Molecular cloning as a tool to improve crop yield.

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Molecular cloning is widely used as a method to transfer a particular trait to any variety of same or even completely unrelated species. It is also used to over express a gene or a group of genes to change its property like yield or content etc. We are interested to improve the salt tolerance of rice and are therefore cloning several genes in order to transform into farmer popular varieties to improve their acceptability to our local farmers in salt affected areas. The most promising genes reported to improve salt tolerance are for the Vacuolar Sodium/Hydrogen Antiporter, DNA helicase and Glyoxalase pathway. Traditional digestion-ligation methods were bypassed using Gateway technology where insert is transferred primarily to an entry vector. The linearized entry vector contains topoisomerase enzyme tagged at the end which transfers the bond to the insert and enables cloning within 5 minute incubation at room temperature. The entry vector contains recombination site flanking the insert site allowing transfer of the insert to the destination vector simply by incubating two vectors with recombinase enzyme. A wide choice of destination vectors allows us to characterize the insert in a different way. Targeted genes were cloned and transgenic plants were regenerated and are being characterized. They are showing promising results upon salinity screening. But commercial binary vectors contain the constitutive CaMV 35S promoter which has bee shown to poorly express in monocots. Constitutive expression also causes yield penalty, an undesirable character for high yielding varieties. Therefore a salt inducible efficient promoter was needed for these destination vectors. We have isolated an upstream region and characterized to be an efficient promoter. The CaMV 35S promoter had to be replaced by traditional digestion-ligation method. Digestion with the same enzyme was not compatible and therefore we modified the traditional process. We filled the digested vector ends and added T- overhang. At the same time we added A-overhang to the amplified insert. This enabled the vector and inserts to be ligated as TA cloning method. We have now produced transgenic plants with the Na/H antiporter driven by the salt-inducible promoter and are comparing salt tolerance of these plants with those that have the same gene driven by the CaMV 35S promoter.