ISOLATION AND INTRODUCTION OF BARLEY YIELD-RELATED SEQUENCES INTO LOCAL ELITE RICE

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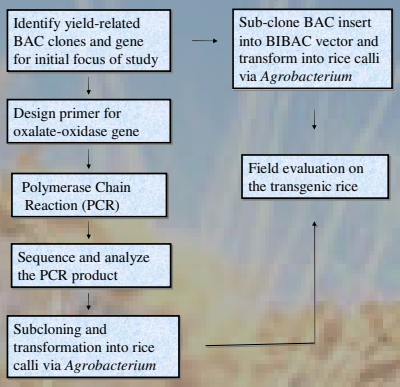
INTRODUCTION

The aims of the current study are to improve yield in local rice cultivars via the introduction of yield related sequences from other cereals. Barley (*Horduem Vulgare*) has been identified as the most suitable source for yield related genes for introduction into local varieties of rice, based on its long history of cultivation, breeding, the current status of genetic and genomic research in this cereal and the availability of suitable DNA sequences and contigs.

A number of yield related regions of the barley genome have been selected, based on published literature and on research data from the North American Barley Genome Project (NABGP) consortium. Ten, Bacterial Artificial Chromosomes (BACs) from a genomic library constructed by members of the NABGP will be used to introduce regions of barley genomic DNA, identified as related to barley yield, into local rice.

One of the selected barley BAC clones (450N4) which has been shown to hybridize to an oxalate-oxidase probe (HvGER-I), Arnis *et al*., 2001, has been the initial focus of the current study. This clone includes the barley oxalate-oxidase (HoXo) gene, a member of the germin-like gene family, thought to be involved in a generalised response to pathogens and abiotic stress (Woo *et al*., 2000).

METHODS & RESULTS



BAC Extraction and Size Estimation

Ten Barley BAC clones were obtained from CUGI at Clemson University U.S.A. Plasmid DNA from the BAC clones was prepared by standard methods and examined by pulse-field gel electrophoresis (CHEF III, 1% agarose), either uncut (Figure 1, left) or after digestion with *Not* I (Figure 2, right). This gave an estimated size of 110kb for the insert of clone 450N4 and three fragments with estimated sizes of 53.35kb, 30.9kb, 21.05kb for clone 450N4 after digestion with *Not* I . Addition of the three fragments produces a total size of 105.3kb, which is very close to the average BAC clone size, 106kb, for the barley BAC library and the estimated size of entire BAC 450N4 from gel.

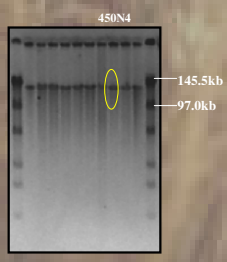


Fig 1 : Pulsed field (CHEF III) gel showing BAC450N4 with an estimated size of 110kb.

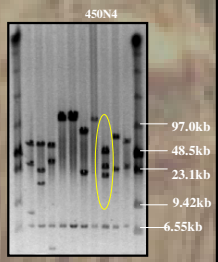


Fig 2 : BAC clone 450N4 digested with *Not* I (circled in yellow).

BAC insert - BIBAC cloning

Not I digested inserts from BAC clone 450N4 were ligated into vector BIBAC2 and transformed into *E.coli* DH10B via electroporation. Eleven transformant clones were extracted and analyzed with pulse- field gel electrophoresis (CHEF III, 1% agarose). Clone 1 and clone 6 (circled in yellow, below) were found to have inserts of 53.35kb.

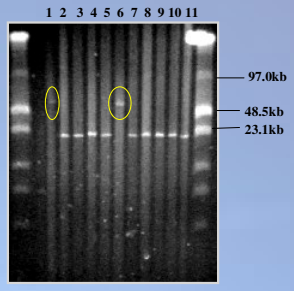


Fig 3 : BAC insert-BIBAC construct digested with *Not* I.

PCR amplification of the barley oxalate-oxidase (HoXo)

The barley oxalate-oxidase (HoXo) gene was initially amplified from BAC clone 450N4 by PCR using primers designed from the barley oxalate-oxidase mRNA. (denaturation 94°C, annealing 53°C, extension 72°C, 29 cycles). The resulting PCR product was sequenced and used to design a more specific pair of primers which were used to amplify the barley oxalate-oxidase gene for subsequent cloning. (denaturation 94°C, annealing 60°C, extension 72°C, 29 cycles)

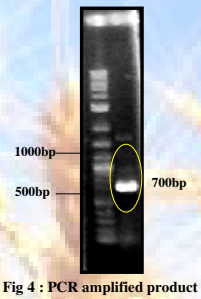


Fig 4 : PCR amplified product of HoXo gene (as used for cloning).

Sequence analysis

Comparative sequence analysis of the PCR product with the reported barley oxalate oxidase cDNA sequence showed that HoXo has a nucleotide identity of 96% and expected value of 0 with *Hordeum vulgare* mRNA for oxalate-oxidase (HvOxOa). Sequence results also indicated that barley oxalate-oxidase gene amplified from the genomic BAC clone, has no intron in the open reading frame as suggested by Zhou *et al*., 1998.

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CATGGTACTAAGCTTGCTACATAGCAAGCAATGGGGTTACTCTAAAAACCTNAGGGGCTG
GCCTGTTCACCATGCTGCTCCTTGCTCGGCCATCATGGTTACGGACCCTGACCTTTTACA
GGATTTTTGTTTCGCGGACCTTGGAGGCAAGGCGTTCTCGGNGAACGGCAATACGTGTAA
AGCCCATGTCAGAGCGGCGACGACTTCTTTTTCTCGTCCAAGCTGACCAAGGCCGGCCA
ACACGTCCACCCGAACGGCTCGGCCCGTGACGGAGCTCGACGTGGCCGAGTGGCCCGGTA
CGAACACGCTGGGCGTGTCCATGAACCGTGTGGACTTCGCGCCGGGCGGCACCAACCCGC
CGCACATCCACCCGGCGTCAACCGAGATCGGCATGGTGATGAAGGTGAGCTCCTCGTTG
GAATCCTCGGCAGCTTTGACTCCGGAAACAAGCTCTACTCCAGGGTGGTGGGTGCCGGAG
AGACTTTCGTCATCCCGCGCGGCCTCATGCACTTCCAGTTCAACGTTGGTAAGACGGAAG
CCTACATGGTTGTGTCCTTCAACAGCCAGAACCCTGGCATCGTCTTCGTGCCGCTCACAC
TCTTCGGTTCCAACCCGCCCATCCCCACACCGGTGCTCACCAAGGCTCTTCGGGTGGAGG
CCGGGGTCGTGGAACTTCTCAAGTCCAAGTTCGCCGGTGGGTCTTAACTTCCATGAGCCC
CAAATGATCAATATGAATATGTAATTCTATATATCCATGTATGCTGCGAATTTAATAGTA
CTCGACAGGAGACTATTA
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Fig 5 : DNA sequence of the PCR product. Sequence in purple indicates the coding sequene (CDS) for the barley HoXo gDNA.

Rice Tissue Culture

Immature seeds of local elite rice cultivar (MR 81) were used to induce calli for use in rice transformation. Induced and healthy calli is in yellowish and around 1cm in diameter after 3-4 weeks.

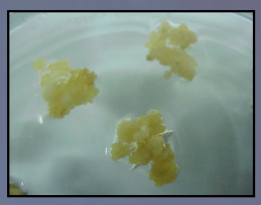


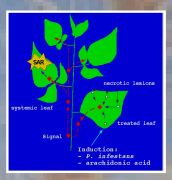
Fig 6 : Induced calli from immature seed after 25 days.

DISCUSSION

Oxalate-oxidases are known from a number of plant, fungal, and bacterial species (Pundir, 1991). Of these, only the wheat and barley enzymes have been classified as germin-like oxalate-oxidases (Dumas et al., 1993). A role of oxalate-oxidase in plant defence has been proposed, based on the capacity of the enzyme to produce H2O2, an active oxygen species(AOS).

$$\text{oxalate} \xrightarrow{\text{oxalate-oxidase}} H_2O_2 + CO_2$$

The generation of the AOS has been suggested to be involved in plant defence responses in several way: in cross-linking of lignin and proteins during cell wall modification, in signal transduction leading to gene regulation, hypersensitive cell death and systemic acquired resistance (SAR) and as an antimicrobial agent, which directly inhibits pathogen development. A study on oxalate-oxidase in barley showed accumulation of oxalate-oxidase transcripts in the leaves in both compatible and incompatible interactions (Zhou *et al*., 1998).



Systemic acquired resistance (SAR) is a general defense response in plants that is characterized by the expression of pathogenesis-related (PR) genes. SAR can be induced after a hypersensitive response to an avirulent pathogen or by treatment with either salicylic acid (SA) or 2,6-dichloroisonicotinic acid (INA).

CONCLUSION

Oxalate-oxidase from barley is a candidate gene to introduce into local rice cultivars as it may protect against compatible and incompatible pathogens (biotic) and various types of abiotic stress to prevent yield-loss.

ACKNOWLEDGEMENT

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