Barley is among the five most important crop plants of the world. Cultivated barley, *Hordeum vulgare* L., belongs to the tribe *Triticeae* in the grass family, *Poaceae*, which is the largest family of monocotyledonous plants. It is a short season, high yielding, early maturing grain which can be grown in widely varying environments. Harsh winter conditions hinder cultivation of winter barley. Barley is considered as one of the sturdy crop as it survive in almost all weather conditions. But this plant is known to be susceptible to various bacterial and fungal diseases. Leaf blight is one of the major diseases of the barley and is found everywhere where the crop is grown.

### 2.1 Origin and distribution of barley

It is a very old crop thought to be originated in wild state in Middle East and in the lands of Ethiopia, cultivated for more than 10000 years now (Singh and Singh, 1991). The geographical origin of barley has been the subject of much debate; however the general consensus appears to be the Nile Valley (Badr et al., 2000). Barley was one of the earliest crops domesticated and is considered one of the foundations of modern agriculture. Archeological remains suggest that barley was first domesticated in the Fertile Crescent around 10,000 years ago, along with wheat (Langridge and Barr, 2003).

At present barley cultivation is widespread in many areas with contrasting environmental conditions. Barley is grown over a broader environmental range than any other cereal and much of the world's barley is produced in regions with climates unfavorable for the production of other major cereals (Langridge and Barr, 2003). Its wide distribution is a result of the wide genetic variation within the crop, with particular varieties adapted to specific environments (Frischbeck, 2002). The major barley producing states in India are Rajasthan, Uttar Pradesh, Madhya Pradesh, Punjab and Haryana (Mishra, 2004). It is considered as the best choice crop under rainfed condition and saline alkaline soils (Singh and Singh, 1991).

### 2.2 Cultivation of barley

Barley is a sturdy crop, able to withstand many different growing conditions. However, it is least tolerant of hot, humid conditions, which makes it unsuitable for the subtropical regions. Barley is a short season, early maturing grain with a high yield potential, and may be found on the fringes of agriculture, in widely varying environments (Harlan 1976). Barley is a temperate as well as a
tropical crop and widely adaptable to various climatic conditions. It is considered as one of the most drought- and salt-tolerant crops. But for effective yields, it needs appropriate irrigation and rich fertile soil. Extremely dry weather or wet weather can damage the crop even if the land is properly ploughed. A slightly dry weather is a bit favourable for the crop at the time of harvesting. The crop takes about four months to mature. Barley seeds begin to germinate at a comparatively low temperature (1 to 3° C). However, optimal temperatures for barley germination are 18 to 25° C. Germinating plants are sensitive to unfavorable environmental conditions (lack of moisture, low temperatures, soil crust formation, redundant moistening, and excessive seed planting depth). In India, the sowing period of barley is around October and November, which makes it a rabi crop and harvesting period is around March and April.

2.3 Production of barley

Barley is grown in over 100 countries of the world. World production of barley in 2013 was 130 m tones and it covers 50.56 m hectares of the total world production area as stated by United States Department of Agriculture. The world production figure of crop in 2008-2009 was 140 million metric tones with EU- 27 was the largest producer of barley accounting for 43 per cent of the world production, followed by Russia (12 per cent), Canada (8 per cent), Turkey (5 per cent) and Ukraine (4 per cent). The world production remains quite stable since the 1970s, as there are no big changes in the world production figures (Singh and Singh, 1991).

Barley production in India is mere 1.33 million tones out of a total grain production of 219 million tones. In 2007-08, the total area for barley cultivation was 0.77 million hectare, while the production was estimated at 1.31 million tones. With an increase in area under cultivation, it is believed that barley cultivation would accelerate in the near future. Although the feed portion would remain stable, the food, seed and industrial use would go up substantially. Uttar Pradesh is the leading producer of barley in the country followed by Punjab, Madhya Pradesh, Haryana, Bihar, Himachal and West Bengal. Traditionally, the six-row type barley is grown in the country. Since barley was considered a crop for marginal and problematic soils under rain fed condition, the quality of the produce was not meeting the industrial requirements. Barley cultivation in India is now becoming more oriented towards industrial utilization, with changing scenario in South East Asia with respect to beer, malt whiskies and other malt product consumption. A good crop of barley could be obtained only by adapting of high yielding and disease resistant varieties. Varieties are different for different regions, soil conditions, sowing conditions and for specific purpose like feed, malt, green forage etc. Uttar Pradesh and Rajasthan are the two major producers of barley in
the country. These two states together provide 64 per cent of the total area and 72 per cent of the total production of barley in India. Barley is essentially a crop of North India. Uttar Pradesh is the leading producer of barley and accounts for 36.65% of the total area and 40.11% of the total production of barley in India. K 508, K 501, NB 2, RD 2552, RD 2035, BH 902, BH 393, RD 2508, RD 2624, RD 2660, PL 419, NDB 1173, DWR 28, DWRUB 52, RD 2668, DWRB 73, DWRUB 64, RD2715, K560 and K 603 are major barley varieties grown in U.P. Rajasthan is the second largest producer of barley accounting for 27.71 per cent of the total area and 31.79 per cent of the total production of the crop in the country. Different varieties are being grown in Rajasthan are RD 2552, RD 2035, BH 902, BH 393, RD 2508, RD 2624, RD 2660, PL 419, NDB 1173, DWR 28, DWRUB 52, RD 2668, DWRB 73, DWRUB 64 and RD2715. Punjab contributes 3.75% of the total area and 6.05% of the total production of barley in the country. RD 2552, RD 2035, PL 426, BH 902, BH 393, RD 2508, RD 2624, RD 2660, PL 419, NDB 1173, DWR 28, DWRUB 52, RD 2668, DWRB 73 and DWRUB 64 are the major crop verities grown in Haryana and Punjab. Other important producers of barley include states like Madhya Pradesh contribute 11.69% by area and 7.3% by production of India. JB 1, RD 2715, PL 751 and JB 58 are the barley varieties grown in Madhya Pradesh.

2.4 Use of barley

Barley is often considered as a commercial crop because of its varied utility in food, feed and brewing industries. It has become an important crop due to its demand for manufacture of alcoholic beverages, such as beer, whisky and non alcoholic malted food products like baby food, cocoa malt drinks, and vinegar and in ayurvedic medicines (Mishra et al, 1982). Germinating barley seeds produce two enzymes, alpha-amylase and beta-amylase, which hydrolyze starch to dextrin and fermentable sugars during the commercial malting process. This sugar provides the basic ingredient for the production of beer and other alcoholic beverages. Barley finds a wide range of usage.

Animal feed: Barley, being a good source of protein, is used as a feed for the livestock. For use as an animal feed, it is rolled, grounded, flaked or pelleted. These products are given in the form of grain, silage or straw to dairy and beef cattle. The byproducts of malted barley are also used in the form of animal feed.

Malted barley: Barley is malted for use in alcoholic beverages like beer and wine. Apart from this, the malted barley is also used as flavour, sweetener, malt extract, malt flour, etc. To get the malted barley, the barley kernels are soaked and dried and are then germinated or sprouted in a controlled
environment. Barley starch consists of 15 to 20% amylose and 80 to 85% amylopectin. Amylose is the most valuable component for brewing trade. Its quantity determines the value of barley as a source of malt. Quality of barley as a source of malt is mostly determined by the extract content of finely ground malt and its activity. If the extract content is high (over 78%), this barley variety is a valuable source of malt.

**Human consumption:** A wide variety of barley products can be made using the different parts of the plant are known to be suitable for human consumption. These include porridge, muesli and cookies made of barley flakes, cereals made of barley bran, and muffins, cookies, breads and pasta made of barley flour. The grain or kernel of the plant is used to make flour, flakes, etc. The barley grain in this case, is milled by crushing the seed kernel and segregating the inside part of the kernel, known as endosperm, where the food is stored from the outside part known as bran. To make the flour, the endosperm is then grounded in a fine manner.

**Other Uses:** Apart from being used as an eatable, barley is used in fields like industries and agriculture. Barley grain can also be polished or pearled by removing the hull to obtain pearl barley and pot barley which is an inedible from kernel. The straw of barley is used to make dry fodder for the livestock, which is obtained by removing the head that hold the grain kernel. It is also used in making building material, paper, newsprint and fibreboard. The starch present in barley is used in making paper, paper starch based detergents, biodegradable plastics, etc.

**2.5 Major limitations on crop production**

The worldwide production of barley has increased over last two decades, due to advances in conventional plant breeding techniques resulting in production of new, higher yielding and better adapted cultivars. Despite this, many environmental factors continue to threaten barley production and challenge growers. Although, Barley is considered as one of the sturdy crop as it survive in almost all weather conditions but many biotic and abiotic factors affect the growth of the crop. Abiotic factors such as drought, excess rainfall and temperature extremes significantly impact on grain yield and quality. In addition, biotic factors including weeds, pests and diseases also contribute to a significant decrease in crop yield. The total loss potential of pests in barley production worldwide was estimated to account for about 50% of the attainable yield. Weeds had the highest share (23%) in this loss rate, followed by fungal pathogens—especially *Pyrenophora teres, Rhynchosporium secalis, Puccinia hordei, B. graminis f.sp. hordei* and *C. sativus*—(15%), animal pests (7%) and viruses (3%). Globally, the main foliar and root diseases affecting barley
include net-from net blotch, spot form net blotch, spot blotch, powdery mildew, leaf rust, stem rust, crown rot, scald and common root rot (Vock, 1978). In India major problems which affect the yield of crop are aphid, spot blotch and leaf rust. A new race of stem rust, which attacks all of our previously resistant varieties, has appeared in the eastern Prairie and the northern great plains. Some of the major diseases of barley which affect the yield of crop are explained below:

**Basal Glume Blotch:** The bacterium that causes this disease is known as Pseudomonas Atrofaciens. A dull brownish-black discolored area is found at the base of the glumes that cover the kernel and is seen to be more prominent in the inside part and that on the outside part of the afflicted glume. Depending on the force with which the disease affects the crop, the discolouration of the base varies from light brown to charcoal black. The leaves, in this case, show small, water soaked spots which get enlarged and turn yellow in colour as the time passes. In the end, they turn brown in colour as the tissues die. However, these diseases can be cured by making use of clean, fresh seeds.

**Bacterial Blight:** It is caused by a bacterium known as Xanthomonas campestris. In this disease, small, pale green spots appear in the lesion, i.e. the abnormal tissues. These lesions then expand, and begin to appear as dead spots. The bacteria that causes this infection remains deep rooted in the soil and water and is spread by rains that are driven by wind. Deep afflicted infections are caused by splashing of bacterial ooze by drop rains.

**Covered smut:** it is caused by fungal pathogen Ustilago hordei. This disease first becomes noticeable at heading time. Hard, black masses of smut spores, each covered with a grayish membrane, are found in the place of kernels in affected heads. Each smutted head contains millions of tiny spores which are spread to healthy seeds while the grain is harvested or in storage. Spread also occurs through spores that lie dormant in the soil. The barley seedling becomes infected between germination and emergence from the soil. Covered smut may be controlled by treating the seed with an appropriate fungicide.

**Net Blotch:** Net Blotch is yet another disease in which the leaves appear to be afflicted the most. It is caused by the fungus known as Pyrenophora Teres, which leave certain spots on the leaves of barley. The spots in the leaves appear in a netted pattern, appearing in longitudinal lines of brown
pigments. These elongated areas finally cover the whole leaf, thereby rendering it useless. Some of the methods used in preventing this disease include crop rotation, seed treatment, etc.

**Powdery Mildew:** Caused by fungus known as *Erysiphe Graminis*. When severe, it may reduce yields by 25 percent or more. The infections appear on the upper surface of the leaves and leaf sheaths. On these leaves, there are certain gray, fluffy threads of the fungus, which destroy the whole plant slowly. Mildew is more severe on tender, rank-growing plants. Thick seeding, heavy application of nitrogen fertilizer, and other factors that promote a heavy, leafy growth are not recommended. While fungicides are an effective way of resisting these diseases, they are not always economical. There are, however, some resistant varieties, available to control their disease.

**Scab:** Scab, also known as Fusarium head blight, sometimes causes severe damage to barley. The scab head blight develops in warm, humid weather. A pink, moldy growth often develops around the base of the infected flower, and black fruiting bodies may be found on the glumes. Kernels of diseased heads are grayish brown and light in weight. The crown tissues of the barley plant are invaded largely by fungus threads from diseased plant residue in the soil. The use of clean seed and treatment of diseased seed with fungicides are effective methods of controlling seedling infection. Sanitation, crop rotation, and early seeding help reduce crown infection and head blight.

**Stem rusts:** Stem rusts sometimes injure barley seriously. It is caused by fungus named *Puccinia graminis hordei*. The disease is recognized by pustules, (blister-like spots), that break through the surface of stems, leaves, and leaf sheaths, and often of the chaff and beards. In severe attacks, the kernels are badly shriveled. Rusted stems turn brown, become dry and brittle, and soon break over. The use of early varieties and cultural practices such as early seedling and the use of phosphate fertilizers which hasten ripening may help the barley escape rust.

This study will focus specifically on the impact of diseases, namely leaf blight on commercial barley production, resulting from infection with the fungal pathogen *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*).

### 2.6 Leaf Blight

Leaf Blight (Spot blotch) is the most important fungal diseases adversely affecting barley yield and quality, and they constitute an increasing threat to intensive crop growing systems worldwide.
(Kumar et al., 2002). Yield reductions due to Leaf Blight can range from 15% to 40% and result from the reduction in the number of heads, reduced seed size and fewer seeds per head (Steffenson, 1997). Leaf blight occurs predominantly in East India, Southeast China, Southeast Australia, Southeast Brazil, Eastern Europe, Northwest Africa and North America. In India, Leaf Blight predominantly occurs in eastern region where localized yield losses of up to 30% at adult plant stage have been reported (Tyagi et al., 2008). Now it has become a major disease of barley crop in North Eastern Plain Zone and North Western Plain Zone.

2.6.1 The Fungal pathogen
Leaf Blight of barley is caused by the hemibiotrophic fungal pathogen Bipolaris sorokiniana, which is also the causal agent of seedling blight and head blight of wheat, barley and many grasses. Bipolaris sorokiniana is a cereal pathogen of increasing global concern and occurs predominantly in East India, Southeast China, Southeast Australia, Southeast Brazil, Eastern Europe, Northwest Africa and North America (Kumar et al., 2002). This fungal pathogen has a wide range of hosts within wild and cultivated Poaceae and is extremely variable in pathogenicity towards barley (Steffenson, 1997).

Bipolaris sorokiniana is an asexual fungus (Alcorn, 1988) reproduces by means of conidia which develop from single or clustered conidiophores. The mycelium of B. sorokiniana is deep olive brown. Conidiophores (50-150 x 6.0-8 m) are single or clustered, simple, erect and septate. The conidia (30-134 x 12-30 m) are olive brown, curved to straight, fusiform to broadly ellipsoidal, 3-10 septate and have rounded ends and a prominent basal scar. The conidia have bipolar germination (Sivanesan, 1987). Extended periods (e.g., longer than 16 hr) of warm (above 20°C), moist weather are conducive to epiphytotic development. Incubation period depends on environmental conditions and takes 3-6 days. Early and heavy infections of the flag leaf result in the greatest losses in yield. Besides causing leaf blight, this pathogen can attack roots, heads and grains.

2.6.2 Infection and symptoms of disease
The fungal pathogen affects all parts of the plant and produces a variety of symptoms that can vary according to a number of factors, including host genotype and growth stage, pathogen isolate and environment. Most infections are initiated by soil-borne conidia or existing mycelium or conidia in plant residue; however inoculums can also be carried in the seed (Steffenson, 1997). Secondary
conidia of *B. sorokiniana* form on infected tissue above the soil level and are dispersed by wind and splashing water. Fungal infection comprises several phases: conidial germination, formation of appressoria, penetration and colonisation (Alcorn, 1988). *B. sorokiniana* produces toxins which interact with the host membranes resulting in cell death and leakage of metabolites (Kumar *et al.*, 2002). The phytotoxins induce both chlorosis and necrosis in plant tissue.

In seedling stage, Leaf Blight infection starts as dark brown-to-black spots on leaf sheaths that cover the young shoot and progresses from lower to upper leaves during crop development. If infection occurs early enough in the crop cycle and conditions remain favourable for disease development, complete defoliation is possible, resulting in major yield reductions (Kumar *et al.*, 2002). On susceptible adult plants lesions are round to oblong (up to 20mm) with chlorotic margins. The lesions may coalesce to form blotches that cover and kill large portions of the leaf, with severely infected leaves senescing prematurely (Steffenson, 1997).

### 2.6.3 Disease management

Due to the long viability of the conidia and the fact that many native grasses can support the pathogen, it is impossible to entirely eliminate the pathogen from a field. High temperatures and humidity favour the outbreak of the disease, thus if weather conditions are conducive during this growth stage an epidemic may rapidly develop (Kumar *et al.*, 2002). Integrated strategies for controlling *B. sorokiniana* on barley include soil and residue management, chemical control, crop rotation and resistance breeding. Primary inoculum in crop residue can be reduced by rotation with non-susceptible crops or by tillage practices which facilitate rapid breakdown of residue (Steffenson, 1997). Wildermuth and McNamara (1991) demonstrated that rotation of wheat with lucerne resulted in the reduction of soil populations of *B. sorokiniana*. However, following a second crop, soil populations of *B. sorokiniana* were effectively restored to their previous levels, thus demonstrating the limited effectiveness of crop rotations in the control of *B. sorokiniana*. Due to fact that the pathogen can be seed borne, the use of pathogen-free seed or fungicide-treated seed is beneficial (Steffenson, 1997). Foliar applications of fungicides can significantly reduce the level of infection; however several applications are often required to achieve adequate control (Sharma-Poudyal *et al.*, 2005). Thus the economics of fungicide application depends on the susceptibility of the variety and the value of the potential yield loss. Effective control strategies for Leaf Blight need to be developed. The use of resistant cultivars offers the most economically and environmentally
sound means of control and should be considered as a major component of integrated disease management (Arabi, 2005; Williams, 2003).

2.7 Plant disease resistance

Resistance can be defined as a plant’s ability to impede the growth or development of a pathogen once contact has been initiated. A plant may achieve resistance through active and/or passive defense mechanisms, involving biochemical, physiological or morphological characteristics. Two broad resistance types have been identified in crop plants: major gene resistance and multigenic resistance (Ayliffe and Lagudah, 2004; Guest and Brown, 1997).

Major gene resistance (also known as vertical resistance) is generally controlled by a single gene, referred to as an \( R \)-gene. These \( R \)-genes can be remarkably effective in controlling disease and can confer complete resistance (Ayliffe and Lagudah, 2004). In general, \( R \) genes function to recognise specific “elicitor” molecules produced by the invading pathogen which results in a rapid signal cascade and an active defence response (Wise, 2000). This form of resistance is explained by the gene-for-gene concept, first proposed by Flor (1956), where for each genetic locus conditioning resistance (\( R \) gene) or susceptibility in a host, there is a corresponding locus in the pathogen controlling avirulence (\( avr \) gene) or virulence. However, each \( R \)-gene confers resistance to only a subset of races of the pathogen. Thus, major gene resistance is prone to breakdown as new virulent pathotypes evolve through mutation to the \( avr \) gene, which results in the absence of an active defense response in the host. Vertical resistance has been popular in conventional breeding programs due to the ease in which the \( R \) gene can be detected and transferred through cross-breeding (Wise, 2000).

Multigenic resistance (also referred to as horizontal resistance) is controlled by multiple genes, each segregating according to Mendel’s laws. This type of resistance is referred to as quantitative, in that the plants that possess it show various degrees of susceptibility to the disease (Guest and Brown, 1997). Unlike major-gene resistance, individual genes contributing to horizontal resistance condition only a partial resistance. This form of resistance does not completely prevent a plant from becoming damaged, however it slows the infection process and decreases spore production, thus diminishing disease severity and spread of the pathogen to other plants. Horizontal resistance is generally effective against all races of a pathogen, and is often referred to as non-race specific resistance. When managed properly multi-gene resistance can be very effective in controlling plant
diseases, due to less selection pressure in favour of specific pathotypes (Guest and Brown, 1997). Multigenic resistance is the preferred type of resistance for plant breeding programs, mainly due to its robustness over a long period of time (durable resistance) in disease prone systems. However currently little is known about the complex interactions between the multigenic host and pathogen and due to the (sometimes) large number of genes involved it is much more difficult to breed varieties with this form of resistance (Wise, 2000).

2.8 Selection for Resistance

Conventional breeding involves the ability to identify plants containing the desired gene combinations for the trait of interest, the generation of genetic variation through hybridisation and the identification of superior recombinants from the pool of genetic variation (Lamkey and Lee, 1993). Conventional plant breeding selection methods are based on plant performance characteristics (morphological markers) such as height, seed size and colour. These traditional selection methods are time consuming, often involving many generations, and are very dependent on environmental conditions (Francia et al., 2005). Effective control strategies for Leaf Blight need to be developed. The use of resistant cultivars offers the most economically and environmentally sound means of control and should be considered as a major component of integrated disease management (Arabi, 2005; Williams, 2003).

2.8.1 Phenotypic assessments for leaf blight resistance

The identification of resistant parental lines and the introgression of resistance into elite germplasm are considered a priority within Indian barley breeding programs. This form of integrated management relies on accurate phenotypic screening methods for resistance. Phenotypic screening for leaf blight resistance within a barley population is often conducted at the seedling stage in the greenhouse and at the adult plant stage in the field. A comprehensive rating scale to assess the infection responses (IRs) of barley cultivars to the leaf blight disease at the seedling stage of plant development has been proposed by Fletch and Steffenson (1999). The rating scale was developed based on the evaluation of a large and diverse set of host accessions and pathogen isolations, to encompass the full range of lesion type variation observed over a 9 year period. The 1-9 rating scale is based on the relative size of lesions and presence of necrosis and chlorosis observed on barley seedlings infected with isolates of \textit{C. sativus}. The infection responses of progeny within a segregating population can be broadly classified into qualitative categories of either resistant or susceptible genotypes. The resulting frequency ratio can be examined for Mendelian inheritance.
patterns, to indicate the number of genes controlling a trait (Steffenson et al., 1996; Bilgic et al., 2005). Using a similar method, Steffenson et al. (1996) identified a single gene (denoted the Rcs5 gene) controlling seedling spot blotch resistance in the DH Steptoe/Morex (S/M) population. Based on the phenotypic analysis of spot blotch glasshouse trials, Steffenson qualitatively separated individual lines into general categories of resistant and susceptible genotypes based on lesion size and type. On the basis of this classification scheme the population segregated 76:74 ($\chi^2=0.03$, p=0.87) for resistance: susceptibility, indicating the presence of a single resistance gene. From this analysis Steffenson concluded that seedling resistance to spot blotch was monogenetically inherited in this genetic background. Previous study investigated the number of genes conferring adult plant resistance to the spot blotch infection in the S/M population (Steffenson, 1996). Chi-square analysis of the segregating progeny indicated that adult plant resistance was controlled by more than one gene (Steffenson et al., 1996). Prediction of the response of progeny to the spot blotch infection is assisted by knowledge of the heritability of the trait. Heritability is the proportion of phenotypic variation in a population that is attributed to the genetic variation among individuals (Falconer and Mackay, 1996). Heritability analysis estimates the relative contributions of genetic and non-genetic factors to the total phenotypic variation in a population trial. Relatively few studies have been conducted on the heritability of leaf blight infection response. Kutcher et al., 1994 reported heritability estimates of 43 and 61% for spot blotch resistance in the cultivar cross Fr926-77/Deuce and heritability estimate of 73 and 78% in the cultivar cross Virden/Ellie. This suggests that the heritability of leaf blight resistance is moderate to high.

2.8.2 Marker Assisted Selection

Major efforts have been devoted to the incorporation of genetic resistance in cereal breeding programs to offset yield losses caused by fungal pathogens (Wise, 2000). Marker assisted selection (MAS) utilizes molecular marker technology to identify regions of a genome that are strongly associated with highly desirable traits, such as disease resistance, to assist selection strategies in cereal breeding programs. Genetic markers act as “flags” to reveal genetic differences (polymorphisms) between individuals or species. Genetic markers may be either located within the genes themselves, referred to as perfect markers (Paterson, 1996b), or are located in close proximity or “linked” to a gene controlling a trait (Falconer and Mackay, 1996). All genetic markers occupy a specific locus on a chromosome and most 15 markers do not affect the phenotypic expression of an individual (Collard et al., 2005). There are 3 major types of genetic markers: morphological; biochemical; and molecular. Morphological (classical) markers are
phenotypic markers or traits, such as flower colour, growth form or seed size (Collard et al., 2005). These markers formed the basis of selection in traditional breeding programs. Biochemical markers are those which reveal polymorphisms between individuals based on their chemical characteristics, for example different molecular forms or isozymes of an enzyme (Arus and Moreno-Gonzalez, 1993). The major disadvantage of morphological and biochemical markers are that they are often limited in number and may be strongly influenced by the environment or the developmental stage of a plant (Chelkowski et al., 2003; Winter and Kahl, 1995).

Molecular markers reveal sites of variation in DNA and arise from different classes of DNA mutations (point mutations, insertions or deletions) or errors in replications of tandem DNA (Collard et al., 2005). Unlike biochemical and morphological markers, molecular markers eliminate the influence of environment on gene expression and are potentially unlimited in number. In addition, many molecular markers are selectively neutral because they are usually located in non-coding regions of a genome. Due to these advantages and their abundance they are the most widely used marker type in genomic research. Numerous DNA-based genetic marker analysis methods have been developed over the last two decades (Table 2.1).

**Table 2.1** Common markers used in cereal breeding (adapted from Korzun, 2003)

<table>
<thead>
<tr>
<th>Marker Type</th>
<th>RFLP</th>
<th>RAPD</th>
<th>AFLP</th>
<th>SSR</th>
<th>DArT</th>
<th>SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA required (μg)</td>
<td>10</td>
<td>0.02</td>
<td>0.5-1.0</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>PCR-based</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Number of loci</td>
<td>1-3</td>
<td>1-50</td>
<td>20-100</td>
<td>1-3</td>
<td>300-600</td>
<td>1</td>
</tr>
<tr>
<td>Ease of use</td>
<td>Difficult</td>
<td>Easy</td>
<td>Easy</td>
<td>Easy</td>
<td>- #</td>
<td>Easy</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>High</td>
<td>Unreliable</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Cost per analysis</td>
<td>High</td>
<td>Low</td>
<td>Moderate</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

# DArT is a commercial marker (Triticarte Pty, Ltd) and their overall cost is low if consumables and man hours are included in a cost per data point analysis.

A) **Simple Sequence Repeats**: Simple sequence repeats (SSRs) are a class of marker that relies on the high rate of polymorphism observed at microsatellite loci (Korzun, 2003). These are tandem repeats of two or more bases that are widespread in eukaryotic genomes. Variation in the number
of repeats is observed by developing locus-specific primers that anneal to sequences flanking the repeat region, and use of the polymerase chain reaction (PCR) to amplify the intervening DNA fragments. The major advantages of microsatellites are their ease of use, low cost of analysis and their ability to detect genetic differences even among closely related individuals (Korzun, 2003). The first two advantages are critical for the widespread use of DNA markers in large scale breeding programs. The third advantage is of vital importance in modern plant breeding programs, where crosses are often made between elite parental lines that are genetically quite similar.

B) Expressed Sequence Tags: Expressed sequence tags (ESTs) are short sequences of the genome obtained from the analysis of complementary DNA (cDNA) from mRNA, and have been instrumental in gene discovery and gene sequence determination (Rudd, 2003). EST-SSRs are molecular markers derived from ESTs. SSR-EST markers are functional, in that they may assist the role of genetic markers by assaying variation in known functional genes. Their other advantage is that their development cost is very low due to the abundance of EST sequence information in public databases.

C) Single Nucleotide Polymorphisms: Single nucleotide polymorphisms (SNPs) are DNA sequence variations that occur when a single nucleotide (A, T, C, or G) in the genome sequence differs between members of a species. SNPs can occur in both coding (gene) and noncoding regions of the genome and are the most common feature underlying genetic variation within species. This form of genetic variation can be screened by means of a wide range of technologies, usually based on primer extension or on the ligation of oligonucleotide ends. The development of SNP scoring technologies has led to an increase in throughput capacity, however this method requires the DNA sequences of sets of loci for both parents of a cross (Korzun, 2003). In addition, it is rarely cost-effective to perform SNP discovery for marker assisted selection in crop breeding, as a large number of markers are required for the identification of QTLs.

D) Diversity Array Technology: Due to the limitations of existing marker technologies, hybridisation-based methods using nucleic acids immobilised on solid-state surfaces have been developed. Diversity Array Technology (DArT) is a new DNA hybridisation-based genotyping technology that allows whole genome scanning using a microarray platform. DArT uses an array of individualised clones from a genomic representation prepared from amplified restriction fragments. Labelled genomic representations of individuals to be genotyped are hybridised to the array.
Polymorphisms are scored based on the presence/absence of hybridisation to individual array elements. This allows high-throughput screening of hundreds of molecular markers simultaneously and is especially suited to genome-wide analysis. Thus, DArT can detect and type DNA variation at several hundred genomic loci in parallel. In addition, DArT markers require low quantities of DNA per sample, and are low cost. The polymorphisms detected by DArT include single nucleotide polymorphisms, insertion-deletions and methylation changes (Wenzl et al., 2004).

The ability to select desirable individuals based on their genotypic configuration, thereby eliminating the need for phenotypic testing, is an extremely powerful application of DNA markers and QTL mapping (Young, 1996). Marker assisted selection (MAS) is an indirect selection method relying on markers outside the target gene. Selection is not done based on the phenotype but based on a genotype of a marker that is linked to the gene affecting the phenotype. In theory, MAS is more effective than phenotypic selection when correlation between the marker genotype scores and the phenotypic values is greater than the square root of heritability of the trait, assuming that the heritability of the marker is 1 (Dudley 1993). In barley many resistance genes for biotic stress have been identified by utilising various molecular marker techniques (Table 2.2).

MAS allow for early generation phenotypic selection, and enables quicker and often more reliable identification of those plants containing genes of agronomic importance (Wise, 2000). In addition, MAS can allow a faster response to a breakdown in resistance, rapid introgression of multiple genes from diverse germplasm, gene pyramiding, and selection of rare recombinants between tightly linked resistance genes (Michelmore, 2003). Although the number of successful examples for applying MAS in barley breeding is still rather limited (Rae, S. J., 2007 and Thomas, W. T. B., 2003), the recent implementation of high-throughput genotyping platforms (Illumina, DArT, and SFP identification by using Barley 1 GeneChip affymetrix array) in barley will significantly increase the identification of marker trait associations, and the subsequent identification of potential candidate genes. Finally, this will allow treating QTLs as monogenic traits and thus spurring their marker assisted manipulation in breeding programs. In combination with a wide range of mapping populations developed for specific agronomic traits, this comprehensive resource of markers now allows the identification of polymorphisms in functionally defined sequences (Luo et al. 2007, Wenzl, P. et al., 2006).
Table 2.2 Qualitative and quantitative resistance genes mapped with molecular markers in barley

<table>
<thead>
<tr>
<th>Disease</th>
<th>Source of resistance(R)</th>
<th>R gene</th>
<th>Ch</th>
<th>Closest marker</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Viral)</td>
<td>BaMMV</td>
<td>10247</td>
<td>Ym8</td>
<td>4H</td>
<td>RFLP</td>
</tr>
<tr>
<td></td>
<td>Bulgarien</td>
<td>Ym9</td>
<td>4H</td>
<td>RFLP</td>
<td>Bauer et al. 1997</td>
</tr>
<tr>
<td></td>
<td>Russia 57</td>
<td>Ym11</td>
<td>4H</td>
<td>RAPD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Franka</td>
<td>Ym4</td>
<td>3H</td>
<td>RFLP</td>
<td>Weyen et al. 1996</td>
</tr>
<tr>
<td></td>
<td>Ragusa</td>
<td>Ym4</td>
<td>3H</td>
<td>RAPD</td>
<td>Bauer &amp; Graner 1995</td>
</tr>
<tr>
<td></td>
<td>BaYMV/Res.Ym No1</td>
<td>Rym5</td>
<td>3H</td>
<td>STS (RFLP), SSR, CAPS</td>
<td>Graner et al. 1999</td>
</tr>
<tr>
<td></td>
<td>Ethiopian b.</td>
<td>Yd2</td>
<td>3H</td>
<td>STS(AFLP)</td>
<td>Paltridge et al. 1998</td>
</tr>
<tr>
<td></td>
<td>Shannon</td>
<td>Yd2</td>
<td>3H</td>
<td>CAPS</td>
<td>Ford et al. 1998</td>
</tr>
<tr>
<td>(Fungal)</td>
<td>Stem rust</td>
<td>Rgp1</td>
<td>7H</td>
<td>STS (RAPD)</td>
<td>Horvath et al. 1995</td>
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<tr>
<td></td>
<td>Chevron</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Q21861</td>
<td>PphQ</td>
<td>5H</td>
<td>RAPD</td>
<td>Poulsen et al. 1995</td>
</tr>
<tr>
<td></td>
<td>Q21861</td>
<td>PphQ</td>
<td>1H</td>
<td>STS (RFLP)</td>
<td>Borovkova et al. 1997</td>
</tr>
<tr>
<td>Leaf rust</td>
<td>Vada</td>
<td>6 QTL</td>
<td>3H</td>
<td>AFLP</td>
<td>Qi et al. 1998</td>
</tr>
<tr>
<td></td>
<td>H. v. spont.</td>
<td>Rph16</td>
<td>2H</td>
<td>STS (RFLP)</td>
<td>Ivandic et al. 1999</td>
</tr>
<tr>
<td>Strip rust</td>
<td>ICARDA/CIMMYT line</td>
<td>2 QTL</td>
<td>5H</td>
<td>RFLP</td>
<td>Chen et al. 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4H</td>
<td>RFLP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G. Zweiz.</td>
<td>Mlo</td>
<td>4H</td>
<td>RFLP</td>
<td>Hinze et al. 1991</td>
</tr>
<tr>
<td>Powdery mildew</td>
<td>Ingrid NIL</td>
<td>Mlg</td>
<td>4H</td>
<td>RFLP</td>
<td>Görg et al. 1993</td>
</tr>
<tr>
<td></td>
<td>Pallas NIL</td>
<td>Mla</td>
<td>1H</td>
<td>RFLP</td>
<td>Jahoor et al. 1993</td>
</tr>
<tr>
<td></td>
<td>Vada</td>
<td>MILa</td>
<td>2H</td>
<td>RFLP</td>
<td>Giese et al. 1993</td>
</tr>
<tr>
<td></td>
<td>H. v. spont.</td>
<td>Mlt</td>
<td>7H</td>
<td>RFLP</td>
<td>Schönfeld et al. 1996</td>
</tr>
<tr>
<td></td>
<td>Ingrid NIL</td>
<td>Mlf</td>
<td>7H</td>
<td>RFLP</td>
<td>Simons et al. 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mlj</td>
<td>5H</td>
<td>RFLP</td>
<td></td>
</tr>
<tr>
<td><strong>Scald</strong></td>
<td><strong>Mlo</strong></td>
<td><strong>4H</strong></td>
<td><strong>AFLP</strong></td>
<td></td>
<td></td>
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<td>---</td>
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<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E224/3</td>
<td>Rh4, Rh10</td>
<td>3H</td>
<td>RFLP</td>
<td>Barua et al. 1993</td>
<td></td>
</tr>
<tr>
<td>H. v. spont.</td>
<td>Rrs13</td>
<td>6H</td>
<td>RAPD</td>
<td>Abbott et al. 1995</td>
<td></td>
</tr>
<tr>
<td>Atlas</td>
<td>Rh2</td>
<td>1H</td>
<td>RFLP</td>
<td>Schweizer et al. 1995</td>
<td></td>
</tr>
<tr>
<td>Triton</td>
<td>Rh</td>
<td>3H</td>
<td>RFLP</td>
<td>Graner &amp; Tekauz 1996</td>
<td></td>
</tr>
<tr>
<td>H. v. spont.</td>
<td></td>
<td></td>
<td>STS (RFLP)</td>
<td>Hakim 1996</td>
<td></td>
</tr>
</tbody>
</table>

| **Spot blotch** | Steptoe/Morex | 2 QTLs | 1H,7H | RFLP | Steffenson et al. 1996 |
| **Leaf stripe** | Proctor | QTL | 7H | RFLP | Pecchioni et al. 1996 |
| **Net blotch** | Steptoe/Morex | 7 QTLs | Except 1H | RFLP | Steffenson et al. 1996 |
| | Igri | Pta | 3H | RFLP | Graner et al. 1996 |
| | Harrington | 4 QTLs | 4H-7H, | RFLP | Spaner et al. 1998 |
| | TR306 | 12 QTLs | except 5,7H | AFLP | Richter et al. 1998 |
| | Arena/Hor9088 | Rpt4 | 7H | RFLP | Williams et al. 1999 |
| Galleon | | | | |

| **(Others)** | Morex | 2 QTLs | 3H | RFLP | El Attari et al. 1998 |
| **Bacterial leaf streak** | TR306 | QTL | 7H | RFLP | Moharamipour et al. 1997 |
| **Aphids** | Sahara 3771 | Ha2 | 2H | RFLP | Kretschmer et al. 1997 |
| **CCN** | Chebec Galleon | Ha4 | 5H | RFLP | Barr et al. 1998 |

**MAS Applications**

The major applications of molecular markers in most breeding programs have been in backcross breeding where loci are tracked to eliminate specific genetic defects in elite germplasm, for the introgression of recessive traits and for the selection of lines with a genomic structure similar to the
recurrent parent (Langridge and Chalmers, 2005). Markers have also improved strategies for gene deployment and enhanced the understanding of genetic control of complex traits (Francia et al., 2005).

In general, marker based breeding systems depend on four main factors (Francia et al., 2005):
1. A genetic map with an adequate number of uniformly spaced polymorphic markers to accurately locate QTLs or major genes;
2. Close linkage between the QTL of interest and adjacent markers;
3. Adequate recombination between the markers and the rest of the genome; and
4. An ability to analyse a large number of individuals from a segregating population in a time and cost effective manner.

**MAS Constraints**

Although molecular markers have been successfully associated with QTLs, in some cases these associations have proved to be of limited usefulness in practical breeding programs. A number of constraints have imposed significant limitations on the efficient utilization of QTL mapping in MAS, including: the identification of major QTLs controlling quantitative traits; uncertainty in the position of small effect QTLs; deficiencies in QTL analysis resulting in the underestimation or overestimation of the number and effect of QTL; and, a lack of validation of QTL marker associations in populations of different genetic backgrounds (Francia et al., 2005; Holland, 2004).

The success of MAS depends on the location of the marker with respect to the gene controlling a quantitative trait. Markers located within the gene of interest are the most sought after but these usually require the target gene to be cloned (Francia et al., 2005). Generally, markers are not located within the target gene and tightly linked flanking markers are required to accurately locate the QTL controlling a trait of interest. Markers located closely on either side of the QTL minimise the chance of double recombination events between the QTL and both flanking markers (Doerge, 2002). Cost-effective marker assisted selection is also essential for plant breeding programs to be effective in the development of disease resistant, high yielding cultivars (Wise, 2000). The cost of employing MAS rather than conventional techniques varies considerably between studies and should be considered on a case by case basis (Collard et al., 2005; Dreher et al., 2003). In some cases phenotypic screening and selection is cheaper than marker assisted selection. However, if phenotypic evaluation is time consuming and laborious, then the utilisation of markers may be the
preferred method of selection. The method of selection ultimately depends on the inheritance of the trait, method of phenotypic assessment and the cost of the required resources (Collard et al., 2005).

2.8.3 Bulked Segregant Analysis
Bulked segregant analysis (BSA), developed by Michelmore et al. (1991), is a method to rapidly identify markers in specific regions of a genome. BSA involves comparing two pooled DNA samples of individuals from a segregating mapping population, originating from a single cross. Within each pool (or bulk) of DNA, individuals have identical genomic regions for the target locus, but random genotypes at loci unlinked to the selected region (Michelmore et al., 1991). In this way, pools of DNA from plants that differ for a particular trait, such as disease resistance or susceptibility, can be analysed to identify polymorphic markers associated with the trait. This method saves both time and money, in those polymorphic markers which can be identified using only a small number of samples (two parents and two bulks).

2.8.4 Marker Genotyping
Once polymorphic markers have been identified by screening across the parents, then the entire mapping population is screened, referred to as marker genotyping. Thus, DNA must be extracted from each individual in the mapping population and the parents (Collard et al., 2005). Generally, the markers will segregate in a Mendelian fashion, allowing parental and recombinant lines to be genotyped (Paterson, 1996a).

2.8.5 Linkage Analysis of Markers
The final step in the construction of a linkage map is to analyze the linkage of markers to determine whether an association exists between the markers and a quantitative trait. The linkage analysis of markers is usually performed by computer programs, such as MapManager QTX (Manly et al., 2001). Linkage between markers is usually expressed in terms of a logarithm of odds (LOD) score, where a LOD score of 3 between two markers indicates that linkage is 1000 times more likely than no linkage. Linked markers are grouped together and represent either an entire chromosome or a chromosomal segment (Collard et al., 2005). It should be noted that distance on a linkage map is not linearly related to the physical distance between markers on a chromosome, as it is measured in terms of the frequency of recombination between the genetic markers (Paterson, 1996a). Recombination frequency is not linearly related to the frequency of crossing over during meiosis.
(Hartl and Jones, 2001). Thus, mapping functions (including the Haldane and Kosambi function) are required to convert recombination fractions into centimorgans (cM) of genetic distance.

QTL analysis aims to detect an association between genetic markers and the genomic region controlling the phenotypic expression of a quantitative trait. Markers are used to partition the mapping population into different genotypic classes according to their genotype at a particular marker locus. It is then determined mathematically whether a significant difference exists between the phenotypic means of the classes ( Tanksley, 1993); where a significant difference indicates that the marker is linked to the trait of interest (Collard et al., 2005). Common methods used to detect QTL are: single marker analysis; simple interval mapping (SIM) (Lander and Botstein, 1989); and composite interval mapping (CIM) (Zeng, 1993). Single marker analysis employs statistical methods, such as analysis of variance (ANOVA), t-test and linear regression, to detect QTL associated with a single marker. A single-factor ANOVA measures the probability that a QTL is present at the same chromosomal location as the marker, and is the quickest way to establish whether a significant association exists between a marker and the expression of a phenotypic trait (Collard et al., 2005), by. The results of this statistical inference are presented as a \( P \)-value, where a \( P \)-value of 0.01 indicates a 1% probability that these results would have been obtained in the absence of a marker-trait association. Lower the \( P \)-value, the higher the probability that a QTL truly exists in the region of the marker (Falconer and Mackay, 1996). Linear regression is the more commonly used statistical method as it provides the coefficient of determination (\( R^2 \)). The \( R^2 \) value for a marker is considered to be the percentage of total phenotypic variance of a trait that is explained by the marker. Single marker analysis does not require a complete linkage map; however QTL locations are detected only in terms of the nearest marker and, therefore, are imprecisely estimated (Falconer and Mackay, 1996). In addition, the size of the QTL effect is confounded with distance of the QTL from the nearest marker (Francia et al., 2005).

Simple interval mapping (SIM; Lander and Botstein, 1989) requires a linkage map, however unlike single marker analysis, SIM analyses intervals between linked markers simultaneously, thus compensating for recombination between the markers and the QTL (Collard et al., 2005; Falconer and Mackay, 1996). The basis of composite interval mapping (CIM) is an interval test that attempts to separate and isolate individual QTL effects by combining interval mapping with multiple regressions. It controls for genetic variation in other regions of the genome, thus reducing background “noise” that can affect QTL detection (Zeng, 1993). To control background variation,
the analysis software incorporates "cofactors" into the model, which are a set of markers that are significantly associated with the trait and may be located anywhere in the genome. They are typically identified by forward or backward stepwise regression, with user input to determine the number of cofactors and other characteristics of the analysis. CIM is more precise and effective at mapping QTLs compared with SIM (Collard et al., 2005).

The results of SIM and CIM are represented by either a likelihood ratio statistic (LRS), or a logarithmic of odds (LOD) score. LRS and LOD profiles reveal the most likely position of a QTL (highest LOD value) in relation to the linkage map (Collard et al., 2005; Falconer and Mackay, 1996). These profiles are usually represented in graphical form, with the test statistic on the y axis and markers comprising linkage groups on the x axis. The most likely QTL position is considered to be the point where the peak LOD score occurs (Falconer and Mackay, 1996), where the peak must exceed a threshold significance level before the QTL can be referred to as statistically significant. Significant thresholds can be determined by carrying out between 500 and 1000 permutation tests to eliminate false positive marker-trait associations.

There are a number of factors which limit QTL detection. Firstly, only QTLs with large phenotypic effects will be detected. Thus, depending on the size of the mapping population, small effect QTLs may fall below the threshold of detection. The second factor is the size of the mapping population. Populations must be relatively large in order to detect minor QTLs against the background of environmental variation in phenotypic expression. Therefore, the larger the population the greater the chance large and small QTLs will be detected. Thirdly, linked QTLs may be recognized as only a single QTL. Combinations of these factors generally contribute to the underestimation of the number of QTLs controlling traits (Asins, 2002).

2.9 Molecular studies for leaf blight in barley

The conventional methods to select resistance genotypes by inoculating plants with leaf blight isolates are time consuming, laborious, destructive and are not always reliable because of substantial environmental influence. The number of lines to phenotype can be substantially reduced (and so the cost) by identifying markers that are closely linked to the gene of interest. However, in leaf blight of barley, it would be important to confirm that the progeny of a new cross between the resistant (linked to the marker) and the new susceptible genotype has (in addition to the marker) the
resistance phenotype due to possible epistatic or modifying effects (Bilgic et al., 2005). Polymerase chain reaction (PCR) based markers, such as microsatellite (SSRs) (Condit and Hubble, 1991), can be used to identify the genetic markers and allow large scale genotyping of individuals at any location. Microsatellites or simple sequence repeats (SSRs) denote a DNA class of mono- up to hexanucleotide sequence repeats dispersed over the whole genome with an accumulation in non repetitive DNA and untranslated 3’- and 5’-regions of genes (Morgante, M., 2002). A high number of alleles are typical for SSR markers, which makes them especially suitable for population studies (Goldstein & Pollock 1997). Up to 37 alleles have been reported in the HVM4 microsatellite locus of barley (Saghai Maroof et al. 1994). Sequence information for SSR amplification is obtained either from gene bank data or by sequencing positive clones probed from DNA libraries with simple sequence repeats. Techniques based on random amplification of microsatellite sequences have also been proposed (Gupta et al. 1994, Wu et al. 1994). Primers based on the conserved regions of sequenced resistance genes have been used for amplifying resistance gene analogs (RGA) in many crop species, including barley (Leister et al. 1996, Chen et al. 1998). Liu et al. (1996), Struss and Pleiske (1998) and Ramsay et al. (2000) have isolated a total of >600 SSRs which can be deployed in genetic studies in barley. Large numbers of SSRs are now becoming available for a selection of crop plants (Bryan et al. 1997; Milbourne et al. 1998; Ramsay et al. 2000) and these provide valuable sets of tools for crop-specific research program.

The process of identification of molecular markers linked with traits of interest has become more efficient by the use of Bulk Segregant Analysis (BSA) (Michelmore et al., 1991). In bulked segregant analysis, DNA pools of individuals of a crossing progeny are made based on their phenotype and screened for differences in the molecular markers (Michelmore et al. 1991). As a result of linkage disequilibrium, segregating markers that are tightly linked to the locus affecting the phenotype will most likely be fixed within the pool, while weaker linkage will result in both marker alleles being present. In barley, BSA has successfully been used for tagging several disease resistance genes with RAPD markers locating 1.6-12 cM from the target locus (Weyen et al. 1996, Borovkova et al. 1997, Poulsen et al. 1995, Barua et al. 1993). BSA has also been proposed for tagging quantitative loci with a major effect: theoretically QTL alleles with phenotypic effects of 0.75-1.0 standard deviations should be detectable in DH populations of 100-200 lines (Wang & Paterson 1994).

Since the 1980s, DNA-based markers emerged as a major tool for genetic studies. Using these markers, a large number of barley genes or QTLs for important traits have been mapped, including
resistance to spot blotch (Manninen et al., 2000, Richter et al., 1998, Spaner et al., 1997, Steffenson et al., 1996). Variation in resistance exists in barley for Leaf Blight (Steffenson et al., 1996; Wilcoxson et al., 1990) however; the degree of resistance in modern cultivars is insufficient (Arabi, 2005). In India, there are currently no commercial resistant cultivars available to growers for this disease. Thus, the identification of parental stocks possessing an adequate level of resistance to leaf blight is required.

Genetic information on the mechanism of resistance in barley and this plant-pathogen interaction is scarce (Arabi, 2005). It is required to develop extensive research strategies to generate information on nature of inheritance and QTL/s involved in leaf blight resistance in Indian barley lines. Leaf blight resistance studies have been done with emphasis on molecular markers in barley. In early research it was reported that three unlinked leaf blight resistance genes conferred field resistance in the cultivar Svanhals (Griffee, 1925 and Bilgic et al., 2005). In an inheritance study it was also reported that resistance to leaf blight in barley cultivars was controlled by one or two genes (Wilcoxson et al., 1990). More recent research has utilized molecular marker technology and QTL analysis to build upon the correlations of Leaf Blight resistance with morphological characters and inheritance reported in earlier studies. Previous research on Steptoe/Morex (S/M), a 6-6-rowed doubled haploid (DH) population, showed that seedling resistance is controlled by a single gene and adult plant resistance by two quantitative trait loci (QTL). In this study by using molecular marker technology, it was reported that seedling resistance to leaf blight was monogenetically inherited and was governed by a single gene (Rcs5) on the short arm of chromosome 7H. It was also found that adult plant resistance was conferred by 2 QTLs; one major QTL on chromosome 1H explaining 62% of the phenotypic variance and a second minor QTL on chromosome 7H, explaining 9% of the phenotypic variance (Steffenson et al., 1996).

To corroborate these results in a 2-6-rowed DH population, composite interval mapping (CIM) was performed on Harrington/Morex (H/M). Instead, a single QTL at or near Rcs5 on chromosome1 (7H) explained nearly all of the phenotypic variance (75%) for disease severity. The 1H QTL has been successfully incorporated into six-rowed malting barley cultivars, originally derived from the breeding line NDB112, and is one of the most successful examples of resistance breeding in the United States (Steffenson et al., 1996). Recent reports have shown that the 1H gene is completely suppressed when introgressed into other genetic backgrounds (H/M and D/M. Three QTLs were found conferring seedling resistance in the D/M population: one near Rcs5 on chromosome 1(7H)
explaining 30%, a second near the centromere of chromosome 1(7H) explaining 9% and a third on
the short arm of chromosome 3 (3H) explaining 19% of the phenotypic variation and three QTLs
explained most of the variation for adult plant resistance: one on the short arm of chromosome
3(3H) explaining 36%, a second on the long arm of chromosome 3(3H) explaining 11% and a third
at or near Rcs5 on chromosome 1 (7H) explaining 20% of the phenotypic variation. These data
demonstrate the complexity of expression of spot blotch resistance in different populations and
have important implications in breeding for durable resistance (Bilgic et al., 2005).

Diversity arrays technology (DarT) -based PCR, expressed sequence tag (EST) and SSR markers
have been mapped across four populations derived from crosses between susceptible parental lines
and four resistant parents (North American two-rowed barley lines; the North Dakota lines
ND11231-12 and ND11231-11 and the Canadian lines TR251 and WPG8412-9-2-1) to determine
the location of resistance loci. Quantitative trait loci (QTL) conferring resistance to leaf blight in
adult plants (APR) were detected on chromosomes 3HS and 7HS. In contrast, seedling resistance
(SLR) was controlled solely by a locus on chromosome 7HS. The phenotypic variance explained
by the APR QTL on 3HS was between 16 and 25% and the phenotypic variance explained by the
7HS APR QTL was between 8 and 42% across the four populations. The SLR QTL on 7HS
explained between 52 and 64% of the phenotypic variance. An examination of the pedigrees of
these resistance sources supports the common identity of resistance in these lines and indicates that
only a limited number of major resistance loci are available in current two-rowed germplasm
(Bovill et al. 2010).

Association mapping of leaf blight resistance in wild barley has also done. Thirteen QTL for spot
blotch resistance were identified with DArT and SNP markers. These QTL were found on
chromosomes 1H, 2H, 3H, 5H, and 7H and explained from 2.3 to 3.9% of the phenotypic variance
(Roy et al., 2010). Two QTL conferring resistance to spot blotch, Rcs-1H-84.6 and Rcs-2H-106-
122 were identified in chromosomes 1H and 2H while doing association mapping using the SNP
markers (Gyawali and Sanjaya, 2010).
The South Asian barley lines were assessed for host plant resistance on field and tissue culture conditions to explore the possibility of screening of disease in *in vitro* conditions. In the finding similar level of chlorosis and disease incidence were recorded in both *in situ* and *ex situ* condition (Chand, 2008).

Cloning of the seedling leaf blight resistance gene, Rcs5 Using a positional cloning approach and molecular markers derived by using synteny with related sequenced genomes of rice and Brachypodium has done and the Rcs5 region was saturated, identified a small interval that is likely to contain Rcs5, and sequence six BACs from the resistant cultivar Morex that should contain Rcs5. Allele sequencing and recombinant analysis limited the number of candidate genes to two Wall-associated kinases, ctg19 and ctg37. These genes represent a novel class of resistance genes in which only WAK1 and WAKL22 from Arabidopsis have been shown to be involved in disease resistance. This research will add to the understanding of disease resistance to hemibiotrophic organisms in crop species (Drader and Thomas Benjamin; 2011).

In India, there are fewer studies reported on Spot Blotch of Barley. Kumar and group in 2002 reviewed the virulence studies, taxanomy and worldwide distribution of *Bipolaris sorokiniana* (teleomorph Cochliobolus sativus). This study stated this as an emerging threat to cereal production including Barley. Current Indian barley varieties are largely susceptible to this disease and attempts are being made to introduce sources of resistance in barley lines. Inheritance studies of spot blotch resistance for cross IBON 18/RD 2508 reported three resistance genes on Rcs-qtl-5H-1, Rcs-qtl-5H-2 and Rcs-qtl-1H-1, respectively. These genes were found closely linked to three SSR markers BMS 32, BMS 90 and HVCMA explaining 28%, 19% and 12% of variation, respectively, as suggested during single marker linear regression analysis of phenotypic and genotypic data (Tyagi et al, 2008).

Not much work has been done so far on leaf blight disease resistance of barley at molecular level. QTL studies in barley have concluded debatable complex inheritance of spot blotch resistance due to different genetic backgrounds of mapping populations with very few reports coming from India. Therefore, it is required to develop extensive research strategies to generate information on nature of inheritance of leaf blight resistance in Indian barley lines. This research work is proposed to study inheritance of leaf blight resistance in barley and characterization of genomic regions involved in leaf blight resistance.