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Article

Mutagenesis and Flowering-Promoting by Sodium Azide In Vitro Culture of *Cymbidium faberi Rolfe*

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Abstracts: *Cymbidium faberi Rolfe* is one of the traditional Chinese orchids with important ornamental value and the cultivation of *Cymbidium faberi Rolfe* mutant strains with different appearances is essential to increase its economic value, but at present, the acquisition of *Cymbidium faberi Rolfe* mutant strains basically relies on natural mutation.The objectives of this research were to mutagenize *Cymbidium faberi Rolfe* protocorm-like bodies (PLBs) and shoots in vitro using sodium azide (NaN_3), and to screen and evaluate mutants in mutagenized *Cymbidium faberi Rolfe* seedlings using morphological characteristics.*Cymbidium faberi Rolfe* PLBs and shoots were used as mutagenic materials, and co-cultivated in induced shoots growth medium with the addition of 0.0 (control), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/L and 0.0 (control), 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 mg/L NaN_3 to induce the mutations, and the mortality rates of the PLBs and shoots were increased with the increase of the NaN_3 concentration.At 14 d of co-cultivation, PLBs and shoots were most efficiently mutagenized with 1.5 mg/L and 4.0 mg/L NaN_3, respectively.After the explants were cultured for 3 months, changes in leaf and flower morphology were observed in some mutants: shorter and thicker leaves, shorter node length, reduced height, and mid-translucent leaves compared with controls; and some *Cymbidium faberi Rolfe* bloomed prematurely, with single flowers with large, thick petal sepals and small inflorescences, and colours include light green throughout, and a few with purple stamens.This suggests that NaN_3 can effectively mutagenise *Cymbidium faberi Rolfe* PLBs and shoots to satisfy people's demand for *Cymbidium faberi Rolfe*'s ornamental properties while increasing its economic value.

Keywords: *Cymbidium faberi Rolfe*; Tissue Culture; NaN_3; Mutagenesis; Early Flowering

1. Introduction

Cymbidium is a terrestrial plant of the genus *Cymbidium Sw.* in the family *Orchidaceae*, native to China, also known as Summer Cymbidium and Nine-section Orchid, etc. (Xu, 2018),and is a second-class protected wild plant in China, with a long history of cultivation, and a tall plant, with fragrant and attractive flowers and elegantly erect leave (Chen, Y. Q. et al., 2005; Zeng et al., 2020), which makes it of high ornamental and high economic value (Chu and Tian, 2022), and has been popular as an important ornamental flower in East Asia (Liu et al., 2017).

The natural fruiting rate of wild *Cymbidium faberi Rolfe* is only 7.5% (Yang, 2017). *Cymbidium faberi Rolfe* capsules, like most Orchidaceae, contain millions of seeds in a single capsule, but the seeds are extremely tiny, and these are devoid of any metabolic mechanism, have no endosperm or cotyledons, and contain only an underdeveloped embryo within the testa, which contains only lipid droplets and a small amount of protein, and lacks the support nutrients needed for germination processes (Pradhan et al., 2014; Soares et al., 2014; Custódio et al., 2016). They require specific fungi for natural germination (Kalimuthu et al., 2007), and although large numbers of seeds are produced,

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few germinate in nature, and wild *Cymbidium faberi Rolfe* resources are becoming increasingly scarce. Therefore, the study of *Cymbidium faberi Rolfe* in vitro tissue culture and the establishment of a regeneration system are extremely helpful for the conservation, innovation and utilization of *Cymbidium faberi Rolfe* germplasm resources. Currently, it has been demonstrated that medium with low inorganic salt concentration is suitable for *Cymbidium faberi Rolfe* seed germination, and coconut juice promotes the induction and proliferation of PLBs (Sun et al., 2008). The addition of banana juice to the medium facilitates the differentiation of PLBs (Yu, 2014). There are fewer systematic studies on *Cymbidium faberi Rolfe* in terms of fast propagation by histoculture. In this paper, a complete system of *Cymbidium faberi Rolfe* regeneration was established from seed germination to proliferation of PLBs, seedling emergence, rooting and final transplantation.

In cultivated plants NaN_3 is probably one of the most effective chemical mutagens found (Türkoğlu et al., 2023), which can cause point mutations, especially AT to CG, which can lead to amino acid changes, alter protein function, and change plant phenotypes (Wannajindaporn et al., 2014), and it is a potent point mutagen with the advantages of high efficiency, no residual toxicity, and low price, which has been widely used in flower breeding (Ma et al., 2010), and also in orchids such as *Phalaenopsis aphrodite Rchb. f.* (Cui et al., 2010), *Dendrobium nobile Lindl.* (Wannajindaporn et al., 2014; Hualsawat et al., 2022) and *Oncidium hybridum* (Cui et al., 2011) in mutagenesis of Orchidaceae. NaN_3 can cause base insertions and/or deletions in replicating DNA molecules and base substitutions, and the mutagenic efficiency of NaN3 is increased when the number of replicating DNA molecules becomes higher during rapid cell division of the mutagenized material, causing an increased likelihood of point mutations (Al-Qurainy et al., 2011; Qian et al., 2017; Zhang, R. C. et al., 2017). The induction of shoots from *Cymbidium faberi Rolfe* PLBs involves a large number of DNA replications, and this time was selected for mutation induction in this study. The concentration of the mutagen and the time of application also play an important role in the mutagenesis process (Salim et al., 2009), and the likelihood of generating mutations increases with each given mutagen concentration level and co-culture time. However, if the concentration is increased to very high levels and the application period is prolonged, negative effects may occur (Jenks et al., 2007), such as an increase in *Cymbidium faberi Rolfe* seedling mortality and browning/damage rates, and, therefore, suitable mutagenic conditions need to be determined for the PLBs tissues.

Cymbidium faberi Rolfe has undergone a long period of natural selection and adaptation during its evolutionary process, and its genome is relatively stable and mostly asexually reproduced, with a small chance of genetic mutation and a relatively low rate of natural mutation. Artificial mutagenesis can increase the mutation rate of plants. In this study, we explored the conditions of *Cymbidium faberi Rolfe* in histoculture, established *Cymbidium faberi Rolfe* regeneration system, and utilized NaN_3 mutagenesis to increase the rate of genetic variation of *Cymbidium faberi Rolfe*, enrich the genetic diversity of *Cymbidium faberi Rolfe*, as well as to provide important materials and means for breeding, to further breed *Cymbidium faberi Rolfe* varieties that are more economical, ornamental, or pest-resistant and to provide support for the enrichment of *Cymbidium faberi Rolfe* germplasm resources.

2. Materials and Methods

2.1. Plant Material and Explant Preparation

Cymbidium faberi Rolfe seeds used in this experimental study were obtained from Tongbai County, Nanyang City, Henan Province. They were aseptically inoculated onto induced seed germination medium and cultured for 3 months to obtain sterile PLBs as explants for mutagenesis. NaN_3 is prepared in the laboratory and the dosage is self-adjusted according to the desired concentration during the test. The whole process of this test needs to be carried out in a sterile environment.

2.2. Building a Cymbidium faberi Rolfe Regeneration System

Seed germination. The seed capsules were sterilized by oscillation with 2% sodium hypochlorite(NaOCl) solution and then rinsed with sterile water for 3 times, the capsules were cut

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open and the seeds were removed with a small sterile spoon and rinsed with sterile water for 3 times, the seeds were transferred to conical flasks and placed on a magnetic stirrer, the seeds were rotary cut with a sterilized blade, and the seeds were inoculated with. Murashige and Skoog(MS) (0.7% agar) medium + 10g/L sucrose + 10g/L glucose + 3g/L peptone + 0.2 mg/L 6-BA + 0.2 mg/L NAA.

PLBs proliferate. Screening of different combinations of plant growth regulators(PGRs) on proliferation of PLBs(Table 1). 1/2MS (0.7% agar) was used as the basic medium with 10 g/L sucrose, 10 g/L glucose, 3 g/L peptone, 1 g/L activated charcoal, 100 ml/L coconut milk, and different concentrations of combinations of PGRs, NAA, 6-BA, 2,4-D, and IBA. 24 vials were connected to each formulation, and each vial was inoculated with six 5-mm unbranched PLBs, placed in a sterile culture room.Observed and recorded after 60 days, mean number of new PLBs, average new length and new diameter.

Table 1. PGR formulation for inducing proliferation of *Cymbidium faberi* Rolfe rhizome.

Treatments	NAA(mg·L^{-1})	6-BA(mg·L^{-1})	2,4-D(mg·L^{-1})	IBA(mg·L^{-1})
B-0	0	0	0	0
B-1	1	0.5	0.1	0
B-2	1	1.0	0.1	0
B-3	1	0.5	0.2	0
B-4	0	0.5	0.1	1

PLBs induced shoots. Aseptic PLBs were inoculated in 1/2MS (0.7% agar) medium + 10 g/L sucrose + 10 g/L glucose + 3 g/L peptone + 1 g/L activated charcoal + 100 ml/L coconut milk + 1 mg/L 6-BA + 1 mg/L KT + 1 mg/L TDZ + 1 mg/L NAA + 0.1 mg/L 2,4-D to induce shoots.

Rooting was induced and transplanted. Seedlings were inoculated in 1/2 MS (0.7% agar) medium + 3 g/L peptone + 15 g/L sucrose + 1 g/L activated charcoal + 100 ml/L coconut milk with different combinations of concentrations of NAA, IAA, and 6-BA to induce rooting of PLBs that had shots. Each vial was inoculated with 6 explants, 12 vials per treatment, 4 vials as one replication, and the experiment was repeated three times. Rooting rate was observed and recorded after 50 days and root growth was recorded. After 80 days of transplanting, open the caps of the bottles of histocultured seedlings for refining before transplanting, take the seedlings out of the bottles after 2 days of refining, rinse the roots, soak them in 0.1% potassium permanganate for 30s, then rinse them under running water and dry the surface water, then transplant them into the orchid-specific substrate and use “Orchid Bacteria King” to pour the fixing water, and the survival rate of *Cymbidium faberi Rolfe* seedlings was recorded 30 days after transplanting.

2.3. NaN_3 Mutagenesis

Addition of 1.0-3.0 mg/L NaN_3 (treatments E1-E6) and 4.0-10.0 mg/L NaN_3 (treatments F1-F7) was used for co-culture mutagenesis, and 0 mg/L NaN_3 (H0, M0) was set as the control group. Well-grown sterile PLBs and newly sprouted shoots were selected for mutagenesis in treatments H0-H6 and M0-M7, respectively, which were inoculated with shoot-inducing medium supplemented with different concentrations of NaN_3. Groups H0-H6 PLBs were treated with 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/LNaN_3; Groups M0-M7 shoots were treated with 0.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 mg/LLNaN_3, and each group was inoculated with 72 explants. The shoots were induced by 1/2MS as the basic medium with sucrose 15g/L, glucose 15g/L, coconut milk 100ml/l, peptone 3g/l, activated charcoal 1g/l, and PGRs NAA1mg/L, 6-BA1mg/L, KT1mg/L, TDZ1mg/L, 2,4-D0.1mg/L. After 60 days of induction culture, the differentiation rate, mortality rate and leaf growth of all treatments were observed and recorded.

2.4. Data Analysis

All experiments were repeated three times. Excel 2021 was used to preliminarily organize the experimental data. SPSS 26.0 software was used for one-way ANOVA. Duncan’s multiple range test

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was used to test the significance of the differences between the means. The significance level was set at 0.05. The results were expressed as mean ± standard deviation.

Index determination method: New branches = number of new growth branches /number of surviving explants; New growth length = new growth length /number of surviving explants; New diameter = new growth diameter / number of surviving explants; Rhizome differentiation rate = number of rhizomes differentiated and sprouted / number of inoculated viable rhizomes × 100%; Mortality = dead explants / inoculated explants × 100%; Leaf growth length = total leaf growth length /number of surviving leaves × 100%.

3. Results

3.1. Effect of PGRs on Proliferation of PLBs

The proliferation culture of *Cymbidium faberi Rolfe* PLBs was carried out using different combinations of PGRs at different concentrations, and the proliferation status of each treatment is shown in Table 1. Newly expanded bumps began to appear on the surface of the PLBs in 20 days of incubation, followed by gradual elongation and growth of new PLBs. The better performers were treatments B-1 and B-2, where the diameter of nascent PLBs was significantly lower than that of the other treatments after increasing the concentration of 2,4-D. High concentrations of 2,4-D inhibited the thickening of PLBs; In the use of growth hormone-like, after replacing NAA with IBA, all indicators of PLBs decreased, and NAA induced better proliferation of PLBs; Treatment B-1 with the addition of 0.5 mg/L 6-BA PLBs was thicker and stronger, after increasing the concentration of 6-BA, the length of newborn PLBs increased, and 6-BA was effective in promoting elongation of PLBs, but the diameter became smaller and the induced PLBs were on the thin side. Combining all the indexes, 1mg/LNAA, 1mg/L6-BA, and 0.1mg/L2,4-D were finally selected as the concentration combination of PLBs proliferating PGRs.

Table 2. Effect of PGRs NAA, 6-BA, 2,4-D and IBA on proliferation of *Cymbidium faberi Rolfe* PLBs.

Treatments	New branches	New growth length (mm)	New diameter (mm)
B-0	0	0.26 ±0.04d	0.04 ±0.05e
B-1	3.10 ±0.23a	4.87 ± 0.01b	0.96 ±0.04a
B-2	2.80 ±0.17a	5.31 ±0.06a	0.82 ±0.03b
B-3	2.47 ±0.14b	4.53 ±0.04c	0.59 ±0.04d
B-4	2.31 ±0.17b	4.76 ±0.05b	0.68 ±0.02c

Note:The values represent the means (±SD).The mean values within a column followed by the same letter are not significantly different at $p < 0.05$ as Analysis of Variance(ANOVA) test.

3.2. Effect of PGRs on INDUCED ROOTIng of Cymbidium faberi Rolfe Seedlings

Rooting and transplanting is a key step in the rapid propagation of *Cymbidium faberi* Rolfe tissue culture. As shown in Table 3, with the increase of NAA concentration and the decrease of 6-BA concentration, the rooting rate gradually increased. After adding IAA, the rooting rate was further improved, indicating that NAA and IAA coordinated with each other and promoted the rooting of *Cymbidium faberi* Rolfe. The rooting rates of the six media were different, and *Cymbidium faberi* Rolfe seedlings that had been rooted by the different formulations were transplanted into orchid-specific substrates, in which the roots induced by the high concentration of NAA were thicker and easier to survive. The combination of 2 mg·L^{-1} NAA, 1 mg·L^{-1} IAA and 1 mg·L^{-1} 6-BA had the highest rooting rate, and the survival rate of yellow-green thick roots was also significantly higher than that of other treatments. One month later *Cymbidium faberi* Rolfe seedlings (Figure 1e) had new shoot growth (Figure 1f), so they were identified as a subsequent induced rooting PGRs.

Table 3. Effect of plant growth regulators on rooting and transplanting survival of *Cymbidium faberi* Rolfe seedlings.

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Treatments	NAA (mg/l)	IAA (mg/l)	6-BA (mg/l)	Rooting rate (%)	Transplant survival rate (%)	Root growth status
D-1	0.2	0	2	8.80 ±0.80e	87.41 ±0.77d	White slender
D-2	0.5	0	2	10.18 ±0.80de	90.29 ±0.44c	White slender
D-3	1	0	2	18.98 ±3.21c	96.64 ±0.37b	Green thick
D-4	0.2	1	2	12.96 ±0.80d	90.47 ±0.24c	White slender
D-5	2	0	1	49.54 ±3.21b	96.21 ±0.77b	White thick
D-6	2	1	1	73.15 ±2.12a	98.31 ±0.37a	Yellow green thick

Note:The letters in the table indicate significant differences at the 0.05 level.

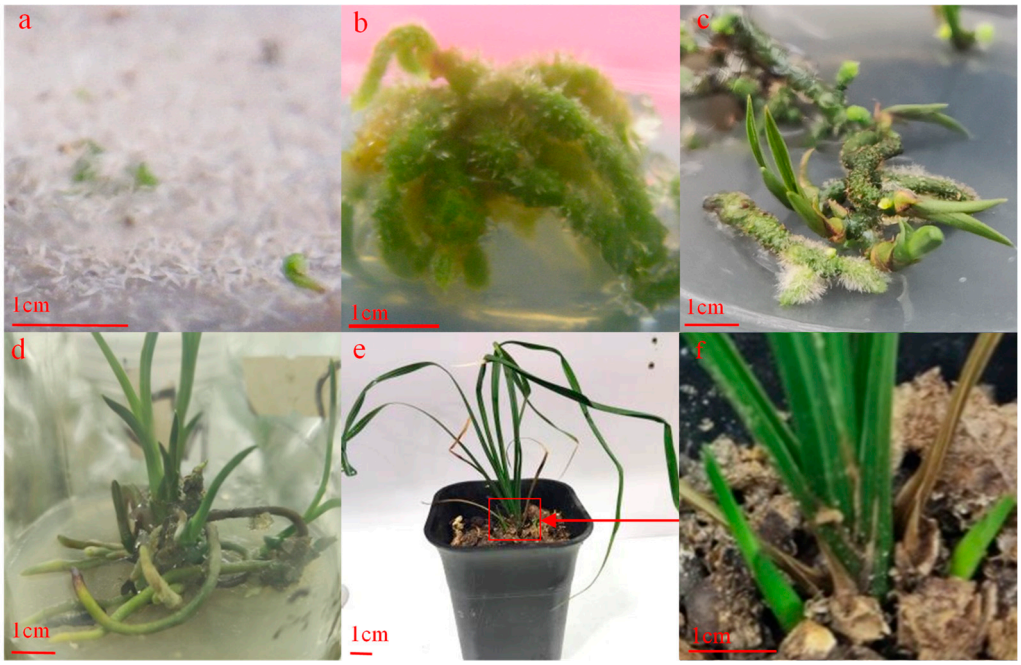

Figure 1. *Cymbidium faberi Rolfe* fast propagation system construction. (a) Sprouting PLBs from *Cymbidium faberi Rolfe* seeds; (b) PLBs expand and begin to multiply; (c) PLBs differentiate into shoots and begin to form seedlings; (d) induce rooting; (e) One month after transplantation; (f) new shoots grow.

3.3. Mutagenesis of PLBs by Low Concentration of NaN_3

In orchid mutagenesis breeding, most researchers use the 50% lethal dose (LD_{50}) as the optimal mutagenic dose. However, there are also cases where the mutagenic effect could not be evaluated with the half lethal dose, and after orchid rhizome mutagenic treatment, sometimes materials treated with different doses survived but growth stagnated, and the use of the half reduction dose (the 50% reduction dose, RD_{50}) as the optimal mutagenic dose has been reported.

When rhizomes were used as explants for mutagenesis (Table 4), rhizome lethality was the highest under 3 mg/L NaN_3 treatment, and although it did not reach half lethality, the differentiation rate was significantly reduced, and the mutagenesis efficiency was low, which made it unsuitable for increasing the concentration of NaN_3 any further. With the increase of 0.5-2.5 mg/L NaN_3 concentration, the mortality rate of rhizomes increased and the differentiation rate decreased significantly. The increase of NaN_3 concentration may inhibit the differentiation of some rhizomes into buds, which is not conducive to the formation of mutant buds. The differentiation rate of rhizomes of 1.5 mg/L NaN_3 was 49.81%, and the amount of rhizome differentiation was close to half of the reduction dose, so it was finally determined that 1.5 mg/L NaN_3 was the most suitable mutagenic concentration for rhizomes as explants.

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Mutant buds in this experiment are celandine buds that are significantly different in appearance from normal celandine buds (Figure 2). There are gold-line striped leaves, yellow-green leaves, albino leaves, spiral leaves and dwarf seedlings.

Figure 2. Cymbidium faberi Rolfe partially mutated plants. (a) Leaf mesophyll penetrating; (b) Leaf veins yellow-green interspersed; (c) Leaf blades curled up; (d) Dwarfed seedlings; (e-h) Mutagenized early flowering.

Table 4. Effect of low concentration NaN_3 on the mutagenesis of *Cymbidium faberi* Rolfe rhizome regeneration buds.

Treatments	NaN_3 (mg/L)	Rhizome mortality (%)	Differentiation rate (%)
E-1	0.0	8.33±0.41d	80.56±0.24a
E-2	0.5	16.67±0.41c	71.17±0.28b
E-3	1.0	19.44±0.48c	58.27±0.34c
E-4	1.5	36.11±0.63b	49.81±0.52d
E-5	2.0	41.67±0.72ab	41.34±0.14e
E-6	2.5	48.61±0.24a	26.28±0.40f
E-7	3.0	48.33±0.41d	10.68±0.40g

Note:The letters in the table indicate significant differences at the 0.05 level.

3.4. Mutagenesis of Shoots by High Concentration of NaN_3

Rhizome-induced buds were used as explants for mutagenesis. The degree of differentiation of buds is relatively high, and the mutagenesis efficiency of low concentrations on already differentiated buds is low, so the selected NaN_3 concentrations are relatively high. As shown in Table 5, the mortality rate of *Cymbidium faberi* Rolfe buds was very high when co-cultured with high concentration of NaN_3. All the shoots treated with 10 mg·$L^{-1}NaN_3$ died, and although some shoots treated with 9 mg·$L^{-1}NaN_3$ survived, their growth was almost stagnant, and the leaf growth was zero in 60 days. Under the treatment of 4~5 mg·$L^{-1}NaN_3$, the *Cymbidium faberi* Rolfe buds were close to the half-lethal dose, among which the leaf growth of 4 mg·$L^{-1}NaN_3$ treatment was close to half of the control group, and the damage to *Cymbidium faberi* Rolfe buds was relatively small, which was the critical point that *Cymbidium faberi* Rolfe buds could withstand for in vitro mutagenesis. Therefore, when using buds as mutagenic materials, 4 mg·$L^{-1}NaN_3$ is the optimal mutagenic concentration.

Table 5. Effect of high concentration of NaN_3 on mutagenesis of *Cymbidium faberi Rolfe* buds.

Treatment	NaN_3(mg/L)	Leaf growth length (mm)	Buds mortality rate (%)
F-1	0.0	16.33±0.88a	0
F-2	4.0	6.04±0.85b	48.61±0.24d

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F-3	5.0	4.38±0.25c	52.78±0.24d
F-4	6.0	3.38±0.33d	66.67±0.42c
F-5	7.0	1.42±0.19e	80.56±1.04b
F-6	8.0	0.96±0.38e	84.72±0.24b
F-7	9.0	0±0f	93.06±0.24a
F-8	10.0	0±0f	1±0a

Note:The letters in the table indicate significant differences at the 0.05 level.

3.5. NaN_3 Promotes Flowering in Cymbidium faberi Rolfe

We found that some of the NaN3-treated PLBs rapidly stepped into flowering after shooting, and some of the material showed early flowering during the co-culture of *Cymbidium faberi Rolfe* shoots with NaN_3 (Figure 2.e-h). Flower shape varies. Some flowers have a normal shape, while others have small or curled petals. The flower color is mostly light green, with some being purple. However, the flowering time is earlier than normal.

4. Discussion

Our results suggest that NaN_3 is effective as a chemical mutagen in the in vitro mutagenesis of *Cymbidium faberi Rolfe*. The viability of *Cymbidium faberi Rolfe* tissues and the mutagenesis rate are affected by NaN_3 concentration due to cytogenetic aberrations and physiological instability (Sato and Gaul, 1967), as well as destabilization between growth regulators and promoter inhibitors (Maherchandani, 1975). An increase in the mutagen concentration increases the mutation rate but causes a decrease in the survival rate of the mutagenized tissues. The addition of low concentrations of NaN_3 results in higher survival rates but also reduces the mutation rate. Therefore, finding a balance between survival and mutation rates and determining the optimal mutagen concentration is crucial for successful mutagenesis. It is common practice to use mutagen concentrations that cause 30% to 50% mortality in plant tissues. Determining the appropriate mutagenic concentrations for different plants or tissues is necessary. The study screened PLBs and shoots of *Cymbidium faberi Rolfe* for mutagenic concentration. The most suitable concentration of NaN3 was found to be 1.5 mg/L when PLBs were used as the mutagenic material, and 9.0 mg/L when shoots were used, while maintaining a reasonable survival rate of the plants.

The Orchidaceae family is the second largest family of flowering plants, with over 27,000 species found in almost all habitats worldwide. The plant is highly valued in the global horticultural market because of its delicate appearance and ecological diversity (Chen, J. T. and Nargar, 2020). *Cymbidium faberi Rolfe* is an important ornamental plant, flowering is a crucial life stage, with most plants blooming once a year, only a few plants bloom in more than one season, and *Cymbidium faberi Rolfe* is particularly challenging to bloom. Typically, new seedlings require over three years to accumulate nutrients, produce flower shoots, and undergo five months of vernalization and dormancy before blooming. In this study, *Cymbidium faberi Rolfe* showed early flowering after NaN3 mutagenesis, which greatly shortened the time required for *Cymbidium faberi Rolfe* to flower, and significantly enhanced its ornamental and economic value.

It was demonstrated that in Spathoglotis plicata, NaN3 produced changes in flower color (Supriya and Amal, 2005). In Browalia speciosa, NaN3 induced changes in flower color, flower shape and leaf shape (El-Mokadem and Mostafa, 2014). In the present study, NaN3 also affected the flower shape, flowering period and color of *Cymbidium faberi Rolfe* flowers. Plant flowering, the transition from nutrient growth to reproductive growth (Chen, D. J. et al., 2018), is a key event in the plant life cycle, and NaN3 caused some *Cymbidium faberi Rolfe* to flower prematurely, and it is speculated that sodium azide, as a respiratory inhibitor, and its azide anion is also a potent proton pump inhibitor (Kleinhofs et al., 1978), which can alter the mitochondrial membrane potential (Zhang, L. et al., 2000), blocking the electron transfer (Vercellino and Sazanov, 2022), leading to a ATP shortage (Naaz et al., 2023), inhibiting nutrient growth and stressing the plant's transition to reproductive growth, causing *Cymbidium faberi Rolfe* to show early flowering.

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In addition, the flowering period is the most critical transition period in the entire life cycle of the plant and is a highly sensitive period to external stresses (Shi et al., 2022). It has been proposed that due to the ability of plants to regulate their own growth and developmental processes in response to changes in the external environment, many plants flower early when they are subjected to unfavorable conditions, such as abiotic stresses such as drought (Sherrard and Maherali, 2006), high salinity (Kolar and Senkova, 2008) and low temperature (Hatayama and Takeno, 2003). This phenomenon is known as "adversity-induced flowering" (Zhang et al., 2016). Environmental stresses can stimulate and regulate gene networks and promote the formation of flower shoots (Song et al., 2013; Verhage et al., 2014), which have an impact on the flowering process of plants. NaN3 can also be regarded as an external stress to *Cymbidium faberi Rolfe*, and it may be that this stress stimulates *Cymbidium faberi Rolfe* to enter the flowering stage earlier.

Cymbidium faberi Rolfe cultured by NaN3 mutagenesis not only showed changes in flowering period, but also brought new variations in flower shape. In the future, it is also possible to change the flower color by adjusting the ph of the medium and the endogenous hormone of the plant, which is of great significance for the cultivation of ornamental plants and the development of the market.

5. Conclusions

In this study, we established a regeneration system for *Cymbidium faberi Rolfe*, induced the formation of PLBs material, and mutagenized it by using NaN_3 to produce mutant plants with predominantly leaf shape variation, and some of them showed test-tube flowering after incubation with added NaN_3. This is the first study on mutagenesis culture of *Cymbidium faberi Rolfe*, and it has produced commercially popular medium translucent, tiger-spotted, and dwarf varieties, which also affected the flower shape and flowering time of *Cymbidium faberi Rolfe*, with the color mostly yellow-green, with some individual labellums with violet color, and in addition, the flowering time has also been advanced. Rarity is precious, because *Cymbidium faberi Rolfe* is mostly yellow-green flowers, once a different color or petal type, it will be highly favored to meet the people's demand for *Cymbidium faberi Rolfe*'s ornamental needs, and greatly improve the economic value of *Cymbidium faberi Rolfe*. This paper also provides ideas for flowering regulation that will help in the future development of flowering time regulation in plants.

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