

Macropropagation and Production of Clonal Planting Materials of *Panax pseudoginseng* Wall.

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Abstract

Panax pseudoginseng Wall., a highly medicinal, herbaceous, long-lived plant, grows in the forest litter of shady primary forest. The species is threatened in the natural habitat due to unsustainable harvesting of rhizome for medicine and habitat destruction. The species has very poor adaptive power to the synthetic environment and fails to grow and propagate. Present study was undertaken to develop suitable low cost propagation technique and produce clonal planting materials through rhizome splitting and root cutting. Rhizomes and roots were cut into segments and sowed in the raised soil bed prepared by mixing decayed wood powder, sand and top black soil at 1:1:3 ratios in a shaded poly house. Of the sowed rhizome segments ~55% segments remained recalcitrant to morphogenetic response in the first year of the study and the response improved in the subsequent years. In the third year ~51% rhizome segments responded positively. The horizontal root cuts mostly remained morphogenetically dormant but inclined root cuts exhibited better response. The morphogenetic response from the roots was comparatively very poor compare to rhizome segments. The plantlets formed both rhizome segments and root cuttings were maintained for three years in the bed/pots followed by transferring in the cultivated plots.

Keywords

Panax pseudoginseng Wall., Macropropagation, Medicinal Plant, Rhizomes Splitting, Root Propagation

1. Introduction

Different species of Ginseng are grown in different parts of the world having its own origin from a particular

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geographical area of any particular country (Shin et al., 2015). *Panax pseudoginseng* Wall., a highly medicinal plant, originates from Nepal and the Eastern Himalayas (India) (Yun, 2001) and is thus known by the common name as the Himalayan Ginseng. Ginseng is also being cultivated in many parts of the world (Schluter & Punja, 2000). Nagaland (India), which is a part of the Eastern Himalayas, is very rich in biodiversity and lie in the Indo-Burma biodiversity hotspots. Different medicinal plants are found in this region and one of the most important medicinal plants is the *Panax pseudoginseng* species plant. *Panax pseudoginseng* is highly medicinal, herbaceous and long lived plant species that grows in the forest litter of shady primary forest. The root of the ginseng plant is widely used for different ailments such as increasing effectiveness of nerve growth factor, inhibition of the multiplication of tumor cells (Radad et al., 2004), for bio modulating action, anti-aging, for creation of new blood vessels (Yue et al., 2007), climacteric syndrome and for diabetes mellitus (Raz et al., 2009). The plant roots of *Panax pseudoginseng* are widely use in local medicines in its dried powdered form for different ailments such as for diarrhea, dysentery, high blood pressure and for impotency (Jamir et al., 2012).

Most of the forest plant species usually regenerate through their seeds or by vegetative means for their continued survival. However, many of these plants are facing threat and their population in the wild is being reduced drastically due to unsustainable harvesting for local medicinal use, removal of natural habitats for “*Slash and Burn Cultivation*”, unplanned developmental activities and other anthropogenic activities. The percentage of plants used for traditional medicine in relation to the total medicinal plants recorded from India is ~20%. Only 20 species of medicinal plant species used in the Indian herbal industry is being cultivated out of more than 400 species (Schippmann et al., 2002). These factors impose a great threat to the existing wild plant species unless appropriate conservation steps are taken up. In order to conserve these economically and medicinally important plants, one of the means is efficient mass propagation for the conservation of these species or else this will pose threat to the existing wild medicinal plants. In order to protect the medicinally important plants, efficient mass propagation for the conservation of this species is required. Mass propagation is important for mass production of both wild species under threat and also for domestic crops. For instance, ginger cultivation in the Northeastern region usually follows the traditional method which is ecologically friendly, low cost and which utilizes the local resources, knowledge and labor (Rahman et al., 2009; Deb et al., 2015). In the past efficient low cost macropropagation protocols are being developed for many threatened medicinal plant species such as *Garcinia afzelii*, *Panax quinquefolius*, *Warburgia salutaris*, *Paris polyphylla* and are propagated now in large scale (Schippmann et al., 2002; Deb et al., 2015). Most effective and economic technique of propagation is through seed, but certain species fails to propagate through seeds due to poor seed setting, long seed dormancy like *Aconitum atrox*, *Panax pseudoginseng* etc. (Banday et al., 2014). One of the effective technique could be root splitting and rhizome splitting for those species having long period of seed dormancy. Important plants having commercial and medicinal value are being cultivated through mass propagation by using their vegetative rhizome parts and roots such as Ginger (Rahman et al., 2009), *Jurinea dolomiaea*, *Aconitum atrox*, *Nardostachys jatamansi* (Banday et al., 2014), stem cuttings in *Strychnos henningsii* (Kipkemoi et al., 2013), through sucker plantlets in *Musa* species (Baiyeri, 2005). The total percentage of species threatened in the Araliaceae family is 16.3 and one of the over-harvested species in Araliaceae family is the *Panax pseudoginseng* (Schippmann et al., 2002). True-to-type copies and preventing extinction of the plant species in the local population can be achieved through macro propagation (Kipkemoi et al., 2013; Deb et al., 2015).

Cultivation of medicinal plants is the only means of meeting the ever increasing current and future demands of the people and the growing industry (Schippmann et al., 2002). The species grows at its best in its natural condition but their population is dwindling due to habitat destruction and anthropogenic activities and it is important to develop farmer friendly propagation protocol to large scale propagation to ensure its population survival. Present study therefore aimed in to experiment the production of clonal planting materials of *Panax pseudoginseng* Wall., an important medicinal and threatened species through different macropropagation techniques.

2. Materials and Methods

2.1. Plant Materials Site Collection

Rhizomes and roots of *Panax pseudoginseng* were collected from the natural forest area of Pangsha village near Indo-Burma border at an elevation of 2278 m above sea level (ASL), 26°14'27.2"N and 95°07'05.7"E under Tuensang district, Nagaland, India and another area at “Chida” and its surrounding forest area of Khezakenoma

village at an elevation of 1874 m ASL, 25°30'11"N and 94°13'37"E under Phek district, Nagaland. Digging of ginseng rhizome and roots are very easy as they are usually found in loose soil of primary forest but it should be done very carefully. Accordingly the present exercise was executed very slowly in order to avoid damages to the rhizome and that nick of any roots is avoided. The collected plants [Figure 1(a)] were kept in the polyhouse set up at Chida where rhizome and root cuttings were performed.

2.2. Rhizome Cuttings in *Panax pseudoginseng*

Panax pseudoginseng has a small neck-like rhizome with bud scale scars/nodes connected with a fleshy root that bears individual small rootlets [Figure 1(b)]. The age of the plants were counted from the number of bud scale scars present on the rhizome. Each individual rhizome was cut according to their ages and ensured that at least minimum two bud scars are present at the resultant cut rhizome [Figure 1(c)]. Healthy rhizomes were selected randomly and rhizomes were cut during November, 2011. The age of the rhizomes were between 5 to 11 years as counted from the bud scale scars on their rhizome. The rhizomes were cut into number of pieces—2 bud scars, 3 bud scars and 5 bud scars which were used for the present study. The segments were mixed and used randomly in the present study.

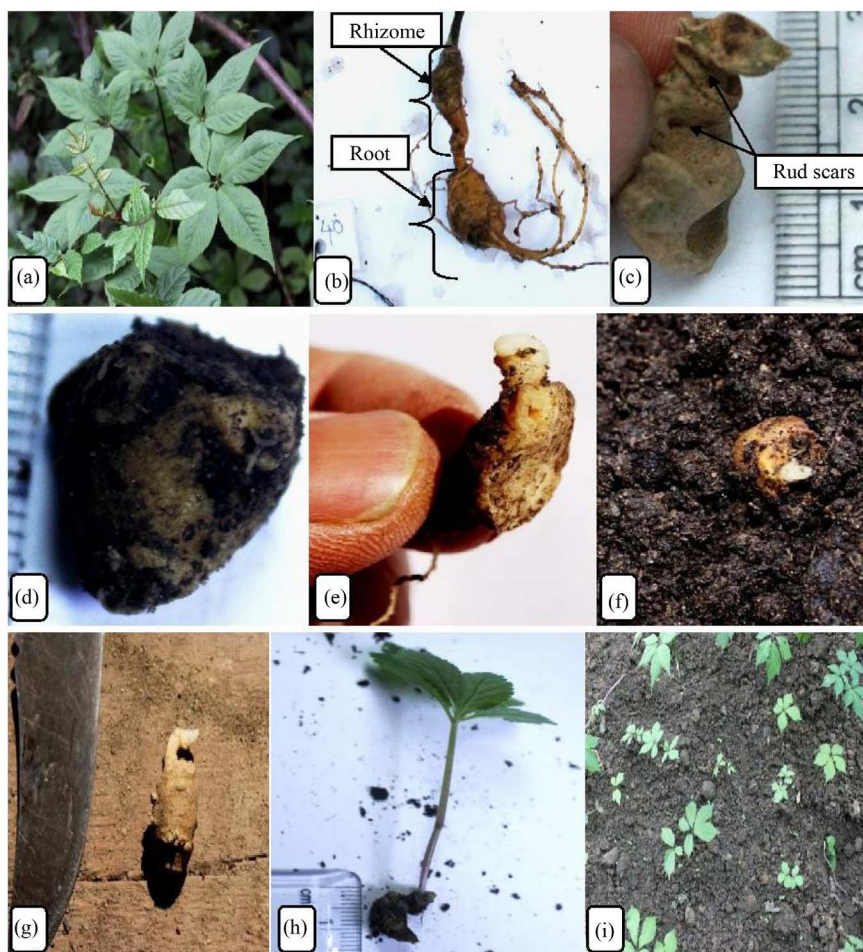


Figure 1. Macropropagation of *Panax pseudoginseng* through rhizome and root cutting. (a) *P. pseudoginseng* plant (above ground); (b) Underground part (rhizome and root); (c) Rhizome segments with multiple buds scars; (d) Horizontal root segment; (e) Inclined root segment with initiation of response; (f) Sprouting of buds from the rhizome segment scar in the bed; (g) Sprouting of shoot buds from whole root; (h) Formation of single leaf plant from rhizome segment; (i) Regenerated plantlets transferred in the cultivated plot from the sowing bed.

2.3. Root Cuttings in *Panax pseudoginseng*

The root cuttings were done in the month of November, 2011. A single root is cut into two equal parts. The roots were cut in two ways—horizontal root cuttings [Figure 1(d)] and inclined root cuttings [Figure 1(e)] and maintained in the raised soil bed. They were monitored for three years from 2012–2014. Horizontal root cutting was done by cutting into two equal halves in the middle-lower cut root and upper cut root. All the roots were first inspected to ensure that they are healthy. Simple traditional method of cutting was used during the experimental process.

2.4. Preparation of Raised Soil Bed for Planting

The raised soil bed was prepared by mixing decayed wood powder, sand and top black soil at 1:1:3 ratio. All the mixer components were first individually sun dried and powdered before being put on-to the bed and ensured that the mixtures are free from any sticks, stones, or insects. Further it was ensured that soil was loose and not tightly compact to facilitate air movement and good water drainage as it is very important for the underground roots and rhizome. The prepared raised soil bed was shaded with roofing made of green poly sheets for regulation of light so that it fulfills the shade requirements.

2.5. Planting of Rhizomes Segments and Roots in the Soil Raised Bed

In the selected sites, propagules such as rhizome segments and root cutting were sowed in their earmarked mounted beds where water can be easily well drained out from the bed during rainy season. Following the traditional local method of macropagation, in the following experimental study, no plant growth hormones were treated on the propagated cut segments. The parts of plant were planted facing the northern slopes as they are usually cooler and have more moisture than south or west facing slopes. The cut rhizome segments and roots were planted just below the soil and those rhizome segments with buds on it are planted by exposing the bud above the soil. The experimental beds were watered each alternate day at regular intervals and constantly monitored against invasion of soil insects, pests and weeds through manual care with hands. However, no inorganic pesticides or herbicides were applied during this following experiment. After monitoring the plants formed from the propagated plant parts of the rhizome segments and root segments for three years period in the prepared soil beds, those plants that sprout out in the year 2015 were gently dug out during the month of March/April and planted in polybags which were later planted in natural forest areas where they were usually found grown wild and also to new locations of similar altitudes so as to maintain and increase their population in the wild.

3. Results

3.1. Regeneration Potential of Rhizome Cuttings of *Panax pseudoginseng*

In the present study with the rhizome cuttings, over 55% of the rhizomes remained recalcitrant to morphogenetic response. The lowest morphogenetic response was registered in 2012 where ~19.44% rhizome segments potentiated morphogenetic response and formed plantlets while in 2014, the response increased to 51.72%. There is a gradual increase in positive plant formation from the cut rhizomes during the three years of study (Table 1). New shoot buds developed from the responding segments which were mostly one-leaf plants [Figure 1(f) & Figure 1(h)] but in the third year, two-leaf plants were also noted in the seed bed along with one-leaf plants. In second year however, a plant with 5 bud scale scars cut rhizome give rise to 3-leaves plant which was stunted in growth and was less than 10 cm in height.

3.2. Root Cuttings in *Panax pseudoginseng*

In general the roots that were cut horizontally remained mostly morphogenetic dormant. Present study was conducted for three years where there was no shoot bud formation but some segments showed formation of rootlets. Inclined root cutting and roots exhibited both rootlet formation and buds [Figure 1(e) & Figure 1(g)]. Only 4.16% root segments formed plantlets from the inclined root cuttings but horizontal root cuttings showed no plant set during the three years of observation (Table 2). While, compare to horizontal root cuts, inclined root cuts exhibited better response but the morphogenetic response is very poor in both the cuttings. It was observed that the plantlets developed were one leaf in inclined root cutting. The roots would be best planted as a whole as some

Table 1. Propagation of *Panax pseudoginseng* through rhizome cuttings.

Year	Total number of cut rhizomes	Number of plants sprouted	Dormancy exhibited by the cut rhizomes	Number of cut rhizomes that died	% rhizome segments formed plants
2012	72	14	52	6	19.44
2013	66	21	37	8	31.81
2014	58	30	25	3	51.72
Mean	65.33	21.66	38	5.66	34.32

Table 2. Propagation of *Panax pseudoginseng* through root cuttings.

Type of cuttings	Total number of cut roots tested	Year	Number of cut roots survived	Number of plants sprouted	Dormancy exhibited by the cut roots	No. of roots that died per year	% of plant set
Horizontal root cuttings	30	2012	30	0	19	11	0
		2013	19	0	12	7	0
		2014	12	0	8	4	0
		Mean per year	-	-	20.33	0	13
Inclined root cuttings	30	2012	30	0	23	7	0
		2013	23	1	18	4	4.34
		2014	19	2	11	6	10.52
		Mean	-	-	24	1	17.33

roots showed sprouting of bud.

The plantlets formed both rhizome segments and root cuttings were maintained for three years in the bed/pots for further growth followed by transferring in the cultivated plots [Figure 1(i)]. The plants were monitored for growth for 3 - 4 months.

4. Discussion

Many plant species have been propagated successfully through macropropagation techniques particularly the rare, endangered and threatened plant species and reintroduce into the wild ameliorating their status in nature. Different parts of the plant such as *Strychnos henningii* stem and root cuttings (Kipkemoi et al., 2013), *Eucalyptus globules* stem cuttings (Wilson, 1993), *Triplochiton scleroxylon* stem cuttings (Leakey, 1983), *Warbugia ugandensis* stem rooting (Kuria et al., 2010), *Paris polyphylla* (Deb et al., 2015) have been used for macropropagation for conservation programme and likewise propagation of *Panax pseudoginseng* through rhizome cuttings was found to be applicable for macro propagation of the plant species. In the past, successful macropropagation through cut rhizome segments of two *Panax* species namely *Panax bipinnatifida* and *Panax sikkimensis* was done through treatment of various plant hormones (Rao, et al., 1998) but in the following experimental study, traditional method of macropropagation was applied without any application of plant growth hormones so that common local can practically apply it conveniently and easily. During the present study attempts were made to propagate and produce clonal planting materials of *Panax pseudoginseng* from rhizomes and roots through cutting technique. However, dormancy of the cut rhizomes was very high. Out of cut rhizomes, ~34% were morphogenetic. It was observed that for successful morphogenetic response rhizome segments should be cut top rhizome part with presence of bud or more than 3 bud scars. Fragments with fewer bud scars supported poorer morphogenesis. This is probably due to smaller size of the segments and lesser reserve food which failed to support morphogenesis. The cut rhizomes mostly sprouted as 1-leaf plants in all the three years of study along with few 2-leaves plants.

The propagation of *Panax pseudoginseng* through root cuttings is not appropriate for mass propagation, even though inclined root cuttings can give some positive results on the plant production. The propagated under-

ground parts for *Panax pseudoginseng* are its rhizomes. The roots would be best planted as a whole though dormancy will be very high. Roots having one or two bud scale scars can still survive very well and formation of buds can take place. Splitting of rhizomes is rare in ginseng as the plant occurs as distinct individuals (Van der Voort et al., 2003) in its natural condition.

Appropriate site habitat for cultivation of the plants is very important to ensure its establishment, growth, reproduction and being disease free. The plant takes long time to mature and bear fruits which is their only natural way of propagation. The plant population in its natural habitat is drastically reduced in recent years with locals becoming more aware of the importance of this medicinal plant leading to rampant harvesting in its natural habitat for local medicinal uses. Therefore, the plant needs immediate conservation and restoration measures to its former range prior than for the commercial purpose in Nagaland, so that the plant is maintained even for the future generations. Locals can play an active role in its restoration through simple macropropagation techniques which will be at their best conveniences. The protocol developed in this present study indicates a good percentage of plant mortality and only around 50% of the cut rhizome segment plants were successfully developed to new plantlets of *Panax pseudoginseng* which shows that success rates were not very high. However, the advantage of this protocol will be that it will go down to the practical applicability of the locals for the regeneration of this medicinally important plants which can also be good technique application in the conservation method of this plant. The protocol developed will generate slow but successful regeneration of the plants which will sustain the needs of the locals, can produce commercially as well as retain the population of the plant species in its natural habitat. Sustainable harvest from wild populations can be a prior conservation option that requires a sound management system and sound scientific information [1] and therefore the locals should adopt sustainable means of harvest of this wild medicinal species. Domestication and propagation of this plant for the purpose of local medicinal uses can be very helpful in protection of the wild populations.

5. Conclusion

In the present study, an attempt was made to propagate and produce clonal planting materials of *Panax pseudoginseng* Wall., a threatened medicinal plant of North East India. The seed propagation of *P. pseudoginseng* is not a very efficient means for producing planting materials as the seeds exhibits physiological dormancy and require over one year for germination. In *Panax pseudoginseng* clonal planting materials could be successfully produced from the rhizome fragments of 3 - 5 bud scars. The plants species produced through 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 the present study is based on traditional method without any usage of inorganic nutrients or different hormone applications so that it will help the locals/farmers in practical application by its easier method, cost effective and convenient in propagating this economically important threatened species.

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