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STUDIES ON REPRODUCTIVE BIOLOGY AND SEED BIOLOGY OF PANAX PSEUDOGINSENG WALL. (ARALIACEAE): A THREATENED MEDICINAL PLANT

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Abstract

Panax pseudoginseng Wall. (Araliaceae) *is a highly threatened medicinal plant. The plant population is declining rapidly owning to high exploitation for medicinal uses along with limitation within the plant itself in its reproductive behavior and seed habit. Sprouting of buds takes place from mid-February and flowers from late May till early June. Centripetal inflorescence is exhibited with continued nature of flowering. Timing of anthesis is between 6:30–7:30 AM and plants are usually self-pollinated. Seeds are dispersed by wind, gravity, small animals and birds. Plant showed positive correlation between plant height and number of leaflets. Berry production starts from 2-leaves plants onwards. Two-seeded fruit was found to be most dominant. Higher stage plants exhibits higher dormancy. Seeds of P. pseudoginseng exhibits long dormancy and demands pre-sowing stratification. Seeds stratified at both warm at 25°C for 3 months followed by cold stratification at 4°C for 4 months exhibited 68.20% germination in the seed bed.*

Keywords: *Dormancy; Medicinal plant; Panax pseudoginseng Wall.; Phenology; Reproductive output; Seed biology; Threatened plants.*

Introduction

The reproductive performance of the plants and its efficiency determines its survival and therefore knowledge on reproductive biology is the key factor in the achievement of plant conservation [1]. Reproductive bottlenecks includes failure of pollination, pre- or post-fertilization barriers leading to no or poor seed set, poor reproductive vigor due to inbreeding depression and very low germination rates impose constraints on the multiplication and survival of the species. Seed germination in nature is strongly influenced by internal as well as external environmental factors. The germinating seeds and seedlings are most vulnerable to predation, desiccation, and developmental stage of embryos, competition and damage as the seeds and seedlings constitute important resource for the herbivores and pathogen.

Ginseng is a well known medicinal herb and there are 11–12 recognized species of ginseng, depending on the method of classification [2]. Ginseng has been using as medicine since 2000 years back and its description can be found even in ancient Oriental Medical Literatures [3]. *Panax pseudoginseng* Wall. (Araliaceae) which is a native to India can be found in China, South Tibet, North Burma, North East India and Nepal [4]. Ginseng is widely used for high blood pressure, diabetes, impotency [5], for asthma, cancer, dysentery [6], for digestion,

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increase overall energy, adaptogen, to increase memory, as an aphrodisiac, for Alzheimer's disease, and for HIV disease [7].

Panax pseudoginseng Wall. is highly threatened due to its over-exploitation for herbal medicinal uses. Moreover the clearing of forest either for cultivation or developmental activities highly pose a threat to this valuable medicinal plant. The limitation of its population size also lies within the plant itself in its reproductive behavior and its seed habits. Plants shows very slow in its growth and high specific in its habitat conditions for its proper growth. The production of very less seeds as compared to its flower production along with its long month's seed dormancy contributes to its less population.

The plant is highly threatened and thus there is an urgent need to conserve this medicinal plant both *ex situ* and *in situ*. Present study was undertaken to understand the reproductive ecology and seed biology of this important species so as to work out the conservation strategy.

Materials and Methods

Study Sites

The present study was conducted in the forest area of Pangsha village, near Indo-Burma border at an elevation of 2278m ASL, 26°14´27.2´´ N and 95°07´05.7´´E under Tuensang district, Nagaland, India and 'Chida' and its surrounding forest area of Khezakenoma village at an elevation of 1874m ASL, 25°30´11´´ N and 94°13´37´´E under Phek district, Nagaland**.** The habitat and morphological aspects of the plant, phenology, leaf morphology in relation to its reproductive output, its dormancy and seed biology were studied in the study areas. Plants were observed from the time of its sprouting till its senescence. The associated plant species which were usually found growing alongside the plant were also noted and account for within a 5m parameter range to study their effect on the growth of *Panax pseudoginseng* and their vigor.

Plant height measurements are usually taken during the middle part of April where almost 90% of the plants have already attained their average height. Plants studied in the selected areas in its natural condition were categorized into different class stages according to the number of leaves present on them and were tagged as numbers 1-leaf, 2-leaves, 3-leaves and 4-leaves. Reproductive output of each stage class was studied taken into account the plant mean leaflets, plant height, flowering and non-flowering plants, pedicel formation and seeds per reproductive plant in all the class stages of the plant. The number of plants in each stage class and their percentage of plants that was dormant the following year were also taken into account by careful study in its natural condition.

Seed Biology

Panax pseudoginseng Wall. seeds were collected from the natural habitat/population in the study areas in the month of October when berries were matured. Seeds were collected from both the umbels as well as those fallen from the mother plants. Care was taken to ensure that the seeds collected from the field are not injured or infected. The collected seeds were wrapped in moistened cotton and put in airtight polybag and transported to the laboratory within two days. In the laboratory seeds were washed thoroughly in running tap water. The outer cover of the berries were removed manually and washed again in running tap water followed by air drying of seeds. The seeds were subjected to differential stratification before sowing in the seed beds as described below:

1. The processed seeds were put in autoclaved sand at ~3–4cm deep in trays. Trays were perforated for good water drainage and to avoid water logging. The trays were maintained at 25^{o}C for three months and watered thrice a week.

2. These trays were then transferred to 4°C in refrigerator for 1–7 months.

3. The stratified seeds were then sowed in two different seed bed viz. (a) mixture of normal top soil, decayed wood powder and sand at 3:1:1 ratio (seed bed 1), and (b) a mixture of normal top soil, decayed cow dung powder and sand at 3:1:1 ratio (seed bed 2).

The stratified seeds were sowed in both the seed beds at a depth of 3–4cm below the soil surface and spacing between the seeds and row was maintained at 12–15cm. The seed bed mixture was sundried before preparing the beds. For each treatment 10 seeds were sowed and repeated thrice. The beds were watered thrice a week and the emergence of radical was used as the marker for seed germination. The seedling developed from the germinated seeds was maintained in the seed bed for one year before transferring to the field/wild.

Results and Discussion

Habit and habitat: *Panax pseudoginseng* grows luxuriously under canopy cover over 80% shade in undisturbed areas with some commonly associated species such as *Artemisia nilagirica* (Asteraceae), *Dicentra* sp. (Fumariaceae), *Girardina heterophylla (*Urticaceae), *Impatiens* sp. (Balsaminaceae), *Pilea umbrosa* (Urticaceae), *Berberis* sp. (Berberidaceae), *Aconitium* sps. (Ranunculaceae), *Smilax* sp. (Smilacaceae), *Schizostachyum dullooa* (Poaceae), *Schima wallichi* (Theaceae), *Alnus nepalensis* (Betulaceae), *Rhododendron arboreus* (Ericaceae), *Quercus* sp. (Fagaceae) and *Taxus baccata* (Taxaceae). Oak tree was the most common tree where ginseng seems to grow nearby its foot trunk. Further it was recorded that wherever these associated species were present, *Panax pseudoginseng* registered healthy growth compare to the areas where these species were absent. Every individual species has its own growth requirements and the genus *Panax* is shade loving plant [8] and primarily grows in primary forest. It was observed that growth and population density of *Panax pseudoginseng* greatly differed in different natural habitats. The observation of the present study is also in agreement with the reports of other workers [8-9]. It was found in all the natural habitats studied that *Panax pseudoginseng* grew luxuriously under canopy cover over 80% shades and altitude above 1850m ASL in undisturbed areas. *Panax pseudoginseng* registered healthy growth compare to poor growth of the plants where these associated species were absent. In the recent past a similar finding was reported with *Aconitum nagarum* and *Paris polyphylla* where they have reported the influence of associated species on flowering of the species [10-11].

Phenology**:** *Panax pseudoginseng* grows to a height of about 50cm in height. Sprouting of buds from the rhizome takes place from mid-February onwards but early and late sprouting can also be seen in some plants within the same area population. Full leaf expansion occurs by second week of April with the bearing of inflorescence from 2-leaves onwards. Centripetal inflorescence is exhibited where flowering occurs blooming from the base of the inflorescence to the tip of the inflorescence (Fig. 1a). Continue nature of flowering is exhibited. The flowers at the outermost edge of the umbel inflorescence open first and subsequently flowering in the inner flower buds flowers last. Flowers are light green in color with green petals of 5, 5-calyx lobed, fused carpels with 5 anthers. The flowers are very small and inconspicuous with just 2–3mm in size (Fig. 1b). The flowers are bisexual. Flowering period starts from late May to early June with 50–65 flowers per umbel. The anthers protrude out and surround the stigmatic lobe (Fig. 1c). The stigmatic lobe is green when young. Ovary is inferior. Anthers produce large amount of pollens and the pollen from the anther falls on the stigmatic lobes once the petal opens and fertilization begins as it reaches the ovary through the style. Anthers within the same flower dehisce in different timing. Insects such as small flies and moths were seen on the flowering plants but were only visitors and not pollinating agents due to absence of pollens on their body parts. The plants are self pollinated. Stigma is usually bilobed. The timing of anthesis is between 6:30–7:30 A.M, followed by the dehiscence of anther from 7:30–8:30 A.M onwards. Fertilized ovary converts into greenish round berries (Fig. 1d). The immature green berries forms in its tightly packed umbel and shortly after 1–2 weeks it forms into matured red berries (Fig. 1e). The fruit production increases with age and size of the plant, the berries usually contain 1–3 seeds. The maturity of berries starts from the outer most edge and proceeds towards the centre of the umbel. The production of seeds in *Panax pseudoginseng* requires a higher

temperature of 18–23˚C which usually occurs during the mid-summer season. Seeds are dispersed by gravity (Fig. 1f), winds, small animals and birds.

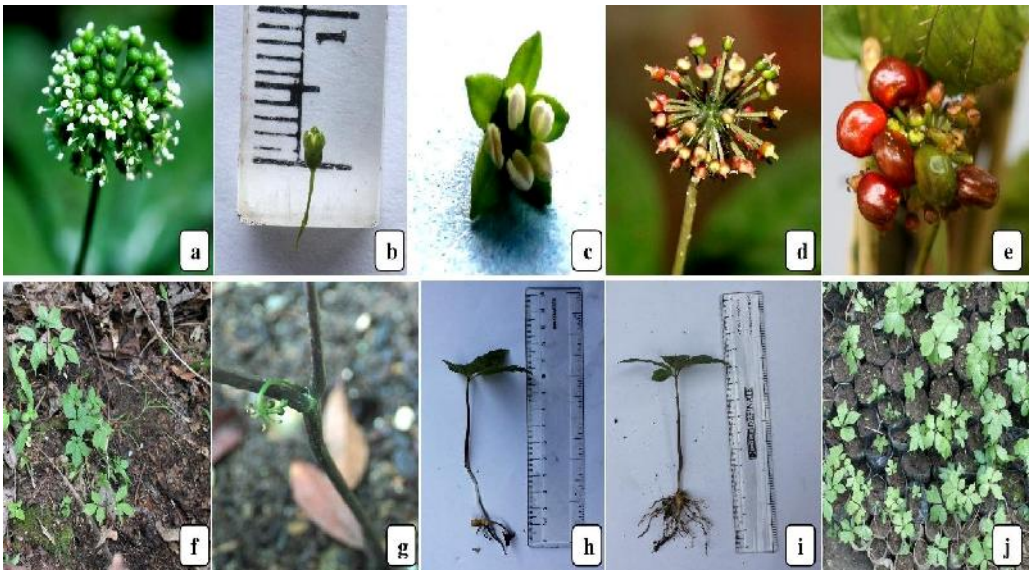

Fig. 1. a. Centripetal inflorescence of *P.pseudoginseng;* **b.** A single flower; **c.** Anthers surrounding stigmatic lobe, **d.** Young green berries, **e.** Matured red berries, **f.** Small ginseng plantlets in wild, **g.** Poor flower production in 2-leaves plants, **h.** Young seedling formed from stratified seed, **i.** One- year old plantlets developed from seed; **j.** Seedlings developed from stratified seeds maintained in polybags.

Panax pseudoginseng generally starts flowering only after they are 3–4 years old but many plants failed to flower even after 5–6 years or did not flower for 2–3 years consecutively. Flowering of plants are influenced by various factors like morpho-physiological and physical environment surrounding the species. These factors influence a lot on the reproductive age and reproductive potentiality of the species. *Panax pseudoginseng* gives flowers when the plants attains two-leaves, however the flowers productions are very less and many a times all the flowers are aborted before fruit formation or fruit production is negligible in 2-leaves plants. The fruit setting of the plant is very poor and many of the berries fails to enlarge or dehisce off before getting matured, this can be perhaps due to weak individual seed stalk which weakens more due to the weight of the berry or may largely attributed to overcrowding in the umbel inflorescence. Most of the fruits are dispersed due to gravity dispersal method and therefore young and small plantlets are found near the parent plant within a range of 1–2m. Some seeds are found at more distances due to the slope of land where the dry twigs and leaves present may aid in its movement. Along with gravity dispersal and dry twigs and leaves, birds and jungle rats also act as the plant dispersal agents and reports of fruits eaten by the bird *Plytes dragopan* in *Panax ginseng* plant has also been reported [12]. One of the documentation from locals is that the ingested seeds release as waste from birds results in high rate of germination, which needs to be further studied.

***Reproductive performance in relation to its plant morphology*:** During the present study effort was put into to study the correlation between the plant and its reproductive outcome. It was found that there was a difference in number of leaflets among the same class stage. Thus average mean was calculated for both the height and the leaflets present in same class stage. It was found that mean plant height was directly correlated with number of leaves. Mean plant height was found to be between 14.2cm (1 leaf) to 40.1cm (4 leaves stage) and there was a positive correlation between plant height and the number of leaflets (Table 1). It was recorded

that the average number of leaflets per seedling was 3.4 and the number of leaflets per plant exhibited an increasing trend from 4.4 to 21.1 from 1 leaf plant to 4 leaves plant. A similar trend was also recorded in plant height where average height of 1 leaf plant was 14.2 cm while the height of 4 leaves plant was 40.1 cm. (Table 1). Seedlings exhibits lesser height as compared to other class stages.

Table 1. Leaf class description: Number of plants (N) in each stage observed during 2011–2014, in relation to the number of leaflets and plant height

Stage	N (Number of plants)	Mean number of Leaflets	Height (cm)
Seedling	192	3.4	6.3
1-leaf	116	4.4	14.2
2-leaves	143	9.1	16.3
3-leaves	177	15.8	27.8
4-leaves	67	21.3	40.1

Reproductive output of each stage class were studied taken into account the number of pedicels formed per plant in all the class stages of the plants and percentage of pedicels producing fruit. No inflorescence developed in one-leaf stage plants as inflorescence and berry production starts only from 2-leaves plants onwards (Table 2), though the production of flowers in 2-leaves plants are usually very less and poor (Fig. 1g). In the present study highest number of pedicels formation per inflorescence was recorded from 4-leaves plant (~46) followed by 3 leaves (~28) and two leaves (~6). Percent pedicel forming drupes and formation of seeds per flowering plants also exhibited a similar trend. However their mortality rate was very high. In *Panax pseudoginseng*, three different types of berries were recorded viz. one-seeded, two-seeded and three-seeded. It was found that number of seeds per berry was dependent on the number of the leaves in the plant. Lower the number of leaves, there was more one-seeded berry and with increase in leaves, number of seeds per berry increases**.** The presence of one-seeded berry was very prominent in 2-leaves plant but there is sizeable decrease in the presence of one-seeded berry as the number of leaves on the plant increases. Likewise, gradual increase in two-seeded and three-seeded berries occurs as the plant acquires more leaves. Two-seeded berry were found to be dominant in the plant in all the studied areas.

Table 2. Correlation between leaf class and reproductive output of each stage class of *P. pseudoginseng*

Stage	Number of plants studies	Pedicels per plant in class	Percentage pedicels forming drupes (%)	Seeds per reproductive plant
2-leaves	26	6.1	16.4	2.1
3-leaves	121	28.3	19.3	8.9
4-leaves	47	46.1	23.7	14.5

Dormancy of plant: In the present study it was found that rhizome dormancy was associated with the leaf class stage. The number of plants in each stage class and their percentage of plants that was dormant the following year were taken into account by careful study in its natural habitat. It was found that dormancy in *Panax pseudoginseng* plants are more likely in the higher stage plants than in lower stage plants. There is a distinct rise in the increase of plant dormancy from 2-leaves plants onwards (Table 3). Though, 4-leaves plants data is too small to be taken into account but the data itself indicates its probability which shows it's more likely to go into the dormancy period than the lower stage plants. The dormant plants often maintain their own class stage; however there is also progress and regress in their class stage after their dormancy. 2-leaves plants are the most frequently emergent plants among the four class stages once the plant breaks its dormancy period. Dormancy exhibited even up to 2 years has being noted.

Table 3. Correlation between leaf class stage and dormancy in the following year for a study period of 4 years under controlled conditions

Year	1-leaf*			2-leaves*			3-leaves*			4-leaves*		
	N	n	%	N	n	%	N	n	%	N	n	%
2011	13	1	7.69	23	2	8.69	14	2	14.28	3	1	33.33
2012	19	1	5.26	21	2	9.52	18	3	16.66	2	0	00.00
2013	24	0	0.00	34	3	8.82	26	3	11.53	2	0	00.00
2014	31	1	3.23	28	2	7.14	17	2	11.76	3	1	33.33
Average %	4.05			8.54			13.55			16.66		

* N: Total plant studied, n: Number of plants remained dormant

Seed Biology

Panax pseudoginseng seeds were collected from the natural habitat/population in the study areas in the month of October when berries mature. Seed beds were prepared as described in materials and methods. The seeds were stratified differentially before sowing in the seed beds. The seeds sowed without stratification remained recalcitrant to germination. Seeds stratified at 25ºC for three months followed by cold stratification at 4ºC for 1 to 7 months proved to be beneficial for seed germination. Under controlled condition ~10% seed germination recorded after 425 days in seed bed 1 while 8% seed germination registered in seed bed 2 after 450 days of sowing. Warm stratification for 3 months followed by cold stratification beyond 3 months was found to promote seed germination in both the seed beds. Of the two seed bed condition studied in the present study, seed bed prepared by mixing normal soil, decayed wood powder and sand at 3:1:1 ratio found to be better for seed germination. Of the different stratifications tested in the present study, warm stratification followed by cold stratification for 4 months before sowing supported optimum seed germination in both the seed beds. Within 14 days after sowing in the seed bed 1 ~68% seeds germinated by emergence of radical followed by formation of seedling while in seed bed 1 ~52% seeds registered germination after 18 days of sowing (Table 4). Though emergence was observed within 14 days of sowing, seedling formation was achieved after ~6 week of sowing seedlings with green leaves formed after 3–4 months. Seed stratified at 4ºC beyond 4 months reduced the germination and seed stratified for 7 months registered only 9% germination. The seedling consists of one-leaf with 3–5 leaflets and leaves were light green immediately after sprouting (Fig. 1h) and slowly turned green as they matured (Fig. 1i). It was found that seedlings maintained in the seed bed 1 were healthy and dark green while seedling maintained in the seed bed 2 exhibited stunted growths. Of the germinated seedlings ~ 27% seedling died within one month due to stem rooting and yellowing of leaves. The seedlings/young plantlets were maintained for 1 year (Fig. 1j) before transferring to the wild. The seedlings were transferred in the wild and monitored for one year.

Table 4. Effect of stratification on seed germination of *Panax pseudoginseng* in different seed beds

Warm stratification period at 25ºC (months)	Cold stratification period at 4ºC (months)	% germination in seed bed 1* (±SE)#	Time for germination (days)	% germination in seed bed 2**(±SE)#	Time for germination (days)
0	0	10.00 (0.5)	425	8.20 (0.3)	425
0	1	0.00	-	0.00	-
0	2	0.00	-	0.00	-
3	3	38.25 (0.4)	12	24.20 (0.2)	17
3	**4**	**68.20 (0.7)**	**14**	**52.40 (0.3)**	**18**
3	5	41.30 (0.5)	11	29.20 (0.2)	14
3	6	26.00 (0.3)	17	14.10 (0.2)	17
3	7	9.10 (0.2)	14	3.10 (0.1)	8

±SE: Standard error from mean.

***** Seed bed 1: Mixture of normal soil, decayed wood manure and sand in 3:1:1.

** Seed bed 2: Mixture of normal soil, decayed cow dung manure and sand in 3:1:1.

The time for emergence of radicals from the germinated seeds, germination time, germination rate, seedling morphology, post germination establishment of seedlings is influenced by various factors and appears to be species specific [10-11]. Seedling survival on

the seed bed and or in the natural habitat is governed by the availability of suitable substrata, light, nutrients, soil moisture etc. [10-11, 13]. A number of species exhibit positive as well as negative correlation between canopy cover, humidity, temperature etc. [10, 14-16]. There are several reports available for enhancement of seed germination by pre-germination treatment of seeds with chemicals, low temperature and systematic stratification [10, 17-19].

Panax pseudoginseng non-stratified seeds registered germination only after ~14 months of sowing which indicates that *Panax pseudoginseng* seeds are morpho-physiological dormant at the time of harvest. Morpho-physiological dormancy is common in many plant species but not as wide as in the genus *Panax* [9, 20]. Dormancy could be due to several factors like embryos not fully developed in the freshly harvested ginseng seeds [20-21]. Besides *Panax*, seed dormancy is also reported in species like *Ginkgo biloba* [22]. In the present study it was necessary to stratify the seeds in two phases for successful germination. Seeds stratified at 25ºC for three months followed by cold stratification at 4ºC for 1 to 7 months proved to be beneficial for seed germination. Under controlled condition ~10% seed germination recorded after 425 days in seed bed 1 while 8% seed germination registered in seed bed 2 after 450 days of sowing. This result is in agreement with the reports with the other species of *Panax* [8-9, 20]. According to *Xiao et. al.* [8] it was necessary to cold stratify the seeds for 18–22 months for successful germination of American ginseng. Warm stratification for 3 months followed by cold stratification beyond 3 months was found to be promotive for seed germination in both the seed beds. Of the two seed bed condition studied in the present study, seed bed prepared by mixing normal soil, decayed wood powder and sand at 3:1:1 ratio found to be better for seed germination. The finding of this work indicates that decomposed cow dung as manure is not an ideal substratum for *Panax pseudoginseng* seed germination.

Conclusion

Panax pseudoginseng is an economically important medicinal plant and the species is extremely threatened. The findings of the present study provide us a clear picture about the natural habitat and their primary requirements for survival, flowering, fruiting, seed setting. Further the findings pinpoints why the species cannot be cultivated easily away from the natural habitat. The findings from the seed biology study will help the commercial growers for propagation of the plant through its seed. The local authority, government agency should join hands to protect this highly threatened economically important medicinal plant. Moreover cultivation of this plant in areas where their growth is favorable should be encourage to the locals so as to meet the needs for local medicinal usage and to stop rampant harvesting of this plants in its natural habitat.

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