

Table 1. Rate of embryo formation and shoot regeneration in each cultivar of anther culture.

Cultivar	year	No. of cultred anthers	Surviving anthers		Calli formation		Embryo formation		Shoot formation	
			Number	(%)	Number	(%)	Number	(%)	Number	from embryos(%) from anthers(%)
‘Danxia’	2013	2700	923	34.2	55	6.0	10	1.1	4	40.0 0.43
	2014	4200	2450	58.3	82	3.3	112	4.6	22	19.6 0.90
	2015	11550	8236	71.3	302	3.7	1062	12.9	565	53.2 6.86
	2016	24150	21198	87.8	783	3.7	1103	5.2	242	21.9 1.14
	Mean			62.9		4.2		6.0		33.7 2.33
‘Fuji’	2013	2550	1986	77.9	75	3.8	32	1.6	12	37.5 0.60
	2014	3700	1645	44.5	66	4.0	42	2.6	7	16.7 0.43
	2015	2000	1550	77.5	31	2.0	15	1.0	2	13.3 0.13
	2016	21450	17550	81.8	581	3.3	159	0.9	9	5.7 0.05
	Mean			70.4		3.3		1.5		18.3 0.30
‘Gala’	2013	5350	2643	49.4	106	4.0	25	0.9	12	48.0 0.45
	2014	17450	9487	54.4	569	6.0	218	2.3	58	26.6 0.61
	2015	46750	34792	74.4	1105	3.2	244	0.7	41	16.8 0.12
	2016	32310	26300	81.4	2089	7.9	361	1.4	58	16.1 0.22
	Mean			64.9		5.4		1.3		26.9 0.35

Surviving anthers (%) = (Number of surviving anthers / Number of cultured anthers) * 100

Calli formation (%) = (Number of Calli formed / Number of surviving anthers) * 100

Embryo formaiton (%) = (Number of embryos formed / Number of surviving anthers) * 100

Shoot formation from embryos (%) = (Number of shoots formed/ Number of embryos formed) * 100

Shoot formation from anthers (%) = (Number of shoots formed / Number of surviving anthers) * 100

Table 2. Effects of sucrose concentration on the cold treatment of root induction media on embryoid root formation.

Cultivar	Sucrose concentration	2009			2010		
		No.of embryos tested	Rooted embryos		No.of embryos	Rooted embryos	
			No.	%		No.	%
ASP	Sucrose 1.5% + Maltose 2.5%	144	9	6.3	200	48	24.0
	Sucrose 4.13%	40	2	5.0	69	5	7.2
	Sucrose 3.00%	40	3	7.5	90	12	13.3
	Sucrose 2.25%	40	2	5.0	63	12	19.0
	Sucrose 1.50%	40	3	7.5	92	30	32.6
Senshu	Sucrose 1.50% + Maltose 2.50%	331	75	22.7	-	-	-
	Sucrose 4.13%	40	4	10.0	-	-	-
	Sucrose 3.00%	40	10	25.0	-	-	-
	Sucrose 2.25%	40	8	20.0	-	-	-
	Sucrose 1.50%	40	10	25.0	-	-	-
SD	Sucrose 1.50% + Maltose 2.50%	1432	112	7.8	-	-	-
	Sucrose 4.13%	180	31	17.2	-	-	-
	Sucrose 3.00%	178	32	18.0	-	-	-
	Sucrose 2.25%	180	37	20.6	-	-	-
	Sucrose 1.50%	160	37	23.1	-	-	-

Rooted embryos (%) = (Number of rooted embryos / Number of embryos tested) * 100

Table 3. Effects of shoot regeneration media for rooted embryos (2009-2011).

Cultivar	Medium components				2009			2010			2011		
					No.of embryos tested	Shoot regeneration		No.of embryos	Shoot regeneration		No.of embryos tested	Shoot regeneration	
	BAP	GA	sucrose%	No.		%	No.		%	No.		%	
‘ASP’	1/2MS	-	-	1.5	-	-	-	31	2	6.5	8	1	12.5
	1/2MS	1mg•L ⁻¹	-	1.5	-	-	-	13	2	15.4	27	2	7.4
	MS	1mg•L ⁻¹	-	3.0	-	-	-	22	3	13.6	11	0	0
	1/2MS	1μM	5μM	1.5	-	-	-	27	7	25.9	-	-	-
	MS	1μM	5μM	3.0	-	-	-	14	0	0	9	1	11.1
‘Senshu’	1/2MS	-	-	1.5	7	5	71.4	-	-	-	21	2	9.5
	1/2MS	1mg•L ⁻¹	-	1.5	11	8	72.7	-	-	-	22	5	22.7
	MS	1mg•L ⁻¹	-	3.0	12	5	41.7	-	-	-	16	1	6.3
	1/2MS	1μM	5μM	1.5	9	5	55.6	-	-	-	18	3	16.7
	MS	1μM	5μM	3.0	-	-	-	-	-	-	24	5	20.8
‘SD’	1/2MS	-	-	1.5	11	7	63.6	-	-	-	-	-	-
	1/2MS	1mg•L ⁻¹	-	1.5	69	10	14.3	-	-	-	-	-	-
	MS	1mg•L ⁻¹	-	3.0	58	7	12.1	-	-	-	-	-	-
	1/2MS	1μM	5μM	1.5	16	0	0	-	-	-	-	-	-
	MS	1μM	5μM	3.0	-	-	-	-	-	-	-	-	-

Shoot regeneration (%) = Number of shoots regenerated / Number of embryos tested) * 100

BAP:1mg•L⁻¹ = 4.4μMGA:1mg•L⁻¹=2.9μM

Table 4. Effects of shoot regeneration media for rooted embryos (2013-2016).

Cultivar	Medium components					2013			2014			2015			2016		
						No. of embryos tested	Shoot regeneration		No. of embryos tested	Shoot regeneration		No. of embryos tested	Shoot regeneration		No. of embryos tested	Shoot regeneration	
							No.	%		No.	%		No.	%		No.	%
‘Danxia’	1/2MS	-	-	-	1.5	2	1	50.0	15	5	33.3	160	93	58.1	60	9	15.0
	MS	0.5	-	-	3.0	2	1	50.0	12	2	16.7	140	76	54.3	60	11	18.3
	MS	0.5	2	-	3.0	2	1	50.0	13	2	15.4	140	82	58.6	40	7	17.5
	MS	0.5		0.1	3.0	2	0	0	14	1	7.1	139	81	58.3	53	8	15.1
‘Fuji’	1/2MS	-	-	-	1.5	9	4	44.4	9	6	66.7	3	2	66.7	20	5	25.0
	MS	0.5	-	-	3.0	6	1	16.7	8	5	62.5	2	2	100	10	3	30.0
	MS	0.5	2	-	3.0	6	2	33.3	8	4	50.0	2	2	100	10	4	40.0
	MS	0.5		0.1	3.0	6	1	16.7	7	6	85.7	2	1	50.0	10	3	30.0
‘Gala’	1/2MS	-	-	-	1.5	5	2	40.0	20	3	15.0	20	9	45.0	40	20	50.0
	MS	0.5	-	-	3.0	5	1	20.0	20	3	15.0	20	11	55.0	40	25	62.5
	MS	0.5	2	-	3.0	4	1	25.0	20	2	10.0	20	7	35.0	40	19	47.5
	MS	0.5		0.1	3.0	4	1	25.0	20	1	5.0	12	5	41.7	51	33	64.7

Shoot regeneration (%) = Number of shoots regenerated / Number of embryos tested) * 100

BAP: $1\text{mg}\cdot\text{L}^{-1} = 4.4\mu\text{M}$ GA: $1\text{mg}\cdot\text{L}^{-1} = 2.9\mu\text{M}$

Table 5. Effects of shoot regeneration media for nonrooted embryos (2009-2011).

Cultivar	Medium components				2009			2010			2011		
					No. of embryos	Shoot regeneration		No. of embryos	Shoot regeneration		No. of embryos	Shoot regeneration	
						No.	%		No.	%		No.	%
‘ASP’	1/2MS	1mg•L-1	-	1.5	-	-	-	40	1	2.5	102	2	2.0
	MS	1mg•L-1	-	3.0	-	-	-	55	0	0	123	5	4.1
	1/2MS	1μM	5μM	1.5	-	-	-	-	-	-	-	-	-
	1/2MS	1μM	5μM	3.0	-	-	-	118	1	0.8	24	0	0
	MS	1μM	5μM	3.0	-	-	-	194	0	0	108	3	2.8
‘Senshu’	1/2MS	1mg•L-1	-	1.5	27	7	25.9	-	-	-	131	3	2.3
	MS	1mg•L-1	-	3.0	27	12	44.4	-	-	-	75	1	1.3
	1/2MS	1μM	5μM	1.5	107	33	30.8	-	-	-	-	-	-
	1/2MS	1μM	5μM	3.0	28	6	21.4	-	-	-	51	0	0
	MS	1μM	5μM	3.0	27	3	11.1	-	-	-	73	5	6.9
‘SD’	1/2MS	1mg•L-1	-	1.5	140	10	7.1	-	-	-	-	-	-
	MS	1mg•L-1	-	3.0	160	4	2.5	-	-	-	-	-	-
	1/2MS	1μM	5μM	1.5	541	25	4.6	-	-	-	-	-	-
	1/2MS	1μM	5μM	3.0	140	7	5.0	-	-	-	-	-	-
	MS	1μM	5μM	3.0	194	7	3.6	-	-	-	-	-	-

Shoot regeneration (%) = Number of shoots regenerated / Number of embryos tested) * 100

BAP:1mg•L⁻¹ = 4.4μMGA:1mg•L⁻¹=2.9μM

Table 6. Effects of shoot regeneration media for nonrooted embryos.

Cultivar	Medium components					2013			2014			2015			2016		
	BAP (mg·L ⁻¹)	GA (mg·L ⁻¹)	IBA (mg ·L ⁻¹)	Sucrose%	No. of embryos tested	Shoot regeneration		No. of embryos tested	Shoot regeneration		No. of embryos tested	Shoot regeneration		No. of embryos tested	Shoot regeneration		
						No.	%		No.	%		No.	%		No.	%	
‘Danxia’	1/2MS	-	-	-	1.5	-	-	-	5	0	0	7	0	0	20	1	5.0
	MS	0.5	-	-	3.0	4	1	25.0	14	0	0	80	21	26.3	185	33	17.8
	MS	0.5	2	-	3.0	2	1	50.0	14	1	7.1	80	39	48.8	185	50	27.0
	MS	0.5		0.1	3.0	-	-	-	12	2	16.7	80	19	23.4	136	30	22.1
‘Fuji’	1/2MS	-	-	-	1.5	-	-	-	-	-	-	-	-	-	3	0	0
	MS	0.5	-	-	3.0	14	5	35.7	3	1	33.3	-	-	-	11	2	18.2
	MS	0.5	2	-	3.0	9	3	33.3	3	0	0	2	0	0	10	1	10.0
	MS	0.5		0.1	3.0	-	-	-	3	0	0	-	-	-	11	1	9.1
‘Gala’	1/2MS	-	-	-	1.5	-	-	-	11	0	0	5	0	0	14	2	14.3
	MS	0.5	-	-	3.0	6	2	33.3	20	2	10.0	12	6	60.0	20	7	35.0
	MS	0.5	2	-	3.0	8	5	62.5	20	3	15.0	12	7	58.3	20	9	45.0
	MS	0.5		0.1	3.0	3	0	0	20	3	15.0	11	9	81.8	20	8	40.0

Shoot regeneration (%) = Number of shoots regenerated/ Number of embryos tested) * 100

BAP:1mg·L⁻¹= 4.4μMGA:1mg·L⁻¹= 2.9μM

Table7 . Expected final shoot formation rate in apple anther culture.

Cultivar	year	No. of embryos tested	Rooted embryos					non-rooted embryos				No. of total shoots	
			Rooted embryos	Root formation(%)	Shoot	Regeneratio n rate	Regeneratio n rate	NO.	Shoot regenerati	Regeneratio n rate	Regeneratio n rate		
‘ASP’	2009	328	19	5.8		2	10.5		309	4	1.3		6
	2010	729	107	14.7	12.1	14	13.1	8.2	622	2	0.3	0.7	16
	2011	2273	357	15.7		4	1.1		1916	10	0.5		14
‘Senshu’	2009	491	107	21.8		47	43.9		384	61	15.9		108
	2010	27	9	33.3	25.9	5	55.6	38.4	18	5	27.8	15.4	10
	2011	445	101	22.7		16	15.8		344	9	2.6		25
‘SD’	2009	2246	427	19.0		74	17.3		1819	64	3.5		138
	2010	35	11	31.4	22.3	2	18.2	13.7	24	1	4.2	2.6	3
	2011	213	35	16.4		2	5.7		178	0	0.0		2
‘Danxia’	2013	9	3	33.3		2	66.7		6	2	33.3		4
	2014	54	9	16.7		3	33.3		45	3	6.7		22
	2015	579	332	57.3	36.1	250	75.3	54.1	247	79	32.0	23.4	329
	2016	838	312	37.2		128	41.0		526	114	21.7		242
	2013	32	9	28.1		4	44.4		23	8	34.8		12
‘Fuji’	2014	32	23	71.9		6	26.1		9	1	11.1		7
	2015	9	7	77.8	52.0	2	28.6	33.1	2	0	0.0	14.3	2
	2016	50	15	30.0		5	33.3		35	4	11.4		9
‘Gala’	2013	22	5	22.7		4	80.0		17	7	41.2		11
	2014	80	9	11.3		2	22.2		71	9	12.7		11
	2015	72	32	44.4	33.8	17	53.1	47.1	40	24	60.0	37.3	41
	2016	171	97	56.7		32	33.0		74	26	35.1		58

Root formation (%) = (Rooted embryos / Number of embryos tested) * 100

Shoot regeneration of rooted embryos (%) = (Number of shoot / rooted embryos) * 100

Shoot regeneration of nonrooted embryos (%) = (Number of shoot / nonrooted embryos) * 100