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Journal of Advanced Scientific Research

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ISSN
0976-9595
Research Article

SEED MATURATION INDICATORS IN *DESMODIUM ELEGANS DC.* IN NAINITAL DIVISION OF KUMAUN CENTRAL HIMALAYA

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Received: 27-12-2021; Accepted: 03-05-2022; Published: 31-05-2022

 https://doi.org/10.55218/JASR.202213414

ABSTRACT

Desmodium is a genus of large flowering plant family Leguminosae. It is widely distributed in temperate and subtropical regions and used as forage plants, for biological control, and in traditional folk medicine. *Desmodium elegans* is a nitrogen fixing shrub generally found in Oak dominated forests approaching a maximum height from 1.8 to 2.5 m. We studied the vegetational parameters and seed maturation timing of *D. elegans* at two sites located in Nainital forest. Density of *D. elegans* ranged between 80 ind./25m^2 and 60 ind./25m^2. Fruits (Pods) of *D. elegans* were collected from north (S1) and south (S2) aspects covering the altitudinal range from 2000 to 2100 m. The mean seed size across the collection dates varied between 6.1±1.0 mm^2 and 18.9±0.2 mm^2 across both the aspects. The change of pod color from light green to dark brown, the range of pod size from 1.6±0.2 cm to 2.6±0.2 cm, the range of pod moisture content between 32.5±1.4 % and 42.8±2.0 % and the seed moisture content between 40.1±1.5 % and 52.1±4.2 % coincided with maximum germination and appear to be indicators of seed maturation.

Keywords: Seed maturation, Seed moisture content, Mean germination, Moisture Content.

1. INTRODUCTION

Desmodium is a genus of large flowering plant family Leguminosae, also known as Fabaceae or pea family with 650 genera and 18000 species, mostly consisting of herbs, undershrubs or shrubs. The genus *Desmodium* is represented by 300-350 species distributed in tropical and temperate areas of the world except Europe and New Zealand [1]. The genus includes pioneer species that resist the xerothermic environment and grow in arid, barren sites. Traditionally, *Desmodium* plants have been used by native people to treat several diseases like jaundice, fever, asthma, cold [2, 3], constipation, cough and convulsion [4]. *Desmodium elegans* DC. is an erect, woody and deciduous shrub growing 1.8 to 2.5 m tall having stalked trifoliate leaves. Flowers are pale purple and arranged in terminal panicles from August-September. Propagation is through seeds from September-December [5]. It is cultivated throughout the tropics up to 1525 m elevation [6]. *D. elegans* has wide applications in folk medicine and various parts of plant have been reported to be used for different purposes, for instance roots were used as carminative, diuretic and tonic and the powdered leaves applying on cuts for healing wounds [5, 7, 8]. It is also used in 'Ayurvedic' preprations like '*Dashmoolarishta*' and '*Dashmoola kwath*' for the post-natal care to avoid secondary complications. Previous studies have revealed that all members of genus *D. elegans* are rich sources of alkaloids. This plant has also been studied for the presence of alkaloids along with wax constituents obtained from the said plant [9, 10]. *Desmodium* species that form nitrogen-fixing symbiosis with rhizobia play an important role in sustainable agriculture. Due to the symbiosis between these plants and rhizobial species, are responsible for an increase in the nitrogen content of the soil-plant system via the mechanism of biological nitrogen fixation (BNF) [11].

Large scale and unrestricted exploitation of *D. elegans* to meet its ever increasing demand by the Indian pharmaceutical industries coupled with limited cultivation and insufficient attempts for replenishment of its wild stock has led to marked depletion in its population and now it is listed as a rare species by the International Union for Conservation of Nature and Natural Resources [12-18].

In life cycle of forest trees, fruit/seed ripening and germination are important events. Knowledge of the

maturation process contributes to the establishment of the ideal time to harvest when seeds have a better physiological quality and a recommended way to study the effect of climatic variations on forest tree species [19]. Change in seed maturity timing can influence regeneration, development and hence impact stand composition and structure [20]. Capsule maturation affects the timing of seed dispersal, and in turn, germination. Seed size and mass, reflects potential food reserves for seedling growth and are considered as important traits determining the successful establishment of individual plants [21].

The knowledge of exact stage of seed collection is of immense importance to avoid the collection of immature and non-viable seeds [22] which can cause nursery and plantation failure. In many species, seed maturation has been related to the physical attributes [23]. Some studies have been made on Himalayan shrubs like *Berberis* species [24] and *Rubus* species [25], but there is little or no information on the seed maturation of this Himalayan shrub *Desmodium elegans*. The present study reports the pod maturation time and seed germination status of *D.elegans* in Nainital Division of Central Indian Himalayan region.

2. MATERIAL AND METHODS

The study area lies between 29°22′-29°24′ N Latitude and 79°25′-79°27′E Longitude along an elevational transect of 2000 to 2200 m in Kumaun Himalaya, India. After a thorough survey, three sites were selected on Northern (S1) and Southern (S2) aspects. The rocks of the study area are mainly sandstones, conglomerates, limestones, quartzite, schist, granites and geniuses [26]. The soil of the study area is mainly sandy with small amount of clay.

The selection of *D. elegans* individuals for collection of pods (seeds) was based on the criteria that the shrub had fair number of pods, freedom from diseases, insect and pest attack and quality of seeds. Five average sized, healthy shrubs of *D. elegans* were collected and marked at a distance of about 100 m from each other at each site. The height was measured for each marked shrub in all the sites. The mean shrub height ranged from 2.5±0.6 to 3.2±1.9 m. The soil moisture content ranged from 27.4 to 19.7%. The water holding capacity ranged from 56.6 to 71.6%.

The shrub layer analysis was carried out by placing 10 quadrats of 5X5 meter. The data was expressed as Density, Frequency and Abundance [28].

The pod collection of *D. elegans* was started from second week of November up to the availability of pods on marked shrubs. Pods were collected at the interval of 7 days directly from the marked shrub. Collected pods were brought back to the laboratory and pods of all shrubs of same collection date were mixed thoroughly to make a composite sample for each site. From the composite sample, four replicates were taken for the determination of pod size; pod fresh weight and pod moisture content. Seeds were manually taken out from the pod (fruit). Seed size was taken as length X width which was expressed in mm^2 and measured with digital vernier caliper. Weight of 100 pods/seeds was measured with digital electronic balance. Number of seeds per 100 gm was also determined. The moisture content of fruits/seeds was expressed on the basis of fresh weight basis by drying at 103±2°C for 16±1hour [29-31] and then reweighted.

Five replicates of 25 seeds each were used to determine germination percent. Seeds collected at each date were germinated on top of the seed germination paper in a dual door seed germinator at 25±1°C. 2 mm radical emergence in seeds was the criteria followed for considering the seeds as germinated. Water was added as required during the experiment. Germination percent was calculated as the sum of total germinated seeds out of tested seeds for each elevation and each collection date within the test period. At the end of germination test, un-germinated seeds were classified as sound seeds and unsound seeds. Results were expressed as germination capacity (GC), the percentage of seeds that had germinated at the end of the test, calculated following [32].

GC % = {(Total germinated seeds+ Total ungerminated sound seeds)/ Total seeds tested} x 100

3. RESULTS AND DISCUSSION

3.1. Vegetational Analysis

In the study area, the distribution of *D. elegans* was found in *Q. leucotrichophora* A. Camus forest where the associated tree species were *Acer oblungum* and *Lyonia ovalifolia*. The associated shrub species were *Berberis asiatica*, *Viburnum cotonifolium*, and *Daphne canabina*. At S1, the total shrub density was 490 ind./25 m^2. The shrub density of *D. elegans* was 60 ind./25 m^2. At S2, the total shrub density was 730 ind./25 m^2. The shrub density of *D. elegans* was 80 ind./25 m^2.

3.2. Pod Characteristics

The color of the pods was light green at initial collection dates which gradually changed to dark brown at last

collection date across the study sites during the study period (Table 1).

Across S1 sites, the mean pod size increased from 1.9±0.1 cm in third week of November to 2.9±0.1 cm in fourth week of December. Like mean pod size, the mean weight of 100 pods increased from 4.0±0.1 g in third week of November and 8.7±1.4 g in third week of December. The mean number of pods per 100 g decreased from 3766.7±5.7 g to 1266.7±3.4 g across the collection dates (Table 1).

Across S2 sites, the mean pod size increased from 1.5±0.2 cm in third week of November to 3.2±0.1 cm in fourth week of December. Like mean pod size, the mean weight of 100 pods increased from 5.3±0.3 g in third week of November to 14.6±0.2 g in third week of December. The mean number of pods per 100 g decreased from 3633.3±1.5 g to 866.6±4.3 g across the collection dates (Table 2).

Table 1: Variation in physical parameters of fruits (pods) and seeds over the collection period of *D. elegans* in S1 site

Collection Dates	Pod Color	Pod Size (cm)	Wt. of 100 Pods (gm)	No. of Pods per 100gm	Seed Size (mm^2)	Wt. of 100 Seeds (gm)	No. of Seeds per 100gm	No. of Seeds per Pod
W3 N	Light Green	1.9±0.1	4.0±0.1	3766.7±5.7	10.3±1.3	0.5±0.1	19966.6±5.2	7.3±0.3
W4 N	Light Green	2.3±0.1	4.5±0.2	3733.3±2.2	11.8±0.7	0.9±0.1	11766.7±3.7	6.0±0.6
W1 D	Dark Green	2.5±0.1	5.2±0.6	2433.3±1.2	14.4±0.2	1.0±0.2	10733.3 ±1.2	6.6±0.3
W2 D	Light Brown	2.6±0.2	8.6±0.4	2266.7±4.2	14.7±1.4	1.6±0.1	10666.7±5.5	5.6±0.3
W3 D	Dark Brown	2.7±0.2	8.7±1.4	2312.8±5.4	15.0±1.1	1.6±0.2	9100.0±4.6	6.0±0.6
W4 D	Dark Brown	2.9±0.1	7.1±1.3	1266.7±3.4	18.9±0.2	1.7±0.2	8800.0 ±5.1	6.6±0.3

Table 2: Variation in physical parameters of fruits (pods) and seeds over the collection period of *D. elegans* in S2 site

Collection Number	Pod Color	Pod Size (cm)	Wt. of 100 Pods (gm)	No. of Pods per 100gm	Seed Size (mm^2)	Wt. of 100 Seeds (gm)	No. of Seeds per 100gm	No. of Seeds per Pod
W3 N	Light Green	1.5±0.2	5.3±0.3	3633.3±1.5	6.1±1.0	0.6±0.1	11266.6±2.2	7.6±1.3
W4 N	Light Green	1.6±0.2	5.1±0.8	3333.3±5.1	7.7±0.3	0.8±0.1	11233.3±6.2	6.6±0.3
W1 D	Dark Green	1.8±0.1	9.3±0.6	3100.2±3.6	8.2±0.1	1.5±0.1	10833.3±5.2	6.0±0.3
W2 D	Light Brown	2.7±0.1	11.6±0.1	2633.3±4.2	8.6±0.2	1.9±0.1	9900.5±3.7	6.0±0.5
W3 D	Dark Brown	2.8±0.1	14.6±0.2	1066.7±3.3	12.3±2.1	2.0±0.1	9533.6±3.3	5.6±0.3
W4 D	Dark Brown	3.2±0.1	14.3±1.1	866.6±4.3	15.4±0.2	2.5±0.3	7766.6±2.6	5.0±0.4

3.3. Seed Characteristics

Across S1 sites, the mean seed size increased from 10.3±1.3 mm^2 in third week of November to 18.9±0.2 mm^2 in fourth week of December. Like mean seed size, the mean weight of 100 seeds increased from 0.5±0.1 g in third week of November and 1.7±0.2 g in fourth week of December. The mean number of seeds per 100 g decreased from 19966.6±5.2g to 8800.0±5.1g (Table 1).

Across S2 sites, the mean seed size increased from 6.1±1.0 mm^2 in third week of November to 15.4±0.2 mm^2 in fourth week of December. Like mean seed size, the mean weight of 100 seeds increased from 0.6±0.1 g in third week of November and 2.5±0.3 g in fourth week of December. The mean number of seeds per 100 g decreased from 11266.6±2.2 g to 7766.6±2.6 g (Table 2).

3.4. Seed Germination

Across S1 sites, the mean germination varied between 30.7±2.7% to 86.7±5.3 %. The maximum germination was 86.7±5.3 % during the first week of December when moisture content of pods and seeds was 36.1±2.3 % and 46.7±1.4 % (Table 3).

Across S2 sites, the mean germination varied between 13.3±1.3% to 37.3±3.5 %. The maximum germination was 37.3±3.5 % during the fourth week of November when moisture content of pods and seeds was 42.8±2.0 % and 63.1±4.2 % (Table 4).

At S1, significant negative co-relation was found between pod size and pod moisture content ($r=0.886$, $p<0.05$), and seed size and seed moisture content ($r=0.891$, $p<0.05$). A significant positive co-relation was found between pod moisture content and seed moisture content ($r=0.8723$, $p<0.05$) (Table 5).

Table 3: Variation in Mean Germination, Germination Capacity and Moisture Content on different collection dates in S1 site.

Collection Number	Mean Germination (%)	Germination Capacity (%)	Moisture Content (%)	
			Pod	Seed
W3 N	62.0±2.3	61.3±5.3	41.1±1.9	53.6±6.4
W4 N	58.7±3.5	82.7±4.4	39.5±1.7	49.3±1.2
W1 D	86.7±5.3	85.3±4.6	36.1±2.3	46.7±1.4
W2 D	76.2±0.7	82.7±3.5	32.5±1.4	40.1±1.5
W3 D	37.3±5.1	68.5±4.0	21.1±3.7	37.0±4.8
W4 D	30.7±2.7	64.7±1.3	15.3±1.7	29.8±2.0

Table 4: Variation in Mean Germination, Germination Capacity and Moisture Content on different collection dates in S2 site

Collection Number	Mean Germination (%)	Germination Capacity (%)	Moisture Content (%)	
			Pod	Seed
W3 N	13. 3±1.3	72.0±6.1	55.7±1.1	55.0±4.1
W4 N	37.3±3.5	84.0±6.1	42.8±2.0	52.1±4.2
W1 D	33.3±3.5	73.3±7.0	32.9±0.2	48.7±3.1
W2 D	18.7±4.8	68.0±5.7	17.6±1.8	41.2±2.6
W3 D	16.9±7.1	61.0±3.3	16.2±4.4	38.5±5.2
W4 D	14.4 ±3.9	57.9±4.1	8.9±1.1	32.1±1.9

Table 5: Co-relations between different parameters of *D. elegans* in S1 and S2 sites

Parameters	S1 Site		S2 Site	
	Pod Moisture Content (%)	Seed Moisture Content (%)	Pod Moisture Content (%)	Seed Moisture Content (%)
Pod Size (cm)	0.7849**		0.7516**	
Seed Size (mm^2)		0.7938**		0.7638**
Pod Moisture Content (%)		0.8611**		0.8798**

** *Correlation is significant at the 0.05 level (p<0.05)*

At S2, significant negative co-relation was found between pod size and pod moisture content (r=0.867, p<0.05), and seed size and seed moisture content (r=0.874, p<0.05). A significant positive co-relation was found between pod moisture content and seed moisture content (r=0.938, p<0.05) (Table 5).

Present work deals with the germination dynamics of *D. elegans* in the Kumaun Central Himalayas. Seed color change is a good indicator of maturity than any other seed parameters which require laboratory facilities [23]. In *D. elegans*, fruit (pod) and seed color change from light green to dark brown was a good indicator of maturity. Color change has also been recommended as a ripeness indicator in the Himalayan wild cherry [*Prunus cerasoides* D. Do, Rox.; [33] and in *Bauhinia retusa* Ham. [34]. Color to be the best indicator of maturity for five species of the Garhwal Himalayas has been reported [35]. Color difference, increase in firmness or brittleness, decreased moisture content and specific gravity or by change in physical dimensions of fruits and seed have been used as an indicator of seed maturity by the seed collectors [36].

Moisture content has also been used as a reliable maturity indicator by numerous researchers. Decline in moisture content percent from maturing seeds is closely related to seed maturity [37]. In *D. elegans* decline in moisture content was a good indicator of maturity. It was observed that the moisture content of S1 was more as compared to the moisture content of S2, due to higher insolation period in S2. Aspect can have a strong influence on temperature. This is because of the angle of the sun in the northern and southern hemispheres which is less than 90 degrees or directly overhead. In the northern hemisphere, the north side of slopes is often shaded, while the southern side receives more solar radiation for a given surface area insolation because the slope is tilted toward the sun and isn't shaded directly by the earth itself. Maturity of seed of *A. butyracea* may be related to changes in fruit color and moisture content of seed, which indicate appropriate dates of collection

to avoid large-scale losses in collecting non-viable seeds of this species, which has a short viability [38]. In *Casuarina equisetifolia* seed maturity is attained when the cone moisture content is below 50% as reported by [39]. In *Pyracantha crenulata* the moisture content of fruit between 30 and 36% and the seed moisture content between 68 and 71% have been adjudged indicators of seed maturation [30]. In *Populus ciliata*, a broad leaved species the drop in moisture content of capsule from 80 to 60% during maturation coincide with the maximum germination in seed as reported by [23]. The correlation coefficient was determined between pod size & pod moisture content and seed size & seed moisture content; and indicated that the moisture content was negatively correlated ($p<0.01$) with pod size and seed size at both the aspects.

The Himalayan biodiversity is severely threatened by natural and anthropogenic and natural reasons or both and these disturbances do not provide time for ecosystem recovery [40]. The regeneration of most of the wild edible species is poor in their natural habitats mainly due to intense biotic pressure on them [41]. To synchronize artificial regeneration of such species an exact knowledge of maturity time is essential to avoid the collection of immature and non-viable seeds [22] which results in low germination percentage and can cause nursery and plantation failure resulting in huge financial losses.

4. CONCLUSION

In studied species, the change in seed color from light green to dark brown and fruit color change from light green to dark brown was a reliable indicator of germination timing. In addition to the color change, the range of pod size (S1: 2.5±0.1 cm to 2.6±0.2 cm ; S2: 1.6±0.2 cm to 1.8±0.1 cm), the range of pod moisture content (S1: 36.1±2.3% to 32.5±1.4%; S2: 42.8 ±2.0% to 32.9±0.2%) and the seed moisture content ranging between (S1: 46.7±1.4% to 40.1±1.5%; S2: 52.1±4.2 % to 48.7±3.1% coincided with maximum germination and appear to be major indicators of seed maturation in *D. elegans*.

5. ACKNOWLEDGEMENTS

We express our sincere thanks to the Head, Department of Forestry and Environmental Science, Kumaun University, Nainital for providing necessary facilities in the department.

Conflict of interest

None declared

6. REFERENCES

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