Paper ID: 1076
Theme 4. Biodiversity, conservation and genetic improvement of range and forage species
Sub-theme 4.1. Plant genetic resources and crop improvement

Nutrient profiling and identification of genetic marker for *Azolla* sp.

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Keywords: Azolla, Molecular marker, Nutrient profiling

Introduction

Azolla (mosquito fern, duckweed fern, fairy moss and water fern) is a genus of seven species of aquatic ferns. They float on the surface of water by means of scale-like leaves, with their roots hanging in the water. *Azolla* is able to fix unlinked nitrogen (N_2) directly from the atmosphere because of its endosymbiotic blue alga *Anabaena azollae* (Van Hove, 1989), and is thus a very promising supply of nitrogen to aquatic ecosystems. In recent years, apart of traditional cultivation of *Azolla* as a bio-fertilizer for wetland paddy, also find increasing use for sustainable production of livestock feed. Since the demand for milk and meat in India is creating new potential in the profitability of animal husbandry as an occupation. Yet, at the same time, there is a substantial decline in fodder availability. The area under forest and grasslands is decreasing as is the amount of various crop residues available for feed, largely due to the introduction of high yielding dwarf varieties. The shortage of fodder is therefore compensated with commercial feed, resulting in increased costs in meat and milk production. Moreover, as commercial feed is mixed with urea and other artificial milk boosters, it has a negative effect on the quality of milk and the health of the livestock. The search for alternatives to concentrates led us to a wonderful plant *azolla*, which holds the promise of providing a sustainable feed for livestock.

Azolla belongs to family Azollaceae which has seven species with a controversial taxonomy. Presently there are two classifications for family Azollaceae based on vegetative and reproductive characters:

1. Two sections: Rhizosperma (*A. nilotica* and *A. pinnata*.) and Azolla (*A. caroliniana, A. Mexicana, A. microphylla, A. filiculoides* and *A. rubra*) (Tan *et al*., 1986), which is mostly accepted.

2. Two subgenera: Tetrasporocarpia (only with *A. nilotica*) and Azolla divided in the sections Azolla (*A. caroliniana, A. microphylla, A. mexicana, A. filiculoides, A. rubra*) and Rhizosperma (*A. pinnata*) (Saunders and Fowler, 1993).

The identification of species without reproductive structures relies on vegetative characters but some are variable, leading to misinterpretations. More recently, the use of DNA sequences of noncoding plastid regions showed the division of the extant species in the sections Azolla and Rhizosperma (Metzgar *et al*., 2007), which was in disagreement with the two subgenera proposal. The molecular methods may be helpful, but until now, they did not provide a conclusive Azolla taxonomy. The whole genome sequence of *Azolla* is also not yet available, that is why, the present study focused on identification of unique molecular microsatellite markers taken from bracken fern (*Pteridium aquilinum*) to study the cross transferability among different *Azolla* sp. and identifying suitable species of *Azolla* for livestock feed.

Materials and Methods

Altogether 102 strains of *Azolla* germplasms (including six species viz. *A. microphilla, A. mexicana, A. carolinniana, A.rubra, A.filicoids* and *A. pinnata*) are maintained at Central Rice Research Institute, Cuttack, India. The nutrient profiling of *Azolla* was done as per methodology of AOAC (2012) and Van Soest *et al*., (1991). The microsatellite markers (total number =50) were selected from bracken fern (*Pteridium aquilinum*) (Der *et al*., 2011) and studied the cross transferability of these markers among *Azolla* sp.

Results and Discussion

To identify suitable species of *Azolla* as livestock feed, we analyzed the nutrient profiling of six species of *Azolla*. Based on nutrient profile data from six *Azolla* species**,** the highest nutrient content (%) in terms of crude protein, ether extract, total antioxidants and minerals (Fe, Cu, Zn, B etc) was found in *A. microphylla* (25.86, 3.90, 76.87, respectively) followed by *A. mexicana* and the lowest value was recorded in *A. caroliniana*. Moreover, the lowest value of neutral detergent fibre (NDF: 29.23) and acid detergent fibre (ADF: 38.04) were also found in *A. microphylla* (Fig. 1 & 2). As per livestock feed

norm (http: // extension.psu.edu /animals/camelids /nutrition /determining forage quality understanding feed analysis), the species having maximum content of crude protein, ether extract, antioxidants and the lowest value of NDF and ADF is considered suitable as cattle feed.

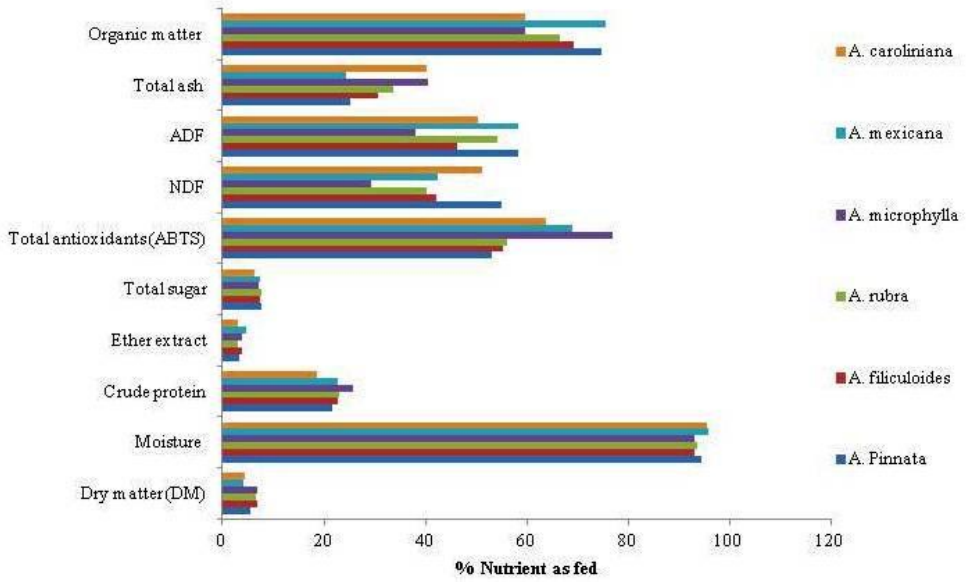

Fig. 1: Major nutrient content (%) in six species of *Azolla*

The identification of *Azolla sp.* based on morphological and physiological aspects is a tedious work. The whole genome sequence of *Azolla* is not yet available, hence the microsatellite markers taken from bracken fern (*Pteridium aquilinum*) to study the cross transferability was conducted. Out of the 50 microsatellite markers analyzed in both the *A. pinnata* and *A. caroliniana*, only five (10%) markers were amplified at 55^{o}C annealing temperature and also showed the clear discrimination and differentiation among *Azolla* species.

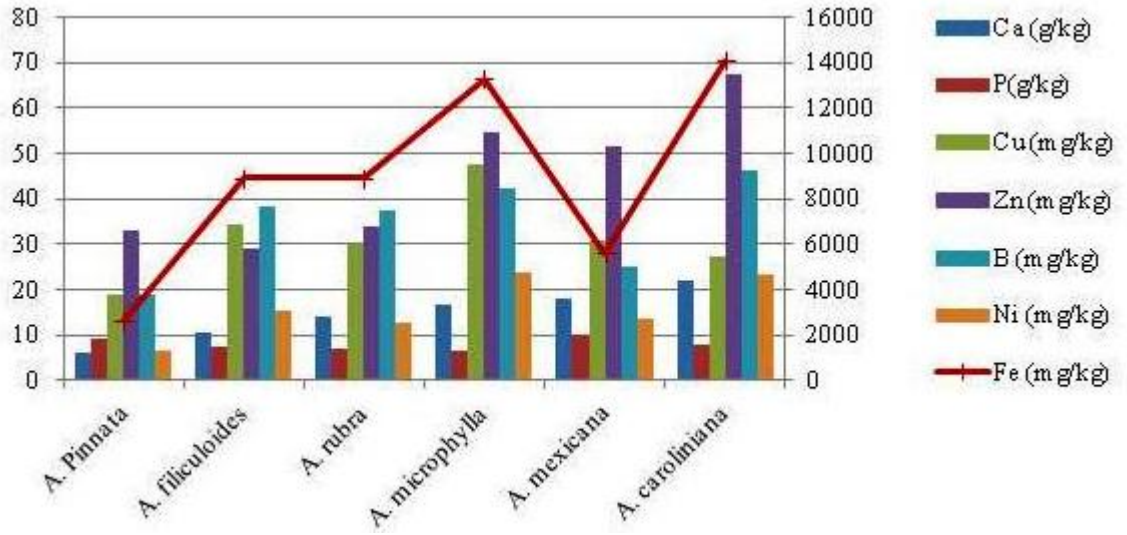

Fig. 2: Comparative mineral content present in six species of *Azolla* (Primary y-axis denotes Ca, P, Cu, Zn, B and Ni concentration and secondary y-axis denotes Fe concentration)

Conclusion

Based on nutrient profile, we can conclude that out of the six species, *A. microphylla* may be considered the most suitable for cattle feed. We also identified five microsatellite markers from 50 markers which can differentiate among *Azolla* sp.

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