RESEARCH PAPER International Journal of Agricultural Sciences, June, 2010, Vol. 6 Issue 2 : 494-496

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

PRASHANT VIKRAM, ALOK KUMAR SINGH AND S.P. SINGH*
Department of Genetics and Plant Breeding, T.D. (P.G.) College, V.B.S.Purvanchal University, JAUNPUR (U.P.) INDIA

ABSTRACT

Several studies reveal that rice variety Nagina-22 has putative genes responsible for drought tolerance and the chromosome regions that possesses of 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

INTRODUCTION

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 Primer3 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

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 three decades. Expression studies with N-22 lines reveal the fact that there are putative genes in it most likely responsible for drought tolerance (Gorantla *et al.*, 2007).

* **Author for correspondence.** & Present Address : Department of Crop Physiology, N.D.University of Agriculture and Technology, Kumarganj, FAIZABAD (U.P.) INDIA

Table1: Sequences and annealing temperature of primers

Name	Forward sequence	Annealing temp.	Reverse sequence	Annealing temp.
N22P1	GGCGGGGCAGAGGAGGAG	67.918	CTCGGGGAACGGGAAGTTGAG	67.321
N22P2	CATCAACGAGCTACCAGTACCA	60.19	AGGAATTTTATTTAGGGGCCAG	59.724
N22P3	TTAGTTGATCTTGGAGCAGTCG	59.517	GTACGTGATGGGGAGGGAG	60.333
N22P4	TATGTGTTAAGAGCAGAGGCCA	59.904	TGATTAGCTTCATCGCCTACAA	59.875
N22P5	AAAGATGCTCCGATGTTTGTCT	60.137	TGCTGGGAATGATGTAAAACTG	59.996
N22P6	AGGCCAGAGAAGGAAGAAGAAG	60.489	TGGTACTGGTAGCTCGTTGATG	60.19
N22P7	AACAAGATCGACGTGAAGGG	60.111	GAGACACAGAACGTACACACGC	60.817
N22P8	GACCTCCTCCATCTTGCTCTC	60.351	GGAGTATGGCTGGACTGCTAAC	60.158
N22P9	AAGGGTGTGTACGCTTGTATCA	59.562	CTCAAGAACATGGCCTCCTC	59.803
N22P10	CTAGGCAAACCACACGAATACA	60.054	CATAGTTGAAGCCGAGAAGAGC	60.522
N22P11	GGGCAACTCTTCTTGTAGTGGT	59.682	GCTGCGGTAGTAGCTGTATCTG	59.227
N22P12	GAAAGCCCAAGAAATGAGCATA	60.566	ACCTAAACAGCCGTAGTCGCT	60.329
N22P13	CACGGCTGGTACTGCAACTA	59.928	TATTTTAGCATCCTCCCCTCAA	59.93
N22P14	TCACCACCGACTACATGACCTA	60.431	GACTCCACGACATACTCAGCAC	59.799
N22P15	CGACGAGCAAGTAGAGCACA	60.347	TGTTTCAAGCTATTCCAACGG	60.117
N22P16	GGGAGTACACAGGCTACAAACA	59.169	TCAGAGGAAGCCAGACATTACA	59.875
N22P17	TATGCTCCAGTGACATCTTTCG	60.27	AAGCCAGGAACACAAACTAGGA	60.165
N22P18	ATGAGGAGATGGAGCAGGAGTA	60.233	GGCCTTAATTTGCTTGCATTAG	60.114
N22P19	GACAGTAGCAGCATCCATACGA	60.3	TTAGTTAAGTCCTTCGCCCTCC	60.928
N22P20	ATCGACAGTAGCAGCATCCATA	59.758	CAATCAGCAACAACATCTATCTCC	60.028
N22P21	ACAGGACGACATGCTCTGG	59.837	TGATACGCACATAGCTCCAAAT	59.636
N22P22	GTTCCACAGGCTCGCGTA	60.991	CGTCATGTGAGTCCCCTACTTT	60.415
N22P23	TATCAACCATGCGTCTCTGTCT	59.763	TCCACACCTACTCCATCATCTG	59.989

ESTs have also been worked out for that (Gorantla *et al*., 2005). 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(Gorantla *et al*., 2005). The aim here was to use this knowledge for the development of SSR markers specific for drought tolerance. Complexity of drought tolerance is a well known fact. Till date there is no any consensus over the mechanism of drought tolerance.

Despite complexity one cannot rule out the possibility of insertion, deletion, duplication and other sources of sequence variations. Single nucleotide polymorphism study of Nagina-22 classified it in "aus" group of cultivars. Chances of insertion, deletion and recombination while due course of evaluation among regions of our interest *i.e.* drought tolerance could not be ruled out. Also interaction among different genes/ loci may be a probable hypothesis. Putative genes in the pathway of drought tolerance coincide with other stress related genes supporting the view that there are interactions between genes for stress tolerance. For mapping, fine mapping and candidate gene analysis one could concentrate on these important regions only. SSRs have been the backbone to create molecular maps for a number of years. These SSRs are useful as molecular markers because their development is inexpensive, they represent transcribed genes and their putative function can often be deduced by a homology search (Varshney *et al*., 2005). SSR primers have been designed using publicly available expressed sequence tags (ESTs) in a number of crops (Thiel *et al.*, 2003; Kota *et al.*, 2001 in barley, Xu *et al.*, 2004 in almond(*Prunus communis* Fritsch.)and peach [*P. persica* (L.) Batsch.], Yu *et al.*, 2004 in *T. aestivum*. and *O.sativa*). This may be an alternate strategy. Expressed Sequenced Tags from Nagina-22 have already been worked out. Simple sequence repeat polymorphism is a cost effective technique with wide application in rice. Simple sequence repeats within ESTs for drought tolerance have been worked out. Evidences of polymorphism are more in the hyper-variable regions so that only those SSRs were taken into account. Such SSR regions can give strong clue about the drought tolerance in populations with donors of 'aus' background like Nagina-22. Also for diversity analysis these can be used but with limited scope. Nagina-22 is also known for high temperature tolerance. Mechanisms of drought and heat

tolerance are reported to be associated with each other as reviewed by some workers (Ian, 2002). SSR primers can be used for the purpose.

REFERENCES

FAO (2006). *FAOSTAT database*. Online Available: http:// faostat. fao.org (5 December, 2006).

Fukai, S. and Cooper, M.(1995). Development of drought-resistant cultivars using physiomorphological traits in rice. *Field Crops Res.,* **40**:67-86.

http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi

Gorantla,M., Babu, P.R., Reddy Lachagari, V.B., Reddy, A.M.M., Wusirika,R.,Jeffrey,L.B.and Reddy,A.R.(2007). Identification of stress-responsive genes in an indica rice (*Oryza sativa* L.) using ESTs generated from drought-stressed seedlings. *J. Experimental Bot.*, **58**(2): 253–265.

Gorantla,M., Babu,P.R., Reddy Lachagari,V.B., Alex Feltus,F., Paterson,A.H. and Reddy,A.R.(2005). Functional genomics of drought stress response in rice: Transcript mapping of annotated unigenes of an *indica* rice (*Oryza sativa* L. cv. NAGINA 22). *Curr. Sci.*, **89**(3): 496-514.

Ian, F. Wardlaw (2002). Interaction between drought and chronic high temperature during kernel filling in wheat in a controlled environment. *Ann. Bot.,* **90**: 469-476.

Khush, G.S.(1997). Origin, dispersal, cultivation and variation of rice. *Plant Mol. Biol.,* **35**:25–34.

Kota, R., Varshney, R.K., Thiel, T., Dehmer, K.J.and Graner, A.(2001). Generation and comparison of EST-derived SSRs and SNPs in barley (*Hordeum vulgare* L.). *Hereditas*, **135**(2-3):145-151.

Thiel, T., Michalek, W., Varshney, R.K.and Graner, A.(2003). Exploiting EST databases for the development and characterization of genederived SSR-markers in barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.*, **106**(3):411-422.

Varshney, R.K., Graner, A.and Sorrells, M.E. (2005). Genic microsatellite markers in plants: features and applications. *Trends Biotechnol*, **23**(1):48-55.

Xu, Y., Ma, R.C., Xie, H., Liu, J.T. and Cao, M.Q.(2004). Development of SSR markers for the phylogenetic analysis of almond trees from China and the Mediterranean region. *Genome*, **47**(6):1091-1104.

Yu, J.K., La Rota, M., Kantety, R.V. and Sorrells, M.E.(2004). EST derived SSR markers for comparative mapping in wheat and rice. *Mol. Genet. Genom.*, **271**(6):742-751.

Received : January, 2010; Accepted : April, 2010