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Maturity indices and dormancy breaking methods of black locust (*Rhobinia pseudoacacia*) seeds under temperate Kashmir condition

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ABSTRACT

Studies were undertaken to ascertain the seed/pod maturation and dormancy behaviour of black locust under temperate Kashmir conditions and it was observed that the seeds were mature enough by 1st September and seed colour changed from chocolate to black. The germination percentage was recorded as 64 per cent. After September, the seed germination decreased. Minimum germination percentage was recorded as 12.07 by the seeds collected on 15th October, indicating that the seeds enter into a state of dormancy. Dormancy release seed treatments of *Robinia pseudoacacia* on germination parameters were studied under laboratory conditions. It was found that seeds pre-treated with boiling water for 1 minute was most effective followed by concentrated sulphuric acid (98%) treatment for 1 minute. Germination was enhanced from 12.07 (untreated) to 82.24 (1 minute dip in boiling water treatment).

Key words : Robinia pseudoacacia, *seed maturation, dormancy, Propagation*

Introduction

Black locust (*Robinia pseudoacacia*), locally called 'Kiker', is fast growing tree. The short rotation of the species makes it a biomass based fuel source of the future. It dominates early forest regeneration in many native forest stands where it occurs (Boring and Swank, 1984). The tree has tremendous potential owing to its early successional, fast growing, dominant nitrogen fixing and heavy surviving ability, Mass propagation by seeds is an easy and very cheap method of propagation. Black locust produces heavy seed crop, but unfortunately, natural regeneration is rarely found in the forests because of seed coat dormancy. Dormancy may be strong to weak, and the extent of dormancy present at any particular moment is referred to as the degree of dormancy; number of techniques have been developed by foresters and researchers for removing the physically imposed dormancy in seeds for increased germination (Bilsland *et al.*, 1984). These methods include treatments with acid, hot water/ temperature, mechanical scarification etc. The very hard impermeable seed coat prevents most seeds from germination (Gosling, 1995). So the seeds of the black locust (*Robinia pseudoacacia*) are to be pretreated to break the dormancy before sowing for raising seedling nursery. Raising of seedling nursery is successful only when mature and viable seeds are collected. The knowledge of exact stage of collection is of immense importance to avoid collection of immature and non-viable seeds (Willians, 1985) which results in large scale wastages of money and labour. Singh and Kachari (2006) reported that the germination

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percentage of seeds increased with decrease in specific gravity and moisture content in cones of Pinus kesiya (Khasi pine). Immature seeds die if they are allowed to dry out (Harrington, 1972). Thus, fruit collection should be started only when the seeds are sufficiently mature corresponding to the indicators of maturity for individual species.

Material and Methods

The study was undertaken in the Faculty of Forestry, SKUAST-K during the year 2008-2009. The experimental site lies between 74.89^0 longitude and 34.08^0 latitude at an altitude of about 1587m amsl. The soil is silty loam type. The climate in general is temperate; with severe winter extending from December to March. The region faces a wide temperature range from a mini-mum of -8.0 ^{0}C in winter to a maximum of 33 ^{0}C in the summers. Winter frost is common and medium to heavy snowfall is also witnessed. The area receives an annual precipitation of 676-1193 mm, with an average of 944.6 mm.

Maturity indices

The optimal time to harvest is when a large amount of viable germinable seeds can be collected. In order to determine the best time for collection of seeds of *Robinia pseudoacacia*, the seeds were collected from phenotypically superior trees from district Srinagar. The seed collection for the study was started from 15th July till their maturation, at an interval of 15 days.

The germination test was performed in petriplates lined with double fold germination paper at bottom. The seeds were placed sparsely in Petri plates and moistened. The plates were incubated at 25±1 ^{0}C in B.O.D incubator for a period of 21 days. The plates were kept moist and inspected regularly. Seeds were considered to have germinated as soon as radical emerged. The following parameters were recorded after each collection date.

Colour: Change in the colour of seeds and Fruit provides simple and reliable criteria for judging seed maturity, which is observed during the course of maturity.

***Seed weight*:** The fresh weight of 100 bold and viable seeds was recorded, using 8 replications with the help of sensitive top pan balance.

***Seed Size*:** The length and width of filled seeds were recorded with the help of calliper. The dimensions of 100 seeds were observed using 8 replications.

***Specific gravity*:** The specific gravity of seeds was determined by water displacement method (Oliver, 1974). The specific gravity of seed was determined by the formula given below:

$$\text{Specific Gravity} = \frac{\text{Fresh seed weight}}{\text{Weight of volume of water displaced by same fresh seed weight}}$$

***Moisture (%)*:** The seeds of *Robinia pseudoacacia* were oven dried at a temperature of 60 ^{0}C till they attained the constant weight. Moisture content was determined by the formula (Schubert and Adams, 1971).

$$\text{Moisture \%} = \frac{\text{Fresh seed weight - Dry seed weight}}{\text{Fresh seed weight}} \times 100$$

***Germination %*:** The germination test was performed in glass petri plates on the top of the germination paper. The seeds were placed sparsely in petri plates and moistened. Four replications of 100 seeds each were used for the test. Germination percentage was recorded at all stages of maturity immediately after collection. The seeds were counted as germinated when radical emerged. The testing period was 21 days. The germination per cent was calculated as per following standard methods:

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

A. Dormancy of the seed

In order to assess the dormancy and germination pattern of *Robinia pseudoacacia* seeds, an attempt has been made. Seeds collected at maturity from identified trees were bulked together to form four seed lots (100 seeds in four replicates were used for taking observations) and were subjected to the following treatments.

***Control*:** None of germination treatment was provided to the seeds.

***Mechanical scarification*:** In this treatment, part of the seed coat was removed with the help of the sharp blade.

***Boiling water treatment*:** In this method seeds were dip into the boiling water for 1 minute.

***Thawing*:** In this method of dormancy release the seeds were alternatively subjected to hot water followed by cold water.

***Acid Scarification*:** Seeds were dip into concentrated

(98%) H_2So_4 for 1 minute. The scarified seeds were thoroughly washed for one hour under running tap water.

The following observations were recorded for above mentioned treatments, Germination Percentage (Ger. %), Germination Value (G.V) and Germination Energy (G.E) as per following standard method:

***Germination Percentage (Ger. %)*:** The germination was carried out in sterilized petridishes lined with double layer of filter paper, moistened with distilled water. The germination was carried out at a temperature of 20±2 ^{0}C and in each treatment four replications were used with 100 seeds in each petridish. The testing period was 21 days.

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

***Germination value (GV)*:** Practical application of the test is mainly related to expected field performance. Czabator (1962) coined the word GV, and related it to mean daily germination and Peak value.

$$\text{G.V.} = \text{Final MDG} \times \text{Peak Value}$$

Where,

$$\text{MDG} = \frac{\text{Cumulative percent of seed germination at the end of test}}{\text{Total number of seeds}} \times 100$$

***Germination Energy (G.E)*:** Germination Energy is defined as the percentage of seeds germinated from 7 to 21 days after sowing. As the testing period was 21 days.

$$\text{G.E.} = \frac{\text{No. of seeds germinated upto peak of germination}}{\text{number of seeds}} \times 100$$

Result and Discussion

A. Maturity indices

Studies undertaken for investigating the maturity of seeds revealed that the tree comes in flowering in late April. The flowers are creamy white or pinkish in colour which appeared in pendulous axillary racemes on current years shoots. The pods appeared in the month of early June and attained full size by the month of July. The colour of the pod changed from green to brownish red and the seed colour changed from green to chocolate and from chocolate to black by the month of September (Plate 1).

The primary requirement for any successful regeneration for all the tree species is that mature seeds should be collected and that too in large quantities for which the knowledge of seed maturation indicators is a must. In order to develop seed maturation indices for better regeneration, seeds were collected regularly from the phenotypically superior trees from 15th July till maturation at an interval of 15 days. The data collected and presented in Table 3 showed that the collection dates have remarkable effect on colour, seed weight, seed size, specific gravity, moisture content and germination percentage.

Colour

During the course of study, it was observed that the seed colour was green in the beginning from 15th July to 15th August and no germination was recorded during this period. The colour of the seed changed from green to chocolate by 15th August and turned black on 1st September after which no further change in colour was observed. With the commencement of colour change germination also commenced and reached 64 per cent on 1st September when the seeds were black. This indicated that the change in colour from green to black is a reliable indicator of maturity in seeds of *Robinia pseudoacacia*. Similar findings were observed in seeds of *Bauhinia retusa* which undergo change in colour from green to brownish red at maturity (Upadhyyay *et al.*, 2006). Similarly, Tompsett and Pritchard (1993) have reported that the colour of seeds change from white to brown in *Aesculus hippocastenum* as the maximum seed fall approaches.

Seed weight and Seed Size

Study further revealed that seed weight and size also varied with the collection period. Initially both seed weight and seed size increased and attained maximum values for both seed weight and seed size by 15th August. The seed weight was recorded as 54.1 g/1000 seeds. The length and breadth recorded for such seeds were 6.99 and 4.37 mm, respectively. The increasing seed weight and size despite the fact that the moisture content recorded a decreasing trend throughout the collection period could be the attribute of the synthesis and accumulation of the food reserves. However, from 15th August onwards both seed weight and seed size started decreasing due to loss of moisture, which is the characteristic feature of maturity. In *Dalbergia sissoo* Joshi (2000)

reported that moisture loss coincides with maturity.

Moisture Content (%)

The moisture percentage of *Robinia pseudoacacia* declined throughout the collection period. The moisture content declined from 75.34 to 6.27 per cent. Maximum germination occurred when seed moisture declined to 8.69 per cent Singh (2006) reported the increase in germination percentage from 0 – 23 percent as the moisture content reduced from 52.19

(a) Green immature seeds and pods

(c) Mature black seeds and pods

(b) Colour change towards maturity

(d) Germinated seeds

Plate 1. Maturity indicators for seed collection of Black Locust (*Robinia pseudoacacia L.)*

Table 1. Maturity indicators for seeds of Black Locust (*Robinia pseudoacacia*) collected on different dates during 2008

S. No.	Date of collection	Colour	Seed weight (g/1000 seeds)	Specific gravity	M.C (%)	Seed Size (mm)		Ger. %
						Length	Breadth	
1.	15th July	Green	29.2	1.08	75.34	5.48	3.37	Nil
2.	1st August	Green	52.8	1.05	64.01	6.83	4.29	Nil
3.	15th August	Chocolate with greenish tinge	54.1	1.04	56.49	6.99	4.37	19.50
4.	1st September	Black	25.3	1.01	8.69	4.64	3.32	64.00
5.	15th September	Black	24.4	1.00	7.45	4.55	3.27	35.50
6.	1st October	Black	23.9	0.98	6.27	4.51	3.15	13.00
7.	15th October	Black	23.7	0.96	6.25	4.50	3.12	12.07

Table 2. Diary of seed lot for dormancy release of *Robinia pseudoacacia*

S. No.	Parameters	Observation
1.	Date of seed collection	15th of October, 2008
2.	Seed weight/1000 seeds (g)	23.7
3.	Seed colour	Black
4.	Specific gravity	0.96
5.	Germination (%)	12.07
6.	Moisture content (%)	6.25
7.	Seed length (mm)	4.50
8.	Seed breadth (mm)	3.12

to 31.63 percent in case of *Celtis australis* (Hack berry), with further loss of moisture content from 8.69 to 6.27, germination decreased which may be attributed to hardening of the seed coat towards the later stage which prevents the imbibition of water and gaseous exchange (Kelly *et al.*, 1992).

Specific Gravity

The specific gravity recorded a deceasing trend and varied between 1.08 and 0.97 over the collection period. The seeds collected on 1st September registered the maximum germination which was recorded as 64 per cent. The specific gravity for such seeds was recorded as 1.01. The relationship between declining specific gravity and increasing seed germination has been reported by a number of workers in various conifer species. Singh, (1998) reported decrease in specific gravity of *Abies pindrow* from 1.04 in August to 0.97 at the maturity in October. In *Celtis australis* (Hackberry) germination percentage increased from 0 – 23 per cent as the specific gravity decreased from 1.22 to 1.03 respectively (Singh, 2006). Majeed *et al.*, (2009) reported increase in germination percentage from 24-80 per cent in the seeds of *Aesculus indica* with the decreased in specific gravity from 1.18 (immature) to 0.82 (maturity). However, with further decrease in specific gravity, the germination was abruptly reduced from 64 to 35.50 per cent by 15th September which further decreased to 12.07 per cent on 15th October. This may be due to hard nature of the seed coat observed in the seeds collected from 15th September onwards. Hard seed coat imposed dormancy which prevents germination has also been reported by (Uniyal and Nautiyal, 1998; Bahar 2007).

Germination percentage

During the course of maturity indices, the germination percentage increased as the seeds advanced towards maturity. It was observed that germination ranged from 19.50 % (15th August) to 64 % (1st September), the increase in the germination can be correlated with the loading of seeds with the carbohydrates, fats, and proteins, which proceed gradually across the season to maturity and is commonly defined by germinability. Mughal and Thapliyal (2006) reported the increase inn the germination percentage of *Cedrus deodara* as the seeds advanced towards maturity from August to October. But again the germination percentage of *Robinia pseudoacacia* started decreasing and reached to a minimum of 12.07 by 15th October. This clearly indicates that the seeds enter into a state of dormancy. Singh (1998) reported the similar trend in *Abies pindrow* from 0.50 in August to 32.14 in October, but decreased after because of hardening physical and physiological dormancy.

B. Dormancy release

During the course of maturity indices, it was observed that maximum germination of 64 per cent was recorded when seeds were collected by 1st September. Thereafter germination percentage started decreasing and reached to a minimum of 12.07 by 15th October. This clearly indicates that the seeds enter into a state of dormancy. The seed colour, seed weight, seed size, specific gravity, moisture content, and germination percentage of such dormant seeds is given in table 2.

In order to improve the germination the seeds

Table 3. Dormancy release of seeds of *Robinia pseudoacacia*

Treatment	Ger. (%)	G.V	G.E
Control	12.07	1.71	8.54
Mechanical scarification (Manual)	26.21	20.88	18.56
Hot water treatment (boiling water for 1 minute)	82.24	161.19	45.47
Thawing (70 ^{0}C and 20 ^{0}C for 1 hr.)	64.24	95.62	35.48
Concentrated sulphuric acid scarification (98%) [1 minute]	74.30	121.97	39.61
CD ($p \leq 0.05$)	11.87	11.80	5.50

collected on 15th October were subjected to various pretreatments as shown in the Table-3. The perusal of the table showed that the seeds of the *Robinia pseudoacacia* behaved differently under different pretreatments. Germination per cent, germination value and germination energy varied from 12.07, 1.71, 8.54 (control) to 82.24, 161.19, 45.47 (Hot water) respectively.

Hot water treatment was most effective pre-treatment which enhanced the germination percentage upto 82.24. The germination value and germination energy for hot water treatment were recorded as 161.19 and 45.47, respectively. This might have happened because immersing seeds in hot water softened the hard seed coat thereby increasing the permeability of the hard seed coat to water and gases (Kelly *et al.*, 1992). Agboola *et al.* (2005) reported 80 per cent germination in case of *Cassia siamea* when seeds were soaked in boiling water for 30 seconds. Similar findings were reported by several authors. Doran and Gunn (1986) reported that the germination of some Australian Acacias was enhanced by immersing seed for 1-5 minutes in boiling water.

The acid scarified seeds also gave encouraging results which enhanced the germination percentage upto 74.30. The germination value and germination energy for such seeds were recorded as 121.97 and 39.61, respectively. The acid scarification may have improved the germination by creating the weak spots on the seed coat through its decomposing action on the seed coat components. These weak spots acted as a passage through which the water entered into the seed and caused expansion of embryonic parts thereby inducing germination (Cavanagh, 1987). Bamel *et al.*, (2007) reported 100 per cent germination in *Acacia leucophloea*, when seeds were treated with concentrated H_2So_4 for 10 minutes.

The mechanical scarification in which the part of the seed coat was removed yielded poor results. The germination per cent for such seeds was recorded as 26.21, germination energy and germination value in this treatment recorded were 20.88 and 18.56 respectively. In seeds of *Acacia holosericea* germination percent showed reduced trend from 96% (Hot water at 70 ^{0}C for 30 minutes) to 56 % (Mechanical Scarification) (Bamel *et al.*, 2007). This might have happened due to fact that mechanical scarification may have injured the embryo which leads to poor germination (Lata and Verma, 1993).

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