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RESEARCH ARTICLE

Study on the Polyploidy Induction of Herbicides on Hengshan Astragalus Membranaceus

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Abstract: Studying medicinal polyploid plants is of great significance for the cultivation of traditional Chinese medicine varieties and the extraction of active ingredients. The polyploidization of *A. membranaceus* (*Astragalus membranaceus*) was induced using two treatment methods: oryzalin and pendimethalin as mutagens. The results showed that in the same method, the induction effect of polyploidy was affected by the concentration of reagents and treatment time; different concentrations and treatment time combinations resulted in mutant plants. The soaking method with an oryzalin concentration of 90μmol/L and a treatment time of 36h resulted in the best induction effect, with a morphological variation rate of 36.96%; that with a pendimethalin concentration of 2000μmol/L and a treatment time of 36h resulted in the best induction effect, with a morphological variation rate of 35.85%. The tissue culture method with an oryzalin concentration of 90μmol/L and a treatment time of 14d resulted in the best induction effect, with a mutation rate of 39.13%; that with a pendimethalin concentration of 1500μmol/L and a treatment time of 7d resulted in the best induction effect, with a mutation rate of 42.31%. Through ploidy identification, the mutant plants conform to the characteristics of polyploid plants.

Keywords: Hengshan *Astragalus membranaceus* (Fisch.) Bunge, Herbicide, Mutagenesis, Polyploid Identification

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Introduction

Astragalus membranaceus, also known as Huangqi, Yuanqi or Beiqi, is the dried root of the Astragalus membranaceus (Fisch.) Bunge. Or Astragalu smembranaceusvar. Mongholicus (Bunge) P.K. Hsiao [1]. There are several types of Astragalus membranaceus (Fisch.) Bunge with the best produced in Shanxi and Mongolia." indicated in Illustrated Catalogue of Plants. Shanxi A. membranaceus is mainly produced in the southern mountainous areas of Hunyuan County and adjacent areas (mainly Datong and Xinzhou in Shanxi Province) in the northern Hengshan Mountains. Hengshan A. membranaceus has been recognized by traditional Chinese medicine practitioners for its long history, excellent quality, and unique therapeutic effects [2]. Hengshan A. membranaceus (also known as Zhengbei Astragalus) belongs to Astragalus membranaceusvar. Mongholicus (Bunge) P.K. Hsiao [3]. It is associated with weeds and shrubs throughout its life, and has strong resistance to stress such as competition, disease resistance, and drought resistance [4]. The excellent characteristics of the germplasm of Hengshan A. membranaceus make its plants have features such as smooth and thick roots, bright color and strong taste,

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high oiliness, sufficient powder, and strong medicinal properties [5, 6]. Metabolomics analysis found that the content of aspartic acid, γ- aminobutyric acid, citric acid, astragaloside IV, and flavonol glucoside in Hengshan A. membranaceus is relatively high, and it is listed as a "high-quality" authentic medicinal herb [7].

With continuous research on the pharmacological effects of A. membranaceus and the development of related products, the market demand for A. membranaceus has been increasing. Through a large number of literatures related to A. membranaceus, Guo et al. found that since the 21st century, the anti-tumor effect of A. membranaceus' has made continuous progress [8]. Especially the anti-tumor effects of A. membranaceus injection, A. membranaceus polysaccharides has promoted the discovery and application of new pharmacological effects of A. membranaceus' anti-tumor effects and enhanced the potential economic value of Astragalus membranaceus resources. The polysaccharides in A. membranaceus of Hengshan have good antioxidant activity and are natural antioxidant substances with development potential. However, due to excessive land use and development, changes in the ecological environment, and large-scale mining, wild A. membranaceus resources are becoming increasingly scarce. Currently, most of A. membranaceus on the market are semi wild cultivated species [9, 10]. Half wild cultivated species, wild varieties, and artificially cultivated varieties are mixed and circulated in the market, resulting in mixed germplasm, decreased quality of cultivated medicinal materials, and severely unstable content of active ingredients. Based on the investigation of the origin of A. membranaceus seeds, Song et al. summarized the current situation of A. membranaceus resource identification and new variety breeding [11]. It lacks unified norms and standards for Hengshan A. membranaceus. The entry of inferior and counterfeit seeds of Mongolian A. membranaceus into the market has caused chaos in the seed market, affecting the yield and quality of cultivated A. membranaceus medicinal materials. The common method to solve the problem of germplasm mixture is to cultivate new superior varieties using the current germplasm in the original medicinal material production areas. The commonly used cultivation method is to double its chromosomes to an increase in yield and income, increase the content of biological activity and pharmacological components, and provide comments on newly added literature.

The principle of this cultivation method is that polyploidy refers to the presence of three or more sets of chromosomes in plants. Due to changes in genetic material, polyploid plants undergo various biological characteristics such as large morphology, strong stress resistance, high bioactive components but low seed setting rate. Polyploidy is prevalent in the biological world, especially in the plant kingdom. Polyploid variations are commonly found in the 147 polyphyletic Chinese medicinal materials recorded in the Chinese Pharmacopoeia. The polyploid morphology of medicinal plants can promote increased yield and income, with higher levels of biological activity and pharmacological components. They have strong resistance and are not suitable for lodging, but are more tolerant to drought and have strong adaptability to the environment. The phenomenon of polyploidy is mostly caused by two main pathways: natural occurrence and artificial induction. The number of natural medicinal polyploids generated by spontaneous doubling is limited and requires a long period of natural selection and adaptation, which cannot meet the breeding needs of medicinal plant polyploids. Chemical reagent induced polyploidization is currently a highly researched, effective, and convenient method for producing plant polyploids. Therefore, artificial induction methods are often used to induce suitable experimental materials, such as germinating seeds, callus tissue, and stem tip tissue, to obtain selectable polyploids. EMS and colchicine are the most widely used mutagens, which have been practiced on species such as wheat, chrysanthemum, wild ginseng, saffron, and drunken fish grass [12-16]. However, recent studies have found that EMS and colchicine are highly toxic, and their safety needs to be considered in practical applications, and cannot be used as mutagens in experiments. Dinitroaniline herbicides can also induce chromosome doubling in plants, with low toxicity, high efficiency, and low cost during induction. They have slowly replaced colchicine as the preferred inducer, and have achieved some success in plants such as watermelon, azalea, wheat, and pear [19-21]. Oryzalin and pendimethalin are herbicides belonging to the dinitroaniline class. Shao et al. have shown that oryzalin can achieve good induction effects with only one percent of the concentration of colchicine [17]. Zhang et al. successfully doubled the induction of Lanzhou lily by using oryzalin, pendimethalin and other mutagens [22]. Zhao studied the vitro induction of Fengwei tea using oryzalin, and EMS [23]. Both studies have confirmed the efficiency and stability of amisulfenolin and pendimethalin inducing polyploidy ofplants compared with traditional induction methods.

The research and development of polyploid induction technology for A. membranaceus has been slow. Zhang and Li used the seeds of A. membranaceusvar. mongholicus and the upper embryos of its leaves as materials to induce polyploidy through tissue culture method using colchicine reagent [24]. It was found that all combinations can induce the production of polyploid plants. When using seeds as materials and treating with a concentration of 100mg/L for 14d, the highest induction rate reached 13.3%. When using the upper embryo of the leaves of the ribbon as the material and treating it with a concentration of 100mg/L for 7d, the highest induction rate reached 10.9%. This experiment used A. membranaceus seeds as materials

and oryzalin and pendimethalin as mutagens to study the mutagenic effects of different treatment methods, mutagen concentrations, and treatment times on Hengshan A. membranaceus seeds. Through morphological, stomatal size, and cytological analysis, the polyploidy of the induced plants was identified to explore the optimal conditions for inducing polyploidy of the seeds of Hengshan A. membranaceus using two herbicides. The aim was to obtain polyploid plants with well-developed roots, enlarged leaves, and short and plump plant types, providing a reference for the development of polyploidy induction techniques for Hengshan A. membranaceus in the future.

Materials and Methods

Experimental Materials

Seeds of Hengshan A. membranaceus (Shanxi BeiyueShenqi Biotechnology Co., Ltd); oryzalin (Shanghai McLean Company, reagents are in powder form); pendimethalin (Shandong Binnong Technology Co., Ltd., reagents are emulsion); dimethyl sulfoxide (DMSO) (China National Pharmaceutical Group Chemical Reagent Co., Ltd).

Seed germination treatment: Full and uniform seeds of Hengshan A. membranaceus were selected and soaked in concentrated sulfuric acid for 10 min, and rinsed with distilled water for 10-20 min [24].

Experimental Methods

Induction of Polyploidy by Soaking Methods

The effect of different concentrations and treatment times of oryzalin on the doubling of the seeds of Hengshan A. membranaceus: an appropriate amount of oryzalin was taken and dissolved in dimethyl sulfoxide, and diluted it with sterile water gradient to obtain the required concentration for the experiment. The germinated seeds were soaked in oryzalin solutions at the concentrations of 60μmol/L, 90μmol/L, and 120μmol/L respectively for 24h, 36h, and 48h for shaking cultivation at 25 ℃, taking a 5% dimethyl sulfoxide solution for a control. Each group was repeated 3 times, with 40 seeds per repetition. The treated seeds were washed clean with running water and cultivated at 25℃. After 7d of cultivation, the germination status of the seeds was counted and the germination rate was calculated. After 10d of cultivation, plants with significant differences in buds between the experimental group and the control group were considered as morphological variation plants. The morphological variation plants were counted and the variation rate was calculated.

The effect of different concentrations and treatment times of pendimethalin on the doubling of the seeds of Hengshan A. membranaceus: An appropriate amount of pendimethalin reagent was taken, adding sterile water to prepare a suitable concentration of mother liquor for later use. The germinated seeds were separately soaked in pendimethalin solutions at the concentrations of 1800μmol/L, 2000μmol/L, and 2200μmol/L respectively for 24h, 36h, and 48h for shaking cultivation at 25℃, taking distilled water treatment as a control. Each group was repeated 3 times, with 40 seeds per repetition. The processed seeds were washed clean with running water and cultivated at 25℃. After 7d of cultivation, the germination status of the seeds was counted and the germination rate was calculated. After 10d of cultivation, plants with significant differences in buds between the experimental group and the control group were considered as morphological variation plants. The morphological variation plants were counted and the variation rate was calculated.

Germination rate=number of germinated seeds/number of treated seeds×100%

Morphological variation rate=number of morphological variation plants/number of sprouts×100%

Polyploidy Induction Using the Tissue Culture Methods

The effect of different concentrations and treatment times of oryzalin on the doubling of the seeds of Hengshan A. membranaceus: an appropriate amount of oryzalin was weighed and dissolved it in a small amount of dimethyl sulfoxide. A suitable concentration of mother liquor was prepared with sterile water and diluted in a gradient to obtain the required concentration for the experiment, autoclave sterilization before use. Mature and plump seeds of A. membranaceus were selected and soaked in 70% alcohol for 10s, and disinfect with 0.1% mercuric chloride solution for 10min. During this process, they were shaked evenly, and disinfected and rinsed the seeds three times with sterile water. The processed seeds were inoculated into 1/2MS medium containing different concentrations (0μmol/L, 60μmol/L, 90μmol/L, 120μmol/L) of sulfasalazine reagent, and transferred them to normal MS medium for cultivation after 7d, 14d, and 21d, respectively.

The effect of different concentrations and treatment times of pendimethalin on the doubling of the seeds of Hengshan A. membranaceus: An appropriate amount of pendimethalin emulsion was taken to prepare a suitable concentration of mother liquor, and diluted in a gradient to obtain the required concentration of 0.22μm filter membrane for the experiment. After filtering and sterilizing, it was added into a 1/2MS medium sterilized by autoclave sterilization for later use. Mature and plump seeds of Hengshan A. membranaceus were selected and soaked in 70% alcohol for 10s, disinfected with 0.1% mercuric solution for 10 minutes, and rinsed four to five times with distilled water. The seeds were inoculated into 1/2MS medium of different concentrations (0μmol/L, 1800μmol/L, 2000μmol/L, 2200μmol/L) of dimethoate, treated for 7d, 14d, and 21d respectively, and then transferred to normal MS medium for cultivation.

All culture media had a pH of 5.8-6.0 and were sterilized under high-pressure at 121℃ for 15 min. The cultivation temperature was 25±2℃ and illuminated for 12h. Each group was treated three times, with 15 seeds per repetition. Normal MS media were used after mutagenesis. Morphological analysis was conducted on the young buds in the experimental group and the control group. Plants with short and strong plant types and enlarged leaves were identified as mutant plants, and the number of morphological mutant plants was counted and the mutation rate was calculated. After the mutagenesis was completed and replaced with normal MS medium for 7d, the survival rate was recorded.

Germination rate = number of germinated seeds/number of treated seeds × 100%

Morphological variation rate = number of morphological variation plants/number of sprouts × 100%

Survival rate = number of survivors/number of sprouts × 100%

These equations are calculated.

Identification Methods of Ploidy

Morphological identification: After 45d of cultivation, the morphological characteristics of the mutant plants in each group and the control group were observed. 30 plants of each group were selected to measure their plant heights with a tape measure, and measure their leaf lengths and widths with a rule. The average plant height and leaf width were taken.

Identification of stomatal characteristics: After 45d of cultivation, leaf stomata were observed in each group of plants. 10 mutant plants from the control group and 10 mutant plants from the treatment group were selected to make leaf epidermis slices. 3 leaves from each sample were taken to observe each leaf 3 times, and take the average. Their images were captured under a 40x microscope.

Chromosome identification: When the embryonic root grows to 1-3 cm, it was cut and about 5mm of the root-tip was pre-treated with a surgical knife. The root-tip was then dissociated in 1mol/L hydrochloric acid solution at 40℃ for 13 min and stained with an improved phenol fuchsin staining solution. After staining, the sample was compressed, examined under a microscope, identified, and photographed.

Data processing method: SPSS software was used for variance analysis on the raw data, and Microsoft Excel 2021 was used to create statistical charts.

Present the intention based on the experimental method (as shown in Fig. 1).

Results and Analysis

The Effects of Two Herbicides on the Seeds of A. Membranaceus Using the Soaking Method

Compared with the control group, oryzalin reagent can promote germination to a certain extent at low concentrations and short-term treatments. As the concentration of oryzalin gradually increased, the germination rate of Hengshan A. membranaceus decreased. The appropriate concentration of mutagen was determined using the half lethal dose as a reference [25]. When the concentration of oryzalin reagent was 90μmol/L, the germination rate of Hengshsan A. membranaceus can reach the half lethal concentration. Different concentrations and treatment times of oryzalin reagent can cause a certain degree of variation of Hengshan A. membranaceus seeds. Taking morphological variation as a preliminary statistical indicator, under the same treatment time, as the concentration of oryzalin reagent induced mutation increased, the morphological variation rate increased. At the same treatment concentration, as the treatment time increased, the morphological variation rate actually decreased (as shown in Table 1).

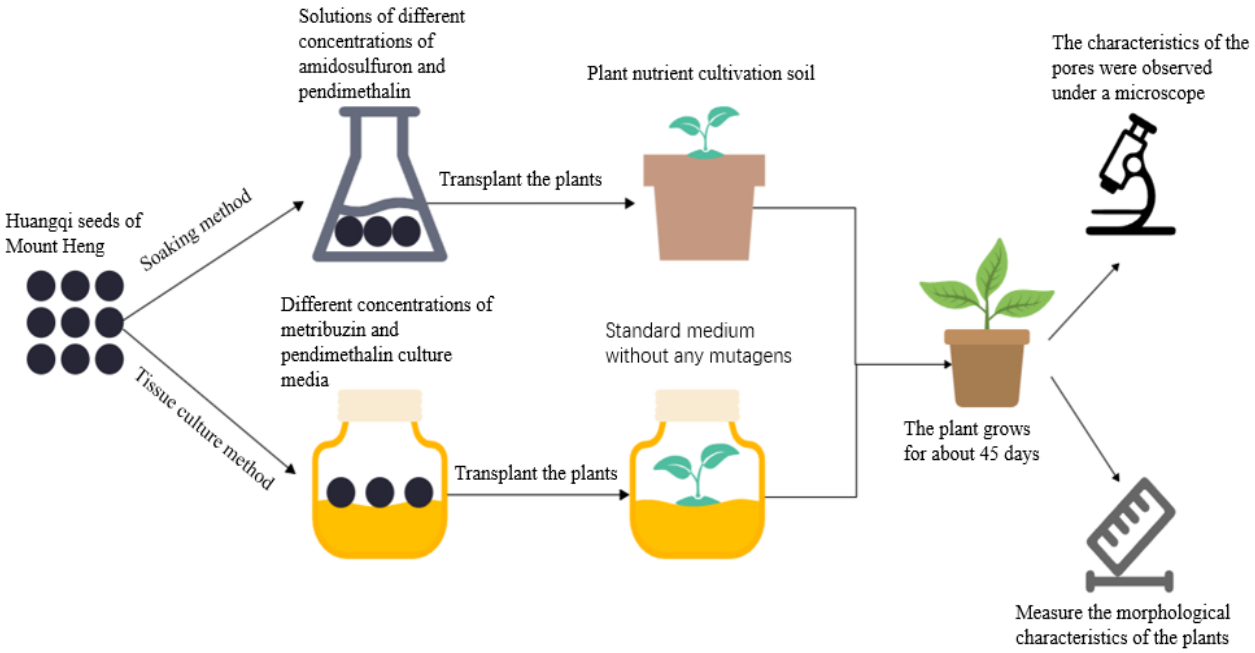

Fig. 1: Flowchart of the research method in this study

Table 1: The effect of oryzalin on the induction rate of Hengshan A. membranaceus using the soaking method

Concentrations (μmol/L)	Treatment time (h)	Inoculated Number (Grain)	Germination number (Grain)	Germination Rate (%)	Morphological Variation (Grain)	Rate of Morphological Variation (%)
0	24	120	70	58.33ab	0	0
0	36	120	86	71.67a		
0	48	120	84	70.00a		
60	24	120	74	61.67ab	18	24.32b
90		120	59	49.17b	21	35.59a
120		120	45	37.50c	14	31.11ab
60	36	120	77	64.17a	26	33.76a
90		120	46	38.33c	17	36.96a
120		120	24	20.00cd	7	29.17ab
60	48	120	71	59.17ab	23	32.37a
90		120	40	33.34c	13	32.50a
120		120	22	18.33db	5	27.78b

Note: Different lowercase letters indicate significant differences between treatments at 0.05 level, the same below

Compared with the control group, the germination rate of Hengshan A. membranaceus seeds decreased with the gradual increase of the concentration of pendimethalin reagent. At a concentration of 2000μmol/L, the germination rate of Hengshan

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A. membranaceus seeds reached the semi-lethal concentration. Different concentrations and treatment times of pendimethalin can cause a certain degree of variation in the seeds of Hengshan A. membranaceus. Under the same treatment time, as the concentration of pendimethalin reagent induced mutation increased, the rate of morphological variation also increased. At the same treatment concentration, as the treatment time increased, the morphological variation rate actually decreased (as shown in Table 2).

Table 2: The effect of pendimethalin on the induction rate of Hengshan A. membranaceus using the soaking method

Concentrations (μmol/L)	Treatment Time (h)	Inoculated Number (Grain)	Germination Number (Grain)	Germination Rate (%)	Morphological Variation (Grain)	Rate Of Morphological Variation (%)
0	24	120	74	61.67ab	0	0
0	36	120	83	69.17a		
0	48	120	85	70.83a		
1800	24	120	62	51.67c	12	19.35cd
2000		120	54	45.00cd	18	33.33ab
2200		120	47	39.17e	10	21.28c
1800	36	120	50	41.67ed	11	22.00c
2000		120	43	35.83e	16	37.21a
2200		120	36	30.00f	10	27.78bc
1800	48	120	39	32.50ef	8	20.51c
2000		120	37	30.83f	12	32.43ab
2200		120	22	18.33g	6	27.28bc

The Effects of Two Herbicides on the Seeds of Hengshan a. Membranaceus Using the Tissue Culture Method

Compared with the control group, different concentrations of oryzalin and different treatment times have a certain inhibitory effect on the germination of Hengshan A. membranaceus seeds. Under the same treatment time, as the concentration of oryzalin reagent increased, the morphological variation first increases and then decreased. As the treatment time increased, at the same concentration of sulfasalazine reagent, the morphological variation first increased and then decreased. After the induction of plants using the tissue culture method, it was found that with the extension of treatment time, there was a significant degree of root rot and damage. Statistical analysis of its survival rate revealed that as the treatment time increased, the survival rate of A. membranaceus seedlings significantly decreased. At high concentration of oryzalin and longer treatment time, the survival rate significantly decreased (as shown in Table 3).

Table 3: The effect of oryzalin on the induction rate of Hengshan A. membranaceus using the tissue culture method

Concentrations	Treatment time	Inoculated number	Germination number	Germination rate	Morphological variation	Rate of morphological variation	Concentrations	Treatment time
(μmol/L	(h)	(Grain)	(Grain)	(%)	(Grain)	(%)	(μmol/L)	(h)
	7	45	37	82.22a	0	0	37	100.00a
0	14	45	34	75.56ab	0	0	34	100.00a
	21	45	33	73.34ab	0	0	32	96.97a
60		45	37	82.22a	8	21.62bc	34	91.89ab
90	7	45	28	62.22c	8	28.57b	22	78.57c
120		45	23	51.11d	7	30.43ab	17	73.91cd
60		45	30	66.67bc	10	33.33ab	21	70.00cd
90	14	45	23	51.11d	9	39.13a	12	52.17e
120		45	18	40.00e	5	27.78c	10	55.56e
60		45	26	57.78cd	7	26.92c	10	38.46f
90	21	45	17	37.78e	7	41.17a	4	23.52g
120		45	14	31.11ef	4	25.57cd	3	21.43g

Compared with the control group, under the same treatment time, the rate of morphological variation increased as the concentration of pendimethalin reagent increased. When the concentration reached 2000 μmol/L (the semi lethal concentration obtained by immersion method), the morphological variation rate of the treatment group significantly decreased. At the same concentration of oryzalin reagent, as the treatment time increased, the rate of morphological variation increased. Through statistical analysis of survival rate, it was found that prolonged treatment time has a significant impact on the survival rate of A. membranaceus seedlings. At the same time, at high concentration of oryzalin and longer treatment time, the survival rate significantly decreased (as shown in Table 4).

Table 4: The effect of pendimethalin on the induction rate of Hengshan A. membranaceus using the tissue culture method

Concentrations	Treatment time	Inoculated number	Germination number	Germination rate	Morphological variation	Rate of morphological variation	Concentrations	Treatment time
(μmol/L)	(d)	(Grain)	(Grain)	(%)	(Grain)	(%)	(μmol/L)	(h)
	7	45	35	77.78ab	0	0.00	34	97.17a
0	14	45	34	75.56b	0	0.00	34	100.00a
	21	45	34	75.56b	0	0.00	34	100.00a
500		45	37	82.22a	10	27.03d	34	91.89ab
1000		45	30	66.70bc	14	46.67ab	30	100.00a
	7							
1500		45	26	57.80c	11	42.31b	20	76.92c
2000		45	23	51.10cd	10	43.48b	16	69.57d
500		45	31	68.90b	12	38.71bc	26	83.87bc
	14							
1000		45	30	66.70b	12	40.00b	17	56.67e

1500		45	29	64.40bcd	16	55.17a	13	44.83f
2000		45	25	55.60c	7	28.00d	10	40.00f
500		45	29	64.40b	11	37.93c	10	34.48g
1000		45	25	55.60c	10	40.00b	7	28.00h
	21							
1500		45	23	51.10cd	9	39.13bc	8	34.78g
2000		45	20	44.40d	6	30.00c	4	20.00h

Identification of Polyploids

Identification of Chromosome

10% of the morphological mutant plants induced by two herbicides was randomly selected for root-tip chromosome slice analysis. As a result, the majority of root-tip chromosomes in plants with morphological variation after treatment with oryzalin doubled in number, exhibiting tetraploid (2n = 4x = 32) chromosome characteristics (as shown in Fig. 2). The number of root-tip chromosomes in a small number of mutant plants did not change and remained diploid (2n = 2x = 16) (as shown in Fig. 2).

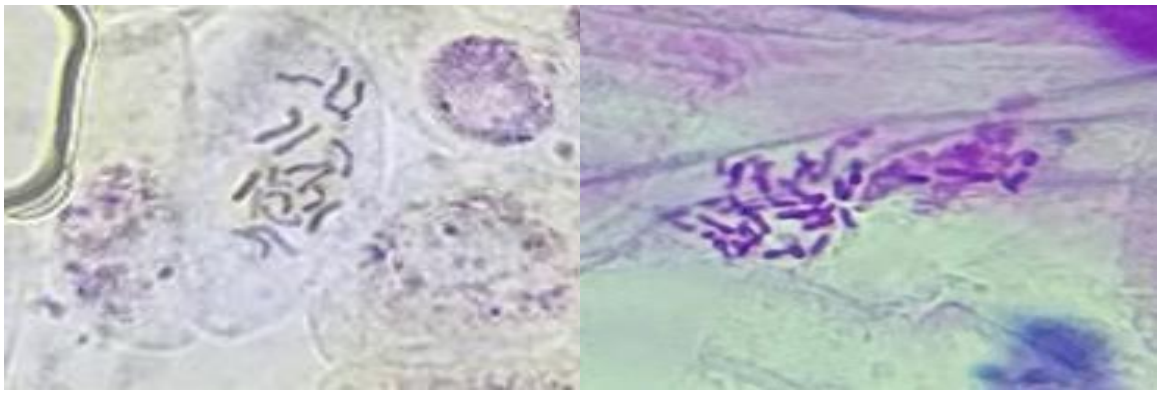

Fig. 2: Chromosomes of normal diploid (2n = 2x = 16) and mutant tetraploid plants (4n = 4x = 32) under 100x magnification

Morphological Identification

30 mutant plants and normal plants were randomly selected for morphological observation. It was found that the mutant plants had significant differences in plant height, leaf length, and leaf width compared to normal plants (as shown in Table 5). The mutant plants had shorter plant types, larger leaves and slower growth, consistent with the morphological characteristics of polyploid plants (as shown in Figs. 3, 4, and 5).

Table 5: Morphological measurement indicators of mutant plants induced by two herbicides and normal plants

	Normal plants	Plant height (cm)	Leaf length (mm)	Leaf width (mm)
		10.46±0.66a	8.74±0.24a	5.46±0. 19a
Pendimethalin	Mutant plants	6.08±0.27b	11.06±0.60b	6.48±0. 18b
	Normal plants	9.82±0.71a	9. 16±0. 17a	7.24±0.28a
Oryzalin	Mutant plants	5.30±0.74b	12.02±0.23b	8.78±0.74a

Note: The data is the mean ± standard deviation, and different lowercase letters indicate significant differences between treatments at the 0.05 level, the same below

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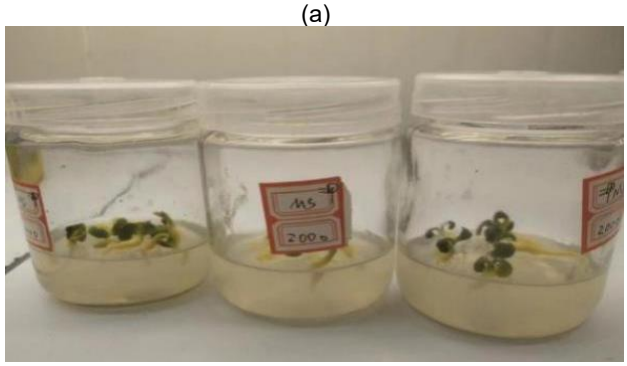
Fig. 3: Normal and variant plants treated by soaking method

Fig. 4: Normal and variant plants cultivated for a period of time

(a)

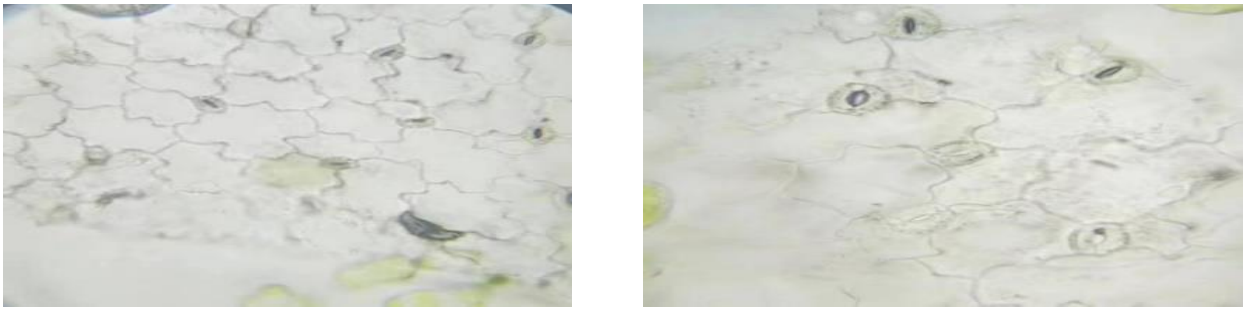


(b)

Fig. 5: Normal plants in the control group (a) and mutant plants in the experimental group (b) using the tissue culture method

Identification of Stomatal Characteristics

The leaf slices of randomly selected mutant plants were observed to measure the length and width of stomata. Comparing with the leaves of normal plants in the control group, there was a significant difference in stomatal size between tetraploid plants and normal plants (as shown in Table 6). The stomata of the mutant plants significantly increased, and the number of stomata in the same field decreased, which is consistent with the characteristics of polyploid plants (as shown in Fig. 6).



(a) (b)

Fig. 6: Stomatal size and number of mutant plants in the control group (a) and experimental group (b) under a 40x magnification

Table 6: Measurement indicators for identifying stomatal characteristics of mutant and normal plants induced by two herbicides

	Control plants	Stomatal length (μm)	Stomatal width (μm)	Number of stomata/field of view
		5.70±0.31c	3.07±0.33b	15.40±0.51a
Pendimethalin	Mutantplants	6.67±0. 17b	4.50±0.14a	9.00±0.55b
	Control plants	6.20±0. 17c	3.11±0.36b	14.40±0.51a
Oryzalin	Mutant plants	7.95±0.28a	4.60±0.34a	8.20±0.67b

Discussion

This experiment used two chemical reagents and different induction methods to produce polyploid mutant plants with short and strong plant types, slow growth, and enlarged leaves. The induction of polyploidy by chemical reagents is a widely studied, effective, and simple method. The selection of chemical mutagens is crucial. Currently, there are over 200 types of chemical reagents that can induce polyploidization, including ethyl methanesulfonate, colchicine, sodium azide, oryzalin, and pendimethalin [24]. Colchicine is the most widely used and efficient chemical inducer. Colchicine binds to microtubule dimers to prevent the addition of other microtubule proteins, hindering the formation of spindles during cell division and causing chromosomes to double due to the inability to divide evenly between the two poles. Scientists have discovered that some dinitrogen herbicides such as dinitrooryzalin and pendimethalin can also inhibit spindle formation through anti microtubule proteins, leading to chromosome doubling. It has low toxicity, with high induction efficiency at low concentrations. Zhang and Li induced A. membranaceus with colchicine at a concentration of 100mg/L for 14d, and the highest induction rate was 13.3% [24]. In this experiment, the induction rate of oryzalin reagent was 36.96% by the soaking method at a concentration of 90μmol/L (35mg/L) for 36h, and 39.17% by the tissue culture method at a concentration of 90μmol/L (35mg/L) for 14d. The induction rate of pendimethalin reagent by soaking method was 37.21% at a concentration of 2000μmol/L (500mg/L) for 36h, and 55.17% when treated with tissue culture at a concentration of 1500μmol/L (375mg/L) for 14d. The induction rates were significantly higher than those of colchicine, indicating that the herbicide has a high induction efficiency at low concentrations. The herbicide has low cost, simple operation, low toxicity, and high safety. During the mutagenesis process of Lanzhou lily by Zhang et al., the test-tube bulbs of Lanzhou lily were soaked and treated with oryzalin at a concentration of 0.002% for 72 h, and the highest mutagenesis rate was 46.7% [22]. The test-tube bulbs of Lanzhou lily were treated by soaking in pendimethalin at a concentration of 3 mmol/L for 48 h, and the highest mutagenesis rate was 23.3%. During the directional mutagenesis process of Fengwei tea by Zhao, it was found that when the stem tips were soaked in 0.005% oryzalin for 12 h, the maximum mutation rate was 22.81% [23]. When mutagenesis was performed on stem segments in vitro, the maximum

variation rate was 20.69% after soaking in 0.005% oryzalin for 6 h. All of them was different from the optimal induction conditions of the results, which can be related to the selected mutagenic materials such as stem tips and seeds, the varieties of different plants and the treatment methods.

Oryzalin and pendimethalin have a certain effect on the germination rate of Hengshan A. membranaceus seeds. As the concentration and the treatment time increased, the germination rate and survival rate decreased, which is consistent with Li [26]. Liu et al. treated Indian pumpkin seeds with Oryzalin by soaking for 3h, and the mutation rate was as high as 83% at a concentration of 50mg/L [27]. Dong et al. used Oryzalin to treat the seeds of Scutellariabaicalensis by soaking for 72h, and the mutation rate was 24.62% at 90μmol/L [28]. In this experiment, the morphological variation rate of pendimethalin reagent reached 37.21% when treated with a concentration of 90μmol/L for 36h using the soaking method; when treated with a concentration of 2000μmol/L for 36h, the morphological variation rate of pendimethalin reagent reached 39.17%. The thickness of seed coat varies in different seeds, leading to different penetration of mutagens into the seeds, which may be the reason for the differences in induction rates in different experiments.

In the tissue culture method, the concentration of reagents and treatment time have a significant impact on the survival rate of Hengshan A. membranaceus. When the treatment time was 21 days and the culture medium was changed, the seedlings of Hengshan A. membranaceus generally showed root rot. In subsequent experiments, the treatment time should be shortened. When the induction time was 21d and the concentration was 90μmol/L, the induction effect of Oryzalin was significant with a morphological variation rate of 41.17%, but the survival rate was low. When the treatment time was 14d, and the concerntration was 1500 μmol/L, the induction effect of pendimethalin was optimal with a morphological variation rate of 55.1 7%, but the survival rate was low. Referring to the two indicators of induction efficiency and survival rate, the most suitable treatment conditions are: Oryzalin was induced for 14 days at a concentration of 90μmol/L, and pendimethalin was induced for 7d at a concentration of 1500 μmol/L. [22] Zhang et al (2025) used oryzalin and pendimethalin. Through tissue culture, it was found that both reagents had certain influences on the survival rate of the materials, among which pendimethalin had a greater impact, which was basically consistent with the results of this paper.

Comparing the induction rates of two herbicides and their induction methods, it was found that the induction rate of pendimethalin reagent on Hengshan A. membranaceus was higher than that of oryzalin reagent in both methods, but the required concentration of pendimethalin reagent was higher. Comparing the costs of the two reagents and the convenience of the experimental operation process, it was found that the cost of the pendimethalin reagent was lower and the operation process was simple, while oryzalin reagent requires other solvents for dissolution. Therefore, pendimethalin is the best chemical inducer in this experiment.

One of the most reliable methods for identifying ploidy is the root-tip chromosome counting method, which has the disadvantage of being cumbersome to produce and difficult to obtain typical slides [29]. Using a scanning cell photometer for early ploidy identification is expensive and cannot be widely used [30]. Yan et al. used FAD method for early ploidy identification of tetraploid in vitro culture [18]. The FAD method is convenient for observation, but it requires a large amount of work and the process is complex. Zhou et al. identified early ploidy through morphological identification [25]. This experiment identifies the morphological characteristics of processed buds using a simple and low-cost method, which solves the disadvantages of large workload and complex production in the above methods. The obtained mutant plants were found to conform to the characteristics of polyploid plants through ploidy identification. Chimerism is a common problem in plant mutagenesis, and a large number of induced polyploid plants are chimeras. Zhou et al. found that rhododendron seedlings treated with colchicine generally had chromosome chimerism [19]. In this experiment, some plants also showed obvious chimerism. Zhao also mentioned the issue of chimerism on the induction of Fengwei tea, and some of the plants he screened out were also chimerism [23].

Conclusion

In this experiment, oryzalin and pendimethalin were used as chemical mutagens. Polyploid induction of Astragalus Hengshan was carried out through two induction methods, and it was found that pendimethalin had a better effect than oryzalin. The soaking method has a short processing time and is easy to operate. The optimal mutagenesis concentration was 2000μmol/L for 36 h of treatment. Hengshan A. membranaceus, as an authentic medicinal herb, has enormous medical and medicinal value. This experiment provides a reference for the breeding work of A. membranaceus, which can help to a certain extent in the selection and breeding of new varieties and solve the problem of the decline in medicinal material quality

caused by the mixture of germplasm in the current market. It lays a foundation for the subsequent industrial development of A. membranaceus and the selection and breeding of high-quality germplasm.

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Authors Contributions

Jianxia Liu: Responsible for conceptualizing, designing, and writing research papers.

Yanmao Gao: Responsible for organizing and writing the data for the article.

Jiachuan Yang: Participated in the data collection in the experiment.

Lizhen Liu and Liqing Zhou: Revised the format and content of the paper.

Runli He: Reviewed the article draft and contents.

Ethics

The authors declare their responsibility for any ethical issues that may arise after the publication of this manuscript.

Conflict of Interest

The authors declare that they have no competing interests. The corresponding author affirms that all of the authors have read and approved the manuscript.

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