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Research Article

Validation of elongated uppermost internode (*eui*) gene with functional markers in spontaneous mutant rice (*Oryza sativa* L.)

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Abstract
To overcome the panicle exertion problems in CMS lines, the inclusion of elongated uppermost internode (*eui*1) gene in wild abortive cytoplasm can become the permanent solution in hybrid rice technology. Elongated uppermost internode gene produces complete panicle exertion in CMS lines. In view of this matter, a spontaneous mutant, Accession 18 shows elongated uppermost internode trait and it is validated with known *eui* gene donor, IR91-1591-3 to investigate the allelic relationship between them using functional molecular markers. A spontaneous mutant Accession 18 revealed 100 per cent allelic relationship with *eui1* gene which is available in the donor, IR91-1591-3.

Keywords
Elongated uppermost internode, panicle exertion, functional markers and mutant

Introduction

The Wild Abortive (WA) Cytoplasmic Male Sterility (CMS) sources linked with panicle exertion problem in rice. Low seed set in hybrid seed production field is major setback in diffusion of hybrid rice because of poor panicle exertion and thus lack of pollination. The uppermost internode elongation trait enhances panicle exertion rate, pollination rate and seed yield increase 30-40% by overcoming seed set problem (Virmani *et al*. 1988 and Krishnasamy *et al*. 2011). The positive and significant association of exertion with internode was confirmed with earlier studies of Kaushal *et al*. 2015 and Lovelin Kaushal *et al*.2018.

Rutger and Carnahan (1981) identified a recessive rice mutant with an upper internode elongation trait derived from the progenies of a japonica rice hybrid and named it elongated uppermost internode (*eui*) mutant. Genetic research indicated that *eui* (elongated uppermost internode) is a recessive gene (Rutger and Carnahan, 1981) that maps to Chromosome 5 (Maekawa and Kita, 1983; Librojo and Khush, 1986; Wu *et al*., 1998).

The phenotype of the *eui* mutant can be used to overcome the sheathed panicle of the rice male sterile line (Shen *et al*., 1987; Yang *et al*., 2002) and to enhance the plant height and panicle neck length of the rice restorer line (Rutger and Carnahan, 1981; Virmani *et al*., 1988). These features increase the plant's pollen dispersal potential to facilitate hybrid rice seed production (Yang *et al*., 2002). Rutger and Carnahan designated the *eui* trait as the Fourth Genetic Element of hybrid rice seed production. Since then, geneticists and plant breeders worldwide have utilized and studied the *eui* mutant.

The two mutants with different upper internode elongation phenotypes of the *indica* rice line, Xieqingzao eB1 has the recessive gene *eui1* mapped to chromosome 5, and is allelic to IR50 *eui* (Ma *et al*., 2004), which derived from mutant 76:4512 (Virmani *et al*., 1988). Another mutant, Xieqingzao eB2, has the recessive gene *eui2* mapped to chromosome 10 and is non-allellic to Xieqingzao eB1 (Yang *et al*., 1999). The *eui1* mutant plays an important role in hybrid rice seed production and its application will bring about large socio-economic benefits (Yang *et al*., 2002). The molecular cloning of the *EUI1* gene will provide valuable information on the function of this protein product and on the mechanism of stem elongation.

Material and Methods

A total of 1569 rice genotypes were evaluated to screen the panicle exertion, of which only nine represented genotypes were taken for polymorphic study. The spontaneous mutant, Accession 18 was developed from the wild type, T18 (Tellahamsa) which has shown the well exerted panicles as like as *eui* donor, IR91-1591-3. Hence, the Accession 18 was taken to characterize the *eui* allele along with international *eui* donor, IR 91-1591-3.

Fifty microsatellite primer pairs covering all the twelve chromosomes along with 14 functional markers were selected for characterization of elongated uppermost internode (*eui*) trait in rice, of which the fourteen functional markers could be selected to assay the allelic difference/similarity between a mutant, Accession 18 and international *eui* donor, IR 91-1591-3. The functional markers were taken to characterize the *eui* allele since those markers had already used to fine map the *eui* gene through *in silico* method (table 1).

Genomic DNA from most of the rice genotypes was isolated from bulked leaf samples (-20 mg each) from five plants using CTAB method. SSR amplification was performed with Eppendorf thermocycler and Taq ampligold DNA polymerase. The PCR reaction was conducted in a reaction volume of 20µl containing: 1x PCR buffer, 200 µM dNTPs, 0.4 µM of each primer, 1.2 mM $MgCl_2$, 1 unit Taq DNA polymerase and 20 ng template DNA. The PCR amplification was performed with a hot start of 94° C for 5 min and then 35 cycles of 1 min denaturation at 94° C, 1 min annealing at 55° C and 2 min extension at 72 °C, and 5 min at 72° C for the final product extension. Amplification products were stored at -20 °C till further use.

Amplification products were denatured at 95° C for 2 min and resolved on 4% denatured polyacrylamide gel, using Aluminum Backed sequencing System Model #535 essentially as described by Chen *et al*. (1997) electrophoresis was performed at constant power of 100 Watt for 3.5 hr including a 1 hr pre-run to warm the gel to 50-60 °C. following electrophoresis, DNA bands were visualized using the Silver Sequence DNA Sequencing System.

Silver staining of the polyacrylamide gels generally revealed a cluster of 2 to 5 discrete bands (stutters) or blurred stutters for most markers. The size (in nucleotide base pairs) of the most intensely amplified band for each microsatellite marker was determined based on its migration relative to a molecular-weight size marker (10bp DNA ladder from Gibco BRL, Gaithersburg, Md).

The frequency of microsatellite polymorphism was calculated based on presence or absence of common bands. The polymorphism information content (PIC) value described by Anderson *et al.* (1993) was calculated as follows:

$$PIC_i = 1 - \Sigma P_{ij}^2$$

Where, $P_{ij}$is the frequency of the j^{th} allele for i^{th} marker and summation extends over n alleles.

The genetic associations among varieties were evaluated by calculating the Jaccard similarity coefficient for pair-wise comparisons based on the proportion of shared bands (allele) produced by primers. Similarity matrices were generated using 'Simqual' sub-program of software, Numerical Taxonomy and Multivariate Analysis System Version 18 (NTSYS-PC). Similarity coefficients were used for cluster analysis of varieties performed using the 'Sahn' sub-program of NTSYS-PC and dendrograms were built by the un-weighted pair-group method with arithmetic average (UPGMA) subprogram of NTSYS-PC.

Results and Discussion

A total of nine rice genotypes were consecutively evaluated for panicle exertion and elongated uppermost internode (*eui*) trait during 2010, 2011 and 2012 (Table. 2). A total of 14 functional markers co-segregated with *eui* trait showed the phylogenetic relatedness for elongated uppermost internode trait among the rice genotypes (Figure.1).

The similarity matrix was generated according to the coefficient obtained through the unweighted pair group method with arithmetic mean (UPGMA) function of NTSYS-PC software. The *eui* donor, Accession 18 showed 100 per cent genetic similarity with IRRI bred *eui* donor, IR91-1591-3 for *eui1* gene. Based on molecular genetic analysis, a spontaneous mutant, Accession 18 has elongated uppermost internode (EUI) gene and it shows allelic similarity to *eui1* gene which is present in the EUI donor, IR 91-1591-3 (Figure 2). The similar finding was noticed in the rice variety, Ishikari and its mutant by Maekawa *et. al* (1989).

The *in silico* isolation of *eui1* gene controlling elongation of uppermost internode was fine mapped by utilizing functional markers by Hongli Ma *et al*. (2006). The inheritance of elongated uppermost

internode and identification of RAPD marker linked to *eui* gene was studied (Gangashetti *et al*., 2004).

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Table 1. Functional Markers used to find out the allelic similarity between EUI donors *viz*., IR 91-1591-3and a mutant, Accession18 for *eui1*gene.

Primer	Forward primer	Reverse primer	Tm	Size (bp)
AC9	F5'-CGTGTGGAAAAAGGAAGCAT-3'	R5'-GTCATGTGTATGGCCGGTTA-3'	60	205
AC24	F5'-ATTCTTCCCGCACTACAC-3'	R5'-AGTGCGACGGACTCTACT-3'	53	157
AC28	F5'-AGCTCAGGTGAAACATCCAC-3'	R5'-ATCCAGAATCCATTGACCCC-3'	58	235
AC29	F5'-GTGGAGGTGAACGACTACCC-3'	R5'-CCCAAATCACACATACAGCC-3'	59	216
AC30	F5'-TCACATACGTCCTGCTCGAC-3'	R5'-TGGTTCAAATTGCTAGAGCC-3'	58	204
AC31	F5'-TAGGCCTCAAACCAAACCAG-3'	R5'-CAGACGCCCGGATTTGTAATC-3'	60	102
AC32	F5'-GATGCATCCTTCAGGTGATT-3'	R5'-CGGTATCTTCGGACGAGTTA-3'	57	169
AC34	F5'-GTCTACTTCCTGGATGACGG-3'	R5'-GAACACATCATCGATTGCAC-3'	57	288
AC40	F5'-GCTCTCCTTCTGCGACCACT-3'	R5'-CAACCTGCGAGCGAGAATAA-3'	61	214
AC41	F5'-GGCCATTAACACTAACACGG-3'	R5'-GGGACCATGTATCATCGAAA-3'	58	260
AC46	F5'-AGACCAACCCATTCCACTC-3'	R5'-GCAGAGTCAGAGAAGGCCAG-3'	57	164
E30531	F5'-GAATGATAGCCCAGTAACC-3'	R5'-TGAGATCCCGTACCATTGAC-3'	52	225
C53357	F5'-GGGGATATTTTTTTCGGGAC-3'	R5'-CCACAAAAACATTGCCTCTC-3'	58	188
E10471	F5'-TGAGATGGAAAATCACACCC-3'	R5'-GCTGAAGTAGCGTTGAGAGG-3'	58	174

Table 2. Phenotypic variations on panicle exertion /elongated uppermost internode (*eui)* between eight rice varieties and the *eui* donors *viz*., IR 91-1591-3 and Accession18.

Genotypes	Plant height (cm)	Days to 50 % flowering	Panicle length (cm)	No. of spikelets per panicle	Uppermost internode length (cm)	Uppermost internode thickness (mm)
IR91-1591-3 (Eui donor)	90	95	25.6	145	9.7	1.91
Acc.18 (mutant)	95	90	28.3	180	10.5	2.30
ADT 42	95	85	25.8	190	8.8	2.21
CB 87 R	95	85	28.5	220	5.8	2.41
IR72	88	82	26.4	225	4.1	2.16
IR66	90	83	23.7	208	2.8	2.19
IR36	85	80	26.2	228	2.9	2.34
TKM 9	90	80	26.9	185	1.7	2.36
IR50	75	85	26.7	218	1.4	2.07

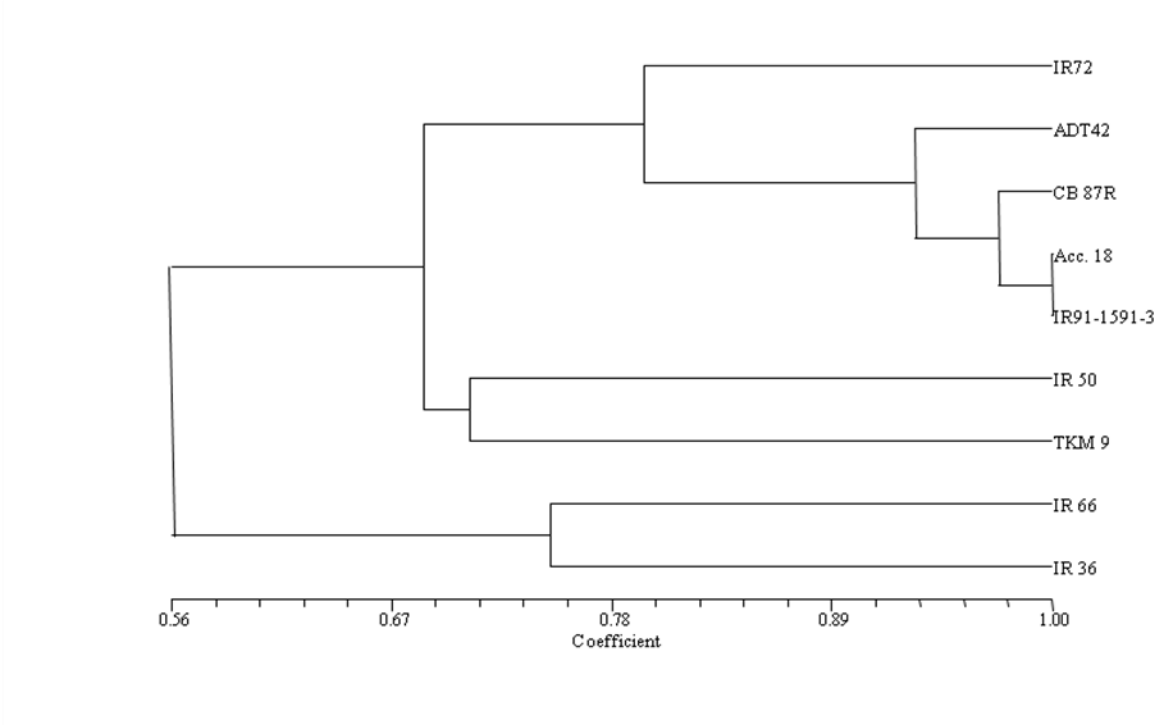
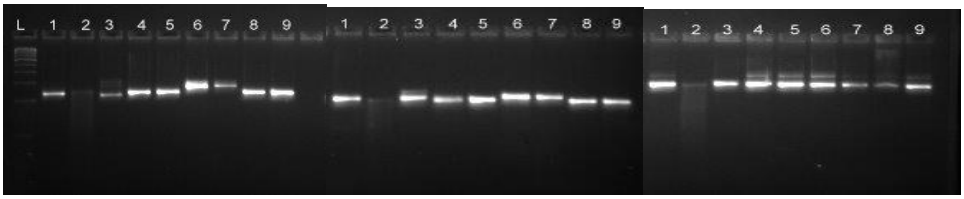


Fig. 1. Phylogenetic tree showing relatedness on panicle exertion /uppermost internode elongation between eight rice genotypes and a *eui1* donors *viz*., IR91-1591-3 and Acc.18. The similarity matrix was generated according to the coefficient obtained through the unweighted pair group method with arithmetic mean (UPGMA) function of NTSYS-PC software.



Label: L- 100 bp ladder, lane 1- IR91-1591-3 (*eui* donor), lane 2- Acc.18 (mutant), lane 3- ADT 42, lane 4- CB 87 R, lane 5- IR72, lane 6- IR66, lane 7- IR36, lane 8- TKM 9 and lane 9- IR50

Fig.2. The genetic polymorphism showing the evidence of allelic relatedness between Accession 18 and IR 91-1591-3 for *eui1* gene expression using 14 functional markers.