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Review Paper

Search for nodulation in rice: research trends

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Abstract

Nitrogen is an essential plant nutrient and the high yielding varieties of rice needs it more. But most of the paddy land is deficient of this component. Therefore the high yielding varieties of rice are dependent on exogenous supply of nitrogen while in consideration to both ecological and economical aspects exogenous supply of nitrogen is not desirable. Hence, in recent times, the biological nitrogen fixation (BNF) is preferred. Among BNFs, rhizobia-leguminous symbiosis fascinated scientists as a model for developing the same or a similar nitrogen acquisition system in cereals including in rice. Therefore, search for legume like symbiosis in rice has been envisaged as one of the major goal of BNF research. Strategies includes, detection of nodules and nodule-like structures in rice in nature, if any; trials on forced nodulation in rice; and exploring the possibility of transferring of nodule genes in (to) rice. Research trends of these have been reviewed in this article.

Keywords: Biological nitrogen fixation, Rice-*Rhizobium* interactions, Nodulation in rice, Forced nodulation, transferring nodule genes.

Introduction

Increasing demand of food crops raised the demand for exogenous nitrogen in paddy soils, mainly in the form of urea-N as the global agriculture now rely heavily on fertilizer. But, the increased use of chemical fertilizer is a global concern as it is undesirable, because (i) its production is energetically costly process (as most of the energy is provided by the consumption of non-renewable fossil fuels) and (ii) considerable pollution is caused through both the production and use of mineral N fertilizer and that too exacerbated by the relatively low efficiency of the uptake of fertilizer by the plants [1,2]. There are evidences that, the rice cultivation which involves excessive use of synthetic fertilizers affects the physiochemical properties of the soil complex, causing environmental pollution[3]. The efficiency of added urea-N has also been found very low, only 30 per cent to 40 per cent and, in some cases, even lowers [4-6].

In this context, in recent time biological nitrogen fixation (BNF) is being advocated, in which the atmospheric nitrogen is converted to ammonia by living organisms. As, it is mediated in nature only by bacteria and certain species of actinomycetes, it is environment friendly and sustainable in nature [7]. But, in true sense, BNF is not a new phenomenon. It was exploited for centuries without recognition of how it was functioning. The Romans practiced mixed cropping with legumes and non legumes following their observations that such a practice enhances the production of the non legumes [8]. In the latter half of the nineteenth century, Boussingault demonstrated it [9]. He reported that, in a mixture of legumes and non legumes the total nitrogen of the plants increased and concluded that legumes were fixing nitrogen from the air for use by the plants [8]. But, until demonstration by Wilforth and Hellriegal in 1988, the idea had not got accepted [8]. Now, we all know that, naturally in the soil, there are a number of aerobic and anaerobic bacteria as well as blue-green algae or cyanobacteria which have ideal biological system for nitrogen fixation [10].

The bacteria, which exert beneficial effects through nitrogen fixation on plant development, can be divided into two groups, *viz*., symbiotic and free-living or associative [11,12]. Again, BNF by symbiotic system, which involve nodulation of legume plants, has got advantage not only over free-living or associative nitrogen fixation, as well as over fixation by blue green algae or cyanobacteria. Further, after several years of key research, it appears that non nodular BNF is likely to have very low potential in terms of the amount of nitrogen fixed relative to the potential of nodular association or *nif* gene transfer to the plant[13]. Therefore, one of the major goals of BNF research in rice has been to search for nodulation (legume-like symbiosis).

In this context, researchers are also of the opinion that, if not all, at least some genetic mechanisms do exist and function in rice, which are instrumental in initiating symbiotic nodulation [14-16]. If it is correct, to ensure such nodulation, efforts should be directed towards firstly in designing a rational search for the phenomenon and thereafter attempting in increasing its frequency through genetics and management[17]. Now the question arises how should we search for nodulation in rice? In this context, there are three approaches, *viz.*, (i) to examine the roots of land races and wild species; (ii) to examine the capacity of rice to support nodulation by rhizobia and other microsymbionts derived from other plant species; and (iii) to examine the rice genome itself for the presence of gene required for N_2 –fixing symbiosis[17].

Does nodulation occur naturally in rice?

In nature, till date, no 'true nodulation' in rice could have been detected. But, possibility cannot be nullified. Why? There are reports that, it possesses at least some genetic information required for symbiotic nodulation [16,18].

The development of symbiotic N_2 fixation between legumes and rhizobia is a multistep process in which genes from both host plant (nodule gene) and bacterium *(nod, nif, fix, exo, lps* and *ndv* genes) play essential role, which is confined only to leguminous plants[19,20,21]. Rhizobia (species of *Rhizobium, Mesorhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium* and *Sinrhizobium*) form intimate symbiotic relationship with legumes by responding chemotactically tó flavonoid molecules released as signals by the legume host [22]. These plant compounds induce the expression of nodulation (*nod)* genes in rhizobia which in turn produce lipochito-oligosaccharide (LCO) signals that trigger mitotic cell division in roots, leading to nodule formation [23, 24].

In nature, from certain locations, where rice is grown as intercropping with legumes or in rotation with legumes, natural endophyotic association between rice and *Rhizobium* have been reported [25-29]. However, there is no conclusive evidence that the benefits involve symbiotic nitrogen fixation, although there is opinion too that, the *Rhizobium* can also fix N in the soil as free-living nitrogen fixer [30-32]. There are also reports that, *Rhizobium* utilises the N from soil by promoting physiological growth response generating changes to the root morphology of the rice plant that favours its uptake [10,31,33- 36]. These findings of natural endophytic association of rice and *Rhizobium* trigger a question, how efficiently the endophytes play role for nitrogen fixation, while no obvious 'symbiotic' structures appears to be present.

A large majority of plants, including rice, wheat and maize possess the genetic information required to pave the way for successful symbiotic root infection involving the reactivation of cortical cells in the presence of a microsymbiont, the accepted penetration of the symbiont in host cells, and the synthesis of a membranes interface with the symbiotic partner to facilitate metabolic exchange [37]. It has been reported too that, rice possess the capacity to form symbiotic (mycorrhizal) association with fungi [38,39]. It also appears that, rice is capable of perceiving *Nod* factors coded for by bacterial *nod* genes, as well as several homologues to legume *ENODs* are present in rice, which (nodule genes) specifically expressed in legumes during early events in infection and nodule formation[40,41,14]. These suggest, there is probability that, nodulation may occur naturally in rice, though till date no 'true nodulation' have been reported in rice[42]. There may be possibility that, formation of nodule might have taken place at low frequency or under unusual condition, and as a result it might have escaped detection in the field [42].

Trials on forced nodulation

In nature 'functional nodulation' in rice has not been documented. In induction too, 'true nodulation', exactly of symbiotic functioning have not been reported, though some morphological response of rice roots have been found, which are able to attach rhizobia to rice root hairs [43]. It has been observed that the recombinant rhizobia containing foreign *nodABCD* genes caused root hair curling and deformation in rice [44].

There are also reports on formation of nodule-like structure/ hypertrophies too [45-51]. All-Mallah *et.al.* were the first to successfully induce nodulation structure on rice roots after treating 2-d-old seedling roots with a cell wall degrading enzyme mixture followed by rhizobial inoculation in the presence of polyethylene glycol[44]. Weber *et.al*. also detected an increase in rhizobial infection of rice root with the application of low concentration of nitrongenase (10^{-4} and 10^{-5} M) [52]. Interestingly, some flavones have shown to enhance the colonization of rice roots and internal localization in xylem of *Azorhizobium caulinodans* strain ORS 571 [53]. Similar rice-rhizobium interactions have also been reported [2,47].

Reviewing the literature, Kennedy and Tchan postulated that, exogenously applied hormones or cell wall degrading enzymes induce nodule-like structure or hypertrophies in rice in response to application of rhizobia to either normal roots or enzyme-treated roots in the presence of polyethylene glycol (glycol) and calcium chloride [54]. Similar results were obtained by applying rhizobia carrying multiple copies of the *nod D* gene to rice and nodule-like structure on rice roots even in the absence of any plant hormone treatment [51,50]. However, except one group no nitrogen fixation has been confirmed on such nodule-like structure or hypertrophies, and in that case too, adequate controls were not presented [48,22,55]. Therefore, there are opinions that, root nodules once formed, may be effective or ineffective for nitrogen fixation[56]. Here, the formation of nodule-like structure or hypertrophies on induction is considered as partial success in causing root modifications, but not enough to extend legume-type nitrogen fixation [57]. Though some genetic mechanisms do exist and function in rice, which are instrumental in initiating symbiotic nodulation, researchers are of the opinion that, the plant does not have the machinery to make the introduced bacterium work [14-16,57]. Therefore, rice would need to be genetically modified to respond to the appropriate rhizobial morphogenic triggers and subsequent Rhizobium-modulated nodule ontogeny requirements [14].

Transferring nodule genes in (to) rice

It is postulated that, if rice contains none of the genetic apparatus required for nodulation, an unknown number of nodule genes and unknown number of regulatory genes would have to be transferred from legumes or from genera nodulated by *Frankia*, such as *Casuarina*, *Alnus*, and *Myrica* [17]. The argument is also that, it may be necessary to modify these genes considerably to render them functional in their new milieu. Since nodules will demand photosynthate from the plant in return for reduced N, it may be necessary to modify the source-sink relations of the rice plant to accommodate nodules. Therefore, it is opined that, knowledge of N metabolism in rice root would also have to be greatly expanded [17].

However, the prospect of transferring nodule gene in (to) rice for developing an intimate symbiotic association between rhizobia and rice is driven by the hypothesis that, rice possesses an incomplete genetic program for nodulation and indeed, recent results support this hypothesis. The development of legume nodules is accompanied by the activation of a unique set of plant genes termed 'nodulin' genes [58]. These are again classified into early and late nodulin genes, which are expressed at the very early stages of nodule development. If rice can be engineered to interact with rhizobia, one requirement will be the presence of the protein called early noduline gene required for nodule development. It has been shown that cDNAs of several legume *ENOD* genes hybridized to DNA from a wide variety of rice genotypes [59]. *ENOD 40* gene homologues ObENOD 40 and *OsNOD 40* from the wild and cultivated rice genotypes *Oryza brchyantha* and *Oryza sativa* respectively have been isolated[60]. Also, using *ENOD 12*-GUS transgenic plants, it has been found that, rice can respond to the addition of rhizobial lipo-chitin nodulation signals, i.e., Nod factors [61]. This surprising result indicates that, rice has the ability to recognize not only the Nod signals but also the signal transduction pathway to couple this recognition to gene transcription.

Conclusion

Till date in nature, no 'true nodulation', *i.e.*, 'functional nodulation' in rice has been recorded. On force too, rice-rhizobia symbiotic relation able to nitrogen fixation could not be formed yet. In spite of that, some of the researchers feel that, excellent opportunity have been created to investigate the possibilities of incorporating nitrogen fixation capacity in (to) rice [62]. Reasons of their assumptions are: (i) recent advances in understanding the symbiotic relationship as applicable to BNF, (ii) evidences that rice possess some developmental sub programmes which are similar to those that leads to development of symbiosis in legumes, and (iii) advent of powerful molecular engineering bio technique [15,62].

But, till date it remains a distant possibility [42]. Why? On one side, though some of the molecular interactions that occur in legume-rhizobia interactions and rice-rhizobia are similar, many differences also exist, and on the other side, though most of the genes necessary for nitrogen fixation in bacteria are well characterised, the transfer of the genes to the plant genome, along with appropriate expression of all these genes beyond current technical ability [14,63].

Therefore, it is advocated that, less developed nitrogen fixation association be preferable goal at this stage, as because, there is a fair degree of probability to induce endophytíc root colonization [57]. Given the technical problems in engineering for true nodulation, which involves a number of required enzymatic and developmental steps, there are opinion too that, enhancing rice nitrogen status by optimizing associations between rice and naturally colonizing endophytic bacteria may be more promising [42].

Several researchers are also of the view that, though some genetic mechanism exist and function in rice, which are instrumental in initiating symbiotic nodulation, the monocot plant such as rice would unlikely to posses the complete complement of genes or genetic programme involved in the nodule ontogeny [14-16]. Therefore, if even a genetic engineering can re-recruit the counterparts of these genes to form functional nodules in rice, it appears that, it is more ambitious and distant goal[54].

There are also views that, though there are many potential obstacles in the transfer of functional nitrogen fixation in rice, there are no theoretical reasons why it cannot be accomplished as it is simply a complex engineering job [64]. It is also fact that, genes controlling nitrogen fixing symbiosis of legumes with specialised bacteria known as rhizobia are presumably the product of many millions of years of evolution[65]. For such evolution to take place for rice-rhizobia symbiosis, if alone left to nature, we have to wait for many more years[64]. Hence, it is opined that, despite the biological obstacles, there are strong and obvious grounds for making an attempt to achieve it and hence, there is postulation that, in principle, it is surmountable [42,66]. Therefore, it is advocated that studies should be further extended at the cellular and molecular levels to identify why such responses do not fully occur in rice [14]. Researchers are hopeful that, man will be able to speed up that process[64].

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