Chapter 6

Summary

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Glutathione synthesis is catalysed by the product of two genes *gshA* and *gshB* sequentially. In first step *gshA* gene encoded y-glutamylcysteine synthetase (GCS) enzyme ligates glutamic acid and cysteine to form y-glutamylcysteine and in the second step, *gshB* encoded enzyme glutathione synthase (GS) catalyses the fusion of y-glutamylcysteine and glycine to make a tripeptide glutathione molecule. Glutathione is one of the most abundant thiols present in prokaryotic and eukaryotic cells. In bacterial, its main role is to maintain the proper oxidation state of protein thiols and protect the cells from oxidative stress. Both bacterial and eukaryotic GCS can be operated by a feed-back loop mechanism, where the glutathione binds the active site of glutamic acid and prevents further binding of glutamic acid. The thiol group of glutathione GSH interacts inside the active site of GCS enzyme.

E. coli ybdK gene encodes for an enzyme YbdK, which has the gamma glutamate-cysteine ligase activity, but it is structurally different with lower kcat as compared to yGCS enzyme. The recombinant plasmid pPAT, bearing *E. coli ybdK* was successfully created by restriction ligation of *ybdK* gene and a low copy number plasmid pBBR1MCS2. Rhizobium bacteria *Sinorhizobium fredii* NGR 234 and *Sinorhizobium meliloti* (NIAMCC B-00836) were transformed with this plasmid, which significantly increased glutathione production in transformed rhizobium compared to their wild type counterpart.

GMO rhizobium capable of producing more glutathione were used as a PGPR for fenugreek seedlings growing in Arsenic and Cadmium contaminated soil. For this, the seeds of fenugreek were coated with GMO and wildtype rhizobium and sown in Arsenic and Cadmium spiked soil.

During individual PGPR experiments we observed that the GMO PGPR were able to reduce heavy metal induced ROS in fenugreek seedlings by lowering the antioxidant enzyme levels, except GR enzyme. Chlorophyll and Carotenoids amount, biomass and growth parameters of the GMO PGPR treated seedlings also increased significantly compared to other wild type PGPR treated seedlings.

Similarly, GMO PGPR consortia was able to reduce heavy metal induced ROS in fenugreek seedlings and enhance the Chlorophyll and Carotenoids amount. Biomass and growth parameters of the GMO PGPR consortium treated seedlings also improved significantly compared to other wild type consortium treated seedlings. The effectiveness of the GMO rhizobia in a consortium with other native PGPR of the soil was more complex, but it was

found that the GMO consortium was more effective at reducing heavy metal-induced ROS and improving the growth parameters of fenugreek seedlings compared to wildtype consortia.

Besides this GMO rhizobia secreting more glutathione were able to synthesize slightly more amount of cadmium sulphide nanoparticles. It was also observed that the Cds nanoparticle aggregates formed by GMO rhizobium were smaller compared to the nanoparticle aggregates formed by wild type rhizobium. Size of aggregates/nanoparticles depends upon the number of glutathione molecule capping the cadmium metal. More capping of glutathione will result in dispersed and smaller aggregates; hence they will not agglomerate. Therefore, it can be said that formation of smaller aggregates is an indicator of elevated excretion of glutathione by GMO rhizobia compared to the wildtype rhizobia. Hence it again proves a successful expression of YbdK in the rhizobial strains.

Looking at its agricultural perspective, the smaller aggregates have more surface to volume ratio compared to larger aggregates, that increases its chances for further modification by numerous other microorganisms present in rhizosphere.