Seed Research, Vol. 45(2): 179-183, 2017

Short Communication

In vivo and *In vitro* Seed Germination in Wood-apple (*Feronia limonia*)

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Feronia limonia (L.) Swingle commonly known as wood-apple or elephant apple is an endemic and endangered monotypic genus of the family Rutaceae. The fruit a rich source of vitamin C is used as a liver and cardiac tonic. *Feronia limonia* flowers profusely in rainy season (July-September) with 30-35% fruit-set. Fruits are berries, round to oval, 7±4 cm in diameter, with a hard scurfy, woody, greyish-white, rind about 6 mm thick, pulp light brown, mealy, odorous, resinous, astringent, acidic, with numerous small, white seeds (45±5/ fruit) scattered in the fruit pulp and ripe in October-November [1]. Mature fruits drop down and crack and the pulp along with seeds are eaten away by birds and squirrels. The pulp is also quickly subjected to rotting due to the infestation of several saprophytic fungi. The development of elite germplasm of *Feronia limonia* through seeds is very difficult due to poor and delayed germination. The seedling growth is very slow and the seedlings often fail to survive. *Feronia limonia* is also propagated by cuttings, but it is a laborious method with poor survival of regenerated saplings. Timber and non-timber tree species with medicinal properties often suffer from extensive exploitation. In order to save these species from extinction there is a need for rapid replacement. Tissue culture is a proven means of producing millions of identical plants under controlled and aseptic conditions, independent of seasonal constraints. It not only provides economy of time and space but also gives greater output and allows further augmentation of elite disease free propagules. Standardised tissue culture method has been used for large scale planting of the hardened *Aegle marmelos* plants under field condition [2]. The micropropagated plants tested for their genetic fidelity were found to show genetic uniformity withtheir mother tree. The plantlets of *Feronia limonia* were raised from shoot tips and nodal explants of the seedlings developed from *in vitro* grown seeds [3]. The regenerated shoots were successfully rooted on MS medium supplemented with 0.5 mg/l NAA. Although, the procedure is time consuming and difficult one, but the *in vitro* raised plantlets were successfully established in soil following the formation of roots with 100% survivability under *ex vitro* condition. Present investigation was undertaken to study the effect of some growth hormones on seed germination and *in vitro* grown seedlings produced directly from the seeds to increase the number of healthy plants of wood-apple.

The seeds of *F. limonia* were collected from mature plants in the months of October and November by extracting the pulp from ripe fruits and washing with running tap water. The seeds were dried in shade and stored in paper bags. The effect of some growth hormones on seed germination was studied by soaking the seeds (100/treatment) in 10, 15, 20 ppm aqueous solutions of GA_3 (gibberellic acid), IAA (Indole-3-acetic acid) and NAA (naphthalene acetic acid) for 1, 2 and 3 h. Seeds soaked in distilled water for 1, 2 and 3 h. served as control These were washed thoroughly with running tap water before sowing.

The treated and untreated seeds were sown in

Received : September 2017 Revised : November 2017 Accepted : December 2017

germination trays containing washed-sterilized river sand in October-November. The trays were kept in the glass house. Germination count was taken daily and seedlings were later transferred to open nursery at two-leaf stage and watered daily. Some treated and untreated seeds were dibbled (2-3/bag) in polythene bags of 45x12 cm size to the depth of ½ -3/4 inch. The bags were filled with loamy soil mixed with farm yard manure and the bags were covered with hay and irrigated at regular intervals. The data on number of days taken for germination and percentage of germination were recorded and statistically analysed [4].

The seeds used for *in vitro* culture were surface sterilized using 2-3 drops of Tween 80 (Himedia Laboratories, India) for 8-10 minutes followed by 4-5 times washing with double distilled water. These were then taken into laminar air flow cabinet for further surface sterilization with Bavistin 0.002% (w/v) for 4-5 minutes. Thereafter, the seeds were treated with 0.1% mercuric chloride (HgCl2) for 1 minute followed by through 4-5 times washing with distilled water to get rid of any traces of chemicals. The hard seed coat of the sterilized seeds was removed and these were cultured to study the effect of GA_3 on seed germination by placing them in test tubes on:

1. Sterilized cotton moistened with 5.0µM GA_3.
2. Agar medium supplemented with 5.0 µM GA_3.
3. MS medium [5] alone.
4. MS medium supplemented with 0.2, 0.5, 1.0, 2.0 mg L^{-1} kinetin (Kn).
5. MS medium supplemented with 0.2, 0.5, 1.0, 2.0 mg L^{-1}benzyle amino purine (BAP).
6. MS medium supplemented with the combination of both 2.0 mg L^{-1}Kn and 2.0 mg L^{-1}BAP.

All the media contained 15 g L^{-1} sucrose, 8 g L^{-1} agar while pH was adjusted at 5.8 before autoclaving at 15 psi (121°C) for 25 minutes. The data on days taken for germination and percentage of germination in hormone treated and untreated seeds are presented in Table 1. Seed germination percentage of control seeds (soaked in distilled water) was lowest (21%) and they took 15±5 days for germination. On the other hand, the percentage of seed germination was enhanced and days taken for germination were reduced by all the treatments with various hormones.

The seeds treated with 10 ppm GA_3 for one hour took 12±3 days to germinate and percentage of germination was significantly enhanced (32%) (Fig. 1 a & b). However, with the increase in the

Table 1. Effect of different concentrations and time period of soaking with GA_3, IAA and NAA on seed germination percentage and number of days taken for germination

S.No.	Chemical	Concentration (ppm)	Period of treatment (hours)					
			1		2		3	
			D	GP	D	GP	D	GP
1.	GA_3	10	*12±3	*32	13±2	*28	14±2	25
		15	13±2	28	14±2	25	15±2	21
		20	14±2	26	16±2	23	20±2	15
2.	IAA	10	13±2	24	13±2	20	13±2	21
		15	15±2	23	13±2	22	14±3	24
		20	17±2	21	14±2	26	15±3	25
3.	NAA	10	13±2	*28	13± 2	23	13±2	22
		15	14±2	26	14±2	21	14±3	24
		20	15±3	25	14±2	20	15±2	21
4.	Control	00	15±3	21				

D: Days taken for germination, GP: Germination Percentage, ±SD; * significance (p=0.05)

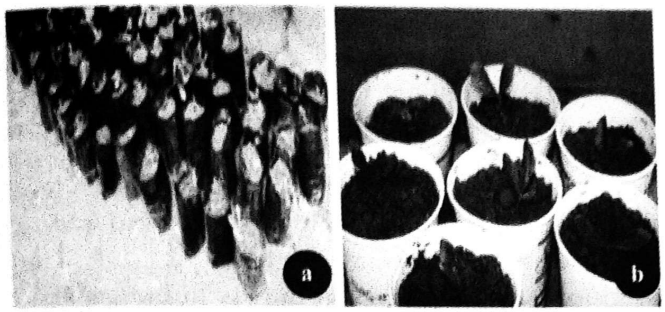

Fig. 1. a. Growth hormone treated seeds of *Feronia limonia* grown in the polythene bags. b. Seedlings raised from 10% GA_3 treated seeds transplanted in pots

period of treatment and concentration of GA_3, the seed germination percentage decreased and the days taken for germination increased. The seeds treated with 20ppm GA_3 for three hours took 20±5 days for germination and there was only 15% germination. Treatments with different concentrations of IAA and NAA for different time periods resulted in non-significant reduction and enhancement, in the number of days taken for germination and germination percentage, respectively (Table 1). The seeds treated with 10 ppm IAA for one hour took 13 days for germination with 24% germination. Similarly, seeds treated with 10 ppm NAA took fewer days for germination and germination percentage was slightly increased as compared to control seeds. With the increase in the concentration and period of treatment with IAA and NAA, the days taken for germination increased and percentage of seed germination decreased.

Effect of growth hormones on seed germination and seedling growth in various plants including tree species have been observed by several workers [6-8]. The effect of growth hormones on seed germination and seedling growth of black gram and horse gram was investigated by [6]. GA_3 (10 ppm) treatment resulted in highest germination percentage as well as enhanced growth of radical and plumule as compared to other treatments [9]. Growth of plumule decreased with the increase in IAA concentration. However, both these hormones failed to show any significant effect on elongation of radical and plumule. GA_3 was more effective on radical and plumule elongation. The effect of plant regulators on seed germination and seedling vigour in *Asparagus sprengeri* was experimented [7]. They found that GA_3 had a significant positive effect on germination percentage as compared to control, IAA, IBA and NAA during light and dark periods. Their results indicated that 50 ppm GA_3 elicited the best response but with the increase in the concentration above 60 ppm, the seed germination declined rapidly and vigour index also decreased during light and dark period. The effect of plant growth hormones *viz.*; IAA, IBA, NAA and GA_3 was assessed on seed germination and seedling growth of *Tetrapleura tetraptera* [8]. Growth parameters e.g. height of the plants, collar diameter and number of leaves were recorded fortnightly. The effect of different concentrations of hormones was highly significant ($p \leq 0.05$) on mean growth of *T. tetraptera* seedlings, seedlings collar diameter and number of leaves. IAA, IBA, and NAA at low concentrations enhanced the seedling growth and could be used for rapid regeneration of *T. tetraptera* in natural forest. Earlier reports on *in vitro* seed culture of *F. limonia* demonstrated that plant regeneration was possible through culture of hypocotyls, node, leaf and cotyledon [3, 9-11]. These workers have reported multiple shoot induction in *F. limonia* in the growth medium supplemented with cytokinin alone.

The seeds placed on cotton moistened with 5.0µM GA_3 took only 8±1 days for germination and seedlings were 5±2 cm long (Fig. 2a). On the other hand, the seeds kept on agar containing 5.0µM GA_3took only 6±1 days for germination and the seedlings were 7±2 cm long (Fig. 1b) and after 60 days the seedlings were 20±5 cm tall in a pot containing loamy soil containing farm yard manure (Fig. 2g).

Following results were observed for seeds cultured on MS basal medium alone or supplemented with Kn and BAP.

1. The seeds cultured on MS basal medium alone took 17±2 days for germination and percentage of germination was 55 (Fig. 2f).
2. The seeds cultured on MS medium supplemented with 0.2, 0.5, 1.0, 2.0 mg L^{-1}Kn took 15±3 days for germination and germination percentage was 68 (Fig. 2c).
3. The seeds cultured on MS medium

supplemented with 0.2, 0.5, 1.0, 2.0 mg L^{-1} BAP took 25±3 days for germination and percentage of germination was 65 (Figs. 2d, 2e).

4. The seeds cultured on MS medium supplemented with the combination of 2.0 mg L^{-1}Kn and 2.0 mg L^{-1} BAP took 15±2 days for germination and there was 72% germination (Fig. 2f).

The seedlings developed *in vitro* in MS medium alone or supplemented with 2.0 mg L^{-1}Kn or 2.0 mg L^{-1} BAP alone or in combination attained a height of 8±2 cm in 20±8 days. The best response of shoot multiplication was observed in the seeds cultured on full strength MS medium supplemented with either 2.0 mg L^{-1}Kn or BAP alone or in combination and also in the medium supplemented with the combination of 0.5 mg L^{-1}Kn and 0.5 mg L^{-1} BAP where shoot primordial were initiated in 10-15 days.

Large numbers of seedlings of *F. limonia* were developed from *in vitro* grown seeds and excised shoot tips from nodal explants [3, 11]. They conducted a series of experiments to induce shoot organogenesis using different auxins and cytokinins individually and in combination, e.g. MS+NAA, MS+Kn, MS+BAP and MS+IAA+BAP. Presence of different concentrations of NAA and IAA promoted callus induction and reduced shoot formation from nodal segments and shoot tip explants. The shoot tip excised from explants was

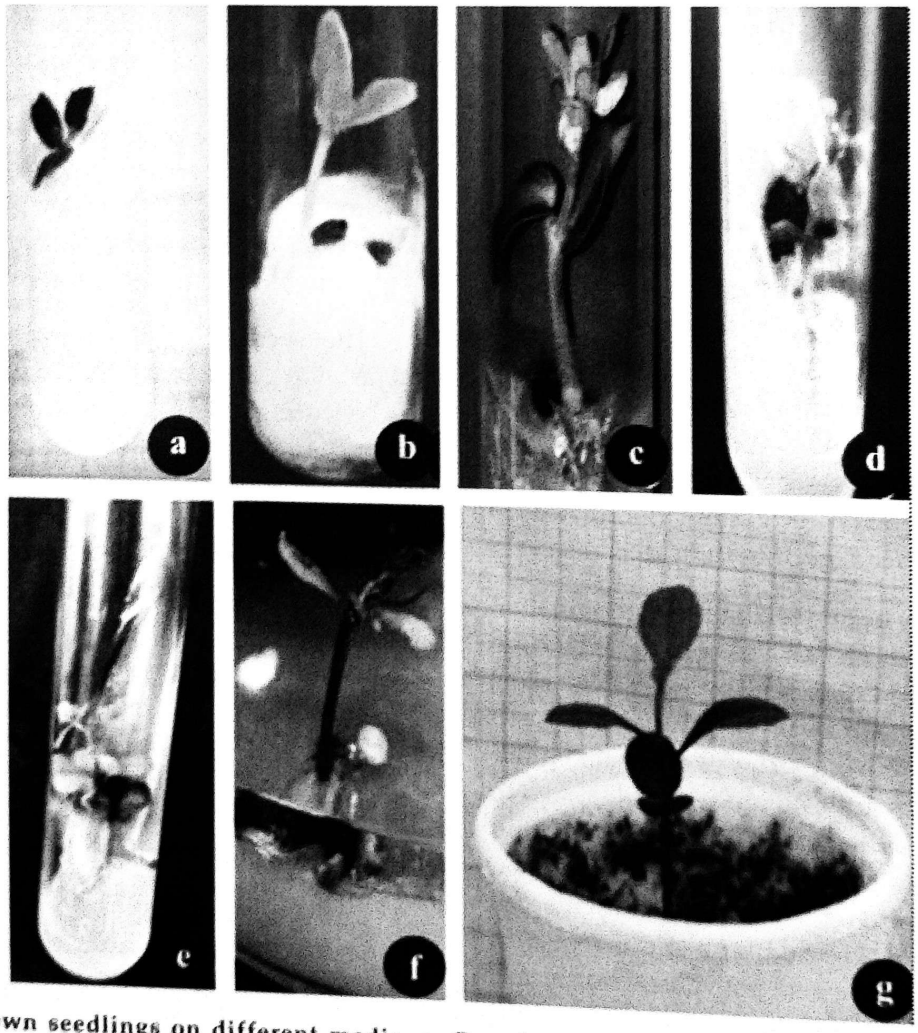

Fig. 2. ***In vitro*** **grown seedlings on different media. a. Germinating seed on cotton moistened with 5.0µM GA_3, b. Germinating seed on Agar + GA_3 (5.0 µM); c, d. Seedlings developed on MS medium supplemented with 2.0 mg L^{-1}Kn; e. Seedling developed on MS medium supplemented with 2.0 mg L^{-1} BAP alone; f. Seedling developed on MS medium supplemented with the combination of both 2.0 mg L^{-1} BAP and 20.0 mg L^{-1} Kn. g.60 days old young plant raised from seeds grown on agar supplemented with 5.0 µM GA_3.**

cultured on MS medium with different concentration of NAA, Kn, IAA and BAP singly or in combinations. Highest number of micro shoots and better plant growth were obtained from explants cultured on MS medium supplemented with 0.2 mg L^{-1} BAP alone. The regenerated shoots were successfully rooted on MS medium supplemented with 0.5 mg L^{-1} mgNAA. The *in vitro* raised plantlets were successfully established in soil following the formation of roots with 100% survivability under *ex vitro* conditions.

Thus, the number of seedlings of *F. limonia* can be enhanced by treating seeds with growth hormones. Seed germination percentage was significantly enhanced (32%) by one hour treatment with 10 ppm GA_3 as compared to 26% germination of control seeds. The number of days taken for germination was also significantly reduced by treatment with 10 ppm GA_3 for one hour (7±2 days) as compared to 10±3 days taken by control seeds. On the other hand, plantlets in *F. Limonia* can be obtained by the direct *in vitro* culture of seedsavoiding the long procedure of culturing the excised shoot tips *in vitro*.

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