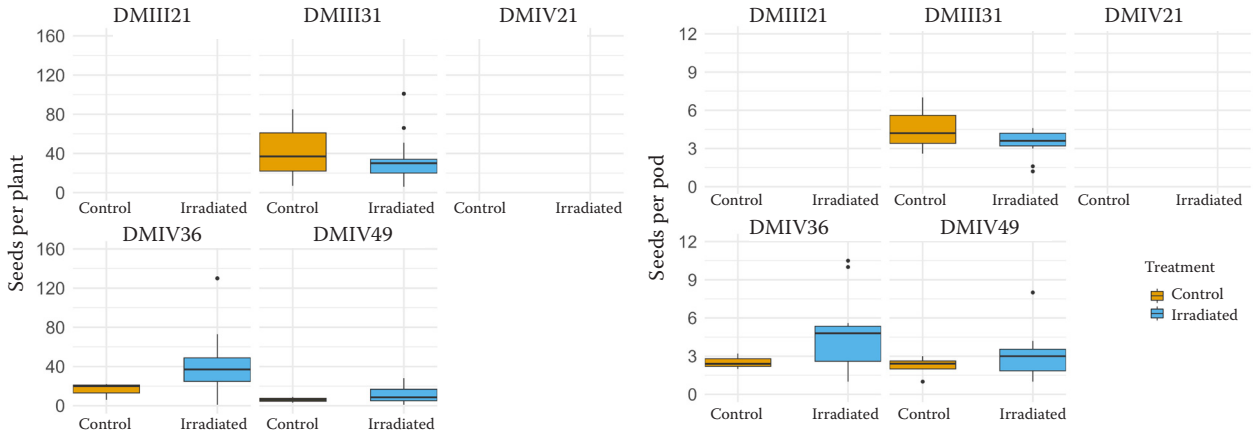

Figure 3. Boxplots showing the effect of gamma irradiation on seeds per plant (left) and seeds per pod (right) in selected *Pisum sativum* families under control and irradiated conditions

Each family is presented separately to visualise treatment response; the median, interquartile range, and outliers are indicated

more than double the seed number compared to controls. Similarly, DMIV36 also exhibited a positive response to irradiation, though to a lesser extent. In contrast, DMIII31 showed a moderate reduction in seed number following irradiation, while families DMIII21 and DMIV21 did not produce seeds under either condition, due to developmental constraints. Regarding seeds per pod, a similar pattern was observed. DMIV49 and DMIV36 demonstrated increased seed number per pod in the irradiated treatment, suggesting a potential improvement in reproductive efficiency. On the other hand, DMIII31 showed a reduction in seeds per pod following irradiation, consistent with its lower total seed output. Again, DMIII21 and DMIV21 failed to produce any pods with seeds, reflecting poor performance under both treatments.

Regarding average pod length, DMIII31 and DMIV36 had plants with slightly longer pods compared to their controls, suggesting a modest positive effect of irradiation on pod elongation. DMIV49 showed a slight reduction in median pod length under irradiation, although with increased variability. Families DMIII21 and DMIV21 did not form pods under either treatment. With regards to pods per plant, a clear increase was observed in irradiated DMIV49 and DMIV36, indicating improved pod set in response to the irradiation treatment. Conversely, DMIII31 showed a marked reduction in the number of pods per plant following irradiation, suggesting

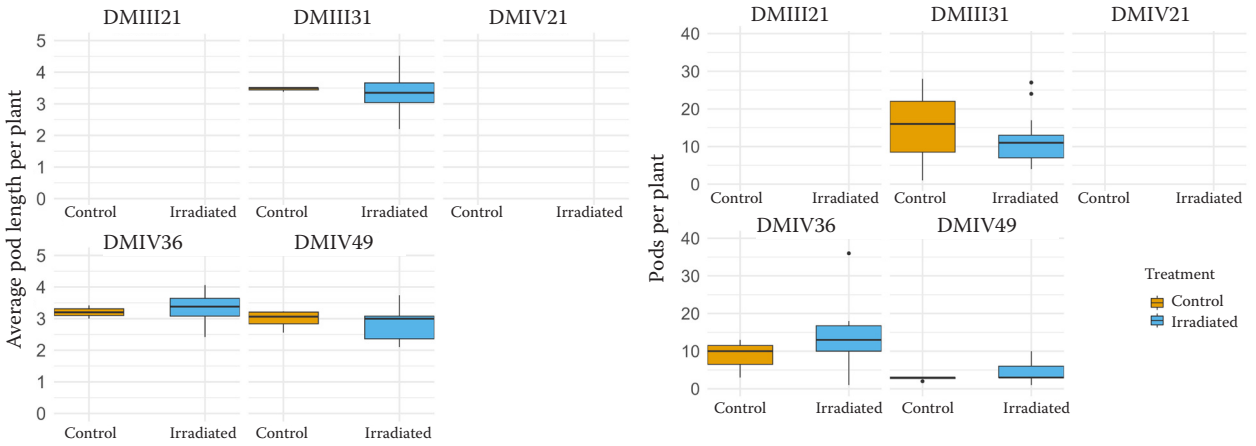

Figure 4. Boxplots showing the effect of gamma irradiation on average pod length per plant (left) and pods per plant (right) in selected *Pisum sativum* families under control and irradiated conditions

Each family is presented separately to visualise treatment response; the median, interquartile range, and outliers are indicated

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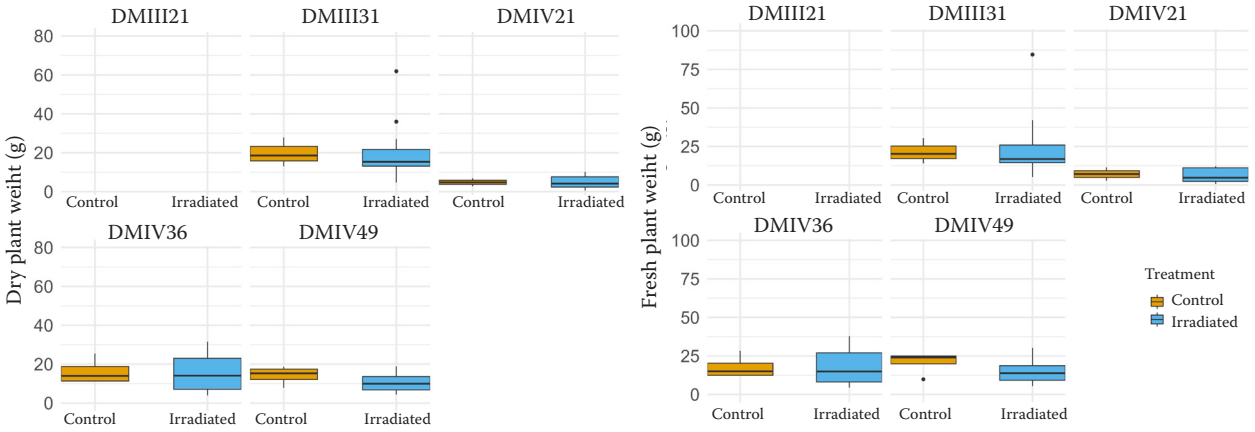

Figure 5. Boxplots showing the effect of gamma irradiation on dry plant weight (left) and fresh plant weight (right) in selected *Pisum sativum* families under control and irradiated conditions

Each family is presented separately to visualise treatment response; the median, interquartile range, and outliers are indicated

a potential negative impact on reproductive capacity. No pods were produced in DMIII21 or DMIV21 in either treatment, consistent with their poor seed productivity (Figure 4, Table 1).

For dry plant weight, families DMIV36 and DMIV21 showed increased biomass under irradiation compared to their respective controls (Figure 5, Table1), suggesting a potential stimulatory effect of the treatment. In contrast, DMIV49 showed a reduction in dry weight following irradiation, indicating possible growth inhibition. DMIII31 showed no notable difference between treatments, while DMIII21 did not produce any measurable dry matter. A similar pattern was observed for fresh plant weight, where DMIV36 and DMIV21 again demonstrated increased biomass under irradiation, DMIV49 showed a decrease, and DMIII31 remained unchanged between treatments. No fresh weight data were available for DMIII21, consistent with its overall poor growth and reproductive performance.

Overall, gamma irradiation had a pronounced effect on yield per plant. In particular, irradiated plants of DMIV36 and DMIV49 exhibited substantially higher yield compared to their controls, suggesting a positive impact of irradiation on seed productivity. DMIII31 showed a slight reduction in yield under irradiation, indicating a possible trade-off between vegetative growth and reproductive output in this genotype. No yield data were recorded for DMIII21 and DMIV21. For thousand seed weight (TSW), irra-

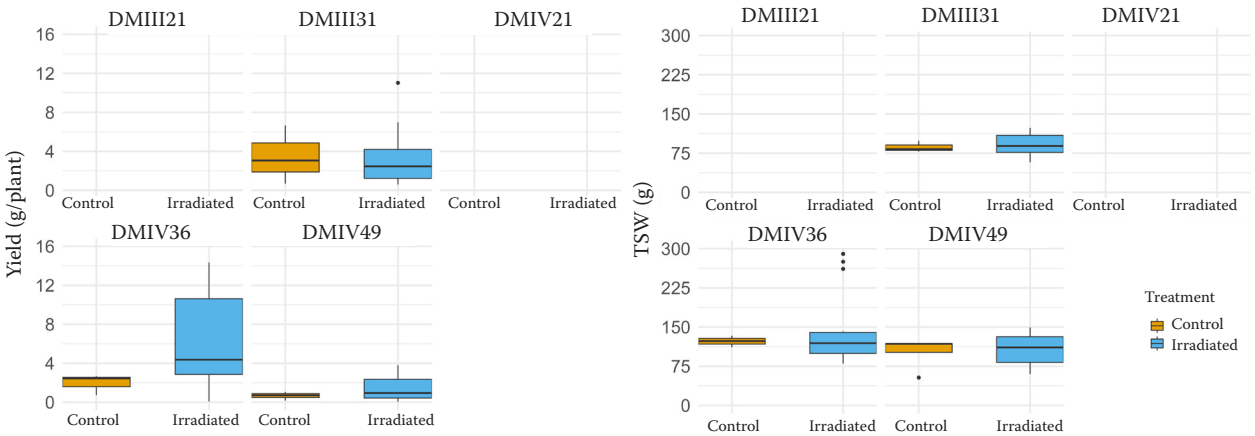

Figure 6. Boxplots showing the effect of gamma irradiation on yield (left) and thousand seed weight (right) in selected *Pisum sativum* families under control and irradiated conditions

Each family is presented separately to visualise treatment response; the median, interquartile range, and outliers are indicated

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diated plants of DMIV49 and DMIII31 outperformed their controls, indicating that gamma irradiation may have enhanced seed filling or individual seed mass in these families. DMIV36 showed similar or slightly improved TSW in the irradiated group, while no data was available for DMIII21 and DMIV21 (Figure 6, Table 1).

To complement these findings, correlations between DSI and the recorded agromorphological traits were assessed. The results of the correlation analysis indicate that tolerance to *Didymella pinodes* is not significantly associated with most agronomic traits. The only strong and statistically significant relationships were the negative correlation between DSI on day 5 and TSW (r = –0.92), and the positive correlation between DSI on day 5 and dry plant weight (r = 0.88). These results indicate that disease progression negatively affects seed development and filling and is associated with increased dry plant weight (Table 2). The strong negative correlation with TSW highlights that increased disease severity can substantially reduce reproductive performance.

DISCUSSION

Detached leaf (ex-planta) bioassays were employed in this study as a rapid, controlled, and reproducible method for assessing pea responses to *Didymella pinodes* infection. This approach allows the direct monitoring of pathogen development on a uniform plant tissue background, minimising environmental variation and enabling precise quantification of disease spread. Similar ex-planta methodologies have been successfully applied for *Ascochyta* blight research in pea and related legumes, providing valuable insights into host–pathogen interactions under standardised conditions (Joshi et al. 2022; Annan et al. 2023; Onfroy et al. 2007). Such assays are particularly useful in early-generation mutant screening, where large populations can be evaluated efficiently before advancing selected lines to more resource-intensive in planta trials. Screening of the M_2 gamma-irradiated *Pisum sativum* cv. Dodoni population revealed pronounced family-specific differences when comparing disease tolerance and agronomic performance. In families DMIV21, DMIV36, and DMIV49, significant reductions in DSI at both three- and five-day post-inoculation indicated that gamma irradiation successfully generated variants with improved tolerance to *Didymella pinodes*. However, these tolerance gains had variable effects on agronomic traits:

Table 2. Pearson correlation coefficients (r) between disease severity (DSI at 3rd and 5th day) and agronomic traits across five *Pisum sativum* families (significant correlations ($P < 0.05$) are shown in bold)

	DSI3	DSI5	Plant height	Number of stems	Plant weight wet	Plant weight dry	No. of pods/plant	Averege pod length/plant	No. of seeds/pod	No. of seeds/plant	TWK
DSI3	1.00										
DSI5	0.76	1.00									
Plant height	–0.08	–0.10	1.00								
No. of stems	0.61	0.62	0.01	1.00							
Plant weight wet	0.73	0.82	0.62	0.47	1.00						
Plant weight dry	0.65	**0.88**	0.45	0.35	**0.97**	1.00					
No. of pods/plant	–0.46	0.50	–0.16	–0.75	**0.95**	**0.92**	1.00				
Average pod length/pant	–0.63	0.32	0.04	–0.86	**0.87**	0.82	**0.98**	1.00			
No. of seeds/pod	–0.41	0.56	–0.22	–0.70	**0.97**	**0.94**	**0.98**	**0.97**	1.00		
No. of seeds/plant	–0.73	0.19	0.18	**–0.93**	0.79	0.74	**0.94**	**0.99**	**0.92**	1.00	
TWK	–0.82	**–0.92**	**0.89**	–0.57	–0.42	–0.50	–0.12	0.08	–0.18	0.22	1.00

TWK – thousand kernel weight

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DMIV49 was exceptional in combining enhanced tolerance with substantial increases in seed number per plant, pods per plant, yield, and thousand seed weight. DMIV36 also showed moderate yield improvements, whereas DMIV21, despite its reduced DSI, failed to produce seeds, suggesting developmental constraints and potential trade-offs between tolerance and reproductive capacity. Gamma irradiation has been shown to induce both beneficial and deleterious mutations in legumes, affecting traits such as disease resistance and yield (Annicchiarico et al. 2021; Parihar et al. 2022). While some studies report enhanced resistance to biotic stresses after mutagenesis (Lee et al. 2023), others highlight the potential for increased susceptibility due to unintended mutations (Liu et al. 2023).

Families DMIII21 and DMIII31 exhibited increased susceptibility after irradiation. DMIII31 maintained or slightly improved thousand seed weight but suffered reductions in overall yield and pod number, whereas DMIII21 did not produce seed at all. These divergent outcomes reflect the well-documented challenge in pea breeding: high resistance to biotic stress and high yield potential do not consistently coincide in the same genotype. Recent field evaluations demonstrate that resistance to Ascochyta blight often comes with trade-offs in agronomic traits such as reduced seed yield or biomass, complicating breeding efforts (Klein et al. 2020; Joshi et al. 2022; Lee et al. 2023). This suggests that high resistance and high yield may not always coexist within the same genotype, aligning with our observations of variable responses across different *Pisum sativum* families. This dynamic underscores the prevailing notion that, under disease pressure, resistance may outweigh agronomic gains in breeding priorities (Annicchiarico et al. 2021; Parihar et al. 2022).

Indeed, Annicchiarico et al. (2021) suggest that traits such as competitive ability and intercropping adaptability, which are critical to field performance, do not always align with stress-resistance loci, further complicating the breeder's task. Conversely, the performance of DMIV49 is particularly valuable because instances where induced disease tolerance is coupled with enhanced yield remain rare. Our previous research has documented that gamma irradiation can generate beneficial variation in pea, especially in biomass and yield traits (Sarri et al. 2024), reinforcing the promise of mutagenesis in expanding breeding potential. Simultaneously, our current results corroborate reports showing that mutagenesis can likewise produce alleles that increase susceptibility, emphasising the necessity of rigorous phenotypic screening for both disease and agronomic outcomes.

Another key observation was that tolerance effects were more pronounced at the early stage of infection (day 3) and became less distinct by day 5. This temporal decline suggests that some mutants may employ delayed or partial resistance mechanisms rather than sustained defence. Such temporal dynamics highlight the need for time-resolved phenotyping in mutation screening. Several genomic and transcriptomic studies have begun to unravel the complex genetic architecture behind disease resistance and yield traits in pea, indicating that simultaneous improvement of both traits requires careful selection of alleles and possibly genomic selection strategies (Annicchiarico et al. 2021; Parihar et al. 2022; Lee et al. 2023). Looking forward, combining mutation breeding with contemporary genomic tools, such as GWAS, marker-assisted selection, and genomic prediction, could enhance the efficiency of selecting genotypes that couple resistance with agronomic strength (Parihar et al. 2022; Lee et al. 2023). In addition to Ascochyta blight resistance, recent work by Sharma et al. (2025) demonstrated the successful development of powdery mildew-resistant pea lines through induced mutagenesis using gamma irradiation and ethyl methanesulfonate (EMS) treatments. Their screening of large M_2 and M_3 populations led to the identification of mutants carrying the well-characterised *er1* and *er2* resistance genes, validated by molecular markers. Meanwhile, optimising mutagenesis protocols for specific genotypes, as described by Pandey et al. (2022), offers a refined approach to improving mutation induction. Ultimately, integrating gamma irradiation with genomic selection pipelines may drive the discovery of dual-purpose genotypes like DMIV49. Field studies emphasise the practical importance of resistance over yield performance under high disease pressure, supporting the prioritisation of breeding for durable disease resistance to ensure crop stability in variable environments (Liu et al. 2016; Lee et al. 2023). Thus, the selected tolerant mutant lines will be evaluated under field conditions in the M_3–M_4 generations to confirm the stability and heritability of their tolerance and agronomic performance, as the M_2 generation is still segregating. Moreover, molecular validation of the identified promising lines was not performed in this study and should be considered in subsequent generations to unravel the genetic changes associated with tolerance or enhanced susceptibility.

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In conclusion, this study highlights the complex interplay between disease resistance and yield traits following gamma-induced mutagenesis. The identification of DMIV49, with its favourable combination of traits, offers a promising path forward. Nevertheless, further multi-generation and multi-environment trials, including in planta inoculation in subsequent generations rather than relying solely on ex-planta assays, along with detailed molecular characterisations, are essential to confirm the stability and elucidate the genetic underpinnings of these phenotypes.

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