2. REVIEW OF LITERATURE

Plants can recognize potential pathogens via two perception systems. One of them, named pathogen or microbe-associated molecular patterns (PAMPs or MAMPs, respectively), detects conserved molecules associated with groups of pathogens through pattern recognition receptors leading to PAMP-triggered immunity. The other evolved to recognize pathogen virulence effectors, usually through intracellular resistance proteins (R proteins), causing effector-triggered immunity (ETI). ETI corresponds to what is classically referred to as gene-for-gene, vertical or race-specific resistance (Dodds and Rathjen 2010).

In studies with bean anthracnose, Barrus (1911) was the first to demonstrate the existence of physiological races of plant pathogens in the interaction of C. lindemuthianum and common bean (Phaseolus vulgaris L.), whereas, Burkholder (1918) provided the first information on the inheritance of disease resistance in plants. Since, C. lindemuthianum is a highly variable pathogen (Balardin et al. 1997; Pastor-Corrales et al. 1995; Rodriguez-Guerra et al. 2003) and resistance in the host is assumed to follow the gene-for-gene theory (Flor 1947). Therefore, genes conferring resistance against different races of C. lindemuthianum have been tagged by using RAPD and SCAR molecular markers in cultivars Cornell (Co-2), AB 136 (Co-6), G 2333 (three genes Co-42, Co-5, Co-7) by different workers (Young and Kelly 1996, 1997; Alzate Marin et al. 1999b). Currently, 14 major race-specific resistance loci to C. lindemuthianum have been reported and are identified by the Co symbol (Co-1 to Co-14) (Kelly and Vallejo 2004; Goncalves-Vidigal et al. 2008, 2009, 2012; Ferreira et al. 2013). Of these, only four loci Co-1, Co-12, Co-13 and Co-14 belong to the Andean gene pool (Kelly and Vallejo 2004; Goncalves-Vidigal et al. 2008, 2009, 2012).

Although breeding the common bean for resistance to anthracnose by traditional method has created several resistant cultivars (Barrus 1911; Singh 1992). But the pyramiding of different race-specific resistance (R) alleles using marker-assisted selection (MAS), retaining defeated genes, and combining QTL of major effect in developing durable resistance clearly assures progress towards developing durable
resistance in common bean (Kelly and Miklas 1999; Miklas et al. 2006; Ragagnin et al. 2009; Garzon et al. 2008; Ferreira et al. 2013; Madakbas et al. 2013; Teran et al. 2013). There have been suggestions that combination of resistance genes of Andean and Mesoamerican origin could provide more durable resistance (Pastor-Corrales et al. 1995; Balardin and Kelly 1998; Kelly and Vallejo 2004). In this direction attempt has been made to study the genetics and molecular mapping of anthracnose resistance genes in Indian landrace KRC5 which possess resistance to eight races of the pathogen. The literature pertaining to the present study has been reviewed under the following heads:

2.1 Genetics of resistance
2.2 Mapping populations for anthracnose resistance genes
2.3 Molecular mapping of anthracnose resistance genes
2.4 Identification of resistant gene(s) using molecular marker
2.5 Linkage map

2.1 Genetics of Resistance

The genetics of anthracnose resistance has been studied for a long time, since this host/pathogen interaction was the first report of race-cultivar specificity (Barrus 1911; McRostie 1919). Early history before the discovery that led to search for disease resistance comprises rediscovery of Mendelism in 1900 that initiated breeding for disease resistance and Biffen’s work on inheritance of resistance in a Mendelian manner in 1905. In a pioneering analysis of resistance H.H. Flor studied interaction both in the plant and the pathogen simultaneously and formulated the most convincing explanation of the observed phenomenon known as ‘gene-for-gene hypothesis (Flor 1947, 1955, 1971). Resistance was classified as vertical or horizontal by Plank (1963) i.e monogenic resistance that is based on single gene major gene whereas quantitative resistance governed by two or more genes with minor effect. Later, two more kind of resistance viz., seedling and adult plant resistance were recognized in the host (Manners 1969) followed by the concept of durable resistance by Johnson (1981).

Under favorable conditions susceptibility or resistance in each host-pathosystem is predetermined by the genetic material of the host and the pathogen. Resistance mechanism in the common bean and extremely high level of genetic resistance, controlled by a single or few major genes have been reported in number of cultivars (Muhalet et al. 1981; Pastor-Corrales et al. 1994).
Such studies are based on the interpretation of results obtained from F₂ segregating populations derived from two types of crosses: R × S or R × R (R is resistant and S is susceptible). Based on these R × S crosses the inference is used to elucidate the number and mode of action of genes conferring resistance to \( C. lindemuthianum \), while those for R × R crosses are used to identify the specific genes involved in the reaction against this pathogen (allelism tests). The existence of single dominant gene in kidney bean cultivars controlling resistance to different races of anthracnose pathogen has been reported by workers. McRostie (1919) discovered first resistance gene \( Co-1 \) (\( A \)) in the Andean variety Michigan Dark Red Kidney. Later, Mastenbroek (1960) found an anthracnose resistance gene \( Are \) gene in the black common bean cv. Cornell 49-242. However, it was renamed from \( Are \) to \( Co-2 \) by Young and Kelly (1996). Bannerot (1965) discovered \( Co-3 \) (\( Mexique 1 \)) in the Mesoamerican variety Mexico 222, an allele for \( Co-3 \) locus was reported in the Mesoamerican variety Mexico 227 (Fouilloux 1975). The \( Co-4 \) (\( Mexique 2 \)) gene was discovered differential variety TO by Bannerot (1969). \( Co-6 \) gene was discovered by Schwartz et al. (1982) in the Mesoamerican differential variety AB 136.

The nature of resistance genes in highly resistant G2333 differential cultivar was firstly studied by Pastor-Corrales et al. (1994), suggested the presence of two independent dominant genes in this cultivar. A 15:1 (resistant: susceptible) ratio was observed after inoculation of an F₂ population of G 2333/Pijao with race 521. Young and Kelly (1996) demonstrated that Sel 1360 cultivar carried a single dominant gene that proved to be the same locus as the \( Co-5 \) gene in differential cultivar TU. Later, Vellejo and Kelly (2009) reported that G 2333 carries a second allele at the \( Co-5 \) gene different from that possessed by TU, which is designated \( Co-5^2 \).

Alzate-Marín et al. (2000) studied the inheritance pattern of resistance present in common bean cultivar AB 136 in segregating populations derived from crosses with cultivar Ruda (susceptible to most \( C. lindemuthianum \) races). They inoculated two progenitors, populations F₁ and F₂, F₂;3 families and backcross-derived plants with race 89 of \( C. lindemuthianum \) under environmentally controlled greenhouse conditions. The results observed indicated that a single dominant gene, \( Co-6 \), controls the common bean resistance to this race, giving a segregation ratio between resistant and susceptible plants
of 3:1 in the F2, 1:0 in backcrosses to AB 136 and 1:1 in the backcross to Ruda. The segregation ratio of F2;3 families derived from F2 resistant plants was 1:2 (homozygous to heterozygous resistant).

Arruda et al. 2000 studied the inheritance of resistance to anthracnose in the cultivar TO (carrying the Co-4 gene) in F1, F2, F2;3, BC1s, and BC1 population from Ruda x TO cross by inoculating with race 65 of C. lindemuthianum. The phenotypic ratios (resistant/susceptible) 3:1 in the F2 population, 1:1 in the BC1s, and 1:0 in the BC1r, confirmed the presence of monogenic dominant resistant gene in the cultivar TO.

Melotto and Kelly (2000) studied the genetic of resistance in the Andean bean cultivar Kaboon and Perry Marrow, and based on the segregation ratio (3R:1S) observed in two F2 population, they demonstrated that Kaboon carries one major dominant gene conferring resistance to race 7 and 73 of C. lindemuthianum. They found that gene in Kaboon is independent from the Co-2 gene and is an allele of the Co-1 gene present in Michigan Dark Red Kidney (MDRK) cultivar and proposes the symbol Co-I2 for this new allele present in Kaboon. Upon inoculation with the less virulent Andean race 5, the segregation ratio -of 57R:7S (p=0.38) in the F2 progeny of Cardinal and Kaboon indicated that Kaboon must possess other weaker dominant genes with complimentary mode of action. As Cardinal is not known to possess genes for anthracnose resistance. Perry Marrow, a second Andean cultivar with resistance to a different group of races was shown to possess another resistance allele at the Co-1 locus and was assigned gene symbol Co-I3. In their study involving R x R cross between Perry Marrow and MDRK or Kaboon, no susceptible F2 plants were found upon inoculation with race 73. These finding supports the presence of a multiple allelic series at the Andean Co-I locus, which have major implication in breeding for durable anthracnose resistance in common bean.

Alzate-Marin et al. (1998) found segregation ratios of 15:1 in the F2 of cross between Ruda and G2333 and 3:1 in the backcrosses to Ruda, when tested against two races i.e. race 73 and 89 of the C. lindemuthianum, indicated the presence two independent resistance loci in G2333 for each of the two races.

Mendoza et al. (2001) investigated resistance to C. lindemuthianum race 1472 in P. vulgaris line A193 in segregating F2 and F2;3 populations from a cross between A193 and Florde Mayo Bajio. They found that resistance in line A193 to race 1472 is governed by a
single dominant gene. They suggested that resistance in A193 is conditioned by the Co-1 gene upon inoculating the crosses between A193 and cultivars Michigan Dark Red Kidney and Perry Marrow with race 1472. However, inoculation of the cross A193 x Perry Marrow with *C. lindemuthianum* race 2, demonstrated that resistance to race 2 in Perry Marrow is also conditioned by a single dominant gene and is distinct from the resistance present in A193 or Michigan Dark Red Kidney.

Alzate-Marin et al. (2003) studied inheritance of anthracnose resistance gene in AND 277 by analyzing segregating population derived from cross with susceptible cultivar Perry Marrow. The presence of single dominant gene was indicated by segregation ratio of 3R:1S in F$_2$. The allelism test showed a segregation ratio of 15:1 for the cross involving AND 227 and Ouro Negro indicating the presence of two independent dominant gene as Ouro Negro is known to carrying Co-10. The segregating population developed by crossing AND 227 with MDRK, Kaboon did not show any segregation, indicating there by the presence of an allele of Co-1 gene in AND 227 cultivar.

Mendez-Vigo et al. (2005) based on allelism tests indicated that resistance to race 38 of anthracnose in genotypes 'A1220', 'A1231', and 'BAT 93' and in the differential cultivars 'PI 207262' and 'Mexico 222' is determined by different dominant alleles at the same locus. Therefore, suggested that the so far considered as different genes Co-3 (described as present in 'Mexico 222') and Co-9 (described as present in 'BAT 93') are alleles of the same gene.

Pathania et al. (2006) in their studies on inheritance of resistance in exotic accession G 2333 and Indian accession, KRC-5 showed that two independent dominant genes conferred resistance in G 2333 to race 3 and 515 and a single dominant gene controlled resistance in KRC-5 to race 775, thereby indicating easy transfer of resistance from these sources to the locally adapted susceptible cultivars.

Goncalves-Vidaligal and Kelly (2006) reported that the anthracnose resistance gene in cv. Widusa is independent of Co-2, Co-3, Co-4, Co-5, Co-6, Co-9, and Co-10 genes. They observed segregation patterns in six F$_2$ populations of a cross between Widusa x Michigan Dark Red Kidney (MDRK), Michelite, BAT 93, Mexico 222, Cornell 49-242, and TO cultivars, that supported an expected 3R:1S ratio suggesting that Widusa carries a single dominant gene conditioning resistance to races 7, 65, 73, and 453 of *C.
and proposed its name as $Co-I^5$. Allelism tests conducted using $F_2$ populations derived from crosses between Widusa and Cornell 49-242 ($Co-2$), Mexico 222 ($Co-3$), TO ($Co-4$), TU ($Co-5$), AB 136 ($Co-6$), BAT 93 ($Co-9$), and Ouro Negro ($Co-10$), inoculated with races 7, 9, 65 and 73 a segregation ratio of 15R:1S was found. A lack of segregation was observed among 200 $F_2$ individuals from the cross Widusa/MDRK, and among 138 $F_2$ individuals from the cross Widusa/Kaboon inoculated with race 65, suggested that Widusa carries an allele at the $Co-I$ locus.

Goncalves-Vidigal et al. (2007) studied the genetic of resistance in *P. vulgaris* L. cultivar Michelite to races 8 and 64 of *C. lindemuthianum*. $F_2$ population of cross made between Michelite and Mexico 222 cultivars was inoculated with race 64. The segregation of $F_2$ population fitted in a ratio of 3R:1S, showing the presence of a dominant gene in Michelite. Allelism tests were also conducted with $F_2$ populations derived from crosses between Michelite and AB 136, AND 277, BAT 93, Cornell 49-242, G 2333, Kaboon, Mexico 222, Michigan Dark Red Kidney (MRDK), Ouro Negro, Perry Marrow, PI 207262, TO, TU, and Widusa. All the cultivars (except Mexico 222) were resistant to race 64. While $F_2$ derived from the Michelite x Mexico 222 was inoculated with race 8. Additionally, allelism tests indicated that the gene present in Michelite is independent from $Co-I$, $Co-2$, $Co-3$, $Co-4$, $Co-5$, $Co-6$, $Co-7$, $Co-9$ and $Co-10$ genes and named this new anthracnose resistant gene as $Co-11$.

Rodriguez et al. (2007) studied $F_2$ population of 85 individuals from a cross between Spanish landrace Andecha (Andean origin) and Mesoamerican genotype A252. Resistance to races 6, 31, 38, 39, 65 and 357 of *C. lindemuthianum* was evaluated in $F_3$ families derived from the corresponding $F_2$ individuals. The intermediate resistance to race 65 proceeding from Andecha was explained by a single dominant gene located on linkage group B1 corresponding to the $Co-I$ gene. The recombination between the resistance specificities proceeding from A252 agrees with the assumption that the total resistance to race 6, 31, 38, 39, 65, and 357 is organized in two clusters. One cluster located on B4 and other on B11 linkage group.

Kun et al. (2009) studied inheritance of resistance in Andean Chinese common bean (*P. vulgaris*) landraces by inoculating 108 $F_2$ plants derived from a cross between *cv. Red Flower* (R) and *cv. Jing* (S) with race 81. The segregation ratio of resistant to
susceptible plants in the F₂ population was found to be 3:1 based on the chi-square test, indicating that resistance in Red Flower to anthracnose race 81 was controlled by a single dominant gene, and thus temporarily named it as Co-F2533.

Goncalves-Vidigal et al. (2009) studied inheritance of resistance in common bean landrace Jalo listras pretas (JLP); the source of a new Andean anthracnose resistance gene. The F₂ population derived from JLP/Mexico 222 and JLP/ Cornell 49242 were inoculated with race 64 and 73, respectively. Segregation for resistance in both F₂ populations fits a 3:1 resistance/ susceptible ratio suggesting that anthracnose resistance in JLP is controlled by a single dominant gene.

Chen et al. (2011) in their study using F₂ plants and F₃ families derived from a cross between red common bean cultivar F2322 (resistant) and Jing common bean cultivar F0777 (susceptible) to race 81, where Chinese cultivar red bean showed to carry a single dominant gene for resistance to anthracnose (C. lindemuthianum), designated tentatively as Co-F2322.

Goncalves-Vidigal et al. (2011) reported two broad spectrum resistance alleles, Co-14 and the Phg-1 that confer resistance to 21 and 8 races of the anthracnose (ANT) and angular leaf spot (ALS) pathogens in Andean common bean AND 277. They elucidated the inheritance of resistance in AND 277 to both pathogens using F₂ populations from the AND 277 x Ruda and AND 277 x Ouro Negro crosses and F₂:₃ families from the AND 277 x Ouro Negro cross. Co-segregation analysis revealed that a single dominant gene in AND 277 confers resistance to races 65, 73, and 2047 of the ANT and to race 63-23 of the ALS pathogens.

Goncalves-Vidigal et al. (2012) studied genetic analysis of anthracnose resistance in the Andean cultivar ‘Pitanga’. They crossed the Pitanga cultivar with the Michelite, Michigan Dark Red Kidney, Cornell 49-242, Mexico 222, PI 207262, TU, AB 136, G 2333, BAT 93, SEL 1308, H1 line, Ouro Negro, Jalo Vermelho and Jalo Listras Pretas cultivars to obtain F₁ and F₂ populations. These populations were inoculated with races 23, 64, 65, 73 and 2047 of C. lindemuthianum and segregation ratio of 3 resistant: 1 susceptible was found which showed that resistance is controlled by a single dominant gene. The results from allelism tests in fourteen F₂ populations fitted a 15 resistant: 1
susceptible ratio, which indicated that the resistance present in the ‘Pitanga’ cultivar is dominant, independent from genes and alleles that were already characterized (Co-1, Co-2, Co-3, Co-4, Co-5, Co-6, Co-7, Co-9, Co-10, Co-11, Co-12 and Co-13). The authors suggested the symbol Co-14 to represent the R-gene present in the ‘Pitanga’ cultivar.

Goncalves-Vidigal et al. (2013) in their study performed a genetic co-segregation analysis of resistance to ANT and ALS using an F₂ population from the Ruda x Ouro Negro cross and the F₂:3 families from the AND 277 x Ouro Negro cross. Where, Ouro Negro is resistant to races 7 and 73 of the ANT and race 63-39 of the ALS pathogens. Conversely, cultivars AND 277 and Ruda were susceptible to races 7 and 73 of ANT, respectively. Both cultivars were susceptible to race 63-39 of ALS. Co-segregation analysis revealed that Co-10 and Phg-ON were inherited together, conferring resistance to races 7 and 73 of ANT and race 63-39.

Sousa et al. (2014) reported that a common bean breeding line MSU 7-1 contains the Co-5 and Co-7 anthracnose resistance genes. The phenotypic analyses of F₂ population derived from the Mexico 222 (S) x MSU 7-1 (R) cross upon inoculations with race 64 of C. lindemuthianum segregated in a 3R:1S ratio, indicating that only Co-5 confers resistance to race 64 once the Co-7 gene has been defeated by this race.

2.2 Mapping populations for anthracnose resistance genes

The choice of plant material is one of the major factors determining the success of a gene mapping work. The wide inter-sub specific crosses between two parents showing a higher level of DNA polymorphism than intra-sub specific crosses and thereby contributing to increased mapping efficiency (McCouch and Tanksley 1991). The trait to be studied in a mapping population needs to be polymorphic between parental lines at the genotypic as well as phenotypic level and its significant heritability is essential for gene mapping.

Various populations, such as near isogenic lines (NILs), recombinant inbred lines (RILs), doubled haploid lines and F₂ populations, are used for gene analysis and mapping. For cultivars that carry more than two R genes, it is preferable to use advanced populations such as NILs and RILs populations, as well as monogenic F₃ and F₄ lines but segregating distortions is seems to be a common feature in RILs populations. The comparison to other types of mapping populations, RILs populations derived from single
seed descent have undergone several rounds of meiosis and natural selection during the inbreeding process, resulting in a high proportion of distorted markers (Haussmann et al. 2002; Singh et al. 2007). There has been greater proportion of distortion for interspecific crosses compared to intraspecific ones.

Despite these constraints, there has been reports of use of RILs in mapping disease resistance genes against different diseases in *Phaseolus* (Schneider et al. 2001; Miklas et al. 2000), their use in development of genetic linkage map containing six genes and nine quantitative trait loci (QTL) comprising resistance to one bacterial, three fungal, and two viral pathogens of bean.

Since, bulked segregant analysis (BSA) is an approach for quickly identifying molecular markers linked to the gene or genomic region of interest in conditions where NILs are not available for mapping project (Michelmore et al. 1991). The method involves comparing two pooled DNA samples of individuals from a segregating population originating from a single cross. Within each pool or bulk, the individuals are identical for the trait or gene of interest but are arbitrary for all other genes. Two pools contrasting for a trait (e.g. resistant and susceptible) are analyzed to identify markers that distinguish them. Markers that are polymorphic between the pools will be genetically linked to the loci determining the trait used to construct the pools. The only pre-requisite for employing BSA in gene mapping is the existence of a population resulting from a cross that segregates for the gene of interest. Bulked segregant analysis overcomes several problems associated with the use of NILs to identify markers linked to particular genes. There is minimal chance that regions unlinked to the target region will differ between the contrasting bulks. In contrast, even after five backcrosses, only half of the loci polymorphic between the NILs are expected to map to the selected region (Muelbauer et al. 1988; Gepts (1993); Nodari et al. (1993a) and Freyre et al. (1998). Near-isogenic lines require many backcrosses to develop and are therefore time consuming to generate; in contrast bulked segregants can be made immediately for any locus or genomic region once the segregating population has been constructed.
2.3 Molecular mapping of anthracnose resistance genes

In the beginning of study employing identification of anthracnose resistance gene in common bean cultivars/landraces against different races of \( C.\ lindemuthianum \), different workers have reported major genes in various lines that are tagged using different molecular markers and are reviewed below:

Young and Kelly (1996) identified Random Amplified Polymorphic DNA (RAPD) markers designated OQ4_{1440} and B355_{1000} generated by decamer primers tightly linked in coupling phase with \( \text{Are} \) gene flanked at 4.0 ± 1.9cM distance between two markers in the Andean and Middle American genetic background varieties.

Young and Kelly 1997 identified RAPD markers linked to three independent resistance loci in common bean by means of bulked segregant analysis and heterogeneous inbred populations. They reported that RAPD marker OF10_{530} could facilitate introgression of the \( \text{Co}-1 \) gene across the Andean and Middle American \( \text{Phaseolus} \) gene pool. An RAPD marker OAB3_{450} was linked in coupling phase to the \( \text{Co}-5 \) (Mexique 3) allele, whereas OAH1_{780} (coupling phase) and OAK20_{890} (repulsion phase) RAPD markers were linked to the \( \text{Co}-6 \) locus.

Young et al. (1998) identified two independently assorting dominant genes conditioning resistance to bean anthracnose \( (C.\ lindemuthianum) \) in an \( F_2 \) population derived from the highly resistant \( P.\ vulgaris \) differential cultivar, G2333. Where one gene was allelic to the \( \text{Co}-4 \) gene in the differential cultivar TO and was named \( \text{Co}-4^2 \), the second gene was assigned the temporary name \( \text{Co}-7 \) until a complete characterization with other known resistance genes can be conducted. They identified two RAPD markers linked to the \( \text{Co}-4^2 \) allele \( i.e. \) one RAPD, OAS13_{950}, co-segregated with no recombinants in two segregating populations of 143 \( F_2 \) individuals, whereas the second RAPD, OAL9_{740}, mapped at 3.9 cM from the \( \text{Co}-4^2 \) allele.

Alzate Marin et al. (1999b) identified RAPD markers OPZ04_{560} linked in coupling phase to the resistance gene in \( \text{cv. AB136} \) conferring resistance to 4 races/pathotypes of \( C.\ lindemuthianum \). They suggested that single gene or complex locus of linked resistance genes present in cultivar AB 136 confers resistance to all four pathotypes (89, 73, 81 and 64).
Alzate-Marin et al. (1999b) identified a Random Amplified Polymorphic DNA (RAPD) marker (OPH18_{1200C}) linked to race 73 resistance in a BC$_3$F$_{2:3}$ populations derived from crosses between Ruda and G2333. They used previously identified RAPD marker (OPAS13$_{950C}$), which was linked to gene $Co-4^2$ to generate a genetic map where the two markers were located at 5.6 (OPH18$_{1200C}$) and 11.2 (OPAS13$_{950C}$) cM of the $Co-4^2$ gene. They also suggested the presence of $Co-5$ resistance gene against race 89 in same BC$_1$F$_{2:3}$ populations based on amplification with RAPD molecular marker OPAB$_{450C}$.

Alzate-Marin et al. (2000) identified RAPD molecular marker (OPAZ20$_{940}$) linked to anthracnose resistance gene $Co-6$ in coupling phase at 7.1 cM in F$_2$ population. Arruda et al. (2000), identified six random amplified polymorphic DNA markers linked to the $Co-4$ resistance gene to *C. lindemuthianum* in common bean. Of these identified markers four were in the coupling phase *viz.*, OPY20$_{830C}$ (0.0 centimorgan [cM]), OPC08$_{900C}$ (9.7 cM), OPI16$_{850C}$ (14.3 cM), and OPJ011$_{380C}$ (18.1 cM); and two markers OPB031$_{800T}$ (3.7 cM) and OPAl8$_{830T}$ (17.4 cM) were in repulsion phase. Marker OPY20$_{830C}$ and OPB031$_{800T}$ used in association as a codominant pair, allowed the identification of the three genotypic classes with a high degree of confidence.

Silva and Santos (2001) identified a Random Amplified Polymorphic DNA (RAPD) molecular marker linked to the $Co-7$ allele in cultivar G2333 to select *C. lindemuthianum*-resistant bean plants in segregating populations. The population F$_1$ [ESAL 696 (G2333 x ESAL 696)], in which the line ESAL 696 was the carrier of the $Co-5$, was used. The segregation of one-half-resistant plants and one-half susceptible plants after inoculation with race 2047, confirmed the genetic control due to only the $Co-4^2$ gene. BSA in back cross population showed the linkage of RAPD primer OPL04 with $Co-4^2$ allele (1000 bp). In the co-segregation analysis, this marker was closely linked to a resistance allele, and became an excellent marker for the indirect selection of plants carrying the $Co-4^2$ allele in segregating populations.

Mendoza et al. (2001) identified three AFLP markers *viz.*, ECAG/MACC-1, EACA/MAGA-2, EAGG/MAAC-8 linked in repulsion to the *Co-1* locus by screening the A193 x Flor de Mayo F$_2$ population with 314 RAPD, AFLP and RFLP markers.
Mendez-Vigo et al. (2005) developed a genetic map including the resistance gene linked markers, that were previously described as SB12, OY171100, SAH18, SW12 and SI19 in $F_2$ segregating population of 72 individuals derived from the cross 'Andecha' x 'A493'. Goncalves-Vidigal and Kelly (2006) reported the presence of dominant R- gene in cv. Widusa and named it as $Co-I^S$. They identified an RAPD marker $AL8_{1500}$ linked to the $Co-I^S$ gene in repulsion phase linkage at a distance of 1.2 cM.

Geffroy et al. (2008) mapped two closely linked Andean resistance genes ($Co-x$, $Co-w$) at the end of linkage group (LG) B1 and mapped one Mesoamerican resistance genes ($Co-u$) at the end of LG B2 in recombinant inbred lines (RILs) population of BAT93 and JaloEEP558. They confirmed the complexity of the previously identified B4 resistance gene cluster, because four of the seven tested strains revealed resistance specificity near $Co-y$ from Jalo EEP558 and two strains identified resistance specificity near $Co-9$ from BAT93. They confirmed that resistance genes found within the same cluster confers resistance to different strains of a single pathogen, such as the two anthracnose specificities $Co-x$ and $Co-w$ clustered at the end of LG B1. Clustering of resistance specificities to multiple pathogens such as fungi ($Co-u$) and viruses (I) was also reported at the end of LG B2.

Kun et al. (2009) mapped a novel anthracnose resistance gene (temporarily named $Co-F2533$) against bean anthracnose race 81 in mapping population of 108 $F_2$ plants using SSR markers in cross between bean cv. Red Flower (R) and cv. Jing (S). They detected four SSR markers on the B6 linkage group linked to the resistance gene $Co-F2533$, with distances of 6.6, 18.4, 20.9 and 30.9 cM, respectively. The sequence of appearance of the resistance gene and the markers on the chromosome were as Clon1429, $Co-F2533$, BM170, BMD37 and Clon410.

Goncalves-Vidigal et al. (2011) reported $Co-14$ and $Phg-1$ are tightly linked (0.0 cM) on linkage group Pv01 in Andean common bean AND 277. With use of synteny mapping between common bean and soybean, they identified two new molecular markers, CV54201_{14450} and TGA1.1570 linked at 0.7 and 1.3 cM, respectively, from the $Co-14/Phg-1$ locus in coupling phase. In another study Goncalves-Vidigal et al. (2013), found genes co-segregation of $Co-10$ and $Phg-ON$ with tight linkage at a distance of 0.0
cM on chromosome Pv04. They reported the existence of a resistance gene cluster at one end of chromosome Pv04, which also contains the Co-3 locus and ANT resistance quantitative trait loci.

Richard et al. (2014) fine mapped the Co-x resistance gene in Andean cultivar Jalo EEP58 on chromosome 1 that conferred resistance to the highly virulent strain 100. They used 181 recombinant inbred lines derived from the cross between JaloEEP58 and BAT93 to fine map the Co-x R- gene using markers developed from genome sequence of Andean genotype ‘G19833’. They analyzed key recombination events in RIL positioned Co-x at one end of chromosome 1 to a 58 kb region of the G19833 genome sequence.

Recently, full genome of an Andean common bean landrace cv. G19833 has been sequenced and annotated (available since July 2012 via www.phytozome.org; Schmutz et al. 2014). Now the access to the complete genome sequence provides an opportunity to rapidly develop locus-specific markers that will have a revolutionary impact on fine mapping strategies in common bean.

Campa et al. (2014) reported that Cornell 49242 genotype carries a complex cluster of resistance genes at the end of linkage group (LG) Pv11 corresponding to the previously described anthracnose resistance cluster Co-2. Specific resistance genes to races 3, 6, 7, 19, 38, 39, 65, 357, 449 and 453 were identified, with one of them showing a complementary mode of action in this position. In addition, they also found Cornell 49242 had an independent gene on LG Pv09 showing a complementary mode of action for resistance to race 453.

Sousa et al. (2014) reported the molecular marker G12333250, in linkage group Pv07, was linked in coupling phase to Co-5^2 at a distance of 1.2 cM. The presence or absence of this marker was also determined in the G2333, TU, H1 and PI 207262 cultivars. It was found that the G12333250 molecular marker was present in G2333 and absent in the other cultivars. Because the allele and marker are physically linked in a cis configuration, the Co-5^2 resistance allele present in MSU 7-1 and G 2333 cultivars can be monitored with great efficiency using g12333250.

2.4 Identification of resistant gene(s) based on molecular marker analysis

Adam-Blondon et al. (1994) identified nine molecular markers, five RAPDs (Random Amplified Polymorphic DNA) and four RFLPs (Restriction Fragment
Length Polymorphism), discriminating the resistant and susceptible members in four pairs of near-isogenic lines (NIL) carrying Are gene in different genetic backgrounds (Coco, Early-wax, Processor and Slender-White). A backcross progeny of 120 individuals was analyzed to map these markers in relation to the Are locus with five markers linked to the Are gene within a distance of 12.0 cM.

Alzate-Marín et al. (2002) identified RAPD marker OPB03_{450C} distantly (15.4 cM) related to Co-5 gene present in cultivar TU using segregating populations derived from cross between cultivar Ruda and TU.

Dongfang et al. (2008) verified morphologically evaluated 21 dry bean accessions grown in Manitoba against race 23, 31, 73, 81, 105 of C. lindemuthianum with RAPD and SCAR markers. The combined analysis of genotypes estimated with markers and race reactions showed possible presence of gene Co-1, Co-1^2, Co-1^3, Co-3 or another unidentified resistance genes in various combinations. They concluded that the molecular tests sometimes support the proposed resistance genotypes based on the results of the race inoculations, but false positive results often occurred with molecular markers for genes Co-2 and Co-4. Virulence pattern information allowed the detection of specific resistance genes in certain cultivars, but did not always rule out the presence of other genes.

Beraldo et al. (2009) identified three parents and nine derived lines carrying four resistance genes against three C. lindemuthianum races viz., 31, 65 and 89. Thereby, demonstrated the effectiveness of the markers in the ongoing breeding programmes while evaluating six SCAR markers in 118 bean accessions. They found the resistance gene Co-6 was the most frequent followed by Co-4^2, Co-3^3, Co-5 and Co-4 gene, respectively.

Chen et al. (2011) in their study constructed molecular genetic linkage map using three SSR markers (BMc 32, C871, Pvm 98) and two CAPs markers (g1224 and g683) linked to the resistance gene on B1 linkage group of common bean at a distance of 26.06, 3.58, 13.56, 3.81, and 12.75 cM, respectively.

Rocha et al. (2012) assessed the efficiency of SCAR markers in selecting plants resistant to anthracnose and angular leaf spot, at the time of bulk formation. They selected twenty six superior families of F_4 generation for disease resistance identified by
SCAR markers and found that eighteen of these families were resistant to race 65 and 453 of *C. lindemuthianum* and five were resistant to the race 63.23 of *Pseudocercospora griseola*. They explained the importance of SCAR markers while selection of resistance trait during generation advancement.

Geetha et al. (2013) screened the French bean germplasm against *C. lindemuthianum* using SCAR markers involving 69 accessions including two resistant lines and susceptible varieties each *i.e.* D-line, L-line and Kanchana and Jawala, respectively with fourteen SCAR markers specific to anthracnose disease resistance. Of these 14 SCAR primers, 5 of them, SAS13, SF10, SC08, SZ04 and SBB14, produced amplification with good monomorphic bands and were use to determine resistant accession comprising multigene combinations.

### 2.5 Linkage maps

**Prior to the availability of full genome sequence of common bean genotype “G19833”,** linkage maps were developed by amplifying various genomic regions using different approaches. These linkage maps were used as reference in determining the relative position of markers to much accuracy in same cross and linkage group of gene of interest. Bean/Cowpea Collaborative Research Support Program (B/C CRSP) scientists have developed integrated consensus maps of the 11 linkage groups (LGs) in bean (*Phaseolus vulgaris* L.). In addition to molecular markers, these maps included locations of defense genes and phenotypic traits for disease and insect resistance, seed size, color and storage proteins, pod color and those traits associated with the domestication syndrome in bean. Map locations of major resistance genes in bean have revealed the presence of gene clusters on LGs B1, B4, B7, and B11 for resistance to bean rust, anthracnose, common bacterial blight and white mold. Now the complete genome sequence information has provided the opportunity for rapid development of locus-specific markers and has therefore a revolutionary impact on fine mapping strategies. However, microsatellites and gene-derived markers are still under represented in the core molecular linkage map of common bean compared to other types of markers. The important linkage map was developed are described below:

The various maps developed by different groups include the Florida map (Vallejos et al. 1992), Davis map (Nodari et al. 1993a; Freyre et al. 1998), the Parismap (Adam-Blondon et al. 1994) and the Nebraska–Wisconsin maps (Jung et al. 1999). These
primitive maps mainly based on RFLP markers. The genetic map of *P. vulgaris* developed by Adam-Blondon (1994) covering 567.5 cM of the bean genome using one hundred and fifty-seven markers (51 RFLPs, 100 RAPDs, 2 sequence characterized amplified regions and 4 morphological markers) in order to locate anthracnose resistance genes and genes involved in plant defense mechanisms. The backcross population (BC$_1$) of 128 individuals, derived from a cross between two European bean genotypes *viz.* Ms8EO2 and Cornell were used for map generation. Finally, 7 genes involved in plant defense mechanisms were located on this map with clusters of 2-10 markers in every linkage group. The linkage map ‘Pv Freyre 1998’ with included (PvB1 to PvB11) were based on RAPD markers in RIL population of the cross cv. BAT93 x Jalo EEP558 that segregates for multiple host–microorganism interactions (Nodari et al. 1993b; Geffroy et al. 1999). For each of the LGs, a minimum of two (or more) markers from the different maps were mapped in the BJ population.

Six genetic and one physical map developed by various bean working group have proved very useful for relative positioning of markers, these maps includes (1) Pv COS Cook (UC Davis) 2009, (2) Pv Freyre integrated linkage map – 1998, (3) Pv McClean NDSU—2007, (4) *Phaseolus vulgaris* Consensus genetic map 2011, (5) PvConsensus_Galeano Fernandez 2011a and (6)BAT93_x_JALOE558 (Waugh et al. 2015), whereas the only physical Maps includes “Pv Jackson Purdue FPC 2007”. In addition to core maps developed, small linkage maps for one or few agronomical traits were also developed and few of them are reviewed below:

Miklas et al. (2000) constructed a genetic linkage map in common bean (*P. vulgaris*) using mapping population consisted of 79 F$_{5:7}$ recombinant inbred lines (RIL) derived from a 'Dorado'/XAN176 cross. They developed a 930 cM (Kosambi) map comprising eleven linkage group with one linked trait, and seven linked pairs. Linkage group comprises 147 randomly amplified polymorphic DNA (RAPD) markers, two sequence characterized amplified region (SCAR) markers, one inter simple sequence repeat (ISSR) marker, two seed coat color genes R and V, the Asp gene conditioning seed brilliance, and two rust (*Uromyces appendiculatus* var. *appendiculatus*) resistance genes *i.e* one conditioning resistance to races 53 and 54 and the other conditioning resistance to race 108.
Rodriguez-Suarez et al. (2007) developed a genetic map of common bean using 197 markers using a F₂ population comprising of 85 individuals from the cross between Spanish landrace Andecha (Andean origin) and the Mesoamerican genotype A252. The resulting map covers about 1,401.9 cM, with an average marker distance of 7.1 cM and includes molecular markers linked to resistance genes for anthracnose, bean common mosaic virus, bean golden yellow mosaic virus, common bacterial blight, and rust. They concluded that most anthracnose resistance Co- genes, previously described as single major genes conferring resistance to several races, could be organized as clusters of different genes conferring race-specific resistance.

Hanai et al. (2010) extended the core map of common bean over 11 linkage groups with EST-SSR, RGA, AFLP, and putative functional markers in 'BAT93' x 'Jalo EEP558' cross. The integration of 282 new markers into the common bean core map has been done by placing markers with putative known function in some existing gaps including regions with QTL for resistance to anthracnose and rust. On an average, the polymorphism information content was 0.40 and the mean number of alleles per locus was 2.7.

Campa et al. (2011) evaluated resistance to the eight races (3, 7, 19, 31, 81, 449, 453, and 1545) of the pathogenic fungus C. lindemuthianum (anthracnose) in F₃ families derived from the cross between the anthracnose differential bean cultivars Kaboon and Michelite. They conducted molecular marker analyses in the F₂ individuals to map and characterize the anthracnose resistance genes or gene clusters present in Kaboon. The analysis of the combined segregations indicated that the resistance present in Kaboon against these eight anthracnose races is determined by 13 different race-specific genes grouped in three clusters viz., Co-1 (races 81 and 1545), Co-3/9 in LG 4 (races 3, 7, 19, 449, 453, and 1545) and (unknown location) races 449, 453, and 1545, respectively.

Microsatellite or Single Sequence Repeat (SSR) markers have proven to be powerful tool in studies of genetic variation, dissecting quantitative trait loci, genetic mapping and molecular breeding in (Phaseolus vulgaris L.) bean genomics research. These polymorphic SSR (microsatellite) markers are more widely distributed in the common bean genome. A large number of SSR markers have been developed for common bean are listed in table 2.1.
Table 2.1. Summary of the simple sequence repeat markers identified

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Type of marker</th>
<th>Genic/Genomic</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>PVM</td>
<td>SSR-EST</td>
<td>Genic</td>
<td>(Hanai et al. 2010)</td>
</tr>
<tr>
<td>BMd</td>
<td>SSR</td>
<td>Genic</td>
<td>(Blair et al. 2012)</td>
</tr>
<tr>
<td>BMe</td>
<td>SSR-EST</td>
<td>Genic</td>
<td>(Blair and Hurtado 2013)</td>
</tr>
<tr>
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<td>SSR-BES</td>
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<td>(Cordoba et al. 2010)</td>
</tr>
<tr>
<td>BMb</td>
<td>SSR-BES</td>
<td>Genomic</td>
<td>(Cordoba et al. 2010)</td>
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<tr>
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<td>(Cordoba 2010)</td>
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<td>(Guerra-Sanz 2004)</td>
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<td>(Yaish et al. 2003)</td>
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<td>SSR</td>
<td>Genic</td>
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</tr>
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<td>SSR</td>
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<td>SSR</td>
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<td>SSR</td>
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<td>(Hanai et al. 2010)</td>
</tr>
<tr>
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<td>Genomic</td>
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<tr>
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<tr>
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<tr>
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<td>(Galeano 2012)</td>
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