चावल में सर्वोपरी परव का अनुवंशिक विश्लेषण

Genetic analysis of uppermost internode in rice

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Genetic analysis of uppermost internode in rice

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CERTIFICATE

This is to certify that the thesis entitled **"**Genetic analysis of uppermost internode in rice**"** submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of the requirements for the award of **Master of Science** in **Genetics and Plant Breeding,** embodies the results of *bona-fide* research work carried out by **Ms. Harshitha B S, Roll No. 21137** under my guidance and supervision, and that no part of this thesis has been submitted for any other degree or diploma.

It is further certified that any help or source of information that has been availed in this connection has been duly acknowledged by him.

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My Parents, Family and Friends

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"AGRICULTURE is our wisest pursuit, because it will in the end contribute most to real wealth, good morals and happiness."

-Thomas Jefferson

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Abbreviations

%	Percentage
ANOVA	Analysis of variance
bp	base pairs
BSA	Bulked segregant Analysis
C.D	Critical Difference
cM	Centi Morgan
cm	Centi-metre
CTAB	Cetyl- Tri Methyl Ammonium Bromide
DF	Degrees of Freedom
DNA	Deoxyribo Nucleic Acid
dNTPs	Di-Nucleotide Tri-Phosphate
EDTA	Ethylene Di-Amine Tetra Acetate
EUI	Elongated Uppermost Internode
F_1	Filial Generation 1
F_2	Filial Generation 2
$F_{2:3}$	F_2 derived F_3
g	grams
GGE	Genotypic and GE interaction
HvSSR	Hyper variable simple sequence repeats
IRG	International Rice Germplasm
Kb	Kilo base pairs
mb	Mega base pairs
mha.	Million hectares
MSS	Mean Sum of Squares
µl	micro litre
ml	milli litre
M. Wt.	Molecular Weight
µg	micro gram
ng	nano gram
PCR	Polymerase Chain Reaction
PER	Panicle Exertion Ration
pH	Power of Hydrogen
PL	Panicle Length
rpm	revolution per minute
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RM	Rice Microsatellites
SMA	Single Marker Analysis
SNP	Single Nucleotide Polymorphism

SSLP	Simple Sequence Length Polymorphisms
SSR	Simple Sequence Repeats
STMS	Sequence tagged micro-satellite
TAE	Tri Acetic acid EDTA
Taq polymerase	*Thermus Aquaticus* Polymerase
TE	Tris EDTA
UIL	Uppermost Internode Legth

1. Introduction

Over the last century, the global population has quadrupled. The population is expected to reach 9.7 billion by 2050 globally, thus requires food production to hike by 60.0–110.0% to meet up the food demand (Tilman *et al*., 2011). Therefore, provide adequate food to supply the burgeoning population is a major task for agriculture.

Rice (*Oryza sativa* L.) being the major staple food crops of the world that fulfill the daily nutrient need for more than half of the global population (Muthayya *et al*., 2014). Rice is mainly grown on 159 mh annually, where the major contribution in rice production is occupied by 200 million smallholding farmers in Asia and Africa (Mottaleb *et al*., 2012). In India Rice is grown in around 44 mh of land with annual production of 110 mt. Current difference of rice cultivated area and production between India and China is about 15 mha and 45 mt, respectively. This difference is mainly because China grows hybrid rice on more than half of the total rice cultivated area. In India hybrid rice is cultivated on 2.8 mha area which is just 6.2% of total rice cultivated area with the production of 3.8 mt annually. Increased seed cost and compulsory seed replacement in every season, as against the low additional income due to the marginal yield advantage over normal varieties have made the farm level adoption of hybrid rice in the country slow

Hybrid rice cultivation consist of three parental lines namely CMS line, maintainer line and restorer line. In CMS lines, particularly in wild abortive (WA) type cytoplasm, incomplete panicle exsertion remains a major problem where 30 % to 35 % of the spikelet's remains inside in the flag leaf (Gaganshetii *et al.*, 2004). Panicle enclosure in male sterile line is happened due to the hindrance of GA_3 supply to the panicle which results in less elongation of uppermost internode. Incomplete panicle exsertion caused less out-crossing which results in poor seed setting. To overcome this problem generally a large amount of GA_3 application is preferred which increased the cost of cultivation and reduced seed quality and storage life (Honnaiah, 2003). GA_3 also adversely affect environment and also enhanced the probability of rice kernel smut (Tsuda *et al*., 2011). Therefore, to reduce the cost of cultivation and increase the storage with quality of the hybrid seed, there is need to search an alternative to GA_3 application in hybrid rice seed production.

The Plant architecture specially the culm plays a significant role which impact the plant height, resistance to lodging, panicle exsertion and yield. Culm is made up of a series of nodes and internodes. Internode is the area between the two nodes. The elongation of these internodes leads to the initiation of panicle primordial till heading. The uppermost internodes elongation causes the panicle to be exserted from the flag leaf (Dunand and Saichuk, 2014).

Rutger and Carnahan (1981) recognized a recessive tall mutant with an elongated uppermost internode originated from a japonica line and named as *eui* mutant. The main characteristic of this mutant are the elongated uppermost internode by almost doubling the uppermost internode length and thereby augment the length of the panicle by 12% and has small effect on the subsequent internodes. This finding drawn attention of many researchers and considered this as fourth genetic element in hybrid rice apart from A line, B line and R line. There are two way to utilize this *eui* gene in hybrid breeding program. One is to develop tall restorer line possessing *eui* gene which improves pollination efficiency and other is to develop male sterile lines having *eui* gene to overcome the poor panicle exsertion (Virmani *et al*., 1988; Zuhua *et al*., 1987 and Liang *et al*., 1992). Study on genetic analysis revels that *eui* mutant is governed by single recessive gene and it is allelic to the original EUI mutant (Maekawa and Kita, 1983; Maekawa *et al*., 1989). This gene has been incorporated into in many CMS lines to get better panicle exsertion in China and also transferred in *indica* restorer line like IR50.

Yang *et al* in 1999 found two EUI mutants from segregating M_2 population obtained by gamma rays treatment. Either of these two mutant considered as *eui* (t) was allelic to the original EUI mutant, while second one was found nonallelic to the original *eui* and *eui1*, named as *eui2(t)*. There are two loci for EUI gene. Both genes had a significant effect on plant height related trait without affecting the pollen or spikelet fertility. In general, *eui1* has greater effect over *eui2*. The expression of both the genes seems to be dominated by genetic background.

One locus is mapped on chromosome 5 through trisomic analysis (Librojo and Khush, 1986) and linked to a RFLP marker RG435 at distance of 33.6 cM (Wu *et al*., 1998). This gene has three alleles *Eui, eui* and *eui1* (t). The other locus has two alleles, *Eui2* and *eui2* (t) which is located on chromosome 10 linked to the RM304, RM271, RM269 and RM258 with distance of 1.4, 35.1, 12.9 and 12 cM, respectively. Many simple sequences repeat markers have been identified linked to *eui1* namely RM7446, RM3870, RM6054, RM5970, RM3620, RM7801 and RM3476 at the distance ranged from 1.0 to 7.1 cM (Khera *et al*., 2009).

Xu *et al*., 2004 fine mapped using an F_2 population having 5000 plants obtained from NILs of 307T with Zhenshan 97. In his results using map-based cloning, a 98-kb interval possessing the *Eui* locus flanked by markers M0387 and M01 was found. Further Zhu *et al*., 2006 isolated the *Eui* gene and studied the biochemical and biological functions of the gene. He reported that the *eui* plant accumulates bioactive GAs in the uppermost internode at the heading stage. They cloned the gene using map based cloning technique and elaborated that *Eui* gene encodes the P450 and resulted that EUI acts as GA-deactivating enzyme. Further Luo *et al*., 2006, cloned the *EUI1* using map-based cloning which encodes the putative cytochrome P450 monooxynase and found the amount of GA is more in young panicle as compared to other tissues. Over expression *EUI1* gave gibberellins-deficient like phenotype and therefore, proposed that *EUI1* is negatively correlated in gibberellins mediated regulation of cell elongation in the uppermost internode in rice. Inactivation of *Eui1* in *eui1*mutants caused the accumulation of GAs in uppermost internode and consequently elongates the internode followed by plant height (Luo *et al*., 2006; Zhu *et al*., 2006).

However, it's been almost 30 year when the first *eui* mutant was identified but, neither of cms line or restorer line have been developed having *eui* gene and practice due to the lack of the effectiveness of the gene (Yang, 1998; Yang *et al*., 1999), and the number of e-type 'A' lines is also very limited (Zhang *et al*., 2003), which restrict the development of e-hybrid. Therefore, it is necessary to identify effective *eui* gene(s) and its nature of action which can further be incorporated in several promising 'A' lines or R line using marker assisted back crossing (MABB) technique.

Keeping this in view the present study was proposed for "**Genetic analysis of uppermost internode in rice**".

Objectives:

1. Morphological and molecular characterization of different rice genotypes for uppermost internode.
2. Genetic analysis of uppermost internode in rice.
3. Mapping of gene using Bulk Segregant Analysis (BSA).

2. Review of Literature

15 to 20 % Heterosis is obtained in hybrid rice varieties over the inbred lines. This happens when large quantity of exogenous GA_3 is applied during the hybrid seed production because of the panicle enclosure trait of cytoplasmic male sterile lines. The application of huge quantity of exogenous GA_3 makes not only hybrid rice cultivation costly but also gives poor storage performance which makes quality of hybrid seed down. Panicle enclosure occurs when some part of uppermost internode remains inside of the flag leaf sheath. Therefore, the two-character uppermost internode and flag leaf sheath are closely related to panicle enclosure. The culm of rice plants is made up of nodes and internodes. The elongation of internode mainly the uppermost internode is a major trait associated with the panicle exsertion, panicle length and culm length. Rice internodes are categorized in three parts, Intercalary zone, elongation and differentiation zone (Kende *et al*., 1997).Cell division takes places in the base of the internode which called as IM where cell division takes place and new internodes forms which displaced into EZ where they elongate till the final length and end in the AZ where secondary wall forms (Kende *et al*., 1997). The uppermost internode is the longest followed by lower internodes. When crops progress from vegetative to reproductive phase, only four to five uppermost internodes elongate from the base to uppermost internode (Chonan 1993).

Among the three parental lines in hybrid rice, panicle inclusion occurs only in CMS line but not in its B line and restorer lines, indicating the impact of the sterile cytoplasm. In 1981, Rutger and Carnahan identified a recessive tall phenotype and considered it fourth genetic element of hybrid rice apart from the CMS line, B line and R line. This recessive *eui* gene elongate the uppermost internode double the normal wild type plants without influencing the other characters such as pollen or spikelet fertility.

2.1 Identification of *eui* (Elongated Uppermost Internode) gene

Internode is an important trait in rice plant. First report on internode elongation was observed by Okuno and Kawai (1978) who came with the recessive rice mutants taken from Japanese line Norin 8 by γ ray. They studied eight induced long culm mutants which showed significant higher culm length from their original cultivars. Based on the length and internode numbers to culm length they divided the long culm mutants into four parts namely upper

elongation, lower elongation, multi nodes and normal types. In 1981, Rutger and Carnahan found a recessive gene for elongated uppermost (*eui*) which gives tall phenotype in rice. Rutger proposed 12% increase in uppermost internode with no effect on the other internodes. In his experiment he measured internode of P_1 just double of the length of internode, when compared to another parent. The comparative study between the *eui/eui* (tall genotype) and *Eui/Eui* (short genotype) provided excellent information of the effect of the gene. Thus, genotypes having *eui/eui* were taller, than the genotypes having *Eui/Eui* and had about 10 to 12% longer panicles.

Regarding the existence of *eui*, since in either parents it was not noticed, in the study *eui* might have come from spontaneous mutation. The *eui/eui* genotype having length of internode elongation just double to the other parents which is lower internode pattern of the induced long-culm mutant LM-1, (Okuno and Kawai, 1978). The *eui/eui* genotype quite different from the initial four internode of the dominant dwarfs, (Mallick *et al*., 1980; Talahashi and Takeda 1969).

The first *eui* mutant was found in California in 1976 (Mackill *et al*., 1992) and this mutant became the source for this trait in many countries for breeding program. For the test of allelism 3 mutants were taken from M-202 and CM-101 and from 83Y45 with original *eui* mutant, indicating that they are allelic to the original *eui* gene (Mackill *et al*., 1992). According to Maekawa *et al*., 1989 uppermost internode elongating gene of their mutant was allelic to original *eui* of P1-1 and further supported by Librojo and Khush, 1986 by trisomic analysis.

Since *eui* was designed by Rutger and Carnahan in 1981, many tall mutants have been recognized in diverse rice cultivars and all were governed by single recessive gene (Yuankun and Zonghong *et al*., 1988; Maekawa *et al*., 1989; Sun *et al*., 1994). Using M_2 population the maintainer line Xieqingzao B (XQZ B) by gamma irradiation, in 1999, Yang's group obtained two *eui* mutants, each possessed diverse length and height. Testing for allelic nature of the gene found non allelic to each other. Therefore, one named as *eui1* due to its allelic nature to the earlier identified *eui* gene whereas the second, named *eui2*, had quite different phenotypes. Similarly, many researchers have found *eui1* and *eui2* in different population with same nature of gene action (Hong-Li *et al*., 2004; Virmani *et al*., 1988; Yang *et al*., 1999).

Soon after finding of Rutger and Carnahan in 1981 on elongated uppermost internode, several germplasm carrying long panicle exsertion, elongated uppermost internode and

uppermost internode identified (Yuankun and Zonghong, 1988; Wu and Zhang, 1983; Yang, 1999)

2.2 Genetics of inheritance of *eui* gene

Hybrid rice seed production cost is increasing due to external application of GA_3 for complete panicle exsertion. So, identification and studying the inheritance of gene controlling the uppermost internode elongation fetches important role in the area of hybrid rice seed production. The first study on variations in internode length in rice was carried out by Okuno and Kawai (1978) in Japan. They induced mutation for internode length and selected six mutant lines and studied the variation in length of internode. The mutants were classified into different classes based on the internode length and number of internodes as upper-elongation types, lower elongation type, multi node type and normal type. The result from the study shows that each mutant gene can affect the respective internode elongation after the internode elongation initiation**.** In 1981 Rutger and co-workers have identified a recessive mutant in *japonica* line76: 4512 that governs the uppermost internode elongation. Normally tall phenotype in hybrid is controlled by dominant gene, but he identified a recessive mutant that is responsible for elongation. He considered this as a fourth factor in hybrid rice production along with CMS line, B and R lines. In his study he made a cross between76: 4512 and ED7 and 616 F_2 plants were generated and he observed a segregation ratio of 3:1 for dwarf and tall phenotype respectively. This confirms that the gene controlling the elongation was recessive in nature. He observed a 12% increase in length of the panicle in lines with homozygous recessive condition for the gene of interest. This was the first breakthrough in the study of uppermost internode elongation. Identification of *eui* attracts attention from various parts and it can be used as an alternative to GA_3 application in hybrid seed production. The identified *eui* is whether allelic to *Sd1* or non-allele was unknown at that time. A study in Xieqingzao eB1 and Xieqingzao eB2 was carried out by Maekawa *et al* (1983) reported the possible interaction of *eui*with the other genes for elongation of internode. Rutger *et al* (1986) studied inheritance of semi-dwarf gene and eui gene to know the allelic nature of identified *eui* mutant. The independent segregation of semi-dwarf gene and *eui* allele confirms that they are non-allelic in nature.

Virmani *et al* (1988) observed a 12% increase in uppermost internode and panicle length in lines positive for *eui* gene. They also assumed that this gene was non-allelic to *sd1* gene (semi-

dwarfing). They transferred this gene into restorer line and it helps in easy pollen dispersal and also facilitates its harvesting before bulk harvesting of male sterile line. Yuankun and Zonghong (1988) identified a recessive tall phenotype called Grlc (Guiitou recessive long-culm). They reported that the identified phenotype has tall and special culm structure and it was different from the checks LM1 (Japan) and 76:4512 (America). The checks are elongated uppermost internode phenotype which was same as original *eui* and controlled by recessive gene. The Grlc type was reported to have good culm structure and some character similar to semi-dwarf rice varieties.

A group of scientists from China, Zongtan and Zuhua *et al* (1991) (Zhejiang Agricultural University, Hangzhou) studied the inheritance of panicle exertion and interaction with the male sterility. They confirmed that the panicle exertion was controlled by recessive *eui* gene in japonica line and they also confirmed that the uppermost internode length and height of the plants positively correlated with presence of *eui* gene. They also studied the interaction between the male sterile cytoplasm and *eui* gene in Zhenshan97A line and concluded that the interaction between the *eui* gene and WA male sterile cytoplasm eliminates the negative effect of MS cytoplasm on panicle exertion. They also transferred this gene into A line using backcrossing and observed increased panicle exertion in introgressed lines. Liang *et al* (1992) studied the inheritance panicle exsertion in rice at Institute of Genetics and Crop Breeding, FAC, Fuzhou, China. Different F_2 and BC population was generated and the results revealed that panicle exsertion was governed by recessive gene and the correlation of this trait also studied with the other traits related to culm. It indicated the positive correlation with the plant height, panicle length, first and second internode length but no correlation with the third and fourth internode length. The gene was further transferred into MS line and observed the improvement in panicle exsertion. Chen *et al.,* (1998) studied the recessive tall mutants in rice for its allelism, their relationship among various semi dwarfs, normal tall. The results from F_1 and F_2generation confirm that the tall phenotype was controlled by single recessive mutant. The phenotypic ratio in F_2 was observed as 3:1 (Semid warf: Tall) but in few crosses it was also observed interaction of three genes. One more gene for uppermost internode elongation was identified by Yang (1998). They identified two kind of mutants in M_2 population of Xieqingzao B (XQZ B) treated with gamma radiation. The mutants were designated as eB1 and eB2 and mutants were observed for effect on elongation in different internode. The eB1 mutant was having effect on first and

lower internode but eB2 was having effect on only first internode. From the segregation study of F_2 plants it was observed that the phenotypic ratio well fits with 3:1 ratio of normal mendelian ratio and controlled by single recessive gene. It was also confirmed that the *eui* gene of Xieqingzao eB-2 was different from the Xieqingzao eB-1 and named it as *eui2* and study revealed that its different from original gene and mapped on chromosome 10 by Yang *et al* (2000).The report from the study makes it possible to develop A line and R lines using different *eui* genes and would reduce the external application of GA_3

A group of scientists from China, Yang *et al.,,* (2002) studied the implication of inducing *eui* mutation in A, B, R line and its role in hybrid breeding. An inheritance study was conducted in induced mutants of maintainer line named as eB1 and eB2. The results showed that the elongation in eB1 was governed by a single recessive gene and allelic to earlier identified *eui* gene. But the recessive mutant in eB2 was recessive and non-allelic to the original *eui* and they considered that mutant as *eui2(t)***.** Gangashetti *et al.,,* (2004) studied the inheritance of *eui* gene in IR91-1591-3 rice. Genetic study was carried out using *non-EUI* (IR58025A, IR58025B) and *EUI* (IR91-1591-3) lines. A large F_2 population and test crosses were performed to study the inheritance of *eui* gene. The F_2 plants segregation for *non-EUI* and *EUI* phenotype was in the ratio of 3:1 and fits well with the *chi-square* value. The results were confirmed by developing $F_{2:3}$ and test crosses. The F_3 progenies were consist of 113 *non-EUI*, 251 segregating and 109 *EUI* families and it fits well with 1:2:1 genotypic ratio for elongated uppermost internode (*eui*) and the test cross ratio was 1:1. Thus the results indicated that the elongation was governed by single recessive gene and supported the earlier observation of Rutger and Carnahan (1981).

A study was conducted by Hong-Li *et al.,,* (2004) on mapping and genetic analysis of *eui1* gene in mutant Xieqingzao eB1. The mutant genotypes for the study was developed using gamma radiation in an elite *indica* maintainer line Xieqingzao B (XQZ B). The mutant line showed significant difference from the wild type for height and panicle length with internode length. The analysis on the genetics of the gene clearly says that the trait is controlled by single recessive gene *eui1*andwhich is allelic to original IR50*eui*. A study on new genetic behavior of elongated uppermost internode was carried out by Zhang *et al.,,* (2004) showed that an *eui* mutant found in Luoshami (wild type) contributes to variation in length of the internode. All combinations of crosses were performed and inheritance analysis from the crosses showed that

the gene was segregating in 3:1 ratio in F_2 population. The mutation was also causing significant increase in flag leaf size and grain length. Mackill *et al*., 1992 conducted a test of allelism for *eui* mutants independently identified in different cultivars M-202, CM101 and the breeding line 83Y45 with original *eui* mutant. All the F_2 and F_3 plants were tall and *eui* type indicating that the identified mutants in different cultivars were allelic in nature. It also confirms the recurrent mutation for *eui* gene in California cultivars.

A study was conducted by Wang *et al*., (2007) on use of *eui* gene from a mutant 02428h in breeding program. In this study the inheritance pattern of *eui* was studied in F_2, BC_1F_2, BC_2F_2, BC_3F_2 population. The phenotypic segregation in two F_2 population was 680:239, 238:86 for semi dwarf and tall phenotype fits to the 3:1 by *chi-square* test. Backcross population also showed similar results and confirmed that the segregation of *eui* was in accordance with the mendelian laws of inheritance and in agreement with the earlier reports. As it was a single recessive gene, its transfer using backcrossing is easy as compared to *QTL* introgression. A study from Khera *et al.,*, (2009) in a cross between IR58025B and IR91-1591-3 shows recessive inheritance of *eui* gene. Around 109 normal and 42 elongated internode plants were observed from 151 F_2 plants and segregated in 3:1 ratio for normal and elongated phenotype ($P > 0.5$). Recently a study was conducted by Thangapandian *et al.,*, (2019), to know the inheritance of *eui* gene in spontaneous mutant, Accession 18 and to check the allelic relationship with IR91-1591-3. The mutant was crossed with four parents and different F_2 plants were generated. The chi-square result shows the monogenic recessive type inheritance of elongated uppermost internode trait. The phenotypic data was in well agreement with 3:1 ratio of normal segregation in F_2 generation. The test of allelism confirms that the mutant was allelic to the original *eui* gene in IR91-1591-3.

2.3 Mapping *gene(s)* or *qtls* for elongated uppermost internodes (*eui*), uppermost internodes (*ui*) and panicle exertion ratio (per)

2.3.1 Mapping *eui1* gene

A mutant was identified with tall phenotype which was found recessive in nature from the cross between two japonica cultivars Rutger *et al*. in 1981 and designated it as *eui* and considered it as fourth genetic element in hybrid rice breeding program. After the identification of the *eui* gene, researcher started introgression it to the restorer lines (Virmani *et al*, 1988).

Yang *et al*., in 1999 obtained two *eui* mutant genes by mutating the *indica* rice cultivar and designated it as eui*1* (allelic in nature) and *eui2* (non-allelic in nature).

Wu *et al*., (1998) has tagged the *eui* gene using RFLP markers. He used a pair of NILs, 307T possessing *eui* gene and Zhensahn 97 with sd1 gene. In segregating F_2 population, a total of 63 RFLP probes were used to map the *eui* gene, two markers namely RG435 and RG493 were found to be linked with *eui* gene on chromosome 5. Gangashetti *et al*., (2004) used 81 polymorphic RAPD markers using bulked segregants analysis. Two bulks were pooled along with the parents using 81 primers, where he found two primers polymorphic between the bulk and finally OPAG01 produced distinct band of 1000bp between the EUI and non EUI parents and their respective bulk in both the population. Further this marker was validated in 34 CMS lines. The 1000bp band was not observed in 31 CMS lines and their respective maintainer lines. In the same year Hong-Li *et al*., (2004) mapped *eui* gene using SSR markers from (XQZ eB1XAJNT) F_2 population. He has selected 100 long culm and 400 short culm plants.

Ma *et al*., (2006) used silico analysis to map the *eui1* gene and identified its candidate gene further fine map the gene. They used linked marker AC9 from their previous study which was 7.9cM way from the *eui* gene. Finally, they concluded that the gene governing the elongation of the uppermost internode in rice is 9804 bp long comprises of two exons and one intron.

Gangashetti *et al*., (2006) utilized STS markers for mapping of gene linked to the elongated uppermost internode (*eui1*). In their study they have converted previously linked RAPD markers to sequenced tagged site markers by molecular cloning and nucleotide sequencing. Based on the result found in the study they reported that internode elongation in IR91-1591-3 is govern by *eui1* gene located on chromosome 5 which was earlier map by Librojo and Khush in 1986. The STS markers amplified a product of 1051bp and 1100bp in *EUI* parents and only 1100bp in non-*EUI* parent where the marker and gene were found at the distance of 3.4cM. With linkage analysis they found only one polymorphic marker sMRF19 located at 2.4cM from the gene of interest.

Khera *et al*., (2009) have mapped the *eui1* gene using simple sequence repeat markers. The F_2 population comprised of 151 plants derived from the cross between IR58025B and IR91-1591-3. A total of 28 SSR primers were used for the polymorphism survey, of which 15 were further selected between the parents. Later bulked segregants analysis was performed using 15

markers between the bulk and the parents. 15 polymorphic markers were found associated to the bulks and used for the genotyping of the 151 F_2 plants. Mapmaker was used for linkage analysis which revealed that primer RM3870 and RM3476 were the closest markers linked to *eui1* gene at the distance of 3.0 and 1.0cM respectively on chromosome 5.

Xu *et al*., (2004) found a segment of 98 kb containing the rice *Eui* gene controlling the elongation of uppermost internode. They used previous information of Wu *et al*., (1998), who reported that *Eui* is located on chromosome 5, they did linkage analysis with population of 198 F_2 plants that revealed the distance between the *Eui* gene and markers (M01980 and C62) is 6 cM.

2.3.2 Mapping *eui2* gene

In 1999, Yang *et al*, has identified two mutant using gamma ray's radiation as designated as XQZ eB-1 and XQZ eB-2. Both of the mutants showed longer internode elongation over the wild type. As there was different in uppermost internode elongation between both mutants, Yang has suggested that both the mutant is different in their mechanism. He later found that XQZ eB-2 is non allelic to the original *eui* and named it as *eui2*. Soon after a year after identification of *eui2* for elongated uppermost internode Yang *et al*., 2000 tagged *eui2* using microsatellite markers. Selected F_2 plants were selfed to produce F_3 families from cross between XQZeB-2 (*eui2* parent) and AJNT (Non *eui2* parent) to identify the markers linked to the gene of interest and locate the *eui2* gene. A total of 186 SSR primers were screened for the mapping of the gene, fifty-four homozygous plants were surveyed from F_3 families. With the linkage analysis it was revealed that primers RM304, RM269, RM258 and RM271 were linked to *eui2* gene on long arm of chromosome 10 with the genetic distance of 1.4cM, 12.9cM, 35.1cM and 12.0cM respectively. Thus, it was confirmed that the *eui2* gene is located on chromosome 10. Another group Yang S.L., 2001 has confirmed the location of *eui2* gene on chromosome 10 and validated the markers linked to the *eui2* gene with the same crosses involved in the earlier report by Yang *et al*., 2000.

In the year of 2003, Yang *et al*., has done mapping and tagging of novel elongated of uppermost internode gene *eui2* using AFLP, RFLP and SSR markers. According to his statement his group identified four AFLP polymorphic markers EM436, EM444, EM521 and EM527 using bulked segregants analysis in F_3 family. Among these four markers EM521 was found tightly linked to the *eui2* (t). In the mean while four SSR markers namely RM269, RM258, RM304 and RM271 with the genetic distance of 12, 12.9, 33.1 and 1.4cM, respectively were found by

microsatellite analysis. Finally, he concluded from microsatellite results and mapping of RFLD markers EM527, that *eui2* (t) on chromosome 10. It has been confirmed that *eui2* gene is located between the markers RM304 and RM258 at the distance of 2cM and 12cM respectively.

Zhu *et al*., 2003 did fine mapping for elongated uppermost internode *eui2* (t) gene. He stated that rice *eui2* (t) mutant obtained by mutagenesis technique and observed drastic increase in uppermost internode elongation at the time of heading stage. In his study he speculated that five genes encoding product may be involved in the dehydrogenation of gibberellin metabolic process.

2.3.3 Mapping of gene(s) for other traits related to uppermost internode elongation

Jian *et al*., (2008) improved panicle exsertion using 98 backcrossed inbred lines, derived from a backcross of Nipponbare/Kasalath//Nipponbare and identified three *qtls* on chromosome 1,3 and 4 respectively. 3 QTLs were detected on chromosomes 1, 3, and 6 for the uppermost internode length, whereas four QTLs on chromosomes 1, 3, 5, and 10 for panicle enclosure length with explained phenotypic variances 6.8–17.8%. Duan *et al*., 2012 has identified a mutant *esp2* from Minghui-86. He found *ESP2* as key gene for development of uppermost internode in rice. Dang *et al*., (2017) investigated panicle exsertion length in CSSL population having sixty-six lines and a natural population of 540 cultivars and found seven QTLs across two environments with the phenotypic variance explained 10.22 to 50.18%. The results on sequencing showed that the shorter PEL contained the A base, and longer PEL contained the G base at the 1,475 bp location of the *EUI2* gene. Nagai *et al*., 2014 identified 2, major QTLs on chromosome 3 and 9 causing length of the internode and number of elongated internodes, among these three QTLs, one has acted as enhancer which was located on chromosome 3.

2.5. Map based cloning/molecular characterization of *eui* genes

Gibberellins are a group of terpenoid hormones which regulates the growth such as elongation of stem, leaf expansion, germination and flowering. The *eui* gene in some genotypes leads to elongation of uppermost internode after heading stage. This gene does not have any role before heading stage. Hence the gene is stage specific and can be used in hybrid breeding. Only few are biologically active gibberellic out of more than 130 GAs identified (Yamaguchi RC., 2008). The major biologically active forms of GAs are GA_1, GA_3, GA_4 and GA_7.

Normally in *EUI* genotypes, the GAs during heading stage is mainly catabolized by the product of *EUI*. Internode elongation response in relation to GA was studied and *EUI* was

characterized by Luo *et al*., 2006. The EUI gene was mapped on chromosome on chromosome number 5 and it was further fine mapped by Xu *et al*., (2004). An F_2 population developed from 307T (*eui/eui*) and Zhenshan 97 (EUI/EUI) was used and high-resolution map was constructed and mapped between the markers M0387 and M01.The *EUI1* locus was further narrowed down to 34kb sequence between marker MP37 and MP45 (Luo *et al*., 2006). RT-PCR results showed that *EUI* gene expression was more in the uppermost internode after heading stage and overexpression of transgene *EUI* in different transgenic lines showed severe dwarfness and failed to produce seeds. Itoh *et al*., (2002) said that gibberellin is a key destabilizer of DELLA protein also known as SLR1, which is not found positive regulator of GA signaling pathway. DELLA proteins positively regulate the expression of *GA_{20}Oxidase* and *GA_3Oxidase* which catalyzes the late step of GA signaling pathway. The results from the studies showed that the transgene *EUI* positively regulates *GA-20-Oxidase* and *GA-3-Oxidase* and negatively regulates the *GA-2-Oxidase*. The molecular cloning also confirms that the *EUI* gene encodes P450 cytochrome oxidase, CYP714DI. The protein family P450s known to be involved in biosynthesis/catabolism of plant hormones (Schuler and Werck-Reichhart, 2003; Nelson *et al.,,* 2004). The *EUI* gene product is involved in inactivation of many gibberellin synthesizing enzymes and reduces the biologically active GA in the internode. In contrast to this, *eui* mutants are carrying non-functional P450 monoxygenage that unable to inhibit the GA synthesizing enzymes. As a result plants homozygous for eui mutant allele are not having any significant difference for uppermost internode length till heading stage and have a significant difference for internode elongation after heading stage (Luo *et al.,,* 2006).

The *EUI* gene encodes an enzyme called P450 monoxygenase that catalyses 16α, 17-epoxidation of non-13-hydroxylated GAs. Hence it causes the deactivation or reduced biological activity of GA_4 in rice. The *EUI* gene product is also assumed to catalyse epoxidation of multiple GA substrates, including GA precursors such as GA_{12} and GA_9 and bioactive GA_4. To study the effect of *EUI* gene product a transgene driven by constitutively expressing CaMV 35S promoter was constructed (Zhu and Chen, 2006). The *EUI* transformed plants were found to be extremely dwarfed and failed to produce seeds whereas the *eui* plants were having elongated internode and cells were enlarged at uppermost internode. The *eui* mutants downregulate the GA catabolizing enzyme such as $GA_{20}ox_2$and $GA_3ox_2$and upregulation of GA_2ox_1that results in higher level of bioactive GA in mutant plants. The results also showed the negative regulation of DELLA

proteins which are negative regulators of GA signaling. The DELLA proteins are responsible for dwarfism and binding of DELLA protein domain to DNA inhibits the genes responsible for elongation (Sun and Gubler, 2004). Many studies also confirmed that 16α, 17-epoxidation might be involved in inactivation of biologically active GA in many crops such as *Pisum sativum* fruits (Santes *et al*., 1995), *Malus domestica* seeds (Hedden *et al*., 1993), *Lupinus alba* seeds (Gaskin *et al*., 1992). A study was conducted by Yaish *et al*., (2010) on a transcription factor which controls interaction between abscisic acid and gibberellin in rice. The interaction between the plant hormones plays a very important role in growth and development. A transgenic plant of *Oryaza sativa* kaybonnet variety was developed to study the overexpression of gene construct for *OsAP39*. The gene construct *OsAP39* is a transcription factor which controls the interaction between the ABA and GA. Overexpression of this gene leads to reduction in yield and biomass. Global Transcriptome analysis also confirms the upregulation of *OsNCED-1* which involved in biosynthesis of ABA and leads to increase in endogenous ABA level. The gene construct was reported to upregulate the *EUI1* gene for uppermost internode elongation. *EUI1* is known for inactivating GA by 16α, 17 epoxidations of non 13-hydroxylated GAs. This study confirms the direct control of *OsAP39* gene on expression of *OsNCED-1* and *EUI1*.

2.5. Introgression of *eui* gene into male sterile lines, maintainer lines and restorer lines for uppermost internode elongation.

After identification of gene for elongated uppermost internode (*eui*) by Rutger *et al*., 1981, it became opportunities for plant breeders to transfer this recessive trait into the CMS lines, maintainer lines and restorer lines for complete panicle exsertion. As it was known that *eui* is a recessive gene, thus after each backcross selfing is required which was indeed a difficult task to do through conventional breeding. Virmani *et al*., 1988 has started a program in 1982 soon after a year of *eui* gene was identified. He decided to transfer the gene into a indica restorer line IR50. Because if the gene was incorporated into the parental parent of hybrid rice, plant would be taller as *eui* gives tall phenotype. So, a taller restorer line would be standard requirement for pollination as pollen dispersal take place on the semi dwarf male sterile line, resulting the F_1 hybrid would be semi dwarf. He has crossed original *eui* genetic stock collected from Dr. Rutger in 1982 with IR50, a semi dwarf indica restorer line. In F_2 population he has selected plants with elongated uppermost internode and again backcrossed with IR50 in three consecutive seasons to till BC_3F_1 than selfing was done to obtained BC_3F_3 families, in every backcross plant resembling

IR50 possessing *eui* trait were selected. Homozygous *eui* families were selected and designated as IR50-*eui*. Comparative analysis was done between IR50 and IR50-*eui*, where IR50-*eui* plants were taller than IR50 but had lower tillering and yield too were not statistically different. There are two way for application of *eui* gene in hybrid rice breeding program. To develop the restorer lines with *eui* gene to increase pollen load and another is to develop male sterile lines with *eui* gene to overcome the panicle enclosure issues in CMS lines (Virmani *et al*., 1988; Zuhua., 1987). According to the statement given by Yang *et al*.,, 2002, that most of the breeding plans were executed based on the backcrossing method and hybridization technique for transfer of *eui* gene into maintainer and restorer lines which takes long period of breeding and low breeding efficiency due to gene recombination and segregation in the progenies. Therefore, he has directly induced maintainer and restorer lines to obtain their mutant and further developed CMS line with *eui* (eA) and restorer line with *eui* (eR) and develop hybrid using mutant parents, designated as e-hybrid. eB1 or eB2 carrying *eui1* and *eui2* gene respectively were used for hybridization and backcrossing to corresponding A line and found only two type of group, one having more plant height and exserted panicle and other was similar to the CMS line. Further backcrossing was done with eB line to obtained eA line with those plants showing more plant height and elongated uppermost internode. Similarly, restorer lines with *eui* gene were developed by induced mutagenesis (Yang *et al*.,, 2002; Yang RC., 1998). In China, these genes have been transferred to promising CMS lines, which caused better panicle exsertion and confirmed that this gene can eliminate the application of GA_3 (Zuhua 1987; He *et al*., 1994).

For the purpose of improving the panicle exsertion, the *eui* gene from the donor parent, was transferred to a TGMS line through backcrossing. In each backcross generation the plants were selfed and taken to study of segregation of *eui* gene in further generation to improve breeding efficiency and finally in the BC_3F_1 plants like recurrent parent were selected for the developing selfed progeny to purify the *eui* gene (Wang *et al*., 2007). In their study they found that the TGMS line carrying *eui* gene had higher panicle exsertion (Wang *et al*., 2007). A significant improvement of having 20.8, 10.7 and 13.6% in panicle exsertion, panicle length, and number of spikelets per panicle respectively was observed in the improved IR 58025eA line (with *eui* gene) (Priyadarshi *et al*. 2016). Priyadarshi *et al*., 2017 revealed in his study, a strong correlation among different internode length related traits. Several researchers have been involved in introgression of the *eui* gene into different female parents to improve the panicle

exsertion in cytoplasmic/thermo sensitive genic male sterile lines (Yang *et al*., 2000, Zhang *et al*., 2002; 2002; Rongbai and Pandey 2002; Zhou *et al*., 2002). Therefore, parents carrying *eui* genes provides an option to overcome of the use of GA_3 in hybrid breeding program as presently, a significant quantity of GA_3 is being used for getting high panicle exsertion of WA-CMS lines.

3. Materials and Methods

Successful demonstration of hybrid seed production by China using WA cytoplasm, many countries started hybrid production in rice. But incomplete panicle exsertion that causes 30-40% of the panicle to be remains inside the flag leaf, is a major problem in hybrid seed production. A recessive gene *eui* for elongation uppermost internode (Rutger and Carnahan, 1981) in *japonica* line acts as 4th element in rice hybrid seed production. Many studies related to Genetic analysis of recessive mutant *eui* has been carried out and few mutants which are allelic to *eui* were identified (Maekawa, M. and Kita, F. 1983). There are two genes identified for elongation of uppermost internode of which one mapped on chromosome 5 and other is on chromosome10. So, it is important to study the genetics of elongation of uppermost internode, its molecular characterization and identification of major genes. Identification of molecular marker linked to major genes helps in its introgression and development of the parental lines with maximum outcrossing potential. So, the present study was started with the title "Genetic analysis of uppermost internode in rice". The materials and methodology followed during the present study is presented in this chapter. Field experimentation for taking morphological data was set up in two different location such as ICAR-IARI, New Delhi during *kharif*-2018 &2019 and Rice Breeding and Genetics Research Centre (RBGRC), ICAR-IARI, Aduturai, Tamil Nadu during *rabi* 2019-20. The molecular work was carried out at Division of Genetics, ICAR-IARI, New Delhi.

3.1 Plant materials

In the present study for morphological and molecular characterization of the uppermost internode elongation, different IRG (International Rice Germplasm) lines introduced from International Rice Research Institute, Manila, Philiphines were used. The screening for internode elongation of rice genotypes was done at two location for three seasons such as *kharif* 2018 at Division of Genetics, ICAR-IARI, New Delhi, *rabi*2018-19 at RBGRC, ICAR-IARI, Aduturai, Tamil Nadu and *kharif* 2019 at Division of Genetics, ICAR-IARI, New Delhi. After phenotyping for internode elongation, the molecular screening also carried out at Division of Genetics, IARI. Out of 348 IRG lines screened, the lines with highest internode length was selected for making crosses and developing F_2 population for genetic analysis and mapping gene(s)/QTL(s) for uppermost internode. The female line used in the present study was RTN10B, having average

uppermost internode length of 21.8 cm. RTN10B was crossed with the IRG213 line selected after screening for internode elongation. The average length of uppermost internode of IRG line was 42.3 cm. The crossing work was carried out during *kharif* 2018 between RTN10B and IRG213 at Division of Genetics, IARI, New Delhi. Sufficient F_1 seeds were generated and the F_1 seeds were sown in RBGRC, Aduturai, Tamil Nadu during *rabi*2018-19. The F_1 plants were allowed for selfing to generate F_2 seeds. The F_2 seeds were sown at Division of Genetics, IARI, during *kharif* 2019 for genetic analysis and mapping gene/QTLs for uppermost internode.

3.2 Morphological and Molecular screening for uppermost internode elongation

The IRG lines introduced from International Rice Research Institute (IRRI), Philiphines were having varying length of internode. Almost 300 rice genotypes were screened for morphological characters such uppermost internode length, length of the panicle, length of panicle exserted out of flag leaf, panicle exsertion ratio, plant height, number of grains per panicle and plant height. There are already 2 gene *eui1* and *eui2* mapped for internode elongation on chromosome number 5 and 10 respectively. The lines were also screened for presence and absence of the previously reported genes using markers linked to the gene of interest. SSR markers RM5970 and RM3476 were used for molecular screening of the genotypes.

3.3 Genetic analysis of uppermost internode

Based on morphological and molecular screening characterization, few IRG lines were selected and used as male parent to cross with RTN10B which is used as female parent having average internode length of 21.8. The F_2 population includes 217 plants were phenotyped for uppermost internode length, panicle length, length of exserted panicle from flag leaf and other parameters. Phenotyping was done for parents RTN10B and IRG213. In each plant, five panicles were selected randomly for taking data and average of five panicle was taken for all the parameters considered. Panicle exserted rate was calculated according to procedure given by Gangashetti *et al*. (2004). The panicle exsertion ratio (PER) was calculated as PER (%) = (Length of exserted panicle/Total length of panicle) × 100. The panicle length was measured from panicle base to panicle tip in centimetres. The F_2 plants were classified into different classes based on the morphological data and chi-squire (χ^2) analysis was carried out to know the goodness of fit at 1% and 5%. Chi-squire value (χ^2) = $\sum$(Expected-Observed)^2/Expected

3.4 Mapping of Gene(S)/ QTLs for elongation of uppermost internode

3.4.1 Development of mapping population

The mapping of gene(s)/QTLs for elongated uppermost internode in IRG (International Rice Germplasm) lines introduced from IRRI, Philiphines, was done using F_2mapping population developed from the cross between two contrasting parents, RTN10Bhaving incomplete panicle exsertion and IRG213 having complete panicle exsertion. The F_2 mapping population is a segregating population and each plant is different from other plant genotypically. So, in order to recover all the recombinants large F_2 mapping population of 217 plants was grown at IARI, New Delhi. Morphological data of all the parameters was taken and leaf samples were also collected from all the 217 plants.

3.4.2 Polymorphism survey and mapping of gene(s)/QTL

Polymorphism survey between RTN10B and IRG213 was carried out using 1358 SSR markers that are uniformly distributed throughout the 12 chromosomes of rice (*Oryza sativa*). The selected markers are well enough to find the polymorphism at molecular level between the parents. The identified polymorphic markers were diagrammatically represented by GGT (graphical genotyping of the chromosome) to know the position of polymorphic markers on chromosome and to know the extent of genomic coverage on different chromosome. The identified polymorphic markers were further used in BSA (Bulked Segregant Analysis) to know the putatively linked markers to the trait of interest. Care was also taken to fill up the gaps, if any, after polymorphism survey to reduce the possibility of missing putatively linked marker during BSA. GGT2.0 tool was used to prepare the graphical genotyping of the chromosome.

3.4.3 Bulked Segregant Analysis (BSA)

Genotyping of large F_2 population using polymorphic markers between the parents to map the gene(s)/QTLs for the trait of interest is a laborious and time-consuming process. So, in order to identify putatively linked marker/s to the trait of interest, a method proposed by Michelmore *et al.,* (1991), Bulk segregant analysis (BSA), was performed. Based on the morphological data of F_2 population, 10 plants each from two extremes were selected; DNA was isolated from 10 plants having low internode elongation and 10 plants of higher internode elongation. 10 plants were used to prepare the bulks because usage of 10 plants for bulking reduces the probability of identifying unlinked marker as linked one. Here after identified polymorphic marker(s) between the parents were used to evaluate the bulks along with the

parents. The molecular marker which shows polymorphism between the parents as well as between the bulks are considered as putatively linked marker to the trait of interest. This marker(s) was further used to genotype the large F_2 population for mapping gene for uppermost internode elongation. The genotypic data and phenotypic data were used for linkage analysis. The percentage of recombination between the marker and the gene of interest gives us the genetic distance between the marker and the gene. QTL cartographer and MAPMAKER was used in the present study to map and identify the genetic distance between the marker and gene.

3.5 Isolation of plant genomic DNA

In the present study the genomic DNA of all the genotypes was isolated by using CTAB method given by Murray and Thompson (1980). CTAB method of DNA isolation is less expensive and easy method to isolate genomic DNA from plant cell. The principle in DNA isolation is purification of DNA by disrupting cell wall and separating the DNA from other cell debris. Various chemicals and proteins (enzymes) were used to remove RNA, secondary metabolites, polysaccharides and phenolic compounds. The name of the chemical reagents used and their function is given in detail in the following paragraph.

1. **CTAB buffer**: For disruption of plant cell
2. **β-mercaptoethanol**:Reducing agents and denaturation of proteins by cleaving disulfide bond
3. **EDTA**:Chelating agents and binds to Mg^{2+} ions and inhibits DNase functioning
4. **Tris**: pH stabilizer and maintains the pH at 8
5. **The cations Na^+ or K^+**:Increases DNA stability in aqueous solution
6. **Ethanol**: Proteins gets dissolved in ethanol
7. **Chloroform**: Increases the viscosity
8. **Isoamyl alcohol**: Anti-foaming agent

DNA isolation was done by collecting young leaf tissue of 55-60 days old seedlings and following protocol was used.

1. Leaf samples were grounded into fine powder using pestles and mortars which was sterilized and pre cooled along with liquid nitrogen

2. Tubes containing polypropylene were taken and powdered leaf tissues were filled into tubes along with a ml of DEB which is pre warmed and the tubes were mounted on stand

3. The tubes were suspended in hot water bath of about 65-70° C for 1 hour and intermittent shaking was given at regular interval of 15-20 minutes

4. Tubes were taken out of hot water bath after 1 hour and kept tubes at room temperature for cooling

5. Same amount of chloroform and isoamyl alcohol (24:1) was added into tubes after cooling using micro pipette. Tubes were shaken gently for thorough mixing of reagent and the sample

6. Centrifugation was done for phase separation of solution at 10,000 rpm for 10 min at 4° Celsius. The supernatant was taken into fresh tubes carefully

7. Chloroform and Isoamyl alcohol (24:1) were added for the second time to the tubes where supernatant was transferred. Then the solution was centrifuged again at 10000 rpm for 10min at 4° Celsius. The supernatant taken in fresh and sterilized tubes

8. Pre chilled iso-propanol of 0.6 ml was added into tubes containing supernatant from second centrifugation and the tubes were shaken gently till a fibrous mass was visible and the tubes were incubated at -20°C for one hour

9. DNA precipitate was obtained after centrifugation at 5000rpm for 10 min at 4°C. The supernatant was removed off without disturbing the DNA present at the bottom of the tubes

10. The DNA pellet was washed with 70% ethanol for two times and kept the tubes overnight for drying at room temperature

11. The DNA pellet present in the tubes was dissolved by adding 100-200 µl of TE buffer (pH 8.0) into tubes.

12. RNase of 10 mg/ml was added into tubes containing DNA at the rate of 2 µl/100 µl of crude DNA, after 7-8 hours of addition of buffer. The solution in was kept for incubation in hot water bath of 37°C for one hour and intermittent shaking was also given

13. The DNA was purified by adding equal amount of chloroform: isoamyl alcohol in 24:1 to aqueous phase and mixed gently for 5-6 min and centrifugation was done at 10000 rpm.

14. Phase separation was done and aqueous phase occupies bottom portion of the tube and 3M sodium acetate (pH 5.2) of $1/10^{th}$ volume was added to the tubes. Pre chilled ethanol was also added and gentle shaking was given and kept for incubation at -20°C for 2 hours

15. The solution was centrifuged for 5 min at 10000rpm. After phase separation, the supernatant was removed off and DNA pellets was collected and washed twice with 70% ethanol. Then the

pellets were allowed for drying at room temperature for overnight and it was dissolved in 100 µl TE buffer (pH 8.0)

16. The DNA was quantified after loading 2 µl of DNA samples on agarose gel of 0.8% in 1x TAE buffer and diluted λ uncut gDNA was used as standard in a parallel well

17. The banding pattern and band intensity of various samples were compared with the standard and concentration of DNA was determined

18. New dilutions were made based on the concentration of each sample and the process was repeated till all the samples reach a standard concentration of ~25 ng/µl in a uniform

3.6 Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) assay was carried out by using SSR marker for elongated uppermost internode. We made10 µl volume of PCR reaction mix consisting of 25 ng of DNA, 10x PCR buffer (10 mM Tris, pH 8.4, 50 mMKCl, 1.8 mM MgCl2), 2 mMdNTPs (MBI, Fermentas, Lithuania, USA), 5 pmol each of forward and reverse primer and 3 U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bengaluru, India). The make-up of the PCR mix volume is presented in table 3.3. The Polymerase chain reaction (PCR) was carried out in a thermal cycler (Applied Biosystem, Somerset, UK) by adapting the following thermal protocol mentioned in table 3.4.

3.7 Resolution of amplified PCR products:

Gel electrophoresis technique is widely used for resolution of nucleic acid and Proteins of various size based on shape, size and net electric charge of molecules. Here we separate amplified DNA fragments based on size principle. Agarose gel of concentration 3.5% (w/v) was prepared by dissolving 17.5 g weighed agarose powder in 500 ml of 1xTAE [490 ml double distilled water + 10 ml of 50x TAE buffer (100 ml of 0.5M EDTA: PH 8.0; 242.2g Tris base Mwt. 121.14; 57.1ml Glacial acetic acid: Mwt. 61.83; make volume to 1000 ml using de ionized Milli-Q water) in 1000 ml conical flask. The suspension was heated in microwave oven at 900 watt for 10 minutes till appearance of clear solution. The heated solution was allowed to cool down at room condition with intermittent shaking and after cooling we added 24 µl (0.05 µl/ml of 1x TAE) of Ethidium Bromide stock solution (10 mg/ml of double distilled water). After gentle shaking, the gel was poured onto gel casting tray and kept it for solidification of gel. The solidified gel along with casting tray was immersed inside the gel tank containing 1x TAE (pH

8.0). To each PCR product of 10 µl was added with 1 µl 6x loading dye by using multichannel pipet (0.25% bromophenol blue; 40% sucrose; 0.25% xylene cyanol FF). After adding the dye samples were loaded into individual wells with the help of 10 µl multichannel pipettes. In parallel, gel was loaded with 50 bp size reference ladder (Fermentas, Lithuania, USA). The electric supply was adjusted at 5 Volts/cm of run and the total duration of electrophoresis varied from 1.5 to 2.5 hours. After optimum run of DNA fragments the gel slabs were visualized under UV trans-illuminator and documented in gel documentation system (Bio-Rad Laboratories Inc., USA).

4. Results

In rice, incomplete panicle exsertion form the flag leaf is one of the major limitations of WA type CMS lines. Therefore, application of GA_3 is an essential component for hybrid rice seed production and which ultimately increases the cost of cultivation.

Culm length is very important component, which determined the plant height. Total length of the calm is divided in three major sections, *viz*., first internode or uppermost internode, second internode and third internode. The uppermost internode is directly correlated to the panicle exsertion. Few *QTL*s and two genes *eui1* and *eui2* have been reported for elongated uppermost internode. In India 105 hybrid rice varieties have been released by public and private sectors but there are no such reports about the direct exploitation of the two genes *viz.*, *eui1* and *eui2* for elongated uppermost internode in any of the any of the CMS line.

Therefore, to understand the genetic nature of uppermost internode and mapping *QTL*s/genes for uppermost internode, present study was carried out on "Genetic analysis of uppermost internode in rice", with following objectives:

1. Morphological and molecular characterization of rice genotypes for uppermost internode.
2. Genetic analysis of uppermost internode in rice
3. Mapping genes for uppermost internodes using BSA.

The results of the present study undertaken to meet the above objectives are presented herewith in the ensuing section.

4.1 Molecular screening of rice genotypes for elongated uppermost internode using gene linked markers

Rice genotypes (IRG lines) collected from International Rice Research Institute (IRRI), Philippines were characterized for elongated uppermost internode (*eui1*) using two gene linked markers RM3476 and RM5970. The marker RM3476 was present at distance of 1.2 cM and RM5970 at a distance of 5.2 cM. The IRG lines which showed a banding pattern of 150 bp for RM3476 and 120 bp for RM5970 were considered as *eui* types**.** On the other hand, lines showed 130 and 110 bp for RM3476 and RM5970 respectively were considered as non *eui* types (Fig. 4.1). Based on these differences in amplified fragment size of RM3476 (150/130) and RM5970 (120/110), IRG lines were classified into *eui* and *non-eui* type (Table 4.1). A total of 348 IRG

lines were characterized, in which 29 lines were classified as *eui* type based on RM5970 marker and remaining 319 were found non-*eui* type. Whereas based on screening with RM3476 marker, 23 lines were classified as *eui* type and 325 lines were non-*eui* type. Out of 348 lines, 23 lines were common *eui1* type based on allelic status of both the markers while in 6 IRG lines there was a mismatch between the two markers *i.e.* lines which shows *eui* type banding pattern for RM3476 showed non *eui* type banding pattern for RM5970 that may due to their genetic distance from the gene. Allelic status of the genotypes obtained from the screening with two gene linked markers is presented in (Table 4.2).

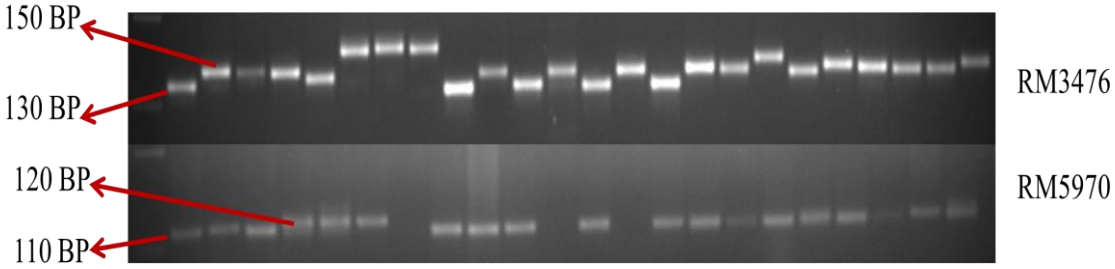


Fig. 4.1 Molecular screening of IRG lines for *eui1* gene using gene linked markers.

Table 4.1: Different fragment size reported for *eui1* gene linked markers RM3476 and RM5970.

Gene	Marker	Chromosome	Allele	Expected fragment size (bp)
eui1	RM5970	5	*eui*	120
			Non-eui	110
eui1	RM3476	5	*eui*	150
			Non-eui	130

Table 4.2: Genotyping of IRG lines for *eui1* gene based on two gene linked markers RM3476 and RM5970.

IRG No	Gene Status (*eui1*)		IRG No	Gene Status (*eui1*)		IRG No	Gene Status (*eui1*)	
	RM5970	**RM3476**		**RM5970**	**RM3476**		**RM5970**	**RM3476**
IRG1	*non-eui*	*non-eui*	IRG36	*non-eui*	*non-eui*	IRG71	***NA***	*non-eui*
IRG2	*non-eui*	*non-eui*	IRG37	*non-eui*	*non-eui*	IRG72	***NA***	*non-eui*
IRG3	*non-eui*	*non-eui*	IRG38	*non-eui*	*non-eui*	IRG73	***eui***	***eui***
IRG4	*non-eui*	*non-eui*	IRG39	*non-eui*	*non-eui*	IRG75	*non-eui*	*non-eui*
IRG5	***eui***	*non-eui*	IRG40	*non-eui*	*non-eui*	IRG76	*non-eui*	*non-eui*
IRG6	*non-eui*	*non-eui*	IRG41	*non-eui*	*non-eui*	IRG77	*non-eui*	*non-eui*
IRG7	*non-eui*	*non-eui*	IRG42	*non-eui*	*non-eui*	IRG78	*non-eui*	*non-eui*
IRG10	***eui***	*non-eui*	IRG43	*non-eui*	*non-eui*	IRG79	*non-eui*	*non-eui*
IRG11	*non-eui*	*non-eui*	IRG44	*non-eui*	*non-eui*	IRG80	*non-eui*	*non-eui*
IRG12	***eui***	***eui***	IRG45	*non-eui*	*non-eui*	IRG81	*non-eui*	*non-eui*
IRG13	***eui***	***eui***	IRG46	*non-eui*	*non-eui*	IRG82	***eui***	***eui***
IRG14	***eui***	***eui***	IRG47	*non-eui*	*non-eui*	IRG83	*non-eui*	*non-eui*
IRG15	***eui***	***eui***	IRG48	*non-eui*	*non-eui*	IRG84	*non-eui*	*non-eui*
IRG17	*non-eui*	*non-eui*	IRG49	*non-eui*	*non-eui*	IRG85	*non-eui*	*non-eui*
IRG18	*non-eui*	*non-eui*	IRG51	*non-eui*	*non-eui*	IRG86	*non-eui*	*non-eui*
IRG19	***eui***	***eui***	IRG52	*non-eui*	*non-eui*	IRG87	*non-eui*	*non-eui*
IRG20	*non-eui*	*non-eui*	IRG53	*non-eui*	*non-eui*	IRG88	*non-eui*	*non-eui*
IRG21	*non-eui*	*non-eui*	IRG54	*non-eui*	*non-eui*	IRG89	*non-eui*	*non-eui*
IRG22	*non-eui*	*non-eui*	IRG55	*non-eui*	*non-eui*	IRG90	*non-eui*	*non-eui*
IRG23	*non-eui*	*non-eui*	IRG56	***eui***	*non-eui*	IRG91	*non-eui*	*non-eui*
IRG24	*non-eui*	*non-eui*	IRG57	*non-eui*	*non-eui*	IRG92	*non-eui*	*non-eui*
IRG25	*non-eui*	*non-eui*	IRG58	*non-eui*	*non-eui*	IRG93	*non-eui*	*non-eui*
IRG26	*non-eui*	*non-eui*	IRG59	*non-eui*	*non-eui*	IRG94	***eui***	***eui***
IRG27	*non-eui*	*non-eui*	IRG60	*non-eui*	*non-eui*	IRG95	*non-eui*	*non-eui*
IRG28	*non-eui*	*non-eui*	IRG62	*non-eui*	*non-eui*	IRG96	*non-eui*	*non-eui*
IRG29	*non-eui*	*non-eui*	IRG63	*non-eui*	*non-eui*	IRG97	*non-eui*	*non-eui*
IRG30	*non-eui*	*non-eui*	IRG64	*non-eui*	*non-eui*	IRG98	*non-eui*	*non-eui*
IRG31	*non-eui*	*non-eui*	IRG65	*non-eui*	*non-eui*	IRG99	*non-eui*	*non-eui*
IRG32	*non-eui*	*non-eui*	IRG66	*non-eui*	*non-eui*	IRG100	*non-eui*	*non-eui*
IRG33	***eui***	***eui***	IRG67	*non-eui*	*non-eui*	IRG101	*non-eui*	*non-eui*
IRG34	***eui***	***eui***	IRG69	*non-eui*	*non-eui*	IRG102	*non-eui*	*non-eui*
IRG35	***eui***	***eui***	IRG70	***eui***	***eui***	IRG103	*non-eui*	*non-eui*

Table 4.2 ***(Contd...)***

IRG No	Gene Status (*eui1*)		IRG No	Gene Status (*eui1*)		IRG No	Gene Status (*eui1*)	
	RM5970	**RM3476**		**RM5970**	**RM3476**		**RM5970**	**RM3476**
IRG104	*non-eui*	*non-eui*	IRG140	*non-eui*	*non-eui*	IRG176	*non-eui*	*non-eui*
IRG105	*non-eui*	*non-eui*	IRG141	*non-eui*	*non-eui*	IRG177	*non-eui*	*non-eui*
IRG106	*non-eui*	*non-eui*	IRG142	*non-eui*	*non-eui*	IRG178	*non-eui*	*non-eui*
IRG107	*non-eui*	*non-eui*	IRG143	*non-eui*	*non-eui*	IRG179	*non-eui*	*non-eui*
IRG108	*non-eui*	*non-eui*	IRG144	*non-eui*	*non-eui*	IRG180	*non-eui*	*non-eui*
IRG110	***eui***	*non-eui*	IRG145	*non-eui*	*non-eui*	IRG181	*non-eui*	*non-eui*
IRG112	*non-eui*	*non-eui*	IRG146	*non-eui*	*non-eui*	IRG182	*non-eui*	*non-eui*
IRG113	*non-eui*	*non-eui*	IRG147	*non-eui*	*non-eui*	IRG183	*non-eui*	*non-eui*
IRG114	*non-eui*	*non-eui*	IRG148	*non-eui*	*non-eui*	IRG184	*non-eui*	*non-eui*
IRG115	*non-eui*	*non-eui*	IRG149	*non-eui*	*non-eui*	IRG185	*non-eui*	*non-eui*
IRG116	*non-eui*	*non-eui*	IRG152	*non-eui*	*non-eui*	IRG186	*non-eui*	*non-eui*
IRG117	*non-eui*	*non-eui*	IRG153	*non-eui*	*non-eui*	IRG187	*non-eui*	*non-eui*
IRG118	*non-eui*	*non-eui*	IRG154	*non-eui*	*non-eui*	IRG189	***eui***	***eui***
IRG120	***eui***	***eui***	IRG155	*non-eui*	*non-eui*	IRG190	*non-eui*	*non-eui*
IRG122	*non-eui*	*non-eui*	IRG156	*non-eui*	*non-eui*	IRG191	*non-eui*	*non-eui*
IRG123	*non-eui*	*non-eui*	IRG157	*non-eui*	*non-eui*	IRG192	*non-eui*	*non-eui*
IRG124	*non-eui*	*non-eui*	IRG158	*non-eui*	*non-eui*	IRG193	*non-eui*	*non-eui*
IRG125	*non-eui*	*non-eui*	IRG159	*non-eui*	*non-eui*	IRG194	*non-eui*	*non-eui*
IRG126	*non-eui*	*non-eui*	IRG160	*non-eui*	*non-eui*	IRG195	*non-eui*	*non-eui*
IRG127	*non-eui*	*non-eui*	IRG161	*non-eui*	*non-eui*	IRG196	*non-eui*	*non-eui*
IRG128	*non-eui*	*non-eui*	IRG162	*non-eui*	*non-eui*	IRG197	*non-eui*	*non-eui*
IRG129	*non-eui*	*non-eui*	IRG163	*non-eui*	*non-eui*	IRG198	*non-eui*	*non-eui*
IRG130	*non-eui*	*non-eui*	IRG164	*non-eui*	*non-eui*	IRG199	*non-eui*	*non-eui*
IRG131	*non-eui*	*non-eui*	IRG165	*non-eui*	*non-eui*	IRG200	***eui***	***eui***
IRG132	*non-eui*	*non-eui*	IRG166	*non-eui*	*non-eui*	IRG201	*non-eui*	*non-eui*
IRG133	*non-eui*	*non-eui*	IRG167	*non-eui*	*non-eui*	IRG202	*non-eui*	*non-eui*
IRG134	***eui***	*non-eui*	IRG168	*non-eui*	*non-eui*	IRG203	*non-eui*	*non-eui*
IRG135	***eui***	*non-eui*	IRG169	*non-eui*	*non-eui*	IRG204	*non-eui*	*non-eui*
IRG136	*non-eui*	*non-eui*	IRG170	*non-eui*	*non-eui*	IRG205	*non-eui*	*non-eui*
IRG137	*non-eui*	*non-eui*	IRG173	*non-eui*	*non-eui*	IRG206	*non-eui*	*non-eui*
IRG138	*non-eui*	*non-eui*	IRG174	*non-eui*	*non-eui*	IRG207	*non-eui*	*non-eui*
IRG139	*non-eui*	*non-eui*	IRG175	*non-eui*	*non-eui*	IRG208	*non-eui*	*non-eui*

Table 4.2 ***(Contd...)***

IRG No	Gene Status (*eui1*)		IRG No	Gene Status (*eui1*)		IRG No	Gene Status (*eui1*)	
	RM5970	**RM3476**		**RM5970**	**RM3476**		**RM5970**	**RM3476**
IRG209	*non-eui*	*non-eui*	IRG243	*non-eui*	*non-eui*	IRG278	*non-eui*	*non-eui*
IRG210	*non-eui*	*non-eui*	IRG244	*non-eui*	*non-eui*	IRG279	*non-eui*	*non-eui*
IRG211	*non-eui*	*non-eui*	IRG245	*non-eui*	*non-eui*	IRG280	*non-eui*	*non-eui*
IRG212	*non-eui*	*non-eui*	IRG246	*non-eui*	*non-eui*	IRG281	*non-eui*	*non-eui*
IRG213	*non-eui*	*non-eui*	IRG247	*non-eui*	*non-eui*	IRG282	*non-eui*	*non-eui*
IRG214	*non-eui*	*non-eui*	IRG248	*non-eui*	*non-eui*	IRG283	*non-eui*	*non-eui*
IRG215	*non-eui*	*non-eui*	IRG249	*non-eui*	*non-eui*	IRG284	*non-eui*	*non-eui*
IRG216	*non-eui*	*non-eui*	IRG250	*non-eui*	*non-eui*	IRG285	*non-eui*	*non-eui*
IRG217	*non-eui*	*non-eui*	IRG251	*non-eui*	*non-eui*	IRG286	***eui***	***eui***
IRG218	*non-eui*	*non-eui*	IRG252	*non-eui*	*non-eui*	IRG287	*non-eui*	*non-eui*
IRG219	*non-eui*	*non-eui*	IRG253	*non-eui*	*non-eui*	IRG288	*non-eui*	*non-eui*
IRG220	*non-eui*	*non-eui*	IRG255	*non-eui*	*non-eui*	IRG289	*non-eui*	*non-eui*
IRG221	*non-eui*	*non-eui*	IRG256	*non-eui*	*non-eui*	IRG290	*non-eui*	*non-eui*
IRG222	*non-eui*	*non-eui*	IRG257	*non-eui*	*non-eui*	IRG291	*non-eui*	*non-eui*
IRG223	*non-eui*	*non-eui*	IRG258	*non-eui*	*non-eui*	IRG292	*non-eui*	*non-eui*
IRG224	*non-eui*	*non-eui*	IRG259	*non-eui*	*non-eui*	IRG293	***eui***	***eui***
IRG225	*non-eui*	*non-eui*	IRG260	*non-eui*	*non-eui*	IRG294	*non-eui*	*non-eui*
IRG227	*non-eui*	*non-eui*	IRG261	***eui***	***eui***	IRG295	*non-eui*	*non-eui*
IRG228	*non-eui*	*non-eui*	IRG262	***eui***	***eui***	IRG296	*non-eui*	*non-eui*
IRG229	*non-eui*	*non-eui*	IRG263	*non-eui*	*non-eui*	IRG297	*non-eui*	*non-eui*
IRG230	*non-eui*	*non-eui*	IRG265	*non-eui*	*non-eui*	IRG298	*non-eui*	*non-eui*
IRG232	*non-eui*	*non-eui*	IRG266	*non-eui*	*non-eui*	IRG299	*non-eui*	*non-eui*
IRG233	*non-eui*	*non-eui*	IRG267	***eui***	***eui***	IRG300	*non-eui*	*non-eui*
IRG234	*non-eui*	*non-eui*	IRG268	*non-eui*	*non-eui*	IRG301	*non-eui*	*non-eui*
IRG235	*non-eui*	*non-eui*	IRG269	*non-eui*	*non-eui*	IRG302	*non-eui*	*non-eui*
IRG236	*non-eui*	*non-eui*	IRG270	*non-eui*	*non-eui*	IRG303	*non-eui*	*non-eui*
IRG237	*non-eui*	*non-eui*	IRG271	*non-eui*	*non-eui*	IRG306	*non-eui*	*non-eui*
IRG238	*non-eui*	*non-eui*	IRG272	*non-eui*	*non-eui*	IRG307	*non-eui*	*non-eui*
IRG239	*non-eui*	*non-eui*	IRG273	*non-eui*	*non-eui*	IRG308	*non-eui*	*non-eui*
IRG240	*non-eui*	*non-eui*	IRG275	*non-eui*	*non-eui*	IRG309	*non-eui*	*non-eui*
IRG241	*non-eui*	*non-eui*	IRG276	*non-eui*	*non-eui*	IRG310	*non-eui*	*non-eui*
IRG242	*non-eui*	*non-eui*	IRG277	*non-eui*	*non-eui*	IRG311	*non-eui*	*non-eui*

Table 4.2 ***(Contd...)***

IRG No	Gene Status (*eui1*)		IRG No	Gene Status (*eui1*)	
	RM5970	**RM3476**		**RM5970**	**RM3476**
IRG312	*non-eui*	*non-eui*	IRG341	*non-eui*	*non-eui*
IRG313	*non-eui*	*non-eui*	IRG342	***eui***	***eui***
IRG314	*non-eui*	*non-eui*	IRG343	***eui***	***eui***
IRG315	*non-eui*	*non-eui*	IRG344	*non-eui*	*non-eui*
IRG316	*non-eui*	*non-eui*	IRG345	*non-eui*	*non-eui*
IRG317	*non-eui*	*non-eui*	IRG346	*non-eui*	*non-eui*
IRG318	*non-eui*	*non-eui*	IRG348	*non-eui*	*non-eui*
IRG321	*non-eui*	*non-eui*	IRG349	*non-eui*	*non-eui*
IRG322	*non-eui*	*non-eui*	IRG350	*non-eui*	*non-eui*
IRG323	*non-eui*	*non-eui*	IRG351	*non-eui*	*non-eui*
IRG324	*non-eui*	*non-eui*	IRG354	*non-eui*	*non-eui*
IRG325	*non-eui*	*non-eui*	IRG355	*non-eui*	*non-eui*
IRG326	*non-eui*	*non-eui*	IRG356	*non-eui*	*non-eui*
IRG328	*non-eui*	*non-eui*	IRG357	*non-eui*	*non-eui*
IRG329	*non-eui*	*non-eui*	IRG358	*non-eui*	*non-eui*
IRG330	*non-eui*	*non-eui*	IRG359	***eui***	***eui***
IRG331	*non-eui*	*non-eui*	IRG360	*non-eui*	*non-eui*
IRG332	*non-eui*	*non-eui*	IRG361	*non-eui*	*non-eui*
IRG333	*non-eui*	*non-eui*	IRG362	*non-eui*	*non-eui*
IRG335	*non-eui*	*non-eui*	IRG364	*non-eui*	*non-eui*
IRG336	*non-eui*	*non-eui*	IRG365	*non-eui*	*non-eui*
IRG337	*non-eui*	*non-eui*	IRG366	*non-eui*	*non-eui*
IRG338	*non-eui*	*non-eui*	IRG367	*non-eui*	*non-eui*
IRG339	*non-eui*	*non-eui*	IRG368	*non-eui*	*non-eui*
IRG340	*non-eui*	*non-eui*	IRG371	*non-eui*	*non-eui*

4.2 Morphological characterization of rice genotypes for uppermost internode and panicle exsertion ratio

Based on the molecular characterization data, a representative sample of 150 IRG lines were selected for morphological screening. These lines include 29 *eui* type IRG lines and 121 *non-eui* type lines. A total of 150 IRG lines were characterized for uppermost internode length, panicle length and panicle exsertion ratio. The 150 IRG lines were extensively evaluated during *Kharif* 2018 and 2019 in ICAR-IARI, New Delhi and during *Off Season* 2018-19 in IARI-RBGRC, Aduthurai. Observations on uppermost internode length, panicle length, and panicle exsertion ratio were taken from five random plants in each IRG line and mean of 5 plants were taken. Panicle exertion ratio was calculated by taking ratio of length of the panicle and length of panicle from the base of the flag leaf.

4.2.1 Performance of rice genotypes at ICAR-IARI, New Delhi.

In Delhi, IRG lines were evaluated for two seasons during *Kharif* 2018 and 2019 for three traits *viz.,* uppermost internode length (UIL), panicle length (PL) and panicle exsertion ratio (PER%). Analysis of variance showed that there was significant variation among the IRG lines for all three traits (Table 4.3 & 4.4).

The phenotypic data of Delhi during *Kharif* 2018 showed that the length of uppermost internode (UIL) ranges from 20.5 cm to 53 cm with an average of 35.75 cm in which lowest uppermost internode length was found in IRG62 and highest in IRG27. During *Kharif* 2019, IRG7 was showed lowest length of 19.8 cm and IRG343 showed highest length of 48.3 cm whereas the average length was 34.70.

Panicle length in Delhi during *Kharif* 2018 was ranged from 15.60 cm to 32.12 cm with an average of 24.24 cm in which IRG29 was having lowest panicle length and IRG143 showed highest panicle length. During *Kharif* 2019 at Delhi, lowest panicle length was found in IRG29 with a length of 14.9 cm and highest in IRG143 with length of 31.62 cm whereas the average length was observed 24.14 cm.

Observations on panicle exsertion ratio during *Kharif* 2018 at Delhi was found lowest of 92% exertion in IRG46 and highest of 118.50% in IRG120 with an average of 102.18 %. During *Kharif* 2019, a lowest of 92.65 % panicle exsertion was observed in IRG185 and IRG126 and

highest of 111.30 % in IRG343 with an average of 103.05 %. Mean performance of IRG lines for all these traits are presented in Table 4.6

4.2.2 Performance of rice genotypes at RBGRC, Aduthurai.

In Aduthurai, IRG lines were evaluated during *Off Season* 2018-19 for three traits *viz.*, uppermost internode length (UIL), panicle length (PL) and panicle exsertion ratio (PER%). Analysis of variance showed that there was significant variation among the IRG lines for all three traits (Table 4.5).

The phenotypic data of Aduthurai during *Off Season* 2018-19 showed that IRG29 was recorded lowest uppermost internode length and IRG 128 was recorded highest internode length of 26.55 cm and 60.16 cm with an average of 41.21 cm. Panicle length was varied from 17.45 cm(IRG142) to 46.80 cm (IRG117) with an average of 27.63 cm. Whereas panicle exsertion ratio was ranged from 60.77 to 164.54 % with an average of 117.02 % (Table 4.6).

4.2.3 Performance of rice genotypes across three environments.

Pooled ANOVA was calculated based on 3 environments data and it was observed that all the traits under study *viz.,* uppermost internode length (UIL), panicle length (PL) and panicle exsertion ratio (PER%) were showing significant genotype and environment (GE) interaction (Table 4.7). Pooled analysis of variance based on combined multi-environment data also suggested the presence of significant difference among IRG lines for all characters under study. Based on overall mean, uppermost internode length ranged from 23.23 cm (IRG 7) to 47.75 (IRG 199) cm with an average of 37.22 cm, whereas panicle length was ranged from 17.57 cm (IRG 29) to 34.7 cm (IRG 117) with an average of 25.34 cm. Panicle exsertion ratio was ranged from 88.14 % (IRG 118) to 123.38 % (IRG 267) with an average of 107.42 %. Mean performance of IRG lines across the locations is presented in (Table 4.8).

Table 4.3 ANOVA for uppermost internode, Panicle length and Panicle exsertion ratio in Delhi, *Kharif* 2018.

Source		UIL	PL	PER
	df	MSS	MSS	MSS
Genotype	149	83.36**	13.20**	34.4**
Replication	1	22.02**	37.49**	116.638**
Error	149	2.27	2.7	6.31
CV (%)		4.20	6.80	2.50
CD (0.05)		2.11	2.32	3.51

**p≤ 0.00 and *p≤ 0.05

Table 4.4 ANOVA for uppermost internode, Panicle length and Panicle exsertion ratio in Delhi, *Kharif* 2019.

Source		UIL	PL	PER
	df	MSS	MSS	MSS
Genotype	149	59.08**	15.49**	30.62**
Replication	1	16.14**	23.18**	8.55**
Error	149	1.04	2.59	4.95
CV (%)		3.00	6.70	2.20
CD (0.05)		2.09	3.18	4.40

**p≤ 0.00 and *p≤ 0.05

Table 4.5 ANOVA for uppermost internode, Panicle length and Panicle exsertion ratio in Aduthurai, *Off Season* 2018-19

Source		UIL	PL	PER
	df	MSS	MSS	MSS
Genotypic	149	86.37**	29.66**	764.88**
Replication	1	898.21**	1.09**	191.04**
Error	149	3.41	3.37	
CV (%)		4.50	6.60	3.70
CD (0.05)		3.65	3.63	8.54

**p≤ 0.00 and *p≤ 0.05

Table 4.6 Mean performance of all traits in three different environments

IRG No	UIL			PL			PER %		
	Delhi		Adt	Delhi		Adt	Delhi		Adt
	2018	2019	2018-19	2018	2019	2018-19	2018	2019	2018-19
IRG3	37.10	28.20	32.30	18.20	18.00	21.14	108.10	105.45	138.10
IRG4	33.12	27.90	33.65	22.40	21.60	27.10	103.27	102.80	106.00
IRG5	36.08	34.40	34.25	17.40	16.60	24.10	106.00	106.80	109.75
IRG6	40.48	42.10	38.64	28.10	27.60	25.98	108.40	106.55	105.30
IRG7	22.65	19.80	27.25	18.60	16.00	25.25	98.80	99.85	101.15
IRG10	39.59	33.90	32.22	22.60	19.00	22.53	103.60	106.45	143.23
IRG11	25.06	29.60	33.79	23.90	24.30	31.51	101.55	104.30	133.43
IRG12	36.10	36.30	41.95	26.30	24.90	25.81	106.30	104.25	134.45
IRG13	33.50	34.50	43.21	21.10	19.70	25.00	103.50	107.85	134.54
IRG14	36.10	37.80	40.79	20.60	19.00	26.51	105.20	100.85	147.59
IRG15	37.60	30.20	38.34	24.20	22.70	23.57	102.60	104.55	136.57
IRG17	34.85	27.90	41.50	24.90	23.00	28.38	97.15	96.35	131.92
IRG18	42.90	42.20	47.55	25.90	24.20	25.75	106.45	103.00	104.00
IRG19	36.60	34.10	43.30	17.60	15.40	32.40	103.35	105.65	142.49
IRG20	34.30	24.35	45.34	22.20	20.90	31.78	96.95	100.55	122.69
IRG21	42.40	32.90	37.75	23.10	22.80	25.25	103.69	107.40	140.85
IRG22	35.70	29.30	35.80	22.10	20.80	25.00	97.62	97.25	99.30
IRG23	39.90	35.50	42.10	25.30	23.70	27.26	105.55	108.85	104.55
IRG24	38.60	39.90	43.20	25.70	25.60	25.75	109.40	105.75	110.00
IRG25	37.40	40.10	40.54	23.90	24.00	25.49	101.95	107.90	143.82
IRG26	36.30	33.90	37.74	22.80	23.70	24.75	101.40	100.50	128.48
IRG27	53.00	44.30	44.70	22.30	21.50	29.13	97.75	107.55	124.02
IRG28	35.40	26.50	33.55	22.50	23.20	23.20	101.14	109.05	113.35
IRG29	33.00	36.50	26.55	15.60	14.90	22.20	100.17	104.45	111.70
IRG30	42.10	35.40	42.09	26.80	27.50	25.68	104.70	101.78	123.20
IRG31	27.90	28.30	38.73	27.50	29.50	23.40	95.70	102.40	128.36
IRG32	26.40	24.60	39.10	23.10	21.10	23.47	98.65	98.10	147.19
IRG33	34.80	33.90	37.05	29.20	28.70	30.75	117.85	107.60	109.50
IRG34	34.50	32.30	43.92	24.90	27.20	23.65	114.10	104.00	132.97
IRG35	32.10	37.50	37.23	27.20	26.40	23.77	103.50	103.95	135.46
IRG36	31.88	33.60	49.50	24.50	27.30	29.02	102.40	104.65	140.83
IRG37	37.30	30.20	49.22	24.10	25.10	27.92	103.30	104.10	131.88
IRG38	41.80	37.50	39.77	25.30	24.90	34.83	101.30	101.45	104.25
IRG39	39.00	36.50	40.60	26.20	25.40	28.55	100.50	107.38	111.35
IRG40	36.40	34.70	43.33	25.00	25.30	27.50	99.85	101.70	123.00

Table 4.6 ***(Contd...)***

IRG No	UIL			PL			PER %		
	Delhi		Adt	Delhi		Adt	Delhi		Adt
	2018	2019	2018-19	2018	2019	2018-19	2018	2019	2018-19
IRG41	41.50	33.70	53.25	25.20	25.00	32.20	100.49	104.20	125.29
IRG42	41.45	37.50	37.58	26.20	27.10	25.17	102.05	104.40	138.52
IRG46	40.20	33.60	43.67	23.40	25.50	26.22	92.00	102.10	142.61
IRG47	44.40	39.70	39.20	24.00	25.00	26.97	103.02	105.35	127.68
IRG49	35.70	35.60	42.17	23.40	23.50	29.33	99.60	99.90	119.71
IRG51	42.90	39.60	38.25	23.50	27.30	24.83	102.00	103.63	130.96
IRG52	40.50	38.10	43.17	20.60	20.60	25.17	102.50	101.00	131.97
IRG53	46.10	34.20	36.55	28.50	26.60	31.25	103.20	109.65	119.50
IRG54	43.20	41.30	43.60	24.00	22.20	26.10	101.50	101.28	106.25
IRG55	45.85	40.30	31.58	27.40	25.40	24.43	101.42	104.35	125.85
IRG56	41.10	45.70	43.23	24.00	24.30	26.83	103.40	102.20	128.81
IRG62	42.40	31.20	41.74	23.30	23.90	25.77	101.82	101.63	105.41
IRG63	21.70	34.60	38.25	22.60	25.00	24.60	102.38	109.20	114.65
IRG64	31.60	32.30	38.04	22.60	24.30	25.37	101.82	96.45	120.97
IRG65	37.30	40.50	43.40	28.30	26.40	24.46	100.50	99.58	132.10
IRG67	33.30	30.90	49.50	22.90	24.00	30.05	99.69	101.95	109.90
IRG69	35.34	32.00	38.00	23.50	23.10	25.85	101.00	104.10	109.00
IRG70	31.95	31.50	50.52	25.20	23.50	29.62	102.02	103.35	135.09
IRG71	30.55	29.40	35.65	23.10	22.60	25.30	101.42	99.83	102.30
IRG72	31.05	33.10	57.15	21.50	20.60	34.46	104.20	102.50	113.22
IRG73	33.40	33.50	43.48	25.60	27.40	29.77	101.10	106.95	124.69
IRG75	34.90	38.80	42.29	25.20	25.90	26.78	102.68	108.05	133.07
IRG76	20.80	26.30	33.15	25.40	25.00	25.50	96.95	104.60	104.48
IRG78	48.80	44.60	40.30	22.60	23.20	26.18	109.00	104.65	118.30
IRG80	46.20	37.75	38.25	23.30	23.60	23.50	101.90	102.60	107.45
IRG81	46.20	44.60	39.70	23.50	23.30	32.03	104.96	100.35	106.69
IRG82	31.00	33.00	35.50	23.40	22.50	25.50	104.75	98.85	105.85
IRG94	32.00	31.30	37.33	27.10	26.40	25.16	108.20	103.35	105.99
IRG95	30.24	38.80	46.85	24.00	24.70	30.68	107.35	109.15	141.36
IRG96	32.84	32.70	46.67	24.90	24.60	29.30	100.87	104.30	132.89
IRG97	36.30	30.80	54.77	24.10	25.00	29.44	103.54	105.50	144.45
IRG98	42.00	39.50	51.58	28.00	25.50	31.97	100.32	99.60	124.87
IRG105	32.29	24.10	52.05	22.00	24.70	29.42	97.80	104.55	131.23
IRG106	34.60	31.80	47.15	23.70	26.20	29.70	98.08	100.60	116.24
IRG110	31.50	33.00	36.95	25.20	22.00	28.80	106.75	105.10	108.30

Table 4.6 ***(Contd...)***

IRG No	UIL			PL			PER %		
	Delhi		Adt	Delhi		Adt	Delhi		Adt
	2018	2019	2018-19	2018	2019	2018-19	2018	2019	2018-19
IRG112	34.60	33.60	39.21	23.40	25.80	24.77	105.60	106.15	105.61
IRG116	31.10	31.10	45.15	24.60	23.40	30.53	100.39	102.35	94.63
IRG117	24.40	28.80	34.11	27.80	29.50	46.80	97.45	102.25	80.46
IRG118	39.60	43.90	52.27	25.00	23.80	32.38	102.86	100.80	60.77
IRG120	33.82	40.30	38.72	20.90	20.50	30.33	118.50	108.00	95.59
IRG123	39.60	43.00	44.44	29.50	29.50	26.52	104.56	96.75	112.61
IRG124	32.52	38.60	38.80	26.50	26.70	28.78	102.15	101.35	105.35
IRG125	24.30	29.80	40.63	20.60	21.80	29.72	98.00	96.40	102.72
IRG126	37.20	30.90	53.55	28.80	29.40	25.75	100.66	92.65	113.37
IRG127	44.70	43.10	43.80	27.70	25.80	28.35	103.30	103.05	132.92
IRG128	38.20	39.40	60.16	25.50	28.80	23.91	102.55	99.85	164.54
IRG129	36.90	36.00	36.41	26.20	28.00	27.42	101.30	95.10	103.87
IRG131	42.35	36.40	48.07	26.90	24.40	25.77	99.78	102.43	123.36
IRG132	40.80	35.10	27.78	28.40	26.20	25.82	97.90	103.35	103.09
IRG134	41.85	37.00	43.25	26.00	25.30	32.48	103.40	108.95	122.49
IRG135	36.70	41.25	50.33	23.20	23.40	24.44	104.10	108.00	128.27
IRG136	42.60	40.00	29.46	26.60	26.00	35.05	101.95	104.00	81.14
IRG137	37.80	36.00	38.23	24.90	22.70	22.21	102.80	101.15	76.72
IRG138	39.50	38.50	27.75	26.30	28.10	28.60	103.55	107.35	105.61
IRG140	38.50	37.40	43.95	24.15	24.15	22.68	96.50	101.20	133.04
IRG142	41.60	42.30	32.08	26.00	27.10	17.45	103.65	103.55	100.71
IRG143	38.80	36.70	38.02	32.12	31.62	29.54	99.30	99.23	162.27
IRG145	45.50	41.00	42.56	26.40	25.90	26.77	99.26	104.90	102.64
IRG146	37.20	36.80	42.60	24.30	24.50	23.53	96.90	96.80	120.98
IRG147	32.95	27.50	39.45	25.20	25.00	25.49	99.86	105.93	103.45
IRG148	32.20	31.50	34.29	28.90	27.40	24.69	100.11	97.50	108.35
IRG149	36.10	38.00	32.51	24.20	24.70	24.52	99.40	99.20	114.79
IRG152	40.80	36.10	38.84	26.40	26.40	22.85	103.15	104.55	144.49
IRG153	44.90	44.30	31.97	25.50	26.50	25.92	100.85	97.30	103.74
IRG155	29.30	31.80	50.40	24.10	22.48	31.88	100.31	103.25	153.77
IRG157	25.20	27.20	39.69	24.70	25.20	27.90	97.49	107.90	104.56
IRG160	33.50	32.10	38.40	23.10	24.20	27.75	97.09	100.80	112.79
IRG161	46.70	43.30	43.95	24.80	22.70	32.52	104.85	105.70	123.27
IRG162	20.50	31.20	47.42	21.80	22.80	30.52	99.42	102.25	115.47
IRG163	31.60	30.90	33.00	24.40	23.50	24.78	101.65	99.35	82.80

Table 4.6 ***(Contd...)***

IRG No	UIL			PL			PER %		
	Delhi		Adt	Delhi		Adt	Delhi		Adt
	2018	2019	2018-19	2018	2019	2018-19	2018	2019	2018-19
IRG164	43.40	35.90	48.23	21.65	23.30	25.73	102.20	105.10	154.80
IRG185	35.40	33.00	33.24	24.50	24.00	27.33	99.50	92.65	100.68
IRG186	33.60	33.50	28.57	24.00	24.75	21.55	100.98	101.40	115.08
IRG187	26.00	35.10	36.45	23.50	25.00	25.53	97.05	100.70	105.72
IRG189	43.90	43.70	34.55	20.90	20.10	28.07	106.55	107.80	97.71
IRG190	26.00	27.70	44.75	23.95	22.45	26.23	93.45	96.80	82.31
IRG191	23.88	24.80	44.54	22.45	21.20	28.51	96.10	97.80	111.97
IRG192	34.45	28.70	39.24	22.75	22.50	28.50	104.30	103.65	122.77
IRG193	36.95	35.90	41.31	22.75	23.85	25.73	101.40	103.40	118.40
IRG194	24.90	22.10	36.48	22.95	23.30	26.24	96.80	94.05	116.04
IRG195	42.00	37.30	39.32	22.70	24.35	25.90	103.40	108.50	91.02
IRG196	34.95	31.00	39.32	24.50	25.20	32.94	98.16	103.55	116.25
IRG197	28.20	23.90	48.00	23.70	23.90	28.25	96.95	97.75	133.79
IRG199	44.80	47.20	51.24	27.60	26.70	31.40	108.25	108.75	113.30
IRG200	32.10	32.90	51.24	22.55	23.05	32.46	118.35	110.00	121.91
IRG201	35.80	36.80	54.74	23.50	22.75	31.58	100.92	97.65	144.28
IRG223	41.80	41.30	40.90	26.95	28.10	27.70	100.47	104.20	120.26
IRG224	31.50	31.90	53.65	22.50	24.50	31.73	101.85	104.65	147.03
IRG225	38.60	34.90	43.83	24.00	23.50	29.71	101.90	102.63	123.53
IRG227	34.20	30.70	42.57	21.60	24.10	26.41	96.60	98.00	115.66
IRG230	28.80	28.10	51.31	21.70	23.35	30.36	98.80	102.85	135.12
IRG239	35.05	32.30	44.59	22.70	22.50	29.92	105.44	105.05	132.15
IRG241	34.55	29.80	38.30	20.70	19.80	23.82	103.13	104.90	103.48
IRG242	31.90	25.85	32.67	17.00	16.65	25.85	104.53	106.15	115.64
IRG243	36.20	31.90	43.58	23.10	23.75	27.10	96.53	97.40	133.06
IRG245	40.10	34.10	35.17	23.95	22.40	27.23	94.80	97.50	116.91
IRG247	39.15	37.30	29.59	22.95	23.00	21.75	103.45	103.55	112.86
IRG248	44.20	38.60	48.67	25.90	25.10	26.67	100.38	99.05	147.17
IRG250	31.00	26.50	41.43	20.10	21.20	29.50	98.47	96.75	117.56
IRG255	29.00	31.35	38.67	25.55	25.20	32.80	103.35	108.35	71.31
IRG256	25.70	39.45	41.68	25.40	25.50	26.19	102.80	103.25	108.86
IRG257	48.50	44.20	42.70	25.75	24.40	33.42	107.50	109.00	88.96
IRG258	22.40	36.45	40.32	23.70	23.50	35.95	99.55	96.85	68.47
IRG262	33.40	36.60	41.44	26.55	26.60	25.67	107.90	109.75	105.64
IRG263	24.90	31.20	44.21	24.50	23.70	29.00	103.61	105.35	116.17

Table 4.6 ***(Contd...)***

IRG No	UIL			PL			PER %		
	Delhi		Adt	Delhi		Adt	Delhi		Adt
	2018	2019	2018-19	2018	2019	2018-19	2018	2019	2018-19
IRG265	24.20	36.85	50.01	21.70	23.10	41.82	105.30	108.55	63.98
IRG266	42.82	38.70	50.15	25.10	23.15	34.03	103.32	106.80	84.70
IRG267	32.00	32.70	51.58	25.20	25.15	25.78	103.08	106.20	160.87
IRG268	44.90	40.30	37.43	24.65	23.90	33.42	99.50	98.47	80.21
IRG283	24.00	26.70	34.40	23.85	22.95	24.39	99.58	97.50	82.81
IRG286	30.90	34.80	50.20	24.10	24.70	32.24	105.00	105.50	127.49
IRG293	30.20	29.90	34.20	22.90	23.90	23.25	107.00	103.25	103.86
IRG342	32.50	35.60	35.10	26.50	25.70	26.75	102.22	103.65	104.20
IRG343	35.70	48.30	43.75	31.75	31.20	32.05	110.10	111.30	113.90
IRG359	41.00	36.30	40.00	23.90	23.45	24.30	104.00	106.15	103.50
Mean	35.75	34.70	41.21	24.24	24.14	27.63	102.18	103.05	117.02
Min	20.50	19.80	26.55	15.60	14.90	17.45	92.00	92.65	60.77
Max	53.00	48.30	60.16	32.12	31.62	46.80	118.50	111.30	164.54
CD (0.05)	**2.11**	**2.09**	**3.65**	**2.32**	**3.18**	**3.63**	**3.51**	**4.40**	**8.54**

Table 4.7 Pooled ANOVA for uppermost internode, panicle length and panicle exsertion ratio across the environment

Source		Traits		
		UIL	PL	PER %
	df	MSS	MSS	MSS
G	149	113.84**	30.73**	290.3
E	2	3665.22*	1183.83**	20799**
GxE	298	57.49**	13.81**	269.8**
Rep within Environment	3	312.13**	20.59**	10.55**
Pooled error	447	2.25	2.91	10.00
CV (%)		**4**	**6.7**	**2.9**

** $p \leq 0.00$ and * $p \leq 0.05$

Table. 4.8 Mean performance of the 150 IRG lines across the environments

IRG lines	UIL	PL	PER	IRG lines	UIL	PL	PER
IRG3	32.53	19.11	117.22	IRG41	42.82	27.47	109.99
IRG4	31.56	23.7	104.02	IRG42	38.84	26.16	114.99
IRG5	34.91	19.37	107.52	IRG46	39.16	25.04	112.24
IRG6	40.41	27.23	106.75	IRG47	41.1	25.32	112.02
IRG7	23.23	19.95	99.93	IRG49	37.82	25.41	106.4
IRG10	35.24	21.38	117.76	IRG51	40.25	25.21	112.2
IRG11	29.48	26.57	113.1	IRG52	40.59	22.12	111.82
IRG12	38.12	25.67	115	IRG53	38.95	28.78	110.78
IRG13	37.07	21.93	115.3	IRG54	42.7	24.1	103.01
IRG14	38.23	22.04	117.88	IRG55	39.24	25.74	110.54
IRG15	35.38	23.49	114.57	IRG56	43.34	25.04	111.47
IRG17	34.75	25.43	108.48	IRG62	38.45	24.32	102.95
IRG18	44.22	25.28	104.48	IRG63	31.52	24.07	108.74
IRG19	38	21.8	117.16	IRG64	33.98	24.09	106.41
IRG20	34.66	24.96	106.73	IRG65	40.4	26.39	110.73
IRG21	37.68	23.72	117.31	IRG67	37.9	25.65	103.85
IRG22	33.6	22.63	98.06	IRG69	35.11	24.15	104.7
IRG23	39.17	25.42	106.32	IRG70	37.99	26.11	113.49
IRG24	40.57	25.68	108.38	IRG71	31.87	23.67	101.18
IRG25	39.35	24.46	117.89	IRG72	40.43	25.52	106.64
IRG26	35.98	23.75	110.13	IRG73	36.79	27.59	110.91
IRG27	47.33	24.31	109.78	IRG75	38.66	25.96	114.6
IRG28	31.82	22.97	107.85	IRG76	26.75	25.3	102.01
IRG29	32.02	17.57	105.44	IRG78	44.57	23.99	110.65
IRG30	39.86	26.66	109.89	IRG80	40.73	23.47	103.98
IRG31	31.64	26.8	108.82	IRG81	43.5	26.28	104
IRG32	30.03	22.56	114.65	IRG82	33.17	23.8	103.15
IRG33	35.25	29.55	111.65	IRG94	33.54	26.22	105.85
IRG34	36.91	25.25	117.02	IRG95	38.63	26.46	119.29
IRG35	35.61	25.79	114.31	IRG96	37.4	26.27	112.69
IRG36	38.33	26.94	115.96	IRG97	40.62	26.18	117.83
IRG37	38.91	25.71	113.1	IRG98	44.36	28.49	108.26
IRG38	39.69	28.34	102.33	IRG105	36.15	25.37	111.19
IRG39	38.7	26.72	106.41	IRG106	37.85	26.53	104.97
IRG40	38.14	25.93	108.18	IRG110	33.82	25.33	106.72

UIL- Uppermost internode length (cm), PL- Panicle length (cm) and PER- Panicle exsertion ratio

Table 4.8 (*Contd*…)

IRG lines	UIL	PL	PER	IRG lines	UIL	PL	PER
IRG112	35.8	24.66	105.79	IRG164	42.51	23.56	120.7
IRG116	35.78	26.18	99.12	IRG185	33.88	25.28	97.61
IRG117	29.1	34.7	93.39	IRG186	31.89	23.43	105.82
IRG118	45.26	27.06	88.14	IRG187	32.52	24.68	101.16
IRG120	37.61	23.91	107.36	IRG189	40.72	23.02	104.02
IRG123	42.35	28.51	104.64	IRG190	32.82	24.21	90.85
IRG124	36.64	27.33	102.95	IRG191	31.07	24.05	101.96
IRG125	31.58	24.04	99.04	IRG192	34.13	24.58	110.24
IRG126	40.55	27.98	102.23	IRG193	38.05	24.11	107.74
IRG127	43.87	27.28	113.09	IRG194	27.83	24.16	102.3
IRG128	45.92	26.07	122.31	IRG195	39.54	24.32	100.97
IRG129	36.44	27.21	100.09	IRG196	35.09	27.55	105.99
IRG131	42.27	25.69	108.52	IRG197	33.37	25.28	109.5
IRG132	34.56	26.81	101.45	IRG199	47.75	28.57	110.1
IRG134	40.7	27.93	111.61	IRG200	38.75	26.02	116.75
IRG135	42.76	23.68	113.46	IRG201	42.45	25.94	114.28
IRG136	37.35	29.22	95.7	IRG223	41.33	27.58	108.31
IRG137	37.34	23.27	93.56	IRG224	39.02	26.24	117.84
IRG138	35.25	27.67	105.51	IRG225	39.11	25.74	109.35
IRG140	39.95	23.66	110.25	IRG227	35.82	24.04	103.42
IRG142	38.66	23.52	102.64	IRG230	36.07	25.14	112.26
IRG143	37.84	31.09	120.27	IRG239	37.31	25.04	114.21
IRG145	43.02	26.36	102.27	IRG241	34.22	21.44	103.84
IRG146	38.87	24.11	104.89	IRG242	30.14	19.83	108.77
IRG147	33.3	25.23	103.08	IRG243	37.23	24.65	109
IRG148	32.66	27	101.99	IRG245	36.46	24.53	103.07
IRG149	35.54	24.47	104.46	IRG247	35.35	22.57	106.62
IRG152	38.58	25.22	117.4	IRG248	43.82	25.89	115.54
IRG153	40.39	25.97	100.63	IRG250	32.98	23.6	104.26
IRG155	37.17	26.15	119.11	IRG255	33.01	27.85	94.34
IRG157	30.7	25.93	103.32	IRG256	35.61	25.7	104.97
IRG160	34.67	25.02	103.56	IRG257	45.13	27.86	101.82
IRG161	44.65	26.67	111.27	IRG258	33.06	27.72	88.29
IRG162	33.04	25.04	105.71	IRG262	37.15	26.27	107.76
IRG163	31.83	24.23	94.6	IRG263	33.44	25.73	108.38

UIL- Uppermost internode length (cm), PL- Panicle length (cm) and PER- Panicle exsertion ratio

Table 4.8 (Contd...)

IRG lines	UIL	PL	PER	IRG lines	UIL	PL	PER
IRG265	37.02	28.87	92.61	IRG286	38.63	27.01	112.66
IRG266	43.89	27.43	98.27	IRG293	31.43	23.35	104.7
IRG267	38.76	25.38	123.38	IRG342	34.4	26.32	103.36
IRG268	40.88	27.32	92.72	IRG343	42.58	31.67	111.77
IRG283	28.37	23.73	93.3	IRG359	39.1	23.88	104.55
Mean	37.22	25.34	107.41	Mean	37.22	25.34	107.41
Min	23.23	17.57	88.14	Min	23.23	17.57	88.14
Max	47.75	34.7	123.38	Max	47.75	34.7	123.38
SE	3.09	1.15	6.70	SE	3.09	1.15	6.70
CD (0.05)	**8.61**	**4.22**	**18.66**	**CD (0.05)**	**8.61**	**4.22**	**18.66**

UIL- Uppermost internode length (cm), PL- Panicle length (cm) and PER- Panicle exsertion ratio

4.2.4 GGE biplot analysis

The combined ANOVA showed that uppermost internode length was significantly affected by environment and GxE which explains 40% and 40.44% variation. The genotype explains only 17% of total variation. For PER, environment and GxE explains 26.12% and 48.56% of total variation and genotype accounts for 25%. So the variation partitioned through GGE biplot to know the stable genotypes across the environments and to identify the suitable genotypes for each environmental condition. The GGE for uppermost internode length showed that principal component 1 (PC1) and principal component 2 (PC2) explains 89.1% of total variation in which PC1 accounts for 56.05% and PC2 accounts for 33.05% variation. Both the Delhi and Aduthurai environments remain distinct (Fig. 4.2) and we found that IRG128 was performing well at RBGRC, Aduthurai, whereas, IRG27, IRG257 were found to be well performed at Delhi in both the seasons. Ranking genotype showed that IRG199 ranks first followed by IRG118, IRG98, IRG266 and IRG18 (Fig. 4.3). The IRG199 line was found to be stable across the environments. GGE biplot for PER showed that the PC1 and PC2 together explains about 85.1% of total variation in which PC1 accounts for 51.53% and PC2 accounts for 33.57% variation. The IRG200 was found to be produced well exserted panicles at Delhi during both the seasons (Fig. 4.4). The ranking of genotype showed that IRG95 was stable in both the environments. From GGE biplot analysis it was confirmed that the genotypes were performing significantly different at different locations.

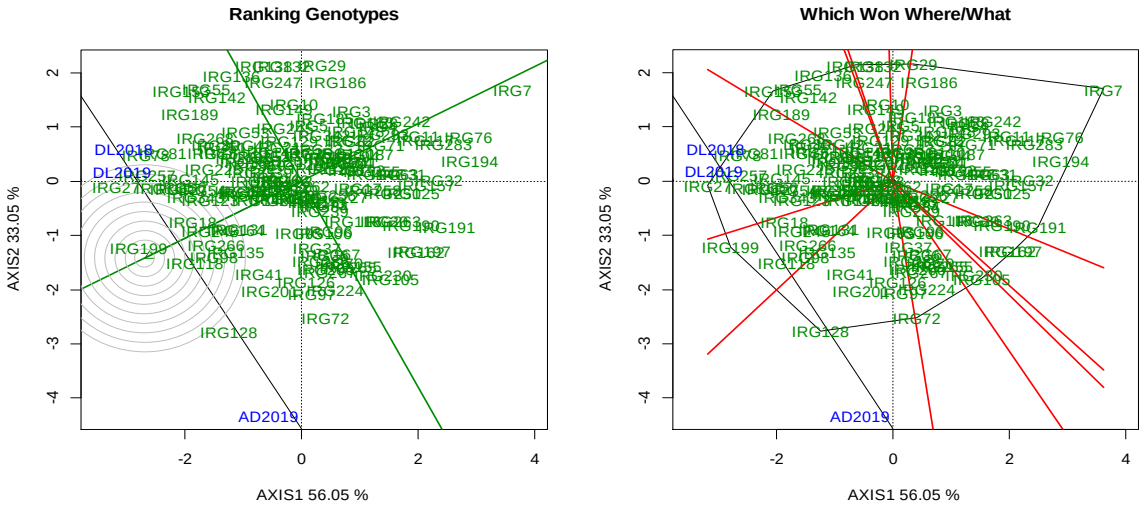


Fig 4.2 GGE biplot representing the stable genotypes across the environments with regards to uppermost internode length

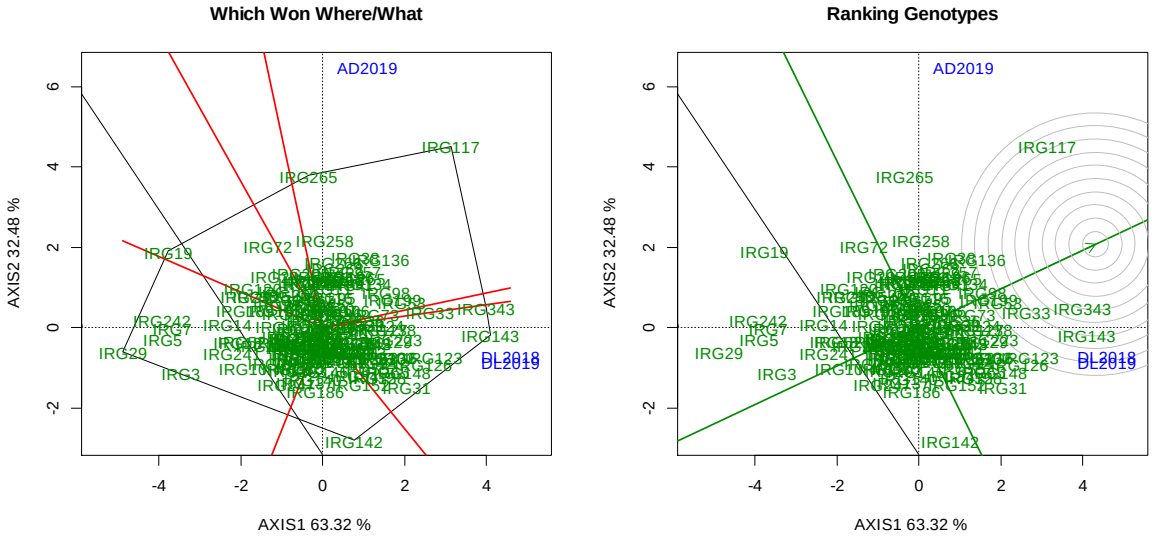
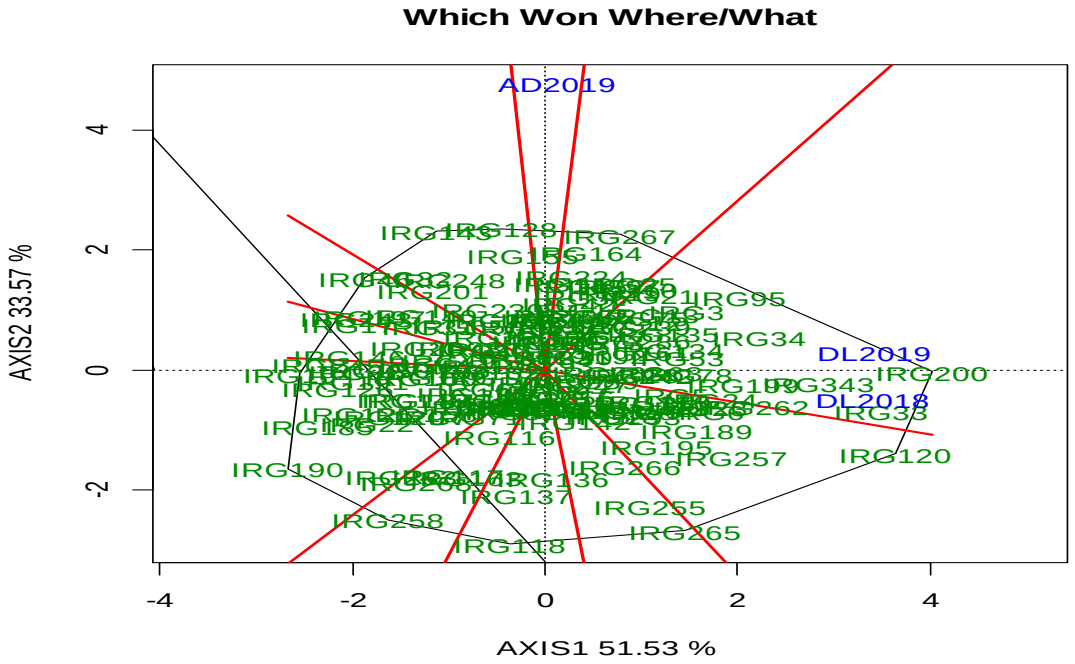


Fig. 4.3 GGE biplot representing the stable genotypes across the environments with regards to panicle length



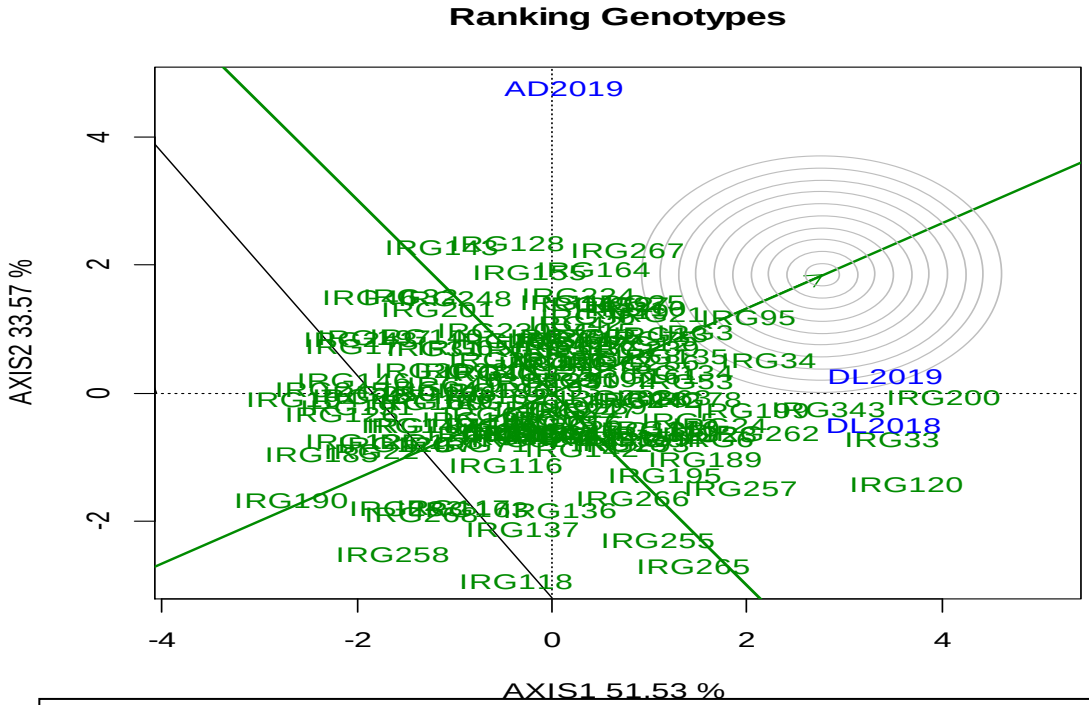


Fig. 4.4 GGE biplot representing the stable genotypes across the environments with regards to panicle exsertion ratio

4.3 Parental polymorphism survey

Parental polymorphism survey is necessary to get the polymorphic markers at close regular intervals to calculate genome recovery percentage of the recurrent parent. A set of 1308 molecular markers were used in the present study for polymorphism survey between the parents IRG213 and RTN10B. These 938 DNA markers include of 794 Rice Microsatellite (RM) markers, 27 Hypervariable Simple Sequence Repeat (HvSSR) and 117 Rice Genic-Non-Coding Microsatellite (RGNMS) markers. A total of 89 polymorphic markers were found out of 1308 markers between the parents IRG213 and RTN10B. Chromosome wise total number of markers utilized for polymorphism, number of polymorphic markers identified, percent polymorphism and name of the polymorphic markers are given in (Table 4.9). The molecular markers per chromosome used for polymorphism survey ranged from 60 on chromosome 10 to 144 on chromosomes 1, 5, 6 and 8. The number of polymorphic markers per chromosome ranged from 3 on chromosome 9 to 17 on chromosome 6. Maximum polymorphism percentage was found in chromosome 6 followed by chromosome 1; whereas minimum polymorphism percentage was found in chromosome 9 followed by chromosome 4, 3, 11 and 12. GGT showing the physical position of polymorphic markers used in this study are depicted in Fig. 4.5. A representative gel picture of parental polymorphism survey is given in Fig 4.6.

4.4 Development of mapping population

For genetic analysis and mapping qtls/genes, we have developed a mapping population by hybridizing the two distinct parents IRG213 having long uppermost internode and RTN10B having short uppermost internode. A total of 37 F_{1s} plants were generated in 2018 at IARI, New Delhi and further grown in RBGRC, Aduthurai during *Off Season* 2018-2019. All 37 F_1 plants were phenotyped for uppermost internode and best single plant was selected to generate F_2 mapping population. The best selected F_1 plant was selfed and a F_2 population of 323 plants was grown in IARI, New Delhi during *Kharif* 2019. The phenotyping of both the parents along with the F_1s, F_2 population were done with respect to uppermost internode length.

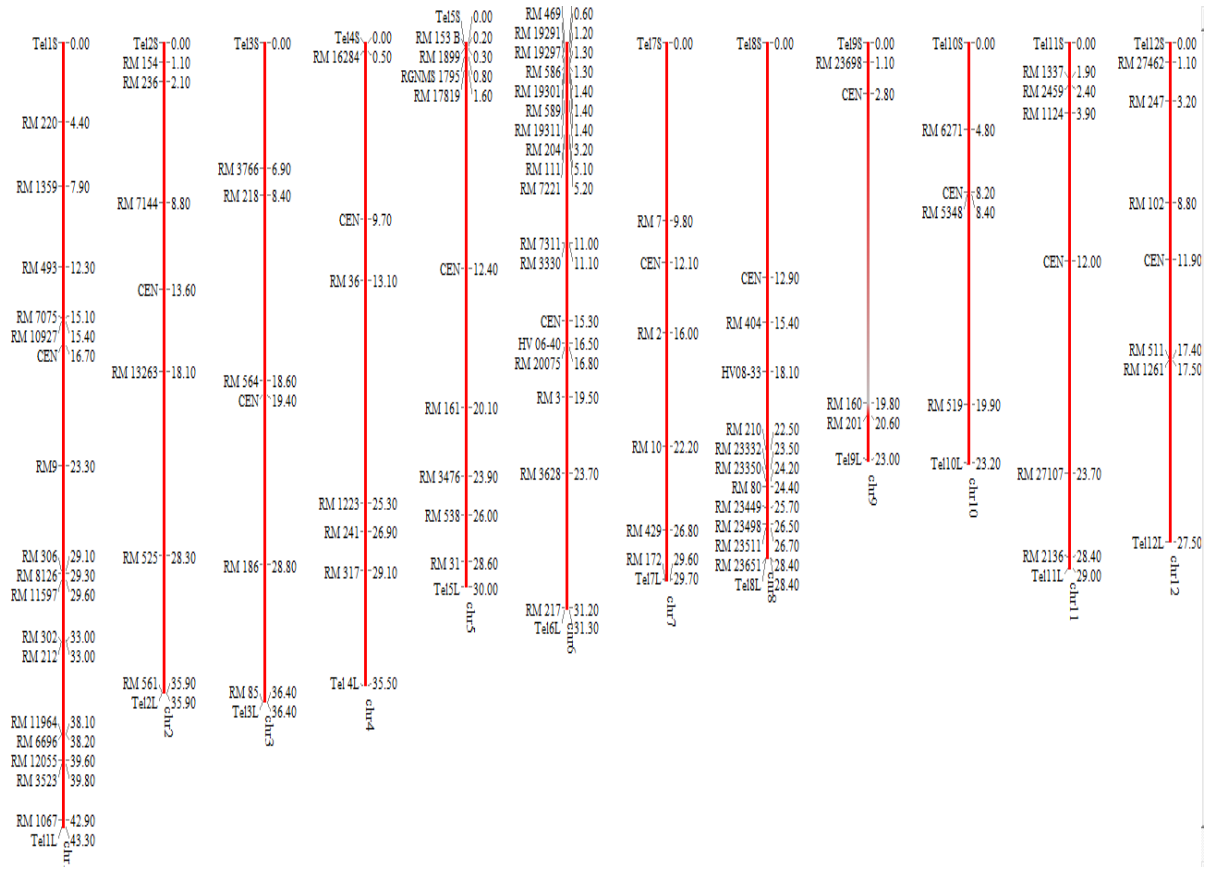


Fig 4.5 Graphical representation of polymorphic markers distributed throughout the 12 chromosomes

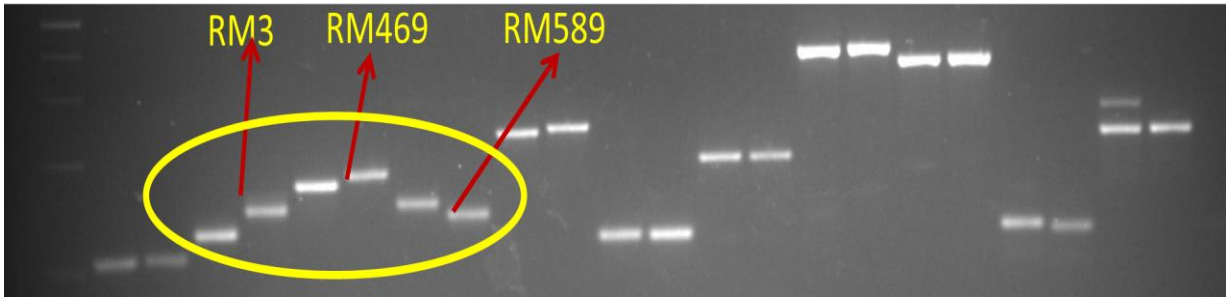


Fig 4.6 Parental polymorphism survey between the parents

4.5 Phenotyping of parents along with F_1s and F_2 plants

Parents along with the F_1s and F_2 population were characterized for uppermost internode length at IARI, New Delhi during *Kharif* 2019. A total of 10 plants each for IRG213 and RTN10B, 37 F_1 plants and 217 F_2 plants were phenotyped for uppermost internode. Length of uppermost internode was taken from first node to panicle node. For IRG213, the values of uppermost internode were ranged from 39 cm to 46 cm with an average of 42.9 cm whereas in RTN10B, uppermost internode length was ranged from 18 to 26 cm with an average of 21.4 cm. Intermediate type of pattern was observed in F_1s where the values were ranged from 21.8 to 39.7 cm. In F_2, values for uppermost internode were ranged of 12.3 to 46.7 cm with an average of 31.9 cm.

4.6 Genetic analysis of uppermost internode

Genetic analysis for the target trait *i.e* uppermost internode was carried out in F_2 population derived from the crosses between RTN10B and IRG 213, where RTN10B having shorter internode and IRG 213 having longer internode. A total of 217 F_2 plants were phenotyped for the trait and the uppermost internode length was ranges from 12 cm to 46 cm. The F_2 plants were normally distributed around the mean *i.e* 32.49 cm with and skewed towards IRG213 parent (Table 4.10). The population also consists of transgressive segregants which fall out of the range of parents. A total of four transgressive segregants was found in F_2 which falls towards RTN10B parents with range of 12 cm to 15.63cm. The phenotypic data was classified into different classes to know the number of genes governing the trait. The F_2 plants were grouped into 3 classes with plants having uppermost internode length of 19 cm to 27 cm as parent RTN10B type, plants having uppermost internode length of 27.1 cm to 37 cm as intermediate to both the parents and plants having uppermost internode length of 37.1 cm to 46.7 cm as IRG213 type. A total of 49 Plants were found similar to RTN10B type, 117 plants were intermediate type and 51 plants were IRG213 type. The phenotypic data of F_2 plants were in the ratio of 1:2:1 and based on goodness of fit analysis it was observed that the trait is following the aforesaid pattern of segregation with chi-square value of 1.14 (*P* value 0.57) which clearly indicated that the target trait is governed by a partial dominant gene (Table 4.11). Based on the variance of the both parental lines and F_1 population, environmental variance was estimated and it was observed to be 5.91 cm. The variance generated in the F_2 population is a result of both environmental as well as genotype. So, it's considered as total phenotypic variance for the trait

of interest and it was found to be 34.57. Genotypic variance was estimated by subtracting the mean of the P_1, P_2 and F_1 from F_2 variance and it was found 28.66 cm. Broad sense heritability was calculated for the trait by taking ratio of genetic variance and total phenotypic variance and it was observed as 82.90%. This result indicates that the uppermost internode elongation trait is highly heritable from one generation to other generation.

Table 4.9 List of polymorphic markers between the parents

Chromosome	No. of markers screened	Polymorphic markers	Polymorphism (%)
1	144	16	11.11
2	96	6	6.25
3	96	5	5.20
4	96	5	5.20
5	144	8	5.55
6	144	17	11.80
7	96	5	5.2
8	60	10	6.94
9	96	3	3.12
10	60	4	6.66
11	96	5	5.20
12	96	5	5.20
Total	**1308**	**89**	

Table 4.10 Descriptive statistic of parents and F_2 population

Parents	Range	Mean	Variance	SD	Kurt	Skew	Heri
RTN10B	18-26	21.4	5.8	2.41	-	-	-
IRG213	39-46	42.8	6.0	2.54	-	-	-
F_2	19-46	33	34.57	5.88	-0.73	-0.21	82.90

Table 4.11 Chi square analysis

Type	Observed	Expected	Ratio	*Chi-square value*
P1 type	49	54	1	0.67
Intermediate	117	109	2	0.45
P2 type	51	54	1	0.02
Total	217	217		1.14
P value				*0.566*

P1: RTN10B (Short upper most internode); P2: IRG213 (Long upper most internode)

4.7 Bulked Segregant Analysis (BSA)

The polymorphic primers between RTN10B and IRG213 have been used to carry out Bulked Segregant analysis. A total of 89 polymorphic markers were observed to be polymorphic between the parents. The polymorphic markers were evenly distributed throughout 12 chromosomes of rice genome and these SSR markers were sufficient enough to identify polymorphism between the bulks. Based on the phenotypic data, 10 plants were selected from both the extremes having parental range of uppermost internode length and selected plants were distinct and contrasting for the trait. The selected plants DNA was isolated and the samples DNA were quantified using nano-drop machine. A light wavelength of 260 nm, 280 nm and 230 nm was passed through samples and DNA quantity and purity was estimated. Equal amount of DNA from high bulk plants was taken and DNA was pooled and prepared high bulk. Same procedure was followed for low bulk. The bulks were screened for polymorphism using polymorphic markers which are polymorphic between the parents. A total of 89 polymorphic markers were used for screening. Among the 89 markers, 88 markers were found monomorphic and unable to differentiate the bulks except Hv-SSR04-60 (Fig 4.7). This SSR marker was found to be polymorphic between the parents as well as in the bulks. The high bulk showed double band whereas, low bulk showed single bands. The banding pattern of the marker between the bulks showed the clear polymorphism and it was found to be putatively linked to the trait. Hv-SSR 04-60 marker is present on chromosome number 6. The Hv-SSR 04-60 was further used for genotyping the individual F_2 plants to estimate the association between the marker and the trait.

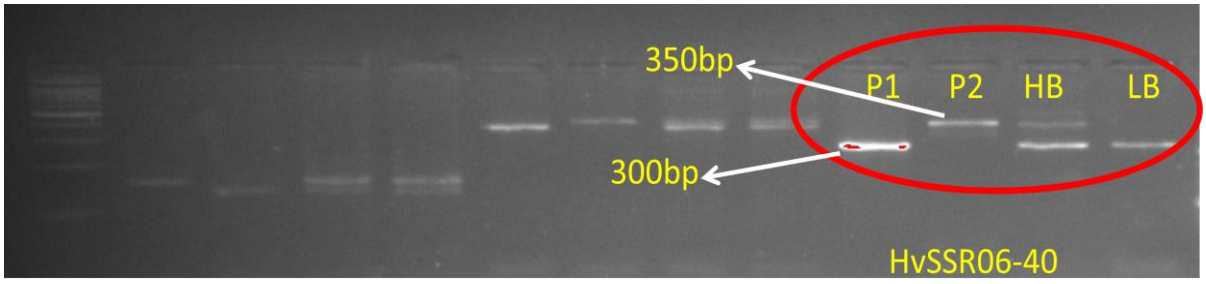


Fig 4.7 Identified putatively linked marker HvSSR04-60 in Bulked Segregant Analysis

4.8 Genotyping of F_2 population and mapping gene for uppermost internode

Bulked segregant analysis showed that the marker HvSSR06-40 was polymorphic between the low bulk and high bulk as shown in the Fig 4.7. This marker (HvSSR06-40) was further used for F_2 population genotyping to know the marker and trait co segregation and to know the genetic distance between them. Genotypic data of the 217 F_2 plants showed that the marker was segregating in 1:2:1 (Table 4.12). Out of 217 plants 49 plants were of RTN10B type *i.e.* homozygous line showing single band of RTN10B type. A total of 51 plants were IRG213 type showing single band *i.e.* homozygous whereas 117 plants were heterozygous in nature having combination of both the parental bands. Most of the plants were correlated for phenotypic data and genotypic data. *i.e.* plants showing RTN10B banding pattern were having uppermost internode length ranging from 19-26 cm, whereas heterozygous plants were ranging from 27-36 cm and homozygous IRG213 types were having range of 36-46 cm. *Chi-square* analysis was carried out to know the goodness of fit of genotypic segregation of Hv-SSR06-40. A *chi-square* value of 1.14 (p=0.57) was obtained which was less than the table value at 2 degree of freedom and 5% significance. Hence the trait is showing monogenic inheritance.

Single marker analysis (SMA) was performed using genotypic data in *MAPMAKER* to know the genetic distance between the marker and gene of interest. The gene was designated as *EUI3 (t)*. Based on the results from *MAPMAKER*, we found that the genetic distance between marker HvSSR06-40 and *EUI3 (t)* gene was 13.7 cM. Further to refine the genetic distance between the marker and gene, three more polymorphic markers *viz.*, RM3330, RM7311 and RM111 located adjacent to the HvSSR06-40 on chromosome 6 were selected and genotyped all 217 F_2 plants. Single marker analysis of these marker showed that the genetic distance of gene from RM3330 was 38.4 cM and this marker is present on the other side of the gene. Therefore, the gene *EUI3(t)* found to present in the marker interval RM3330 and HvSSR06-40. The genetic distance between the marker RM7311 and RM3330 was found to be 6.6 cM and this maker present opposite side of *EUI3(t)*. The genetic distance of the marker RM111 and RM7311 was found 26.8 cM and RM111 present away from the *EUI3(t)*. The HvSSR06-40 was found very closely linked to *EUI3(t)*with a LOD value of 61.62 and R^2 value of 72.90. The linkage map of the markers used for single marker analysis and the genetic distance from each other is given in Fig 4.8.

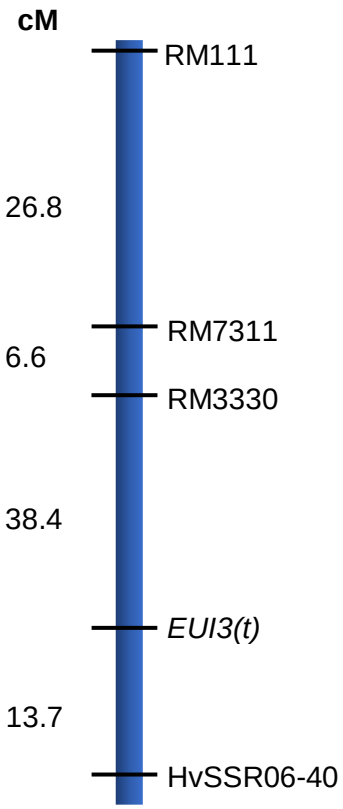


Fig 4.8. Linkage map showing the distance between the markers and the gene

5. Discussion

Incomplete panicle exsertion in WA based CMS line is one of the major limitations for hybrid rice seed production and seed yield. Enclosure of panicle within the flag leaf sheath that leads to low level of cross fertilization and low hybrid seed set. So, there is need of application of exogenous GA_3 for complete emergence and to increase seed yield. Exogenous GA_3 application leads to increase in cost of seed production and also poor seed quality. To enhance the seed setting one can concentrate on improvement of the panicle exertion and increase in uppermost internode length. Therefore, identification of gene(s) governing these traits is the first step. During 1981, Rutger and Carnahan identified a recessive mutant *eui1* type that controls the tall phenotype of rice and called it as fourth genetic element of hybrid rice. Plants positive for *eui1* have elongated uppermost internode which was double that of the normal wild types and they have reported that it doesn't influence any other characters.

Elongated uppermost internode governed by *eui1* was a spontaneous mutant identified in *japonica* line and leads to tall phenotype. Till now two *eui* genes namely, *eui1* and *eui2* were identified and mapped on chromosome number 5 and 10 respectively. *Eui*1 is considered to be most effective in increasing the panicle exsertion which enhanced cross fertilization in hybrid seed production, seed setting and hybrid seed yield. It also reduces the requirement of exogenous application of GA_3. Incorporation of identified major genes into male sterile line, maintainer line and restorer line can increase the seed yield substantially and minimizes the cost of hybrid seed production. Many scientists at IRRI and china has transferred these genes in the background in *indica* lines which was parental lines in hybrid seed production (He and Shen, 1994; Virmani and Wan, 1998). After Rutger and Carnahan (1981) identification of *eui1* mutants, many alleles for internode length and tall culm have been identified. Maekawa *et al.,* 1989 reported that the GA content was significantly high in *eui* mutants compared to wild types. Therefore, they concluded that the main function of *eui* was to increase the endogenous level of GA_3 production by producing P450 monoxygenase enzyme.

Understanding the effect of *eui* gene on GA biosynthetic pathway was first studied in Arabidopsis (Olszewski *et al.,* 2002, Sun and Gulber 2004). In rice also GA_3 signalling was studied by inducing the mutation and altering the signalling pathway (Ikede *et al*., 2001). Induction of mutation and identification of key components in biosynthetic pathway is very important (Sasaki 2002). The precursor for GA_3 production is geranylgeranyl diphosphate

(GGDP). Conversion of GGDP into biologically active GA involves three enzymes namely, terpene cyclase, cytochrome 450 monoxygenase and oxoglutarate dependant dioxygenase (2ODDs) (Zhu and Chen, 2006). *EUI1* encodes a putative cytochrome 450 monoxygenase, one of the key enzymes for endogenous GA synthesis which regulates internode elongation in rice.

Morphological and molecular characterization of rice genotypes.

WA is the widely used male sterile cytoplasm in rice for hybrid seed production in India and other countries. But the main constrain associated with the WA cytoplasm is the incomplete panicle exsertion that reduces the out-crossing and seed setting (Devi *et al.,* 2011). Therefore, identification of good germplasm or genetic stock to solve the problem of incomplete panicle exsertion in A line is one important aspect in the area of hybrid seed production. Characterization of the rice lines for uppermost internode is very important in future breeding programme for hybrid development in rice. In the present study, 348 IRG lines were selected and showed high variation for uppermost internode, PER and panicle length. So, grouping of the rice lines into different classes helps the breeder for its direct use in breeding programme. But till now no separate classification of rice lines based on uppermost internode length are available. Therefore, a set of 348 IRG lines were screened by two markers namely, RM3476 and RM5970 which are linked to *eui1* gene on chromosome number 5 and classified IRG lines into *eui* and non *eui* types. Further, a subset of 150 IRG lines was made. All 29 lines which having *eui1* based on genotyping was included in the subset. The subset of 150 lines was evaluated in three different environments for three traits namely, uppermost internode length (UIL), panicle length (PL) and panicle exsertion ratio (PER). Many stable IRG lines were identified for all three traits. The PER and UIL were found to be positively correlated and this can be a great advantage in rice hybrid seed production. The IRG lines in the present study, showed significant variation for uppermost internode where few lines were normal for UI length till heading stage and we found a rapid elongation of uppermost internode after heading stage. Few lines were found to be same as that of the normal wild type plant where there is less elongation and incomplete panicle exsertion. This results in accordance with the results of He and Shen *et al.,* 1994 where they studied the difference in elongation between the mutant type and wild type. The sole reason behind the elongation of the internode may be increase in level of GA during reproductive stage and increase in cell elongation.

The morphological data of *eui* type lines in the present study showed long internode length and more panicle exsertion compared to non *eui* lines. But some non *eui* type IRG lines were also found with long internode length like *eui* types. This indicates different molecular mechanism/gene may be governing the trait. Therefore, utilising of those non *eui* lines with elongated uppermost internode may provide an anecdote for identification of novel gene(s). The molecular mechanism behind the UIL and PER in non *eui* types in the present study is not known. Therefore, mapping and determining the molecular basis of uppermost internode elongation in non *eui* types lines is needed.

Both *eui* and non *eui* types were evaluated in different environmental condition and we identified best performing lines as well as location specific lines. Based on multi environment evaluation, it was observed that all three traits were highly influenced by environment. GxE interaction was also significant for all three traits, which indicated that the IRG lines under the study showed differential behaviour with changing environment. This is quite obvious like many others quantitative traits. The lines performed better in both the environments can be used in future breeding programme. IRG199, IRG118, IRG98 and IRG266 were found promising across the environments for UIL, whereas IRG95 was found stable for PER.

Genetic analysis

Understanding the inheritance of uppermost internode and identifying the number of genes controlling the trait with nature of gene action is very important to exploit the trait in breeding programme. Zongtan and Zuhua (1991) studied the inheritance pattern of *eui* and confirmed that the trait is controlled by single recessive gene. Gangashetti *et al.,* (2004) studied the inheritance pattern of elongated uppermost internode in F_2 population derived from IR58025A, a non *eui* type parent and IR91-1591-3, an *eui* parents. They observed that the inheritance pattern of *eui* was monogenic and recessive in nature. It was also confirmed in $F_{2:3}$ progenies. In the present study inheritance pattern of uppermost internode elongation was carried out using F_2 population developed from the cross between two contrasting parents with respect to uppermost internode length *viz*., RTN10B and IRG213. The F_1 plants developed from the cross showed average uppermost internode length of 31cm which is intermediate between the two parents. This result follows the pattern of inheritance observed for flower colour in Snapdrag on by Mendel's successors. They observed 1:2:1 ratio for red flower, pink flower and white flower. When the trait is controlled by partial dominant gene

the heterozygotes will be intermediate to the parental type. Whereas if the trait is controlled by dominant gene, the phenotype of the homozygous dominant and heterozygote will be same and another genotype will show contrasting phenotype and same in case of recessive gene. In incomplete dominant gene action, the phenotype of the heterozygote will be just above the mid parental value and neither the parent one nor the parent two gen is dominant (Rheinberger 2000). The F_1 of the RTN10B and IRG213 was intermediate between the parents which show that neither gene from RTN10B nor the gene from IRG213 is sufficient enough to produce their respective phenotype in heterozygote. This indicates that the gene responsible for uppermost internode is controlled by partially dominant in nature. Many genes controlling the plant characters are partial dominant in nature. Khan *et al* (2000) studied the inheritance pattern of genes controlling the plant height, 1000 grain weight, peduncle length, grain yield per plant and number of tillers per plant in wheat. They observed the partial dominant nature of gene action of aforesaid traits and mapped the candidate genes on different chromosome of wheat. Study of heading days and plant stature in rice by Li *et al.,,* (1995) showed that the three out of four QTL for plant namely *QPh2a, QPh3a* and *QPh8a* and one QTL for heading days *QHd9a* exhibited partial dominance. Many studies in different crops identified many partial dominant genes and selection for these genes will be easy in early segregating population as heterozygotes are significantly different from the homozygous dominant genotypes.

The F_2 plants were developed from the F_1 to know whether the trait is monogenic, digenic or polygenic in nature. The plants in F_2 were classified based on uppermost internode length and they were grouped in to three different classes namely Class 1(19-26 cm) towards RTN10B type, Class II (27-36 cm) towards intermediate type and Class III (36-46 cm) towards IRG213 type. Maximum number of plants falls in intermediate type and based on marker-based screening majority of those plants were heterozygous in nature. Among the 217 plants, 4 and 2 plants were found out layers towards short and long internode parents, respectively, which may be transgressive segregants or may be due to the environmental variations. This confirms the partial dominant nature of gene action and also shows that the trait is monogenic in nature. Based goodness of fit, it was observed that F_2 plants were fit in 1:2:1 ration of segregation. *Chi-square* value was also non-significant at both 5% and 1% level of significance with a *p* value of 0.57. Thangapandian *et al.,* (2019) studied the inheritance pattern of *eui* in spontaneous mutant of Accession 18 and it was observed that the gene is monogenic and allelic to the previously reported *eui* mutant. Rutger and Carnhan

(1981) were also initially confirmed the monogenic inheritance of *eui* gene. Traits with monogenic pattern of inheritance have significant advantages in plant breeding as selection of these traits in early segregating population is very easy and transfer of these genes through backcrossing is very simple (Tsunematsu *et al.*, 2000). Many monogenic traits were identified into elite varieties to develop insect, disease resistance varieties. Zongton and Zuhua (1991) explained the recessive nature of inheritance of panicle exsertion and *eui* and showed that the PER due to *eui* gene was positively correlated with the plant height and uppermost internode length. He also introgressed *eui* monogenic trait into male sterile line and observed significant increase in the uppermost internode length. Yi *et al*., (2004) studied the inheritance pattern of *eui* and reported that the trait is monogenic as well as digenic in nature. In one of the cross, they observed a ratio of 13:3 for uppermost internode length and they called it as inhibitory gene action. They proposed two genetic models to explain the inheritance pattern of uppermost internode length. Model one explains the dominant nature of *eui* gene as monogenic and other model explains the inhibited model of *eui* gene.

Traits controlled by major genes are highly heritable compare to quantitative traits and effect of environmental on phenotype is very less. In the present study uppermost internode trait is monogenic and its heritability was found high. This results in accordance with the previous studies on differences between the qualitative traits and quantitative traits.

Mapping gene(s) for uppermost internode length

Identification of markers linked to the economically important traits for its use in any breeding programme is very important (Yang *et al.*, 2000). In the present study, a marker was identified on chromosome 6 which is present 13.5 cM away from the gene. The marker and the trait association were found significant at *p* value of 0.00 which represents the strong association. The first gene for internode elongation was identified and isolated by Okuno and Kawai, (1978) in Norin 8 rice cultivar by inducing mutation by gamma rays. The first recessive rice internode elongation mutant was isolated from the Japanese rice cultivar Norin 8 by gamma ray treatment (Okuno and Kawai, 1978). Later on, gene for uppermost internode elongation (*eui1*) identified by (Rutger and Carnhan 1981). They called this as fourth genetic element in hybrid seed production as it has positive correlation with the panicle emergence ratio and seed setting percentage. This spontaneous mutant was later mapped on chromosome 5 linked to marker RM3476 at a genetic distance of 1.5 cM (Librojo and Khush, 1986). In the present study F_2 population developed from IRG213 and RTN10B

was used for mapping and a marker HvSSR06-40 was found linked with the gene, which has been named as '*eui3*(t)'. In the present study, eui3*(t)* gene is the third gene identified for elongated uppermost internode after *eui1* and *eui2*. Second gene, *eui*2 was mapped on chromosome 10 which has low phenotypic variance explained compare to *eui1*. Yang *et al.*, (1999) identified *eui*2, a recessive gene (*eui*-2) in Xinquing ZhaoB, a maintainer line by using gamma rays, which was mapped on chromosome 10 using SSR markers. Many mutants were identified for elongated uppermost internode in different sources and allelic studied revealed that those mutants were allelic to the previously reported *eui*1 and *eui*2 (Rutger and Carnhan 1981; Yang *et al.*, 1999). As the *eui* gene controlling the trait is recessive in nature, identification of marker linked to the trait is very important; otherwise the transfer of gene using backcross will be difficult as there is need of selfing after every generation of backcrossing. In contrast, *eui3(t)* which has been identified through the present study is partial dominant and phenotypic selection for the gene will be easily compared to earlier reported recessive gene during backcrossing.

The *eui3(t)* gene found linked very strongly to the marker HvSSR06-40 with LOD value of 61.62 which shows the very less rate of false association. As the marker present at a distance of 13.5 cM which need to be refined using high density marker in the region. Mapping of genes and identification of candidate genes helps in understanding the molecular mechanism behind the uppermost internode and panicle exsertion. Panicle exsertion and uppermost internode length are significantly correlated and breeding for uppermost internode may have significant effect on panicle exsertion and seed setting (Khera *et al.*,2009). GWAS study in *japonica* and *indica* lines for PE and UI found 12 loci for PE and 17 novel loci for UI. Two target candidate genes namely CYP734A4, OsLIS-L1 were identified in *qPE14* and *qUI14* respectively. The candidate genes help in preventing the brassinosteroids degradation and increase the UI and PE. The fine mapping of the *eui3(t)* and revealing the molecular basis behind the elongated uppermost internode, sequencing the candidate gene and development of functional or gene-based marker is necessary.

The phenotypic variance explained by *eui*1 was more compared to *eui2*. So practical implication was more in case of *eui1* compare to *eui2*. Therefore, identification of genes for elongated uppermost internode with more phenotypic variance is very important. In the present study, *EUI3(t)*explains phenotypic variance of 72.90 and we considered this major gene on chromosome 6. Genes/QTLs can be classified into major and minor QTLs based on phenotypic variance explained. Those QTLs with more than 10% phenotypic variance can be

considered as major QTLs (Brondani *et al.*,2002). So, the gene identified in the present study is a major gene and the broad sense heritability was found to be very high. The genomic region of the gene was further refined using three more markers namely, RM3330, RM7311 and RM111. The F_2 population was screened and these markers also found linked to the gene but association was very less. The *EUI*3(t) gene was mapped in the interval of HvSSR06-40 and RM3330. Therefore, in future further fine mapping of the gene is needed.

6. Summary and Conclusion

Rice (*Oryza sativa*) is one of the major cereals and most important food crop of India as well as world and which feeds over one third of the population in the earth. In India hybrid rice is cultivated on 2.8 mha area which is just 6.2% of total rice cultivated area with the production of 3.8 mt annually. Currently difference of rice cultivated area and production between India and China is about 15 mha and 45 mt, respectively. This difference is mainly because China grows hybrid rice on 50% of the total rice cultivated area in the country. Increased seed cost and the repeated requirement to use new seed in every season, as against the low additional income due to the marginal yield advantage over normal varieties have made the farm level adoption of hybrid rice in the country slow. To overcome this, there is a need to boost heterosis while reducing seed production cost. However, almost all the cytoplasmic male sterile lines as the female (A line) of hybrid rice have natural tendency to 'panicle enclosure' in which panicles are partially or fully enclosed in the leaf sheath (Yin *et al.*, 2007). Panicle enclosure hinders pollination between the 'A' line and the fertility restorer line as the male (R line) in hybrid seed production and therefore decreases seed yield (Zhang *et al.*, 2009). A recent study demonstrated that the occurrence of cytoplasmic male sterility (CMS) in the 'A' line caused a deficiency of indole-3-acetic acid in the panicle, which consequently caused a decrease of gibberellins A1 (GA1) in the uppermost internode (UI) by down-regulating the expression of OsGA3ox2 (Yin *et al.*,2007). The reduced GA1 resulted in a shortened UI, and therefore, the panicle could not be pushed out of the flag leaf sheath. In hybrid rice seed production, exogenous '920' (the active ingredient is GA_3) mostly applied to eliminate or alleviate panicle enclosure of the 'A' line; however, this usually causes a series of problems. Exogenous application of GA_3 leads to increase production costs, decrease seed quality and also responsible environmental pollution.

In 1981, Rutger and Carnahan identified a tall rice mutant with elongated uppermost internode which showed that the trait was controlled by single recessive gene and named it as *eui*. This recessive gene is therefore regarded as the fourth genetic element of hybrid seed production in addition to the other three genetic elements *viz.*, CMS, maintainers and restorers. Based on the allelic and non-allelic nature of the gene, Yang *et al.*, 1999 referred it as *eui1* and *eui2*, respectively. *Eui1* encoded P450 monooxygenase CYP714D1, which could deactivate GAs in rice (Luo *et al.*, 2006; Zhu *et al.*, 2006). Inactivation of *Eui1* in *eui1*

mutants caused the accumulation of GAs in uppermost internode and consequently increased internode elongation and plant height (Luo *et al.*, 2006; Zhu *et al.*, 2006). The putative encoding product of *Eui2* was an epoxide hydrolase, which was inferred to precipitate in the catabolism of GAs (Ma, 2007).

So, by considering all the constrains associated with the hybrid seed production, problem of panicle exsertion, poor pollination percentage and poor seed setting, the present study was planned. A set of 348 IRG lines were screened initially by linked markers against *eui1* for uppermost internode. Based on molecular screening 29 *eui1* positive lines along with another 121 non *eui1* were selected and evaluated in three different environments. Based on environment wise ANOVA, it was observed that significant genotypic effects for all three traits in each trial. Pooled ANOVA for the all the three traits under this present study showed that the genotypes were highly significantly and we found significant GxE for uppermost internode, panicle length and PER. Genotypes under this study showed differential behavior or expression with changes of environment. GGE biplot results showed that the IRG lines were significantly different from each other and genotypes were ranked based on their performance for different traits.The GGE for uppermost internode length showed that principal component (PC1) and PC2 explains 89.1% of total variation in which PC1 accounts for 56.05% and PC2 accounts for 33.05% variation. Ranking genotype showed that IRG199 ranks first followed by IRG118, IRG98, IRG266 and IRG18. GGE biplot for PER showed that the PC1 and PC2 together explains about 85.1% of total variation in which PC1 accounts for 51.53% and PC2 accounts for 33.57% variation. The ranking of genotype showed that IRG95 was stable in all environments.

The IRG lines were also screened for previously reported genes by using linked markers *viz.*, RM3476 and RM5970. A total of 348 IRG lines were characterized using both the markers, in which 29 lines were classified as *eui1* type based on RM5970 marker, whereas by RM5970, 23 lines were identified as *eui1* type. Among them, 23 lines were common *eui1* type by both the markers while in 6 IRG lines there was a mismatch between the two markers and that may be due to their genetic distance from the gene. Phenotypic variation and *eui* gene presence, the IRG lines can be used for further breeding program to identify closely linked marker, to understand the molecular mechanism behind the elongation and its introgression into male sterile line. Based on the molecular data, one IRG line which was negative for *eui* but having long uppermost internode length was selected and crossed with the RTN10B line. Phenotypic studies in F_2 population of 217 plants shows that the trait is

controlled by partially dominant gene. The F_1 plants were intermediate to the parents used for hybridization. The F_2 was fitting well with a ratio of 1:2:1 and the uppermost internode length were ranged from 12 cm to 46 cm. We also observed that the trait was highly heritable with broad sense heritability of 82.90%.

In order to identify the gene(s) controlling the trait, bulked segregant analysis followed by single marker analysis was performed. Based on the screening markers, HvSSR06-40 located on chromosome 6, was identified as putatively linked to the trait and single marker analysis confirms the significant association between the marker and the trait. Number of recombination between the marker and gene, we further concluded that the marker is present at a genetic distance of 13.7 cM. To refine the genetic distance few more markers were used for screening and the study reveals that, the gene present in the marker interval of RM3330 and HvSSR06-40. The LOD value was observed to be very high 61.62 and R^2 value of 72.90.

Based on our results we concluded that,

- IRG lines were characterized into *eui* and non-*eui* type based on molecular screening and based on multi environment evaluation it was observed sufficient variability present for the uppermost internode length with significant GxE interaction.
- Studied the inheritance of uppermost internode elongation and found to be controlled by single partially dominant gene.
- Mapped a gene for uppermost internode which is linked to marker Hv-SSR 06-40 chromosome 6 with a LOD value of 61.62.

The identified novel gene in the present study needed to be fine mapped and identification of candidate gene or gene-based marker for its use in future breeding programme. There is need to clone the gene and revealing the molecular mechanism behind the uppermost internode elongation and functional characterization of the gene. This helps the plant breeders to use this gene in future breeding program for its introgression into male sterile line and to enhance the level of pollination and seed setting rate. This could be a better strategy for increasing the hybrid seed production in rice to feed the burgeoning human population.

सार

संकर धान में वाइल्ड अबोर्टिवे साइटोपल्ज़ं, नर बंध्यता के लिए एक मुख्य स्त्रोत है किन्तु इन्कम्प्लीट पनिकल एक्सेरसेन, वाइल्ड अबोर्टिवे साइटोपल्ज़ं से जुडी हुई एक बड़ी समस्या है, जिसके कारण संकर धान में बीज कम बनते है. रुट्गर ने १९८१ में अलोंगेटेड अपरमोस्ट इन्तेर्नोड के लिए अप्रभावी म्युटेंट की खोज की जिसके कारण संकर धान में बीजो की संख्या बढ़ी तथा उन्होंने इसे संकर धान का चौथा आनुवंशिक तत्व कहा. अभीतक, अलोंगेटेड अपरमोस्ट इन्तेर्नोड के लिए दो म्युटेंट की खोज हो चुकी है जो क्रमशः ***eui1* और *eui २* है.** हमने अपने वर्तमान शोध में दो आंडविक चिनको क्रमसः आर ऍम ३४७६ और आर ऍम **5970** की सहायता से ३४८ आई आर जी लाइन्स का वर्गीकरण अपरमोस्ट इन्तेर्नोड के लिए किया. जिसमे हमने पाया की २९ लाइन्स में अलोंगेटेड अपरमोस्ट इन्तेर्नोड के लिए ***eui १*** जीन है. जिसके आधार पे १५० लाइन्स का चयन हुआ और उन सभी लाइन्स को उनके मॉर्फोलॉजी के आधार पे ३ ट्राइट्स क्रमसः अपरमोस्ट इन्तेर्नोड, पानिकल लेंथ और पनिकल एक्सेरसेन रासीओ के लिए तीन अलग अलग वातावरण में विश्लेषण किया गया. चयन किये हुए डाटा के आधार पे हमने ये पाया की आई आर जी १९९ तीनो वातावरण में सबसे प्रभावी लाइन है जबकि आई आर जी १२८ केवल आदुथुरई और आई आर जी २५७ दिल्ली में प्रभावशाली है. आनुवंशिक वर्गीकरण के फलस्वरूप तथा रूपात्मक और आनुवंशिक विश्लेषण के आधार पर हमने दो लाइन्स आई आर जी २१३ और आर टी येन १०ब का चयन आनुवंशिक चित्रण के लिए किया गया. दोनों पैतृक लाइन्स के क्रॉस के फलस्वरूप प्रथम फिटियल जनरेशन प्राप्त हुई जिसमे से एक पौधे का चयन किया जिसमे अपरमोस्ट इन्तेर्नोड की लम्बाई अधिक थी, तथा उसे स्वपरागित करके एफ२ जनरेशन प्राप्त की गई. इस एफ२ पापुलेशन के २१७ पौधों का आधयान करके हमने ये रिजल्ट्स पाया की ये १:२:१ के रासीओ में आ रहे है जो यह निर्धारित करत है की ये ट्रेट केवल एक हे जीन से कण्ट्रोल है जोकि आंशिक रूप से प्रभावी है. अब आनुवंशिक चित्रण के लिए हमने बी यस से टेक्निक का प्रयोग किया जिसके लिए हमने ८९ पोलीमॉर्फिक मार्कर्स को दोने पैतृक लाइन्स और अधिक और काम बल्क वाले पौधो के बिच चलाया. ८९ पोलीमॉर्फिक चिन्हको में केवल एक चिनाहक़ एच वि यस यस आर ०६-४० बल्क में भी पोलीमॉर्फिक प्राप्त हुआ इसलिए हुनमे इस चिन्हक को २१७ एफ्२ पापुलेशन में प्रयोग किया. प्राप्त हुए रूपात्मक और आनुवंशिक डाटा के आधार पर हमने एक जीन ***eui3(t)*** की खोज की जो इस चिन्हक से १३.७ cM की दुरी पे था. जिसका लोड वैल्यू ६१.६२ और आर स्कैर वैल्यू ७२.९० प्राप्त हुआ.

Abstract

Wild abortive (WA) type of male sterile source is the most widely used in hybrid seed production in rice. The incomplete panicle exsertion is a major problem associated with the WA cytoplasm that reduces the pollination level and seed setting**.** Uppermost internode length is very crucial for panicle exsertion from the flag leaf. There are several genes/QTLs governing the trait have been reported by different groups but two recessive genes *viz.*, *eui1* and *eui2* are most exploited among them. The *eui1* was cloned and molecular mechanism behind the elongation has been revealed. In the present study 348 IRG lines were screened using reported marker for *eui1* (RM3476 and RM5970) and IRG lines were classified into *eui* and *non eui* types. Among them 29 lines were found *eui* type and 325 were found *non eui* type. Within the non *eui* types, huge variation of uppermost internode length was observed. A subset of 150 IRG lines were evaluated in three different environments and characterized morphologically and found that IRG199 one of the stable lines. On the other hand, based on the morphological and molecular data, IRG213 with long uppermost internode length but non *eui* was selected and inter-crossed with RTN10B, a maintainer line with short uppermost internode length to study the genetic basis behind the elongation of uppermost internode. F_1 was found intermediate between the parents. This indicates the partial dominance nature of gene controlling the trait. The F_2 plants were distributed normally around the mean of 32.49 cm. The F_2 plants were classified into three classes and fitting well to 1:2:1 ratio of partial dominance gene. The *chi*-square value was found non-significant and null hypothesis was accepted. Therefore, the trait was found to be controlled by the single partially dominant gene. Bulked segregant analysis (BSA) was performed by making bulk of extreme plants to identify the putatively linked marker. A set of 1308 molecular markers were used for parental polymorphism survey and 89 markers were identified as polymorphic markers between the parents were used to screen the bulks and found HvSSR06-40 on chromosome 6 as polymorphic marker between the bulks. This marker was further used for genotyping of 217 F_2 plants and found a significant association between the marker and trait. Based on the recombination frequency of marker loci with the *eui3(t)*,the marker was mapped at a genetic distance of 13.7cM from the *eui3(t)*. To refine the genetic distance, few more markers

namely, RM3330, RM7311 and RM111, flanking to the HvSSR06-40 were selected and F_2 plants were genotyped. The *eui3(t)* was found present in the marker interval of RM3330 and HvSSR06-40 with a LOD value of 61.62 and R^2 value of 72.90. The identified gene in the present study needed to be fine mapped and identification of candidate gene or gene-based marker for its use in future breeding programme. There is need to clone the gene and revealing the molecular mechanism behind the uppermost internode elongation and functional characterization of the gene. This helps the plant breeders to use this gene in future breeding program for its introgression into male sterile line to increases the level of pollination and seed setting rate. This could be a better strategy for increasing the hybrid seed production in rice to feed the burgeoning human population.

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