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Sequence analysis of Nagina-22 drought tolerant ESTs for drought specific SSRs

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SUMMARY

Several studies reveal that rice variety Nagina-22 have putative genes responsible for drought tolerance and the chromosome regions that possess these genes have been also identified as site for drought tolerance QTLs.This knowledge was exploited for the development of SSR markers specific for drought resistance.A total of 31 SSR primers were designed out of which 23 gave amplicons and the length of primers ranged from 18-24.Source EST sequences gave hits on chromosome 1,2,3,5,6,9 and 12.

Key words : Drought, Rice, SSR markers, QTLs

Rice is the most important food crop of world grown under 149mha area(FAO, 2006). Rice is mostly grown in Asia where it is estimated to provide 35-60 % calorie intake (FAO, 2006;Khush, 1997). Rice breeding for drought have made little progress to date (Fukai and Cooper, 1995). This is due to the fact that drought resistance is a trait controlled by many genes having different effects, and is dependent on types and severity of drought. Complexity of drought involves an interaction between the genes involved in yield potential per se (which are numerous) and the genes for drought resistance.

Nagina-22 a selection from landrace Rajbhog in Nepal is a well known drought tolerant cultivar in northern India. It has been used as a drought tolerant donor for drought breeding since last three decades in India. Expression studies with N-22 lines reveal the fact that there are putative genes in it which are most likely responsible for drought tolerance (Gorantla et al., 2007). Transcriptome analysis clearly depict that regions that are supposed to have those genes coincide with QTLs identified for drought tolerance as reviewed by earlier workers. Major QTLs for drought tolerance are dispersed throughout the genome.It would be worthwhile therefore to concentrate on already established regions for identification of candidate genes for drought tolerance.

MATERIALS AND METHODS

SSR Primer designing:

Expressed sequenced tags of Nagina-22 have been downloaded (Gorantla *et al*., 2007).Total of 503 ESTs were analyzed using Web Troll software for the presence of simple sequence repeats in them. Primers flanking to the repeats were designed using Primer plus software. For primer designing both number and length of repeats were considered. Only those sequences which were sizeable in number and size of repeat were used for designing primers. Parameters of the primers were kept default. Total of 31 primers were designed. These primers were checked for hairpin structure, dimmers etc.

DNA extraction and primer validation:

DNA of Nagina-22 was extracted using CTAB method. PCR reaction was carried out with all 31 SSR primers using DNA of Nagina-22. For PCR amplification 50 pmol of each primer and 50 ng of genomic DNA were added to 25 µl of PCR reaction mixture with 1 x reaction buffer (Promega), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP and 0.5 units of T*aq* DNA polymerase. Forty cycles of PCR, consisting of denaturation at 94^0 C for 1 min, reannealing at 60^0C for 1.0 min, and extension at 72^0C for 45 seconds, were performed in a DNA thermal cycler (PTC-200, MJ Research, USA). PCR product was run on 1.5% agrose gel.

RESULTS AND DISCUSSION

The results obtained from the present investigation are presented in Table 1.

Out of 31 primers designed 23 gave amplicons from the N22 DNA. Length of primers range from 18-24 (Table1). Annealing temperature of most primers was 59-60. Repeat motif were di, tri, tetra, penta and hexamer.Product sizes of primers ranged from 112 to 370bp. Source EST sequences gave hits on chromosomes 1,2,3,5,6,9 and 12.

Nagina-22 is a well known drought tolerant cultivar. It has been used as a drought tolerant donor since last

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