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Sporulation and Spore Germination in the Tissue Culture of Cheilanthes Fragrans

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ABSTRACT

The concentration of spores .05 MS It was used for each Petri dish was considered as an example to finding the effect kind of spore on the growth of spores some of them scratch with sand and spore put on the centrifuge after that petri dish was divided into two parts using a sampler to a normal spores and spores to other parts of the scratches were added at a concentration equal by using a sampler concentration 20 micro liters of medium .05MS Was added. Spores were selected include the leaves of fern spores from fresh normal from laboratory or fern spores of normal in the natural environment. Our results showed that the number of petri dishes have growth prothallus without scratched spore after four test repetitions was 70.6±1.5
and number of Petri dishes have growth prothallus from scratched spore was 65.7±0.6 spore culture in the Ms tissue media showed that scratched of spore no need ,camper between of spore determined that normal spore easy grow in the laboratory, all spore type produce normal Prothallus.
KEYWORDS: *Cheilanthes fragrans*, Fern, Biotechnology, Spore, Tissue culture

1. INTRODUCTION

Cheilanthes fragrans is a desiccation tolerant plant or a resurrection fern. This plant can withstand lack of rains for years and again can resume its viability and life cycle with 10 ml of rain or more. Finding the time of desiccation induction in life cycle of this fern by in vitro regeneration of callus, gametophyte, and saprophyte have been possible by tissue culture techniques this plant original provenances of seeds were respectively N.S.W., Australia, and Transvaal, South Africa, were grown in 15 cm pots containing a coarse sandy loam peat mixture (3:1:1) in a greenhouse at 20–250C under natural light (Donald et al., 2013).

Man has been using plants as a source of food, medicines and many other necessities of life since ancient times. Even to this day the primitive tribal societies that exist depend on the plant life in their surroundings. Though there were investigations of the edible economic values of the higher plants, especially the pteridophytes and angiosperms have been unfortunately ignored. The pteridophytes are used in Homoeopathic, Ayurvedic, Tribal and Unani medicines and provide food, insecticides and ornamentations. (M. Mannar,et al.,2008)

The pteridophytes constitute the primitive vascular plant groups which are found scattered all over the world. Although, not much consideration has been given towards the utility of pteridophytes yet these possess equal economic importance including medicinal ones. Biotechnology of reproduction of fern *Cheilanthes fragrans* is resurrection fern that grows in the mountain, with a height of around 25-10 cm with short rhizome. Fern Class Petridophyta, a member of Adiantaceae family, is a desiccation tolerant fern and regenerates by spore. *Cheilanthes fragrans* is a drought tolerant plant to various physiological, biochemical and morphological characteristics (Ghasempour and Maleki; 2003).

Cheilanthes fragrans raw material quality has three benefits; first at all nutritional values for example eating jam of fern's rhizome with honey has been used for prevent pregnancy or abortion. (Ghasempour ,2007) Fresh rhizomes of Male fern have been used for removing worms such as *Taenia saginata*. The result of my research can produce in vitro fern for the Commercial and large scale. Second benefit that is the medicinal values of whole plant of *Cheilanthes fragrans* for Cold and sore throats described in India, also ferns in Iranian traditional medicine are used for the treatment of diseases such as anti tumor, anti bacterial , anti fungal, Leprosy, melancholy (mental and physical symptoms of depression or despondency) involved , exactly we want find which material or secondary material has this effect.

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2. MATERIAL AND METHOD

2.1. Techniques

Modern plant tissue culture is performed under aseptic conditions under filtered air. Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so surface sterilization of starting materials (explants) in chemical solutions (usually Sodium or calcium hypochlorite or mercuric chloride) is required. Mercuric chloride is seldom used as a plant sterility today, unless other sterilizing agents are found to be ineffective, as it is dangerous to use, and is difficult to dispose of. Explants are then usually placed on the surface of a solid culture medium, but are sometimes placed directly into a liquid medium, particularly when cell suspension cultures are desired. Solid and liquid media are generally composed of inorganic salts plus a few organic nutrients, vitamins and plant hormones. Solid media are prepared from liquid media with the addition of a gelling agent, usually purified agar. The composition of the medium, particularly the plant hormones and the nitrogen source (nitrate versus ammonium salts or amino acids) have profound effects on the morphology of the tissues that grow from the initial explants. As cultures grow, pieces are typically sliced off and transferred to new media (MS) to allow for growth or to alter the morphology of the culture. The skill and experience of the tissue culturist are important in judging which pieces to culture and which to discard. As shoots emerge from a culture, they may be sliced off and rooted with auxins to produce plantlets which, when mature, can be transferred to potting soil for further growth in the greenhouse as normal plants bioprocessing of plant cell cultures is needed for mass production of targeted compounds). Plants were watered and fertilized regularly with a half-concentration modified MS. A few weeks before commencement of experimental treatment, plants were transferred to a controlled environment chamber (CEC) maintained at 280C and 260 μ molm2s-1 photosynthesis photon fluxes with photoperiod of 16 h.

2.2. Choice of explants

The tissue obtained from the plant to culture is called an explants. In many species explants of various organs vary in their rates of growth and regeneration, while some do not grow at all. The choice of explants material also determines if the plantlets developed via tissue culture are haploid or diploid. Also the risk of microbial contamination is increased with inappropriate explants. Thus it is very important that an appropriate choice of explants be made prior to tissue culture.

The specific differences in the regeneration potential of different organs and explants have various explanations. The significant factors include differences in the stage of the cells in the cell cycle, the availability of or ability to transport endogenous growth regulators, and the metabolic capabilities of the cells. The most commonly used tissue explants are the meristematic ends of the plants like the stem tip, auxiliary bud tip and root tip. These tissues have high rates of cell division and either concentrate or produce required growth regulating substances including auxins and cytokinins. We used spore of *Cheilanthes fragrans* because this fern produce a lot of spore and we can find spore in the four seasons of the year.

2.3. Plant material

Sporophytes of the Cheilanthes fragrans were obtained from in Taq -e Bostan, Shahbazi Mountains of Kermanshah and cultured in a greenhouse at the Razi University.

2.4. Spore germination and gametophyte development

Fresh spores of ferns were domesticated in the laboratory, the best way is shake the frond of this fern on the paper after that collect brown dust contain enough spores. Loop as well as by UV microscopy of spores was examined. Mature sporophylls (frond) were collected, warped in paper bag, and t dried in room temperature for one week to release spore these spores were then transferred to centrifuge tubes and stored at 4◦C in the dark for use. Spores (6 mg) were wetted with 0.1 g/l Tween solution for about 30 min, collected on filter paper, dipped in 70% (v/v) ethanol for 30s, surface-sterilized in 0.1 g/l aqueous mercuric chloride solution for 2–3 min, and rinsed six times (about 3 min per rinse) with sterile distilled water. Finally, spores were rinsed from the filter paper, suspended in 40 ml sterile distilled water (Wu, H., Chen et al., 2009).About 1 ml of spore suspension was distributed with a sterilized pipet onto each Petri plate (9 cm diameter), which contained 20 ml culture medium, and then sealed with saran wrap strips.

3. RESULT AND DISCUSSION

Cheilanthes fragrans is resurrection fern that grows in the mountain, with a height of around 25-10 cm with short rhizome. Fern Class Petridophyta, a member of Adiantaceae family, is a desiccation tolerant fern and

regenerates by spore. Cheilanthes fragrans is a drought-tolerant plant to various physiological, biochemical and morphological characteristics (Ghasempour and Maleki; 2003). Desiccation tolerance is common in lower plants and in mature seeds, but is less common in vegetative tissues of higher plants. Most species of this ferns grow in the gap and Shade of rocks (Gaff and Latz, 1978).Tissue culture requires to understanding the cause of this tolerance. Resurrection plants are able to withstand severe water loss, and some are even able to equilibrate the leaves with air to 0% (v/v) relative humidity (Gaff, 1971). Mature tissue of resurrection plants such as leaves and roots are able to remain in the air-dried state for months by reaching a quiescent state which is comparable with dormancy in seeds in several aspects. Resurrection plants take immediate advantage of rainfall after dry periods: they resurrect, grow and reproduce before other species can do so (Scott, 2000).

Using different concentrations of MS medium in the following research is confirming.

Effects of light, macronutrients strength of Murashige and Skoog (MS), in the culture medium on spore germination and gametophyte development of Adiantum Cheilanthes fragrans were investigated our result showed new approach for that how we can doing tissue cultur of fern to find out cycle life of fern . Additionally, sporophyte formation and early growth of gametophytes in a medium consisting of clay and peat (v/v = 1:2) was higher than those in a medium consisting of natural habitat. These findings indicated that requirements for nutrients for spore germination and early gametophyte development of Cheilanthes fragrans.

Sinese was relatively low, but these increased with further gametophyte development, formation and growth of sporophytes. The effects of shoot physiological status, medium ingredients, and culture conditions on in vitro floral morphogenesis are species-specific. The ability of explants to form flowers in vitro depends on numerous factors internal and external chemical and physical, all of which virtually interact in various complex and unpredictable ways .In general, spore cultures of ferns require surface sterilization before germination procedures although sterilization affected the ability of the spore to defend itself from fungal infections Sterilization can be done using trophosporophylla and calcium hypochlorite .For tissue culture of Cheilanthes fragrans we can do in vitro tissue culture without some plan regulators for this method we find new fern leaf normal spores in vitro and then mixing them with sand and Centrifuge their 1000 rate and 10 minutes Spores by abrasion and scratch vigor had both grown more scratch, but the number of spores without harming the spores that can be linked when they scratch; spores created in the laboratory and in natural environments, the ability to both grow so they do not need hibernation; Fresh and dried spores, spores were grown in both the presence of cold stress is essential for the growth of fern spores. Cold stress is needed for spore germination.Growth regulators may also use the same GA3 decrease is observed in addition to the very different Prothallus is created so that a string of unusual Protallus Antheridia was seen only. Archegonia not are observed.

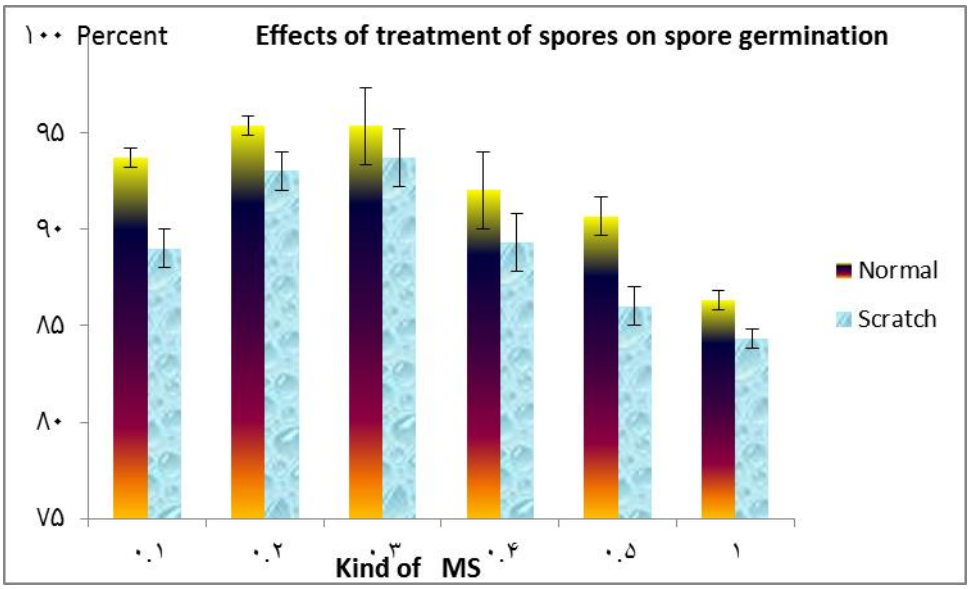
To obtain fresh spores of ferns was domesticated in the laboratory. A solution that is easier on paper than waving fern leaves are brown dust that may contain enough spores. Loop as well as by UV microscopy of spores was examined, Environment needed for growth of spores from the environment was Ms. Spores obtained from the plant for disinfection of spores mixed with water HLO. Fresh and dried spores, spores were grown in both the presence of cold stress is essential for the growth of fern spores. Concentration Of medium in each Petri dish was (20ml) and spore suspension was 1ml Scratched stress isn't needed for spore germination; effects of treatment of spores on spore germination Scratched spore compare normal showed that normal spore germination was 70.6%±1.5 and Scratched 65.7% ±0.6 (Table. 1). Spores and sporangium developed under the leaves. In the early stages sporangium is white، then green and finally turns to brown (Fig. 1) The results demonstrated that: spores can grow no scratches easily, and when scraping due to damages caused by reducing levels of a Prothallus. Growth regulators may also use the same scratches decrease is observed in addition to the very different Prothallus is created so that a string of unusual Protallus was seen. (Graf. 1) portals developing showed that Sporulation and spore germination in the tissue culture of Cheilanthes fragrans that crate normal spore with normal surface so that is more effective because grow of spore in this type of fern depend on water and we found that rainfall for this plant not regular (Fig. 2)

Fig. 1. Stages of sori formation

Fig. 2. Effects of treatment of spores on spore germination

Table. 1

MS (l) kind	Percent growth prothallus in Without Scratch					Percent growth prothallus in With Scratch (Normal)				
	Repetition			Average	Error	Repetition			Average	Error
0.1	24	22	20	22	±2	28	26	30	28	±2
0.2	66	64	64	64.6667	±1	69	71	72	70.66667	±1.5
0.3	66.2	65	66	65.7333	±0.6	70	68	66	68	±2
0.4	35	37	36	36	±1	38	36	37	37	±1
0.5	32	30	32	31.3333	±1	33	33	34	33.33333	±0.5
1	21.6	22	21	21.5333	±0.5	23	22	23	22.44447	±0.5



Graph. 1. For total number of growing portal from laboratory spore scratched.

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