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IR spectrum and *In vitro* studies of endemic plant *Ipomoea clarkei* hook.f.

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Abstract
Ipomoea clarkei Hook. f. is rare, climbing herb and restricted to India. The conservation of the rare plants in recent years was extensively done by the reintroduction of in-vitro propagated plantlets to their natural habitat. The Ipomoea clarkei Hook.f.is one of the ignored, rare and an endemic terrestrial Species of Western Ghats demands attention to the conservation. In-vitro propagation using tissue culture is a significant method for rapid multiplication upon the natural germination of seeds and vegetative means. Propagation by using Plant Tissue Culture of such plant aims to conserve the plant by rehabilitation and reduce the pressure on the ecosystem. In present investigation it was observed development of multiple shoots on MS basal medium supplemented with 0.5 mg/lit BAP and combination 0.1 mg/lit NAA from nodal segment.

Keywords: ipomoea, IR spectroscopy, Ms media, tissue culture, Western Ghats

1. Introduction
Ipomoea is one of the major genera of family Convolvulaceae. It represented by over 650 species and mainly spread to tropical regions in the world (Mabberley 2008) [3]. Genus *Ipomoea* represented about 65 species in India, (Shimpale *et al*., 2012 [14]; Shimpale *et al*., 2014 [13]; Sarvalingam *et al*., 2014) [12], out of which three species and one variety, i.e. *Ipomoea clarkei* hook. f. (Maharashtra), *Ipomoea laxiflora* H.J. Chowdhery & Debt (Western Himalaya, Uttarakhand), *Ipomoea salsettensis* Santapau & Patel (Maharashtra) and *Ipomoea deccana* Austin var. *lobata* (C.B. Clarke) Johari (Karnataka, Kerala and Maharashtra) are endemic to India (Singh *et al*., 2015) [15]. Plants have been used for the treatment of diseases from centuries. Natural product chemistry, especially phytochemistry, has become a topic of interest for most of the researchers due to the advantages of the plant derived medicinal compounds over the traditional ways of using herbal plants. *In vitro* of Cultivation of explant (all plant parts) under aseptic conditions is known as plant tissue culture. *Ipomoea clarkei* is propagated by seeds, but, in nature seeds germinate slowly and persist dormant for a long time (Sangle, 2019) [11]. *In vitro* methods for bulky multiplication would be a feasible choice, and has been reported for several Himalayan medicinal herbs (Giri *et al*., 2012 [2]; Chandra *et al*., 2006 [1]; Nadeem *et al*., 2000) [6]. Tissue culture technique is a great tool to multiply difficult to propagate, rare or endangered and useful species for commercial cultivation as well conservation (Nandi *et al*., 2002). The present research work has concluded the *in vitro* micropropagation is one of best methods to produce huge number of pathogen free and virus free plantlets within a small period of time.

Materials and Methods
Surface sterilization Culture media and inoculations of plant material.
The seeds of Ipomoea clarkei were collected from Malshej Ghat, Pune district located in ranges Western Ghats (19°20.349'N 073° 44.232'E) and raised in botanical garden of Rajaram college, Kolhapur. Plant material washed using liquid detergent for removing dust and waxy particles from the surface of plant material. The plant materials are then washed with distilled water and flask are transfer to laminar air flow cabinet. Then plant material was surface sterilized by using 0.1% Hgcl2.for 1 to 2 min. After surface sterilization plant material was rinsed 3-4 times with sterile distilled water to remove all traces of chemicals and was dissected under aseptic conditions with a sharp scalpel blade. Explants (Leaf, Nodal sector, Stem) were excised into 2–3 cm size pieces and inoculated in culture bottles containing Murashige and Skoog's medium (Murashige and Skoog, 1962). with different combinations and stocks of various compounds as mentioned in observation table. The success in cell, tissue and organ culture technique is related to the selection or development of the media composition.

IR spectroscopy
The application of IR spectroscopy is to identification of compounds by matching spectrum of unknown compound with the reference spectrum (fingerprinting) for identification of functional groups in unknown substances. The synthesized extract was characterized by FTIR spectroscopy instrument Alpha Bruker ATR. 1mg. sample is required for the analysis and it takes estimate time to obtain spectrum from a routine i. e. 1 -5 minutes. The frequency region of IR instrument is 600-4000 cm^{-1}. IR spectra are obtained by detecting change in transmittance (or absorbance) intensity as function of frequency.

Result and Discussion
Multiple shoot initiation from explants was observed after 30 days of inoculation and multiple shoot proliferation was obtained within 40-45 days after subculture. The responses of shoot regeneration to various growth regulators such as BAP and NAA are presented in Table number 1.

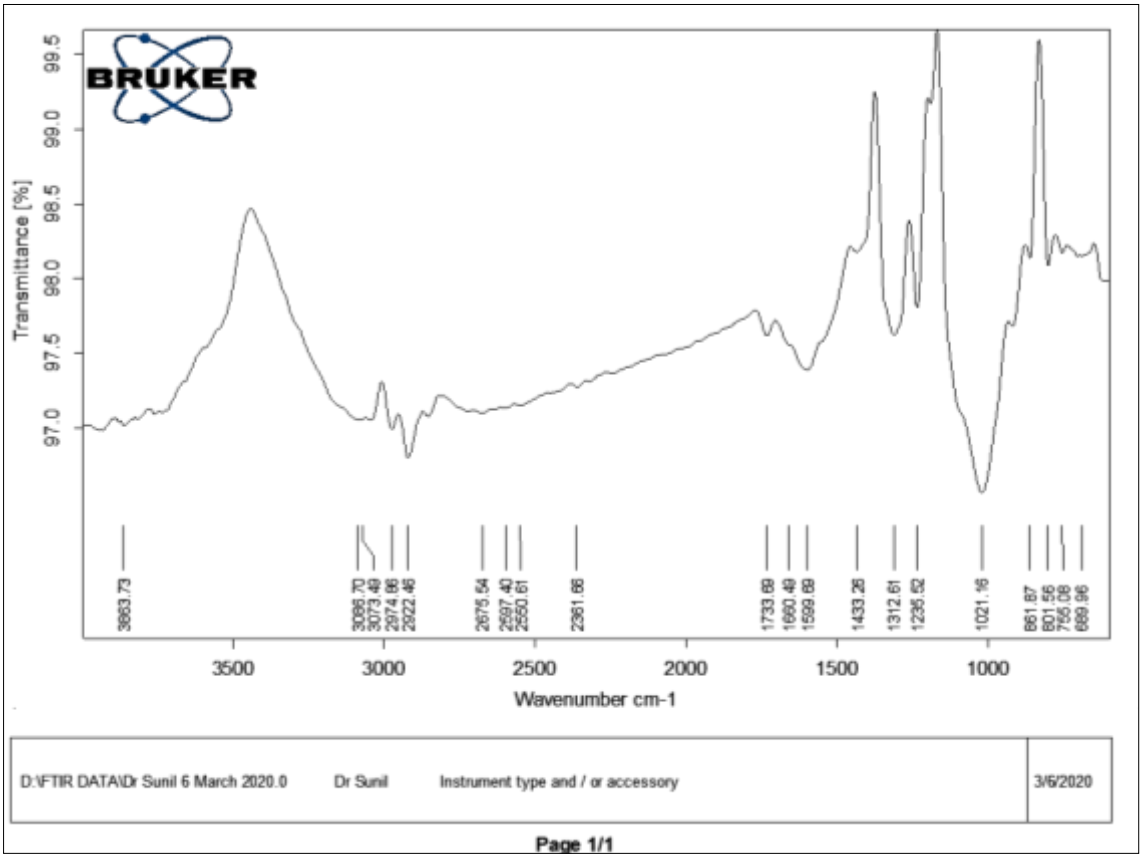

Fig 1: IR Spectrum of sample extract of plant material *Ipomoea clarkei.* Hook. f.

Table 1: The effects of plant growth regulators on shoot regeneration from explant of *Ipomoea clarkei*. [(Values are mean ± SE) and SD, 10 cultures per treatment of 3 replications]

Sr. No.	M.S. media with plant growth regulators	Response of shoot regeneration (%)	Mean number of shoots/ culture X ± SE	SD (σ)
1	M.S.+ 0.5 mg/ lit BAP	86.66 %	3.47 ± 0.09	0.29
2	M.S. +1. 0 mg/ lit BAP	63.33 %	1.90 ± 0.05	0.16
3	M.S.+ 1.5 mg/ lit BAP	43.33 %	0.93 ± 0.04	0.12
4	M.S.+ 2.0 mg/ lit BAP	46.33 %	0.87 ± 0.04	0.12
5	M.S.+ 2.5 mg/ lit BAP	26.66 %	0.27 ± 0.01	0.05
6	M.S.+ 3.0 mg/ lit BAP	00.00 %	0.00 ± 0.00	0.00
7	M.S.+ 0.5 mg/ lit BAP + 0.1 mg/lit NAA	43.33 %	0.83 ± 0.03	0.09
8	M.S.+ 0.5 mg/ lit BAP + 0.2 mg/lit NAA	23.66 %	0.23 ± 0.01	0.05
9	M.S.+ 0.5 mg/ lit BAP + 0.3 mg/lit NAA	16.66 %	0.17 ± 0.01	0.05
10	M.S. + 0.5 mg/lit. BAP + 0.1 mg/lit. AC	76.66 %	1.53 ± 0.06	0.21
11	M.S. + 0.5 mg/lit. BAP + 0.2 mg/lit. AC	83.33 %	1.67 ± 0.08	0.26
12	M.S. + 0.5 mg/lit. BAP + 0.3 mg/lit. AC	73.33 %	0.73 ± 0.01	0.05

Table 2: The effects of 2, 4-D plant growth regulators on callus formation from explant of *Ipomoea clarkei*. [(Values are mean ± SE) and SD, 10 cultures per treatment of 3 replications]

Sr. No.	M.S. media with plant growth regulators	Response of callus formation (%)	Degree of callus development
1	M.S.+ 0.1 mg/ lit 2,4-D	00.00%	--
2	M.S.+ 0.3 mg/ lit 2,4-D	10.00%	+
3	M.S.+ 0.5 mg/ lit 2,4-D	13.33%	++
4	M.S.+ 1.0 mg/ lit 2,4-D	23.00%	++
5	M.S.+ 2.0 mg/ lit 2,4-D	06.66%	+
6	M.S.+ 3.0 mg/ lit 2,4-D	00.00%	-

The number of plus signs (+) denotes the degree of callusing, -- denotes no response. Scoring of callus response: + is 5%-10%; ++ is 11%-30% and +++ is indicates 31%-50%;

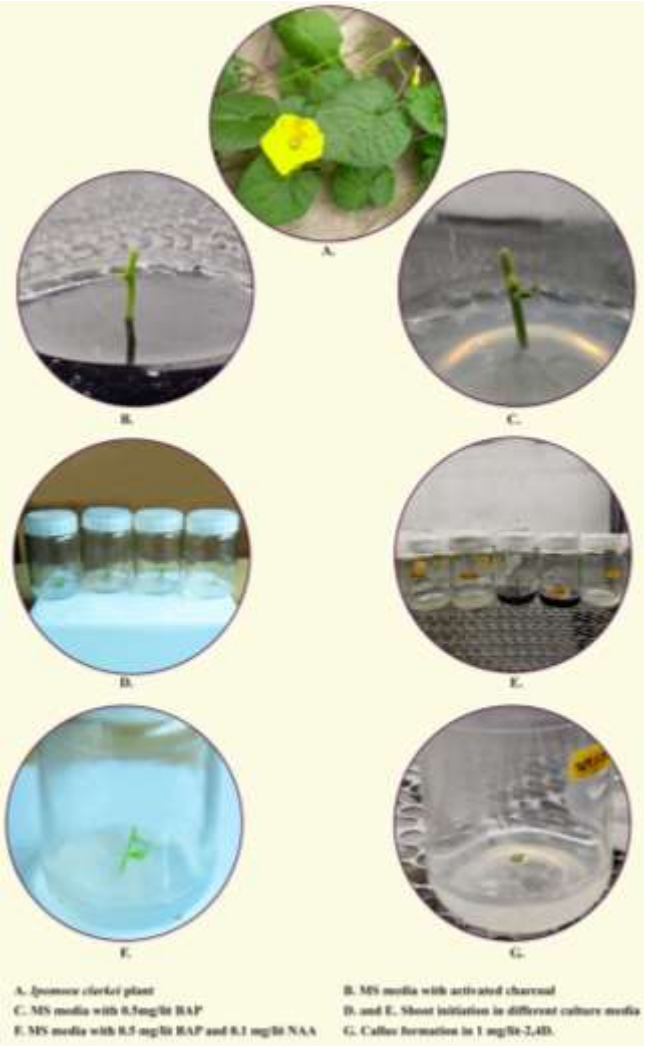

Fig 2

Shoot initiation and elongation

Experimental plant material was poorly efficiently regenerated from leaf and stem port. Explants were capable of directly developing multiple shoots on MS basal medium supplemented with 0.5 mg/lit BAP and combination 0.1 mg/lit NAA from nodal segment. Thomas and Hoshino (2015) [17]. describe an effective regeneration protocol for huge scale multiplication of *T. bicolor*. and high frequency shoot induction was accomplished from internodal derived callus. Cheruvathur *et al*., (2015) [4]. observed highest shoot induction response on 3.0 mg/L BA and 0.5 mg/L NAA and 100% cultures responded with an average number of 3.2 shoots per explant. in *Ipomoea sepiaria* Roxb.

Combination with Activated charcoal

Activated charcoal is generally used in tissue culture media for providing a dark environment in the medium and adsorption of plant growth regulators and other organic compounds (Pan and Staden, 1998) [8]. MS medium with growth regulator 0.5 mg /lit. BAP with increasing order 0.1, 0.2 and 0.3 mg/lit. Indicate desirable regeneration of shoot. Activated Charcoal The presence of activated charcoal significantly reduced the percentage of the callus induction and biomass accumulation in the callus induction medium (Sutee and Thanawat, 2018) [16].

Callus initiation

Thomas and Hoshino (2015) [17]. were observed optimum callusing when internode explants were cultured on MS medium augmented with 4. µM 2, 4-dichlorophenoxy acetic acid (2, 4-D) and 0.5µM kinetin (Kn). Table no 2. Demonstrate MS with concentration of 0.5 and 1.0 mg/ lit 2, 4-D callus initiation from leaf explant.

Analysis of IR spectroscopy

Paul and Sinha (2016) [9]. Determined *I. quamoclit* have numerous biological activities which is encouraging to find its new therapeutic uses. The stretching frequencies obtained from sample were 801, 1021, 1235, 1599, 1733, 2922, 2974 cm^{-1}. The figure no. 2 showing the possible functional group is aromatic compound, C-O-bond, ester carbonyl, diketones / amines, C=C-stretching bond, C-H alkane, C-H alkane respectively. Similar work carried out by Vaishali Agme-Ghodke *et al.*, (2016) [18]. in *Centella asiatica.* IR Spectrum detected occurrence of the herbal medicinal values which is helpful for identification of these phytochemicals which can have medicinal properties. Sangle and Dongre (2020) [10]. analysed HR-LCMS of ethanolic leaves extracts of *Ipomoea clarkei* and confirmed presence of secondary metabolite which belongs to a class of Fatty acid, Sterioisomer, Carbaxomide, Metabolite, Glycoside, Enzyme, Pigment xanthonoid, Glucuronide, Metabolite.

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