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Seed germination in *Prunus cerasoides* D. Don influenced by natural seed desiccation and varying temperature in Central Himalayan region of Uttarakhand, India.

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Abstract: *Prunus cerasoides* D. Don the Himalayan wild cherry is one lesser known multipurpose tree species of Himalaya. The tree prefers to grow on sloping grounds between the altitudes of 1200-2400 m, on all types of soils and rocks. The tree is used as a medicinal plant in Himalayan region. The fruit is edible and the pulp is used to make a cherry brandy. The species has poor germination and seedling establishment in natural habitat. The over exploitation of seeds of the species coupled with relatively hard seed coat has adversely affects the germination of seeds in their natural habitat. The information about the seed maturity and technique of germination enhancement is scanty. The present study was conducted to assess the exact maturity time and optimum temperature for enhancement of germination in seed of *P. cerasoides*. The fruit/seeds were collected from six sites covering the altitudinal range of 1350 – 1810 m during the period (2003-2004). The colour change of fruit from dark green to red was a useful indicator of seed maturity. Maximum germination coincided with 50.24 ± 0.19 % fruit and 30.11 ± 0.57 % seed moisture content. Negative correlation existed between germination and seed moisture content (r = 0.294; P< 0.01). Significantly higher germination occurred when seeds were placed above the paper at 25º C.

Key words: Seed moisture content; Maturation; Temperature; Germination percent; Germination capacity

Introduction

The lesser known woody perennials like *Diploknema butyracea, Myrica esculenta, Rhododendron arboreum, Prunus cerasoides, Grewia oppositifolia, Berberis* species etc can play a paramount role not only in meeting farm based fodder and fuel wood needs but also in creating an income generating systems at the village level in hilly regions (Tewari, 1997). Among the multipurpose tree species (MPT) of Himalayas, *Prunus cerasoides* D. Don (Family-Rosaceae) commonly known as Paddam or Himalayan wild cherry is one lesser known and studied MPT of Kumaun. This is the undercanopy species which commonly occurs in association of *Quercus leucotrichophora, Pinus roxburghii, Aesculus indica* etc. in the rocky and sloping areas between 1200 and 2400 m. (Troup, 1921). Because of its multipurpose value the species is very beneficial for the upliftment of local people. It is one of 31 multipurpose species which is used as a medicinal plant in Himalayan region (Samant et.al., 1998). The bark is used in psycho-medicines. The juice from the bark applied on body swelling and contusions. Kernels used as a remedy for stones and leaves are crushed with twigs and bark and soaked in water, taken internally to stop abortion and other female disorders (Tewari, 2005). Tree is mainly used as rootstock for cultivation of cherries, apart from being of medicinal value. The plant is known to exude gums. The gum possesses antioxidant property (Malsawmtluangi *et al.,* 2014). The tree contains 83 % Moisture, 3.11 % Ash, 7.32 % Fiber, 0.319 % Vitamin C and 0.133 mg/g Chlorophylls (Sundariyal and Sundariyal, 2001). In Kumaun region it is highly used for ethnobotanical purposes. The well-seasoned timber of this species used to make ornamental furniture, walking sticks etc. which is durable and liable to either fungus or insect attack. Bark is used for tannin (Troup, 1921).

The fruit is edible and the pulp is used to make a cherry brandy. The kernels contain oil similar to that of bitter almond. The off season autumn / winter flowering in the species is also very useful for beekeeping. *Prunus cerasoides* has been identified as an excellent framework tree species for restoring evergreen forest in seasonally dry tropical forestlands (Pakkad *et al.,* 2003).

Seed maturation has been related to the physical attributes in many species (Pandit *et al.,* 2002). In many pine species generally maturity is reached when moisture content of cone is below 50% (Tewari, 2005). Shah (2005) also observed that seed maturity in *Myrica esculenta* is attained when moisture content of seed is 30%. Knowledge of exact stage of seed collection can be feasible and of immense important to avoid the collection of immature and nonviable seeds which result in nursery and plantation failures. However, dormancy in seeds can severely limit germination (Tewari *et al.,* 2011).

The regeneration of *Prunus cerasoides* is very poor in its natural habitat (Tewari *et al.,* 2011). Pakkad *et al.,* (2004 b) has reported around 40% germination in *Prunus cerasoides* in the nursery. Literature reveals that the regeneration of wildest edible species *Baccaurua sapida* (Sundariyal and Sundariyal., 2001) and *Myrica esculenta* (Pandey, 2002) is poor in natural habitat. Seeds of *Prunus cerasoides* have hard seed coat and possess mechanical dormancy (Baskin and Baskin, 2001). Shah (2005) has also reported mechanical dormancy in *Myrica esculenta.*

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This adversely affects the germination of this species.

The effect of environment on seed germination is very complex because of interaction of external and internal factors which modify the rate and magnitude of germination. However, among the various environmental factors that influence the seed germination, water, temperature and light are of paramount importance (Rao, 1984). Temperature plays a significant role during the process of seed germination because various biochemical reactions in the food reserves of the seed depend on temperature (Kumar and Bhatnagar, 1976). Combined effect of temperature and media was also studied by Pandit, (2002) in seeds of *Cupressus torulosa*. Maximum and minimum temperatures vary with the species. Variation in temperatures for seed germination within or between species has been reported in most of the Himalayan species (Thapaliyal and Gupta, 1980; Semwal and Purohit, 1980; Thapaliyal *et al.,* 1985). Thapaliyal *et al.,* (1991) has reported that 20º C and 25 º C temperature on Top of the paper (TP) and between the papers (BP) enhances germination of *Alnus nitida*.

In *Prunus cerasoides* there has been little or no focused studies on propagation techniques. The studies on wild edible plant are very limited (Tewari and Dhar, 1997). Tewari (2005) has worked on nursery techniques of *Prunus cerasoides*. Pakkad *et al.,* (2004 a) also worked on the genetic variations and gene flow in *Prunus cerasoides*. The present study was designed for assessing the exact maturity time and optimum temperature for attaining the maximum germination in the seeds of selected species.

Material and Methods

Study area

The study area, lies between 1350 - 1810 m on the Southern extremity of the lesser Himalayan zone in Kumaun, it lies at 29º24' N latitude and 79º28' E longitude. The climate of this area is subtropical monsoon type with warmer temperatures towards lower elevation and cool temperature towards higher elevation. Rainfall is governed by southwest monsoon and the average annual rainfall during the study ranged from 2000-2200 mm. The climatic data were taken from Aryabhatt Research Centre for Observational Sciences, Nainital. The mean maximum temperature ranged from 12.3º C (January) and 27º C (May) and mean minimum temperature from 4.5º C (January) to 16.7º C (August) during the study period. After a thorough survey of Nainital district, six sites with suitable representation of *Prunus cerasoides* were selected between 1350 m and 1810 m altitude (Table 1).

Table 1: Site characteristics of *Prunus cerasoides* in Nainital district.

Sites	Altitude (m)	Aspect	Associate species
Shyamkhet I (S1)	1760	Northern	*Myrica esculenta, Acer caesium, Pyrus communis*
Shyamkhet I (S2)	1810	North-eastern	*Myrica esculenta, Acer caesium, Pyrus communis*
Shyamkhet I (S3)	1730	Northern	*Myrica esculenta, Acer caesium, Pyrus communis*
Khamari (S4)	1350	North-eastern	*Acer caesium, Pyrus communis*
Mangoli (S5)	1375	Northern	*Pyrus communis*
Khurpatal (S6)	1425	North-eastern	*Acer caesium, Pyrus communis*

Seed Maturity

Ten medium sized healthy trees of *Prunus cerasoides* were marked with paint at each selected site at distance varying between 80 to 100 m from each other. The selected trees had a clear bole, disease free, had good number of flower/fruit and compact crown. The height, girth and crown of each selected tree were measured. The height was measured with Ravi multimeter and girth and crown area (Length x Width) of each selected tree was measured with meter tape. The fruits were collected from all selected sites subsequently at 10 days interval (during last week of February – Mid April). The seeds collected from each tree, mixed and five replicates (100 fruits/seeds in each replicate) were taken and depulped in the laboratory and dried under shade for 48 hours. All the fruit/seed parameters [length (mm), width (mm)] were measured with a digital vernier (Model no. CD -1206" CS, accuracy ± 0.02 mm Mitutoyo Co.) and weight of 100 fruits/seeds (g) was measured using electronic balance (WENSAR). Moisture content of fruit/seed was determined on the fresh weight basis by drying the material at 103 ± 2º C for 16 ± 1 h (ISTA, 1993) and then reweighed. Seeds were then surface sterilized with 0.1% $HgCl_2$ and rinsed thoroughly under running tap water. The Petri-dishes and germination paper were sterilized at high temperature (130º C) for 4 hours to make it free from fungal infection. Five replicates of 100 seeds were used. The petri-dishes were lined with germination paper and 100 seeds were then placed on them. The petri-dishes were kept in a seed germinator (20º C) under dark condition for each collection date. Daily observation was taken and germination was counted when visible protrusion of radical (1mm) occurred. The germination was monitored for 90 days with water being added at regular interval. After completion of experiment germination percent and germination capacity was calculated (Shah et. al., 2010).

Germination test

The experiment was carried out on fully ripened fruits which had started to fall down. After depulping, sterilization and washing three replicates each of 100 seeds were used.

The Petri-dishes were lined with germination paper. To enhance the germination percent (as it was found low in the test of maturity indices) the seeds were placed on two conditions i.e. above paper (AP) and between papers (BP) and allowed to germinate at different temperatures i.e. Room temperature (16 ° - 22 ° C T1), 20° C (T2) and 25° C (T3) in dual chamber seed germinator. Daily observations were made for germination at varying temperatures following the procedure used in studying maturity indices.

Statistical test:

The data of maturity indices was statistically analyzed for multiple analyses of variance (ANOVA) to show the significant difference between sites and dates. In germination test ANOVA showed significant difference between sites, temperature and conditions (Snedecor and Cochran, 1967).

CD was calculated as-

CD = S.Em* t0.05 (t0.05 is t value at 5% level of significance)

Where S. Em is the standard error of difference calculated as S.Em. = √ Me / r (Lavania, 2004).

Results

Tree characteristics

Among all the six sites the mean tree height varied between 17.6 ± 0.62 m (S3) and 22.2 ± 3.04 m (S6). Mean tree diameter at breast height (dbh) varied between 50 ± 0 cm (S5) and 72 ± 5.6 cm (S1). Mean crown cover varied between 20.6 ± 1.02 m2 (S2) and 39.3 ± 1.76 m2 (S5) (Table 2).

Table 2: Tree characteristics for different sites (± SE)

S.No.	Sites	Mean tree height (m)	Mean tree diameter (cm)	Crown cover (m2)
1	Shyamkhet (S1)	20.7 ± 0.84	72 ± 5.6	36.8 ± 2.5
2	Shyamkhet (S2)	17.6 ± 0.65	55.6 ± 12.33	20.6 ± 1.02
3	Shyamkhet (S3)	17.6 ± 0.62	56 ± 12.22	34 ± 3.05
4	Khamari (S4)	18.9 ± 1.63	60 ± 10	30.3 ± 3.17
5	Mangoli (S5)	18.1 ± 1.57	50 ± 0	39.3 ± 1.76
6	Khurpatal (S6)	22.2 ± 3.04	53.3 ± 13.33	24 ± 2.30

Fruit/Seed characteristics

The green colour of fruit changed with each collection date and pale red to red at final collection in second week of April. Across all the sites the fruit/seed length, width and weight of 100 fruits increases gradually with each collection date. The mean fruit length ranged between 4.21 ± 0.19 mm and 15.69 ± 0.29 mm, mean fruit width between 5.57 ± 0.12 mm and 11.53 ± 0.2 mm and mean weight of 100 fruits ranged between 18.76 ± 1.03 g and 114.57 ± 2.1 g. The fruit parameters (length, width and weight of 100 fruits) varied significantly (P<0.01) across sites and dates of collection. The mean seed length ranged between 7.46 ± 0.1 mm and 12.87 ± 0.29 mm, mean seed width between 4.31 ± 0.09 mm and 8.91 ± 0.08 mm and mean weight of 100 seeds ranged between 20.37 ± 0.18 g and 48.08 ± 0.68 g. The seed length/width varied significantly (P<0.01) across sites and dates of collection. The weight of 100 seeds was varied significantly (P<0.01) across dates of collection. The fruit moisture content 50.24 ± 0.19 % and seed moisture content 30.11 ± 0.57 % at fifth collection in S6 site coincide with maximum germination (20 ± 8.8 %). Negative correlation existed between germination and seed moisture content (r = 0.294; P< 0.01) (Fig. 1). There was no relation between other physical parameters and maturity.

Germination test

At room temperature the mean germination in seeds placed on AP, varied between 6.66 ± 2.66% and 53.33 ± 13.33 %. On BP germination varied between 0 ± 0% and 7.49 ± 4.16 % across all the sites (Table 3).

Table 3: Variation in germination of *Prunus cerasoides* seeds subjected to three different temperatures (T1 = room temperature), T2 = 20° C and T3 = 25° C) and two conditions, above paper (AP) and between paper (BP). The values are mean of two years. MG = Mean germination; GC = Germination capacity.

Site	Temperature	Condition	M.G %	G.C %
S1	T1 (room)	AP	16.66 ± 16.66	68.33
		BP	0 ± 0	74.00
	T 2 (20º C)	AP	6.3 ± 0.3	70.83
		BP	9.33 ± 4	76.16
	T 3 (25 º C)	AP	59.16 ± 0.83	77.50
		BP	30.83 ± 2.5	73.33
S2	T 1 (room)	AP	25 ± 15	60.00
		BP	0 ± 0	57.50
	T 2 (20º C)	AP	17.5 ± 2.5	72.65
		BP	9.5 ± 0.5	71.65
	T 3 (25 º C)	AP	63.33 ± 6.67	72.50
		BP	46.66 ± 3.33	75.00
S3	T 1 (room)	AP	53.33 ± 13.33	72.50
		BP	0 ± 0	67.50
	T 2 (20º C)	AP	1 ± 1	72.00
		BP	2.6 ± 0.35	70.00
	T 3 (25 º C)	AP	63.33 ± 6.67	75.83
		BP	41.33 ± 2	71.00
S4	T 1 (room)	AP	6.66 ± 2.66	60.83
		BP	0.33 ± 0.33	59.50
	T 2 (20º C)	AP	8.83 ± 1..17	59.15
		BP	2.33 ± 2.33	55.00
	T 3 (25 º C)	AP	46.66 ± 0	61.16
		BP	22.83 ± 3.83	60.83
S5	T 1 (room)	AP	33.33 ± 33.33	75.00
		BP	10.66 ± 4.33	70.00
	T 2 (20º C)	AP	5.99 ± 1.66	70.80
		BP	1.83 ± 1.83	65.00
	T 3 (25 º C)	AP	54..99 ± 1.66	70.83
		BP	29.99 ± 3.33	60.83
S6	T 1 (room)	AP	30.99 ± 17.66	60.83
		BP	7.49 ± 4.16	52.50
	T 2 (20º C)	AP	5 ± 5	55.00
		BP	6.5 ± 0.5	57.50
	T 3 (25 º C)	AP	33.33 ± 13.33	63.33
		BP	29.99 ± 3.33	55.83

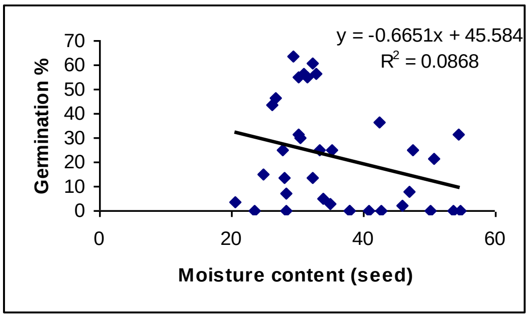

Fig. 1: Relationship between germination and moisture content of seed in *P.cerasoides* across all the sites.

At 20º C the mean germination in seeds placed on AP, varied between 1± 1 % and 17.5 ± 2.5 %. On BP germination varied between 1.83 ± 1.83 % and 9.5 ± 0.5 % across all the sites (Table 3).

Table 4: Analysis of variance (ANOVA) for variations in germination on *Prunus cerasoides* seeds of different sites subjected to different temperatures (T 1= room temperature), T 2 = 20º C and T 3 = 25 º C) and conditions (AP = Above paper, BP = Between paper).

Characters	Source of variation	df	Mean square	F. value	Sig. / non sig.
Germination (Year 1)	Site	5	731.437	4.823	.001*
	Temperature	2	16035.954	105.732	.000*
	Condition	1	2241.333	14.778	.000*
	Site x Temperature	10	430.498	2.838	.005**
	Site x Condition	5	204.178	1.346	.255 NS
	Temperature x Condition	2	495.583	3.268	.044 NS
	Site x Temperature x Condition.	10	373.461	2.462	.014 NS
Germination (Year 2)	Site	5	563.526	12.451	.000*
	Temperature	2	15295.009	337.942	.000*
	Condition	1	10800.000	238.625	.000*
	Site x Temperature	10	533. 831	11.795	.000*
	Site x Condition	5	247. 156	5.461	.000*
	Temperature x Condition	2	4739.194	104.712	.000*
	Site x Temperature x Condition.	10	319.683	7.063	.000*
	(Germination Year 1)			(Germination Year 2)	
Site	*			*	
CD at 5 %	10.53			5.73	
Temperature	*			*	
CD at 5 %	35.26			19.27	

NS = non-significant
*** = Significant at 1% (P<0.01)**
**** = Significant at 5% (P<0.05)**

At 25º C the mean germination in seeds placed on AP, varied between 33.33 ± 13.33 % and 63.33 ± 6.67 %. On BP the germination varied between 22.23 ± 3.83 % and 46.66 ± 3.33 % across all the sites (Table 3). Across all sites the maximum germination occurred at 25º C when seeds had been placed on above paper. Germination varied significantly (P<0.01) across all the sites, temperatures and conditions (Table 4).

Discussion

Physical indices have been widely used, particularly since the seed collectors lend themselves to field estimation. Several workers throughout the world have investigated colour as a workable indicator for several species (Shah, 2005). Distinct colour changes have been associated with seed maturity in hard wood fruits. The physical characters of fruit/seed have played a significant role in maturity indices (Tewari, 2005). Various mature and immature fruits and seeds can be distinguished in various ways e.g. by colour difference, increased firmness, brittleness, decreased moisture content, specific gravity and by change in physical dimensions (Shah *et al.,* 2010). The fruit maturation of *P.cerasoides* became apparent with the change in its colour from green to pale green and finally red with maturity. The germination was maximum when the colour of fruit turned pale green. Upadhyay *et al.,* (2006) also found colour change to be one of the best criteria for determining maturity in *Bauhinia retusa.* Moisture content and specific gravity are other two physical parameters that are interrelated and more objective. Both have been reported as reliable maturity indices by numerous researchers (Shah, 2005).

Decline in moisture content appears to be a good indicator of seed maturity in *P.cerasoides.* The moisture content of the seeds declined as fruits matured (Tewari, 2005). Decline in fresh weight moisture content percent from maturing seeds is closely related to seed maturity (Pandit *et al.,* 2002). The maximum germination (20 ± 0 %) at 30.11 ± 0.57 % seed moisture content was observed in *P.cerasoides.* Shah *et al.,* (2006) have reported that moisture content of 23.4 - 36.1 % can be associated with optimum germination in *Pyracantha crenulata* seeds.

Negative correlation existed between germination and seed moisture content (r = 0.294; P< 0.01) in *P.cerasoides.* There was no relation between other physical parameters and maturity in *P.cerasoides.* Edwards (1980) reported significant correlation between maturity and physical parameters but several studies have reported no correlation between the two parameters (Tewari, 2011). Edwards (1969) could not find any relationship between physical parameters and maturity.

The absorption rate is dependent upon degree of seed coat permeability as the seed coat indirectly inhibit rate of absorption by mechanically preventing seed tissue from expanding in pace with moisture uptake. The presence of an impermeable seed coat may prevent water uptake by seeds and so prevent germination, as seed do not resume physiological activity until they imbibe a certain amount of water. *Prunus cerasoides* is one such species having hard seed coat dormancy. Pakkad *et al.,* (2004 b) found around 40% germination in seeds of *Prunus cerasoides* in the nursery. The hard seed coat mechanical dormancy is usually found in *Prunus* genera due to stony endocarp (Heit, 1967) (Baskin and Baskin, 2001) occurs in several other species like *Acacia, Prosopis, Ceranotia, Robinia* and *Cassia* etc. (Pant, 2002). Shah *et al.,* (2010) has reported this kind of dormancy in *Myrica esculenta*. Seeds of some species germinate better at constant temperature and others on alternate temperatures (Anon, 1966; Bonner, 1972; Kumar and Gopal, 1974). Temperature and substratum play a significant role during the process of seed germination because various biochemical reactions in the food reserves of the seed depend on temperature (Kumar and Bhatnagar, 1976). The availability of optimum moisture and oxygen also depends on the media used for germination (Pant, 2002). Temperature regimes regulate seed germination by affecting enzymatic activities, reaction rates and changes in the physical state of cellular components. The result of the present study revealed that *P. cerasoides* seeds germinated best at 25º C when kept above the paper (AP). The combined effect of temperature and media on seed germination was also studied by Kotoky *et al.,* (2000) in seeds of *Anthocephalus chinensis* which was statistically significant. Shah, (2005) also found maximum germination on top of the paper at 25º C in *Myrica esculenta*. International Seed Testing Association (1993) has recommended that top of the paper as substratum and temperature between 20 - 30º C is general. Pant, (2002) found 25º C as the best temperature for germination of *Alnus nepalensis.* Similarly, Pandit, (2002) reported high germination at 25 º C on top of the paper for *Cupressus torulosa.* Shah *et al.,* (2006) has reported maximum germination on top of the paper at 25º C in *Pyracantha crenulata.* In *P. cerasoides* fruit colour pale red and fruit moisture content 50.24 ± 0.19% and seed moisture content 30.11 ± 0.57 % appear to be a reliable indicator of seed maturity. Seed kept above the paper (AP) at 25º C temperature is the best method for enhanced seed germination.

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