CHAPTER - I
INTRODUCTION

1.1 STATEMENT OF PROBLEM

Castor (*Ricinus communis* L.) belongs to the family Euphorbiaceae, is an important non edible oilseed crop. Castor (*Ricinus communis* L., 2n = 2x = 20) is an industrially important non-edible oilseed crop widely cultivated in the arid and semi-arid regions of the world. Its local name is arind or arindi. The genus *Ricinus* is monotypic and *communis* is the only species with the most polymorphic forms known. Several of these forms were designated as species (*R. communis, R. macrocarpus, R. microcarpus*) but they are inter crossable and fertile and are not true species. All the varieties investigated cytologically are diploids and it is presumed to be a secondary balanced polyploid with a basic number of x = 5 (Singh, 1976). Many of the morphological differences might be due to genic differences, cryptic inversions, duplications etc., rather than changes in the whole chromosome complement.

Castor is believed to have most probably originated in Ethiopian-East African region. There are four centers of diversity for castor viz., Ethiopian-Eastern African, North-West, South –West and Arabian Peninsula and Sub-continent of India and China. In India, it is known from very early days and is referred in *Susruta Samhita* written over 2,000 years ago. Its monoecious nature favours cross-pollination and it is up to the extent of 50 per cent. It is essentially a semi-tropical, intermediate perennial plant, but it has naturalized as annual or seasonal crop plant throughout the world in frost free zones. It has an ability to grow under low-rainfall and low-fertility conditions, and hence it is most suitable for dry land farming.

India is the world leader in castor production and export. In India, Castor is generally cultivated in *kharif* season. It is cultivated in about 30 countries on commercial scale. Among those, India, Brazil, China, Russia, Thailand and Philippines are the principal castor growing countries. Total world production of castor seed was 19.96 lakh tonne during the year 2014-15, from an area of 14.94 lakh ha with 1336 kg/ha productivity (FAOSTAT, 2016). Total area under castor crop in
India for the year 2015-16 was 10.61 lakh hectares with production of 17.52 lakh tonne with 1652 kg/ha (Anon., 2017). The major castor growing states in India are Gujarat, Andhra Pradesh, Rajasthan, Karnataka, Orissa, Tamilnadu and Maharashtra. Gujarat is leading castor growing state, where the crop was grown on 7.14 lakh hectares with production of 14.13 lakh tonne and productivity is 1979 kg/ha during 2015-16 (Anon., 2017). In Gujarat, Mehsana, Banaskantha, Sabarkantha, Gandhinagar, Ahemdabad and Kutch are major castor growing districts. Being the largest producer, India is also largest exporter of castor seed oil and exports 80% of its total castor oil to China, which is the world’s largest importer of castor oil followed by USA, Japan, Thailand and other European countries. India’s export of castor oil and derivatives are estimated to be over Rs. 4000 crores per annum and the whole world is highly dependent on India for the supply of this oil.

With the availability of short stature early hybrids, its cultivation in middle Gujarat is increasing year by year. The crop ecological condition of middle Gujarat is different in comparison to semi-arid regions, where soils are fertile with good rainfall and assured irrigation facilities. The castor crop is an important contingent crop for middle Gujarat under the condition of natural calamity such as excess rainfall or drought as pre-rabi or rabi crop.

The seed of castor contains more than 45% oil, this oil is rich (80-90%) in an unusual hydroxyl fatty acid, ricinoleic acid. Castor oil is the only oil soluble in alcohol, presenting high viscosity and requiring less heating than other oils during the production of biodiesel. Due to its unique chemical and physical properties, the oil is used as raw material for numerous and varied industrial applications. Castor oil is used as a lubricant in all moving parts of the machinery and particularly high speed engines and aeroplanes. Hydrogenated castor oil is used in polishes, varnishes, transparent paper, linoleum, plasticizers, ointments, waxes, printing ink, cosmetics, hair dressing, soaps etc. Castor oil is also used as purgative. It is used in many veterinary, medicinal and lighting purposes. Due to the presence of poison "Ricin", it is not suitable for feed to the cattle. It is useful as a trap crop because root contain "ricin" (poisonous alkaloids) which kills nematodes entered into roots (Bozza et al. 2014). It is used as windbreak crop for sugarcane, papaya and banana crops. In eri silk-producing areas, leaves are fed to eri worms. The plant stalks are used for fuel purpose or preparing paper pulp. Castor plant is also grown on the border of the
nursery for natural insect control. Castor seed and castor cake are highly poisonous to man and animal because it contains a toxic alkaloid ricinine and ricin. The castor cake is a good source of organic manure as it contains nitrogen 4.5%, $\text{P}_2\text{O}_5$ 1.75% and $\text{K}_2\text{O}$ 1.5% and also controls white ants and nematodes.

Fusarium wilt caused by *Fusarium oxysporum* f.sp. *ricini* is one of the major yield losing disease in castor. Cultivating wilt resistant cultivars is an effective strategy to control the disease. Utilization of diverse sources of stable resistance is a prerequisite for durable resistance breeding. The present experiment was conducted to identify genetically diverse resistant sources in castor germplasm. Genetic diversity among 20 germplasm lines was assessed using molecular markers. Resistant accessions are valuable in *Fusarium* wilt resistance breeding programme. They would serve as base diverse material for *Fusarium* wilt resistance breeding, wilt resistant gene pool construction and molecular tagging of resistant genes. The pathogen is primarily soil-borne and survives in the form of micro-conidia, macro conidia and chlamydospores. Seed-borne nature of the pathogen has also been reported. Seeds from wilted castor plants carried inoculum at the micropylar end in 2-19 per cent seeds and seed infection was confined to testa, tegmen and endosperm. *F. oxysporum* f.sp. *ricini* was found seed borne in 10.8 per cent seeds of castor variety Aruna collected from wilted plants. Thus, seeds from infected area may also play an important role in the dissemination of the pathogen in the new areas. Infected seeds played important role in the perpetuation and spread of the pathogen (Dange *et al.*, 2006). Cultivars resistant to this fungus are the most practical way to control this disease. Both compatible and incompatible responses induce alterations in plant metabolism; only in the latter the plant is able to efficiently block pathogen penetration without suffering excessive damage.

Molecular markers have been tried to characterize different genotypes in different crops. Different genetic markers based on DNA polymorphism like Random Amplified Polymorphism DNA (RAPD), Simple Sequence Repeats (SSR), Amplified Fragment Length Polymorphism (AFLP) and Restriction Fragment Length Polymorphism (RFLP) were used. Major advantage with RAPD is that prior sequence information is not required. It is used to target genetic markers of near isogenic lines. RAPD uses ten base pair primer to amplify the random portion of genome (Williams *et al.*, 1990). It allows the analysis of individual and large number of markers in
relatively short time, as only a few primers allow the generation of sufficient data to obtain a robust estimate of diversity index and have allowed the resolution of complex taxonomic relationships. Genetic diversity parameters (average number of alleles per locus, per cent polymorphism, average heterozygosity and marker index) were calculated for ISSR, RAPD and ISSR+RAPD approaches in various varieties (Dangi et al., 2004). The microsatellites or SSRs are amongst the most widely used DNA marker for various purposes such as diversity, genome mapping and varietal identification (Singh et al., 2011).

Molecular investigations are essential for collection, conservation and its utilization in future breeding programmes. The knowledge of genetic diversity in a crop species is fundamental to its improvement. The use of various molecular marker methods which are independent of environmental conditions such as RAPD, ISSR and SSR offers significant advantages for species identification in that they are rapid, relatively cheap and eliminates the need to grow plants up to maturity. The use of molecular techniques in genetic diversity studies is supported by the finding that evolutionary forces such as natural selection and genetic drift produce divergent phylogenetic branching which can be recognized because the molecular sequences on which they are based share a common ancestor. The present study is aimed at analyzing the genotypes to molecular markers and classifying the relationship and variability using RAPD, ISSR and SSR data among the plant taxa with numerical taxonomic techniques.

1.2 OBJECTIVES OF STUDY

The present investigation on “MOLECULAR SCREENING AND CHARACTERIZATION OF CASTOR GENOTYPES FOR FUSARIUM WILT RESISTANCE” was undertaken with the following objectives:

1. To screen castor genotypes for fusarium wilt resistance by artificial infection method.
2. To study the genetic diversity in different castor genotypes using molecular markers.
3. To find out the phylogenic relationship among castor genotypes differing in susceptibility and resistance to fusarium wilt.
1.3 SIGNIFICANCE OF THE STUDY

Microbial screening gives idea about level of susceptibility and resistance towards various biotic stress conditions. Resistant and susceptible genotypes were screened for Fusarium wilt resistance and on the basis of that, resistant and susceptible genotypes were selected for further molecular analysis.

DNA-based molecular analysis tools are ideal for germplasm characterization and phylogenetic studies. RAPD has proven to be quite efficient in detecting genetic variations and used for diversity assessment and for identifying germplasm in a number of plant species and have been used in population genetic studies. RAPD, ISSR and SSR techniques has the advantage of high sensitivity and are ideal and effective techniques of molecular biology. The core technology is that random oligonucleotide sequences are used as primers for the polymerase chain reaction to amplify genomic DNA in order to get the fingerprints which show polymorphisms of generated DNA.

1.4 LIMITATIONS OF THE STUDY

Present experiment is planned for molecular characterization of 20 castor genotypes through molecular markers. The selections of genotypes are based on importance for cultivation and crop improvement programs. The polymorphism pattern, diversity analysis and unique bands recognized for the identification and characterization of diverse castor genotype is restricted to analysis of 20 castor genotypes. If the number of genotypes is increased in future then the polymorphism pattern, diversity and unique bands may change.