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Comparative mutagenic frequency, efficiency, effectiveness and rate of ethyl methane sulphonate and sodium Azide in groundnut (*Arachis hypogaea* L.)

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Abstract
The present investigation was carried out with an objective of assessing the response of four selected groundnut genotypes *viz.*, Smruti, Devi, ICGV 7220 and ICGV 2266 to doses of mutagenic treatments. Mutagenic concentrations of 0.2%, 0.3% and 0.4% of EMS and sodium Azide were taken respectively. The frequency, effectiveness and relative efficiency of mutagenic treatments in M_1 generation were calculated based on the seedling characters. The genotypes showed varied response to different concentrations of the two mutagens. SA produced a greater number of mutants with increase in concentration. Smruti showed reduced mean seedling height. Genotype vigour index was high for ICGV 7220. Efficiency of mutagen varied according to genotype. Effectiveness ranged from 1.975 (V3E2) to 8.148 (V1N1). Mutation rate (in terms of efficiency and effectiveness) was highest in SA treated Smruti (7.099) M_1 generation compared to EMS (3.447).

Keywords: EMS, sodium Azide, M_1 generation, effectiveness, efficiency, mutation, groundnut

Introduction
Groundnut (*Arachis hypogaea* L) belongs to family Fabaceae subfamily Faboideae (Krapovickas and Gregory, 1994) [11]. Groundnut is the principal oilseed crop of India. Cultivated groundnut is an allo-tetraploid (2n=4x=40) and a self-pollinated crop. Mutation breeding is a source of increasing the genetic variability in groundnut. The choice of mutagen in the present study is chemical mutagens i.e. ethyl methane sulphonate (EMS) and sodium Azide (SA). EMS generates random mutations in genetic content through nucleotide substitution producing point mutations (Okagaki *et al*., 1991) [13]. Sodium azide is classified as a "super mutagen" (Swaminathan, 1969) [17]. The biological effect induced by chemical mutagen is usually measured in terms of percentage of lethality and seedling injury in M_1 generation (Konzak *et al*., 1965; Khan and Wani, 2006) [10, 9]. The quantitative determination of M_1 mutation frequency is done by determining biological injury using seedling height and survival, as these characters are correlated to M_1 mutation frequency (Etsuo, 2004) [4] which enables predicting efficiency of mutagens and identifying desirable mutants. Varietal response towards different mutagen doses was also observed by (Khursheed *et al*., 2015; Raina *et al*., 2017) [12, 14] in *Hordeum vulgare* and faba bean respectively. Effectiveness and efficiency both are the important parameters to evaluate the usefulness of the mutagens. The selection of effective and efficient mutagens is very essential to recover a high frequency and spectrum of desirable mutations (Solanki & Sharma, 1994) [16]. Mutagenic effectiveness can be defined as measure of frequency of mutations induced by a unit dose of mutagens (Konzak *et al*., 1965) [10] whereas; mutagenic efficiency is the measure of proportion of mutations in relation to undesirable changes like lethality, injury, sterility etc. Mutation frequency of mutagens is a measure of comparison of mutagenic efficiency (Freese, 1963) [5]. The usefulness of any mutagen in plant breeding depends not only on its effectiveness but also upon its efficiency. The higher efficiency of a mutagen indicates relatively less biological damage. A highly effective mutagen may not necessarily show high efficiency and vice versa.

Materials and Methods
Well adapted groundnut varieties *viz.*, Smruti and Devi and two germplasm entries from ICRISAT *viz.*, ICGV 7220 and ICGV 2266 were taken and genetically pure, fully matured uniform seeds (moisture content ≈ 9%) of four groundnut varieties were used for carrying out the mutagenic treatments.

Four hundred and fifty seeds were taken for each mutagenic treatment and controls for each variety used and soaked in distil water for overnight (16 hours). Soaking of seeds prior to mutagenic treatment allows permeability of the mutagen and DNA synthesis, which allows producing high mutation frequency with minimum chromosome damage (Gaul *et al.*, 1972) [6]. EMS and sodium azide solutions were freshly prepared at 0.2%, 0.3% and 0.4% concentrations respectively with 0.1 M phosphate buffer (PBS). Seeds were soaked in mutagenic solution for six hours and thoroughly washed with water and sown in the field in Randomised Block Design (RBD) with three replications (150 seeds per variety per treatment) with inter row and intra row spacing of 30 cm and 15 cm respectively. The experiment was conducted in the EB-II section of Department of Plant Breeding and Genetics, College of Agriculture, OUAT, Bhubaneswar. In M_1 generation seedling characters as germination percentage, survival percentage, lethality, height reduction, number of visible mutations or aberrations were recorded and mutagenic parameters were calculated.

Results and Discussion

Germination percentage was recorded at 14 DAS seedling stage. Germination percentage of treated genotypes ranged from 68% (V3E3) to 92% (V1E1). The trend of germination percentage was decreasing with increasing concentration of mutagen as reported by (Singh *et al*., 1988) [15] and supported by the work of (Badigannavar and Murty, 2007; Burghate *et al.*, 2013 and others) [2, 3]. ICGV 7220 (V3E2) however showed increased seedling germination rate.

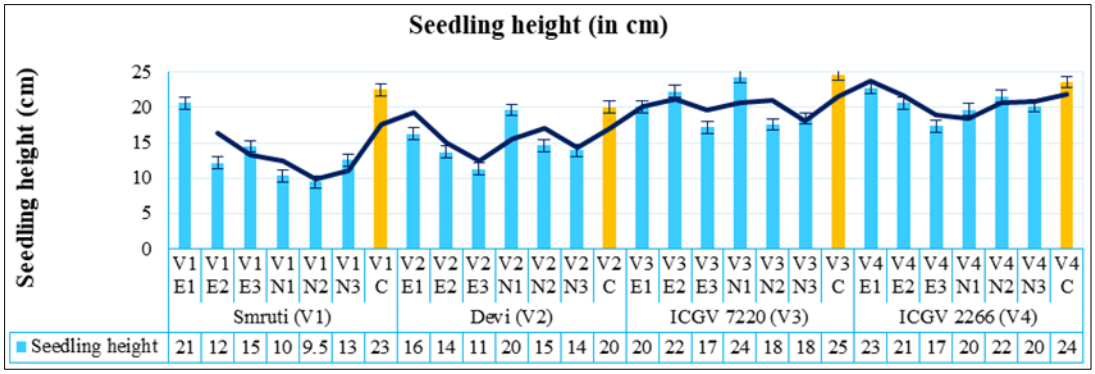

Fig 1: Seedling height in response to doses of chemical mutagens in selected groundnut genotypes

Fig 2: Dwarf mutants in Smruti and Devi

The reduction in germination percentage can be due to damage at cellular physiological level or molecular level. Survival percentage was recorded at crop maturity stage that varied from 56% to 83%. Reduction in survival was observed with increasing dose of mutagens on genotypes. The findings were in conformity with the reports of (Wang *et al.*, 2020; Kavera and Nadaf, 2008) [18, 8].

Lethality is an indication of the sensitivity of a genotype to a mutagenic dose (Gaul, 1958) [7]. Lethality (%) increased with increase in the concentration of mutagen. Highest lethality was observed in ICGV 7220 at 0.4% concentration in both the mutagens. SA showed higher percentage of lethality than EMS. Production of higher number of mutants was seen in case of sodium azide with highest mutants in Devi at 0.4%. The type of mutants observed were genetic aberrations, crinkled and necrotic mutants. The primary effect of mutagenic treatment is reduced seedling height in M_1 generation (Badigannnavar and Murty, 2007) [2]. The seedling height reduction was also influenced by variety. Treated seedlings exhibited remarkable height reduction over control (Table 1, Figure 1). Lowest mean of 9.47 cm was observed in V1N1. Higher doses of mutagens showed drastic height reduction from control in Smruti and Devi. Smruti (SA treated) and Devi (EMS treated) exhibited dwarf mutants of 2cm or less (Figure 2). Vigour index showed a gradual decrease with increasing concentrations. Mutagenic treatments of 0.3% showed some interesting results of either high or reduced vigour. ICGV 7220 showed high vigour index compared to other genotypes. Recovery index exhibited similar trends. Seedling injury percentage was observed to be high in Smruti (Figure 3).

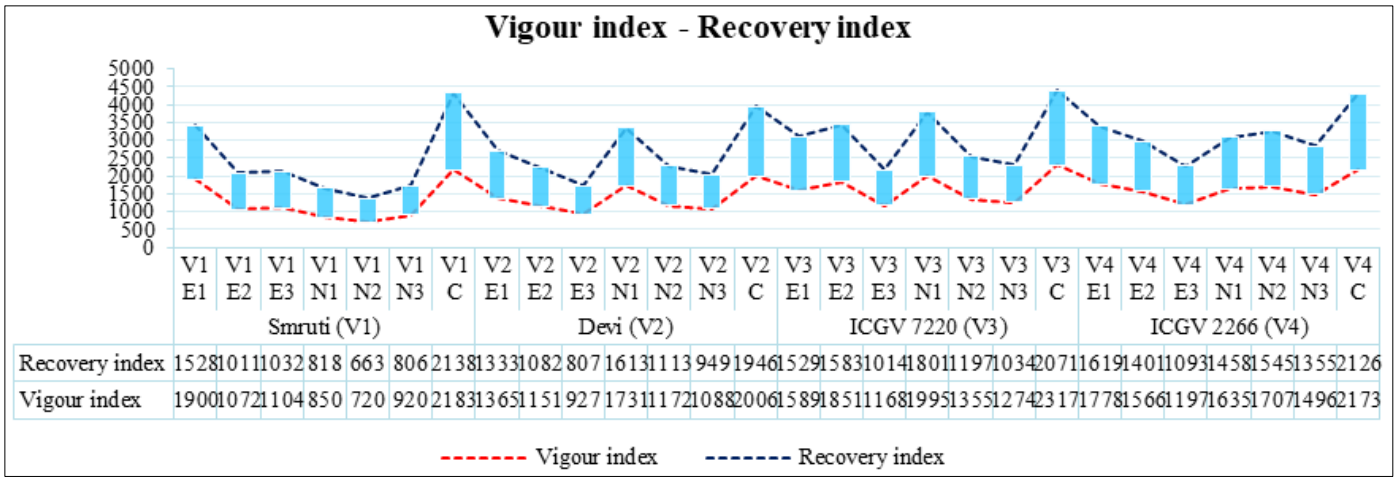

Fig 3: Variation in Vigour-Recovery index in selected groundnut genotypes

Table 1: Seedling characters and effectiveness, efficiency and rate of mutation of EMS and SA in Smruti, Devi, ICGV 7220 and ICGV 2266 groundnut genotypes in M_1 generation

Genotype	Treatments	Seeds sown	Germination (%)	Survival (%)	Lethality (%)	Total mutants	Mutagenic frequency	Seedling height (in cm)	Vigour index	Recovery index	Injury (%)	Effectiveness	Efficiency (Lethal)	Efficiency (Height Reduction)	Efficiency (Vigour Index)	Efficiency (Relative)	Mutation rate (in terms of effectiveness)	Mutation rate (in terms of efficiency)
Smruti (V1)	V1E1	450	92	74	26	17	0.038	20.65	1899.8	1528.1	8.22	3.148	0.0015	0.020	0.0020	0.008	4.084	0.0119
	V1E2	450	88	83	17	37	0.082	12.18	1071.8	1010.9	45.87	4.568	0.0048	0.008	0.0077	0.007		
	V1E3	450	76	71	29	49	0.109	14.53	1104.3	1031.6	35.42	4.537	0.0038	0.014	0.0099	0.009		
	V1N1	450	82	79	21	44	0.098	10.36	849.5	818.4	53.96	8.148	0.0047	0.008	0.0115	0.008	7.099	0.0151
	V1N2	450	76	70	30	57	0.127	9.47	719.7	662.9	57.91	7.037	0.0042	0.010	0.0176	0.011		
	V1N3	450	73	64	36	66	0.147	12.60	919.8	806.4	44.00	6.111	0.0041	0.015	0.0159	0.012		
	V1C	450	97	95				22.50	2182.5	2137.5								
Devi (V2)	V2E1	450	84	82	18	19	0.042	16.26	1365.8	1333.3	18.94	3.519	0.0023	0.011	0.0031	0.006	3.447	0.0093
	V2E2	450	84	79	21	26	0.058	13.70	1150.8	1082.3	31.70	3.210	0.0028	0.009	0.0050	0.006		
	V2E3	450	82	71	28	39	0.087	11.30	926.6	806.8	43.67	3.611	0.0030	0.010	0.0094	0.007		
	V2N1	450	88	82	18	28	0.062	19.67	1730.9	1612.9	1.94	5.185	0.0035	0.160	0.0036	0.056	6.440	0.0425
	V2N2	450	80	76	24	56	0.124	14.65	1172.0	1113.4	26.97	6.914	0.0052	0.023	0.0106	0.013		
	V2N3	450	78	68	32	78	0.173	13.96	1088.9	949.3	30.41	7.222	0.0054	0.028	0.0159	0.017		
	V2C	450	100	97				20.06	2006.0	1945.8								
ICGV 7220 (V3)	V3E1	450	79	76	24	24	0.053	20.12	1589.5	1529.1	18.38	4.444	0.0022	0.012	0.0034	0.006	3.220	0.0091
	V3E2	450	83	71	29	16	0.036	22.30	1850.9	1583.3	9.53	1.975	0.0012	0.015	0.0019	0.006		
	V3E3	450	68	59	41	35	0.078	17.18	1168.2	1013.6	30.30	3.241	0.0019	0.010	0.0067	0.006		
	V3N1	450	82	74	26	34	0.076	24.33	1995.3	1800.6	1.29	6.296	0.0029	0.238	0.0038	0.082	5.340	0.0494
	V3N2	450	77	68	32	42	0.093	17.60	1355.2	1196.8	28.60	5.185	0.0029	0.013	0.0069	0.008		
	V3N3	450	69	56	44	49	0.109	18.46	1273.7	1033.7	25.11	4.537	0.0025	0.018	0.0085	0.010		
	V3C	450	94	84				24.65	2317.1	2070.6								
ICGV 2266 (V4)	V4E1	450	78	71	29	21	0.047	22.80	1778.4	1618.8	3.47	3.889	0.0016	0.057	0.0026	0.020	3.385	0.0154
	V4E2	450	76	68	32	23	0.051	20.60	1565.6	1400.8	12.79	2.840	0.0016	0.017	0.0033	0.007		
	V4E3	450	69	63	37	37	0.082	17.35	1197.2	1093.1	26.55	3.426	0.0022	0.013	0.0069	0.007		
	V4N1	450	83	74	26	11	0.024	19.70	1635.1	1457.8	16.60	2.037	0.0009	0.006	0.0015	0.003	3.076	0.0115
	V4N2	450	79	71	28	26	0.058	21.61	1707.2	1545.1	8.51	3.210	0.0020	0.029	0.0034	0.011		
	V4N3	450	74	67	33	43	0.096	20.22	1496.3	1354.7	14.39	3.981	0.0029	0.028	0.0064	0.012		
	V4C	450	92	90				23.62	2173.0	2125.8								
Minimum		68	56	10	11	0.024	9.47	719.7	662.9	1.28	1.975	0.0009	0.0062	0.0015	0.0029	3.076	0.0061	
Maximum		100	97	44	78	0.173	24.65	2317.1	2137.5	57.91	8.148	0.0054	0.2383	0.0176	0.0817	7.099	0.0330	

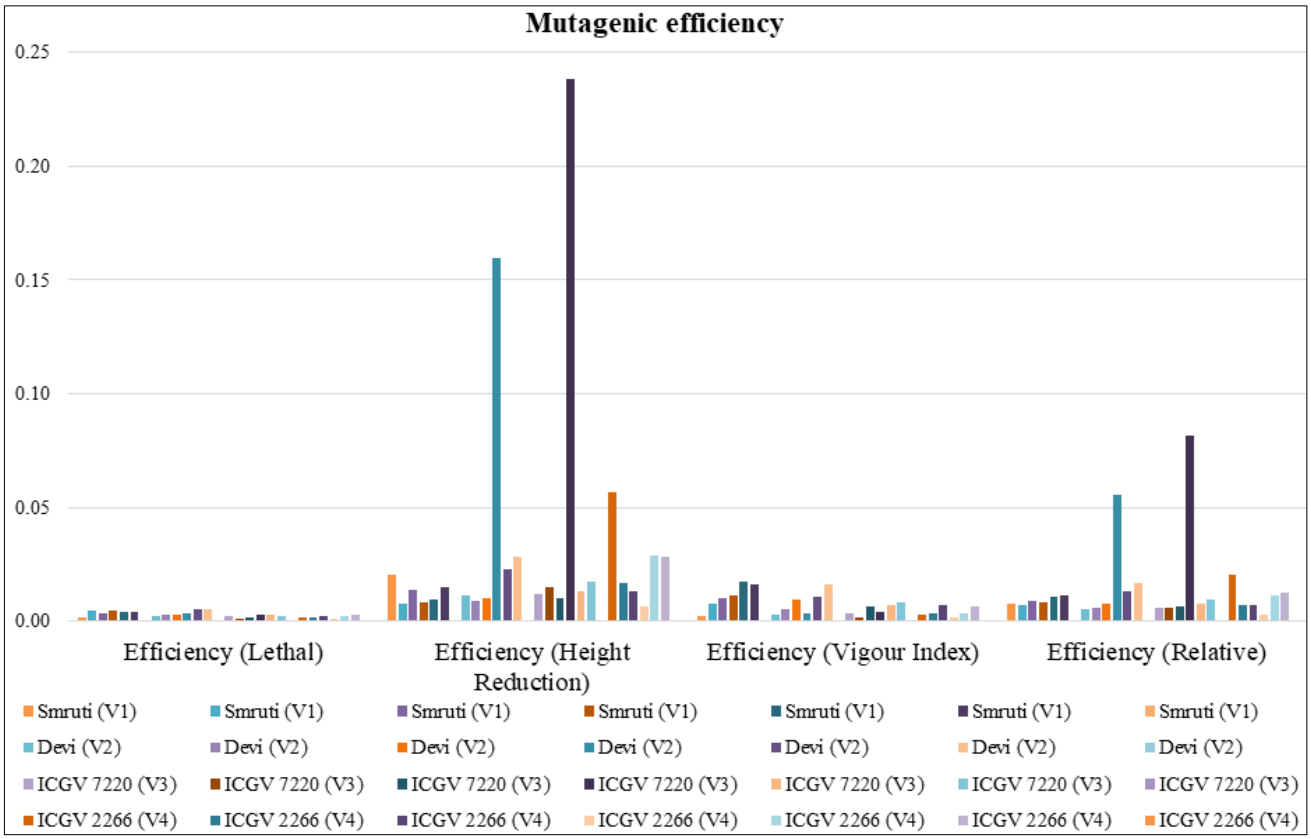

Fig 4: Mutagenic efficiency of EMS and SA in selected groundnut genotypes

Mutagenic frequency, efficiency, effectiveness and mutation rate

The mutagenic frequency ranged from 0.024% (V4N1) to 0.173% (V2N3) with sodium azide treatments. The findings were supported by (Swaminathan, 1969; Al Qurainy and Khan 2009) [17, 1].

Efficiency$_{Lethal}$: The results were comparable among treatments in genotypes irrespective of concentration. Efficiency for sodium azide was high in all varieties. V4N1 showed the least efficiency of 0.0009.

Efficiency Height reduction: the trend was decreasing for EMS where as it kept increasing in SA.

Efficiency Vigour index: with increase concentration of mutagen the efficiency increased.

Relative efficiency: the mutagenic concentration showed different effect on different varieties (Table 1, Figure 4) (Khursheed *et al*., 2015; Raina *et al.*, 2017) [12, 14].

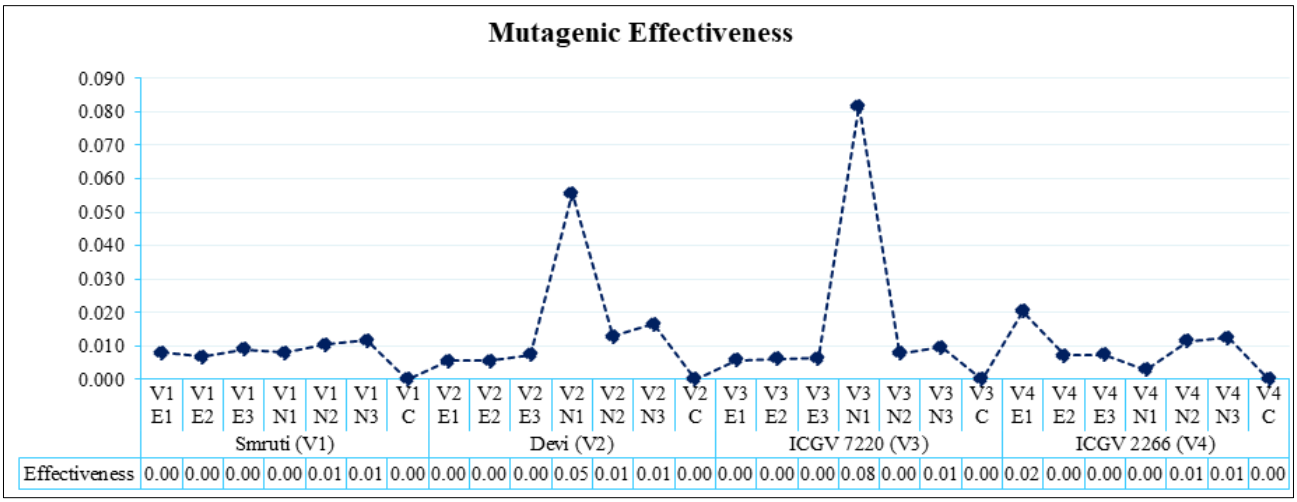

Fig 5: Effectiveness of EMS and SA in selected groundnut genotypes

Effectiveness was high with increase in concentration of mutagen in Smruti for EMS, and SA in Devi, comparable in EMS treated Devi M_1 plants and ICGV 2266 with sodium azide. Rest all treatments showed decreasing efficiency with increase in mutagen concentration. Effectiveness ranged from 1.975 (V3E2) to 8.148 (V1N1) (Table 1, Figure 5).

Mutation rate (in terms of efficiency and effectiveness) was higher in SA treatment in Smruti (highest = 7.099), Devi and ICGV 7220, whereas EMS rate if mutation was high in case of ICGV 2266 (3.385) (Figure 6), (Swaminathan, 1969) [17].

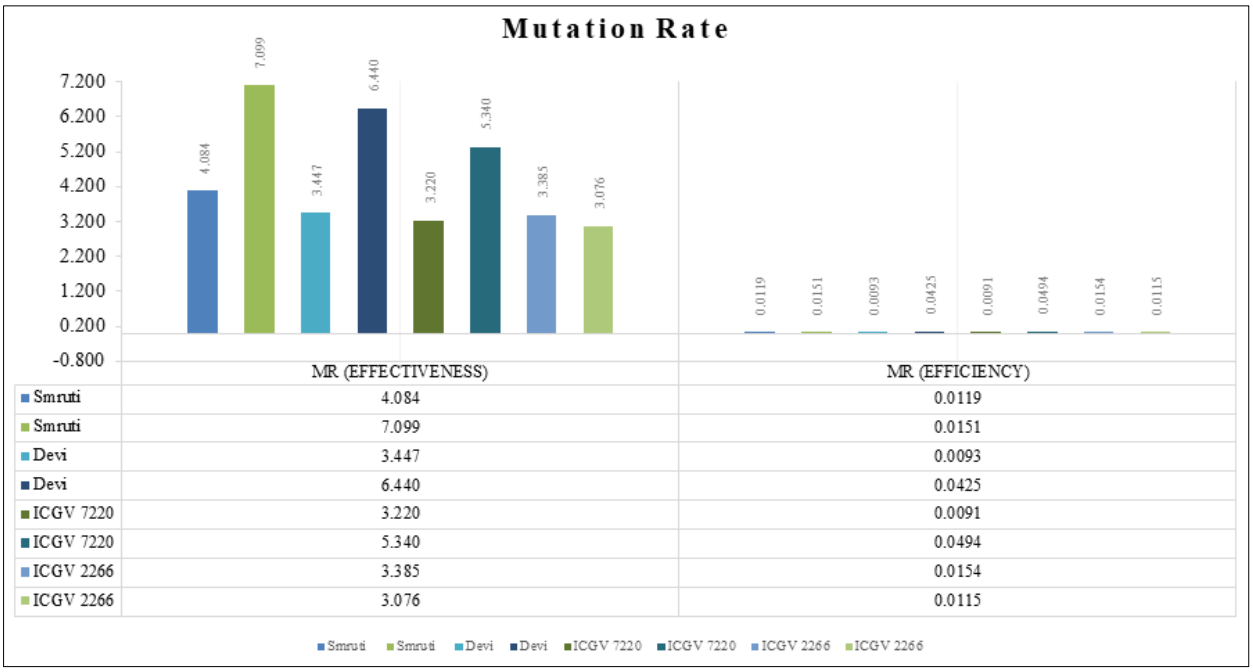

Fig 6: Rate of mutation in selected groundnut genotypes with EMS and SA

Conclusion

Sodium Azide was more effective mutagen in case of Smruti, Devi and ICGV 7220, whereas EMS was more effective in ICGV 2266**.** Smruti and Devi performed well with mutagens in producing viable mutant population for further studies. The efficiency of mutagen used was influenced by the genotype and rate of mutation was for SA than EMS.

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