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Propagation of *Tectaria coadunata* (Wall. ex Hook. & Grev.) C.Chr by Spores

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Abstract

This study explores the spore germination and formation of sporophyte of *Tectaria coadunata* (Wall. ex Hook. & Grev.) C.Chr from its spores. *Tectaria coadunata* is a perennial evergreen edible fern species belonging to the family Tectariaceae. It is locally known as *kali niuro* in Nepali language and it is a very common wild vegetable in Nepal. We carried out the successful propagation of *Tectaria coadunata* from spores using two propagating media: coco-peat and soil mixture (soil, sand and compost manure). The temperature recorded from the spore sown time to development of sporophyte phase was 18-27°C while the general florescent light regime for 14-16 per day was supplied. To keep the propagating media humid and moist it was regularly monitored and maintained. The research is now in acclimatize phase and success of this study will be shared soon. Moreover, the work is successful in laboratory under control condition of certain factors like light and moisture and it is under progress to exploit its capacity to get survived in natural field, and the findings could be used as an alternative method of raising the plant in semi-natural environment.

Keywords: Cultivation, Edible fern, Kali niuro, Spore propagation

Introduction

Fern spores are tiny and have very few resources within them to support further growth and development (Mehltreter, 2014). The spore plays the dominant role in fern life cycle because it is the propagule that determines the potential dispersal and biogeographic limits of a fern species (Walker & Sharpe, 2010).The propagation of ferns from their spores is an expensive but very slow process (Dobbie, 1929). Fern germinate from spores and these develop into gametophyte which is haploid (Ranker & Haufler, 2008). As the complete plant body is made after the fertilization of haploid gametophytic plant and it is determined by the external factors such as light, moisture, soil nutrients/type and other environmental factors. Only some microhabitats or "safe sites" allow the spores to germinate and gametophytes to develop and to reproduce successfully into sporophyte.

The memorial work by Okada (1929) on the germination and viability of fern spores is considered as the first in-depth detailed study of these phenomena. The spores of the ferns need light to germinate as it does not germinate in dark (Pérez García et al., 1994). The gametophytes produced from green spores appear to develop more quickly than those from non-green spores. The rapid germination and shorter viability of green spores occur because of their constant respiration and lack of dormancy (Lloyd & Klekowski, 1970). Fern gametophytes developed from spores have been considered as ideal experimental organisms in scientific research and as model multicellular systems (Hickok et al., 1987; Miller, 1968).

For the propagation of spores in artificial medium many researchers have included metabolic sugars but no clear trends have resulted from such studies. Hurel-Py (1955) found that sugars inhibited germination of *Alsophila australis*. Camloh (1993) reported that the inclusion of sucrose in liquid medium produced no stimulation on germination of *Pteridium bifurcatum* spores. However, Sheffield et al. (2001) found that the germination of gametophytes of species namely *Pteridium aquilinum*, *Athyrium filix-femina*, *Dryopteris expansa* and *Anemia phyllitidis* were

significantly enhanced by inclusions of sucrose in artificial media.

Tectaria coadunata (Wall. ex Hook. & Grev.) C.Chr. is a perennial evergreen edible fern species belonging to the family Tectariaceae (the halberd fern family). The plant prefers to grow at open places/slopes, damp fields and moist places. It is native to Eastern Tropical Africa, Tropical and Subtropical Asia. The plant *Tectaria coadunata* is locally known as kali niuro in Nepali language and it is very common wild vegetable in Nepal and is mostly collected from forest in the spring or rainy season by local people and it can also be purchased from local market. Their tender leaves or shoots are consumed for food (Bhattarai & Rajbhandary, 2017) and also possess medicinal value (Bhagat & Shrestha, 2010). The young shoots are rich in minerals like Iron (Fe), Magnesium (Mg) and Potassium (K) but low in Sodium (Na). The rhizomes extracts of this plant contain major chemical compound like Decenediol, Dodecanoic acid and Palmitic acid (Marahatta et al., 2019).

The primary aim of this study was to germinate the fern spores from mother plant of *Tectaria coadunata* in order to develop the protocol for its propagation that are not yet done in Nepal. This species was selected as, it has wild edible value and also possess medicinal properties. The research was performed in the general laboratory of Plant Research Centre, Makawanpur, Hetauda. The germination trial has been carried under control condition of light and moisture. The germinated sporophytes are kept and planted in open field in different environmental conditions to acclimatize in natural field. The implications of the success of this study will also be shared soon and once the propagation methods have been consolidated and the technique transferred to communities, people will be able to grow these species for economic benefit in their own fields or gardens.

Materials and Methods

The study was carried in the laboratory of Plant Research Centre, Hetauda and all the experimental procedures were performed under control condition of light and moisture. The mature light brown colored spores of *Tectaria coadunata* was collected from its mother plant. The mother plant was collected from the field of Lamidanda (on the way of Tribhuwan Rajpath) of Makawanpur district. The collected plant was planted and grown at Brindaban Botanical garden, Makawanpur.

Two media: one having coco-peat (with NPK: 0.41%, 0.81% and 1:32%, pH 6.7) and other with soil (mixtures of soil, sand and compost manure in 1:1:1 ratio, pH 6.5) were taken for propagating media. Six plastic pots for each media were taken and were filled with both mixture media and it was sterilized with boiling water to remove and kill the pathogens or germs of the mixture. The sterilized pots were then kept for 24 hrs to cool enclosing with plastic foil and sealed. For the light source, fluorescent light were used (CFL light of 15 voltage power) and light regime were kept for 14-16 hrs per day. To maintain humid and moisture, normal water was used.

Collection of Spores

The fronds having mature spores were cut and secured in an envelope to capture the spores**.** The fronds were kept in room for about two days for drying of spores which would ease the dehiscence process of spores. The fronds of spores were dried in laboratory devoid of air and sunlight. After two days of drying, the spores were tapped off with the help of small pencil/measuring scale collected in a plane paper sheet. The paper was shaken to make a thin layer of distribution of spores in it because it will be difficult for high density of the spores to grow in crowd.

Sowing of spores

For the spore sowing technique (Ensoll & Matthews, 2004) was applied. The mature spores of *T. coadunata* were collected in a paper then it was sown to both sterilized media pots. The pots were covered with plastic to trap the water evaporating and maintaining high humidity inside it to prevent contamination as well. The six replications for each propagating media (Total 12 pots) were made and all those pots were placed in a tray containing shallow layer of water. The temperature at the time of sowing

spores was noted 18-21°C. The pots were placed under the exposure of 12-14 hrs light regime.

Germination of spores

The germination of spores was indicated by the green emerging prothalii. High humidity condition inside the propagating plastic tray was maintained all the time by keeping medium moist and covering the containers. A green mat started to appears across the surface of propagation plastic pots which indicates the gametophyte formation phase. Regular humid condition and light regime was maintained for the further growth of prothalii and the first stage of gametophytes during which it attaches itself to surface of medium and developed into two leaves structure.

Patching off gametophytes

With the help of tweezers, the thick layered of gametophytes measuring up to 2 mm in diameter was patched off from the carpet of gametophytes. The gametophytes were then planted gently on the surface of sterilized pots of propagating media of coco-peat and soil mixture. A gap of 6-8 mm was left between the patches to allow the gametophytes to grow after its fertilization. The pots were again sealed with plastic and placed under fluorescent light of 12 hrs regime and temperature noted about 25-27°C.

Fertilization and formation of sporophytes

The fertilization process (the union of male and female gametes) was facilitated by gently spraying very fine mist of water over the gametophytes mat using water sprayer bottle. This misting process was done in every two days of interval and regular moisture was maintained. After 97 days of spore sown, the sporophyte formation started to appear. The moisture of propagating plastic pots was regularly maintained by spraying water.

Hardening off sporophyte

The hardening off sporophyte was noted once they were beginning to push against the covering of sealed plastic. The sealed plastic of the propagating pots were removed and the growth of sporophyte was allowed in uncovered way. In this condition the sporophyte gets acclimatized to low humidity condition. The misting process was done at regular to maintain the humidity of stabilizing sporophytes. The light regime of 12-14 hrs was applied and recorded temperature was 27°C.

Results and Discussion

The current experiment described the details in the propagation of *Tectaria coadunata* from its spores using propagating media of coco-peat and soil mixture. It yielded detail information from the spore collection to its germination and development of gametophytes and sporophyte (formation and stabilization) and step-wise photographs were captured in each phases of germination (Figure 1, 2 and 3, Table 1) respective propagating media. The spores of *Tectaria coadunata* were sown on 17th February, 2021 to both the media.

Spore germination in coco-peat media

The germination of spores started after the 28 days of spore sown. The temperature recorded at the time spores sown was 21°C and regular light regime of 12-15 hrs per day was supplied. The gametophytes with green thick carpet of mat started at the surfaces of propagating media and after 69 days of spore sown the gametophytes measuring up to 3 mm in diameter were patched off in new pots with same media. The fertilization of gametophytes were occurred after 97 days of spore sown and sporophyte formation time were recorded after 112 days of spore sown and stabilizing and hardening off sporophyte were observed after 162 days of spore sown period.

Spore germination performance in soil mixture

The germination of spores started after the 34 days of spore sown. The temperature recorded at the time spores sown was 21°C and regular light regime of 12-15 hrs per day was supplied. The gametophytes with few green layer of mat appears after 75 days of spore sown at the surfaces of propagating media and after 75 days of spore sown the gametophytes measuring up to 3 mm in diameter were patched off in new pots with same media after 98 days. The

fertilization of gametophytes was occurred after 135 days of spore sown and the complete sporophyte started to form after 167 days of spore sown.

In the both propagating media, the spore germination and formation of sporophyte are successful with regular supply of 12-16 hrs light and moisture. The influence of light and moisture supplement the germination pattern of fern (Banks, 1999; Raghavan, 1989; Wada, 2008). The relative success of spore germination when compared with each other, the germination is quite faster and vigorous for coco-peat propagating media than that of soil. It may be due to sufficient amount of nutrients present in coco-peat to support the germination of spores. The higher value of pH also might have stimulated the growth in coco-peat media. The factors like sucrose and gibberellic acids enhance the germination of spores (Nester & Coolbaugh, 1986). The fertilization for coco-peat media took about 97 days to form sporophyte. However it was quite earlier than that observed by De Brum and Randi (2006) for *Rumohra adiantiformis* (133 days) and the observations made by Ravi et al. (2015) for *Pteris tripartite* (about 150 days). The complete sporophyte plant was obtained after 112 days of spore sown in coco-peat media while it took 167 days for soil mixture. The sporophyte formation period was comparatively longer for both media than that observed by Apuan et al. (2016) for *Pteris melanocaulon* Fée (77 days) D. A. Apuan et al. (2016) for *Pteris vittata* L. (57 days).

Table 1: Germination performance of *Tectaria coadunata* spores sown on 17th February, 2021

Propagation media	Germination of Spores(DAS)	Formation of gametophytes (DAS)	Patching off gametophytes (DAS)	Fertilization of gametophytes (DAS)	Formation of sporophyte (DAS)	Hardening of sporophyte (DAS)
Coco-peat	28 (Prolific)	53	69	97	112	162
Soil, sand and compost manure(1:1:1)	34 (Few)	75	98	135	167	207

Note: DAS = Days after sowing

A

B

Figure 1: *Tectaria coadunata* **A.** Mother plant, **B.** Ventral view of leaflet with sori

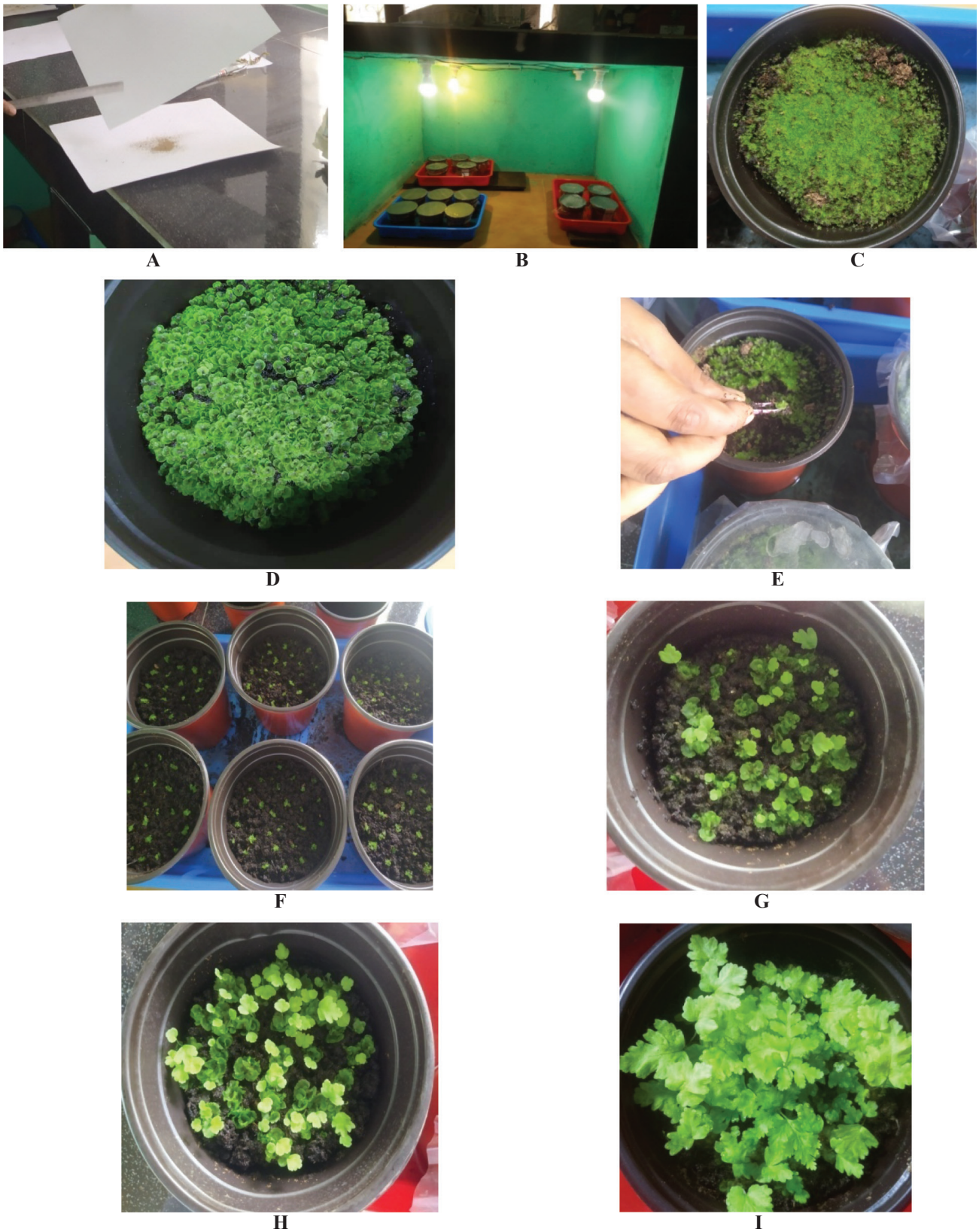

Figure 2: Showing different Phases of development and Sequence of events from the collection of spores to sporophyte formation of *Tectaria coadunata* in coco-Peat propagating media, **A.** Spore collection, **B.** Spore sown pots kept under light, **C.** Emerging prothalli from germinated spores (after 28 days of spore sown), **D.** Gametophytes formation (after 28 days of spore sown), **E&F.** Patching off gametophyte (after 69 days of spore sown),**G.** Fertilization of gametophytes (after 97 days of sporophyte), **H.** Formation of sporophyte (after 112 days of spore sown), **I.** Hardening off sporophytes (after 162 days of spore sown)

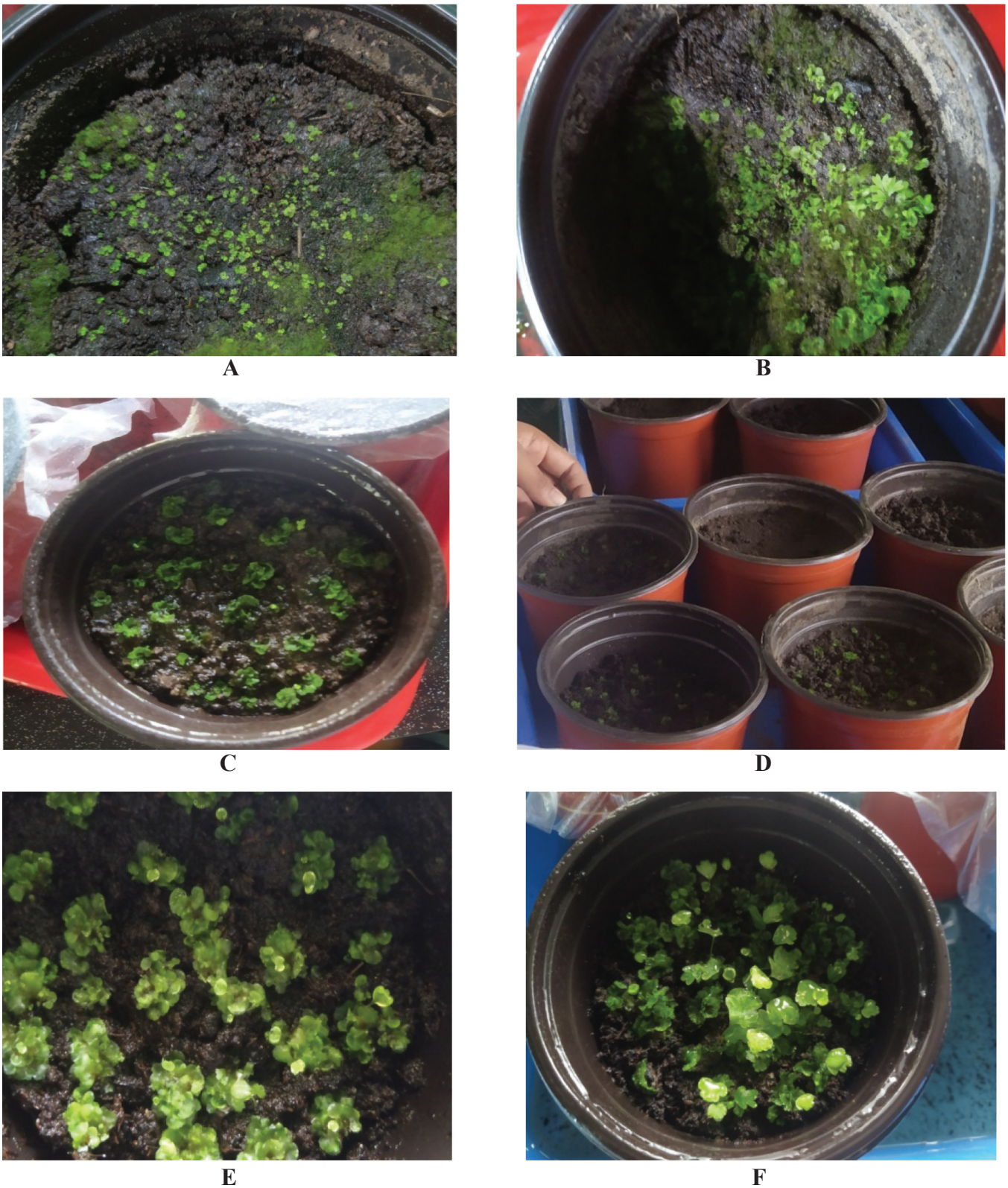

Figure 3: Showing different Phases of development and Sequence of events from the collection of spores to sporophyte formation of *Tectaria coadunate* in Soil mixture propagating media, **A.** Emerging prothalli from germinated spores (after 34 days of spore sown), **B.** Gametophytes formation (after 75 days of spore sown), **C.** Gametophytes after 87 days of spore sown (after 98 days of spore sown), **D.** Patching off gametophytes, **E.** Fertilization of gametophytes (after 135 days of spore sown), **F.** Formation of sporophytes (after 167 days of spore sown)

Conclusion

The study on propagation of spores of *Tectaria coadunata* (Wall. ex Hook. & Grev.) C.Chr was successful in the laboratory of Plant Research Centre, Makawanpur in both the propagating media coco-peat and soil mixture. The coco-peat media shows higher and prolific rate of germination of spores while it is much lower in soil mixture media. The temperature recorded from the spore sown time to development of sporophyte phase was at the range of 18-27^{o}C. For the source of light, general light regime for 12-16 hrs per day was maintained and by supplying normal water humidity of propagating pots were regularly monitored and kept moist.

Authors Contributions

Chandrakala Thakur and Raghu Ram Parajuli conceived and planned the experiment. Chandrakala Thakur and Prativa Budhathoki performed the experiment in lab and collected data. The overall manuscript was prepared by Chandrakala Thakur consulting with Sangeeta Rajbhandary. Sangeeta Rajbhandary and Raghu Ram Parajuli edited and reviewed the manuscript. Chandrakala Thakur as a corresponding author is the guarantor for this article.

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