DOI: http://doi.org/10.4038/vingnanam.v17i1.4187

Seed germination and methods to break seed dormancy in Sri Lankan endemic wild rice species *Oryza rhizomatis*

Gowri Rajkumar

Department of Botany, Faculty of Science, University of Jaffna, Sri Lanka

Email: gowrir@univ.jfn.ac.lk

Abstract – Wild species *Oryza rhizomatis* which has been reported only from Sri Lanka. Therefore, it is endemic to the country and was discovered in 1989 for the first time. It is distributed in the dry zone of Sri Lanka during the period of December to May. *O. rhizomatis* is adapted to specific areas and highly resistant to drought, temperature, soil type and water quality. Studies related to assessment of viability, germination potential and dormancy breaking methods of *O. rhizomatis* were carried out to check the viability and germination potential of the seeds of this species and compared with commercial rice cultivar Bg352.Tetrazolium chloride test revealed that *O. rhizomatis* seeds used in the study were found to be 80% viable. However, only 5% of seeds germinated under the optimum laboratory conditions. From this study, it was confirmed that *O. rhizomatis* seeds have dormancy thus proper treatments are required to break the dormancy as compared to Bg352.This study revealed that the mechanical, chemical and heat treatment as a combination is the most appropriate method for breaking the dormancy to get maximum germination of seeds of *O. rhizomatis*.

Keywords: Bg352, germination, dormancy, *Oryza rhizomatis*, Tetrazolium chloride, viability

1. INTRODUCTION

Oryza rhizomatis is a Sri Lankan endemic wild rice species (Liyanage *et al*., 2002) and it was discovered in 1989 by Vaughan., 1990. *O. rhizomatis* is found in primary and secondary forests but not in rain forests such as Sinharaja. *O. rhizomatis* is adapted to specific areas and highly resistant to drought, temperature, soil type and water quality. *O. rhizomatis* has the best adaptability to survive in the adverse environmental condition (drought) of dry zone because of its thick root system and underground branched rhizome. *O. rhizomatis* is the only species which has the branched underground rhizome and this feature makes it a perennial plant (Tao *et al*, 2001).

O. rhizomatis is reported to have tolerance to biotic and abiotic stresses prevailing in the country (Vaughan, 1990, Shah *et al.*, 2009, Rajkumar *et al*., 2017, Rajkumar *et al*., 2011). Seeds of *O. rhizomatis* may have special structures to survive in adverse environmental conditions and they may have the dormancy to tide over unfavorable conditions, in addition to the rhizome which contributes to perennial habit of the plant (Tao *et al*., 2001).

O. rhizomatis occurs in the region of maximum diversity of the major rice pest, the brown plant hopper, *Nilaparvata lugens* (Vaughan, 1990) and it could possibly have new sources of resistance to this pest. *O. rhizomatis* is also resistant to Bacterial Leaf Blight (BLB) of rice caused by *Xanthomonas oryzae* (Shah *et al.*, 2009). This plant is found in high salinity areas like Putallam in Sri Lanka and the ability to survive in high soil salinity indicates the possibility of carrying genes that make the pant tolerant to salinity (Rajkumar *et al*., 2011). *O. rhizomatis* possesses some desirable nutritional attributes, which are not found in Bg352. Therefore, this variety could be considered as a healthy food source with respect to having lower fat and carbohydrate and higher protein contents than in Bg352 (Rajkumar *et al*., 2014).

Seed viability test is very important to understand the quality of seeds, which has a great influence on the quality of planting stocks. Studies carried out to find out prediction of viability, germination and dormancy breaking methods for the seeds of *O. rhizomatis*. In this study Bg352 seeds were selected as the control for all the experiments as Bg352 is a popular high yielding commercial variety (Jeyawardena *et al*., 2010 and Rajkumar *et al*.,2011)

2. MATERIALS AND METHODS

2.1 Materials

Seeds were collected from *O. rhizomatis* plants grown in the plant house of Rice Research and Development Institute (RRDI) of the Department of Agriculture, Sri Lanka and seeds of popular commercial variety (Bg352) were collected from the research field of RRDI to use for tests. Studies related to germination of *O. rhizomatis* were carried out at the Central Seed Testing Laboratory, Department of Agriculture, Gannoruwa**,** Sri Lanka.

2.2 Purity test

Purity of the samples was checked in accordance with the guidelines established for seed certification at the seed testing laboratory. Purity analysis was performed for the above two sets of seeds and obviously empty, infested or necrotic seeds were discarded. The samples of *O. rhizomatis* and Bg352 were examined and It is divided into four components: pure seeds, other seeds, weed seeds and inert substances. The experiments were carried out on fully developed, completely intact, seemingly mature seeds. Percentage of each component and purity of samples were calculated for each seed sample. Seeds were stored at room temperature (24-25^{o}C) until used for the experiments. Purity Percentage was calculated according to the following formula.

Purity (%) = Pure seed (g) / Working sample

2.3 Viability test

Eight replicates of 50 seeds from each seed lot of four hundred seeds were subjected to various treatments.

Pretreatment: The seeds were soaked in distilled water (3-18 hrs) before staining to fully hydrates all the tissues. This process allowed the activation of germination enzymes and made the seed tissues less fragile.

Tetrazolium (TZ) Test: A longitudinal cut was made through the middle of the embryonic axis (approximately three quarters of the length of the endosperm) of all the seeds. The seeds were then completely immersed in the TZ solution (1%) in a Petri dish on two layers of Watman No. 1 filter paper moistened with distilled water, and covered with aluminum foil to prevent the photo transformation of the TZ solution. These were incubated at 30 ±1^{o}C temperature in darkness for 2-36 hrs as given below. Adequate solution was used to cover the seeds in order to permit its adsorption.

Treatment 1- Seeds soaked overnight (~18 hrs) in water and then 2 hrs in 1% TZ; Treatment 2- Seeds soaked overnight (~18 hrs) in water and then 8 hrs in 1% TZ; Treatment 3 - Seeds soaked overnight (~18 hrs) in water and then 18 hrs in 1% TZ; Treatment 4- Seeds soaked overnight (~18 hrs) in water and then 24hrs in 1% TZ; Treatment 5- Seeds soaked 3 hrs in water and then 36 hrs in 1% TZ; Treatment 6- Seeds soaked 12 hrs in water and then 12hrs in 1% TZ; Treatment 7- soaked overnight (~18 hrs)in wet filter paper and then 22 hrs in 1%TZ

Seeds were transferred to pre-wetted filter papers using a pair of forceps. Internal seed tissue was inspected under a light microscope. The viability was measured using the colour of the embryo after treatment. Viability of each seed was interpreted based on the topographical staining pattern of the embryo and the concentration of colouration. Bg352 was used as a control for each treatment.

2.4 Breaking of dormancy

The following treatments were used to break dormancy of wild rice seeds prior to planting. Cultivar, Bg352 was used as a control for each treatment.

Treatment 1- without any pretreatment; Treatment 2 -Seeds were heated at 50 ºC for 96 hrs (Heat treatment); Treatment 3-Seed husk removed and dehusked seeds soaked for 24 hrs in water (Mechanical treatment); Treatment 4- Preheated at 50ºC for 24 hrs, removed husk and dehusked seeds soaked in water for 24 hrs (Heat treatment followed by mechanical treatment); Treatment 5- Preheating at 50ºC for 24 hrs removed husk and soaked in 0.1 M HNO_3 for 24 hrs (Heat treatment followed by mechanical and chemical treatment); Treatment 6 - Preheated at 50ºC for 96 hrs, removed husk and soaked in 0.1M HNO_3 for 24 hrs (Heat treatment followed by mechanical and chemical treatment).

After the above treatments seeds were planted separately as described below.

2.5 Germination test

Germination test was carried out in 8 replicates of 50 pure seeds each randomly drawn from the fresh seed lots of *O. rhizomatis* and Bg352. Properly graded, sterilized and impurities free sand was used as a substrate to plant the seeds. Suitable amount of water was added to moisten the sand. Themoist sand (2in thickness) was placed in plastic germination boxes (25×19×4inches). Fifty seeds were planted at equidistance over the sand bed contained in each germination box and covered with another moist sand layer of approximately 1/4in thickness. Lids were replaced and transferred to seed germinator for facilitating the specific conditions of temperature (20 - 30ºC), relative humidity (90%) and light intensity 750 and 1250 lux (from cool white lamps) for 8 hrs light and 16 hrs dark period respectively.

The emergence of radicle, equal to the length of the seed was taken as the criterion for germination (Phartyal *et al.,* 2001). First and the final counts were taken after 5^{th} and 14^{th} days respectively. Seedlings and seeds were categorized into normal seedlings, abnormal seedlings, dead seeds, fresh ungerminated and hard seeds. Germination Rate (GR) was calculated using the formula GR = $\frac{n}{N} \times 100$n = number of germinated seeds and N= Number of seeds planted.

Mortality rate(MR) was also calculated using the formula MR = m/N x 100 (where m= number of dead seeds, N = Total number of seeds planted).

Statistical analysis was performed using Minitab version 14 statistical software.

Graphical representation of the data and the mathematical regression model fitted were done using Minitab (version 14) for personal computers. The viability estimated by the germination test was compared with the T test results through Analysis of Variance (ANOVA). Both experiments (viability and the germination tests for both *O. rhizomatis* and Bg352) were conducted as factorial experiments, replicated 8 times with 50 seeds for each treatment.

Normality tests were carried out to determine whether the residuals were well modeled by a normal distribution. The null hypothesis of the normality test showed that there is no significant departure from normality (and the decisions were taken at 0.05 level of significance).

Analysis of variance (ANOVA) was conducted on percentage viability and percentage germination for all viability and germination experiments to determine the significance of the factors namely, species (wild and cultivar) and treatments.

A Tukey's multiple comparison test was used to determine the significance of the treatment differences for each viability and germination

experiment separately. Correlation between treatments of viability and treatments of germination were determined by calculating Pearson correlation.

3. RESULTS

3.1 Purity analysis

Samples used for experiments had 99.60% purity for *O. rhizomatis* and 98.72% purity for Bg352. Since the samples showed high percentage of purity, they were considered as pure enough for the experiments.

3.2 Viability test

Figure 1 : Appearance of red coloured viable embryos of *O. rhizomatis* after treatment 4.4.

Figure 2: Appearance of red coloured viable embryos of Bg352 after treatment

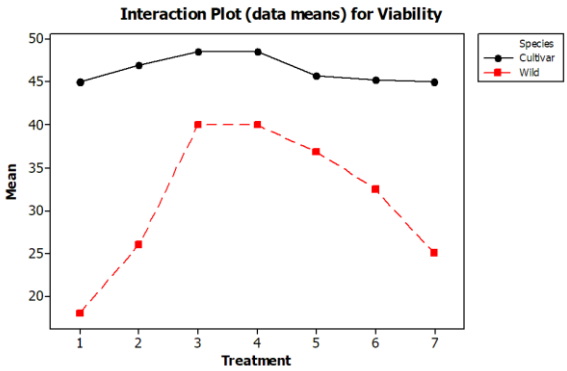

Figure 3 : Mean viability of *O. rhizomatis* (wild) and Bg352 (cultivated) for different treatments.

Residuals are normally distributed at 0.05 level of significance (p-value >0.05) for the viability experiments according to the probability plot of SRES.

Mean viability was calculated for each viability treatment separately for cultivar and *O.rhyzomatis* species. Analysis of variance indicated that there was a significant difference (P = 0.000) between two rice samples and treatment at 0.05level of significance for viability treatments. According to the interaction plot (Fig 3 data means for viability), it appears that the mean viability was high for Bg352 than *O. rhizomatis*. There were no more significant differences (P = 0.000) between treatment means for each treatment for cultivar Bg352. However, there was a significant difference between treatment means for *O. rhizomatis* rice species (P = 0.000). For both Bg352 and *O. rhizomatis* treatment 3 and 4 gave maximum viability whereas Treatment 1 gave minimum viability.

The results obtained for the viability test showed the following: Viability of *O. rhizomatis*rice species is significantly lower than Bg352.There is no significant difference among treatments for the Bg352 variety tested. Treatment 1 gave lowest mean viability for the *O.rhizomatis* rice species. For *O. rhizomatis* viability under treatment 2 is lower than that of other treatments

except treatment 1 and treatment 7. There is no significant difference between the treatments 2 and 7 for the *O. rhizomatis* rice species. Treatment 3 and 4 gave the best result with no significant difference for *O. rhizomatis* rice species. For *O. rhizomatis* viability under treatment 3 is higher than that of treatment 6 and treatment 7.

3.3 Dormancy breaking and germination analysis

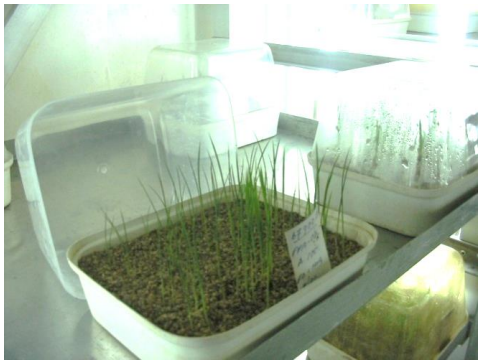

Figure 4: seedlings of Bg352on sand in a germination box.

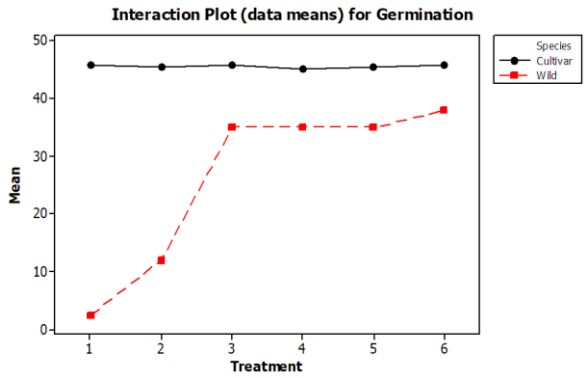

Figure 5 : Mean germinability of Bg352 and *O. rhizomatis* species after different dormancy breaking treatments.

Residuals were normally distributed at 0.05 level of significance (P>0.05) for the germination experiment according to the probability plot of SRES. Analysis of variance indicated that there was a significant difference between species and treatment at 0.05 for germination experiments.

According to the interaction plot (Fig 5, data means for germination), the mean germination of cultivar was significantly different (P = 0.000) from the *O. rhizomatis* rice species. There were no more significant differences between treatment means for each treatment for cultivar Bg352. But it seems that there was a significant difference between treatment means for *O. rhizomatis* rice species.

The results obtained for the germination experiment showed the following: Germination of Bg352 is significantly higher than that of *O. rhizomatis*. There is no significant difference among treatments for the Bg352.Germination after treatment 1 gave lowest mean germination for *O. rhizomatis* rice species. Germination after treatment 3 is same as that of treatment4andtreatment 5 for *O. rhizomatis* species. Germination after treatment 6 gave maximum mean germination for *O. rhizomatis* rice species.

Therefore, it can be recommended that pretreatment was not required for Bg352 variety whereas pretreatment alone or heat treatment alone were not sufficient to break the dormancy of *O. rhizomatis* rice species. Therefore, it was recommended to follow any of the treatment 3, 4, 5 or 6 to break the dormancy of *O. rhizomatis* in order to germinate the seeds.

Germination experiment using filter paper and sand showed that filter paper method gave better germination than sand for *O.rhiomatis*. However, further experiments need to be done to confirm the results.

3.4 Correlation between viability and germination treatments

According to the Pearson correlation, maximum correlation coefficient existed between germination treatment 4 and the viability treatment 4 (0.917) for the *O. rhizomatis* species.

For Bg352, germination treatment1 with viability treatment 4 (0.7540) and germination treatment 4 with viability treatment 1 (0.7480) resulted high correlation value.

4. DISCUSSION

Seed viability testing is important for understanding the quality of seeds, which has great impact on the quality of planting stock. If the quality of the seed is poor at the time of sowing, then no amount of post-sowing case will be of any avail. It is therefore imperative to have certainty about the quality and viability of seeds before commencing sowing and planting work. However, in this work it was important to assess the quality of seeds in terms of purity as samples were to be used for various experiments such as seed germination, and dormancy breaking studies. In this study Bg352 seeds were selected as a control for all the experiments as Bg352 is a common high yielding commercial variety with white medium bold grains. It is cultivated in 16.63% of the total extent of rice cultivation of Sri Lanka (Jeyawardena *et al*., 2010).

The results indicate that for *O. rhizomatis* seeds, pre-moistening of overnight in water and then 18 hrs in 1% TZ (treatment 3) and pre-moistening overnight in water and then 24 hrs in 1% TZ (treatment 4) resulted maximum viability. These two methods are more suitable for viability testing of the *O. rhizomatis* rice species, it does not tally with the general agreement of ISTA (International Seed Testing Association, 1999) rules prescribed for rice seed testing where 18 hrs pre-moistening followed by 2 hrs staining in 1% TZ is used. According to the protocol of recommended by ISTA, mean viability of *O. rhizomatis* species was very low. However, the cultivated variety Bg352 gave maximum mean viability. In summary since the cultivated variety responded with no significant difference for treatments, any treatment can be used to test the viability but treatment 3 or 4 is recommended for viability test of *O. rhizomatis* according to the results obtained.

Phartyal (2003) showed that the viability increased with increasing the time of TZ staining for *Acercaesium* and *Ulmuswallichiana* seeds. This work supported our result as similar trend was observed.

There were no reports for successful seed dormancy breaking treatments and germination testing methods for seeds of *O. rhizomatis*. Therefore, this study was conducted to develop a suitable method to break the dormancy of *O. rhizomatis*to optimize seed germination.

O. rhizomatis is distributed in the dry zone of Sri Lanka and has the best adaptableness to endure in the adverse environmental situations such as drought, adverse temperature, soil nature and water quality (salinity). Seeds of *O. rhizomatis* may have a special structure to survive in these adverse environmental conditions as they may possess the dormancy to tide over unfavorable conditions for a considerably long period which will allow the spread of the species.

Naredo *et al.* (1998) indicated that *O. rhizomatis* species possess stronger seed dormancy than the other wild species and *O. rhizomatis* showed 0% germination without any pretreatment for dormancy breaking. In our study *O. rhizomatis* showed 5% germination (mean germination 2.5) without any pretreatment.

From this study, it was confirmed that *O. rhizomatis* seeds have dormancy thus proper treatments are required to break the dormancy as compared to Bg352. After removal of seed husk there were significant amount of increase in germination. It is evident that *O. rhizomatis* seeds have water impermeable seed coats, making the seeds become physically dormant. The sturdy inhibitory effect exerted by seed coat on seed germination caused by numerous possible mechanisms, including mechanical constraint, inhibition of water and oxygen intake, and retaining or synthesis of chemical

inhibitors (Taiz and Zeiger, 2002). Removal of seed coat (mechanical scarification) led to break the dormancy and to increase germinability due to enhanced facilitation of water uptake and gaseous exchange resulting in increased embryo growth. Removal of seed husk is very effective in breaking seed dormancy. However, it is labor intensive and may also have the risk of damaging the embryos.

Certain temperatures are required to stimulate germination (≥50ºC, Daws *et al.* 2006). Pearson *et al*. (2002) reported poor germination (<20%) after exposing seeds to diurnal temperature fluctuations. Gaewood (1986) reported that 80ºC to 85ºC for 10 min resulted in 0% germination compared to control values of 20%. Culshaw *et al.* (2002) found that excessive hot water treatments can result in rapid seed ageing and death. In our experiment it was evident that heat treatment at 50ºC under different incubation times resulted in breaking dormancy of O. *rhizomatis* rice seeds tested. This heat treatment was more pronounced to break the dormancy when the seeds were incubated for a long time (96 hrs). The best combination to incubate the seeds was 96 hrs at 50 ºC.

Chemical treatments have been considered to be effective in removing seed dormancy of many species including rice (Footitt and Cohn, 1995; Naredo *et al.*, 1998; Cohn, 2002). Chemical treatment (softening the hard seed coat with concentrated HNO_3) was used to remove exogenous dormancy along with other treatments. This study revealed that the mechanical, chemical and heat treatment as a combination is the most appropriate method for breaking the dormancy to get maximum germination of seeds of *O. rhizomatis*.

Effectiveness of tetrazolium test in determining the seed viability of *O. rhizomatis* assessed against the germination test, showed viability treatment, where seeds were pre-moistened overnight in water followed by 24hrs staining in 1% TZ and dormancy breaking method of preheating at 50 ºC for 24 hrs, removal of husk and soaking in water for 24 hrs gave maximum correlation for optimum results.

5. CONCLUSION

O. rhizomatis seeds used in the study were found to be 80% viable. However, only 5% of seeds germinated under the optimum laboratory conditions. Experiments carried out to break dormancy showed that seeds subjected to heat treatment at 50 ºC for 96 hrs followed by removing husks and soaking them in 0.1 M HNO_3 for 24 hrs increased the germination up to 76%. Therefore, these studies provide rapid, effective and valuable techniques to estimate seed viability, breaking of dormancy and for optimum germination of the seeds of *O. rhizomatis*. In addition, the current studies provide the way for successful cultivation and multiplication of this endemic species, which is vital for varietal development.

6. REFERENCES

1. Culshaw, CMP., Espinosa, HW., Pritchard and Engels, J. (2002). Thermal scarification of hard seeds by wet heat treatments risks accelerated seed ageing: evidence from five woody taxa. International union of forestry research organization tree seeds meeting., China: 34-39.
2. Daws, MI., Orr, D., Burslem, DFRP and Mullins, CE. (2006). Effect of high temperature on chalazal plug removal and germination in *Apeiba tibourbou* Aubl. Seed Science and Technology. 34:221-225.
3. Footitt, S and Cohn. (1995). Seed dormancy in red rice *Oryza sativa*. Plant Physiology. 107:1365-1370.
4. Gaewood, NC. (1986). Effects of acid and hot water pre-treatments and seed burial on the germination of tropical moist forest seeds. Turrialba. 36:479-484.
5. Jeyawardena, SN., Muthynayake, MMP and Abeysekera, SW. (2010). Present status of

varietal spread of rice (*Oryza sativa* L.) in Sri Lanka. Annals of the Sri Lankan Department of Agriculture. 12:247-256.

6. Liyanage, ASU., Hemachandra, P., Edirisinghe, DK., Senevirathna, SK. and Takahashi, J. (2002). Surveying and mapping of wild rice species of Oryza in Sri Lanka. Japanese Journal of Tropical Agriculture. 46:14-22.
7. Naredo, MEB., Juliano, AB., Lu, BR., Guzman, FD. and Jackson, MT. (1998). Responses to seed dormancy-breaking treatments in rice species (Oryza L.). Seed Science and Technology. 26:675-689.
8. Phartyal, SS., Thapliyal, RC., Nayal, JS. and Joshi G. (2003). Seed dormancy in Himalayan maple (*Acer caesium*): Effect of stratification and phyto-hormone. Seed Science and Technology. 31:1-11.
9. Rajkumar G., Silva R, Weerasena J., Kumudu K. (2014). Comparison of General Nutritional composition of wild rice *O. rhizomatis* D.A. Vaughan and the commercial variety Bg352. Tropical Plant Research. 1:2:08–10.
10. Rajkumar G., Weerasena J., and Kumudu K. (2017). DNA Sequencing and Bioinformatics Analysis of Clone pOr78 from the Species-Specific Suppression Subtractive Hybridization Library Constructed from Endemic Wild Rice Species *O. rhizomatis*. International Journal of Applied Sciences and Biotechnology. 5(2): 133-140
11. Rajkumar G., Weerasena J., Fernando K., Liyanage A., Silva R. (2011). Genetic differentiation of Sri Lankan traditional rice (*Oryza sativa*) varieties and Wild rice species by AFLP markers. Nordic Journal of Botany. 29:2:238–243.
12. Rajkumar G., Weerasena J., and Kumudu K. and Liyanage A. (2011). Assessment of genetic diversity among Sri Lankan rice varieties by AFLP markers. Plant Genetic Resources: Characterization and Utilization. 9(2); 224–228.
13. Shah, SMA., Rahman, HU., Abassi, FM., Akhtar, MA., Rafi, A. and Khan, IA. (2009). Resistance characterization of wild relatives of Rice in response to bacterial blight. Pakistan Journal of Botany. 41:2:917-925
14. Taiz, L. and Zeiger, E. (2002). Plant Physiology, Chapter 23. Abscisic Acid: A Seed Maturation and Antistress Signal, 3rd ed. Sinauer Associates, Inc, Sunderland, MA. p. 538-558.
15. Tao, D., Hu, F., Yang, Y., Xu, P., Li, J. and Wen G. (2001).Rhizomatous individual was obtained from interspecific BC_2F_1 progenies between *Oryza sativa* and *Oryzalongistaminata*. Rice Genetics Newsletter.18:11.
16. Vaughan D.A. (1990). A new rhizomatous *Oryza* species (Poaceae) from Sri Lanka. Botanical Journal of the Linnean Society. 103:159-163.