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Establishment of a Callus Culture System of *Populus euphratica, Populus alba* cv. Pyramidalis and *Populus maximowiczii*×*Populus plantierensis*

Hailong SHEN*,**, Shin WATANABE*** and Yuji IDE****

Introduction

Reforestation at deteriorated land in arid and semi-arid regions is an urgent problem in the world. For successful reforestation, trees having higher tolerance for environmental stresses such as drought, soil salinity and soil alkalinity must be developed.

Populus euphratica Oliv. is a relatively fast-growing species naturally distributed in semi-arid regions from Europe to Central Asia (FAO,1979). Its natural forest is widely distributed in Northwest China, especially in Xinjiang Uygur Autonomous Region as one of the center of its distribution (WEI, 1988). This poplar shows high tolerance for drought, salinity and alkalinity (KANG *et al.*, 1996 a, c; WEI, 1988; LUO and ZHOU, 1991). According to these fact, we consider that *P. euphratica* is one of key species for the breeding of trees having higher tolerance in arid and semi-arid regions in the world.

Therefore, we have conducted the researches on analyzing tolerance mechanism and developing new cultivars with high tolerance for drought, salinity and alkalinity using *in vitro* culture system of *P. euphratica* and other poplars (KANG *et al.*, 1992; KANG *et al.*, 1995; KANG *et al.*, 1996a, b, c).

In this paper we report the establishment of a callus culture system of *P. euphratica, Populus alba* cv. Pyramidalis and *Populus maximowiczii*×*Populus plantierensis* (FS51) to develop new cultivars through callus culture in the future investivation. *P. alba* cv. Pyramidalis is a fast-growing species distributed in almost same regions of Northwest China with *P. euphratica* (XU, 1988). *P. maximowiczii*×*P. plantierensis* is a fast-growing hybrid for temperate forest. We have been using these two poplars as control materials on the course of our breeding project.

Materials and Methods

1. Callus formation from leaf explants and its subculture

The leaves of *in vitro* subcultured plants of the three poplars were taken as test materials (KANG *et al.*, 1992; IDE *et al.*, 1994; WATANABE, in press). The plantlets were cultured on 1/2MS medium (macro inorganic elements of MS medium (MURASHIGE and SKOOG, 1962) were reduced to a half) solidified by 0.8% agar and containing 0.01 mg/*l* NAA and 2% sucrose. The leaves were cut into about 5 cm^2 pieces and put onto media in 50 m*l* Erlenmeyer flask. Two or three explants were cultured in a flask. Each flask contained 20 m*l* of medium. MS medium containing 2% sucrose and supplemented with different combinations of BAP (6-benzylaminopurine), NAA (1-naphthaleneacetic acid) and

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2,4-D (2,4-dichlorophenoxyacetic acid) was used as shown in Table 1. Media were adjust their pH to 5.6 before autoclaving and solidified by 0.6% agar. The culture were kept at 25℃ under dark condition for 42 days.

Considering the results of initial culture, medium supplemented with 0.2 mg/*l* BAP and 1.0 mg/*l* 2,4-D was used for callus subculture. Calli were subcultured on the medium for 34, 36 and 30 days for the first, the second and the third subculture at 25℃ under dark condition. Well-grown calli were selected after the third subculture.

2. Plant regeneration from subcultured callus

After the second subculture, several calli originated from different initial hormonal conditions were selected. Calli were cut into about 5 cm^3 pieces and placed on 20 m*l* of 1/2 MS medium in 25 mm×120 mm culture tubes. Media were supplemented with BAP, NAA, 2,4-D and GA_3 (gibberellic acid) in various combination as shown in Table 3. They were kept at 25℃ under 6,600 lux fluorescent light for 16 h photoperiod for 70–90 days.

The regenerated shoot were cut from callus and transplanted to 20 m*l* of 1/2 MS media in 25 mm×120 mm culture tubes. Media were supplemented with 0.01 mg/*l* NAA or hormone free. They were kept at 25℃ under 6,600 lux fluorescent light for 16 h photoperiod.

All media used for plant regeneration were contained 2% sucrose and solidified by 0.8% agar. Their pH were adjusted to 5.6 before autoclaving.

Results and Discussion

1. Callus formation from leaf explants and its subculture

Calli were formed on almost all media tested except for media without NAA and 2,4-D irrespective of species (Table 1). BAP was not always necessary for callus formation from leaf explants for these three poplars. However NAA or 2,4-D was essential.

Calli were induced on media containing both BAP and NAA or 2,4-D in *Populus deltoides* (SAITO, 1989), *Populus*×*euramericana* (SAITO, 1980), *Populus canadensis* (SATO, 1989) and *Populus alba* (SAITO, 1989). Explants were taken from field plants in these reports. There may be some difference on the effect of BAP for callus formation between field plant and cultured plant.

The reflection to each hormonal combination for the three species were different. Although all explants formed callus on media supplemented with NAA or 2,4-D in *P. euphratica*, culture period needed for 100% explants to form callus varied from 13 to 34 days and callus volume at 42 days of culture varied from 1 to 11 mm^3 with the hormonal condition. Percentage of explants forming callus were lower on media with BAP than on media without BAP in *P. alba* cv. Pyramidalis. And another index of callus formation performance such as days for 100% explants to form callus and callus volume also showed the inhibitory effect of BAP for callus formation. In *P. maximowiczii*×*P. plantierensis*, 2.4-D seemed to be suppressive on callus formation.

In the case of callus regeneration from protoplasts of *P. euphratica*, 1/2 MS medium supplemented with 0.5 mg/*l* BAP and 0.1 mg/*l* 2,4-D was appropriate (KANG *et al.*, 1996b). In this experiment combinations of BAP and 2,4-D were also effective for callus formation not only in *P. euphratica* but also in *P. alba* cv. Pyramidalis and *P. maximowiczii*×*P. plantierensis*.

Roots or shoots were formed simultaneous to the callus formation on media supplemented with NAA. In spite of rapid callus formation, these organogenesis disturb continuous callus culture. Therefore we did not use these media for the subculture.

Fragile, yellowish and well-grown calli were obtained after the third subculture

Table 1. Effect of hormonal condition on callus formation from leaf explant of *in vitro* subcultured plant*

Species			*P. euphratica*				*P. alba* cv. Pyramidalis				*P. maximowiczii*×*P. plantierensis* (FS-51)			
Hormonal conditions (mg/*l*)			% of explants formed callus**	Days for 100% explants to form callus	Callus volume** (mm^3)	Organs formed together**	% of explants formed callus**	Days for 100% explants to form callus	Callus volume** (mm^3)	Organs formed together**	% of explants formed callus**	Days for 100% explants to form callus	Callus volume** (mm^3)	Organs formed together**
BAP	NAA	2,4-D												
0	0	0	0				0			shoot	0			shoot
0	0.5	0	100	21	11	root	100	13	8	shoot	100	21	4	
0	1.0	0	100	21	5	root	100	10	8	shoot	100	17	5	
0	0	0.5	100	21	4		100	17	13		88		3	
0	0	1.0	100	17	4		100	13	14		100	13	8	
0	0	5.0	100	34	2		100	17	8		89		3	
0.4	0	0	0				0			shoot	0			shoot
0.4	0.5	0	100	28	6	shoot	80		1		100	28	3	
0.4	1.0	0	100	21	10	shoot	100	34	3		100	34	3	
0.4	0	0.5	100	21	9		50		1		67		1	
0.4	0	1.0	100	28	5		86		2		100	28	5	
0.4	0	5.0	100	34	2		87		1		11		1	
0.8	0	0	0				0			shoot	0			shoot
0.8	0.5	0	100	13	9	shoot	50		2		100	34	4	
0.8	1.0	0	100	28	6	shoot	100	34	2		100	34	3	
0.8	0	0.5	100	28	4		50		1		22		1	
0.8	0	1.0	100	21	5		33		1		88		2	
0.8	0	5.0	100	34	1		10		1		0			

* Basal medium was MS solidified by 0.6% agar and 6–7 explants were cultured for each hormonal condition.

** Data were obtained 42 days after the initiation of culture.

Table 2. Callus growth after three times of subculture on MS solid medium with 0.2 mg/*l* BAP and 1.0 mg/*l* 2,4-D

Hormonal condition at initial callus formation (mg/*l*)			*P. euphratica*	*P. alba* cv. Pyramidalis	*P. maximowiczii* × *P. plantierensis*
BAP	NAA	2,4-D			
0	0.5	0	±	±	±
0	1.0	0	±	±	±
0	0	0.5	±	+	+
0	0	1.0	±	+	+
0	0	5.0	±	+	±
0.4	0.5	0	+	±	±
0.4	1.0	0	+	+	±
0.4	0	0.5	±	±	+
0.4	0	1.0	±	+	+
0.4	0	5.0	±	±	+
0.8	0.5	0	+	+	±
0.8	1.0	0	±	+	+
0.8	0	0.5	+	±	+
0.8	0	1.0	±	+	±
0.8	0	5.0	±	±	±

± Callus growth was not so good.
+ Fragile, yellowish and well grown callus was obtained.
Data were taken at the end of third subculture.

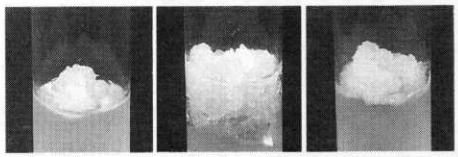

Fig. 1. Callus growth after 10 times of subculture on MS medium with 0.2 mg/*l* BAP and 1.0 mg/*l* 2,4-D. *P. euphratica*, *P. alba* cv. Pyramidalis and *P. maximowiczii*×*P. plantierensis* from left to right. After 20 days of subculture. Diameter of tubes are 25 mm.

(Table 2). There was no relation between the initial hormonal condition and callus growth in subculture. Then among these well-grown calli we could select calli for continuous subculture. And they kept rapid growth even after 10 times of subculture (Fig. 1).

2. Plant regeneration from subcultured callus

Shoot regeneration was observed irrespective of species. However, the regeneration is rare in *P. alba* cv. Pyramidalis (Table 3). Shoots were developed on the medium supplemented with BAP. 2,4-D inhibited shoot regeneration in spite of BAP concentration. GA_3 seemed to induce multiple shoot formation. Adventitious shoots were easily formed on the medium containing only BAP in the stem culture of popular hybrids (IDE *et al.*, 1994). Limited calli regenerated shoots on media containing only BAP in this experiment. It might be an effect of hormonal condition in previous culture or caused by the loss of regeneration ability during subcultures. Then we have to make clear the change of regeneration ability in the course of subculture.

Regenerated shoots of these three poplars rooted on 1/2 MS medium supplemented with 0.01 mg/*l* NAA (Fig. 3). *P. euphratica* rooted within 30–40 days of transplanting to the

Table 3. Shoot regeneration from second subcultured callus

Hormonal condition at initial callus formation (mg/l)				*P. euphratica**					*P. alba* cv. Pyramidalis**					*P. maximowiczii*×*P. plantierensis**			
BAP				0.4	0.4	0.8	0.8		0	0.4	0.8	0.8		0	0.4	0.8	
NAA				0.5	1.0	0	0	Total	0	0	0	1.0	Total	0	0	0	Total
2,4-D				0	0	0.5	1.0		5.0	1.0	0.5	0		1.0	5.0	1.0	
No. of calluses cultured for each hormonal condition shown below				1	3	1	1	6	2	1	1	1	5	3	1	1	5
Hormonal condition for shoot regeneration (mg/l)																	
BAP	NAA	2,4-D	GA_3														
0	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0	0.02	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0	0.02	0	2.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0	0.02	0.1	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0	0.05	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.2	0	0	0	—	—	—	—	—	—	—	—	—	—	2–3***	1–6	—	3
0.2	0.02	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.2	0.02	0	2	1–M	1–7***	—	—	2	—	—	—	—	—	—	—	—	—
0.2	0.02	0.1	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.2	0.05	0	2.0	—	—	—	—	—	—	—	—	—	—	3–2***	1–2	—	4
0.5	0	0	0	1–1	1–3***	—	—	2	—	—	—	—	—	—	1–4	—	1
0.5	0.02	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.5	0.02	0	2.0	1–M	1–1	1–2	—	3	—	—	—	—	—	1–2	1–6***	—	2
0.5	0.02	0.1	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.5	0.05	0	0	1–5	1–2	—	—	2	—	—	—	—	—	2–3***	—	—	2
1.0	0	0	0	—	—	—	—	—	—	—	—	—	—	1–6	—	—	—
1.0	0.02	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1.0	0.02	0	2.0	—	—	1–M	—	1	1–3	—	—	—	1	—	1–1	—	1
1.0	0.02	0.1	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1.0	0.05	0	0	1–1	—	1–M	—	2	—	—	—	—	—	—	—	—	1

* Data were taken after 70 days of culture.
** Data were taken after 90 days of culture.
*** Shoot longer than 5 mm was developed.
Data was shown as No. of callus which developed shoot-average number of induced shoots on a callus. M means more than 10 shoots developed. —; No shoot differentiation

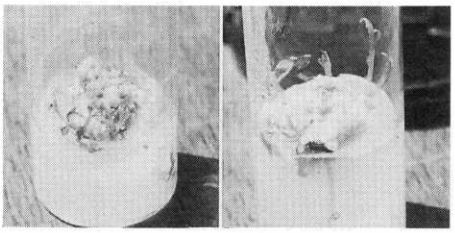

Fig. 2. Shoot regeneration from second subcultured callus.
Left; *P. euphratica* cultured on the medium with 0.5 mg/*l* BAP and 0.05 mg/*l* NAA.
Right; *P. maximowiczii*×*P. plantierensis* cultured on the medium with 0.5 mg/*l* BAP, 0.02 mg/*l* NAA and 2.0 mg/*l* GA_3.

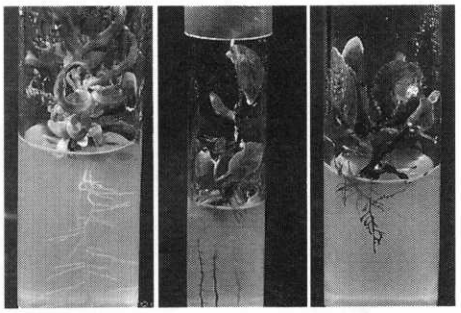

Fig. 3. Successfully regenerated plantlets.
P. euphratica, P. alba cv. Pyramidalis and *P. maximowiczii*×*P. plantierensis* from left to right.
After 86 days of transplanting to the rooting medium.
Diameter of tubes are 25 mm.

rooting medium. *P. alba* cv. Pyramidalis and *P. maximowiczii*×*P. plantierensis* rooted within 15–20 days. Rooting was also observed on hormone free medium within 30 days in *P. maximowiczii*×*P. plantierensis*.

Although a few samples planted in each hormonal combination for shoot regeneration in this experiment owing to the limited quantity of callus obtained, we could show the basic protocol of callus culture of *P. euphratica, P. alba* cv. Pyramidalis and *P. maximowiczii*×*P. plantierensis* in this experiment. This technique will be provide an effective way of *in vitro* breeding of poplar cultivar for higher stress tolerance.

Summary

A basic protocol of callus culture of *Populus. euphratica, Populus alba* cv. Pyramidalis and *Populus maximowiczii*×*Populus plantierensis* (FS-51) was established. Callus was induced on MS medium containing BAP, NAA and 2,4-D in combination or alone. The callus could be subcultured on MS medium supplemented with 0.2 mg/*l* BAP and 1.0 mg/*l* NAA. When callus was cultured on the medium containing BAP in combination with NAA or GA_3, adventitious shoots were regenerated. The shoots were successfully rooted on 1/2 MS medium. Callus culture and plant regeneration were possible in all species tested. However, there were some differences in the hormonal condition suitable for callus formation and shoot regeneration among species.

Key words: *Populus euphratica, Populus alba* cv. Pyramidalis, *Populus maximowiczii*×*Populus plantierensis*, Callus culture, Plant regeneration

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Populus euphratica, *Populus alba* cv. Pyramidalis, *Populus maximowiczii*×*Populus plantierensis* のカルス培養系の確立

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要　旨

Populus euphratica（コトカケヤナギ，中国名，胡楊），*Populus alba* cv. Pyramidalis（中国名，新疆楊），*Populus maximowiczii*×*Populus plantierensis*（品種名，FS-51）の3種類のポプラについてカルス培養の基本的手法を確立した。BAP, NAA, 2,4-Dを単独あるいは組み合わせて添加したMS培地上でカルス誘導が可能であった。誘導されたカルスは，BAPを0.2 mg/*l*, NAAを1.0 mg/*l*含むMS培地上で継代培養可能であった。また，継代2回目のカルスをBAPを単独あるいはNAAまたはGA_3を組み合わせたMS培地上でシュートが再生した。これらのシュート

は，1/2MS 培地にさしつけることにより発根して植物体が再生した。三種類すべてでカルスの培養と植物体の再生が可能であったが，種によって適当なホルモン条件は異なっていた。

キーワード：*Populus euphratica, Populus alba* cv. Pyramidalis and *Populus maximowiczii*×*Populus plantierensis*，カルス培養，植物体再生

The applicability of the shear stress/shear strain formula was examined by comparing with the relationships predicted from the equivalent stress/equivalent plastic strain relationship.

We concluded that the shear stress/shear strain relationship can be well formulated when the torsional moment/shear strain relationship is derived by the power function.

Establishment of a Callus Culture System of *Populus euphratica, Populus alba* cv. Pyramidalis and *Populus maximowiczii*×*Populus plantierensis*

Hailong SHEN, Shin WATANABE and Yuji IDE

A basic protocol of callus culture of *Populus euphratica, Populus alba* cv. Pyramidalis and *Populus maximowiczii*×*Populus plantierensis* (FS-51) was established. Callus was induced on MS medium containing BAP, NAA and 2,4-D in combination or alone. The callus could be subcultured on MS medium supplemented with 0.2 mg/*l* BAP and 1.0 mg/*l* NAA. When callus was cultured on the medium containing BAP in combination with NAA or GA_3, adventitious shoots were regenerated. The shoots were successfully rooted on 1/2 MS medium.

A Study on Landscape Formation and the Influence of Hang-Zhou West Lake in China

Yue SHEN

This research elucidates the landscape composition and landscape formation of Hong-Zhou West Lake (H-Z. W. L), and studies the landscape forming of other landscape areas based on H-Z. W. L. The main method of the research was to make out DTM according to topographical maps and perform quantitative analysis. In conclusion, the features of the landscape composition of H-Z. W. L are 1) both collected and expanded landscape, 2) three-layer structural landscape, 3) a skillful combination of man-made landscape and natural landscape. The formation methods are 1) promoting a layer-structure, 2) laying out atmosphere, 3) creating a typical landscape. In the lates projects of landscaping, the landscape composition and formation methods of H-Z. W. L were referred to and a common landscape appeared.