

The Effects of Ethyl Methanesulphonate and Sodium Azide on Growth in 'G.G.Gerbing' azalea (Rhododendron)

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Introduction

- Mutagens have become an important tool in generating new variability, which can be used to improve yield and quality in many crops and ornamental plants^[1].
- Ethyl methanesulphonate (EMS) and sodium azide (SA) have been the most commonly used mutagens in plant breeding programs, primarily because they are the chemical mutagens that create point mutation in the genome of plants and do not disturb desirable attributes^[2].

Objectives

The main objective of the experiment was to determine the effectiveness and optimum doses of EMS and SA to induce the changes in growth and physiology responses in azalea.

Materials and Methods

- ❖ 'G.G.Gerbing' azalea plants were procured from Magnolia Gardens Nursery, Magnolia, TX, and were transplanted to 6 inch pots filled with Berger growing media (BM7, Berger,) on 16 Dec. 2015 in greenhouse at College Station, TX.
- ❖ Uniform, disease and pest free plants were selected for the treatments. Each plant contained 6 buds, and the extra buds were manually removed.
- ❖ Total of 126 plants were selected, the buds were wrapped in absorbent cotton and treated with EMS and SA solutions for 1d, 2d, and 3d, which were prepared in 0.1 M phosphate buffer adjusted to pH 7.4 and pH 3.0, respectively. The treatment concentrations were 0 mM, 30 mM, 60 mM, 90 mM, 120 mM and 150 mM of EMS and 2 mM, 4 mM, 6 mM, 8 mM and 10 mM of SA.
- ❖ During the establishment stage, plants were irrigated as needed using a nutrient solution containing 200 mg·L⁻¹ (21N-7P-7K).
- ❖ Environmental condition: D/N temperature : 24.2°C /19.7 °C; D/N RH: 30%/41% ; 22.6 mol·m⁻²·d⁻¹ DLI.

- ❖ Measured parameters in Exps: Visual rating of buds (from 0 to 10, where 0 is "no symptom of damage and 10 is "100% symptom of damage"), new bud length, new leaf number, abnormal leaf number, and SPAD reading.



Figure 1. Rating standard from 0 to 10.

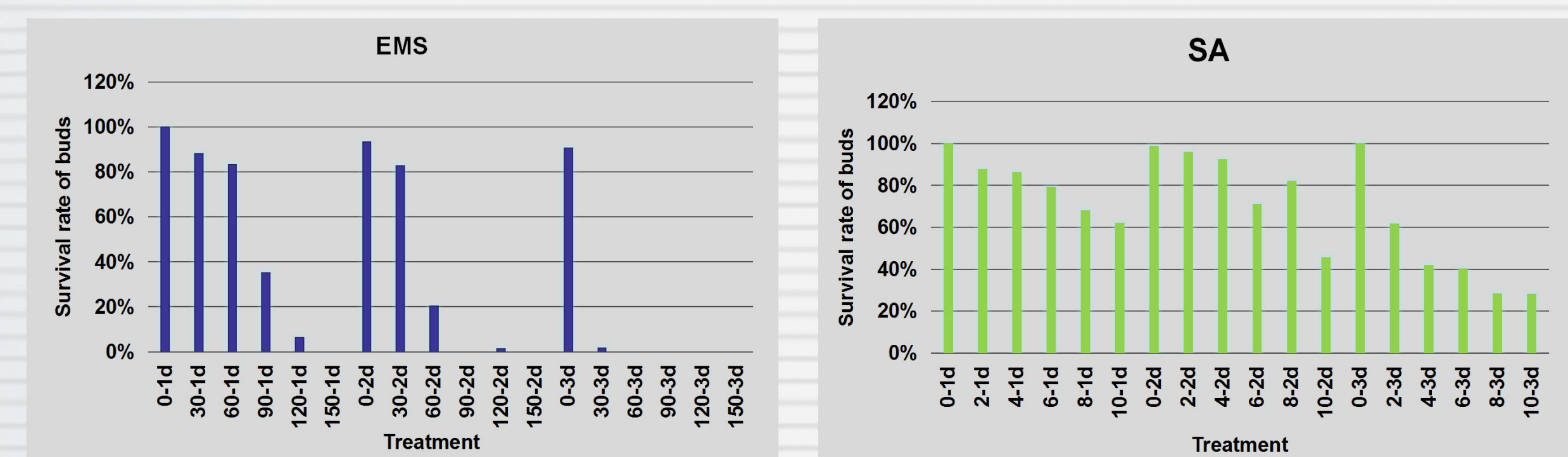


Figure 2. Survival rate of buds subjected to EMS and SA treatments.

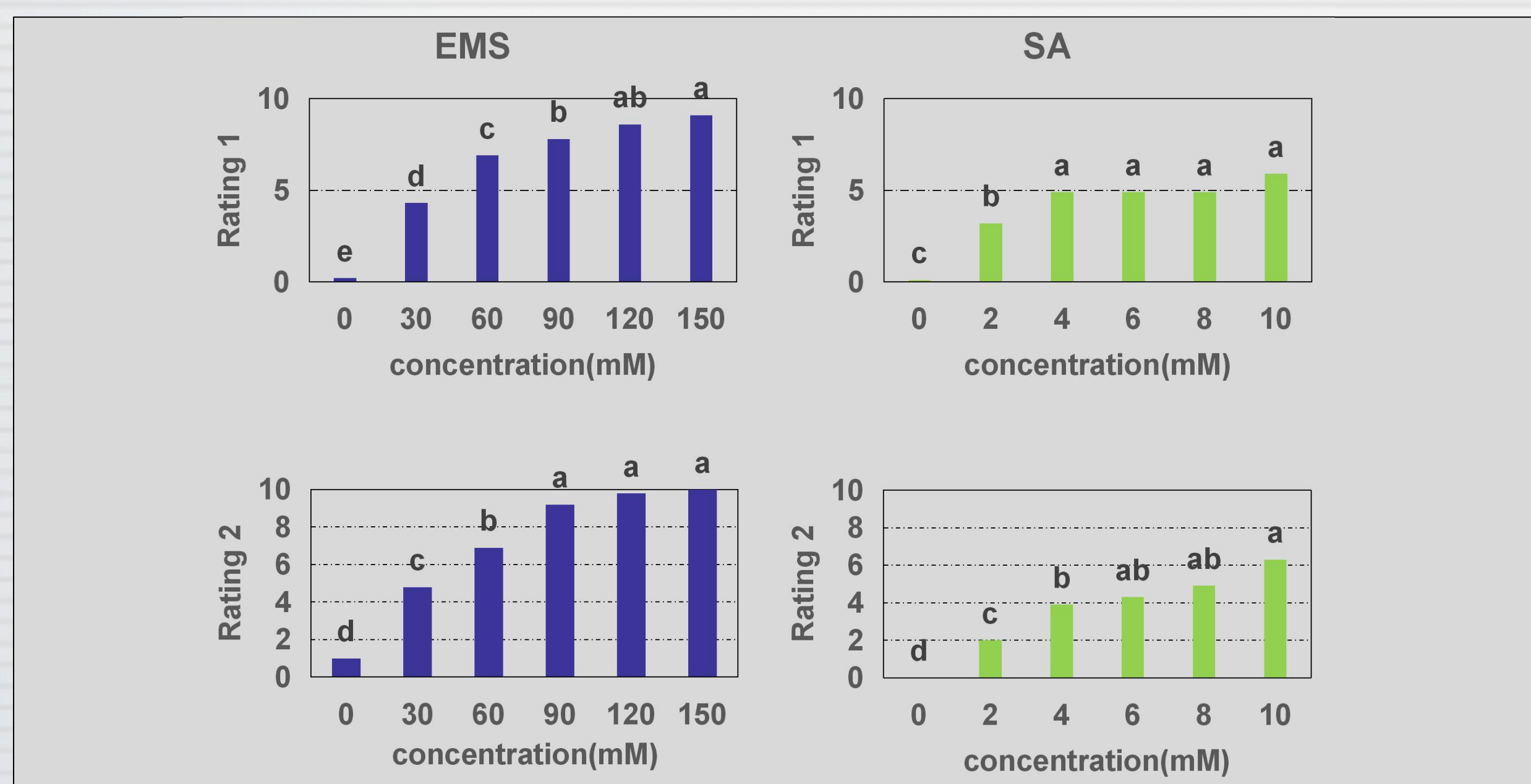


Figure 3. Rating scores among EMS and SA treatment solutions. Rating 1/ rating 2 were evaluated 1 week/4 months after treatment, respectively.

Table 1. Bud length and leaf number among different EMS and SA treatment times. (b = bud length, l = leaf number) b1/b2/b3/b4, l1/l2/l3/l4 mean that bud length, leaf number were evaluated 4/8/12/16 weeks after treatment, respectively.

Mutagen	Time	b1	b2	b3	b4	l1	l2	l3	l4
EMS	1d	0.6b	4.6b	7.8a	9.6a	3.1a	10.3a	14.6a	18.2a
	2d	0.9a	5.9a	8.8a	10a	3.6a	11.3a	15.9a	18.6a
	3d	0.5b	2.9c	5.5b	6.3b	0.35a	6.3b	11.8b	17a
SA	1d	1.1b	6.5a	10.4a	20.1a	3.4a	10.8a	15.5a	11.7a
	2d	1.3a	6.3a	9.3ab	18.7a	3.9a	11.3a	14.8a	10.3a
	3d	0.9b	4.9b	8.7b	18.6a	3.4a	9.4b	14.5a	10.3a

Table 2. Bud length and leaf number among different EMS and SA treatment solutions. (b = bud length, l = leaf number) b1/b2/b3/b4, l1/l2/l3/l4 mean that bud length, leaf number were evaluated 4/8/12/16 weeks after treatment, respectively.

Mutagen	Rate	b1	b2	b3	b4	l1	l2	l3	l4
EMS	0	0.9b	4.7a	8a	9a	4ab	9.9a	15.2a	17.6a
	30	0.7bc	5.4a	7.9a	10.1a	3.8ab	10.1a	14.3a	19.5a
	60	0.5bc	4a	6.9a	8.3a	2.5bc	9.9a	13.6a	17.8a
	90	0.2b	3.2a	7.8a	11a	1.7c	9.8a	13.3a	18.5a
	120	1.6a	3.5a	9.3a	7.3a	5a	7a	17a	16a
	150	-	-	-	-	-	-	-	-
SA	0	1a	5.6ab	9.9a	12a	3.7ab	10.1a	15.1a	20.1a
	2	1.1a	501b	7.9a	8.7b	3.3ab	10.5a	14a	18.3a
	4	1a	5.9ab	9.1a	10.8ab	4.3a	11.1a	15.1a	18.8a
	6	1.2a	6.6a	10.5a	11ab	3.5ab	10.6a	15.4a	18.7a
	8	1.2a	7a	10.6a	11.3ab	3.1b	11.6a	15.8a	18.9a
	10	1.3a	6.9a	10.4a	11.6ab	3.4ab	10.3a	14.9a	20.8a

Figure 4. Abnormal bud and abnormal leaves.

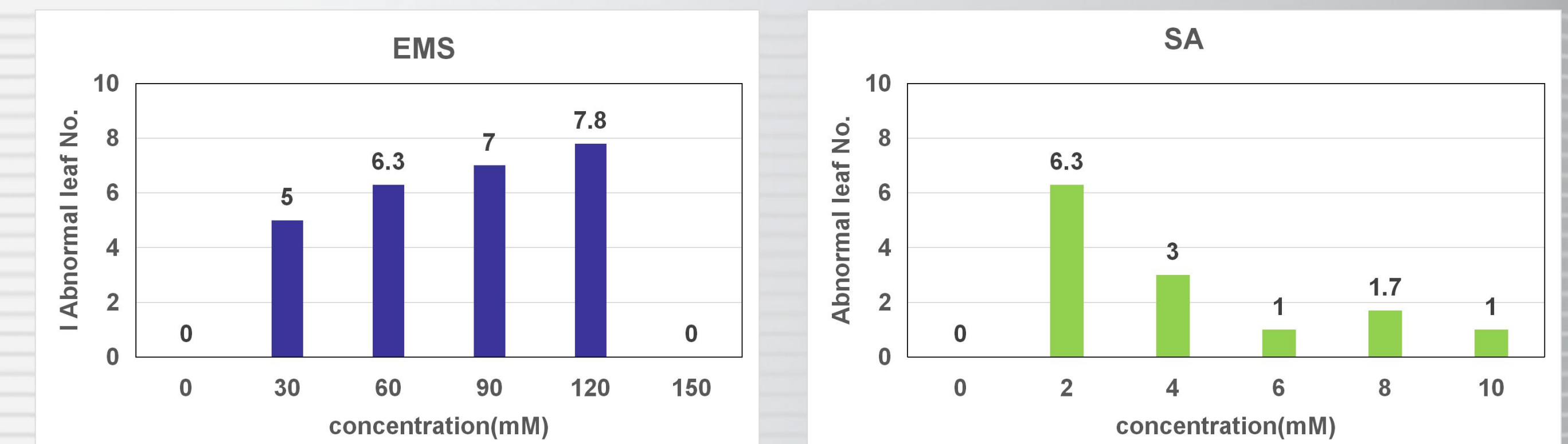
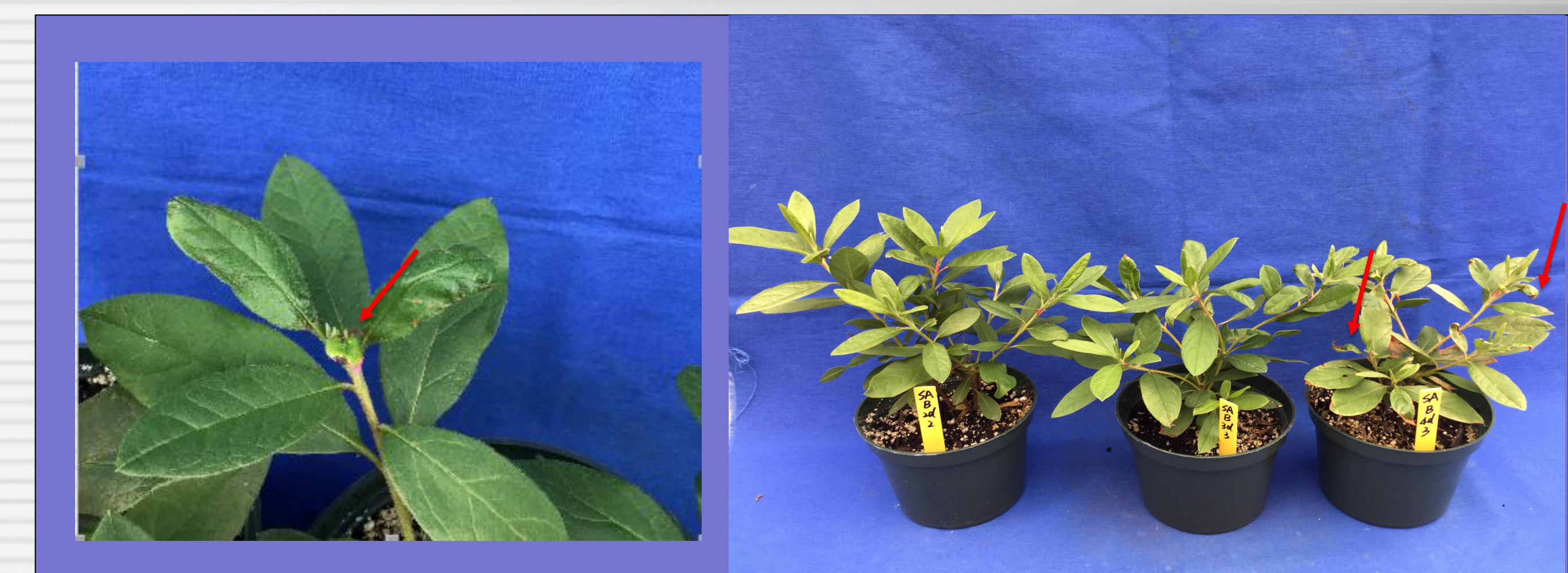


Figure 5. Abnormal bud and abnormal leaves.



Results

- The median lethal dose (LD50) is about 60-90 mM for EMS and 8-10 mM for SA (Figure 2).
- Plants treated with 60-90 mM EMS or 8-10 mM SA have the medium visual damage rating score (5-7). This rating result further supports the LD50 conclusion (Figure 3).
- The average bud length and leaf number between LD50 condition and control are similar. The anova test shows no significant difference; this may be caused by the mutagen enlarging the ingroup variation (Table 1, 2).
- The abnormal leaf number of SA decreased (from 6.3 to 1) as the concentration of the solution increased (from 2 mM to 8mM). The abnormal leaf number in EMS treatment increased (from 5 to 7.8) as the concentration of the solution increased (from 30 mM to 120mM) (Figure 4).
- There was no significant difference in SPAD reading or branch number among all EMS and SA treatment solutions (data not shown).

Conclusion

- Plants can be treated with EMS at rates up to 90 mM for 2d, and with SA at rates up to 10 mM for 3d as the optimum doses of chemical mutagens.
- This are just the preliminary experiment results. After flowering, the flower number, flower size, flower color data, etc. will help further optimize the doses of EMS and SA mutagens.

Literature Cited

- ✓ [1] Chaudhari, Ajey Karan, Sandhya Verma, and B. R. Chaudhary. "Ethyl Methanesulphonate and Sodium Azide Effects on Seedling Growth and Chlorophyll Mutations in Psoralea corylifolia IC 111228." Journal of Crop Improvement 29.5 (2015): 602-618.
- ✓ [2] Al-Qurainy, Fahad, and Salini Khan. "Mutagenic effects of sodium azide and its application in crop improvement." World Applied Sciences Journal 6.12 (2009): 1589-1601.