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Production of clonal planting material and propagation of *Paris polyphylla* Smith var. *polyphylla* through rhizome splitting

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Abstract
Mass propagation is important for mass production of both wild species under threat and also for domesticated crops. *Paris polyphylla* Smith var. *polyphylla*, a vulnerable medicinal plant of North East, India can be macropropagated through rhizome cuttings/ splitting and group rhizome fragmentation. Preparation of raised soil bed and application of appropriate procedures in planting the cut and fragmented plant parts was followed. The mean obtained in three years of study: For sprouted shoot buds from cut rhizomes mean was found to be 49.33% while in group rhizome fragmentation it was 75%.

Keywords: Rhizome fragmentation, macropropagation, *Paris polyphylla* Smith var. *polyphylla*, raised soil bed, traditional method.

Introduction
Forest plant species usually regenerates through their seeds or by vegetative means for their continued survival. Many of the plants are facing threat and their population in the wild reduced drastically due to unsustainable harvesting for local medicinal use, removal of natural habitats for 'Jhum Cultivation' and different anthropogenic activities. The percentage of plants used for medicinal purpose in relation to the totally available medicinal plants is found to be highest in India where ~20% of the total species being used as medicinal plants. However, most of these medicinal plants are being harvested from the wild and only ~20 species medicinal plants are cultivated though over 400 species are being used for the purpose [1]. Mass scale propagation is therefore important for mass production of both wild species under threat and also for domesticated crops. Propagation of plants through different macropropagation techniques like cutting, layering, rhizome splitting, seed propagation, suckers etc are some of the widely used techniques for commercially important plants and threatened species [2-3]. Species such as *Garcinia afzelii*, *Panax quinquefolius*, *Saussurea costus*, *Warburgia salutaris* etc. are threatened species but are now cultivated[1] and thereby reduces the risk of population being threatened further. Important plants having commercial and medicinal values are being cultivated through mass propagation by using their vegetative rhizome parts such as Ginger [4], *Jurinea dolomiaea* through rhizome cuttings [5], tuberous roots of *Aconitum atrox* [5], splitting of roots of *Nardostachys jatamansi* [5], stem cuttings of *Strychnos henningsii* [3], stem cuttings of *Casuarina cunninghamiana* [6], stem cuttings of *Gongronema latifolia* [7], stem cuttings of *Shorea guiso* [8] and through sucker plantlets in *Musa* species [9].

Paris polyphylla is an important medicinal plant and its rhizome is used mainly for medicinal purpose for treatment of different diseases. The plant contains saponin steroids polyphyllin D, dioscin and balanitin7 [10]. The rhizome is used as antihelmintic, antispasmodic, expectorant, scabies, rashes, or itching problems, to treat liver cancer [11-14]. Due to overexploitation of the species from the wild for medicinal uses *Paris polyphylla* population are under threat. Production of true-to-type individuals and preventing extinction of the plant species in the local population can be achieved through macropropagation [3]. The problem related to threatened species can be overcome by introducing clonal planting materials produced either through conventional technique in the nursery or through *in vitro* technique. Present study was aimed to produce clonal planting materials of *Paris polyphylla* Smith var. *polyphylla* through cost effective technique i.e., rhizome splitting/fragmentation.

Materials and methods

Plant materials site collection: The plant materials were collected from Nagaland, India from four natural habitats – forest of Pangsha village, Tuensang district (2278 m ASL, 26˚14´27.2´´N Latitude and 95°07´05.7´´E Longitude), 'Chida' and its surrounding forest area, Phek district (1874 m ASL, 25˚30´11´´N Latitude and 94°13´37´´E Longitude), Aradura hill forest area, Kohima district (1396 m ASL, 25˚38´49.5´´N Latitude and 94°06´25.8´´E Longitude) and forest of Longkum village, Mokokchung district (1404.51 m ASL, 29˚15´98.8´´N Latitude and 94°24´03.5´´E Longitude). After collection, plants were kept at Chida area where rhizome cuttings/splitting and group rhizome fragmentation was performed.

Rhizome cuttings: A single rhizome may give rise to multiple off-shoots as the plant ages; *Paris polyphylla* Smith var. *polyphylla* often gives rise to multiple shoot buds (Fig. 1a). The shoot buds sometimes bifurcates in different directions from one end point. This type of rhizome can give rise to two different directional shoot buds (Fig. 1b). This form of rhizomes can be propagated through cuttings where rhizome with multiple buds are separated and planted. Middle parts of rhizome are also cut into pieces without having buds from large rhizomes. Basing on the nature of the growing tips (single or double buds) rhizome pieces were cut accordingly and used for the present study (Fig. 1c).

Group rhizome fragmentation: *Paris polyphylla* Smith var. *polyphylla* rhizome sometimes occurs in masses due to aggregation of large group of rhizomes (Fig. 1d). This may be due to large number of seeds falling in a particular site from their mother plant and where most seeds are healthy and viable. The roots of *Paris polyphylla* var. *polyphylla* are long and wavy and so when a number of rhizomes are found in very close proximity in the soil together they become entwined to each other forming large masses of rhizomes due to dense aggregation of individual rhizomes. This might hamper the healthy growth of the individual plants. Therefore the rhizomes need to be individually separated and at the same time, rhizomes that show compound buds are selected and used in cutting propagation. Rhizome splitting was simply done by breaking off the masses of rhizomes into each individual rhizome and sowed in the prepared bed in the polyhouse. The selected aggregated masses of rhizomes were obtained from those plants that were flowering in group in the previous season.

Preparation of raised soil bed for planting

For the present study soil mixture was prepared by mixing decayed wood, sand and top black soil at a ratio of 1:1:3. The three different soil components were individually sun dried and any sticks, stones, or insects present on them were removed. The soil after mixing was made sure it is loosely formed and well-drained by watering to see that water drains well out from them. The experiment was performed during the month of November after the above ground part dies off. The experiment was done in poly-house with good ventilation and drainage. Artificial shade was provided in such a way that it fulfills the shade requirements.

Planting of rhizomes segments and roots in the soil raised bed: In the selected site, soil mixtures prepared were put on dug soils. The plant parts meant for propagation were sowed in their distinct beds made on a mounded a bit high than the normal ground level so that water is well drained during the rainy season. If any depressions are found on the soil, it is quickly covered with more organic soil mixtures. The plant is grown facing the northern slopes as they are usually cooler and have more moisture than south or west facing slopes. Before planting, the soil beds were watered to moisten the soil. The cut rhizomes were planted just below the soil and those rhizomes with buds on it are planted by exposing the bud a little less than a 0.5 cm outside the soil. The experimental beds are watered three times a week as the propagation process is done during the dry season of the year. Continuous monitoring of the soil bed was done and weeds were removed from the soil whenever necessary. The present study was conducted for three years during 2011-2014.

Results

Rhizome cuttings: In the study with rhizome cuttings, it was observed that ~50% rhizomes remained recalcitrant while the remaining segments registered morphogenetic response and formed shoot buds (Table 1). Most of the shoot buds occurred from those rhizomes is those that have a bud with a part of rhizome on it as cut material but middle cut parts of rhizome exhibits high dormancy. The shoot buds was converted into plants in the raised soil bed within 4-5 months (Fig. 1e) but majority of them were non-flowering in nature. On the regenerated plants of ~49%, only 7% were reproductive plants that bear the inflorescence. During the present study it was found that the rhizome length and the number of buds are very important. When multiple buds are present in a single off-shoot, the length of the rhizome must be taken into consideration, sometimes the off shoot rhizome is found to be very short even when multiple shoot buds are present and in this case the rhizome is planted as having all the multiple off-shoots. Appropriate pieces should be cut to achieve the optimum morphogenetic response and the existence of a bud in the rhizome is an added advantage during cuttings as most dormant rhizomes are from those cut parts where the bud was absent.

G**roup rhizome fragmentation:** For regeneration of plants from the group rhizome, it was necessary to split the individual rhizome from the aggregated rhizome masses to optimize the multiplication (Fig. 1f). Of the total separated rhizomes planted, ~75% rhizomes could sprout successfully and formed plants (Table 2, Fig. 1g) while the remaining rhizomes failed to register morphogenetic response. Mortality rate and dormancy exhibition by the plant was very low. The regenerated plants were maintained in the bed for 6 months before transferring to the field. The regenerated plants started flowering (Fig. 1h).

Table 1: Propagation of *Paris polyphylla* Smith var. *polyphylla* through rhizome cuttings

Study year	No. of segments sowed	No. of segments formed reproductive plants	No. of segments formed non-reproductive plants	No. of segments remained dormant	% segments sprouted & formed plants
2011-2012	100	09	40	51	49
2012-2013	100	04	39	57	43
2013-2014	100	08	48	44	56
Mean	100	7	42.33	50.66	49.33

Table 2: Propagation of *Paris polyphylla* Smith var. *polyphylla* through group rhizome fragmentation

Year of study	No. of rhizome pieces tested	No. of rhizome sprouted	No. of dormant rhizome	Rhizome died	% rhizome sprouted
2011-2012	43	32	10	01	74.41
2012-2013	62	46	15	01	74.19
2013-2014	51	39	10	02	76.47
Mean	52	39	11.66	1.33	75

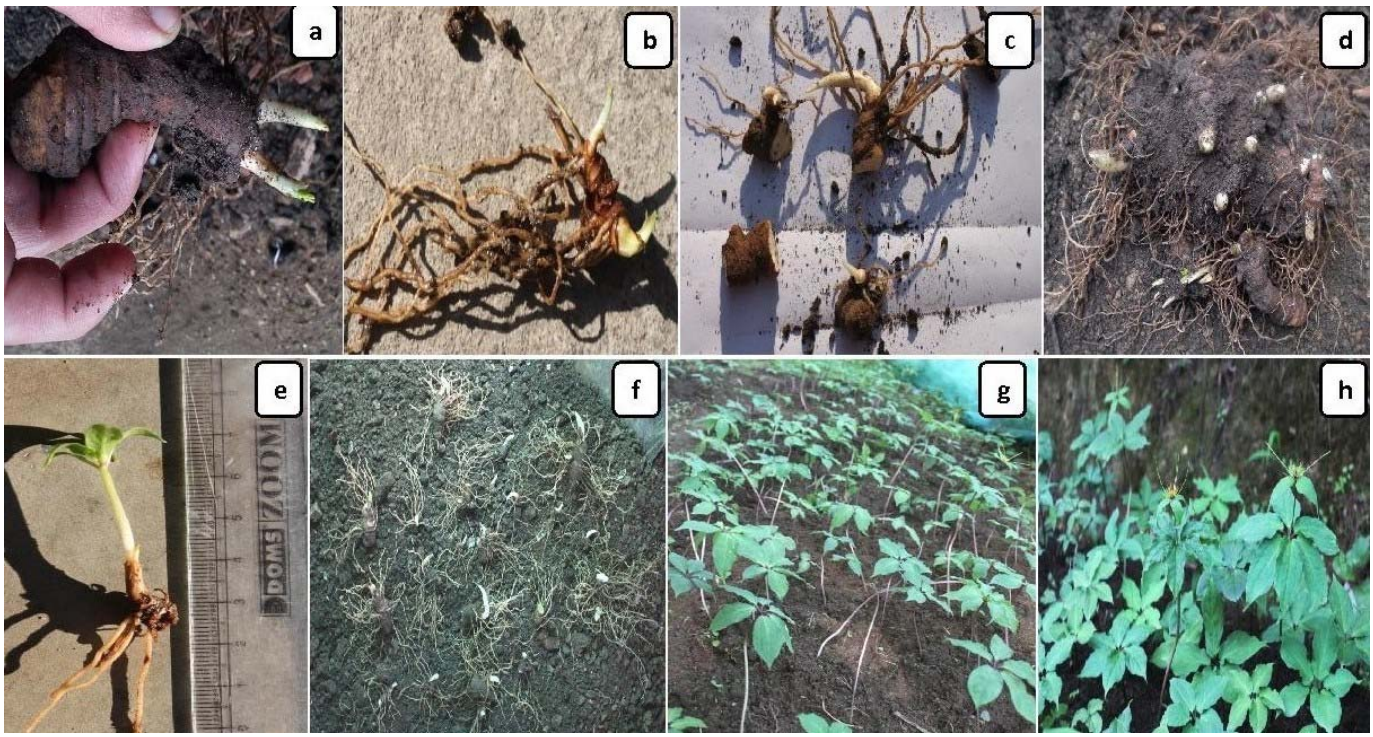

Fig 1: Different stages of macropropagation of *Paris polyphylla* through rhizome splitting. a. Sprouting of rhizome produces multiple shoot buds; b. Sprouting of shoot buds from opposite directions of rhizome; c. Fragmented rhizomes ready for sowing; d. Composite rhizome showing multiple shoot buds; e. Formation of plantlets from the rhizome fragment; f. Separated shoot buds from the composite rhizome; g. Regenerated plants in the nursery; h. Well-developed plants developed from the rhizome fragments at flowering stage.

Discussion

During the present study attempts were made to propagate and produce clonal planting materials of *Paris polyphylla* Smith var. *polyphylla* from rhizomes through cuttings and group fragmentation.

Many of the important medicinal plant species in the wild are facing crisis to species extinction due to unsustainable harvesting, therefore cultivation of medicinal plants is the only means of meeting the ever increasing current and future demands of the people and the growing industry[1] and the problem related to threatened species in the wild can be overcome by introduction of any rare, vulnerable or threatened medicinal plants into the wild through mass propagation in the nurseries or through *in vitro* propagation. Propagation through rhizome cutting/splitting is convenient, cost-effective and ensures large scale cultivation and for its long term process of conservation, systematic cultivation is important [5]. For instance, ginger cultivation in the Northeastern region of India usually follows the traditional method which is ecologically friendly, low cost and which utilizes the local resources, knowledge and labor [4]. Likewise, *Paris polyphylla* Smith var. *polyphylla* can be propagated efficiently from the underground cut rhizomes and it was found to be more efficient than propagation through the seeds. Moreover, the traditional technique applied was efficient without any hormonal usage as this will help in practical application for the local poor farmers. The disadvantages however will be slower growth rate, presence of high dormancy and even mortality rate of the cut rhizomes will be a bit higher if no synthetic hormones are being used.

In the present study, rhizome cuttings and fragmentation show good plant growth on the prepared raised soil bed and the plant grows well when more appropriate spacing is given to them. Some of the cut rhizomes shows flowering but majority of them were in the non-flowering stage. *Paris polyphylla* Smith var. *polyphylla* grows well in slope areas. Naga Hills forest with 1300 m and above is an ideal place for its propagation. However, planting should not be done in too slope areas as it drains off the top soil during heavy rains.

For cultivation of this plant species, appropriate site habitat is very important to ensure its establishment, growth, reproduction and being disease free. The plant propagated through its seeds takes long time to mature and bear fruits which is their only natural way of propagation. Domestication and macropropagation of this plant for local medicinal uses can be helpful in protection of the wild population. Further, the locals should adopt sustainable means of harvest of this wild medicinal species from its natural habitat through selective harvest by harvesting only those plants that do not produce inflorescence. Sustainable regeneration of the plant species in the wild is required so proper conservation measures, legislation laws and acts needs to be impose especially for protecting this medicinal plant species.

Conclusion

In the present study an attempt was made to propagate and produce clonal planting materials of *Paris polyphylla* Smith var. *polyphylla*, a vulnerable medicinal plant of North East India. Fragmentation and cutting of rhizomes could be successfully used for producing clonal planting materials in this selected plant species. Plants species produced through this traditional macropropagation have been introduced into their natural habitats in the studied area locations and also to other new locations of high altitudes. The protocols developed in this present study will help the locals to propagate these economically important threatened species and workout the conservation strategies of these species in their natural habitats.

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References

1. Schippmann U, Leaman DJ, Cunningham AB. Impact of Cultivation and Gathering of Medicinal Plants on Biodiversity: Global Trends and Issues. *In:* Biodiversity and the Ecosystem Approach in Agriculture, Forestry and Fisheries. Food and Agriculture Organization (FAO), 2002.
2. Kuria MW, Ngumi VW, Njenga PK, Odee DW. *Ex vitro* rooting of *Warbugia ugandensis* (Sprague) ssp. Ugandensis Sprague. Eastern African Journal of Botany. 2010; 2(1):243–248.
3. Kipkemoi NK, Kariuki NP, Wambui NW, Justus O, Jane K. Macropropagation of an endangered medicinal plant, *Strychnos henningsii* (gilg), (Loganiaceae) for sustainable conservation. International Journal of Medicinal Plant Research. 2013; 2(7):247-253.
4. Rahman H, Karuppaiyan R, Kishore K, Denzongpa R. Traditional practices of ginger cultivation in Northeast India. Indian Journal of Traditional Knowledge. 2009; 8(1):23-28.
5. Banday A, Nawchoo IA, Kaloo ZA, Shabir PA, Rather AA. Efficient propagation of an endangered medicinal plant *Jurinea dolomiaea* Boiss in the North Western Himalaya using rhizome cuttings under *ex situ* conditions. Journal of Plant Breeding and Crop Science. 2014; 6(9):114-118.
6. Karoshi VR, Hegde GV, Hiremath SM. Macropropagation of *Casuarina cunninghamiana* Miq via mistless polytunnel (hydropit). Journal of Tropical Forestry. 2000; 16:79-81.
7. Agbo CU, Obi IU. Macro-propagation technique for different physiological ages of *Gongronema latifolia* Benth cuttings. African Journal of Biotechnology. 2006; 5(13):1254-1258.
8. Patricio HP, Castaneto YT, Vallesteros AP, Castaneto ET. Macropropagation of *Shorea guiso* using stem cuttings. Journal of Tropical Forest Science. 2006; 18(3):198-201.
9. Baiyeri KP, Response of *Musa* species to Macro-propagation. II: The effects of genotype, initiation and weaning media on sucker growth and quality in the nursery. African Journal of Biotechnology. 2005; 4(3):229-234.
10. Verma P, Mathur AK, Jain SP, Mathur A. *In vitro* conservation of twenty three overexploited medicinal plants belonging to the India Sub-Continent. Scientific World Journal. 2012; 2:1-10.
11. Li F, Jiao P, Yao S, Sang H, Qin S, Zhangn Y *et al*. *Paris polyphylla* Smith extract induces apoptosis and activates cancer suppressor gene Connexin 26 expression. Asian Pacific Journal of Cancer Prevention. 2012; 13:205-209.
12. Madhu KC, Phoboo S, Jha PK. Ecological study of *Paris polyphylla* Sm. Ecoprint. 2010; 17:87-93.
13. Bhattarai KR, Ghimire MD. Cultivation and sustainable harvesting of commercially important medicinal and aromatic plants of Nepal. Herbal Research Development and Forestry of Nepal. 2006, 369-372.
14. Jamir NS, Lanusunep, Pongener N. Medico-herbal medicine practiced by the Naga tribes in the state of Nagaland (India). Indian Journal of Fundamental and Applied Life Sciences. 2012; 2(2):328-333.