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Effect of chemical mutagens and X-rays on morphological and physiological traits of tulips

Efecto de mutágenos químicos y rayos X en las características morfológicas y fisiológicas de tulipanes

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Abstract. The tulip (*Tulipa* sp.) is an ornamental plant produced by bulbs. Wild tulips are native to several areas of Iran, especially the Alborz and Zagros mountains. Endemic Iranian tulips are the origin of some commercial cultivars of tulip, and mutagens are commonly used to produce new traits and varieties. In this trial, X-rays were applied at 60, 70 and 80 kV; sodium azide (SA) at doses of 0.01%, 0.02%, and 0.03%, and diethyl sulfate (DES) at doses of 0.1%, 0.2% and 0.4 on Kess Nelis and Jan Van Ness varieties of tulip. Morpho-physiological traits of the treated plants were measured. The results showed that application of DES increased the fresh weight and number of bulbs, and decreased the number of offsets, main stem length, leaf area, and total fresh weight. The results showed that SA had a positive effect on most traits. All three doses of DES increased the flower longevity and all had an inverse effect on the anthocyanin content of the petals.

Keywords: *Tulipa*; Mutagenic; Sodium azide; Diethyl sulfate; X-ray.

Resumen. El tulipán (*Tulipa* sp.) es una planta ornamental producida por bulbos. Los tulipanes silvestres son nativos en varias áreas de Irán, especialmente las zonas montañosas de Alborz y Zagros. Los tulipanes iraníes endémicos son el origen de algunos cultivares comerciales de tulipanes, y comúnmente se usan mutágenos para producir nuevas variedades y características. En este estudio, se aplicaron rayos X a 60, 70 y 80 kV; azida sódica (SA) a dosis de 0,01%, 0,02% y 0,03%, y sulfato de dietilo (DES) a dosis de 0,1%, 0,2% y 0,4% en las variedades de tulipanes Kess Nelis y Jan Van Ness. Se midieron las características morfofisiológicas de las plantas tratadas. Los resultados mostraron que la aplicación de DES incrementó el peso fresco y número de bulbos, y redujo el número de bulbos reproductores, la longitud del tallo principal, el área foliar, y el peso fresco total. Los resultados mostraron que SA tuvo un efecto positivo en la mayoría de las características. Las tres dosis de DES incrementaron la longevidad de las flores y todos tuvieron un efecto inverso en el contenido de antocianinas de los pétalos.

Palabras clave: Tulipán; Mutagénico; Azida sódica; Sulfato de dietilo; Rayos X.

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INTRODUCTION

Tulips originated in central Asia, Siberia, Mongolia and China. The Netherlands has the largest area under tulip production (10800 ha; 88%) and produces 4.32 billion tulip bulbs, of which 2.3 billion (53%) are used as starting material to produce cut flowers (Buschman, 2005). Mutations are hereditary changes that are the major source of genetic variation (Ali Beik, 2011). Mutations can be caused by physical or chemical factors (Yazdi-samadi & Tabatabai, 2004). Physical mutagenic agents include X-rays, gamma rays, beta particles, neutron radiation and UV radiation. Chemical mutagenic substances are, for example, diethyl sulfate, methane sulfonate, ethylamine, *N*-nitroso-*N*-ethyl-urethane, *N*-nitroso-*N*-ethylurea, bromouracil, maleic hydrazide and sodium azide (Akrami, 2009). Diethyl sulfate ($C_4H_{10}SO_4$; DES) is an alkylating agent. Alkylating agents are known as very strong mutagens (Mirmohammadi-Meybodi & Mirlohi, 1999). Salarian (2005) examined the effect of DES and gamma rays on gladiolus. He found that the number of cormlets was significantly affected by the mutagens. Sodium azide (NaN_3; SA) is a base analogue. Base analogs are chemical compounds with chemical formulas having a number of similarities with the organic base. Under specific conditions, it can be substituted into nucleic acid structures instead of the main base (Mirmohammadi-Meybodi & Mirlohi, 1999). In Al-Qurainy and Khan (2009) experiment, the mutant plants treated with SA showed increased yield and longer vase life. Bhate (2000) studied the effect of chemical materials on morphological characteristics of two cultivars of *Ipomoea purpurea*. Both cultivars were significantly affected by three chemicals (ethyl methane sulfonate, SA and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine). X-rays can affect one or both strands of the DNA double helix. A break in one strand of a DNA helix can be repaired, but breaks that occur in both strands are not easily reconstructed and can cause detectable mutations (Akrami, 2009). X-rays treatments caused a significant decline in plant height, fresh weight and chlorophyll *a* and *b* contents of *Vicia faba* (Al-zahrani, 2012). Myodo (1952) observed that x-ray treatment was harmful for plant growth; however, treatment at different growth stages increased the weight of the mother bulbs and number of bulblets over the results for the control. Mutagenesis has been also successfully employed to generate desirable and valuable changes in tulip flower traits. Many varieties such as Estella Rijnveld revealed that mutagenesis (mainly X-rays) is a powerful tool for the improvement of tulips (Popescu, 2012). Since mutations lay the foundation for genetic potential by creating a new variety that would not otherwise naturally appear, it was decided in the present study to create changes using X-rays and chemical mutagens to develop morphological traits in tulips.

MATERIALS AND METHODS

Plant material and experimental treatments. Dutch precooled tulip bulbs from the cultivars Kess Nelis (KN, red, yellow lips) and Jan Van Ness (JVN, yellow) were purchased from a private institute in Isfahan, Iran. Dethyl Sulfate [0.1%, 0.2% and 0.4 % (v/w)] and SA [0.01%, 0.02% and 0.03% (v/w)] solutions were prepared. The bulbs were placed in moist hemp bags for 48 h at 20 to 22 °C to stimulate the meristem cells and raise their water content. They were then placed in a solution containing different concentrations of DES and SA for 8 h. The bulbs were washed with running water for 2 h after treatment. For the X-ray treatment, stimulated bulbs were irradiated with 60, 70 and 80 kV doses of X-ray for 0.1 ms.

Experimental design and traits. This experiment was carried out as a factorial test with 10 treatments in 3 replications. Garden soil, sand and cocopeat (2:2:1) were applied as the substrate. The measured traits were the number of offsets and leaves, diameter and length of the main stem, leaf area, leaf longevity, total fresh and dry weights, fresh and dry bulb weights, bulb number and diameter, flower longevity, and total chlorophyll, carotenoid and anthocyanin contents. The leaf area was obtained using the formula K (LW) as suggested by Palaniswamy and Gomez (1974). In this formula, L is leaf length at maximum width, W is leaf width and K equals 0.74. Leaf longevity was the number of days from bulb germination to leaf yellowing. Matter was dried in an oven at 105 °C for 24 h. In this experiment, flower longevity was the number of days from flower opening to the end of its life. Measurements of total chlorophyll, carotenoid and anthocyanin content were performed as suggested by Mazumdar and Majumder (2003).

Statistical analysis. Analysis of variance of the data was carried out using MSTATC software, and mean comparisons were done using a Tukey HSD test.

RESULTS

Analysis of variance (Table 1) showed that the simple effects of tulip variety, mutagen and their interaction were significant for the number of offsets ($P<1\%$). As shown, the Kess Nelis (KN) cultivar produced 19% more offsets than did Jan Van Ness (JVN). Sodium azide (0.02%) produced the maximum number of offsets; the lowest number of offsets was obtained for 0.1% and 0.2% DES, but this was not significantly different from the control (water) and the 70 kV X-ray treatment. The interaction of 0.01% SA and KN produced the highest number of offsets (5.67 offset/pot); the lowest number of offsets (2.17 offset/pot) was obtained for JVN and 0.1% DES. The results of this trial indicate that the different mutagens have different effects on specific cultivars of tulip.

Kess Nelis treated with 0.01% SA produced about three times the number of offsets than JVN treated with 0.1% DES. The two cultivars of tulip showed different results under similar treatments (like DES). The KN cultivar produced 3.33 offsets, and the JVN cultivar produced 2.17 offsets on the 0.1% DES treatment.

Table 1 shows the significant effect of trial factors and their interactions on the number of leaves. A comparison of means indicates that KN produced more leaves (Table 2). The greatest number of leaves per plant was obtained for 0.02% SA, and the lowest number of leaves was observed for 70 kV X-rays (Table 3). The highest number of leaves per plant was obtained for the combined treatment of SA (0.02%) + KN; the lowest number of the leaves per plant was found for DES (0.1%) + JVN and 80 kV X-rays + JVN (Table 4).

Table 1. Analysis of variance of experimental factors effects on growth traits of tulip.
Tabla 1. Análisis de varianza de los efectos de los factores experimentales sobre características del crecimiento de tulipanes.

		Mean Squares													
SOV	df	Offsets No	Leaves No	Diameter of main stem (mm)	Length of main stem (cm)	Leaf area (cm^2)	Leaf longevity (day)	Total fresh weight (g)	Total dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Bulb fresh weight (g)	Bulb dry weight (g)	Bulbs No	Bulb diameter (mm)
Replication (R)	2	0.09 ns	0.38 ns	0.03 ns	1.94 ns	31.20 ns	2.52 ns	22.39 ns	3.21 ns	4.95 ns	0.18 ns	1.92 ns	3.02 ns	0.32 ns	10.31 ns
Cultivar (C)	1	7.70**	467.60 **	**5.37	**97.03	**56495.49	180.27**	11179.35**	1241.60**	188.19**	1.07*	5060.38 **	911.90 **	6.67 **	75.98 **
Mutagen (M)	9	2.62 **	17.79 **	*0.39	*21.47*	*1026.01	144.37 **	287.35 **	15.09 **	148.78**	0.95 **	71.62 **	19.86 **	3.04 **	5.61 ns
AB	9	1.75 **	20.63 **	0.29 ns	17.12 **	1092.84*	223.12 **	271.12 *	18.43 **	36.27**	0.25 ns	49.76**	19.15**	4.04**	3.11 ns
Error	38	0.38	1.71	0.18	2.85	380.82	17.99	95.26	5.00	7.79	0.19	14.97	2.96	0.60	7.43
CV%	-	17.77	13.67	4.24	5.42	13.53	3.34	9.5	12.5	17.39	33.72	9.69	14.55	14.68	15.21

ns: Not significant; * and ** significant at 5% and 1% levels, respectively.

Table 2. Mean comparisons of growth traits of tulip cultivars.
Tabla 2. Comparación de promedios de características de crecimiento entre cultivares de tulipán.

Treatment	Offsets No	Leaves No	Diameter of main stem (mm)	Length of main stem (cm)	Leaf area (cm^2)	Leaf longevity (day)	Total fresh weight (g)	Total dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Bulb fresh weight (g)	Bulb dry weight (g)	Bulbs No	Bulb diameter (mm)
KN cultivar	3.83a	12.35 a	10.29 a	32.41 a	113.54 b	125.43 b	89.05 b	13.35 b	14.27 b	1.16 b	30.75 b	7.93 b	5.6 a	16.8 b
JVN cultivar	3.12b	6.77 b	9.69 b	29.87 b	174.91 a	128.9 a	116.35 a	22.45 a	17.82 a	1.42 a	49.12 a	15.72 a	4.93 b	19.05 a

Table 3. Mean comparisons of mutagen effects on growth and morphophysiological traits of tulips.
Tabla 3. Comparaciones de promedios de los efectos de mutágenos sobre el crecimiento y características morfofisiológicas de tulipanes

Treatment	No offset	No leaves	Diameter of main stem (mm)	Length of main stem (cm)	Leaf area (cm^2)	Leaf longevity (day)	Total fresh weight (g)	Total dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Bulb fresh weight (g)	Bulb dry weight (g)	Bulbs No	Flower longevity (day)	Anthocyanin content (mg/kg)
1- Water	3.08 c	9.00 cd	9.69 bc	31.38 a-d	153.6ab	126.7 a-d	96.21 ab	17.60 ab	14.34 c	0.76 b	41.22 a-d	12.31 abc	4.33 b	bc 11.67	6.44 a
2- 0.1% DES	2.75 c	7.83 cd	10.27 a	29.72 bcd	131.8ab	120.2 cd	95.87 ab	17.11 ab	15.75 bc	1.38 ab	41.05 a-d	12.06 bc	5.00 ab	a 16.00	4.33 c
3- 0.2% DES	2.75 c	10.33abc	9.54 c	28.88 cd	136.1ab	133.8 ab	102.2 ab	17.70 ab	14.99 c	1.18 ab	45.48 a	11.98 bc	5.17 ab	a 16.08	5.30 abc
4- 0.4% DES	3.25 cd	9.17 cd	10.09 abc	28.29 d	120.8 b	128 abc	94.22 b	17.85 ab	3.51 d	0.67 b	43.34 ab	13.21 ab	4.50 b	a 16.75	6.11 ab
5- 0.01% SA	4.42 ab	11.75ab	10.32 a	33.21 a	161.7 a	126.8 a-d	114.9 a	19.31 ab	16.94 abc	1.75 a	37.70 bcd	11.99 bc	6.33 a	a 16.67	5.52 abc
6- 0.02% SA	4.58 a	12.83 a	9.84 abc	29.88 bcd	153.1ab	134.8 a	113.0 ab	18.26 ab	21.52 a	1.40 ab	34.98 d	9.06 c	5.83 ab	b 13.33	4.58 bc
7- 0.03% SA	3.67 abc	10.08 bc	9.94 abc	34.04 a	144.1ab	126.5bcd	104.3 ab	21.17 a	19.16 abc	1.24 ab	42.75 abc	15.48 a	5.67 ab	a 16.67	5.02 abc
8- 60 K.V X-Ray	3.75 abc	8.17 cd	10.08 abc	32.42 ab	160.4 a	129.0 ab	104.1 ab	18.05 ab	20.53 ab	2.00 a	39.78 a-d	11.02 bc	6.17 a	a 16.00	4.81 abc
9- 70 K.V X-Ray	2.83 c	7.42 d	10.22 ab	31.48 a-d	139.9ab	126.3bcd	99.38 ab	16.92 ab	16.58 abc	1.20 ab	35.75 cd	11.74 bc	4.50 b	bc 12.25	3.91 c
10- 80 K.V X-Ray	3.67 abc	9.00 cd	9.96 abc	32.08 abc	140.9ab	119.5 d	102.8 ab	15.05 b	17.12 abc	1.31 ab	37.27 bcd	9.41 c	5.17 ab	c 10.67	4.89 abc

† Means followed by the same letters in each column are not significantly different at 5% level according to Tukey test.

Table 4. Mean comparisons of interaction of experimental factors on growth traits of tulip.
Tabla 4. Comparaciones de promedios de la interacción de los factores experimentales en las características de crecimiento de tulipanes.

Cultivar	Treatment	No offsets	No Leaves	Length of main stem (cm)	Leaf area (cm^2)	Leaf longevity (day)	Total fresh weight (g)	Total dry weight (g)	Root fresh weight (g)	Bulb fresh weight (g)	Bulb dry weight (g)	No bulbs	Flower longevity (day)	Chlorophyll content (mg/mL)
KN cultivar	1- Water	3.67 bcd	12.00 c-f	31.75 b-e	120.7 c-g	132.3 abc	81.25 def	12.25 gh	10.60 de	33.40 e-h	8.22 efg	5.00 b-e	6.33 g	4.51 ab
	2- 0.1% DES	3.67 bcd	10.33 d-g	33.10 a-d	120.0 c-g	114.3 de	78.12 ef	11.92 h	11.59 cde	30.21 gh	7.91 fg	5.67 a-e	13.67 de	4.18ab
	3- 0.2% DES	3.33 cd	14.67 abc	28.25 def	102.7 efg	138.3 ab	94.81 b-f	15.24 fgh	11.58 cde	37.02 d-h	9.63 d-g	5.33 a-e	13.17 de	b 4.10 b
	4- 0.4% DES	3.00 cd	9.50 d-h	27.33 ef	63.07 g	123.3 cd	66.35 f	12.91 gh	0.43 f	29.17 gh	8.40 efg	5.00 b-e	13.50 de	4.71 ab
	5- 0.01% SA	3.00 cd	16.50 ab	35.75 ab	130.5 b-f	132.0 cd	98.44 b-e	14.26 fgh	19.15 a-d	24.91 h	8.27 efg	7.33 ab	15.00 bcde	5.52 a
	6- 0.02% SA	5.67 a	18.00 a	30.08 c-f	126.0 b-f	129.7 abc	103.8 a-e	11.23 h	17.74 a-d	26.39 h	5.59 g	7.67 a	11.00 ef	4.57 ab
	7- 0.03% SA	5.33 ab	13.50 bcd	38.17 a	124.3 c-g	129.3 abc	96.18 b-f	15.42 e-h	21.85 ab	34.08 e-h	7.22 fg	5.00 b-e	15.00 bcde	4.64 ab
	8- 60 K.V X-Ray	4.33 abc	8.33 f-i	33.33 a-d	139.6 a-f	130.0 abc	86.32 c-f	13.29 fgh	19.40 a-d	30.00 gh	7.37 fg	5.33 a-e	14.00 cde	4.89 ab
	9- 70 K.V X-Ray	3.33 cd	8.00 f-i	32.25 b-e	92.56 fg	128.7 abc	93.95 b-f	16.12 d-h	15.25 b-e	31.18 fgh	10.06 d-g	5.00 b-e	7.83 fg	5.31 ab
	10- 80 K.V X-Ray	2.50 cd	12.67 b-e	34.08 abc	116.0 d-g	105.3 e	91.27 b-f	10.88 h	15.14 b-e	31.14 gh	6.59 g	4.67 cde	4.33 g	4.86 ab
JVN cultivar	1- Water	4.17 abc	6.00 hi	31.00 b-f	186.6 ab	121.0 cd	111.2 a-d	22.95 a-d	18.08 a-d	45.05 a-d	16.40 bc	3.67 e	17.00 abcd	4.47 ab
	2- 0.1% DES	2.50 cd	5.33 i	26.33 f	143.5 a-f	126.0 bcd	113.6 abc	22.31 a-e	19.91 abc	51.90 abc	16.22 bc	4.33 de	18.33 ab	4.86 ab
	3- 0.2% DES	2.17 d	6.00 hi	29.52 c-f	169.5 a-d	129.3 abc	109.7 a-d	20.16 a-f	18.40 a-d	53.94 ab	14.33 bcd	5.00 b-e	19.00 ab	4.79 ab
	4- 0.4% DES	2.50 cd	8.83 e-i	29.25 c-f	178.4 abc	132.7 abc	122.1 ab	22.79 a-d	6.59 ef	57.51 a	18.02 b	4.00 de	20.00 a	**5.41 ab**
	5- 0.01% SA	3.50 bcd	7.00 ghi	30.67 b-f	193.0 a	130.7 abc	131.4 a	24.35 abc	14.74 b-e	50.50 abc	15.71 bc	5.33 a-e	18.33 ab	**4.18 ab**
	6- 0.02% SA	3.17 cd	7.67 ghi	29.68 c-f	180.1 abc	140.0 a	122.1 ab	25.30 ab	25.29 a	43.57 b-e	12.52 c-f	4.00 de	15.67 bcd	**5.29 ab**
	7- 0.03% SA	3.83 a-d	6.67 ghi	29.92 c-f	163.8 a-e	123.7 cd	112.5 abc	26.91 a	16.47 bcd	51.43 abc	23.73 a	6.33 a-d	18.33 ab	**4.54 ab**
	8- 60 K.V X-Ray	3.00 cd	8.00 f-i	31.50 b-f	181.2 abc	128.0 abc	121.8 ab	22.80 a-d	21.66 ab	49.55 abc	14.66 bcd	7.00 abc	18.00 abc	**4.89 ab**
	9- 70 K.V X-Ray	4.17 abc	6.83 ghi	30.72 b-f	187.2 ab	124.0 cd	104.8 a-e	17.73 c-h	17.91 a-d	40.32 c-g	13.41 b-e	4.00 de	abcd 16.67	**4.41 ab**
	10- 80 K.V X-Ray	3.17 cd	5.33 i	30.08 c-f	165.8 a-d	133.7 abc	114.4 abc	19.21 b-g	19.11 a-d	43.40 b-f	12.22 c-f	5.67 a-e	17.00 abcd	**4.36 ab**

†Means followed by the same letters in each column are not significantly different at 5% level according to Tukey test.

The results indicated that JVN was more sensitive to all mutagens, reacted strongly under all treatments and produced fewer leaves. It appeared that this cultivar generally produced fewer leaves. The results also indicated that different mutagens had different effects on different cultivars of tulip (Table 4). Analysis of variance showed that the effect of cultivars and mutagens was significant ($P<0.01$ and $P<0.05$, respectively) on main stem diameter, but that their interaction was not significant (Table 1). The main stem diameter for KN was greater than that for JVN (Table 5). Sodium azide (0.01%) produced the largest main stem diameter, but it was not significantly different from the results for 0.1% DES. Diethyl sulfate (0.2%) produced the smallest main stem diameter (Table 3).

There were significant differences between the factors and their interaction on length of the main stem ($P<0.01$; Table 1). The length of the main stem for KN was greater than that for JVN (Table 5). The greatest main stem length was observed for 0.03% SA but this was not significantly different from the results for 0.01% SA. The smallest stem length was recorded for 0.4% DES (Table 2). The greatest stem length was recorded for SA 0.01% + KN, and the smallest stem length was recorded for JVN + 0.1% DES (Table 4).

Differences in leaf area were significant between cultivars ($P<0.01$), mutagens and their interaction ($P<0.05$; Table 1). Jan Van Ness leaf area was 35% larger than KN leaf area (Table 2). Sodium azide (0.01%) produced the largest leaf area. Diethyl sulfate (0.4%) decreased leaf area (Table 3). The largest leaf area was produced by the combined treatment of SA (0.01%) + JVN (Table 4). Table 1 indicates that analysis of variance showed significant differences ($P<0.01$) between cultivars, mutagens and their interaction for leaf longevity. Leaf longevity for JVN was greater than that on KN (Table 2). The greatest leaf longevity was recorded for the combined treatment of SA 0.02% + JVN, and the lowest longevity was recorded for KN + 80 kV X-rays (Table 4). The total fresh weight (TFW) and total dry weight (TDW) of plants showed that they were significantly affected by cultivars, mutagens and their interaction (Table 1). A comparison of means (Table 2) showed that JVN recorded higher total fresh and dry weights than KN. The highest TFW recorded for the interaction of variables was obtained for SA 0.01% + JVN, and the lowest TFW was recorded for KN + 0.4% DES (Table 4). The highest TDW for the interaction was recorded for SA 0.03% + JVN, and the lowest TDW was recorded for KN + 80 kV X-rays (Table 4).

Analysis of variance showed a significant difference between cultivars, mutagens and their interactions for fresh and dry bulb weights (Table 1). Jan Van Ness recorded the highest fresh and dry weights. The heaviest bulbs were recorded for the 0.2% DES treatment, and the lightest bulbs were recorded for the 0.02% SA treatment (Table 3). The heaviest bulbs for the interaction were recorded for 0.4% DES + JVN, and the lightest bulbs belonged to KN + 0.01% SA (not significantly different from 0.02% SA; Table 4). The highest dry weight of bulbs (27.7 g) was recorded for the combined treatment of 0.03% SA + JVN; the lowest dry weight of bulbs was recorded for KN + 0.02% SA (Table 4). The results showed that DES increased the fresh bulb weight. Sodium azide decreased the fresh bulb weight but increased the dry bulb weight. X-rays also decreased the dry bulb weight (Table 3). Analysis of variance showed a significant difference between the factors and their interactions on the number of bulbs (Table 1). Ken Nelis produced more bulbs than JVN. The highest number of bulbs (6.3) was recorded for 0.01% SA, but this was not significantly different from the results for 60 kV X-rays. The lowest number of bulbs was produced by the control group (Table 3). The greatest number of bulbs for the combined treatment was recorded for SA 0.02% + KN; the lowest number of bulbs for the combined treatment was recorded for JVN + control (Table 4). The results indicated that SA treatments increased the number of bulbs (Table 3). Salarian (2005) assessed the effect of DES and gamma rays on gladiolus and found that the number of cormlets was significantly affected by the mutagen. The control produced the lowest number of bulbs and gamma rays produced the highest number of bulbs. There were no significant differences between the control and DES treatments. There were significant differences for cultivars, mutagens and their interaction for flower longevity. Flower longevity for JVN was longer than that for KN. Diethyl sulfate (0.4%) produced the longest flower longevity, but there were no significant differences from the other DES doses or the 0.01%, 0.03% SA and 60 kV X-rays. The shortest flower longevity was recorded for bulbs treated with 80 kV X-rays (Table 3). The longest flower longevity (20 d) in the interaction treatments was recorded for the 0.2% DES + JVN treatment, and the shortest longevity (4.3 d) was recorded for the JVN + 80 kV X-ray treatment (Table 4). The total chlorophyll content differed significantly on the interaction effects; there was no significant difference by cultivar and mutagen. Anthocyanin was only evaluated for the KN tulip with red petals. The results showed a significant difference for mutagens. Control plants produced the highest anthocyanin content; the lowest anthocyanin content was produced by bulbs treated with 70 kV X-rays and 0.1% DES (Table 3).

DISCUSSION

Based on the present experiment, different plants showed different responses to mutagens. The two cultivars of tulip showed different results under similar treatments (like DES). The KN cultivar produced more offsets than the JVN cultivar for the 0.1% DES treatment. Sheikh et al. (2012) studied the effect of SA on biological damage to and diversity of wheat, and found that the treatments had adverse effects on the number of tillers per plant. In addition, the results indicate that JVN was more sensitive to all mutagens, reacted strongly under all treatments and produced fewer leaves. Salarian (2005) found that the effect of DES was not significant for gladiolus spike length. In the present study, the results under DES were not significantly different for stem length compared to the control. Al-zahrani (2012) reported the lack of effect for low doses of X-rays on height of faba bean plants, which agrees with the results of this study for tulips. Sander and Muehlbauer (1977) reported that SA was an effective mutagen in pea plants when used at 10^{-3} molar and pH of 3.

The results indicated that leaf area decreased as the concentration of DES increased, but trial cultivars subjected to similar treatments showed different results. For 0.01% SA, the KN leaf area was 130.5 cm^2, while JVN leaf area was 193 cm^2. Hossein-Ava and HajNajari (2008) found that mutagenic compounds (DES and dimethyl sulfate) caused morphological changes in leaf size. KN and JVN subjected to 80 kV X-rays recorded longevity values of 105.3 and 133.7 d, respectively. Al–Qurainy and Khan (2009) reported that mutants treated with SA survived better when exposed to adverse conditions, and showed increases in yield, resistance to stress and vase life. Anil Kumar et al. (2013) reported that ethyl methane sulfonate (0.1% and 0.3%) on mulberry cultivars increased strength of the stem. Diethyl sulfate was shown to increase flower longevity; however, overall flower longevity was higher for JVN than for KN treatments. Diethyl sulfate and gamma rays significantly affected the harvest time of cut gladiolus flowers. Radiation decreased the harvest time of flowers more than did DES (Salarian, 2005).

Increasing the DES concentration decreased TFW, but this mutagen had no effect on TDW. Sodium azide increased both the TFW and TDW. Total dry weight decreased as X-ray intensity increased; however, X-rays did not significantly affect TFW (Table 3). Al-zahrani (2012) stated that high doses of X-rays (162 kV) decreased the fresh weight of *Vicia faba*, but low-dose X-rays had no significant effect on fresh weight. In the present study, SA increased both TFW and TDW.

The results of the present study showed that, although the number of bulbs increased for the DES treatment, differences were not significant in comparison to values in the control group; these results are consistent with those from Salarian (2005). Anwarkhan et al. (2011) studied the use of mutagens to increase the cormlets in saffron and observed that gamma rays (2 kR) increased the number of cormlets. Bulb diameter showed significant differences between cultivars; the highest bulb diameter was recorded for JVN (Table 2). Salarian (2005) stated that the effect of DES was not significant on bulb diameter in gladiolus, and these results agree with our results. In our experiment, control plants produced the highest anthocyanin content. Broertjes and Van Harten (1978) obtained pink flowers from red, white and yellow mutants after irradiation of chrysanthemum cuttings. The highest chlorophyll content was obtained for 0.01% SA + KN, and the lowest for 0.2% DES + KN (Table 4). Al- Qurainy (2009) reported that total chlorophyll content was not significant for SA-treated plants at 60 d after sowing *Eruca sativa.* Konzak et al. (1975) found that SA produced high frequencies of chlorophyll mutations when used alone and it had a synergistic effect on mutation yield following combination with N-methyl-N′-nitrosourea (MNH). They found that the mutagenic efficiency of SA was higher, probably because it caused less physiological damage. The treatments and their interaction had no significant effects on carotenoid content. Al-zahrani (2012) found that the effect of X-rays was not significant on the carotenoid pigment of *Vicia faba.* Salarian (2005) found that DES did not modify gladiolus flower color.

CONCLUSIONS

Table 3 showed that DES increased the fresh weight and number of bulbs for tulips, but this treatment decreased the number of offsets, main stem length, leaf area, and total fresh weight. Diethyl sulfate had no significant effect on total dry weight. The results showed that SA had a positive effect on the number of offsets, leaf number, diameter of main stem, leaf area, total fresh and dry weights and number of bulbs; but decreased the fresh bulb weight. The X-ray treatment increased the diameter of the main stem, leaf area and number of bulbs, but decreased the number of leaves, total dry weight, and fresh and dry bulb weights. The X-ray treatment had no significant effect on length of the main stem and total fresh weight. The effect of SA was relatively positive for the experimental traits.

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