"Autoecology and *In Vitro* Seed Germination Study in *Peucedanumgrande* C.B.Clarke"

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Abstract

Peucedanumgrande C. B. Clarke (Baphali) belongs to family of Apiaceae formerly known as Umbelliferae. It is locally known as Baphali in Maharashtra and Duqu in Unani literature. The plant grows normally to the height of 5 to 6 feet and is a monsoon specific perennial. Germination begins in July and plants reach reproductive stage in September with formation of clusters of umbels. Baphali has a great economic value for herbal and Unanimedicinal formulations. Itis a wonder plant with amazing pharmacological properties. This plant possesses more than adozen of important bioactivities like antidote, carminative, demulcent, diaphoretic, anti-inflammatory, stimulant, expectorant, deobestruent, lithotriptic, maturative, diuretic, emmenogouge, spermatogogue, semen procreator, gastric tonic, aphrodisiacand anthelmintic.

Peucedanumgrande is an endemic species in Western Ghats of Maharashtra.*Peucedanumgrande* is threatened due to unregulated and overharvesting collection from nature and lack of systematic cultivation. Highest but negligible percentage of normal seed germination was achieved with mechanical scarification. Also, maximum percentage of *in vitro* seed germination wasfound on ½ MS with 5mg/l GA_3.

Keywords: Autoecology, *Peucedanumgrande*, GA_3, Seed germination and *Invitro*seed germination.

Introduction:

*Peucedanumgrande*C. B. Clarkeis commonly known as Wild carrot or Baphali in Maharashtra and Duqu in Unani literature. This plant grows mostly in Hilly region and in between the rocks in rainy season. It is about one to two meter tall in height. Synonyms of Baphali are *Heracelumgrand*e (Dalzell and A. Gibson) P.K.Mukh and *Pastinacagrande* Dalz.

Duqu is distributed worldwide in Europe, Asia, North East Africa, South West America, Iran and India. *P.grande* can be found occasionally scattered in moist hilly slopes and shady places in Ghats in tall grasses (Adiba*et al.,* 2013).

Baphali is a perennial plant with primary and secondary spreading branches on main stem which is hollow inside (fistular). Leaves are glabrous, radical leaves bipinnate with terminal leaflet. Lowest pair is ternate with serrate margins.

Al QanoonFilTib (Canon of Medicine) describes duqu as seeds of wild carrot. Duqu is a wild carrot and its roots are medicinally useful has been mentioned by IbneRushd (1980).Roots are large and perennial, fistular (hollow) stem emits strongscent on crushing, leaves are bipinnate, mostly radical. Yellow flowers are produced in umbels which turn into fruits/ mericarps at maturity.Blooms are in July- August.

Fruits are elliptical/obovate 10 -13 mm long, reddish yellow having strong lemon like odor.

Flowers are formed in umbels more than 10 rayed. Fruits are obovoid and have prominent ridges on dorsal surface. Geographical distribution in Maharashtra is as Latitude (DMS): 19° 58´- 16° 49´NLongitude (DMS): 72° 58´- 74° 43´E and Altitude ranges between 600 to 1200 m above sea level. Fruits/ mericarpsposses small quantity of oil, producing carrot oil like smell(Adiba*et al.,* 2013).

Umbels are picked by local people in September- October. Therefore, we can't find a single plant in November except its deep root system in rocks. This plant has been extensively harvested by local people and is being sold in the local market. Buyers are from others states and make medicinal formulations of Unani and Homeopathic medicines. It is having high market value, as seeds(dried fruits) are sold at the rate of Rs700 to 1000 per kg.

Fruits have number of important phyto-constituents like flavonoids, coumarin likebergapten etc. Root infusions are used from long time by traditional medicine practitioners to treat digestive illness like indigestion and bloating.

This plant possesses many biological activities like antidote, carminative, demulcent, diaphoretic, anti-inflammatory, stimulant, expectorant, deobestruent, lithotriptic, maturative, diuretic, emmenogouge, spermatogogue, semen procreator, gastric tonic, aphrodisiac and anthelmintic(Adiba*et al.,* 2013).

Unani literature reveals that Hakim Kabeeruddinin his book MoghzanulMufradathhas mentioned that duqu are seeds of wild carrot. Hakim Naseer Ahmed Tariq in his book BastanulMufradath describes that leaves of duqu are similar to carrot leaves.

Duqu is useful as gastric tonic, deobestruent, antiflatulence, expectorant, diuretic, and nephroprotective (Aslam*et al.,* 2012).

Fruits/ mericarps as well as roots are used medicinally and possesspotent biological activities. It has hot and dry temperament. InUnani system of medicine, fruits are used in various forms like decoction and powder syrups for different disorders. India has reported 10 species(Adiba*et al*.,2013).

In last decade,few significant bioactivities of duquhave been evaluated in animal models by researchers from Department ofIlmulAdviaUniversity of Delhi. Many pharmacological activities are yet to be explored. The methanolic extractof fruits of *Peucedanumgrande* showed presence of coumarins and Nephthyllabdanoate Diarabinoside. It showed nephroprotective activity against gentamicin induced nephrotoxicity in wistar rats (Aslam*et al*.,2012).

Though this plant is having significant curative potential for various visceral organ related issues in human beings, it is not being systematically cultivated.Conversely, it is being extensively harvested from natural habitat.Root infusions of Baphali(*Peucedanumgrande*) are used to treat indigestion(Tayde and Patil, 2005).

The natural forests which are primary source of herbal ethno medicines have either been completely vanished or seriously damaged. Therefore, there is urgent need to protect the remaining forest resources knowing their traditional applications in various diseases from long ago(Okafor and Ham,1999).

Considering the pharmacological importance and economical potential of Baphali, we had undertaken this study and successfully standardized the protocol for normal seed germination and*in vitro* germination of Baphali by overcoming all constrains in its germination. This protocol willdefinitely be helpful to commercialize the production of this medicinal plant species. Autoecology helps to explain the distribution and regulation of a plant species in nature, why some are abundant, others are rare and some remain rare over generations(Anjah and Oyun, 2009).

Material and Methods:

Peucedanumgrande is adapted to hill slopes and rocks.This is clear from the areas and habitats it occupies, which are hill slopes and rich in vegetation. Its roots grow deep and spread out into rocks.

The plant has been located at hills of Trimbakeshwar village of Nashik district. Auto ecological study of *Peucedanumgrande* from Bramhagiri Hills and Anjaneri Hills of Trimbakeshwar village of Nashik is carried out.

Collection and authentication of plant material: Seeds and live specimens of *Peucedanumgrande*were collected from Bramhagiri and Anjaneri Hills of Trimbakeshwar in Nashikdistrict in the month of August. It was authenticated by using Regional Flora. The herbarium sheets of this medicinal plant under study were prepared and deposited in Herbarium Center of School of Life Sciences, S.R.T.M.University, Nanded (MS), India.

Autoecology of *P.grande*: Following parameters like height of the main stem, number of branches/plant, number of leaves/plant, diameter of main stem, leaf area/plant, length of root, diameter of root, fresh and dry weight of stem, leaves, roots and fruits (umbels)/ plant have been studied. Readings taken are of 100 plantsand values are average ±standard error for each parameter.

Seed germination study: Fresh seeds of *Peucedanumgrande* were collected from its uniform population occurring on natural habitats (Trimbakeshwar and Anjaneri Hills) at Nashik, Maharashtra, India. Collected seeds were air dried in shade and were stored in dry glass bottle. Dried Neem leaf powder was used as insecticide for its preservation.

Air dried seeds were used for seed germination and germination percentage was expressed on the basis of seedlings emerged out of total number of seeds tried for germination.Protrusion of radical through seed coat is considered as an index for germination.Germination experiments were done in laboratory conditions in Petri plates (10 cm diameter) in between two moist blotting papers. For each treatment, five replicates of 50 seeds were used and the mean values of germination are given in the text.

For mechanical scarification, needle was used to break the seed coat. Mechanical scarification was also done by rubbing seeds in between two sand papers. For chemical and physical scarification, H_2SO_4 and hot water was used respectively. After scarification, seeds were well washed under running tap water.

***In vitro*germination study:**

Pre-treatment for *in vitro*seed germination: Seeds inoculated on plane full and half MS yielded very less percentage of germination. This may be due to recalcitrance or presence of inhibitors in its seed coats or due to certain dormancy period. Hence, different seed pre-treatments were applied before seed inoculation on MS media.

a) Hot water treatment: Seeds were subjected to hot water exposure for 3 to 4 minutes before inoculation.

b) Immersing in solution of GA_3: Seeds were soaked in 5% GA_3 solution for 6 to 8 hours

c) Flushing the seeds overnight under running tap water: Seeds were loosely tied in muslin cloth and hanged in running tap water for 8 to 10 hours.

d) Removal of seed coats before inoculation.

The overnight flushed seeds were washed and seed coat of each seed was removed by mechanical method.

Culture conditions for *in vitro*seed germination: For *in vitro* seed germination, different types of media were tried like full strength MS (Murashige and Skoog,1962), half strength MS medium andKnudson medium.Different media and all the glassware required was steam sterilized in an autoclave at 15lbs for 20 min. Aseptic inoculation of the surface sterilized seeds was done in laminar air flow. Growth room conditions were maintained at 25±2°C temperature. 2000 lux light intensity was provided by fluorescent tube lights with 16 hours photoperiod and 08 hours darkness.
Germination response was alsostudied on ½ strength MS medium fortified with different concentrations of gibberellic acid (GA_3),BAP,Ascorbic acid and Charcoal.
Surface Sterilization of Seeds: Seeds were cleaned, sorted and washed with distilled water for removal of dust particles. Seeds were immersed in 0.1% bavistin solution for 1 to 2 minutes to kill the fungi and its spores, if any.Then, seeds were surface sterilized with 0.1% $HgCl_2$ for two to three minutes and thereafter rinsed with sterile water 4 to 5 times to remove traces of mercuric chloride. Finally, sterilized seeds were blot dried on sterile filter paper before transferring to MSmedium.

Results:

Autoecological observations in plants of *Peucedanumgrande* (Baphali) growing at Trimbakeshwar of Nashik district:

Peucedanumgrande C.B. Clarke is an erect, perennial and highly branched herb(Plate I Fig.a to d). It grows in gravel soil in rock crevices at Bramhagiri and Anjaneri hills of Trimbakeshwarnear Nashik, Maharashtra, India.It grows erect with main stem which is fistular (hollow inside). It produces primary and secondary branches. It was observed that plant has average height of 151±0.3 cm with diameter of stem 2.6±0.2 cm. The average number of leaves per plant were 120±0.2 with mean leaf area 25±0.4 cm. Leaves are ternate and cauline with serrate margins. It possesses a strong main root about 19 to 20 cmin length which grows deep in soil and rocks. Roots emit strong odor. Umbels start blooming in August and lasts up to September end. Each plant has an average of 60± 0.3 umbels.Each umbel has about an average 70 to 80 fruits. Fresh weight of the umbel is recorded as 3.8±0.4 gram.Fruits emits strong odor on crushing. In early vegetative stage, local people collect the leaves and use them as vegetable.
The seeds loose viability after a year's storage. It means sterility/ dormancy is increasing with time. Dry weight of umbel is recorded as 0.08± 0.2 perplant.The analysis of growth parameters of plants growing in nature at Trimbakeshwar village ofNashik is given in Table No. 1.

Table No.1.Autoecological observations in plants of *Peucedanumgrande* (Baphali) growing at Trimbakeshwar of Nashik district:

Sr. No	Parameters	Average ±SE
1.	Height of the plant (cm)	151.2±0.3
2.	Diameter of main stem (cm)	2.6±0.2
3.	No of primary branches/plant	14.0±0.3
4.	No of secondary branches/plant	20.0±0.4
5.	Total no of branches /plant	34.0±0.4
6.	Total no leaves/ plant	120.0±0.2
7.	Fresh weight of main stem/plant (gm)	230.0±0.4
8.	Fresh weight of all leaves/plant (gm)	65.0±0.2
9.	Dry weight of main stem/plant (gm)	25.0±0.3
10.	Dry weight of all leaves/plant(gm)	12.5±0.2
11.	Mean leaf area (cm^2)	25.0±0.4
12.	Diameter of seed (cm)	0.4±0.0

13.	Length of main root /plant (cm)	19.0±0,2
14.	Diameter of main root (Apical Middle Basal) (cm)	2.2 1.2 0.8
15.	Fresh weight of main root/plant (gm)	85.0±0.3
16.	Dry weight of main root/plant (gm)	10.0±0.2
17.	Total dry biomass/plant(gm)	47.5±0.1
18.	Total number of umbels/plant	60.0±0.3
19.	Fresh weight of each umbel (gm)	3.8±0.4
20.	Dry weight of each umbel(gm)	0.08±0.2
21.	Average number of fruits/umbel	75.0±0.5
22.	Average length of fruit/ Mericarp (cm)	1.2±0.1
23.	Average length of umbel (cm)	5.2±0.3
24.	Total dry biomass including umbels/plant(gm)	52.5±0.1

PLATE I

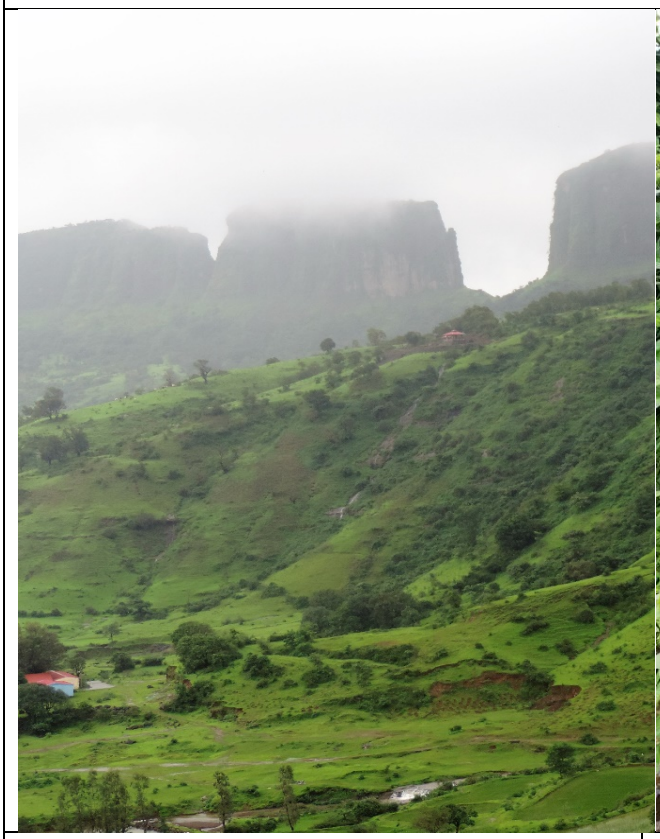

Fig.a. Locality of Baphali (*Peucedanum grande*) at Anjaneri Hills at Nashik, India.

Fig. b.Baphali (*Peucedanumgrande*) growing in natural habitat

Fig.c.Umbels of Baphali (*Peucedanumgrande*)

Fig.d. Roots of *Peucedanum grande*

Seed germination study in *P. grande*:

Mature seeds without any treatment failed to germinate. Seeds with mechanical scarification with needle or sand paper showed one to two percent seed germination.However, this percentage of seed germination was negligible.Hot water treatment also did not yield any positive result.Chemical scarification with sulfuric acid also had negative impact on seed germination. The results are summarized in Table No.2.

Table No 2: Seed germination study in*P. grande:*

Sr. No	Treatment	No of days required for germination	Percentage of seed germination (%)
1	Untreated	No germination	0.0
2	Mechanical i) Needle scarification ii) sand paper scarification	 30 35	 2 1
3	Hot water treatment	No germination	0
4	Chemical treatment with H_2SO_4	No germination	0

In vitro* seed germination study in *P. grande:

Effect of MS, ½ MS, and Knudson medium on seed germination of *Peucedanumgrande:*

In vitro seed germination was tried by using different plant tissue culture media like MS medium, ½ MS medium and Knudson medium. Seed germination results were taken after 30 to 45 days of inoculation.

Seeds of *P. grande* inoculated on full MS showedjust 2% seed germination within 45 days of inoculation. Further increase in time for seed germination did not have any positive impact on seed germination.

Seeds inoculated on ½ MS alone increased percentage of seed germination to 5%.The time required for seed germination also reduced from 45 days to30 days.Seeds

inoculated on specific medium like Knudson medium showed just 1% seed germination within 35 days of inoculation.

Therefore, it is concluded that the best percentage of seed germination can be achieved on ½ MS medium only.The percentage of seed germination may also be enhanced by supplementingdifferent hormones. The results are summarized in Table No.3.

Table No. 3: Effect of MS, ½ MS, and Knudson medium on seed germination of *Peucedanumgrande:*

Sr. No	Medium used	No of days required for seed germination	Percentage of seed germination(%)
1	Full MS	45	2
2	½ MS	30	5
3	Knudson	35	1

Effect of ½ MS with BAP at different concentrations on seed germination of *P. grande*:

To study the effect of growth hormone like BAP on germination, seeds were inoculated on ½ MS medium containing different concentrations of BAP.

Seeds inoculated on ½ MS + 0.1 mg/l BAP showed just 3% germination within 30 days of inoculation. Further increase in concentration of BAP to 0.5, 1.0, 1.5, 2.0 mg/l did not have any positive effect on seed germination. The results are summarized in Table No. 4.

Table No. 4: Effect of ½ MS with BAP at different concentrations on seed germination of *P. grande*.

Sr. No	Medium +BAP	No of days required for seed germination	Percentage of seed germination (%)
1	½ MS+0.1 mg/l BAP	30	3
2	½ MS+0.5 mg/l BAP	30	2
3	½ MS+1.0 mg/l BAP	30	2
4	½ MS+1.5 mg/l BAP	30	2
5	½ MS+2.0 mg/l BAP	30	1

Effect of ½ MS with Ascorbic acid at different concentrations on seed germination of *P. grande:*

Ascorbic acid is a standard antioxidant. This antioxidant may inhibit formation of germination inhibitory compounds formed after oxidation.

Therefore, seeds were inoculated on ½ MS + Ascorbic acid at different concentrations to study its effect on seed germination.

Seeds inoculated on ½ MS + 0.1 mg/l Ascorbic acid overall did not enhance seed germination. Further increase in concentration of Ascorbic acid also did not have any positive effect. The results are summarized in Table No. 5.

Table No.5: Effect of ½ MS with Ascorbic acid at different concentrations on seed germination of *P. grande:*

Sr. No	Medium+ Ascorbic acid	No of days required for seed germination	Percentage of seed germination (%)
1	½ MS+ 0.1 mg/l	35	2
2	½ MS+ 0.5 mg/l	35	2
3	½ MS+ 1.0 mg/l	35	2

Effect of ½ MS containing Charcoal at different concentration on seed germination of *P. grande*:

Activated charcoal is often used in plant tissue culture to improve cell growth and development and to promote seed germination. Activated charcoal is involved in many stimulatory and inhibitory activities in micro propagation, seed germination and embryogenesis,etc.

Therefore, the *P.grande* seeds were inoculated on ½ MS supplemented with three different concentrations of Charcoal (1%, 2% and 3%).

Seeds inoculated on ½ MS+1% charcoal showed only 2% germination after 35 days of inoculation. Similar percentage was observed after increase in charcoal concentration to 2 %. Further increase in charcoal percentage didn't enhance seed germination.In fact, it reduced the percentage of germination. The results are summarized in Table No.6.

Table No. 6: Effect of ½ MS containing Charcoal at different concentrations on seed germination of *P. grande*:

Sr. No	Medium + Charcoal	No of days required for germination	Percentage of seed germination (%)
1	½ MS+ 1% Charcoal	35	2
2	½ MS+ 2% Charcoal	35	2
3	½ MS+ 3% Charcoal	35	1

Effect of ½ MS containing different concentrations of GA_3 on seed germination of *P. grande:*

Pre-treated seeds were inoculated on full and half MS with (0.5, 1, 2,3,4,5 mg/l) GA3 and observations were recorded for 21 days of inoculation. Seeds subjected to overnight flushing, removal of seed coats andinoculated on ½ MS containing 5mg/l GA_3 resulted in maximum germination percentage of 55%. Either increase or decrease in concentration of GA_3 reduced the percentage of seed germination. Results are summarised in Table No.7.

Table No.7: Effect of ½ MS containing different concentrations of GA_3 on seed germination of *P. grande:*

½ MS + GA_3 (mg/l)	Flushing seeds under tap water	Removal of seed coat	No of seeds germinated ±SE	Percentage of seed germination (%)
0.5	Y	------	02±00	02
1.0	-----	Y	01±00	01
1.5	Y	Y	00±00	00
2.0	Y	Y	9.0±0.3	10
2.5	Y	Y	10.8±0.2	12
3.0	Y	Y	18.0±0.1	25
4.0	Y	Y	22.5±0.2	30
5.0	Y	Y	49.5±0.2	55
6.0	Y	Y	18.0±0.3	20

PLATE II

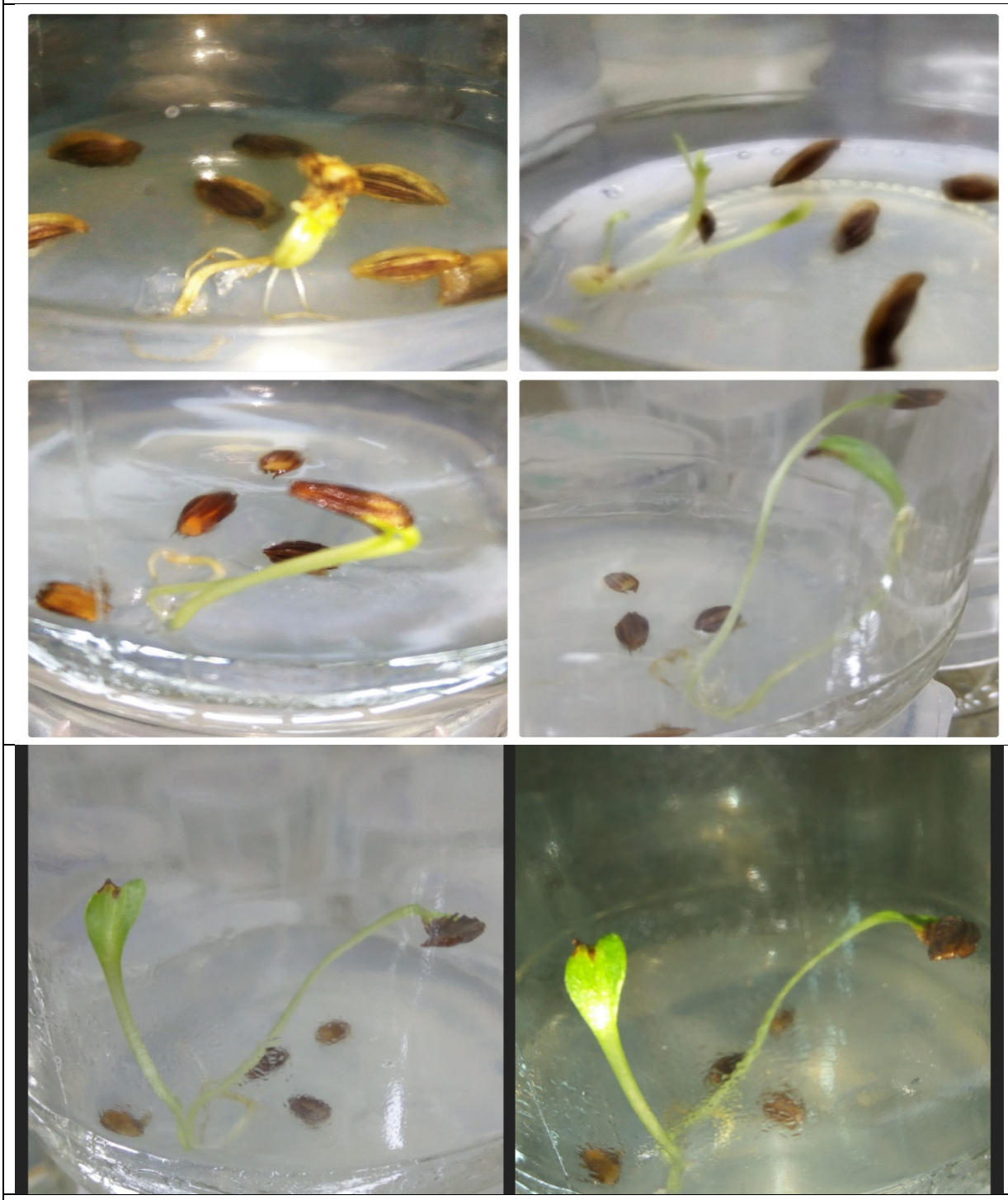

Fig.e.*In vitro* seed germination in Baphali (*Peucedanum grande*) on ½ MS containing 5mg/l GA_3

Discussion:

Autoecological study:

P. grande grows on moist hilly slopes in tall grasses in Western Ghats of Maharashtra.The plant starts its vegetative growth in June -July, flowers and forms fruits between August- September and dry up by October end.

The plant is already confined to specific locations (Western Ghats) and its population is rapidly depleting due to its overharvesting for medicinal formulations in Unani system of medicines.

From the autoecological study of *Peucedanumgrande* from Bramhagiri Hills and Anjaneri hills of Nashik district, it was observed that this plant species is picked up in early growth stages for vegetable purpose by local people. From those plants which reach reproductive stage immature fruits are extensively picked and sold in local market as per the demand of Unani medicine industry. Similar autoecologicalstudies were reported by Kure (2020) and Surwase (2000).

Seed germination studies in *P. grande*:

Seed germination of *P. grande* has been found to be extremely difficult on account of several barriers such as presence of inhibitors in seed coat, non-availability of fully mature seeds and poor germination percentage due to high sterility.

Fully mature seeds of duqu exhibit poor germination due to presence of germination inhibitors in seed coat.

For standardization of seedgermination protocol, seeds of *P. grande* were subjected to various seed treatments like physical scarification with needle, chemical exposure like H_2SO_4, hot water treatment, flushing overnight under running tap water then removal of seed coats to facilitate protrusion of embryos.

In present study, chemical and physical treatments did not remove dormancy of *P.grande*seeds.

In *Echinacea puppurea* (Bishnoi*et al*., 2010) and*Flemingiatuberosa* and *Merremiarhyncorhiza* (Surwase, 2000), percentage of seed germination was enhanced by mechanical scarification.

Similarly, Sanya*et al*. (1970) observed that seeds of *Vernoniaanthelmintica* germinate only after leaching with abundant water. Similar result has also been observed in *Vernoniaanthelmintica*by Kure (2013).

It was found that seed germination of *P. grande* is affected by the factors like age of seed and seed pretreatments followed. Role of GA_3 has been found in wakening up the embryo.

***In vitro*seed germination studies in *P.grande*:**

Low *in vitro* germination efficiency is a problem with many species of Apiaceae.*P. grande* seeds show high sterility. The seeds inoculated on full MS alone failed to germinate.

Flushing seeds overnight under running tap water and then removal of seed coats and inoculation on ½ MS supplemented with GA3 (5mg/l) resulted in germination in 21days in *in vitro* conditions with 55% result.

Similarly in *Ferula ferulaeoides*, there was no germination on Full MS. Seeds were subjected to different treatments including various levels of GA_3, BAP and cold stratification at 4°C for different times(Surani*et al*., 2016). Germination of seeds was observed on MS media supplemented with GA_3. There was no influence of BAP on *F. ferulaeoides* seed germination.

On the contrary, *Tavernieracuneifolia,* (Jamdhade*et al.,* 2012) and *Pterocarpussantalinus* (Vipranarayana*et al.,*2010) showed optimum germination in ½

MS alone.Seeds of *P. grande*showed maximum percentage of seed germination in ½ MS+ 5mg/l GA_3.

Whereas, application of GA_3 did not break dormancy during two months of incubation in *Sorbusalnifolia*(Tang, 2019).

However, seeds of *Bupleurumlatissimum,* a critically endangered plant from Apiaceae, are difficult to germinate, even when treated with GA_3 (Bae, 2015).

Conclusions:

P.grande is an important medicinal plant endemic to Western Ghats of Maharashtra. It is reported that seeds possess high pharmacological potential for curing vital ailments of kidneys, GI tract and genital organs in human beings. Considering the importance of herbal ethno medicines, there is need for the systematic cultivation of this plant species to get high production of its fruits which are rich source of phytochemicals, without harming its confined natural habitat and so as to commercialize the medicinal formulations from this plant with almost no side effects. Seeds of *P. grande* are highly dormant for germination when stored for long time. Normal seed germination cannot be achieved by using different methods. However, *in vitro* seed germination can be achieved on ½ MS +GA_3.This protocol may be utilized for multiplication of this species.

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