



Effect of depth of seed sowing and orientation on germination and seedling growth performance of *Prinsepia utilis* Royle seeds

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ABSTRACT :

In this paper, effect of depth of seed sowing and seed orientation on germination percent and seedling growth performance of freshly collected and stored seeds of *Prinsepia utilis* Royle (Bhekal) was studied. The seeds were sown in three orientations with micropyle end kept in horizontal, upward and downward positions and sown at four different depths viz. 1cm, 2cm, 3cm and 4cm in nursery beds filled with fine sand. After 35 days of sowing, seedlings were evaluated for seed and seedlings parameters such as germination percent, mean germination time (MGT), germination value (GV), seedling length, collar diameter, number of leaves, total biomass, vigour index, sturdiness quotient (SQ), volume index, quality index (QI) and root shoot ratio. Collected data where ever required was arc-sin transformed before being subjected to a two-way Analysis of Variance (ANOVA). The findings reveal that fresh seeds can be sown either horizontal or upward at 2cm depth for better germination and seedling growth performance in the nursery. Even if slightly delayed in sowing, horizontal seed orientation may be preferred for sowing upto 2cm depth to get maximum germination as well as a better quality seedling.

Key words: Seed storage, depth, orientation, seed, seedling performance, germination.

Introduction

P. utilis Royle locally known as bhekal, bekoi, and bekuli in Uttarakhand is one of the important shrub species of tropical and temperate Asia. It thrives well in varying habitats, including from the hill slopes to the vertical cliffs and from the mountain valleys to the barren lands. All the parts of plant are used owing to its multiple uses like having potential source of edible seed oil, fuel wood, fodder, and medicines and are an integral part of ritualistic practices. Seed yields about 50% fatty edible oil. Its oil has rubefacient properties, applied externally to treat rheumatism and muscular pain resulting from fatigue (Chauhan, 1999) and externally to treat coughs and colds (Manandhar, 2002). The seed cake paste is applied to treat ringworm or eczema, and hot oilcake relieves stomach aches. Indigenous people used its root, stem, and leaf to cure skin ailments and diseases (Pu *et al.*, 2015). It is best suited plant for colonized at the degraded areas in Garhwal Himalayas (Maithani *et al.*, 1986) and could be useful in the reclamation of the degraded wastelands in Himalayas (Bhagat and Singh, 1991). Hence nursery techniques of this species need to be improved in order to raise quality nursery stock for carrying out plantation to combat the degradation of land and soil erosion.

Depths of sowing and orientation of seed affect significantly seed germination and seedling growth performance of the species in the nursery (Kumar and Mishra, 2007). Neither seeds should be sown too deeper or too shallow in the nursery. If seeds are sown too shallow, there are chances of losing seeds due to attack by ants, birds etc. (Dwivedi, 1993). Besides this, seed growers tend to sow many seeds per hole of stored seeds to compensate for deteriorated seeds leading to great wastage (Omondi, 1998). The depth of sowing and seed orientation affects germination and seedling performance of many species. Guo *et al.* (2010) evaluated the effect of soil burial depth on seed germination and seedling development of *Prunus armeniaca* and revealed that the seed germination and seedling establishment rates were

higher at 4 cm depth of sowing. Similarly Gurunathan and Srimathi (2011) in *Jatropha* sowing depth at 4 cm showed higher germination and a higher number of quality seedlings. Adeogun and Usman (2012) worked on *Adanonia digitata*, *Acacia senegal*, *Delonix rigia*, *Balanites aegyptiaca*, *Khaya senegalensis*, *Senna siamea*, *Prosopis juliflora*, and *Ziziphus spinachristi* and found the highest germination at 3 cm sowing depth in the nursery. Cano *et al.* (2012) studied the effects of sowing depth on seed germination and seedling emergence of Mexican oaks (*Quercus affinis*, *Q. castanea*, *Q. coccolobifolia*, *Q. laeta*, *Q. maxicana*, *Q. polymorpha*, *Q. tinkhamii*, and *Q. viminea*) and observed a significant difference in germination and seedling establishment for all the species. They reported sowing seeds at higher depth is beneficial for *Quercus* seed germination. Abedi and Herfehdest (2013) evaluated the effects of sowing depth (3, 6, and 9mm) on seed germination and seedling growth of Bamboo (*Dendrocalamus hamiltonii*) and observed higher growth parameters at 6 mm sowing depths.

Orientation of the seed at the time of sowing also affects seed germination. Kevin *et al.* (2015) studied on *Lagenaria siceraria* and found the highest germination at 2cm depth with horizontal orientation. Bhatt (2011) observed a maximum 80% germination in *Calamus prasinus* seeds sown in inverted position and maximum % germination in seeds of *C. stoloniferous* and *C. thwaitesii* sown in vertical position. Ahn *et al.* (2017) investigated the effect

of seed sowing orientation on germination viability on peanut (*Arachis hypogaea*) sprouts. The vertical orientation with hypocotyl end-down and hypocotyl-end-up orientations showed the highest (91.7%) and lowest (25%) germination rates, respectively. Zhang *et al.* (2015) and Lal *et al.* (2020) found similar results for seed germination and seedling performance in Litchi (*Litchi chinensis*) with significant reduction in seedling emergence with increased sowing depth. Mishra *et al.* (2013) also observed on half-strength medium in horizontal position recorded to produce maximum germination (78.23%), shoot number (0.86) and root number (7.99).

Based on above facts, the present studies were conducted in *P. utilis* (bhekal) to determine the optimum depth of sowing and proper seed orientation for better germination and production of quality planting stock.

Materials and Methods

The fully matured fruits of *P. utilis* were directly collected from diseased free healthy plants near Sahiya village, Chakrata Forest Division, Uttarakhand, India situated between 30°37'00.25" N, 77°52'28.09" E above 1200m msl. There is northern mixed deciduous forest and subtropical Himalayan lower mostly planted Chir pine forest. The climate is subtropical (Champion and Seth, 1968). Fruits were brought to Forest Tree Seed Laboratory, FRI (Dehradun), India for further seed processing and carrying out various experiments.

The fruits were kept in tied gunny bags for overnight after collection from the field in the laboratory. After one night fruits became soft and ready for depulping. Fruits were depulped by macerating gently with hands and washed out with running freshwater. The cleaned seeds were spread over the floor under the electric fan for one day in the laboratory. Every four hours interval, seeds were shaken by hand for uniform drying till equilibrium moisture content (EMC) which obtained after 3 days of shade drying. The seeds were then stored in airtight plastic containers and kept at ambient room temperature.

Seeds were sown in nursery bed in four replications with 100 seeds using four different depths of sowing viz. 1.0 cm, 2.0 cm, 3.0 cm and 4.0 cm and three different orientations viz. horizontal, upwards, and downwards in completely randomized design (CRD) (Plate 1). Before the sowing, beds were watered by hand using Hazara. Immediately after sowing of seeds, beds were watered again carefully and continued daily as per requirement till termination of experiments.

Seeds germination was recorded daily up to 35 days. The seed was counted germinated when cotyledon had emerged about 1cm above the ground. 10 x 4 seedlings were uprooted at the end of germination and wash out with fresh running water carefully. Uprooted seedlings were measured immediately for total seedling length (cm), shoot length (cm), root length (cm) collar diameter (mm) and total no. of leaves. Fresh and dry weights were also taken. Uprooted seedlings were dried in oven at 103 °C for 17 hrs for biomass and measured on dry weight basis. Volume index was calculated by multiplying of diameter (mm) and height. Vigour index (Abdul Baki and Anderson, 1973), quality index (Dickson, 1960), sturdiness quotient (Ritchie, 1985), and root shoot ratio (on dry weight basis) were also calculated.

Moisture determination of seeds was carried out with four replications. Percent moisture of the seeds was taken up on a wet basis by oven dry method at 103 °C for 17 hrs as per ISTA (2010).

Calculated by the following formula: -

$$M.C (\%) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Germinated seeds were counted when radicle emerged about 1 cm. Germination percent was calculated by using the following formula: -

$$G (\%) = \frac{\text{Total germination}}{\text{No of seeds sown}} \times 100$$

Germination Value (GV) was calculated using Djavanshir and Poubeik (1976) formula, which is more closely related to the survival of plants in field nurseries. The formula is given below:-

Where,

GV= Germination Value

GV= $\frac{\sum DGS}{N(GP/10)}$

DGS= daily germination speed, obtained by dividing the cumulative Germination percentage by the number of days since sowing

$\sum DGS$ = The total obtained by adding every DGS figure obtained from the daily counts

N = The number of daily counts, starting from the first germination date.

MGT is the time taken to complete germination under various treatments was determined as Mean Germination time (MGT) in days.

The following formula calculates the MGT: -

$$MGT = \frac{\sum D * G + \text{No. Of seed not germinated} \times [\text{Days taken for termination of experiment} + 1]}{\text{No of seed sown}}$$

Collected data was analyzed in SPSS by using scheffe formula. Statistics analysis was carried out to test whether any significant difference exists in effect of treatments on each of the characters separately through ANOVA. The P value <0.05 were taken as significant.

Results and discussion

Effect of orientation and depth of sowing on germination of fresh and stored seeds.

Fresh seeds sown in horizontal orientation showed significantly maximum mean germination (79.44%) as compared to upward orientation and downward orientation with 71% and 62.75% respectively (Table 1). Similar trend was observed in stored seeds. The maximum mean germination percentage was observed in horizontal orientation (53.88%) followed by upward and downward orientation with 37.38 and 33.88 percent. However, there was non significant difference between upward and downward orientation (Table 2). Downward orientation showed lower mean germination in both the cases. Similar observations were reported by Kevin *et al.* (2015) in *Lagenaria siceraria* and Lal *et al.* (2020) in litchi. According to them horizontal sowing proved to be the best orientation sowing methods. While contradictory results were reported by Singh *et al.*, 2017 in *Cinnamomum tamala*. Ahn *et al.*, 2017 in peanut; Bhat (2011) in species of rattans and reported maximum germination percentage in downward orientation.

Germination percentage was significantly affected by depth of sowing. Fresh seeds sown at 1cm and 2cm depth significantly exhibited maximum mean germination percentage (88.08 and 85.00 respectively) as compare to 3cm (72.50%) and 4 cm (38.67%) depth of sowing (Table 1). However, there was non significant difference between 1cm and 2cm depth of sowing in term of mean germination percentage of fresh seeds. Seeds sown at 3cm and 4cm depth showed significantly lower mean germination percentage as compare to 1 and 2 cm depth (Table 1). Present investigation supports earlier researchers who stated that as a thumb rule, seed should be sown at a depth that approximate three to four time of their diameter. However, large seeds need only a sowing depth of twice their diameter. Our results are also in accordance to Fredrick *et al.* (2018) on *Dialium guineense*, Koger *et al.* (2004) on *Caperonia palustris*, Rusdy and Sjahril, 2015 in *Centrosema pubescens*, Opande *et al.* (2017), and Chima *et al.* (2017), in *C. brevidens* and *A. muricata* respectively. They reported fewer seeds were germinated at depth increased. Grace and Mbogue, (2020) and Zhang *et al.*, (2015) also support our findings, they noted higher germination emergence at minimum depth of sowing in *Aframomum citratum* (Pereira) K. Schum and *Litchi chinensis* and seedling growth generally decreased with increase in sowing depth. The interaction of depth and orientation significantly affect the seed germination percentage as fresh and stored seeds.

The maximum germination percentage was observed at 1cm depth with horizontal orientation (94.25) non significantly followed by 2cm (92.00) depth with horizontal orientation. Horizontal orientation showed maximum germination at 1cm, 2cm, 3cm and 4cm depth and remained at par with each other in fresh seeds. At 4cm depth, all orientation showed significantly lower germination percentage while upward and downward orientation remained non-significant. The stored seeds also showed significant difference in interaction of depth and orientation. Horizontal orientation showed maximum germination percentage at 1cm, 2cm, 3cm and 4cm of depth followed by upward orientation. Interaction with best orientation and depth gave higher germination in horizontal orientation upto 3cm depth (Table 2). Mean germination time was affected by orientation. Seed germinated fastest in horizontal orientation followed by upward orientation and downward orientations. However, horizontal gave early germination in both the cases. Similar results were reported by Agboola *et al.* 1993 in *Dalbergia sissoo*. The present study is in conformity with the earlier study of Guo *et al.*, 2010. They reported that deeply buried seeds might suffer from rotting due to higher soil humidity in deeper soil zone than that in shallower. But Arkoh *et al.*, 2018 and Aikins *et al.* (2006) reported contrary to this study. They reported *Dioscorea* placing in any orientation with variable depth condition will germinate equally. Our results are in concurrence with Singh *et al.*, 2017; Bhat, 2011; Koger *et al.* (2004); and Kevin *et al.* (2015).

A significant difference was observed with respect to the mean GV of fresh seeds. Horizontal orientation exhibited significantly highest mean GV (19.57) followed by upward and downward orientation with 16.40 and 8.26 respectively. Depth of sowing significantly affected the mean GV. It was significantly decreased as the depth increased. The horizontal orientation of stored seeds also showed significantly higher mean GV with 10.56 as compared to upward and downward orientation of GV 4.43 and 2.75 respectively. Sowing depth showed higher mean GV at 1cm (8.13) and 2 cm (7.83), reduced as the depth increased at 3 and 4cm depth with 5.02 and 2.69 respectively.

The interaction of depth and orientations affected the GV. There was a significant difference in GV obtained from all interactions. Fresh seeds sown horizontal at 1cm depth exhibited maximum GV (33.42) followed by 1cm horizontal (29.90) and 2cm horizontal (28.09). Downward orientation at all depths shows reduced GV with increased depth. All the orientations showed poorer GV at 4cm depth of sowing (Table 1). Horizontal orientations showed significantly higher GV (19.57) of stored seeds followed by upward orientation (16.40). Horizontal orientation shows an insignificant difference at 1 and 2 cm of the depth of sowing. Horizontal orientation exhibited higher GV followed by upward orientation at all the depths as compare to downward orientations. All orientations show poorer GV at 4cm depth of sowing (Table 2). The similar results were reported by Li *et al.* (2015) in *Bromus japonicus*. Ramirez *et al.* (2012) also reported maximum seedling percentage at 1cm depth in *Bidens alba*, which was in line of our study. In contrast, *Citrullus lanatus*, Ramirez *et al.* (2014) reported that seed sown on the soil surface did not germination.

Effect of orientation and depth of sowing on seedling performance of fresh and stored seeds.

Non-significant difference was observed at all depths and orientations with respect to seedling length of fresh and stored seeds (Table 1 and 2). Orientation affected the mean number of leaves. Upward orientation shows significantly higher number of leaves (11.7) followed by horizontal orientation (10.75) as compared to downward (10.62). However, the depth of sowing did not show a significant effect on the mean number of leaves up to 4cm in fresh seeds. Contrary, stored seeds do not show any significant difference in term of orientation. However, mean number of leaves in stored seeds increase with increasing depth of sowing (Table 2). Significantly maximum total biomass was produced by fresh seeds in horizontal orientation (1.94g) followed by upward and downward orientation with 1.51g and

1.42g respectively. However, no significant was observed in term of depth. Depth and orientation showed non significant effect on biomass in stored seeds. Vigour index is directly correlated with total germination percentage and total biomass (Abdul Baki and Anderson, 1973). Horizontal orientation

showed maximum mean vigour index followed by upward and downward orientation of fresh and stored seeds. However, fresh seeds show non significant difference between 1, 2, and 3cm of depth, while 4 cm depth significantly showed least vigour index. However, non significant effect of depth was found in stored seeds. Similar results were observed by Sikuku *et al.*, 2018 in *Senna spectabilis*. He reported that seedling vigour index is significantly affected by sowing depth. There is a significant decrease in seedling vigour index with an increase in sowing depth. In contrast to our studies, Shiva *et al.*, (2003) showed maximum vigour index in downward orientation. Both fresh and stored seeds in horizontal orientation showed minimum value of sturdiness quotient (Table 1 and 2). However, no significant difference was recorded in stored seeds in all orientation. The minimum sturdiness quotient (good value) showed at minimum depth and increased with increasing sowing depth. Horizontal orientation showed maximum volume index in both fresh and stored seeds (Table 1 and 2). It was maximum at lower depth as increased in depth. However, no significant difference in was observed in fresh and stored seeds upto 2 and 3 cm respectively. Orientation and depth of seeds showed non significant difference with respect to quality index of fresh and stored seeds (Table 1 and 2). The present findings corroborate the findings of Ginwal *et al.* (2001, 2004) who reported higher values of Dickson quality produced better seedlings. Horizontal orientation showed significantly higher root shoot ratio in fresh seeds. However, non significant effect of orientation and depth was observed in stored seeds (Table 1 and 2).

Conclusion

Fresh or stored seeds of *Prinsepia utilis* may be sown in micropyle horizontal whereas stored seeds must be sown upto 2cm depth for higher productivity in nursery for seed growers.



Plate 1. Morphology of *P. utilis* seedling at various depth and orientation

A- Upward orientation, B- Downward orientation and C- Horizontal orientation

Table 1. Effect of depth and orientation on seed and seedling performance of fresh *P. utilis* seeds. Values of germination percentage, mean germination time (MGT) and germination value (GV) are means of four replication of 100 seeds. Seedling traits are average of 10 randomly selected seedlings after 35 days of growth in each replication. Values in parenthesis are sin transformed.

Sowing depth	Orientation	Germination (%)	Mean Germination Time (days)	Germination Value	Seedling length (cm)	Number of leaves	Collar Diameter (mm)	Total biomass (g)	Vigour index	Sturdiness Quotient	Volume index	Quality index	Root Shoot ratio
1	Upward	89.00 (70.73)	18.06	29.90	16.74	11.10	1.80	09.76	58.91	1.34	119.75	0.37	3.06
	Downward	81.00 (64.41)	23.75	17.07	16.55	10.00	1.92	08.74	64.47	1.02	083.73	0.32	3.50

	Horizontal	94.25 (76.33)	17.15	33.42	18.19	10.90	2.50	07.43	91.17	1.75	165.20	0.40	2.99
	Mean	94.25 (70.49)	19.65	26.80	17.16	10.67	2.07	08.64	71.52	1.37	122.89	0.36	3.18
2	Upward	88.00 (69.90)	20.12	25.22	16.88	10.90	1.99	08.86	67.82	1.65	145.11	0.36	3.22
	Downward	75.00 (60.38)	28.71	08.49	16.76	09.70	1.79	09.61	61.32	1.37	104.80	0.31	3.78
	Horizontal	92.00 (75.01)	18.51	28.09	18.46	11.20	2.39	08.06	87.96	2.18	200.43	0.48	2.53
	Mean	85.00 (68.43)	22.45	20.60	17.37	10.60	2.06	08.85	72.37	1.73	150.11	0.39	3.17
3	Upward	77.00 (61.84)	26.73	09.36	17.57	10.60	2.23	07.98	78.65	1.51	111.48	0.45	2.50
	Downward	63.00 (52.80)	29.81	06.18	16.99	11.00	1.99	08.80	67.01	1.90	119.29	0.37	3.27
	Horizontal	77.50 (61.92)	24.68	12.54	18.91	11.80	2.63	07.28	99.28	1.60	120.90	0.35	3.17
	Mean	72.50 (58.86)	27.07	09.36	17.82	11.13	2.28	08.02	81.65	1.67	117.22	0.39	2.98
4	Upward	30.00 (33.02)	33.57	01.13	16.80	14.20	2.26	07.63	75.16	1.56	048.24	0.42	2.61
	Downward	32.00 (33.92)	33.58	01.32	18.42	11.80	1.75	10.98	64.42	1.39	047.32	0.38	3.90
	Horizontal	54.00 (47.33)	30.95	04.23	17.79	9.10	2.45	07.42	86.48	2.25	121.78	0.34	3.50
	Mean	38.66 (38.09)	32.70	02.23	17.67	11.70	2.15	08.68	75.35	1.73	072.45	0.38	3.34
Mean	Upward	65.00 (58.87)	24.62	16.40	17.00	11.70	2.07	08.56	70.13	1.52	106.15	0.40	2.85
	Downward	56.66 (52.88)	28.96	08.26	17.18	10.63	1.86	09.54	64.31	1.42	088.79	0.34	3.61
	Horizontal	79.43 (65.15)	22.82	19.57	18.34	10.75	2.49	07.55	91.22	1.94	152.08	0.39	3.05
C.D. at 5%	Orientation	3.06	1.00	1.44	1.84	1.61	0.63	1.57	4.41	0.77	6.67	0.34	0.86
	Depth	2.84	0.93	1.34	1.71	1.50	0.59	1.46	4.10	0.72	6.21	0.32	0.80
	Interaction	4.02	1.32	1.90	2.42	2.12	0.83	2.07	5.80	1.02	8.78	0.45	1.14

Table 2. Effect of depth and orientation on seed and seedling performance of seeds. Seeds were stored for 30 days in airtight plastic containers under ambient root temperature. values of germination percentage, mean germination time (MGT) and germination value (GV) are mean of four replications of 100 seeds. Seedling traits were means of 10 randomly selected seedlings after 35 days of growth in each replication. Values in parenthesis are arcsin transformed.

Sowing depth	Orientation	Germination (%)	Mean Germination Time (days)	Germination Value	Seedling length (cm)	Number of leaves	Collar Diameter (mm)	Total biomass (g)	Vigor index	Sturdiness Quotient	Volume index	Quality index	Root Shoot ratio
1	Upward	44.50 (41.86)	27.61	06.70	18.26	09.50	1.81	10.18	66.92	1.35	60.07	0.35	3.29
	Downward	39.25 (38.76)	29.70	04.48	18.72	09.10	2.01	09.45	74.61	1.39	55.45	0.40	2.80
	Horizontal	59.50 (50.53)	23.80	13.21	18.92	09.80	1.99	09.73	74.74	1.39	84.06	0.37	3.01
	Mean	47.75 (43.72)	27.03	08.13	18.63	09.47	1.94	09.79	72.09	1.38	66.52	0.37	3.03
2	Upward	41.75 (40.28)	27.94	06.07	16.99	10.00	2.22	07.82	76.14	1.72	71.58	0.32	3.76
	Downward	36.25 (36.98)	31.02	03.10	17.81	10.50	2.06	09.16	73.00	1.75	65.89	0.37	3.19
	Horizontal	60.00	23.17	14.31	17.55	11.00	1.93	09.08	68.54	1.60	97.28	0.52	2.36

		(50.84)											
	Mean	46.00 (42.70)	27.37	07.83	17.45	10.50	2.07	08.69	72.56	1.69	78.25	0.40	3.10
3	Upward	36.25 (36.98)	30.71	03.36	16.89	10.80	1.95	08.76	65.76	2.19	77.95	0.42	3.06
	Downward	33.00 (34.92)	32.16	02.13	15.19	10.30	1.83	08.57	55.54	1.56	55.42	0.47	2.50
	Horizontal	54.75 (47.78)	25.36	09.56	18.91	11.80	2.63	07.28	99.28	1.60	89.92	0.35	3.17
	Mean	41.33 (39.89)	29.41	05.02	17.00	10.97	2.14	08.20	73.53	1.78	74.43	0.41	2.91
4	Upward	27.00 (31.31)	32.39	01.60	19.14	10.70	1.92	10.81	72.41	1.78	47.25	0.39	3.09
	Downward	27.00 (31.28)	32.96	01.30	16.55	10.20	1.69	10.26	57.13	1.75	48.60	0.47	2.80
	Horizontal	41.25 (39.98)	27.76	05.16	17.02	11.00	2.07	08.63	70.75	1.77	73.44	0.38	3.10
	Mean	31.75 (34.19)	31.04	02.69	17.57	10.63	1.89	09.90	66.77	1.77	56.43	0.41	3.00
Mean	Upward	37.38 (37.60)	29.66	04.43	17.82	10.25	1.98	09.39	70.31	1.76	64.21	0.37	3.30
	Downward	33.87 (35.48)	31.46	02.75	17.07	10.03	1.90	09.36	65.07	1.61	56.34	0.43	2.82
	Horizontal	53.88 (47.28)	25.02	10.56	18.10	10.90	2.16	08.68	78.33	1.59	86.17	0.40	2.91
C.D. at 5%	Orientation	2.62	1.11	1.46	1.86	1.52	0.63	1.65	4.56	0.80	5.64	0.31	0.89
	Depth	2.44	1.03	1.35	1.73	1.42	0.58	1.53	4.24	0.74	5.25	0.29	0.83
	Interaction	3.45	1.46	1.92	2.44	2.01	0.83	2.17	6.00	1.05	7.42	0.41	1.18

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