CHAPTER 1. INTRODUCTION

Cultivated groundnut or peanut (Arachis hypogaea L.) is an allotetraploid crop (2n=4x=40, AABB) having two subspecies, spp. hypogaea and spp. fastigiata (Krapovickas and Rigoni, 1960). It is one of the major annual legume and oil seed crops of the world and is particularly adapted to arid and semi-arid dry parts of the tropical and temperate regions covering more than 100 countries (Mishra et al., 2015). It grows on 24.78 million ha worldwide, with a total production of 40.56 metric tons, and an average productivity of 1.64 metric tons ha\(^{-1}\) (USDA FAS, 2017). Although it originated from South America, the majority of groundnut is produced in Asia (12.40 M ha area and 11.54 M tons of production) and Africa (11.87 M ha and 29.95 M tons), whereas the remaining comes from North America, Caribbean countries, Europe and Oceania (FAOSTAT, 2014). Africa and Asia together account for 95% global groundnut area with 91% of the global production. It is cultivated predominantly by small farms under low input conditions and ranks fourth and third as a source of edible oil and protein, respectively (Bosamia et al., 2015).

Globally, 49% of groundnut produced is crushed for extraction of oil and 41% is used for food purposes. Apart from oil, groundnuts are consumed as whole kernels and widely used for the production of groundnut butter, confections, roasted groundnuts, snack products, cereal bars, breakfast cereals, extenders in meat product formulation, soups and desserts. It provides a spectrum of nutrients, including oil, vegetable protein, fibre, phytosterols, tocopherols and phenolic compounds and is an extremely high energy source (5.64 calorie/g) (Arya et al., 2016).

Groundnut oil contains about 12 fatty acids, of which, oleic acid (C18:1, Δ9); a mono unsaturated fatty acid (MUFA) and linoleic acid (C18:2, Δ9, Δ12); a poly unsaturated fatty acid (PUFA) constitute around 80% of groundnut oil composition. Further, palmitic acid, contributing about 10%, whereas, rest 10% is constituted of up to 9 other fatty acids (Bishi et al., 2015). The nutritional quality, flavor, and shelf-life of groundnut seeds and groundnut products are reliant on the relative proportion of various fatty acids like saturated, unsaturated (MUFA and PUFA) present in its oil (Derbyshire, 2014). The high concentration of linoleic acid in groundnut oil is responsible for low oxidative and frying stability of oil which results in rancidity,
off-flavors, and short shelf life of manufactured food products (Mondal et al., 2010). The food industry has adopted the use of partially hydrogenated vegetable oils so as to provide stability and longer shelf life and adequate functionality for a range of product applications with high flavor, low-price, and consistent availability (Schwingshackl and Hoffmann, 2012). A numerous Chemical and epidemiological studies have also shown a strong association between partially hydrogenated fats or trans fatty acids and cardiovascular disease risk (Mozaffarian et al., 2006). Further, during storage, there are breaks in carbon double bonds and oxidized lipids produce acids, aldehydes, ketones and hydrocarbons which are associated with atherosclerosis or hardening and narrowing of the arteries (Cohn, 2002).

Oleic acid has 10-fold higher auto-oxidative stability than linoleic acid; therefore, high O/L groundnut has a longer shelf life (O’Keefe et al., 1993). Due to its thermo-oxidative stability, neutral flavour and odour, these types of oils have very high demand in different industries for food (e.g. fried products, spray coating, in non-dairy creamers, bakery and margarine), cosmetic (emulsions, soaps and detergents, foams) and oleo chemic industries (e.g. bio-based lubricants and plastic; Graces, paints, inks and adhesives pharmaceuticals) (Abiodun, 2017). Oleic acid has lower crystallization temperature than SFAs and better oxidative stability, which makes high oleic groundnut as a perfect candidate for superior biodiesel production (Moser, 2012).

A diet rich in oleic acid is a unique way to reduce the systolic blood pressure, thus reduction in the risk of heart diseases (Teres et al., 2008). Besides, it also promotes a healthier ratio of high density lipoprotein (HDL) to low density lipoprotein (LDL) (O’Byrne et al., 1997), reduces triacylglycerol (Pelkman et al., 2004), slows down the atherosclerosis (Yu et al., 2008) and reduces triacylglycerol and blood glucose levels (Vassiliou et al., 2009).

Henceforth, a concentrated effort has been put forth in groundnut breeding programs to elucidate the molecular and biochemical characterisation of the genes controlling fatty acid biosynthesis and to generate high oleic groundnut cultivars. Normal groundnut genotypes contain about 36 to 70% oleic while 15 to 43% linoleic acid (Knauft et al., 1993). However Norden et al. (1987) had identified the first high oleic mutant line; F435 with about 80% oleic acid and 2% linoleic acid. Since then, over 90 groundnut cultivars with the high oleic trait have been derived through
traditional breeding, chemically induced mutagenesis and marker-assisted selection (MAS). Studies following the discovery of the high oleic groundnut line ‘F435’ were targeted to elucidate the underlying mechanisms of the genes controlling for the high oleic trait. In groundnuts, two homeologous genes, ahFAD2A and ahFAD2B having 99% sequence similarity, are reported to regulate the desaturase activity (Jung et al., 2000b; Lopez et al., 2000). The open reading frames (ORF) of these genes consist of 1,140 bp, encoding 379 amino acids. The presence of single base pair (bp) substitution (448G>A) mutation at 448 bp position in ahFAD2A gene, results in a missense amino acid from aspartic acid to asparagine (D150N). While, 1-bp insertion (441_442insA) mutation in ahFAD2B gene, at 442 bp position results in frame-shift mutation, which generates a premature stop codon (Jung et al., 2000b; Lopez et al., 2000). All these mutations in ahFAD2 affect the histidine motifs which are involved in the metal ion complex required for oxygen reduction (Lopez et al. 2000; Yu et al. 2008). This leads to the altered ahFAD2 gene expression, resulting in reduced enzymatic activity, which results in high oleic acid content in the mutant genotypes (Jung et al., 2000a; Chu et al., 2009).

To enhance the efficiency of high-oleic acid groundnut breeding program, different molecular assays for accurate genotyping of ahFAD2 genes have been developed which includes; cleaved amplified polymorphic sequences (CAPS) markers for ahFAD2A (Chu et al., 2007) and ahFAD2B alleles (Chu et al., 2009), real-time PCR (Barkley et al., 2010; 2011), allele-specific PCR (AS-PCR) assays (Chen et al., 2010; Yu et al., 2013) and Kompetitive Allele Specific PCR (KASP) assay (Zhao et al., 2017). These tools have been successfully utilised for the screening of groundnut germplasm collections (Chu et al., 2007; Wang et al., 2011d, 2013b; Mukri et al., 2012) as well as marker-assisted selection (MAS) studies (Chu et al., 2009; Janila et al., 2016). However, no such efforts for characterization as well as the development of Indian groundnut cultivars and advanced breeding lines with high oleic acid content have been reported till date. Although Indian vegetable oil economy is world’s fourth largest after the USA, China and Brazil. India ranks first occupying 5.50 M ha area under groundnut cultivation and second in production (6.92 M tons) in the world, after China (17.00 M tons) (USDA FAS, 2017).

Looking at the unavailability of high oleic groundnut cultivars in India, the present investigation was aimed to find the relationship between ahFAD2 allele
polymorphism and its fatty acid composition, especially its O/L fluxes in Indian groundnut genotypes and to improve the oleic acid content of Indian groundnut cultivar through cost and time effective, robust molecular breeding approach.

Higher productivity is an ultimate target in any cultivated crop breeding. Several biotic and abiotic constraints limit the insight of the full genetic potential of improved groundnut varieties. Among the biotic stresses, early leaf spot (*Cercospora arachidicola* Hori), late leaf spot (LLS) caused by *Cercosporidium personatum* and rust *Puccinia arachidis* are economically important and widespread.

These foliar diseases are more prevalent diseases in groundnut growing regions across the world causing yield loss and economic losses (Janila *et al.*, 2013). The extent of economic loss of $326 million by early leaf spot, $467 million by rust and $599 million by LLS was estimated (Monyo *et al.*, 2009). These diseases damage the plant by reducing the leaf area available for photosynthesis and by stimulating leaflet abscission leading to heavy defoliation and ultimately yield. Besides, adversely affecting the productivity, they also affect the quality of the seeds and fodder, making it unsuitable for consumption. Henceforth, several popular groundnut varieties have been phased out of farmer’s fields in the recent past due to heavy yield losses caused by foliar fungal diseases (Varshney *et al.*, 2014).

Though chemical control i.e., use of fungicides are available to control these diseases, but that upsurges financial burden on farmers, thereby, increasing the production cost and reduction in the marginal income. Moreover, the application of fungicides also has detrimental effects on human health, soil, underground water, and the environment. As the control measures using fungicides are not cost-effective and environment-friendly, breeding new cultivars with genetic resistance is the sustainable and environment-friendly approach.

Conventional breeding has been successful for introgressing diseases resistance in groundnut breeding programs. The development of disease resistant genotypes not only requires high skill for characterization of the resistance but also thorough and repeated screening under disease epiphytotic, which is laborious, error-prone and time-consuming. Due to co-occurrence and defoliating nature of foliar diseases, it is difficult to differentiate resistant and susceptible cultivars for both the diseases in field conditions by conventional screening techniques. Even identified
also, the resistant sources often suffer from undesirable traits like low productivity, long duration and poor adaptability besides poor yield and seed traits. Hence, conventional breeding would not alone give the expected results.

With the advent of molecular markers, that are superior to morphological and protein markers facilitated the identification target trait(s) to select the best line in a breeding program. These are neutral, occur throughout the genome, co-dominant, can be monitored in any tissue and any stage of the plant, not influenced by environment and normally follow the Mendelian segregation ratio. The different user-friendly markers and QTLs have been reported for these foliar fungal diseases and these identified markers have been compared with field disease performance and validated in different studies (Mishra et al., 2015). The earlier studies identified two major quantitative trait locus (QTL) for LLS resistance and one QTLs for rust using the recombinant inbred line (RIL) population of TAG 24 x GPBD 4 (Khedikar et al., 2010; Sujay et al., 2012). They reported the common QTL for LLS as well as rust on LG AhXV flanked by GM2009 and GM1954 markers (20.6 cM) which contribute up to 67.98 and 82.96% phenotypic variation, respectively. Another genomic region (29.3 cM) is on LG AhXII and is flanked by GM1573/GM1009 and seq8D09 which contributes up to 62.3% phenotypic variation. The applicability of these linked markers of QTL on LG AhXV was validated covering a set of resistant and susceptible genotypes as well as on mapping populations (Yeri et al., 2014; Gajjar et al., 2014; Sukruth et al., 2015; Yol et al., 2016; Kolekar et al., 2016).

Further, Varshney et al. (2014) introgressed this QTL region on linkage group AhXV having up to 82.62% phenotypic variation from cultivar ‘GPBD 4’ into three rust susceptible varieties (‘ICGV 91114’, ‘JL24’ and ‘TAG 24’) through marker-assisted backcrossing (MABC). This MABC scheme employed molecular markers including one dominant (IPAHM103) and three co-dominant (GM2079, GM1536, GM2301) markers present on the same QTL region.

In the light of the above facts, present study on “Marker assisted selection for oil-stability and foliar diseases resistance in groundnut” was undertaken with the following specific objectives

1. Genetic and biochemical characterization of groundnut genotypes for ahFAD2 allele polymorphism and high oleic: linoleic acid (O/L) ratio.
2. Identification of SNPs in the *ahFAD2* gene(s) in selected genotypes.

3. Marker assisted selection for mutant *ahFAD2* allele (for high O/L ratio) and foliar
diseases resistance alleles in selected groundnut genotypes.