UNIVERSITI PUTRA MALAYSIA

CRYOPRESERVATION OF EXCISED EMBRYOS OF RAMBUTAN (NEPHELIUM LAPPACEUM L.) USING VITRIFICATION TECHNIQUE

FLORENCE C. GINIBUN

FP 2001 18

CRYOPRESERVATION OF EXCISED EMBRYOS OF RAMBUTAN (*NEPHELIUM LAPPACEUM* L.) USING VITRIFICATION TECHNIQUE

By

FLORENCE C. GINIBUN

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Agricultural Science in the Faculty of Agriculture Universiti Putra Malaysia

February 2001

Dedicated To:

My Beloved Parents:

Camillus Ginibun and Jovinia Polycarpus

My Beloved Sisters:

Janet, Rose, Rovina and Linda

My Beloved Relatives and Friends

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Agricultural Science.

CRYOPRESERVATION OF EXCISED EMBRYOS OF RAMBUTAN (*NEPHELIUM LAPPACEUM* L.) USING VITRIFICATION TECHNIQUE

By

FLORENCE C. GINIBUN

February 2001

Chairman : Associate Professor Hor Yue Luan, Ph. D.

Faculty : Agriculture

The present study evaluates the effects of various loading solutions, concentrations of glycerol and vitrification solutions and their time of exposure on the vitrification of rambutan embryos in liquid nitrogen.

In the initial study to evaluate the effects of loading solutions on survival, excised embryos were exposed to four loading solutions. The two most promising loading solutions were LB (1.5 M glycerol + 0.4 M sucrose + 5 % DMSO), which gave 44.0 % viability and 32.4 % survival and LA (2.0 M glycerol + 0.4 M sucrose) which gave 39.3 % viability and 28.1 % survival after freezing.

The effects of different concentrations of glycerol (0 – 2.0 M) in the most promising loading solutions were evaluated further. For loading solution LB, 1.5 M glycerol gave highest survival of 22.7 %. For loading

solution LB, 1.5 M glycerol gave highest survival of 22.7 %. For loading solution LA, 0 M glycerol or the use of only 0.4 M sucrose gave the highest viability of 76.0 % and survival of 59.0 %. Hence, loading solution with only 0.4 M sucrose (LA without glycerol) was established in this study as the most effective loading solution for rambutan embryos.

The effects of exposure time (0 – 16 hours) to the best loading solution on survival of rambutan embryos were further investigated. It was found that 8 hours duration gave the highest viability (47.7 %) and survival (32.8 %).

Having confirmed the best loading treatment, the study further evaluates the effects of six vitrification solutions on survival of rambutan embryos in liquid nitrogen. The results show that after freezing, L Solution gave the highest viability (46.0 %) and survival (24.0 %). L Solution was therefore selected as the most effective vitrification solution.

In optimizing the time of exposure, excised rambutan embryos were exposed to L Solution for 0 to 90 minutes before LN exposure. The highest viability (55.6 %) and survival (40.3 %) after vitirification were achieved at 60 minutes exposure. Longer exposure to L Solution for up to 90 minutes reduced survival to 16.0 %.

This study concludes that 0.4 M sucrose loaded for 8 hours, followed by exposure to L Solution for 60 minutes was optimum for the vitrification of excised rambutan embryos, which yielded 55.6 % viability and 40.3 % survival.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Pertanian

PENGKRIOAWETAN EMBRIO RAMBUTAN (*NEPHELIUM LAPPACEUM* L.) MELALUI TEKNIK VITRIFIKASI

Oleh

FLORENCE C. GINIBUN

Februari 2001

Pengerusi Penyelia : Prof. Madya Hor Yue Luan, Ph.D.

Fakulti : Pertanian

Kajian ini menilai kesan pelbagai larutan 'loading', kepekatan larutan gliserol, larutan vitrifikasi dan tempoh pendedahannya ke atas penvitrifikasian embrio rambutan di dalam cecair nitrogen.

Dalam kajian menilai kesan larutan 'loading' ke atas kemandirian embrio rambutan, embrio didedahkan kepada empat larutan 'loading'. Dua jenis larutan 'loading' yang memberi kesan ialah LB (1.5 M gliserol + 0.4 M sukrosa + 5 % DMSO) di mana 44.0 % viabiliti dan 32.4 % kemandirian diperolehi dan LA (2.0 M gliserol + 0.4 M sukrosa) memberi hasil 39.3 % viability dan 28.1 % kemandirian setelah disejukbekukan.

Kesan ke atas perbezaan kepekatan gliserol (0 – 2.0 M) dalam larutan 'loading' yang paling berpotensi dikaji seterusnya. Dalam larutan 'loading' LB, 1.5 M gliserol memberikan kemandirian yang tertinggi sebanyak 22.7 %. Dalam larutan 'loading' LA, 0 M gliserol atau

penggunaan hanya 0.4 M sukrosa, memberi viabiliti yang tertinggi sebanyak 76.0 % dan kemandirian sebanyak 59.0 %. Oleh yang demikian, larutan 'loading' dengan hanya 0.4 M sukrosa (LA tanpa gliserol) terbukti di dalam kajian ini sebagai larutan yang paling efektif terhadap embrio rambutan.

Kesan tempoh pendedahan (0 – 16 jam) larutan 'loading' yang terbaik ke atas kemandirian embrio rambutan seterusnya dinilai. Di dapati bahawa tempoh pendedahan selama 8 jam memberikan viabiliti (47.7 %) dan kemandirian (32.8 %) yang tertinggi.

Setelah mengenalpasti rawatan 'loading' yang terbaik, kajian seterusnya menilai kesan ke atas enam jenis larutan vitrifikasi terhadap kemandirian embrio rambutan dalam cecair nitrogen. Keputusan menunjukkan bahawa selepas penyejukbekuan, larutan L memberikan viabiliti (46.0 %) dan kemandirian (24.0 %) yang tertinggi dimana ianya lebih baik daripada larutan PVS2. Oleh yang demikian, larutan L dipilih sebagai larutan vitrifikasi yang paling efektif.

Untuk menentukan tempoh pendedahan yang optima, embrio rambutan dirawatkan dengan larutan L selama 0 sehingga 90 minit sebelum didedahkan ke dalam cecair nitrogen. Viabiliti (55.6 %) dan kemandirian (40.3 %) yang tertinggi selepas penvitrifikasian diperolehi pada tempoh pendedahan 60 minit. Tempoh pendedahan yang lebih

panjang ke atas larutan L sehingga 90 minit mengurangkan kemandirian sebanyak 16.0 %.

Kajian dapat disimpulkan bahawa rawatan dengan 0.4 M sukrosa selama 8 jam diikuti dengan pendedahan kepada larutan L selama 60 minit adalah yang optima untuk penvitrifikasi embrio rambutan di mana menghasilkan sebanyak 55.6 % viability dan 40.3 % kemandirian.

ACKNOWLEDGEMENTS

Firstly, I would like to thank God Almighty for giving me the inspiration to finish my thesis in the given time. It is my pleasure to take this opportunity to express my deepest appreciation and gratitude to my supervisor Associate Prof. Dr. Hor Yue Luan, of Department of Crop Science, Universiti Putra Malaysia for his constant encouragement, advice, guidance and friendship throughout my master's programme to the completion of this thesis.

My grateful appreciation is also due to my supervisory committee members, Associate Prof. Dr. Saleh bin Kadzimin of Department of Crop Science, Universiti Putra Malaysia and Dr. Baskaran Krishnapillay from Forestry Research Institute Malaysia (FRIM) for their comments and suggestion to improve my study.

I would like to thank Mr. Ong Choon Hoe and Puan Norafidah Yusoff, laboratory assistants of Seed Technology Research Laboratory, for their assistance and guidance in the laboratory during this study.

Special thanks also go to the staff of Field 5, Universiti Putra Malaysia and the Department of Agriculture, Serdang for their kind assistance and the supply of the rambutan fruits for this study.

My deepest thanks and love towards my parent, sisters and relatives for their love, support, prayer and assistance during my course of study in Universiti Putra Malaysia.

Last but not least, I would like to express my warmest gratitude to my beloved Syed Huzal bin Syed Jaffar and to all my friends especially Miss Wong Lay Yieng, Miss Cynthia P. Cossall, Miss Sam Yen Yen, Miss Shubashini, Mr. Hendry Joseph, Mr. Philip Sipen, Mr. Khairul Naim, Mr. Thaddeus Kasun and Mr. Peter Lintar for their kind support and friendship.

TABLE OF CONTENTS

		Page
DEDICATION		ii
ABSTRACT		iii
ABSTRAK		vi
ACKNOWLEDGEMENTS		ix
APPROVAL SHEETS		xi
DECLARATION FORM		xiii
LIST OF TABLES		xvii
LIST OF FIGURES		xxi
LIST OF ABBREVIATIONS		xxv
CHAPTER		
I	**INTRODUCTION**	1
II	**REVIEW OF LITERATURE**	6
	Taxonomy of Rambutan	6
	Germplasm Conservation	8
	Seed Classification and Behavior in Storage	9
	Storage Behavior of Recalcitrant Seeds	12
	Cryopreservation of Recalcitrant Seeds	13
	Cryopreservation of Embryos	14
	Cryoprotective Agents	18
	Vitrification	21
	Loading	23
	Unloading	25
	Freezing in Liquid Nitrogen	26
	Thawing and Recovery	28
III	**MATERIALS AND METHODS**	31
	Study Layout	31
	Experimental Materials	32
	Experimental Procedures	33
	Excision of Embryos	33
	Glassware and Cleaning for *In-vitro* Studies	35
	Preparation of MS Stock Solutions	35
	Preparation of MS Basal, MS Culture and Stabilisation Medium	36
	Preparation of Loading Solutions	37
	Preparation of Different Concentrations of Glycerol in Loading Solution	37
	Preparation of Vitrification Solutions	38
	Preparation of Unloading Solution	39

Vitrification Procedure 39
Incubation of Cultures 42
Measurement and Observations 42
Percentage Moisture of Excised Embryos 42
Percentage Viability and Survival of Excised Embryos in MS Medium 43
Experiments 45
Experiment 1: Effects of Different Loading Solutions on Survival of Excised Embryos of Rambutan in Liquid Nitrogen..... 45

Experiment 2: Effects of Glycerol Concentrations in the Loading Solution LB (1.5 M Glycerol with 0.4 M Sucrose and 5 % DMSO) and LA (2 M Glycerol and 0.4 M Sucrose) on Survival of Excised Embryos of Rambutan in Liquid Nitrogen... 47

Experiment 2A: Effects of Glycerol Concentrations in Loading Solution LB on Survival of Excised Embryos of Rambutan in Liquid Nitrogen..... 47

Experiment 2B: Effects of Glycerol Concentrations in Loading Solution LA on Survival of Excised Embryos of Rambutan in Liquid Nitrogen..... 49

Experiment 3: Effects of Exposure Time to Loading Solution (0.4 M Sucrose) on Survival of Excised Embryos of Rambutan in Liquid Nitrogen..... 50

Experiment 4: Effects of Different Vitrification Solutions on Survival of Excised Embryos of Rambutan in Liquid Nitrogen..... 51

Experiment 5: Effects of Exposure Time to L Solution on Survival of Excised Embryos of Rambutan in Liquid Nitrogen..... 53

Statistical Analysis 54

IV RESULTS AND DISCUSSIONS 55

Experiment 1: Effects of Different Loading Solutions on Survival of Excised Embryos of Rambutan in Liquid Nitrogen..... 55

Experiment 2A: Effects of Glycerol Concentrations in Loading Solution LB on Survival of Excised Embryos of Rambutan in Liquid Nitrogen..... 64

Experiment 2B: Effects of Glycerol Concentrations in Loading Solution LA on Survival of Excised Embryos of Rambutan in Liquid Nitrogen..... 73

Experiment 3: Effects of Exposure Time to Loading Solution (0.4 M Sucrose) on Survival of Excised Embryos of Rambutan in Liquid Nitrogen..... 82

Experiment 4: Effects of Different Vitrification Solutions on Survival of Excised Embryos of Rambutan in Liquid Nitrogen..... 91

Experiment 5: Effects of Exposure Time to L Solution on Survival of Excised Embryos of Rambutan in Liquid Nitrogen..... 99

V **SUMMARY AND CONCLUSION** 107

REFERENCES 110

APPENDICES 122

APPENDIX A
Murashige and Skoog (1962) Inorganic Salts and Vitamin 122

APPENDIX B
Additional Tables (Morphological Categorisation) 123

APPENDIX C
Statistical Analysis..... 129

BIODATA OF AUTHOR 146

LIST OF TABLES

Table		Page
1	Percentage viability and survival of excised rambutan embryos after exposure to different loading solutions, after PVS2 dehydration (-LN) and after LN exposure (+LN).....	56
2	Moisture content of excised rambutan embryos after exposure to different loading solutions and dehydration in PVS2.....	59
3	Percentage viability and survival of excised rambutan embryos after exposure to different concentrations of glycerol in the loading solution LB after PVS2 dehydration (-LN) and after LN exposure (+LN).....	65
4	Moisture content of excised rambutan embryos after exposure to different concentrations of glycerol in the loading solution LB and after dehydration in PVS2.....	68
5	Percentage viability and survival of excised rambutan embryos after exposure to different concentrations of glycerol in the loading solution LA, after PVS2 dehydration (-LN) and after LN exposure (+LN)	74
6	Moisture content of excised rambutan embryos after exposure to different concentrations of glycerol in the loading solution LA and after dehydration in PVS2.....	77
7	Percentage viability and survival of excised rambutan embryos after loading in 0.4 M sucrose for different duration, followed by PVS2 desiccation (-LN) and LN exposure (+LN)	83
8	Moisture content of excised rambutan embryos after loading in 0.4 M sucrose for different duration and desiccation in PVS2.....	86
9	Percentage viability and survival of excised rambutan embryos after exposure to different vitrification solutions before (-LN) and after (+LN) freezing in liquid nitrogen.....	92
10	Moisture content of excised rambutan embryos after exposure to different vitrification solutions.....	95

11	Percentage viability and survival of excised rambutan embryos after exposure to L Solution for different duration before (-LN) and after (+LN) freezing in liquid nitrogen.....	100
12	Moisture content of excised rambutan embryos after exposure to L Solution for different duration.....	103
13	Morphological categorisation for survival evaluation of excised rambutan embryos after exposure to different loading solutions, after PVS2 dehydration (-LN) and after LN exposure (+LN)	123
14	Morphological categorisation for survival evaluation of excised rambutan embryos after exposure to different concentrations of glycerol in the loading solution LB, after dehydration in PVS2 (-LN) and after LN exposure(+LN)...	124
15	Morphological categorisation for survival evaluation of excised rambutan embryos after exposure to different concentrations of glycerol in the loading solution LA, after dehydration in PVS2 (-LN) and after LN exposure (+LN)...	125
16	Morphological categorisation for survival evaluation of excised rambutan embryos after loading in 0.4 M sucrose for different duration followed by dehydration in PVS2 (-LN) and after LN exposure (+LN)	126
17	Morphological categorisation for survival evaluation of excised rambutan embryos after exposure to different vitrification solutions before (-LN) and after LN exposure (+LN)	127
18	Morphological categorisation for survival evaluation of excised rambutan embryos after exposure to L Solution for different duration before (-LN) and after exposure (+LN)	128
19	ANOVA table of percentage viability of excised rambutan embryos after exposure to different loading solutions (a), after dehydration in PVS2 (-LN) (b) and after LN exposure (+LN) (c)	129
20	ANOVA table of percentage survival of excised rambutan embryos after exposure to different loading solutions (a), after dehydration in PVS2 (-LN) (b) and after LN exposure (+LN) (c)	130

21 ANOVA table of percentage viability of excised rambutan embryos after exposure to different concentrations of glycerol in the loading solution LB (a), after dehydration in PVS2 (-LN) (b) and after LN exposure (+LN) (c) 131

22 ANOVA table of percentage survival of excised rambutan embryos after exposure to different concentrations of glycerol in the loading solution LB (a), after dehydration in PVS2 (-LN) (b) and after LN exposure (+LN) (c)..... 132

23 ANOVA table of percentage viability of excised rambutan embryos after exposure to different concentrations of glycerol in the loading solution LA (a), after dehydration in PVS2 (-LN) (b) and after LN exposure (+LN) (c) 133

24 ANOVA table of percentage survival of excised rambutan embryos after exposure to different concentrations of glycerol in the loading solution LA (a), after dehydration in PVS2 (-LN) (b) and after LN exposure (+LN) (c) 134

25 ANOVA table of percentage viability of excised rambutan embryos after loading in 0.4 M sucrose for different duration (a), followed by dehydration in PVS2 (-LN) (b) and after LN exposure (+LN) (c) 135

26 ANOVA table of percentage survival of excised rambutan embryos after loading in 0.4 M sucrose for different duration (a), followed by dehydration in PVS2 (-LN) (b) and after LN exposure (+LN) (c)..... 136

27 ANOVA table of percentage viability of excised rambutan embryos after exposure to different vitrification solutions before (-LN) (a) and after (+LN) (b) freezing in liquid nitrogen..... 137

28 ANOVA table of percentage survival of excised rambutan embryos after exposure to different vitrification solutions before (-LN) (a) and after (+LN) (b) freezing in liquid nitrogen..... 137

29 ANOVA table of percentage viability of excised rambutan embryos after exposure to L Solution for different exposure time before (-LN) (a) and after (+LN) (b) freezing in liquid nitrogen..... 138

30 ANOVA table of percentage survival of excised rambutan embryos after exposure to L Solution for different exposure time before (-LN) (a) and after (+LN) (b) freezing in liquid nitrogen..... 138

31 ANOVA table of moisture content of excised rambutan embryos after exposure to different loading solutions (a) and dehydration in PVS2 (b)..... 139

32 ANOVA table of moisture content of excised rambutan embryos after exposure to different concentrations of glycerol in the loading solution LB (a) and after dehydration in PVS2 (b)..... 139

33 ANOVA table of moisture content of excised rambutan embryos after exposure to different concentrations of glycerol in the loading solution LA (a) and after dehydration in PVS2 (b)..... 140

34 ANOVA table of moisture content of excised rambutan embryos after loading in 0.4 M sucrose for different exposure time (a) and dehydration in PVS2 (b)..... 140

35 ANOVA table of moisture content of excised rambutan embryos after exposure to different vitrification solutions... 141

36 ANOVA table of moisture content of excised rambutan embryos after exposure to L Solution for different exposure time 141

LIST OF FIGURES

Figure		Page
1	Matured unripe rambutan fruits of variety R7.....	32
2	Rambutan seed of variety R7.....	33
3	Rambutan embryos attached to the cotyledons.....	34
4	Excision of rambutan embryos.....	34
5	Test materials in cryovial secured to cryocanes.....	40
6	Freezing of test materials using LN in a cryogenic tank	41
7a	Morphological categorization (A - D).....	44
7b	Morphological categorization (E - H).....	44
8a	Development of excised rambutan embryos after exposure to different loading solutions (from left: Fresh control, Control 1, LA, LB; 8 weeks, after loading).....	61
8b	Development of excised rambutan embryos after exposure to different loading solutions (from left: LC, LD, Control 2; 8 weeks, after loading).....	61
9a	Development of excised rambutan embryos after exposure to different loading solutions and PVS2 before freezing in LN (from left: Fresh control, Control 1, LA, LB; 8 weeks, -LN).....	62
9b	Development of excised rambutan embryos after exposure to different loading solutions and PVS2 before freezing in LN (from left: LC, LD, Control 2; 8 weeks, -LN).....	62
10a	Development of excised rambutan embryos after exposure to different loading solutions and PVS2 after freezing in LN (from left: Fresh control, Control 1, LA, LB; 8 weeks, +LN)..	63
10b	Development of excised rambutan embryos after exposure to different loading solutions and PVS2 after freezing in LN (from left: LC, LD, Control 2; 8 weeks, +LN).....	63

11a Development of excised rambutan embryos after exposure to different concentrations of glycerol in loading solution LB (from left: Fresh control, Control 2, 0.0 M, 0.5 M; 8 weeks, after loading)..... 70

11b Development of excised rambutan embryos after exposure to different concentrations of glycerol in loading solution LB (from left: 1.0 M, 1.5 M, 2.0 M; 8 weeks, after loading)... 70

12a Development of excised rambutan embryos after exposure to different concentrations of glycerol in loading solution LB before freezing in LN (from left: Fresh control, Control 2, 0.0 M, 0.5 M; 8 weeks, -LN)..... 71

12b Development of excised rambutan embryos after exposure to different concentrations of glycerol in loading solution LB before freezing in LN (from left: 1.0 M, 1.5 M, 2.0 M; 8 weeks, -LN)..... 71

13a Development of excised rambutan embryos after exposure to different concentrations of glycerol in loading solution LB after freezing in LN (from left: Fresh control, Control 2, 0.0 M, 0.5 M; 8 weeks, +LN)..... 72

13b Development of excised rambutan embryos after exposure to different concentrations of glycerol in loading solution LB after freezing in LN (from left: 1.0 M, 1.5 M, 2.0 M; 8 weeks,+LN)..... 72

14a Development of excised rambutan embryos after exposure to different concentrations of glycerol in loading solution LA (from left: Fresh control, Control 2, 0.0 M, 0.5 M; 8 weeks, after loading)..... 79

14b Development of excised rambutan embryos after exposure to different concentrations of glycerol in loading solution LA (from left: 1.0 M, 1.5 M, 2.0 M; 8 weeks, after loading)... 79

15a Development of excised rambutan embryos after exposure to different concentrations of glycerol in loading solution LA before freezing in LN (from left: Fresh control, Control 2, 0.0 M, 0.5 M; 8 weeks, -LN)..... 80

15b Development of excised rambutan embryos after exposure to different concentrations of glycerol in loading solution LA before freezing in LN (from left: 1.0 M, 1.5 M, 2.0 M; 8 weeks, -LN)..... 80

16a Development of excised rambutan embryos after exposure to different concentrations of glycerol in loading solution LA after freezing in LN (from left: Fresh control, Control 2, 0.0 M, 0.5 M; 8 weeks, +LN)..... 81

16b Development of excised rambutan embryos after exposure to different concentrations of glycerol in loading solution LA after freezing in LN (from left: 1.0 M, 1.5 M, 2.0 M; 8 weeks, +LN)..... 81

17a Development of excised rambutan embryos after exposure to loading solution (0.4 M sucrose) for different duration (from left: 0, 0.5, 1, 2, 4 hr; 8 weeks, after loading)..... 88

17b Development of excised rambutan embryos after exposure to loading solution (0.4 M sucrose) for different duration (from left: 6, 8, 12, 16 hr; 8 weeks, after loading)..... 88

18a Development of excised rambutan embryos after exposure to loading solution (0.4 M sucrose) for different duration before freezing in LN (from left: 0, 0.5, 1, 2, 4 hr; 8 weeks, -LN)..... 89

18b Development of excised rambutan embryos after exposure to loading solution (0.4 M sucrose) for different duration before freezing in LN (from left: 6, 8, 12, 16 hr; 8 weeks, -LN)..... 89

19a Development of excised rambutan embryos after exposure to loading solution (0.4 M sucrose) for different duration after freezing in LN (from left: 0, 0.5, 1, 2, 4 hr; 8 weeks, +LN)..... 90

19b Development of excised rambutan embryos after exposure to loading solution (0.4 M sucrose) for different duration after freezing in LN (from left: 6, 8, 12, 16 hr; 8 weeks, +LN)..... 90

20a Development of excised rambutan embryos after exposure to different vitrification solutions before freezing in LN (from left: Fresh control, After loading, PVS, PVS2; 8 weeks, -LN)..... 97

20b Development of excised rambutan embryos after exposure to different vitrification solutions before freezing in LN (from left: PVS3, L Solution, Towill, Watanabe; 8 weeks, -LN)..... 97

21a Development of excised rambutan embryos after exposure to different vitrification solutions after freezing in LN (from left: Fresh control, After loading, PVS, PVS2; 8 weeks, +LN)..... 98

21b Development of excised rambutan embryos after exposure to different vitrification solutions after freezing in LN (from left: PVS3, L Solution, Towill, Watanabe; 8 weeks, +LN).... 98

22a Development of excised rambutan embryos after exposure to L Solution for different duration before freezing in LN (from left: Fresh control, 0, 15, 30 min; 8 weeks, -LN)..... 105

22b Development of excised rambutan embryos after exposure to L Solution for different duration before freezing in LN (from left: 45, 60, 75, 90 min; 8 weeks, -LN)..... 105

23a Development of excised rambutan embryos after exposure to L Solution for different duration after freezing in LN (from left: Fresh control, 0, 15, 30 min; 8 weeks, +LN)..... 106

23b Development of excised rambutan embryos after exposure to L Solution for different duration after freezing in LN (from left: 45, 60, 75, 90 min; 8 weeks, +LN)..... 106