

## **IN VITRO PROPAGATION OF *POTENTILLA FULGENS* HOOK (*BAJRADANTI*) – A HIGH VALUE MEDICINAL HERB FOR COMMERCIAL CULTIVATION**

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### **ABSTRACT**

A rapid *in vitro* clonal propagation protocol is standardized for *Potentilla fulgens*, a high value medicinal herb of Indian Himalayas. The best promising results were obtained by culturing the tender petiole segments for adventitious shoot proliferation in Murashige and Skoog basal medium supplemented with a combination of benzyl adenine (BA, 1.5 mg/l) and indole acetic acid (IAA, 0.5 mg/l). The best rooting (92.3%) was obtained while culturing the *in vitro* raised shoots in MS medium modified with IAA (1.5 mg/l). Rooted plantlets were hardened in sterilized peat moss in plastic pots. Spray of quarter strength MS salts solution on alternate days is found reducing the mortality of plants considerably during the hardening process. As a result it is possible to obtain 30-35 rooted plant from a single explant within three months, and hence is useful for commercial cultivation.

**Keywords:** Benzyl adenine; Indole acetic acid; clonal propagation ; *Potentilla fulgens*

**Abbreviations:** BA–6-Benzyl adenine, IAA-indole-3-acetic acid, MS- Murashige & Skoog's medium

### **INTRODUCTION**

*Potentilla fulgens* Hook of family Rosaceae is a high value annual medicinal herb growing at an altitude of 2000 to 3000 meters (above msl) in the Northwest Himalayas. Locally known as "*Bajradanti*", is endemic to the Indian Himalayas (Samant et al. 1998) Out of 120 species of *Potentilla* reported globally (Hooker, 1986) only 50 are found in India (Anonymous, 1998).

The aerial parts of *P. fulgens* contain many phenolic and flavanone compounds including ursolic acid, which are having medicinal properties. Leaves are astringent, antispasmodic and the decoction is used in the treatment of mouth cancer as well as for the strengthening of

gums. The roots are used to check the diarrhoea and in the treatment of mouth ulcer (Fig. 1E).

The over exploitation due to the above multifarious applications may result a considerable shrinkage in the habitat of *P. fulgens*. Conventional propagation of *P. fulgens* is quite slow, reason being longer time for seed set and its poor viability and also localized to specific niche of Himalayas, which hinder the process of its large-scale multiplication. This species therefore, needs to be propagated on a large scale to fulfill the demand of raw material for preparation of various useful herbal products. Micropropagation may be one of the alternatives that could be helpful in mass multiplication of *P. fulgens* for its commercial

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cultivation. Therefore, an experiment was planned to work out a suitable protocol for propagation of *P. fulgens*. So far no report is available on micropropagation of *P. fulgens*.

**MATERIALS AND METHODS****SURFACE STERILIZATION OF EXPLANT MATERIALS**

The different types of the explants, leaf discs (0.75cm in diameter), shoot tips and petioles (0.75 cm in length) were taken from the freshly collected plants from their wild habitat. The explants were washed with detergent extran (neutral pH) to remove the dust, and then thoroughly washed in tap water for 5 to 6 times followed by rinsing in sterile distilled water. The explants were surface disinfected with 0.1% HgCl<sub>2</sub> for 10 minutes followed by three times washing with sterile distilled water and surface drying under aseptic airflow conditions.

**ASEPTIC CULTURE ESTABLISHMENT**

The *explants* were cultured in Murasighe and Skoog (1962) medium supplemented with 4 different concentrations of BA (0.5 mg/l, 1.0 mg/l, 1.5 mg/l and 2.0 mg/l) in combination with 7 different concentrations of IAA (0.0 mg/l, 0.1 mg/l,

0.2 mg/l, 0.3 mg/l, 0.4 mg/l, 0.5 mg/l and 0.6 mg/l).

The cultures were incubated in a culture room at 25 ± 1°C, under a cyclic photoperiod of 16 hours of cool white fluorescence light (5500 to 6000 lux) followed by 8 hrs dark period. The vessels used in experiment were glass conical flasks (150 ml) for shoot regeneration and glass tubes (150X25 mm) for root development in micro shoots. Each conical flask and culture tube contained 50 ml and 10 ml shoot and root media respectively. MS basal salts were supplemented with 3% sucrose and 0.8% agar (HIMEDIA Pvt. Ltd.) and the pH of culture medium was kept at 5.8.

**SHOOT REGENERATION AND ROOTING** - During the period of incubation, *explants* were observed for percent response to shoot regeneration, days to shootlet initiation and number of shoots per *explant*. Observations were also recorded for root induction percentage in shoots, number of roots per shoot and root length in the rooting medium.

**ACCLIMATIZATION** - Seven-week-old rooted plantlets were transferred to sterilized peat moss in plastic pots (6 X 6 cm) and hardened inside a mist chamber containing RH (80 ± 5%) for 2 weeks. One-fourth strength of MS salts were sprayed on alternate day basis during the period of acclimatization. The hardened plants were then transferred to the mixture of sand, soil and oak forest humus (1:1:1 ratio) in thermocol pots (8 x 7 cm) inside the glass house having controlled temperature (25 ± 1°C) and relative humidity (70 – 80 %) for one week. During this process of hardening water was sprinkled thrice a day on the plantlets.

**STATISTICAL ANALYSIS** - Factorial design with three replications was used to assess the response of three types of *explant* materials (first factor) for shoot regeneration efficiency in 28 combinations of BA and IAA (second factor). Rooting medium consisted 11 levels of IAA (0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5 and 1.6 mg/l) and experimented in randomized block design (RBD) with three replications.

## RESULTS AND DISCUSSION

**RESPONSE OF EXPLANTS TO SHOOT REGENERATION** - Data on percent response to shoot regeneration, days for initiation of shoot regeneration and number of shoots per *explant* revealed significant differences among *explant* sources and combination of growth regulators. Mean square for interaction of *explant* sources and combinations of BA and IAA was also found to be highly significant for all the characters.

**RESPONSE OF PETIOLE SEGMENTS TO ADVENTITIOUS SHOOT REGENERATION** - Petiole segments significantly ( $p < 0.01$ ) regenerated maximum percentage of shoots, produced highest number of shoots per explant and took minimum duration. Perusal of the table 1 indicated minimum of 31.67% response by petiole segments to shoot regeneration with BA (0.5 mg/l) alone while maximum response of 88.67% was observed at BA (1.5 mg/l) and IAA (0.5 mg/l) with a mean response of 50.51% shoot regeneration over 28 combinations of BA and IAA. The earliest shoot regeneration observed in 39.67 days with BA and IAA having concentrations 1.5 mg/l and 0.5 mg/l respectively and was found significantly lower than the remaining of the combinations. The number of shoots per *explant* varied from a minimum of 12.33 with BA (0.5 mg/l) alone to maximum of 35.33 with BA (1.5 mg/l) and IAA (0.5 mg/l) (Figure 1-A).

**RESPONSE OF LEAF DISHES TO ADVENTITIOUS SHOOT REGENERATION** - Interaction of *explant* sources with 28 combinations of BA and IAA revealed that in case of leaf *explant*, growth regulator BA alone or in combinations of BA and IAA did not response to shoot regeneration. The combination of BA (1.5 mg/l) and IAA (0.5 mg/l) gave 41.33% response to shoot regeneration, which was highest and statistically significant to other combinations. Days to initiation of shoot regeneration were recorded minimum of 54.33 days using BA (1.5 mg/l) and IAA (0.5 mg/l) and maximum of 82 days with BA (2.0 mg/l) and IAA (0.2 mg/l). There was no shoot regeneration in MS medium having BA (0.5 mg/l) and IAA (0.3 mg/l) whereas maximum shoots (10.33) per *explant* were observed with BA (1.5

mg/l) and IAA (0.5 mg/l) (Table 1).

**RESPONSE OF SHOOT TIPS TO SHOOT PROLIFERATION**-Shoot tip *explants* responded minimum 27.33% shoot proliferation in MS modified with BA (0.5 mg/l) and IAA (0.1 mg/l) and maximum of 70% in the same medium modified with BA (1.5 mg/l) and IAA (0.5 mg/l). Minimums of 48.67 days were taken for shoot initiation with BA (1.5 mg/l) and IAA (0.5 mg/l), which was significantly lower than the days taken to shoot initiation with other combinations. The number of shoots obtained per *explant* varied from a minimum of 8.33 with BA (2.0 mg/l) alone to a maximum of 24.33 shoots per *explant* with BA (1.5 mg/l) and IAA (0.2 mg/l), (Table 1). Effect of cytokinins and auxins in combination to multiple shoot formation was earlier observed by Yuan et al. (1994).

Thus, combination of BA (1.5 mg/l) and IAA (0.5 mg/l) was found the most suitable combination of hormones for maximum number of shoot regeneration per petiole *explant*. Adventitious shoots regeneration from leaves (Escalettes and Dosba, 1993; Camloha et al., 1994) and cotyledons (Compton and Gray, 1994; Han, 1994; Hossain et al., 1994) were earlier reported in different plant species.

**ROOTING IN MICROSHOOTS** - Analysis of variance for root induction revealed the significant differences among the treatments for all the three characters. The IAA (8.56 $\mu$ M) significantly resulted into maximum rooting (92.33%) in microshoots (Table 2). Number of roots per microshoot varied from a minimum of 2.8 with IAA (0.6 mg/l) to a significantly higher value of 20.67 roots per shoot with IAA (1.5 mg/l) (Figure 1-B & C). Significantly higher root length of 9.87 cm was also observed with IAA (1.5 mg/l) whereas IAA (0.6 mg/l) again showed minimum response. Thus, MS medium supplemented with IAA (1.5 mg/l) was identified to be the best rooting medium for an early root induction in microshoots of *P. fulgens*. Previous workers have also reported the role of IAA in the development of lateral roots (Bhalero et al., 2002; Casimiro et al., 2001; Marchant et al., 2002; Reed et al., 1998).

**ACCLIMATIZATION**-A maximum survival frequency (91.12%) was observed when

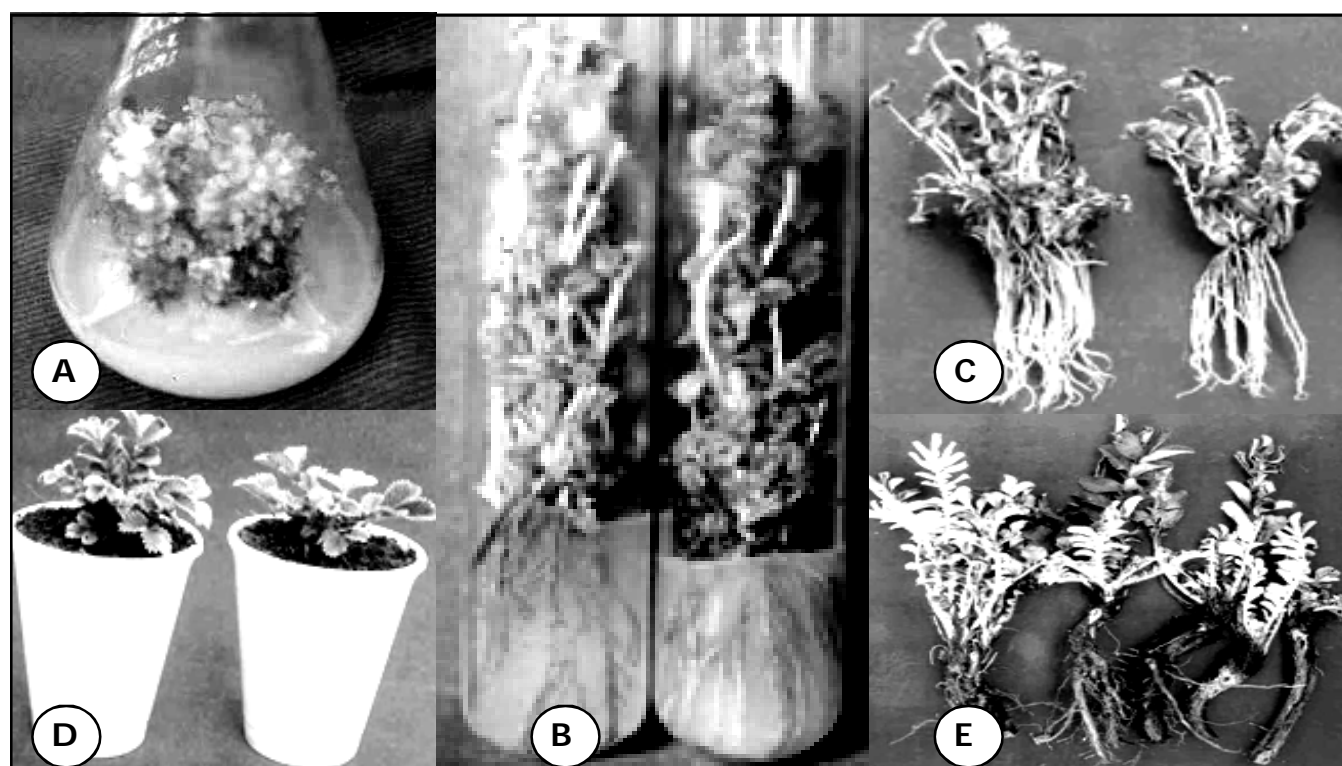
**TABLE 1. RESPONSE OF DIFFERENT *EXPLANTS* TO VARIOUS COMBINATIONS OF BA AND IAA IN *P. FULGENS***

| PHYTO-HORMONE<br>(mg/l)<br>BA X IAA | PERCENTAGE RESPONSE<br>TO SHOOT REGENERATION |                     |               | DAYS FOR INITIATION OF<br>SHOOT REGENERATION |                     |               | NUMBER OF SHOOTS<br>PER <i>EXPLANT</i> |                     |               |
|-------------------------------------|--|---------------------|---------------|--|---------------------|---------------|--|---------------------|---------------|
|                                     | Leaf<br>dishes                               | Petiole<br>segments | Shoot<br>tips | Leaf<br>dishes                               | Petiole<br>segments | Shoot<br>tips | Leaf<br>dishes                         | Petiole<br>segments | Shoot<br>tips |
| 0.5 X 0.00                          | 0.00 h                                       | 31.67 k             | 28.00 i       | -  | 74.33 i             | 79.00 h       | 0.00 f                                 | 12.33 i             | 09.67 f       |
| 1.0 X 0.00                          | 0.00 h                                       | 41.33 j             | 43.67 f       | -  | 66.00 h             | 66.67 e       | 0.00 f                                 | 15.67 h             | 12.33 e       |
| 1.5 X 0.00                          | 0.00 h                                       | 63.67 e             | 52.00 e       | -  | 54.00 e             | 63.00 d       | 0.00 f                                 | 24.33 e             | 13. 67 d      |
| 2.0 X 0.00                          | 0.00 h                                       | 50.33 h             | 47.00 f       | -  | 62.00 g             | 69.67 f       | 0.00 f                                 | 19.00 g             | 08.33 f       |
| 0.5 X 0.1                           | 0.00 h                                       | 33.67 k             | 27.33 i       | -  | 60.67 f             | 78.33 h       | 0.00 f                                 | 13.67 h             | 09.00 f       |
| 1.0 X 0.1                           | 0.00 h                                       | 42.67 i             | 40.67 g       | -  | 55.00 e             | 73.33 g       | 0.00 f                                 | 18.33 g             | 11.67 e       |
| 1.5 X 0.1                           | 0.00 h                                       | 64.33 e             | 56.33 d       | -  | 50.67 d             | 69.33 f       | 0.00 f                                 | 24.67 d             | 14.00 d       |
| 2.0 X 0.2                           | 0.00 h                                       | 52.00 g             | 53.33 d       | -  | 55.33 e             | 71.67 f       | 0.00 f                                 | 21.00 f             | 10.33 e       |
| 0.5 X 0.2                           | 0.00 h                                       | 35.67 k             | 33.67 h       | -  | 56.33 e             | 68.67 f       | 0.00 f                                 | 20.00 f             | 09.33 f       |
| 1.0 X 0.2                           | 0.00 h                                       | 54.67 f             | 49.33 e       | -  | 51.33 d             | 65.00 e       | 0.00 f                                 | 23.67 e             | 10.67 e       |
| 1.5 X 0.2                           | 9.00 g                                       | 70.33 d             | 63.33 c       | 80.67 f                                      | 47.00 c             | 61.33 d       | 2.67 d                                 | 26.33 d             | 24.33 a       |
| 2.0 X 0.2                           | 7.00 g                                       | 66.33 e             | 59.00 c       | 82.00 f                                      | 48.33 c             | 66.00 e       | 1.67 e                                 | 23.67 e             | 11.67 e       |
| 0.5 X 0.3                           | 0.00 h                                       | 40.67 j             | 38.67 g       | -  | 54.00 e             | 62.67 d       | 0.00 f                                 | 23.00 e             | 10.33 e       |
| 1.0 X 0.3                           | 15.67 f                                      | 58.33 f             | 55.67 d       | 71.67 e                                      | 51.67 d             | 58.33 c       | 3.67 c                                 | 25.00 d             | 12.33 e       |
| 1.5 X 0.3                           | 22.33 d                                      | 75.33 c             | 67.33 b       | 67.33 c                                      | 43.00 b             | 57.00 c       | 6.67 b                                 | 27.33 c             | 14.67 d       |
| 2.0 X 0.3                           | 18.00 e                                      | 68.33 d             | 62.00 c       | 70.33 d                                      | 54.33 e             | 61.33 d       | 4.67 c                                 | 25.33 d             | 13.33 d       |
| 0.5 X 0.4                           | 18.33 e                                      | 57.33 f             | 45.33 f       | 66.33 c                                      | 53.00 d             | 63.67 d       | 6.00 b                                 | 22.00 f             | 11.33 e       |
| 1.0 X 0.4                           | 20.00 d                                      | 71.67 d             | 59.33 c       | 64.33 c                                      | 46.67 c             | 58.33 c       | 6.67 b                                 | 28.67 c             | 15.00 d       |
| 1.5 X 0.4                           | 25.00 c                                      | 84.67 b             | 74.33 a       | 57.00 a                                      | 41.00 a             | 53.33 b       | 8.00 b                                 | 31.33 b             | 16.67 c       |
| 2.0 X 0.4                           | 20.67 d                                      | 76.67 c             | 64.33 c       | 65.00 b                                      | 48.67 c             | 56.67 c       | 5.67 c                                 | 27.67 c             | 13.67 d       |
| 0.5 X 0.5                           | 34.33 b                                      | 64.67 e             | 51.00 e       | 67.00 c                                      | 55.00 e             | 59.67 c       | 4.00 c                                 | 25.00 d             | 13.00 d       |
| 1.0 X 0.5                           | 36.87 b                                      | 77.00 c             | 62.67 c       | 60.00 b                                      | 47.33 c             | 54.33 b       | 8.67 a                                 | 29.00 c             | 17.33 c       |
| 1.5 X 0.5                           | 41.33 a                                      | 88.67 a             | 70.00 b       | 54.33 a                                      | 39.67 a             | 48.67 a       | 10.33 a                                | 35.33 a             | 21.33 b       |
| 2.0 X 0.5                           | 34.00 b                                      | 71.67 d             | 59.67 c       | 64.33 c                                      | 48.00 c             | 58.33 c       | 6.67 b                                 | 33.00 b             | 13.67 d       |
| 0.5 X 0.6                           | 22.33 d                                      | 46.33 i             | 35.67 h       | 68.33 d                                      | 59.00 f             | 64.00 d       | 3.67 c                                 | 20.33 f             | 10.67 e       |
| 1.0 X 0.6                           | 28.33 c                                      | 50.67 h             | 38.67 g       | 65.67 c                                      | 56.00 e             | 59.00 c       | 5.00 c                                 | 22.67 e             | 12.33 e       |
| 1.5 X 0.6                           | 34.33 b                                      | 54.33 g             | 49.33 e       | 62.00 b                                      | 48.67 c             | 59.00 c       | 7.00 b                                 | 26.67 d             | 17.00 c       |
| 2.0 X 0.6                           | 27.67 c                                      | 45.33 i             | 41.67 g       | 67.33 c                                      | 57.67 f             | 64.67 e       | 4.33 c                                 | 22.33 e             | 13.33 d       |
| *CD at 1%                           |  | 3.80                |               |  | 3.13                |               |  | 2.27                |               |

\*for interaction of *explant* and combination of phytohormones

**TABLE 2. EFFECT OF DIFFERENT CONCENTRATIONS OF IAA ON ROOTING IN MICROSHOOTS.**

| PHYTOHORMONE IAA (mg/l) | PERCENTAGE OF SHOOTS SHOWING ROOT REGENERATION | NUMBER OF ROOTS/SHOOT | ROOT LENGTH (cm) |
|-------------------------|--|-----------------------|------------------|
| 0.6                     | 15.33 i  | 2.80 g                | 1.77 f           |
| 0.7                     | 17.00 h  | 3.93 g                | 2.10 f           |
| 0.8                     | 19.00 g  | 4.33 g                | 2.33 f           |
| 0.9                     | 28.67 f  | 6.03 f                | 2.50 f           |
| 1.0                     | 40.33 e  | 7.63 f                | 3.07 e           |
| 1.1                     | 50.33 d  | 9.20 e                | 3.60 e           |
| 1.2                     | 65.67 c  | 11.40 d               | 4.87 d           |
| 1.3                     | 78.00 b  | 14.27 c               | 5.80 c           |
| 1.4                     | 84.67 b  | 17.17 b               | 7.63 b           |
| 1.5                     | 92.33 a  | 20.67 a               | 9.87 a           |
| 1.6                     | 82.67 b  | 15.53 c               | 7.30 b           |
| CD at 1%                | 5.086  | 1.596                 | 0.78             |



**Fig 1.** *In vitro* shoot regeneration in *Potentilla fulgens*: **A.** Shoot proliferation in MS medium supplemented with BA (1.5 mg/l) and IAA (0.5 mg/l) **B.** Rooted shoots in MS medium containing IAA (1.5 mg/l) **C.** *In vitro* raised rooted seedlings **D.** Hardening of tissue culture raised plantlets in peat moss **E.** Root yield in tissue culture raised plants of *Potentilla fulgens*.

rooted plantlets were transferred to peat moss for a period of 2 weeks (Figure 1-D) followed by a transfer to the mixture of sand, soil and oak forest humus (1:1:1 ratio). The most congenial environmental conditions found for

acclimatization of microshoots  $25 \pm 1^{\circ}\text{C}$  temperature and 70 – 80 % relative humidity. A sprinkle of quarter strength of MS medium on the alternate day was found significantly useful in reducing the mortality of plantlets.

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