

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Seed Storage Behaviour of New Zealand's Threatened Vascular Plants

A thesis presented in partial fulfilment of the requirements
for the degree of

Doctor of Philosophy (PhD)

in

Horticultural Science

at Massey University, Palmerston North,
New Zealand

Myoung Joo Park

2013

ABSTRACT

At least 38% of New Zealand's approximately 2300 vascular plant taxa (species and subspecies) are threatened *in situ* and are vulnerable to extinction. It has become increasingly important that a number of different complementary conservation approaches and methods are used to insure against this loss of species. However, limitations exist for the development of *ex situ* storage techniques due to a lack of basic research on seed biology, including seed storage behaviour and germination requirement of many rare and threatened species.

Seven New Zealand native species, *Carmichaelia williamsii*, *Clianthus puniceus*, *Clianthus maximus*, *Hibiscus diversifolius*, *Myosotidium hortensium*, *Tecomanthe speciosa* and *Dysoxylum spectabile* are a priority for investigation for *ex situ* conservation strategies because of their conservation priority and lack of corresponding techniques for their long term *ex situ* conservation. This study reports on seed storage behaviour, dormancy mechanisms and potential *ex situ* conservation strategy including conventional seed storage and cryopreservation of these New Zealand indigenous species to facilitate germplasm storage.

C. williamsii, *C. puniceus*, *C. maximus*, *H. diversifolius*, *M. hortensium* and *T. speciosa* were found to tolerate low seed moisture content (<5%) and thus can potentially be stored for long periods under conventional seed bank conditions, whilst *Dysoxylum spectabile* displayed essentially recalcitrant seed storage behaviour i.e. loss of viability when desiccated below 25%. There is a need to consider alternative *ex situ* conservation strategies, such as cryopreservation, to conserve this species.

Both *C. maximus* and *M. hortensium* displayed 'essentially' orthodox seed storage behaviour i.e. tolerance to desiccation and low temperature. However, viability of *M. hortensium* seeds declined after 12 months in all storage treatments and may be defined as sub-orthodox.

This study demonstrated embryonic axis cryopreservation in *D. spectabile* using a method based on rapid desiccation, encapsulation-dehydration and vitrification. This is the first report of the successful cryopreservation of New Zealand native species. In *D. spectabile*, encapsulation-dehydration or vitrification-based cryopreservation gave higher levels of survival (20%) than rapid desiccation cryopreservation. However, further optimisation of the protocol for plantlet recovery is needed to improve efficiency before it can be considered suitable for conservation purposes.

It is essential to understand dormancy mechanisms and to have effective methods of seed germination so seed banks can increase their efficiency and enhance their role in biodiversity conservation. However, germination requirements for many native species are either incomplete or not available. Unlike *T. speciosa* and *D. spectabile*, seeds of *C. williamsii*, *C. puniceus*, *C. maximus*, *H. diversifolius* and *M. hortensium* were dormant and that this dormancy was found to be of function of the seed coat. Dormancy in *C. williamsii*, *C. puniceus*, *C. maximus* and *H. diversifolius* is a result of the seed coat preventing water uptake by the dry seed. While physical constraint of embryo growth by the seed coat is the cause of dormancy in *M. hortensium* seeds.

The findings of this study will facilitate the *ex situ* conservation of New Zealand native species, to complement *in situ* conservation and help secure the future of the New Zealand's native flowering plants.

ACKNOWLEDGEMENTS

I am indebted to my supervisors Prof. Warren Williams, Dr. Bruce McKay, Mr. Craig McGill and Dr. Jayanthi Nadarajan for their continuous support. This thesis would not have been possible without their enthusiastic approach, help and kind encouragement.

My gratitude is also expressed to the Institute of Natural Resources for hosting and supporting me over the period of my PhD studies.

I would like to acknowledge the financial, academic and technical support of the Massey University and its staff, particularly in the award of a Massey University Doctoral scholarship that provided the necessary financial support for this research, Education New Zealand for providing financial support in the form of the New Zealand International Doctoral Research Scholarship (NZIDRS).

Much of the work contained in Chapter 4.3 was done in the Millennium Seed Bank Project (Royal Botanic Garden, Kew). Thanks to all the staff in Seed Conservation Department for their expertise and supervision. I also wish to thank the Sir Victor Davies Foundation for providing the funding which allowed me to undertake this research at the Millennium Seed Bank, Wakehurst Place, West Sussex, UK.

My Korean employer, Seed and Variety Service, to grant me long-term leave for this PhD.

Finally, thanks to my family and friends for their support, patience and understanding.

TABLE OF CONTENTS

ABSTRACT	II
ACKNOWLEDGEMENTS	IV
TABLE OF CONTENTS	V
LIST OF TABLES	VIII
LIST OF FIGURES	XI
CHAPTER 1 INTRODUCTION	1
CHAPTER 2 LITERATURE REVIEW	4
2.1 Rare and threatened vascular plants in New Zealand	4
2.2 Priority species	8
2.2.1 <i>Carmichaelia williamsii</i> Kirk.	9
2.2.2 <i>Clianthus spp.</i>	11
2.2.3 <i>Hibiscus diversifolius</i> Jacq.	13
2.2.4 <i>Myosotidium hortensium</i> (Decne.) Baill.	14
2.2.5 <i>Tecomanthe speciosa</i> W. R. B. Oliv.	15
2.2.6 <i>Dysoxylum spectabile</i> Hook.	16
2.3 Seed storage behaviour	17
2.4 Seed dormancy	23
2.4.1 Classification of dormancy	24
2.4.2 Dormancy classes.....	25
2.4.3 Dormancy mechanisms.....	27

2.5 <i>Ex situ</i> conservation approaches.....	29
2.5.1 Seed bank storage for orthodox seeds	30
2.5.2 Cryopreservation for non-orthodox seeds	32
CHAPTER 3 DESICCATION SENSITIVITY AND DORMANCY	35
3.1 Desiccation sensitivity	35
3.1.1 Introduction	35
3.1.2 Materials and methods.....	36
3.1.3 Results	40
3.1.4 Discussion.....	45
3.2 Seed germination	49
3.2.1 Introduction	49
3.2.2 Materials and methods.....	50
3.2.3 Results	54
3.2.4 Discussion.....	61
CHAPTER 4 <i>EX SITU</i> CONSERVATION APPROACH.....	67
4.1 Seed storage of <i>Clanthus maximus</i>	69
4.1.1 Introduction	69
4.1.2 Materials and methods.....	69
4.1.3 Results	72
4.1.4 Discussion.....	76
4.2 Seed storage of <i>Myosotidium hortensium</i>.....	79
4.2.1 Introduction	79
4.2.2 Materials and methods.....	80

4.2.3 Results	83
4.2.4 Discussion.....	90
4.3 Cryopreservation of <i>Dysoxylum spectabile</i>	94
4.3.1 Introduction	94
4.3.2 Materials and methods.....	98
4.3.3 Results.....	105
4.3.4 Discussion.....	128
CHAPTER 5 GENERAL DISCUSSION	139
5.1 Overview of main findings/outcome	139
5.2 Future research studies	142
5.3 Conservation options in New Zealand threatened species	143
CHAPTER 6 REFERENCES	147

LIST OF TABLES

Table 2.1	Summary statistics for threatened and uncommon vascular plant in New Zealand.....	4
Table 2.2	Characteristics of seeds with deep, intermediate and non-deep physiological dormancy.	26
Table 3.1	Seed material, number of seeds and chosen target and actual moisture content reached for species used in the desiccation experiment.....	39
Table 3.2	Characteristics of <i>Carmichaelia williamsii</i> , <i>Clianthus puniceus</i> , <i>Clianthus maximus</i> , <i>Hibiscus diversifolius</i> , <i>Myosotidium hortensia</i> , <i>Tecomanthe speciosa</i> and <i>Dysoxylum spectabile</i> on receipt	41
Table 3.3	Change in germination in <i>Tecomanthe speciosa</i> as seed moisture declined compared with control germination	44
Table 3.4	Change in germination in <i>Dysoxylum spectabile</i> as seed moisture declined compared with control germination	44
Table 3.5	Hardseededness percentages of fresh and after drying on silica gel and changes of germination of without scarification and after scarification <i>Carmichaelia williamsii</i>	54
Table 3.6	Hardseededness percentages of fresh and after drying on silica gel and changes of germination of without scarification and after scarification the native species, <i>Clianthus puniceus</i> and <i>C. maximus</i>	55
Table 3.7	Hardseededness percentages of fresh and after drying on silica gel and changes of germination of without scarification and after scarification <i>Hibiscus diversifolius</i>	55
Table 3.8	Percentage of normal and abnormal seedlings, and dead seeds of <i>Myosotidium hortensia</i> after 63 days.	58
Table 4.1	Changes in moisture content during storage at different temperatures and moisture contents of <i>Clianthus maximus</i>	72

Table 4.2	Comparison of percentage normal germination after desiccation to different seed moisture content and after 210 days' hermetic storage at different temperatures and moisture contents of <i>Clianthus maximus</i>	73
Table 4.3	Hardseededness percentages of before and after 210 days' hermetic storage at different temperatures and moisture contents and changes of germination of without scarification and after scarification of <i>Clianthus maximus</i>	74
Table 4.4	Sampling schedule and treatments for the <i>Myosotidium hortensia</i> storage experiment.....	81
Table 4.5	Changes of moisture content during storage at different temperatures and moisture contents of <i>Myosotidium hortensia</i>	83
Table 4.6	Changes of moisture content during storage at different temperatures and moisture contents of <i>Myosotidium hortensia</i>	87
Table 4.7	Germination and time taken for 50% germination of developing seeds of <i>Dysoxylum spectabile</i>	106
Table 4.8	Changes in moisture content, percentage survival of excised embryos of <i>Dysoxylum spectabile</i> after various periods of desiccation.....	108
Table 4.9	Changes in moisture content, percentage of normal seedlings and time to 50% germination (T_{50}) of <i>Dysoxylum spectabile</i> during desiccation.....	109
Table 4.10	Rewarming thermodynamic properties of embryos of <i>Dysoxylum spectabile</i> equilibrate at different RH.	114
Table 4.11	Rewarming thermodynamic properties of embryos of <i>Dysoxylum spectabile</i> after different drying period.....	117
Table 4.12	Water composition of embryo of <i>Dysoxylum spectabile</i> following dehydration treatments.	119
Table 4.13	Rewarming thermodynamic properties of alginate-encapsulated embryos of <i>Dysoxylum spectabile</i> after different drying period.....	120

Table 4.14	Water composition of encapsulated embryo of <i>Dysoxylum spectabile</i> following dehydration treatments	122
Table 4.15	Changes in moisture content, survival of desiccated embryonic axes of <i>Dysoxylum spectabile</i> before and after 24hr cryo-storage.	123
Table 4.16	Changes in moisture content, survival of desiccated alginate encapsulated embryos of <i>Dysoxylum spectabile</i> before and after 24hr cryo-storage ...	124

LIST OF FIGURES

Figure 2.1	Composition of New Zealand flora	5
Figure 3.1	A single of seed of <i>Carmichaelia williamsii</i> , <i>Clianthus puniceus</i> , <i>Clianthus maximus</i> , <i>Hibiscus diversifolius</i> , <i>Myosotidium hortensium</i> , <i>Dysoxylum spectabile</i> and <i>Tecomanthe speciosa</i>	42
Figure 3.2	Germination of seeds during desiccation: (A) <i>Carmichaelia williamsii</i> , (B) <i>Clianthus puniceus</i> and <i>Clianthus maximus</i> , (C) <i>Hibiscus diversifolius</i> , (D) <i>Myosotidium hortensia</i>	43
Figure 3.3	Water uptake of <i>Myosotidium hortensia</i> seed over time	56
Figure 3.4	Cumulative germination of control, decoated seeds were left without a seed coat, had their seed coat returned, seeds were pricked with a fine needle at the centre, and that Vaseline [®] applied to pricked area of <i>Myosotidium hortensia</i> seeds	57
Figure 3.5	The rate of oxygen uptake of intact seeds, decoated seeds without a seed coat, seeds were pricked with a fine needle at the centre, and that Vaseline [®] applied to pricked area of <i>Myosotidium hortensia</i>	59
Figure 3.6	Cumulative germination percentages of <i>Tecomanthe speciosa</i> and <i>Dysoxylum spectabile</i> seeds	60
Figure 3.7	The rate of carbon dioxide evolution of intact seeds, decoated seeds without a seed coat, seeds were pricked with a fine needle at the centre, and that Vaseline [®] applied to pricked area of <i>Myosotidium hortensia</i>	64
Figure 3.8	Intact seeds and seeds were pricked with fine needle at the cotyledon area showing the seed coat rupturing during germination in <i>Myosotidium hortensia</i>	65
Figure 4.1.	Scanning electron micrographs of seed coat of <i>Clianthus maximus</i> at 10% moisture and following desiccation to 2.5% moisture	75

Figure 4.2	The effect of desiccation and subsequent hermetic storage on the germination of seeds of <i>Myosotidium hortensia</i>	85
Figure 4.3	Germination capacity of seeds of <i>Myosotidium hortensia</i> during ultra-drying	86
Figure 4.4	The effect of ultra-drying and subsequent hermetic storage on the germination of seeds of <i>Myosotidium hortensia</i>	88
Figure 4.5	Comparison of percentage of normal germination and total germination of <i>Myosotidium hortensia</i> seeds stored hermetically for 12 months at 20°C , 0°C and -20°C at two moisture contents	89
Figure 4.6	Changes in moisture content, fresh weight and dry of weight of developing seeds of <i>Dysoxylum spectabile</i>	105
Figure 4.7	Changes in moisture content, fresh weight and dry of weight of developing embryonic axes of <i>Dysoxylum spectabile</i>	106
Figure 4.8	Effect of desiccation on subsequent ability of <i>Dysoxylum spectabile</i> seeds to germinate at different maturity stage seed lots	107
Figure 4.9	Changes in moisture content and percentage survival of embryos of <i>Dysoxylum spectabile</i> after various periods of desiccation.....	110
Figure 4.10	Changes in moisture content and percentage survival of alginate encapsulated embryos of <i>Dysoxylum spectabile</i> after various periods of desiccation.....	111
Figure 4.11	DSC cooling and warming thermograms for control <i>Dysoxylum spectabile</i> embryonic axes.....	112
Figure 4.12	DSC cooling and warming thermograms for control <i>Dysoxylum spectabile</i> cotyledon	109
Figure 4.13	Rewarming thermodynamic properties of embryos of <i>Dysoxylum spectabile</i> equilibrate at different RH	110

Figure 4.14	DSC rewarming thermograms of seed samples of <i>Dysoxylum spectabile</i> equilibrate at different RH	111
Figure 4.15	DSC cooling thermograms of seed samples of <i>Dysoxylum spectabile</i> equilibrate at different RH	112
Figure 4.16	DSC warming thermograms for embryonic axes of <i>Dysoxylum spectabile</i> after different desiccation periods.....	114
Figure 4.17	Summary profiles of osmotically active and inactive water contents calculated as percentage of total water content of embryonic axes of <i>Dysoxylum spectabile</i> following dehydration treatment.....	115
Figure 4.18	DSC warming thermograms for alginate-encapsulated embryos of <i>D. spectabile</i> after different desiccation periods.....	117
Figure 4.19	Summary profiles of osmotically active and inactive water contents calculated as percentage of total water content of alginate-encapsulated embryos of <i>Dysoxylum spectabile</i> after different dehydration treatments	118
Figure 4.20	Effects of sucrose preculture on survival rate of <i>Dysoxylum spectabile</i> before and after 24hr cryo-storage.....	121
Figure 4.21	Effects of sucrose preculture and exposure time to PVS2 and PVS3 on survival rate of <i>Dysoxylum spectabile</i> before and after 24hr cryo-storage	123