

DNA MARKER ASSISTED GENETIC ANALYSIS OF EARLY VIGOUR RELATED TRAITS IN RICE (*Oryza sativa* L.)

Thesis submitted to the
University of Agricultural Sciences, Dharwad
in partial fulfillment of the requirements for the
Degree of

DOCTOR OF PHILOSOPHY

In

GENETICS AND PLANT BREEDING

By

JAYATEERTHA R. DIWAN

**DEPARTMENT OF GENETICS AND PLANT BREEDING
COLLEGE OF AGRICULTURE, DHARWAD
UNIVERSITY OF AGRICULTURAL SCIENCES
DHARWAD – 580005**

MARCH, 2006

ADVISORY COMMITTEE

Dharwad

(M.V.C GOWDA)

NOVEMBER, 2005

MAJOR ADVISOR

Approved by:

Chairman : _____

(M.V.C. GOWDA)

Co-chairman: _____

(V.V. SHENOY)

1. _____

(P.M. SAILMATH)

2. _____

(H.M. VAMADEVAIAH)

3. _____

(V.V. ANGADI)

CONTENTS

Chapter No.	Title	Page No.
I.	INTRODUCTION	
II.	REVIEW OF LITERATURE	
III.	MATERIAL AND METHODS	
IV.	EXPERIMENTAL RESULTS	
V.	DISCUSSION	
VI.	SUMMARY	
VII.	REFERENCES	
	APPENDICES	

LIST OF TABLES

Table No.	Title	Page No.
1	Early vigour related traits present on different chromosomes and their association with different markers	
2	Information on parents of new population generated	
3	ANOVA for early vigour related traits in IR64/Azucena – DH	
4	Mean, range and genetic parameters for early vigour related traits in IR64/Azucena – DH population	
5	Phenotypic and genotypic correlations among early vigour related traits in IR64/Azucena DH population	
6	QTLs associated with early vigour related traits in IR64/ Azucena – DH population identified by interval mapping (MAPMARKER / QTL v. 1.1)	
7	Mean, range and genetic components for early vigour related traits in F_2 population of BPT5204/ A67 and BPT2504/ Dodiga	
8	ANOVA for early vigour related traits in parents and F_3 population of BPT5204/ A67 cross	
9	ANOVA for early vigour related traits in parents and F_3 population of BPT5204/ Dodiga cross	
10	Mean, range and genetic parameters for early vigour related traits in F_3 population of BPT5204/ A67	
11	Mean, range and genetic parameters for early vigour related traits in F_3 population of BPT2504/ Dodiga	
12	Phenotypic/ Genotypic correlation in BPT 5204/A67 F_3 families	
13	Phenotypic/ Genotypic correlation in BPT5204/Dodiga F_3 families	
14	Phenotypic correlations in $F_2:F_3$ family of cross BPT5204/ A67	
15	Phenotypic correlations in $F_2:F_3$ family of Cross BPT5204/ Dodiga	
16	Heritability estimates of shoot length trait in $F_2:F_3$ analysis	
17	Polymorphism for different SSR loci in the parental lines of crosses studied (P-Polymorphic, M-Monomorphic)	
18	Goodness of fit to 1:2:1 marker segregation in F_2 population of the cross BPT5204/ A67	
19	Goodness of fit to 1:2:1 marker segregation in F_2 population of the cross BPT5204/ Dodiga	
20	Association between marker and early vigor related traits in $F_2:F_3$ of BPT5204/ Dodiga	
21	Association between marker and early vigor related traits in $F_2:F_3$ of BPT5204/ A67 cross	

LIST OF FIGURES

Figure No.	Title	Between pages
1	Frequency distribution of early vigour related parameter in IR 64 / Azucena – DH population	
2	QTL map of early vigour related traits in R 64 / Azucena DH population	
3	Performance of parental lines under laboratory (A) and net house (B and C) for early vigour	
4	Variability for root and shoot length in F ₂ individuals of BPT-5204 / A67 under lab and net house conditions	
5	Variability for root and shoot length in BPT 5204 Dodiga – F ₂ lines	
6	Frequency distribution of root length (RL6, RL10 & RL14) in F ₂ : BPT5204 / A-67 and BPT5204 / Dodiga	
7	Frequency distribution of shoot length (SL6, SL10 & SL14) in F ₂ : BPT5204 / A67 and BPT5204 / Dodiga	
8	Variability for root and shoot length in F ₃ families of BPT5204 / Dodiga (A) and BPT5204 / A-67 (B) under laboratory	
9	Frequency distribution for morphological traits in BPT5204/A67 F ₃ families (P ₁ –BPT5204 P ₂ – A67)	
10	Frequency distribution for morphological traits in BPT5204/Dodiga F ₃ families (P ₁ – BPT5204 P ₂ – Dodiga)	
11	Simple sequence repeat (SSR) patterns in Parents, F ₁ and F ₂ of the cross BPT5204 / A-67	
12	Simple sequence repeat pattern (SSR) in parents F ₁ , F ₂ of the cross and BPT5204 / Dodiga	

APPENDICES

Appendix No.	Title	Page No.
1	Preparation of stocks for CTAB method of DNA Extraction	
2	List of primers used in the study	

I. INTRODUCTION

Rice (*Oryza sativa* L.) is the world's most important food crop and a primary source of food for more than half the world's population. More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's people live. Rice accounts for 35-75% of the calories consumed by more than 3 billion Asians. It is planted on about 154 million hectares annually (11% of the world's cultivable land). We will have to produce 40% more rice by 2030 to satisfy the growing demand without affecting the resource base adversely. This increased demand will have to be met from less land, with less water, less labour and fewer chemicals. To meet the challenge of producing more rice from available lands, we need varieties/ hybrids which grow better under adverse conditions and have high yield potential (Khush, 2005).

The weeds, which reduce yield considerably under upland condition, need to be controlled to obtain better yield levels. Apart from heavy weed infestation, moisture stress, erratic rainfall, rapid loss of soil water and heavy labour costs add to the misery of rainfed upland rice farmers. The weeds can be controlled through hand weeding or through herbicide. However, both are difficult to practice under rainfed ecology due to economic and ecological considerations.

Early vigour trait is important for optimum stand establishment and increasing weed competitiveness in rice cropping systems. Several morpho-physiological quantitative traits such as germination rate and seedling growth are associated with early vigour in rice and performance is generally determined by genotype and modified by the environment (Perry, 1972). The seed vigour in rice also affects many of its agronomic traits and grain yield as well (Pandey *et al.*, 1989). Vigour is under genetic control (Perry, 1972) and can be incorporated in the genetic background of high yielding varieties (Seshu and Krishnasamy, 1987).

DNA based markers are increasingly being recognized as useful tools for various applications in plant breeding; since they are not influenced by the environment (Lee, 1995). DNA markers detect genetic variation among genotypes and are useful as landmarks to tag functional genes. RAPD are dominant markers, while RFLP and AFLP are difficult to perform. Therefore, Simple Sequence Repeat (SSR) markers are a better choice as they are abundant, easy to detect and codominant. The breeding for increasing early seedling vigour via conventional strategies has not been very successful for a long time (Li and Rutger, 1980; Redona and Mackill, 1996a) because of complex inheritance and quantitative nature. DNA marker and genome mapping techniques have become powerful tools for genetic analysis of quantitative trait loci (QTLs) controlling complex traits (Lander and Botstein, 1989; Tanksley, 1993) and have been extensively used for genetic dissection of agriculturally important traits in rice (Xiao *et al.*, 1996; Li *et al.*, 1997, 2001; Yano *et al.*, 1997; Yu *et al.*, 1997). The results of QTL mapping are largely dependent on mapping populations, i.e., only genes segregating in the mapping populations could be identified. Therefore, to identify more valuable QTLs for early vigour, it is surely essential to use diverse mapping populations. This would not only advance our understanding of the genetic diversity for seedling vigour in rice, but also favour the pyramiding of more favourable alleles by marker-assisted selection (MAS) in molecular breeding programs. There is need to improve early vigour of semi-dwarf rice cultivars. The increasing importance of direct seeding in many Asian countries (Dingkuhn *et al.*, 1992) has made it critical to improve the seedling vigour of rice cultivars.

QTL maps could assist plant breeders in devising more efficient conventional breeding strategies for these characters as well as in developing marker assisted selection schemes where selection for the quantitative trait would be based on a set of molecular markers. Determination of the number, location and magnitude of effects through SSR linkages of QTLs underlying seedling vigour could elucidate the genetic basis of the trait leading to improved breeding and selection efficiency.

Antarsali(A67) and Dodiga are land races adapted to upland condition in Northern Karnataka. They are known for high seedling vigour, but possess low tiller number and moderate grain quality. BPT5204 (Samba Mahsuri) is a semi-dwarf, late maturing variety with good grain quality and high yield potential under irrigated conditions, but suffers from low seedling vigour. The crosses BPT5204/A67 and BPT5204/Dodiga are expected to give

recombinants combining early vigour, high yield and good grain quality and thus useful for cultivation in upland conditions.

The generation of molecular map for an entirely new population is more time consuming and resource intensive, therefore, the use of a reference population (IR64/Azucena-DH) saturated with molecular markers to know the chromosomal regions associated with the trait and validating in the new populations is a more useful approach. Therefore, the present study was carried out with the following objectives.

Objectives

1. To assess variability and association for early vigour related traits in DH population of IR64/Azucena,
2. To assess variability for early vigour related traits in BPT5204/A67 and BPT5204/Dodiga in F₂ and F₃ generations, and
3. To identify and validate SSR markers associated with the early vigour related traits.

II. REVIEW OF LITERATURE

Rice has served as model species for genetic investigations because of its small genome size, dense genetic maps, relative ease of genetic transformation and synteny with other cereals. Therefore, genetic studies on early vigour related traits in different mapping populations will help to understand the genetic basis in a better way and would be useful for incorporation of needed traits in the desired background. The studies on early vigour and its importance, especially under upland rice ecology are reviewed herein.

Weeds cause significant yield reduction. The extent of yield loss ranges between 15-20% in the transplanted rice, 30-35% in the direct seeded lowland rice and 50-90% in the upland rice (Mishra and Vaishya, 2000).

Mishra (2000) reported rice varieties that suppress either weed-growth or compete with weeds without sacrificing the yield. The morpho-physiological traits useful for better competitive ability were rapid and uniform germination, faster growth at seedling stage, early prolific root growth, good vegetative vigour, height and more canopy, especially during the early stages crop growth.

Gibson and Fischer (2004) reported weed competitiveness of rice cultivars as a tool for crop based weed management. They reviewed the literature on varietal differences in weed competitive ability among rice cultivars, the plant traits that confer the competitive ability, the potential for inclusion of competitive rice cultivars in weed management and have suggested areas for further research in that direction.

Seedling-vigour is important for optimum stand establishment and increasing weed competitive ability in rice cropping systems (Zhang *et al.*, 2005). High seedling vigour helps the genotypes to suppress the weeds, which is a serious problem in large rainfed and upland tracts of tropics where dry seeding is practiced (Kanbar *et al.*, 2006).

Previous studies have suggested positive response of transplanted rice to nursery fertilizer application due to increased seedling vigour or increased nutrient content. Seedling vigour was responsible for the response of transplanted seedlings to nursery treatments. The response of rice yield to nursery treatments is largely due to increased seedling vigour and can be affected by a range of nutritional as well as non-nutritional treatments of seedlings that increase seedling dry matter, nutrient content and nutrient concentration. Impairment of leaf growth and to a lesser extent root growth in the nursery depressed seedling vigour after transplanting. However, rather than increasing stress tolerance, seedling vigour was more beneficial when post-transplant growth was not limited by nutrient or water stresses.

2.1 SEED AND SEEDLING VIGOUR

High vigour is a characteristic of high quality seed (Chowdhury and Singh, 2000). Seed vigour is a highly complex phenomenon. It is not a single measurable property like germination, but a concept for describing several characteristics associated with various aspects of performance of the seed (Perry, 1981); both in the field (Perry, 1978) and in storage (Hampton and Coolbear, 1990). Seedling vigour is plant's ability to emerge rapidly from soil or water (Heydecker, 1960).

Seed vigour is the "Sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence" (Anon., 1993). The seedling vigour has been associated with seed size and density as well as other parameters of the germinating seed (Pandey *et al.*, 1989 and Shenoy *et al.*, 1990). The characters like germination percent, germination rate, coleoptile and mesocotyl lengths were useful in prediction of seedling establishment in anaerobic condition (Yamaochi and Winn, 1996).

The maintenance of optimum population in field is important in obtaining maximum yield and in reducing cost of seed and seed vigour has influence in maintaining optimum plant population (Perry, 1980). Seed vigour is also important when seeds are to be sown in harsh environments (Hampton and Hill, 1990).

Seedling vigour plays a critical role under direct seeded cultivation of rice. It helps in better plant establishment and successful competition with weeds. Vigour in rice affects many agronomic characters and grain yield (Pandey *et al.*, 1989). Siddique (1986) reported that compared with high vigour seeds reduction in yield levels by 7.89 and 14.14 % were noticed when medium and low vigour seeds were used, respectively. Reddy *et al.* (1996) studied effect of initial germination levels on vigour, growth and yield in rice and observed decrease in grain yield from 6.74 to 5.26 t/ha with a decrease in initial germination from 91 to 47 %.

Proper vigour tests are necessary to assess the performance of a seed lot accurately. The test should provide a reproducible result, which accurately describes the potential for rapid and uniform emergence under

field conditions (Anon., 1993). Teckrony and Egli (1977) proposed that vigour indices should be developed taking germination tests with one or more vigour tests, which should be compared with the field emergence. Shenoy *et al.* (1990) studied the association of a number of seed related traits with the field emergence in 51 cultivars of rice. Among those, first and final counts of standard germination, germination rate, vigour index, germination at 15°C, germination in the presence of nonanoic acid and amylase activity showed significantly positive correlation with field emergence. Seed vigour was measured as per cent field emergence. Other vigour related variables, such as, standard germination, rate of germination, vigour index, total dehydrogenase activity, root and shoot length, seedling dry weight were found to be significantly correlated with field emergence (Seshu and Dadlani, 1993). The physiological and biochemical properties of seed can also be used in measuring seed vigour, the most common being electrical conductance of seed leachate and total dehydrogenase activity (Perry, 1981; Anon., 1983).

2.2 GENETIC VARIATIONS FOR EARLY VIGOUR RELATED TRAITS

The phenotyping of the trait is very important for better study of genetics. Seed germination rate, seedling shoot length and dry matter weight, were investigated by the paper-roll tests with rice recombinant inbred lines derived from a cross between Lemont (*japonica*) and Teqing (*indica*) by Zhang *et al.*, 2005.

2.2.1 First count of germination

The first count of germination indicates the speed of germination of a seed lot and in turn the vigour of seeds. It showed a very high range (18 – 100%) in a study of 51 cultivars by Shenoy *et al.* (1990). Menezes *et al.* (1994) reported that first count is an efficient and rapid method of assessment of field emergence.

2.2.2 Final count of germination

Final count of germination indicates total germination percentage. Sharma and Sharma (1993) reported that percentage germination had high heritability and high genetic advance. Ramadevi (1998) reported low variability for final count with phenotypic coefficient of variability of 13.37 % and genotypic coefficient of variability of 12.27 %. However, the heritability was high (88.30%).

2.2.3 Rate of germination

Among the many components of seed vigour, rate of germination is recognized as very important (Pollock and Roos, 1972). Cultivar differences for germination rate were observed by Krishnasamy and Seshu (1989). Germination rate showed high variability in the studies conducted by Shenoy *et al.* (1990). Significant differences among cultivars were also reported by Pandey *et al.* (1994). In an evaluation of 69 cultivars, significant variability was observed for rate of germination along with high heritability (Subbarao *et al.*, 1996). Akram *et al.* (1993) reported role of additive and non-additive types of gene action in the inheritance of this trait. However, additive gene effects were more prominent than dominant ones, leading to high narrow-sense heritability.

2.2.4 Root length

Li *et al.* (1990) observed heterosis for root length in a study involving parents and F₁ hybrids of five crosses of *indica* type; all the F₁s showed better root length compared to their respective parents. Akram *et al.* (1993) reported involvement of additive and dominance effects in controlling this trait. However, the dominance effect was more prominent with low heritability. Similar findings were also reported by Qing *et al.* (1996), Redona and Mackill (1996a) and Price *et al.* (1997). Jiakui *et al.* (1996) studied heterosis, genetic variance and heritability of root characters and observed significant heterosis for these characters and inferred that they are mainly controlled by non-additive gene effects.

Pandey *et al.* (1992) found highly significant variation for root growth/length taken on the 14th day after germination in *indica* types, whereas, the variability in *japonica* types was non-significant for this character. Significant variability for root length was also reported by Subbarao *et al.* (1996).

Chunhai *et al.* (1996) reported pleiotropic effects of *sd1*, semidwarf gene on root traits and observed direct and indirect effects of this gene on some of the seedling traits.

2.2.5 Shoot length

High variability for shoot length was observed by Pandey *et al.* (1992, 1994). Akram *et al.* (1993) reported role of additive and non-additive type of gene actions in inheritance of seedling shoot length. However, additive gene effects were more prominent leading to high narrow sense heritability. Li and Rutger (1980) also reported that under cool temperature (18 °C), shoot length of seedling grown for 14 days was regulated by additive and dominance effects in F₁ and F₂ generations. According to Redona and Mackill (1996a), heritability value differed with different populations. Both high (0.80) and low (0.26) heritability values were obtained in two populations derived from different crosses. Subbarao *et al.* (1996) has reported moderate heritability for shoot length.

Shoot length was a good indicator of green house and field seedling vigour even at higher temperatures. Therefore, it is useful trait for seedling vigour in warm and tropical conditions (Redona and Mackill, 1996a). Despite modest gains through traditional breeding, difficulty exists in obtaining high level of seedling vigour in modern semidwarf cultivars (McKenzie *et al.*, 1994).

2.2.6 Seedling dry weight

Considerable variation for seedling dry weight was reported by Chen *et al.* (1986) in a study with 242 rice varieties. Highly significant differences among *indica* types for seedling dry weight was observed by Pandey *et al.* (1992, 1994). Lee *et al.* (1986) reported that dry weight of 12 days old rice-seedling was under the control of additive and dominance effects, with additive effects being higher. High narrow sense heritability value of 65.24% was observed for this trait.

Blanco and Akita (1988) observed heterosis for seedling dry weight in *indica/indica* and *japonica/japonica* crosses but not in *japonica/indica* crosses. Sharma and Sharma (1993), reported high heritability for seedling dry weight and its association with yield. High heritability for seedling dry weight was also reported by Subbarao *et al.* (1996).

2.2.7 Vigour index

Ramadevi (1998) reported about high phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV) and heritability for vigour index trait. Makhasi (2001) reported high heritability and moderate genetic advance for vigour index trait in upland rice germplasm.

Moderately high heritability was noticed for seedling vigour, which indicated that a major portion of phenotypic variability was controlled by genes with additive and/or additive x additive effects. Seedling vigour was found to be highly correlated with earliness and larger seed size, but correlation with mature plant height was low (Li and Rutger, 1980).

2.3 MOLECULAR MARKERS

2.3.1 Bio-chemical markers

In cereals, α -amylase is the primary enzyme involved in the conversion of insoluble starch to the simple sugars that provide energy for the growing seedling (Fincher, 1989). Analysis with isoelectric focussing revealed at least three isoforms of α -amylase in extracts of germinating rice seeds (Daussant *et al.*, 1983; Perata *et al.*, 1992), encoded by various members of α -amylase gene families (Mitsui *et al.*, 1993). In wheat and barley, Southern blot analysis of genomic DNA probed with α -amylase cDNA clones indicates that the isozymes of α -amylase are encoded by a multigene family (Lazarus *et al.*, 1985; Khursheed and Rogers, 1988). In barley, two different sub-families (AMY1 AND AMY2) have been identified based on isoelectric points of the isozymes encoded by them (high PI and low PI groups) (Khursheed and Rogers, 1988). Ranjhan *et al.* (1991) identified location of α -amylase genes on chromosomes of rice. The five groups of genes are located on chromosomes 1, 2, 6, 8 and 9. Krishnasamy and Seshu (1993) reported the appearance of high mobility band in the α -amylase isozyme pattern in fast germinating cultivars on the third day after germination and in cultivars with slow germination rate, it appeared on fourth or fifth day after germination. The positive association between high mobility of α -amylase isozyme and one of the component of early vigour shoot length, was reported by Diwan and Shenoy (2001).

2.3.2 DNA markers

DNA markers are being increasingly utilized for various purposes viz., cultivar development, quality control of seed production, measurement of genetic diversity for conservation management, marker-assisted selection (MAS) in crop improvement. Molecular markers have played a role in DNA fingerprinting for varietal identification and protection of intellectual property rights (IPR).

Molecular markers allow selection for the traits to be done on the basis of a simple laboratory tests on a small amount of plant tissue, rather than direct measurement of the character itself (Christopher *et al.* 2004).

There are various kinds of molecular markers available including Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLPs), microsatellites (Simple Sequence Repeats, SSRs) and Single Nucleotide Polymorphisms (SNPs). There are merits and demerits of each type of marker depending on the specific objectives of particular study. Among the marker systems available SSRs have been proved to be more useful in marker assisted selection (Yang *et al.*, 1996).

SSRs are tandem repeats of di, tri or a higher number of nucleotide units in the DNA of plants. SSRs are abundantly distributed throughout the nuclear genome of all plant species, which make them useful for both genetic mapping and for characterization of cultivars or populations. SSRs are co-dominant and highly informative. They generally display higher levels of polymorphism (Beckmann and Soller, 1990; Brown *et al.*, 1996; Senor *et al.*, 1998) and are amenable to automated genotyping strategies. They can be amplified by PCR and efficiently detect DNA polymorphism (Pejic *et al.*, 1998). SSR marker technology has been developed and used for genome mapping and DNA fingerprinting in different plant species such as rice (Wu and Tanksley, 1993; Yang *et al.*, 1996); wheat (Roder *et al.*, 1995); barley (Saghai-Marouf *et al.*, 1994) and sorghum (Dean *et al.*, 1999).

A total of 2414 new di-, tri- and tetra-nucleotide non-redundant SSR primer pairs, representing 2240 unique marker loci, have been developed and experimentally validated for rice (*Oryza sativa* L.) (McCouch, 2002). In combination with the pool of 500 previously mapped SSR markers, this makes available a total of 2740 experimentally confirmed SSR markers for rice or approximately one SSR every 157 kb of rice genome.

Various studies are available in understanding the suitability of SSR markers for different objectives. Microsatellite markers are known for higher order profiling of DNA, giving closer look on the genetic aspects of

the individuals. Even though reports say that AFLP can detect polymorphism to the tune of 81 per cent in land races of rice (Singh *et al.*, 1999), comparatively use of SSR markers is much easier, simpler and more sensitive in polymorphism studies (Zhou and Gustafson, 1995). Chao *et al.* (2000) are of the opinion that SSR markers have high resolution as they observed 83 per cent of polymorphism using SSR markers compared to Expressed Sequence Tags (ESTs, 54%) in the same genetic stocks.

Since SSR markers are efficient enough in detecting polymorphism, can be utilized for testing distinctness, uniformity and stability (DUS) of varieties. But Blair *et al.* (1999) reported that ISSR markers are also equally efficient in detecting polymorphism in varieties. These markers can not only be utilized for studying polymorphism and also be best exploited for fingerprinting and cultivar identification (Nybor *et al.*, 1994; Zhou and Gustafsen, 1995; Singh *et al.*, 1999).

SSRs or microsatellites are most suited to routine applications in breeding programs. Analysis of SSRs utilizes the polymerase chain reaction (PCR). PCR is relatively simple, quick and safe and requires only small quantities of DNA.

Potential characters for application of molecular marker assisted selection are identified by considering current improvement strategies, breeding objectives and criteria for identification of traits suited to application of molecular markers. The traits selected as candidates for development of molecular markers were fragrance, disease resistance including rice bacterial blight, blast and sheath blight, grain extension on

cooking, predisposition to chalkiness, gelatinization temperature, minor fragrance components and seedling vigour/early establishment (Christopher *et al.*, 2004).

2.4 MAPPING POPULATION

The choice of the mapping population depends primarily on the objectives of the work. One could efficiently use, when available, mapping populations that have already been scored for many markers. Recombination frequencies between pairs of markers can be scored in many different segregating populations. Those derived from true F_1 are most straight forward, because only two alleles are segregating. They include F_2 , Backcross (BC), Recombinant Inbred Line (RILs) or Double Haploid (DH) lines. F_2 populations detect more distant linkage and offer better resolution of tighter linkages because recombination in both mega- and micro-gametophytes can be detected. The major drawback of the F_2 and BC is that they are ephemeral. However, this can be overcome by construction of RILs, which takes more time. The recombinant lines can be produced by doubled-haploid procedure as the time required to reach homozygosity is shorter. But, this cannot be applied to all genotypes. F_2 populations efficiently map co-dominant markers, but map dominant markers less efficiently. The selection of parents is most important, they should show sufficient DNA sequence polymorphism.

2.4.1 IR64/Azucena – Double Haploid (DH) mapping population

One hundred and thirty five double haploid (DH) lines were developed from the cross between IR64, an *indica* variety adapted to irrigated conditions and Azucena, an upland tropical *japonica* variety (Guiderdoni *et al.*, 1992). IR64 is a semi-dwarf, heavy tillering, high yielding and widely grown variety in rice growing regions of Asia, while Azucena is a tall, shy tillering, low yielding and long grained variety from the Philippines.

The molecular map for this DH population was earlier generated by Huang *et al.* (1994) using 135 RFLP markers covering all the 12 chromosomes. This was further updated by adding 40 new isozyme and/or RAPD markers with total up to 175 markers with an average distance of 10.3 cM between the markers. Maheswaran *et al.* (1997) added 208 AFLP markers to the same population, distributed over all the 12 chromosomes. Temnykh *et al.* (2001) developed and integrated a set of 200 Class I SSR markers into the microsatellite map of rice, providing immediate links between the genetic, physical, and sequence-based maps. This contribution brought the number of microsatellite markers that have been rigorously evaluated for amplification, map position, and allelic diversity in *Oryza* spp. to a total of 500.

There are over sixty-seven documented mapping populations and maps in rice and more are being generated. Rice genome sequence maps are also available. Now, it is possible to map precise location of QTLs and know genome sequence controlling trait. Researchers working across continents and habitats have mapped over 2500 traits.

2.4.2 Population size

The size of the mapping population will greatly affect resolution of a map and ability to determine marker order. Generally, populations of 50-100 plants are used for mapping in plants, human and mice. However, fine mapping of QTLs requires a larger size.

2.5 QUANTITATIVE TRAIT LOCUS (QTL)

Quantitative trait loci are marvelous genetic entities demonstrating greater utility in genetic understanding of complex traits and promise greater impact in crop improvement endeavors. They have given us clearer view of the genetics of agronomically important, and several other traits too. A quantitative trait could be now considered as effect of series of monogenes acting 'in concert' or unison so as to influence a single trait. Each gene pair (locus) interacts with the environment and causes effect/s that other/s add/s to or subtract/s from the effects of other gene pairs that influence the trait. The cumulative effect or trait manifestation of a QTL is thus a combination of the effects of each gene pairs (either in homozygous and in heterozygous condition) with the action of environment. It is now possible to map QTLs on rice genomic sequence and find out reading frames, overlapping genes, copies of genes at the locus (Shashidhar, 2004).

The detection of linkage between a QTL and genetic markers provides more powerful and robust method of identifying QTLs. Linkage maps moderately or densely populated with several types of highly informative markers have now been developed. These maps provide the basic tools with which to study variation underlying quantitative traits. Each QTL will only explain a proportion of genetic variance and usually an even smaller proportion of the total variance (Collard *et al.*, 2005). The most likely position of QTL may appear to be between pair of markers, but it could be as far as 20 cM away (Kearsey and Farquhar, 1998).

The QTLs classified based on phenotypic variance explained. The QTL responsible for more than 50% of the phenotypic variation are designated as 'major genes', where as loci controlling 25-50% of the variation would be major QTL according to Mackill and Ni (2001). In rice, a total of 7056 QTLs have been reported from 1994 to October 2004 (www.gramene.org).

2.5.1 Seedling vigour QTLs

The phenotype data, together with a linkage map consisting of 198 marker loci, was used to conduct composite interval mapping by QTLMapper v 1.0 to simultaneously map both the main-effect and the epistatic QTLs for seedling vigor in rice by Zhang *et al.* (2005). Totally, 13 putative main effect QTLs and 19 pairs of epistatic loci with $R^2 \geq 5\%$ were identified. Almost all of these QTLs or interactions individually explained only around 5–10% of the phenotypic variation observed for the traits. The majority (68%) of these main effect and epistatic loci were clustered in seven chromosome regions, each spanning 12–28 cM and containing three or more detectable loci.

The multiple seedling-vigor-related traits, either the main-effect QTLs or the epistatic interactions sharing the same map location had their additive or epistatic effects in the same direction, which agreed well with the positive correlations among the traits. The results demonstrated that seedling-vigor in rice could be controlled by many loci, most of which had small effects, but, relatively, epistasis as a genetic factor was much more important than the main-effects of QTLs. Along with the results

reported previously, this study revealed the extensive genetic diversity for seedling-vigor in rice.

In addition, the QTL qSV-7 on chromosome 7 was found to have the largest main-effect on multiple seedling-vigor-related traits, and therefore, could be used as a potential target to be genetically manipulated by MAS in rice seedling-vigor breeding programs (Zhang

et al., 2005). The genome wide approach of insertional mutagenesis in rice using transposon and /or *Agrobacterium* T-DNA as tags was followed to identify functional part of genome controlling seedling vigour. The common QTLs across different populations were used in functional genomics study in a project at CSIRO, Australia, by Upadhyaya (2005).

2.6 MARKER-ASSISTED SELECTION (MAS) FOR EARLY VIGOUR RELATED TRAITS

MAS for early vigour traits is more relevant, because seedling vigour QTLs is controlled by many loci with small effects as shown by Zhang *et al.* (2005). Therefore, breeding through conventional strategies is not very successful. The improvement for vigour was not very successful in both temperate (Mc Kenzie *et al.* 1994) and tropical (Herd, 1991) conditions. This may be due to association of vigour trait with characters like tallness, lodging susceptibility, large grain size and earliness that are selected against during the breeding process (Redona and Mackill, 1996a).

The loose relationship between genotype and phenotype in parents, making identification of superior donors and breeding lines based on phenotype alone difficult (Redona and Mackill, 1996b). Low realized heritability for shoot length suggests that early generation selection may not be effective and also conventional breeding may not be effective or difficult (Redona and Mackill, 1996a). Genetic basis of seedling vigour could be more complex than characters like plant height. Additive and varying degree of dominance effects were observed at all loci for seedling vigour traits (Redona and Mackill, 1996b).

Epistasis for complex traits appears to be more pronounced in self pollinated crops like rice than out crossing spp. like Maize (Li *et al.*, 2001). This pronounced epistasis could result in differential expression of the alleles at such QTLs in different genetic background. This might be another reason for previous failure in breeding for increased seedling vigour using conventional approach (Li and Rutger, 1980; Redona and Mackill, 1996b). From plant breeding point of view it is preferable to identify and use donors of seedling vigour traits within the subspecies or same varietal groups. Use of wide crosses often result in sterility problems in the hybrid and its progenies, disrupting favourable linkage blocks, gene combinations and linkage drag. The reduced recombination and distorted segregation resulting from wide hybridization in rice may also cause difficulties in selection for desired recombinants during the breeding process.

The different sets of seedling vigour genes may be present in *japonica* and *indica* cultivars (Redona and Mackill, 1996c). The presence and distribution of QTLs across genome in various populations reveal genetic diversity in rice for vigour-related traits. Therefore, to identify genes from diverse origins the QTL mapping approach is useful.

The tagging of positive alleles for seedling vigour from both parents with molecular markers and using them in selection could be more effective breeding strategy for improvement of vigour. The linked markers may be utilized in screening parental materials and breeding lines for desired genotypes at marker loci in order to introgress and pyramid favourable QTL alleles in new population.

Some QTLs or genomic regions were commonly detected across very deferent populations but others largely dependent on population used. Vigour QTLs on chromosome 1 were identified by Zhang *et al.* (2005), Redona and Mackill (1996b) and Cui *et al.* (2002). The QTLs on chr. 5 were reported by Cui *et al.* (2002), Zhang *et al.* (2005) and Upadhyaya, (2005). The QTL on chromosome 6 was reported by Zhang *et al.* (2005) and Cui *et al.* (2002). The QTLs on chromosome 2, 8 and 12 were identified by Zhang *et al.* (2005). Xu *et al.* (2004) identified QTLs for seedling characters in population of cross Zhenshan 97 and Minghui 63 at different time intervals. They indicated that different genes or QTLs control the same traits during different periods.

Miura *et al.* (2004) reported backcross breeding with MAS is effective for introducing genes for adaptability to direct sowing from donor to desired background. They carried out genetic studies on germination of seed and seedling establishment for breeding of improved rice varieties suitable for direct seeding culture. Pyramiding of desirable alleles can be done

through MAS. Major effect traits affected by empirical breeding, while MAS is useful to manipulate genes with minor effects (Yin *et al.*, 1997 and Stuber *et al.*, 1999).

The QTLs contributing to vigour may also contribute for stress tolerance with varied gene expression, which has been described as G x E interaction (Moll *et al.*, 1978 and Tanksley, 1993). These interactions were gene specific.

The grain size is important from consumer point of view. The increase in grain size may reduce popularity of cultivar. Cui *et al.* (2002) revealed that chromosomal regions of C246-C1447 on chromosome 5 and C952-waxy-C1496, which were closely associated with seedling vigour and α -amylase, were not linked to seed weight. Therefore, identification of breeding lines that carry these chromosomal regions using MAS may improve seedling vigour without increasing grain size.

With respect to crop improvement, transgressive variation represents a potential source of novel genetic variation. Since most important traits of domestic plants are conditioned by polygenic systems, detection of allele dispersion can be useful in the selection of favorable new germplasm. The transgressive segregants were obtained for seedling vigour related traits in CT9993/IR62266-DH population (Kanbar *et al.*, 2006).

The QTLs identified, mapping populations used and chromosomes to which QTLs were traced are given in Table 1.

Table 1. Early vigour related traits present in different chromosomes and their association with different markers.

Trait	Chromosome No.	No. of QTLs	Marker	Mapping population		Reference
				Cross	Type	
Shoot length	1, 3, 5, 9	4	RFLP	Labelle/ Black gora	F ₂ -F ₃	Redona and Mackill (1996b)
Root length	1, 2,	2	RFLP	Labelle/ Black gora	F ₂ -F ₃	Redona and Mackill (1996b)
Coleoptile length	3, 6	2	RFLP	Labelle/ Black gora	F ₂ -F ₃	Redona and Mackill (1996b)
Mesocotyl length	3, 7, 1, 5	5	RFLP	Labelle/ Black gora	F ₂ -F ₃	Redona and Mackill (1996b)
Germination rate	3, 5, 6, 7	8	RFLP, SSR	Zhenshan 97/ Minghui 63	RILs	Cui <i>et al.</i> (2002)
Total dry weight	1, 3, 5, 6, 9	8	RFLP, SSR	Zhenshan 97/ Minghui 63	RILs	Cui <i>et al.</i> (2002)
Shoot dry weight	1, 3, 5, 6, 9	7	RFLP, SSR	Zhenshan 97/ Minghui 63	RILs	Cui <i>et al.</i> (2002)
Root dry weight	1, 5, 10	4	RFLP, SSR	Zhenshan 97/ Minghui 63	RILs	Cui <i>et al.</i> (2002)
Maximum root length	1, 5, 6	4	RFLP, SSR	Zhenshan 97/ Minghui 63	RILs	Cui <i>et al.</i> (2002)
Shoot dry weight	1, 2, 3, 5, 10, 11	6	RFLP, SSR	Zhenshan 97/ Minghui 63	RILs	Xu <i>et al.</i> (2004)
Root dry weight	2, 3, 4, 5, 10, 11	7	RFLP, SSR	Zhenshan 97/ Minghui 63	RILs	Xu <i>et al.</i> (2004)

Table 1. Contd...

Trait	Chromosome No.	No. of QTLs	Marker	Mapping population		Reference
				Cross	Type	
Total dry weight	2, 3, 5, 10, 11	5	RFLP, SSR	Zhenshan 97/ Minghui 63	RILs	Xu <i>et al.</i> (2004)
Maximum root length	3, 5, 7, 10, 11	6	RFLP, SSR	Zhenshan 97/ Minghui 63	RILs	Xu <i>et al.</i> (2004)
Germination rate	1, 2, 3, 4, 5, 6, 7, 12	33	RFLP, SSR	Lemont / Teqing	RILs	Zhang <i>et al.</i> (2005)
Shoot length	1, 2, 3, 4, 5, 7, 8, 9	12	RFLP, SSR	Lemont / Teqing	RILs	Zhang <i>et al.</i> (2005)
Dry matter weight	1, 5, 7, 9, 11, 12	6	RFLP, SSR	Lemont / Teqing	RILs	Zhang <i>et al.</i> (2005)
Total plant length	1, 2, 12	3	RFLP, AFLP, SSR	CT9993 / IR62266	DH	Kanbar <i>et al.</i> (2002)
Root dry weight	4	1	RFLP, AFLP, SSR	CT9993 / IR62266	DH	Kanbar <i>et al.</i> (2002)
Low temperature germination	3, 4	3	RFLP, SSR	Italicalivorno / Hayamasari	BILs	Fujino <i>et al.</i> (2004)

III. MATERIAL AND METHODS

The experiments were conducted at the Department of Genetics and Plant Breeding, College of Agriculture, University of Agricultural Sciences, Dharwad. The material used for the study and the methods followed are presented here.

3.1 MATERIAL

3.1.1 Double Haploid (DH) population

3.1.1.1 Genotypes

The 121 DH lines derived from cross a between IR64 and Azucena, were originally developed by Guiderdoni *et al.* (1992) maintained at International Rice Research Institute (IRRI), Philippines and Barwale Foundation (formerly known as Mahyco Research Foundation), Hyderabad were used.

3.1.1.2 Molecular map

The matrix of the genotypic data of the 269 SSR markers spanning 12 chromosomes of rice was downloaded from www.gramene.org.

3.1.1.3 Phenotyping Methods

Laboratory assessed parameters of vigour

Standard germination tests

Seeds were treated with dry heat at 50°C for four days to eliminate possible dormancy. Two sets of 25 seeds of each line of the DH population were germinated in rolled paper towels at ambient temperature (25° ± 3°C) in dark. Seeds showing 2 mm radicle length were considered as germinated. The following parameters were measured according to ISTA (Anonymous, 1993).

First count

Seeds germinated by 48 hours (h) in the standard germination test were counted and expressed as percentage.

Final count

Seeds germinated by 72 h in the standard germination test were counted and expressed as percentage.

Rate of germination

Rate of germination was calculated as the ratio of the first count to the final count of germination and expressed as percentage.

$$\text{Rate of germination (\%)} = \frac{\text{First count of germination}}{\text{Final count of germination}} \times 100$$

Shoot length

Five normal seedlings were randomly selected from each replication of the standard germination test on the 14th day and length of shoots was measured from collar region to the tip of top most leaf and expressed in centimetre.

Root length

The length of roots was measured from collar region down to the tip of longest root in five samples per replication and expressed in centimetre.

Seedling dry weight

The seedlings used for measuring root and shoot lengths on 14th day were dried in hot air oven to constant weight and weighed. The total weight of seedlings in milligrams divided by number of seedlings gave average weight of each seedling and this was expressed in milligrams per seedling.

Vigour Index

Vigour Index (VI) was calculated by multiplication of the rate of germination with seedling dry weight.

VI = Rate of germination (%) X seedling dry weight (mg/seedling)

3.2 GENERATION AND EVALUATION OF NEW POPULATIONS

3.2.1 Parents for generating crosses

The genotypes used for the study comprised of high and low-vigour types obtained from the screened germplasm maintained at Agricultural Research Station, Mugad of University of Agricultural Sciences, Dharwad. The two high vigour genotypes used were Dodiga and Antarsali (A67). The Dodiga and A67 are land races with wide adaptability and suitable for upland conditions, where weeds and moisture stress are major problems. The low-vigour genotype was Samba Mahsuri (BPT5204), a late maturing, high yielding, good grain quality variety (Table 2). The new populations are expected to be useful for studies on other traits, viz., seed quality, grain type, plant height, flowering, tiller number and drought tolerance apart from early vigour.

Table 2. Information on parents of new population generated

3.2.2 Methods

The experiments were carried out during *Kharif* 2003, *Kharif* 2004 and Summer 2005.

3.2.2.1 *Kharif*, 2003

- a) Planting male and female parents was taken up in pots with different dates of sowing to ensure synchronization of flowering to effect the crosses.
- b) The following two crosses viz., BPT5204/Dodiga and BPT5204/A67 were effected, representing combinations of low and high-vigour types at the screen-house of ARS, Mugad.

Emasculation was done everyday during flowering season, after 4 PM by cutting top 1/3rd portion of each spikelet with scissors and removing six anthers with the help of forceps. Panicles with emasculated spikelets were covered with butter paper bag. Hand pollination was carried out next day morning between 7.30 to 10 AM and crossed panicles were continued to be covered with butter-paper bags to avoid out-crossing and properly labelled.

3.2.2.2 *Kharif*, 2004

The F₁ seeds of the two crosses were pre-germinated and carefully transplanted to the pots. The panicles were bagged to ensure self-pollination at the time of flowering. The F₂ seeds were harvested from true F₁ (based on morphology) plants and dried properly. The seeds were kept at 50^o C for five days to break dormancy.

Table 2. Information on parents of new population generated

Sl. No	Variety	Pedigree	Vigour	Duration (Days)	Seed character	Reaction to diseases	Grain Quality	<i>Height</i>	Yield	<i>Special features</i>
1	Sambha mahsuri (BPT 5204)	Bapatla IET 11521 GEB 24 X TN-1 X Mahsuri in 1986	Low	145-150	MS fine grain	Blast Susceptible	Excellent	Semidwarf	6-8 t/ha	TBP area, Irrigation required
2	A67	Land races from UAS, Dharwad, ARS, Mugad collection	High	135	MB	Blast Tolerant	Medium	Tall	3.5 t/ha	Drought tolerant, Highly adaptable
3	Dodiga	Land races from UAS, Dharwad, ARS, Mugad collection	High	130	MB	Durable resistance to blast	Medium	Tall	3.0 t/ha	Drought tolerant, Highly adaptable

3.2.2.3 Summer, 2005

The F_2 seeds were sown in polythene bags filled with soil and maintained till 28th day. The seedlings were carefully transplanted into field and maintained till harvest to obtain F_3 seeds.

3.2.3 Phenotyping of Parents and F_2 populations in laboratory

3.2.2.1 Root length (RL6)

The length of roots were measured from collar region down to the tip of longest root of the each seedling on sixth day after seeding (DAS) and expressed in centimetre (cm).

3.2.2.2 Shoot length (SL6)

The length of shoots were measured from collar region to the tip of top most leaf of each seedling on sixth DAS and expressed in centimetre (cm).

3.2.2.3 Root length (RL10) and Shoot length (SL10) of each seedling were measured on tenth DAS.

3.2.2.4 Root length (RL14) and Shoot length (SL14) of each seedling were measured on fourteenth DAS.

3.2.4 Phenotyping in Parents and F_2 populations in net house

The seeds of parents BPT5204, A67, Dodiga and the two F_2 populations, viz., BPT5204/A67 and BPT5204/Dodiga were sown in polythene bags filled with soil. Single seedling was maintained in each polythene bag. The following parameters were recorded at various stages.

3.2.4.1 Shoot length (SL14), shoot length (SL21) and shoot length (SL28)

The length of shoots was measured from soil surface to the tip of top most leaf of the each seedlings on fourteenth DAS, twenty first DAS and twenty eighth DAS and expressed in centimetre (cm).

3.2.4.2 Root length (RL28)

The length of roots was measured from collar region down to the tip of longest root of the each seedling on twenty eighth DAS and expressed in centimetre (cm).

3.2.5 Phenotyping in Parents and F_3 populations in laboratory

The F_2 plants grown in glasshouse were transplanted into field on 28th DAS. The F_3 seeds were harvested from the F_2 plants, which produced sufficient quantity of seeds.

The seeds were treated at 50°C for five days to break residual dormancy. The F_3 families were analysed for root and shoot lengths. Two replications of ten seeds each were germinated and observations on individual seedlings were taken and expressed as family means. Root length (RL10), shoot length (SL10), root length (RL14) and shoot length (SL14) observations were taken as described earlier on 10th and 14th DAS, respectively.

3.3 MOLECULAR ANALYSIS

The molecular analysis was carried out using SSR markers in parents, F_1 s and F_2 plants.

3.3.1 Isolation of genomic DNA

The genomic DNA was isolated from fresh leaves following C-TAB procedure with minor modifications as explained below.

3.3.1.1 C-TAB method of Plant DNA extraction (Appendix I)

Appendix I. Preparation of stocks for CTAB method of DNA Extraction

1. The 2 g of fresh leaf sample was taken and ground with liquid nitrogen to make fine powder
2. Added to prewarmed (65°C) extraction buffer (10 ml) in 50 ml centrifuge tube
3. Kept in water bath at 65°C for 10-15 min with intermittent mixing
4. Samples cooled to room temperature.
5. Equal volume of chloroform: isoamyl-alcohol added and mixed by inverting
6. Spun at 15,000 rpm for 5-10 min.
7. Took supernatant to fresh tube carefully
8. Equal volume of prechilled isopropanol added and mixed gently
9. Allowed for 1-2 h at –20°C/overnight
10. DNA was spooled or spun down at 15,000 rpm for 5-10 min
11. DNA spool or pellet re-suspended in Tris-EDTA

3.3.2 Quality-check for DNA

Extracted DNA samples were run through 0.8% agarose gel (1X TAE) and after series of dilutions all samples were brought to uniform concentration of 20-25 ng/μl. Then these samples were tested for amplification using RAPD primer and run through 1.4 % agarose gel (1X TAE).

3.3.3 Testing of polymorphism using SSR markers

The 42 SSR markers were chosen based on previous reports and used to assess polymorphism between parents. The details of primers are presented in Appendix II

Appendix II. List of primers used in the study

3.3.3.1 PCR reaction and conditions for the amplification of SSRs

The PCR conditions followed were according to information present in manual for microsatellites published by Mahyco Research Foundation, Hyderabad (MRF, 2003).

The standard PCR reagents in total volume of 15 μl were

Component	Quantity (μl)
Deionised distilled water	7.8
PCR buffer (10x)	1.5
MgCl ₂ (25 mM)	1.0
dNTP (mix) (10 mM each)	1.0
Forward primer (5 pM)	0.6
Reverse primer (5 pM)	0.6
DNA template (20-25 ng/μL)	1.5

Taq polymerase (1U/ μ L)	1.0
Total	15.0

3.3.3.2 PCR cycling

PCR reaction was carried out using model Master Cycler Gradient 5331 (Eppendorf, Germany). The cycler was programmed as under.

Step	Temperature ($^{\circ}$ C)	Duration HH:MM:SS	Cycles
Initial denaturation	94	00:05:00	1
Denaturation	94	00:00:30	} 35 cycles
Primer annealing	55	00:00:30	
Primer extension	72	00:01:00	
Complete primer extension	72	00:05:00	1
Dump	15	Until removed	1

3.3.3.3 Visualization of amplified products in agarose gel

One per cent agarose gel was casted in TAE (1x) buffer in a horizontal frame. Eight microlitre of amplified samples were loaded with loading dye, run under 70 V. After ethidium bromide staining, gels were viewed and documented in gel documentation system.

3.3.3.4 Polyacrylamide gel electrophoresis

Four per cent polyacrylamide gels (vertical) were used for better separation and visualization of PCR amplified microsatellite products. Since polyacrylamide gels have better resolution for amplified products, denaturing gels were casted in Sequi-Gen GT nucleic acid electrophoresis cell unit as per the protocol published by Mahyco Research Foundation, Hyderabad Manual (MRF, 2003).

Glass plates were prepared before making the gel solution. Both glass plates (outer glass plate inner glass plate) were cleaned thoroughly with warm water and detergent then with deionised water. Second rinse was given with alcohol.

Fresh binding solution was prepared by adding 3 μ L of bind silane to 1 ml of 0.5 % acetic acid in 95 % ethanol in a 1.5 mL micro-centrifuge tube. Mixture was poured on to the large glass plates and spread using tissue paper evenly on entire surface of the plate.

Repel silane (250 μ L) was added to 750 μ L of 0.5 % acetic acid in 95 % ethanol in a 1.5 mL micro-centrifuge tube. Mixture was poured on to the notched plate and spread using tissue paper.

3.3.3.5 Assembling and pouring the gel

Spacers of 0.4 mm thickness were placed along the side edges of bind silane treated surface. Large plate was put on small plate so that treated surfaces faced each other. It was taken care to see that spacers fit well against the edge of the plates, so that there were no gaps or leakage. Clamps were put on both sides in precision caster base for screening both sides so that there was no leakage from bottom side.

For casting each gel, 80 ml of 4 % acrylamide gel solution was prepared just prior to pouring. For each 80 mL of solution, 60 μ L of TEMED (N-N-N-N-Tetramethylethylene diamine) and 600 μ L of 10 % ammonium per sulphate (APS) were added to initiate the polymerization process. The contents were mixed gently by swirling, but bubbles were avoided. Before pouring, assembly was kept on the bench top so that it made 45 degree angle with bench top. Then gel was pushed from the bottom loading hole provided using 100 ml large syringe with maximum care to avoid air bubbles.

Shark tooth comb of 0.4 mm thickness (49 wells) was inserted with straight side facing the gel at the top of the gel. Care was taken to produce flat, 4-5 mm depth surface at the top side so as to facilitate insertion of comb and loading. Later assembly was kept for polymerization for 30-40 min.

3.3.3.6 Electrophoresis

- After the polymerization process, assembly was detached from the clamp and precision caster base and was placed in universal base against the back wall. IPC was locked to the base in vertical position by fitting stabilizer bar.
- TBE (5x) was poured in upper tank in the IPC unit and the rest was poured in bottom chamber (1.8 L of buffer was prepared fresh each time).
- Comb was removed and excess polyacrylamide gel was removed with a razor blade. Tissue paper was used to clean the glass plates with buffer.
- Air bubbles and nonpolymerised acrylamide on the top of gel were removed by squirting with 5x TBE.
- The gel was pre run to achieve gel surface temperature of approximately 45 to 50°C with following conditions of power pack settings. Temperature 50°C, constant power of 100 W.
- SSR loading dye (3x STR dye) was added to PCR products and samples were denatured by heating to 95°C for 4 min and immediately cooled on ice.
- After pre run, urea was flushed out from the well area using syringe and shark tooth comb was carefully inserted into the gel so that teeth were just touching the surface of gel.
- Five μ l of each samples were loaded into the wells for facilitating the sizing of the various alleles. 100 bp marker ladder was loaded after denaturing in the first and last wells.
- Gel was run using the same conditions as the pre run. It was run till the dye reached bottom of the gel.

3.3.3.7 Visualization of SSR bands by silver staining

After electrophoresis, clamps were loosened and buffer was removed. Glass plates were separated without damaging the gel. Gel was affixed to rectangular plate.

DNA fragments separated were detected following the staining protocol. Each solution was prepared in separate containers. The same solutions were used four times over a period of 48 h except for the developer, which was freshly prepared during the staining process.

- Gel was rinsed with distilled water 3 to 5 min and placed in a shallow tray.
- Gel was soaked in 2 L of 2 % acetic acid (fix solution) for 20 min.
- The gel was rinsed in water for 2 times each with 2 min.
- The gel was kept for staining with 2 litre of 1 per cent silver nitrate for 20 min.
- A quick water wash was given for 10-15 second.
- Developer solution was added to the tray and agitated until the bands appeared.

- Developer was removed and plate was placed in fixer or stop solution for 5 min.
- Gel was placed in 2 litres of impregnate solution for 15 min.
- Gel was given water wash for 5 minutes and kept for drying overnight.

All the steps were done with constant shaking.

The gels were scanned with BIO-RAD scanner.

3.4 STATISTICAL ANALYSIS

3.4.1 DH population

The percentage data (such as germination counts, etc., were arc-sine transformed before subjecting to statistical treatments. The analysis of variance (ANOVA) for the parameters observed was done using DESIGN software. The laboratory generated data were analysed applying completely randomized design (CRD). The genetic parameters and correlation matrix for vigour parameters were generated using SPAR computer package.

The estimates of phenotypic and genotypic co-efficient of variation were obtained by using formula given by Singh and Chaudhary, (1977).

$$PCV \% = \frac{\sqrt{V_p}}{\bar{X}} \times 100$$

$$GCV \% = \frac{\sqrt{V_g}}{\bar{X}} \times 100$$

Where, PCV = Phenotypic coefficient of variation

GCV = Genotypic coefficient of variation

V_p = Phenotypic variance

V_g = Genotypic variance

$\bar{X}$ = Mean

3.4.2 Phenotypic correlation

Phenotypic correlation co-efficients were estimated by using the formulae by Singh and Chaudhary (1977).

$$r_p = \frac{\text{Covp (x, y)}}{\sqrt{\text{Varp (x) Varp (y)}}}$$

Where,

r_p = Phenotypic correlation co-efficient

Covp (x, y) = Phenotypic covariance between characters x, y

Varp (x) = Phenotypic variance in character x

Varp (y) = Phenotypic variance in character y

3.4.3 Genotypic correlation

$$r_g = \frac{\text{Covg (x, y)}}{\sqrt{\text{Varg (x) Varg (y)}}}$$

Where,

r_g	=	Genotypic correlation co-efficient
$Covg(x, y)$	=	Genotypic covariance between characters x, y
$Varg(x)$	=	Genotypic variance in character x
$Varg(y)$	=	Genotypic variance in character y

The observed value of correlation coefficient was compared with the tabulated value for (n-2) degrees of freedom for test of significance.

3.4.4 Heritability and genetic advance

Heritability in broad-sense which is the heritable variation was computed using the following formula.

$$\text{Heritability } [h^2_{bs}] = \frac{V_G}{V_P} \times 100$$

Where,

V_G	=	Genotypic variance
V_P	=	Phenotypic variance

The extent of genetic advance (GA) to be expected from selecting five per cent of the superior progeny ($i = 2.06$) using the following formula.

$$GA = i\sigma_p \cdot h^2$$

Where,

i	=	Intensity of selection
σ_p	=	Phenotypic standard deviation
h^2	=	Broad sense heritability

Genetic advance as per cent mean (GAM) was calculated as follows.

$$GAM (\%) = \frac{GA}{\bar{X}} \times 100$$

Where,

$\bar{X}$ = Grand mean of the trait

3.4.5 Mapmaker

Mapmaker/EXP and Mapmaker/QTL are companion programs. The linkage analysis and map construction was done using Mapmaker/EXP, while chromosomal location of putative QTLs were determined by using Mapmaker/QTL program (Lander *et al.*, 1987; Lincoln *et al.*, 1992). The simple interval mapping was followed for identifying QTLs.

The phenotypes of double haploid lines were superimposed on already available genotypes to identify QTLs. The proportion of trait variation accounted for by each QTL was also estimated. The QTLs with LOD scores of 2.5 and above were considered as putative.

3.4.6 F_2 population

The phenotypic variance, covariance and standard deviation were calculated using MS-EXCEL program. The genotypic variance and covariance were calculated by subtracting

parental values from F_2 population values. The phenotypic and genotypic coefficients of variation, heritability and genetic advance were computed as explained above.

3.4.7 F_3 population

The analysis of variance (ANOVA) and correlation matrix for components were performed using SPAR1 programme. The laboratory generated data were analysed applying Completely Randomized Design (CRD). The estimates of phenotypic and genotypic coefficients of variation, heritability and genetic advance were calculated as explained above.

3.4.8 F_2 : F_3 population

The correlation between individual F_2 parameters observed at green house and their corresponding F_3 family mean parameters at laboratory were computed in MS-EXCEL and the regression was calculated for the significantly correlated traits. The regression gives narrow-sense heritability for the trait.

3.4.9 Realised heritability

The actual values were standardised before calculating realised heritability (Falconer, 1960)

Standardised value = Variable minus population mean divided by standard deviation

$$= \frac{X_i - \bar{X}}{SD}$$

Where,

X_i	=	Individual value
$\bar{X}$	=	Population mean
SD	=	Standard deviation

The mean of all standardised values is zero and standard deviation is 1.

$$H = R/S$$

S = Selection differential, i.e., Average of top 20% of F_2 genotypes

R = Response to selection, i.e., Average F_3 family means of corresponding F_2 genotypes.

3.4.10 Chi-Square test

The segregation pattern of the markers was studied using chi-square test. The SSR are co-dominant markers, hence the goodness of fit was tested for 1:2:1 segregation (Gomez and Gomez, 1984).

3.4.11 Single-Marker Analysis

The genotype of F_2 plants and phenotype of corresponding F_3 family were used to know association between markers and the trait. The analysis was done using IRRISTAT program. The percent phenotypic variance explained by association was calculated using linear regression approach.

IV. EXPERIMENTAL RESULTS

The investigations on DNA marker assisted genetic analysis of early vigour related traits in rice were conducted with a double haploid population (IR64/Azucena) and two $F_2:F_3$ populations, viz., BPT5204 (Low)/A67 (High) and BPT5204 (Low)/Dodiga (High) and their corresponding parents.

The results obtained are presented in following tables and figures.

4.1 IDENTIFICATION OF QTLs FOR EARLY VIGOUR RELATED TRAITS IN IR64/AZUCENA- DH POPULATION

4.1.1 Phenotyping

4.1.1.1. Analysis of variance (ANOVA)

The DH population showed highly significant variation for early vigour related parameters, viz., first count of germination, final count of germination, rate of germination, root length, shoot length, seedling dry weight and vigour index (Table 3).

Table 3. ANOVA for early vigour related traits in IR64/ Azucena – DH population

4.1.1.2 Components of variance

The details of different components of variance are furnished in Table 4. The IR64 parent was superior for first count of germination, final count of germination, rate of germination and shoot length with higher means. The Azucena had higher means for root length, seedling dry weight and vigour index. The DH means for counts and rate of germination were just higher than the high parent while the means for root length and shoot length were just lower than low parent. The means of seedling dry weight and vigour index fell within parental range. A much wide range beyond parental values was observed for all the traits. The magnitude of variation as revealed by PCV and GCV was moderate (10-20%) for all the traits except final count of germination (7.9 and 5.5%) and GCV for rate of germination (8.9%).

Table 4. Mean, range and genetic parameters for early vigour related traits in IR64/ Azucena DH population

The broad-sense heritability, which indicates heritable portion of the trait was high (70.1 to 81.1%) for all the traits except final count of germination (47.1%) for which it was moderate. The seedlings dry weight recorded highest broad sense heritability (81.1%) followed by shoot length, vigor index, root length, first count of germination and rate of germination.

The genetic advance was high for vigour index (28.94%) followed by root length (28.26%), seedling dry weight (27.20%), shoot length (24.63%), first count of germination (20.68%) and moderate for rate of germination (15.46%) and low for final count of germination (7.58%).

4.1.1.3 Pattern of distribution in DH population for different traits

The frequency distribution of DH lines for vigour parameters is depicted in Fig. 1. All the traits except final count of germination and rate of germination exhibited normal and nearly normal distribution. The normal distribution was observed for shoot length and seedling dry weight, while nearly normal distribution was shown by first count of germination, root length and vigour index. The distribution was skewed towards higher values for final count of germination and rate of germination. Significant transgressive segregants in both directions were observed for root length and shoot length, while beyond Azucena (high parent) for vigour index.

Fig 1. Frequency distribution of early vigour related parameter in IR 64 / Azucena - DH population

Table 3.ANOVA for early vigour related traits in IR64/Azucena DH population

Source of variance	DF	Mean sum of squares of traits [@]						
		FIRC (%)	FINC (%)	RGER (%)	RTLN (cm)	SHLN (cm)	SDWT (mg)	VI
Genotype	120	237.36**	81.68**	159.84**	11.60**	8.63**	15.47**	157433.60**
Error	120	41.73	29.40	32.05	1.79	1.27	1.61	23723.73
CV (%)		7.75	5.42	6.33	9.70	8.15	7.12	9.68

** Significant at 1% level of probability by 'F' test

@ FIRC- First count of germination RTLN- Root length
 FINC- Final count of germination SHLN- Shoot length

RGER- Rate of germination SDWT- Seedling dry weight

VI- Vigour index (RGER x SDWT)

Table 4. Mean range and genetic parameters for early vigour related traits in IR64/ Azucena DH population

Parameter@	Parents		DH population						
	IR64	Azucena	Minimum	Maximum	Population Mean	PCV (%)	GCV (%)	Heritability (bs)	GAM (%)
FIRC (%)	82.0	60.0	56.0	100.0	83.4	14.2	11.9	70.1	20.68
FINC (%)	93.0	81.0	66.0	100.0	93.3	07.9	05.5	47.1	07.58
RGER (%)	88.2	74.1	64.5	100.0	89.3	10.9	08.9	66.6	15.46
RTLN (cm)	14.0	17.1	08.5	22.1	13.8	18.7	16.0	73.2	28.26
SHLN (cm)	16.6	14.3	09.2	23.5	13.8	16.1	13.9	74.3	24.63
SDWT (mg)	14.8	21.8	12.0	22.3	17.8	16.4	14.7	81.1	27.20
VI	1305.4	1615.4	1073.4	2508.4	1590.9	18.9	16.3	73.8	28.94

@ Abbreviations as listed in Table 3

PCV - Phenotypic coefficient of variability

GCV - Genotypic coefficient of variability

GAM - Genetic advance over mean

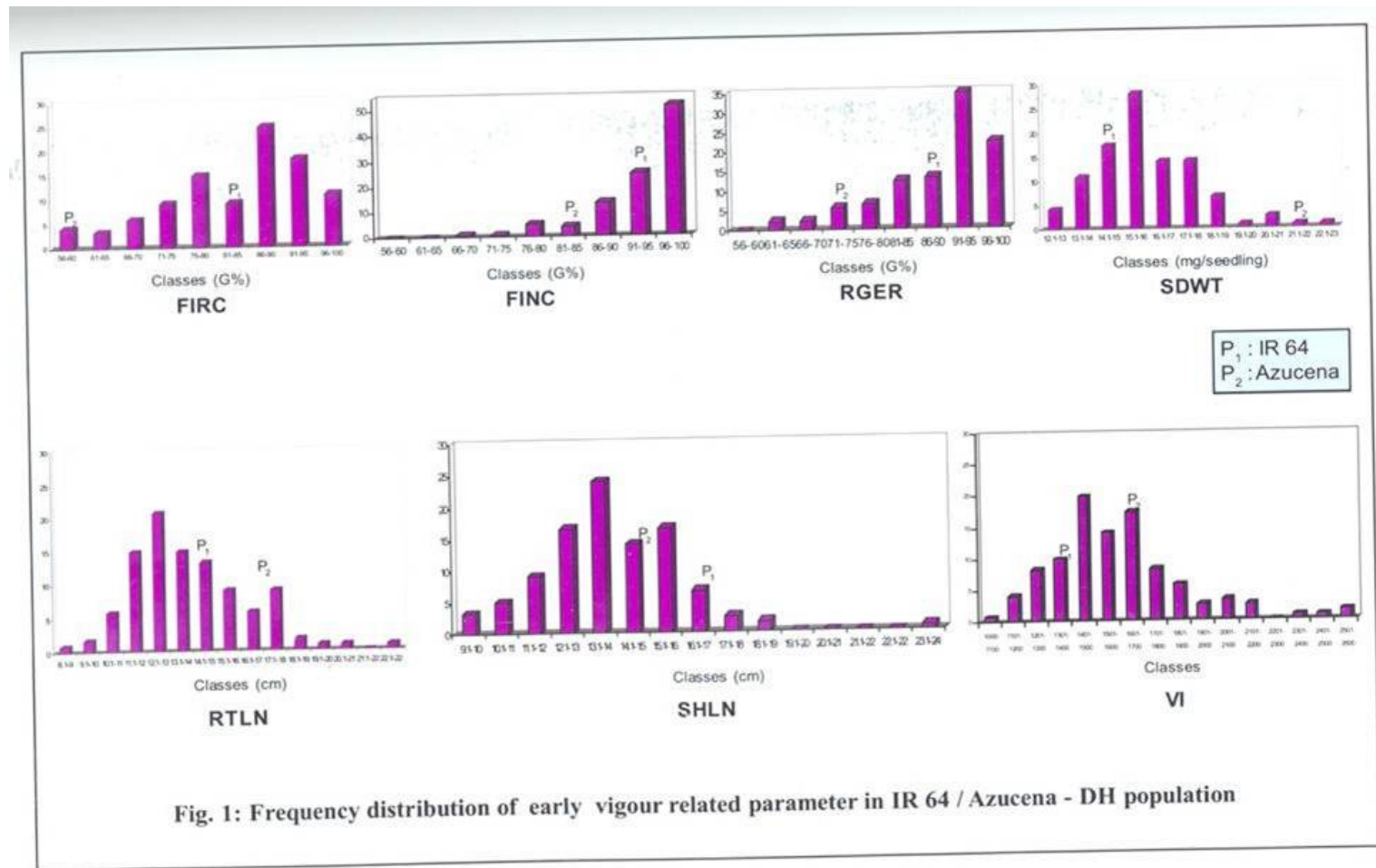


Fig. 1. Frequency distribution of early vigour related parameter in IR 64 / Azucena – DH population

4.1.1.4 Correlation among early vigour related traits

The phenotypic and genotypic correlation coefficients for early vigour related traits are furnished in Table 5. The correlation was positive and significant among counts and rate of germination. The first count of germination, rate of germination and seedling dry weight were significantly correlated with vigour index.

Table 5. Phenotypic and genotypic correlations among early vigour related traits in IR64/ Azucena - DH population

4. 2 QTL ANALYSIS BY INTERVAL MAPPING

The QTLs with flanking SSR markers were identified through interval mapping using MAPMAKER program at a threshold LOD score of 2.5 for all the traits. The QTLs associated with six vigour related traits along with percent phenotypic variance, LOD score and additive effect are presented in Table 6. The chromosomal position of QTLs is depicted in Fig 2. As many as 22 QTLs were identified for different vigour related traits and they were distributed on chromosomes 1 to 6.

Fig 2. QTL map of early vigour related traits in IR 64 / Azucena DH population

Table 6. QTLs associated with early vigour related traits in IR64 /Azucena-DH population identified by interval mapping (MAPMAKER/QTL v. 1. 1)

4. 2.1 First count of germination (FIRC)

Two QTLs were identified contributing to this trait. These were located on chromosome 1 (RM5-RM306) near to RM5 with phenotypic variance 12.3% and major QTL on chromosome 2 (RM341-RM106) near to RM106 with higher phenotypic variance (49.4%) and LOD score 2.8.

4.2.2 Final count of germination (FINC)

Single QTL was identified on Chromosome 1 (RM5-RM306) near to RM5 with 2.5 LOD score and 10.5% phenotypic variance.

4. 2. 3 Rate of germination (RGER)

Five QTLs were distributed over four chromosomes (2, 3, 4 and 5). Among these one was major gene and others were major QTLs. The QTL on chromosome 2 (RM341-RM106) has peak LOD of 4.5 and phenotypic variance of 56.2%. Among the two QTLs identified on Chromosome 4, the region between RM307-RM335 had higher LOD score (3.1) and phenotypic variance (32.4%) with its position more near to RM307. All the QTLs exhibited negative additive effects.

4. 2. 4 Shoot length (SHLN)

All the six QTLs were located on chromosome 1. The QTL (RM243-RM23) near to RM243 exhibited higher LOD score of 3.1 and 15.1% phenotypic variance. The region RM84 to RM86 contained three QTLs. The additive effect of these QTLs was negative.

4.2.5 Seedling dry weight (SDWT)

Three QTLs were identified for this trait and were located on Chromosome 2 and 5. The major QTL (RM327-RM27) located near RM327 on Chrom 2 exhibited high phenotypic variance 40.2% with LOD score 2.6. The additive weight (2.97) for this QTL was positive. However, QTL (RM87-RM334) near to RM87 on chromosome 5 contributed 17.1% to phenotypic variance with LOD 2.9. The additive effect was negative for this QTL.

4. 2. 6 Vigour Index (VI)

A total of five QTLs were identified, two QTLs each located on chromosome 5 and 6, one QTL on chromosome 2. The contribution of major QTL (RM3-RM162) on chromosome 6 was highest with phenotypic variance 39.2% and LOD score 4.4. The position of QTL was 8

Table 5. Phenotypic and genotypic correlations among early vigour related traits in IR64/Azucena - DH population

Parameters @	FIRC	FINC	RGER	RTLN	SHLN	SDWT	VI
FIRC	1.00						
	1.00						
FINC	0.64**	1.00					
	0.71**	1.00					
RGER	0.82**	0.09	1.00				
	0.90**	0.33**	1.00				
RTLN	0.17	0.13	0.14	1.00			
	-0.20	0.13	-0.19	1.00			
SHLN	0.00	0.03	0.03	0.01	1.00		
	0.03	0.02	0.05	0.07	1.00		
SDWT	0.10	0.03	0.12	0.02	0.05	1.00	
	0.14	0.09	0.15	0.01	0.05	1.00	
VI	0.41**	0.04	0.49**	0.05	0.02	0.80**	1.00
	0.39**	0.14	0.42**	0.09	0.01	0.83**	1.00

@ Abbreviations as indicated in Table 3

** Significant at 1% level of probability
 Bold letters indicate genotypic correlation

Table 6: QTLs associated with early vigour related traits in IR64/Azucena- DH population identified by interval mapping (MAPMAKER/QTL v. 1. 1)

Trait @	Marker Interval	Chrom No.	Peak LOD	Phenotypic Variance (%)	QTL position	Distance between the markers (cM)	Additive effect	No. of QTLs
SHLN	RM 84- RM323	1	2.8	13.1	0.0	01.5	-1.08	6
	RM323- RM220	1	2.8	13.3	0.0	01.6	-1.12	
	RM220-RM86	1	2.6	12.2	0.0	01.1	-1.11	
	RM272-RM259	1	2.7	13.7	8.0	08.3	-1.29	
	RM259-RM243	1	2.7	13.7	0.0	00.8	-1.30	
	RM243-RM23	1	3.1	15.1	0.0	01.3	-1.35	
SDWT	RM327-RM27	2	2.5	40.2 (MQ)	0.0	43.9	2.97	3
	RM153-RM159	5	2.6	13.8	0.0	01.3	-1.13	
	RM87-RM334	5	2.9	17.1	0.0	06.5	-1.79	
FIRC	RM5-RM306	1	2.6	12.3	0.0	02.8	0.02	2
	RM341-RM106	2	2.8	49.4 (MQ)	36.0	53.3	-0.05	
FINC	RM5-RM306	1	2.5	10.5	0.0	02.8	0.01	1
RGER	RM341-RM106	2	4.5	56.2 (MG)	28.0	53.3	-8.74	5
	RM231-RM7	3	2.6	47.8 (MQ)	16.0	41.1	-9.20	
	RM307-RM335	4	3.1	32.4 (MQ)	0.0	16.1	-10.35	
	RM303-RM317	4	2.5	25.5 (MQ)	0.0	01.3	-7.99	
	RM87-RM334	5	2.5	28.0 (MQ)	6.0	06.5	-8.98	
VI	RM174-RM145	2	2.5	16.3	0.0	02.6	-191.40	5
	RM178-RM26	5	2.6	18.2	4.0	10.7	-196.60	
	RM87-RM334	5	2.5	16.0	0.0	06.5	-196.20	
	RM3-RM162	6	4.4	39.2 (MQ)	8.0	20.0	356.03	
	RM136-RM3	6	3.6	28.5 (MQ)	24.0	25.1	335.30	

@ Abbreviations as listed in Table 4.1

Bold letters indicate common genomic regions controlling different traits

MQ- Major QTL (25-50% phenotypic variance)

MG- Major gene (> 50% phenotypic variance)

cM away from RM3. The additive effects were both positive and negative for QTLs controlling the trait.

The congruence of QTLs was observed on chromosome 5 in the region RM87-RM334 for three traits, viz., rate of germination, seedling dry weight and vigour index. The QTLs for both first count of germination and rate of germination were located near to RM106 on chromosome 2.

4.3 GENERATION OF NEW POPULATIONS

4.3.1 Choice of parents

The parents BPT5204, Dodiga and A67 were differing for various agronomical as well as seed quality characters apart from early vigour related traits. The ovule parent (BPT5204) is a low vigour, semi-dwarf, late maturing variety with good grain quality. The pollen parents (A67 and Dodiga) are land races adapted to upland rice cultivation and characterized by high vigour, tall and medium duration with moderate grain quality. The performance of parental lines shown in Figure 3.

4.3.2 Phenotyping F_2 population along with parents in laboratory for early vigour related traits

The phenotyping of 350 F_2 seedlings of BPT5204/A67 and 320 F_2 seedlings of BPT5204/ Dodiga along with 25 seedlings of each parent was carried out and results are furnished in Table 7. The early vigour related parameters comprised of root length and shoot length taken at 6th, 10th and 14th DAS.

Table 7. Mean, range and genetic components for early vigour related traits in F_2 population of BPT5204/ A67 and BPT5204/ Dodiga

4.3.2.1 Parents

The BPT5204 has exhibited distinctly low means for all the parameters as compared to A67 and Dodiga. The CV was high at initial stage (RL6 and SL6) and decreased in later stages.

4.3.2.2 F_2 population

The F_2 means were in between two parental means for all the traits in both the populations. The range was wide and more number of classes found in F_2 compared to parents (Table 7 and Figures 3 & 4). The genetic variation was substantial for all the traits in both the populations. The PCV and GCV values for the two traits, viz., RL6 and SL6 were highest followed by RL10 & SL10 and RL14 & SL14 in both the populations. Heritability was high and ranged from 80 to 87% for different parameters in the two populations except for SL6 in BPT5204/A67 (69%) and BPT5204/Dodiga (59%). The genetic advance was high (30.19 to 63.18%) for different traits revealing substantial genotypic variation in the two populations.

Fig 3. Performance of parental lines under laboratory (A) and net house(B and C) for early vigour

Fig 4. Variability for root and shoot length in F_2 individuals of BPT-5204 / A67 under lab and net house conditions

4.3.2.3 Pattern of distribution for root and shoot lengths

The frequency distribution for root and shoot length parameters at various stages in parents and F_2 s is depicted in Figures 6 & 7. The individuals spread over less number of classes in parents while more number of classes in F_2 population. The normal, nearly normal and slight bimodal distribution was observed for different parameters in parents and F_2 populations.

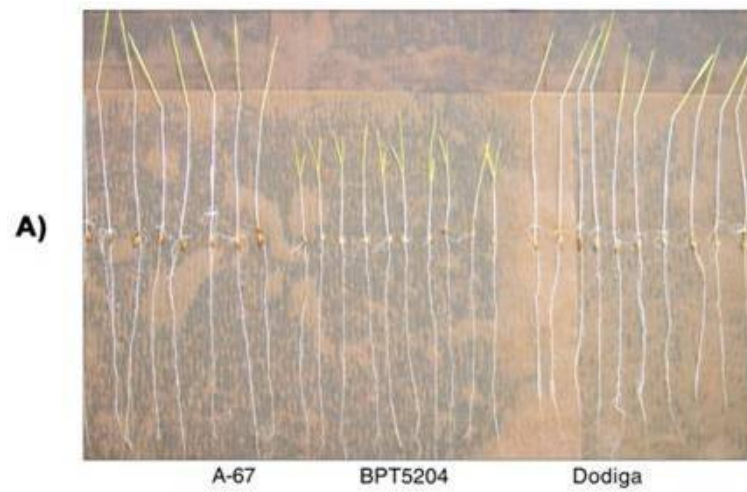


Fig. 3. Performance of parental lines under laboratory (A) and net house (B and C) for early vigour

Table 7. Mean range and genetic components for early vigour related traits in F₂ population of BPT5204/ A67 and BPT5204/ Dodiga

POPULATION	COMPONENTS	RL6	RL10	RL14	SL6	SL10	SL14
BPT5204/A67 F ₂ N= 350	Mean (cm)	9.97	13.75	14.40	5.41	11.89	12.72
	Min (cm)	1.50	1.50	2.80	0.50	0.50	2.50
	Max (cm)	15.50	21.80	22.00	11.00	20.50	20.50
	PCV (%)	35.50	22.10	18.47	36.57	28.75	21.70
	GCV (%)	33.08	20.13	16.74	30.47	26.57	19.50
	Herit (bs)	86.0	83.00	83.00	69.00	85.00	80.00
	GAM(%)	63.18	37.90	31.45	51.95	50.58	35.97
BPT5204/Dodiga F ₂ N=320	Mean (cm)	10.47	14.88	16.20	5.58	13.05	14.23
	Min (cm)	1.50	2.50	2.80	1.50	2.00	2.50
	Max (cm)	16.00	23.00	24.00	10.10	20.80	22.40
	PCV (%)	33.82	22.98	17.34	30.99	25.29	19.26
	GCV (%)	31.43	21.36	15.92	23.82	23.22	17.22
	Herit (bs)	86.00	87.00	84.0	59.00	84.00	80.00
	GAM (%)	59.99	41.08	30.19	37.88	43.90	31.76
PARENTS BPT5204 N=25 A67 N=25 Dodiga N=25	Mean (cm)	7.09	9.78	11.48	3.00	7.36	8.35
	CV (%)	17.06	10.73	7.66	22.66	18.48	10.89
	Mean (cm)	13.12	15.76	17.14	8.01	14.86	17.09
	CV (%)	10.14	8.62	7.64	16.98	7.94	8.19
	Mean (cm)	12.50	18.18	19.32	6.46	16.98	18.26
	CV (%)	11.28	7.15	5.64	17.37	8.12	7.11

RL6 - Root length at 6th DAS SL6 - Shoot length at 6th DAS
 RL10 - Root length at 10th DAS SL10 - Shoot length at 10th DAS
 RL14 - Root length at 14th DAS SL14 - Shoot length at 14th DAS



Fig. 4. Variability for root and shoot length in F_2 individuals of BPT 5204 / A67 under lab and net house conditions

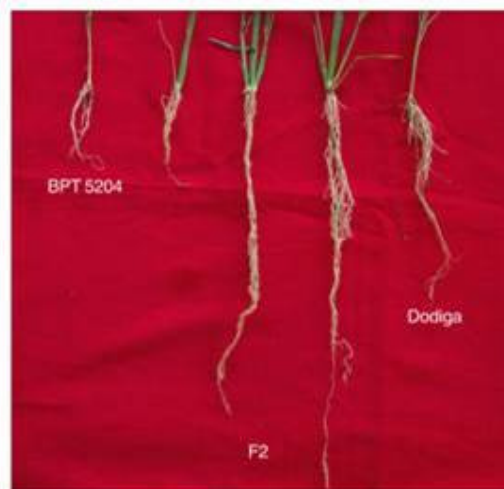


Fig. 5. Variability for root and shoot length in F_2 individuals of BPT 5204 / Dodiga under lab and net house conditions

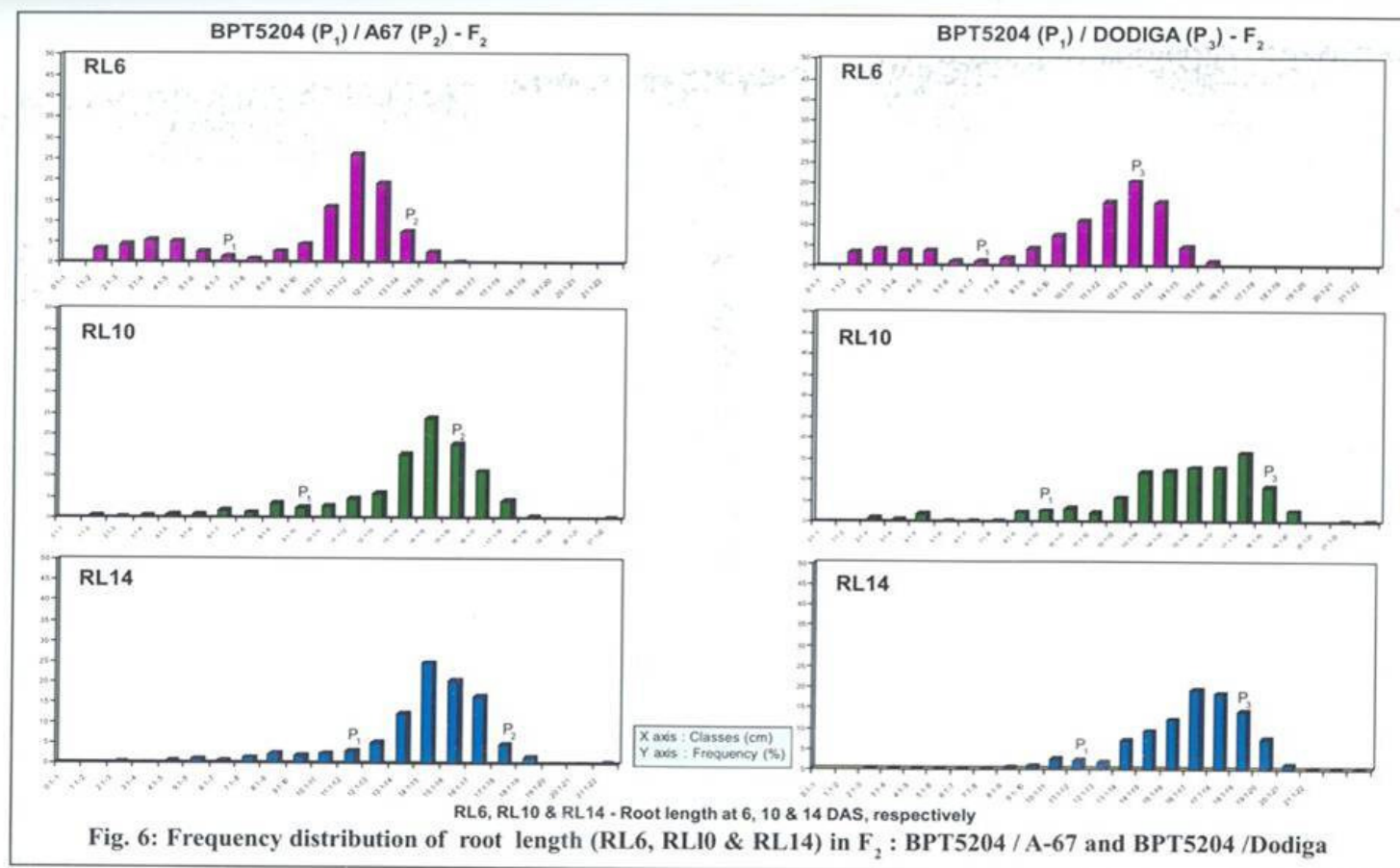


Fig. 6: Frequency distribution of root length (RL6, RL10 & RL14) in F₂ : BPT5204 / A-67 and BPT5204 / Dodiga

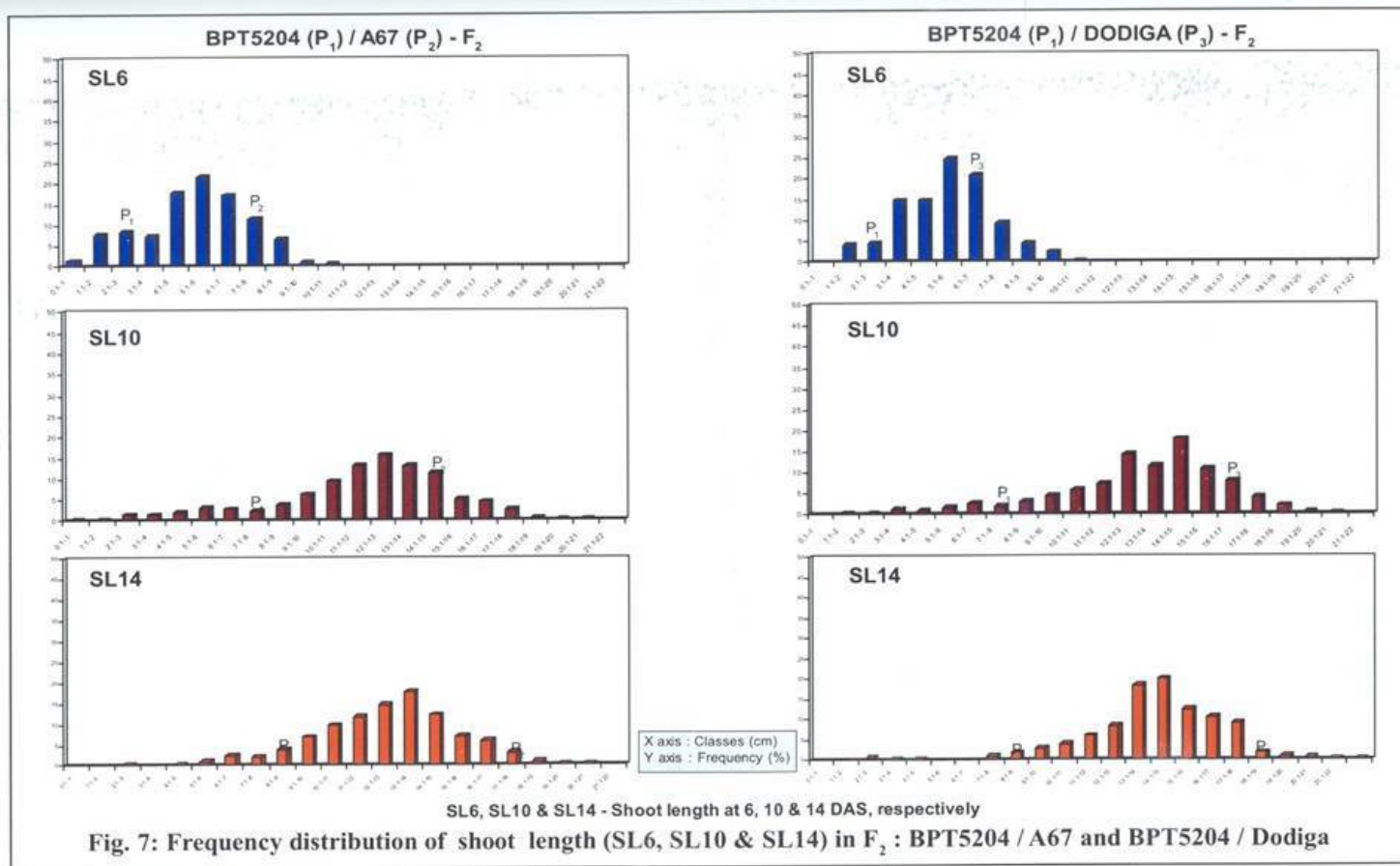
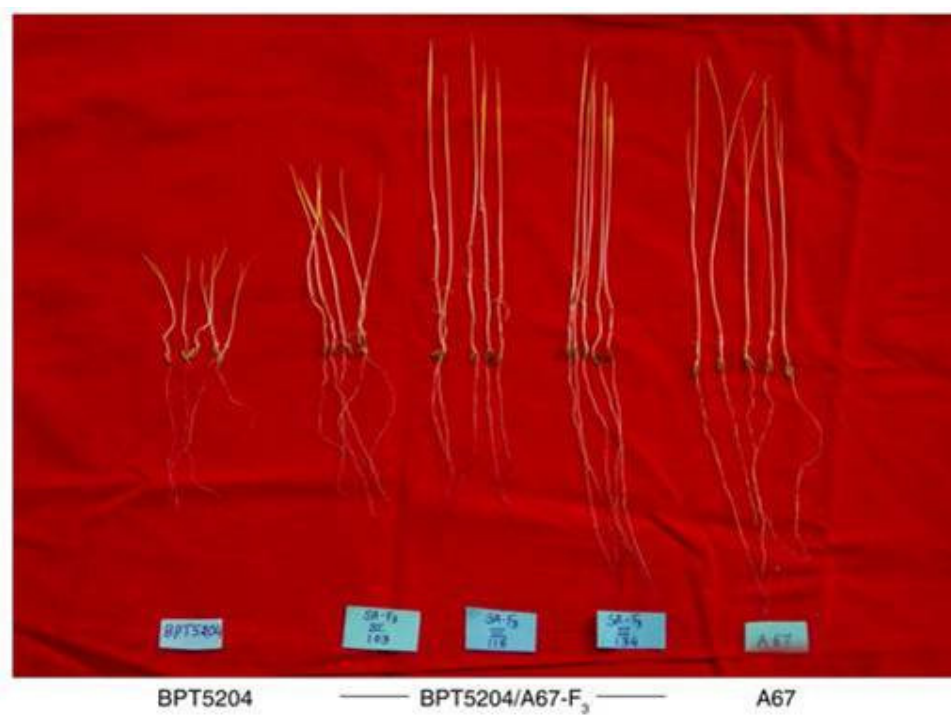


Fig. 7: Frequency distribution of shoot length (SL6, SL10 & SL14) in F_2 : BPT5204 / A67 and BPT5204 / Dodiga



A)



B)

Fig 8. Variability for root and shoot length in F_3 families of BPT 5204 / Dodiga (A) and BPT2504 / A67 (B) under laboratory conditions

Table 8. ANOVA for early vigour related traits in parents and F₃ population of BPT5204/ A67 cross

Source	df	Mean sum of squares for [@]			
		RL10	SL10	RL14	SL14
Genotype	108	6.01**	14.37**	8.88**	7.94**
Error	108	1.27	0.94	1.11	0.90
CV (%)		9.71	8.08	7.88	6.81

** Significant at 1% level of probability by 'F' test

Table 9. ANOVA for early vigour related traits in parents and F₃ population of BPT5204/ Dodiga cross

Source	df	Mean sum of squares for [@]			
		RL10	SL10	RL14	SL14
Genotype	73	9.8**	9.82**	13.44**	8.25**
Error	73	1.17	0.86	1.46	1.16
CV (%)		9.38	9.77	7.93	7.78

** Significant at 1% level of probability by 'F' test

@ RL10- Root length measured at 10th DAS
 SL10- Shoot length measured at 10th DAS
 RL14- Root length measured at 14th DAS
 SL14- Shoot length measured at 14th DAS

Table 10. Mean range and genetic parameters for early vigour related traits in F₃ population of BPT5204/ A67

Parameter @	Mean				Variability					
	BPT5204	A67	Population Mean	CD (5%)	Range		PCV (%)	GCV (%)	Heritability (bs)	GAM(%)
					Min	Max				
RL10 (cm)	10.79	14.95	11.62	2.23	7.37	16.98	16.43	13.25	65.0	22.03
SL10 (cm)	8.25	15.80	12.04	1.92	6.86	18.42	22.99	21.52	87.6	41.52
RL14 (cm)	13.37	16.82	13.33	2.08	8.84	18.98	16.75	14.78	77.9	26.85
SL14 (cm)	10.12	17.88	13.88	1.87	7.69	19.09	15.15	13.53	79.7	24.85

@ Abbreviations as listed in Table 9

Table 11. Mean, range and genetic parameters for early vigour related traits in F₃ population of BPT5204/ Dodiga

Parameter @	Mean				Variability					
	BPT5204	Dodiga	Population Mean	CD (5%)	Range		PCV (%)	GCV (%)	Heritability (bs)	GAM(%)
					Min	Max				
RL10 (cm)	10.79	14.29	11.56	2.16	7.06	15.67	20.26	17.96	78.5	32.78
SL10 (cm)	8.25	17.42	9.50	1.85	6.19	17.42	24.33	22.28	83.9	42.00
RL14 (cm)	13.37	16.13	15.26	2.41	10.19	21.41	17.89	16.03	80.3	29.61
SL14 (cm)	10.12	19.35	13.84	2.14	10.12	19.35	15.67	13.60	75.3	24.34

@ Abbreviations as listed in Table 9

Fig 6. Frequency distribution of root length (RL6, RL10 & RL14) in F_2 : BPT5204 / A-67 and BPT5204 /Dodiga

Fig 7. Frequency distribution of shoot length (SL6, SL10 & SL14) in F_2 : BPT5204 / A67 and BPT5204 / Dodiga

4.3.3 F_3 Population

Hundred and seven F_3 families of BPT5204/A67 and seventy two F_3 families of BPT5204/ Dodiga along with parents were evaluated for early vigour related parameters, viz., root length at 10th day (RL10), shoot length at 10th DAS (SL10), root length at 14th DAS (RL14) and shoot length at 14th DAS (SL14). The performance is depicted in Figure 8.

Fig 8. Variability for root and shoot length in F_3 families of BPT 5204 / Dodiga (A) and BPT 5204 / A-67 (B) under laboratory conditions

4.3.3.1 Analysis of Variance (ANOVA)

The Analysis of Variance (ANOVA) revealed significant differences for all the traits in both the populations (Tables 8 & 9).

Table 8. ANOVA for early vigour related traits in parents and F_3 population of BPT5204/ A67 cross

Table 9. ANOVA for early vigour related traits in parents and F_3 population of BPT5204/ Dodiga cross

4. 3.2 Components of Variance

The range, mean, PCV, GCV, heritability, and GAM are presented in (Tables 10 & 11).

Table 10. Mean, range and genetic parameters for early vigour related traits in F_3 population of BPT5204/ A67

Table 11. Mean, range and genetic parameters for early vigour related traits in F_3 population of BPT5204/ Dodiga

The mean of BPT5204 for all the parameters were significantly low compared to A67 and Dodiga. Shoot length at 10th DAS (SL10) was high (12.04) in BPT5204/A67 as compared to BPT5204/Dodiga (9.50), while it was on par at 14th DAS (SL14). Root length at 10th day was comparable in two populations but higher at 14th day in BPT5204/ Dodiga (15.26) as compared to BPT5204/A67 (13.33). The range was wider and beyond parents for all the parameters in BPT5204/A67 and root parameters in BPT5204/Dodiga.

The magnitude of variation as revealed by PCV and GCV was high (21.52-24.33) for SL10 in both the crosses, while it was moderate (13.25- 20.26) for other traits, viz., RL10, RL14 and SL14 in both the populations.

Heritability was high (> 60%) for all the parameters but highest heritability was recorded for SL10 (87.6 and 83.90) populations, respectively. Similarly genetic advance was also highest (41.52 and 42.00) for SL10 in BPT5204/A67 and BPT5204/Dodiga respectively. The GAM for other parameters was also high (22.03- 32.78) in both the populations.

The frequency distribution of F_3 families for root and shoot length in both the populations is shown in Figures 9 & 10. The pattern of distribution was normal and nearly normal in BPT5204/A67 but not in BPT5204/Dodiga for different traits. The distribution was bimodal for root length at both the stages and skewed towards lower values for shoot traits in BPT5204/Dodiga.

Fig 9. Frequency distribution for morphological traits in BPT5204/A67 F_3 families (P_1 - BPT5204 P_2 - A67)

Fig 10. Frequency distribution for morphological traits in BPT5204/Dodiga F_3 families (P_1 - BPT5204 P_2 - Dodiga)

4. 3. 3 Association studies

4. 3. 3. 1 Association among the traits in F_3

The Phenotypic/ Genotypic correlation coefficients among root and shoot lengths recorded at different days, viz., Root length at 10th DAS (RL10), Shoot length at 10th DAS (SL10), Root length at 14th DAS (RL14) and Shoot length at 14th DAS (SL14) in both the populations are presented in Tables 12 and 13.

Table 12. Phenotypic/ Genotypic correlation in BPT5204/A67 F_3 families

Table 13. Phenotypic/ Genotypic correlation in BPT5204/Dodiga F_3 families

The association was positive and significant in root length and shoot length measured at different stages (10th DAS and 14th DAS) in both the crosses. Though correlation was positive between root and shoot length at 10th DAS (RL10 and SL10), but non-significant at 14th DAS in both the crosses.

4. 3. 3. 2 Association among morphological traits in $F_2:F_3$

The correlation among root and shoot traits in F_2 observed in net-house and F_3 observed in laboratory are furnished for BPT5204/A67 (Table 14) and BPT5204/Dodiga (Table 15) crosses. The positive and significant correlation was found among shoot length parameters measured at different stages within F_2 and F_3 generations in both the crosses. Shoot length at 28th DAS in F_2 was significantly associated with shoot length at 10th DAS but not at 14th DAS in F_3 generation. Correlation was not significant for root length between two generations.

Table 14. Phenotypic correlations in $F_2:F_3$ family of cross BPT5204/ A67

Table 15. Phenotypic correlations in $F_2:F_3$ family of cross BPT5204/ Dodiga

4.3.4 Heritability

The narrow-sense heritability as an estimate of standardized coefficient of regression and realized heritabilities were computed between SL28 F_2 -SL10 F_3 in two crosses (Table 16). The narrow sense heritability was slightly higher (29.2) in BPT5204/A67 than BPT5204/Dodiga (24.1). In contrast, realized heritability was higher in BPT5204/Dodiga (48.7) compared to BPT5204/A67 (15.5).

Table 16. Heritability estimates of shoot length trait in $F_2:F_3$ analysis

4. 4 GENOTYPING

4.4.1 Parents

The parents were screened for 42 SSR primers distributed across 11 chromosomes (except chromosome No. 10). The parents BPT5204 and Dodiga were polymorphic for 24 (57.14 %) primers, while BPT5204 and A67 were polymorphic for 20 (47.61 %) primers (Table 17).

Table 17. Polymorphism for different SSR loci in the parental lines of crosses studied (P- Polymorphic, M- Monomorphic)

4.4.2 F_2 generation

Ten plants each of high and low vigour segregants in F_2 were assessed individually and in bulks for SSRs that were polymorphic between parents. The markers RM178 and RM163 in BPT5204/Dodiga and RM220 and RM253 in BPT5204/A67 were screened to assess association between marker and early vigour (Figures 11 and 12).

Fig 11. Simple sequence repeat (SSR) patterns in Parents, F_1 and F_2 of the cross BPT 5204 / A-67

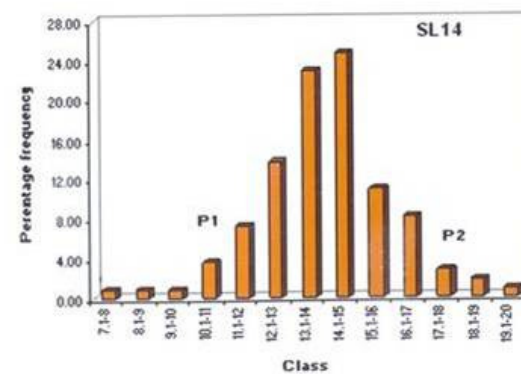
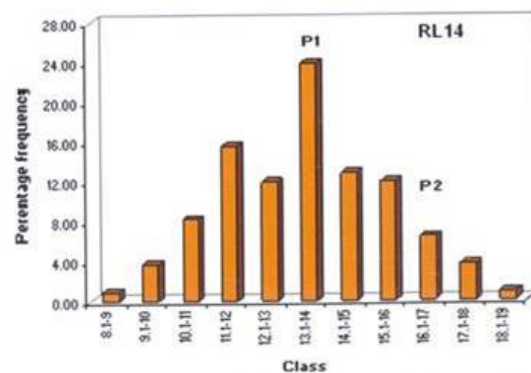
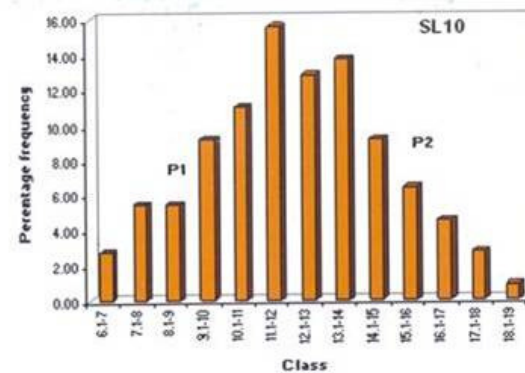
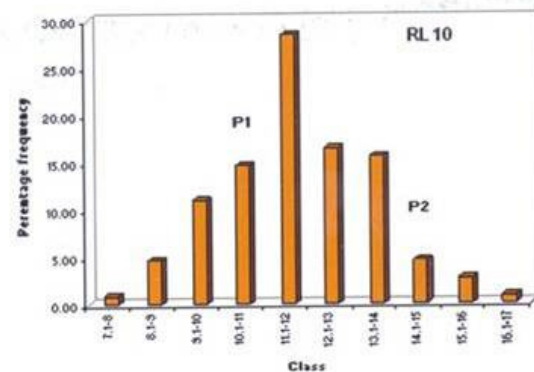


Fig. 9: Frequency distribution for morphological traits in BPT5204/A67 F_3 families (P_1 - BPT5204, P_2 - A67)

Fig. 9: Frequency distribution for morphological traits in BPT5204 / A67 F_3 families (P_1 – BPT5204, P_2 – A67)

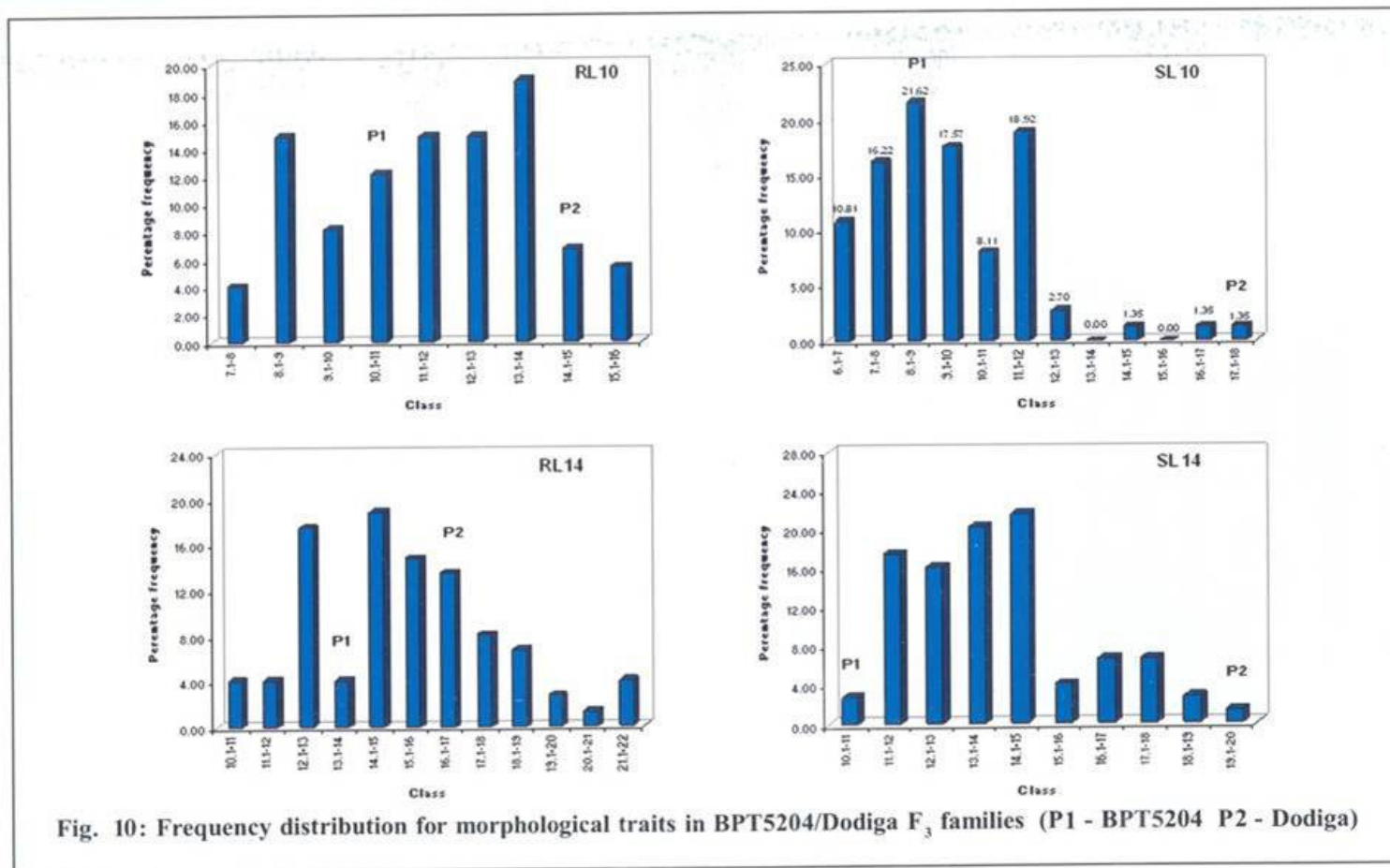


Fig. 10: Frequency distribution for morphological traits in BPT5204 / Dodiga F₃ families (P1 – BPT2504 P2 – Dodiga)

Table 12: Phenotypic/ Genotypic correlation in BPT5204/A67 F₃ families

Parameters @	RL10	SL10	RL14	SL14
RL10	1.00			
	1.00			
SL10	0.26**	1.00		
	0.26**	1.00		
RL14	0.76**	-0.16	1.00	
	0.75**	-0.25**	1.00	
SL14	0.19*	0.76**	-0.01	1.00
	0.22*	0.79**	-0.08	1.00

*, ** Significant at 5% and 1% level of probability by 'F' test
 Bold letters indicate genotypic correlation

Table 13. Phenotypic/ Genotypic correlation in BPT5204/ Dodiga F₃ families

Paramete @	RL10	SL10	RL14	SL14
RL10	1.00			
	1.00			
SL10	0.43**	1.00		
	0.42**	1.00		
RL14	0.78**	0.11	1.00	
	0.81**	0.10	1.00	
SL14	0.07	0.63**	0.09	1.00
	0.02	0.63**	0.04	1.00

** Significant at 1% level of probability by 'F' test
 Bold letters indicate genotypic correlation

@ Abbreviations as mentioned in Table 9

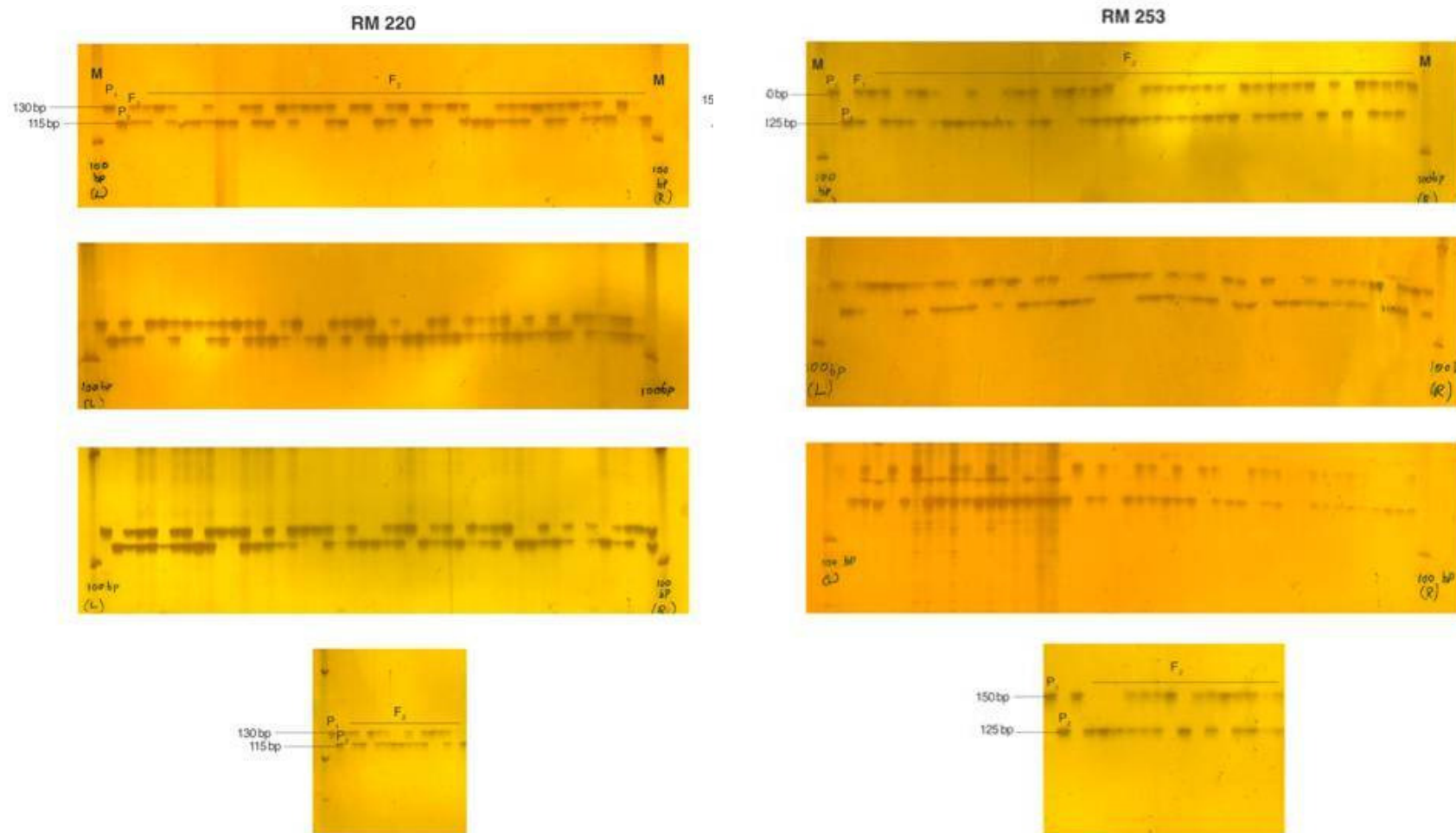


Fig. 11. Simple sequence repeat (SSR) patterns in Parents, F_1 and F_2 of the cross BPT 5204 / A-67

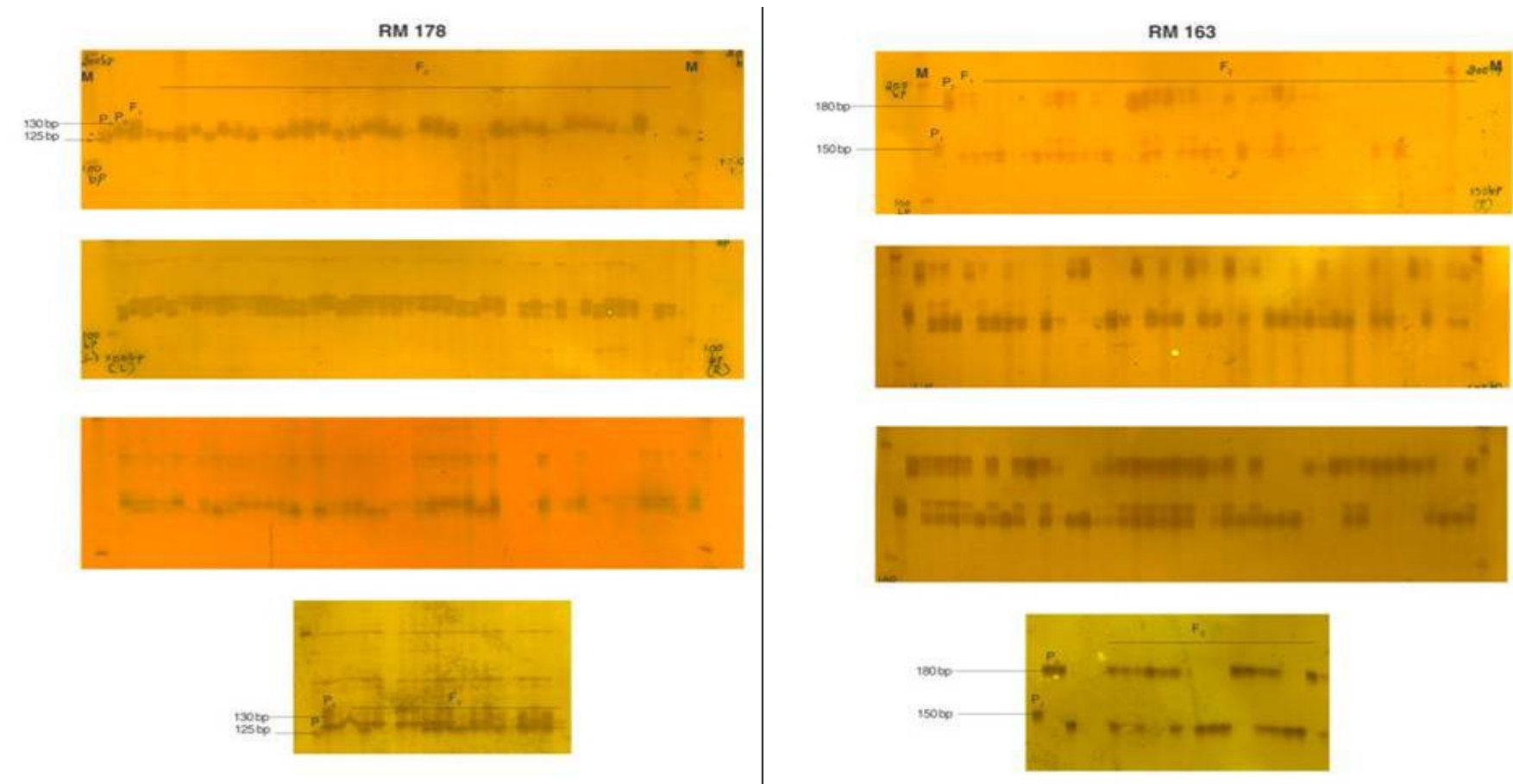


Fig. 12. Simple sequence repeat pattern (SSR) in parents F₁, F₂ of the cross BPT 5204 / Dodiga

Table 14 Phenotypic correlations in F₂:F₃ family of cross BPT5204/ A67

Parameters @	SL14-F ₂	SL21-F ₂	SL28-F ₂	RL28-F ₂	RL10-F ₃	SL10-F ₃	RL14-F ₃	SL14-F ₃
SL14-F ₂	1.00							
SL21-F ₂	0.71**	1.00						
SL28-F ₂	0.61**	0.62**	1.00					
RL28-F ₂	0.33**	0.16	0.58**	1.00				
RL10-F ₃	0.08	0.07	0.10	0.16	<i>1.00</i>			
SL10-F ₃	0.04	0.14	0.30**	0.34**	<i>0.25**</i>	<i>1.00</i>		
RL14-F ₃	0.05	0.04	0.11	0.06	<i>0.80**</i>	<i>0.16</i>	<i>1.00</i>	
SL14-F ₃	0.08	0.10	0.17	0.15	<i>0.16</i>	<i>0.76**</i>	<i>0.03</i>	<i>1.00</i>

Bold letters indicate correlations within F₂

Italic letters indicate correlations within F₃

Bold italic letters indicate correlations between F₂:F₃

Table 15. Phenotypic correlations in F₂:F₃ family of cross BPT5204/ Dodiga

Parameters @	SL14-F ₂	SL21-F ₂	SL28-F ₂	RL28-F ₂	RL10-F ₃	SL10-F ₃	RL14-F ₃	SL14-F ₃
SL14-F ₂	1.00							
SL21-F ₂	0.72**	1.00						
SL28-F ₂	0.78**	0.80**	1.00					
RL28-F ₂	0.51**	0.60**	0.68**	1.00				
RL10-F ₃	0.15	-0.21	0.04	0.12	<i>1.00</i>			
SL10-F ₃	0.12	0.18	0.27*	0.18	<i>0.42**</i>	<i>1.00</i>		
RL14-F ₃	-0.27*	-0.27*	-0.21	0.07	<i>0.79**</i>	<i>0.11</i>	<i>1.00</i>	
SL14-F ₃	0.06	0.22	0.11	0.01	<i>0.01</i>	<i>0.61**</i>	<i>0.04</i>	<i>1.00</i>

Bold letters indicate correlations within F₂

Italic letters indicate correlations within F₃

Bold italic letters indicate correlations between F₂:F₃

@ SL14 - Shoot length at 14th DAS

SL21 - Shoot length at 21st DAS

SL28 - Shoot length at 28th DAS

RL28 - Root length at 28th DAS

*, ** - Significant at 5% and 1% level of probability

Table 16. Heritability estimates of shoot length trait in $F_2:F_3$ analysis

Cross	Narrow sense heritability (%)	Realised heritability (%)
BPT5204/ A67	29.2	15.5
BPT5204/ Dodiga	24.1	48.7

Table 17. Polymorphism for different SSR loci in the parental lines of crosses studied (P- Polymorphic, M- Monomorphic)

N0	Primer	Chrom No	Between BPT5204 and Dodiga	Between BPT5204 and A67
1	RM1	1	P	P
2	RM14	1	P	P
3	RM226	1	P	P
4	RM212	1	M	M
5	RM243	1	P	P
6	RM23	1	P	M
7	RM220	1	P	P
8	RM29	2	M	M
9	RM250	2	M	M
10	RM347	3	P	P
11	RM203	3	P	M
12	RM426	3	P	M
13	RM554	3	M	M
14	RM7	3	P	P
15	RM570	3	M	M
16	RM55	3	M	P
17	RM335	4	P	M
18	RM303	4	P	P
19	RM178	5	P	M
20	RM26	5	P	M
21	RM163	5	P	P
22	RM249	5	P	P
23	RM159	5	M	M
24	RM87	5	M	M
25	RM3	6	M	M
26	RM162	6	M	M
27	RM253	6	M	P
28	RM585	6	P	P
29	RM225	6	M	M
30	RM214	7	M	P
31	RM10	7	M	M
32	RM256	8	M	M
33	RM210	8	M	M
34	RM105	9	P	P
35	RM201	9	P	P
36	RM242	9	M	M
37	RM257	9	P	M
38	RM410	9	P	P
39	RM224	11	P	P
40	RM144	11	M	M
41	RM19	12	P	P
42	RM247	12	P	P

Fig 12. Simple sequence repeat pattern (SSR) in parents F_1 , F_2 of the cross and BPT 5204 /Dodiga

4. 4. 2. 1 Marker Segregation

The selected markers were assessed for segregation and goodness of fit was tested for 1:2:1 ratio using chi-square test in both the populations.

BPT 5204/ A67 cross

The segregation of two markers RM253 (chromosome 5) and RM220 (chromosome 1) was assessed and it fits well into expected 1:2:1 ratio (Table 18). The probability value (0.50-0.75) indicated nonsignificant difference between the expected and observed numbers.

Table 18. Goodness of fit to 1:2:1 marker segregation in F_2 population of the cross BPT5204/ A67

BPT5204/ Dodiga cross

The segregation of two markers RM178 (chromosome 5) and RM163 (chromosome 5) were assessed and only RM178 showed the expected 1:2:1 ratio, while segregation of RM163 did not fit 1:2:1 ratio (Table 19).

Table 19. Goodness of fit to 1:2:1 marker segregation in F_2 population of the cross BPT5204/ Dodiga

4. 5 DNA MARKER- TRAIT ASSOCIATION

The association between F_2 genotype by SSR markers and their corresponding F_3 phenotype was studied by single-marker analysis (SMA) using IRRISTAT program. The results are presented in Tables 20 and 21.

Table 20. Association between marker and early vigor related traits in $F_2:F_3$ of BPT5204/ Dodiga cross

Table 21. Association between marker and early vigor related traits in $F_2:F_3$ of BPT5204/ A67 cross

4.5.1 BPT 5204/A67 cross

The comparison of means for the shoot and root lengths at different stages between three groups (Two homozygotes and an heterozygote) revealed that the shoot length trait is associated with the marker RM253 at both stages (0.014 and 0.01) as probability value was near to 0.01. Its contribution to phenotypic variance as measured by R^2 indicated 9.6% and 10.1% for SL10 and SL14, respectively, while association was not evident between RM220 and the traits. The two markers were found not associated with the root length at both the stages.

4. 5. 2 BPT5204/Dodiga cross

The comparison of means for the shoot and root lengths at different stages between three groups (Two homozygotes and an heterozygote) revealed the association of RM178 with root length at 10th DAS (0.038) and shoot length at 10th (0.003) and 14th (0.029) DAS. Its contribution to phenotypic variance as measured by R^2 value for shoot length was 18% (SL10) and 12.1% (SL14) respectively. The phenotypic variance was found to be 11.2% for root length at 10th DAS.

Table 18. Goodness of fit to 1:2:1 marker segregation in F₂ population of the cross BPT5204/ A67

Marker	No. of individuals	Group	Observed	Chi square for 1P ₁ :2H:1P ₂	Probability
RM 220	142	GP ₁	33	1.154	0.50-0.75
		GH	68		
		GP ₂	41		
RM 253	132	GP ₁	29	0.651	0.50-0.75
		GH	69		
		GP ₂	34		

GP₁- Group of F₂ showing BPT5204- banding
 GH- Group of F₂ showing Heterozygous banding
 GP₂- Group of F₂ showing A67 - banding

Table 19. Goodness of fit to 1:2:1 marker segregation in F_2 population of the cross

BPT5204/ Dodiga

Marker	No. of individuals	Group	Observed	Chi square for $1P_1:2H:1P_2$	Probability
RM 178	117	GP ₁	29	0.282	0.75-0.90
		GH	61		
		GP ₂	27		
RM 163	131	GP ₁	49	10.77	0.005
		GH	54		
		GP ₂	28		

GP₁- Group of F_2 showing BPT 5204 banding

GH- Group of F_2 showing Heterozygous banding

GP₂- Group of F_2 showing Dodiga- banding

Table 20. Association between marker and early vigor related traits in F₂:F₃ of PT5204/
Dodiga cross

Characters		Marker			
		RM178		RM163	
		Mean	Probability	Mean	Probability
RL10	P ₁	10.35	0.038* (11.2%)	11.83	0.815
	H	11.67		11.24	
	P ₂	11.41		11.78	
SL10	P ₁	8.08	0.003** (18.0%)	9.16	0.654
	H	9.24		9.31	
	P ₂	9.89		9.85	
RL14	P ₁	14.14	0.096	15.38	0.816
	H	15.55		14.88	
	P ₂	14.72		15.33	
SL14	P ₁	12.64	0.029* (12.1%)	13.31	0.46
	H	13.73		13.87	
	P ₂	14.26		14.39	

GP₁- Group of F₂ showing BPT5204- Low Vigour banding

GH- Group of F₂ showing Heterozygous banding

GP₂- Group of F₂ showing Dodiga - High Vigour banding

Values in parenthesis indicate coefficient of determination (Phenotypic variance)

Table 21. Association between marker and early vigor related traits in F₂:F₃ of BPT5204/ A67 cross

Characters	Group	Marker			
		RM253		RM220	
		Mean	Probability	Mean	Probability
RL10	GP ₁	11.6790	0.857	11.6700	0.056
	GH	11.5436		11.2040	
	GP ₂	11.8933		12.0787	
SL10	GP ₁	10.7797	0.014* (9.6%)	11.5867	0.655
	GH	12.6260		12.1433	
	GP ₂	12.5550		12.5070	
RL14	GP ₁	13.3758	0.394	13.3262	0.082
	GH	12.9096		12.7423	
	GP ₂	13.7571		13.6163	
SL14	GP ₁	13.0050	0.01** (10.1%)	13.6827	0.541
	GH	14.0453		13.8202	
	GP ₂	14.7133		14.3687	

GP₁- Group of F₂ showing BPT5204- Low Vigour banding

GH- Group of F₂ showing Heterozygous banding

GP₂- Group of F₂ showing A 67 - High Vigour banding

Values in parenthesis indicates coefficient of determination (Phenotypic variance)

V. DISCUSSION

The loss due to weeds constitutes a major problem in upland condition along with water scarcity. The problem of weeds can be overcome by manual weeding or through use of herbicides. However, due to adverse weather conditions, pollution hazard, poor economic condition of the farmer, high cost and non-availability of labour, the afore-said methods may not be always most effective. The early vigour trait in the rice crop has ability to suppress weed growth and the trait can be used to upgrade existing cultivars through proper breeding programmes. But, the early vigour is a very complex trait and the improvement through traditional breeding is rather difficult hence application of new tools like molecular marker assisted selection can enhance the efficiency of breeding programmes.

Improvement through breeding depends on the availability of genetic variability. The utility of such material in further improvement depends on the knowledge of target trait, and extent of variability and character association. The information obtained on the linkage relationship of QTLs for different traits and establishing their association with appropriate DNA markers would make indirect selection through marker-assisted selection (MAS) more effective.

DNA based markers are increasingly being recognized as useful tools for assessing genetic diversity among the germplasm, since they are not influenced by the environment (Lee, 1995). A number of marker systems such as RAPD, AFLP, SSR, ISSRs, CAPs, STMS, etc. are available for molecular studies. Among them SSR markers have been proved to be more useful in marker assisted selection (Yang *et al.*, 1996).

In the present investigation, IR64/Azucena-DH population was evaluated for early vigour related traits. The phenotypic data and SSR genotypic data were superimposed to identify QTLs controlling the trait. The markers associated with the traits in DH population as well as from other investigations (Redona and Mackill, 1996b and Zhang *et al.*, 2005) were employed to validate in the new F₂:F₃ populations of BPT5204/A67 and BPT5204/Dodiga representing low and high vigour parents.

5.1 IR64/Azucena- DH population

The phenotyping of trait is very important for good genetics. The vigour parameters are sensitive to temperature conditions. Therefore, in the present study temperature range of 25-30°C was maintained, which is optimum for rice germination and early growth (Chapman and Paterson, 1962). The vigour parameters were measured during initial 15 days, as early stages are critical for weed competition (Li and Rutger, 1980 and Ramadevi, 1998). The morpho-physiological parameters, viz., first count of germination, final count of germination, rate of germination, root length, shoot length, seedling dry weight and vigour index were chosen as important traits related to early vigour (Redona and Mackill, 1996b; Ramadevi, 1998 and Zhang *et al.* 2005). The IR64/Azucena population has nearly saturated molecular map (Temnykh, 2001) with wide genome coverage with SSR markers. The early vigour related traits are governed by polygenic inheritance; hence saturated molecular map is necessary to map and identify maximum number of QTLs contributing to the traits.

5.2 Genetic variability

The Analysis of variance and wide range indicates significant variability for all the early vigour related traits in the population. However,

the amount of variation differed among different traits with moderate variability (10-20%) for most of the traits and it was low (5.5 to 7.9 %) for final count of germination. Substantial amount of genetic variation for seedling vigour has been observed in cultivated rice including *indica*, temperate japonica and tropical japonica (Krishnsamy and Seshu, 1987; Yamauchi *et al.*, 1993; Redona and Mackill, 1996a; Yamauchi and Winn, 1996) subspecies. The different sets of genes controlling vigour may be present in *japonica* and *indica* cultivars (Redona and Mackill, 1996c). Since DH is derived from *indica* (IR64)/*japonica* (Azucena) cross, it is expected to show variation for seedling vigour related traits. Earlier studies have also revealed substantial variability in the DH population for root parameters, (Yadav *et al.*, 1997) and heading date, growth and yield parameters (Hittalmani *et al.*, 2003).

The populations mean for counts and rate of germination was high as compared to parents. Such a response was observed in the case of mesocotyl length in the cross Labelle/Italica Livorno (Redona and Mackill, 1996c) and it was ascribed to moderate parental diversity.

The amount of genetic variation alone will not be of much use for the breeder unless supplemented with the information on heritability, which gives the measure of heritable portion of the total variation. Burton and Devane (1953) suggested that GCV along with heritability could provide a better picture of the amount of advance to be expected by selection process.

The root length and vigour index exhibited high heritability (73.2 and 73.8) and genetic advance (28.26 and 28.94) indicating scope for improvement in these traits. Yadav *et al.* (1997) also reported high heritability (77.0%) for root length in this population.

The distribution of DH lines (Figure 1) was normal for shoot length and seedling dry weight, but skewed for germination parameters. Zhang *et al.* (2005) reported a similar trend in Lemont/Tequing. The distribution was also normal for root length and shoot length in Black Gora/Labelle (Redona and Mackill, 1996b).

Significant transgressive segregants were observed for root and shoot length in the DH population. Redona and Mackill (1996b) and Zhang *et al.* (2005) obtained similar results in different populations. The transgressive segregants in both the directions indicate that neither parents carried all the positive or the negative alleles. The transgressive segregants may be due to complementary action of additive genes that were dispersed among the parents (Xu *et al.*, 1998).

5.3 QTL mapping

The interval mapping was followed to identify QTLs linked to early vigour traits. The use of linked markers in this method compensates for recombination between the markers and the QTL and it is considered to be statistically more powerful (Yadav *et al.* 1997). A total of 22 QTLs were identified for early vigour related traits in the present study.

Apart from identification of QTLs, additive effect for each QTL was also computed. It reveals effect of replacement of a female allele (IR64) by a male (Azucena) allele. The additive effect could be either positive or negative. A positive value indicates increase in effect, i.e., Azucena has positive allele, while negative value indicates decrease in effect, i.e., and IR64 has positive allele.

5.3.1 Germination

Totally eight QTLs were identified for germination parameter viz., first count (2), final count (1) and rate (5) of germination. The QTLs controlling first and final counts of germination were located on chromosome 1 in the region (RM5-RM306) near to marker RM5. Interestingly, the α -amylase gene *amy1B/A* influencing germination is located at 13 cM away from RM5 towards centromere on the long arm of chromosome 1 (Temnykh *et al.*, 2001). The additive effect for the locus RM5-RM306 was positive indicating contribution of positive allele by the japonica variety Azucena. Thomson *et al.* (2003) reported QTL controlling germination in the same region in cross *O. rufipogon* and US cultivar Jefferson and the positive allele was contributed by Jefferson, a japonica variety. This gives evidence about the presence of genes controlling the germination in the region. Krishnasamy and Seshu (1993), reported about relation between alpha-amylase and seedling vigour.

The RM341-RM106 region on chromosome 2 is common for the first count and rate of germination. High correlation (0.82) between the first count and rate of germination compared to final count and rate of germination (0.33) also reveals greater contribution of first count towards rate of germination, which is due to shared QTL. The gene *Amy 1A/C* contributing to germination is also located on the same arm (long arm) (Temnykh *et al.*, 2001). With 56.2% (rate of germination) and 49.4% (first count of germination) contribution towards variation, this QTL is found to be a major determinant of the trait.

Other QTLs for the trait are present on chromosome 3 (RM231-RM7), chromosome 4 (RM307-RM335, RM303-RM317) and chromosome 5 (RM87-RM334). Zhang *et al.* (2005) also reported presence of main effect and epistatic QTLs for rate of germination on chromosome 4 in Lemont/Teqing. However, under salt stress Raghvendra Prasad *et al.* (2000) have found QTLs contributing to germination on chromosome 6 in IR64/Azucena. All the loci, controlling rate of germination have negative additive effect indicating the contribution of positive alleles by IR64. The female parent IR64 is also found superior for rate of germination compared to Azucena.

5.3.2 Morphological traits

Six QTLs contributing for variation in shoot length were located on the long-arm in the region RM84-RM23 of chromosome 1 and all were found to be minor QTLs as they contributed 10-15% of the phenotypic variance in the trait. Shoot length trait is controlled by many loci as reported by Redona and Mackill (1996a), Li and Rutger (1980). However, shoot length QTLs were located either on other chromosomes or short arm of chromosome 1 in different populations (Redona and Mackill, 1996b and Zhang *et al.* 2005).

Seedling dry weight is controlled by three QTLs. A single QTL (RM327-RM27) on chromosome 2 contributes highest phenotypic variance of 40.2% and positive additive effect reveals contribution of Azucena towards increase in phenotype. Two QTLs on chromosome 5 (RM153-RM159, RM87-RM334) contribute 13.8% and 17.1% to phenotypic variance, respectively. However, the negative additive effect of the QTLs indicated IR64 as contributor of positive alleles. Total seedling dry weight QTL (RM26-C1447) on chromosome 5 in Zhenshan97/Minghui 63 (Cui *et al.* 2002) is located close to RM87-RM334. Seedlings dry weight QTLs on chromosome 5 have also been reported by Zhang *et al.* (2005) in Lemont/Teqing but at different positions.

Vigour index is conditioned by five QTLs distributed on chromosome 2 (RM174-RM145), 5 (RM178-RM26, RM87-RM334) and 6 (RM3-RM162, RM136-RM3). The two major QTLs (39.2%, 28.5 %) on chromosome 6 are from Azucena, while minor QTLs (16.0-18.2%) on chromosome 2 and 5 are from IR64. The QTLs on RZ70-RZ225 for seedling vigour and dry matter in IR64/Azucena (Raghavendra Prasad *et al.*, 2000) corresponds to RM178-RM26 on chromosome 5.

The congruence of QTLs on the chromosome 5 (RM87-RM334) for rate of germination, seedling dry weight and vigour index may be due to either linkage or pleiotropy. Multiple QTLs controlling seedling vigour on chromosome 5 was reported by Zhang *et al.* (2004) and Upadhyaya (2005). The additive effect of all the three QTLs at this locus is negative indicating the contribution from IR64 parent. This signifies the plural selection efficiency by selecting markers closely associated with these traits. The vigour index is significantly correlated to rate of germination and seedling dry weight and often phenotypically correlated traits map together (Albert *et al.*, 1991; Paterson *et al.*, 1991; Shashidhar *et al.*, 1999; Hittalmani *et al.*, 2002).

Compared with results of Temnykh *et al.* (2001) and Thompson *et al.* (2003), the QTL (RM5-RM306) controlling germination exhibited better performance in different genetic backgrounds, however some QTLs are specific to the mapping population. These reveal extensive genetic diversity for early vigour related traits, even though some common genomic regions are found controlling the trait.

Molecular marker technology is capable of identifying close relationships and would help in discerning between pleiotropy and tight linkage or overlapping genes. In a given genotype, genes acting in both positive and negative direction would control expression of any quantitative trait. Plant breeding is directed towards accumulating favourable genes/alleles for traits by exerting selection. Identifying the undesirable/desirable alleles at different loci could efficiently do this task. Molecular marker technology is potential enough to provide such information. This gives ample scope to exercise selection for desirable alleles at genotype or molecular level.

5.4 New populations

Two new populations were generated using BPT 5204 as female and Dodiga and A 67 as male parents. BPT5204 is a high yielding, late maturing, semidwarf variety with medium fine grain and good cooking quality but suffers from low seedling vigour. Dodiga and A67 are land races adapted to upland cultivation, with high vigour, tall and medium bold grains. The populations are expected to segregate for plant height, tiller number, maturity and grain quality characters. But, our emphasis in the present study was on early vigour related traits. The populations will also be useful for drought studies as one of the parents A67 exhibited drought tolerance at seedling stage (Makhasi, 2001). BPT5204/Dodiga was earlier studied for physio-biochemical traits influencing drought tolerance (Mohankumar *et al.*, 1999).

The modern semi-dwarf cultivars lack in early vigour, hence genotypes in BPT5204 background with high early vigour are desirable for upland condition. Li and Rutger (1980) also indicated possibility of combining semi-dwarf with high vigour. The F_1 s generated from the two crosses were checked for hybridity with respect to morphological characters. The F_2 seeds from the true F_1 s were used for further advancement.

The permanent populations such as DH and RI populations are often used owing to their inherent advantages, although F_2 populations and F_3 families are still widely used to locate desired loci. The parents belonging to *indica* subspecies and intra sub-specific crosses can reduce sterility and linkage drag that result from wide hybridization and can be considered useful from point of view of practical application. The significant parental differences for early vigour related traits, i.e., root and shoot lengths at various stages indicated the proper choice of material for the study.

5.4.1 Variability in F_2 Population

The root and shoot length were measured on individual seedlings at three stages (6th, 10th and 14th DAS). Shoot length is important determinant for vigour (Peterson *et al.*, 1978) and root length is important for vigour as well as stress tolerance (Yadav *et al.*, 1997). The variance and range in the both populations were high compared to the parents because of segregation and distribution of alleles contributing to the phenotype. Substantial amount of genetic variation was found in both the populations. The heritability was moderate to high (59-85%) for root and shoot lengths. The relatively higher variation and low heritability in SL6 in both the populations could be due to high error coefficient of variation at 6th DAS as revealed in the parents. The moderate to high heritability was also obtained in Li and Rutger (1980) for seedling height measured on 14th DAS. Same trait at two different stages was measured by and the difference in genetic components and distribution pattern of individuals at different stages was ascribed to time specific QTLs (Xu *et al.*, 2004)

5.4.2 Variability in F_3 population

The root and shoot length were measured in parents and F_3 population at two different stages, i.e., 10th DAS and 14th DAS. Significant variability was found for all the parameters in both the crosses (BPT5204/A67 and BPT5204/Dodiga), because, they were developed by choosing phenotypically divergent parents for early vigour related traits. The presence of wide variability within the population indicates their suitability for molecular mapping as well as genetic studies.

Population mean for different parameters were within parental limits in both the crosses. Heritability in conjunction with genetic advance will be more effective and reliable in predicting the response to selection. Broad sense heritability and GAM were highest for SL10 in both the populations. Therefore, selection at 10th day for SL would be more effective compared to 14th day. High GAM was due to predominance of additive genetic variation for shoot length (Li and Rutger, 1980). Transgressive segregants (Fig. 9 and 10) obtained for root length in both the crosses indicated the contribution of favorable alleles by both the parents.

The phenotypic as well as genotypic correlations among morphological characters measured at different stages were high as expected. The correlation between root and shoot traits at initial stages was high but reduced in the later stages. This may be due to shared

metabolic pathways during the early heterotrophic stage, which eventually may become relatively independent of each other when the seedling switches over to autotrophic phase during the later developmental stages (Cui *et al.*, 2002).

The growth conditions under laboratory and net house were different. The shoot length parameters measured at different stages (SL14, SL21 and SL28) in the net house were also correlated. The shoot length measured at 28th day in F₂ in net house was associated with shoot length measured in F₃ at 10th DAS, but not with 14th DAS in laboratory. (Tables 14 and 15). This indicates that shoot length on 10th day represents the field performance at 28th DAS. Jones *et al.* (1976) also reported correlation between laboratory parameters and field performance for shoot length.

5.5 Heritability

The heritability estimates vary with the method of estimation and genetic background. The heritabilities estimated from variance components (87.6%, 83.9%) were higher than those obtained by parent-offspring regression (29.2%, 24.1%). Narrow sense heritability estimated based on F₂:F₃ regression Labelle/Italica livorno was 26% for shoot length (Redona and Mackill, 1996a). Low narrow sense heritability as compared to broadsense heritability could be due to predominance of non-additive components. The realized heritability BPT5204/A67 (15.5%) was low as compared to BPT5204/Dodiga (48.7%) revealing better response to selection in the latter cross. Redona and Mackill (1996a) also reported differences among the crosses for heritability. The low heritable traits are also difficult to improve by conventional selection strategies. Therefore, MAS has relevance in the improvement of such complex traits.

5.6 Molecular diversity in the parents

The molecular diversity is also important for mapping studies along with phenotypic diversity. The SSR markers were used to assess parental polymorphism at molecular level. Marker validation is testing effectiveness of marker in determining the target phenotype in independent populations and different genetic backgrounds. This helps to know whether marker could be used in routine screening for MAS (Collard, 2005). For marker to be useful in plant breeding, they should reveal polymorphism in different populations derived from a wide range of genotypes (Langridge *et al.*, 2001). Therefore, the markers already identified using DH populations, RILs and F₃ populations in different genetic background were used for validation in the F₂:F₃ populations of BPT5204/A67 and BPT5204/Dodiga in this study.

Forty-two SSR primers associated with early vigour trait in rice (Redona and Mackill, 1996b; Zhang *et al.*, 2005 and the current study on DH population) were targeted for analysis. The flanking markers of particular trait as well as nearby locations were chosen for assessing polymorphism. No QTLs for early vigour related traits were reported on chromosome 10, hence no markers were chosen from that chromosome. Twenty-four in BPT5204/Dodiga (57.14%) and twenty markers in BPT5204/A67 (47.61%) were found to be polymorphic indicating molecular diversity among the parents. Initial screening employing ten F₂ plants each of low and high vigour was done to assess putative association of markers with the trait in both the crosses. Only two markers each in BPT5204/Dodiga (RM178 and RM163) and BPT5204/A67 (RM220 and RM253) were found associated with the early vigour trait indicating that very few markers may be effective across populations. Thus screening based on existing information on other populations may not be efficient strategy to identify potential QTLs.

5.6.1 Marker segregation

Marker segregation was observed in large number (117-142) of F₂ plants. The Simple Sequence Repeat (SSR) markers used in this study are codominant and they are expected to segregate in 1:2:1 ratio. The goodness of fit was tested using chi-square test (Gomez and Gomez, 1984). The markers RM220 and RM253 in BPT5204/A67 and RM178 in BPT5204/Dodiga segregated in Mendelian fashion. However, the marker RM163 exhibited distorted segregation. Xu *et al.* (1997) and Sayed *et al.* (2002) also reported distorted segregation for several SSR markers in rice.

5.6.2 Association study

The association between marker and trait was estimated by using single marker analysis (SMA). The phenotypic data of 109 F_3 families and corresponding marker data of F_2 individuals of BPT5204/A67 was used for analysis. RM253 marker on chromosome 6 was associated with shoot length at both the stages (SL10 and SL14) in BPT5204/A67. This marker is near to qSV-6 (C76-RM253) controlling epistatic QTLs for germination rate and shoot length (Zhang *et al.*, 2005) in Lemont/Teqing population. In IR64/Azucena (current study) major QTL (RM136-RM3) controlling vigour index was located in nearby region. The phenotypic variance explained was 9.6% (SL10) and 10.1% (SL14), respectively. The trait is controlled by many factors/QTLs with small contribution from each (Li and Rutger, 1980; Redona and Mackill, 1996 a & b; Zhang *et al.*, 2005). The shoot length QTLs (5) in IR64/Azucena also explained phenotypic variance in the range of 13-15%.

RM220 marker was not associated with either root or shoot length in BPT5204/A67 population, even though initial screening of marker with ten high and low F_2 plants showed possible association.

The phenotypic data of 75 F_3 families and corresponding marker data of F_2 individuals was used for assessing association between RM178 and RM163 markers on chromosome 5 with root and shoot lengths at both the stages (10th and 14th DAS) in BPT5204/Dodiga. The RM178 has contributed 18.0% (SL10) and 12.1% (SL14) to the phenotypic variance, in shoot length and 11.2% for root length at 10th DAS.

The QTL (RM178-RM26) on chromosome 5 was associated with vigour index in IR64/Azucena (current study) and multiple QTLs controlling seedling dry weight, rate of germination and vigour index are also located in the nearby region (RM87-RM334). Cui *et al.* (2002) reported QTLs controlling seedling vigour related traits (germination rate, root dry weight and root activity) in the C246-RM26 region and root length QTL in RM26-C1447 region in Zhenshan 97/Minghui 63. The root length QTL in that study also explained variance ranging from 11.4-15%. Significant correlation (0.42) between RL10 and SL10 in BPT5204/Dodiga population could be due to the association of this marker with both traits. Even though RM163 marker was located on the same chromosome, it was not associated with either root or shoot lengths, which may be due to segregation distortion of the marker.

The information obtained on the new populations (BPT5204/A67 and BPT5204/Dodiga) with respect to markers is limited. About 50% of the 42 markers selected based on other populations showed polymorphism in the new populations and only four exhibited putative association with early vigour trait. Among them only two markers, one each in a population, were useful for identifying early vigour QTLs. Intensive studies (Redona and Mackill, 1996b; Cui *et al.* 2002; Zhang *et al.* 2005; current study) on early vigour have revealed existence of many QTLs (20-32) in different populations. But such information has not proved useful in the new populations with breeding potential due to highly population specific nature of QTLs and differential marker polymorphism in the parents. Thus, an approach based on information on other populations has not proved successful. Since seedling vigour trait has proved to be complex in nature and lack of success in early generation selection, identification of majority of the QTLs for the trait becomes important for marker-assisted selection to be successful.

A linkage with more number of markers will be useful in identifying QTLs controlling the trait. Initially, polymorphic anchored markers representing each arm of the chromosomes could be employed. A saturated linkage map will be useful to identify QTLs for other potential traits, viz., Plant height, maturity, tiller number, grain quality and drought tolerance for which the parents are polymorphic. Development of permanent populations (RILs) will help in the success of such a strategy.

VI. SUMMARY

DNA marker assisted genetic analysis for early vigour related traits in rice was undertaken at the Department of Genetics and Plant Breeding, College of Agriculture, UAS, Dharwad and Barwale Foundation (formerly Mahyco Research Foundation), Hyderabad. The experiments were aimed at studying genetics of early vigour related traits and identifying QTLs controlling early vigour related traits using SSR markers. The markers thus identified are expected to be useful in Marker Assisted Selection (MAS) for early vigour related traits. IR64/Azucena-DH was used as reference population for identification of QTLs controlling early vigour with bracketing SSR markers. QTLs were identified by interval mapping approach using MAPMAKER program. The associated markers along with other reported markers in different populations were used for validation in BPT5204/A67 and BPT5204/Dodiga populations. First count of germination, final count of germination, rate of germination, root length, shoot length, seedling dry weight and vigour index parameters were used in the evaluation of DH lines. The root and shoot lengths were measured at various stages (6th, 10th and 14th DAS) in F₂ and 10th, 14th DAS in F₃ populations of both the crosses. Simple correlation analysis was performed to study inter-trait association. The frequency graphs were used for discerning the pattern of distribution for different traits. The F₂ genotype and F₃ phenotype for root and shoot lengths were used for assessing association between marker and trait by Single Marker Analysis (SMA) using IRRISTAT program.

The important experimental findings are summarized here.

- The IR64/Azucena-DH population exhibited significant variation for early vigour related traits. Parents showed moderate divergence for early vigour related traits. A much wide range beyond parental values was observed for all the traits. The magnitude of variation as revealed by PCV and GCV was moderate (10-20%) for all the traits except final count of germination (7.9 and 5.5%) and GCV for rate of germination (8.9%). The broad sense heritability, which indicates heritable portion, was high (70.1 to 81.1%) for all the traits except final count of germination (47.1%) for which it was moderate.
- Significant transgressive segregants in both the directions were observed for all the traits in DH population, which may be due to complementary action of alleles and favorable alleles contributing to the trait may be dispersed in both the parents.
- The correlation was positive and significant among counts and rate of germination. The first count of germination, rate of germination and seedling dry weight were significantly correlated with vigour index.
- Twenty-two QTLs were identified for different vigour related traits and they were distributed on chromosomes 1 to 6. The congruence of QTLs was observed on Chrom 5 in the region RM87-RM334 for rate of germination, seedling dry weight and vigour index. The QTLs for both first count of germination and rate of germination were located near to RM106 on chrom 2. The congruence may be due to tight linkage or pleiotropy. The chromosome 5 is important for early vigour related traits. The additive effect was both positive and negative indicating both the parents contributing to the trait.
- The parents for generating new crosses, viz., BPT5204, A67 and Dodiga differed significantly for early vigour related traits along with other agronomical characters.
- Substantial genetic variation was found for root and shoot lengths measured at 6th, 10th and 14th DAS in BPT5204/A67 and BPT5204/Dodiga crosses in F₂ generation. The PCV and GCV for the two root and shoot length at 6th DAS as compared to 10th and 14th DAS. Heritability was high and ranged from 80 to 87% for different parameters in the two populations except for SL6 in BPT5204/A67 (69%) and BPT5204/Dodiga (59%). The genetic advance was high (30.19 to 63.18%) for different traits revealing scope for selection.

- Significant variability was found for the traits, viz., RL6, SL6, RL10 and SL10 in both the crosses in F₃ generation. Highest heritability and genetic advance was recorded for shoot length at 10th DAS.
- The frequency distribution was normal for shoot length in BPT5204/A67 while, it was bimodal in BPT5204/Dodiga. Significant transgressive segregants were observed for root length in both the populations. The segregants were within parental range for shoot length indicating that the parents were extremes for the trait.
- The phenotypic and genotypic correlation for RL10-RL14 and SL10-SL14 were higher in both the populations. The correlation between RL10 and SL10 was significant at 10th DAS, but non-significant at 14th day, which coincides with switching over from heterotrophic to autotrophic stage.
- The narrow sense heritability for shoot length was 29.2% in BPT5204/A67 and 24.1% in BPT5204/Dodiga. The realized heritability for shoot length was higher in BPT5204/Dodiga (48.7) compared to BPT5204/A67 (15.5). Low heritability indicates difficulty for improvement through traditional breeding; hence markers would be useful in selection and pyramiding of favorable alleles.
- Forty-two SSR markers were screened in the parents, BPT5204, A67 and Dodiga. Out of these fifty percent of the markers were polymorphic between male and female parents.
- RM253 (Chrom 6) was associated with the shoot length at 10th and 14th DAS with 9.6% and 10.1% contribution to total phenotypic variance in BPT5204/A67. RM178 (Chrom 5) was associated with shoot length (18%) and root length (11.2%) at 10th DAS in BPT5204/Dodiga. This indicated that chromosome 5 and 6 are active locations for early vigour traits viz., root and shoot length.

Already existing information in rice was useful only to limited extent in the new populations due to differences in polymorphic markers and predominance of population specific QTLs for the trait. The codominant and user-friendly SSR markers would be useful as selection tools for diagnosing presence of favorable alleles and for introgression into desired background.

VII. REFERENCES

- AKAGI, A.K., 1996, Microsatellite DNA markers for rice chromosomes. *Theoretical Applied Genetics*, **93**: 1071
- AKRAM, M., SESHU, D.V. AND KHAN, A.M., 1993, Inheritance of seedling vigour in rice. *Oryza*, **30**:179-185.
- ALBERT, B.S.B., EDWARDS, M.D., STUBER, C.W., 1991, Isoenzymatic identification of quantitative trait loci in crosses of elite maize inbreds. *Crop Science*, **31**: 267-274.
- ANONYMOUS, 1983, *Hand Book on Seed Testing*. Association of Official Seed Analysts, NE, and USA, **50**: 88.
- ANONYMOUS, 1993, International rules for seed testing. Supplement to *Seed Science and Technology*, **21**: 1-288.
- ATCHLEY, W.R. AND ZHU, J., 1997, Developmental quantitative genetics, conditional epigenetic variability and growth in mice. *Genetics*, **147**: 765-776.
- BECKMANN, J.S. AND SOLLER, M., 1990, Towards a unified approach to genetic mapping of eukaryotes based on sequence tagged micro satellite sites. *Biotechnology*, **8**: 930-932.
- BLAIR, M.W. AND MCCOUCH, S.R., 1999, Microsatellite and sequence-tagged site markers diagnostic for the rice bacterial blight resistance gene *xa5*. *Theoretical Applied Genetics*, **95**:174-184.
- BLANCO, L.C. AND AKITA, S., 1988, Physiological mechanism of heterosis in seedling growth of F₁ rice hybrids. In *IV Scientific Meeting of the Federation of Crop Science Societies of the Philippines*, April 27-30, 1988, IRRI, Los Banos, Philippines.
- BROWN, S.M., HOPKINS, M.S., MITCHELL, S.E., SENIOR, M.L., WANG, T.Y., DUNCAN, R.R., GONZALEZ-CANDELAS, F. AND KRESOVICH, S., 1996, Multiple methods for identification of polymorphic simple sequence repeats (SSRs) in sorghum [*Sorghum bicolor* (L.) Moench]. *Theoretical and Applied Genetics*, **93**: 190-198.
- BURTON, G.W. AND DEVANE, E.M., 1953, Estimating heritability in tall fescue (*Festuca, circumclinaceae*) from replicated clonal material. *Agronomy Journal*, **45**: 478-481.
- CHAO, Y.G., ISHII, T., TEMNYAKH, S., CHEN, X., LIPOVICH, L., MCCOUCH, S.R., PARK, W.D., AYRES, N. AND CARTINHO, S., 2001, Diversity of microsatellites derived from genomic libraries and gene bank sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **100**(5): 713-722.
- CHAPMAN, A.L. AND PETERSON, M.L., 1962, The seedling establishment of rice under water in relation to temperature and dissolved oxygen. *Crop Science*, **2**: 391-395
- CHEN, C.L., 1997, Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **95**: 553.
- CHEN, C.L., SUNG, F.J.M. AND LI, C.C., 1986, Physiological and genetic studies on seedling vigour in rice (*Oryza sativa*): Relation between seed weight, seedling respiration rate and growth of rice seedlings. *Journal of the Agricultural Association of China*, **135**: 10-16.
- CHO, 2000, Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **100**: 713.
- CHOWDHURY, R.K. AND SINGH, R.K., 2000, Rice seed supply in eastern India. In. Rainfed Rice- A source book of best practices and strategies in Eastern India (Ed., Singh, V. P and Singh, R. K), IRRI, Manila, Philippines, p. 292.
- CHRISTOPHER, M., GIOVANNI CORDEIRO, DAN WATERS AND ROBERT HENRY, 2004, Marker assisted selection in rice improvement. A report for the Rural Industries Research and Development Corporation.
- CHUNHAI, S., ZONUTAN, S. AND GUANY, C.W., 1996, Effects of semi dwarf gene *Sd1* on plant and root traits of seedling in *indica* rice. *Chinese Rice Research Newsletter*, **4**: 2.

- COLLARD, B.C.Y., JAHUFER, M.Z.Z., BROUWER, J.B. AND PANG, E.C.K. 2005, An introduction to markers, quantitative trait loci (QTL) mapping and marker assisted selection for crop improvement: The basic concepts. *Euphytica*, **142**: 169-196.
- CUI, K.H., PENG, S.B., XING Y. Z., XU, C.G., YU, S.B. AND ZHANG, Q., 2002, Molecular dissection of seedling vigor and associated physiological traits in rice. *Theoretical and Applied Genetics*, **105**: 745-753.
- DAUSSANT, J., MIYATA, S., MITSUI, T. AND AKAZAWA, T., 1983, Enzymatic mechanism of starch break down in germinating rice seeds (15): Immunochemical study on multiple forms of amylase. *Plant Physiology*, **71**: 88-95.
- DEAN, R.E., DAHLBERG, J.A., HOPKINS, M.S., MITCHELL, S.E. AND KRESOVICH, S., 1999, Genetic redundancy and diversity among "Orang" accession in the US national sorghum collection as assessed with simple sequence repeat (SSR) markers. *Crop Science*, **39**: 1215-1221.
- DINGKUNH, M., DE DATA, S.K., PAMPLONA, R., JAVELLANA, C. AND SCHNIER, H. F., 1992, Effect of late-season N-fertilization on photosynthesis and yield of transplanted and direct-seeded tropical flooded rice. A canopy stratification study. *Field Crop Research*, **28**: 235-249.
- DIWAN, J.R. AND SHENOY, V.V., 2001, Inheritances of α -amylase and its association with early vigour related traits in rainfed upland rice. *Current Science*, **80**(2): 284-286.
- FALCONER, D.S., 1960, *Introduction to Quantitative Genetics*. 1st Ed. Oliver and Boyd, Edinburgh, UK.
- FINCHER, G.B., 1989, Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains. *Annual Review of Plant Physiology Plant Molecular Biology*, **40**: 305-346.
- FUJINO, K., SEKIGUCHI, H., SATO, T., KIUCHI, H., NONOUE, Y., TAKEUCHI, Y., ANDO, T., LIN, S.Y. AND YANO, M. 2004. Mapping of quantitative trait loci controlling low-temperature germinability in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **108**: 794-799.
- GIBSON, K.D. AND FISCHER, A.J., 2004 Competitiveness of rice cultivars as a tool for crop based weed management. *Weed Biology and Management* (edited Inderjit), pp. 517-537.
- GOMEZ, K.A. AND GOMEZ, A.A., 1984, *Statistical Procedure for Agricultural Research*. John Wiley and Sons, New York, USA, p.680.
- GUIDERDONI, E., GALLINATO, E., LUISTRO, J. AND VERGARA, G., 1992, Anther culture of tropical *japonica x indica* hybrids of rice. *Euphytica*, **62**: 219-224.
- HAMPTON, J.G. AND COOLBEAR, P., 1990, Potential versus actual seed performance - Can vigour provides an answer? *Seed Science and Technology*, **18**: 215-228.
- HAMPTON, J.G. AND HILL, M.J., 1990, Herbage seed lots: Are germination data sufficient? *Proceedings of the New Zealand Grassland Association*, **51**: 59-64.
- HANSON, G.H., ROBINSON, H.F. AND COMSTACK, R.E., 1956, Biometrical studies of yield in segregating populations of Korean *Lespedeza*. *Agronomy Journal*, **48**: 267-282.
- HERDT, R. W., 1991. Research priorities for rice biotechnology. In: Khush, G. S., Toenniessen, G.H. (eds) *Rice Biotechnology*. CAB Int. Oxon, UK, pp. 19-54.
- HEYDECKER, W., 1960, Can we measure seedling vigour ? *Proceedings of International Seed Testing Association*, **25**: 498-512.
- HITTALMANI, S., HUANG, N., COURTOIS, B., VENUPRASAD, R., SHASHIDHAR, H.E., ZHUANG, J.Y., ZHENG, K.L., LIU, G. F., WANG, G.C., SIDHU, J.S., SRIVANTANEYAKUL, S., SINGH, V.P., BAGALI, V.P., PRASANNA, H.C., MCLAREN, G. AND KHUSH, G. S., 2003, Identification of QTL for growth and grain yield related traits in rice across nine locations of Asia. *Theoretical and Applied Genetics*, **107**: 679-690.
- HITTALMANI, S., SHASHIDHAR, H.E., BAGALI, P., HUANG, N., SIDHU, J. S., SINGH, V.P. AND KHUSH, G.S. 2002. Molecular mapping of quantitative trait loci for plant

- growth, yield and yield related traits across three diverse locations in a doubled haploid rice population. *Euphytica*, **125**: 207-214.
- HUANG, N., MCCOUCH, S. R., MEW, T., PARCO, A. AND GUIDERDONI, E., 1994. Development of an RFLP map from a double-haploid population in rice. *Rice Genetics. Newsletter*, **11**: 134-137.
- JIAKUI, Z., ZUOLIAN, Y., YIN, G., GUANGHUA, H., ZHENGLIN, Y. AND KAIFENG, J., 1996, Genetic studies on root growth ability in seedling stage of rice. *Chinese Journal of Rice Science*, **16**: 51-53.
- JONES, D. B. AND PETERSON, M.L., 1976. Rice seedling vigor at sub-optimal temperatures. *Crop Science*, **16**: 102-105.
- KANBAR, A., JANAMATTI, M., SUDHEER, E., VINOD, M.S. AND SHASHIDHAR, H.E., 2006. Mapping QTLs underlying seedling vigour traits in rice (*Oryza sativa* L.). *Current Science*, **90**: 24-26.
- KANBAR, A., SHASHIDHAR, H.E. AND HITTALMANI, S., 2002, Mapping of QTL associated with root and related traits in DH population of rice (*Oryza sativa* L.). *Indian Journal of Genetics and Plant Breeding*, **62**(4): 287-290.
- KEARSEY, M.J. AND FARQUHAR, A.G.L. 1998. QTL analysis in plants; where are we now. *Heredity*, **80**: 137-142.
- KHURSHEED, B. AND ROGERS, J.C., 1988, Barley α -amylase genes. *Journal of Biological Chemistry*, **263**: 18953-18960.
- KHUSH, G. S., 2005. What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology*, **59**: 1-6.
- KRISHNASAMY, V. AND SESHU, D.V., 1989, Seed germination rate and associated characters in rice. *Crop Science*, **29**: 904-908.
- KRISHNASAMY, V. AND SESHU, D.V., 1993, α -amylase activity in relation to seed vigour in rice (*Oryza sativa*). *Seed Research*, Special **2**: 42-48.
- LANDER, E. S. AND BOTSTEIN, D., 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, **139**:1421-1428.
- LANDER, E. S., GREEN, P., ABRAHAMSON, J., BARLOW, A., DALY, M. J., LINCOLN, S. E. AND NEWBURG, L., 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics*, **1**: 174-181.
- LANGRIDGE, P., LAGUDAH, E., HOLTON, T., APPELS, R., SHARP, P. AND CHALMERS, K., 2001. Trends in genetic and genome analysis in wheat: A review. *Australian Journal of Agricultural Research*, **52**: 1043-1077.
- LAZARUS, C.M., BAULCOMBE, D.C. AND MERTIENSSEN, R.A., 1985, Alpha-amylase genes of wheat are two multigene families which are differentially expressed. *Plant Molecular Biology*, **5**: 13-24.
- LEE, C.C., LI, C.C. AND SUNG, F.J.M., 1986, Physiological and genetic studies on seedling vigour in rice (*Oryza sativa*) II. Inheritance of alpha-amylase activity and seedling vigour in rice *Journal of Agricultural Association of China*, **135**: 17-24.
- LEE, M., 1995, DNA markers in plant breeding programmes. *Advances in Agronomy*, **55**: 265-234.
- LI, C.C. AND RUTGER, J.N., 1980, Inheritance of cool temperature seedling vigour in rice and its relationship with other agronomic characters. *Crop Science*, **20**: 295-298.
- LI, P., WANG, Y.R. AND LIU, H.X., 1990, Physiological basis of high yielding heterosis in F_1 hybrids of *indica* rice. *Scientia Agricultura Sinica*, **23**: 39-44.
- LI, Z. K., LUO, L.J., MEI, H.W., WANG, D.L., SHU, Q.Y., TABIEN, R., ZHONG, D.B., YING, C.S., STANSEL, J.W., KHUSH, G.S. AND PATERSON, A.H., 2001, Over-dominant epistasis loci are the primary genetic basis of inbreeding depression and heterosis in rice. Biomass and grain yield. *Genetics*, **158**: 1737-1753.
- LI, Z.K., PINSON, S.R.M., PATERSON, A.H., PARK, W.D. AND STANSEL, J.W., 1997. Epitasis for three grain yield components in rice (*Oryza sativa* L.). *Genetics*, **145**: 453-465.

- LINCOLN, S., DALY, M. AND LANDER, E., 1992. Mapping genes controlling quantitative traits with MAPMAKER/QTL. *Whitehead Institute Technical Report*, 20pp
- MACKILL, D. J. AND NI, J., 2001, Molecular mapping and marker-assisted selection for major-gene traits in rice. In. Rice Genetics IV. Proceedings of the Fourth International Rice Genetics Symposium, 22-27, October 2000, Los Banos, Philippines. (Ed. Khush, G. S., Brar, D. S. and Hardy, B.), p. 488.
- MAGHIRANG, Z. LI, Y. XING, Q. ZHANG, I. KONO, M. YANO, R. FJELLSTROM, G. DECLERCK, D. SCHNEIDER, S. CARTINHOURE, WARE, D. AND L. STEIN., 2002, Development and Mapping of 2240 New SSR Markers for Rice (*Oryza sativa* L.). *DNA Research*, **9**:199 - 207.
- MAHESWARAN, M. SUBUDHI, P.K., NANDI, S., XU, J.C., PARCO, A., YANG, D.C. AND HUANG, N. (1997). Polymorphism, distribution and segregation of AFLP markers in a double haploid rice population. *Theoretical and Applied Genetics*, **94**: 39-45.
- MAKHASI, J. 2001. Variation for drought tolerance and isozymes at seedling stage in rainfed upland rice (*Oryza sativa* L.). *M.Sc (Agri.) Thesis*, University of Agricultural Sciences, Dharwad.
- McCOUCH, S.R., 2002, Development and Mapping of 2240 New SSR Markers for Rice (*Oryza sativa* L.). *DNA Research*, **9**: 199-207
- McKENZIE, K. S., JOHNSON, C. W., TSENG, S. T., OSTER, J. J. AND BRANDON, D.M., 1994. Breeding improved rice cultivars for temperate regions-a case study. *Australian Journal of Experimental Agriculture*, **34**:389-905.
- MENEZES, N.L., De SILVEIRA, T.L.D. AND Da PASINTTO, P.R., 1994, Comparison of methods for rapid evaluation of physiological quality in rice seeds. *Revista Brasileira de Sementes*, **16**: 121-127.
- MISHRA, G.N., 2000, Weed management in different rainfed rice farming systems. In. Rainfed Rice- A source book of best practices and strategies in Eastern India (Ed., Singh, V. P. and Singh, R. K.), IRRI, and Manila, Philippines. p. 292.
- MISHRA, G. N. AND VAISHYA, R. D., 2000, Cost effectiveness of weed management for rice. In. Rainfed Rice- A source book of best practices and strategies in Eastern India (Ed., Singh, V. P. and Singh, R. K.), IRRI, and Manila, Philippines, p. 292.
- MITSUI, T., UEKI, Y. AND IGAGUE, I., 1993, Biosynthesis and secretion of α -amylase by rice suspension - cultured cells: purification and characterization of α -amylase isozyme H. *Plant Physiological Biochemistry*, **31**: 863-874.
- MIURA, K., LIN, S.Y., ARAKI, H., NAGAMINE, T., KUROKI, M., SHIMIZU, H., ANDO, I. AND YANO, M. 2004. Genetical studies on germination of seed and seedling establishment for breeding of improved rice varieties suitable for direct seeding culture. *Japan Agricultural Research Quarterly*, **38**: 1-5.
- MOHANKUMAR, H.D., 1999, Genetic analysis of physio-biochemical traits influencing drought tolerance in rice (*Oryza sativa* L.). *Ph. D. Thesis*, University of Agricultural Sciences, Dharwad, p.50.
- MOLL, R. H., COCKERHAM, C.C. STUBER, C.W. AND WILLIAMS, W.P., 1978, Selections, responses, genetic-environmental interactions and heterosis with recurrent parent selection for yield in maize. *Crop Science*, **18**: 641-645.
- MRF, 2003, Laboratory manual for marker assisted selection for bacterial leaf blight resistance and elongated uppermost internode genes in rice. Mahyco Research Foundation, Banjara Hills, Hyderabad
- NYBOM, H., BURG, W.J., VAN DER AND BINO, R.J., 1994, DNA fingerprinting for cultivar identification and plant breeding research. *Technological Advances in Variety and Seed Research*, **3**: 73-74.
- PANAUD, J. S., 1996, Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). *Molecular General Genetics*, **252**: 597.
- PANDEY, M.P., SESHU, D.V. AND AKBAR, M., 1989, Genetic control of embryo size and implications of that trait on seed vigour in rice. *IRRI Saturday Seminar*, June 17, 1989, IRRI, Los Banos, Philippines.

- PANDEY, M.P., SESHU, D.V. AND AKBAR, M., 1992, Genetic variation and association of embryo size to rice seed and seedling vigour. *Indian Journal of Genetics and Plant Breeding*, **52**: 310-320.
- PANDEY, M.P., SESHU, D.V. AND AKBAR, M., 1994, Genetics of embryo size and its relationship with seed and seedling vigour in rice (*Oryza sativa* L.). *Indian Journal of Genetics and Plant Breeding*, **54**: 258-268.
- PATERSON, A.H., DAMON, S., HEWITT, J.D., ZAMIR, D., RABINOWITCH, H.D., LINCOLN, S.E. AND TANKSLEY, S.D., 1991, Mendelian factors underlying quantitative traits in Tomato: comparison across species, generations and environments. *Genetics*, **127**:181-197.
- PEJIC, I., AJMONE-MARSAN, P., MORGANTE, M., KOZUMPLICK, V., CASTIGLIONI, PL., TARAMINO, G. AND MOTTO, M., 1998, Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs and AFLPs. *Theoretical and Applied Genetics*, **97**: 1248-1255.
- PERATA, P., POZUETA-ROMERO, J., AKAZAWA, T. AND YAMAGUCHI, J., 1992, Effect of anoxia on starch breakdown in rice and wheat seeds. *Planta*, **188**: 611-618.
- PERRY, D.A., 1972, Seed vigour and field establishment, *Horticultural Abstracts*, **42**: 334-342.
- PERRY, D.A., 1978, Report of the vigour test committee 1974-1977. *Seed Science and Technology*, **6**: 159-181.
- PERRY, D.A., 1980, Seed vigour and field establishment. *Advances in Research and Technology of Seeds*, **5**: 9-42.
- PERRY, D.A., 1981, Introduction, In *Hand Book of Vigour Test Methods*, International Seed Testing Association, Zurich, Switzerland, pp.3-7.
- PETERSON M.L., JONES, D.B. AND RUTGER, J. N., 1978, Cool temperature screening of rice lines for seedling vigour. *Riso*, **27**: 269-274.
- PETERSON, M L., JONES, D.B. AND RUTGER, J.N., 1978, Cool temperature screening of rice lines for seedling vigour. *Riso*, **27**: 269-274
- POLLOCK, B.M. AND ROOS, E.F., 1972, Seed and seedling vigour, In *Seed Biology*, Ed. T.T. Kozlowski, 1: 313-387.
- PRICE, A.H., TOMOS, A.D. AND VIRI, D.S., 1997, Genetic dissection of root growth in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **95**: 132-142.
- QING, S.C. AND XU, Z.W., 1996, Inheritance and correlation of root characters and leaf water potential in rice. *Scientia Agricultura Sinica*, **28**: 42-48.
- RAGHAVENDRA PRASAD, S., PRASHANTH, G., BAGALI, SHAILAJA HITTALMANI AND SHASHIDHAR, H. E., 2000. Molecular mapping of quantitative trait loci associated with seedling tolerance to salt stress in rice (*Oryza sativa* L.). *Current Science*, **78**: 162-164.
- RAMADEVI, R., 1998, Evaluation and genetic characterization of early vigour related traits in rainfed upland rice (*Oryza sativa* L.). *M.Sc (Agri.) Thesis*, University of Agricultural Sciences, Dharwad.
- RANJHAN, K., LITTS, J.C., FOOLAD, M.R. AND RODRIGUEZ, R.L., 1991, Chromosomal localization and genomic organization of α -amylase genes in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **82**: 481-488.
- REDDY, S.K.N., REDDY, B.M. AND ANKAIAH, R., 1996, Effect of initial germination levels on vigour, growth and yield in rice. *Seed Research*, **22**: 22-25.
- REDONA, E.D. AND MACKILL, D.J., 1996a, Genetic variation for seedling vigour traits in rice. *Crop Science*, **36**: 285-290.
- REDONA, E. D AND MACKILL, D. J., 1996b. Mapping quantitative trait loci for seedling vigour in rice using RFLPs. *Theoretical and Applied Genetics*, **92**:395-402.
- REDONA, E. D AND MACKILL, D. J., 1996c, Molecular mapping of quantitative trait loci in *japonica* rice. *Genome*, **39**:395-403.
- RODER, M.S., PLASCHKE, J., KONIG, S.O., BORNER, A., SORRELS, M.E., TANKSLEY, S.D. AND GANAL, M.W., 1995, Abundance variability and chromosomal location in microsatellites in wheat. *Molecular and General Genetics*, **246**: 327-333.

- SAGHAI MAROOF, M.A., BIYASHEV, R.M., YANG, G.P., ZHANG, Q. AND ALLARD, R.W., 1994, Extraordinarily polymorphic microsatellite DNA and barley species diversity chromosomal locations and population dynamics. *Proceedings of National Academy Sciences, USA*, **91**: 5466-5470.
- SAYED, H., KAYYAL, H., RAMSEY, L., CECCARELLI, S. AND BAUM, M. 2002. Segregation distortion in double haploid lines of barley (*Hordeum vulgare* L.) detected by simple sequence repeat markers. *Euphytica*, **225**: 265-272.
- SENIOR, M.L., MURTHY, J.P., GOODMAN, M.M. AND STUBER, C.W., 1998, Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. *Crop Science*, **38**: 1088-1098.
- SESHU, D.V. AND DADLANI, M., 1993, Seed vigour in rice (*Oryza sativa* L.): Its assessment and impact on crop performance. *Seed Research, Special Volume*, **2**: 700-706.
- SESHU, D.V. AND KRISHNASAMY, V., 1987. A genetic approach to reduction of yield losses in rice due to seed quality as affected by the post-harvest environment. *Paper Presented at the Third Annual Convention of the Federation of the Crop Science Society of the Philippines*, UPLB College, Laguna, Philippines.
- SHAHID, M., LATIF, T., IQBAL, M. AND KHAN, M.A., 1994, Genetic studies on drought tolerance in rice. *Sarhad Journal of Agriculture*, **10**(6): 671-674.
- SHARMA, R. N. AND SHARMA, S. M., 1993, Genetic analysis of seed and seedling vigour of rice (*Oryza sativa* L.). *Advances-in-Plant-Sciences*, **6**: 313-320.
- SHASHIDHAR, H. E., 2004. QTL: Identification and applications in improving drought tolerance in rice. *International Symposium on Rainfed Riceecosystem – Prospective and Potential* from 11-12 October, 2004, Indira Gandhi Agricultural University, held at Raipur, Chattisgarh, India.
- SHASHIDHAR, H.E., HEMAMALINI, G.S. AND HITTALAMANI, S., 1999, Molecular marker assisted tagging of morphological and physiological traits at the peak vegetative stage: two contrasting moisture regimes. In: O'Toole, J.C., Hardy, B. (eds). *Genetic improvement of rice for water-limited environments*. IRRI, Los Banos, Manila, Philippines, pp.239-256.
- SHENOY, V.V., DADLANI, M. AND SESHU, D.V., 1990, Association of laboratory assessed parameters with field emergence in rice. The nonanoic acid stress as a seed vigour test. *Seed Research*, **18**: 60-69.
- SIDDIQUE, S.B., 1986, Variability in seed dormancy and tolerance to accelerated ageing of seed in rice (*Oryza sativa* L.). *Ph.D. Thesis*, Cornell University, USA, p.146.
- SINGH, K.N., NANDI, R., SHANMUGASUNDARAM, P., SADASHIVAM, S., HUANG, N., BRAR, D.S. AND KUSH, G.S., 1999, High resolution DNA fingerprinting of Indian rice varieties (*Oryza sativa* L.) by AFLP. *Genetic Resource and Crop Evaluation*, **46**(5): 427-433.
- SINGH, R.K. AND CHAUDHARY, B.D., 1977, *Biometrical Methods in Quantitative Genetic Analysis*. Kalyani Publishers, New Delhi.
- STUBER, C.W., POLACCO, M. AND SENIOR, M.L. 1999. Synergy of empirical breeding, marker assisted selection and genomics to increase crop yield potential. *Crop Science*, **39**: 1571-1583.
- SUBBA RAO, L.V., KUMAR, S. AND VANISHREE, G., 1996, Genetic variability for seedling characters among rice (*Oryza sativa* L.) cultivars. *Seed Research*, **24**: 124-128.
- TANKSLEY, S. D., 1993. Mapping polygenes. *Annual Review of Genetics* **27**: 205-233.
- TECKRONY, D.M. AND EGLI, D.B., 1977, Relationship between laboratory indices of soybean seed vigour and field emergence. *Crop Science*, **17**: 573-577.
- TEMNYKH, 2000, Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **100**:697.
- TEMNYKH, S., 2001, Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): Frequency, length variation, transposon associations, and genetic marker potential. *Genome Research*, **11**:1441.

- TEMNYKH, S., GENEVIEVE DECLERCK, ANGELIKA LUKASHOVA, LEONARD LIPOVICH, SAMUEL CARTINHOOR AND SUSAN McCOUCH, 2001, Computational and Experimental Analysis of Microsatellites in Rice (*Oryza sativa* L.): Frequency, Length Variation. *Transposon Associations and Genetic Marker Potential. TAG*, **11**(8): 1441-1452.
- THANH, D., THANH, N.D., ZHENG, H.G., DONG, N.V., TRINH, L.N., ALI, M.L. AND NGUYEN, H.T., 1999, Genetic variation in root morphology and microsatellite DNA loci in upland rice (*Oryza sativa* L.) from Vietnam. *Euphytica*, **105** (1): 53-62.
- THOMSON, M.J., TAI, T.H., MCCLUNG, A.M., LAI, X.H., HINGA, M.E., LOBOS, K.B., XU, Y., MARTINEZ, C.P. AND McCOUCH, S.R., 2003, Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. *Theoretical and Applied Genetics*, **107**: 479-493
- UPADHYA, N. M., 2005, Mining genes controlling seedling vigour in cereals. Project report, CSIRO, Australia. p 4.
- WU, K. S. AND TANKSLEY, S. D., 1993, Abundance, polymorphism and genetic mapping of microsatellites in rice. *Molecular General Genetics*, **241**: 225-235.
- XIAO, J, S GRANDILLO, SN AHN, S. R., McCOUCH AND TANKSLEY, S. D., 1996, Genes from wild rice improve yield. *Nature*, **384**: 223.
- XU, C.G., LI, X.Q., XUE, Y., HUANG, Y. W., GAO, J. AND XING, Y.Z. 2004., Comparison of quantitative trait loci controlling seedling characteristics at two seedling stages using rice recombinant inbred lines. *Theoretical and Applied Genetics*, **109**: 640-647.
- XU, Y., Mc COUCH, S.R. AND SHEN, Z., 1998, Transgressive segregation of tiller angle in rice caused by complementary gene action. *Crop Science*, **38**:12-19.
- XU, Y., ZHU, L., XIAO, J., HUANG, N. AND McCOUCH, S. R., 1997, Chromosomal regions associated with segregation distortion of molecular markers in F₂, backcross, double haploid and recombinant inbred populations in rice (*Oryza sativa* L.). *Molecular General Genetics*, **253**: 535-545.
- YADAV, R., COURTOIS, B., HUANG, N. AND McLAREN, G., 1997, Mapping genes controlling root morphology and root distribution in a double-haploid population of rice. *Theoretical and Applied Genetics*, **94**: 619-632.
- YAMAOCHI, M. AND WINN, T., 1996, Rice seed vigour and seedling establishment in anaerobic soil. *Crop Science*, **36**: 680-686.
- YAMAUCHI, M., AGGILAR, A.M., VAUGHAN, D.A. AND SESHU, D.V., 1993. Rice germplasm suitable for direct sowing under flooded soil surface. *Euphytica*, **67**: 177-184.
- YANG, W., DE OLIVEKIA, A.C., GODWIN, I., SCHERTZ, K.F. AND BENNETZEN, J.L., 1996, Comparison of DNA marker technologies in characterizing the plant genome diversity. *Crop Science*, **36**: 1669-1676.
- YANO, M., HARUSHIMA, Y., NAGAMURA, Y., KURATA, N., MINOBE, Y. AND SASAKI, T., 1997, Identification of quantitative trait loci controlling heading date of rice using a high density linkage map. *Theoretical and Applied Genetics*, **95**: 1025-1032.
- YIN, X., KROFF, M.J., AGGARAWAL, P.K., PENG, S., AND HORIE, T., 1997. Optimal preflowering, phenology of irrigated rice for high yield potential in three Asian environments: A simulation study. *Field Crops Research*, **51**: 19-27.
- YU, S. B., LI, J. X., XU, C.G., TAN, Y. F., GAO, Y. J., LI, X. H., ZHANG, Q.F. AND SAGHAI MAROOF, M.A., 1997. Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. *Proceedings of National Academy Science, USA*, **94**: 9226-9231.
- ZHANG, Z., SHUN QU, XIN., WAN, SHAN, CHEN, LI-HUA AND ZHU YING-GUO, 2004, Comparison of QTL controlling seedling vigour under different temperature conditions using recombinant inbred lines in Rice (*Oryza sativa*). *Annals of Botany*. www.aob.oupjournals.org
- ZHI-HONG ZHANGA, SI-BIN YUB, TING YUA, ZHENG HUANGA AND YING-GUO ZHUA, 2005, Mapping quantitative trait loci (QTLs) for seedling-vigor using recombinant inbred lines of rice (*Oryza sativa* L.). *Field Crops Research*, pp. 161-170.

ZHOU, Z. AND GUSTAFSON, J.P., 1995, Characterization of micro satellite sequences in rice and their application for DNA fingerprinting. *Induced Mutations and Molecular Techniques for Crop Improvement, Proceedings*, Vienna, pp.205-214.

APPENDIX

Appendix – I . Preparation of stocks for CTAB method of DNA Extraction

1 M Tris base (pH 8.0)

Tris base 121.1g/lt. --- 1M

DDW upto 1000 mL

0.5 M EDTA (pH 8.0)

372.24g/lit ----- 1M

4 M NaCl

58.44g/lit ----- 1M

T10E1 (pH 8.0)

Tris base – 0.121 g/100 mL

EDTA - 0.0372 g/100 mL

50X TAE (for 250 mL)

Tris base – 60.5 g

Acetic acid glacial - 14.25 mL

0.5 M EDTA - 25 mL (pH 8.0)

Volume made up to 250 mL with DDW

PH adjusted to 8.0 with conc. HCl. Autoclaved and stored at room temperature.

Chloroform: Iso amyl alcohol (24:1) (v/v)

70 % Ethanol

Extraction Buffer for CTAB (100 mL)

10% CTAB	-	20 mL
----------	---	-------

1M Tris base	-	10 mL
--------------	---	-------

4M NaCl	-	35 mL
---------	---	-------

Sterile Water - make up to 100 mL

Autoclave and add

Mercapto ethanol	-	1 mL
------------------	---	------

PVP	-	0.1% (100 mg)
-----	---	---------------

Appendix II: List of primers used in the study

Sl.No	SSR	Chrom No.	Repeat type	Forward primer	Reverse primer	Anneal temp	Reference
1	RM1	1	(AG)26	gcgaaaacacaatgcaaaa a	gcgttggttgacctgac	55	Panaud <i>et al.</i> ,1996
2	RM14	1	(GA)18	ccgaggagaggagttcgac	gtgccaatctcctcgaaaaa	55	Panaud <i>et al.</i> ,1996
3	RM226	1	(AT)38	agctaaggctctgggagaaac c	aagtaggatggggcacaag ctc	55	Akagi,1996; Wu&Tanksley,1993
4	RM212	1	(CT)24	ccacttcagctactaccag	caccattgtctctcattatg	55	Chen <i>et al.</i> ,1997
5	RM243	1	(CT)18	gatctgcagactgcagttgc	agctgcaacgatgtgtcc	55	Chen <i>et al.</i> ,1997
6	RM23	1	(GA)15	cattggagtggaggctgg	gtcaggctctgccattctc	55	Chen <i>et al.</i> ,1997
7	RM220	1	(CT)17	ggaaggtaactgtttccaac	gaaatgcttcccacatgtct	55	Chen <i>et al.</i> ,1997
8	RM29	2	(GA)7	cagggaccacctgtcatac	aacgttggtcatatcggtgg	55	Chen <i>et al.</i> ,1997
9	RM250	2	(CT)17	ggttcaaaccaagctgatca	gatgaaggcctccacgcag	55	Chen <i>et al.</i> ,1997
10	RM347	3	(GGC)5(AT)7	cacctcaaacttttaaccgcac	tccggcaagggatacggcgg	55	Temnykh <i>et al.</i> ,2000;Cho <i>et al.</i> ,.2001
11	RM203	3	(AT)21	cctatcccattagccaaacatt gc	gatttacctcgacgccaacct g	55	Akagi, 1996;Wu&Tanksley,1993;Temnykh <i>et al.</i> , 2001
12	RM426	3	(CA)10	atgagatgagttcaaggccc	aactctgtacctccatcgcc	55	Temnykh <i>et al.</i> , 2001
13	RM554	3	(GA)14	gttcgtcgtctctcgtctc	cccaaaaatctgtgcctctc	55	Temnykh <i>et al.</i> , 2001
14	RM7	3	(GA)19	ttcgccatgaagtctctcg	cctcccatcatttcgttgtt	55	Panaud <i>et al.</i> ,1996
15	RM570	3	(AG)15	gttctcaactcccagtgcg	tgacgatgtggaagagcaag	55	Temnykh <i>et al.</i> ,2001

16	RM55	3	(GA)17	ccgtcgccgtagtagagaag	tcccggttattttaaggcg	55	Chen <i>et al.</i> ,1997
17	RM335	4	(CTT)25	gtacacacccacatcgagaa g	gctctatgcgagtatccatgg	55	Temnykh <i>et al.</i> ,2000
18	RM303	4	[AC(AT)2- 10]9(GT)7(ATGT)6	gcatggccaaatattaaagg	ggttggaaatagaagttcggg	55	Temnykh <i>et al.</i> ,2000
19	RM178	5	(GA)5(AG)8	tcgcgtgaaagataagcggc gc	gatcaccgtccctccgcctgc	67	Temnykh <i>et al.</i> ,2000
20	RM26	5	(GA)15	gagtcgacgagcggcaga	ctgcgagcgacggtaaca	55	Chen <i>et al.</i> ,1997
21	RM163	5	(GGAGA)4(GA)11C(G A)20	atccatgtgcgccttatgagga	cgctacctccttcacttactagt		Wu & Tanksley,1993;Temnykh <i>et al.</i> ,2001

Appendix I. Contd...

Sl.No	Primers	Chrom No.	Repeat type	Forward primer	Reverse primer	Anneal temp	Reference
22	RM249	5	(AG)5A2(AG)14	ggcgtaaaggttttgcattg	atgatccatgaaggtcagc	55	Chen <i>et al.</i> ,1997;Xu <i>et al.</i> ,1998
23	RM159	5	(GA)19	ggggcactggcaagggtaga gg	gcttggtctctctctctctctc ctc	55	Temnykh <i>et al.</i> , 2000
24	RM87	5	(CTT)3T(CTT)11	cctctccgatacaccgatg	gcgaaggtacgaaaggaaa g	55	Temnykh <i>et al.</i> ,2000
25	RM3	6	(GA)2GG(GA)25	acactgtagcggccactg	cctccactgctccacatctt	55	Panaud <i>et al.</i> ,1996
26	RM162	6	(AC)20	gccagcaaaaccagggatcc gg	caaggtcttgtagcggtgcgg	61	Temnykh <i>et al.</i> , 2000; Akagi <i>et al.</i> ,1996
27	RM253	6	(GA)25	tcctcaagagtgcataacc	gcattgtcatgtcgaagcc	55	Temnykh <i>et al.</i> ,2001
28	RM585	6	(TC)45	cagtcctgtccgtttgtg	ctgtgactgacttggtcatagg	55	Temnykh <i>et al.</i> ,2001
29	RM225	6	(CT)18	tgcccatatggtctggatg	gaaagtggatcaggaaggc	55	Chen <i>et al.</i> ,1997
30	RM214	7	(CT)14	ctgatgatagaaacctcttc	aagaacagctgactcaca	55	Chen <i>et al.</i> ,1997
31	RM10	7	(GA)15	ttgtcaagaggagcatcg	cagaatgggaaatgggtcc	55	Panaud <i>et al.</i> ,1996
32	RM256	8	(CT)21	gacaggagtgattgaaggc	gttgattcgccaagggc	55	Panaud <i>et al.</i> ,1996
33	RM210	8	(CT)23	tcacattcggtagcattg	cgaggatggtgttcactg	55	Chen <i>et al.</i> ,1997
34	RM105	9	(CCT)6	gtcgtcgacccatcggagcca c	tggtcgagggtgggtagcgggt c	55	Temnykh <i>et al.</i> , 2000
35	RM201	9	(CT)17	ctcgttattacctacagtacc	ctacctccttttagaccgata	55	Chen <i>et al.</i> ,1997
36	RM242	9	(CT)26	ggccaacgtgtgtatgtctc	tatatgccaagacggatggg	55	Chen <i>et al.</i> ,1997

37	RM257	9	(CT)24	cagttccgagcaagagtactc	ggatcggacgtggcatatg	55	Chen <i>et al.</i> ,1997
38	RM410	9	(TA)13	gctcaacgtttcgttctg	gaagatgcgtaaagtgaacg g	55	Temnykh <i>et al.</i> , 2001
39	RM224	11	(AAG)8(AG)13	atcgatcgatcttcacgagg	tgctataaaaggcattcggg	55	Chen <i>et al.</i> ,1997
40	RM144	11	(ATT)11	tgccctggcgcaaattgatcc	gctagaggagatcagatggt agtgcattg	55	Temnykh <i>et al.</i> , 2000
41	RM19	12	(ATC)10	caaaaacagagcagatgac	ctcaagatggacgccaaga	55	Panaud <i>et al.</i> ,1996
42	RM247	12	(CT)16	tagtgccgatcgatgtaacg	catatggttttgacaaagcg	55	Chen <i>et al.</i> ,1997

DNA MARKER ASSISTED GENETIC ANALYSIS OF EARLY VIGOUR RELATED TRAITS IN RICE (*Oryza sativa* L.)

JAYATEERTHA R. DIWAN 2006 Dr. M. V. C. GOWDA
Major Advisor

ABSTRACT

Upland rice plays a major role in the sustainable food security of the country. Early vigour trait is important in the drill-sown rice for weed suppression as well as initial establishment. The IR64/Azucena was used as a reference population to identify QTLs for early vigour related traits. The SSR markers flanking QTLs in IR64/Azucena and other populations were used for validation in the new populations (BPT5204/A67 and BPT5204/Dodiga). Twenty-two QTLs distributed on chromosomes 1 to 6 were identified for different vigour related traits. The QTLs controlling germination were located on chromosome 1 in the region RM5-RM306 near to α -amylase genes which control germination. The congruence of QTLs was observed on Chromosome 5 in the region RM87-RM334 for rate of germination, seedling dry weight and vigour index. The additive effect was both positive and negative indicating contribution from both parents. Transgressive segregants were observed for different traits in all the populations revealing contribution of favourable alleles from both parents. The narrow sense heritability for shoot length was comparable in the two crosses viz., BPT 5204/A67 (29.2%) and BPT 5204/Dodiga (24.1%) while realized heritability was higher in BPT5204/Dodiga (48.7%) as compared to BPT 5204/A67 (15.5%). Out of 42 Simple Sequence Repeats (SSR) markers assessed, 24 were polymorphic between BPT 5204 and Dodiga and 20 markers were polymorphic between BPT 5204 and A67. The polymorphic markers were screened in F₂:F₃ populations. The single marker analysis was employed to assess the association between marker and trait. RM253 (Chromosome 6) was associated with the shoot length at 10th and 14th DAS with 9.6% and 10.1% contribution in BPT5204/A67. RM178 (Chromosome 5) was associated with shoot length (18%) and root length (11.2%) at 10th DAS in BPT5204/Dodiga.