Among the world’s major food crop, potato acquires third position after rice and wheat. It is highly efficient in converting sunlight into nutritious food in the form of tuber. It is rich source of carbohydrates, proteins, phosphorus, calcium, Vitamin C, Vitamin A and has high protein calorie ratio. Potato is viewed to have the potential for alleviating hunger and malnutrition of the developing world especially in view of the shrinking arable land. In India, there is sound increase in potato productivity since independence due to the development of 43 high yielding potato cultivars and improved package of practices for different agro-climatic zones by Central Potato Research Institute (CPRI). At present India ranked third in potato production after China and Russia (http://faostat3.fao.org, 2014). Today, India produces about 46.39 million tones of potato annually from an area of 2.02 million hectares ranking 3rd on global scale (http://faostat3.fao.org, 2014). But the productivity of potato is attenuated by several biotic as well as abiotic stresses. Amongst biotic stresses, oomycetous fungus Phytophthora infestans induced late blight disease is the most destructive and dreadful disease of potato. It is also the decimator of the potato cultivation that led to the worldwide losses estimated annually over $ 6.7 billion (Haverkort et al., 2008). *P. infestans* caused infamous Irish potato famine in 1845, which led to the social turbulence in Europe. Since the tragedy of great Irish famine in 1840’s phytopathologists have focused much attention in breeding programmes for the production of disease resistant potato cultivars.

Haas et al. (2009) explained that the genome of *P. infestans* (240Mb) were several times larger than *Phytophthora ramorum* (65Mb) and *Phytophthora sojae* (95Mb) genome. Comparison analysis revealed that *P. infestans* genome goes through a rapid turnover and widespread extension of effector proteins that are placed in highly extended and dynamic regions of the genome. *P. infestans* has the significant capability to alter morphological, biochemical and physiological pathways in its host via the secretion of virulence or avirulence molecules, known as effectors (Haas et al., 2009). These effectors encourage infection by modulating defense pathways and stimulate disease in susceptible plants. *P. infestans* is hemibiotrophic pathogen which needs living host in early stage of infection followed by extensive necrosis of host tissues. Pathogen establishes association with host cells through haustoria to facilitate
the translocation of effector proteins into the host in biotrophic phase. In host plants, two classes of effectors i.e. Apoplastic effectors are secreted in the extracellular space of plant cells and cytoplasmic effectors are secreted inside the plant cell presumably through infection vesicles and haustoria that protrude inside the host (Kamoun, 2006). Cytoplasmic effectors carry N-terminal signal peptides followed by RXLR and LXLFLAK, remarkably conserved motifs. Effector (protein) needs the presence of the RXLR- dEER motif (arginine, any amino acid, leucine, arginine) at the N-terminus through which they invaginate to alter host defenses while the C terminal domain operates inside the plant cells carrying the effector activity (Whisson et al., 2007; Birch et al., 2008). RXLR proteins are the important weapons of the molecular armory of oomycetes. During infection phase, effector genes of *P. infestans* have different patterns of temporal expression. Haas et al. (2009) has demonstrated that the RXLR effectors (Avrblb1, Avr3a and Avr4) with known avirulence activity and 79 of other predicted RXLR effectors, show a distinct expression pattern and are transcriptionally up regulated in biotrophic phase.

Disease management mostly based upon the application of fungicides. To control late blight, the fungicides, which are used for treatment of infected potato crops, include chlorothalonil, copper preparations such as Bordeaux mixture, Maneb, Meatalaxyl. Application of fungicides is expensive and therefore farmers in developing countries cannot afford the most modern fungicides. In addition to the high rate of fungicides, the use of these harmful chemicals has harmful impacts on the environment. For this reason, there is need for the development of crop that illustrate durable genetic resistance either through genetic engineering or classical breeding methods which provides best perspective for environmentally sound, economical and efficient control of late blight. Host resistance is the most attractive mean from both economical and environmental perspectives for management of late blight disease.

Plants have developed profound defence system to identify and respond to pathogens. There are two types of defence system: constitutive defence system and inducible defence system. Constitutive defence provide the general protection during the life span of a plant while inducible responses are triggered by cytoplasmic immune receptors encoded by resistance (*R*) genes and plasma membrane pattern recognition receptors (PRRs). Defence first layer involve pathogen associated patterns (PAMPs) which are detected by receptors present in membrane to trigger defence
responses against pathogen. This mechanism is known as PAMP-Triggered Immunity (PTI). In comparison, R proteins recognize effector proteins secreted by the pathogen inside the plant cell. As a result, second layer of defence comprises of plants resistance (\(R\)) genes to stimulate ETI (Effector Triggered Immunity) which caused hypersensitive response (HR) resulting in programmed cell death confined to a small area (Staskawicz et al., 1995; Dangl and Jones, 2001). Till date, 11- \(R\) genes from Solanum demissum that provide resistance against \(P. infestans\) have been introduced in susceptible potato cultivars through breeding. But new races of Phytophthora evolved in the mid 1900’s have overpowered the resistance mediated by \(R1-R11\) genes. Due to the continuous research efforts of 46 years, breeders have been able to introduce resistance genes (\(Rpi-blb2\)) to Solanum tuberosum obtain from the wild cross among \(S. tuberosum\) and \(S. bulbocastanum\) (Vleeshouwers et al., 2011). In continuation of breeding efforts of the CPRI, the transgenic approach to combat the late blight disease, \(RB\) gene from \(S. bulbocastanum\) also been introgressed into Indian potato cultivars but the stability of \(RB\) transgenic is a major concern. Due to the sexual recombination the novel virulent strains of \(P. infestans\) has emerged which make this pathogen more complex and difficult to control. Genome of \(P. infestans\) has extensive extension of effector genes to avoid detection by \(R\) genes. As a result, due to the stability and durability concern \(R\) genes have lost popularity. Transgenic approach can be use for the management of late blight pathogen but in case of transgenics, encouragement of genetically modified organisms (GMOs) is relatively not easy due to communal concern. In gene transfer techniques there could be gene exchange among non target organisms.

To minimize the possibility of gene exchange among organisms, strategies such as RNAi mediated resistance is employed. In eukaryotes, gene silencing mechanism known as RNA interference (RNAi) has become the major focus of scientific community all around the world. RNAi is considered to be a “biosafe” technology due to the abolition of certain threats measured to be linked with transgenics. There is no protein introduced, and the RNAi construct is used to silence the gene of interest. RNAi is manifested by small noncoding RNAs (sRNAs) of about 20–30 nucleotides. siRNA (small interfering RNAs) and miRNA (micro RNAs) are the two foremost classes of sRNAs which have been at the vanguard of research related to RNA biology. During recent years, the microRNAs have opened a novel possibility
for the genetic improvement of eukaryotes through manipulation of miRNAs by different methods. One method of manipulating the miRNAs is to modify the expression of genes through artificial microRNAs (amiRNAs). amiRNAs are modified for silencing of any desired target. In both plants and animals, miRNA precursor can be tailored for the expression of a small RNA having sequence which is not related to the miRNA usually produced via precursors (Zeng et al., 2002; Alvarez et al., 2006; Niu et al., 2006; Schwab et al., 2006). The most important turning point is development of turnip yellow mosaic virus (TYMV) and turnip mosaic virus (TuMV) resistant transgenic Arabidopsis through gene silencing mediated by amiRNAs (Niu et al., 2006). In another study Qu et al. (2007) developed transgenic tobacco resistant to cucumber mosaic virus (CMV) through the expression of amiRNAs against suppressor protein of virus. Transgenic Nicotiana tabacum resistant to Potato virus Y (PVY) and Potato virus X (PVX) was produced by employing amiRNAs targeting the silencing suppressor HC-Pro and the TGBp1/p25 (p25) of PVY and PVX respectively (Ai et al., 2011). Zhang et al. (2011) have demonstrated that the tomato plants harbouring amiRNAs against cucumber mosaic virus genome showed highly effective viral resistance. Recently, transgenic wheat resistant to wheat streak mosaic virus by polycistronic amiRNA was developed (Fahim et al., 2012). Transgenic tomato plants resistant to tomato leaf curl New Delhi virus (ToLCNDV) was developed through silencing mediated by amiRNAs (Vu et al., 2013). RNAi based host induced gene silencing (HIGS) or host induced RNA interference (RNAi) allows the silencing of plant pathogen genes by expressing an RNAi construct against specific genes endogenous to the pathogen in the host plant. Reduced growth of root knot nematodes as well as Lepidoptera and Coleoptera insects during host induced RNAi has been reported (Huang et al., 2006; Baum et al., 2007; Mao et al., 2007). Recently, study by Nowara et al. (2010) suggested the exchange of small non coding RNAs between cereal hosts and Blumeria graminis. Host induced RNAi of chitin synthase gene provide resistance against Fusarium seed blight and head blight in wheat (Wei et al., 2015). Transgenic lettuce plants were developed through host-induced gene silencing targeting vital genes of Bremia lactucae, biotrophic oomycete (Govindarajulu et al., 2015). Avr3a (RXLR effector) from P. infestans is recognized by parallel R3a (resistance) protein of potato. Two isoforms of Avr3a have been identified that differ by two amino acids i.e. Avr3a<sup>K80I103</sup> and Avr3a<sup>E80M103</sup> referred to as AVR3aKI and AVR3aEM respectively. AVR3aKI
stimulates potato resistance protein R3a to activate effector-triggered immunity (ETI) (Armstrong et al., 2005). In the absence of R3a both forms suppresses host cell death triggered by elicitin infestin1 (INF1) of *P. infestans* through the stabilization of ubiquitin E3-ligase, CMPG1 (Bos et al., 2010). CMPG1 is essential for cell death stimulated by INF1 (González et al., 2006). Suppression through AVR3aKI is stronger in comparison to AVR3a EM (Bos et al., 2006; Bos et al., 2009). As the cytoplasmic effector protein, *Avr3a* has been reported to modulate host processes during infection by suppressing hypersensitive cell death, amiRNA-mediated silencing of gene encoding Avr3a protein of *P. infestans* in transgenic host background will be aimed for silencing of the targeted gene and consequently imparting late blight resistance in potato. Based on the background specific objectives of the study are:

1. Data base mining for identification of *P. infestans* RXLR effector gene(s) essential in pathogen virulence.
2. Identification of effective amiRNAs targeting different regions of the effector gene(s) using bioinformatics tool.
4. Potato transformation.
5. Evaluation of late blight resistance under glasshouse condition.
6. Molecular characterization of transgenic lines.