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Gamma irradiation effect on some morphological and chemical characters of Sudani and Masri Roselle varieties

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Abstract: Roselle or Karkadaih (*Hibiscus sabdariffa* L.) is one of the most important medicinal plants used for various nutritional, medicinal and pharmaceutical purposes. Seeds of two Roselle varieties (Sudani and Masri) were sown for the plant parent generation, then the harvested seeds were exposed (in dry and in soaked seed categories) to gamma rays with different doses. The irradiated seeds were re-sown for M_2 and M_3 generations to evaluate the responsibility to gamma radiation. All the studied morphological characters indicated significant variability between varieties in the plant parent generation except the number of main branches/plant. The Sudani plant parents exhibited the higher values for all characters than of Masri variety. Moreover, in the M_2 and M_3 generations all the morphological characters were affected significantly by the seed categories (dry or soaked) radiation doses and their interaction as well as the varietal variation. Gamma rays improved the values of all morphological traits than of control. The dose of 40 Gray in the soaked seed category and 240 Gray in the dry seed category stimulated the highest trait values. Furthermore, genotypic and phenotypic coefficients of variation, broad sense heritability and expected genetic advance estimation presented higher values in M_2 than M_3 for most characters. On the other hand, the phytochemical screening of Roselle sepals showed greater acidity, anthocyanins, phenolics and antioxidant activity for Sudani than Masri variety. The responsibility of soaked seed category was more sensitive than of dry seed category. Total soluble solids and pH values had slight responsibility to gamma rays. Irradiation dose of 60 Gray in soaked seed category and 240 in dry seed category stimulated the highest values for most evaluated chemicals in Masry variety. While 20 Gray gave the highest sugars, anthocyanins and antioxidant activity and 60 Gray gave the maximum acidity and phenolics at the soaked seed category. No characterized doses effects were noticed in the dry seed category of Sudani variety. All the morphological and chemical results indicated that there is a store of genetic variability between the studied varieties that can be exploited for the improvement of Roselle yield through the selection and/or the hybridization between Sudani and Masri to produce a new variety that can share the valuable characters.
Key words: Roselle, *Hibiscus sabdariffa*, varieties, generations, Gamma irradiation, sepals phytochemical screening.

Introduction

Genus *Hibiscus* under Malvaceae family consists of about 300 species. More than half of them originated in the parts of central and eastern of Africa[1,2]. *Hibiscus sabdariffa* L. also known as Roselle or

Karkadaih is one of the most important species of *Hibiscus* in Egypt. Roselle is best grown in tropical and sub-tropical regions[3, 4].

Roselle is cultivated for its leaves, seeds and calyces. Nutritionally young leaves of Roselle contain nutrients such as phosphorus, calcium, magnesium and potassium[5]. The leaves are consumed as a green vegetable and prepared like spinach[6]. However, the seed of Roselle is a valuable food resource on account of its protein, calorie and also substantial amount of fiber and valuable micro-nutrients[7]. The seeds contain 17 to 20% edible fixed oil which is similar in its properties to cotton seed oil [8, 9, 10]. On the other hand, the color extract from the dry calyces is rich in anthocyanin[11], amino acids, organic compounds, mineral salts[12] and source of vitamin C[13]. Calyces extract is also a potential source of natural colorant to replace red synthetic coloring agents for carbonated soft drinks, jams, juices, jellies, sauces, chutneys, wines, preserves and other acidic foods[2, 6, 14]. In fact, some Roselle varieties/cultivars are identified according to calyxes anthocyanin content. For example, the Sudani Roselle variety has dark red calyces, while the calyx has light red color in Masri variety[9, 10].

In several countries, Roselle is also considered to be one of the most famous folk medicinal plants. Where, many chemical components present in Roselle have potential health benefits and support the ethno medicinal use of Roselle in promoting cardio-vascular health and preventing hypertension[15], pyrexia and liver disorders[16], microorganism growth limitation[17], as well as a diuretic, digestive and sedative[18]. The red varieties of Roselle have antioxidant and cyclooxygenase inhibitory activity[19]. Also, Roselle inters in pharmaceutical and cosmetic industries[20].

On the other hand, irradiation induces several cytological, genetic[21], morphogenetic[22], biochemical[23] and physiological alteration in cell and tissues of plants[24, 25]. Gamma ray treatments to plants with high doses disturb the leaf gas-exchange, hormone balance, water exchange and enzyme activities[26, 27]. These effects include changes in the plant cellular structure and cell metabolism such as alteration in photosynthesis, dilation of membranes of thylakoids, modulation of the antioxidant systems and accumulation of phenol compounds. Irradiation has proven an adept mean of encouraging the expression of recessive genes and producing new genetic variations[28]. Irradiation also been successfully used for mutation in breeding of various plants[22, 29, 30]. Mutation induction is one approach for creating genetic variation in the plants[31]. The technology of mutation induction has become an established tool in plant breeding in order to supplement existing germplasm and to improve cultivars in specific traits. Improved varieties of many crops have been released to forms as a result of induced mutation which have been used directly as new cultivars or in cross breeding programs[32]. Also, gamma radiation significantly affected the plant active ingredient biochemical contents such tannins and phenols…etc.[33]

The aim of the present work is to evaluate the response of some important breeding characters and sepal biochemical screening for Sudani and Masri Roselle varieties affected by different doses of gamma irradiation in dry and soaked categories.

Materials and Methods

Materials

Air dried seeds of two Roselle (*Hibiscus sabdariffa* L.) varieties; Sudani and Masri were obtained from the Genetics and Breeding of Medicinal and Aromatic Plants Group, Genetics and Cytology Dept., National Research Centre (NRC), Egypt.

Seed radiation

The seeds of each Sudani and Masri Roselle varieties were divided into two categories; dry category where the air dry seeds were exposed to radiation directly, and Soaked category where the seeds has been soaked in water for 10 hours before exposing to rays. The seeds were exposed to gamma irradiation under Gamma Cobalt 60 Apparatus at the Nuclear Research Centre. The applied doses were 20, 40, 60 and 80 Gray for the soaked seeds category, and 80, 160, 240, and 320 Gray for the dry seeds category. The doses rate of gamma rays was one Gray per 1.613 second. Unexposed seeds were used as control.

Cultivation method

Irradiated and non-irradiated (control) seeds were sown in a new reclaimed sandy land at Wadi El-Natroun Village, Behira Governorate on April for three successive summer seasons 2012-2014. A randomized complete block design with three replications was used. Each replicate had five lines 3.5 m length and 60 cm in between. The distance between hills was 40 cm and each hill was thinned at one plant. Normal agronomic recommended practices of Roselle growing were followed to obtain maximum yield. A representative random sample of 10 individual plants from each plot were selected for recording the data of five traits: plant height (PH) cm, number of main and total branches per plant (NMB and NTB, respectively), number of capsules per plant (NC) and air dry sepals weight per plant (DSW) g.

Statistical analysis

The general statistical procedures were applied using version 11 of SPSS software[34]. The statistical procedures were practiced according to standard methods given by[35]. The analysis of variance (ANOVA) and broad sense heritability (h^2b) were generally assigned according to[36]. The phenotypic and genotypic coefficients of variance (PCV and GCV %) were computed according to[37]. The expected genetic advance (GA %) was computed according to[38].

Phytochemical screening

Total anthocyanins

Total anthocyanins were determined according to [39], with some modifications. 0.1 g for each replicate, was added to 20 mL of methanol containing HCl (0.5%, v/v), homogenized for 3 min at 1500 rpm using a homogenizer Ultraturrax Turratec TE102E (Tecnal, Brazil), and held at 4 °C for 1 h in the darkness. The slurry was centrifuged at 17,600 × *g* for 15 min at 4 °C. The absorbance of the supernatant was recorded at 515 nm. Total anthocyanins content was calculated using the extinction coefficient equal to 3.6×10^4 mol^{-1} m^{-1}. Total anthocyanin content was expressed as mg pelargonidin-3-glucoside eq. g^{-1} DW.

Water extraction

Powdered sepals were subjected to extraction with water. One gram of powdered sample was shacked with 100 ml of distilled water for 12 hrs using shaking incubator at room temperature. Solids were separated by centrifugation and filtration. Total soluble solids, pH, total titratable acidity, soluble sugars, total phenolics, antioxidant activity, were then determined in the extracts.

Total soluble solids, pH and total titratable acidity (TTA)

Total Soluble solids were determined by evaporating water from 20ml of water extract using oven at 80 ^{0}C. Determination of pH for water extract was carried out using pH meter. Total titratable acidity was determined by titrating 20 ml of water extract. Titration was carried out to pH 8.2 using a 0.1 mol L^{-1} NaOH solution according to[40]. Results were expressed as mg citric acid /g of dry weight (DW).

Soluble sugars

Soluble sugars in water extract were determined by phenol-sulfuric acid method according to[41].

Total phenolics

Total phenolics was determined using Folin-Ciocalteu's reagent according[42]. Briefly, 1 ml of the extract was mixed with 1.5 ml of deionised water followed by 0.25 ml of Folin-Ciocalteu's reagent and allowed to react for 6 min. Then, 2.5 ml of 7% sodium carbonate was added and allowed to stand for 1 hr, and then the absorption was measured colorimetrically at 765 nm. Total phenolic was expressed as mg gallic acid/g dry sample using standard curve of gallic acid solution.

Antioxidant activity

Antioxidant was determined using Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity according to the method of[43] with some modifications. The stock solution was prepared by dissolving 24 mg 1, 1-diphenyl-2-picrylhydrazyl (DPPH) with 100 ml methanol and then stored at −20 °C until needed. The working solution was obtained by mixing 10 ml stock solution with 45 ml methanol to obtain an absorbance of 1.1±0.02 units at 515 nm using the spectrophotometer. Water extracts (750 µl) were allowed to react with 1500 µl of the DPPH solution for 5 min in the dark. Then the absorbance was taken at 515 nm. The standard curve was linear between 25 and 800 µmol Trolox. Results are expressed in µmol Trolox /g DW. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve.

Results and Discussion

Data of five morphological characters of the plant parents generation (the first season) of Sudani and Masri Roselle varieties were recorded, analyzed and illustrated in Table (1).

Table 1. Analysis of variance for five quantitative characters of the plant parent generation of two Roselle varieties

Source of variance	Df	Plant height	No. main branches /plant	No. total branches /plant	No. Capsules /plant	Dry sepals weight /plant
Varieties	1	16245.00**	26.45	396.05**	3892.05*	426.43**
Replicates	9	264.89	4.72	16.45	527.38	39.46
Error	9	546.56	7.78	5.61	692.27	35.61

The traits of plant height (PH), number of total branches (NTB), number of capsules (NC) and dry sepals weight (DSW) per plant indicated significant differences between the studied Roselle varieties. While the varieties differed non-significantly in the trait of number of main branches per plant (NMB). The plant parents of Sudani variety exhibited the higher range and mean values for all characters than of Masri variety. The plant parents of both varieties presented considered coefficient of variance CV% (more than 10%) for all studied traits except PH trait in Sudani variety (Table, 2).

Table 2. Four statistical items of five quantitative characters for the plant parent generation of Masri and Sudani varieties

variety	Items	Plant height	No. main branches /plant	No. total branches /plant	No. Capsules/plant	Dry sepals weight /plant
Sudani	Range	170-220	9-18	20-32	57-118	21.95-40.25
	X	202.5	12.2	25.3	88.9	27.7
	SE	4.73	0.92	1.33	7.04	1.71
	CV%	7.38	23.76	16.67	25.03	19.50
Masri	Range	100-190	7-13	13-19	30-92	11.5-29.5
	X	145.5	9.9	16.4	61.0	18.48
	SE	7.67	0.64	0.66	8.51	2.14
	CV%	16.66	20.45	12.6	44.12	36.64

The significant differences of average value addition to the considered CV% values for the morphological characters between the plant parent generation of Sudani and Masri Roselle varieties indicated that there is a store of genetic variability that can be exploited for the improvement of Roselle yield and thus suggesting the possibility of evolving higher Roselle yield variants through proper selection[2]. The ranges of PH trait (170-220 and 100-190 cm for Sudani and Masri, respectively) are near to that of [2, 4]. Meanwhile, [44, 45] found that Roselle plant is about 3.5 m tall. [9] recorded average of 150 and 147 cm for the height of Sudani and Masri Roselle plants, respectively. However, the range of Masri NTB trait was (13-19) lower than (18.47-36.73) of [4] and (22.22-24.5) of [2]. Meanwhile, NTB range of Sudani variety (20-32) was nearest to[4] and wider than[2]. But, [9] found average of 5.7 and 4.3 branches per plant for Sudani and Masri Roselle varieties. A great diversity between the varieties was also recorded in both NC and DSW traits agree with the finding of[2,46,47]. This diversity indicated the possibility to increase calyx fruit production (quantity and weight) through selection.

Data of the above studied morphological characters (after radiation exposing) for M_2 and M_3 generations were analyzed and shown in Table (3). From this table, each of seed categories, radiation dose and their interaction affected significantly in all traits for both M_2 and M_3 generations. Also, NMB presented significant differences between varieties in both generations. The varieties affected significantly by radiation for NTB in M_2, and PH in M_3. The varieties interacted significantly with seed categories for NC and DSW in M_2 as well as NMB and NC for M_3. Meanwhile, the relationship of varieties with radiation doses was significant only for NTB in M_2 and NMB in M_3. Furthermore, the triple relationship among varieties, seed categories and irradiation doses had non-significant interaction in all traits for both generations except PH in M_2 which was significant (Table, 3).

Table 3. Analysis of variance for five quantitative characters of two generations (M_2 and M_3) of two Roselle varieties treated with five radiation doses for two radiation seed categories

G.	Source of variance	Df	Plant height	No. main branches /plant	No. total branches /plant	No. Capsules /plant	Dry sepals weight /plant
M2	Replicates	2	3.63	2.23	15.05	31.85	3.73
	Varieties (V)	1	79.3	16.03*	1530.15**	163.35	19.66
	Radiation Category (C)	1	9101.97**	673.37**	728.02**	2394.02**	290.01**
	Dose (D)	4	5303.45**	306.65**	1529.86**	13607.69**	1665.04**
	V x C	1	0.06	1.33	0.41	904.81**	110.65**
	V x D	4	51.16	2.56	159.11**	22.39	1.77
	C x D	4	764.32**	46.89**	258.23**	3055.47**	372.98**
	V x C x D	4	119.22*	0.98	17.04	219.61	28.50
	Residual	38	41.69	2.86	10.93	96.59	11.73
M3	Replicates	2	9.05	1.40	1.25	42.02	8.63
	Varieties (V)	1	7526.40**	228.15**	36.82	109.35	18.60
	Radiation Category (C)	1	504.60**	36.82**	331.35**	770.41**	78.44*
	Dose (D)	4	6120.71**	268.39**	3105.23**	14174.23**	1726.78**
	V x C	1	106.67	25.35*	14.01	442.82*	46.45
	V x D	4	99.69	17.86**	16.32	107.14	13.76
	C x D	4	699.14**	40.77**	496.27**	1513.54**	176.31**
	V x C x D	4	61.29	2.31	11.18	86.53	13.36
	Residual	38	56.31	4.51	40.95	99.12	12.14

Mean values of the five morphological traits of M_2 and M_3 generations for Masri and Sudani Roselle varieties treated with five radiation doses in a soaked seed category are shown in Table (4). The table shows that all gamma radiation doses stimulated the higher values than that of control (untreated plants) for all characters, generations and varieties to prove that all used doses improved the morphological traits of Roselle plants but with different responses. The dose of 40 Gray stimulated the highest morphological traits value for both generations and varieties. The highest radiation dose (80 Gray) stimulated the lowest morphological traits value comparing with the other doses. Generally, doses arrangement was 40, 60, 20 and 80 Gray, respectively related to the morphological traits improving. On the other hand, the plants of M_3 generation gave the higher morphological traits values than those of M_2 generation. Sudai was the better variety for all characters than Masri variety at M_2, while inverse pattern was noticed for PH, NMB and NTB of M_3 generation.

The same result pattern was noticed in the dry seed category (Table, 5). Gamma radiation improved the values for all studied traits in both generations and varieties than control. The dose of 240 Gray gave the highest trait value more than 160, 320 and 80 Gray, respectively. The plants of M_3 generation had the higher mean value in all traits than those of M_2 generation, except the mean value of NMB in Masri variety addition to PH and NMB traits of Sudani variety which had higher values in M_2 plants for all radiation doses.

Table 4. Mean value of five morphological traits of M_2 and M_3 generations for Masri and Sudani Roselle varieties treated with five radiation doses in a soaked seed category

Traits	Radiation doses	M_2 generation		M_3 generation	
		Masri variety	Sudani variety	Masri variety	Sudani variety
Plant height (cm)	Control	118.3 ± 4.41	111.3 ± 3.67	147.7 ± 6.23	139.7 ± 2.60
	20 Gy	144.0 ± 2.0	150.3 ± 2.19	190.0 ± 2.89	165.0 ± 2.89
	40 Gy	168.3 ± 4.41	183.0 ± 4.58	223.3 ± 9.28	203.3 ± 3.33
	60 Gy	153.7 ± 3.76	160.0 ± 1.15	207.7 ± 5.36	176.3 ± 2.73
	80 Gy	132.0 ± 1.15	123.3 ± 3.76	172.7 ± 2.67	158.3 ± 1.67
Number of main branches/plant	Control	8.3 ± 0.33	8.3 ± 0.88	12.0 ± 0.58	11.0 ± 1.53
	20 Gy	14.3 ± 0.33	15.3 ± 0.33	17.3 ± 0.33	15.3 ± 0.33
	40 Gy	18.7 ± 1.20	22.0 ± 0.58	27.3 ± 1.20	24.3 ± 3.93
	60 Gy	15.3 ± 0.33	17.3 ± 0.88	23.0 ± 1.00	17.0 ± 0.00
	80 Gy	12.3 ± 1.67	12.7 ± 0.67	15.0 ± 0.58	14.0 ± 0.58
Number of total branches/plant	Control	13.7 ± 0.88	17.3 ± 0.67	30.3 ± 3.84	30.3 ± 1.86
	20 Gy	20.3 ± 0.88	26.3 ± 2.85	61.3 ± 2.19	58.0 ± 2.31
	40 Gy	30.0 ± 2.00	48.0 ± 2.00	83.3 ± 1.20	78.0 ± 2.89
	60 Gy	23.3 ± 0.33	41.3 ± 4.18	70.0 ± 2.08	65.7 ± 0.88
	80 Gy	17.7 ± 0.67	21.7 ± 0.33	48.3 ± 3.38	48.7 ± 1.45
Number of capsules/plant	Control	42.0 ± 3.61	59.7 ± 4.37	72.3 ± 11.05	61.3 ± 1.33
	20 Gy	73.3 ± 1.86	93.0 ± 0.58	116.3 ± 1.20	120.3 ± 4.26
	40 Gy	120.0 ± 3.46	122.7 ± 10.4	163.0 ± 2.52	170.0 ± 0.00
	60 Gy	98.0 ± 1.15	99.0 ± 1.53	148.7 ± 9.84	151.3 ± 3.76
	80 Gy	57.7 ± 4.33	72.0 ± 2.52	106.0 ± 4.73	89.7 ± 5.17
Dry sepals weight (g)	Control	14.7 ± 1.26	20.9 ± 1.53	25.3 ± 3.87	21.5 ± 0.45
	20 Gy	25.7 ± 0.68	32.6 ± 0.20	39.1 ± 2.04	42.2 ± 1.39
	40 Gy	42.0 ± 1.18	42.9 ± 3.64	57.0 ± 0.89	59.2 ± 0.15
	60 Gy	34.4 ± 0.46	34.7 ± 0.52	52.0 ± 3.44	52.9± 1.30
	80 Gy	20.2 ± 1.53	25.2 ±0.89	37.1 ± 1.65	31.5 ± 1.87

Table 5. Mean value of five morphological traits of M_2 and M_3 generations for Masri and Sudani Roselle varieties treated with five radiation doses in a dry seed category

Traits	Radiation doses	M_2 generation		M_3 generation	
		Masri variety	Sudani variety	Masri variety	Sudani variety
Plant height (cm)	Control	142.0 ± 7.51	147.0 ± 3.79	154.3 ± 6.36	136.0 ± 4.93
	80 Gy	155.7 ± 1.20	154.3 ± 0.33	173.0 ±1.53	150.0 ± 2.89
	160 Gy	184.3 ± 2.96	182.0 ± 4.16	201.7 ± 4.41	170.0 ± 2.89
	240 Gy	193.0 ± 1.53	199.7 ± 5.49	211.7 ± 1.67	187.0 ± 5.86
	320 Gy	164.7 ±2.91	168.0 ± 3.61	185.0 ± 2.89	157.3 ± 2.33
Number of main branches/plant	Control	12.0 ± 1.73	12.0 ± 0.58	12.0 ± 0.58	8.7 ± 0.88
	80 Gy	18.7 ± 1.33	18.7 ± 0.33	14.0 ± 0.00	12.3 ± 0.67
	160 Gy	24.0 ± 1.00	25.0 ± 0.58	22.3 ± 0.88	15.3 ± 0.33
	240 Gy	27.7 ± 1.67	29.3 ± 1.45	26.7 ± 2.19	17.3 ± 0.33
	320 Gy	21.7 ± 0.33	22.7 ± 0.88	18.3 ± 0.33	13.7 ± 0.33
Number of total branches/plant	Control	14.7 ± 2.33	16.7 ± 1.67	37.0 ± 3.51	33.3 ± 3.18
	80 Gy	22.7 ± 0.33	27.3 ± 2.67	42.3 ± 0.67	45.0 ± 3.00
	160 Gy	33.0 ± 2.52	45.3 ± 1.20	60.0 ± 1.53	59.3 ± 2.19
	240 Gy	42.3 ± 2.73	64.7 ± 1.33	77.0 ± 9.50	72.7 ± 4.37
	320 Gy	26.3 ± 1.20	36.3 ± 2.03	48.7 ± 1.76	51.7 ± 1.20
Number of capsules/plant	Control	50.3 ± 8.41	39.0 ± 3.79	73.3 ± 9.77	76.0 ± 4.16
	80 Gy	77.7 ± 3.84	65.3 ± 6.77	93.7 ± 1.20	97.7 ± 0.67
	160 Gy	109.7 ± 4.70	119.7 ± 11.14	120.3 ± 7.31	134.3 ± 5.33
	240 Gy	162.7 ± 9.33	162.0 ± 4.62	154.3 ± 7.88	162.7 ± 3.84
	320 Gy	92.7 ± 4.26	84.7 ± 4.37	101.7 ± 2.40	113.3 ± 7.26
Dry sepals weight (g)	Control	17.7 ± 2.89	13.7 ± 1.31	25.9 ± 3.39	26.6 ± 1.47
	80 Gy	27.2 ± 1.33	22.8 ± 2.35	32.9 ± 0.30	34.2 ± 0.24
	160 Gy	38.3 ± 1.60	41.9 ± 3.89	42.1 ± 2.53	47.1 ± 1.54
	240 Gy	56.9 ± 3.27	56.7 ± 1.62	53.9 ± 2.71	57.0 ± 1.33
	320 Gy	32.5 ± 1.49	29.6 ± 1.53	35.5 ± 0.76	39.7 ± 2.39

These results proved that all the used gamma doses enhanced (with different responses) the traits of Roselle plants comparing with the control plants. This stimulatory effect of gamma rays is due to the fact that mutagens stimulate the role of enzyme and growth hormone responsible for growth and yield, in addition to stimulate the cell division, alteration of metabolic processes that affect synthesis of nucleic acids[22, 27, 48]. Improvement of Roselle plant characters as a result of gamma radiation was recorded by[49] in cotton plant as well as[50, 51, 52] in okra plant. The superior gamma dose in each category (40 and 240 Gray in the soaked and dry seed categories, respectively) stimulated the maximum values for all studied traits. [53] assumed the stimulation of gamma radiation to its impact on the auxins balance within the plant tissues. The dose of 80 Gray was the weaken dose compared with the other gamma doses might be due to reduced mitotic division in meristematic tissues and reduced moisture content[27, 54, 55, 56]. [26] suggested that gamma radiation disturb the leaf gas-exchange, hormone balance, water exchange and enzyme activities. These effects include changes in the plant cellular structure and cell metabolism such as alteration in photosynthesis, dilation of membranes of thylakoids, modulation of the antioxidant systems and accumulation of phenolic compounds. Generally, the studied characters were beter enhanced in M_3 plants than in M_2 plants to confirm that the Roselle plant can be improved and enhanced its income through selection. However, Sudani was the best variety in M_2 generation, while Masri was the best one for PH, NMB and NTB traits in M_3 generation. These results can be utilized for improving the Roselle plant in Egypt through the hybridization between Sudani and Masri to produce a new variety that could share the valuable characters.

On the other hand, Table (6) shows the assessed genotypic and phenotypic coefficients of variation (GCV% and PCV%, respectively) as well as broad sense heritability (h^2_b) and expected genetic advance (GA%) for the five studied traits of M_2 and M_3 generations for Masri and Sudani Roselle varieties exposed to different gamma radiation doses in case of soaked seeds. Both varieties presented greater GCV% in M_2 than M_3 generation for all traits except NC and DSW of Sudani variety affected by 20 and 80 Gray and PH of Masri variety affected by 80 Gray. However, the dose of 60 Gray stimulated the highest GCV% (9.98 and 10.55%) for NMB at M_2 of Masri and Sudani, respectively. While, the lowest GCV% value (1.62 and 1.57%) related to PH character for Masri M_2 and Sudani M_3, respectively. Higher PCV% was also assessed for M_2 than M_3 generation for both varieties in all traits and doses, except for Sudani NMB exhibited low PCV% affected by 20, 60 and 80 Gray addition to Masri DSW affected by 40 Gray. The greatest PCV% was affected by 80 Gray and related to NMB (25.23%) of Masri M_2 and (24.74%) of Sudani M_3. While the lowest PCV% (5.89 and 6.24% for Masri and Sudani, respectively) was exhibited for PH affected by 40 Gray in the M_3 generation (Table 6).

Exposing to 40 Gray of gamma rays produced the highest h^2_b for PH, NMB and NTB traits (17.68, 35.51 and 37.16%, respectively) in Sudani M_2 and (14.57, 29.82 and 36.84%, for the same respecting) in Masri M_3 generation. The maximum h^2_b for NC and DSW traits (38.24 and 38.24%, respectively) were obtained by 40 Gray in Masri M_2 and (37.15 and 36.89%, respectively) for Sudani M_3. The lowest h^2_b values were obtained affecting by 80 Gray for PH, NC and DSW (3.72, 11.07 and 11.07%, respectively) in Masri M_2 and for PH, NTB, NC and DSW (3.47, 7.83, 6.43 and 6.40%, respectively), in Sudani M_2. The lowest h^2_b of NMB was exhibited by exposing to 80 Gray in M_3 of both Masri (7.69%) and Sudani (8.33%), addition to 3.39% of the lowest h^2_b caused by 60 Gray in Masri M_2. Furthermore, higher GA values were recorded in M_3 generation for Masri PH, NTB and NC and Sudani NTB, NC and DSW, while Sudani PH and NMB presented the higher GA at M_2 generation. The dose of 40 Gray gamma rays stimulated the highest GA for Masri NC and DSW (6.50 and 3.84%, respectively) in M_2 and for PH, NMB and NTB (3.95, 2.54 and 5.26%, respectively) in M_3. Same stimulation was recorded for Sudani PH and NMB (4.23 and 2.62%) at M_2 and NTB, NC and DSW (4.82, 7.56 and 4.44%, respectively) at M_3 generation. Gamma dose 80 Gray exhibited the lowest GA values for all traits of both varieties and both generations (Table 6).

The resulted genetic parameters for the irradiated dry seeds of both varieties are shown in Table (7). Both varieties presented higher GCV% in M_2 than M_3 generation for NTB, NC and DSW affected by all gamma radiation doses. Meanwhile Sudani PH and NMB had the lower GCV% in M_2 affected by all gamma radiation doses. The dose of 80 and 320 Gray for PH trait and 240 Gray for NMB of Masri variety exhibited the higher GCV% in M_3 generation. The trait of NMB had the highest GCV% (8.33%) for Masri affected by 160 Gray in M2 and (9.79%) for Sudani affected by 240 Gray in M_3 generation. While, the lowest GCV% was exhibited by the lowest dose (80 Gray) for PH of both Masri and Sudani (1.37 and 1.01%, respectively) in M_2 generation. Higher PCV% was assessed for Masri PH and NTB affected by all gamma doses in M_2. While higher PCV% was obtained for Masri NMB affected by all gamma doses (except by 240 Gray) as well as NC and DSW (except by 80 Gray) in M_3 generation. However, Sudani PH and NMB had the higher PCV% affected by all gamma doses in M_3. Higher PCV% was obtained for Sudani NTB affected by all radiation doses (except by 240 Gray) as well as NC and DSW (except by 160 and 240 Gray) in M_2 generation. The maximum PCV% was obtained by 80 Gray in NMB trait of both Masri (25.42%) and Sudani (25.58%) in M_3 generation. Meanwhile NC trait showed the lowest PCV% (5.76 and 5.52% affected by 240 Gray for both Masri and Sudani, respectively at M_2 generation.)

Table 6. Four genetic items estimated for five quantitative characters of M_2 and M_3 generations for Masri and Sudani Roselle varieties treated with four radiation doses in a soaked seed category

Traits	variety	Item	M_2 generations				M_3 generations			
			20G	40G	60G	80G	20G	40G	60G	80G
Plant height (cm)	Masri	GCV	2.03	2.43	2.23	1.62	1.98	2.25	2.15	1.67
		PCV	7.82	6.90	7.42	8.40	6.69	5.89	6.23	7.23
		h^2_b	6.75	12.35	9.07	3.72	8.71	14.57	11.93	5.34
		GA	1.57	2.96	2.13	0.85	2.28	3.95	3.18	1.37
	Sudani	GCV	2.39	2.67	2.52	1.62	1.76	2.26	1.98	1.57
		PCV	7.41	6.35	7.06	8.71	7.38	6.24	6.99	7.63
		h^2_b	10.39	17.68	12.73	3.47	5.69	13.18	8.03	4.25
		GA	2.38	4.23	2.96	0.77	1.43	3.44	.2.04	1.06
Number of main branches /plant	Masri	GCV	9.89	9.96	9.98	9.38	7.69	8.27	8.33	6.67
		PCV	22.44	18.35	21.31	25.23	21.45	15.15	17.21	24.04
		h^2_b	19.42	29.48	21.92	13.81	12.85	29.82	23.42	7.69
		GA	1.28	2.08	1.47	0.88	0.98	2.54	1.91	0.57
	Sudani	GCV	9.98	9.72	10.55	9.55	7.82	8.66	8.32	7.14
		PCV	21.31	16.31	19.71	24.61	23.04	16.17	21.21	24.74
		h^2_b	21.92	35.51	28.63	15.05	11.50	28.71	15.38	8.33
		GA	1.47	2.62	2.01	0.97	0.84	2.32	1.14	0.59
Number of total branches /plant	Masri	GCV	7.31	7.77	7.68	6.52	5.24	5.05	5.20	5.07
		PCV	19.64	14.58	17.64	21.90	10.40	8.31	9.43	12.47
		h^2_b	13.84	28.38	18.93	8.85	25.42	36.84	3.39	16.53
		GA	1.14	2.56	1.60	0.71	3.34	5.26	4.13	2.05
	Sudani	GCV	6.59	6.66	6.85	5.59	5.24	5.11	5.23	5.08
		PCV	17.13	10.93	12.18	19.97	10.84	8.71	9.88	12.39
		h^2_b	14.78	37.16	31.62	7.83	23.35	34.42	28.03	16.83
		GA	1.37	4.02	3.28	0.70	3.02	4.82	3.75	2.09
Number of capsules /plant	Masri	GCV	4.41	4.25	4.41	3.96	3.29	3.37	3.39	3.16
		PCV	9.88	6.87	7.95	11.91	8.02	6.21	6.65	8.62
		h^2_b	19.89	38.24	30.77	11.07	16.87	29.48	26.05	13.44
		GA	2.97	6.50	4.94	1.57	3.24	6.15	5.31	2.53
	Sudani	GCV	3.58	3.73	3.66	2.81	3.69	3.54	3.62	3.43
		PCV	9.05	7.32	8.62	11.09	7.48	5.81	6.32	9.38
		h^2_b	15.68	26.02	17.99	6.43	24.29	37.15	32.86	13.38
		GA	2.72	4.82	3.16	1.06	4.50	7.56	6.47	2.32
Dry sepals weight /plant (g)	Masri	GCV	7.45	7.18	7.45	6.70	5.49	5.70	5.74	5.34
		PCV	16.68	11.62	13.41	20.13	13.98	14.42	11.24	14.57
		h^2_b	19.98	38.24	30.89	11.07	15.38	15.64	26.02	13.45
		GA	1.76	3.84	2.93	0.93	1.73	2.65	3.14	1.50
	Sudani	GCV	6.06	6.31	6.18	4.75	6.22	5.99	6.12	5.79
		PCV	15.28	12.39	14.55	18.75	12.63	9.86	10.69	15.82
		h^2_b	15.73	25.97	18.04	6.40	24.30	36.89	32.75	13.41
		GA	1.61	2.84	1.88	0.62	2.67	4.44	3.81	1.38

GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, h^2_b = Broad sense heritability%, GA = Genetic advance

Table 7. Four genetic items estimated for five quantitative characters of M_2 and M_3 generations for Masri and Sudani Roselle varieties treated with four radiation doses in a dry seed category

Traits	variety	Item	M_2 generations				M_3 generations			
			8oG	16oG	240G	320G	8oG	16oG	240G	320G
Plant height (cm)	Masri	GCV	1.37	2.04	2.14	1.67	1.44	1.97	2.07	1.73
		PCV	7.78	6.78	6.53	7.43	7.32	6.47	6.22	6.93
		h^2_b	3.12	9.03	10.69	5.06	3.88	9.29	11.03	6.22
		GA	0.78	2.32	2.78	1.28	1.01	2.50	2.99	1.64
	Sudani	GCV	1.01	1.88	2.10	1.57	1.44	1.98	2.20	1.69
		PCV	7.92	6.92	6.42	7.59	7.91	7.14	6.61	7.60
		h^2_b	1.63	7.35	10.68	4.55	3.32	7.69	11.11	4.96
		GA	0.41	1.91	2.82	1.16	0.81	1.92	2.83	1.22
Number of main branches /plant	Masri	GCV	7.99	8.33	8.26	8.28	5.85	8.31	8.29	7.92
		PCV	20.17	16.67	14.99	17.98	25.42	17.61	15.40	20.52
		h^2_b	15.67	25.00	30.35	21.21	5.29	22.23	28.99	14.89
		GA	1.22	2.06	2.60	1.70	0.39	1.80	2.46	1.15
	Sudani	GCV	7.99	8.32	8.20	8.32	8.91	9.69	9.79	9.43
		PCV	20.17	16.16	14.39	17.38	25.58	21.58	19.66	23.51
		h^2_b	15.67	26.52	32.47	22.93	12.12	20.18	24.81	16.10
		GA	1.22	2.21	2.82	1.86	0.79	1.37	1.74	1.07
Number of total branches /plant	Masri	GCV	7.20	7.48	7.17	7.48	3.15	4.62	4.74	4.06
		PCV	18.36	13.82	11.56	16.39	14.72	11.14	9.21	13.13
		h^2_b	15.37	29.33	38.49	20.84	4.57	17.17	26.49	9.54
		GA	1.32	2.73	3.88	1.85	0.59	2.36	3.87	1.26
	Sudani	GCV	6.88	6.81	6.18	7.04	4.39	4.97	4.98	4.79
		PCV	16.48	11.31	8.84	13.28	13.55	10.92	9.37	12.15
		h^2_b	17.45	36.33	48.93	28.11	10.48	20.66	28.28	15.55
		GA	1.62	3.83	5.76	2.79	1.32	2.76	3.97	2.01
Number of capsules /plant	Masri	GCV	3.89	4.06	3.76	4.06	2.78	3.29	3.37	3.03
		PCV	9.92	7.63	5.76	8.66	9.55	7.84	6.49	8.95
		h^2_b	15.36	28.25	42.69	21.93	8.49	17.61	26.92	11.44
		GA	2.44	4.87	8.24	3.63	1.57	3.42	5.55	2.14
	Sudani	GCV	4.54	4.33	3.95	4.61	2.75	3.28	3.30	3.11
		PCV	10.58	6.78	5.52	8.69	9.34	7.27	6.30	8.30
		h^2_b	18.36	40.82	51.25	28.08	8.69	20.36	27.55	14.06
		GA	2.61	6.83	9.44	4.26	1.63	4.10	5.81	2.72
Dry sepals weight /plant (g)	Masri	GCV	6.55	6.84	6.35	6.83	4.64	5.52	5.67	5.04
		PCV	16.80	12.94	9.75	14.64	16.15	13.29	11.01	15.20
		h^2_b	15.19	27.96	42.48	21.79	8.25	17.25	26.48	11.00
		GA	1.43	2.86	4.85	2.13	0.90	1.99	3.24	1.22
	Sudani	GCV	7.63	7.32	6.68	7.78	4.65	5.55	5.58	5.27
		PCV	17.94	11.47	9.34	14.73	15.78	12.28	10.63	14.02
		h^2_b	18.11	40.69	51.12	27.89	8.69	20.43	27.58	14.11
		GA	1.53	4.03	5.58	2.50	0.97	2.43	3.44	1.62

GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, h^2_b = Broad sense heritability%, GA = Genetic advance

Greater h^2_b was estimated for all traits (except PH) of both Roselle varieties in M_2 than M_3 generation affected by all gamma doses. But, the greater h^2_b for PH was estimated in M_3 of both varieties affected by all radiation doses. The highest h^2_b value was computed in NC trait affected by 240 Gray for both Masri (42.69%) and Sudani (51.25%) in M_2 generation. However, the higher GA% was received for all traits (except PH) of both varieties in M_2 than M_3 generation affected by all gamma doses. The greater GA% for PH was obtained in M_3 of both varieties affected by all gamma doses. The maximum GA% was obtained for NC trait affected by 240 Gray for both Masri (8.24%) and Sudani (9.44%) in M_2 generation. Hence the lowest GA% was estimated for Masri NMB (0.39%) affected by 80 Gray in M_3 and for Sudani PH (0.41%) affected by 80 Gray in M_2 generation (Table 7).

In general, greater GCV% values were computed in M_2 than M_3 generation for most of studied traits of both Roselle varieties affected by the different gamma ray doses in both soaked and dry categories. Irradiated application in soaked seed category gave higher PCV% in M_2 than M_3 for all traits of both varieties except Sudani NMB and Masri DSW. M_3 had the higher PCV% in dry seed category for Masri NMB, NC and DSW, as well as Sudani PH and NMB. Opposite to PCV% of Masri PH and NTB addition to Sudani NTB were higher in M_2 generation. Gamma exposed dry seed category presented greater h^2_b in M_2 for all characters of both varieties except PH trait. Same trend was noticed in soaked seed category for Masri NC and DSW as well as Sudani PH and NMB, but higher h^2_b was noticed for Masri PH and NTB as well as Sudani NC and DSW in M_3 generation. GA% results had approximately the same response of h^2_b to gamma radiation. In this conception, [57, 58, 59] found greater GCV%, h^2_b and GA% in the second season. They attributed this difference to lesser effect of the environmental factors. On the other hands, highest GCV% exhibited in NMB affected by 60 Gray for both varieties at M_2 generation. NMB in M_2 of Masri and M_3 of Sudani had the highest PCV% affected by 80 Gray. [57] found that the trait of seed yield/plant exhibited the highest GCV% in the first and second seasons. Whereas, the plant height trait exhibited the lowest GCV% value in both seasons. Furthermore, the highest value of heritability broad sense (h^2_b) was achieved in the trait of NC affected by 40 Gray in Masri M_2 and Sudani M_3. Therefore, it can be indicated that this trait (NC) possessed a wide range of genetic variability, so it can be improved by the mass selection. [60] concluded that more variable conditions reduce heritability, whereas uniform conditions increase it. Meanwhile, [57, 61, 62] concluded that any plant character depends on many components which are greatly influenced by environment exhibits low heritability. However, NC character had the highest GA% affected by 40 Gray in M_2 generation of both varieties, whereas the lowest GA% was obtained by Masri NMB and Sudani PH affected by 80 Gray. These results indicate that the GA% for a trait depends on the amount of genetic variability of such trait. Similar conclusions have been drawn by [57] who stated that there was no definite trend between genetic coefficients of variation and heritability or between heritability and genetic advance. Therefore, conjunction of heritability estimates with genetic advance in a selection program is essential.

Phytochemical screening of Roselle calyces (sepals) for M_3 generation of Masri and Sudani varieties response to gamma irradiation was presented in Table (8). The responsibility of Soaked seed category to gamma irradiation was higher than dry seed category for most evaluated chemicals in both Roselle varieties. Moreover, Sudani variety had the higher values for most evaluated chemicals in both soaked and dry seed categories. Total soluble solids (ranged from 47.5 to 56.5 g/100gDW) and pH value (ranged from 2.0 to 2.3 pH.) had slight response to gamma ray exposing in both varieties and categories. Irradiation doses 60 Gray in soaked seed category and 240 Gray in dry seed category stimulated the highest values for total acidity, soluble sugars, total anthocyanins, total phenolics and antioxidant activity in Masri variety. However the dose of 20 Gray stimulated the highest values of sugars, anthocyanins and antioxidant activity, but the highest acidity and phenolics were achieved by 60 Gray in Sudani variety at soaked seed category. Meanwhile, the dry seed irradiated category of Sudani variety did not show general dose effect, where 320 Gray stimulated the highest phenolics and antioxidant activity, the highest sugars was obtained by 240 Gray and the highest acidity was obtained by 80 Gray (Table 8).

Generally, greater acidity, anthocyanins, phenolics and antioxidant activity were recorded for Sudani variety comparing with those of Masri variety. The responsibility of soaked seed category to gamma irradiation was higher than dry seed category in both Roselle varieties. Irradiation doses 60 Gray in soaked seed category and 240 Gray in dry seed category stimulated the highest values for most evaluated chemicals in Masri variety. In Sudani variety the highest sugars, anthocyanins and antioxidant activity were stimulated by 20 Gray, but the highest acidity and phenolics were achieved by 60 Gray at soaked seed category, while the dry seed category did not show general dose effect. These results are in agreement with [63] who found greatest anthocyanins, sugars and pH in the sepals of Sudani variety comparing with Masri and White Roselle varieties. They returned the differences in sepal chemicals to the varietal genetics and environmental conditions which exerted a great influence on the metabolism of biochemical components. Furthermore [33, 64, 65] reported that gamma irradiation can modify the tannin and the phenol contents. This modification is very favorable, since this anti-nutritional factor had the capacity for decreasing protein digestibility [66]. On the other hand, gamma irradiation resulted in a significant tendency to decreasing DPPH (Diphenyl-2-picrylhydrazyl) radical-scavenging activity of different methanolic extracts [33]. However [53] reported that the dose of 40 Gray had the capacity to enhance total flavones content and coloring matters (anthocyanins) in Roselle plants.

Table 8. Seven chemical evaluations of the plant calyces (DW) for M3 generation of Masri and Sudani Roselle varieties treated with five radiation doses in soaked and Dried seed categories

Category	Variety	Dose	Total soluble solids g/100g	pH	Total acidity mg acid/g	Soluble sugars g/100g	Total anthocyanins mg/g	Total phenolics mg/g	Anti-oxidant activity µmol/g
Soaked seed	Masri	Control	56.0	2.1	127.8	10.4	4.5	38.8	93.2
		20 Gy	47.5	2.2	61.5	5.1	3.1	32.1	75.9
		40 Gy	53.0	2.1	84.5	10.0	5.0	39.2	97.9
		60 Gy	55.0	2.2	115.6	15.7	6.2	64.1	183.4
		80 Gy	54.4	2.2	57.0	15.8	6.1	56.3	145.0
	Sudani	Control	55.8	2.1	104.1	15.0	6.2	61.3	169.0
		20 Gy	56.5	2.2	54.1	14.2	6.4	59.3	179.5
		40 Gy	54.2	2.1	113.3	13.6	5.8	47.7	112.8
		60 Gy	51.2	2.2	128.3	13.7	6.3	64.0	150.4
		80 Gy	50.9	2.1	65.5	13.6	6.2	57.7	163.2
Dried seed	Masri	Control	51.5	2.1	82.0	6.1	2.5	29.3	54.8
		80 GY	48.2	2.0	103.7	14.2	4.3	39.8	86.4
		160 Gy	51.2	2.0	96.0	7.8	2.2	31.4	67.2
		240 Gy	53.1	2.0	120.3	15.3	5.3	39.5	110.4
		320 Gy	50.0	2.0	74.9	8.3	4.3	37.2	102.7
	Sudani	Control	51.3	2.1	103.5	12.9	6.2	61.1	163.7
		80 GY	52.6	2.0	127.4	10.1	5.7	46.6	93.1
		160 Gy	51.9	2.2	77.5	11.8	6.2	58.7	163.2
		240 Gy	55.1	2.3	49.3	14.2	6.2	60.7	176.7
		320 Gy	49.7	2.1	70.4	12.6	6.2	64.8	188.1

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