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# Effect of Sowing Time on Germination and Early Seedling Growth of *Quercus floribunda* Lindl.

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## Abstract

*Quercus floribunda* (Tilonj oak) is among the five species of *Quercus* and an evergreen tree found in Kumaun Himalayan region. Timing of germination is a crucial event determining the success of seedling establishment and survival. The aims of the study were to investigate the effect of sowing date on the germination and morphological responses of Tilonj oak (*Quercus floribunda*), cultivated during the month of August-September, with supplementary irrigation. The experiment was conducted by sowing seeds at two dates with one month interval in glass house conditions at DSB Campus, Nainital, Uttarakhand, India. Present study revealed that higher germination percentage (46.67 %) was recorded at sowing time $S_2$ as compared to the sowing time $S_1$ (32.86 %). Germination percentage as well as seedling growth were affected by sowing date though the differences were insignificant. At both the sowing dates, highest germination percentage was recorded for large seeds (32.86-46.67 %) followed by medium (31.43-33.33 %) and lowest germination (6.67-7.14 %) was recorded for small size seeds. In all parameters assessed, sowing time $S_2$ had the best performance in combination with large seed size. Thus, September is suitable month for best germination, growth and seedling vigour and large seed size is recommended to silviculturists and tree planters. The information on seed germination and seedling growth is vital both for conservation and rehabilitation of degraded lands.

**Key Words:** germination, morphological response, degradation, seedling growth, conservation

## Introduction

Seedling establishment and survival is successfully determined by the time of germination (Gerhardt 1996). In addition, seedling growth also need ample amount of light on the soil surface (Montgomery and Chazdon 2002). Due to the constant change in climate, it is very difficult to choose appropriate time for sowing of seeds (Laux et al. 2010; Folberth et al. 2012), especially for the Oak species, because these are vulnerable to the climate change (Bargali et al. 2014; 2015). Rehabilitation of any kind of degraded land needed information regarding seed germination and seedling growth that played very vital role (Bargali and Bargali 2016). Greven et al. (2004) reported that timing of harvest is an important factor since both seed immaturity and weathering reduce seed quality. Choice of appropriate sowing date is important, because every crop needs specific climate or season to take out maximum resources from the soil (Sumathi and Srimathi 2013).

The genus *Quercus* is one of the most important groups of woody plants in many regions in the Northern Hemisphere (Nixon 2002) that produces great acorn size

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variation within a species and even in the individual tree level. Oak forests are considered as established and climax communities (Singh and Singh 1992) and are the basis of age-old subsistence agriculture (Bargali et al. 2013; Bargali et al. 2014) however; very prone or sensitive to climate change (Bisht and Kuniyal 2013). Presently, because of constant anthropogenic pressures these forests are being depleted for their goods and services. According to Singh and Rawat (2010) there are very few patches of intact oak forests left in the Himalayan region today and some alien species such as *Eupatorium adenophorum, Lantana camara* have very deteriorating impact on these patches than more aggressive species such as *Pinus roxburghii*. Perpetual grazing from cattle and other livestock has very negative impact on regeneration of oaks (Bargali et al. 2013; 2014, 2015; Pandey et al. 2017).

Even though seed is a renewable natural resource, once it is exhausted, it may take a long time to regenerate. Seed wastage due to frugivory, inadequate regeneration, low establishment of seedlings and trampling driven damages to seedlings by browsing animals are attributed as key reasons for degradation of Oak forests (Bargali 1997; Bisht et al. 2013). Over the years original vegetation modified by human activities have lost its original form. To correct this situation, regeneration/plantation of ecologically and economically important species must be established to regain the lost glory. Generally, seed germination is controlled by many internal and external factors while seed size and sowing time are among them. One of the important character of seed is its size that has pivotal role in influencing the germination, growth and biomass of the nursery seedlings which leads to the structure and output of crop (Shahi et al. 2015 a,b; Vibhuti et al. 2015 a,b; Awasthi et al. 2016; Pantola et al. 2017). Apart from seed size, there is also substantial local variation in the microclimatic conditions due to difference in sowing time that characterize a species regeneration niche. This leads to local variation in the earliest time when seed germinates and could affect differential success among seedlings within a population. Therefore, in the present study an attempt was made to investigate whether variation in sowing time could lead to differences in germination and seedling growth in Tilonj oak (*Quercus floribunda* Lindl.).

## Materials and methods

### Seed Source

Acorns of *Q. floribunda* (Tilonj oak) were collected at different stage of maturity from natural oak forest around Nainital town of Uttarakhand state, India (between 29° 21' – 29° 24' N latitude and 79° 25' – 79° 29' E longitude) in August 2015 to September 2015. After collection, seeds were brought to the laboratory in polythene bags and air dried and defective acorns were discarded by visual inspection. Healthy seeds were selected and classified into three size classes viz. small (SS), medium (MS) and large (LS) (Table1, Fig.1).

### Germination Experiment

To analyse the effect of sowing time, seeds were sown at two dates [4 August ($S_1$) and 4 September ($S_2$)] with one month interval. Only those seeds were used in the experiment that sank to the bottom when submerged in the water. For each size class at each sowing date, 15 seeds per size class were used. Seeds were sown in plastic pots filled with sterilized sieved forest soil and kept in glass house. The pots were arranged in a Randomized Complete Block Design (RCBD) replicated three times. A seed was considered germinated when visible protrusions of plumule were 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 maximum temperature ranged between 11°C to 36°C and glasshouse received full sunlight. Cumulative germination percentage was evaluated daily and final value was obtained after 50 days. The observation recorded were germination percentage (GP), Germination rate (GR) and Mean daily germina-

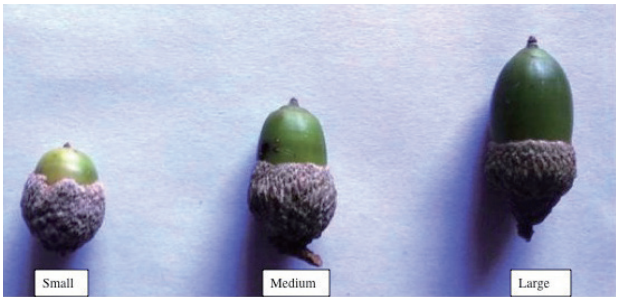


**Fig. 1.** Size class category of *Q. floribunda* seeds.

**Table 1.** Seed size class of *O.floribunda*

| Size Classes | Length (cm) | Width (cm) | Fresh weight (g) |
|---|---|---|---|
| Small (SS) | 1.83±0.36 | 1.00±0.04 | 1.06±0.07 |
| Medium (MS) | 2.93±0.28 | 1.69±0.42 | 4.35±0.22 |
| Large (LS) | 3.75±0.03 | 1.73±0.04 | 5.63±0.24 |

tion (MDG).

## Seedling Growth

To analyze the effect of seed size on seedling growth, harvests were conducted at one and two months 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 (root, stem, leave and cotyledon). All plant parts were dried in oven at 60°C for 48 hours and weighed whereas fresh weight was taken immediately. Statistical analysis was performed using SPSS. After final count, germination percentage (GP) and germination rate (GR) was calculated by the following formulae (Raun et al. 2002).

Germination Percentage (GP):

$$GP = \frac{n}{N}$$

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

Germination Rate (GR):

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

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

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):

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

Where, W2 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 Vigor index (SVI):

This index was determined following Abdul and Anderson (1970) method:

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 Sumithra et al. (2006);

RWC % = 100 x [(FW – DW) / FW]

Where, FW= Fresh weight

DW= Dry weight

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

| | df | GP | RL | SL | DW |
|---|---|---|---|---|---|
| Size class | 2 | 891.643ns | 675.473* | 156.563* | 0.567* |
| Sowing time | 1 | 58.069 ns | 229.477 ns | 93.029 ns | 1.188* |

df= degree of freedom, GP= germination percentage, RL= root length, SL= shoot length, DW= dry weight

## Results and Discussion

The analysis of variance (ANOVA) showed that the seedling dry mass was the only trait that was significantly affected by sowing time while seed size significantly affected most of the traits except seed germination (Table 2).

### *Effect on seed germination*

In the present study, higher germination percentage (6.67 - 46.67 %) was recorded at sowing time $S_2$ as compared to the sowing time $S_1$ (7.14 - 32.86 %). At both the sowing dates, seed germination increased with increasing seed size (Table 3) indicated that sowing time could not alter the effect of seed size. Cumulative germination percentage showed positive correlation with seed size class (Fig. 2). Germination rate showed fluctuating pattern at sowing time $S_1$ while at sowing time $S_2$ it increased with increasing seed size. First germination was observed one (1) day after sowing in the large seed size at sowing time $S_2$ while at sowing time $S_1$ germination started after 2 days of sowing in the large and medium size seeds (Table 3). Mean daily germination (MDG) was higher (0.13 - 0.64) in seeds at sowing time $S_1$, as compared to the sowing time $S_2$ (0.08 - 0.30).

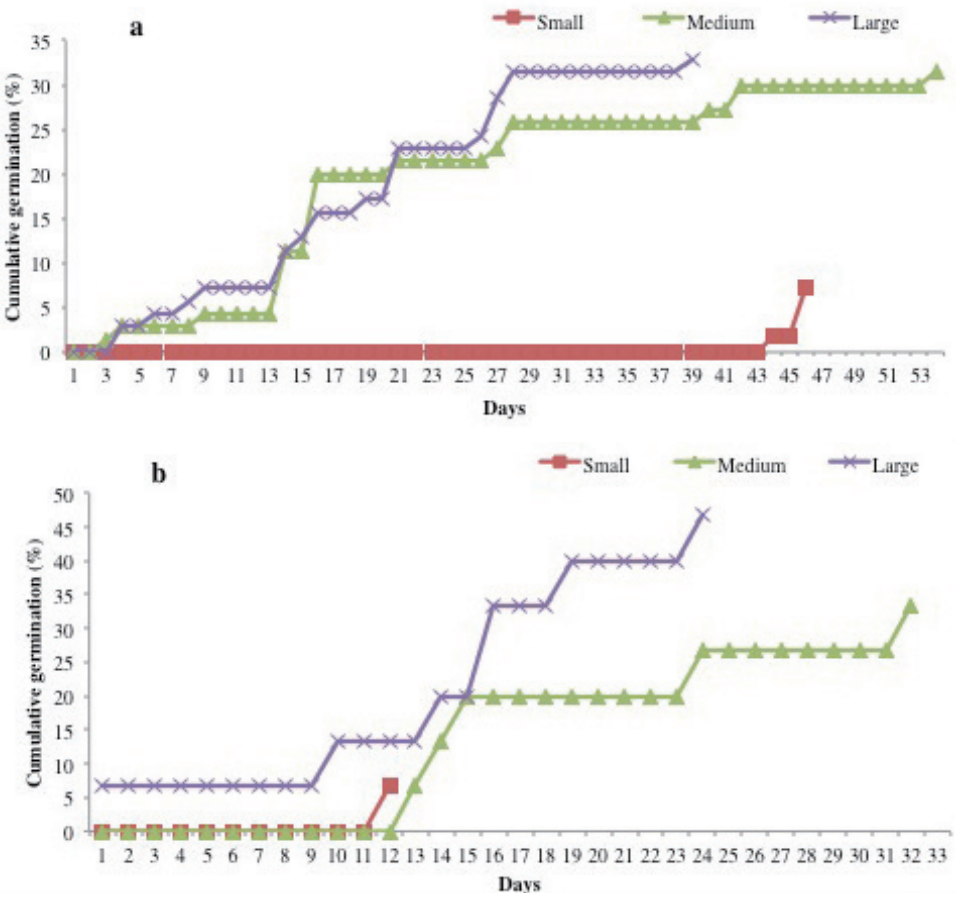


**Fig. 2.** Effect of sowing time on cumulative seed germination in *Q. floribunda*.

**Table 3.** Effect of sowing time on different germination parameters in *Q. floribunda*

| Seed size | Sowing time | GP | GR | I | C | MDG |
|---|---|---|---|---|---|---|
| Small | S1 | 7.14 | 0.13 | 30 | 32 | 0.13 |
| | S2 | 6.67 | 0.08 | 12 | 12 | 0.08 |
| Medium | S1 | 31.43 | 2.79 | 2 | 56 | 0.39 |
| | S2 | 33.33 | 0.31 | 13 | 30 | 0.17 |
| Large | S1 | 32.86 | 2.59 | 2 | 36 | 0.64 |
| | S2 | 46.67 | 1.39 | 1 | 23 | 0.30 |

GP: Germination percentage, GR: Germination rate, I: Initiation of germination, C: Completion of initiation, MDG: Mean daily germination.

## *Effect on morphological growth parameters*

In the present study, at both the sowing dates seedling raised from the small size seeds could not survive so only two seed size classes were available for comparing seedling growth parameters. Root and shoot length increased with increasing seed size, and were higher in sowing time $S_2$ as compared to sowing time S1. Result of this study showed that sowing time had significant ($p > 0.05$) effect on seedling height. The shortest seedling (7.40 cm) was obtained from medium seed size at sowing time $S_1$ (Table 4).

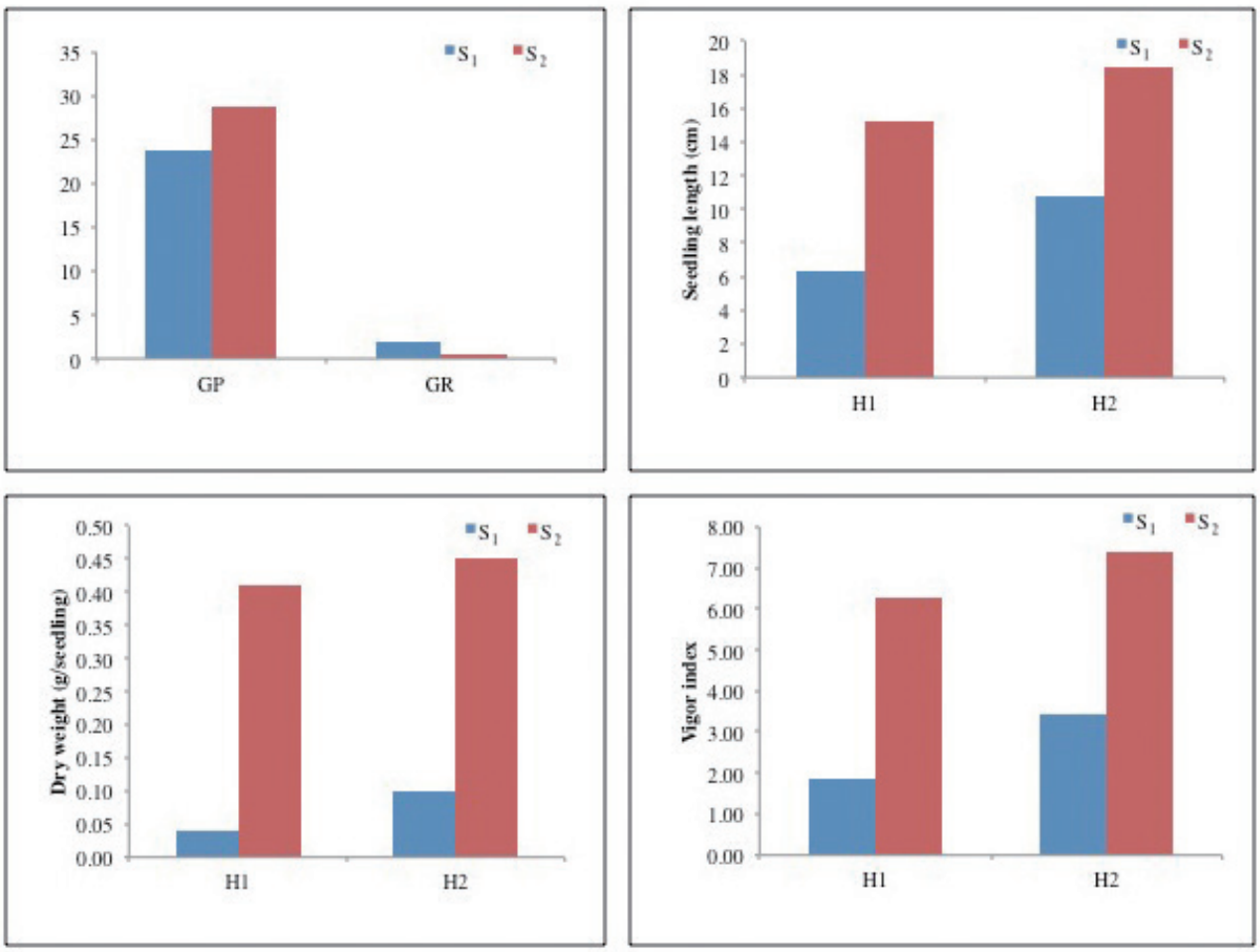


**Fig. 3.** Effect of sowing time on seed germination and seedling growth parameters.

$S_1$= Sowing time 1(August), $S_2$= Sowing time 2 (September), GP= Germination percentage, GR= Germination rate, H1= Harvest I, H2= Harvest II

**Table 4.** Effect of sowing time on different morphological growth parameters in *Q. floribunda*

| Seed size | Sowing date | Root length (cm) | | Shoot length (cm) | | Total seedling length (cm) | | Leaf no. (Leaf no. seedling$^{-1}$) | | Leaf area (cm$^2$ seedling$^{-1}$) | | Dry weight (g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | H1 | H2 | H1 | H2 | H1 | H2 | H1 | H2 | H1 | H2 | H1 | H2 |
| Small* | S1 | - | - | - | - | - | - | - | - | - | - | - | - |
| | S2 | - | - | - | - | - | - | - | - | - | - | - | - |
| Medium | S1 | 4.25±0.029 | 11.10±0.237 | 3.15±0.033 | 3.93±0.094 | 7.40±0.166 | 15.03±0.164 | 3±0.013 | 6±0.094 | 7.74±0.053 | 11.38±0.177 | 0.05±0.001 | 0.14±0.002 |
| | S2 | 11.25±0.246 | 18.40±0.172 | 8.25±0.026 | 9.10±0.156 | 19.50±0.203 | 27.50±0.601 | 5±0.015 | 7±0.057 | 32.28±0.329 | 41.75±0.692 | 0.64±0.009 | 0.75±0.009 |
| Large | S1 | 7.35±0.149 | 13.10±0.136 | 4.03± 0.027 | 4.25±0.053 | 11.60±0.223 | 17.13±0.089 | 3±0.023 | 5±0.081 | 11.25±0.124 | 13.3±0.051 | 0.06±0.001 | 0.16±0.001 |
| | S2 | 17.70±0.295 | 18.75±0.468 | 8.65±0.162 | 9.17±0.148 | 26.35±0.069 | 27.92±0.029 | 5±0.045 | 7±0.134 | 42.68±0.852 | 53.65±0.707 | 0.59±0.001 | 0.61±0.002 |

*Seedling could not survive.

**Table 5.** Effect of sowing time on different physiological growth parameters in *Q. floribunda*

| Seed size | Sowing date | R:S | | LWR | | RWC (%) | | RGR (g day$^{-1}$) | AGR (g day$^{-1}$) | Seed vigor | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | H1 | H2 | H1 | H2 | H1 | H2 | | | H1 | H2 |
| Small* | S1 | - | - | - | - | - | - | - | - | - | - |
| | S2 | - | - | - | - | - | - | - | - | - | - |
| Medium | S1 | 1.85 | 0.73 | 0.43 | 0.40 | 47.37 | 39.13 | 0.034 | 0.003 | 2.33 | 5.39 |
| | S2 | 1.98 | 0.89 | 0.19 | 0.35 | 62.53 | 31.9 | 0.005 | 0.004 | 6.5 | 9.17 |
| Large | S1 | 0.38 | 0.79 | 0.39 | 0.55 | 57.14 | 33.33 | 0.036 | 0.004 | 3.18 | 4.94 |
| | S2 | 1.25 | 0.89 | 0.04 | 0.39 | 28.24 | 36.5 | 0.002 | 0.001 | 12.3 | 13.02 |

R:S=Root: Shoot ratio, LWR= Leaf: weight ratio, RWC= Relative water content , RGR= Relative growth rate, AGR=Average growth rate. *Seedling could not survive.

**Table 6.** Correlation matrix of different germination parameters

| Variables | GP | GR | RGR | AGR |
|---|---|---|---|---|
| GP | 1 | 0.445 | 0.179 | 0.412 |
| GR | 0.445 | 1 | 0.912 | 0.459 |
| RGR | 0.179 | 0.912 | 1 | 0.636 |
| AGR | 0.412 | 0.459 | 0.636 | 1 |

*Values in bold are different from 0 with a significance level alpha=0.05.*

**Table 7.** Eigen values of different germination parameters

| | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| Eigenvalue | 2.575 | 0.866 | 0.558 | 0.001 |
| Variability (%) | 64.364 | 21.662 | 13.962 | 0.012 |
| Cumulative % | 64.364 | 86.026 | 99.988 | 100.000 |

**Table 8**. Factor loading of different germination parameters

| | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| GP | 0.564 | 0.806 | -0.180 | 0.006 |
| GR | 0.911 | -0.169 | -0.377 | -0.014 |
| RGR | 0.906 | -0.421 | -0.041 | 0.015 |
| AGR | 0.779 | 0.104 | 0.618 | -0.006 |

**Table 9.** Correlation matrix of physiological growth parameters

| Variables | RL | SL | DW |
|---|---|---|---|
| RL | 1 | 0.942 | 0.857 |
| SL | 0.942 | 1 | 0.945 |
| DW | 0.857 | 0.945 | 1 |

Values in bold are different from 0 with a significance level alpha=0.05.

**Table 10**. Eigen values of different physiological growth parameters

| | F1 | F2 | F3 |
|---|---|---|---|
| Eigenvalue | 2.830 | 0.143 | 0.028 |
| Variability (%) | 94.327 | 4.756 | 0.917 |
| Cumulative % | 94.327 | 99.083 | 100.000 |

**Table 11**. Factor loading of physiological growth parameters

| | F1 | F2 | F3 |
|---|---|---|---|
| RL | 0.961 | 0.270 | -0.067 |
| SL | 0.991 | -0.004 | 0.134 |
| DW | 0.962 | -0.265 | -0.071 |

More leaf number per seedling was observed at sowing time $S_2$ as compared to $S_1$ sowing time at both the harvests. At both the sowing time and harvests, seed size has no significant effect on leaf number. The result showed that large seeds produced seedlings with mean number of leaves of 5-7 while the medium size seeds produced seedlings with the mean values of 6-7 (Table 4). At both the sowing dates leaf area of seedling increased with increasing seed size. At harvest I, leaf area varied from 7.74 to 42.68 $cm^2$ $seedling^{-1}$, whereas at harvest II, it ranged between 11.38 and 53.65 $cm^2$ $seedling^{-1}$and was always greater for sowing time $S_2$.

Sowing time and seed size had significant effect on seedling dry weight (Table 4). At sowing time $S_1$ dry weight of seedling increased with increasing seed size, whereas at sowing time $S_2$, it decreased with increasing seed size. At harvest I, highest (0.64 g $seedling^{-1}$) and lowest (0.05g $seedling^{-1}$) seedling dry weight was recorded for medium size seeds at sowing time $S_2$ and $S_1$, respectively. At harvest II, seedling dry weight varied from 0.14-0.75 g seed ling-1 in medium size seeds and from 0.16-0.61 g seedling-1 in large size seeds and was always higher for sowing time $S_2$ (Table 4).

***Effect on physiological growth parameters***

At harvest I, root: shoot ratio decreased with increasing seed size at both the sowing dates. In contrast, at harvest II, it increased with increasing seed size class at both the sowing dates. With increasing seed size class, leaf weight ratio (LWR) increased at both the sowing dates at harvest II, whereas it declined at both the sowing dates at harvest I (Table 5).

At each sowing date relative water content (RWC) gen-

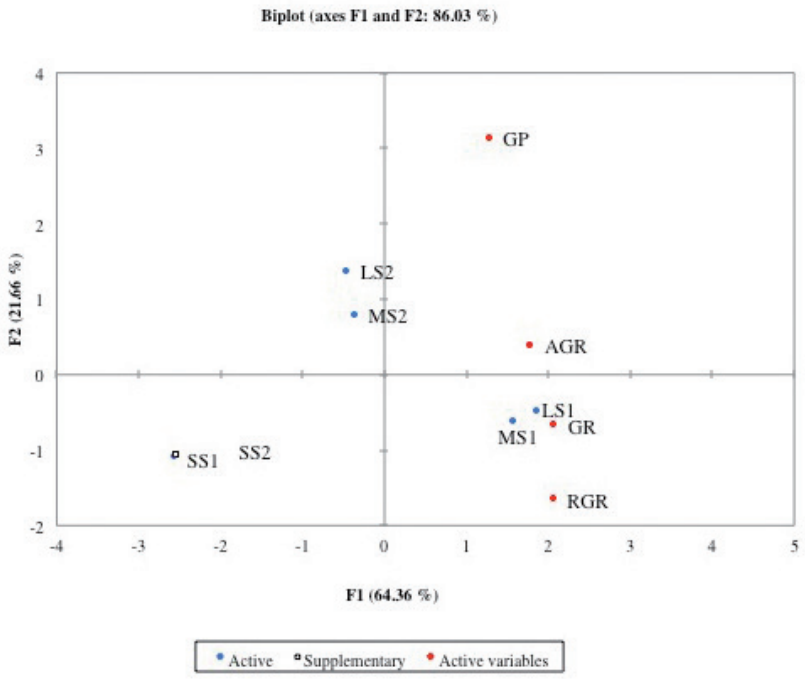


**Fig. 4.** Principal component analysis (PCA) of different germination parameters.
SS1=small seeds at sowing time 1; SS2= small seeds at sowing time 2; MS1= medium seeds at sowing time 1; MS2= medium seeds at sowing time 2; LS1= large seeds at sowing time 1; LS2= large seeds at sowing time 2

erally decreased from harvest I to harvest II and showed fluctuating trend in relation to sowing time and seed size (Table 5). RGR increased with increase in seed size. Highest relative growth rate (RGR : 0.036 g $day^{-1}$) was observed in large size seeds whereas, lowest RGR (0.034 g $day^{-1}$) was reported for medium size seeds. Average growth rate (AGR) showed the same growth pattern as described for RGR. Maximum AGR (0.004 g $day^{-1}$) was observed in large seeds whereas, minimum AGR (0.003 g $day^{-1}$) was reported for medium size seeds. Large seed size showed strong vigor index. It ranged between 2.33-12.3 at harvest I, whereas it ranged from 4.94 to 13.02 at harvest II (Table 5).

In the present experiment, both germination and early seedling growth rate of *Q. floribunda* were affected by sowing time and seed size (Fig. 3). This could be attributed to availability of more food reserves in large seeds which enhanced their viability, hence resulted in prompt and highest germination percentage. Similar observation has been made by Owoh et al. (2011), who reported that seeds with large dimensions are likely to have large embryo which enhances good germinability. Murali (1997) also reported that the seed size often controls the germination and initial seedling growth in many tree species. Sowing time could not alter the effect of seed size though the germination percentage was higher at sowing time $S_2$. Same results were observed by Ahirwar (2012) in *Alangium lamarckii*. This study indicated that large size seeds gave best germination only when they were sown in their favourable sowing season. Sowing season $S_2$ was more suitable for the germination of seeds of *Q. floribunda* indicating that under similar environmental conditions seed maturation with time resulted in better germination ability. Hence seed grading is an essential step to improve the quality of nursery stock as well as their performance at field conditions.

Principal component analysis (PCA) was carried out with 4 germination parameters. Relative growth rate may be considered as most potential performer as it showed excellent correlation with germination rate and average growth rate (Table 6). The PCA axis F1 accounted for 64.4 % and variation in quality indicator composition while PCA axis 2 accounted for 21.7 % variation (Table 7).

In the first PC (Principal Component), a highest loading was observed with germination rate, the next higher loading for relative growth rate closely related indicator (Table 8). The components contributing to maximum variance always become first PC and hence in this case three quality indicators with highest loading were selected for PC

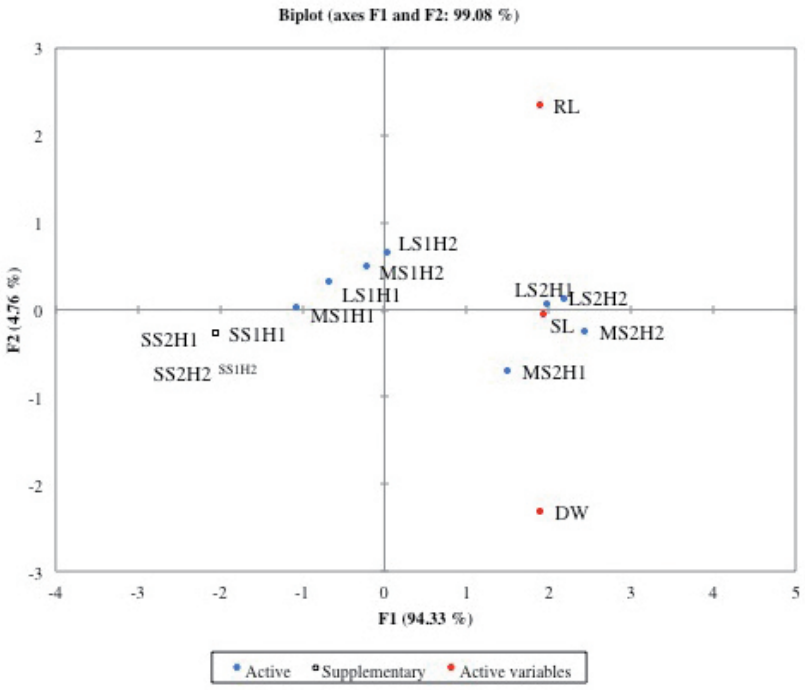


**Fig. 5.** Principal component analysis (PCA) of morphological growth parameters.
SS1H1=small seeds at sowing time 1 at harvest I; SS2H1= small seeds at sowing time 2 at harvest I; SS1H2= small seeds at sowing time 1 at harvest II; SS2H2= small seeds at sowing time 2 at harvest II; MS1H1= medium seeds at sowing time 1 at harvest I; MS2H1= medium seeds at sowing time 2 at harvest I; MS1H2= medium seeds at sowing time 1 at harvest II; MS2H2= medium seeds at sowing time 2 at harvest II; LS1H1=large seeds at sowing time 1 at harvest I; LS2H1= large seeds at sowing time 2 at harvest I; LS1H2= large seeds at sowing time 1 at harvest II; LS2H2= large seeds at sowing time 2 at harvest II

analysis while, two were selected in second components with highest loading. The PCA ordination of germination parameters of seeds on the basis of sowing date is presented in Fig. 4.

The first component highest loading values were found with germination rate (0.911) and relative growth rate (0.906) (Table 8).

Ypc1= 0.911 (GR) + 0.906 (RGR) +……

Second component (Ypc2) the highest loading was found with germination percentage (0.806) followed by relative growth rate (-0.421).

Ypc2 = 0.806 (germination percentage) + -0.421 (RGR) + ….....

A correlation analysis and PCA were carried out between different variables (root length, shoot length and dry weight of seedlings) of seed size and their sowing time (Fig. 5). The Pearson correlation (Table 9) showed positive and significant (always lower than $P<0.05$) correlation between variables. F1 explains 94.3 % and the F2 explain 99.1 % of total variance (Table 10). Table 11 indicatedss the loading value of the principal components. These loadings explain the contribution of each variable in principal components. The bold number means the variables load on that component (loading > 0.8). Table 11 showed high loadings of shoot length (0.991) and seedling dry weight (0.062) with positive effect. Rathinavel (2018) also analyzed the Cluster analysis by using Ward's method of minimum variance exhibited a distinct pattern of group formation for different seed growth parameters. Similarly Sudrajat (2015) also described the principal component analysis of various fruit, seed and seedling revealed that most of the geographically distant populations are genetically close.

The relationship between leaf area and total plant weight may also influence total seedling growth and survival. Larger investment in leaf area per unit of total plant carbon enhances growth rate (Newhouse and Madgwick 1968; Farmer 1980; Reich et al. 1992), even more than an increase in photosynthetic rate does (Schulze 1983).

## Conclusion

It can be concluded that sowing time and seed size greatly affected seed germination, seedling length and dry mass in tilonj oak (*Q. floribunda*). In all parameters assessed, sowing time $S_2$ had the best performance in combination with large seed size. Thus, large seed size with sowing time September is recommended to silviculturists and tree planters for best germination and seedling vigour.

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