

Appendices

Appendix 1

Percentage of plasmolysed cells in samples of 10 cortical (C) or epidermal (E) cells from the three cross-sections of thallus (T) soaked in seawater (30 ppt) with the respective mannitol concentrations at 30 minutes. Five samples were taken for each cross-section.

Mannitol Concentration (M)	Cell Type	Number of plasmolysed cells per five samples of 10 cells			
		T1	T2	T3	Average (%)
0.5	C	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0
	E	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0
0.6	C	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0
	E	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0
0.7	C	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0
	E	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0
0.8	C	2, 1, 2, 2, 1	3, 2, 1, 1, 2	0, 3, 3, 0, 2	17±9.8
	E	1, 1, 1, 1, 0	0, 0, 0, 0, 0	0, 1, 0, 0, 0	3±4.9
0.9	C	4, 1, 2, 2, 5	5, 7, 3, 2, 1	3, 5, 0, 0, 1	28±20.9
	E	1, 1, 1, 1, 0	1, 1, 1, 0, 1	0, 0, 1, 0, 0	6±5.1
1.0	C	4, 6, 3, 3, 4	4, 7, 6, 6, 4	6, 2, 6, 7, 4	48±15.7
	E	1, 0, 0, 0, 0	4, 2, 3, 4, 2	4, 2, 1, 2, 0	16±15.4
1.1	C	4, 3, 3, 3, 2	8, 6, 5, 5, 6	2, 6, 8, 6, 7	49±20.1
	E	0, 0, 1, 1, 1	7, 5, 3, 3, 1	3, 2, 1, 2, 2	21±18.8
1.2	C	4, 7, 7, 5, 2	10, 7, 6, 9, 7	9, 10, 10, 6, 7	71±23.1
	E	1, 1, 1, 0, 1	2, 1, 0, 2, 2	4, 3, 1, 1, 1	14±10.6

Appendix 2

Effect of time and single enzyme concentrations on protoplast yield.

Enzyme: Abalone Acetone Powder

Time: 2 hours.

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	5.34	5.66	4.0	$5.0 \times 10^4 \pm 8807$
2	1.66	1.32	1.34	$1.44 \times 10^4 \pm 1908$
3	1.0	0.0	0.32	$0.44 \times 10^4 \pm 5107$

Time: 1.5 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	5.0	3.0	4.5	$4.17 \times 10^4 \pm 10408$
2	2.0	5.0	5.5	$4.17 \times 10^4 \pm 18930$
3	4.0	1.5	2.0	$2.50 \times 10^4 \pm 13229$

Time: 1 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	2.0	0.5	1.5	$1.33 \times 10^4 \pm 7638$
2	1.0	0.0	2.0	$1.50 \times 10^4 \pm 10000$
3	0.5	2.0	1.5	$1.34 \times 10^4 \pm 7638$

Time: 0.5 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^3$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	1.0	0.0	0.5	$0.75 \times 10^3 \pm 5000$
2	0.5	0.5	0.5	$0.5 \times 10^3 \pm 0$
3	0.5	0.5	0.5	$0.5 \times 10^3 \pm 0$

Enzyme: Cellulase

Time : 2 hours

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	2.5	2.5	3.25	$2.75 \times 10^4 \pm 4330$
2	3.0	2.75	4.0	$3.25 \times 10^4 \pm 6614$
3	1.5	2.0	1.0	$1.50 \times 10^4 \pm 5000$

Time: 1.5 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	0.5	0.5	2.0	$1.0 \times 10^4 \pm 8660$
2	0.5	0.5	0.5	$0.5 \times 10^4 \pm 0$
3	1.0	2.0	0.5	$1.16 \times 10^4 \pm 7638$

Time: 1 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	0.5	0.5	1.0	$0.67 \times 10^4 \pm 2887$
2	0.5	0	0.5	$0.34 \times 10^4 \pm 2887$
3	0.5	0	0	$0.167 \times 10^4 \pm 2887$

Time: 0.5 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	0.5	0.5	0.5	$0.5 \times 10^4 \pm 0$
2	0	0	0.5	$0.167 \times 10^4 \pm 2887$
3	1.0	0.5	0.5	$0.67 \times 10^4 \pm 2887$

Enzyme: Pectinase

Time: 2 hours

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	2.0	2.5	2.5	$2.34 \times 10^4 \pm 2887$
2	2.25	2.5	2.0	$2.25 \times 10^4 \pm 2500$
3	2.0	2.25	1.75	$2.0 \times 10^4 \pm 2500$

Time: 1.5 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	1.5	2.0	1.0	$1.5 \times 10^4 \pm 5000$
2	2.0	1.0	1.5	$1.5 \times 10^4 \pm 5000$
3	1.5	1.5	1.0	$1.33 \times 10^4 \pm 2887$

Time: 1 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	1.5	1.0	1.0	$1.17 \times 10^4 \pm 2887$
2	0.5	1.0	0	$0.5 \times 10^4 \pm 5000$
3	0	0.5	2.0	$2.5 \times 10^4 \pm 10408$

Time: 0.5 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	1.25	1.0	0.75	$1.0 \times 10^4 \pm 2500$
2	1.0	0.5	0.5	$0.67 \times 10^4 \pm 2887$
3	0.5	0	0.5	$0.34 \times 10^4 \pm 2887$

Enzyme: Pectolyase

Time: 2 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	2.0	1.0	0.5	$1.67 \times 10^4 \pm 7638$
2	1.0	0	1.5	$0.83 \times 10^4 \pm 7638$
3	1.0	1.5	1.5	$1.33 \times 10^4 \pm 2887$

Time: 1.5 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	1.0	0.5	0.0	$0.5 \times 10^4 \pm 5000$
2	0.5	0	0.5	$0.33 \times 10^4 \pm 2887$
3	1.0	1.0	0.5	$0.83 \times 10^4 \pm 2887$

Time: 1 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	0.5	0.5	0	$0.33 \times 10^4 \pm 2887$
2	0	1.0	0.5	$0.5 \times 10^4 \pm 5000$
3	1.5	0.0	1.0	$0.83 \times 10^4 \pm 7638$

Time: 0.5 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	0.75	1.25	1.0	$1.0 \times 10^4 \pm 2500$
2	1.0	0.5	1.0	$0.83 \times 10^4 \pm 2887$
3	0.5	2.0	1.0	$1.17 \times 10^4 \pm 7638$

Enzyme: Agarase

Time: 2 hours

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	0.5	1.0	1.0	$0.83 \times 10^4 \pm 2887$
2	1.0	1.0	1.5	$1.17 \times 10^4 \pm 2887$
3	1.0	0.5	1.0	$0.83 \times 10^4 \pm 2887$

Time: 1.5 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	0	0	0	0
2	1.5	1.0	2.0	$1.5 \times 10^4 \pm 5000$
3	2.0	1.25	2.0	$1.75 \times 10^4 \pm 4330$

Time: 1 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	0	0	0	0
2	2.0	1.0	1.5	$1.5 \times 10^4 \pm 5000$
3	0	0	0	0

Time: 0.5 hour

Enzyme Concentration (% w/v)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0

Appendix 3

Effect of time and combination of enzymes - 1% each of abalone acetone powder, cellulase, pectinase, pectolyase and agarase on protoplast yield.

Time (hour)	Number of Viable Protoplasts per Gram of Fresh Weight in Each Replicate ($\times 10^4$)			Average Number of Viable Protoplasts per Gram of Fresh Weight from Three Replicates
	R1	R2	R3	
0.5	0	0	0	0
1	3.0	4.0	2.0	$3.0 \times 10^4 \pm 10000$
1.5	3.5	2.5	2.5	$2.8 \times 10^4 \pm 5774$
2	3.0	3.0	3.0	$3.0 \times 10^4 \pm 0$

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