



NRRI Research Bulletin No. 19

# Azolla Germplasms at NRRI

Conservation,  
Characterization  
and Utilization

Upendra Kumar and AK Nayak



भाकृअनुप - राष्ट्रीय चावल अनुसंधान संस्थान

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ICAR - National Rice Research Institute

Cuttack- 753 006, Odisha, India







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Cuttack- 753 006, Odisha, India





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## Foreword



*Azolla* is an aquatic fern that grows on the surface of fresh water ponds, lakes or streams. It is considered as “Green Gold Mine” because of its potential use as bio-fertilizer, compost, livestock feed, bio-accumulator of heavy metals for waste-water treatment and bio-fuel. It is fast-grow fern with high nutritional value and nitrogen-fixing ability. Presence of symbiotic cyanobionts in dorsal leaf cavity of *Azolla* enables it to fix atmospheric nitrogen and thus serves as potential biofertilizers in agricultural crops including rice.

One hundred and two strains of *Azolla* germplasms are collected from worldwide and are being maintained at ICAR-NRRI, Cuttack, Odisha since 1975. The bulletin entitled “*Azolla* Germplasms at NRRI: Conservation, Characterization and Utilization” provide glimpses of *Azolla* germplasms collection at NRRI; their morphological features to distinguish different strains of *Azolla* through microscopic pictures and significance, cultivation practices and various use of *Azolla* in agriculture and industries.

I appreciate the efforts of the authors in bringing out this bulletin and hope that farmers, researchers, students, planners and extension agents will find this publication useful.

(H Pathak)  
Director, NRRI

Place: Cuttack, Odisha  
Date: 23<sup>rd</sup> May, 2019





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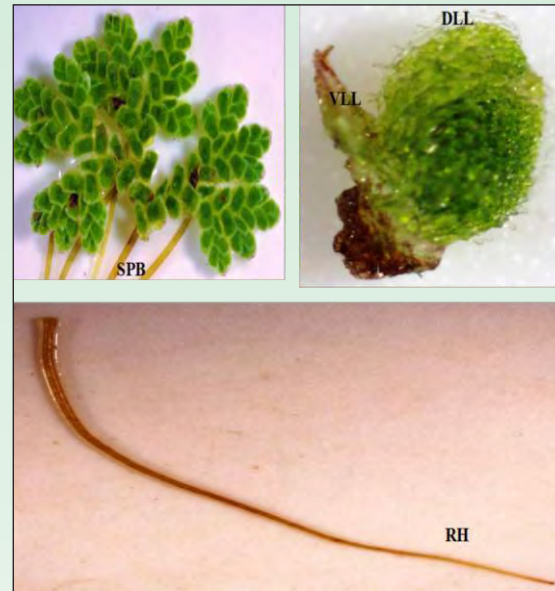
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### Non-sporocarp producing strains

#### UYCC-2



- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. caroliniana*



Non-sporocarp producing strains

## Preface

*Azolla* is a genus of aquatic small floating fern and grouped into two sections namely Euazolla (*A. caroliniana*, *A. filiculoides*, *A. mexicana*, *A. microphylla* and *A. rubra*) and Rhizosperma (*A. pinnata* and *A. nilotica*). *Azolla* is named as “Green Gold Mine” because of possessing multifaceted characters which made it dominance in the field of agriculture and industries, and “Superorganism” due to symbiotically presence of cyanobionts in its dorsal leaf cavities that helps *Azolla* to enable it to fix atmospheric nitrogen (N) upto 1100 kg of N ha<sup>-1</sup> year<sup>-1</sup>, the highest in all kinds of biofertilizer available to date.

We are maintaining, conserving and utilizing a total of 102 strains of *Azolla* germplasms at ICAR-NRRI, Cuttack, Odisha and these were collected from different countries of the world. While maintaining large number of germplasms, we were facing difficulties to differentiate those strains which belonged to section either Rhizosperma or Euazolla, hence an attempt was made to characterize those stains to distinguish at species level with ease.

The present bulletin is an effort to explore the differentiation of 102 strains of *Azolla* sp. through a unique set of morphological markers. Besides, we also try to highlights the significance and various role of *Azolla* in agricultural crops including rice.

We are expressing our sincere gratitude to all the researchers who have been associated with the collection and conservation of *Azolla* since its inception at the ICAR-National Rice Research Institute, Cuttack.

Authors



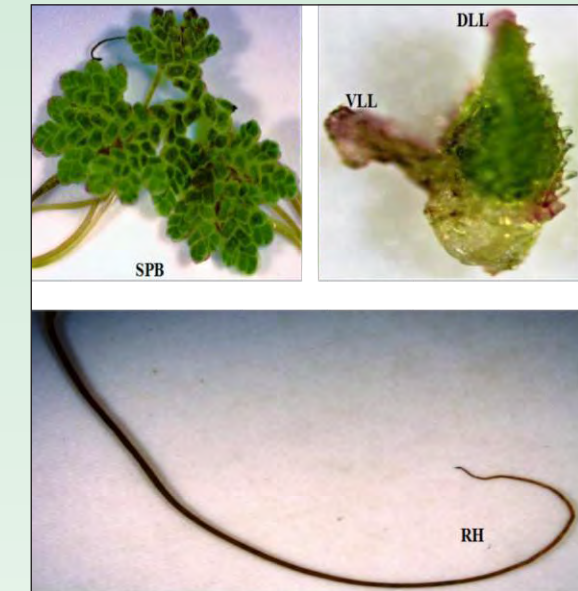




### Non-sporocarp producing strains

SLPI-2

USMM-2



- Star shaped branched floating stem and highly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

- Star shaped branched floating stem and highly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. mexicana*

Resembled as .....*A. mexicana*

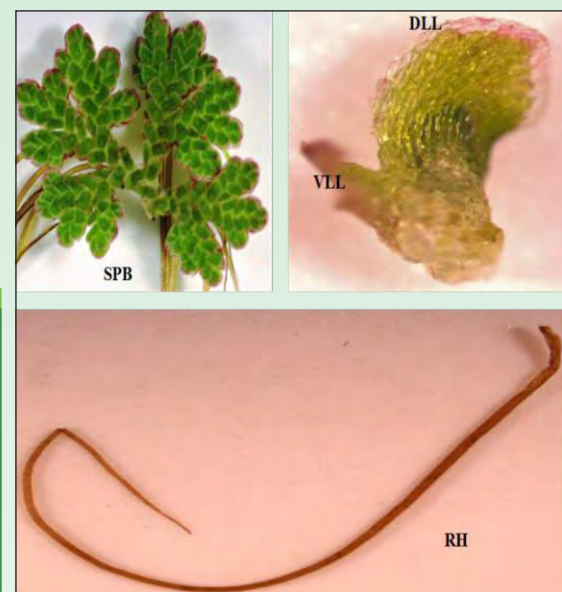
Non-sporocarp producing strains





### Non-sporocarp producing strains

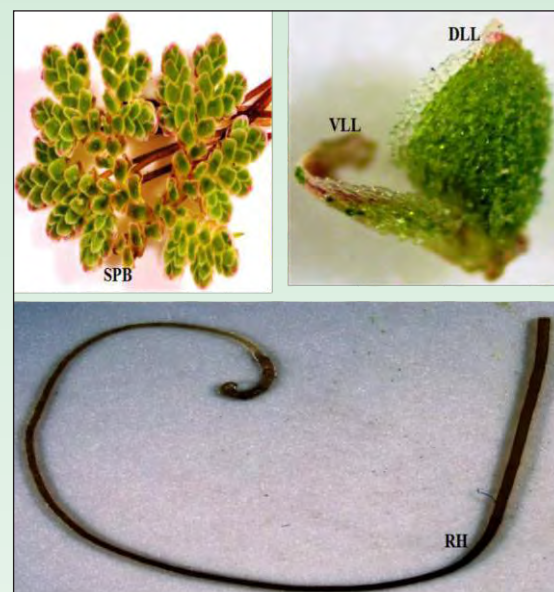
**R-99**



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

**R-100**



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

Non-sporocarp producing strains

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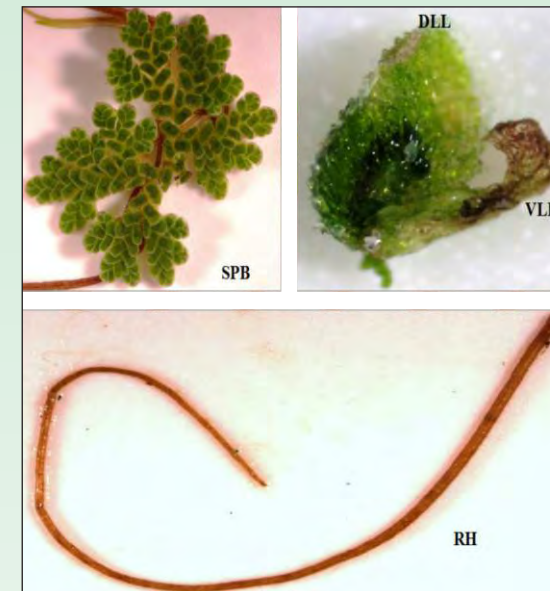
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## Terminology

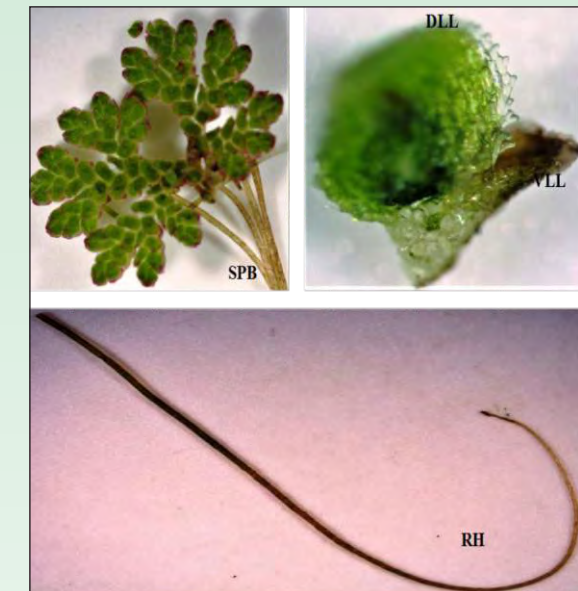
Fron	Leaf and leaflike part of <i>Azolla</i>
Acuminate	Tapering end of the dorsal leaf lobe of <i>Azolla</i>
Rhizomes	Planar stem from which leaves produced dorsally and roots ventrally
Sporophytic plant body (SPB)	The plant body which produces haploid spores (micro and megasporocarp)
Vegetative plant body (VPB)	The plant body includes roots, stems and leaves used for asexual reproduction
Dorsal leaf lobe (DLL)	The upper thick and photosynthetically active lobe among the two leaf lobes of <i>Azolla</i>
Ventral leaf lobe (VLL)	The thin lower leaf lobe of <i>Azolla</i>
Root hairs (RH)	Large number of elongated macro and microscopic outgrowth from the outer layers of cells in a root
Sporocarp (SC)	Structures within which the spores are produced either in the form of microsporangia (microsporocarp) and megasporangia (megasporocarp)
Microsporocarp	Male spore producing structure
Megasporocarp	Female spore producing structure
Trichomes	Small unicellular and glandular outgrowth of epidermis of leaf
Indusium	Tip or cap of the megasporangium
Imbrication	Overlapping of leaves of <i>Azolla</i>
Massula	A hardened layer of cytoplasm formed around the maturing microspore of <i>Azolla</i>
Glochidia	Tubular outgrowths with anchor shaped tips of microspore to cling to the female megaspores for facilitating the fertilization.
Involucre	Sporocarps are attached to the plant by short stalks and, prior to maturation, remain enclosed by a common, single celled protective layer which originates from the dorsal leaf lobe
Planar	Branched floating stem of <i>Azolla</i> on the plane surface

## Non-sporocarp producing strains

R-97



R-98



- |   |   |
|---|---|
| <ul style="list-style-type: none"> <li>▪ Star shaped branched floating stem and highly imbricated leaves</li> <li>▪ Rounded dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe</li> <li>▪ Thin root hairs on the entire root length</li> <li>▪ Sporocarp not produced in natural climatic condition at Cuttack, Odisha</li> </ul> | <ul style="list-style-type: none"> <li>▪ Star shaped branched floating stem and highly imbricated leaves</li> <li>▪ Rounded dorsal leaf lobe and acute angled with translucent ventral leaf lobe</li> <li>▪ Thin root hairs on the entire root length</li> <li>▪ Sporocarp not produced in natural climatic condition at Cuttack, Odisha</li> </ul> |
|---|---|

Resembled as .....*A. caroliniana*

Resembled as .....*A. caroliniana*

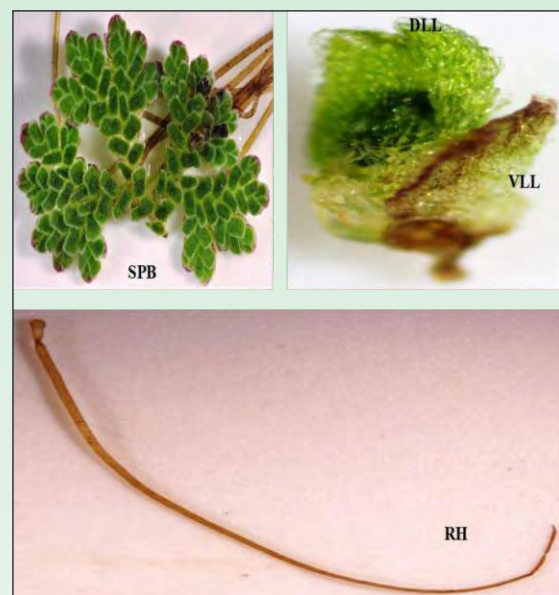
Non-sporocarp producing strains





### Non-sporocarp producing strains

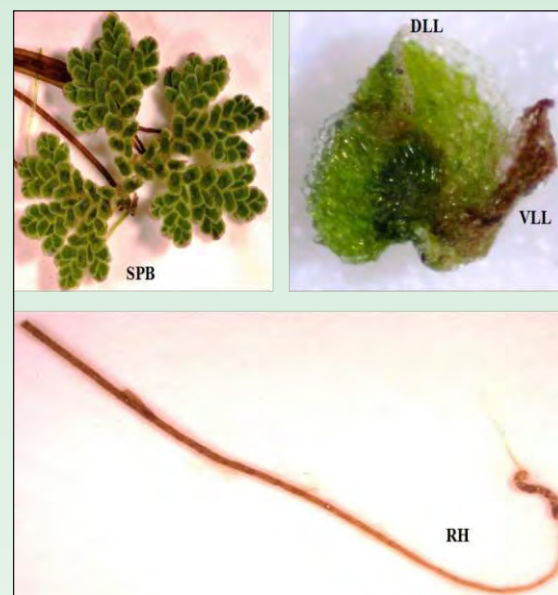
**R-95**



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

**R-96**



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

Non-sporocarp producing strains



### Introduction

*Azolla* is considered to be mysterious fern as geologists believed that it helped in cool down the warmth earth's temperature which persisted over 50 million years ago. *Azolla* bloom was the main cause for this transition which lasted for 800,000 years, where the *Azolla* plants sucked up the atmospheric CO<sub>2</sub> making it absent to act as green house gas and thus decreased the global temperature. This great event is commonly known as '*Azolla* event' which has been even portrayed by fossil records dating around early to mid- Cretaceous period (Kempen et al., 2012). *Azolla* has seven extant species which are grouped into two subgenus *Azolla* and *Tetrasporocarpia* (*A. nilotica* De Caisne) on the basis of morphology and reproductive structures. Under subgenus *Azolla* two sections are grouped together (i) Rhizosperma (*A. pinnata* R. Brown) and (ii) Euazolla (*A. caroliniana* Willdenow, *A. filiculoides* Lamarck, *A. mexicana* Presl., *A. microphylla* Kaulfuss and *A. rubra* R. Brown) (Carrapiço, 2006; Carrapiço, 2010a).

*Azolla* is a tiny elegant water fern which grows on the surface of stagnant freshwater ditches. However, according to the map of Small and Darbyshire (2011), modern distribution of present *Azolla*'s species had the following occurrences prior to their dispersal by humans, result of invasive nature. *A. caroliniana*: eastern North America, and the Caribbean; *A. filiculoides*: southern South America through western North America and Alaska; *A. microphylla*: tropical and subtropical America; *A. mexicana*: northern South America through western North America; *A. nilotica*: upper reaches of the Nile to Sudan; *A. pinnata*:

most of Asia and the coast of tropical Africa.

*Azolla* germplasm collection was initiated at National Rice Research Institute (NRRI) since 1975. Altogether 102 strains belonging to six major species (*A. caroliniana*, *A. filiculoides*, *A. mexicana*, *A. microphylla*, *A. pinnata* and *A. rubra*) of *Azolla* are continuously maintained till date at Microbiology net house of Crop Production Division, NRRI, Cuttack (Odisha), India (Kumar et al., 2019). These germplasms were collected from all over the world including different states of India and are being currently maintained in both soil-based (in earthen pots) and in hydroponics conditions (in glass beakers) (Kar et al., 1999; 2000).

*Azolla* requires some macro and micro nutrients for its growth. In soil based condition, they get those nutrients directly from the known amount of soil which is being replaced in every 3-4 months from each earthen pots. Mature *Azolla* germplasms are being thinned at fortnight interval from each earthen pot. Whereas in the hydroponics medium, an optimum levels of these nutrients are provided by exogenously for better growth of each *Azolla* germplasms in glass beakers and being thinned at the interval of 25-30 days.

Among these nutrients, macro-elements are phosphorous (P), potassium (K), calcium (Ca) and magnesium (Mg), where as micro-elements are copper (Cu), zinc (Zn), molybdenum (Mo), boron (Bo), manganese (Mn) and iron (Fe). Some pests also affect the *Azolla* species that can be effectively controlled by application of carbofuran and other insecticides such as BHC, phorate and thimet (Pereira and Carrapiço, 2009).

Some strains of *Azolla* species sporulate



under specific climatic conditions. At NRRI, Cuttack, almost all strains of *A. pinnata* and *A. microphylla* are forming sporocarps during November to March of each year, when day length is short and temperature is relatively low at night. P-deficiency and overcrowding are one of the reasons to enhance sporulation (Kar et al., 2000).

Isolation of *Anabaena* was attempted along with algal-free *Azolla* was produced by sequential treatment of antibiotics and characterized. A new association between alga-free *Azolla* and *Aphanothece* sp. was developed (Forni et al., 1991; Satapathy and Chand, 2008).

The taxonomy of *Azolla* is a long-term debate within the scientific community. It is easy to identify the two sections of *Azolla* (Rhizosperma and Euazolla) based on morphological features but there are very limited morphological markers which can distinguish the differences of species within these two sections. Hence, in the present bulletin, an attempt was made to differentiate the 102 strains of *Azolla* sp. through a unique set of morphological markers. Besides, we also highlighted the significance and various role of *Azolla* in agricultural crops including rice.

### Significance of *Azolla*

#### *Azolla*: A 'Green Gold Mine'

*Azolla* is known to be 'green gold mine' as it is commonly used as azobiofer in rice and also used in various fields such as livestock feed, waste water treatment, azobiofuel, mosquito control, weed control, helps in removal of contaminants and heavy metal pollutants. It also used as antimicrobial drugs for bacteria and fungi. It also has other uses like in medical purpose

against dental caries and as a component in a space diet (Ahluwalia et al., 2002; Carrapiço, 2010a).

#### Important role of *Azolla* in different commercial fields

##### *Azolla* as azobiofer

*Azolla* has a unique characteristic of fixing environmental nitrogen, which fixes 1100 kg N ha<sup>-1</sup> year<sup>-1</sup> whereas legumes fixes 400 kg N ha<sup>-1</sup> year<sup>-1</sup> (Huang et al., 2010). This helps it to be known azobiofer in paddy, coffee and even in integrated farming. The research on use of *Azolla* as biofertilizer in rice field was started at National Rice Research Institute, Cuttack since 1975 (Singh and Subudhi, 1978; Singh et al., 1981). Although the early societies recognized the efficiency of *Azolla*, almost 2000 years back before the concept was successfully converted into effective practice (Kumar et al., 2015).

##### *Azolla* as livestock feed

The use of *Azolla* as livestock feed is due to its high nutritional value where it contains proteins, essential amino acids, vitamins and minerals. It also contains 4.8-6.7% dry weight crude fat, with 6.1-7.7% and 12.8-26.4% total fat for the polyunsaturated acids omega 3 and omega 6, respectively (Kumar et al., 2019). Hence, it is used in poultry, aquaculture, and cattle feeding, where they easily digest them (Chandewar et al., 2017).

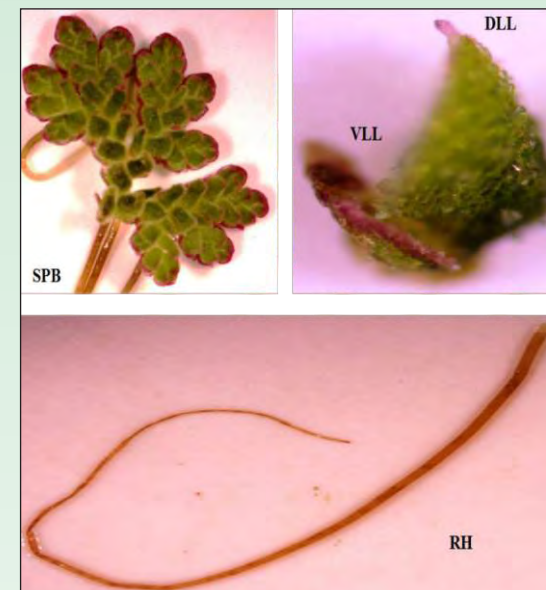
##### *Azolla* as wastewater treatment

*Azolla* has ability to thrive well in partially treated domestic wastewater and in effluents from wastewater stabilization ponds despite the high ammonium content of the medium. This confirms the use as bio-filter for the removal of both phosphorous and nitrogen (Golzary et al., 2018).



### Non-sporocarp producing strains

**R-90**



**R-87**



- Star shaped branched floating stem and highly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

Resembled as .....*A. rubra*

Non-sporocarp producing strains





### Non-sporocarp producing strains

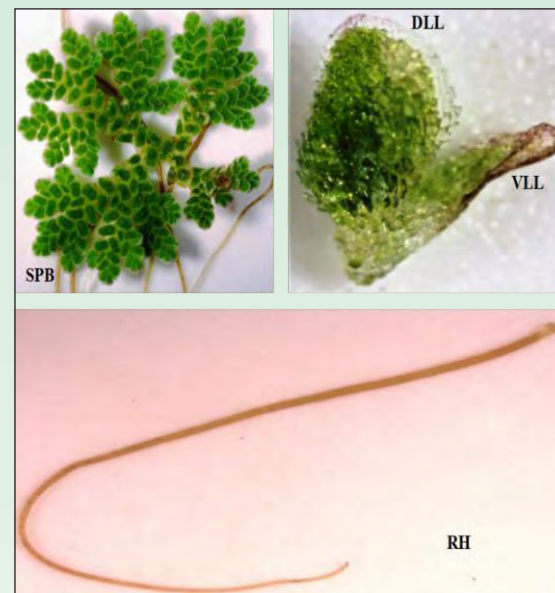
**R-83**



- Star shaped branched floating stem and moderately imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. microphylla*

**R-93**



- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. caroliniana*

Non-sporocarp producing strains



#### **Azolla as antimicrobial drug**

*Azolla* has antimicrobial ability mainly against several bacteria and fungi. Reports indicated that the organic and aqueous extract of some *Azolla* species shows positive result against infections caused by gram positive bacteria and fungus (*Candida albicans*), thus it acts as complementary antibacterial and antifungal drugs respectively (Abraham et al., 2015; Pereira et al., 2015).

#### **Azolla as anti dental caries**

The phytochemicals present in organic and aqueous extract of some *Azolla* species showed highly inhibitory effect against oral isolates which proved it can be a complementary medicinal treatment against dental caries (Selvi et al., 2017; Kekuda, 2014).

#### **Azolla as azobiofuel**

*Azolla* has a unique chemical composition which includes cellulose, hemicellulose, starch and lipids which tends it as renewable bio-fuels under the name of azobiofuel. Several workers also suggested that *Azolla* has potential economical raw material source for bio-diesel production due to its readily availability and probable low cost. Other studies are also done showing its potential as a biogas, hydrogen fuel source and as a source of bio-oil (Miranda et al., 2016).

#### **Azolla: A 'superorganism'**

*Azolla* is perpetually associated with a cyanobiont which gives a distinct uniqueness from other organisms (Kumar et al., 2019). Cyanobiont and *Azolla* evolve continuously together generation to generation without any interruption inside the leaf cavity of vegetative plant body and

the indusium cap of megasporocarp of sporophyte. One of the studies showed that other than cyanobiont, bacteriobionts are also present in all stages of leaf development in close association with the primary branched hairs, or *Azolla's* epidermal cells. Thus, the relation of *Azolla*-Cyanobiont-Bacteriobionts makes it as 'superorganism' (Carrapiço, 2010a).

#### **Cultivation of Azolla**

##### **Azolla production by inoculum**

*Azolla* production is done by increasing biomass from inoculums which is the easiest way to cultivate. As *Azolla* multiplies faster, hence its cultivation becomes easy and even becomes more comfortable for an ordinary farmer. There are mainly two methods of *Azolla* multiplication: (i) standing water method and (ii) nursery method (Mandal, 2018).

##### **Standing water method**

Under this method, a pond or a field with shallow standing water is chosen. The depth of water required for *Azolla* cultivation varies between 5-10 cm. For the rapid growth of *Azolla*, application of super phosphate (4-8 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) is recommended. Once the bed is ready 100-200 g of *Azolla* inoculum is introduced into standing water. *Azolla* multiplies to form a carpet on the water surface within three weeks, which can be collected and used immediately or dried and preserved for later use. The process is repeated to produce more *Azolla* culture (Senthilkumar and Manivannan, 2016).

##### **Nursery method**

*Azolla* can also raised in small nursery plots of 50-100 m<sup>2</sup> size with strong bunds all around so that water can be stand up to a





height of 5-10 cm. However, in a newly constructed nursery plot retaining water is a problem due to high percolation rate. To control this, puddling is adopted in the paddy field. Compacting the soil can also control percolation. Plastering the bottom and sides with a mixture of cow dung and fine clay is yet another effective method of controlling percolation. Permanent *Azolla* nurseries can

be constructed with brick and cement. Spreading polythene sheets at the bottom of the nursery beds can also control percolation. Small nursery beds are advantageous compared to large plots as wind causes drifting of *Azolla* towards one side in large plots (Mandal, 2018).



**Method of utilization of *Azolla***

*Azolla* could be used as both green manure (compost) and dual crop with rice, but dual cropping (inter-cropping) was more practicable and economical.

***Azolla* as compost**

*Azolla* biomass is incorporated into the field either directly or in dried form. When it is incorporated directly into the soil, it decomposes rapidly within 7-10 days. However, nitrogen availability extends from one week to ten weeks. Experiments have shown that 34% of the total nitrogen is available two weeks after incorporation,

63% after 4 weeks, 76% after 6 weeks and 85% after 8 weeks. Application of *Azolla* in the green form produces better result than dry form. This green manuring supplied 20-40 kg N ha<sup>-1</sup> to rice crop (Singh et al., 1981).

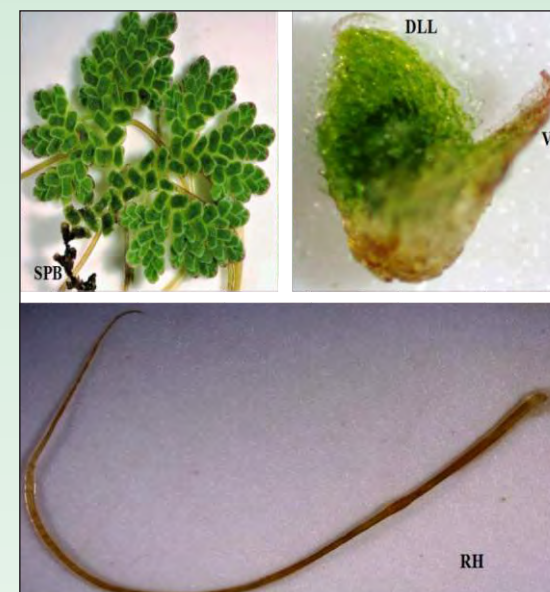
***Azolla* as inter-crop**

One week after the planting of rice seedlings, fresh *Azolla* at the rate of 200-300 g m<sup>-2</sup> should be applied in standing crops. *Azolla* biomass is formed in three weeks. Water is then drained out and *Azolla* is incorporated into the soil using implements. Split application of super phosphate at 8-10 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and carbofuran at 75-90 g ha<sup>-1</sup>

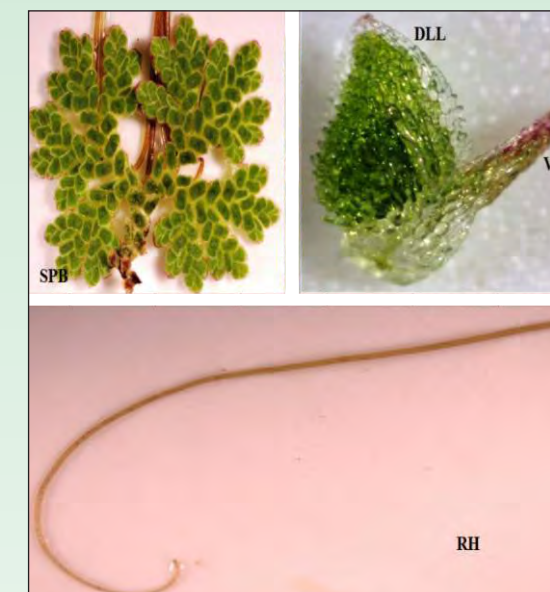


**Non-sporocarp producing strains**

**R-67**



**R-82**



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

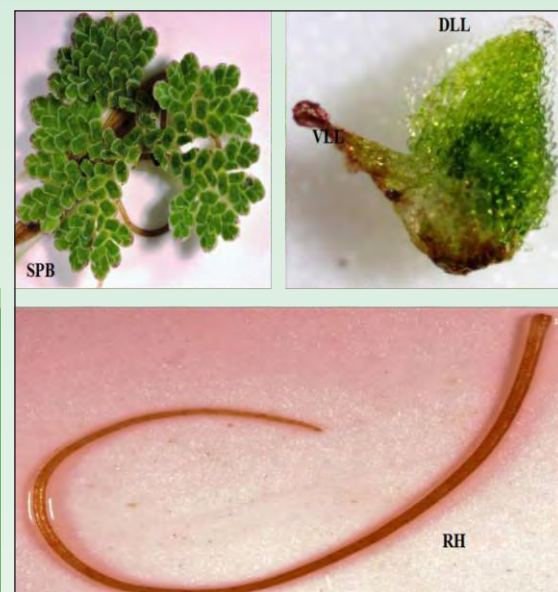
Non-sporocarp producing strains





### Non-sporocarp producing strains

**R-57**



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

**R-65**



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

Non-sporocarp producing strains



ensured rapid fern growth. The requirement of Phosphorus to *Azolla* could be met from recommended dose of P for rice by applying half the dose of P during *Azolla* cultivation. Dual cropping supplied 20-30 kg N ha<sup>-1</sup> (Singh and Singh, 1990).

#### Azobiofer in Rice cultivation

Azobiofer used in the paddy field either as monocrop or as intercrop. It is efficiently used in the countries of Asia (Yadav et al., 2014). Reports indicated that azobiofer enhanced the rice yield by 23%, 112% and 216% over unfertilized controls when applied as a monocrop, intercrop with rice, both as a monocrop and an intercrop, respectively (Peters et al., 1978; Roy et al., 2016; Ripley et al., 2003). *Azolla* has quick decomposition rate in soil and thus it speeds up the efficient availability of its nitrogen to rice plant. The quick multiplication rate and rapid decomposing capacity of *Azolla* have become an important factor to use as green manure cum bio-fertilizer in the rice field. *Azolla* suppresses the weeds in rice field by



forming a thick mat which is its great advantage (Cheng et al., 2010; Janiya and Moody, 1984). The azobiofer basal application (10-12 t ha<sup>-1</sup>) increases soil nitrogen by 50-60 kg ha<sup>-1</sup> and reduces 30-35 kg of nitrogenous fertilizer requirement of the rice crop. The release of green *Azolla*

twice as dual cropping in rice crop (500 kg ha<sup>-1</sup>) enriches soil nitrogen by 50 kg ha<sup>-1</sup> and reduces nitrogen requirement by 20-30 kg ha<sup>-1</sup>. Use of azobiofer increases rice yield by 20-30% (Raja et al., 2012). Besides successful use of azobiofer under paddy cultivation in Asia, it is also spreading the rest of the world like Italy (Bocchi and Malgioglio, 2010) and Africa (Carrapico et al., 2002).

#### Morphology of Azolla

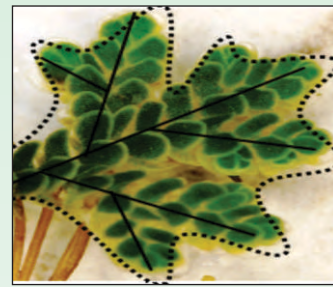
*Azolla* shows two distinct phases in the life cycle: sporophytic and gametophytic; which follow each other in regular succession. In sporophytic phase, *Azolla* reproduces vegetatively and each frond consists of leaves, rhizome and roots. Leaves occur in two rows alongside the rhizome and each leaf has a thin ventral and thick dorsal lobe. This dorsal leaf lobe mainly photosynthesizes which contains chlorophyll a, chlorophyll b, and carotenoids. These pigments mainly give green colour to the plant but sometimes under stress it produces anthocyanin pigment which gives reddish colour. The dorsal lobe has an ellipsoidal cavity, measuring approximately 0.15 × 0.30 mm, which is hydrophobic, that opens to the external environment, located in the adaxial epidermis of the leaf cavity. Within the dorsal leaf lobe, there is a specialized ovoid cavity containing the cyanobacterium, *Anabaena azollae*. The roots are hydrophilic, mainly solitary with or without root hairs grow from the rhizome hanging downwards into the water. These roots are mainly adventitious and chlorophyllous during the early stages. The older roots turn brownish in colour due to partial decomposition and finally gets detaches from the plant. In gametophytic phase, structure is generally consisting of sporophytes which are triangular to polygonal in shape and their diameter ranges between 0.5 cm and 7 cm with heterospores.



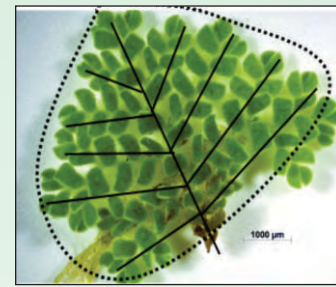


It produces both the male (micro) and female (mega) sporocarps on the same plant. The sporocarps are formed at the lateral-branch points on the ventral side of the sporophytes, replacing the ventral lobe of the first leaf. The sporocarps are attached to the plant by short stalks and prior to maturation; remain enclosed by a common, single celled protective layer called involucre or hood which originates from the dorsal leaf lobe (Carrapiço, 2006).

Megasporocarps of *Azolla* are oval shaped with pointed ends and their size at maturity is about 0.75-1.00 mm in length and 0.5 mm in breadth. Each mega-sporocarp contains a mega-sporangium (consisting of a megaspore and the megaspore apparatus), distally-located colony of *Anabaena* and food reserves. Prior to maturation, the mega-sporocarp is enclosed by a two layered indusium, with a pore located at the distal end. Following maturation and release of the



a. Star shaped branched floating stem



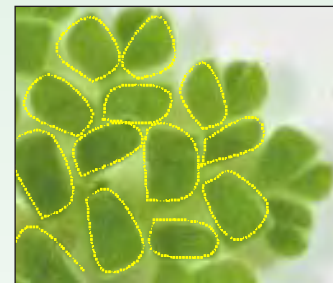
b. Triangular shaped branched floating stem



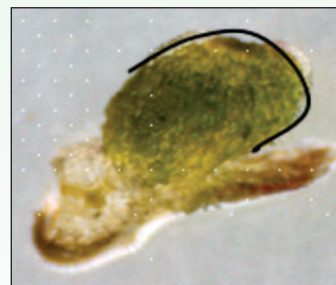
a. Highly imbricated leaves



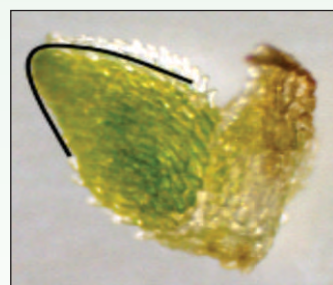
b. Moderately imbricated leaves



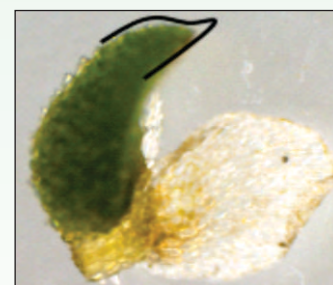
c. Slightly imbricated leaves



a. Highly imbricated leaves



b. Moderately imbricated leaves



c. Slightly imbricated leaves



### Non-sporocarp producing strains

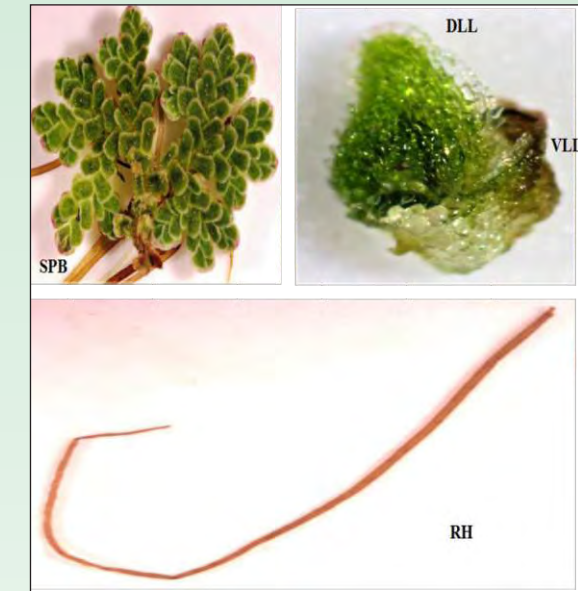
R-59



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

R-63



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

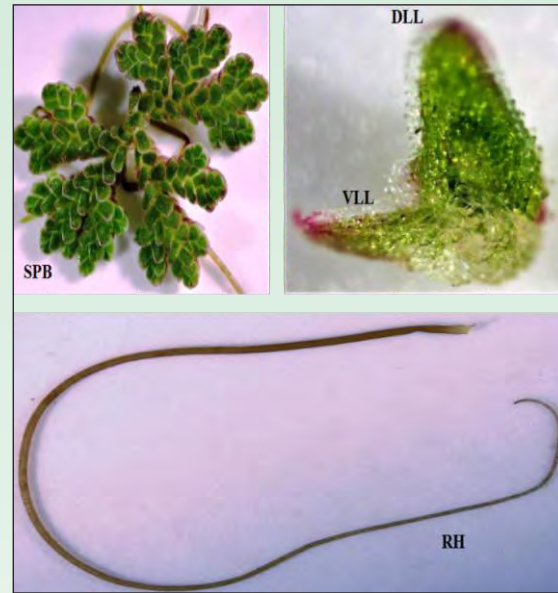
Resembled as .....*A. rubra*





### Non-sporocarp producing strains

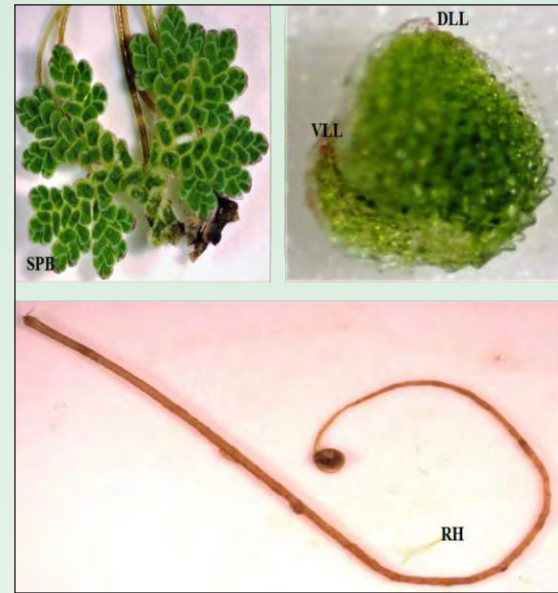
R-56



- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. caroliniana*

R-64



- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

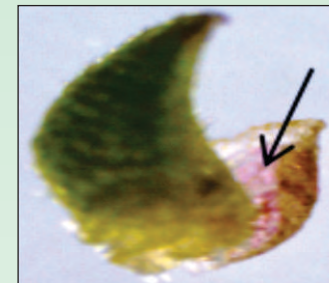
Resembled as .....*A. caroliniana*

Non-sporocarp producing strains

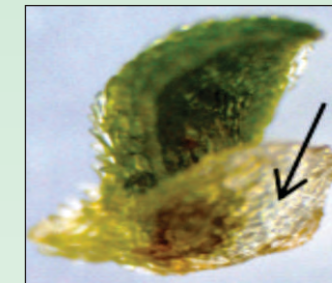


megaspore into water column, the proximal portion hardens, due to the lignification and deposition of tannin. The distal portion remains

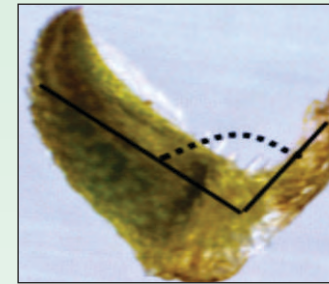
associated with the megaspore apparatus until it gets displaced by the emerging sporophyte embryo (Calvert et al., 1983).



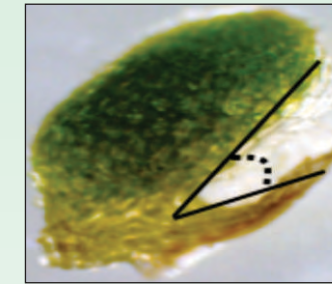
a. Pink tinted ventral leaf lobe



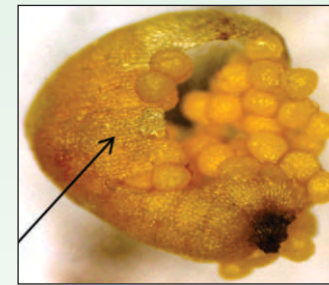
b. Translucent ventral leaf lobe



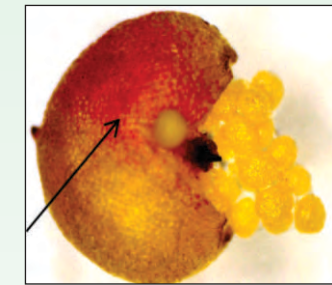
a. Obtuse angled between DLL and VLL



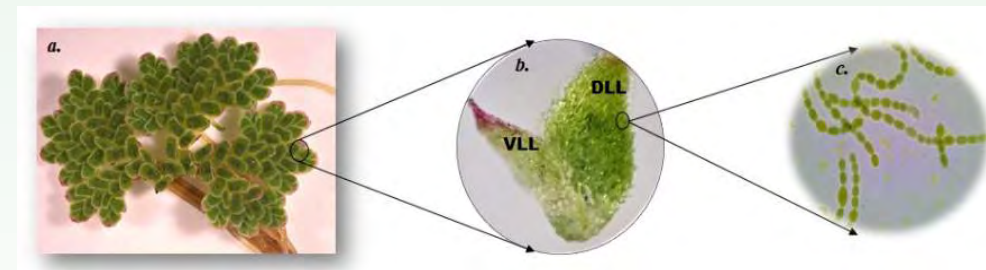
b. Acute angled between DLL and VLL



a. Yellow translucent microsporocarp



b. Red tinted microsporocarp



Overall view of *Azolla* and its cyanobionts- a. *Azolla* frond; b. Dorsal (DLL) and ventral (VLL) leaf lobe of *Azolla* and c. Cyanobacteria present inside leaf cavity of *Azolla*.

Representation of basic structure of *Azolla*



Micro-sporocarps of *Azolla* are globular in shape and yellowish, pink or red in coloured measuring about 2 mm in length and 1.5 mm in breadth. They are surrounded by a membranous indusium, which decays soon after maturation. Each micro-sporocarp contains 50-200 stalked microsporangia. When released into water column, the microsporangia are attached to a placentum by thin stalks. Each fragile mega-sporangium contains 3-10 massulae that are composed of hardened mucilage containing high concentrations of sporopollenin. There are 32 or 64 microspores encased within each massula (Perkins et al., 1985).

### Cytology of *Azolla*

Smallest chromosomes reported among the ferns are in *Azolla* species (Saunders and Fowler, 1993). The report shows chromosomal counts are done for various species that they represent in diploid, triploid, and tetraploid populations. The diploid chromosomal numbers i.e.  $2n = 44$  were reported in *A. caroliniana*, *A. filiculoides*, *A. microphylla*, *A. mexicana*, *A. pinnata*, and *A. rubra* but exceptionally diploid chromosomal count in *A. nilotica* is  $2n = 52$  (Stergianou and Fowler, 1990; Nayak et al., 1989; Saunders and Fowler, 1993).

### Reproduction

Reproduction in *Azolla*, are of two types: vegetative reproduction and sexual reproduction. Vegetative reproduction in *Azolla* is mainly by formation of an independent plant from the oldest lateral branch at the base of the stem by fragmentation and sexually is by producing both male and female gamete, fuse together to form a zygote which then further develops into an individual. Sexual reproduction is not very common and seems to be influenced by

environmental factors (Carrapiço et al., 2010a).

### Fertilization and Germination

The sporocarps after maturation get separated from the mother sporophyte and sink to the bottom of water body. It is not clear whether a dormant period is required prior to the fertilization and germination (Yuan et al., 1987).

Within the mega-sporocarp, the megaspores germinate into the female gametophyte, each of which bears one or more archegonia containing a single egg cell (oocyte). Following attachment of the massulae to the mega-sporocarp, the antherozoids move through the gelatinized massulae to fertilize the oospores with the archegonia. The zygote formation occurs within the megaspore apparatus under water surface (Lumpkin, 1993; Konar and Kapoor, 1974; Perkins et al., 1985).

A fertilization takes place inside the megaspore, the young embryo is protected from external environment. The germination of *Azolla* sporocarps is gently influenced by light and temperature. As the embryo grows, the hardened distal indusium of the megaspore apparatus hinges to one side and the plantlet gets released. It floats on water surface after one or two leaves have been formed and initially grow in the vertical direction. There is no cavity for the symbiont on the initial cotyledons. The *Anabaena* cells surviving under the indusium become entrapped in the shoot apex of the germinating spore and grow in harmony with the fern. A young *Azolla* plantlet attains the full size within 1-2 months (Watanabe, 1982; Perkins et al., 1985).

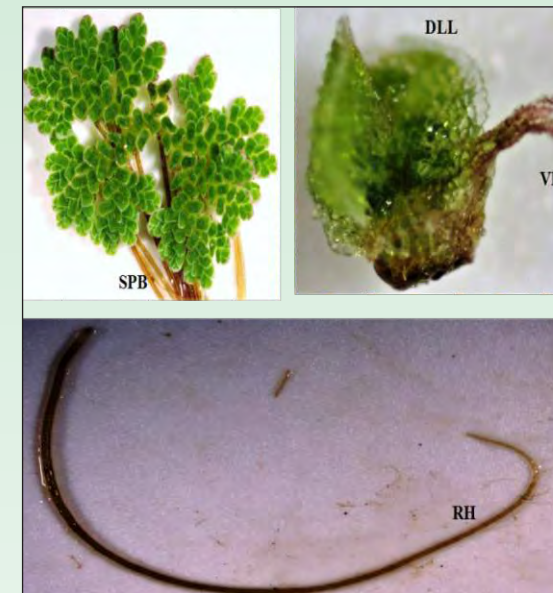
### Life-cycle

Like Bryophytes, Pteridophytes too

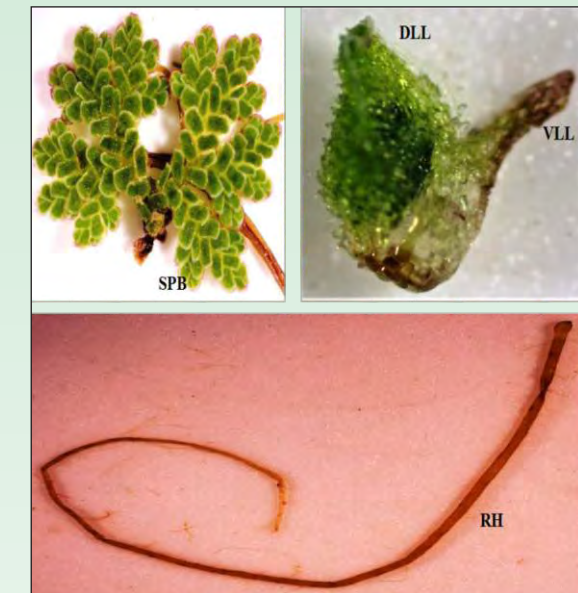


## Non-sporocarp producing strains

**R-54**



**R-45B**



- |  |  |
|--|--|
| <ul style="list-style-type: none"> <li>▪ Star shaped branched floating stem and highly imbricated leaves</li> <li>▪ Highly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe</li> <li>▪ Thin root hairs on the entire root length</li> <li>▪ Sporocarp not produced in natural climatic condition at Cuttack, Odisha</li> </ul> | <ul style="list-style-type: none"> <li>▪ Star shaped branched floating stem and highly imbricated leaves</li> <li>▪ Highly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe</li> <li>▪ Thin root hairs on the entire root length</li> <li>▪ Sporocarp not produced in natural climatic condition at Cuttack, Odisha</li> </ul> |
|--|--|

Resembled as .....*A. rubra*

Resembled as .....*A. rubra*

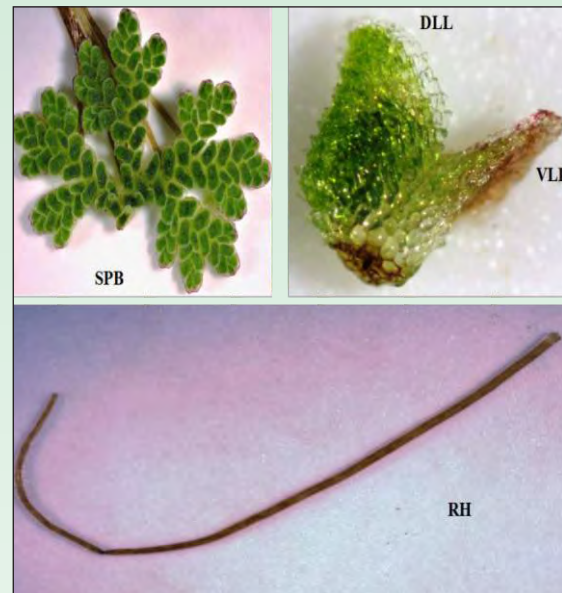
Non-sporocarp producing strains





### Non-sporocarp producing strains

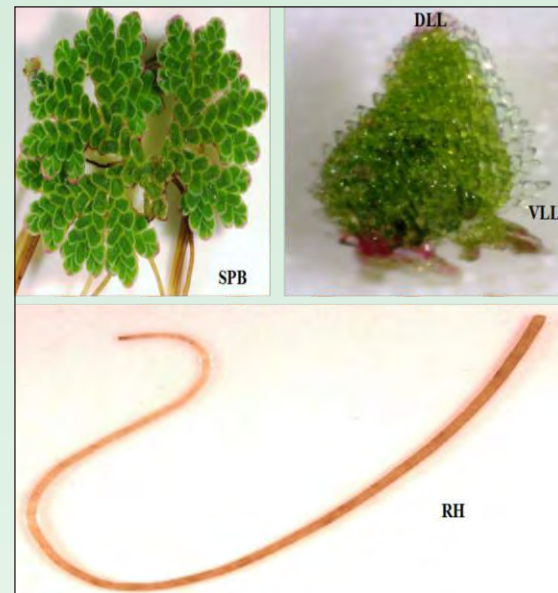
**R-52**



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminated dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

**R-53**



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminated dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

Non-sporocarp producing strains



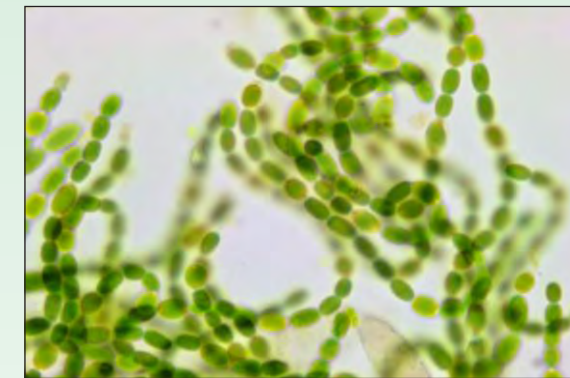
have (Smith and Allen, 1955) two generations life cycles and heteromorphic in nature. Under normal conditions, a gametophyte produces motile male gametes (antherozoids) and non-motile female gametes (eggs). Fusion between an egg cell and antherozoid results in the formation of a zygote which is diploid. The zygote divides into mitotic divisions and forms the sporophyte. On sporophyte, a number of haploid, non-motile spores are produced by meiosis. The life cycle is then completed when a spore germinates and produces a haploid gametophyte by mitotic divisions. In this life-cycle, the sporophyte is independent of the gametophyte and is the dominant generation (Konar and Kapoor, 1974). Hence, *Azolla* also shows the same type of gametophytic as well as sporophytic generations but sporophytic generation is observed less frequently that means sporulation occurs regularly in a few *Azolla* strains but rarely or no sporulation in others (Lumpkin and Plucknett, 1985).

The sexual reproduction involving the production of sporocarps which is observed less frequently but the sporulation occurs regularly in a few *Azolla* and rarely in the others. This sporulation can be induced by application of various hormones. However, the formation of sporocarps is often follows the exposure to adverse conditions (Kar et al., 2002). In temperate region, the sporocarp formation was induced by a combination of high intensity, relatively high temperature and short day length. Whereas in tropical region, a combination of low temperature, low intensity and short day length induces sporulation (Kar et al., 1999).

#### ***Azolla* Cyanobiont**

In 1873, Strasburger named the cyanobacterial endophyte of *Azolla* as

*Anabaena azollae*. The classification and phylogeny of *Azolla* cyanobiont have often been controversial to identifying the cyanobiont as belonging to *Anabaena* or *Trichormus*. On the basis of morphological and biochemical characteristics the endophyte are recorded to be *Anabaena*. However, using molecular DNA probes study showed that the cyanobacterial symbiont of *Azolla* is closely related to Nostocaceae family (Preferably *Cylindrospermopsis* genera under cultivated cyanobionts) (Kumar et al., 2019).



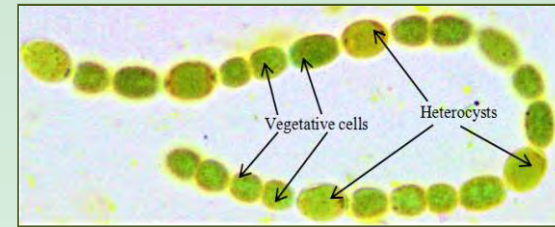
Kingdom	: Eubacteria
Phylum	: Cyanobacteria
Class	: Cyanophyceae
Order	: Nostocales
Family	: Nostocaceae
Genus	: <i>Anabaena</i>
Species	: <i>azollae</i>

The cyanobiont *A. azollae* is a gram negative bacterium having uniform vegetative cells with numerous thylakoid with phycobilisomes, carboxysomes, cyanophycin and glycogen granules. At the apical meristem of *Azolla* has no nitrogenase activity due to absence of heterocysts at the earlier stage. But at the mature cavities, the



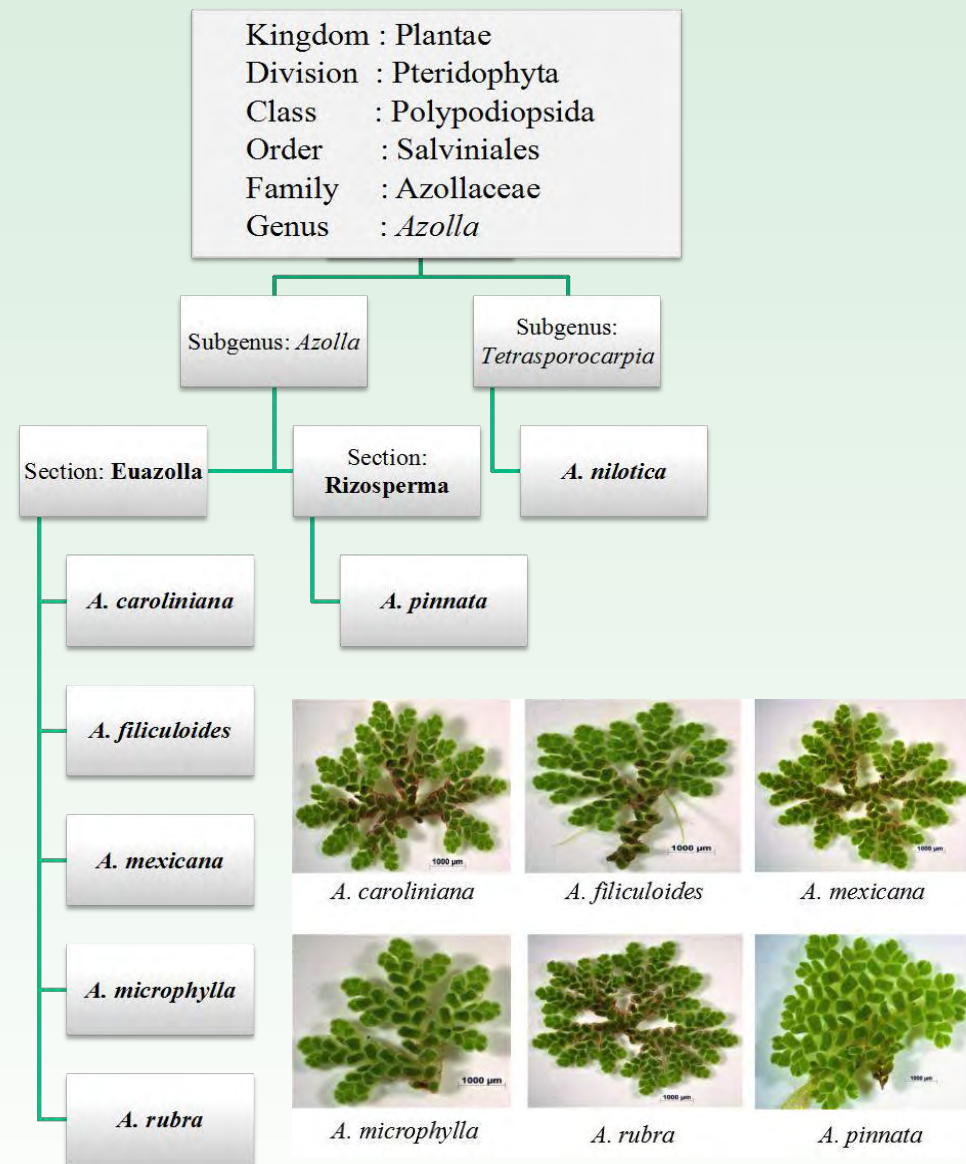


cyanobiont filaments contain vegetative cells with fewer carboxysomes and thylakoid membranes distributed throughout the cytoplasm and heterocysts with nitrogenase activity (Lindblad, 1985). The cyanobiont filaments are dense in mature foliar cavity than the apical cavity. The cyanobiont has chlorophyll a, carotenoids and phycobiliproteins where as



the *Azolla* contains chlorophyll a, b and carotenoids (Pereira and Vasconcelos, 2014).

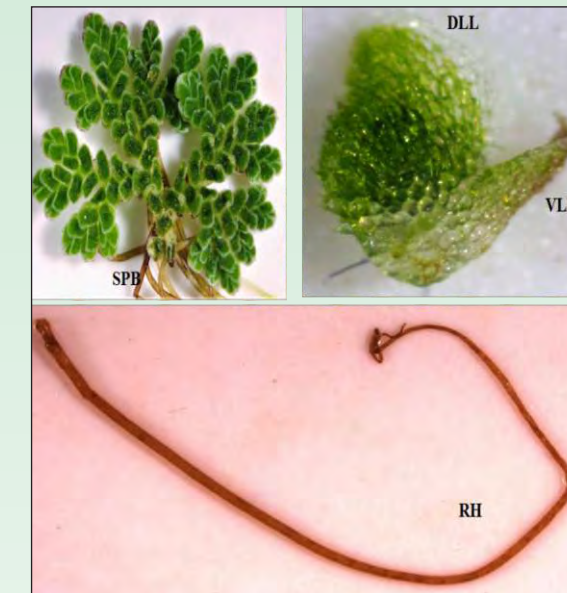
**Azolla Taxonomy**



**Non-sporocarp producing strains**

**R-50**

**R-51**



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

Resembled as .....*A. rubra*

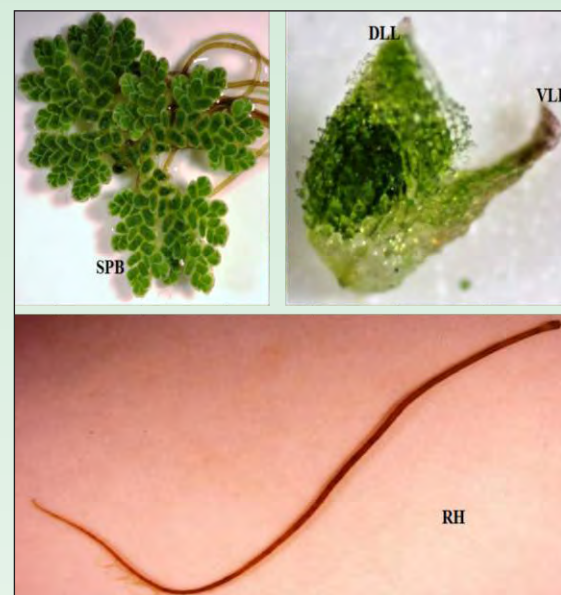
Non-sporocarp producing strains





### Non-sporocarp producing strains

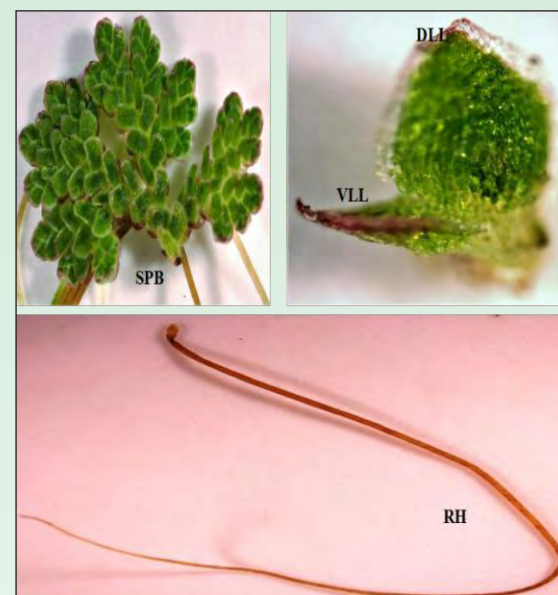
**R-48**



- Star shaped branched floating stem and highly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

**R-49**



- Star shaped branched floating stem and highly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

Non-sporocarp producing strains



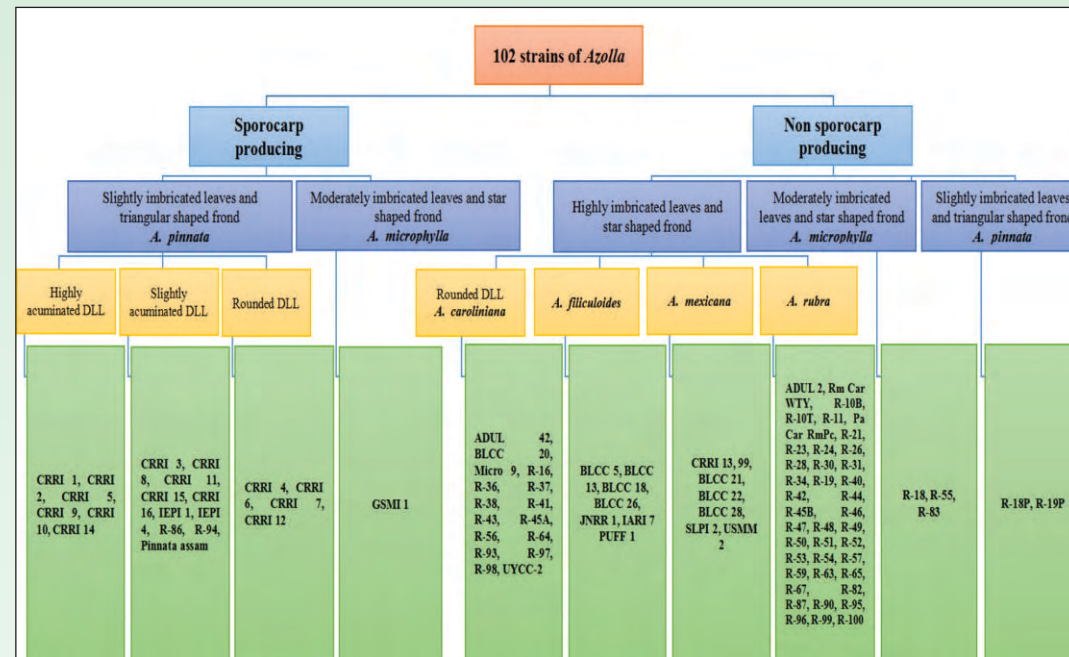
### List of 102 strains of *Azolla* collected from worldwide

(Kar et al., 1999; 2000)

Strain No.	Country	Strain No.	Country
Adul 2	New Zealand	R 26	India
Adul 42	Egypt	R 28	India
BLCC 13	India	R 30- 31	India
BLCC 18	Brazil	R 34	India
BLCC 20- 22	Brazil	R 36- 38	India
BLCC 26	India	R 40- R 44	India
BLCC 28	Brazil	R 45A- R 45B	India
BLCC 5	India	R 46- R 57	Philippines
CRR1 1- CRR1 16	India	R 59	Thailand
GSMI 1	Ecuador	R 63- R 65	Bangladesh
IARI 7	India	R 67	India
IEPI 1	Indonesia	R 82- R 83	India
IEPI 4	Indonesia	R 86	India
JNNR 1	India	R 87	India
Micro 9	India	R 90	India
Pa Car Rm Pc	USA	R 93- 100	India
Pa Car WTY	USA	SLPI 2	Sri Lanka
Pinnata Assam	India	USMM 2	India
PUFF 1	Peru	UYCC 2	India
R 10 B	Nepal	99	India
R 10 T	India		
R 11	India		
R 16	India		
R 18	India		
R 18 P	India		
R 19	India		
R 19 P	India		
R 21	India		
R 23- 24	India		
		<b>Major species</b>	<b>Country</b>
		<i>A. caroliniana</i>	Philippines
		<i>A. filiculoides</i>	Philippines
		<i>A. mexicana</i>	Philippines
		<i>A. microphylla</i>	Philippines
		<i>A. pinnata</i>	India
		<i>A. rubra</i>	Philippines



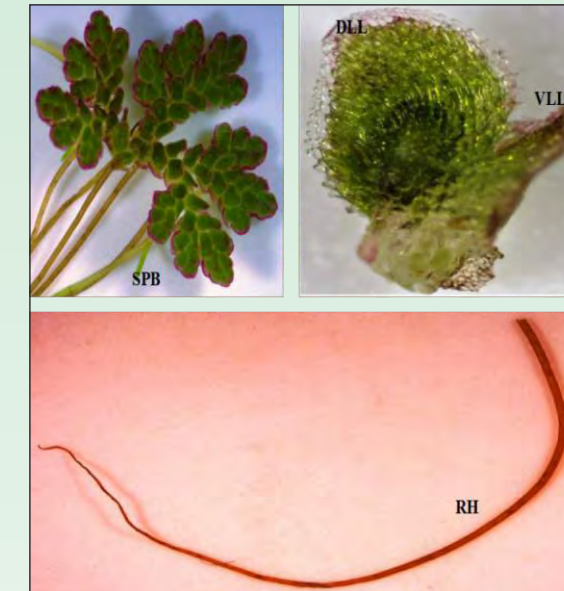
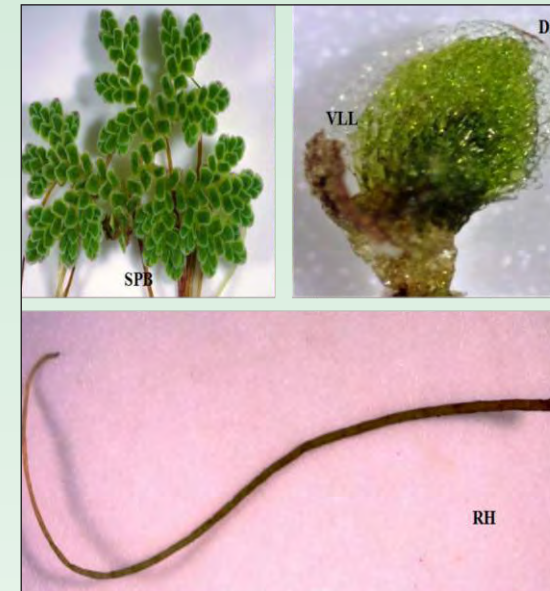
### Segregation of 102 strains of *Azolla* germplasms based on morphological characteristics



### Non-sporocarp producing strains

R-46

R-47



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

Resembled as .....*A. rubra*

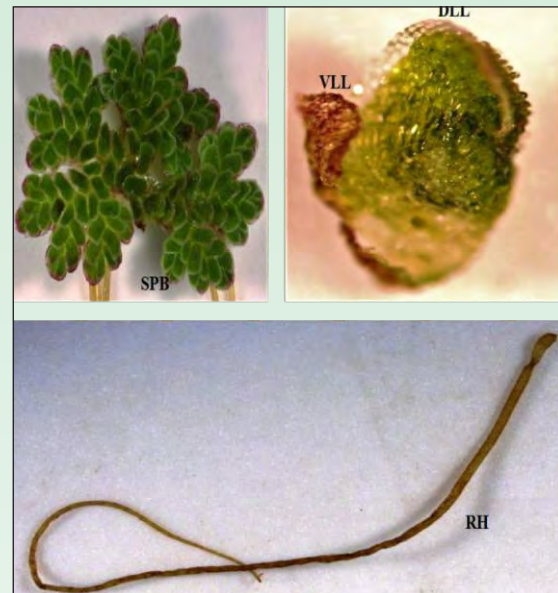
Non-sporocarp producing strains





### Non-sporocarp producing strains

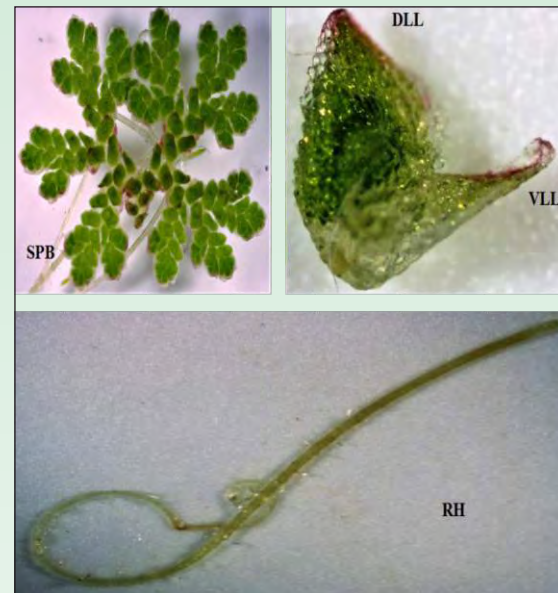
R-45A



- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. caroliniana*

R-55



- Star shaped branched floating stem and highly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

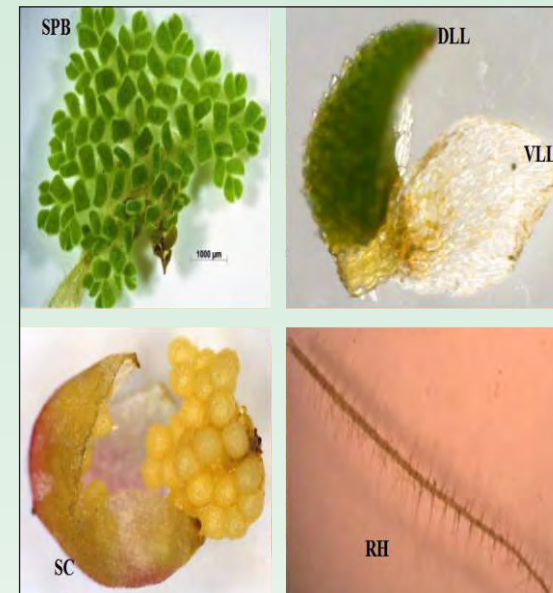
Resembled as .....*A. microphylla*

Non-sporocarp producing strains



### Sporocarp producing major species

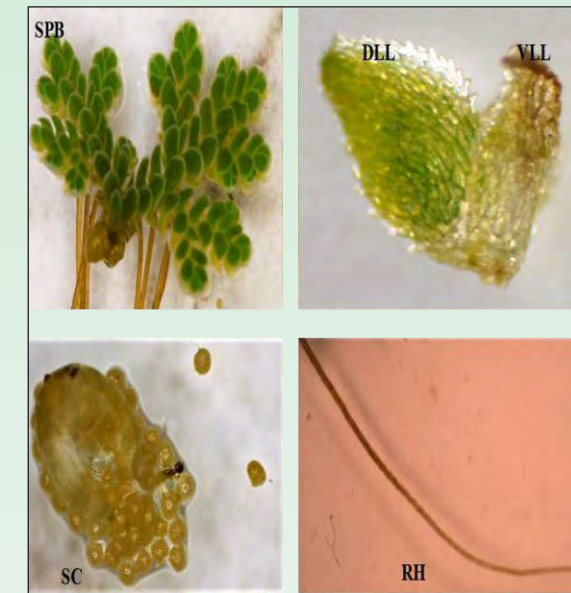
*Azolla pinnata*



- Planar habitat at maturity
- Dorsal and ventral leaf lobes grow upper side of the rhizome
- Triangular shaped branched floating stem with slightly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Sporocarp produced under natural climatic condition of Cuttack, Odisha during month of November to March
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs grow laterally from the side of the entire root length.

.....*A. pinnata*

*Azolla microphylla*



- Planar habitat at maturity
- Dorsal and ventral leaf lobes grow upper side of the rhizome
- Star shaped branched floating stem with moderately imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Sporocarp produced under natural climatic condition of Cuttack, Odisha during month of November to March
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thin root hairs grow laterally from the side of the entire root length

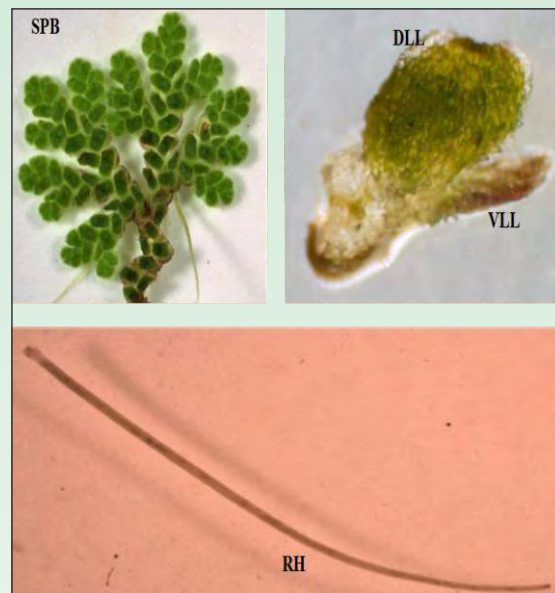
.....*A. microphylla*

Sporocarp producing major species



### Non-sporocarp producing major species

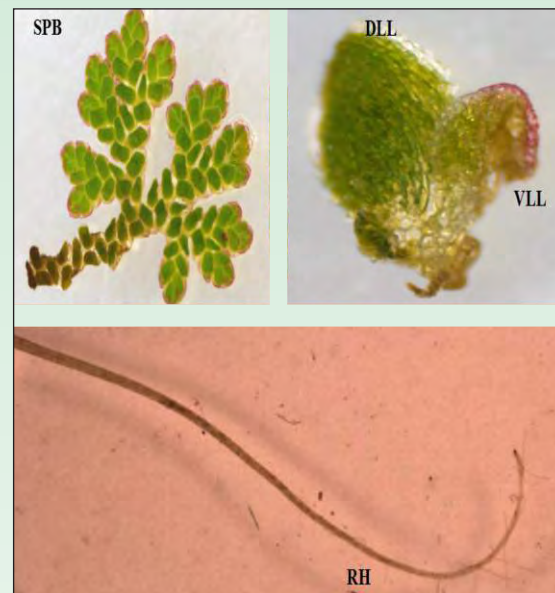
*Azolla caroliniana*



- Planar habitat at maturity
- Star shaped branched floating stem with highly imbricated leaves
- Dorsal and ventral leaf lobes grow upper side of the rhizome
- Round shaped dorsal leaf lobe and acute angled with pink tinted coloured ventral leaf lobe
- Thin root hairs grow laterally from the side of the entire root length
- Sporocarp formation does not take place under natural climatic condition at Cuttack, Odisha

..... *A. caroliniana*

*Azolla filiculoides*



- Planar habitat at maturity
- Star shaped branched floating stem with highly imbricated leaves
- Dorsal and ventral leaf lobes grow upper side of the rhizome
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted coloured ventral leaf lobe
- Thin root hairs grow laterally from the side of the entire root length
- Sporocarp formation does not take place under natural climatic condition at Cuttack, Odisha

..... *A. filiculoides*

Non-sporocarp producing major species



### Non-sporocarp producing strains

R-42



- Star shaped branched floating stem and highly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as ..... *A. rubra*

R-44



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as ..... *A. rubra*

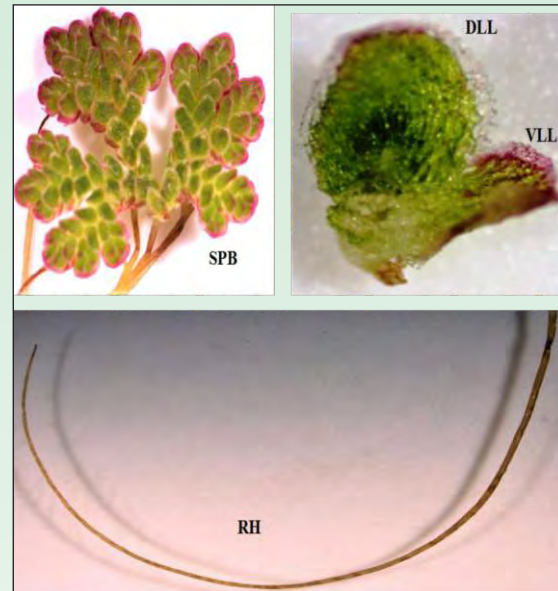
Non-sporocarp producing strains





### Non-sporocarp producing strains

R-41



- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. caroliniana*

R-43



- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

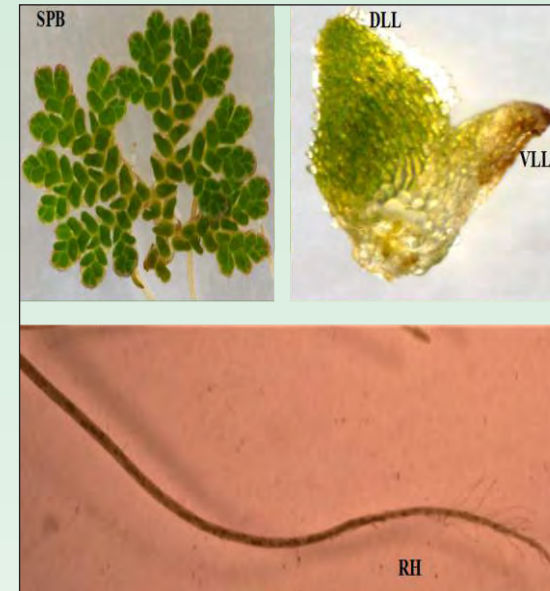
Resembled as .....*A. caroliniana*

Non-sporocarp producing strains



### Non-sporocarp producing major species

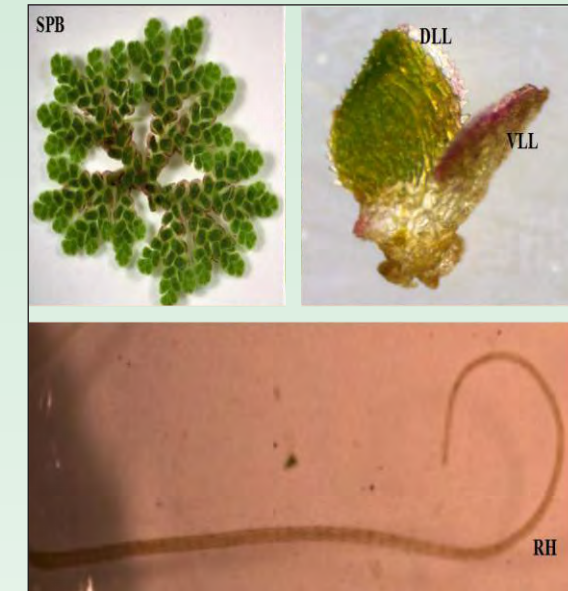
*Azolla mexicana*



- Planar habitat at maturity
- Leaves with dorsal and ventral lobes grow upper side of rhizome
- Star shaped branched floating stem with highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent coloured ventral leaf lobe
- Thin root hairs grow laterally from the side of the entire root
- Sporocarp formation does not take place under natural climatic condition at Cuttack, Odisha

.....*A. mexicana*

*Azolla rubra*



- Planar habitat at maturity
- Leaves with dorsal and ventral lobes grow upper side of rhizome
- Star shaped branched floating stem with highly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with pink tinted coloured ventral leaf lobe
- Thin root hairs grow laterally from the side of the entire root
- Sporocarp formation does not take place under natural climatic condition at Cuttack, Odisha.

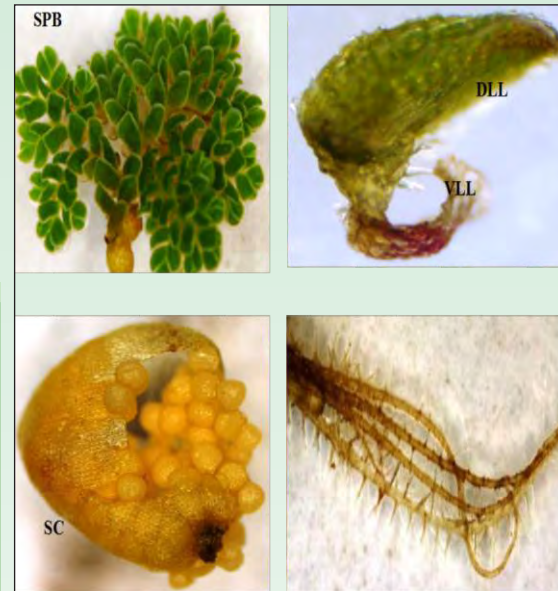
.....*A. rubra*

Non-sporocarp producing major species



### Sporocarp producing strains

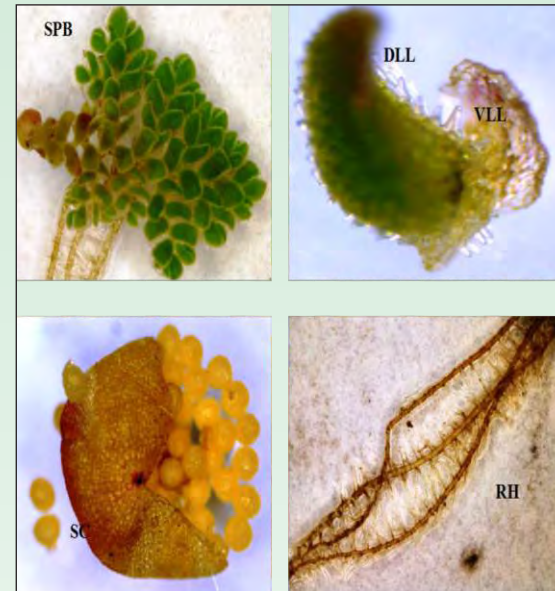
CRRI-1



- Triangular shaped branched floating stem and slightly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Wall of microsporocarp made up of translucent yellow parenchymatous layer containing microspores
- Thick root hairs on the entire root length

Resembled as .....*A. pinnata*

CRRI-2



- Triangular shaped branched floating stem and slightly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

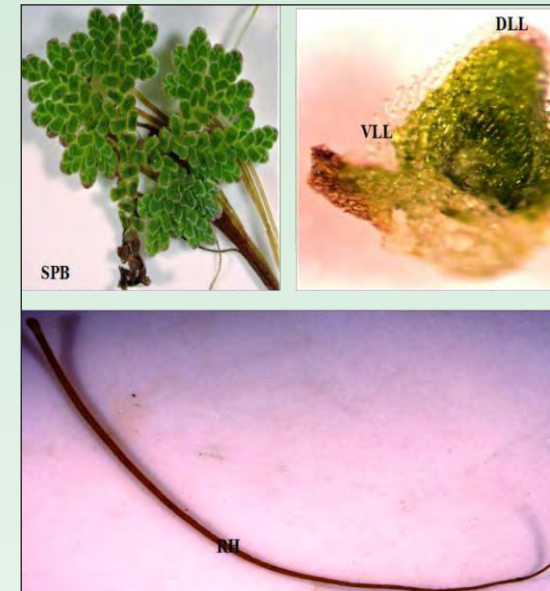
Resembled as .....*A. pinnata*

Sporocarp producing strains



### Non-sporocarp producing strains

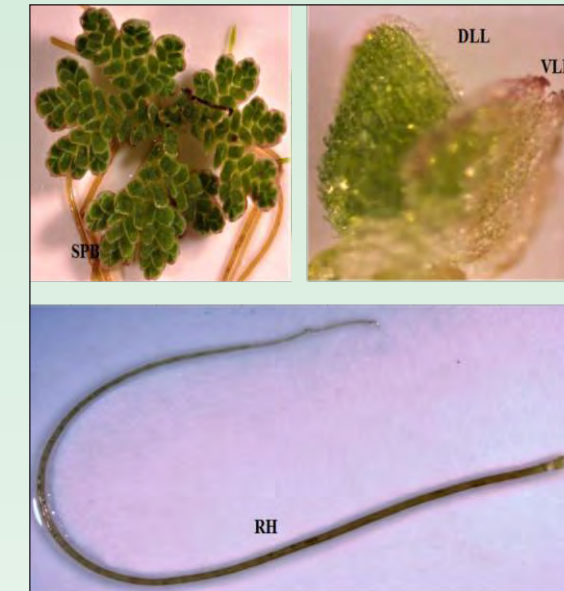
R-19



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

R-40



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

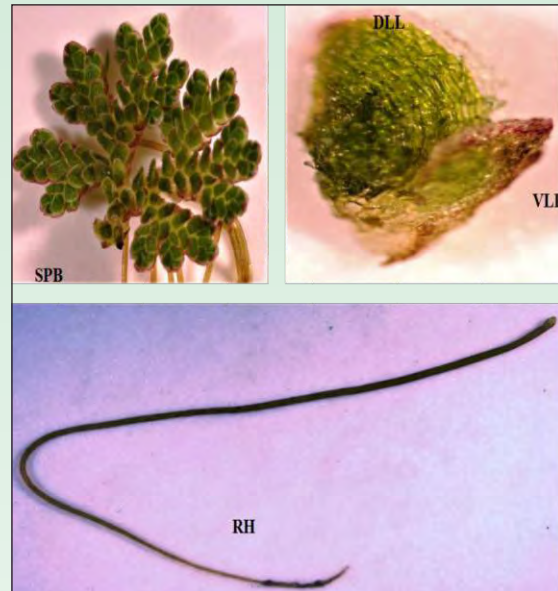
Resembled as .....*A. rubra*

Non-sporocarp producing strains



### Non-sporocarp producing strains

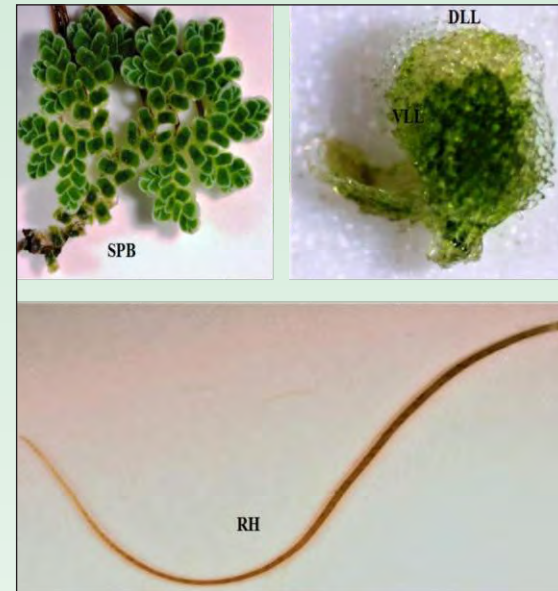
R-36



- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. caroliniana*

R-37



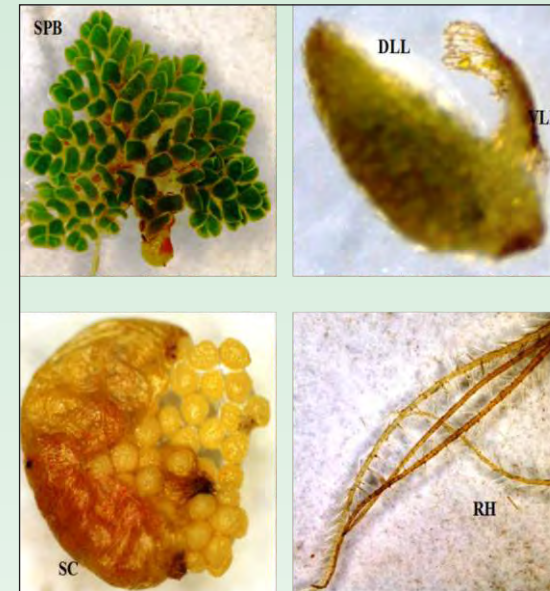
- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. caroliniana*

Non-sporocarp producing strains

### Sporocarp producing strains

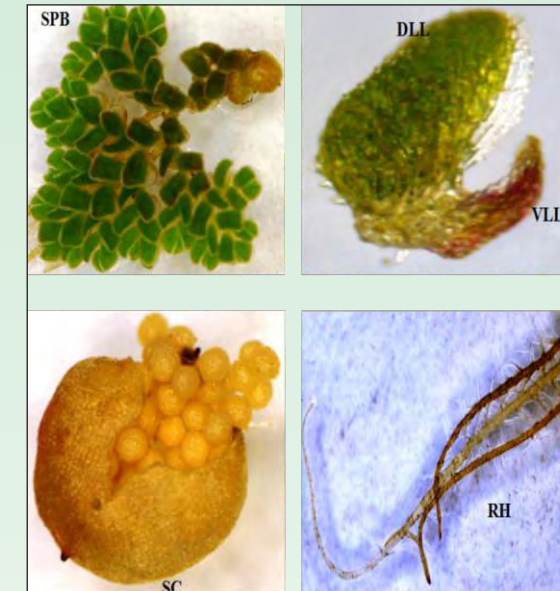
CRRI-3



- Triangular shaped branched floating stem and slightly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

Resembled as .....*A. pinnata*

CRRI-4



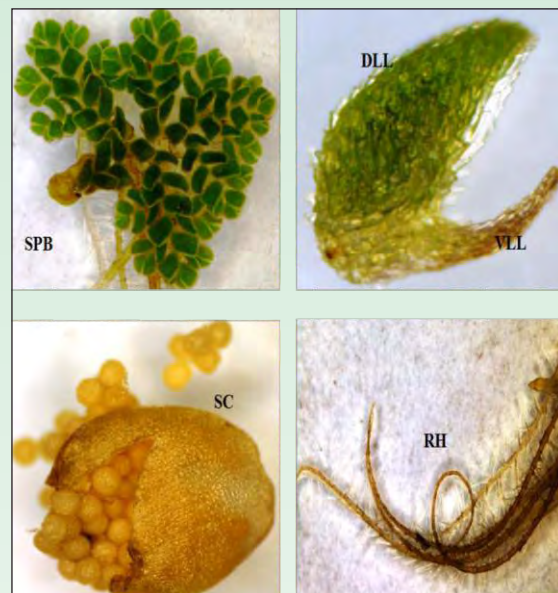
- Triangular shaped branched floating stem and slightly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

Resembled as .....*A. pinnata*

Sporocarp producing strains

### Sporocarp producing strains

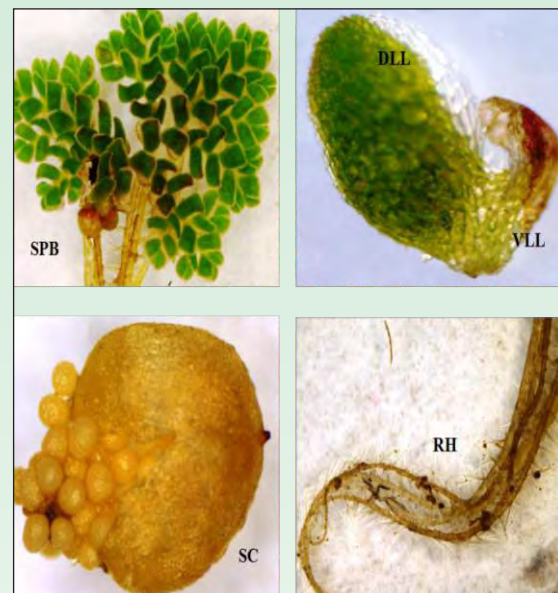
**CRRI-5**



- Triangular shaped branched floating stem and slightly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

Resembled as .....*A. pinnata*

**CRRI-6**



- Triangular shaped branched floating stem and slightly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Wall of microsporocarp made up of yellow translucent parenchymatous layer containing microspores
- Thick root hairs on the entire root length

Resembled as .....*A. pinnata*

Sporocarp producing strains

### Non-sporocarp producing strains

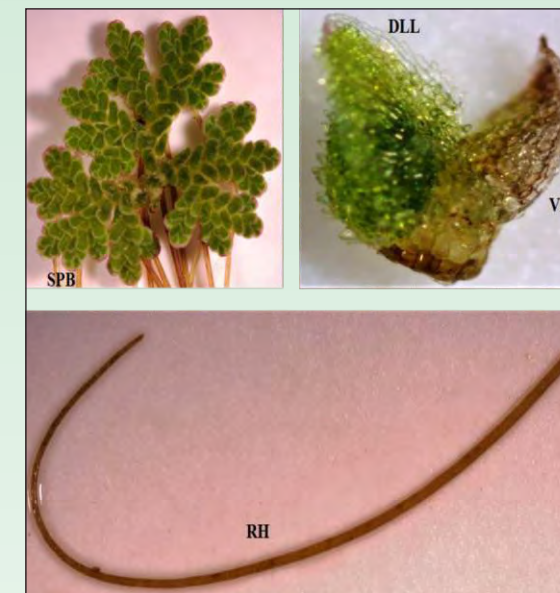
**R-31**



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

**R-34**



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

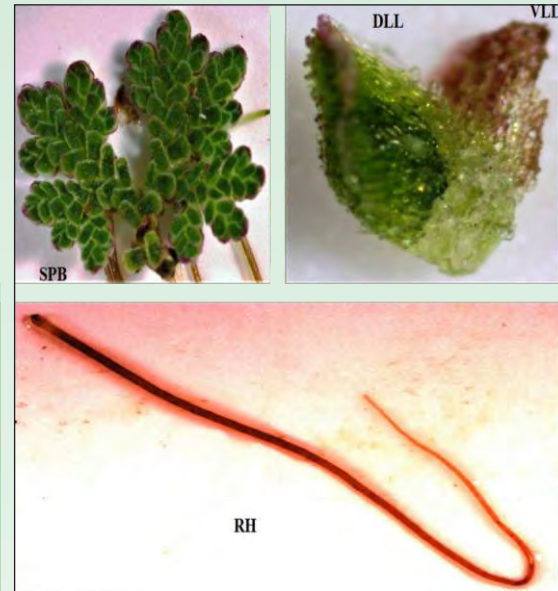
Non-sporocarp producing strains





### Non-sporocarp producing strains

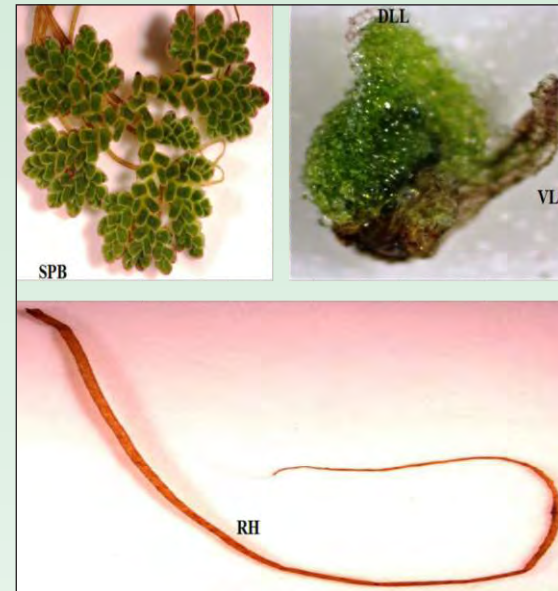
R-28



- Star shaped branched floating stem and highly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

R-30



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

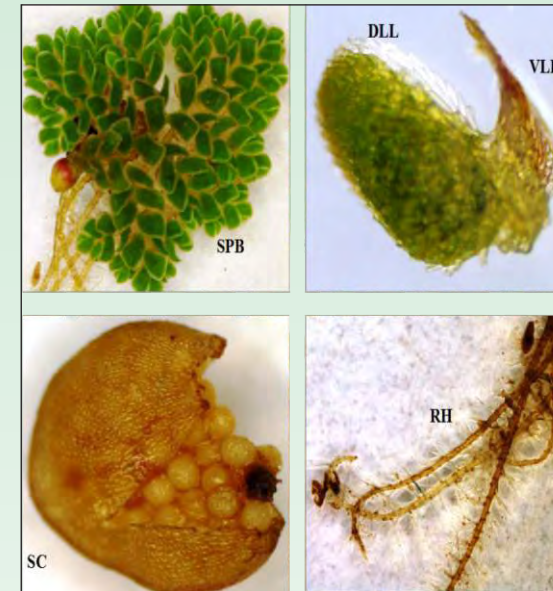
Resembled as .....*A. rubra*

Non-sporocarp producing strains



### Sporocarp producing strains

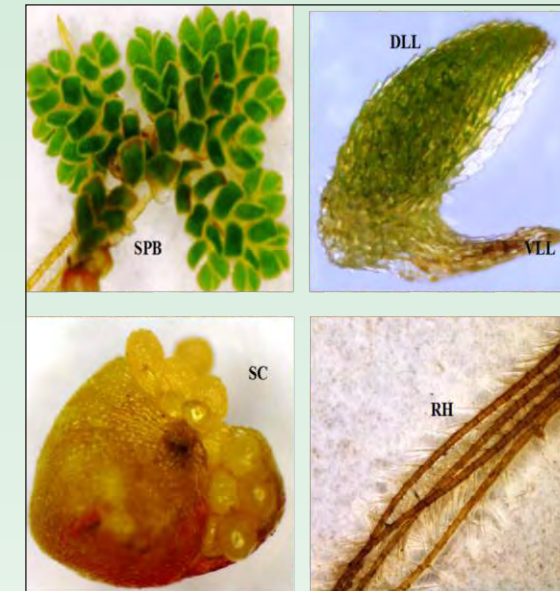
CRR1-7



- Triangular shaped branched floating stem and slightly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Wall of microsporocarp made up of yellow translucent parenchymatous layer containing microspores
- Thick root hairs on the entire root length

Resembled as .....*A. pinnata*

CRR1-8



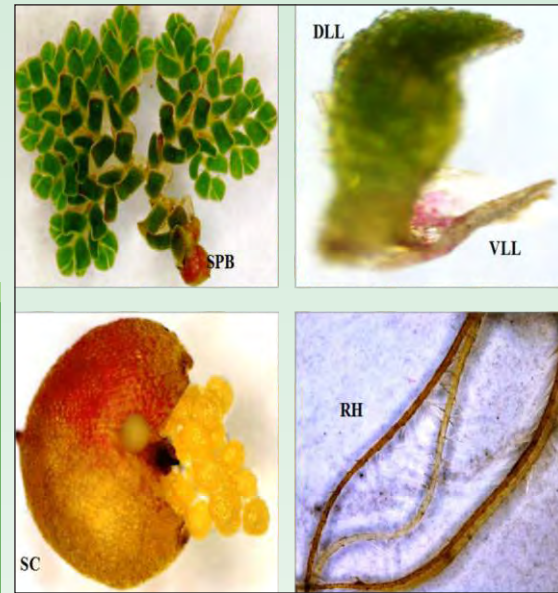
- Triangular shaped branched floating stem and slightly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

Resembled as .....*A. pinnata*

Sporocarp producing strains

### Sporocarp producing strains

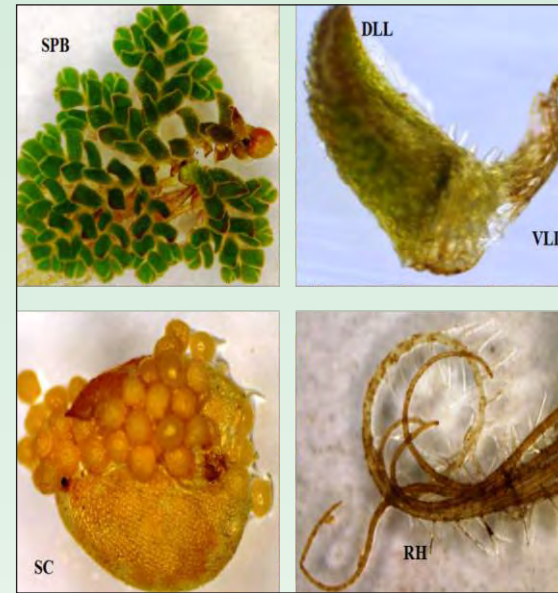
CRRI-9



- Triangular shaped branched floating stem and slightly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

Resembled as .....*A. pinnata*

CRRI-10



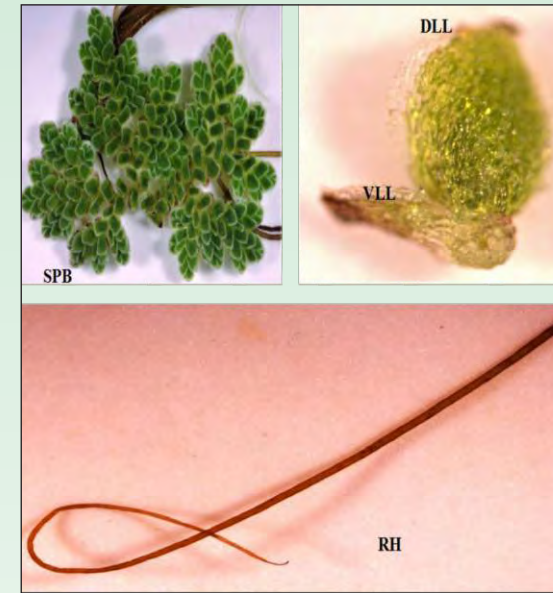
- Triangular shaped branched floating stem and slightly imbricated leaves
- Highly acuminate dorsal leaf lobe and obtuse angled with translucent ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

Resembled as .....*A. pinnata*

Sporocarp producing strains

### Non-sporocarp producing strains

R-24



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and obtuse angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

R-26



- Star shaped branched floating stem and highly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

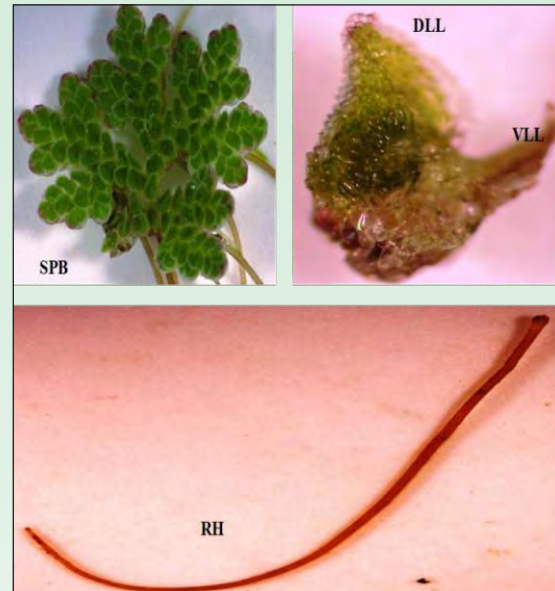
Non-sporocarp producing strains





### Non-sporocarp producing strains

R-21



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

R-23



- Star shaped branched floating stem and highly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

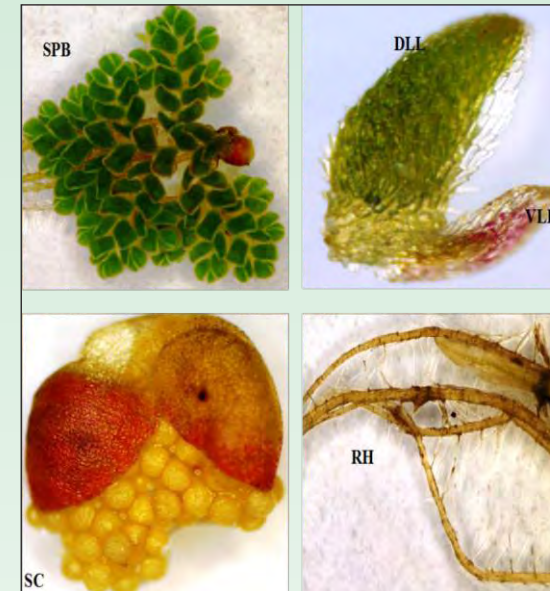
Resembled as .....*A. rubra*

Non-sporocarp producing strains



### Sporocarp producing strains

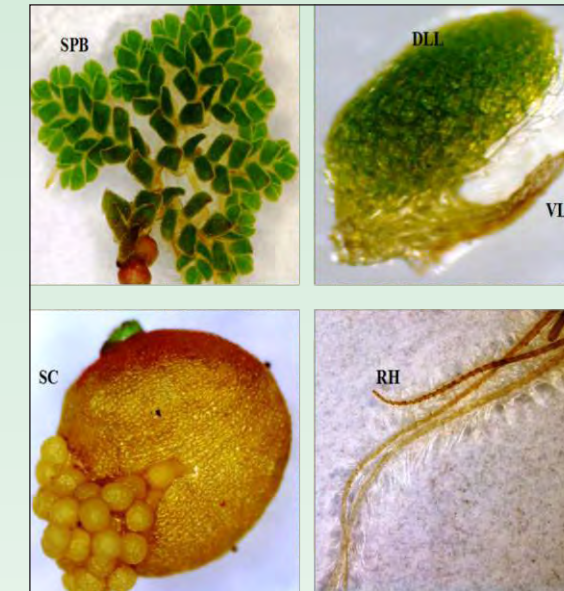
CRR1-11



- Triangular shaped branched floating stem and slightly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

Resembled as .....*A. pinnata*

CRR1-12



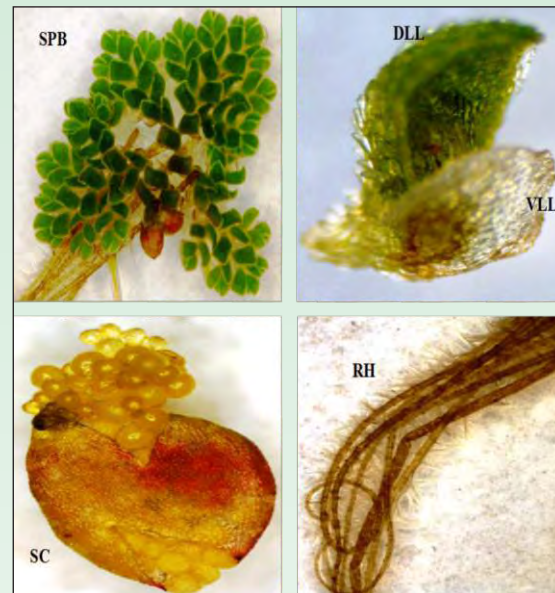
- Triangular shaped branched floating stem and slightly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Wall of microsporocarp made up of yellow translucent parenchymatous layer containing microspores
- Thick root hairs on the entire root length

Resembled as .....*A. pinnata*

Sporocarp producing strains

### Sporocarp producing strains

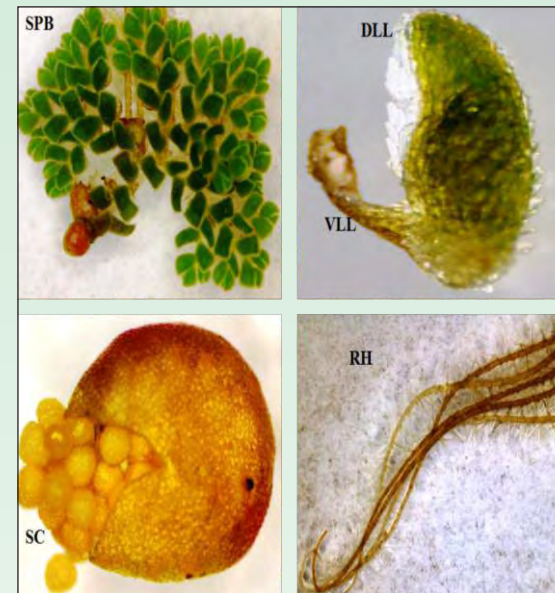
CRRI-14



- Triangular shaped branched floating stem and slightly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

Resembled as .....*A. pinnata*

CRRI-15



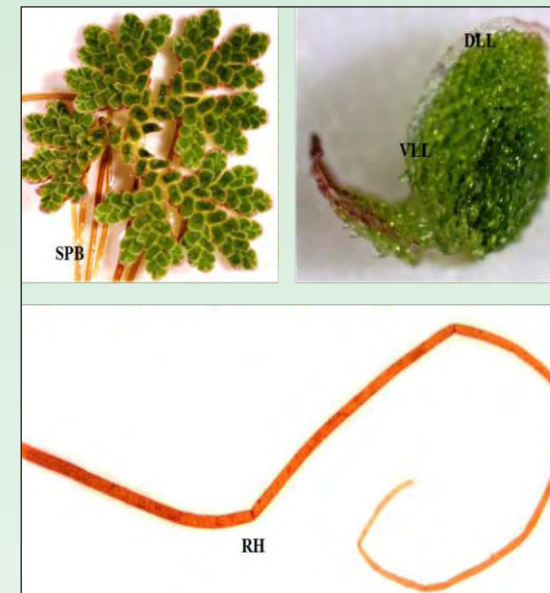
- Triangular shaped branched floating stem and slightly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

Resembled as .....*A. pinnata*

Sporocarp producing strains

### Non-sporocarp producing strains

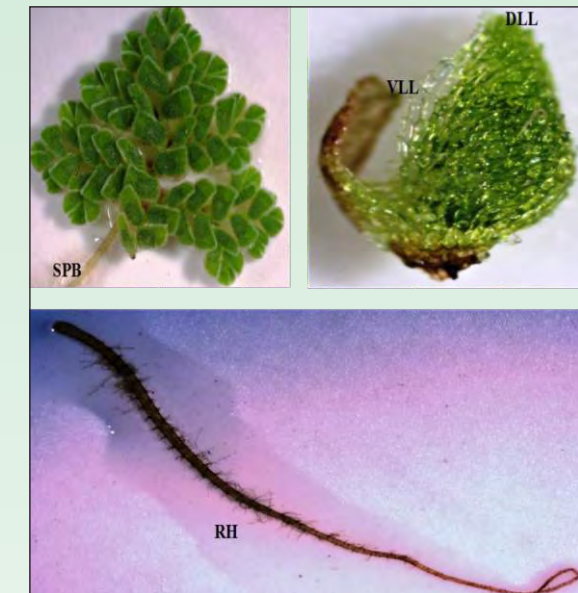
R-38



- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. caroliniana*

R-19P



- Triangular shaped branched floating stem and slightly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thick root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. pinnata*

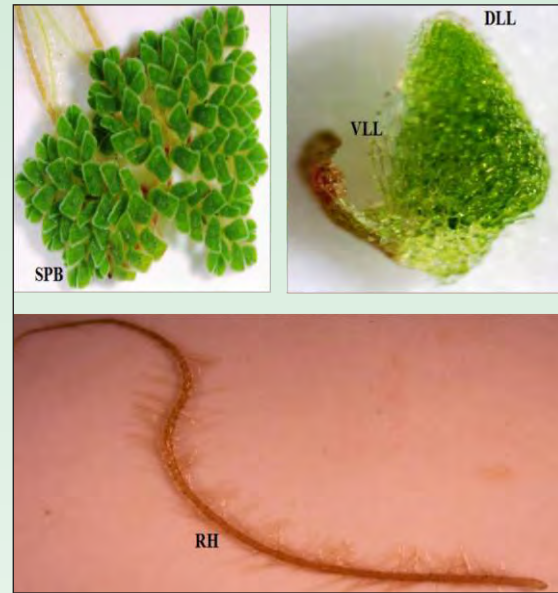
Non-sporocarp producing strains



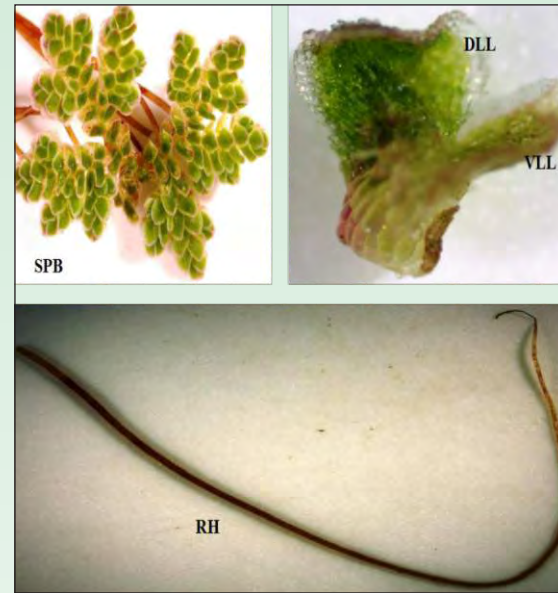


### Non-sporocarp producing strains

R-18P



R-18



Non-sporocarp producing strains

- Triangular shaped branched floating stem and slightly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thick root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

- Star shaped branched floating stem and moderately imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

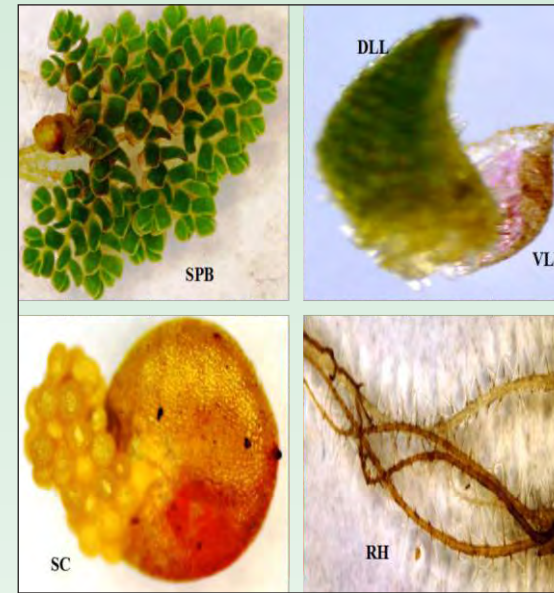
Resembled as .....*A. pinnata*

Resembled as .....*A. microphylla*

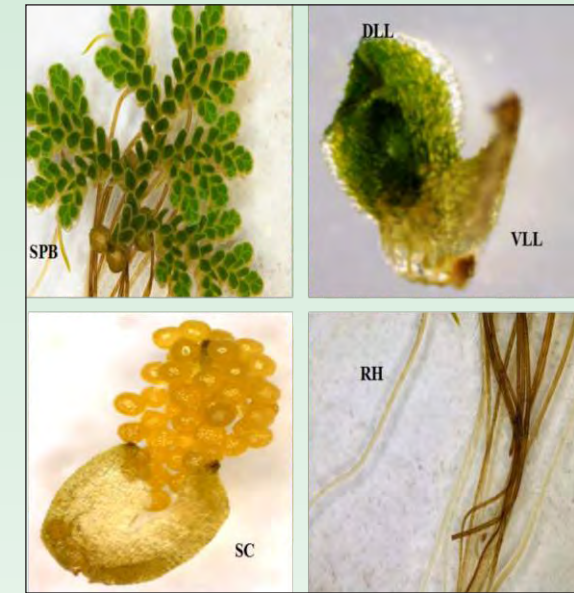


### Sporocarp producing strains

CRR1-16



GSMI-1



Sporocarp producing strains

- Triangular shaped branched floating stem and slightly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

- Star shaped branched floating stem and medium imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Wall of microsporocarp made up of yellow translucent parenchymatous layer containing microspores
- Thin root hairs on the entire root length

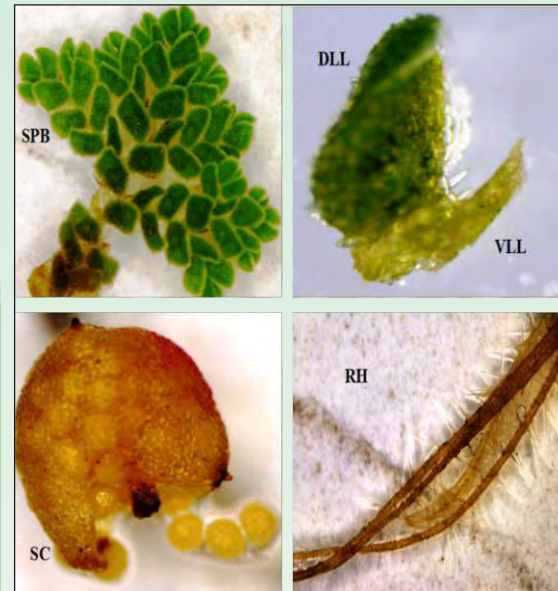
Resembled as .....*A. pinnata*

Resembled as .....*A. microphylla*



### Sporocarp producing strains

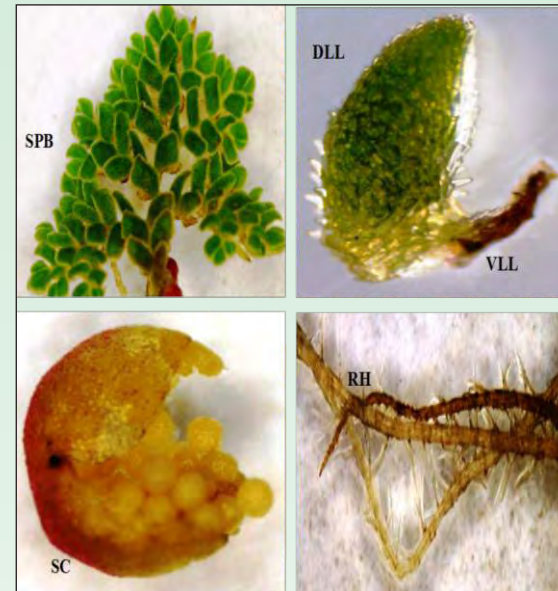
IEPI-1



- Triangular shaped branched floating stem and slightly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

Resembled as .....*A. pinnata*

IEPI-4



- Triangular shaped branched floating stem and slightly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

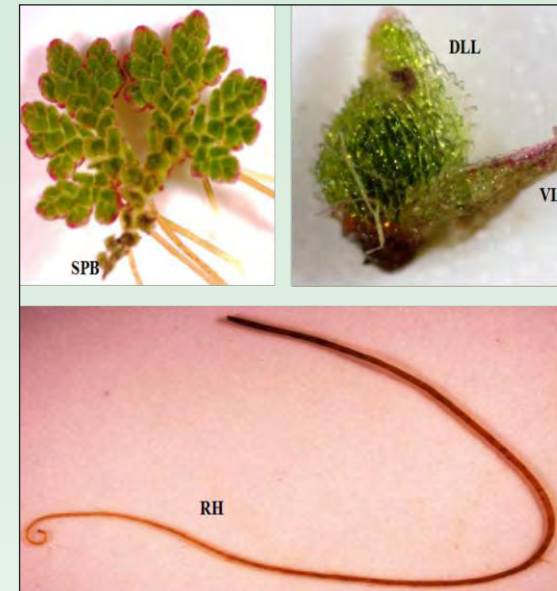
Resembled as .....*A. pinnata*

Sporocarp producing strains



### Non-sporocarp producing strains

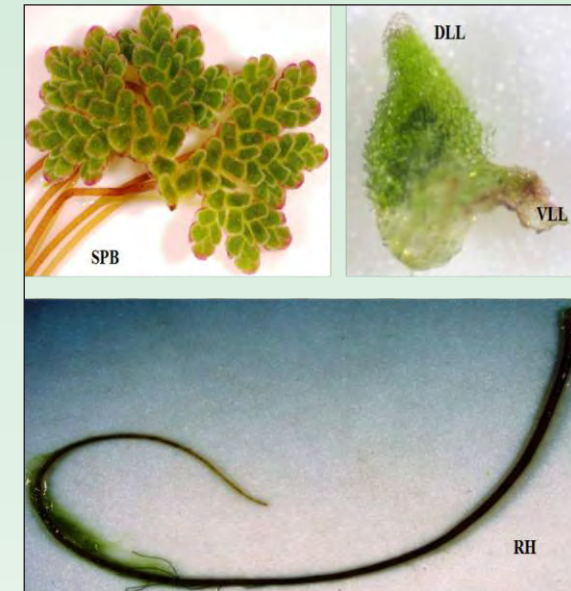
R-11



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

Pa Car RmPc



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

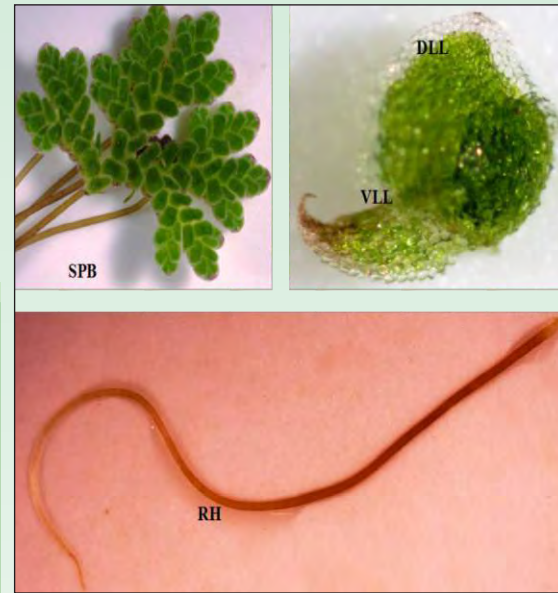
Non-sporocarp producing strains





### Non-sporocarp producing strains

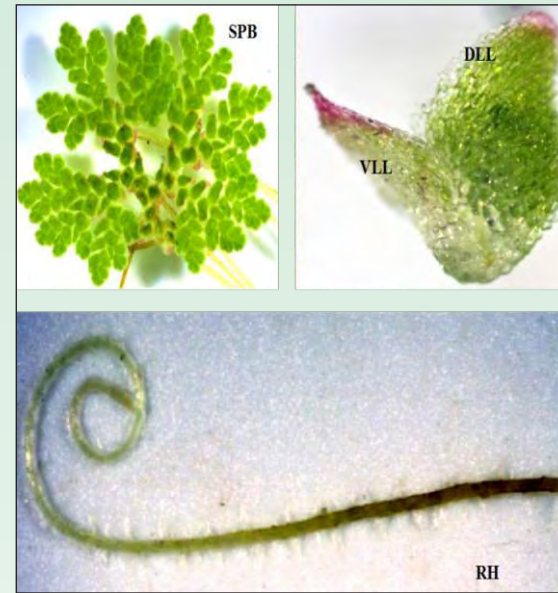
R-10B



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

R-10T



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

Non-sporocarp producing strains



### Sporocarp producing strains

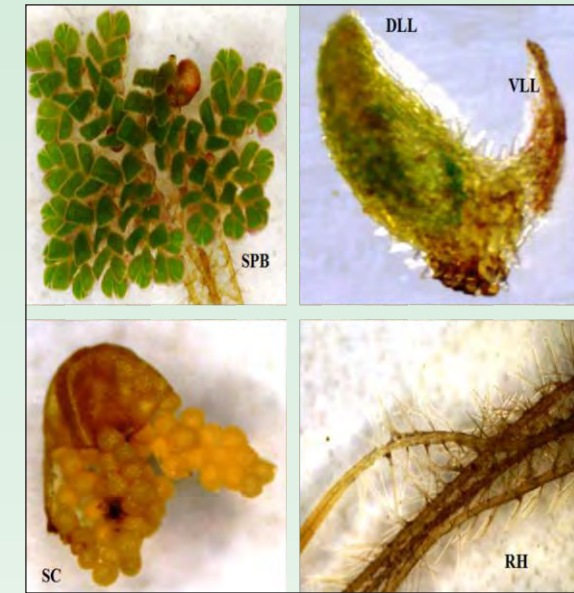
R-86



- Triangular shaped branched floating stem and slightly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

Resembled as .....*A. pinnata*

R-94



- Triangular shaped branched floating stem and slightly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

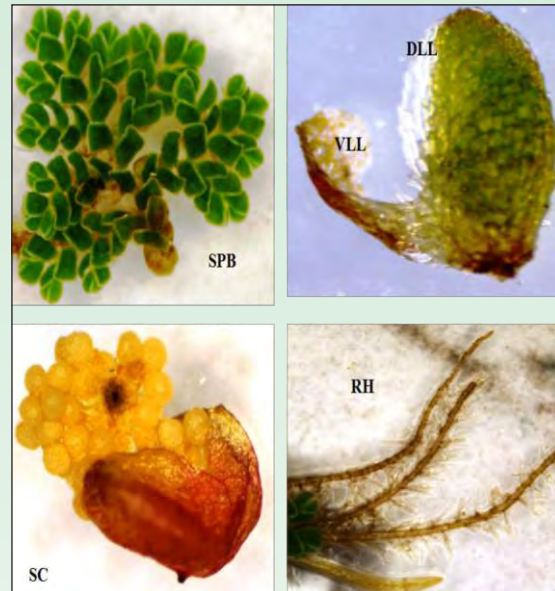
Resembled as .....*A. pinnata*

Sporocarp producing strains



### Sporocarp producing strains

#### Pinnata Assam



- Triangular shaped branched floating stem and slightly imbricated leaves
- Slightly acuminated dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Wall of microsporocarp made up of red tinted parenchymatous layer containing microspores
- Thick root hairs on the entire root length

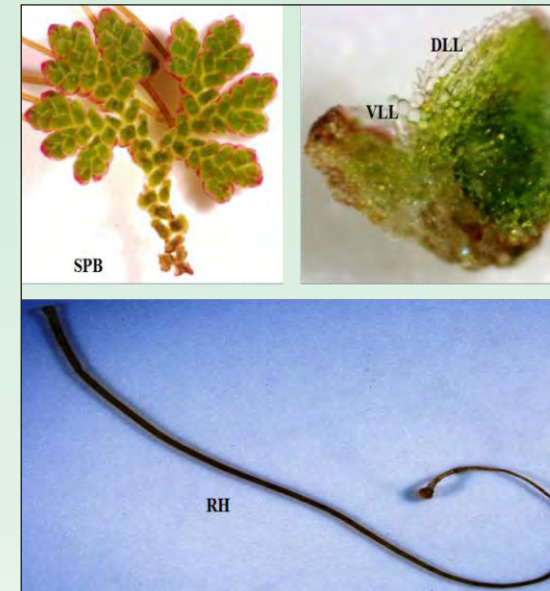
Resembled as .....*A. pinnata*



Sporocarp producing strains

### Non-sporocarp producing strains

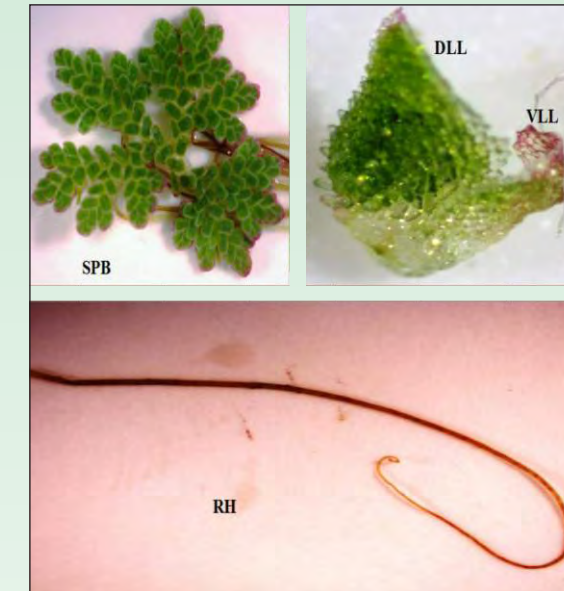
#### Rm Car WTY



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminated dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. rubra*

#### PUFF-1



- Star shaped branched floating stem and highly imbricated leaves
- Highly acuminated dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

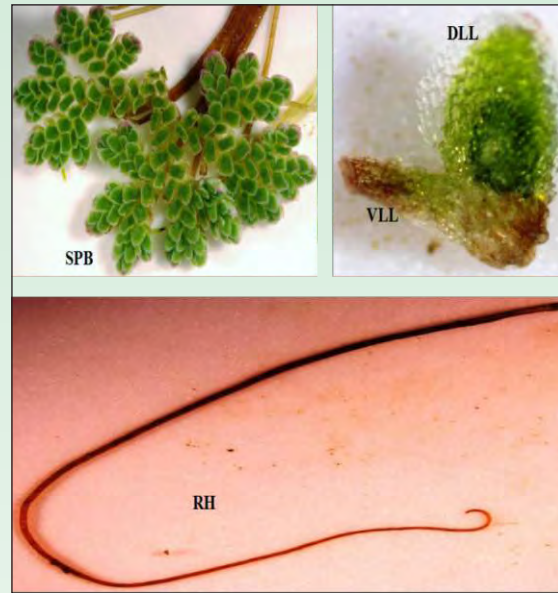
Resembled as .....*A. filiculoides*

Non-sporocarp producing strains



### Non-sporocarp producing strains

Micro-9



- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. caroliniana*

R-16



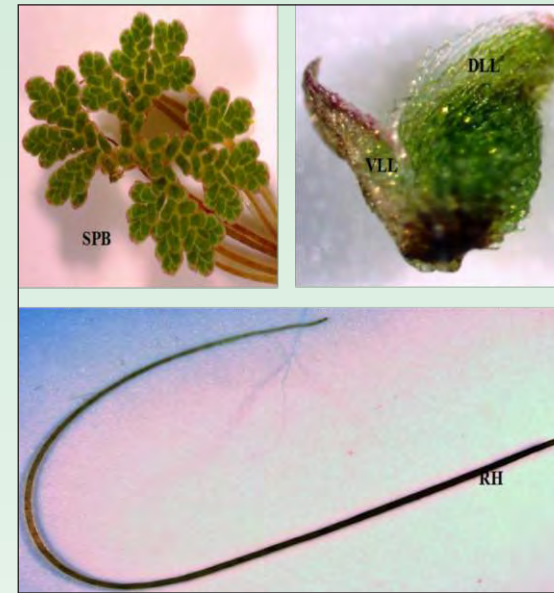
- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. caroliniana*

Non-sporocarp producing strains

### Non-sporocarp producing strains

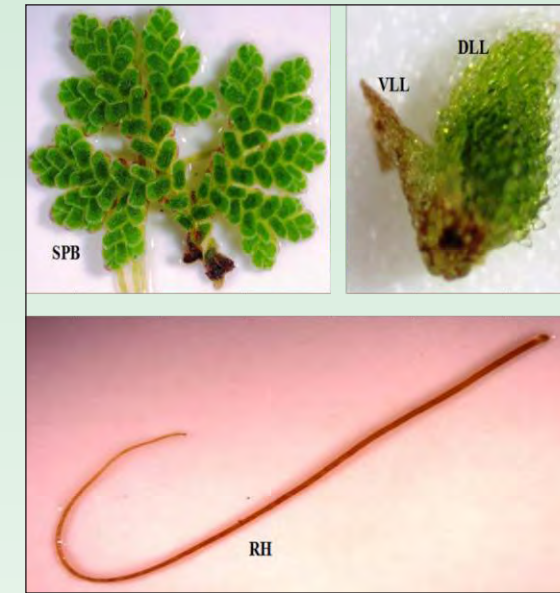
CRR1-13



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. mexicana*

ADUL-2



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

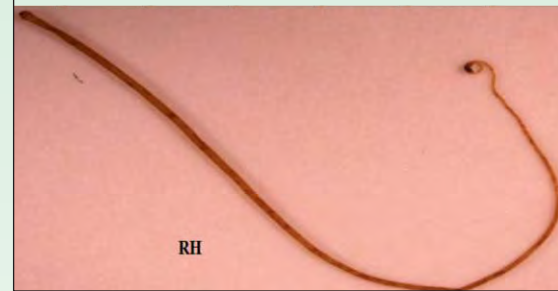
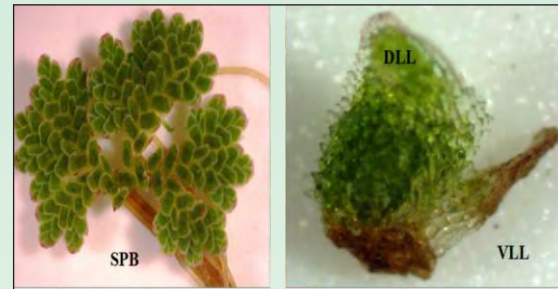
Resembled as .....*A. rubra*

Non-sporocarp producing strains



### Non-sporocarp producing strains

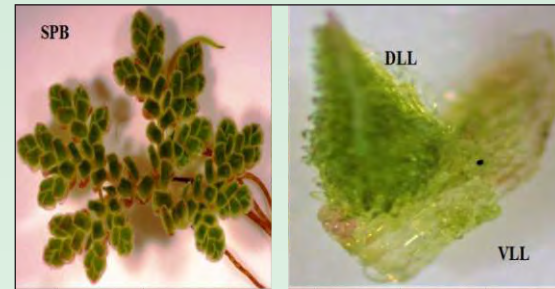
JNNR-1



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. filiculoides*

99



- Star shaped branched floating stem and highly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

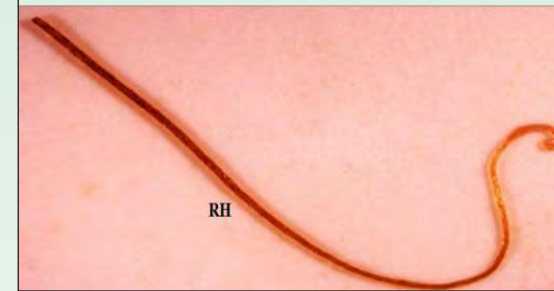
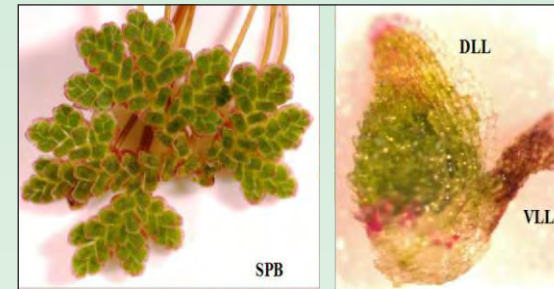
Resembled as .....*A. mexicana*

Non-sporocarp producing strains



### Non-sporocarp producing strains

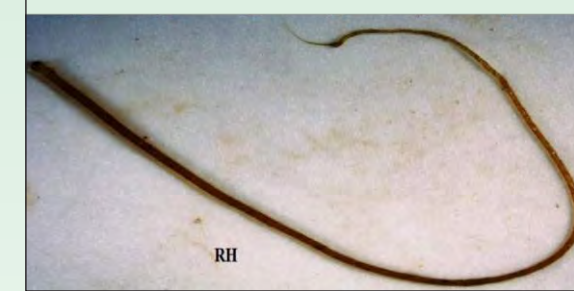
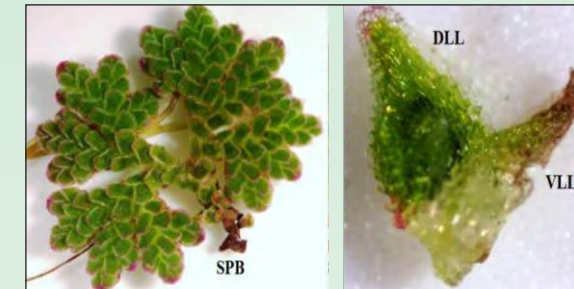
IARI-7



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. filiculoides*

BLCC-18



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

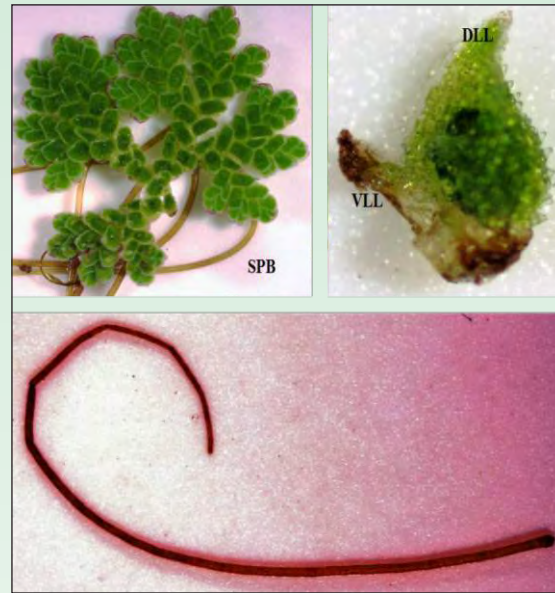
Resembled as .....*A. mexicana*

Non-sporocarp producing strains



### Non-sporocarp producing strains

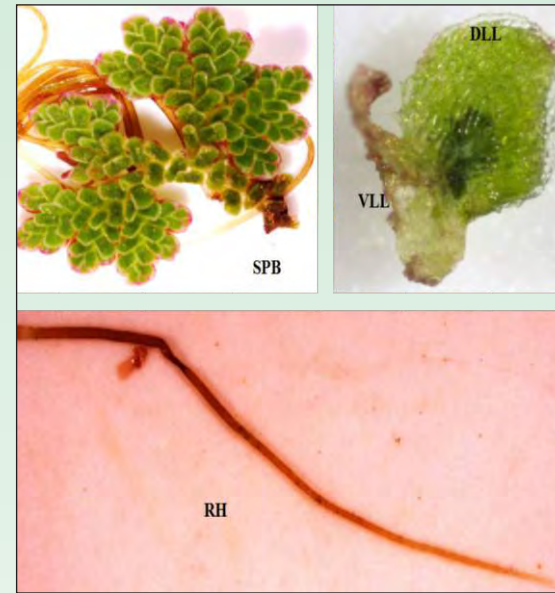
BLCC-26



- Star shaped branched floating stem and highly imbricated leaves
- Highly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. filiculoides*

BLCC-28



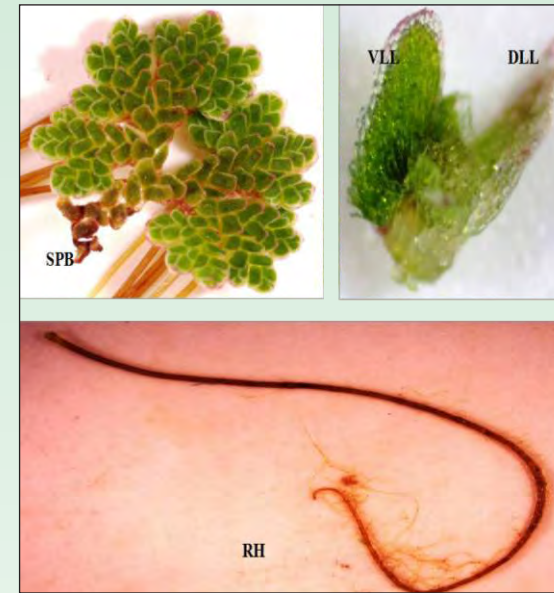
- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. mexicana*

Non-sporocarp producing strains

### Non-sporocarp producing strains

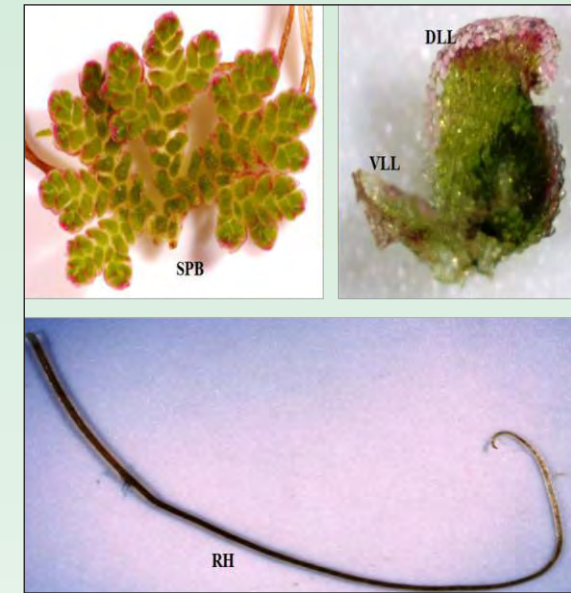
BLCC-5



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. filiculoides*

BLCC-13



- Star shaped branched floating stem and highly imbricated leaves
- Slightly acuminate dorsal leaf lobe and acute angled with pink ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

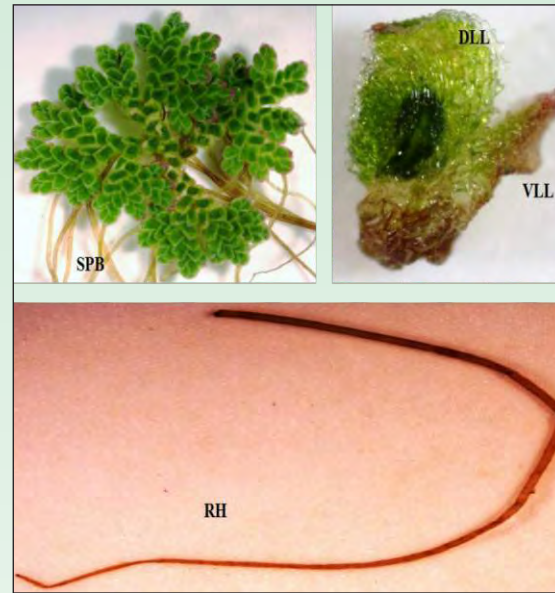
Resembled as .....*A. filiculoides*

Non-sporocarp producing strains



### Non-sporocarp producing strains

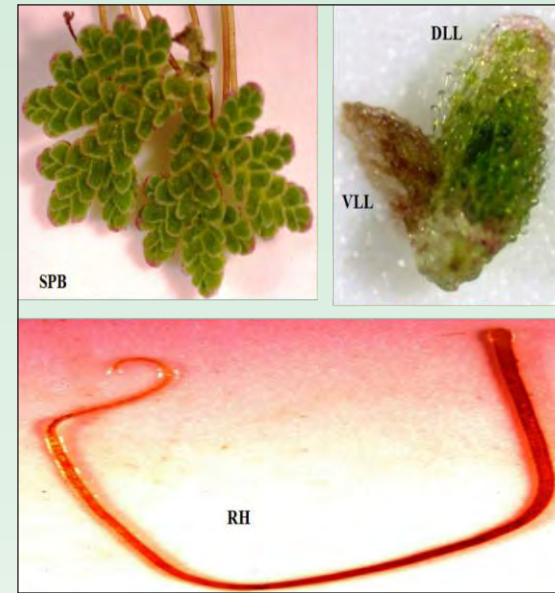
ADUL-42



- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. caroliniana*

BLCC-20



- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

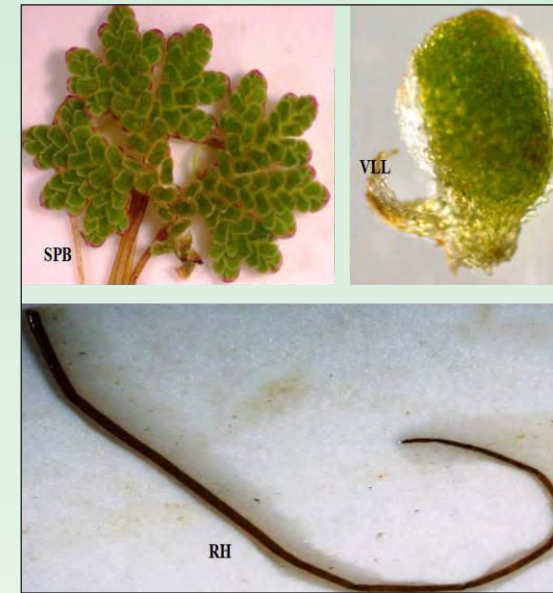
Resembled as .....*A. caroliniana*

Non-sporocarp producing strains



### Non-sporocarp producing strains

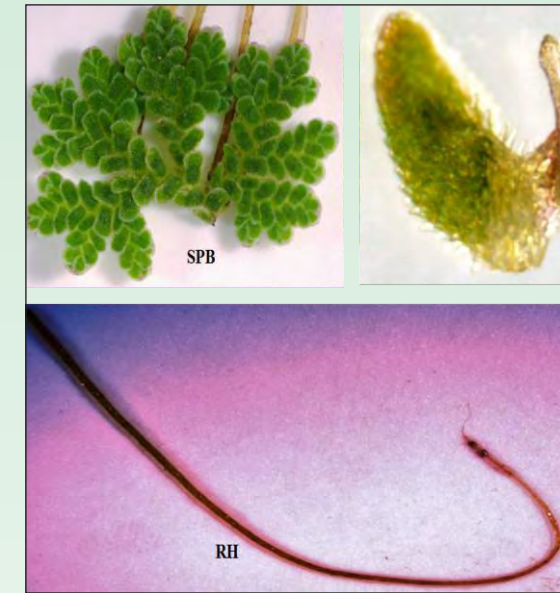
BLCC-21



- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with translucent ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. caroliniana*

BLCC-22



- Star shaped branched floating stem and highly imbricated leaves
- Rounded dorsal leaf lobe and acute angled with pink tinted ventral leaf lobe
- Thin root hairs on the entire root length
- Sporocarp not produced in natural climatic condition at Cuttack, Odisha

Resembled as .....*A. caroliniana*

Non-sporocarp producing strains