Transforming plant biology to translational research applications – National Botanical Research Institute

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Understanding the structural and functional fabric of plant life is a major facet of plant science research. In the face of several unprecedented challenges, including the impending issues of climate change, environmental pollution, biodiversity loss, genetic erosion, malnutrition and diseases, plant science demands a synthetic and systems approach to find affordable solutions to the above challenges. The challenge before plant biologists is, therefore, not limited only to discovering and documenting the diversity in plants, fungi and microbes, but also in identifying the useful properties in them. A premier plant science research centre under the patronage of the Council of Scientific and Industrial Research (CSIR), the Lucknow-based National Botanical Research Institute (CSIR-NBRI) is known for its excellence in enriching the knowledge base on plant diversity of India and its systematic documentation, conservation and sustainable utilization through traditional and advanced biotechnological approaches. CSIR-NBRI has a wholesome expertise in plant biodiversity, biotechnology and bioinformatics. The Institute has developed technologies and knowhow for the preparation of a number of value-added herbal products as well as microbial technologies for the development of biofertilzers, bioinoculants and biopesticides. Its translational research on microbial consortia-based bio-inoculants, executed along with the Directorate of Agricultural Research, Govt of Uttar Pradesh won the prestigious CSIR S&T Award for Rural Development for the year 2011.

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THE year 2014 witnessed CSIR–NBRI, Lucknow catching up momentum with the emerging trends in transdisciplinary research, especially in prospecting plant and microbial diversity for identifying potential candidate species/strains as new sources of biochemicals, bioenergy and bioinoculants as well as in understanding the molecular mechanisms behind plant development and biosynthesis of secondary plant metabolites and their application in human health. Highlights of a few such exemplary researches carried out at the Institute during 2014 are presented here.

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Scenedesmus abundans: a potential feedstock for high-quality diesel production

Over exploitation of fossil fuels due to increase in human population has raised several environmental issues. At present about 90% of global energy demand is fulfilled by the burning of fossil fuels. If this trend continues, it is predicted that the finite fossil fuel reserve will be completely exhausted in near future. To overcome global energy crises, several renewable sources of energy have been explored in the last few decades. Biofuel, which is non-toxic, renewable and biodegradable in nature, has gained much popularity as an alternative to fossil fuels.

Algae are considered promising feedstock for biofuel production. They have several benefits over other energy crops, e.g. they do not compete with other food crops for growth, they do not require large land area for growth and biomass production, and they can be grown on sewage and effluent water and can thus mitigate the problem of wastewater treatment. Therefore, in its quest for new and eco-friendly sources of biofuels, CSIR-NBRI identified a freshwater green micro-alga, *Scenedesmus abundans* (Kirchner) Chodat (Figure 1 *a*), as a potential candidate for high-quality biodiesel production[1].

S. abundans was isolated from the algal collection made by the CSIR–NBRI team from Dal Lake, Srinagar, Kashmir in 2011. The algal culture was established and maintained through optimal growth conditions. Biochemical analysis revealed that *S. abundans* produced lipid content of 37% and 67% of dry cell weight in nutrient replete and deprived conditions (nitrogen limitation) respectively, which was much higher than the lipid content in other species of *Scenedesmus* (*S. dimorphus* – 26%; *S. obliquus* – 21%; *S. quadricauda* – 18%)[2]. The fatty acid profiles of *S. abundans* also reveal the abundance of fatty acids with carbon chain length of C16 and C18. Oleate (C 18 : 1), palmitate (C 16 : 0), linolenate (C 18 : 3), linoleate (C 18 : 2), palmitoleate (C 16 : 1) and

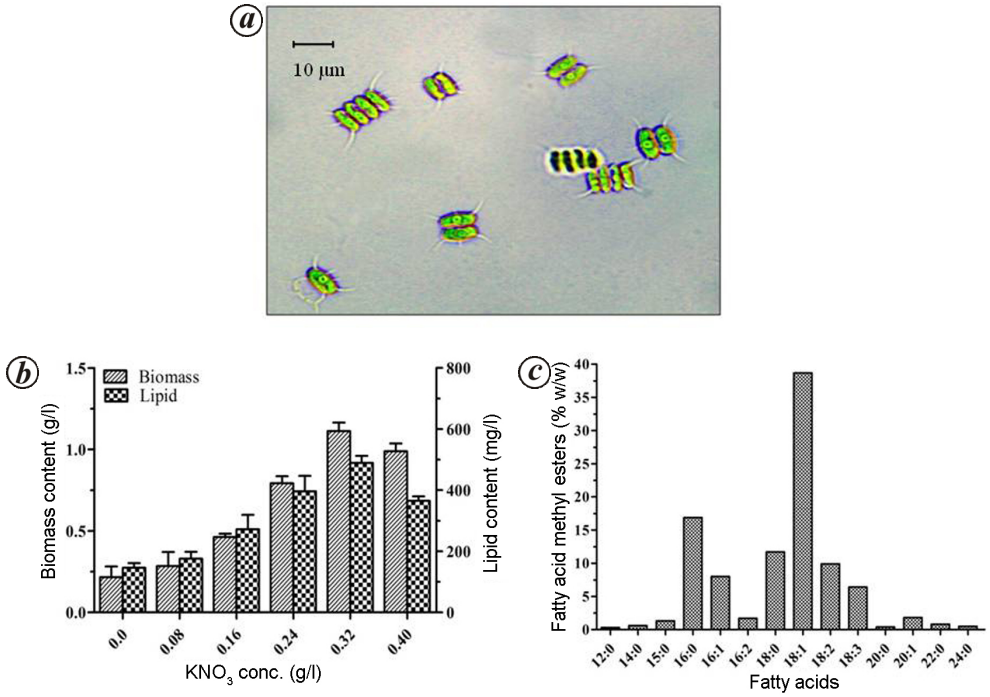

Figure 1. ***a***, Photomicrograph of *Scenedesmus abundans*. ***b***, Comparison of biomass and lipid content of *S. abundans* cultured in different nitrogen concentrations using modified CHU-13 medium. ***c***, Fatty acid methyl esters profile of *S. abundans* grown in large scale using photobioreactor. (***b***, ***c***, Source: Mandotra *et al.*[1].)

stearate (C 18 : 0) contribute about 90.6% of the total fatty acid methyl esters content (Figure 1 *b* and *c*). The biodiesel properties such as cetane number, degree of unsaturation, iodine value and saponification value meet the criteria of National Petroleum Agency (ANP255), European biodiesel standard EN14214, Germany's standard DIN51606 and South African standards SANS1935.

The higher biomass and lipid content in *S. abundans* with suitable fatty acid profile suggest that the alga can be grown on large scale in algal raceway ponds for industrial production of biodiesel. This report was the first experimental evidence showing the potential of this alga as a biodiesel source.

Rapid synthesis of gold nanoparticles by biocontrol agent *Trichoderma*

Nanoparticles have a wide range of applications in pharmaceuticals, drug delivery, imaging, sensors, catalysis, optics, and nanotechnology has become an outstanding substitute in every aspect of human welfare. Gold nanoparticles have attracted significant scientific interest as a new generation of antimicrobial agents because of increasing resistance of bacteria towards antibiotics and potent biocatalysis property. Among several sources such as plants, fungi, bacteria, actinomycetes and agro waste, fungi are considered a better source for higher productivity of nanoparticles because of extracellular secretion of proteins and secondary metabolites. Rapid synthesis of nanoparticles in a short span of time has several advantages. The few reports available on rapid synthesis of nanoparticles are based on pathogenic bacteria or fungi, which may cause serious problems during their further applications. To avoid these complications, scientists at CSIR–NBRI successfully developed a rapid, clean and eco-friendly method for biosynthesis of gold nanoparticles using cell-free extract of the non-pathogenic *Trichoderma viride* and recombinant *Hypocrea lixii* at different reaction temperatures[3].

The study reported extracellular biosynthesis of gold nanoparticles in only 10 min with cell-free extract of *T. viride* at 30°C and 100°C, where *H. lixii* also biosynthesized gold nanoparticles within minutes only at 100°C. Synthesized particles were capped and stabilized by biomolecules present in cell-free extract of both the fungi. Further, the particles were characterized by visual observations, UV–Vis spectroscopy, zeta sizer, transmission electron microscopy (TEM), selected area electron diffraction (SAED) pattern and energy dispersive X-ray spectroscopy (EDAX). A characteristic surface plasmon resonance peak of gold nanoparticles synthesized from both *Trichoderma* sp. was observed between 500 and 600 nm. SAED pattern confirmed the crystalline nature of biosynthesized gold nanoparticles, while the presence of bioelements along with gold was visualised by EDAX spectroscopy. TEM studies revealed that majority of particles were 20–30 nm in size when synthesized by *T. viride* at 30°C after 10 min of reaction, while particles were smaller in size when synthesized at 100°C.

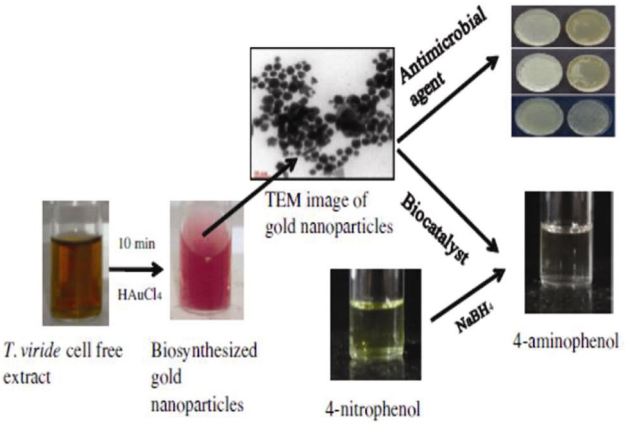

Figure 2. Biocatalytic and antimicrobial properties displayed by biosynthesized gold nanoparticles from cell-free extract of *Trichoderma* sp. (Source: Mishra *et al.*[3].)

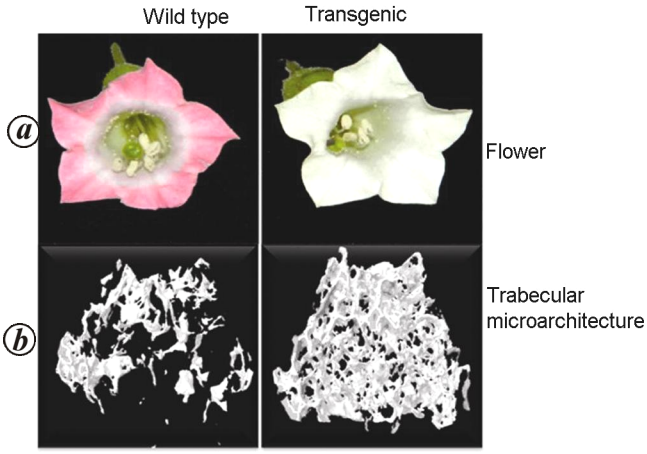

Figure 3. Metabolic engineering for isoflavones biosynthesis and improved bone health. ***a***, White flowered phenotype of the transgenic tobacco line co-expressing the *Arabidopsis* transcription factor (*AtMYB12*) and soybean isoflavone synthase (*GmIFS1*) genes accumulated enhanced flavonol (genistein) content. ***b***, Oestrogen-deficient (ovariectomized) mice fed with leaf extract from transgenic plants exhibit significant conservation of trabecular microarchitecture as analysed using microcomputed tomography.

The biosynthesized nanoparticles served as an efficient biocatalyst, which reduced 4-nitrophenol to 4-aminophenol in the presence of $NaBH_4$ and showed antimicrobial activity against pathogenic bacteria. On addition of biosynthesized gold nanoparticles, the absorbance at 400 nm decreased continuously for 35 min and became constant thereafter, illustrating the transformation of 4-nitrophenol to 4-aminophenol (Figure 2). The particles also demonstrated antibacterial activity against *Pseudomonas syringae*, *Escherichia coli* and *Shigella sonnei.* A significant reduction of 53%, 47% and 55% for *E. coli*, *S. sonnei* and *P. syringae*, respectively, was observed with 25% of supplemented nanoparticles. Cotton immobilized assay with sterile water and poly vinyl pyrrolidone (PVP) demonstrated higher antimicrobial activity with PVP, which can further be impregnated on contact lens, socks and bandages to avoid microbial contamination. This was the first report of rapid synthesis of gold nanoparticles by *Trichoderma* sp. which can act as an antimicrobial agent as well as an efficient biocatalyst.

Transgenic plants with increased osteoprotective activity

Secondary plant metabolites play a crucial role in pollination, dispersal and defence systems in plants. These metabolites, including various alkaloids, flavonoids and phytosterols are beneficial for human health, and are being used as anti-cancerous, anti-inflammatory, immunosuppressive, immuno-stimulant and anti-osteoporosis agents. Synthesis and accumulation of these molecules is species as well as chemotype-specific and under strict regulation of gene expression. Majority of food products consumed by humans are deficient in these phytoceuticals. Therefore, there is an urgent need to scale up biosynthesis of these secondary plant products in plants synthesizing these molecules or develop strategies for their synthesis in plants which do not synthesize these molecules through pathway engineering.

Among secondary plant products, isoflavones, a subgroup of flavonoids, are synthesized almost exclusively in the plant family Leguminosae. Isoflavones are reported to have shown antioxidant, anti-mutagenic, anti-carcinogenic, anti-osteoporotic and anti-proliferative activities in human and animal systems. Various attempts have been made in the past to synthesize these molecules in commonly consumed food crops through metabolic engineering. Earlier efforts, based on expression of isoflavone synthase (*IFS*) genes from leguminous plants in non-legume plant species had limited success, in terms of isoflavone content in the transgenic tissue. Such studies paved the way for pathway engineering to provide enhanced substrate flux for IFS enzyme in target plants.

In an earlier study, scientists at CSIR-NBRI showed that the expression of *Arabidopsis* transcription factor *AtMYB12* in tobacco resulted in enhanced expression of genes involved in phenylpropanoid pathway, leading to several fold higher accumulation of flavonols[4]. In order to overcome substrate limitation for IFS, further experiments were carried out on activation of multiple genes of flavonoid pathway by *Arabidopsis* transcription factor *AtMYB12*. The team at NBRI successfully developed transgenic tobacco lines constitutively co-expressing *AtMYB12* and *GmIFS1* (soybean *IFS*) genes, and their phytochemical and molecular analyses were carried out[5]. The leaves of transgenic lines accumulated substantial amount of genistein (up to 0.058 ± 0.007 mg/g FW), not reported as yet in any transgenic plants developed, in addition to flavonols. The health benefits of the engineered transgenic lines were examined through experimental

trials on estrogen-deficient (ovariectomized, Ovx) mice fed with leaf extract from transgenic plants. Extracts from transgenic plants co-expressing *AtMYB12* and *GmIFS1* (but not wild-type extract) exhibited significant conservation of different parameters for bone health (Figure 3 *a* and *b*). These novel findings establish strategy for successful pathway engineering of isoflavones in crop plants (like tomato, banana, rice, etc.) and provide a direct evidence of improved osteoprotective effect of transgenic plant extracts.

Core promoter elements regulate light-mediated gene expression in plants

Structural architecture of the core promoter plays an important role in regulated expression of genes. The promoter of a gene is composed of two types of promoter elements – core promoter elements and proximal promoter elements – and their interaction is essential for controlling gene expression. The TATA-box present in the core promoter region, is required for accurate transcription initiation and it determines the level as well as selectivity of gene expression in plants. The light signalling regulates a series of physiological and biochemical changes a plant shoot undergoes in response to sunlight, called as de-etiolation, which is an important phenomenon for plant development. The phytochromes (PhyA–E) are photoreversible and are found in two forms, Pfr (active) and Pr (inactive). The PhyB perceives light signals and activates the transcription factor HY5 which directly binds to light responsive elements (LREs) located upstream to the TATA-box in the promoter and synergistically regulates the gene expression. The light-regulated promoters can be distinctly grouped into either TATA-box-containing or TATA-less/Inr-containing promoters. Although various studies have been performed to understand light-mediated regulation of gene expression, little is known about the mechanism of regulation in TATA-less promoters and discrete role of TATA-box and Inr elements.

Scientists at CSIR-NBRI are involved in studies enumerating the role of TATA-box in transcriptional regulation[6,7]. In an interesting experimental study using TATA-box containing light activated/light repressed and Inr containing light activated/light repressed promoters, scientists at NBRI observed that the TATA-box and Inr (Initiator) elements have distinct mechanisms which are involved in light-mediated regulation, and these elements are not swappable using either native promoters or their swapped versions of core promoter elements[7]. The results showed that mutations in either functional TATA-box or Inr elements led to the formation of nucleosomal structure. The study further explored nucleotide polymorphism in the TATA-box or Inr element in *Arabidopsis* ecotypes and found that nucleotide variation in core promoters can affect gene expression. It was also found that the light-activated promoters contain different specific regulatory motif, present downstream of transcription start site (TSS), which serves as a key factor in regulating light promoters that are parallel with these elements (Figure 4 *a–d*). The study concludes that the TATA-box or Inr element does not act in isolation. There is probable involvement of other distinct core promoter elements in concurrence with the TATA-box or Inr element to impart selectivity to light-mediated transcription.

The new model of light-mediated gene expression provides a better understanding on the molecular mechanisms behind plant development and elucidates the role of core promoter elements in the light-regulated transcription with regard to the TATA-box-containing and TATA-less promoters. The results highlight unexpected complexity of the core promoter and the involvement of a probable interaction between the TATA-box/Inr and other cis-regulatory elements in the core promoters for light-mediated transcription regulation.

Molecular evidences on arsenic transport and tolerance in rice

Arsenic (As), a metalloid ubiquitous in the terrestrial environment, is considered a potential carcinogenic element and causes serious health risk to people worldwide. The East Asian countries are suffering greatly from As toxicity. One major route of As exposure to human is due to contaminated food chain, especially consumption of rice grains. Rice grain accumulates high amount of As when grown either in As-contaminated soil or irrigated with As-polluted water. Therefore, rice cultivars with less As in the grain are highly desirable for cultivation in extreme As environments.

In order to reduce As intake via consumption of contaminated rice grain, identification of the mechanisms for As accumulation and detoxification in rice is a prerequisite. In previous experimental studies conducted at CSIR-NBRI, several genes that are differentially expressed during As exposure were identified[8]. These genes include those related to defence and stress-responsive, transporters, heat-shock proteins, metallothioneins, sulphate-metabolizing proteins, and those involved in regulatory processes and networks. However, their direct involvement in As transport and accumulation was not known. A recent study published in 2014 by NBRI reported involvement of a member of natural resistance-associated macrophage protein (OsNRAMP1) transporter in As, in addition to cadmium (Cd) accumulation through functional genomics approach[9]. Expression of *OsNRAMP1* in yeast mutant (*fet3fet4*) complemented iron (Fe) uptake and exhibited enhanced accumulation of As and Cd. Surprisingly, heterologous expression of *OsNRAMP1* in *A. thaliana* provided tolerance in addition to enhanced As

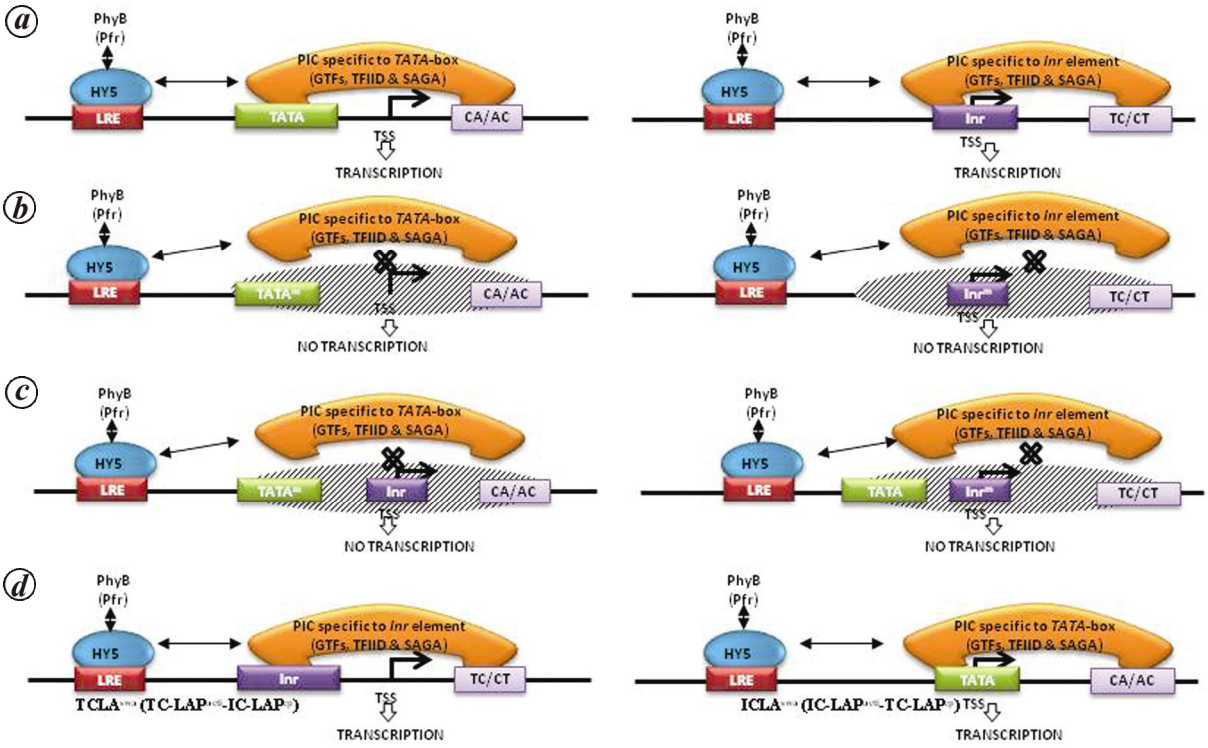

Figure 4. ***a–d***, Selective activation models of the core promoter elements of light-regulated promoter. ***a***, The assemblies of transcription factors and complex that identify the promoter lead to the activation of gene expression in plants. In the natural promoter of TATA/Inr type, the light signal mediated by upstream activators operates on TATA+/Inr− or TATA−/Inr+ to activate transcription initiation. Transcription initiation starts with cooperative interaction between upstream sequences, transcription factor, and PIC complex, which, in turn, removes the repressive nucleosome structure over core promoter elements. ***b***, When the core promoter region contains mutated TATA/Inr, transcription initiation is stopped. ***c***, In TATA/Inr swap or vice versa, transcription initiation is not established due to lack of interaction between downstream of the TSS and PIC along with TATA/Inr in the promoter. ***d***, Interchanging the core promoter sequences either with proximal promoter of TATA/Inr light-activated promoter re-establishes the transcription. LRE, Light-responsive element; TC, TATA-box-containing; IC, TATA-less Inr-containing; LAP, Light-activated promoter; acti, Activator region (proximal promoter) and cp, Core promoter sequences. (Source: Srivastava *et al.*[7]; reproduced by permission of Oxford University Press.)

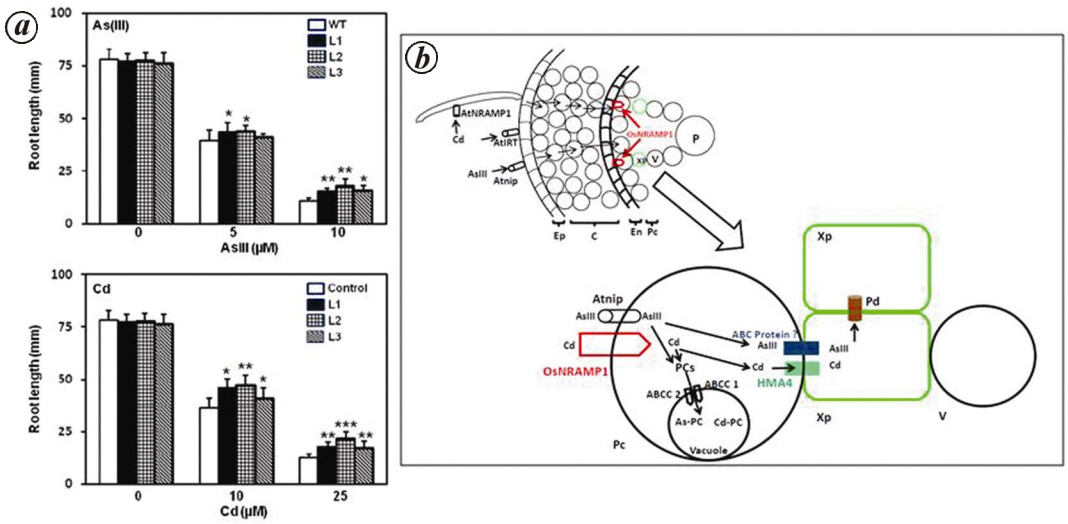

Figure 5. ***a***, ***b***, Expression of *OsNRAMP1* in *Arabidopsis* leads to tolerance for heavy metals and cellular localization. ***a***, Root length of *Arabidopsis thaliana* plants after growth for 10 days on ABIS medium supplemented with As (III) (5 and 10 µm) and Cd (10 and 25 µm). Seeds of *A. thaliana* (WT; accession Col-0) and three transgenic lines (L1–L3) were germinated on ABIS medium supplemented with As (III) (5 and 10 µm) and Cd (10 and 25 µm). ***b***, Schematic view of transverse section of the transgenic root. Symplastic route for entry and efficient loading of As (III) and Cd into vascular tissues by *OsNRAMP1*, especially in xylem. Ep, Epidermis;C, cortex; En, Endodermis; Pc, Pericycle; Xp, Xylem parenchyma; V, Xylem vessel, and Pd, Plasmodesmata. (Source: Tiwari *et al.*[9].)

and Cd accumulation in root and shoot. To establish correlation between enhanced accumulation and tolerance, further experiments were carried out to study localization of OsNRAMP at cellular level through expressing OsNRAMP : GFP fusion protein in *Arabidopsis*[9]. Cellular localization revealed that OsNRAMP1 resides on the plasma membrane of endodermis and pericycle cells and may assist in xylem loading for root to shoot mobilization (Figure 5 *a* and *b*). This was the first report demonstrating role of NRAMP in xylem-mediated loading and enhanced accumulation of As and Cd in plants. This study hypothesizes that modulated expression of *OsNRAMP1* in rice might be helpful in developing varieties with low As and Cd content in grain and minimize the risk of food chain contamination.

Plant science research at CSIR-NBRI continues to be a challenging area, especially in search of new and alternate plant and microbial resources as well as innovative and sustainable technological solutions for agriculture, health, energy and food security, and environmental and biodiversity conservation.

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