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Research Article

Seed Size Variation in Quercus floribunda Lindl. and its Effect on Germination and Seedling Growth

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Abstract

Population of a species growing in different ambience may show differential germination and seedling growth efficiencies, therefore a consideration of seed provenance is essential for a successful restoration effort. Experiment was conducted to examine, how seed size effect the seed germination and seedling growth of a temperate tree species (Tilonj oak: *Quercus floribunda*). The seeds were categorized in different categories on the basis of their length, width and weight. Germination percentage, germination rate and mean daily germination showed maximum value in large seed size class (S_5). Germination and initial seedling growth are often curb by seed size in many tree species. Different size of seeds having different levels of starch and other food storage may be one factor that influences the expression of germination and growth of the plants.

Keywords: Germination Percentage; Germination Rate; Seedling Growth; Seed Size

Introduction

Various streams of plant life got affected by seed size [1]; it influences the dispersal, seed water relations, emergence, establishment, survival and growth of seedlings. Seed size is one of the principle factor that affect the germination and seedling growth along with many internal and external factors [2,3]. Small seeds have a better chance to enter into the soil easily than large seeds and thus, facilitate the buildup of persistent soil seed bank, crucial for regeneration of species following disturbance. On the other hand, a greater seed reserve may enhance the abilities of larger seeds to persist by providing for metabolic requirements during quiescence period, until suitable light or moisture conditions arise. Larger and heavier seeds are relatively less abundant but produce seedlings with greater competitive ability than those produced by small seeds, enabling them to establish and survive under various stresses such as competition [4], moisture [5,6], disturbances [7], defoliation and herbivory [8]. Germination, growth and biomass of the nursery seedlings are drastically affected by seed size which leads to the future crop [9].

Quercus floribunda Lindl. (Family Fagaceae), commonly known as Tilonj/ Moru oak is a major forest forming species of Indian Himalayan region. It is a large evergreen tree with a dense crown of shining green foliage, a diameter of up to 1.6m and a straight bole up-to 45m long. Its natural range of distribution is the temperate region of the Western Himalaya at an altitude of 2100-2700m in cool moist areas. It avoids very dry situations and regenerate in dense pure patches. It is frost-hardy species but does not tolerate drought. Anthropogenic disturbances have change the climate and species distribution pattern as well as phenological attributes [10-12]. In *Quercus floribunda* seed maturation takes place during rainy season (July-September) however, in recent year due to the change in climate the rainfall pattern has also been changed, resulting in low seed germination in this species [13].

Q. floribunda occupies an important place in the Himalayan region due to its significant contribution in soil and water conservation that help to sustain forest ecosystem [14-16]. It is a genus that produces great acorn size variation within a species and even in the individual tree level. Most of the species need only one season to complete the cycle (from flower until the seed maturity). This genus (*Quercus*) is one of the main woody tree species in North hemisphere [17]. For age old subsistence agriculture, oak is one of the vital tree species [18-21]. Oaks generally fail to regenerate in the regions where cattle are regulatory used for browsing [21]. The seed size often controls the germination and initial seedling growth in many tree species. Different size of seeds having different levels of starch and other food storage may be one factor which influences the expression of germination and growth of the plants. Germination may be dependent on the ability of seed to utilize reserves more efficiently, by mobilization of seed reserves for germination traits. Seed grading based upon their size and weight is a common practice to regulate the germination and subsequent seedling growth. Acorn (seed) size is important for seedling establishment and growth in oaks. For successful restoration efforts, knowledge about reproductive limitations of target species is essential for the formulation of effective management strategies when restoring oaks by seedling. Several attempts made to achieve effective afforestation have been inadequately rewarding because of the lack of or insufficient quantity/quality seed. Seed size is a parameter for predicting germination and seedlings growth rates, both in the nursery and for a brief period following plantation establishment [22]. A successful plantation cannot be established unless healthy nursery seedlings or stocks are produced. This also may depend on the viabilities and seed sizes [23]. Therefore, in the present study, an attempt has been made with the objective to determine the effect of seed size on seed germination and early seedling growth in *Q. floribunda.*

Materials and Methods

Study Site and Seed Collection

Fresh and current year mature acorns (seeds) of *Q. floribunda* were collected from natural oak forest around Nainital district of Uttarakhand state, India (between 29^{o} 21'- 29^{o} 24' N latitude and 79^{o} 25' - 79^{o} 29' E longitude) from August 2015 to September 2015. In the Himalayan region, distribution of *Quercus floribunda* ranged from 2100-2700 m, descending to about 1700 m in cool Moist area [24]. In Nainital, this species is common in China peak and near DSB Campus (Figure 1). After collection, seeds were brought to the laboratory in polyethylene bags and air dried and defective acorns were discarded by visual inspection. To analyse the effect of seed size on seed germination and seedling growth, seeds were classified into six size classes as S_1 to S_6 (Figure 2) on the basis of their length width and weight (Table 1).

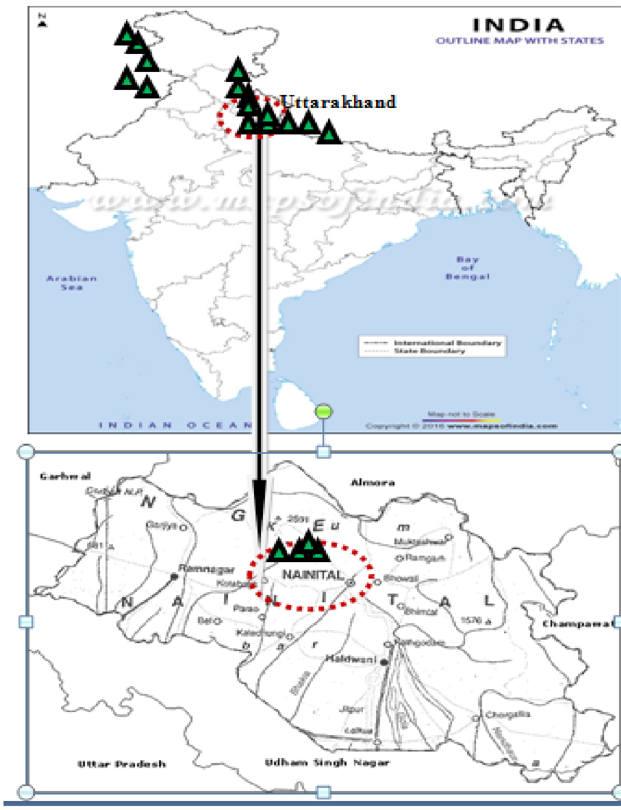


Figure 1: Map showing distribution of *Quercus floribunda*.

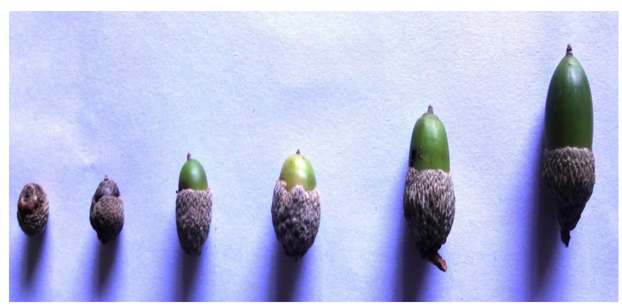


Figure 2: Size class category of Tilonj oak seeds.

Size Classes	Length (cm)	Width(cm)	Weight (g)
Size Class 1	1.83±0.04	1.00±0.04	1.06±0.07
Size Class 2	2.32±0.36	1.43±0.42	2.35±0.18
Size Class 3	2.93±0.28	1.69±0.42	4.35±0.22
Size Class 4	3.35±0.03	1.59±0.10	5.59±0.34
Size Class 5	3.75±0.03	1.73±0.04	5.63±0.24
Size Class 6	4.13±0.06	1.67±0.15	7.11±0.35

Table 1: Seed size class of *Q. floribunda*.

Experiment

Only those seeds were used in the experiment that sank to the bottom when submerged in the tap water. 3 sets of 15 seeds were taken for each size class (= 45 seeds). Seeds were sown in plastic pots filled with sterilized sieved forest soil. A seed was considered germinated when visible protrusions of plumule observed. Germinated seeds were transferred from plastic pots to polythene bags (22cm x 9cm) for further observation. These polythene bags were kept in glass house and watered regularly. The mean minimum and mean maximum temperature ranged between 11˚C to 36˚C and glasshouse received full sunlight. To analyse the effect of seed size, germination percentage (GP), Germination Rate (GR) and Mean Daily Germination (MDG) were observed.

Seedling Growth

To analyse the effect of seed size on seedling growth, harvests were conducted at one (harvest I) and two months (harvest II) from the date of sowing. Root and shoot length, leaf number and total leaf area were measured for each seed size category at each harvest. After harvesting, seedlings were separated into component parts (roots, stem, leaves and cotyledons). All plant parts were dried in oven at 60˚C for 48 hours and weighted, whereas fresh weight was taken immediately. Statistical analysis was performed using SPSS.

After final count, Germination Percentage (GP) and GerminationRate(GR)wascalculatedbythefollowingformulae[25].

- **Germination Percentage (GP): GP =** $\frac{n}{N}$**x100**

Where,

n is the number of germinated seeds and N is the total number of seeds.

- **Germination Rate (GR):**

$$GR = \frac{\text{Number of germinated seeds}}{\text{Day of first count}} + \text{-------------} + \frac{\text{Number of germinated seeds}}{\text{Day of final count}}$$

Mean Daily Germination (MDG): MDG = $\frac{N}{D}$

Where,

N is the total number of germinated seeds and D is the number of days to final germination.

- **Leaf Area (LA):**

LA= Leaf Length X Leaf width

- **Relative Growth Rate (RGR):**

$$RGR\ (g/day) = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

Where,

W_2 is the total seedling weight at harvest II

W_1 is the total seedling weight at harvest I and

t_2 - t_1 is the days between harvest I and harvest II.

- **Average Growth Rate (AGR):**

$$AGR = \frac{W_2 - W_1}{t_2 - t_1}$$

Where,

W_2 is the total seedling weight at harvest II

W_1 is the total seedling weight at harvest I and

t_2 - t_1 is the days between harvest I and harvest II.

- **Root: Shoot Ratio: R: S=** $\frac{\text{Dry weight of root}}{\text{Dry weight of Shoot}}$
- **Leaf Weight Ratio (LWR):**

$$LWR = \frac{\text{Leaf dry weight}}{\text{Total seedling dry weight}}$$

- **Seed Vigor or VigorIndex (SVI):**

This index was determined following [26]:

Vigor index = (germination percentage × means of seedling length (root + shoot)/100).

- **Relative Water Content (RWC):**

The water content respective to the fresh weight was calculated as described by [27].

RWC % = 100 x [(FW – DW) / FW]

Results

Effect of Seed Size on Germination Indices

Germination percentage revealed a mixed pattern along with size class gradient (S_1- S_6). Maximum germination was recorded in size class 5 (46.67 %), while minimum in size class 1 (6.67%) (Table 1). The Analysis of Variance (ANOVA) showed that the shoot length of seedling was the only trait that was significantly affected by seed size while harvesting time significantly affected germination percentage (Table 2).

	df	GP	RL	SL	DW
Size class	5	137.010^{ns}	87.870^{ns}	21.586*	0.136^{ns}
Harvesting time	1	3559.374*	40.077 ^{ns}	1.129 ^{ns}	0.036^{ns}
df= degree of freedom, GP= germination percentage, RL= root length, SL= shoot length, DW= dry weight					

Table 2: Analysis of variance on seed germination and seedling growth parameters as affected by seed size.

As in germination percentage, germination rate also showed fluctuating pattern with each seed size class. Maximum germination rate (6.56) was observed in S_5 seed size class, whereas minimum (0.08) in S_1seed size class (Table 3). Seedlings that emerged from the larger seeds showed better survival than those from the smaller seeds. Days taken to initiate and complete the germination processes varied in different size classes. Initiation time varied from 1 to 13 days, however completion time ranged between 12-32 for all 6 size class (S_1, S_2, S_3, S_4, S_5 and S_6) (Table 3).

Mean Daily Germination did not show any correlation with size classes. Maximum (40) and minimum (8.33) mean daily germination was observed at $S_2$and S_1seed size class, respectively (Figure 4). Highest cumulative germination was observed in S_2 size class, whereas minimum in S_1 size class (Figure 3).

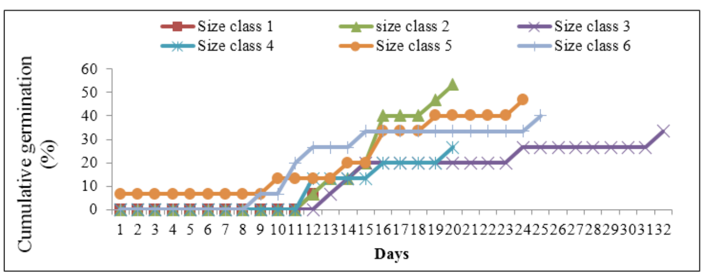


Figure 3: Effect of size class on cumulative germination in *Q. floribunda*.

Effect of Seed Size on Morphological Growth Parameters

In the present study, seedling emerged from S_1 size class could not survive so only five seed size classes were available for comparing seedling growth parameters. Shoot and root length showed fluctuating pattern in each size classes at both the harvest periods. Maximum shoot length (8.65 cm) and root length (17.70 cm) was observed in size class 5, whereas minimum shoot length (4.40 cm) and root length (11.25 cm) in size class 2 and size class 3, respectively at harvest period I. At harvest period II, shoot length varied from 7.13 cm (S_6) to 9.15 cm (S_5), whereas root length observed between 15.5 cm (S_6) - 20 cm (S_2) (Figure 4).

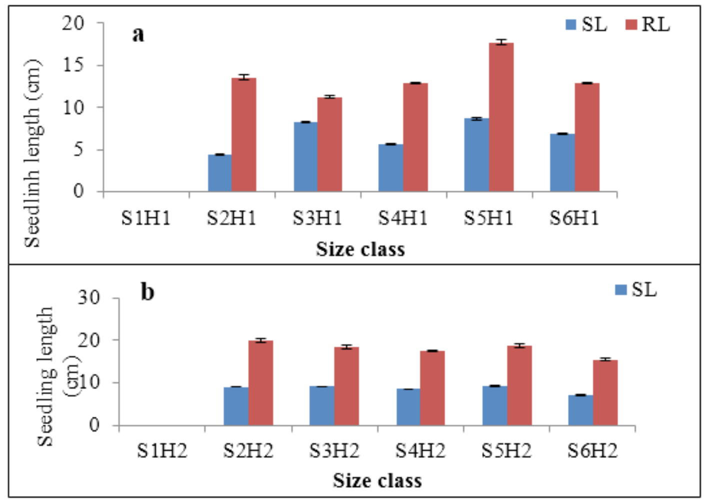


Figure 4: Effect of seed size on shoot length and root length of *Q. floribunda* seedlings (a=harvest I, b=harvest II).

At harvesting period, I, leaf number varied from 3 (S_4) - 6 (S_6) whereas at harvest period II, it ranged from 6 (S_2, S_4 and S_6) - 7 ($S_3$and S_5) (Table 4). At harvest period I, maximum leaf area (42.68 $cm^2 seedling^{-1}$) was recorded in size class 5 and minimum in size class 2 (19.45 $cm^2 seedling^{-1}$), whereas at harvest period II, highest leaf area was observed in size class 5 (53.65 $cm^2 seedling^{-1}$) and minimum in size class 4 (29.96 $cm^2 seedling^{-1}$) i.e. maximum leaf area was found at harvest period II (H II) as compared to the harvest period I (H I). Fluctuation occurred in all size class at both the harvest periods. Maximum dry weight of seedling (0.75 g) was observed in size class S_3 and S_4 at harvest II while, minimum (0.18 g) in size class S_2 at harvest I. Maximum seedling length (29.0 cm) was observed in S_2at harvest II, while minimum (17.9 cm) was in S_2at harvest I (Table 4).

Size class	Leaf Number (Leaf no. seedling^{-1})		Leaf Area (cm^2 seedling^{-1})		Dry weight of seedling (g)		Total seedling length (cm)	
	H I	H II	H I	H II	H I	H II	H I	H II
S_1	-	-	-	-	-	-	-	-
S_2	5±0.085	6±0.106	19.45±0.142	38.22±0.786	0.18±0.004	0.58±0.012	17.90±0.332	29.00±0.418
S_3	5±0.059	7±0.167	32.28±0.118	47.89±0.049	0.64±0.015	0.75±0.018	19.50±0.248	27.50±0.430
S_4	3±0.002	6±0.049	25.84±0.054	29.96±0.281	0.59±0.007	0.75±0.016	18.47±0.104	26.00±0.239
S_5	5±0.101	7±0.044	42.68±0.267	53.65±1.256	0.59±0.015	0.61±0.014	26.35±0.481	27.90±0.616
S_6	6±0.122	6±0.02498	29.59±0.323	39.67±0.991	0.54±0.004	0.62±0.001	19.75±0.172	22.63±0.358
*Seedling could not survive								

Table 4: Effect of seed size on different morphological growth parameters in *Q. floribunda.*

Effect of Seed Size on Physiological Growth Parameters

At harvest II, S_5 size class showed maximum (2.55) and S_2 showed minimum (0.8) root- shoot dry weight ratio. However, highest (1.15) and lowest (0.71) root- shoot ratio was observed in S_4 and S_2 seed size classes, respectively at harvest I (Table 5). Maximum leaf weight ratio was occurred in S_2 as well as S_5 seed size classes at harvest II, which was 0.39 and minimum in S_5 seed size class at harvest I, which was 0.04. Relative growth rate was highest (0.039 g day^{-1}) in S_2 size class, while lowest (0.001 g day^{-1}) in S_5 size class. Same results were observed for average growth rate. Size class 2 (S_2) showed maximum vigour index (15.47) at harvest II, whereas minimum vigour index (4.93) was observed in size class 4 at harvest I. (Table 5).

Size class	R:S		LWR		RGR (g day-1)	AGR (g day-1)	Seed vigor	
	H I	H II	H I	H II			H I	H II
S_1	-	-	-	-	-	-	-	-
S_2	0.71	0.8	0.36	0.39	0.039	0.013	9.55	15.47
S_3	0.89	1.98	0.19	0.35	0.005	0.004	6.5	9.17
S_4	1.15	1.68	0.22	0.3	0.008	0.005	4.93	6.93
S_5	0.89	2.55	0.04	0.39	0.001	0.001	12.3	13.02
S_6	0.98	1.29	0.27	0.38	0.005	0.003	8.01	8.94

Table 5: Effect of seed size on physiological growth parameters in *Q. floribunda*.

Effects on Relative water content

In general, relative water content (RWC) showed a fluctuating pattern along with size class gradient. Maximum value of RWC (62.53%) was observed in size class 3 at harvest II, while minimum value of RWC (25%) was observed in size class 2 at harvest I (Figure 5).

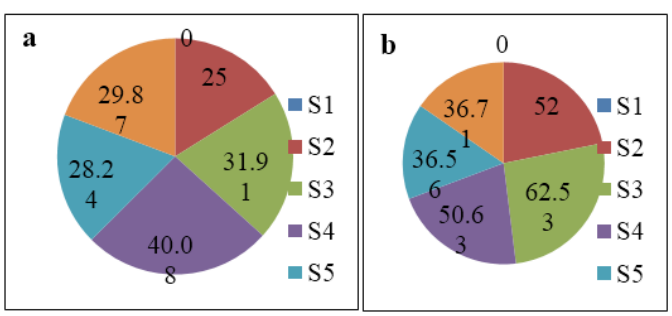
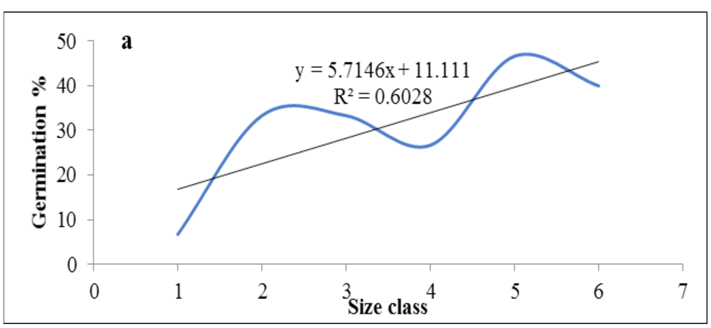


Figure 5: Effect of seed size on Relative water content in *Q. floribunda* seedlings.

Discussion

Seed size significantly affected germination parameters of *Q. floribunda* seedlings. Maximum and minimum germination percentage was observed for S_5 and S_1 size class, respectively. Identical pattern was also observed for germination rate and mean daily germination (Table 3) as well. The better germination reported by the large seed size due to availability of more food reserves in large seeds that enhanced their viability, hence earliest and highest germination percentage of the large seeds. According to [13], *Q. floribunda* showed good/fair regeneration, as this species produce large-sized seeds compared to the other species. Large and heavier seeds produce seedlings with greater competitive ability than those of small seeds enabling them to become established and survive under various stresses such as disturbance (biotic and abiotic). Germination percentage showed positive correlation ($R^2 = 0.602$) with size classes. Similar results were also observed by [28,29] and in *Gmelina* species and *Anacadium occidentale* (cashew), respectively. Seedlings were unable to survive for smallest (S_1) seed size class. Least leaf number was estimated for S_4 size class at harvest I, while highest for S_3 and S_5 at harvest II. The minimum emergence time taken by large sized seeds as compared to the small sized seeds that showed comparative similarity to the *Alangium lamarckii* by [30].

Maximum leaf area was observed in S_5 at harvest II while minimum in S_1 size class at harvest I. Seedling weight was highest in S_3 and S_4 size class at harvest II, while least was in S_2 size class at harvest I. Linear relationship was not observed for the root and shoot length in the present study with respect to seed size classes. In contrast, [31] showed positive correlation with seed size in *Acacia nilotica*. Dry weight of seedling showed positive correlation (R^2=0.606) with seed size at harvest I, as compared to harvest II (R^2= 0.371). Similar trend revealed by seedling length with seed size class also (Figure 6).



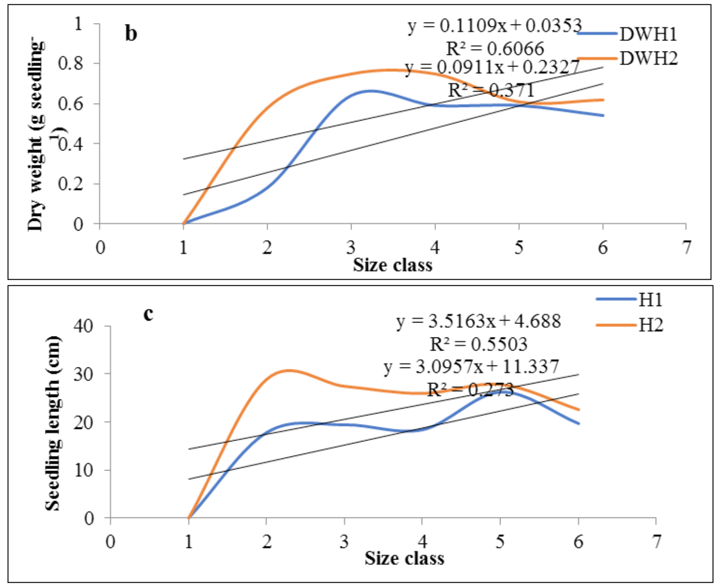


Figure 6: Relationship between seed size and (a) germination percentage, (b) dry weight of seedling and (c) seedling length.

The production of seedlings for plantation in afforestation, reforestation and forest plantation programmes are largely dependent upon the germination of available seeds. Natural regeneration of plant species depends upon the production of viable seeds, subsequent germination and successful establishment of seedlings. Knowledge of seed germination and seedling establishment is not only important for understanding the community processes such as plant recruitment and succession, but it is also required for the success of the efforts on augmentation, introduction and reintroduction of species population in restoration efforts. Population of a species growing in different environments may show differential germination and seedling growth efficiencies and therefore a consideration of seed provenances is essential for a successful restoration effort.

Conclusion

The present study indicated that large seed sized (S_5) showed best germination at a shorter period of time than the other small seed size classes. It was observed that the seeds of S_5size class germinated faster than the small sizes. The best seed sizes to use by villagers and forest department were the large sized seeds because of their fast germination. The seed size is a considerable and significant factor in the germination and early seedling growth. Varied degree of size classes in seeds having different level of carbohydrates and other food storage may be one factor which affects the germination and growth of plants. The overall result showed that the seed grading is an essential step to improve the quality of nursery stock as well as their performance at field condition. Further, it is suggested to use bigger sized seeds to get higher and quicker seed germination and early seedling growth in *Q. floribunda*.

Acknowledgement

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