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RHIZOPHORE IN *SELAGINELLA*: AN EVOLUTIONARY ENIGMA

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ABSTRACT: Therhizophore of *Selaginella* is a unique evolutionary innovation which combines the features of the shoot as well as the root. The debates about the interpretation of the true nature of this organ are still on. With the advancement of molecular techniques in recent years, the results from the recent research have apparently begun to shift the paradigm. This review attempts to present the current status of the opinions on this enigmatic structure in the light of new findings and points to the goals of future research in this area.

KEYWORDS: Rhizphore; Auxin; *KNOX* genes; *WOX* genes; Stem Cell

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1.INTRODUCTION

Selaginella is an extant model lycophyte which is considered an evolutionary relic and plays a significant role in mapping the evolutionary history of vascular plants [2]. Its genome has also been sequenced recently [3].To cope with the harsh terrestrial condition, it has evolved various adaptive features including perhaps the rhizophore, a root-like structure. There have been considerable debates since the turn of the twentieth century about the morphological nature of rhizophore, the chlorophyll-less structure found at the shoot branching points of the stem in *Selaginella*, with some considering them as shoots and others as roots. Still others were content with terming it organ *sui generis,* a unique structure sharing properties of both the root and the shoot. On the one hand, it is achlorophyllous and positively geotrophic like the root; on the other, unlike the root, it originates exogenously and like the shoot, it gives rise to roots at its tips usually when it comes into contact with the soil or other supports. The conjectures are compounded by the lack of fossil records for the origin of the rhizophore, compelling us to turn our attention to the molecular analyses of the living

lycophytes [12].Despite tremendous technological advances, our understanding of the true nature of this organ remains doubtful. This review article attempts an overview of the topic by presenting various aspects of the arguments, focusing mainly on the latest developments in this area.

Root Nature: Morpho-anatomically, the rhizophore bears resemblance with the root as it is usually devoid of leaves and lacks chlorophyll. This superficial similarity,which could be due to phenotypic plasticity and adaptations for different functions, may not help one to draw conclusion as to the actual nature of the organ. The rhizophore dichotomizes at the tip like the the shoot apex, but the resulting roots also bifurcate to form roots. Unlike the branching pattern in the shoot apex, the rhizophore and the dichotomising roots developed at its apex typically show a growth arrest in their root primordia which again remain buried endogenously for a short duration [18].Consequently, scientists attempted to address the question by investigating the more reliable and relatively stable physiological features to make their judgement. Indeed, radio-labelling experiments conducted to study the direction of movement of auxin (Indeleacetic Acid) showed that the hormone moves acropetally in the rhizophore as in the root which underscores the root nature of this organ [27, 19]. Histological sectioning combined with three-dimensional reconstruction in *Selaginella* have also revealed a vascular tissue architecture in the rhizophore that is supportive of acropetal auxin transport in it from the shoot branches [16]. Auxin is implicated in root initiation and in euphyllophytes it acts via downstream regulators such AS2/LOB-domain (ASL/LBD) proteins, although its counterparts are not found in lycophytes including *Selaginella*, suggesting an alternative mechanism [4]. However, auxin, a small molecule, is too ubiquitious in its roles and often distribution in plant for one to arrive at the conclusion that the rhiophore is root,especially on the basis of the direction of the flow of auxin alone. In addition, its all-encompassing influence on plant life makes it harder to be considered as a definitive marker for either the shoot or the root. Furthermore, being a hormone, auxins typically trigger signal transduction cascades rather than acting as the actual workhorses like the downstream transcriptional regulators. With the availability of the data on the genes expressed in *Selaginella* roots [13], it may be worthwhile to draw comparisons of gene expression profiles from the shoot and the rhizophore to address the ambiguity surrounding the rhizophore.

Shoot Nature: The argument put forward in favour of considering rhizophore as shoot initially came from morphological and anatomical evidence that it is of exogenous origin and forms root at its tip. Further support for this is furnished by the fact that the angle meristem at the branch point, which normally forms the rhizophore,   in case of injury to the shoot apical meristem(SAM), makes shoot *in vitro* instead [7,25-26, 28-30]. Excision of the apical meristems of branchlets bearing dorsal angle meristem causes it to develop into a shoot suppressing its normal developmental fate of rhizophore formation [7]. This may be caused by changes in the levels and/or the direction of flow of auxins and sugars from the angle or the apical meristem following its excision as found in case

of the regulation of apical dominance in Arabidopsis [15]. In fact, the application of inhibitors of polar auxin transport (PAT) also results in rhizophore to shoot fate transition [22]. Further research should shed light on the probable involvement of auxin or energy signalling pathways in this developmental switch. These studies imply a developmental switch in the stem cells of the angle meristem allowing for change of fate from rhizophore to shoot. A clearer picture may emerge as to the molecular underpinnings of the switch from the rhizophore to the shoot fate, with future research comparing the transcriptome, proteome, non-coding RNA expression data from the angle meristems on the branchlets bearing intact apical meristems and those with excised apical meristems at different time points. More recent works at the molecular level provides a huge impetus to the shoot argument.Knotted related homeobox (KNOX) transcription factors perform myriad functions in plant including stem cell maintenance [9, 11]. They have been classified into two groups: the Class I and Class II KNOX proteins. While the Class I proteins act as transcriptional activators, Class II proteins generally perform repressive functions [6, 23]. *Selaginella* genome harbours five *KNOX* genes and a pseudogene [17]. The Class I *KNOX* genes, the expression of which is known to be associated with the indeterminate growth pattern, are expressed highly in *Selaginella* in both the shoot and the rhizophore [10, 14]. These findings indeed make a strong case for the shoot nature of the rhizophore.*WUSCHEL (WUS)-related homeobox (WOX)*genes are important regulators in stem cells [7]. WOX protein family members generally have WUS box motif in addition to the homeobox (HB) domain [24]. WOX13 is an ancient protein containing homeobox and acid domain but lacking canonical WUS box [5]. As a result, it is non-functional in stem cell maintenance in Arabidopsis, in spite of serving other important purposes [20]. However, in the model moss *Physcomitrella patensWOX13*-like genes have been implicated in stem cell fate [21]. *Selaginella moellendorffii* genome contains six different *WOX* genes whereas *Selaginella kraussiana* genome encodes eight members of the WOX family [7, 17]. In *Selaginella krausiana SkWOX13b* transcript level was found to be particularly high in the rhizophore tip in intact seedlings, underscoring its importance in rhizophore development [7]. Identification of the interacting protein partners and a detailed functional characterization of such *WOX* genes in *Selaginella* could reveal genetic networks involved in the fate choice of the angle meristem or the developmental aspects of the rhizophore.

2. CONCLUSION

It is apparent that regarding the rhizophore as shoot is slowly gaining traction with disproportionately more evidence from the molecular viewpoint favouring the notion, turning upside down the original idea that the rhizophore(meaning the root-bearer) has more root-like attributes or that it is an amalgamation of the root and the shoot or that it is neither(an organ *sui generis*).It is also likely that the rhizophore, despite essentially being shoot, shows some of the features of the root for adaptive edge. Although the undifferentiated plant cells are totipotent in that they have the capacity to take on the fate of any types of tissue occurring in the fully grown plant, the molecular

bases are not that clear. Through plant tissue culture techniques, callus, a mass of undifferentiated tissue, is routinely induced *in vitro* to form either root or shoot by mainly altering the ratios of auxins and cytokinins in the culture media, suggesting how the fate of the angle meristem might be regulated in a similar fashion. Yet, there might be subtle differences in the mechanistic details in the in vivo situation, as the angle meristem is situated in a different tissue microenvironment in the intact plant, being surrounded mostly by differentiated tissues which could guide the fate choice in the angle meristem cells, in contrast to the undifferentiated callus tissues *in vitro*. Again, in Arabidopsis, molecular feedback loops govern the Shoot Apical Meristem(SAM) and Root Apical Meristem(RAM), through fundamentally similar yet distinct ways, in the root and the shoot [8]. Interestingly, both the shoot and root forming abilities seem to be present in the *Selaginella* angle meristem that forms the rhizophore. Therefore, how the same meristem can acquire either fate in a context dependent manner remains a mystery. Notably, it has been possible to in *Selaginella microphylla* to convert RAM into SAM *in vitro* by the application of auxin [1]. Further research may help to entangle some of these knots and tell whether similar mechanisms are at play in the *Selaginella* angle meristem and Arabidopsis meristems.

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