Construction of sterile system of *Buchloe dactyloides* (Nutt.) Engelm.

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Abstract. The stems of male and female strains of Bison grass (Buchloe dactyloides (Nutt.) Engelm.) were taken as explants, and 1 / 2MS and MS medium were used, and different concentrations of NAA, KT and 6-BA were added to study the rapid propagation technology of ferison grass. Results indicated that 75% alcohol was treated for 30s with another 0.1 g · L^{-1}The disinfection effect of mercury solution for 12 min; The MS + 1.5 mg · L-1 NAA + 0.4 mg · L-1 KT female strain showed the best induction effect, The MS + 1.5 mg · L-1 NAA + 0.6 mg · L-1 KT male strain had the best induction effect; 1 / 2 MS + 2 mg + L-1 NAA + 1.5 mg · L-1 6-BA the female and male strains had the best proliferation effect; The highest number of roots and root root rate of 1 / 2 MS + 1.5 mg · L-1 female and male NAA strains were combined.

Key words: Bison grass bird strike against rapid propagation.

1. Introduction

Bison grass (Buchloe dactyloides (Nutt.) Engelm.) Also known as buffalo grass, buffalo grass [1], Is a perennial herb of the genus grass and one of the oldest grasses growing in the American grasslands [2]. Bison grass plants are slender, 5-25 cm tall, with developed stolons and sometimes rhizomes. Monothecious or heteroecious plants. Male inflorescence contains 2 florets, sessile, into two rows closely covered with tile arranged on one side of the ear; female inflorescence contains 1 floret, often 4-5 clusters to form a head [3]. Although buffalo grass is a perennial warm season carbon four plant, it is highly adaptable, grows rapidly, has soft branches and leaves, and has strong competitiveness with weeds [4], A homogeneous lawn can be formed without manual pruning [5]. In the case of severe dry early in 2-3 months, the evaporation amount is much lower than that of other warm season and cold season lawn plants [6]. In the northern region, generally only need to pour green water, frozen water, less pruning times, not easy to occur diseases and insect pests, even if the extensive maintenance and management, but also grow well [7]. Because of its small amount of application, the pollution to the environment is also very little [8]. In the 1940s, China introduced buffalo grass from North America, which was first widely promoted in Gansu [9]In the late 1950s, Beijing and other places, have received good results[10]At present, it has become one of the main grass plants of landscaping in North China, northeast China, northwest China and other regions[9]It is the leading role in ecological restoration, green space construction and difficult sites in China.

Bird strike prevention is an important part of the operation guarantee of the flight area. The root cause of the bird strike disaster in the airport lies in the attraction of birds by the airport environment. Therefore, reducing the ecological factors that attract birds in the airport environment is the fundamental method to solve the bird strike disaster in the airport [11]. Bison grass has low plants and thin leaves, which is not easy to attract birds to nest. There has natural clonal mutations. If all-male or all-female plants can be planted and non-seeding, the harm of airport bird strike may be fundamentally solved by reducing the density of insects and birds in the airport environment.

In this experiment, the stems of male and female strains of buffalo grass were used as explants, and the appropriate medium and hormone ratio concentration were screened to establish and optimize the tissue culture rapid propagation technology system of buffalo grass, so as to provide the operation process and data reference for the tissue culture rapid propagation production, and provide the theoretical basis for the mass production of buffalo grass tissue culture and its application to the lawn management in airports and highways.

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2. Materials and Methods

2.1 Test materials, main reagents and instruments

Male and female buffalo grass plants with segmented stem segments, sterile water, 75% alcohol (Chengdu Cologne Chemicals Co., LTD.), 0.1 g · L-1Mercury-liter solution (Guizhou Sifang Technology Co., LTD.), MS culture medium (Hangzhou Mumu Biotechnology Co., LTD.), 1 / 2 MS medium (Hangzhou Mumu Biotechnology Co., Ltd.), KT (Xi'an Fumar Biotechnology Co., Ltd.), NAA (Xi'an Fumar Biotechnology Co., Ltd.), Sucrose (Chengdu Cologne Chemical Co., Ltd.), Agar (Chengdu Cologne Chemical Co., Ltd.), Ultra-clean table (YJ-875 model of Suzhou Tianqi Purification Technology Co., LTD.), Autoclave pot (Xiamen Zhi Micro Instrument Co., Ltd. GR60DA model), Electronic universal furnace (Tianjin Tester Instrument Co., LTD. DK-98-model), Intelligent light incubator (V2.6 model of Zhejiang Top Yunnong Technology Co., Ltd.).

2.2 Test method

2.2.1 Equipment disinfection

After the disinfection of the ultraviolet lamp in the inoculation room, first wipe the table of the ultra-clean working table with 75% alcohol, then put the disinfectant and inoculation plate needed for implant disinfection and inoculation into the ultra-clean working table, and open the ultraviolet lamp and strong wind of the ultra-clean working table, and turn off the ultraviolet lamp after 20 minutes.

2.2.2 Effects of different disinfection methods on sterilization and growth of explant

The male and female stems of buffalo grass were collected and washed with tap water to remove the surface soil. Cut it at about 0.5 cm before and after the separation interval, and treat it into a small section of about 1.5 cm long, then put the cut stem into a clean beaker, rinse it with tap water, and then disinfect as follows: 75% alcohol for 30 seconds, rinse with sterile water for 3 times, and then use 0.1 g · L-1The mercury solution was disinfected for 8,12 and 16 minutes, and washed with sterile water 5 times. The above three treatment groups were individually inoculated on MS medium with 20 vials of each treatment. The incubation temperature was (25 ± 2) ℃, and the light intensity was 1500-2000 lx, with 12 h · d^{-1}. They were grown for 15d and observed for growth.

2.2.3 Effect of different culture formulations on lateral bud induction of bison grass

The germinated stems of sterile male and female lateral buds were taken as explants and inoculated on the induction media of different formulations (see Table 1), with 30 vials of each treatment. The incubation temperature (25 ± 2) was ℃, first dark culture for two days, then placed in a light intensity of 1500-2000 lx, 12 h · d^{-1}Under the conditions, the cells were cultured for 20d to observe their growth.

Table 1. Induction medium formulation

Processing number	Type of culture medium	NAA (mg·L^{-1})	KT (mg·L^{-1})	saccharose (g·L^{-1})	agal-agal (g·L^{-1})
1	MS	1	0.2	30	20
2	MS	1	0.4	30	20
3	1/2MS	1	0.6	30	20
4	1/2MS	1.5	0.2	30	20
5	MS	1.5	0.4	30	20
6	MS	1.5	0.6	30	20
7	MS	2	0.2	30	20
8	1/2MS	2	0.4	30	20
9	MS	2	0.6	30	20

2.2.4 Effect of different culture formula on bison grass proliferation

Ssterile male and female buds were taken and inoculated onto proliferation media of different formulations (see Table 2), with 30 bottles of each treatment. The incubation temperature (25 ± 2) was ℃, first dark for 5 days, and then placed at 1500 lx for 12 h · d^{-1}Under the conditions, they were cultured for 15d and observed for proliferation.

Table 2. Formulations of the proliferation medium

Processing number	Type of culture medium	NAA (mg·L -1)	6-BA (mg·L -1)	saccharose (g·L^{-1})	agal-agal (g·L^{-1})
1	1/2MS	1.5	1	30	20
2	1/2MS	1.5	1.5	30	20
3	1/2MS	1.5	2	30	20
4	1/2MS	2	1	30	20
5	1/2MS	2	1.5	30	20
6	1/2MS	2	2	30	20
7	1/2MS	2.5	1	30	20
8	1/2MS	2.5	1.5	30	20
9	1/2MS	2.5	2	30	20

2.2.5 Effect of different culture formulas on buffalo grass rooting

The sterile male and female sterile proliferation shoots were taken and inoculated onto the rooting media of different formulations (see Table 3), with 30 bottles of each treatment. The incubation temperature (25 ± 2) was ℃, first dark for 5 days, and then placed at 1500 lx for 12 h · d^{-1}Under the conditions, they were cultured for 15d and observed for rooting.

Table 3. Rooting medium formulation

Processing number	Type of culture medium	NAA ($mg \cdot L^{-1}$)	saccharose ($g \cdot L^{-1}$)	agal-agal ($g \cdot L^{-1}$)
1	1/2MS	0	15	20
2	1/2MS	0.5	15	20
3	1/2MS	1	15	20
4	1/2MS	1.5	15	20

3. Results and analysis

3.1 Effects of different disinfection methods on sterilization and growth of explant

From Table 4, the explants were transferred through 75% alcohol for 30s, 0.1 $g \cdot L^{-1}$The 16 min treatment of the mercury liter solution had the best disinfection effect, but the mercury liter was more toxic, and the long sterilization treatment may lead to plant death.75% alcohol 30s, 0.1 $g \cdot L^{-1}$12 min liters of mercury solution was used as a disinfection method for explants.

Table 4. Effects of different disinfection methods on the growth of bison grass explants

Disinfection time	Experimental phenomenon	Total number of plants	Number of contaminated strains	Number of dead plants	Sterile survival	Aseptic survival rate of (%)
8 min	The stems were partially dead and slightly contaminated	20	6	1	13	0.65
12 min	The stem section is partially dead and seriously polluted	20	2	2	16	0.8
16 min	A large number of stems die, and some of them are seriously polluted	20	1	10	9	0.45

Fig. 1 Disinfection effect

3.2 Effects of different media on explant induction

Using the stems of sterile male and female band segments, the germination of buffalo plants on different media was calculated under the same culture conditions. The results are shown in Table 5. The test results showed that neither the MS medium ion concentration nor the KT concentration had a significant effect on the induction efficiency of male and female strains, and the NAA concentration had a very significant effect on the germination and had greater effects on females. Female strains with medium 5 (MS + 1.5 mg · L-1 NAA + 0.4 mg · L-1 KT) showed the best induction, and males with medium 6 (MS + 1.5 mg · L-1 NAA + 0.6 mg · L-1 KT).

Table 5. Induced growth status of upper bison grass explants in different media after one week

Processing number	Type of culture medium	NAA (mg·L -1)	KT (mg·L -1)	Number of inoculated female explants	Number of inoculated male explants	Average number of shoots in female plants	Average number of buds produced in males
1	MS	1	0.2	30	30	1.17	1.26
2	MS	1	0.4	30	30	1.24	1.37
3	1/2MS	1	0.6	30	30	1.39	1.42
4	1/2MS	1.5	0.2	30	30	1.57	1.68
5	MS	1.5	0.4	30	30	1.84	1.73
6	MS	1.5	0.6	30	30	1.73	1.87
7	MS	2	0.2	30	30	1.39	1.27
8	1/2MS	2	0.4	30	30	1.3	1.29
9	MS	2	0.6	30	30	1.25	1.33

Media Type (mean value ± SD)	Average number of shoots in female plants	Average number of buds produced in males
1/2MS(n=3)	1.42±0.14	1.46±0.20
MS(n=6)	1.44±0.28	1.47±0.26
F	0.009	0.002
p	0.927	0.963

* $p<0.05$ ** $p<0.01$

NAA (mean value ± SD)	Average number of shoots in female plants	Average number of buds produced in males
1(n=3)	1.27±0.11	1.35±0.08
1.5(n=3)	1.71±0.14	1.76±0.10
2(n=3)	1.31±0.07	1.30±0.03
F	15.029	33.371
p	0.005**	0.001**
* p<0.05 ** p<0.01		
KT (mean value ± standard deviation)	Average number of shoots in female plants	Average number of buds produced in males
0.2(n=3)	1.38±0.20	1.40±0.24
0.4(n=3)	1.46±0.33	1.46±0.23
0.6(n=3)	1.46±0.25	1.54±0.29
F	0.095	0.215
p	0.91	0.812
* p<0.05 ** p<0.01		

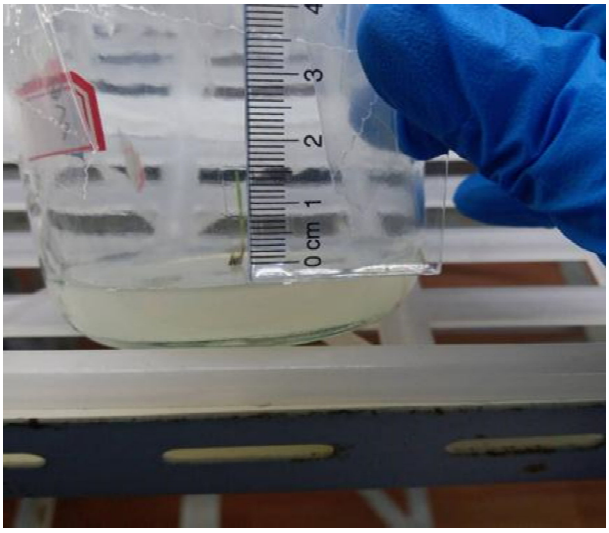

Fig. 2 Induced culture

3.3 The effect of different concentrations of hormone ratio on the proliferation of male and female strains of bison grass

Table 6. Effects of different hormone concentration ratios on bison grass proliferation

Processing number	Type of culture medium	NAA ($mg \cdot L^{-1}$)	6-BA ($mg \cdot L^{-1}$)	Number of inoculated female explants	Number of inoculated male explants	Average number of shoots in female plants	Average number of buds produced in males
1	1/2MS	1.5	1	30	30	4.47	4.53
2	1/2MS	1.5	1.5	30	30	5.03	5.25
3	1/2MS	1.5	2	30	30	4.15	4.03
4	1/2MS	2	1	30	30	6.27	6.01
5	1/2MS	2	1.5	30	30	7.66	7.73
6	1/2MS	2	2	30	30	6.03	6.29
7	1/2MS	2.5	1	30	30	3.53	3.65
8	1/2MS	2.5	1.5	30	30	4.33	4.5
9	1/2MS	2.5	2	30	30	3.03	2.97

Results of a two-way ANOVA for female strains

Differential source	quadratic sum	*df*	mean square	*F*	*p*
6-BA	2.578	2	1.289	22.650	0.007**
NAA	14.411	2	7.205	126.609	0.000**
Residual	0.228	4	0.057		

R^2: 0.987

**p <0.01

Results of a two-way ANOVA for males

Differential source	quadratic sum	*df*	mean square	*F*	*p*
6-BA	3.243	2	1.622	16.734	0.011*
NAA	13.924	2	6.962	71.837	0.001**
Residual	0.388	4	0.097		

R^2: 0.978

**p <0.01

The female and male strains of buffalo grass were used as the test material, and they were inoculated on the culture media with different concentrations of hormone ratio under the same culture conditions. The growth situation is shown in Table 6. The results showed that male and female plants could proliferate normally after halving MS ion concentration, and the concentration of NAA and 6-BA had significant effects on female and male stems, and NAA had more influence on proliferation efficiency. Medium # 5 (1 / 2 MS + 2 mg + L-1 NAA + 1.5 mg · L-1 6-BA) had the best proliferation effect.

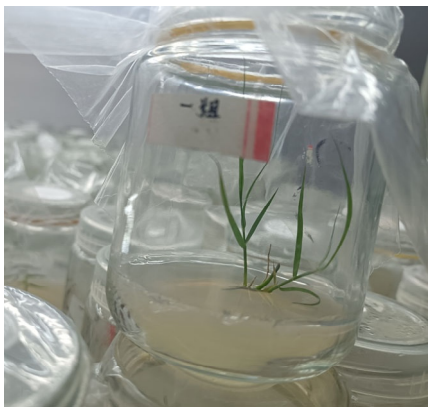

Fig. 3 Proliferation culture

3.4 Effect of different concentrations of hormones on the rooting of bison grass

Table 7. Effects of different hormone concentrations on the rooting of regenerated plants

Processing number	Type of culture medium	NAA ($mg \cdot L^{-1}$)	saccharose ($g \cdot L^{-1}$)	agal-agal ($g \cdot L^{-1}$)	Average number of female roots	The root rate of female strains	Average number of male plant roots	The root rate of male strains
1	1/2MS	0	15	20	2.5	76.3	2.3	67.5
2	1/2MS	0.5	15	20	3.3	87.5	4.3	82.1
3	1/2MS	1	15	20	3.5	84.2	3.2	83.3
4	1/2MS	1.5	15	20	4.3	85.7	3.7	86.7

		Average number of female roots	The root rate of female strains	Average number of male plant roots	The root rate of male strains
NAA	correlation	0.978*	0.651	0.990*	0.894
	p price	0.022	0.349	0.010	0.106

* p <0.05 ** p <0.01

The induced proliferative shoots were individually transferred to four different rooting media, and the adventitious root formation was observed after 20d of culture, and the results are shown in Table 7. The results showed that NAA concentration significantly affected the number of male and female plants, but not the rooting rate. Medium # 4 (1 / 2 MS + 1.5 mg · L-1 NAA) had the highest combined root number and root rate.

Fig. 4 Rooting culture

4. Conclusion and discussion

4.1 Conclusion

From the results of this trial, 75% alcohol was treated for 30s with 0.1 g · L-1The disinfection effect of mercury solution for 12 min; The MS + 1.5 mg · L-1 NAA + 0.4 mg · L-1 KT showed the best female strain induction results, MS + 1.5 mg · L-1 NAA + 0.6 mg · L-1 KT was the best for the male induction effect; 1 / 2 MS + 2 mg + L-1 NAA + 1.5 mg · L-1 6-BA the female and male strains had the best proliferation effect; 1 / 2 MS, combined the highest number and highest roots in male and female + 1.5 mg · L-1 NAA plants.

4.2 Discussion

4.2.1 Effect of dark treatment on preventing browning

Browning is a common phenomenon in the process of plant tissue culture, which mainly occurs in the starting stage of explant culture, and often occurs in the process of plant callus subgeneration, suspension cell culture, and the separation and culture of protoplasts[12]. Andy Wing Keung Chin[13]Found that in bison grass mature embryo culture. This experiment also found that selecting dark culture for 5 days could effectively prevent the browning of callus.

4.2.2 Simplified test of culture medium

In order to reduce the cost and facilitate the future production and promotion, on the basis of the original culture medium, the simplified test of the culture medium can be carried out in the future. The sucrose components in the culture medium can be changed to white granulated sugar, and the distilled water prepared in the culture medium can be changed to tap water, but the amount should be added appropriately.

5. Looking forward to

With the continuous development of warm season lawn grass, buffalo grass has become an important research object, and the development and utilization of its research results are continuously enhanced. In recent years, buffalo grass has appeared dioecy variation in planting, and if large areas of pure female or pure male cultivation can be achieved, it is possible to reduce the risk of airport bird strike from the perspective of the food chain.

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