

**APPENDIX A**  
**(Murashige and Skoog medium)**

<b>Medium composition</b>	<b>mg l<sup>-1</sup></b>
NH <sub>4</sub> NO <sub>3</sub>	1650
KNO <sub>3</sub>	1900
CaCl <sub>2</sub> .2H <sub>2</sub> O	440
MgSO <sub>4</sub> 7H <sub>2</sub> O	370
KH <sub>2</sub> PO <sub>4</sub>	170
KI	830
H <sub>3</sub> BO <sub>3</sub>	6200
MnSO <sub>4</sub> .4H <sub>2</sub> O	22300
ZnSO <sub>4</sub> .7H <sub>2</sub> O	8600
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	250
CuSO <sub>4</sub> .5H <sub>2</sub> O	25
CoCl <sub>2</sub> .6H <sub>2</sub> O	25
FeSO <sub>4</sub> .7H <sub>2</sub> O	27850
Na <sub>2</sub> EDTA.2H <sub>2</sub> O	37250
Myo-inositol	100
Nicotinic acid	500
Pyridoxine-HCl	500
Thiamine-HCl	100
Glycine	2

## APPENDIX B

### (Starch analysis reagents)

#### 1) Acetate buffer

Dissolve the following in 5.72 ml of acetic acid (99.8%):

- a)  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (0.2198 g)
- b) NaOH (first dissolve approximately 10 pellets in 20 ml water)

Adjust pH to 5 with NaOH and then make up to one litre with distilled water.

#### 2) Glucose oxidase colour solution

Dissolve the following in 500 ml distilled water in a two litre volumetric flask.

- a) 24.8 g disodium hydrogen orthophosphate (AR grade)
- b) 12.4 g sodium dihydrogen orthophosphate (AR grade)
- c) 4.0 g benzoic acid (GPR grade), dispersed in a small volume of ethanol
- d) 0.2 g 4-amino-antipyrine (Sigma Chemical Co., A-4382)
- e) 3.0 g p-hydroxybenzoic acid (Sigma Chemical Co., H-5376)
- f) 0.04 g glucose oxidase (Sigma Chemical Co., G-2133)
- g) 0.01 g peroxidase (Sigma Chemical Co., P-8375)

Afterwards, make up the solution to two litres and stored at 4°C in the dark. Only remove the solution from refrigerator before use.

### 3) Glucose standard solution

Add 1 ml of glucose solution (reagent 5) to 9 ml distilled water and vortex. This solution is used in the standard for test tubes 1 to 8. For test tubes 9 and 10, the undiluted glucose solution (reagent 5) is used.

The glucose standard curve is drawn from the glucose standard solution according to the volumes listed below.

Test Tube	Glucose Standard Solution ( $\mu\text{l}$ )	Glucose Oxidase Colour Solution (ml)	Glucose Concentration ( $\text{mg } 5 \text{ ml}^{-1}$ )
1	100	4.90	0.01
2	200	4.80	0.02
3	300	4.70	0.03
4	400	4.60	0.04
5	500	4.50	0.05
6	600	4.40	0.06
7	800	4.20	0.08
8	1000	4.00	0.10
9	150	4.85	0.15
10	200	4.80	0.20

## APPENDIX C

### Abbreviated analysis of variance (ANOVA) tables

**Table C1.** Analysis of variance of cotyledon fresh mass of *P. cynaroides* zygotic embryos germinated on MS medium (Figure 2.2).

Source	DF	SS	MS	F-value	Pr > F
Treatment	11	318583.16	28962.11	29.01	< 0.0001
Error	132	131778.45	998.32		
Total	143	450361.61			
Media	2	196948.43	98474.21	98.64	< 0.0001
Light	1	1730.39	1730.39	1.73	0.1903
Temperature	1	217.02	217.02	0.22	0.6418
Media*Light	2	11122.59	5561.30	5.57	0.0048
Media*Temperature	2	6203.23	3101.62	3.11	0.0480
Light*Temperature	1	2020.34	2020.34	2.02	0.1572
Media*Light*Temperature	2	1157.90	578.95	0.58	0.5614
CV = 25.73		R <sup>2</sup> = 0.71			

**Table C2.** Analysis of variance of root fresh mass of *P. cynaroides* zygotic embryos germinated on MS medium (Figure 2.3).

Source	DF	SS	MS	F-value	Pr > F
Treatment	11	775.06	70.46	48.92	< 0.0001
Error	132	190.12	1.44		
Total	143	965.17			
Media	2	546.88	273.44	189.85	< 0.0001
Light	1	2.07	2.07	1.43	0.2332
Temperature	1	31.96	31.96	22.19	< 0.0001
Media*Light	2	23.46	11.73	8.14	0.0005
Media*Temperature	2	18.36	9.18	6.37	0.0023
Light*Temperature	1	5.81	5.81	4.03	0.0466
Media*Light*Temperature	2	2.68	1.34	0.93	0.3964
CV = 27.18		R <sup>2</sup> = 0.80			

**Table C3.** Analysis of variance for the mean number of somatic embryos formed on *P. cynaroides* explants on MS medium after 8 weeks in culture (Table 4.1).

Source	DF	SS	MS	F-value	Pr > F
Treatment	3	242.28	80.76	40.22	0.0001
Error	58	116.46	2.01		
Total	61	358.74			
CV	21.59				
R <sup>2</sup>	0.67				

**Table C4.** Analysis of variance for the effect of pre-treatments on the mean root dry mass of *P. cynaroides* cuttings after 90 days (Figure 5.3).

Source	DF	SS	MS	F-value	Pr > F
Treatment	5	143489.14	28697.83	8.72	< 0.0001
Error	60	197414.15	3290.24		
Total	65	340903.29			
CV	73.75				
R <sup>2</sup>	0.42				

**Table C5.** Analysis of variance for the effect of pre-treatments on the number of roots formed between 1 and 10 mm in length (Group 1) (Table 5.1).

Source	DF	SS	MS	F-value	Pr > F
Treatment	5	266.27	53.25	5.98	0.001
Error	24	213.60	8.9		
Total	29	479.87			
CV	52.03				
R <sup>2</sup>	0.55				

**Table C6.** Analysis of variance for the effect of pre-treatments on the number of roots formed between 11 and 20 mm in length (Group 2) (Table 5.1).

Source	DF	SS	MS	F-value	Pr > F
Treatment	5	313.60	62.72	24.60	< 0.0001
Error	24	61.20	2.55		
Total	29	374.80			
CV	38.02				
R <sup>2</sup>	0.84				

**Table C7.** Analysis of variance for the effect of pre-treatments on the number of roots formed between 21 and 30 mm in length (Group 3) (Table 5.1).

Source	DF	SS	MS	F-value	Pr > F
Treatment	5	342.80	68.56	24.93	< 0.0001
Error	24	66.00	2.75		
Total	29	408.80			
CV	34.55				
R <sup>2</sup>	0.84				

**Table C8.** Analysis of variance for the effect of pre-treatments on the number of roots formed between 31 and 40 mm in length (Group 4) (Table 5.1).

Source	DF	SS	MS	F-value	Pr > F
Treatment	5	45.77	9.15	10.36	< 0.0001
Error	24	21.20	0.88		
Total	29	66.97			
CV	35.69				
R <sup>2</sup>	0.68				

**Table C9.** Analysis of variance for the effect of pre-treatments on the number of roots formed between 41 and 50 mm in length (Group 5) (Table 5.1).

Source	DF	SS	MS	F-value	Pr > F
Treatment	5	294.80	58.96	16.38	< 0.0001
Error	24	86.40	3.60		
Total	29	381.20			
CV	52.70				
R <sup>2</sup>	0.77				

**Table C10.** Analysis of variance for the effect of pre-treatments on the number of roots formed that were >51 mm in length (Group 6) (Table 5.1).

Source	DF	SS	MS	F-value	Pr > F
Treatment	5	122.7	24.54	17.32	< 0.0001
Error	24	34.00	1.41		
Total	29	156.70			
CV	62.64				
R <sup>2</sup>	0.78				

**Table C11.** Analysis of variance of dry root mass of cuttings during rooting period (60, 90 and 120 days after planting) (Figure 5.4).

Source	DF	SS	MS	F-value	Pr > F
Treatment	5	226888.63	45377.73	7.73	< 0.0001
Error	35	205569.28	5873.41		
Total	40	432457.92			
CV	51.73				
R <sup>2</sup>	0.52				

**Table C12.** Analysis of variance of total phenolic content in the basal part of *P. cynaroides* cuttings taken from motherplants (0 days) (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	677.68	677.68	86.13	< 0.0001
Error	6	47.21	7.87		
Total	7	724.89			
CV	8.53				
R <sup>2</sup>	0.93				

**Table C13.** Analysis of variance of total phenolic content in the basal part of *P. cynaroides* cuttings during rooting, after 60 days (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	669.32	669.32	84.91	< 0.0001
Error	6	47.29	7.88		
Total	7	716.62			
CV	4.93				
R <sup>2</sup>	0.93				

**Table C14.** Analysis of variance of total phenolic content in the basal part of *P. cynaroides* cuttings during rooting, after 90 days (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	1086.41	1086.41	38.79	0.0008
Error	6	168.05	28.01		
Total	7	1254.46			
CV	7.3				
R <sup>2</sup>	0.87				



**Table C15.** Analysis of variance of total phenolic content in the basal part of *P. cynaroides* cuttings during rooting, after 120 days (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	81.78	81.78	2.49	0.1654
Error	6	196.80	32.80		
Total	7	278.59			
CV	7.02				
R <sup>2</sup>	0.29				

**Table C16.** Analysis of variance of total phenolic content in the middle part of *P. cynaroides* cuttings taken from motherplants (0 days) (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	644.19	644.19	52.97	< 0.0003
Error	6	72.96	12.16		
Total	7	717.16			
CV	11.72				
R <sup>2</sup>	0.90				

**Table C17.** Analysis of variance of total phenolic content in the middle part of *P. cynaroides* cuttings during rooting, after 60 days (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	502.90	502.90	82.96	< 0.0001
Error	6	36.37	6.06		
Total	7	539.27			
CV	3.67				
R <sup>2</sup>	0.93				

**Table C18.** Analysis of variance of total phenolic content in the middle part of *P. cynaroides* cuttings during rooting, after 90 days (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	790.92	790.92	100.06	< 0.0001
Error	6	47.43	7.90		
Total	7	838.34			
CV	3.96				
R <sup>2</sup>	0.94				

**Table C19.** Analysis of variance of total phenolic content in the middle part of *P. cynaroides* cuttings during rooting, after 120 days (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	795.28	795.28	47.22	< 0.0005
Error	6	101.04	16.84		
Total	7	896.32			
CV	6.00				
R <sup>2</sup>	0.89				

**Table C20.** Analysis of variance of total phenolic content in the top part of *P. cynaroides* cuttings taken from motherplants (0 days) (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	22.18	22.18	2.47	< 0.1674
Error	6	53.96	8.99		
Total	7	76.14			
CV	7.41				
R <sup>2</sup>	0.29				

**Table C21.** Analysis of variance of total phenolic content in the top part of *P. cynaroides* cuttings during rooting, after 60 days (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	3.16	3.16	0.13	< 0.7318
Error	6	146.97	24.49		
Total	7	150.13			
CV	6.06				
R <sup>2</sup>	0.02				

**Table C22.** Analysis of variance of total phenolic content in the top part of *P. cynaroides* cuttings during rooting, after 90 days (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	9.19	9.19	0.29	< 0.6081
Error	6	188.51	31.42		
Total	7	197.71			
CV	5.33				
R <sup>2</sup>	0.05				

**Table C23.** Analysis of variance of total phenolic content in the top part of *P. cynaroides* cuttings during rooting, after 120 days (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	0.82	0.82	0.03	< 0.8795
Error	6	196.34	32.72		
Total	7	197.16			
CV	5.49				
R <sup>2</sup>	0.004				

**Table C24.** Analysis of variance of total phenolic content in the leaves of *P. cynaroides* cuttings taken from motherplants (0 days) (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	105.70	105.70	2.11	< 0.1968
Error	6	300.89	50.15		
Total	7	406.58			
CV	8.14				
R <sup>2</sup>	0.26				

**Table C25.** Analysis of variance of total phenolic content in the leaves of *P. cynaroides* cuttings during rooting, after 60 days (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	17.05	17.05	0.27	< 0.6188
Error	6	372.17	62.03		
Total	7	389.22			
CV	5.61				
R <sup>2</sup>	0.04				

**Table C26.** Analysis of variance of total phenolic content in the leaves of *P. cynaroides* cuttings during rooting, after 90 days (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	13.39	13.39	0.21	< 0.6592
Error	6	373.70	62.28		
Total	7	387.09			
CV	6.58				
R <sup>2</sup>	0.03				

**Table C27.** Analysis of variance of total phenolic content in the leaves of *P. cynaroides* cuttings during rooting, after 120 days (Figure 5.5).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	6.26	6.26	0.38	< 0.5619
Error	6	99.75	16.63		
Total	7	106.02			
CV	3.50				
R <sup>2</sup>	0.06				

**Table C28.** Analysis of variance of starch content in the basal part of *P. cynaroides* cuttings taken from motherplants (0 days) (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	101.11	101.11	12.49	< 0.0241
Error	4	32.37	8.09		
Total	5	133.48			
CV	9.31				
R <sup>2</sup>	0.76				

**Table C29.** Analysis of variance of starch content in the basal part of *P. cynaroides* cuttings during rooting, after 60 days (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	9.20	9.20	0.64	< 0.4681
Error	4	57.39	14.35		
Total	5	66.59			
CV	11.04				
R <sup>2</sup>	0.14				

**Table C30.** Analysis of variance of starch content in the basal part of *P. cynaroides* cuttings during rooting, after 90 days (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	142.79	142.79	149.40	< 0.0003
Error	4	3.82	0.96		
Total	5	146.61			
CV	3.20				
R <sup>2</sup>	0.97				

**Table C31.** Analysis of variance of starch content in the basal part of *P. cynaroides* cuttings during rooting, after 120 days (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	260.70	260.70	44.92	< 0.0026
Error	4	23.21	5.80		
Total	5	283.91			
CV	6.30				
R <sup>2</sup>	0.92				

**Table C32.** Analysis of variance of starch content in the middle part of *P. cynaroides* cuttings taken from motherplants (0 days) (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	13.35	13.35	0.95	< 0.3856
Error	4	56.38	14.10		
Total	5	69.73			
CV	11.97				
R <sup>2</sup>	0.19				

**Table C33.** Analysis of variance of starch content in the middle part of *P. cynaroides* cuttings during rooting, after 60 days (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	57.54	57.54	2.78	< 0.1705
Error	4	82.63	20.66		
Total	5	140.19			
CV	12.50				
R <sup>2</sup>	0.41				

**Table C34.** Analysis of variance of starch content in the middle part of *P. cynaroides* cuttings during rooting, after 90 days (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	140.17	140.17	136.93	< 0.0003
Error	4	4.09	1.02		
Total	5	144.26			
CV	3.30				
R <sup>2</sup>	0.97				

**Table C35.** Analysis of variance of starch content in the middle part of *P. cynaroides* cuttings during rooting, after 120 days (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	273.11	273.11	79.47	< 0.0009
Error	4	13.75	3.44		
Total	5	286.85			
CV	4.90				
R <sup>2</sup>	0.95				

**Table C36.** Analysis of variance of starch content in the top part of *P. cynaroides* cuttings taken from motherplants (0 days) (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	56.61	56.61	4.19	< 0.1102
Error	4	54.10	13.53		
Total	5	110.71			
CV	11.62				
R <sup>2</sup>	0.51				

**Table C37.** Analysis of variance of starch content in the top part of *P. cynaroides* cuttings during rooting, after 60 days (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	174.21	174.21	47.72	< 0.0023
Error	4	14.60	3.65		
Total	5	188.81			
CV	5.28				
R <sup>2</sup>	0.93				

**Table C38.** Analysis of variance of starch content in the top part of *P. cynaroides* cuttings during rooting, after 90 days (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	207.92	207.92	12.47	< 0.0242
Error	4	66.70	16.67		
Total	5	274.61			
CV	13.45				
R <sup>2</sup>	0.76				



**Table C39.** Analysis of variance of starch content in the top part of *P. cynaroides* cuttings during rooting, after 120 days (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	96.16	96.16	7.41	< 0.0529
Error	4	51.89	12.97		
Total	5	148.05			
CV	9.14				
R <sup>2</sup>	0.65				

**Table C40.** Analysis of variance of starch content in the leaves of *P. cynaroides* cuttings taken from motherplants (0 days) (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	93.22	93.22	4.62	< 0.0982
Error	4	80.80	20.20		
Total	5	174.02			
CV	10.17				
R <sup>2</sup>	0.54				

**Table C41.** Analysis of variance of starch content in the leaves of *P. cynaroides* cuttings during rooting, after 60 days (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	127.70	127.70	9.44	< 0.0572
Error	4	54.13	13.53		
Total	5	181.83			
CV	7.71				
R <sup>2</sup>	0.70				

**Table C42.** Analysis of variance of starch content in the leaves of *P. cynaroides* cuttings during rooting, after 90 days (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	290.51	290.51	15.35	< 0.0173
Error	4	75.71	18.93		
Total	5	366.23			
CV	10.97				
R <sup>2</sup>	0.79				

**Table C43.** Analysis of variance of starch content in the leaves of *P. cynaroides* cuttings during rooting, after 120 days (Figure 5.6).

Source	DF	SS	MS	F-value	Pr > F
Treatment	1	1695.12	1695.12	7.44	< 0.050
Error	4	911.52	227.88		
Total	5	2606.64			
CV	23.31				
R <sup>2</sup>	0.65				

**Table C44.** Analysis of variance of root length of *P. cynaroides* explants cultured on MS medium containing 3,4-dihydroxybenzoic acid concentrations (Table 6.1).

Source	DF	SS	MS	F-value	Pr > F
Treatment	7	78.09	11.15	5.63	< 0.0001
Error	40	79.25	1.98		
Total	47	157.33			
CV	24.97				
R <sup>2</sup>	0.50				

**Table C45.** Analysis of variance of root fresh mass of *P. cynaroides* explants cultured on MS medium containing 3,4-dihydroxybenzoic acid concentrations (Table 6.1).

Source	DF	SS	MS	F-value	Pr > F
Treatment	7	34.66	4.95	10.39	< 0.0001
Error	38	18.12	0.48		
Total	45	52.78			
CV	67.30				
R <sup>2</sup>	0.66				