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Determination of lethal dose and growth reduction of EMS and sodium azide treated dragon fruit (*Hylocereus* spp.) cultivars

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ABSTRACT

An exotic vine cactus, dragon fruit (*Hylocereus* spp.) commonly known as 'pitaya' is a weather resilient crop, rich in vitamin C and antioxidants. The chances of crop improvement through conventional breeding is a limitation in dragon fruit, hence induced mutation can be exploited for creating genetic variability. The present study was carried out during 2019 to 2022 at Central Horticulture Experimental Station (ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka), Hirehalli, Karnataka to induce the variability in two dragon fruit cultivars, viz. Hirehalli Red and Hirehalli White. The experiment was laid out in completely randomized design (CRD) comprises of 15 treatments with different dosage of EMS (Ethyl Methane Sulphonate) (0.2%, 0.5%, 0.8%, 1.5%, 2%, 2.25%, 2.50%, 2.75% and 3.00%) and sodium azide (0.02%, 0.03%, 0.04%, 0.05% and 0.06%) and untreated seeds were sown as control with 3 replications of each. The outcome of the variance analysis showed highly significant difference (P<0.05) for the evaluated traits. The lethal doses (LD_{50}) for EMS in Red was 2.685% and in White was 2.791%. Mutagenesis with sodium azide showed the lethal doses (LD_{50}) of 0.052 and 0.053% in Red and White cultivars, respectively. The GR_{50} value in the cv. Red was 0.68, 0.86, 0.48% and in White was 0.98, 1.08 and 0.89% for seedling height, shoot and root length, respectively. The determined LD_{50} and GR doses obtained from this research can be exploited in mutation breeding programme of dragon fruit to develop a cultivar with novel traits.

Keywords: Dragon fruit, EMS, Growth reduction, Lethal dose, Sodium azide

Induced mutation has great potential in bringing about genetic improvement and has contributed considerably to plant breeding (Sigurbjornsson 1977). Variation is an essential component in fruit crops breeding which can be created by induction of mutation and they have been crucial in developing better cultivars of perennial fruit crops. One such attempt was made in dragon fruit (*Hylocereus* spp.) cultivars to obtain the mutants with desirable traits. Dragon fruit is an excellent tropical fruit with exotic characters locally known as pitaya grouped under Cactaceae family and its home land is southern and central region of Mexico and America. Of all the 14 *Hylocereus* spp. known to exist worldwide, *H. undatus* is cultivated in various states of India (Arivalagan *et al*. 2021). Dragon fruit doesn't perform well under high intense rainfall as it affects reproductive growth of the plant (Karunakaran and Arivalagan 2019). These crops hold high potential for diversification as a commercial fruit in India also have huge export potential and this is mainly due to nutraceutical benefits, consumer preference, present market demand and premium price (Al-Mekhlafi *et al.* 2020).

Induced mutation can broaden the genetic base and helps to develop population with desired traits like compatibility, dwarf stature, early flowering, better fruit quality, biotic and abiotic stress tolerance. Recent past, plant breeders have been using chemical mutagens like sodium azide and Ethyl Methane Sulfonate (EMS) to induce variability. Due to the potency and ease of use, chemical mutagen like EMS, alkaline sulfonate, is extensively used for induction of mutation (Wattoo *et al*. 2013). Sodium azide (SA) is commonly considered as a relatively safe and effective that is both affordable and non-carcinogenic (Salvi *et al*. 2014). The LD_{50} (lethal dose 50%) varies with plant species, type and stage at which lethality is analysed. Induced mutation as a strategy to obtain the desirable mutant this study was conducted to create genetic variability with desirable traits of dragon fruit.

MATERIALS AND METHODS

The present study was carried out during 2019 to 2022 at Central Horticulture Experimental Station (ICAR-Indian

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Institute of Horticultural Research, Bengaluru, Karnataka), Hirehalli (28◦38′ N, 77◦11′ E and an altitude of 842 m amsl), Karnataka. Hirehalli Red and Hirehalli White seeds of dragon fruit were choosen for the experiment. The experiment was conducted in completely randomized design (CRD) comprises of 15 treatments with 3 replications. Freshly extracted seeds (100) were soaked in distilled water for 8 h followed by treatment with different dosage levels of EMS (Ethyl Methane Sulphonate), viz. 0.2%, 0.5%, 0.8%, 1.5%, 2%, 2.25%, 2.50%, 2.75% and 3.00% for 5 h. For sodium azide treatment, pre-soaked seeds were treated with 0.02%, 0.03%, 0.04%, 0.05% and 0.06% concentration for 5 h with intermittent shaking at room temperature of 25±2°C. Treated seeds were washed to remove the residues. The untreated seeds were sown as control. 1-month old seedlings were transplanted into portrays containing cocopeat and FYM (farmyard manure) (1:1). The parameters, viz. germination [30 DAS (days after sowing)], survival (60 DAS) and survival reduction percentage (90 DAS) and growth parameters (90 DAS) were recorded. Seed vigour indices were computed based on the formulae proposed by Abdul-Baki and Anderson (1973). The LD_{50} value was calculated based on probit analysis.

Seed vigour index-I = Seed germination (%) × Seedling length (cm)

Seed vigour index-II = Seed germination (%) × Seedling dry weight (g)

Statistical analysis: The analysis of variance (ANOVA) in completely randomized design (CRD) using Microsoft excel in Windows 10 was computed using the mean data. The difference was evaluated at 5% level of significance. The median lethal dose (LD_{50}) was calculated based on the number of seeds germinated at different mutagen doses and probit analysis was done in USD polo software (Shankarganesh *et al*. 2022).

RESULTS AND DISCUSSION

Probit analysis determined LD_{50} value of the EMS mutagen for the cultivar Red and White based on the percent of mutant plant population that germinated after 30 days of sowing. The study showed significant difference (P<0.01) among the treatments compared to control.

Germination and survivability: Highest seed germination was observed in control of Red (98.83%) and White cultivars (99.75%). The germination percentage of treated seeds in Red cultivars ranged from 44.50% (3% EMS)–92.67% (0.2% EMS) and 45.99% (0.06% sodium azide)–73.28% (0.02% sodium azide). In White cultivars, the germination percentage ranged between 45.69% (3% EMS)–94.32% (0.2% EMS) and 48.27% (0.06% sodium azide)–72.35% (0.02% sodium azide). The dragon fruit cv. Red and White showed a differential response in terms of seed germination or varying sensitivity of cultivars to chemical mutagens. Increased growth inhibitors chromosomal abnormalities and disruption of growth promoters might have contributed to the lower germination percentage caused by a higher dose of EMS (Jayakumar and Selvaraj 2003, Nurmansyah *et al*. 2018). Similar reports were also reported by Saini and Gill (2009), Sharma *et al*. (2013), Kaur and Rattanpal (2010) in rough lemon.

Highest survival percentage was recorded at 60 days after sowing in untreated seeds of Red (99.62%) and White (99.15%) cultivars. In EMS treatment, the seedling survival percentage ranged from 49.56% (3% EMS)–92.48% (0.2% EMS) in Red cultivar and 45.28% (0.06% sodium azide)–90.27% (0.02% sodium azide) in White cultivar. Similar results were obtained in Red and White cultivar showing highest survival percentage (85.60% and 89.23%) in 0.02% sodium azide. In Red cultivar, the percentage survival reduction over control in EMS treatment ranged from 6.79–49.87% and in sodium azide it varied from 13.14–27.77%. Similarly, survival reduction percentage ranged from 8.11–53.48% in EMS treatment and 9.93–25.58% in sodium azide treated population in White cultivar (Table 1). Higher survival percentage was observed in the cultivars exposed to lower and moderate EMS dosage as compared with those exposed to higher dosage. According to Kiong *et al.* (2008), a large mutagenic dosage leads to increased chromosomal damage causing decline in survival percentage. The results are in conformity with Dhatt *et al.* (2000) in acid lime, Singh *et al.* (2010) in papaya, Kaur (2015) in rough lemon.

Table 1 Effect of chemical mutagen on germination, survival and survival reduction percentage in dragon fruit cultivars

Treatment	Germination (%)				Survival (%)				Survival reduction over control (%)			
	Red		White		Red		White		Red		White	
	20 DAS	30 DAS	20 DAS	30 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
Control	98.83	98.83	99.75	99.75	99.62	99.62	99.15	99.15	0.00	0.00	0.00	0.00
EMS												
0.2%	92.67	92.39	94.60	94.32	93.69	92.48	92.20	90.27	5.95	6.79	7.01	8.11
0.5%	87.33	86.55	85.29	84.52	83.90	82.13	83.31	81.56	15.78	17.17	15.98	16.90
0.8%	73.00	72.64	78.72	77.54	78.86	76.41	68.57	67.13	20.84	22.91	30.84	31.45
1.5%	62.33	61.90	71.67	70.02	69.96	66.81	58.33	57.11	29.78	32.55	41.16	41.55
2.0%	58.17	58.11	64.95	63.32	64.22	61.08	51.25	50.18	35.53	38.31	48.31	48.55

Contd.

Table 1 (*Concluded*)

2.25%	52.50	52.34	56.04	54.52	61.79	58.64	50.43	49.37	37.97	40.75	49.14	49.36
2.50%	50.50	50.25	50.83	49.36	60.10	56.91	49.03	48.00	39.67	42.49	50.55	50.74
2.75%	47.67	47.33	49.67	48.13	57.33	54.18	47.99	46.99	42.45	45.23	51.59	51.76
3.0%	44.50	44.10	48.20	45.69	52.78	49.56	46.25	45.28	47.02	49.87	53.35	53.48
Sodium azide												
0.02%	73.50	73.28	72.57	72.35	86.73	85.60	90.41	89.23	12.67	13.14	8.92	9.93
0.03%	65.67	65.34	65.59	65.26	84.08	82.82	88.55	87.22	15.34	15.94	10.80	11.96
0.04%	56.33	55.94	55.93	55.42	78.47	76.98	85.08	83.46	20.99	21.82	14.30	15.74
0.05%	50.17	49.98	50.92	50.36	75.45	73.87	79.72	78.04	24.04	24.96	19.70	21.18
0.06%	46.50	45.99	48.90	48.27	72.75	71.08	75.38	73.65	26.76	27.77	24.05	25.58
SEM	0.57	0.56	0.73	0.72	1.04	0.99	0.73	0.72	1.04	1.03	0.76	1.01
CD (P=0.05)	1.67	1.65	2.13	2.10	3.01	2.89	2.13	2.09	3.04	3.01	2.20	2.94
CV	1.56	1.55	1.92	1.92	2.41	2.38	1.79	1.78	7.26	6.75	4.64	4.91

DAS, Days after sowing; EMS, Ethyl Methane Sulphonate.

The maximum viable mutants with minimum damage can be obtained by determining the LD_{50} values (Laskar *et al*. 2017). The response curve for different dose of EMS and sodium azide based on probit units (Fig. 1). The lethal dose value depends on genotype, seed hardiness, seed maturity, nature of treatment and moisture content. The regression equation showed the correlation between the EMS and sodium azide mutagen dose and the germination percentage of the Red and White cultivars of dragon fruit. The probit curve analysis showed that the LD_{25} for EMS treatment was 0.853 and 0.943% and LD_{50} value was 2.685 and 2.791% in Red and White cultivars, respectively. The lethal dose for sodium azide treated Red cultivar was 0.019% (LD_{25}) and 0.052% (LD_{50}) and in White cultivars, the lethal dose was 0.017% (LD_{25}) and 0.053% (LD_{50}).

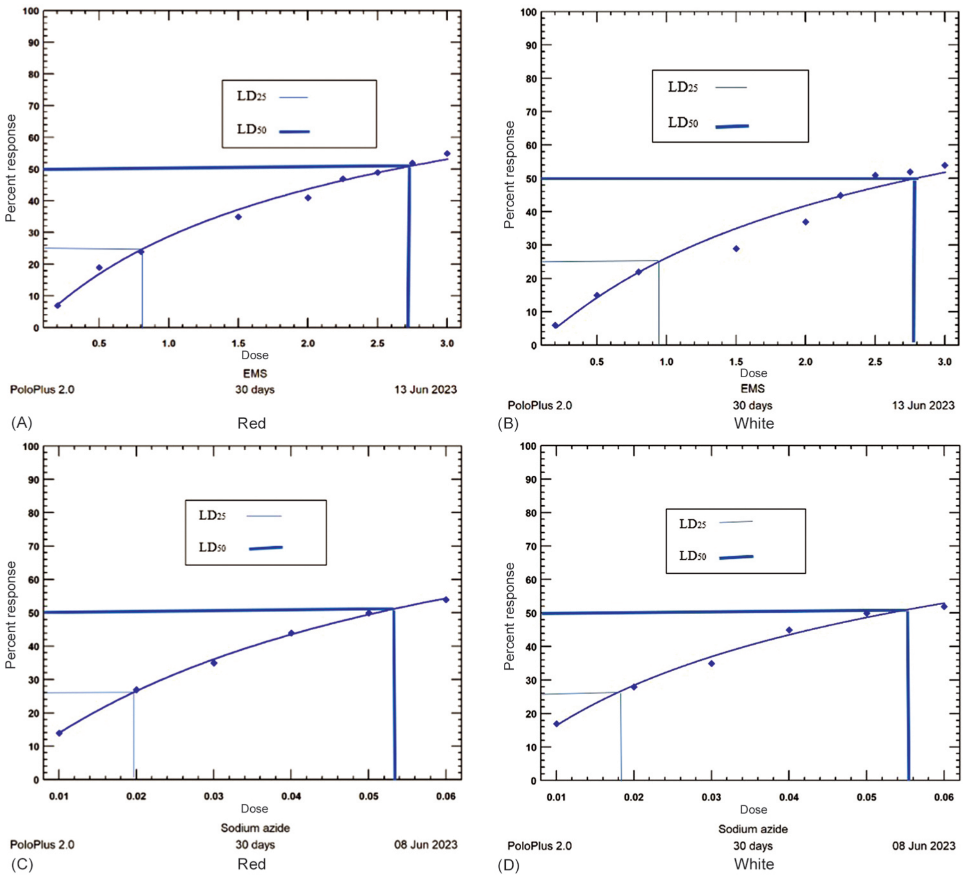

Fig. 1 Probit curves of EMS population (A, B) and sodium azide population (C, D) in dragon fruit cultivars.

Growth parameters: Reduced concentration of EMS (0.1%) and sodium azide (0.02%) showed triggering effect on seedling height, shoot and root length (Table 2). The seedling height measured after 90 days of germination was highest in control in both Red (35.39 cm) and White cultivars (63.73 cm) compared to other treatments. At the higher dosage level of chemical mutagens, the seedling height was 4.65 cm in 3% EMS and 10.05 cm in 0.06% sodium azide in Red cultivars, respectively. While in White cultivars the seedling height was 3.14 cm in 3% EMS and 20.21 cm in 0.06% sodium azide treatment. To detect the biological effects of chemical mutagen, seedling height was considered as an index.

The papaya seeds treated with EMS and sodium azide showed inhibitory effect on germination percentage as reported by Singh *et al*. (2010). Probit analysis showed a linear reduction in germination at higher dose of EMS and sodium azide supported by a well-fitted R^2 co-efficient, which is as same as correlation coefficient forming the reliability of the study.

Similarly, all the growth parameters showed inverse relationship with the dosage of mutagen. In comparison to the control, mutagenic population has substantially reduced shoot length. Untreated plants recorded a mean maximum shoot length of 20.98 cm in cv. Red and 33.38 cm in cv. White. At higher dosage levels of EMS (3%) and sodium azide (0.06%) in red cultivars there was reduced shoot length of 3.67 and 6.72 cm, while in White cultivars it was 2.25 and 14.07 cm, respectively. In the mutagenic population, the length of shoot dramatically decreased in contrast to the growth in dosage of the EMS-treated plants (Shailendra *et al*. 2010). Declined plant height was due to influence of mutagen on the physiological system i.e. short internodal length. An inverse relationship in shoot length with EMS dosage was also reported by Dhakshanamoorthy *et al*. (2010) in jatropha and Deepthi and Remesh (2016) in okra.

Higher dosage of EMS declines the gibberellic acid level and increases the level of abscisic acid which leads to reduction in growth traits of seedlings. The findings were similar in dwarf mutant reported by Bai *et al*. 2016. The reduced mean growth parameters as result of higher doses level was also confirmed in apple (Sharma and Sharma 1996), and rough lemon (Kaur and Rattanpal 2010).

The root length was highest in control i.e. 14.42 cm and 30.35 cm in Red and White, respectively. The results showed drastic reduction in root length and it was quite prominent at higher mutagenic concentrations. Among mutagen treatments, the highest dose of EMS (3%) and sodium azide (0.06%) resulted in lowest root length i.e. 0.98 cm and 3.33 cm in Red cultivars. Similarly, the root length was 0.89 cm and 6.14 cm in White cultivars, respectively. The reduced shoot and root length which might be due to sensitivity of dragon fruit at higher doses of EMS and sodium azide (Wani *et al.* 2017).

Growth reduction: Growth reduction (GR) percentage in EMS and sodium azide treated dragon fruit cultivars Red and White was determined using the regression equation. The seedling height, shoot and root length data were used to determine growth reduction at 25% (GR_{25}), 50% (GR_{50}) and 75% (GR_{75}) (Supplementary Fig. 1, 2).

The present investigation revealed that among the two mutagens studied, EMS at higher concentration had highly significant negative effect compared to sodium azide. Higher dose of EMS had deleterious effect on growth parameters, as evidenced by decrease in plant height, shoot and root length. The GR_{25} values in EMS treated seeds for seedling height, shoot and root length were 0.25%, 0.31%, 0.19% in cv. Red and 0.53%, 0.57%, 0.50% in cv. White, respectively. In Red cultivars, the GR_{50} value was 0.68%, 0.86%, 0.48% and for White cultivar was 0.98%, 1.08% and 0.89% for seedling

Table 2 Growth parameters of dragon fruit cultivars treated with EMS and sodium azide

Treatment	Seedling height (cm)		Shoot length (cm)		Root length (cm)	
	Red	White	Red	White	Red	White
Control	35.39	63.73	20.98	33.38	14.42	30.35
EMS						
0.2%	28.85	54.71	17.88	29.04	10.96	25.67
0.5%	20.17	45.00	13.03	24.21	7.14	20.79
0.8%	15.22	32.96	10.70	18.49	4.52	14.47
1.5%	10.44	17.24	7.69	10.41	2.75	6.83
2.0%	8.27	12.43	6.20	7.61	2.07	4.82
2.25%	7.64	9.35	5.77	6.39	1.88	2.96
2.50%	6.58	6.71	4.97	4.70	1.61	2.02
2.75%	5.81	4.30	4.41	3.02	1.40	1.27
3%	4.65	3.14	3.67	2.25	0.98	0.89
Sodium azide						
0.02%	22.18	38.52	12.29	25.28	9.89	13.24
0.03%	15.40	34.82	8.89	23.44	6.51	11.38
0.04%	13.16	28.84	7.96	19.53	5.19	9.30
0.05%	11.52	25.96	7.14	17.84	4.38	8.12
0.06%	10.05	20.21	6.72	14.07	3.33	6.14
SEM	0.55	1.49	0.35	0.66	0.27	1.07
CD (P=0.05)	1.60	4.34	1.01	1.94	0.78	3.12
CV	6.27	9.76	6.59	7.25	9.10	17.67

EMS, Ethyl Methane Sulphonate.

height, shoot and root length, respectively. The GR_{75} value for all the three parameters, viz. seedling height, shoot and root length were 1.81%, 2.37% and 1.18% in Red cultivar, whereas 1.82%, 2.04% and 1.62% in White cultivars.

Similarly, in sodium azide treated population the GR_{25} value for seedling height, shoot and root length was 0.017%, 0.018%, 0.016% in cv. Red and 0.022%, 0.029%, 0.016 % in cv. White, respectively (Fig. 2, 3). The GR_{50} value for Red cultivar was 0.036%, 0.043%, 0.030% and for White cultivar it was 0.052%, 0.064% and 0.037% for seedling height, shoot and root length, respectively. Parallelly, GR_{75} values for all the three parameters, viz. seedling height, shoot and root length was 0.077%, 0.100% and 0.059% in Red cultivar, whereas 0.121%, 0.143% and 0.085% in White cultivars. The present study revealed that increased EMS and sodium azide dosage had direct negative impact on plant tissue and mutation can be lethal showing delayed and stunted growth.

Seed vigour indices: Suppression in physiological and enzymatic process due to induction of mutation by EMS and sodium azide had adverse effects on seed vigour index. In the present investigation, growth parameters decreased with raise in the dosage of EMS and sodium azide (Table 3).

Seedling growth and survival potential are the best indicators of seed vigour. The seedling height helps to calculate seed vigour index-I, while vigour index-II is computed using seedling dry weight. Of these two indices, vigour index-I followed a negative trend with mutagen doses and it ranged from 206.71 (3% EMS)–2673.49 (0.2% EMS) and 467.03 (0.06% SA)–1629.84 (0.02% SA) in cv. Red. The vigour index-I ranged from 151.54 (3% EMS)–5175.40 (0.2 % EMS) and 990.29 (0.06 % SA)–2798.14 (0.02 % SA) in cv. White.

In Red cultivar, seedling vigour index-II ranged from 25.38 (0.2% EMS)–596.44 (3% EMS) and 114.69 (0.06% SA)–400.26 (0.02% SA). In White cultivar, vigour index-II ranged from 2.88 (3% EMS)–1050.10 (0.2% EMS) and 115.12 (0.06% SA)–568.94 (0.02% SA). The highest value for all the above parameters was observed in control. This differential response of cultivars to chemical mutagens shows variation in seedling parameters.

Biomass of seedlings depends on growth and development (Aknabi *et al*. 2010). Minimum shoot and root length at higher dosage of mutagen led to reduced seedling fresh and dry weight. The reduction in shoot and root length and vigour indices at higher dosage of EMS and Sodium azide may be due to altered enzymatic activity, decrease in mitotic index and imbalance in plant hormonal levels. The findings are in line with Thanga *et al.* (2017) in blackgram. Changes in morphological traits were most likely brought about by chemical mutagen, phenotypically constructive multidirectional polygene mutations. The

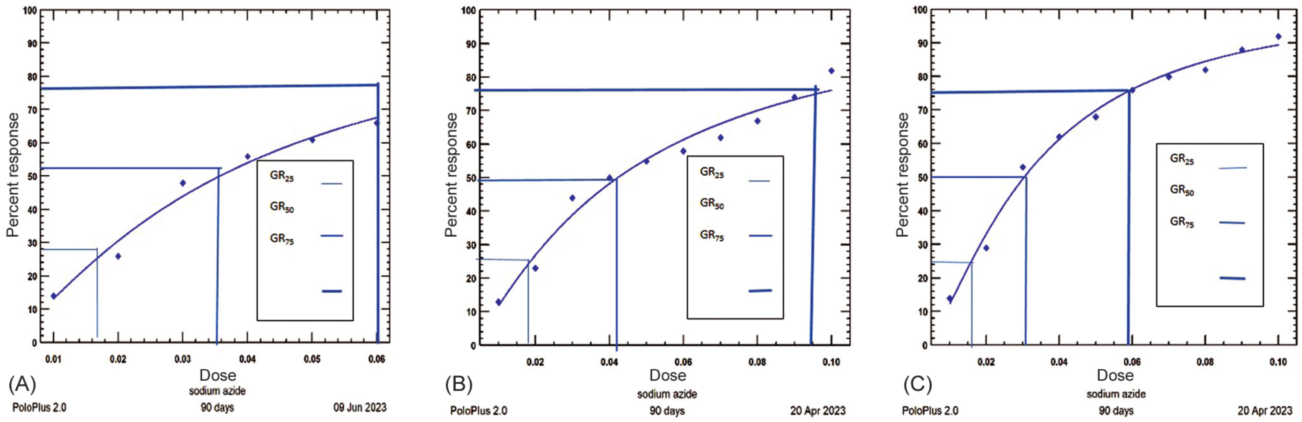

Fig. 2 Effect of sodium azide doses on growth reduction of seedling height (A); shoot length (B); root length (C) of Red cultivar of dragon fruit.

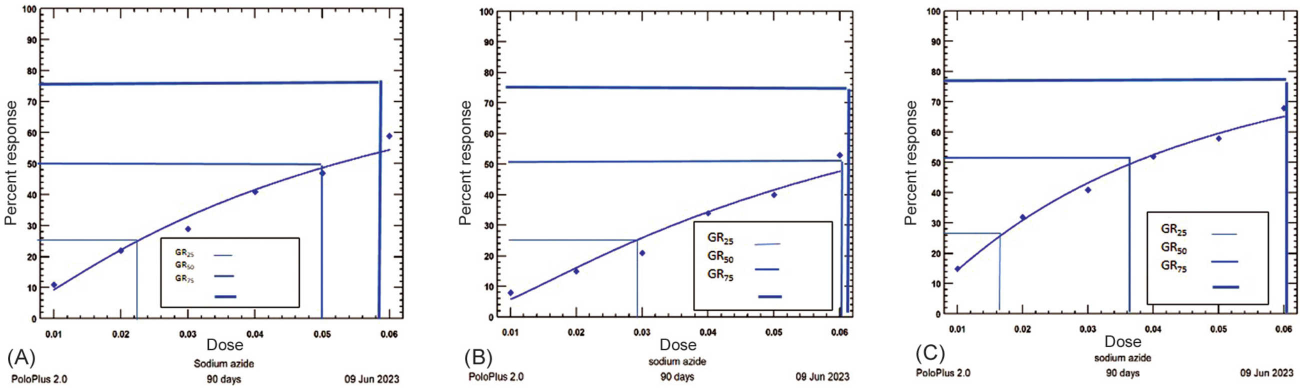

Fig. 3 Effect of sodium azide doses on growth reduction of seedling height (A); shoot length (B); and root length (C) of White cultivar of dragon fruit.

Table 3 Effect of chemical mutagens on vigour indices of dragon fruit cultivars

Treatments	Seed vigour index-I		Seed vigour index-II	
	Red	White	Red	White
Control	3498.33	6357.06	885.83	1573.49
EMS				
0.2%	2673.49	5175.40	596.44	1050.10
0.5%	1761.30	3839.14	306.90	514.84
0.8%	1111.09	2593.30	150.05	202.46
1.5%	651.38	1235.81	73.52	32.61
2.00%	481.06	811.47	64.04	15.81
2.25%	401.46	525.05	56.43	10.01
2.50%	332.04	341.78	52.71	6.54
2.75%	276.69	212.52	33.98	4.08
3.00%	206.71	151.54	25.38	2.88
Sodium azide				
0.02%	1629.84	2798.14	400.26	568.94
0.03%	1010.60	2285.77	248.18	413.83
0.04%	741.75	1612.51	182.16	252.19
0.05%	577.75	1321.87	141.88	177.26
0.06%	467.03	990.29	114.69	115.12
SEM	36.97	112.04	32.68	23.81
CD (P=0.05)	107.29	325.16	94.85	69.10
CV	6.07	9.62	25.48	12.52

EMS, Ethyl Methane Sulphonate.

loci varying within the genotype for the same or different traits may have varied degrees of sensitivity, which could be the reason for varying response of plants to differential chemical treatments.

Each genotype has a distinctive genetic structure, physiological and enzymatic activities that makes them respond differently when exposed to a certain mutagen. The lethal doses (LD_{50}) for EMS in Red were 2.685% and in White was 2.791%, for sodium azide the lethal doses (LD_{50}) were 0.052% and 0.053% in Red and White cultivars, respectively. The hypothesis of the study proved the interaction of specific cultivar with mutagen that depicts the chances of achieving viable and unique mutants with wide genetic variability. The findings of the present investigation will serve as frame work for breeders to develop useful mutation with noticeable genetic variability in the gene pool of dragon fruit.

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