

Problem statements

S. plicata is considered as an **endangered** species and has difficulty in production of new variation.

Orchids have **delayed development** under its wild habitat.

In vitro mutation induction through chemical mutagen in the breeding of orchids (*S. plicata*) has **not been commonly used**.



Spathoglottis plicata

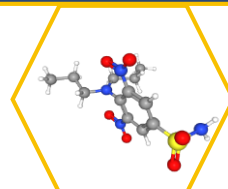
Introduction

Tissue culture techniques



Convenient and easier (large number of plantlets in short time)
Low- cost and large-scaled propagation
Commercial and conservation
(Huy et al.,2019)

Chemical mutagen



Oryzalin (Herbicide)
Mitotic inhibitor agent

86% -100% of chromosome doubling with low dosed
30% - 100% of surviving rate (proved its level of toxicity)
(Chung et al., 2014)
(Thao et al., 2003)

Results

Table 1. Effect of oryzalin treatment for 5 days on plant morphological characteristic after 1 month of culture. Mean values \pm SE (n = 6)

Characteristics	Treatments			
	Control	3 mgL ⁻¹	5 mgL ⁻¹	9 mgL ⁻¹
Plant length (cm)	7.67 \pm 0.12	7.65 \pm 0.26	7.63 \pm 0.34	7.62 \pm 0.45
Leaf length (cm)	5.58 \pm 0.26	6.03 \pm 0.32	5.38 \pm 0.31	5.81 \pm 0.29
Leaf width (cm)	0.30 \pm 0.00	0.29 \pm 0.00	0.40 \pm 0.03	0.29 \pm 0.03
No. of leave	5.33 \pm 0.21	4.18 \pm 0.17	4.50 \pm 0.22	4.33 \pm 0.21
Root length (cm)	6.33 \pm 1.03	1.80 \pm 0.42	1.60 \pm 0.29	1.32 \pm 0.19
Root width (cm)	0.14 \pm 0.01	0.14 \pm 0.01	0.18 \pm 0.01	0.18 \pm 0.01
No. of root	5.33 \pm 0.49	4.00 \pm 0.37	4.50 \pm 0.56	4.16 \pm 0.70
Fresh weight (g)	0.38 \pm 0.03	0.20 \pm 0.02	0.31 \pm 0.02	0.17 \pm 0.02
Dried weight (g)	0.039 \pm 0.00	0.023 \pm 0.00	0.038 \pm 0.00	0.029 \pm 0.00

Materials & methods

In vitro seedlings were multiplied in half MS media supplemented with 0.5 mgL⁻¹NAA and 2.0 mgL⁻¹ BAP

Genetic variations will be detected using flow cytometry analysis and chromosome counting

Seedlings were transferred into liquid half MS media with oryzalin (3,5 and 9 mgL⁻¹) in dark

Morphological characteristics were collected after 1 month of culture

Flasks were placed on rotary shaker at 150 rpm for 5 days

After treatment the treated seedlings were transferred into half MS media for development

Conclusion

In this study, the results obtained by the morphological characteristics. *S. plicata* with 3 mgL⁻¹ and 5 mgL⁻¹ oryzalin for 5 days treatment have been demonstrated as effective protocols in development of leaves width and leaves length. *In vitro* mutation induction of *S. plicata* is hoped could improve the development of mutant lines at vegetative and reproductive stages.

Future work

Treatment with other concentration of oryzalin for 1, 2, 3, and 4 days will be conducted in future. The ploidy number of treated plants will be labelled and further grown in the orchid house at ambient temperature.

References

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- Thao, N. T. P., Ureshino, K., Miyajima, I., Ozaki, Y., & Okubo, H. (2003). Induction of tetraploids in ornamental *Alocasia* through colchicine and oryzalin treatments. *Plant cell, tissue and organ culture*, 72(1), 19-25.

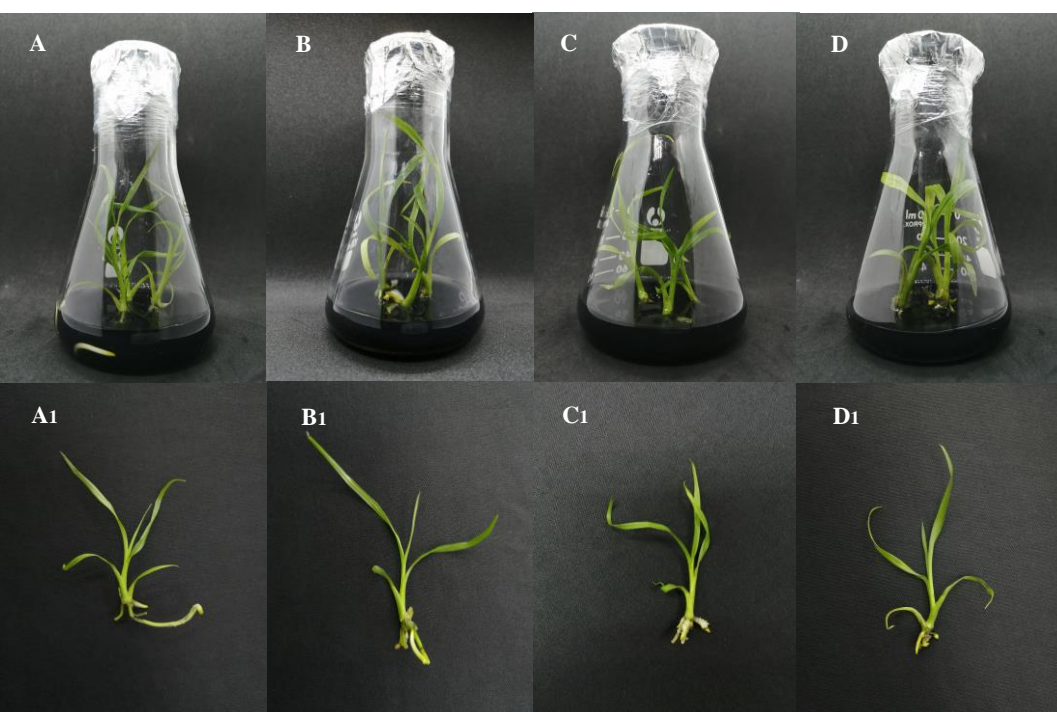


Fig. 2. Plant development of *S. plicata* of 1-month culture. Control; A, A1. Oryzalin 3 mgL⁻¹ ; B, B1. Oryzalin 5 mgL⁻¹; C, C1. Oryzalin 9 mgL⁻¹ ; D, D1.