

Table 3.2.1: Composition of culture medium, Murashige and Skoog (MS) Medium (1962)

Chemical	Formula	Concentration
<u>Macronutrients (10X)</u>		
Ammonium nitrate	NH_4NO_3	16.5
Potassium nitrate	KNO_3	19.0
Calcium chloride	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	4.4
Magnesium sulfate	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	3.7
Potassium dihydrogen orthophosphate	KH_2PO_4	1.7
<u>Micronutrients (100X)</u>		
Manganese sulphate	$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	2.23
Zinc sulphate	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.86
Potassium iodide	KI	0.086
Cupric sulphate	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.0026
Sodium molybdate	$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.025
Cobalt (ous) chloride	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.0026
Boric acid	H_3BO_3	0.62
<u>Vitamin source (100X)</u>		
Nicotinic acid	$\text{C}_6\text{H}_5\text{NO}_2$	0.05
Thiamine hydrochloride	$\text{C}_{12}\text{H}_{17}\text{ClN}_4\text{OS} \cdot \text{HCl}$	0.01
Pyridoxine hydrochloride	$\text{C}_8\text{H}_{12}\text{N}_2\text{O}_2 \cdot 2\text{HCl}$	0.05
Glycine	$\text{C}_2\text{H}_5\text{NO}_2$	0.2

<u>Iron source (100X)</u>		
Sodium EDTA	$\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_8\text{Na}_2\text{H}_2\text{O}$	2.78
Ferrous sulphate	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	3.72

Myo-inositol	$\text{C}_6\text{H}_{12}\text{O}_6$	0.1 g (freshly add)
Sucrose		30 g
Phytigel		2 g

Table 3.2.2: Table below showing forty-nine different Plant Growth Regulators (PGRs) combinations in this 7 x 7 factorial experiment and (number) coded for the different PGRs combinations.

BAP ($\mu\text{M/l}$)	NAA ($\mu\text{M/l}$)						
	0	0.5	1.0	5.0	10.0	20.0	40.0
0	0,0 (1)	0.5,0 (2)	1.0,0 (3)	5.0,0 (4)	10.0,0 (5)	20.0,0 (6)	40.0,0 (7)
0.5	0, 0.5 (8)	0.5, 0.5 (9)	1.0,0.5 (10)	5.0, 0.5 (11)	10.0, 0.5 (12)	20.0, 0.5 (13)	40.0, 0.5 (14)
1.0	0, 1.0 (15)	0.5, 1.0 (16)	1.0,1.0 (17)	5.0, 1.0 (18)	10.0, 1.0 (19)	20.0, 1.0 (20)	40.0, 1.0 (21)
5.0	0, 5.0 (22)	0.5, 5.0 (23)	1.0,5.0 (24)	5.0, 5.0 (25)	10.0, 5.0 (26)	20.0, 5.0 (27)	40.0, 5.0 (28)
10.0	0,10.0 (29)	0.5, 10.0 (30)	1.0,10.0 (31)	5.0, 10.0 (32)	10.0,10.0 (33)	20.0,10.0 (34)	40.0,10.0 (35)
20.0	0, 20.0 (36)	0.5, 20.0 (37)	1.0, 20.0 (38)	5.0, 20.0 (39)	10.0, 20.0 (40)	20.0,20.0 (41)	40.0,20.0 (42)
40.0	0,40.0 (43)	0.5, 40.0 (44)	1.0,40.0 (45)	5.0, 40.0 (46)	10.0, 40.0 (47)	20.0,40.0 (48)	40.0,40.0 (49)

Table 3.5.1 List of *Cryptocoryne* sp. that been used in the study.

	Name	Location
1.	<i>Cryptocoryne</i> sp. (1)	Sarawak, Malaysia
2.	<i>Cryptocoryne nuri</i>	Felda Kahang, Johor, Malaysia
3.	<i>Cryptocoryne auriculata</i>	Sg. Entabai, Sarawak
4.	<i>Cryptocoryne nuri</i>	Kahang, Johor, Malaysia
5.	<i>Cryptocoryne willisii</i>	Sri Lanka (tissue culture)
6.	<i>Cryptocoryne willisii</i>	Sri Lanka (Tissue culture and radiated)
7.	<i>Cryptocoryne parva</i>	Sri Lanka
8.	<i>Cryptocoryne striolata</i>	Sarawak
9.	<i>Cryptocoryne schulzei</i>	Hutan Panti, Johor
10.	<i>Cryptocoryne cordata</i>	Johor
11.	<i>Cryptocoryne purpurea</i>	Tasek Bera, Pahang
12.	<i>Cryptocoryne affinis</i>	Jerantut, Pahang
13.	<i>Cryptocoryne pallidinervia</i>	Sibu, Sarawak
14.	<i>Cryptocoryne lingua</i>	Sarawak
15.	<i>Cryptocoryne</i> sp. (2)	Sri Lanka
16.	<i>Cryptocoryne wendtii</i> Brown	Sri Lanka
17.	<i>Cryptocoryne wendtii</i>	Sri Lanka
18.	<i>Cryptocoryne uenoi</i>	Sri Aman, Sarawak
19.	<i>Cryptocoryne ferruginea</i>	Bau, Sarawak
20.	<i>Cryptocoryne keei</i>	Bau, Sarawak

Appendix

Table 3.5.2 The IRAP primers used in the phylogenetics study of selected *Cryptocoryne* sp. experiment.

Name		Retrotransposon source	Sequence	Accession, position
LTR6149	61	BARE-1→	CTCGCTCGCCCACTACATC AACCGCGT TTATT	Z17327 1993-2012
LTR6150	62	BARE-1←	CTGGTTCGGCCCATGTCTA TGTATCCACACATGTA	Z1 7327 418-439
5'LTR1	63	BARE-1←	TTGCCTCTAGGGCATATTT CCAACA	Z17327 1-26
5'LTR2	58	BARE-1←	ATCATTCCCTCTAGGGCAT AATTC	Z17327 314-338
3'LTR	69	BARE-1→	TGTTTCCCATGCGACGTTC CCAACA	Z17327 2112-2138
Sukkula	66	<i>Sukkula</i> →	GATAGGGTCGCATCTTGG GCGTGAC	AY054376 4301-4326
Nikita	58	<i>Nikita</i> →	CGCATTTGTTCAAGCCTAA ACC	AY078073 AY078074 AY078075 1-22
Reverse TY1	42	W1,W3,W7,W8 ←	CCYTGNAYYAANGCNCT	AF416815 AF416816 AF416817 AF416818 1-17
Reverse TY2	36	W1,W3,W7,W8 →	TRGTARAGRAGNTGRAT	

Table 3.5.3 Forty-five combinations of primer A and primer B for IRAP-PCR phylogenetics of selected *Cryptocoryne* sp. experiment.

Primer A Primer B	LTR6149	LTR6150	5'LTR1	5'LTR2	3'LTR	Sukkula	Nikita	Reverse TY1	Reverse TY2
LTR6149	49/49	49/50	49/1	49/2	49/3	49/ Suk	49/ Nik	49/ R1	49/ R2
LTR6150		50/50	50/1	50/2	50/3	50/ Suk	50/ Nik	50/ R1	50/ R2
5'LTR1			1/1	1/2	1/3	1/ Suk	1/ Nik	1/ R1	1/ R2
5'LTR2				2/2	2/3	2/ Suk	2/ Nik	2/ R1	2/ R2
3'LTR					3/3	3/ Suk	3/ Nik	3/ R1	3/ R2
Sukkula						Suk/ Suk	Suk/ Nik	Suk/ R1	Suk/ R2
Nikita							Nik/ Nik	Nik/ R1	Nik/ R2
Reverse TY1								R1/ R1	R1/ R2
Reverse TY2									R2/ R2

Table 3.6.1 The IRAP Primers used in the experiment for mutation detection.

Name		Retrotransposon source	Sequence	Accession, position
LTR6149	61	BARE-1→	CTCGCTCGCCCACT ACATCAACCGCGT TTATT	Z17327 1993-2012
LTR6150	62	BARE-1←	CTGGTTCGGCCCAT GTCTATGTATCCAC ACATGTA	Z17327 418-439
5'LTR1	63	BARE-1←	TTGCCTCTAGGGCA TATTTCCAACA	Z17327 1-26
5'LTR2	58	BARE-1←	ATCATTCCCTCTAG GGCATAATTC	Z17327 314-338
Sukkula	66	<i>Sukkula</i> →	GATAGGGTTCGCAT CTTGGGCGTGAC	AY054376 4301-4326
Nikita	58	<i>Nikita</i> →	CGCATTTGTTCAAG CCTAAACC	AY078073 AY078074 AY078075 1-22

Table 3.6.2 Twenty-one combinations of primer A and primer B for IRAP-PCR for mutation detection.

Primer A Primer B	5'LTR1	5'LTR2	LTR6149	LTR6150	Sukkula	Nikita
5'LTR1	1/1	1/2	1/49	1/50	1/SUK	1/NIK
5'LTR2		2/2	2/49	2/50	2/SUK	2/NIK
LTR6149			49/49	49/50	49/SUK	49/NIK
LTR6150				50/50	50/SUK	50/NIK
Sukkula					SUK/SUK	SUK/NIK
Nikita						NIK/NIK

Appendix

Table 4.2.1 Results of mean number of shoots on various concentration of NAA and BA

No. of shoots

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	10	2.50	1.080	.342	1.73	3.27	1	4
2	10	3.90	.994	.314	3.19	4.61	3	6
3	10	1.50	1.650	.522	.32	2.68	0	4
4	10	1.00	.000	.000	1.00	1.00	1	1
5	10	.90	.316	.100	.67	1.13	0	1
6	10	2.30	.823	.260	1.71	2.89	1	4
7	10	1.60	.966	.306	.91	2.29	0	3
8	10	4.30	1.418	.448	3.29	5.31	1	6
9	10	2.30	.483	.153	1.95	2.65	2	3
10	10	2.90	1.197	.379	2.04	3.76	1	4
11	10	3.10	1.729	.547	1.86	4.34	1	7
12	10	2.90	.738	.233	2.37	3.43	2	4
13	10	1.90	.876	.277	1.27	2.53	1	4
14	10	2.40	.843	.267	1.80	3.00	1	4
15	10	6.80	1.751	.554	5.55	8.05	5	10
16	10	3.20	1.398	.442	2.20	4.20	1	5
17	10	2.10	1.524	.482	1.01	3.19	0	4
18	10	2.00	.816	.258	1.42	2.58	1	3
19	10	2.00	1.155	.365	1.17	2.83	1	4
20	10	1.70	.823	.260	1.11	2.29	1	3
21	10	2.10	.876	.277	1.47	2.73	1	4
22	10	3.70	.949	.300	3.02	4.38	2	5
23	10	2.60	1.578	.499	1.47	3.73	0	4
24	10	4.60	2.989	.945	2.46	6.74	1	10
25	10	2.60	1.174	.371	1.76	3.44	1	4
26	10	2.30	.823	.260	1.71	2.89	1	3
27	10	2.00	1.333	.422	1.05	2.95	1	5
28	10	1.50	.707	.224	.99	2.01	1	3
29	10	2.90	2.283	.722	1.27	4.53	0	6
30	10	3.50	2.273	.719	1.87	5.13	0	7
31	10	4.50	1.900	.601	3.14	5.86	1	7
32	10	5.40	1.955	.618	4.00	6.80	3	9
33	10	4.50	2.635	.833	2.61	6.39	1	9
34	10	4.20	2.150	.680	2.66	5.74	0	8
35	10	4.40	1.955	.618	3.00	5.80	2	8
36	10	4.30	1.829	.578	2.99	5.61	2	7
37	10	4.60	1.955	.618	3.20	6.00	1	8
38	10	3.40	1.955	.618	2.00	4.80	1	7
39	10	4.20	2.300	.727	2.55	5.85	1	8
40	10	2.10	1.524	.482	1.01	3.19	0	4
41	10	2.60	1.174	.371	1.76	3.44	1	4
42	10	3.20	1.751	.554	1.95	4.45	1	6
43	10	6.40	3.204	1.013	4.11	8.69	2	13
44	10	3.80	1.229	.389	2.92	4.68	2	6
45	10	3.40	1.578	.499	2.27	4.53	1	5
46	10	3.30	2.541	.803	1.48	5.12	0	8
47	10	2.90	1.524	.482	1.81	3.99	1	5
48	10	2.70	1.160	.367	1.87	3.53	1	4
49	10	1.50	1.179	.373	.66	2.34	0	3
Total	490	3.07	1.984	.090	2.90	3.25	0	13

Table 4.4.2.1 Results of mean number of lengths of normal type *C. xwillisii*.

Length (cm)

Sampel	Mean	N	Std. Deviation	Std. Error of Mean	Minimum	Maximum
N1	5.220	5	1.5959	.7137	3.5	6.8
N2	4.071	7	1.3671	.5167	3.0	6.0
N3	5.100	6	1.9860	.8108	2.5	8.0
N4	6.625	4	3.2755	1.6378	2.5	9.5
N5	6.167	6	1.1255	.4595	4.5	8.0
N6	5.460	5	1.0526	.4707	4.0	6.8
N7	5.600	5	1.0840	.4848	4.0	6.5
N8	4.883	6	1.4838	.6058	2.8	6.5
N9	6.040	5	2.2098	.9882	2.6	8.5
N10	6.660	5	.7765	.3473	6.0	8.0
N11	5.750	4	1.0408	.5204	4.5	7.0
N12	5.875	4	.7500	.3750	5.0	6.5
N13	5.275	4	1.6840	.8420	3.0	6.6
N14	3.650	4	.6245	.3122	3.0	4.5
N15	6.000	3	1.0000	.5774	5.0	7.0
N16	4.333	6	.6831	.2789	3.5	5.0
N17	4.125	4	1.1087	.5543	3.0	5.5
N18	5.767	3	1.3650	.7881	4.3	7.0
N19	3.375	4	.2500	.1250	3.0	3.5
N20	4.125	4	.2500	.1250	4.0	4.5
Total	5.179	94	1.5715	.1621	2.5	9.5

Table 4.4.2.2 Results of mean number of lengths of mutant G1 *C. xwillisii*.

Length (cm)

Sampel	Mean	Std. Deviation	Minimum	Maximum	Std. Error of Mean	Variance
G1	6.500	1.4760	4.0	9.0	.3811	2.179
G2	6.650	1.5693	4.0	9.0	.4194	2.463
G3	6.885	1.3253	4.0	8.5	.3676	1.756
G4	6.630	1.1265	4.5	8.0	.3562	1.269
G5	5.760	1.2280	4.0	7.0	.5492	1.508
G6	9.643	2.5774	4.0	11.5	.9742	6.643
G7	6.633	1.6573	3.5	8.0	.6766	2.747
G8	8.000	2.2638	5.0	10.5	1.0124	5.125
G9	6.967	2.4089	3.2	9.5	.9834	5.803
G10	8.700	1.5652	7.0	11.0	.7000	2.450
G11	7.733	1.8991	6.0	10.5	.7753	3.607
G12	7.350	1.5871	4.8	9.0	.6479	2.519
G13	7.375	.6292	6.5	8.0	.3146	.396
G14	6.100	.6856	5.0	6.8	.3066	.470
G15	8.200	1.6416	6.5	10.5	.7342	2.695
G16	7.225	1.0012	6.0	8.4	.5006	1.003
G17	7.200	1.7536	4.5	9.0	.7842	3.075
G18	5.000	.9354	4.0	6.0	.4183	.875
G19	5.325	.5377	4.8	6.0	.2689	.289
G20	5.183	1.1771	3.8	6.5	.4806	1.386
Total	6.924	1.7956	3.2	11.5	.1540	3.224

Table 4.4.2.3 Results of mean number of lengths of mutant D1 *C. xwillisii*.

Length (cm)

Sampel	Mean	N	Std. Deviation	Std. Error of Mean	Minimum	Maximum	Variance
D1	3.067	6	.0816	.0333	3.0	3.2	.007
D2	3.111	9	.2205	.0735	3.0	3.5	.049
D3	3.000	5	.7071	.3162	2.5	4.0	.500
D4	2.700	5	.4472	.2000	2.5	3.5	.200
D5	4.500	5	.8660	.3873	3.0	5.0	.750
D6	5.000	5	.7071	.3162	4.0	6.0	.500
D7	3.767	3	.9292	.5364	3.0	4.8	.863
D8	3.340	5	.6542	.2926	2.5	4.0	.428
D9	3.329	7	.9250	.3496	2.5	4.5	.856
D10	3.186	7	1.0107	.3820	2.0	4.5	1.021
D11	3.220	5	.7259	.3247	2.5	4.2	.527
D12	2.913	8	1.0973	.3880	2.0	4.8	1.204
D13	3.429	7	1.0226	.3865	1.8	4.8	1.046
D14	4.400	5	.4183	.1871	4.0	5.0	.175
D15	2.575	8	.6453	.2282	2.0	3.6	.416
D16	4.500	6	.3162	.1291	4.0	5.0	.100
D17	3.850	10	.6258	.1979	3.5	5.5	.392
D18	3.900	7	.9327	.3525	2.5	5.5	.870
D19	3.900	5	.4183	.1871	3.5	4.5	.175
D20	3.682	11	.3371	.1016	3.5	4.5	.114
Total	3.526	129	.8943	.0787	1.8	6.0	.800

Appendix

Data for Mutagenesis Study Using Gamma Irradiation.

Table 4.4.1.a. Data on number of survival of radiated *C. xwillisii* after 2 months.

Radiation	13 Dec 2006			
Place	MINT Bangi			
Dose	No. of sample/bottle	No. of bottles	No. of sample	No. of survival
0	3	5	15	15
10	3	5	15	10
20	3	5	15	15
30	3	5	15	9
40	3	5	15	7
50	3	5	15	10
60	3	5	15	12
70	3	5	15	7
80	3	5	15	13
		Total	135	98

Radiation	12 Jan 2007			
Place	MINT Bangi			
Dose	No. of sample/bottle	No. of bottles	No. of sample	No. of survival
0	5	3	15	0
150	5	3	15	0
200	5	3	15	0
250	5	3	15	0
300	5	3	15	0
400	5	3	15	0
500	5	3	15	0
600	5	3	15	0
700	5	3	15	0
800	5	3	15	0
900	5	3	15	0
		Total	165	0

Appendix

Radiation	7 Feb 2007			
Place	Physic Dept., UM			
Dose	No. of sample/bottle	No. of bottles	No. of sample	No. of survival
0	5	3	15	14
100	5	6	30	0
150	5	3	15	3
200	5	3	15	0
250	5	3	15	0
300	5	3	15	3
400	5	3	15	0
500	5	3	15	0
600	5	3	15	0
700	5	3	15	0
800	5	3	15	0
		Total	180	20

Radiation	14 Feb 2007			
Place	Physic Dept., UM			
Dose	No. of sample/bottle	No. of bottles	No. of sample	No. of survival
0	5	5	25	10
10	5	5	25	10
20	5	5	25	10
30	5	5	25	0
40	5	5	25	0
50	5	5	25	0
60	5	5	25	0
70	5	5	25	0
80	5	5	25	0
100	5	5	25	0
120	5	5	25	0
		Total	275	30

Appendix

Radiation	7 Mac 2007			
Place	Physic Dept., UM			
Dose	No. of sample/bottle	No. of bottles	No. of sample	No. of survival
0	5	3	15	15
100	5	3	15	0
150	5	3	15	0
200	5	3	15	0
250	5	3	15	0
300	5	3	15	0
400	5	3	15	0
500	5	3	15	0
600	5	3	15	0
700	5	3	15	0
800	5	3	15	0
		Total	165	15

Radiation	7 Mac 2007	
Place	Physic Dept., UM	
Dose	No. of sample	No. of survival
0	15	14
10	45	34
15	45	13
20	39	20
25	43	17
30	25	9
Total	140	107

Appendix

Radiation	26 Mac 2007	
Place	Physic Dept., UM	
Dose	No. of sample	No. of survival
0	15	12
10	15	14
15	25	24
20	30	25
25	25	12
30	15	2
35	15	2
40	15	0
50	15	0
60	15	0
70	15	0
80	15	0
90	15	0
100	15	0
Total	245	91

Radiation	2 April 2007			
Place	Physic Dept., UM			
Dose	No. of sample/bottle	No. of bottles	No. of sample	No. of survival
0	5	1	5	3
10	5	1	5	2
15	5	2	10	6
25	5	2	10	2
30	5	2	10	1
35	5	2	10	0
40	5	2	10	1
50	5	2	10	1
60	5	2	10	0
70	5	2	10	0
80	5	2	10	0
90	5	2	10	0
100	5	2	10	0
		Total	120	16

Appendix

Radiation	4 May 2007	
Place	Physic Dept., UM	
Dose	No. of sample	No. of survival
0	15	13
10	15	15
15	15	12
20	15	11
25	15	3
30	15	4
35	15	0
40	15	4
50	15	1
60	15	2
70	15	3
80	15	6
Total	245	74

Radiation	15 May 2007	
Place	Physic Dept., UM	
Dose	No. of sample	No. of survival
0	15	14
10	15	15
15	15	11
20	15	4
25	15	11
30	15	7
35	15	3
40	15	2
50	15	0
60	15	0
70	15	0
80	15	0
90	15	0
100	15	0
Total	245	67