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Morphological Characteristics of Gamma-Irradiated *Desmanthus virgatus* Mutants Adapted to High Salinity Conditions

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ABSTRACT

Gamma-ray irradiation can induce plant mutations, resulting in stable genetic changes that persist in future generations. This study aims to assess the morphological characteristics of the potential mutant of Hedge Lucerne (*Desmanthus virgatus*) that has developed adaptation to high salinity conditions as a result of irradiation using gamma-ray. A total of 36 candidate mutants first generation (M1) of *D. virgatus* were used in this study, and they had developed adaptation to high salinity conditions (8.4 dS/m) and were observed 64 wk after planting. The collected data underwent a descriptive statistical analysis and succeeded by applying the Shapiro-Wilk test to assess the normality of the data distribution. A concentration index greater than one (>1.00) signifies a high value of the plant's morphological characteristic. All levels of gamma irradiation groups produced an excellent survival response in the mutant candidate. The high diversity in morphological characteristics was reflected in the number of compound leaves, the width of compound leaves, and the number of leaves per pinnae, flowers, and pods. The dominant color of the lower leaves was 7.5 Green Yellow (5/6), and the middle leaves were 7.5 Green Yellow (5/6), while the dominant color of the upper leaves was 7.5 Green Yellow (6/8). The mutant candidate of *D. virgatus* exhibited the best characteristics, as determined by the concentration index, were those subjected to 200 Gray and 300 Gray irradiation. Fifteen superior mutant candidates were identified, namely GDV100.1, GDV100.2, GDV100.4, GDV100.5, GDV200.2, GDV200.3, GDV200.7, GDV200.9, GDV200.10, GDV300.3, GDV300.4, GDV300.5, GDV300.12, GDV300.13. and GDV 500.1. The three best mutant candidates (M1) were GDV100.2, GDV200.3, and GDV500.1. The three best mutant candidates (M1) were GDV100.2, GDV200.3, and GDV500.1.

Keywords: *D. virgatus*, Gamma-ray iradiation, Morphological, Salinity

Introduction

The *Desmanthus virgatus* plant is a legume plant that originates from Tropical America and is spread from Mexico, Central America, and the Caribbean to South America (Nielsen, 1992; Luckow, 1993; Cowan, 1998; Mountara *et al.*, 2021). This plant has good nutritional content. According to Mahata *et al.* (2010), the potential production of *D. virgatus* is 7.59 tons DM/ha/year, crude protein 27.62%, crude fat 1.95%, crude fiber 16.75%, calcium 0.52%, and phosphorus 0.17%. *D. virgatus* plants can be harvested four times yearly Tillman *et al.* (1991). However, *D. virgatus* has limited tolerance to salinity stress. According to Nufus *et al.* (2022), the in-vitro culture of *D. virgatus* exhibits a salinity stress tolerance level of 2.500 ppm.

The Division of Science and Technology of Feed and Pasture Plants IPB University has a collection of *D. virgatus* plants resulting from gamma-ray irradiation that are resistant to high salinity up to 8.4 dS/m as superior mutant candidates (M1). Plant mutations through gamma-ray irradiation can produce stable genetic modifications for subsequent generations. Gamma-ray irradiation has high radiation energy, so it can penetrate plant tissue and cause chromosome modifications, resulting in morphological, anatomical, and physiological changes in plants (Lelang *et al.*, 2016) and producing more stable genetic variations (Sattar *et al.*, 2021).

The collected mutant candidates were able to adapt to high salinity conditions (8.4 dS/m) compared to non-mutant D. virgatus plants, which could only adapt to a salinity level of 2,500 ppm (3.33 dS/m). Further evaluation and selection are needed to measure the morphological characteristics of *D. virgatus* (M1) plants in media with high salinity to be determined as superior plant candidates. Namely, it has a characteristic value higher than the population average value. Hayati *et al.* (2009) defined superior plants as plants with characteristics higher than the population average. This research aims to evaluate the morphological traits of *D. virgatus* mutant candidates adapted to high salinity conditions from irradiation using gamma-ray to identify M1 mutant candidates exhibiting superior characteristics.

Materials and Methods

Time and place of research. This research was carried out from January to May 2023 at the Agrostology Unit Field Laboratory, Science and Technology Division of Forage Plants and Pasture, Faculty of Animal Science, IPB University.

Research tools and materials. The tools used in this research included a 0.5 mm scale meter, digital caliper, ruler, 0.75 cm sieve, laptop, and Munsell Plant Tissues Color Chart. The materials used were 36 candidate mutants first generation (M1) of *D. virgatus* aged 48 wk after planting (WAP) adapted to high salinity conditions (8.4 dS/m) (Abrol *et al.*, 1988). Mutants first generation (M1) of D. virgatus resulted from different gamma-ray irradiation levels (100, 200, 300, 400, and 500 gray) irradiated on D. virgatus seeds. D. virgatus seeds were grown and collected on saline media (8.4 dS/m) until 48 wk after planting (WAP). No non-mutant plants (control) were produced due to high salinity stress.

Media preparation and planting mutant candidates in saline soil. *D. virgatus* mutant candidates aged 48 wk were transferred to saline soil media with a volume of 5 kg per polybag. Saline soil comes from the coast of Ciparage Jaya Village, Karawang, West Java, with an Electrical Conductivity (EC) of 8.4 dS/m (High Salinity). Media preparation begins with filtering using a 0.75 cm sieve. Next, it is homogenized with 20 tons of manure per ha (50 g per polybag), inorganic fertilizer according to Rizki *et al.* (2022) recommendations: Urea 200 kg/ha (0.5 g per polybag), KCl 100 kg/ha (0.25 g per polybag), and SP36 150 kg/ha (0.375 g per polybag).

Plant maintenance. Plants are watered daily in the morning according to the field capacity of the planting medium while maintaining salinity status (preventing water from escaping from the planting medium). Routine weed control is carried out by manually pulling out weeds that grow as quickly as possible.

Measurement of survival and morphological characteristics. Survival and Morphological Characteristics evaluation was conducted at the age of 48 WAP to the age of 64 WAP. The survival measurement was quantified by the ratio of the number of surviving plants to the total number of plants, expressed as a percentage. Measurements encompassing various parameters such as vertical height, stem diameter, branch and twig count, compound leaf quantity and dimensions (length and width), pinnae count and length, leaves per pinnae, and the number of flowers and pods were conducted when the plants reached 64 wk after planting (WAP). Plant height measurements were determined from the base of the stem to the highest shoot. The diameter of the stem is quantified at the base of the giant stem. The number of branches was measured on the branches attached to the main stem. The number of twigs was measured on the branch attached to the branch. The number of compound leaves was calculated from all the compound leaves of the plant. The number of pinnaes was counted on each compound leaf. The length of a compound leaf was calculated from the base of the leaf axil to the tip of the compound leaf. The width of a compound leaf was measured from the outer side of the compound leaf. The length of the pinnae was measured from the base of the pinnae's armpit to the tip of the pinnae. The number of leaves per pinnae was calculated based on the total leaves of each pinnae. The number of flowers was calculated based on each plant's total number of flowers. The number of pods was calculated based on the total pods from each plant.

Leaf color measurements refer to Prihantoro *et al.* (2023b). Leaf color was measured manually based on the level of similarity of leaf samples from each *D. virgatus* mutant candidate with leaf color standards from the Munsell Plant Tissues Color Chart.

Morphological characteristic concentration index. The concentration index of morphological characteristics were measured to obtain superior plant candidates from mutant candidates, referring to a modification of the Syamsu (2006) method. Superior plant candidates are mutant candidates with a concentration index greater than one (>1.00). The concentration index was measured by calculating the ratio of sample value per sample average value.

Research design and data analysis. The research design in this study was quantitative descriptive. The analytical process involves the computation of mean values and the application of the Shapiro-Wilk test to assess data distribution. These analyses are conducted using the IBM SPSS Statistics 25 software.

Results and Discussion

Plant survival

Genetic engineering using gamma-ray irradiation can improve plant adaptation under abiotic stress conditions. The survival of candidate mutants of *D. virgatus* resulting from gamma-ray irradiation in high-salinity (8.4 dS/m) (Abrol *et al.*, 1988) soil was 100% for all gamma irradiation levels and is presented in Table 1.

The control plants (0 gray) in this study were unable to survive the high levels of saline stress applied to them (8.4 dS/m). All mutant candidates (100 – 500 gray) were able to survive and adapt to high-salinity soil. These results show that plant products from gamma-ray mutations adapt well to high salinity (8.4 dS/m) up to 64 WAP. Mutation techniques using gamma-ray irradiation can induce stable mutants quickly and more efficiently Parlaongan *et al.*, (2022) and increase abiotic stress tolerance (Katiyar *et al.*, 2022). This shows that irradiation using gamma-ray is an effective mutation technique. This is in line with Caro-Melgarejo *et al.* (2012), who state that irradiation with gamma-ray has proven to be an effective way to mutate plants.

Table 1. Survival of candidate mutants of *D. virgatus* resulting from gamma ray irradiation in high salinity soil

Gamma Irradiation Levels (gray)	48 WAP (n)	64 WAP (n)	Plant Survival* (%)
0 (control)	0	0	0.00
100	5	5	100.00
200	10	10	100.00
300	17	17	100.00
400	1	1	100.00
500	3	3	100.00
Total	36	36	100.00

*Plant age 64 WAP.

Morphological characteristics of *D. virgatus* plant mutant candidates adapted to high salinity results from gamma irradiation

Plant morphological characteristics can be used as information on the diversity of breeding plants. Detailed morphological characteristics of candidate mutants of *D. virgatus* resulting from gamma-ray irradiation in high-salinity soil are presented in Table 2.

Analysis of the distribution of morphological characteristics in candidate mutants of *D. virgatus* showed an abnormal distribution (varied) for the character's number of compound leaves, compound leaves width, and number of leaves per pinnae, flowers, and pods. Meanwhile, other characteristics show a normal (uniform) distribution. The mutant products showed slight variation in the data, indicating that the mutation does not lead to the expression of stem characters, number of pinnae, number of compound leaves, and pinnae length. Some mutagenesis methods can cause plant mutations, but this does not always occur in every plant cell (FAO, 2011). The random nature of gamma mutations causes variations in the distribution of morphological characteristics. Kurniajati *et al.* (2020) stated that irradiation with gamma-ray produces genetic diversity in plants that are distributed randomly at various irradiation doses and observed characteristics. Diversity in plants irradiated with gamma rays arises due to genetic changes/mutations in plant parts induced

Table 2. Morphological characteristics of *D. virgatus* plant mutant candidates

Plant codes*	Stem				Leaf						Flowers and Pods	
	VH (cm)	SD (mm)	NoB (Branch)	NoT (Twigs)	NoCL (Piece)	NoP (Piece)	CLL (cm)	CLW (cm)	PL (mm)	NoLP (Piece)	NoF (Flower)	NoPo (Pods)
GDV100.1	109.60	6.13	13	22	363	10.80	5.97	4.79	24.39	43.60	12	5
GDV100.2	133.45	6.66	15	18	357	11.40	6.15	4.62	24.64	38.40	21	36
GDV100.3	114.95	6.06	8	15	334	11.00	5.99	4.76	25.64	44.00	8	12
GDV100.4	90.10	6.16	20	30	370	11.20	5.38	4.23	20.17	44.80	3	26
GDV100.5	101.80	5.36	10	30	392	11.40	5.18	4.20	24.58	42.00	19	16
GDV200.1	71.90	6.69	11	17	335	10.80	5.81	4.61	23.84	42.40	0	37
GDV200.2	83.45	7.46	11	29	340	10.60	5.87	4.55	27.05	38.80	15	6
GDV200.3	100.70	8.60	11	18	399	11.80	6.27	5.02	24.95	46.44	13	57
GDV200.4	112.15	6.11	18	22	326	8.80	5.02	4.32	21.99	34.44	3	21
GDV200.5	100.30	6.18	10	24	415	11.20	6.46	5.10	23.70	41.80	7	0
GDV200.6	100.45	7.18	7	21	245	13.00	6.91	4.99	27.54	43.80	3	12
GDV200.7	117.60	6.88	13	14	228	13.40	6.37	5.21	25.14	46.00	14	7
GDV200.8	84.70	7.11	4	18	274	13.80	7.94	6.60	31.15	36.67	2	15
GDV200.9	97.30	6.85	9	32	426	9.60	5.77	4.73	24.17	45.40	11	36
GDV200.10	110.10	6.12	16	5	301	11.60	5.85	4.13	22.57	45.20	12	14
GDV300.1	107.45	6.50	7	20	235	12.20	6.38	4.72	23.23	36.89	5	9
GDV300.2	87.45	6.71	15	25	586	9.40	5.73	4.58	21.84	39.00	0	13
GDV300.3	84.95	6.82	10	23	279	12.20	7.36	6.06	32.26	45.60	1	21
GDV300.4	108.00	7.18	10	15	284	11.00	5.51	4.26	25.98	40.00	7	33
GDV300.5	103.30	7.24	14	25	257	13.60	6.54	4.84	25.64	46.80	5	10
GDV300.6	91.80	6.88	8	22	244	10.80	5.28	4.19	22.44	41.80	2	16
GDV300.7	82.05	7.08	14	32	205	10.00	5.56	4.24	24.04	44.80	0	34
GDV300.8	71.20	5.87	10	20	270	12.60	6.89	6.03	26.33	45.33	1	33
GDV300.9	94.70	5.74	13	28	269	10.80	5.87	4.61	22.04	44.60	5	11
GDV300.10	89.35	6.48	6	11	211	10.80	5.90	4.67	27.21	43.20	0	0
GDV300.11	70.80	6.08	13	31	243	9.60	6.19	5.09	29.61	38.80	1	0
GDV300.12	77.60	5.85	11	35	510	10.00	5.00	4.12	18.92	28.20	13	6
GDV300.13	85.85	6.65	4	11	273	14.00	7.68	6.19	29.05	49.60	2	48
GDV300.14	84.00	6.63	6	19	202	11.00	6.40	5.17	29.59	39.80	5	1
GDV300.15	88.30	8.10	13	27	372	10.80	6.42	4.97	25.25	43.80	0	1
GDV300.16	104.75	6.96	14	19	271	11.60	6.07	4.92	23.54	44.20	4	8
GDV300.17	87.15	4.88	11	27	344	10.80	5.98	5.27	24.59	46.80	6	13
GDV400	80.40	5.41	7	14	289	9.40	6.11	5.12	20.28	42.80	4	25
GDV500.1	105.90	6.51	14	29	490	9.40	5.76	4.95	21.27	39.20	30	14
GDV500.2	104.65	5.37	8	23	315	9.20	5.50	3.69	15.12	34.80	1	56
GDV500.3	92.10	6.35	11	25	446	11.00	6.20	4.65	20.36	43.20	4	4
Mean	95.29± 14.26	6.52± 0.75	10.97± 3.68	22.11± 6.87	325± 90.15	11.13± 1.33	6.09± 0.67	4.84± 0.62	24.45± 3.48	42.03± 4.28	6.64± 6.91	18.22± 15.58
Shapiro-Wilk Test	0.560	0.582	0.715	0.876	**0.032**	0.092	0.052	**0.010**	0.814	**0.020**	**0.000**	**0.002**

VH: Vertical height; SD: Stem diameter; NoB: Number of branches; NoT: number of twigs, NoCL: Number of compound leaves; NoP: Number of Pinnaes; CLL: Compound leaf length; CLW: Compound leaf width; PL: Length of pinnae; NoLP: Number of leaves per pinnae; NoF: Number of flowers; NoPo: Number of pods. *Normality test (p<0.05 = not normally distributed, p>0.05 = normally distributed). The candidate mutant plants are Single mutant candidates with high salinity tolerance (8.4 dS/m).

by mutagens. Van Harten (1998) characterizes this phenomenon as physiological damage, which encompasses the disruption of chemical bonds and alterations within the cell nucleus. This includes modifications to the gene structure, deletion of gene or DNA sequences, fractured centromeres, loss or addition of chromosomes, and damage to spindle fibres, among other changes.

Under certain circumstances, plant genetic changes can be recovered naturally by the plant (Susila *et al.*, 2019). This allows the emergence of uniformity in candidate mutants. This is possible because the effects of gamma-ray irradiation are random. The physiological damage inflicted on cell metabolism due to mutations induced by gamma-ray irradiation occurs randomly (Kurniajati *et al.*, 2020), which can accelerate or inhibit plant growth (Anshori *et al.*, 2014). In addition, the uniformity of the morphological characteristics of candidate mutants is also caused by light irradiation gamma, which can select plants with the same characteristic values. Syukur *et al.* (2015), gamma-ray irradiation has a random effect and directly selects plants with the same high expression. The uniformity and diversity of morphological characteristics of *D.virgatus* mutant candidates show that mutations can cause character values to be good or bad. Delastra *et al.* (2021) stated that gamma irradiation can have positive and negative impacts on plant morphological characteristics.

Leaf color of *D. virgatus* plants adapted to high salinity

Chlorophyll is a green pigment found in chloroplasts. The greener the color of the leaves indicates, the higher the chlorophyll content. The results of the leaf color response in *D. virgatus* plants aged 64 WAP is presented in Table 3.

The leaves with the darkest color are owned by the GDV 300.6 plant, with the lower leaves being 7.5 Green Yellow (3/3), the middle part being 7.5 Green Yellow (2/3), and the upper part being 7.5 Green Yellow (3/3). The leaves with the brightest color at the bottom belong to the GDV 200.9 plant with a color of 5 Green Yellow (7/8); in the middle, it belongs to the GDV 100.2 plant with a color of 5 Green Yellow (7/8), and at the top, it belongs to the GDV 100.4 plant with a color of 5 Green Yellow (9/10). The dominant color on the lower and middle leaves is 7.5 Green Yellow (5/6), and the dominant color on the upper leaves is 7.5 Green Yellow (6/8). Differences in leaf color in *D. virgatus* plants can be

Table 3. Leaf color response of *D. virgatus* plants adapted to high salinity aged 64 WAP

Plant codes	Lower leaf color		Middle leaf color		Upper leaf color	
	Codes	Color palette	Codes	Color palette	Codes	Color palette
GDV100.1	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/6)	🟩
GDV100.2	7.5 Green Yellow (4/8)	🟩	5 Green Yellow (7/8)	🟩	5 Green Yellow (6/8)	🟩
GDV100.3	7.5 Green Yellow (4/6)	🟩	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (6/8)	🟩
GDV100.4	7.5 Green Yellow (6/8)	🟩	7.5 Green Yellow (6/6)	🟩	5 Green Yellow (9/10)	🟩
GDV100.5	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (6/8)	🟩
GDV200.1	7.5 Green Yellow (4/4)	🟩	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/6)	🟩
GDV200.2	7.5 Green Yellow (4/6)	🟩	7.5 Green Yellow (4/6)	🟩	7.5 Green Yellow (5/8)	🟩
GDV200.3	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (6/6)	🟩	7.5 Green Yellow (7/8)	🟩
GDV200.4	7.5 Green Yellow (4/4)	🟩	7.5 Green Yellow (4/4)	🟩	7.5 Green Yellow (6/8)	🟩
GDV200.5	7.5 Green Yellow (4/4)	🟩	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/8)	🟩
GDV200.6	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/6)	🟩
GDV200.7	7.5 Green Yellow (4/6)	🟩	7.5 Green Yellow (3/6)	🟩	7.5 Green Yellow (4/6)	🟩
GDV200.8	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/4)	🟩	5 Green Yellow (8/10)	🟩
GDV200.9	5 Green Yellow (7/8)	🟩	7.5 Green Yellow (6/6)	🟩	7.5 Green Yellow (6/8)	🟩
GDV200.10	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/6)	🟩	5 Green Yellow (8/8)	🟩
GDV300.1	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (4/6)	🟩	7.5 Green Yellow (5/6)	🟩
GDV300.2	7.5 Green Yellow (5/4)	🟩	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (7/8)	🟩
GDV300.3	7.5 Green Yellow (6/8)	🟩	7.5 Green Yellow (6/8)	🟩	5 Green Yellow (7/10)	🟩
GDV300.4	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (6/8)	🟩
GDV300.5	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (3/3)	🟩	7.5 Green Yellow (4/8)	🟩
GDV300.6	7.5 Green Yellow (3/3)	🟩	7.5 Green Yellow (2/3)	🟩	7.5 Green Yellow (3/3)	🟩
GDV300.7	7.5 Green Yellow (6/6)	🟩	7.5 Green Yellow (6/6)	🟩	7.5 Green Yellow (6/6)	🟩
GDV300.8	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/8)	🟩	7.5 Green Yellow (6/8)	🟩
GDV300.9	7.5 Green Yellow (3/6)	🟩	7.5 Green Yellow (4/4)	🟩	7.5 Green Yellow (4/6)	🟩
GDV300.10	7.5 Green Yellow (5/6)	🟩	5 Green Yellow (6/6)	🟩	5 Green Yellow (7/10)	🟩
GDV300.11	7.5 Green Yellow (3/4)	🟩	7.5 Green Yellow (3/4)	🟩	7.5 Green Yellow (5/8)	🟩
GDV300.12	7.5 Green Yellow (4/6)	🟩	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/6)	🟩
GDV300.13	7.5 Green Yellow (6/8)	🟩	7.5 Green Yellow (6/6)	🟩	5 Green Yellow (8/10)	🟩
GDV300.14	7.5 Green Yellow (6/8)	🟩	7.5 Green Yellow (6/6)	🟩	5 Green Yellow (7/8)	🟩
GDV300.15	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/6)	🟩
GDV300.16	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (6/6)	🟩
GDV300.17	7.5 Green Yellow (5/4)	🟩	7.5 Green Yellow (5/6)	🟩	5 Green Yellow (7/10)	🟩
GDV400	7.5 Green Yellow (6/6)	🟩	7.5 Green Yellow (6/6)	🟩	5 Green Yellow (7/8)	🟩
GDV500.1	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (6/8)	🟩
GDV500.2	7.5 Green Yellow (6/8)	🟩	7.5 Green Yellow (5/6)	🟩	5 Green Yellow (9/10)	🟩
GDV500.3	7.5 Green Yellow (5/6)	🟩	7.5 Green Yellow (6/6)	🟩	5 Green Yellow (7/8)	🟩
Dominant color	7.5 Green Yellow (5/6) (41.67 %)	🟩	7.5 Green Yellow (5/6) (41.67 %)	🟩	7.5 Green Yellow (6/8) (19.44 %)	🟩

The candidate mutant plants are Single mutant candidates with high salinity tolerance (8.4 dS/m).

caused by differences in chlorophyll content or chloroplasts due to mutation. Maslukah *et al.* (2019) stated that differences in leaf color indicate differences in chlorophyll content in the leaves. Specifically, a higher chlorophyll content correlates with a more pronounced green coloration in the leaf's appearance.

All the colored leaves of the *D. virgatus* mutant candidates are still capable of photosynthesis, as proven by the plant's survival. Changes in leaf color commonly occur due to gamma-ray irradiation, which disrupts chlorophyll synthesis. Hapsari *et al.* (2021) state that changes in leaf color can occur due to disruption of chlorophyll synthesis. A darker color dominates the color of the lower leaves, while a lighter color dominates the color of the top leaves. This usually happens because the ages of the leaves are different. Prihantoro *et al.* (2023a) stated that the older the plant, the darker green the leaf color tends to be. Meanwhile, the bright color of the leaves is dominated by younger leaf tissue.

ConcentrationiIndex of *D. virgatus* plant mutants

Concentration index measurements were performed on stem, leaf, flower, and pod characteristics. Calculation of the concentration index for candidate mutant *D. virgatus* plants shows individual plants with sample values higher than the average. Detailed concentration indices of morphological characteristics of *D. virgatus* mutant candidates are presented in Table 4.

The mutant candidate group subjected to 300 Gray irradiation demonstrated dominance in terms of superior plant characteristics, as evidenced by parameters such as plant height, number of twigs, length and width of compound leaves, length of pinnae, number of leaves per pinnae, and number of pods. Conversely, the mutant candidate group exposed to 200 Gray irradiation exhibited dominance in superior plant traits based on parameters such as stem diameter, number of branches, number of compound leaves, and number of flowers. Based on the number of pinnae, the superior plants were dominated by mutant candidates with 200 Gray and 300 Gray irradiation. Plants with an average concentration index of more than one are indicated by plants GDV100.1, GDV100.2, GDV100.4, GDV100.5, GDV200.2, GDV200.3, GDV200.7, GDV200.9, GDV200.10, GDV300.3, GDV300.4, GDV300.5, GDV300.12, GDV300.13. and GDV500.1. Plants with more than one concentration index show

Table 4. Concentration index of morphological characteristics of *D. virgatus* mutant candidates

Plant codes	Stem				Leaf							Flowers and pods	Mean
	VH (cm)	SD (mm)	NoB (Branch)	NoT (Twigs)	NoCL (Piece)	NoP (Piece)	CLL (cm)	CLW (cm)	PL (mm)	NoLP (Piece)	NoF (Flower)	NoPo (Pods)	
GDV100.1	**1.15**	0.94	**1.18**	0.99	**1.12**	0.97	0.98	0.99	1.00	**1.04**	**1.81**	0.27	**1.04**
GDV100.2	**1.40**	**1.02**	**1.37**	0.81	**1.10**	**1.02**	**1.01**	0.95	**1.01**	0.91	**3.16**	**1.98**	**1.31**
GDV100.3	**1.21**	0.93	0.73	0.68	**1.03**	0.99	0.98	0.98	**1.05**	**1.05**	**1.21**	0.66	0.96
GDV100.4	0.95	0.94	**1.82**	**1.36**	**1.14**	**1.01**	0.88	0.87	0.83	**1.07**	0.45	**1.43**	**1.06**
GDV100.5	**1.07**	0.82	0.91	**1.36**	**1.21**	**1.02**	0.85	0.87	**1.01**	1.00	**2.86**	0.88	**1.15**
GDV200.1	0.75	**1.03**	**1.00**	0.77	**1.03**	0.97	0.95	0.95	0.98	**1.01**	0.00	**2.03**	0.96
GDV200.2	0.88	**1.14**	**1.00**	**1.31**	**1.05**	0.95	0.96	0.94	**1.11**	0.92	**2.26**	0.33	**1.07**
GDV200.3	**1.06**	**1.32**	**1.00**	0.81	**1.23**	**1.06**	**1.03**	**1.04**	**1.02**	**1.11**	**1.96**	**3.13**	**1.31**
GDV200.4	**1.18**	0.94	**1.64**	0.99	**1.00**	0.79	0.82	0.89	0.90	0.82	0.45	**1.15**	0.97
GDV200.5	**1.05**	0.95	0.91	**1.09**	**1.28**	**1.01**	**1.06**	**1.05**	0.97	0.99	**1.05**	0.00	0.95
GDV200.6	**1.05**	**1.10**	0.64	0.95	0.75	**1.17**	**1.13**	**1.03**	**1.13**	**1.04**	0.45	0.66	0.93
GDV200.7	**1.23**	**1.05**	**1.18**	0.63	0.70	**1.20**	**1.05**	**1.08**	**1.03**	**1.09**	**2.11**	0.38	**1.06**
GDV200.8	0.89	**1.09**	0.36	0.81	0.84	**1.24**	**1.30**	**1.36**	**1.27**	0.87	0.30	0.82	0.93
GDV200.9	**1.02**	**1.05**	0.82	**1.45**	**1.31**	0.86	0.95	0.98	0.99	**1.08**	**1.66**	**1.98**	**1.18**
GDV200.10	**1.16**	0.94	**1.46**	0.23	0.93	**1.04**	0.96	0.85	0.92	**1.08**	**1.81**	0.77	**1.01**
GDV300.1	**1.13**	1.00	0.64	0.90	0.72	**1.10**	**1.05**	0.98	0.95	0.88	0.75	0.49	0.88
GDV300.2	0.92	**1.03**	**1.37**	**1.13**	**1.80**	0.84	0.94	0.95	0.89	0.93	0.00	0.71	0.96
GDV300.3	0.89	**1.05**	0.91	**1.04**	0.86	**1.10**	**1.21**	**1.25**	**1.32**	**1.09**	0.15	**1.15**	**1.00**
GDV300.4	**1.13**	**1.10**	0.91	0.68	0.87	0.99	0.90	0.88	**1.06**	0.95	**1.05**	**1.81**	**1.03**
GDV300.5	**1.08**	**1.11**	**1.28**	**1.13**	0.79	**1.22**	**1.07**	**1.00**	**1.05**	**1.11**	0.75	0.55	**1.01**
GDV300.6	0.96	**1.05**	0.73	0.99	0.75	0.97	0.87	0.87	0.92	0.99	0.30	0.88	0.86
GDV300.7	0.86	**1.09**	**1.28**	**1.45**	0.63	0.90	0.91	0.88	0.98	**1.07**	0.00	**1.87**	0.99
GDV300.8	0.75	0.90	0.91	0.90	0.83	**1.13**	**1.13**	**1.25**	**1.08**	**1.08**	0.15	**1.81**	0.99
GDV300.9	0.99	0.88	**1.18**	**1.27**	0.83	0.97	0.96	0.95	0.90	**1.06**	0.75	0.60	0.95
GDV300.10	0.94	0.99	0.55	0.50	0.65	0.97	0.97	0.97	**1.11**	**1.03**	0.00	0.00	0.72
GDV300.11	0.74	0.93	**1.18**	**1.40**	0.75	0.86	**1.02**	**1.05**	**1.21**	0.92	0.15	0.00	0.85
GDV300.12	0.81	0.90	**1.00**	**1.58**	**1.57**	0.90	0.82	0.85	0.77	0.67	**1.96**	0.33	**1.01**
GDV300.13	0.90	**1.02**	0.36	0.50	0.84	**1.26**	**1.26**	**1.28**	**1.19**	**1.18**	0.30	**2.63**	**1.06**
GDV300.14	0.88	**1.02**	0.55	0.86	0.62	0.99	**1.05**	**1.07**	**1.21**	0.95	0.75	0.05	0.83
GDV300.15	0.93	**1.24**	**1.18**	**1.22**	**1.14**	0.97	**1.05**	**1.03**	**1.03**	**1.04**	0.00	0.05	0.91
GDV300.16	**1.10**	**1.07**	**1.28**	0.86	0.83	**1.04**	1.00	**1.02**	0.96	**1.05**	0.60	0.44	0.94
GDV300.17	0.91	0.75	**1.00**	**1.22**	**1.06**	0.97	0.98	**1.09**	**1.01**	**1.11**	0.90	0.71	0.98
GDV400	0.84	0.83	0.64	0.63	0.89	0.84	**1.00**	**1.06**	0.83	**1.02**	0.60	**1.37**	0.88
GDV500.1	**1.11**	1.00	**1.28**	**1.31**	**1.51**	0.84	0.95	**1.02**	0.87	0.93	**4.52**	0.77	**1.34**
GDV500.2	**1.10**	0.82	0.73	**1.04**	0.97	0.83	0.90	0.76	0.62	0.83	0.15	**3.07**	0.99
GDV500.3	0.97	0.97	**1.00**	**1.13**	**1.37**	0.99	**1.02**	0.96	0.83	**1.03**	0.60	0.22	0.92

VH: Vertical height; SD: Stem diameter; NoB: Number of branches; NoT: number of twigs, NoCL: Number of compound leaves; NoP: Number of Pinnaes; CLL: Compound leaf length; CLW: Compound leaf width; PL: Length of pinnae; NoLP: Number of leaves per pinnae; NoF: Number of flowers; NoPo: Number of pods. *Normality test ($p<0.05$ = not normally distributed, $p>0.05$ = normally distributed). The candidate mutant plants are Single mutant candidates with high salinity tolerance (8.4 dS/m).

Table 5. Recapitulation of superior *D. virgatus* mutant candidates

Gamma Iradiation Levels	Mutant Candidates	Superior Mutant Candidates*	
(Gray)	Number of plants	Number of plants	(%)
100	5	4	80.00
200	10	5	50.00
300	17	5	29.41
400	1	0	0.00
500	3	1	33.33
Total	36	15	41.67

*Based on Concentration index calculation.

superior plant morphological characteristic values. This is in line with the theoretical approach of Syamsu (2006), who states that a concentration index value of more than one (>1.00) has a high characteristic value. Gamma-ray irradiation can increase the value of morphological characteristics in mutant plants. Naibaho *et al.* (2023) claim that using gamma-ray irradiation as a physical mutagen can improve plants' quantitative and qualitative characteristics.

Calculation of the average concentration index resulted in 15 of *D. virgatus* mutant candidates with a value of more than one (>1.00) as superior mutant plants out of 36 candidate mutant candidates. Fourteen superior mutant candidates were produced from gamma irradiation levels of 100-300 Gray. The data of this study indicate that an effective dose range of 100-300 Gray of gamma-ray irradiation can enhance the superior morphological traits of *D. virgatus* plants. Normasari *et al.* (2023) state that irradiation using gamma rays produces plants with superior properties. Other research shows that it is expected to use gamma doses ranging from a few to show superior plant characteristics.

Irradiation with 200 Gray and 300 Gray produced the most superior mutant candidates, namely five plants, followed by irradiation at 100 Gray and 500 Gray with four and one plants. This shows that mutagen induction using gamma-ray irradiation can produce superior plants. However, there is an optimal dose range to increase plant genetic diversity. Normasari *et al.* (2023) state that irradiation using gamma rays produces plants with superior properties. In addition, gamma irradiation can produce permanent gene expression to eliminate oxidative stress starting from the first generation of plants. This aims to improve superior plant varieties against biotic and abiotic stress factors (Beyaz and Yildiz, 2017).

Conclusion

The *D. virgatus* mutant candidates exhibit a high degree of variation in morphological characteristics, including the number of compound leaves, width of compound leaves, and the number of leaves per pinnae, flowers, and pods. The dominant color of the lower leaves was 7.5 Green Yellow (5/6), and the middle leaves were 7.5 Green Yellow (5/6), while the dominant color of the upper leaves was 7.5 Green Yellow (6/8). The mutant candidates of *D. virgatus* that exhibited the most desirable characteristics, as determined by the concentration index, were those subjected to 200 Gray and 300 Gray irradiation. Fifteen superior mutant candidates were identified based on concentration index calculation, specifically GDV100.1, GDV100.2, GDV100.4, GDV100.5, GDV200.2, GDV200.3, GDV200.7, GDV200.9, GDV200.10, GDV300.3, GDV300.4, GDV300.5, GDV300.12, GDV300.13. and GDV500.1. The three best mutant candidates (M1) were GDV100.2, GDV200.3, and GDV500.1. The dominance of superior traits was observed in mutant candidates that underwent 200 Gray and 300 Gray irradiation.

Conflict of interest

The authors have no conflict of interest to declare. All authors have seen and agree with the contents of the manuscript.

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Author’s contribution

The authors confirm their contribution to the paper as follows: study conception and design: IP, and PDMHKS; methodology: JR, and IP. data analysis: JR. validation IP, and PDMHKS; writing manuscript-original: JR, IP, and PDMHKS. Writing manuscript-review/revision: JR. All authors reviewed the results and approved the final version of the manuscript.

Ethics approval

This research did not involve the use of animals or human subjects as research samples. Therefore, ethical approval was not required.

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