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Micropropagation of Indian Ginseng (*Panax pseudoginseng* Wall): A Proposition to Save an Endangered Commercial and Medicinal Forest Plant

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INTRODUCTION

The process of deforestation on a global scale has recently caused a great alarm with respect to the survival of hundreds of plants precious to human community. In view of saving the "gene pool" of many wonderful plants, the wealth of medicinal plants of the forests needs a special attention for their preservation through propagation and cultivation. Amongst them, the forest perennial herb ginseng, *Panax pseudoginseng* (family Araliaceae) is one of the most threatened forest medicinal plants of India.

The word "ginseng" is said to mean "the wonder of the world". In Chinese, it also means "man root" for the resemblance of its root to the human body. Panax, the generic name is derived from the Greek "Panakos" (a panacea), in reference to the miraculous virtue ascribed to it by the Chinese, who consider it a sovereign remedy in almost all diseases. As *P. pseudoginseng* is native to India, needs seem to be in demand that means should be developed for its successful micro-propagation in forestry research in India.

DISTRIBUTION

International range of ginseng varies with species. Asiatic or Korean ginseng (*P. ginseng* C. A.. Meyer) is indigenous to north China and Korea and is cultivated in China, Korea, Japan and to a limited extent in USSR. *P. notoginseng* or Chinese ginseng is indigenous to China. American ginseng (*P. quinquefolium* L.) is indigenous to Quebec Manitoba in Canada and northeast USA. Japanese ginseng (*P. japonicas*) grows in the warm temperate regions of China, India, Nepal and Sanchi ginseng (*P. pseudoginseng* Wall.) in the mountains of China, Nepal, noartheast India, north Burma and south Tibet. Besides this, *P. sikkimensis, P. bipinnatifada* and *P. pseudoginseng* are reported from India amongst which is most common (Bennet, 1983, 1984).

STATUS REPORT

P. pseudoginseng was first described by Wallich in 1829. It is also mentioned as Sanchi ginseng due to its prevalence in Yunnan and Kwaangsi provinces in China; sanchi is the highly estimated product extracted from *P. pseudoginseng* (Bennet, 1983). It is frequent in Nepal, Sikkim, Bhutan and Khasi hills (Hooker, 1897). Bennet (1984) reported the occurrence of *P. pseudoginseng* as an undergrowth in Upper Shillong along with *Elsholtzia* sp., *Strobilanthes* sp., *Impatients* sp. etc. on black peat soils in Oak - Rhododendron. Later on Nayar and Sastry (1990) reported it endangered due to deforestation of its habitat and road construction and cultivation. The aerial shoot of this plant usually appears during June and dies by end of September. Mass cultivation in India has not been recorded yet. As the plant is very slow growing, endangered and possesses highly valuable medicinal property, therefore, tissue culture and micropropagation of *P. pseudoginseng* should be urgently developed for conservation of this endangered medicinal plant as well as increased productivity of its ingredients.

THE PLANT

The *Panax* species are perennial herbs, which bloom and bear berries three years after sowing. Yearlings are about 9-15 cm tall, bearing a palmately compound leaf with five leaflets. Four to five year old plants grow about 60 cm in height. The roots corpulent; the leaflets, 15 x 8 cm in dimension, have serrated margins. Usually a three year old plant bloom with a long peduncle at the centre of the whorled leaves. The flowers are polygamous have five petals,

and inflorescence is umble. Flat and oval shape, berries are green in the beginning, and turn red as they ripen. The roots are bifurcate, and the roots and rhizomes vary from 80 to 200 mm in length and 5 to 20 mm in diameter. The fruit is a small berry containing one or two seeds. The seeds are greyish-white in colour. Fresh seed does not yield to germination for 12 to 18 months. It should not be dried but kept for stratification in cool moist soil in between layers of sand and covered with sawdust for a few months. This process of stratification allows the seed embryo to develop.

GENETICS

Panax ginseng and *P. quinquefolium* are tetraploids with 2n = 44. However, strains from different geographical areas with chromosomes number 2n = 48 have also been reported. Till to-date, probably there is no report on chromosome number and karyotype analysis in the case of *P. pseudoginseng* (Husain, 1992).

ECOLOGICAL FACTORS AND CULTIVATION

The ginseng is found over rich, cool, woody and shaded temperate forests. It prefers well drained, deep acidic (pH 5.5 to 6.5), light sandy loam soils, rich in organic matter and potash. Although shade loving, the plating site should be faced north-east to allow exposure to the warm sun. The annual variation for maximum temperature of localities chosen to raise plantations should be around to 20 to 25°c in summer and 9 to 14°c in winter. It grows well in localities receiving 80 to 110 cm rain and these locales are covered with snow in winter.

The ginseng seedlings are generally transplanted during March and early April. Growth rate, diseases and yield much depend on planting density. Generally, planting of 10 to 12 seedlings per square meter are optimum . Shading should be done after approximately 30% seedling growth. Light intensity and retention of rain water are important for good cultivation. For optimum light intensity 3,000 to 5,000 lux of full sunlight must be beds maintained. For transplanting, loosen bed soil is preferable that becomes harder by the second year after transplantation. A large amount of green grass is applied in the third, fourth and fifth year. The sides of beds must be repaired with furrow soil. Weeding must be done to eliminate -harmful insects and to prevent nutrients from leaching away after transplanting. Harvesting or cultivation of ginseng roots is usually done three to five years after transplantation seedling (Atal and Kapoor, 1982).

Samalfield et al. (1995) have reported that trails were established to assess the environment tolerance, adaptability and productivity of *P. ginseng, P. pseudoginseng* and *P. quinquefolium* in New Zealand under artificial shade where plants were grown successfully for four seasons.

ACTIVE COMPOUND

Saponins are plant glycosides with distinctive property of frothing, show haemolysis, and their alcoholic solutions give precipitate with cholesterol. The ginseng saponins are biologically active compounds of great pharmaceutical value. About twelve primary glycosides are found in the Korean ginseng, number of which is slightly fewer in the American ginseng (Hornok, 1992). Ginseng consists of ginsenosides or panaxosides which belong to two sapogenins viz. 20-S-protopanaxadiol (tetracyclic triterpene derivatives) and oleanosides (pentacyclic triterpene derivatives). The saponins are triterpene glycosides (dammarane, triterpenoids and oleanolic acids) which yield an aglycone and a sugar moiety such as xylose, glucose, rhamnose or arabinos in addition to aglycone during acid hydrolysis (Shibata and Saito, 1977). The credit of its excellent pharmacological properties may also go to other constituents including essential oils, e.g. panacene, non-essential oils, panaxynol, organic acids, ginseng-pectin, choline, amino acids, peptides, vitamin B_{12}, minerals and trace elements.

MEDICINAL USE

Ginseng enhances the natural resistance and recuperative powers of the body, having both a stimulant and seductive activity. It is called an "adaptogen" for having the ability to maintain the body a stamina at a regular level without

detrimental side effects.

Ginseng is prescribed as a tonic, stimulant and aphrodisiac, also as a source of vitamins and minerals. It is used in neurathenia, dyspepsia, palpitation and asthma. It is also used for controlling amnesia, headaches, convulsion, dysentery and cancer. Further, in terms of its clinical effects it is claimed to possess analeptic, antihypothermal, antianaemic, antifatigue, tranquilizing and sedative effects.

MARKET VALUE

Ginseng root is marketed in two forms in the world trade: white ginseng (dried and raw) and red ginseng (boiled and re-dried). The market demand of ginseng roots are increased rapidly due to cultivation difficulties and gradual extinction of the wild ginseng. In 1993 financial year, the monthly statistics of the foreign trade of India reported that India earned 802.455 lakh rupees by ginseng exportation. It is marketed in the form of capsules, tablets, granules and is also used in the instant tea and wine by the herbal and health food industries. In cosmetic industry, ginseng is used in lotions, creams and perfumes (Husain, 1992).

GINSENG MICRO-PROPAGATION OUTSIDE INDIA

Many workers outside India have worked on micro-propagation of ginseng species. The effect of 2,4 -D and kinetin was studied on saponin production *P. ginseng* by Kim et al. (1980). Saponin production by cultures of *P. ginseng* transformed with *Agrobacterium rhizogenesis* was studied by Yoshikawa and Furuya (1987). Formation of plantlets from callus culture of *P. ginseng* was reported by Furuya et al. (1986). Bio-transformation of 2-phenylpropionic acid in root culture *P. ginseng* was reported by Furuya et al. (1989). Zhou et al. (1990) have investigated a mass culture of *P. quinquefolium* in a modified MS media free from NH_4NO_3 where the amount of KNO_3 was doubled; the plants were found to be grown rapidly with much higher saponin content as compared to that of plantlets culture in regular medium. Mass production of *P. ginseng embryoids* on media containing high concentration of sugar was reported by Asaka et al. (1994). Further, a number of investigations of culture conditions have been done for better growth and more saponin production in cell lines selected from original

Flow chart of Ginseng cultivation

Seed

Flowering ☛	Seed collection ☛	Stratification ☛	Seed Selection
Mid-May	Mid-July	Late July - early Nov.	November

Nursery Bed

Sowing ☛	Germination ☛	Irrigation ☛	Soil Covering
Early Nov.	Shading early and mid April	Mid-April to late September	Early December

∇ ∇ ∇

Digging up the seedling Early April ◂◂◂◂◂◂◂◂◂◂◂◂ Wintering

Permanent Bed

[Field selection preparation before one or two years]

Transplanting ☛	Sprout shading ☛	Leaves falling ☛	Harvest
Early April	Mid April	Sept - Oct	Sept - Oct

(From Atal and Kapoor, 1982)

callus, where four times higher production of saponin content was reported (Choi et al., 1994; Wijesekera, 1991).

HARVESTING AND YIELD

The plantations are thinned at 6 years of age when 50% old trees are uprooted and debarked. This is repeated at 8 years of age and finally the remaining trees are uprooted at the end of 12 years. Where ever the land is for a new plantation, the trees are cut at 5 cm from the ground level, taking precautions to avoid splitting the stump. These stumps regenerate into several sprouts during the next rainy season. Usually two sprouts are maintained to grow so as to have another cycle of tree growth to remain in the field for the next 8 to 10 years. The bark is removed by ringing the stem at a height of 60 cm from ground and is dried in the open. The drying reduces weight by 60 to 70%. The root is taken out similarly. An average yield of 5 tons of dry bark per hectare is obtained from a plantation. The bark contains around 30 chemically related alkaloids. of which quinine is the most important. The concentration of alkaloids in the collar part (30 to 45 cm length) and the root bark; it declines in the trunk bark and is low in branches and twigs (Wijesekera, 1991).

PROPOSAL AND DISCUSSION

There is a pressing need for production of more ginseng around the globe due to its precious medicinal properties. More than 3500 tons of ginseng roots are produced in the world every year but the scientific data on known aspects of cultivation are not available (Husain, 1992). Most of the methods are based on age old experience of growers. Wijesekera (1991) indicated that non-availability of data on ginseng cultivation prevails due to publication of scanty literature on it and for maintaining strict secrecy on cultivation practices. However, the existing literature describes forest areas of deep rooted deciduous trees with natural or artificial shade and forest soil rich in organic matter and humus in hilly and lower temperate regions are ideal for ginseng growth and cultivation (summer temperature not exceeding 30°C); chilling below 0°C is necessary for seed germination (Husain, 1992). Some locations in the northeast India, especially in Meghalaya and Sikkim, have been reported to contain the natural growth of this commercial and medicinal forest plant for the existence of the favorable ecological factors. Therefore, considering the climatic factors, probable collection spot in North Eastern region, and being a deciduous, endangered forest herb, the works on micro-propagation of *P. pseudoginseng* in this region may be a very attractive proposition for conservation of this famous medicinal plant species in future. Genetic experiments to improve the existing material would also have to be taken up to standard varieties which can give better yield of bio-mass and higher saponin content. The effect of the ginseng constituents at gene expression and molecular level on cellular metabolism and tissue development and functions is yet to be explored.

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KEYWORDS Tissue Culture. Ginseng. Medicinal Plant.

ABSTRACT Panax pseudoginseng, a native plant to India, is reported to be a naturally occurring and highly threatened herb in low temperate regions of deciduous forests in the North-east. Lack of proper maintenance, construction of structures, cultivation of other plants are some of the factors which have put this precious medicinal plant of the era on the verge of extinction; also, the plant is very slow growing in natural conditions. The necessity for development of project infrastructure attempting the survival as well as mass cultivation of this endangered medicinal plant through micro-propagation has been discussed in this article.

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