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Research Note

Revealing the genetic inheritance of elongated uppermost internode (*eui*) gene in spontaneous mutant of rice (*Oryza sativa* L.)

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Abstract
Success of hybrid rice breeding technology is solely depend on hybrid seed yield. Due to incomplete panicle exsertion, use of gibberlic acid (GA_3) spray at proper stage with proper dose enhances the panicle exsertion rate in male sterile lines which incurred huge cost on hybrid seed production in rice. This is the reason for low deployment of hybrid rice in farmer field. To overcome this expenditure, there is a need of CMS lines with complete panicles exsertion. The spontaneous mutant, Accession 18 shows elongated uppermost internode trait but it is treated as unknown donor, hence inheritance of this mutant line is compared with known *eui* gene donor, IR91-1591-3 to investigate the allelic relationship between the available genotypes. A spontaneous mutant Accession 18 possesses elongated uppermost internode traits based on the inheritance and allelic relationship with *eui* gene donor, IR91-1591-3. The eui gene follows mendelien inheritance of single monogenic recessive as it segregates 3:1 ratio of non elongated and elongated trait.

Keywords
Elongated uppermost internode, panicle exsertion, gibberlic acid and CMS line, monogenic recessive, recessive allele

Rice is the stable food and supplies energy to human population. Rice hybrid breeding technology equalizes the ever growing demand of rice production. Improper panicle exsertion causes low yield. Incorporation of elongation of the uppermost internode (*eui*) trait by breeding methodologies is viable solution to hybrid breeding. Revealing the inheritance pattern of eui genes will be highly useful for genetic manipulations. Rutger and Carnahan (1981) found a recessive gene that can cause elongation of the uppermost internode (*eui*), in japonica rice 76: 4512. The finding has drawn worldwide attention and is recognized as the fourth genetic element in hybrid rice seed production. Elongated uppermost internode trait has been widely used in China as an alternative to gibberlic acid (GA_3). Use of GA_3 at proper stage with proper dose enhances the panicle exsertion rate in male sterile lines which accounts for Rs.3000 per hectare during hybrid seed production in rice. The cost of seed production is increased with application of gibberlic acid which is become an additional financial burden to the farmers. To overcome this expenditure, there is a need of CMS lines with complete panicles exsertion. This can be achieved by incorporating the *eui* gene into the background of CMS lines to avoid the use of GA_3. The IRRI bred *eui* gene donor, IR91-1591-3 is predominantly being utilized for improving the male sterile lines in most of the research institutes in India. But this donor shows some undesirable characters especially thin culm production. The spontaneous mutant, Accession 18 was identified with elongated uppermost internode trait which should be confirmed with available *eui* gene. In view of this prime objective, an indigenous mutant, Accession 18 was taken to confirm the inheritance pattern and allelic relationship with *eui* gene donor IR91-1591-3.

The experimental materials comprise four parents and four crosses (Table.1). Hybridization work was done during 2010-11 to study the genetics of elongated uppermost internode trait.

Each individual F_1 plants were selfed in all cross combinations during 2010-11 and the F_2 nursery was raised during 2011-12. The single seedling was transplanted at 20 x 15 cm space in the experiments in F_2 population. Each experiment was independently laid out in the different field. The experimental area was isolated using more than 200metre distance to avoid the influence of GA_3 spray in the F_2 population plot. The polyethylene barrier was also erected to avoid the aerosol dispersal of GA_3 compound.

The panicle exsertion rate (PER) was recorded to measure the length of peduncle between flag leaf sheath and the color of the panicle in all individual F_2 plants. Incomplete panicle exsertion was also counted in all individual F_2 plants of the all cross combinations at the time of heading and maturity. The eui gene expression was more than 90 per cent in individual F_2 plants.

The key concept in experimental genetics are finding goodness of fit of the result obtained from an actual cross or other experiments based on a particular mechanism and a perfect genetic segregation. The geneticist must know how much the experimental result can differ from the hypothetical or calculated figure and still be regarded as statistically close to expectation. In evaluating the results of crosses and determining which modes of inheritance are involved, how much deviation is permissible without casting some doubt as to whether the data agree with a given hypotheses?

The Chi- square (χ^2) test is a valuable tool that aids the investigator in determining goodness of fit. The test takes into account the size of the sample and the deviations from the expected ratio. The Chi-square test was conducted to study the segregation pattern of elongated uppermost internode (*eui*) in the F_2 population of the four cross combinations.

The Chi- square (χ^2) test for confirmation to genetic equilibrium expectation revealed the monogenic recessive gene action of elongated uppermost internode (*eui*) trait in all cross combinations and fully agreed with the Mendelian inheritance pattern (3:1) of the 3 incomplete panicle exsertion : 1 EUI trait and perfectly had the goodness of fit to the expected ratio in the F_2 progenies (Table 2 - 5). The spontaneous mutant of *eui* donor, Accession 18 had highest probability for the goodness of fit to Mendelian inheritance pattern and segregated based on monogenic recessive gene in elongated uppermost internode (EUI) trait.

The observed deviations are very less and might be due to experimental or sampling errors. In all the crosses calculated Chi- square (χ^2) value are far lesser than table Chi- square (χ^2).

Mendelian monogenic recessive segregation pattern was noticed in all the F_2 crosses for elongated uppermost internode (complete panicle exsertion) trait. The segregation pattern of complete panicle exsertion trait in F_2 progenies is on par with the expression of the panicle exsertion trait in both the parents *viz.*, Accession 18 and IR 91-1591-3. It was concluded that elongated uppermost internode trait in Accession 18 was inherited by single recessive gene and it is allelic to the IRRI bred *eui* donor, IR91-1591-3. Genetic analysis of a spontaneous mutant for elongated uppermost internode in the variety Ishikari showed that EUI was controlled by single recessive gene and it is allelic to the original EUI mutant (Maekawa *et. al* 1989).

The spontaneous mutant of *eui* donor Accession 18 and its derived F_2 progenies has highly desirable morphological features than IR91-1591-3. The endogenous GA_3 expression level was much better in native donor than IR91-1591-3 hence; it could be useful to develop better CMS lines in hybrid rice technology in future. CMS plants with *eui* gene do not need $GA_3$for promotion of panicle exsertion (He and Shen, 1991). In China, this gene has been introduced into CMS lines, which would cause better panicle exsertion (Virmani, 1990).

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Table 1. Genotypes and cross combinations involved to study the genetics of *eui* trait.

S.No	Parents/crosses	Duration	Features
1.	Accession 18	90 days	A mutant shows *eui* trait which was developed by irradiation.
2.	IR 91-1591-3	95 days	IRRI bred *eui* donor
3.	IR 79156 B	90 days	Maintainer line, isogenic line of A line IR79156A
4.	IR 73328 B	90 days	Maintainer line, isogenic line of A line IR73328A
5.	IR 79156 B /Acc. 18	90 days	All F_1 show incomplete panicle exsertion
6.	IR 79156 B / IR91-1591-3	90-95 days	All F_1 show incomplete panicle exsertion
7.	IR 73328 B /Acc. 18	90 days	All F_1 show incomplete panicle exsertion
8.	IR 73328 B / IR91-1591-3	90-95 days	All F_1 show incomplete panicle exsertion

Table 2. Segregation pattern of elongated uppermost internode (*eui*) in IR 79156 B/ IR91-1591-3

Progenies	Segregation pattern of panicle exsertion		Expected ratio	χ^2 chi square value (calculated)	Probability
	Incomplete	EUI trait			
F_1	50	0			
F_2	143	52	3:1	0.382	0.05

Chi- square test for conformity to genetic equilibrium expectation for the F_2 progenies of the cross combination IR 79156 B/ IR91-1591-3

Phenotype	Observed (o)	Expected (e)	(o – e)	$(o - e)^2$	$\chi^2 = (o - e)^2 / e$
Incomplete panicle exsertion	143	147.59	- 4.59	- 9.18	- 0.062
EUI	52	47.41	4.59	21.07	0.444
Chi- square (χ^2) value					**0.382**
Probability (0.05%) at 1 degree of freedom					3.84

Table 3. Segregation pattern of elongated uppermost internode (*eui*) in IR 73328 B/ IR91-1591-3

Progenies	Segregation pattern of panicle exsertion		Expected ratio	χ^2 chi square value	probability
	Incomplete	EUI trait			
F_1	50	0			
F_2	172	78	3:1	0.2042	0.05

Chi- square test for conformity to genetic equilibrium expectation for the F_2 progenies of the cross combination IR 73328 B/ IR91-1591-3

Phenotype	Observed (o)	Expected (e)	(o – e)	$(o - e)^2$	$\chi^2 = (o - e)^2 / e$
Incomplete panicle exsertion	172	164	8	64	0.3902
EUI	78	86	- 8	-16	- 0.1860
Chi- square (χ^2) value					**0.2042**
Probability in 0.05 degree of freedom					3.84

Table 4. Segregation pattern of elongated uppermost internode (*eui*) in IR 79156 B/ Accession18

Progenies	Segregation pattern of panicle exsertion		Expected ratio	χ^2 chi square value	probability
	Incomplete	EUI trait			
F_1	50	0			
F_2	129	71	3:1	**0.0037**	0.05

Chi- square test for conformity to genetic equilibrium expectation for the F_2 progenies of the cross combination IR 79156 B/ Accession 18

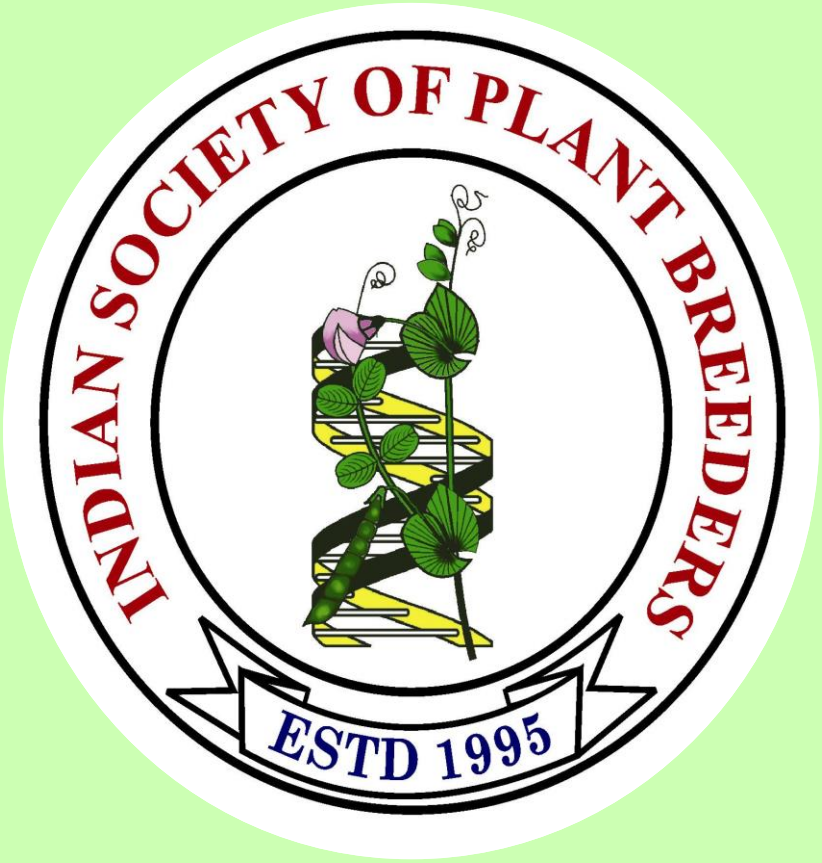
Phenotype	Observed (o)	Expected (e)	(o – e)	$(o - e)^2$	$\chi^2 = (o - e)^2/ e$
Incomplete panicle exsertion	133	133.5	- 0.5	-1.0	- 0.0074
EUI	67	66.5	0.5	0.25	0.0037
Chi- square (χ^2) value					**0.0037**
Probability (0.05%) at 1 degree of freedom					3.84

Table 5. Segregation pattern of elongated uppermost internode (*eui*) in IR 73328 B/ Accession 18

Progenies	Segregation pattern of panicle exsertion		Expected ratio	χ^2 chi square value	probability
	Incomplete	EUI trait			
F_1	50	0			
F_2	163	87	3:1	0.0608	0.05

Chi- square test for conformity to genetic equilibrium expectation for the F_2 progenies of the cross combination IR 73328 B/ Accession 18

Phenotype	Observed (o)	Expected (e)	(o – e)	$(o - e)^2$	$\chi^2 = (o - e)^2/ e$
Incomplete panicle exsertion	163	168.5	- 5.5	- 11.0	- 0.0653
EUI	87	81.5	5.5	30.25	0.0045
Chi- square (χ^2) value					**0.0608**
Probability (0.05%) at 1 degree of freedom					3.84



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