核果類におけるカルス誘導について

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Callus Induction from Leaf Disks of Stone Fruits (*Prunus* spp.)†1

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I Introduction

Prunus includes many species of commercial fruits called stone fruits, such as peach, almond, cherry, plum, apricot and Japanese apricot. Until now, the breeding program in *Prunus* has been carried out by crossing and mutation. Recently, tissue culture technique has been developed as a new tool for cultivar improvement. For example, embryo culture has been already used for breeding of early maturing cultivars in peach (Smith *et al.* 1969). Cell culture also provides a promising approach for cultivar improvement. By inducing genetic variation such as somaclonal variation or promoting cell fusion, breeding programs could be carried out more efficiently. However, only a few studies in callus culture in *Prunus* have been reported. Successful regeneration from callus is also limited in some species such as peach (Hammerschlag *et al.* 1985), almond (Mehra and Mehra 1974), sweet cherry (Seirlis *et al.* 1979), cherry rootstock (Matsuta *et al.* 1983, James *et al.* 1984, Jones *et al.* 1984, Ochatt *et al.* 1987), and wild species (Druart 1980, 1981). In the present report, the various factors affecting callus induction from *Prunus* leaf disks were studied.

II Materials and Methods

Callus was induced from leaf disks of several *Prunus* species grown in a greenhouse (Table 1). Fully expanded leaves were sterilized for 15 minutes in 0.5% sodium hypochlorite containing a few drops of Tween 20 and rinsed three times in sterile distilled water. Leaf disks, without midribs, were excised with a cork borer (5mm diameter) and placed on the culture medium. Culture media were designed to examine the effect of basal media, auxins, cytokinis, light and carbon sources in callus induction. Details of each experiment are presented in the Results. Ten leaf disks were placed in a 100 ml conical beaker containing 20ml culture medium. The culture media were adjusted to pH 5.8 and then 0.8% agar was added before autoclaving. The cultures were incubated in the dark at 28°C. The fresh weight of the induced callus with the leaf disk was measured after given periods of culture.

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Abbreviations: B_5=Basal medium by Gamborg *et al.* (1968); BA=6-Benzylaminopurine; BSA=Bovine serum albumin; CPA=(4-Chlorophenoxy) acetic acid; CH=Casein hydrolysate; 2,4-D=(2,4-Dicholorophenoxy) acetic acid; DTT=Dithiothreitol; IAA=Indole-3-acetic acid; 2iP=(Isopentenyl) adenine; KIN=Kinetin; MS=Basal medium by Murashige and Skoog (1962); NAA=1-Naphthaleneacetic acid; NN=Basal medium by Nitsch and Nitsch (1969); NOA=2-Naphthoxyacetic acid; PIC=4-amino-3,5,6-trichloropicolinic acid, same as Picloram; PVP=Polyvinylpyrrolidone; 2,4,5-T=(2,4,5-Trichlorophenoxy) acetic acid; ZEA=Zeatin

Table 1. List of the *Prunus* species examined.

Subgenus	Species	Cultivar
Amygdalus	*P. persica* (L.) Batsch; peach	Akame
		Hakuho
		Okinawa
	P. triloba Lindl.	
	P. davidiana (Carr.) Franch.	
Cerasus	*P. cerasus* L.; sour cherry	Meteor
	P. avium L.; sweet cherr	Compact Stella
		NY 1193
		Satonishiki
	P. lannesiana wils.	
Prunophora	*P. domestica* L.; European plum	Yellow Egg
	P. salicina Lindl.; Japanese plum	Methley
		White Plum
	P. psinosa L.	
	P. mume Sieb.; Japanese apricot	Gyokuei

III Results

1. Basal media

The first callus derived from the leaf disk emerged from the cut edge and veins, and continued to proliferate, as reported previously (Matsuta *et al.* 1983). Initial weight of the leaf disk ranged from 2.5 to 3.5mg. Fresh weight of induced callus in peaches and

Table 2. Effect of basal medium on callus induction from *Prunus* spp. leaf disks.

Cultivar and species	Phytohormone	Duration of culture (days)	Fresh weight of induced callus (mg)		
			Basal medium		
			NN	MS	B_5
Hakuho	10μM 2,4-D, 1μM BA	36	219.1±19.2[a]	136.3±50.1	241.5±34.7
	10μM NAA, 1μM BA	33	171.5±15.9		105.0±27.9
Okinawa	10μM 2,4-D, 1μM BA	34	26.9±19.1	8.1±6.5	57.5±12.7
P. triloba	10μM 2,4-D, 1μM BA	35	49.4±7.1	30.0±12.2	34.5±38.3
	10μM NAA, 1μM BA	35	208.1±21.7	51.8±16.0	215.8±54.5
P. davidiana	10μM 2,4-D, 1μM BA	33	214.7±30.0		291.4±56.7
	10μM NAA, 1μM BA	33	172.4±35.2		186.9±49.0
Meteor	10μM 2,4-D, 1μM BA	35	289.9±25.1	397.9±34.2	299.8±17.2
	10μM NAA, 1μM BA	35	275.8±65.6	368.3±55.6	330.5±47.0
Compact Stella	10μM 2,4-D, 1μM BA	35	189.6±23.5	228.7±39.5	146.3±12.8
	10μM NAA, 1μM BA	35	165.2±22.8	167.9±41.1	130.2±24.6
NY 1193	10μM 2,4-D, 1μM BA	35	240.6±35.6	272.5±56.3	162.2±19.6
	10μM NAA, 1μM BA	35	262.4±36.0	289.8±52.4	193.0±28.6
Satonishiki	20μM 2,4-D, 1μM BA	35	282.1±50.7		301.3±41.9
P. lannesiana	10μM 2,4-D, 1μM BA	35	78.4±10.9	95.6±28.6	77.7±6.7
Methley	10μM 2,4-D, 1μM BA	33	8.8±2.1		6.4±1.2
	10μM NAA, 1μM BA	33	19.5±5.9		6.9±2.3
White Plum	10μM 2,4-D, 1μM BA	35	3.0±0.02	2.9±0.03	3.6±0.06
P. spinosa	10μM 2,4-D, 1μM BA	35	119.0±18.7	14.6±9.0	38.3±8.0
	10μM NAA, 1μM BA	35	41.8±5.0	7.9±1.4	28.5±3.6

[a]Mean±standard deviation. Each value is the mean of 10 replicates.

cherries increased by more than 50 times compared with the initial weight after one month of culture. The effect of the basal medium on callus induction is shown in Table 2. The amount of induced callus was different among the three basal media used (NN, MS and B_5). In this experiment, 2, 4-D or NAA as auxin was used for callus induction. The effect of the basal medium in callus proliferation was similar for both 2, 4-D and NAA. NN and B_5 basal media were suitable for callus induction in peaches although the use of the MS basal medium resulted in good callus proliferation in cherries. In *P. spinosa*, a large amount of callus was induced in only the NN basal medium when 2, 4-D was used. However, callus induction was difficult on all basal media in other plums.

2. Auxins

The optimum concentration of auxins for callus induction varied with the cultivars and species (Table 3). Leaf disk of 'Hakuho' showed a good callus proliferation at a concentration of 10μM 2, 4-D. In 'Akame', a lower concentration (5μM) was suitable for callus induction for both 2, 4-D and NAA, but the fresh weight of the induced callus was lower than that of 'Hakuho'. The optimum concentration for callus induction in *P. triloba* was different between 2, 4-D and NAA. The optimum callus proliferation was obtained at a concentration of 5μM for 2, 4-D and 10 or 20 μM for NAA. When NAA was used, the fresh weight of the callus was larger than when 2, 4-D was used. The callus of 'Meteor' was able to proliferate in a wide range of concentrations as compared with that of peaches. When 2, 4-D was used for callus induction at all of the examined concentrations (1 to 50μM), a large amount of callus was produced although there were differences in the callus amount among the concentrations. When NAA was used, the range of effective concentrations for callus induction was wider than that for other cultivars and species. Callus of 'Satonishiki' proliferated well at 20 μM 2, 4-D. *P. spinosa* required a higher concentration for callus induction. Good results were obtained at 50 or 20 μM for 2, 4-D and at 50 or 100μM for NAA. In plums except for *P. spinosa*, callus was never produced regardless of the concentration examined. Callus induction in Japanese apricot was limlted but could be obtained at the optimum concentration of 1μM 2, 4-D which was low compared with the optimum concentrations for the other species.

The experiment in *P. lannesiana* indicated that the optimum concentration for callus induction was affected by the auxin types. Among seven auxins, in the case of 2, 4, 5-T callus proliferation was satisfactory at a concentration as low as 1μM, and the amount of callus formed decreased with the increase of the concentration. NAA and 2, 4-D were effective for callus induction at a concentration of 20 or 50μM and PIC at 1 to 50μM. When NOA, CPA and IAA were used, a high concentration of 100μM resulted in the formation of the largest amount of callus.

The optimum concentration of auxin was influenced by the basal medium also. That is, the callus of 'Meteor' proliferated best at 20μM 2, 4-D in NN and MS basal media and at 10 or 5μM in the B_5 basal medium. When NAA was used for callus induction, the optimum concentration also varied with the basal media.

3. Cytokinins

BA concentration affected callus induction (Table 4). In 'Hakuho', there was no difference in the fresh weight of the callus at concentrations ranging from 0.2 to 5μM

Table 3. Effect of auxin concentration on callus induction from *Prunus* spp. leaf disks.

Cultivar and species	Basal medium	Auxin	Fresh weight of induced callus (mg)									
			Auxin concentration (μM)									
			100	50	20	10	5	2	1	0.5	0.2	0.1
Hakuho	NN	2,4-D		5.5±1.0[a]	174.4±30.5	264.5±13.6	203.2±29.8	200.5±29.1	86.9±14.7			
Akame	NN	2,4-D	3.2±4.2	3.8±5.3	27.5±11.8	43.2±20.0	68.4±47.3		41.2±8.9			
	NN	NAA	2.2±3.2	3.1±3.9	20.2±11.2	25.7±10.6	35.1±11.8		13.6±6.1			
P. triloba	NN	2,4-D	6,6±7.0	7.8±1.3	22.0±4.6	61.6±11.3	121.6±52.2		108.2±28.5			
	NN	NNA	43.2±22.1	79.5±42.6	155.6±40.9	178.7±53.6	98.8±24.2		21.6±5.0			
Meteor	NN	2,4-D		238.8±29.8	420.7±62.3	300.6±26.3	245.9±27.8	215.9±11.7	188.9±3.8			
	MS	2,4-D		468.0±57.3	513.5±51.3	410.7±49.7	383.4±41.2	333.5±33.8	209.3±33.8			
	B_5	2,4-D		251.7±52.0	298.5±54.7	321.6±41.0	311.3±42.6	294.4±29.3	240.6±31.0			
	NN	NAA		287.8±29.3	334.9±52.5	335.6±54.2	198.0±90.2	126.2±78.0	78.1±35.9			
	MS	NAA		509.3±79.7	453.0±80.0	388.0±61.1	340.2±96.6	95.2±41.3	48.9±22.4			
	B_5	NAA		358.0±25.3	410.0±66.7	344.5±50.9	298.0±35.9	281.4±37.6	177.0±45.5			
Satonishiki	NN	2,4-D		224.6±49.3	266.5±36.1		164.8±27.3	110.5±21.4	78.2±16.6			
P. lannesiana	NN	2,4-D	12.6±3.3	151.3±29.4	144.6±23.5	91.5±15.9	31.3±8.6		15.6±2.6			
	NN	NAA		218.1±32.7	258.6±27.6	106.7±35.8	133.8±29.3	25.7±4.6	20.1±3.3			
	NN	NOA	122.3±29.4	65.6±15.1	26.4±10.1	20.7±5.2	15.2±1.8		9.4±3.4			
	NN	CPA	142.5±26.2	116.9±29.9	33.8±9.8	23.0±6.1	15.3±14.6		8.5±5.6			
	NN	IAA	100.5±34.0	21.7±7.2	11.3±2.3	11.6±1.7	10.8±2.3		9.1±0.09			
	NN	2,4,5-T	4.3±0.07	9.6±2.5	84.8±12.1	154.2±13.8	231.3±31.6		260.9±28.1			
	NN	PIC	153.6±9.1	224.5±14.4	255.8±24.4	241.7±24.0	243.9±19.2		229.2±14.8			
White Plum	NN	2,4-D		3.6±0.3	2.9±0.01	3.0±0.02	4.1±2.6	11.6±7.9	24.0±9.4			
Yellow Egg[b]	NN	2,4-D		6.7±1.4	30.4±12.8	29.3±11.7	17.5±6.8	14.6±3.7	14.6±1.9			
	NN	NAA		27.5±47.2	20.1±6.8	14.1±2.3	14.5±2.9	14.4±1.5	17.6±2.6			
P. spinosa	NN	2,4-D	46.4±12.9	127.5±17.0	122.3±32.5	99.8±27.8	20.5±5.4	23.2±2.4	12.3±1.2			
	NN	NAA	170.0±17.4	194.0±20.0	97.9±18.1	17.2±4.7	14.0±1.7	12.5±1.1	13.7±2.6			
Gyokuei	NN	2,4-D		5.6±0.07	5.5±0.07	7.7±5.0	45.4±80.7	37.2±20.9	82.9±31.8			
	NN	2,4-D						19.8±12.8	65.6±35.7	39.7±13.0	9.1±0.03	8.0±0.05
	NN	NAA		5.4±0.04	7.1±0.08	20.7±27.6	54.0±66.2	16.3±12.3	12.6±3.5			

[a] Each value is the mean of 20 replicates in 'Akame', *P. triloba*, 'Meteor' and *P. lannesiana* (except data of NAA) and 10 replicates in the other cultivars and species including data of NAA in *P. lannesiana*. Data were collected after 45 days of culture in 'Yellow Egg', 40 days of culture in 'Meteor' and 35 days of culture in the other cultivars and species. Culture media were supplemented with 1μM BA.

[b] Glucose (2%) was used as carbon source instead of sucrose.

Table 4. Effect of cytokinin concentration on callus induction from *Prunus* spp. leaf disks.

Cultivar and species	Fresh weight of induced callus (mg)								
	BA concentration (μM)								
	50	20	10	5	2	1	0.5	0.2	0.1
Hakuho	19.6±7.5[a]	129.5±18.0	219.2±22.3	287.8±57.2	280.0±38.7	264.5±13.6	282.5±25.7	267.1±32.2	
Akame	3.7±9.9		15.2±15.8	26.2±11.8		22.0±16.7	11.7±6.8		9.3±2.5
Meteor	237.6±33.9		246.0±30.0	247.0±18.7		299.6±31.3	318.4±33.0		345.8±33.5
Satonishiki	27.7±15.5	223.2±44.6	278.4±42.8	233.0±29.6	223.2±32.3		211.0±32.2	234.3±49.2	229.4±39.9
P. lannesiana	17.3±10.0	51.8±14.0	69.9±28.4	68.7±15.7	57.4±11.0	116.2±15.8	115.4±14.3	77.8±16.0	8.5±3.2
P. spinosa	10.6±5.9		106.1±11.7	101.2±16.9		101.7±7.0	76.9±7.2		97.9±11.6
Gyokuei				25.7±10.6	17.9±5.8	20.9±10.7	23.8±9.8	33.3±33.5	15.8±16.9

[a] Culture media consisted of NN basal medium supplemented with 10μM 2,4-D except for the cultivars 'Meteor' and 'Gyokuei'. The culture medium of 'Meteor' consisted of B_5 basal medium supplemented with 5μM 2,4-D. The culture medium of 'Gyokuei' consisted of NN basal medium supplemented with 1μM 2,4-D. Each value is the mean of 20 replicates in 'Akame' and 'Meteor' and 10 replicates in the other cultivars and species. Data were collected after 40 days of culture in 'Meteor' and 35 days in the other cultivars and species.

BA, and at a concentration above 10μM the amount of callus formed decreased with the increase of the concentration. The medium lacking cytokinin was able to induce only a small amount of callus even in the presence of 2, 4-D or NAA (data not shown). Callus proliferation was satisfactory at all the examined concentrations in 'Meteor', especially, treatment with 0.1μM BA produced the largest amount of callus on a fresh weight basis. The callus of 'Satonishiki' also showed a good proliferation in a wide range of concentrations from 0.1 to 20μM, with the optimum being 10μM. The medium lacking cytokinin could also induce callus although the amount of callus formed was about half of the amount in 0.1μM. In *P. lannesiana*, the callus was well induced in 0.5 or 1 μM BA, but was not induced in the medium lacking cytokinin. Callus proliferation in *P. spinosa* was satisfactory in the range from 0.1 to 10 μM. Satisfactory callus proliferation could not be obtained in 'Akame' and 'Gyokuei' regardless of the BA concentration.

Cytokinin type also influenced callus induction (Table 5). Among the four cytokinins (BA, KIN, ZEA and 2 iP), ZEA was the most effective for callus induction in *P. lannesiana* in combination with both 2, 4-D and NAA, while KIN gave the lowest fresh weight of callus. Although 2 iP induced a large amount of callus in combination with NAA as in the case of ZEA, the combination with 2, 4-D resulted in a lower callus proliferation than in the case of ZEA. A low concentration of 1μM was more effective than 10μM for callus induction in all the cytokinins.

Table 5. Effect cytokinin type on callus induction from *Prunus lannesiana* leaf disks.

Species	Auxin (10μM)	cytokinin conc.(μM)	Fresh weight of induced callus (mg)			
			Cytokinin			
			BA	KIN	ZEA	2iP
P. lannesiana	2, 4-D	10	59.5±17.2[a]	52.0±17.5	104.4±15.5	84.6±14.5
	2, 4-D	1	112.4±24.4	73, 3±15.2	117.6±32.8	53.8±17.5
	NAA	10	190.4±42.7	151.1±35.3	224.8±21.9	223.7±20.7
	NAA	1		208.4±22.0	257.6±30.2	238.0±24.3

[a] NN basal medium was used as culture medium. Each value is the mean of 10 replicates. Data were collected after 35 days of culture.

4. Light

The following two methods of culture were adopted in media contaning 2, 4-D and NAA: in the first the leaf disks were cultured in the dark and in the other under a photoperiod of 16hr light (about 3000 lux). In all the clones thus tested (Table 6), callus induction was distinctly inhibited in the presence of light.

5. Carbon sources

'Hakuho', 'Meteor' and *P. lannesiana* were able to utilize both sucrose and glucose as carbon sources, and produced a sufficient amount of callus (Table 7). Sorbitol was not effective for callus induction. Glucose was more suitable for 'Hakuho' whereas sucrose was more suitable for 'Meteor'. There was no difference between the use of sucrose and glucose in *P. lannesiana*. All the media with combinations of carbon sources afforded a good callus proliferation in these three cultivars and species. There

Table 6. Effect of light on callus induction from *Prunus* spp. leaf disks.

Cultivar and species	Auxin	Fresh weight of induced callus (mg)	
		dark	light
Hakuho	2, 4-D	323.6±38.0[a]	290.3±42.7
	NAA	218.1±18.5	105.9±21.6
Satonishiki	2, 4-D	225.8±16.6	186.2±16.8
	NAA	161.1±23.6	127.7±24.1
P. lannesiana	2, 4-D	131.9±27.5	29.9±6.6
	NAA	267.4±68.9	112.8±107.6

[a] Culture medium consisted of NN basal medium supplemented with 10μM of either auxin and 1μM BA. Each value is the mean of 10 replicates. Data were collected after 35 days of culture.

Table 7. Effect of carbon source on callus induction from *Prunus* spp. leaf disks.

Cultivar and species	2, 4-D Conc. (μM)	Fresh weight of induced callus (mg)					
		Carbon source					
		Suc[a](20g)	Glu[a](20g)	Sor[a](20g)	Suc(10g)+ Glu(10g)	Suc(10g)+ Sor(10g)	Glu(10g)+ Sor(10g)
Hakuho	10	280.6±41.8[b]	360.4±40.7	35.6±21.9	298.9±34.1	315.2±30.1	293.6±34.8
Meteor	10	231.3±15.5	169.8±11.3	8.2±0.9	205.0±12.7	203.1±26.4	151.7±23.7
Methley	10	4.4±0.7	5.5±2.3	18.2±9.2	3.2±0.5	4.3±1.0	3.6±0.7
		5.1±0.9	15.3±6.0	38.6±17.8	8.3±1.9	19.4±15.8	18.0±6.9
Yellow Egg	10	12.1±3.6	50.4±4.7	12.8±3.4	6.2±0.9	10.6±2.8	25.3±8.9
	1	6.7±0.9	11.1±1.3	8.8±1.0	7.2±0.7	6.5±1.3	9.8±1.5
P. lannesiana	10	52.9±39.9	50.2±24.6	9.3±0.4	70.4±10.0	79.6±11.8	55.0±19.2

[a] Suc=sucrose, Glu=Glucose, Sor=sorbitol.

[b] Culture medium consisted of NN basal medium supplemented with the respective concentratinos of 2, 4-D and 1μM BA. Each value is the mean of 10 replicates. Data were collected after 25 days of cultute in *P. lannesiana* and 35 days of culture in the other cultivars and species.

was no additional effect on callus induction by the combination of carbon sources. In plum, regardless of the carbon source satisfactory results were not obtained.

6. Attempts to induce callus from plums

The leaf disks of plums placed on the culture medium became brown within one week. Then, chemicals such as 3mM DTT, 3mM mercaptoethanol, 1% PVP and 1% BSA were added into the culture medium to prevent the oxidation of phenols. No chemicals enabled to prevent leaf disks browning and to induce callus. The addition of 0.1% CH and 5mM glutamine to the culture medium was not effective for inducing callus.

IV Discussion

Callus induction from *Prunus* species has been mostly achieved by using anther or stem explants (Skirvin 1984), and reports on the use of leaf tissues for callus induction are limited (Mehra and Mehra 1974, Hedtrich 1977). Leaf disk also appears to be an explant that could be used to induce a callus, because a large number of explants can be obtained from one leaf and the physiological characteristics of the leaf disks are more uniform than that of anther or stem. The use of physiologically uniform explants is desirable to determine the optimum conditions for callus induction. Thus, leaf disk was

adopted as an explant for callus induction in these experiments.

Skirvin (1984) showed that the basal media most frequently utilized to induce callus from *Prunus* species were the MS medium and the modified MS medium. However, the high salt concentration of the MS basal medium was reported to be unsuitable for callus induction (Kester *et al.* 1977) and it was found that the use of the MS basal medium with one-third to one-half strength resulted in better callus proliferation in almond than the use of the medium with the full strength. Mehra and Mehra (1974) on the other hand reported that the use of the MS basal medium was suitable for callus induction compared with the NN basal medium which consisted of about half of the salt concentration of the macronutrient elemets contained in MS. In these experiments, the suitability of the basal medium for callus inducton varied with the cultivars and species. MS basal medium induced the largest callus amount among the three basal media examined in cherries. In peaches, however, NN and B_5 basal media were more suitable for callus induction than the MS basal medium.

Phytohormones affected significantly the amount of callus induced, and callus induction in *Prunus* species required the presence of both auxin and cytokinin. The range of auxin concentration suitable for callus induction was limited in most of the cultivars and species. However, in 'Meteor' the range of auxin concentrations for callus induction was wide. Thus, the optimum concentration of auxin for callus induction was specific to each cultivar and species. It is well known that auxin is the phytohormone most related to callus induction. The remarkable changes in the amount of callus formed depending on the auxin concentration, suggested that the auxin concentration was also a major factor for callus induction in *Prunus* species except for 'Meteor'. In addition, the optimum concentration for callus induction was also different among the auxins. These differences appeared to reflect the activity of each auxin for callus induction. The results showed that 2, 4, 5-T actively promoted callus induction as a concentration as low as 1 μM enabled to induce a large amount of callus. In the case of NOA, CPA and IAA a concentration of 100 μM was required for callus induction. NAA and 2, 4-D were moderately effective. PIC acted differently from other auxins for callus induction, and a wide range of concentrations from 1 to 50μM resulted in the formation of a large amount of callus. PIC was an auxin derivative with a chemical structure different from that of the other auxins. So the activity site appeared to be different.

Sorbitol which is the major form of translocation in Rosaceae (Webb and Burley 1962, Bieleski 1969), is metabolized in *Prunus* species. Therefore, sorbitol could be utilized in some species of Rosaceae as a carbon source for callus culture (Coffin *et al*, 1976). It was reported that callus induction was more satisfactory on sucrose than on sorbitol medium in four *Prunus* species (*P. mahaleb, P. nigra, P. padus* and *P. tenella*). The present data supported the observation that sorbitol could not be used as a carbon source for callus induction.

In peaches and cherries, callus induction was easy and the optimum conditions could be clarified. However, callus induction in plums was very difficult to achieve and a small amount of callus was formed. Regardless of the culture medium examined in these experiments callus could not be satisfactorily induced. The addition of 0.1% CH and 5mM

glutamine which were required for callus formation from peach protoplasts (Matsuta *et al.* 1986) was not effective. Therefor it is considered that further investigations should be carried out for callus induction from plum species.

V Summary

The various factors affecting callus induction from leaf disks of several *Prunus* species (Table 1) were analysed.

NN and B_5 media were suitable for callus induction in peaches (Table 2). The use of MS basal medium resulted in good callus proliferation in cherries.

2, 4-D or NAA in the concentration range from 5 to 20μM was effective for callus induction (Table 3). In 'Meteor', low and high concentrations of 2, 4-D or NAA were also effective. Among the auxins examined (2, 4-D, NAA, NOA, CPA, IAA, 2, 4, 5-T and PIC), 2, 4, 5-T was able to induce callus at a low concentration (1 μM). In contrast, a high concentration (100μM) was required for callus induction in the case of NOA, CPA and IAA. PIC was effective over a wide concentration range from 1 to 50 μM. The optimum concentration of auxins was also affected by the basal medium.

In the case of BA a wide range of concentration (0.2 to 20μM) was effective for callus induction as compared with the auxin concentration (Table 4). Among the cytokinins examined (BA, KIN, ZEA and 2iP), ZEA was the most effective for callus induction in combination with 2, 4-D or NAA (Table 5).

The presence of light inhibited callus induction, which was satisfactory in the dark (Table 6).

Sucrose and glucose were effective for callus induction, and a sufficient amount of callus could be obtained (Table 7). Sorbitol was not effective for callus induction. Good results were obtained with any sugar combination.

In peaches and cherries, callus induction was easy and it was possible to analyse the optimum conditions in the current experiments. However, callus induction in plums was very difficult to achieve and a small amount of callus was formed.

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核果類におけるカルス誘導について

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摘　　要

核果類では，組織培養に関する知見が少なく，基礎的な情報の蓄積が十分なされていない．そのため，培養細胞からの植物体再生系についても確立している樹種は少なく，組織培養が育種手段として利用されずにいる．そこで，核果類の葉片を用い，カルス誘導に及ぼす種々の要因について検討を行った．

野生種を含めた核果類15系統（Table 1）の葉片を28°C暗黒下で培養し，一定期間後の形成されたカルス量を測定し，比較検討した．

1. 基本培地

NN, B_5, MS の3種類の基本培地を検討した．モモのグループでは NN 及び B_5 培地がカルス誘導には有効であったが，オウトウのグループでは MS 培地の方が良い結果が得られた（Table 2）．これは，オーキシンとして2,4-D, NAA のどちらを用いた場合にも認められた．

2. オーキシン濃度および種類

主に2,4-D と NAA を用い，0.1～100μM の濃度を検討した．カルス誘導に最適な濃度は，系統によって異なっていたが，2,4-D, NAA をオーキシンとして用いた場合，5～20μM の範囲の濃度が適していた（Table 3）．2,4-D, NAA の他に，NOA, CPA, IAA, 2,4,5-T, PIC の5種類のオーキシンについても最適濃度の検討を行った．2,4,5-T は，オーキシン活性が高く，1μM が有効であったが，NOA, CPA, IAA は活性が低く，100μM と高い濃度がカルス誘導には必要だった．PIC は，反応が異なっており，1～100μM のどの濃度でも多量のカルスを誘導した．また，NN, B_5, MS の3種類の基本培地を用い，濃度との相互関係をみたところ，最適濃度は基本培地によって異なっていた．

3. サイトカイニン濃度および種類

BA を用い，0～50μM の濃度を検討した．カルス誘導に有効なサイトカイニンの濃度は，0.2～20μM の範囲が適しており（Table 4），オーキシン濃度に比べより広い範囲の濃度が有効であった．BA, KIN, ZEA, 2iP の4種類を検討したところ，2, 4-D, NAA のどちらと組み合せた場合も，ZEA が他のサイトカイニンより有効だった（Table 5）．

4. 光条件

照明下（16hr, 3000 lux）及び暗黒下でカルス誘導を行ったところ，暗黒下の方が多量のカルスが得られた（Table 6）．

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5. 炭素源

ショ糖，ブドウ糖，ソルビトールの3種類の炭素源を単独あるいは組合わせて，培養を行った．ショ糖，ブドウ糖はカルス誘導に有効であった（Table 7）が，ソルビトールを用いた場合には，ほとんどカルスは誘導されなかった．また，それぞれ2つを組合わせた場合には，どの組合わせでもカルス誘導は良かった．

6. モモ及びオウトウではカルス誘導に関する要因が明らかになり，カルス誘導系は確立できたが，スモモでは今回検討した条件ではカルス誘導は困難であった．