In vitro plant regeneration of indica rice varieties: Bg-360 and Bg-250

R.M.L.K.Ratnayake and G.H.C.M.Hettiarachchi
Department of Chemistry, University of Colombo.

Rice is the single most important crop in Sri Lanka which needs to improve the yield in order to combat with demands of increasing population. The improvement can possibly be achieved by genetic manipulation and it is highly dependent on the use of *in vitro* techniques. Callus induction and plant regeneration are prerequisites in *in vitro* techniques for the use of biotechnological approaches for rice improvement through genetic transformation. In this study the callus induction and plant regeneration were done with Bg-360, Bg-250 Sri Lankan rice varieties on modified N_6B_5 medium. High variability in callus induction and plant regeneration was observed among the cultivars. The candidate variety Bg-250 showed highest (90%) callus induction frequency than that of the other candidate variety Bg-360 (41.25%). Highly significant regeneration difference was observed in partially desiccated calli of Bg-360 in comparison to non-desiccated Bg 360 calli. It took 5-6 weeks for callus to regenerate into complete plants. The Bg-360 produced 16 and Bg-250 produced 13 plantlets per callus, with the regeneration frequencies of 42.42% and 95% respectively. The shoots regenerated from calli were successfully transferred to rooting medium for one week and plantlets with healthy roots were established in the soil. The results suggested two rice varieties used, Bg 250 showed the highest regeneration efficiency and after desiccation Bg 360 could successfully regenerated into complete plant. In conclusion, with standardized rice regeneration protocol, Bg 360 and Bg 250 are good candidates to transform with Arabidopsis STH2 gene in order to improve their yield.

Interallelic and intragenic recombination at the hypervariable region of merozoite surface protein-1 (MSP-1_{33}) of *Plasmodium vivax* in Sri Lanka

S. Dias[1], S. Longacre[2], P. V. Udagama-Randeniya[1]

[1]Department of Zoology, Faculty of Science, University of Colombo, Colombo 3, Sri Lanka, [2] Laboratoire de Vaccinologie, Department de Parasitologie, Institut Pasteur, Paris Cedex 15, France.

Extensive polymorphism in *Plasmodium* Merozoite Surface Protein-1_{42}, a leading vaccine candidate for malaria, poses serious problems for vaccine development. Ninety five *P. vivax* isolates were investigated at the *Pvmsp-1_{42}* locus from malaria endemic and non-endemic sites in Sri Lanka. All 95 sequences were identical at the amino acid (a.a.) level of PvMSP-1_{19}, while 39 amino acid (a.a) variant positions in PvMSP-1_{33} defined 27 a.a. haplotypes with 19 unique to the island. Regions I (RI) and III (RIII) that flank the hypervariable region (RII) of PvMSP-1_{33} included 2 and 1 unique variant positions, respectively. While, 24 of the 27 PvMSP-1_{42} haplotypes represented 7 basic a.a. sequence types (HVR T1-T7) in the hypervariable region, the remaining 3 haplotypes, generated by interallelic double recombination between T1/T3 and T2/T3, represented HVR T8-T9 and HVR T10, respectively. A further 107 local and global isolates manifested 62 more a.a. haplotypes (H28-H89); 74 of these showed 9 of the 10 HVR types, with the exception of HVR-T7, unique to Sri Lanka. Two novel HVR types, T11 and T12, with a double

recombination between HVR-T1/T3 and HVRT6/T2, are reported for the first time from South America and Thailand, respectively. Intragenic recombination was a critical factor for the manifestation of a.a. haplotypes representing HVR-T3 to T7, and for the generation of H71-H89. In conclusion, under low transmission and unstable malaria conditions prevalent in the island, both interallelic and intragenic recombination appear to be critical for the origin of new PvMSP-1_{42} amino acid haplotypes in local *P. vivax* populations.

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Phylogenetic relationship of *Plasmodium vivax* duffy binding protein gene between Sri Lankan and world wide isolates

P. H. Premaratne[1], B. R. Aravinda[1], A. A. Escalante[2], P. V. Udagama-Randeniya[1]

[1]Department of Zoology, Faculty of Science, University of Colombo, Colombo 03, Sri Lanka, [2]School of Life Sciences, Arizona State University, Tempe, AZ, USA.

Phylogenetic relationship of the region II of the *P. vivax* Duffy Binding Protein gene (*PvdbpII*), a prime vaccine candidate against blood stage of vivax malaria, was analysed between parasite isolates from Sri Lanka and from different geographical areas, worldwide. A gene tree was derived from the aligned nucleotide sequences of a 672 bp region, using the Neighbour-Joining method with 1000 bootstrap replicates, the Tamura's three-parameter distance model as implemented in the MEGA version 4.0 programme. One hundred and seventy five *PvdbpII* sequences found in the GenBank were compiled and compared to 100 Sri Lankan (SL) sequences. The worldwide sequences included those from the reference Salvador-I strain, single sequences each from Vietnam (VIAT), Indonesia (INDO) and India (INDI) and 13, 17, 30 and 111 sequences from South Korea (SK), Colombia (COL), Thailand (Thai) and Papua New Guinea (PNG), respectively.

Twenty three different groups in all were evident in the gene tree. SL isolates were grouped in 6 different clades (*A*-*G*). A majority of these concentrated amongst 3 different clades (A, B and C), with no clustering detected according to the sample collection sites within the country. A single clade (*D*), exclusively contained SL isolates, proving evidence for geographical isolation. Two other clades (*A* & *F*) contained isolates from SL in combination with THAI and SK, respectively. The close phylogeny of the SL sequences with world wide ones were more clearly evident in the gene tree by SL isolates grouping with those from PNG, THAI, COL, INDO and VIAT in clade *B*, and with those from PNG, THAI, COL and from INDI with the reference strain Sal-1 in clade *E.* Interestingly, both these groups lacked SK isolates. The Sri Lankan *PvdbpII* sequences thus appeared to represent a sample from the *PvdbpII* worldwide genetic diversity, rather than from any particular lineage.

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