Screening of Chickpea (*Cicer arietinum* L.) genotypes using gene based markers for *Fusarium* wilt disease and study on important qualitative and quantitative characters

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By

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CERTIFICATE – I

This is to certify that the thesis entitled **"Screening of Chickpea (*Cicer arietinum* L.) genotypes using gene based markers for *Fusarium* Wilt Disease and study on important Qualitative and Quantitative characters"** submitted in partial fulfilment of the requirement for the Degree of **MASTER OF SCIENCE** in **AGRICULTURE (Genetics and Plant Breeding)** of **Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior** is a record of the bona-fide research work carried out by Ms. **SHRISHTIKA RAJPUT** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and Director of Instruction.

No part of the thesis has been submitted for any other degree or diploma or has been published. All the assistance and help received during the course of this investigation has been acknowledged by the scholar.

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This is to certify that thesis the entitled **"Screening of Chickpea (*Cicer arietinum* L.) genotypes using gene based markers for *Fusarium* Wilt Disease and study on important Qualitative and Quantitative characters"** submitted by Miss. **Shrishtika Rajput** to the Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE** in **AGRICULTURE** in the department of **Genetics and Plant Breeding, College of Agriculture, Gwalior** has been accepted after evaluation by the External Examiner and approved by the Student's Advisory Committee after an oral examination of the same.

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Place: Gwalior

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LIST OF ABBREVIATIONS

Abbreviations/ Acronyms	Meaning
Ag.	Agriculture
a.i.	Active ingredient
&	And
et al.	And co-workers
@	At the rate of
CD	Critical difference
CGR	Crop growth rate
Cm	Centi meter
DAS	Days after sowing
DAT	Days after transplanting
dSm^{-1}	Deci Siemens per meter
^{o}C	Degree centigrade
Dist.	District
E	East
EC	Electrical conductivity
Fig.	Figure
FYM	Farm yard manure
G	Gram
Ha	Hectare
H. I.	Harvest index
HW	Hand weeding
i.e.	In reference to; that is
Kg	Kilogram
kg ha^{-1}	Kilogram per hectare

Max.	Maximum
Mg m^{-3}	Mega gram per cubic meter
M	Meter
mg kg^{-1}	Milli gram per kilogram
Min	Minimum
Mm	Milli meter
M.P.	Madhya Pradesh
Mt	Metric tones
Viz	Namely
No.	Number
%	Per cent
PE	Pre-emergence
PoE	Post emergence
RH	Relative humidity
Rs.	Rupees
R.V.S.K.V.V.	Rajmata Vijayaraje Scindia Krishi Vishwa Vidhyalaya
pH	Soil reaction
Q	Quintal
S.E.m (d)	Standard error mean of difference
Sy.	Symbol
Temp.	Temperature
t ha^{-1}	Tonnes per hectare
VC	Vermicompost
Wt.	Weight

CHAPTER I

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is likewise called Bengal gram, Egyptian pea, Garbanzo pea and it belongs to kingdom Plantae; genus Cicer; family Fabaceae; and subfamily Papilionaceae. Chickpea is a self-pollinated, diploid (2n = 2x= 16), Rabi season leguminous crop that performs optimally in 21.1^{O}C to 26.6^{O}C at daylight hours temperatures and 17.77^{O}C to 21.1^{O}C in night time temperatures. The foundation of chickpea is assumed to be south eastern Turkey. In India, chickpea (Chana) is taken into consideration as the „King of Pulses". Chickpea (*Cicer arietinum* L.) isthe 2nd most important legume crop after common bean (*Phaseolus vulgaris* L.) (Gaur *et al.,* 2008; Varshney *et al.,* 2013).Chickpea is cultivated in nearly all the parts of the world covering Asia, Africa, Europe, Australia, North America and South America continents. It is the staple meals in lots of growing nations like South Asia and Sub-Saharan Africa, wherein it's miles fed one specially for its protein and carbohydrate. Whereas, in like Canada and Australia it is extensively cultivated for export purpose and as a crucial exchange commodity. Chickpea is one of the most efficient symbiotic Nitrogen fixer, playing a vital function in the farming system. It is one of the most essential legume plants in sustainable agriculture system due to its low production cost, presence of prolific tap root system, wider adaptation and because it fit in numerous crop rotations. Chickpea consists of about 20.8% protein, 5.6% fat, 1.2% fibre, 59.8% carbohydrate, 3% ash, 0.2% calcium and 0.3% phosphorus. Alongside protein, it's also wealthy in fibre, folic acid and minerals like phosphorus, zinc, iron, calcium and magnesium. It has no anti-dietary factors (Mallikarjuna *et al.,* 2007) and it additionally comprise excessive quantity of carotenoids like β-carotene than genetically engineered „golden rice' (Abbo *et al.,* 2005). On the premise of the seed morphology chickpea s divided into types: the large-seeded kabuli type and the small-seeded desi type. Kabuli type seeds are nearly white in colour and is relatively larger in size and has thin seed coat while the Desi type seeds are brown in colour and is relatively small in size along thick seed coat. The desi type that is consumed in Asia, accounts for nearly 80% of worldwide chickpea production as well as an identical proportion of the whole trade in chickpea while

Chickpeas are a multifunctional grain legume that is widely utilised around the world, particularly as a protein source (Bejiga *et al.*, 2006). Desi chickpea seeds are typically eaten as a dry pulse, whole, split, or ground as dhal or flour, and in sauces such as hummus or soups (Bejiga *et al.*, 2006; van der Maesen, 1989). Chickpeas are primarily grown on residual moisture in the semi-arid tropics during the cool, dry season. The plant is well adapted to tropical climates with moderate temperatures and is successfully cultivated under irrigation in many tropical countries during the cool season (Bejiga *et al.,* 2006). It will benefit from spring rains if the soil is well drained. Well aerated sandy to sandy loam soils and black cotton soils with a pH of 5-7, or even higher, are appropriate, but salinity and sodicity should be avoided (Ecoport,2013; Van der Maesen,1989). Chickpeas can be grown in areas with annual rainfall ranging from 500 to 1800 mm (Bejiga *etal.*, 2006). It is drought tolerant but not tolerant of the humid and hot lowland tropics. Rainstorms during flowering, which might happen during the monsoon season, can affect the crop, which is mostly used for fodder (Van der Maesen, 1989). Crop output can potentially be hampered by early summer heat or cold during flowering (Ecoport, 2013).

The area occupied by chickpea in India is reported to be 10.56Mha which leads to the production of 11.38MT. The productivity of India is around 1078kg/ha (Agriculture Statistics at a glance 2019). Madhya Pradesh is leading state in terms of area and production as it contributes around 34 and 40 per cent share to the total area and production of gram in the country (Annual Report 2017-18, Directorate of Pulse Development). The chickpea occupies around 1.92Mha with a production of 2.48MT. The productivity of Madhya Pradesh is 1288kg/ha. (Directorate of Pulse Development Report 2020)

The genetic diversity of genotypes makes them a vital aid of genes for breeding programs, developing new farming systems, diversification of production and new quality products. Information about genetic diversity enables the choice of

parental genotypes from random populations. Chickpea has excessive variation for various qualitative and quantitative traits i.e. grain colour and shape, colour of flower, pod number, seed coat colour, earliness, insect pest resistance, that can assist breeders to increase or select advanced lines and varieties. The greater genetic variability present within species or populations, the better the probability that at least some of the individuals might be resistant to biotic and abiotic factor, high yielder, and most cost-effective like in nutrient use efficiency.

Chickpea production is predominantly affected by numerous biotic and abiotic stresses. Biotic stresses consists of disease - *Fusarium* Wilt, Collar rot, Dry root rot, Ascochyta blight etc. Insects affecting chickpea includes- Pod borer, army warm, Black aphid, Cut worms and abiotic stresses includes Drought, Cold, Salinity, Water logging etc. Among them *Fusarium* wilt caused by Deuteromycetes fungal *pathogen Fusarium oxysporum f. sp. ciceris* (FOC) is one of the widely disbursed diseases of chickpea and cause yield loss as much as 10–100% depending on varietal susceptibility and climatic conditions (Jimenez- Diaz *et al.,*1989; Patil *et al.,* 2015). The disease is more predominant in the Indian subcontinent, Spain, Ethiopia, Mexico, Tunisia, Turkey, and the United States (Westerlund *et al.,* 1974; Halila and Strange, 1996; Ghosh *et al.,* 2013). Since the disease is soil borne, even without a host, the pathogen can live in the soil for more than five years. There are eight races of the pathogen, with races 1A, 2, 3, 4, 5, and 6 causing wilting symptoms and races 0 and 1 B/C causing yellowing (Jimenez-Diaz *et al.,* 1993). A single recessive gene confers resistance to *F. Oxysporum f. sp. ciceris* (FOC2) (Sharma and Muehlbauer, 2007); resistance to *F. Oxysporum f. sp. ciceris* (FOC2) is conferred by a single recessive gene. Chickpea productivity could be significantly increased if the negative effects of biotic stresses, especially *Fusarium* Wilt, were reduced.

Genetic diversity in plants influences the potential for increased productivity and crop production. Awareness of genetic diversity aids in germplasm marking, gene stock recognition, and the establishment of core collections (Upadhyaya *et al.,* 2007). If the parents chosen for hybridization have a diverse history, the chances of enhancing the characters under consideration are increased. Genotypic variability is, therefore, the element of variation which is due to the genotypic variations amongst individuals within a population and is the primary concern of

plant breeders all over the world (Tadesse *et al.,* 2016). Assessment of genetic variability using suitable tools such as genetic coefficient of variation, heritability estimates, and genetic advance is, therefore, undeniably vital to improve yield as well as the quality of any crop and/or forage in any breeding program (Ali *et al.,* 2008).For long-time, plant breeders have been aware that predominant component of phenotypic variation were accounted for by means of environmental variation than from genotypes and genotypes by means of environmental interactions. Thus, in-depth understanding of genetic variation has paramount significance for yield and its component improvement in any crop as the determined variability is a combined effect of genetic, environments and various interactions between genes and environments, of which only the former one is heritable.

It is a time-saving, reliable, and reproducible approach via Marker Assisted Selection (MAS), DNA markers have immense potential to increase the efficiency and precision of traditional plant breeding. Genomic tools in the form of Molecular Markers have been developed by Molecular Biology to identify certain DNA variants that can be used to assist crop improvement programmes. DNA polymorphism, also known as DNA markers, has a huge potential for increasing productivity.

In chickpea, molecular markers including isozymes, RAPD, ISSR, and RGA showed limited polymorphism (Radhika *et al.,* 2007). Despite this, the first wilt resistance gene for the H1 locus of race 1A was identified and mapped using the allele specific marker CS27700 (Mayer *et al.,* 1997). Following that, resistance genes to various races of *Fusarium oxysporum* were tagged and mapped using various markers such as ISSR, RAPD, and SSR. The markers associated with foc1 (Sharma *et al.,* 2004b; Sharma and Muelhbauer, 2005), foc-01 (Rubio *et al.,* 2003; Cobos *et al.,* 2005), foc-2 (Sharma and Muehlbauer, 2005), foc-3 (Sharma *et al*., 2004b; Sharma and Muehlbauer, 2005), foc-4 (Tullu *et al.,* 1999; Sharma and Muehlbauer, 2005), the second resistance gene for race 4 (Tullu *et al.,*1999) and foc-5 (Sharma and Muehlbauer, 2005) were identified. Except for the H2

locus of race 1A, race 1B/C, and race 6, all resistance genes to *Fusarium* wilt have been identified and mapped.

In light of the foregoing, a study is being planned to estimate the genetic variability, genetic advance, heritability for quantitative and qualitative characters in chickpea, and to screen out the chickpea genotypes for *Fusarium* wilt using gene- based molecular markers.

Objectives:

- To evaluate genetic variability, heritability (Broad sense) and genetic advance for Quantitative and Qualitative characters in chickpea.
- Estimation of Proline, Lipid peroxide, SOD and Carbohydrate in chickpea genotypes.
- Screening of chickpea genotypes against *Fusarium* wilt using gene based markers.

CHAPTER II

REVIEW OF LITERATURE

The chickpea (*Cicer arietinum*) is the world's second most commonly grown legume (2n = 2x = 16). Chickpea seeds are a primary source of human dietary protein in the developing world, where the capacity for symbiotic nitrogen fixation makes it particularly significant for food security. Biotic stresses, such as fungal and viral infections, have a major impact on yield potential.

Fusarium wilt is the major disease limiting chickpea production among the numerous biotic stresses. Even without a host, the pathogen can live in the soil for more than five years. Symptomless carriers of the fungus have been found in crops such as lentil, pigeonpea, and pea (Haware and Nene, 1982). The development of resistant varieties is a cost-effective and environmentally friendly strategy. Mole is a creature that lives underground.

The available, relevant literature related to various aspects of present investigation has been reviewed under the following heads:

1. Importance and status of chickpea crop
2. Chickpea wilt: Economic Impact and Control
3. Genetic diversity for chickpea improvement
4. Marker Assisted Selection for screening of chickpea genotypes
5. Biochemical analysis of chickpea genotypes

1. Importance and status of chickpea crop

Chickpea is an annual herbaceous plant. With diffused, spreading leaves, it resembles a small bush. It is a decent source of carbohydrates and protein, with a higher protein content than other pulses. Legumes are multipurpose crops and are consumed either directly as food or in various processed forms or as feed in many farming systems (Kumara Charyulu & Deb, 2014) Except for sulphur- containing amino acids, chickpeas contain large quantities of all essential amino acids, which can be supplemented by including cereals in the daily diet. The most important ingredient is starch. The most common storage carbohydrate is

starch, which is followed by dietary fibre, oligosaccharides, and simple sugars like glucose and sucrose. Chickpea is high in nutritionally essential unsaturated fatty acids such as linoleic and oleic acids, despite its low lipid content. Chickpea oil contains essential sterols such as sitosterol, campesterol, and stigmasterol.

Chickpea seeds also contain Ca, Mg, P, and, most importantly, K. The seeds are high in fiber and protein and are a good source of iron, phosphorus, and folic acid. Chickpeas are high in essential vitamins including riboflavin, niacin, thiamin, folate, and beta-carotene, which is a precursor to vitamin A. Chickpea seeds, like other pulses, contain antinutritional factors that can be reduced or removed using various cooking techniques. Chickpea has a number of possible health benefits, and when combined with other pulses and cereals, it may be able to reduce the risk of cardiovascular disease, type 2 diabetes, digestive diseases, and certain cancers. Chickpeas are an effective pulse crop with a wide range of nutritional and health benefits (Jukanti *et al.,* 2012)

Cultivated chickpeas are classified into two types based on seed size and colour (Cubero *et al.,* 1987), namely macrosperma (kabuli type) and microsperma (desi type). Kabuli chickpeas are distinguished by white or beige-colored seed with a ram's head shape, skinny testa, and a smooth seed surface. The Kabuli type with large, smoother, light-colored seeds, is grown in temperate regions (Mediterranean countries), while the Desi type is grown in semi-arid tropics (India), usually with yellow to black seed color, smaller in size, and a rough surface (Muehlbauer 1987, Malhotra, 1987). Desi type seeds are small and angular in shape. The colour of the seed ranges from cream to black, brown to yellow, and green. There are 2-3 ovules per pod, but only 1-2 seeds are produced on average per pod. Short plants with small leaflets and purplish flowers that contain anthocyanin. There are two to three ovules in each pod, but only one to two seeds are produced per pod.The plant grows from medium to tall, has giant leaflets, and white flowers, but no anthocyanin. When compared to Desi types, Kabuli types have higher levels of disaccharide and lower levels of fibre. This type's seeds are large (100-seed mass >25 g), and four centres of diversity have been identified in the Mediterranean, Central Asia, the Near East, and India, with a secondary centre of origin in Ethiopia (Vavilov, 1951).

The flowers square measure are typically pink and also the plants show varied degrees of anthocyanin pigmentation, though some Desi sorts have white flowers, however there's no anthocyanin pigmentation on the stem. The Desi sorts account for eighty to eighty fifth of the chickpea space. The split seeds (dal) and floor (besan) square measure invariably made up of Desi sort chickpea. Chickpeas are not only a good source of nutrition, but it also help to improve soil fertility through nitrogen fixation. Chickpea produces nitrogen-fixing root nodules and can enrich the soil with at least 50 kg of nitrogen per hectare each season (Kantar *et al.,* 2007). However, genomic resources for chickpea are still limited. Many approaches are being taken to increase genomic resources and to gain a thorough understanding of the gene content and organisation of the chickpea genome. Jain *et al.* (2013) used a next generation sequencing platform, bacterial artificial chromosome end sequences, and gene bank to generate a draught sequence of a desi-type chickpea genome.

There are genomic resources available, such as transcriptome sequences, whole genome sequences, and EST databases. Garg *et al.* (2011), Jhanwar *et al.* (2012), and Varshney *et al.* (2013). Despite its economic importance and extensive national and international breeding programmes, chickpea productivity has not improved significantly over the years.

Various abiotic and biotic factors are major constraints in realising the full yield potential of chickpea. Because chickpeas are grown with residual soil moisture, they do not require irrigation. As a result, terminal drought and heat stress are significant abiotic constraints to production, especially in the semi-arid tropics. *Fusarium* wilt, Ascochyta blight, Botrytis grey mould (BGM), and *Helicoverpa armigera* are all major biotic constraints. *Fusarium* wilt is another common chickpea disease that causes significant yield loss.

2. Chickpea wilt: Economic Impact and Control

Fusarium wilt is a major abiotic factor that limits chickpea productivity. It is one of the most serious diseases, resulting in significant yield losses. Global legume production is severely challenged by a variety of fungal diseases (Kaiser *et al.,* 2000), of which wilt caused by *Fusarium oxysporum* is one of the most destructive (Wade, 1929; Haware *et al.*, 1978; Armstrong and Armstrong,

1981; Reddy *et al*., 1990; Kraft *et al.,* 1998; Smith *et al.,* 1999; Fall *et al.,*2001). The disease is most common during the dry and warm seasons and has been reported in 23 countries around the world. Chickpea wilt causes significant yield losses in many geographical areas. *Fusarium* wilt has been reported to cause yield losses of up to 10-15% on average, but in severe conditions such as those found in India and Pakistan, yield losses can reach up to 70%. (Grewal and Pal, 1970). Early wilting can result in greater loss than late wilting. Wilting can be observed in highly susceptible cultivars within 25 days of sowing in the field. Seeds obtained from wilted plants were lighter and duller than seeds obtained from healthy plants (Haware and Nene, 1980). Adult plants may exhibit typical wilting that occurs in the field up to the podding stage. The first symptom is drooping petioles and rachis, as well as leaflets. The green colour is gradually fading, and the plant appears dull green. All of the leaves eventually turn yellow and straw-colored. The roots of the wilted plant show no external rotting, drying, or internal discoloration of the pith and xylem when split vertically (Haware *et al.,* 1990). The mechanical plugging of water dusts (xylem) by FOC mycelium, wilt toxins, or hydrolytic enzymes is a mechanism involved in wilt development. *Fusarium oxysporum* is the causative agent of wilt in many plant species and is abundant in the rhizosphere microflora. In India, *Fusarium orthoceras* var. *ciceri*, later identified as *Fusarium oxysporum* f. sp. (FOC) ciceri, was known to cause chickpea wilt (Padwick *et al.,* 1940).

In terms of taxonomy FOC belongs to the *Fusarium* genus, the Nectriaceae family, the Hypocreales order, the Sordariomycetidae class, the Ascomycetes subclass, the Ascomycota phylum, and the Fungi kingdom (Nelson *et al.,* 1981). Pathogen can be grown on potato-sucrose-agar at 25 ° C. In old culture, fungus is delicate, white, colony felted, and wrinkled. *Fusarium* taxonomy based on morphological characteristics such as size and shape of microconidia, colony color. *F. oxysporum* on culture initially produces colourless to pale yellow mycelium that turns pink or purple with age and it has no known sexual stage (Pietro *et al.,* 2003).

Based on disease reactions on a series of differential chickpea cultivars, eight pathogenic races (races 0, 1A, 1B/C, 2, 3, 4, 5, and 6) have been reported in *F. oxysporum f. sp. ciceris* to date, in addition to major symptom forms (Jimenez

Diaz *et al.,* 1993; Haware and Nene, 1982). Race 1 is found throughout Central and Peninsular India, while race 2 is found throughout Northern India. Race 1 was subsequently divided into two races named as race 1A (from India) and race 1 B/C (from Spain) based on variation in reaction on differential host lines (Trapero-cases and Jimenez-Diaz, 1985; Jimenez Diaz *et al.,* 1993). Races 0 and 5 are found in Spain (Jimenez-Diaz *et al.,* 1989) and race 6 in California, USA (Phillips *et al.,* 1988) The more virulent race 3 and race 4, found in Punjab and Haryana states (Haware *et al.,* 1992).Even with isolates of reported races taken from culture collections, the reactions observed on the cultivars were not identical to the earlier study (Haware and Nene, 1982). Sharma *et al.,* (2005) looked into genetic resistance in one of the chickpea cultivars WR 315 that was included in the international differential against pathogen races 1A, 2, 3, 4, and 5 and reported that the resistance was governed by a single gene in the cultivar to each of the races.

Based on the reactions of a collection of known differential cultivars, T Honnareddy and Dubey (2006) identified three new races of the pathogen in India. Sharma and Muehlbauer (2007) discovered sequence tagged microsatellite site (STMS) markers linked to six genes that control resistance to the pathogen's six races (0, 1A, 2, 3, 4, and 5). To isolate potential genes involved in preventing disease progression, a detailed understanding of *Fusarium* and chickpea interaction at a cellular and molecular level is needed. In this direction, Gupta *et al.,* (2009) designed an experiment in which they induced pathogen-induced disease responses in both susceptible and resistant plants and controlled the transcript level expression of stress-induced genes or gene fragments.

3. Genetic diversity for chickpea improvement

Chickpea, like many other widely grown crops, has a limited genetic base as a result of domestication. Recent breeding attempts have been limited to the limited introduction of diverse germplasm over the last 60 years (Varshney *et al.,* 2010). When compared to other nations, India has a higher participation in chickpea. Because of domestication, chickpea, like many other widely grown crops, has a small genetic base. Over the last 60 years, recent breeding

attempts have been limited to the limited introduction of diverse germplasm (Varshney *et al.,* 2010). When compared to other countries, India has a higher participation in chickpea.

There are success stories of chickpea improvement available, such as the development of early maturing chickpea varieties that avoided terminal heat stress, drought stress, and so on. (Gaur *et al.,* 2005). ICCV2 is a notable early maturing chickpea variety that matures in around 85 days and is considered the world's earliest maturing Kabuli chickpea variety. It is very important varieties that are grown in Pakistan, India, and other chickpea-growing countries around the world during the short growing season. Further progress in the chickpea breeding programme resulted in the production of early maturing Kabuli and desi chickpea varieties. ICCV 96029 and 96030 are two super early maturing chickpea varieties that mature in 75 to 80 days and are primarily grown in Myanmar and southern India.

Sharma *et al.,* (1990) evaluated 70 genotypes of chickpea and reported high heritability for days to maturity, plant weight, 100 seed weight and number of branches per plant.

Rao *et al.,* (1994) evaluated sixteen bold seeded chickpea genotypes for developmental traits and reported maximum variability for seed yield followed by harvest index and biological yield.

Rao S. K. et al., (2000) evaluated forty short duration chickpea genotypes for developmental and morphological traits including biological yield and harvest index. Maximum variation was observed for biological yield followed by seed yield. Low variability was observed for days to maturity followed by days to 50% flowering. Biological yield had a positive association with harvest index. Pod bearing length had positive association with 100 seed weight, seed yield and biological yield.

Kumar *et al.,* (2001) studied 26 genotypes for genotypic and phenotypic coefficient of variation for 12 quantitative characters, in which pods per plant exhibited the highest amount of genetic variability, followed by secondary

branches per plant, seed yield per plant, 100 seed weight, primary branches per plant and number of seeds per plant

Noor *et al.,* (2003) observed high heritability with medium to high genetic advance for days to flowering, days to maturity, secondary branches and 100 seed weight whereas, low to medium heritability exhibited for other characters.

Patel and Babbar (2004) evaluated 24 chickpea genotypes each of desi, kabuli and gulabi. High heritability coupled with high genetic advance as percentage of mean was found for biological yield per plant in desi chickpea; seed yield per plant, harvest index, biological yield per plant in kabuli chickpea.

Noor *et al.,* (2003) observed high heritability with medium to high genetic advance for days to flowering, days to maturity, secondary branches and 100 seed weight whereas, low to medium heritability exhibited for other characters.

Honnappa *et al.,* (2018) evaluated Ninety green chickpea (*Cicer arietinum* L.) genotypes for genetic potential, heritability, genetic advance, and traits association of yield contributing characters. Data were recorded on days to 50% flowering, pods per plant, seeds per pod, number of primary branches /plant, number of secondary branches/ plant, 100-seed weight and seed yield /plant.

Sudhanshu Jain *et al.,* (2013) Thirty newly bred and diverse genotypes were tested to assess the heritable variation and yield factors in chickpea and recorded observation on days to 50 % flowering, days to maturity, plant height (cm), primary branches/plant, pods/ plant, seeds/ pod, 100 seed weight (g), harvest index, and seed yield/ plant (g). Seed yield/ plant, harvest index, and 100 seed weight exhibited high heritability and moderate to high estimates of genetic advance as percentage of mean. Plant height, pods/ plant, seeds/ pod, 100 seed weight and harvest index showed positive and significant correlation with seed yield/ plant.

Lokare *et al.,* (2007) found wide range of variation for seed yield per plant, 100 seed weight and number of pods per plant while moderate variation for number of fruiting branches per plant.

Akhtar *et al.,* (2011) demonstrated high phenotypic coefficient of variation for days to flowering, days to maturity, plant height and seed yield than genotypic coefficient of variations, which means that the expression of these traits is more influenced by environmental effects. It is therefore, suggested that the grain yield could be improved by using the 100-seed weight and number of pods per plant as selection criterion in chickpea.

Jerotich *et al.,* (2013) screened several chickpea genotypes to identify and select high yielding and drought tolerant lines with associated morpho-physiological characteristics under field conditions and determined their heritability. Parameters that were measured included phenological growth stages, yield (Kg/ha) and its components which included number of pods/plant, plant height (cm), biomass (Kg/ha) and harvest index (HI).

Ramanappa *et al.,* (2013) revealed that the genotypes exhibited highly significant differences for days to 50% flowering, plant height, number of primary branches, number of pods per plant, days to maturity, 100 seed weight, harvest index and grain yield per plant. Considerable variation between genotypes for qualitative traits such as early plant vigour, growth habit, seed colour, seed shape and texture was also recorded.

Waseem *et al.,*(2014) indicated that dry biomass, grain yield per plant, pods per plant, 100-seed weight and seeds per pod resulted higher genotypic coefficient of variation in genotypes under study.

Saleem *et al.,* (2005) analyzed the means and components of variability (genetic, phenotypic and environmental), heritability (h2bs), genetic advance and interrelationships (genetic and phenotypic) for yield and various other yield components. The results suggested that pods per plant, seeds per plant, 100- seed weight and total weight of plant might be useful while selecting for high yielding genotypes of chickpea.

Anita Babbar *et al.,* (2013) evaluated Forty four promising lines of chickpea and noticed maximum genotypic coefficient of variation for damaged pod percentage, total number of seeds per plant and total number of pods per plant. Days to 50% flowering, days to maturity, plant height, 100 seed weight and seed yield per

plant showing high heritability coupled with medium genetic advance as percentage of mean, whereas, damage pod percentage, number of seeds per plant and number of pods per plant showing medium heritability and high genetic advance as percentage of mean.

Priyanka Joshi *et al.,* evaluated 252 intra-specific recombinant inbred line (RIL) population derived from a cross between desi (ICC 283) and kabuli (ICC 8261) type of chickpea. Traits assessed were days to 50% flowering, maturity, plant height, biological yield, seed yield, harvest index and 100-seed weight. Out of seven characters studied biological yield, seed yield, harvest index and 100- seed weight exhibited high genotypic coefficient of variation and heritability coupled with high genetic advance as percent of mean which revealed that these traits might be under control of additive gene effects and therefore these traits are more reliable for effective selection for developing desirable lines.

Vaghela *et al.,* (2008) study the variability of characters under different parameters in fifty diverse genotypes of kabuli chickpea (*Cicer arietinum* L.). Analysis of variance revealed significant genotypic differences for all the ten characters with wide range of variations. Estimates of genotypic and phenotypic co-efficients of variation were high for seed yield per plant and number of pods per plant. Broad sense heritability was higher for all the traits except plant height. High genetic advance expressed as a percentage of mean was exhibited by seed yield per plant and number of pods per plant.

Rozina *et al.,* (2011) estimates high heritability fordays to 50% flowering followed by biological yield per plant, plant height, 100 seed weight, grain yield per plant and days to maturity.

Tiwari A. (2013) estimated high heritability for 100 seed weight followed by harvest index, total number .of pods per plant, seeds per pod, days to maturity and days to pod initiation

O.K. Tomar *et al.,* (2009) Forty five genotypes of chickpea were evaluated. The genotypic and phenotypic co-efficients of variation were found maximum for number of seeds/plant. The high heritability in broad sense was recorded for all

the traits except days to maturity. The genetic advance as per cent mean was maximum for seeds/plant.

Waseem *et al.,*(2014) analysed three varieties that were sown in triplicate completely randomized block design Significant genotypic and phenotypic correlations were reported for dry biomass, pods per plant, 100-seed weight, grain yield per plant and seeds per pod. Heritability, genetic advance, principle component analysis, principle component biplot and path coefficient analysis indicated that these traits may be used for the development of higher grain yielding chickpea genotypes to improve yield of chickpea.

Pundir *et al.,* (1991) observed 25 diverse genotypes of chickpea and observed high genetic advance as percentage of mean for seed size, seed yield and number of seeds per pod and low for number of pods per plant and days to flowering.

Gupta *et al.,* (1992) estimated high genetic advance as percentage of mean for 100 seed weight and seed yield and moderate for number of seeds per plant, number of pods per plant and number of seeds per pod. However, it was low for plant height, days to 50 percent flowering, number of branches per plant and days to maturity.

Arora and Jeena (2001) evaluated 40 genotypes of chickpea for 18 quantitative characters. High genetic advance was noted for 100 seed weight, followed by primary branches per plant and seeds per plant.

Pratap *et al.,* (2004) assessed 38 genetically diverse early maturing chickpea genotypes in four different environments, revealed that seed yield, 100 seed weight and biological yield showed high genetic advance.

R. N. Arora *et al.,* (2018) Fifty genotypes of kabuli chickpea were studied for assessment of genetic variability. Maximum variability was recorded for number of pods per plant and minimum for number of days to maturity. There were close agreement between GCV and PCV for number of days to flowering, number of days to maturity and 100-seed weight. Highest heritability was observed for 100-seed weight. Genetic advance as percent of mean was maximum for number of

pods per plant. High heritability coupled with high genetic advance was observed in case of number of pods per plant.

A. Tiwari *et al.,* (2016) studied thirty eight chickpea varieties to find out genetic variability, genetic association and path coefficient analysis for grain yield and its attributing characters. Both genotypic and phenotypic variances were highly significant for all the traits with little higher phenotypic coefficient of variations. High heritability coupled with high genetic advance were obtained with harvest index, seeds per pod, total number of pods per plant, seed yield per plant, days to maturity. Genotypic correlation coefficients were higher than the corresponding phenotypic correlation coefficients in most of the traits. Harvest index, 100-seed weight, seeds per pod, number of effective pods per plant, total number of pods per plant, number of primary branches per plant per plant and plant height were the most important characters, which possessed positive association with seed yield per plant.

A.Nishant Bhanu *et al.,* (2017) fifty three germplasm of chickpea were undertaken to determine relationships among yield and some yield components using direct (variability, heritability, and genetic advance) and indirect selection parameters (correlation and path coefficient analysis). Significant genetic variations were observed among the genotypes for days to flower, days to maturity, plant height, number of branches per plant, number of pods per plant, 100-seed weight and seed yield per plant. Correlation studies revealed that seed yield was positively and significantly correlated with number of primary branches, number of secondary branches and number of pods per plant. The path coefficient analysis based on seed yield, as a dependent variable, showed that pods per plant had the greatest direct effect on seed yield (0.81) followed by number of secondary branches.

Akansha Sonwani *et al.,* (2017) examined the genetic variability and correlation existing among 16 genotypes of chickpea. The data were recorded for nine quantitative characters to obtain estimates of variability, heritability, and genetic advance. Significant differences were observed among the genotypes for all the characters studied. Harvest mean performance was estimated for days to

maturity and days to 50% flowering. The high value of GCV and PCV was recorded for number of pods per plant and number of primary branches per plant. High genetic advance as a percent of mean recorded in number of pods per plant.

Padmavathi *et al.,* (2013) studied 30genotypes of kabuli chickpea were used to study genetic variability with high heritability and high genetic advance as per cent of mean was recorded for number of primary branches per plant, biological yield per plant and seed yield per plant.

Jivani *et al.,* (2013) assessed magnitude of high heritability coupled with high GCV and high genetic advance as per cent of mean were observed for seed yield per plant, biological yield per plant, harvest index, number of pods per plant and 100-seed weight.

Tesfamichael *et al.,* (2015) The evaluated 39 kabuli genotypes and documented wide variability with respect to plant height, number of total pods per plant, seed yield per plant and 100 seed weight.

Rao *et al.,* (1994) evaluated genetic diversity of 25 genotypes for 10 characters and grouped them into three clusters. Days to flowering, days to maturity, pods per plant and 100 seed weight accounted for maximum divergence. JG 315, JG 86-8, and JG 86-22 were identified as genetically diverse parents with agronomic value. Cluster III had high yield and small seeded genotype.

Tesfamichael *et al.*, (2015) evaluated 39 kabuli genotypes and noticed highly significant ($p < 0.001$) variations among genotypes and genotype by environment interactions for all studied traits. The top five high yielding genotypes were ICCV 05315, ICC 13461, ICCV 07313, ICC 13764 and ICCV
00302. Genotypes ranking for most agronomic traits varied across environments which indicated a crossover type of genotype by environment interactions. The current study indicated that genotypes with higher number of pods per plant were well adapted to the climatic condition and gave more pods and these could be contributed to high ultimate yield per plant. Therefore, this study indicated there is a potential of obtaining high pod yielding genotypes from the evaluated kabuli germplasm.

Singh *et al.,* (2001) observed that days to maturity and number of primary branches per plant had high indirect effect on seed yield via number of pods per plant although direct effect was negative. Muhammad et al., (2002) studied that number of pods per plant had maximum positive direct effect on seed yield. The other traits in the study also exhibited considerable indirect effect on the seed yield through number of pods per plant. It was concluded that number of pods per plant and 100 seed weight could be used as selection criteria to improve the yield.

Patel (2003) revealed that biological yield and harvest index had high direct effect on seed yield in desi chickpea. Whereas, days to flowering initiation, fifty per cent podding, and secondary branches had positive direct effect on seed yield in gulabi chickpea.

Muhammad *et al.,* (2008) studied various parameters of genetic variability in seventeen elite genotypes and three standard varieties of chickpea for yield and various yield components. The result suggested that increase in days to flowering, days to maturity, number of secondary branches and 100-seed weight might be useful while selecting high yielding genotype of chickpea.

Qureshi *et al.,* (2004) studied two hundred and nineteen chickpea genotypes and found considerable variation between genotypes for qualitative traits such as growth habit, seed shape and texta texture.

Biru Alemu *et al.,* (2017)Studied a total of 16 chickpea materials (8 improved varieties, 7 advanced lines, and one local check) to assess and quantify the genetic variability, estimate heritability and genetic advance for yield and yield contributing characters of released and pipeline chickpea varieties based on agro-morphological traits. The combined analysis of variance (ANOVA) indicated highly significant differences ($P \leq 0.01$) among chickpea genotypes for grain yield and other agronomic traits except for number of seed per pod and branch per plant, indicating the existence of ample genetic variability among present chickpea genotypes.

Arshad *et al.,* (2003) studied genotypic and phenotypic variability heritability, genetic advance and correlation for yield and its components in 24 genotypes of

chickpea. High heritability with low genetic advance of day to 50% flowering, days to maturity and 100 seed weight indicated the influence of dominant and epistatic genes for these traits. High heritability of secondary branches and biological yield coupled with high genetic advance revealed that additive gene effect are important in determining character. Grain yield had positive and significant with plant height, pod per plant, 100 seed weight and biological yield.

Ajinder *et al.,* (2004) studied genetic variability, heritability and genetic advance for grain yield and its components in 30 genotypes of Desi chickpea under normal and late sown conditions in Rabi 2001-2002. The results showed that 100-seed weight had comparable phenotypic and genotypic coefficients of variation under both sowing conditions.

Thakur and Sirohi (2008) evaluated genetic variability, heritability, genetic advance for yield and nine other economic traits in chickpea. The study revealed considerable genetic variability among the genotypes for all the traits. Genotypic and phenotypic coefficients of variation were more or less similar for all the characters. Phenotypic and genotypic coefficients of variation were high for seed yield per plant, biological yield per plant, pods per plant and 100-seed weight

Dwevedi and Gaibriyal (2009) investigated the twenty five genotypes of chickpea and found that moderate to high degree of heritability and genetic advance was observed for number of pods per pant, harvest index and biological yield.

Farshadfar and Sabaghpour (2011) found out that heritability is greater for days to maturity, days to 50% flowering, plant height, number of primary branches and number of secondary branches.

Sweta *et al.,* (2013) observed a high degree of significant variation for all the morphological characters studied except seeds per pod. The phenotypic and genotypic coefficients of variation were found maximum for seed yield per plant followed by pods per plant and seeds per pod whereas minimum for days to maturity. She also reported high heritability with high genetic advance as percent of mean for secondary branches per plant, seed yield per plant, 100-seed weight, pods per plant and plant height that could be improved by simple selection.

Jivani *et al.,* (2013) studied 105 diverse genotypes of chickpea and observed seed yield per plant had direct effect on harvest index followed by biological yield per plant, number of pods per plant and100 seed weight.

Kumar *et al.,* (2015) studied genetic variability as well as direct and indirect effects of yield related traits on seed yield in 25 genotypes of chickpea and recorded the mean values of the characters studied showed a wide spectrum of genetic variation.

Md. Yeasin Ali *et al.,* (2018) studied the yield and quality response of two varieties of chickpea (BARI Chola-5 and BARI Chola-9), at five different sowing dates. The results of two varieties revealed significant variations in days to flowering, maturity, number of grains per plant, number of seeds per grain, the weight of 1000 seeds, grain yield, stover yield, biological yield and harvest index due to different sowing dates. Early and delayed sowings affected the chickpea yield and the quality of grain. Irrespective of variety, early and delayed sowings showed lower performance on all parameters, significantly affected the yield and quality of chickpea.

Lizica Szilagyi (2021) Estimated the degree of genetic diversity in 42 chickpea genotypes based on the multivariate analysis of several quantitative and qualitative traits. Principal Component Analysis (PCA) showed a significant correlation between the number of pods per plant and the number of seeds per plant. The yield was significantly correlated with the number of pods per plant, number of seeds per plant and TSW (thousand seed weight) The first principal component of the PCA analysis, accountable for a 45.5% share of the total variation between genotypes, has been shown to be an association between the following variables: number of pods per plant, number of seeds per plant and seeds yield per plant. These findings can provide practical information for the selection of parental material and thus assist in planning breeding strategies.

Zeeshan Qadeer *et al.,* (2021) Studied for investigation of genetic diversity of forty chickpea germplasm the data were subjected to D^2 statistics, principal component and cluster analysis. Data also showed that number of pods plant^{-1} and 100 grain weight have highest positive contribution in construction of PC1 and PC2. Cluster analysis distributed the chickpea genotypes into four distinct

clusters. Ward's Dendrogram was constructed based on Euclidean distance for grouping of genotypes in clusters. It was noted that Cluster I revealed maximum Euclidean distance followed by cluster II. It was also recorded that the genotypes with higher yield potential were grouped in cluster II. Therefore the members of cluster II possessing higher grain yield potential along with sufficient amount of genetic diversity may be incorporated in chickpea genetic improvement program.

4. Marker Assisted Selection for screening of Chickpea genotypes

The genome is the most fundamental set of chromosomes. In nature, the cultivated chickpea has 16 chromosomes, which are numbered in decreasing order from 1 to 8. 353. The chickpea chromosome is 53 mb long, with sizes ranging from 30.53 to 58.05 according to (Ahmad and Hymowitz1993).

Marker Assisted Selection is an indirect selection process in which a trait of interest is selected using molecular markers in a completely safe environment over a short period of time. Rather than the trait itself, this process is entirely focused on markers linked to the trait of interest. Molecular markers are useful in assessing genetic diversity for the following reasons: Indentifying and removing duplicates;
(ii) creating core collections; and (iii) evaluating genetic relationships between taxa. (iv) choosing a wide range of parental genotypes for (a) studying QTLs for complex traits and (b) establishing mapping populations.

Molecular markers are unique detectable molecules that can be used to distinguish between species, such as a readily detectable DNA or protein sequence whose inheritance can be tracked. A marker is a chromosome element, locus, or DNA sequence that can be identified using cytological, phenotypic, or other molecular techniques. Recent breakthroughs in crop genomics, like as MAS, array-based genotyping, GBS, and NGS, have made molecular tools available to help in breeding. Marker-assisted selection allows for the introduction of a desired chromosomal region into the progeny by accurate phenotyping (MAS), has been effectively employed in the production of better varieties/ germplasm in numerous grain and legume crops.

There are various types of markers, including morphological, biochemical, cytological, and molecular markers (Soregaon *et al.,* 2010). The environment has an effect on morphological and biochemical markers, but the environment has no impact on DNA markers. The molecular markers are not genes of interest, but they function as a sign or flag and are found near the targeted gene. The DNA markers, on the other hand, are closely linked to the targeted gene (Nadeem *et al.,*2018).

Molecular markers are pieces of DNA that code for specific features and can be used to track their inheritance. There are several DNA markers which are used in plant breeding. They have been categorized into hybridization based and PCR based (Jeffreys *et al.,* 1985, Gupta *et al.,* 1999). Several types of DNA markers based on hybridization or PCR have been created and are utilised in plants, including restriction fragment-length polymorphism (RFLP), amplified fragment-length polymorphism (AFLP), randomly amplified polymorphic DNA (RAPD), and randomly amplified polymorphic DNA (RAPD),Sequence-tagged sites (STS), Expressed sequence tags (ESTs), and simple sequence repeats (SSRs) or microsatellites, sequence-characterized amplified regions (SCARs), Sequence-Tagged Microsatellites Marker (STMS) and single nucleotide polymorphisms (SNPs) (Joshi *et al.,* 1999, Nadeem *et al.,* 2018, Adlak *et al.,* 2020)

Since the first use of DNA restriction fragment length polymorphism (RFLP) in human linkage mapping by Botstein *et al.,* (1980), significant progress has been made in the development and improvement of molecular techniques that help to easily find markers of interest on a large scale, resulting in extensive and successful uses of DNA markers in human genetics, animal genetics and breeding, plant genetics and breeding, and Germplasm characterization and management. Lesser in number, confer indistinguishable phenotypes, influenced by the environment, Influenced by the genetic background, Influenced by the ontogeny and No stable inheritance are constraints in using morphological markers and abundant, Ubiquitous, Highly polymorphic, stable inheritance, No environmental influence, No influence of ontogeny of individual and codominant or dominant are the properties of DNA marker. DNA marker are preferred due to contrasting application such as Diversity analysis at molecular level to

characterize the germplasm entries, Markers aided selection for pest resistance in crop improvements, DNA finger printing of crop species from different geographical regions, to establish phylogenetic and taxonomic relationship among individuals, Tagging of major and minor QTLs, Physical mapping and map based cloning of genes for producing transgenic organisms. The various molecular markers such as RAPD, SCAR, DAF, STMS, ISSR, etc. have been widely used in the genetic improvement of chickpea.

Random Amplified Polymorphic DNA (RAPD)

It is a single 8–10 nucleotide long arbitrary primer that produces one to a few amplification items (Williams *et al.,* 1990). PCR artifacts are avoided by using primers with >58% GC content and sequences incapable of internal pairing. The PCR technique allows for the precise amplification of DNA fragments ranging in length from 200 to 3000 bp, which can then be visualised using ethidium bromide staining after electrophoresis. RAPDs provide a rapid assay for nucleotide sequence polymorphism since a single primer enables amplification of several loci distributed across the genome (Tingey *et al.,* 1992). Since each primer amplifies a large number of different fragments, the technique has proven to be a quick way to detect polymorphism. The majority of commercially available primers contain 6– 12 fragments. Hybridization, autoradiography, or high technical expertise are not needed for RAPD analysis. RAPD markers are dominant. Amplification occurs at a locus or does not, resulting in scores with or without bands. As a result, homozygotes and heterozygotes are indistinguishable. RAPD is sensitive to changes in PCR conditions, which causes some of the amplified fragments to shift. If the conditions used are standardised, reproducible results can be obtained.

DNA Amplification Fingerprinting (DAF)

Another technique that uses as few as 5 nucleotides, but usually 7 or 8 nucleotides, and either high or low stringency cycles to produce relatively complex DNA profiles when resolved by polyacrylamide gel electrophoresis and a highly sensitive DNA silver stain (Caetano-Anolles *et al.,*1991). (Bassam *et al.,* 1991). DAF is similar to the random amplified polymorphic DNA (RAPD) technique (Welsh and McClelland, 1990). Despite the low level of polymorphism

in the chickpea genome, DAF outperformed RAPD because banding patterns were highly reproducible and polymorphic. A DAF employs low stringency amplification conditions, allowing primers to anneal arbitrarily at multiple locations on each template strand. Although DNA synthesis begins throughout the template, only sequences with priming sites on opposite strands and in close proximity will be successfully amplified. In DAF, Amplification is arbitrary, but not random. DAF fingerprints have two types of bands: those that are phylogenetic consensuses and those that are individual-specific. This suggests that primer sites are distributed at random along the target genome, flanking both conserved and highly variable regions.

Sequence Characterized Amplified Regions (SCAR)

Cloning and sequencing RAPD fragments produce SCARs markers, which are of particular interest. Centered on the RAPD technique, it is an example of STS. If the sequence is known, primers that are longer than standard RAPD primers (24-mer oligonucleotides) and precisely complementary to the ends of the original RAPD fragment can be designed. A When these primers are used in a PCR, single loci that correspond to the original fragment are found. SCARs (Sequence Characterized Amplified Regions) are the names given to these loci. These markers have many advantages over RAPD and other arbitrarily primed approaches, the most notable of which is that the results are highly repeatable (due to the use of longer primers) and the markers are dominant.

Sequence-Tagged Microsatellites Marker (STMS)

A Primers can be made that are complementary to the short, specific sequences flanking microsatellite repeat loci and guide the amplification of the repeat in a PCR reaction. This is an important method of detecting polymorphisms since the repeat length is highly variable. The following characteristics of these markers make them useful for population studies:

- Usually define a single, multi-allelic locus
- Co-dominant- homozygotes and heterozygotes can be distinguished and
- Highly reproducible results are obtained

Multiplexing (using STMS primers in the same reaction tube) can save time, but this is only possible if the products of the different primers do not overlap in size. STMS markers are a useful method for detecting hybrids, genotype screening, and diversity analysis because they are easy to visualise and inexpensive. Despite their many benefits, STMS have significant drawbacks that have kept them from becoming widely used for a long time. The high costs of cloning, sequencing, and primer manufacturing are the main barriers to widespread usage. Furthermore, radioisotopes and sequencing gels are still used in conventional procedures to identify amplified SSRs. Finally, a variety of issues hamper primer generation efficiency, including clone redundancy and the existence of manufactured chimaeras [Winter *et al.,* (2000), GUPTA P.K *et al.,* (2000)]

Inter simple sequence repeats

ISSRs (Inter Simple Sequence Repeats) are DNA sequences with a few (1-6) base pair repeat length. In contrast to other molecular markers, these sequences are abundant, distributed across the genome, and highly polymorphic. These are PCR-based markers that enable polymorphism in microsatellite and intermicrosatellite loci to be detected without knowing the DNA sequence. These markers were created by Kantety *et al.,*(1995) to evaluate genetic diversity in dent and popcorn. These primers can be anchored to unique genomic sequences flanking the repeat in the 5' or 3' to prevent stuttering. The effects of 3' anchoring are higher than 5' anchoring. Only amplification products are obtained when SSRs in opposite orientation are located within a PCR-able radius, with flanking sequences that fit the oligo's.

Studies related to marker assisted breeding in chickpea for *Fusarium* wilt:

A Several markers were previously mapped on LG2 (CS27700A, TA59, TA96, TA103, TA110, TA194, Tr19, H1B06, H1Fo5, H1F22, H1P09/2, and H6D11) of the chickpea reference chart (Winter *et al.,* 2000; Sharma *et al.*, 2004; Cobos *et al.,* 2006; Lichtenzveig *et al.,* 2006). Two microsatellite markers (H3A12 and TA144) and a RAPD marker (UBC302) were added to this LG by Gowda *et al.,* (2009). Many studies have found markers linked to *Fusarium* wilt resistance genes in chickpea. There are no markers linked to wilt resistance genes for the

H2 locus that confer resistance to race 1A or those that regulate resistance to races 1B/C and 6. Furthermore, no research has been done on the inheritance of resistance to races 1B/C and 6. Soregaon *et al.,* (2007). however, discovered a molecular marker linked to the H2 locus of the *Fusarium* wilt resistance gene for race 1. They used a RIL population generated by crossing K850 (h1h1H2H2, susceptible to late wilt) with WR315 (h1h1h2h2, resistant) and segregating for the H2 locus alone. In susceptible parents, the primer A07C417 amplified an extra 417bp fragment, which co-segregated in susceptible bulk. In RILs, the marker revealed a 1:1 monogenic segregation ratio. The *Fusarium* wilt resistant gene in chickpea was first discovered by Ayyar and Iyer in 1936. Resistance to *Fusarium* wilt was conferred by a single gene with incomplete dominance.

Resistance to *Fusarium* wilt is regulated by a single recessive gene, according to Haware *et al.,* (1980). Race 2 of *Fusarium oxysporum* was described by Haware and Nene (1982). Later, after inoculation with race 2, the phenomenon of late wilting was identified. Mayer *et al.,* (1997) were the first to identify a molecular marker linked to the H1 locus in chickpea (FOC1). They discovered two primers, UBC-170550 and CS-27700, which amplified a DNA fragment associated with *Fusarium*

wilt resistance and susceptibility, respectively. They discovered 7% recombination between the two markers and the wilt resistance locus, as well as 6% recombination between the loci corresponding to the two RAPD markers after examining these primers (UBC-170 and CS-27). After that, they cloned the sequence and produced the CS-27700 and UBC-170550 allele specific associated primers (ASAPs). UBC-170 formed a single fragment for both resistant and susceptible genotypes, while CS-27 amplified a fragment linked to the allele for susceptibility to race 1 (H1 locus) of *Fusarium* wilt.

Tullu *et al.,* (1998) used intraspecific cross rather than interspecific cross in their research. Later, since chickpea is a self-pollinated crop with a moderate genome size, the pattern shifted, and polymorphism in cultivated chickpea was lower. They discovered that 38 out of 100 ISSR primers were polymorphic and segregated in a 1:1 mendelian ratio. They discovered an ISSR marker UBC-

855550 linked to the gene for *Fusarium* wilt race 4 resistance at a distance of 5.2 cM for the first time. It was mapped to linkage group 6 of the cicer genome and co-segregated with CS27700, resistance to *Fusarium* wilt race 1. The marker UBC-855550 is 0.6 cM away from CS-27700 and on the same side of the wilt resistance gene as CS-27700.

The genes for resistance to *Fusarium* wilt races 1 and 4 were found to be closely related in all of the studies described above. Most available chickpea genetic maps (Gaur and Slinkard, 1990; Kazan *et al.,* 1993; Simon and Muehlbauer, 1997; Collard *et al.,* 2003) were established using interspecific crosses due to the low genetic variability within C. arietinum. MAS will not be useful until polymorphic markers for C. arietinum (intraspecific) populations are available. Intraspecific linkage maps saturated with codominant markers are needed for MAS. Such markers will also be useful for map-based cloning because genetic and physical distances between markers will be small, and linkage drag will be low.

Ratnaparkhe *et al.,*(1998) discovered a (ISSR) marker linked to the gene for *Fusarium* wilt race 4 resistance. At a distance of 5.2 cM, the marker in question, UBC- 855500, was discovered to be associated in repulsion with the *Fusarium* wilt resistance gene. It co-segregated with CS-27700, a RAPD marker previously shown to be linked to the gene for resistance to *Fusarium* wilt race 1, and was mapped to linkage group 6 of the *Cicer* genome. Winter *et al.,* (2000) used 130 F6:7 RILs from an inter-specific cross between cultivated chickpea line ICC-4958 and C. reticulatum to create a detailed reference map (PI-489777). The map was created using 354 markers. At an average distance of 6.8 cM between markers, 313 markers covered 2077.9 cM in eight large and eight small linkage categories.

Chickpea germplasm accession resistance to race 3 WR315 was inherited as a single gene, foc 3, which was named after the nomenclature suggested by Tekeoglu *et al.,* (2000) for *Fusarium* wilt resistance genes in chickpea The experiment was carried out on 100 F7 RILs derived from a cross of two *C. arietinum* sections, WR315 (resistant) and C-104 (susceptible). The foc 3 gene was mapped 0.6 cM from STMS markers TA96 and TA27, as well as STS

marker CS27700A, according to the report. On the other hand, another STMS marker, TA194, with a length of 14.3 cM, flanked the gene.

Tekeoglu *et al.,* (2001) used a chickpea map to identify fifty sequence-tagged microsatellite site (STMS) markers and a resistant gene-analog (RGA) locus. The map covers 1,174.5 cM and has an average marker distance of 7.0 cM in nine linkage categories (LGs).

Benko-Iseppon *et al.,* (2003) developed DNA amplification fingerprinting (DAF) markers that were connected to both resistance loci. Between marker R-2609-1 and the race 4 resistance locus, SCAR loci derived from DAF markers closely linked to the FOC-4 resistance locus 2.0 cM in chickpea were found. This locus was flanked by seven other markers ranging in size from 4.1 to 9.0 cM. These are the most closely related markers for this locus that are currently available.

Ravikumar *et al.,* (2003) found that the CS-27700 (ASAP) primer was associated with wilt susceptibility in widely used chickpea parental lines.

Two F6:7 RIL populations were derived from intraspecific crosses with the same parental line (JG62), i.e. Rubio *et al.,* (2003) investigated CA2156 x JG62 (susceptible x resistant) and CA2139 x JG62 (resistant x resistant) to determine the inheritance of resistance to *Fusarium* wilt race „0' of chickpea and the associated RAPD marker.

The RIL population from the CA2156 x JG62 cross had a 1:1 resistant to susceptible ratio, suggesting that resistance was mediated by a single gene with two alleles. A 3:1 resistant to susceptible ratio in the second RIL population (CA2139 x JG62) suggested that two genes were present and that either gene was sufficient to confer resistance. Linkage analysis showed a RAPD marker, OPJ20600, linked to resistance in both RIL populations, which is present in the resistant parent JG62.

Sharma *et al.,* (2004) discovered sequence tagged Microsatellite site (STMS) and sequence tagged site (STS) markers linked to the *Fusarium oxysporum* f.sp. ciceris race 3 resistance gene in chickpea, as well as a correlation between three wilt resistance genes (Foc 1, 3, and 4). It was discovered that foc 3 is linked to two other chickpea wilt resistance genes, foc 1 (syn h1) and foc 4. Foc

3 is 9.8 cM away from foc 1 and 8.7 cM away from foc 4, while foc 1 and foc 4 are just 1.1 cM apart. The A07C417 marker is related to the H2 locus and sensitivity, and they are separated by 21.7 cM, according to the linkage analysis.

The RILs of another cross JG62 (H1H1H2H2, susceptible early wilter) x WR315 (h1h1h2h2 resistant) segregating for both the loci H1 and H2 showed digenic inheritance for wilt resistance (Brinda and Ravikumar, 2005). Consequently, the DNA marker linked to H1 (CS27700) and H2 (A07C417) also showed independent segregation.The identified DNA markers linked to both H1 and H2 of wilt resistance will facilitate marker assisted selection and pyramiding of resistance genes to susceptible varieties.

Cobos *et al.,* (2005) used same RIL populations to develop a chickpea genetic map for locating genes for resistance to *Fusarium* wilt race 0. The presence in both RIL populations of 47 common molecular markers (41 RAPD and six STMS) and three morphological traits enabled joining of maps obtained independently. Integrated map was developed by joint segregation analysis including a total of 160 markers and 159 RILs which resulted in 10 LGs comprising a total of 138 markers and joint map covered a total genetic distance of 427.9 cM. LG3 included a gene for resistance to Foc 0 (Foc 01/foc 01) flanked by RAPD marker OPJ20600 (3 cM apart) and STMS marker TR59 (2 cM apart). To overcome this problem, STMS markers have been developed for chickpea (Huttel *et al.,* 1999; Sethy *et al.,* 2003, 2006, Lichtenzveig *et al.,* 2005, Choudhary *et al.,* 2006). With the advent of STMS markers and availability of sources of resistance to all races in *C. arietinum*, it is possible to map genes using intraspecific populations.

Brinda and Ravikumar, (2005) showed the independent segregation of DNA marker linked to H1 (CS27700) and H2 (A07C417) by using RILs of cross JG62 (H1H1H2H2 susceptible early wilter) x WR315 (h1h1h2h2 resistant). Soregaon *et al.,* (2007) identified molecular marker linked to H2 locus of *Fusarium*F; wilt resistance gene for Race 1.

Halila *et al.,* (2009) worked on tagging and mapping of second resistance gene for *Fusarium* Wilt race 0 in chickpea. Both genes separately confer complete resistance to race 0 of the wilt pathogen. Using a RIL population that segregated

for both genes (CA2139 x JG62) and the genotypic information provided by two markers flanking Foc 01/foc 01 ten resistant lines containing the resistant allele Foc 02/foc 02 were selected. Genotypic analysis using these ten resistant lines paired with ten susceptible RILs, selected in the same population, revealed that STMS markers sited on LG2 were strongly associated with Foc 02/foc 02.

Gowda *et al.,* (2009) mapped the FOC1, FOC2 and FOC3 genes with previously unreported SSR markers that closely flank the genes. They also validated the markers using 16 diverse chickpea genotypes. After linkage analysis, 19 markers showed association with one or more wilt resistance genes. All these markers were located on LG2. Further they carried out validation of markers viz. TA110, TA96, H1B06 and TA194 which were determined to be tightly linked with FOC1, FOC2 and FOC3. They got thirteen genotypes resistant to FOC1 and all of these were determined to carry the allele associated with resistance for the marker TA110. Of the three FOC1 susceptible genotypes, JG62 carried the susceptibility associated TA110 allele. Overall, TA110 correctly identified 14 of the 16 genotypes as either resistant or susceptible to either of the FOC races.

Millan *et al.,* (2010) created a consensus genetic map by combining linkage maps from ten different populations and using STMS as bridge markers. Previously, populations derived from five wide crosses (*C. arietinumx Cicer reticulatum* types) and five narrow crosses (Desi x Kabuli types) were used to map genes for several agronomic traits such as Ascochyta blight, *Fusarium* wilt, rust resistance, flowering time and days to flower.

Varshney, *et al.,* (2013) reported Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement in Desi and Kabuli chickpea has opened the possibility of anchoring genetic maps and positioning QTL on the physical one. They have reported the ~738-Mb draft whole genome shotgun sequence of CDC Frontier, a kabuli chickpea variety, which contains an estimated 28,269 genes. Resequencing and review of 90 cultivated and wild genotypes from ten countries establishes breeding-associated genetic sweeps and balancing selection goals. Candidate genes for disease resistance and agronomic traits, such as those that differentiate the two key market groups of cultivated chickpea desi and kabuli, have been identified.

Ahmad *et al.,* (2014) used PCR-based molecular markers to perform Marker Assisted Selection (MAS) for chickpea *Fusarium oxysporum* wilt resistant genotypes and found that only the TA194 SSR marker showed 85 percent probability linkage to the wilt resistant locus among the total used RAPD/SSR primers. The receiver operating characteristic curve was used to validate the connection of a marker. The use of the sorted wilt resistant genotypes through SSR marker TA194 can make available ample prospect in MAS breeding for yield improvement of the crop.

Mannur, *et al.,* 2019 has reported Super Annigeri 1 and improved JG 74: two *Fusarium* wilt-resistant introgression lines developed using marker-assisted backcrossing approach in chickpea (*Cicer arietinum L.*) To improve Annigeri 1 and JG 74, they introgressed a genomic region conferring resistance against FW race 4 (foc4) through marker-assisted backcrossing using WR 315 as the donor parent.

Biochemical analysis of Chickpea:

Chickpeas are one of the most widely used legumes in the world because they are a good source of dietary protein (Frias *et al.,* 2000). It is a valuable source of protein, starch, fibre, and many essential vitamins and minerals that is grown in more than 50 countries (Roy *et al.,* 2010).

Roy (2010) studied bioactive proteins and peptides in pea, chickpea, and lentil crops. Recent research has shown that protein antinutritional compounds like lectins, protease inhibitors, and the non-antinutritional component angiotensin I-converting enzyme (ACE) inhibitor may have beneficial properties, according to him. Lectins have been linked to the prevention of some cancers, the activation of innate defence mechanisms, and the management of obesity. Protease inhibitors, such as trypsin and chymotrypsin inhibitors, have been shown to minimize the risk of some cancers and have anti-inflammatory properties. Angiotensin-converting enzyme (ACE) inhibitors have been linked to a decrease in heart rate, hypertension.

Biochemical study of chickpea accessions in terms of zinc, iron, total protein, proline, and antioxidant activity was carried out by Bhagyawant *et al.,* (2015).

The aim of this analysis was to compare the biochemical properties of chickpea seeds. They investigated the biochemical properties of 20 different chickpea seed accessions in terms of total protein, proline, and antioxidant activity. Atomic absorption spectroscopy was employed to quantify zinc and iron contents. Significant variation in biochemical composition of chickpea was noted for total protein (144.59 mg/g), proline (15.89 mg/100g) and antioxidant activity (48.2%) in all the accessions that are tested.

Vijeta Singh *et al.,* 2010 suggested the involvement of proline in copper tolerance of four genotypes of *Cicer arietinum (*chickpea). The basis of differential copper tolerance in chickpea genotypes was characterized by analyzing, antioxidant enzymes (superoxide dismutase, ascorbated peroxidase and catalase), phytochelatins, copper uptake, and proline accumulation. Based on hyper accumulation of proline in tolerant genotype (RSG 44) and the reduction and improvement of lipid peroxidation and tolerance index, respectively, by proline pretreatment, we conclude that hyper accumulation of proline improves the copper tolerance in chickpea.

Saiema Rasool *et al.,*(2012) evaluate the effect of NaCl on growth and some key antioxidants in chickpea. Eight genotypes of chickpea were grown hydroponically for 15 days. Salinity showed marked changes in growth parameters (fresh and dry weight of root and shoot). The level of lipid peroxidation was measured by estimating malondialdehyde content. Lipid peroxidation increases with the increase in NaCl concentration in all genotypes but salt-tolerant genotypes (SKUA-06 and SKUA-07) were least affected as compared to other genotypes. Two genotypes of salt-tolerant and salt-sensitive varieties were analyzed further by real time PCR which revealed that the expression of SOD, APX and CAT genes were increased by NaCl in the salt-tolerant variety. The enhancement in tolerance against salt stress indicates that the genes involved in the antioxidative process are triggered by oxidative stress induced by environmental change. The results indicate that NaCl-induced oxidative stress hampers the normal functioning of the cell.

Mamta Rathore *et al.,(*2021) study twenty samples of chick pea in which the biochemical composition of this crop consists of protein was varied

from 22.12% to 24.42%, sulphur containg amino acids ranged from 0.15 to 1.25% and Tryptophan was ranged from 0.63 to 1.38% which was analyzed by NIRS-2500

Sameer S. Bhagyawant*et al.,*(2015)analyzed chickpea seeds and compare their biochemical properties. Biochemical characterization of 20 different chickpea seed accessions vis-a-vis total protein, proline and antioxidant activity was carried out. Significant variation in biochemical composition of chickpea was noted for total protein (144.59 mg/g), proline (15.89 mg/100g) and antioxidant activity (48.2%) in all the accessions that are tested. The overall results indicated that the chickpea seeds exhibit different biochemical properties.

Pradeep Kumar Patel et al., (2012) studied the effect of drought stress on hydrogen peroxide (H2O2) content, lipid peroxidation and antioxidant enzyme activities in four chickpea genotypes viz., Tyson, ICC 4958, JG 315 and DCP 92-3. Enzyme activities of Superoxide Dismutase (SOD), Peroxidase (POX), Catalase (CAT) and Ascorbate Peroxidase (APX) increased in chickpea leaves as a consequence of drought conditions and all the enzyme activities were significantly higher in drought stressed genotypes. Early drought stress (pre-anthesis drought) was found to be more damaging than the late drought stress (post-anthesis drought).

Navkiran Randhawa*et al.,*(2016)studied the influence of moisture stress in in vitro identified tolerant (GL28151, RSG963, PDG3) and sensitive (GL22044, GNG1861, PBG1) chickpea genotypes under field conditions. Osmolytes (in seeds) viz. total soluble sugars, starch, proline, cellular functions; relative water content, membrane permeability index and lipid peroxidation (in leaves), antioxidant enzymes (at pod filling stage) viz. peroxidase, catalase, superoxide dismutase, glutathione reductase were estimated in chickpea seeds under control and stressed conditions. The pronounced cellular damage, lesser alleviation in the content of osmolytes, antioxidant enzymes activity was observed in sensitive genotype GL22044 under stress treatments. High molecular weight protein bands were found either absent or of low intensity in sensitive genotypes (GL22044, GNG1861 and PBG1) under severe stress treatment (WSVFP).

Gurinder kaur *et al.,*2011 studied the role of metabolic controls governing chilling-sensitivity in chickpea, particularly involving proline explored in a set of chickpea plants (cv. GPF2) growing under warm conditions of the glass house. The studies revealed that proline application was significantly effective in reducing the impact of chilling injury on reproductive growth in chickpea. Proline application increased the level of sucrose and trehalose (cryoprotectants) in chilling-stressed plants.

Vaishali Sharma *et al.,* 2017 evaluated Six chickpea genotypes i.e. tolerant (BGD1094, ILC 3279 and L555) and sensitive (GL29095, GL12003 and GNG2171) categorized on the basis of lysimetric screening for moisture stress conditions were evaluated for physiological and biochemical studies. Proline, total soluble sugars, superoxide dismutase, peroxidase and catalase activities increased among all the genotypes but tolerant ones showed higher upheaval and under moisture stress conditions (rainfed) in contrast to sensitive genotypes. Starch content reduced correspondingly under moisture stress with maximum decline (32.36%) observed in GL12003. The accumulation of osmolytes and higher antioxidative enzymatic activity in tolerantgenotypes imparted tolerance to moisture stress in comparison to the sensitive ones.

Khan *et al.,* (2005) investigated the biochemistry of chickpea resistance to *Fusarium* oxysporum wilt disease. Complete phenols in the uninoculated roots of resistant/susceptible test lines did not correlate with wilt resistance, since the susceptible lines provided higher phenolic contents than the resistant lines. The roots of resistant chickpea lines developed antifungal compounds when they were not inoculated, while the susceptible line produced none. When compared to un-inoculated roots, inoculated roots of both resistant and susceptible lines produced higher antifungal activity.

Luo *et al.,* (2002) Antioxidants derived from the fruits *of Chrysophyllum cainito* L. (star apple). For electrophoresis, seed proteins were extracted using Ng & Bushuk's (1987) method and analysed on an SDS-polyacrylamide gel using Laemlli's (1970) method with a 10% monomer concentration. The MW markers employed were: β –lactoglobulin (18400 Da), trypsinogen (24000 Da), pepsin (37400 Da), egg albumin (45000 Da) and bovine albumin (66000 Da).

Physical and biochemical variations in the composition of Algerian leguminous crop plants, Amir *et al.,* (2007) Using the Folin–Ciocalteu reagent, total soluble phenolic content was measured spectrophotometrically at 760 nm (Waterman and Mole, 1994). The content of chlorophyll and carotenoids in leaves was determined using the Hiscox and Israelstam (1979) using dimethyl sulfoxide (DMSO).

Yousef Sohrabi & Gholamreza Heidari & Weria Weisany &Kazem Ghasemi Golezani & Khosro Mohammad(2012 investigated the effects of *Glomus* species on some physiological characteristics of two chickpea types(Pirouz cultivar of Desi type and ILC-482 of Kabuli type)under non-stress (NS) and drought stress. Leaf chlorophyll content was decreased, but leaf proline content and guaiacol peroxidases (POD), catalase (CAT), and ascorbate peroxidase activities were increased as a result of drought stress. The reaction of chickpea cultivars to inoculation by AM species and irrigation levels were different. ILC-
482 showed thatantioxidant enzymes activities were more and thus less MDA compared with Pirouz cultivar.

Sethy *et al.,* (2006) used 25 STMS primer for detecting genetic diversity within 36 chickpea accession. A total of 159 alleles were produced from 25 loci with an average of 6.4 alleles and 4.6 effective alleles per locus. Maximum number of 11 alleles were detected for primer pair NCPGR90 while minimum of 2 alleles were detected for NCPGR86 with the allele varying in size from 131 to 314 bp and found high heterozygocity among the materials.

Varshney *et al.,* (2007) observed that progress towards development of a reasonable number of molecular markers has been very slow in cultivated species of chickpea. One of the main reason for this may be attributed to the low genetic diversity present in the cultivated gene pool of these species.

Bayraktar *et al.,* (2008) indicated that the low genetic difference and high gene flow among populations had a significant effect on the emergence and evolutionary development of *F. oxysporum fsp. ciceris.*

Chaudhary *et al.,* (2009) studied genetic diversity analysis in 30 chickpea accessions and revealed 10 markers to be polymorphic producing a total of 29

alleles and observed heterozygosity average of 0.16 there by exhibiting low level of intra specific polymorphism.

Bharadwaj *et al.,* (2010) found that out of 55 STMS primers pair, 35 generated polymorphism with an average 2.49 amplicons/ primer pair polymorphic information content ranged from 0.48 to 1 and genetic similarity between cultivars ranged from 0.10 to 0.77.

Joshi *et al.,* (2010) describe DNA isolation procedure for chickpea which is rapid and less expensive without involving ultra-centrifugation or column purification steps. The yield of DNA ranged from 0.595 to 5.55 µg/ml and purity ratio was between

1.025 – 2.01, indicating minimum level of contaminating metabolites.

Ahmad *et al.,* (2012) assessed the genetic diversity of 70 accessions of chickpea. For molecular characterization of germplasm 5 PCR based RAPD primers and 3 SSR primers viz., CaSTMS2, CaSTMS15 and CaSTMS21 scored the genetic variability up to 55% by cluster analysis through UPGMA percent disagreement. The primers, TA72 and TA130 were linked with yield related traits, indicated highest dissimilarity index value (0.69) and notable variation in the identified promising lines.

Rizvi *et al.,* (2014) used 51 Sequence tagged microsatellite sites (STMS) primer pairs were employed to assess the genetic diversity and relationships with morphological characters. A total of 32 out of 51 STMS primers were found polymorphic and a total of 121 alleles were generated out of which 102 (83 %) were detected for the 32 polymorphic STMS markers with an average of 2.22 alleles per locus.. Based on the STMS marker analysis by considering the parameters of PIC value (≥0.55), gene diversity (≥0.62) and polymorphic alleles (≥4), six highly polymorphic STMS loci GA11, TA76S, TA89, TS29, TS43 and TS71 were observed which can effectively be used in further molecular studies.

Bagde *et al.,* (2018) conducted biochemical studies on the parents Vijay and ICC 506 EB, as well as the chickpea variety JG-62, to determine its susceptibility to *Fusarium oxysporum* wilt.

Ashraf *et al.,* (2018) profiled two contrasting chickpea genotypes in disease and immune state. The discovery of interaction coordinators that lead to pathway determination regulating different (disease or immune) phenotypes was made possible by an integrative gene-regulatory network. They examined the typical and discrete features of the chickpea gene network during host specific resistance in response to *Fusarium* wilt using functional network analyses based on cDNA microarray temporal datasets consisting of 6072 spots representing 1749 unigenes. The aim was to establish a signal transduction catalogue for chickpea defence and/or disease signalling, as well as a snapshot of transcription. With the above status of knowledge pertains to chickpea for *Fusarium* wilt the present investigation are directed towards enlisted objectives. If is envisaged that it will help in furtherance of knowledge and extend information on Screening of Chickpea (*Cicer arietinum L.*) genotypes using gene based markers for *Fusarium* Wilt Disease and study on important Qualitative and Quantitative characters.

CHAPTER III

MATERIAL AND METHODS

The present investigation entitled Screening of Chickpea (*Cicer arietinum*L.) genotypes using gene based markers for *Fusarium* Wilt Disease and study on important Qualitative and Quantitative characters field work was carried out during Rabi season 2020-21at Research Farm, College of Agriculture, R.V.S.K.V.V. Gwalior (M.P.) and molecular work was carried out at Biotechnology Lab, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, R.V.S.K.V.V. Gwalior.

3.1 Field Experimental site and climatic conditions

The experiment was carried at Research Farm, College of Agriculture, R.V.S.K.V.V. Gwalior (Madhya Pradesh). The experimental area occupied was quite uniform in respect of topography and fertility. Gwalior is situated at 220 43' N Latitude and 760 54' E longitudes and altitude 618m at above the mean sea level. This region has subtropical, semi- arid climate with hot and dry summers and cold winters with occasional showers. The average rainfall is about 23. Mm (Oct to Dec) and 27 mm (Jan- March). The experiment consisted of 40genotypes grown in Randomized Block Design with row to row distance of 30 cm, in two replications during Rabi 2020-19. Fertilizer was applied in the ratio of 20 N: 40 P2O5: 20 K2O kg/ha. Observations were recorded as per the DUS guidelines for chickpea.

3.2 Plant material

Plant material consisted of 40 chickpea genotypes (mentioned in table 3.2) that were received from RAK Krishi College, Sehore Madhya Pradesh.

3.3 Observational procedure:

Five competitive plants were selected randomly from each plot and tagged for recording the observations the following observations were recorded on the five selected plants in each plot.

Table 3.1: Detail of experimental material used for present investigation

Season	Rabi 2020-21
Experimental Design	Randomized Block Design
Number of genotypes	40
Replications	2
Rows per plot	3
Row length	4.0m
Plot size	4.0m X 0.6m
Distance (R-R / P-P)	30 cm x 10 cm
Date of sowing	06 December 2020

3.4 Observations recorded from field material

As per objectives of research title observations were recorded on single plant basis on five randomly selected plants from each plot.

1. **Days to maturity:** Number of days taken from date of sowing to physiological maturity of the plant was recorded as days to maturity.
2. **Days to 50% flowering:** Number of days taken from the date of sowing to the day on which 50% of the plants in a genotype initiate first flower was recorded as days to flowering
3. **100 seed weight**: Hundred seed were counted from the seed lot and weighted in gram**.**
4. **Seed yield per plant (g):** Each plant was harvested from ground level and its seeds were weighted in gram after sun drying.
5. **Biological yield per plant (g):** Each plant was harvested from ground level and weighted in gram after sun drying.
6. **Harvest index (%):** Harvest index was obtained by dividing the total seed yield per plant by biological yield per plant and expressed in percentage.

$$\frac{\text{Seeedyield per plant (g)}}{\text{Biologicalyieldperplant(g)}} \times 100$$

Fig. 3.1: Field view of Chickpea genotypes.

Fig.3.2: Inspection team field visit and symptoms of *Fusarium* wilt in chickpea plant.

S.NO.	Name of genotype	S.NO.	Name of genotype
1	RVSVT PS-2019- 201	21	RVSVTK-2019-106
2	RVSVT PS-2019- 202	22	RVSVTK-2019-107
3	RVSVT PS-2019- 203	23	RVSVTK-2019-108
4	RVSVT PS-2019- 204	24	RVSVTK-2019-109
5	RVSVT PS-2019- 205	25	RVSVTK-2019-110
6	RVSVT PS-2019- 206	26	RVSVTD-2019-1
7	RVSVT PS-2019- 207	27	RVSVTD-2019-2
8	RVSVT PS-2019- 208	28	RVSVTD-2019-3
9	RVSVT PS-2019- 209	29	RVSVTD-2019-4
10	RVSVT PS-2019- 210	30	RVSVTD-2019-5
11	RVSVT PS-2019- 211	31	RVSVTD-2019-6
12	RVSVT PS-2019- 212	32	RVSVTD-2019-7
13	RVSVT PS-2019- 213	33	RVSVTD-2019-8
14	RVSVT PS-2019- 214	34	RVSVTD-2019-9
15	RVSVT PS-2019- 215	35	RVSVTD-2019-10
16	RVSVTK-2019-101	36	RVSVTD-2019-11
17	RVSVTK-2019-102	37	RVSVTD-2019-12
18	RVSVTK-2019-103	38	RVSSG91-13
19	RVSVTK-2019-104	39	RVSSG96-14
20	RVSVTK-2019-105	40	RVG-203

Table 3.2:List of chickpea varieties used in the study.

3.5 Statistical analysis

3.5.1 Analysis of variance:

The data on yield and its contributing traits were statistically analyzed on the basis of model described by Cochran and Cox (1950) for randomized block design. In order to test the significance of treatments Critical difference was computed (Fisher and Yates, 1963).

$Y_{ij} = \mu + t_i + b_j + e_{ij}$

where,

Y_{ij}= value of the variate for the ith treatment in the jth block

(i=1, 2, 3,……, t; j=1, 2, 3, ... , b)

μ = General mean effect

b_j = effect due to j^{th} block

t_i = effect due to i^{th} treatment

eij = Random errors which is assumed to be independently and normally distributed with mean zero and variance e^2 .

Table 3.3:ANOVA table for RBD

Source	Degree of freedom	Mean sum of squares	Variance ratio
Replication	(r-1)	M_r	M_r/M_e
Treatment	(t-1)	M_t	M_t/M_e
Error	(r-1) (t-1)	M_e	-
Total	rt-1	-	-

where, r = Number of replications, t = Number of treatments

3.6 Estimation of heritability and genetic advance:

Heritability in per cent in broad sense was estimated by the following formula given by Singh and Choudhary (1977):

$$\text{Heritability } (h^2) = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} \times 100$$

Heritability values are catagorised as low, moderate and high (Robinson *et al.,* 1949) and are given below,

0-30% - Low

30-60% - Moderate

60% and above - High

The estimates of expected genetic advance from selection, G(s), was obtained by the formula suggested by Robinson *et al.,*(1949).

$G(s) = k \times h^2 \times \sigma p$

where,

k = Selection differential in standard deviation units which is 2.06 for 5% selection intensity,

h^2 = Heritability in broad sense, and

σ_p = Phenotypic standard deviation

Genetic advance was expressed as percentage of mean by using the formula suggested by Johnson *et al.,* (1955).

$$\text{Genetic advance as percentage of mean} = \frac{\text{Genetic advance}}{\text{Grand mean}} \times 100$$

Genetic advance as percent of mean was classified as low, moderate and high (Johnson et al., 1955) and values are given below:

0-10% - Low

10-20% - Moderate

20% and above - High

3.7 Estimation of phenotypic and genotypic coefficients of variation:

The phenotypic and genotypic coefficients of variation in per cent were computed by the following formulae given by Burton (1952).

Phenotypic Coefficient of Variation (PCV) = $\frac{\text{Phenotypic standard deviation}}{\text{Mean}} \times 100$

Genotypic Coefficient of Variation (GCV) = $\frac{\text{Genotypic standard deviation}}{\text{Mean}} \times 100$

The PCV and GCV values are ranked as low, medium and high (Shivasubramanian andMenon, 1973) and are mentioned below:

0-10% - Low

10-20% - Moderate

>20% - High

3.8 Marker validation and screening of Chickpea genotypes for *Fusarium* wilt

Leaf samples were collected from one month old seedling of chickpea genotypes from experimental field. Usually fresh and younger leaves were used for DNA extraction and further molecular marker analysis. The collected samples were placed in cooling pads to transfer and then stored at -80^{0} C deep freezer.

3.9 Isolation of genomic DNA:

Genomic DNA isolation of chickpea genotypes was done by modified CTAB methods (Murray and Thompson 1980, Tiwari *et al.,* 2017). DNA extraction buffer composition used for the study is given below (Table 3.4)

3.10 Polymerase Chain Reaction for markers analysis

3.10.1. Equipment's: PCR machine (Biored-T100 & Hi-Media-Prima-96), PCR Plate, PCR sealing film (Optical sealing film).

3.10.2. Chemicals: Agarose, Chilled ethanol, DNA extraction buffer (Table 3.2), Ethidium Bromide, Ladder (50 &100bp), Loading dye (6x), Master mix, PCI (Phenol: Chloroform: Isoamyl alcohol, 25:24:1), Sodium acetate, TAE buffer (50X), TBE buffer (10X), TE buffer (1x).

Table 3.4: DNA extraction buffer composition used for the study are given below

Chemical	Concentration	Volume (ml)
1 M Tris-CL (pH 8.0)	100mM	5.0
0.5 M EDTA (pH 8.0)	20mM	2.0
4 M NaCl	1.4M	17.5
10% CTAB	2% (w/v)	10.0
B-mercaptoethanol	0.2% (W/v)	0.2
Milli-Q water	-	15.3
Total	-	50

3.10.3. Details of gene specific primers: Total 15 gene based molecular markers reported earlier were used for validations and screening of chickpea genotypes for *Fusarium* wilt. The markers included 2 Randomly Amplified Polymorphic DNA (RAPD), 1 Sequence Characterized Amplified Region (SCAR), 1 DNA Amplification Fingerprinting (DAF), 6 Sequence Tagged Microsatellites (STMS) and 5 Inter Simple Sequence Repeats (ISSR) (Table 3.5)

Table 3.5: Details of primers used for screening of *Fusarium* wilt in chickpea genotypes

Primer Name	Nam Of Marker	Primer sequence		Reference
CS27	RAPD	AGT GGT CGC G		Mayer *et al.*,1997
CS-27A	SCAR	F: ACC TGG TCG CGG GTC AGA GGA AGA	R: AGT GGT CGC GAT GGG GCC ATG GTG	Mayer *et al.*,1997
OP-U17-1	DAF	ACC TGG GGA G		Benko-Iseppon *et al.*,2003
UBC-825	ISSR	ACA CAC ACA CAC ACT		Ratnaparkhe *et al.*,1998
UBC-170	RAPD	ATC TCT CCT G		Tullu *et al.*,1998
TA-59	STMS	F: ATC TAA AGA GAA ATC AAA ATT GTC GAA	R: GCA AAT GTGAAG CAT GTA TAG ATA AAG	Winter *et al.*,1999
TA-96	STMS	F: TGT TTT GGA GAA GAG TGA TTC	R: TGT GCA TGC AAA TTC TTA CT	Winter *et al.*,1999
TR- 19	STMS	F: TCA GTA TCA CGT GTA ATT CGT	R: CAT GAA CAT CAA GTT CTC CA	Winter *et al.*,1999
TA194	STMS	F:TTTTTGGCTTATT AGACTGAC TT	R:TTGCCATAAAA TACAAAATCC	Winter *et al.*,1999
TR29	STMS	F:GCCCACTGAAA AATAAAAAG	R:ATTTGAACCTC A AGTTCTCG	Winter *et al.*,1999
TR31	STMS	F:CTTAATCGCACA TTTACTCTAAA ATCA	R:ATCCATTAAAA CA CGGTTACCTATA A	Winter *et al.*,1999
UBC-811	ISSR	GAGAGAGAGAGAGAGAC		(Ratnaparkhe *et al.*, 1998b).
UBC -841	ISSR	GACACGACACGACACGACAC		(Ratnaparkhe *et al.*, 1998b).
UBC-864	ISSR	ATGATGATGATGATGATG		(Ratnaparkhe *et al.*, 1998b).

3.11 Methods:

3.11.1 Genomic DNA Isolation Principle:

To isolate nuclear DNA, pure and intact nuclei were isolated from fresh plant material. The primary requirement for DNA isolation is to extract DNA from cells in solution. Physical grinding results in physical breakage of cells. Subsequently, addition of extraction buffer cetyl-trimethyl ammonium bromide (CTAB) assists breakage of cell and nuclear membrane made up of lipids, which aids in cell lysis and all the components of cell are dispersed in the buffer. Tris-HCl in the buffer ensures that the pH of the solution is maintained around 8. EDTA forms complexes (chelates) with several kinds of metal ions. Divalent metal cations, such as Mg2+ and Ca2+ are the required cofactor by the majority of DNase. The DNA being extracted is protected from DNase degradation since the enzyme cannot utilize the complexed Mg2+. NaCl being a salt, increases solubility of DNA in the buffer solution and also increases the osmotonicity of the buffer and hence facilitates the process of cell lysis. Phenol Chloroform or chloroform extractions remove proteins by denaturing them and they aggregate in the intermittent phase along with cell debris. For DNA precipitation, isopropanol (0:6 to 1 volume) or ethanol (2 volumes) is used, The RNA is removed by RNase treatment, after precipitation, the pellet was washed with 70% ethanol for removing any salts retained after precipitation.

3.12 Reagents for plant total genomic DNA isolation

0.5 M EDTA (pH 8.0) 186.1 g of sodium salt of EDTA was dissolved in 800 ml of MQ water, pH was adjusted to 8.0 with NaOH pellets. The final volume was adjusted to one liter with MQ water and sterilized by autoclaving.

4 M NaCl 233.8 g of NaCl was dissolved in 800 ml of MQ water. The final volume was adjusted to one liter with MQ water and sterilized by autoclaving.

1M Tris-Cl (pH 8.0) 121.1 g of Tris-Cl salt was dissolved in 800 ml of sterile MQ water. pH was adjusted to 8.0 with concentrated 1N HCl. The final volume was adjusted to one liter with MQ water and sterilized by autoclaving.

10% CTAB 100 gm of CTAB powder was dissolved in sterile MQ water and the volume was adjusted to one liter.

Phenol : Chloroform : Isoamyl alcohol Buffer saturated phenol, chloroform and isoamyl alcohol were mixed in the ratio of 25: 24: 1. The equilibrated mixture was stored under a layer of 0.01 M Tris-HCl (pH 7.6) at 4^{0}C in dark glass bottle.

3.13 Genomic DNA isolation Using Modified CTAB method:

The leaf tissue of chickpea was collected for DNA isolation from either 7-10 day glasshouse grown seedlings or 3-4 week old plants from field. It is always better to collect the samples in morning hours to avoid more impurities. Protocol of Murray & Thompson (1980) with minor modification was used for DNA isolation. Detail protocol is given below:

➢ Pre-warmed (65° C) CTAB extraction buffer was made and freshly 0.2% β mercaptoethanol was added.

➢ Pipette out 700µl of CTAB pre-mixed buffer to the fresh young leaf samples (2-3 leaves), crushed in mortar pestle with DNA Extraction Buffer, transferred in 2.0 ml Eppendorf tubes, mix the content gently and incubate at 65° C for 45-60 min. with occasional mixing by gentle swirling (Liquid Nitrogen or DNA Extraction Buffer can be used for crushing)

➢ Allow the tubes to cool to room temperature and emulsify the mixture with an equal volume (700µl) of Phenol: Chloroform: Isoamyl alcohol (25: 24: 1) for 1-2 min by gentle inversion and centrifuge at 10,000 rpm for 10 min. at room temperature.

➢ Transfer the separate aqueous phase from the contents to fresh clean 2 ml tube

➢ Add 3.5 µl of RNaseA (20 mg/ml) and mix the contents and incubate at 37° C for 30-45 mins.

➢ Add 1/10th volume of 3M Sodium Acetate (pH 5.2) and 2 volume of absolute ethanol to the aqueous solution, mix by quick gentle inversion for five to six times and incubate at -20° C for 30-40 min. (or can leave for overnight at 4° C)

➢ Pellet the DNA by centrifugation of deep well plate at 10,000 rpm for 10 min and discard the supernatant.

➢ Wash the DNA pellet with 500 µl of 70% ethanol by centrifugation at 10,000 rpm for 5 min, air dry properly till ethanol smell goes and dissolve finally in TE (100 mMTris and 50 mM EDTA, pH 8.0) buffer (100-200 µl) or Nuclease free water (NFW) based on DNA pellet size and store at 4 C for complete dissolution. Note: If Liquid nitrogen is not available crushing of leaf tissue can be done in DNA Extraction Buffer. All other steps after crushing would remain same.

Major contaminants of crude DNA preparation are RNA, protein and polysaccharides. Inclusion of CTAB in DNA extraction buffer helps in elimination of polysaccharides to a large extent. The RNA is removed by treating the sample with DNase free RNase (10 mg/ml) denatured at 70^0C. Proteins including RNase can be removed by treatment with Phenol: Chloroform: Isoamylalcohol (25:24:1).

3.14 Quantification of DNA

Quality of DNA was determined by horizontal submarine gel electrophoresis on 0 8% agarose gel. Purity of DNA was checked by taking the ratio of optical density (OD) using spectrophotometer, at 260 nm to that of 280 nm. The samples with OD ratio (260nm/280nm) between 1.7-1.9 were used in subsequent experiments. DNA samples showing the values beyond this range were re- purified. Isolated DNA was quantified in UV spectrophotometer at 260 and 280nm. Concentrated 50 ng/ml solution of double stranded DNA showed absorbance of 1 at 260nm. DNA concentration of sample was calculated as; OD 260 x 50 µg DNA/ ml x D.F /1000 the quantity, quality and integrity of isolated DNA were also checked by gel electrophoresis. 2 µl of DNA sample was isolated from each line and electrophoresis was done along with the λ Hind-III standard DNA in 0.8% gel concentration at 60 volts for 90 min. The DNA was stained by ethidium bromide and observed under UV-Transilluminator. The amount of fluorescence was proportional to the total mass of DNA. After quantification, the DNA was diluted by distilled water. The final concentration of DNA was ~25 ng/µl. Dilution of DNA for PCR reaction the quantified DNAwas diluted

according to the DNA quantify in each sample for PCR amplification upto 25-40 ng/ul in Nuclease Free water.

Dilutions were carried out according to the following formula:

$$\text{Dilution} = \frac{\text{Required concentration of DNA (ng/µl)}}{\text{Available concentration of DNA (ng/µl)}} \times \text{Total Volume required (µl)}$$

3.15 Agarose gel electrophoresis:

The amplified PCR products of different RAPD, DAF, STMS, ISSR, SCAR markers were subsequently resolved on 1.5, 2, and 3 % (W/V) agarose gel (Fig 3.3). Agarose was boiled to dissolve in 1X TBE buffer in a microwave oven. It was cooled to about 50-600C by swirling the flask and added ethidium bromide solution (10 mg/ml), poured on the gel plate fixed in the gel caster. Immediately, combs were inserted in the gel to create wells for loading the PCR product. Gel was allowed to solidify for about an hour, combs were removed carefully and gel was kept submerged in an electrophoresis buffer tank containing 1X TBE buffer. To each PCR product, 1 µl of 6X gel loading dye was added, mixed well and loaded in the wells of the gel. 100-bp DNA ladder (MBI Fermentas, Germany) was used as molecular weight marker. The gel was run for at least 3 h at 80 volts using power pack 1000 (BIO-RAD, USA) and visualized under UV trans- illuminator in a gel documentation system (Zeneth System).

3.16 PCR amplification and thermal profiling:

Genetic diversity analysis was performed using a total of 15 Gene based primers. These markers were assayed for validation of marker and screening of 44 Chickpea genotypes. The primers were synthesized by Eurofins Genomics India Pvt Ltd. Polymerase chain reaction was performed in 10µl reaction mixture comprising of 1X PCR buffer, 0.1 U Taq DNA polymerase, 1 µl dNTP (1 mM), 0.5 µl of forward and reverse primers each 10 pM and 50 ng/µl of genomic. PCR thermal cycles were standardized during validation of markers. All the thermal cycles were similar except the annealing temperature (Table 3.6). Annealing temperature of ISSR markers was 55' C, STMS 54' C and RAPD, DAF, SCAR was 30' C. PCR amplified products of RAPD, SCAR, DAF and ISSR primers were separated on 1.5 per cent whereas PCR amplified product of STMS

primers was separated on 3.0 per cent agarose gel containing 0.5 µg/ml Et-Br at 100 V with standard DNA marker. The separated bands were visualized under UV light and photographed under Bio-Rad Gel documentation system.

Table 3.6: PCR thermal cycles used for amplification of primers

Steps	Temperature	Duration	Cycle	Activity
1	94^{0} C	2 Minute	1	Denaturation
2	92^{0} C	0.45 sec		Denaturation
3	30^{0} C to 55^{0} C	0.50		Annealing
4	72^{0} C	0.50	35	Elongation
5	72^{0} C	5.00	1	Final elongation
6	12 ^{0}C	Infinite		Storage

3.17 Marker analysis:

The genetic profile of 40 Chickpea genotypes was scored on the basis of difference in allele size using 14 Different types markers 2 RAPD, 1 SCAR, 1DAF, **5** ISSR and 5 STMS markers. The major allele frequency, polymorphism information content (PIC) and genetic distance based clustering was performed with Unweighted Pair Group Method for Arithmetic average (UPGMA) tree using Power Marker v3.25 software and the dendrogram was constructed using MEGA 4.0 software (Tamura, Dudley, Nei, and Kumar 2007). STMS data was again

subjected to cluster analysis followed by bootstrap analysis with 1000 permutations for all the genotypes using Mega 4.0 software.

3.18 Estimation of Biochemical parameters of chickpea genotypes

Proline will be estimated as per method given by Bates *etal.,* (1973), Lipid peroxidation by the method given by Hodges *et al.,(*1999), Superoxide Dismutase(SOD) as per method by W.F. Beyer, Y. Fridovich and total sugar was calculated as per method given by Dubois *et al.,* (1956).

3.18.1 Total Sugar Estimation

1. 25mg fresh seed sample of each genotype were collected.
2. Crush the taken sample in 1ml (80% ethanol) in mortar pestle until the leaf completely disappear and makes fine liquid solution.
3. Pour the solution (seed +ethanol) in the 15ml falcon tube and centrifuge at 1000rpm for 10 minutes.
4. Transfer the supernatant in the falcon. Dry the supernatant in glass bottle at 65ˡ C until it gets dried.
5. After drying add 1ml distil water and leave it until it gets dissolved.
6. Heat the falcon tube at 100ˡ C for 30 minutes and cool until comes at room temperature and then take the reading in spectrophotometer.

3.18.2 Proline Estimation

1. 25mg fresh seed sample of each genotype were collected.
2. Crush the seed sample in 1ml (3% homogenized sulpho salicylic acid) solution in mortar pestle very finely.
3. Centrifuge at 1000rpm for 10 minutes and take the supernatant (used as proline content) in 15ml falcon tube.
4. Then add 500µl Ninhydrin acid and 500µl Glacial acetic acid.
5. Heat at 100ˡ C for 60minutes in water bath and terminate at -80ˡ C for 2 minutes.
6. Then add 750 µl toluene and vortex for 1 minute. A pink layer should come after adding, then take the upper layer for absorbance at 520nm in spectrophotometer reading.

3.18.3 Lipid Peroxidation Estimation

1. 25mg fresh seed sample of each genotype were taken and were crushed in mortar pestle very finely.

2. Add 500 µl (0.1%) TCA(TriChloroAcetic acid) and vortex for 5 minutes and centrifuge at 10,000rpm for 10 minutes.

3. Take 100µl of the supernatant in an eppendrof tube and add 200µlof 0.5% TBA.

4. Heat the reaction mixture for 95 C for 30 minutes terminate at -80 C for 2 minutes.

5. After two minutes let it come at room temperature and centrifuge at 10000rpm for 10 mins. and take the supernatant to take absorption at 532nm.

3.18.4 Superoxide Dismutase (SOD)Estimation

1. 25mg fresh seed sample of each genotype were collected.
2. Crush the seed sample in mortar pestle very finely.
3. Add 250µl (0.1%) TCA (TriChloroAcetic acid) and vortex for 5 minutes and centrifuge at 10,000rpm for 20 minutes.
4. Take 160µl supernatant in an eppendrof and add 160µl of Phosphate buffer.
5. Then add 680 µl (1M) potassium iodide and then keep the tube in dark for 1 hour.
6. Later, take the reading at 390nm absorbance in spectrophotometer.

CHAPTER IV

RESULT

During Rabi 2020-21, a field experiment with 40 chickpea advance genotypes was set up in a randomised block pattern with all recommended agronomic procedures entitled as Screening of Chickpea (*Cicer arietinum* L.) genotypes using gene based markers for *Fusarium* wilt disease and study on important qualitative and quantitative characters. The results of the present investigation are presented under the following headings.

- Analysis of variance
- Population mean and Range
- Phenotypic and genotypic coefficients of variation
- Genetic variability, Heritability (broad sense) and genetic advance

4.1 Analysis of Variance

Table 4.1 shows the results of an analysis of variance experiment using a randomised block design. For all yield-attributing characteristics, such as Days to 50% blossoming, Days to maturity, Seed yield per plant, 100 seed weight, Biological yield, and Harvest index, the mean of squares due to genotypes was very significant (p=0.01).Table 4.2 shows the range, mean, phenotypic and genotypic coefficients of variation, heritability estimates, and anticipated genetic progress as a percentage of the mean for the characters investigated.

4.2 Population mean and range

Table 4.2 shows the values of range and estimations of means for various characters. The range values indicated a lot of variation between genotypes for each character.

Days to 50% flowering ranged from 68 days for RVSVT-PS-2019-209 and 77.5 days for RVSVT-PS-2019-208,RVSVTD-2019-3 and RVSVTD-2019-11with a mean value of 74.02 days. Days to maturity ranged from 115.50 days (RVSVT-PS-2019-202) to 126.50 days (RVSVTD-2019-4). The mean value for the trait was 120.75 days. The seed yield per plant ranged from 8.09 g (RVSVTK-2019-

109) to 35.19 g (RVSVTK-2019-108) with a mean value of 18.73 g.The 100 seed weight ranged from 17.00 g for RVSVTK-2019-109 to 39.5 g for RVSVT PS- 2019-215 with a mean value of 29.88 g. Biological yield ranged from 17.27 g(RVSVTK-2019-109) to 63.49g (RVSVT-PS-2019-215). The average value for the trait was 37.90 g. Harvest index ranged from 41.82% (RVSVTK-2019-112) to 64.82 % (RVSVT-PS-2019-206). The average value for the trait was 49.12%.

4.3 Phenotypic and genotypic coefficient of variation

The value of phenotypic and genotypic coefficients of variation, heritability, and genetic progress for distinct characteristics were calculated and provided in Table 4.2 to determine the extent to which the observed variation was attributable to genetic causes.

High Phenotypic Coefficient of Variation PCV% (>20) was observed for characters like seed yield per plant (gm) and biological yield (gm).The moderate Phenotypic Coefficient of Variation (10-20 percent) was observed for trait 100 seed weight while low phenotypic coefficient of variation (<10 percent) was recorded for days to 50% flowering, days to maturity and harvest index.

High Genotypic Coefficient of Variation GCV% (>20) was observed for seed yield per plant(gm) and Biological yield (gm). Moderate Genotypic Coefficient of Variation (10-20 percent) was observed for 100 seed weight and low Genotypic Coefficient of Variation (<10 percent) was recorded days to 50% flowering, days to maturity and harvest index.

4.4 Genetic variability, Heritability (broad sense) and genetic advance:

Table 4.2 shows the estimates of heritability in a broad sense. The magnitude of heritability estimated was high for days to maturity (93.2%) followed by, Days to 50% flowering seed yield per plant, 100 seed weight, biological yield and harvest index (90.8%, 96.8%,97.5%,96.3%,67.9% respectively).

The genetic advance as a percentage of the mean for all characteristics was investigated using Johnson *et al.,* (1955) categorization, and the estimated results are shown in Table 4.2.

Characters with a high genetic advance (>20%) as a percentage of the mean seed yield per plant (gm 62.035), 100 seed weight (33.764), biological yield (51.884). Moderate genetic advance level of genetic advance as per cent of mean is observed in Harvest index (%). The traits that recorded low (<10 percent) genetic advance as per cent of mean were days to 50% flowering (6.389) and days to maturity (4.406).

Table 4.1: Analysis of Variance for Six Yield and Yield Contributing Quantitative Characters in Chickpea

S.NO.	Character	Mean sum of squares		
		Replication	Genotype	Error
1	Days to 50% flowering	22.049**	12.81**	1.178*
2	Days to maturity	31.25**	15.35**	1.04*
3	Seed yield per plant (gm)	5.51*	50.52**	1.28*
4	100 seed weight	191.36**	196.60**	7.28*
5	Biological yield (gm)	0.716	38.67**	12.42**
6	Harvest index(%)	57.08**	67.92**	2.16*

* Significant at p=0.05 ** Significant at p=0.01

Table 4.2:Estimation of range, mean and different genetic parameters for different characters in 40 genotypes of chickpea

S.No	Characters	Mean	Range	PCV (%)	GCV (%)	Broad Sense Heritability	Genetic Advance	Genetic Advance As A Percent Of Mean
1	**Days to 50% flowering**	74.02	68.00-77.00	3.420	3.259	90.8%	4.736	6.398
2	**Days to maturity**	120.75	115.50-126.50	2.295	2.216	93.2%	5.320	4.406
3	**Seed yield per plant (gm)**	18.73	8.09-35.19	31.107	30.606	96.8%	11.622	62.035
4	**100 seed weight**	29.88	17.00-39.5	16.817	16.602	97.5%	10.091	33.764
5	**Biological yield (gm)**	37.90	17.27-63.49	26.15	25.667	96.3%	19.667	51.884
6	**Harvest index(%)**	49.12	41.82-64.82	8.952	7.37	67.9%	6.149	12.519

4.5 CORRELATON COEFFICIENT ANALYSIS

Days to maturity is significant and positively correlated with days to 50% flowering (r=0.703) at 1% level significance. Seed yield per plant is significant and positively correlated with Biological yield (r =0.808) at 1% level of significance. Seed yield per plant is significant and positively correlated with Harvest index (r = 0.620) at 1% level of significance(Table 4.3)

Table4.3.Correlation coefficient among morphological traits of Chickpea genotypes

	DF	DM	HSW	BYD	HI	GYD
DF	1	0.703**	0.158	-0.113	0.214	0.049
DM		1	0.122	-0.039	0.264	0.170
HSW			1	0.241	0.093	0.277
BYD				1	0.266	0.808**
HI					1	0.620**
GYD						1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

DF= Days to 50% flowering, DM= days to maturity, HSW= Hundred seed weight, BYD= biological yield, GYD= Seed yield per plant.

4.6 Cluster Analysis of Morphological Traits

Dendrogram showed two major groups A and B consisting 5 and 35 genotypes respectively. Cluster A can be further divided into sub groups A1 and A2 (2) and (3) respectively. B can be sub grouped into B1 and B2 consisting (1) and (34). Then B2 can be further grouped into B2' and B2' (12) and (22) respectively. B2'can be divided into B2# and B2## Consisting (5) and (7) genotypes respectively. B2'can be further divided into two sub groups B2^ and B2^^ consisting (1) and (21) genotypes respectively. Cluster diagram and 3-dimensional plot analysis was done using NTSYS software which indicates that closely related genotypes based on yield attributing morphology traits are grouping together.

Fig. 4.1: Cluster diagram showing the characterization of chickpea genotypes based onmorphological data.

DM

Fig.4.2 & Fig.4.3: 3-dimensional and 2- dimensional plot showing the characterization and association of chickpea genotypes based on Days to maturity and seed yield per plant.

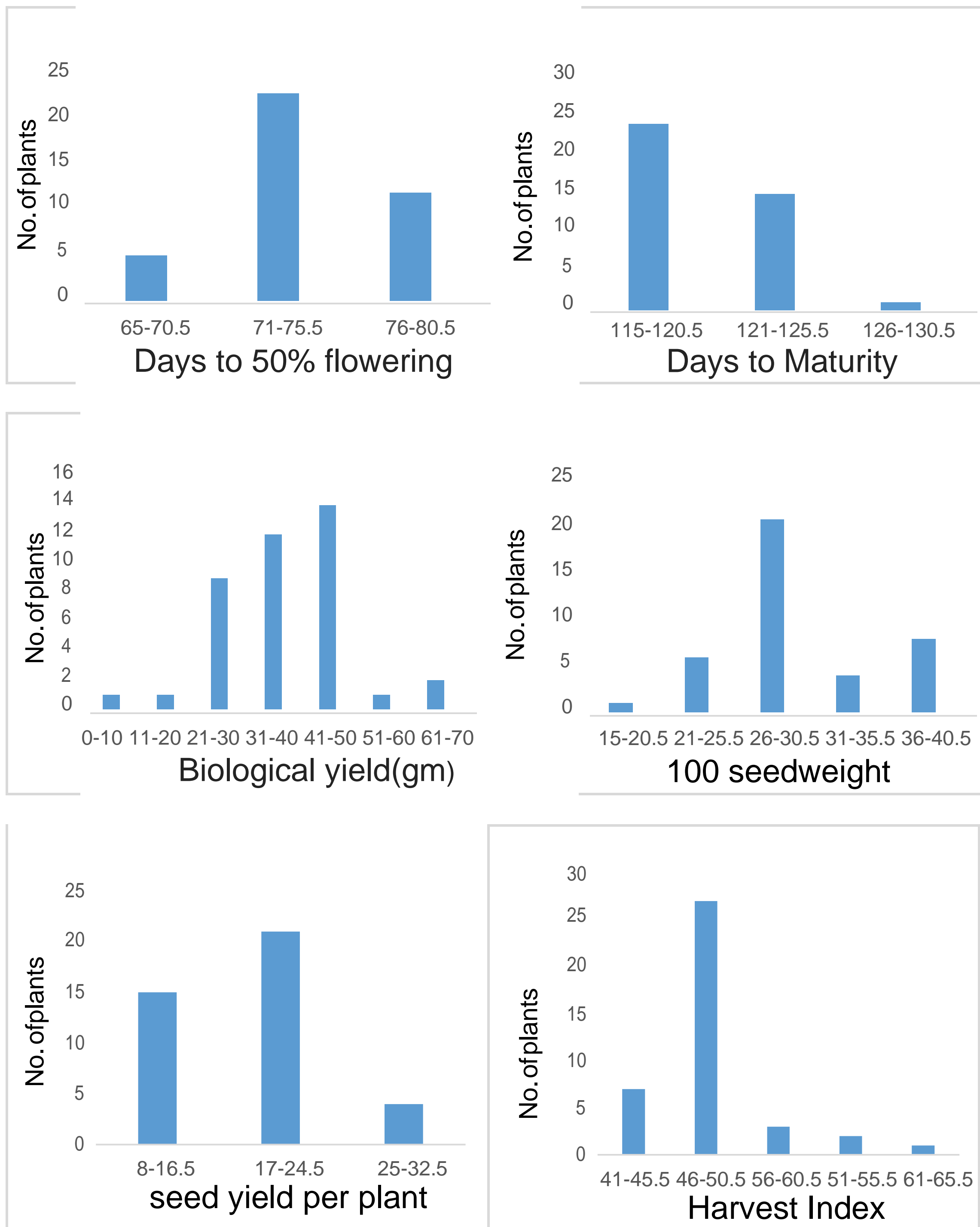


Fig.4.4: Bar diagram of important morphological traits recorded in 40 Chickpea genotypes

4.7 Statistical Analysis of Biochemical traits of chickpea genotypes

All the forty genotypes of chickpea were analysed for various biochemical parameters like Total sugar, Proline, Superoxide Dismutase, Lipid Peroxidation (Table 4.4).The Total sugar ranged from 16.8 mg/g (RVSVT PS-2019- 201) to 24.9 mg/g (RVSVT PS-2019- 214) with a mean value of 20.36mg/g, Proline varied from 1.4 µmol/g (RVSVTK-2019- 104) to 2.91 µmol/g (RVSVT PS-2019- 213)with a mean value of 2.16 µmol/g. Superoxide Dismutase (SOD) ranged from 8.2 nmol/g (RVSVT PS-2019- 211) to 20.1nmol/g (RVSVT PS-2019- 205) with a mean value 12.99 nmol/g. and Lipid Peroxide varied from 1.04 nmol/g (RVSVTK-2019-101) to 1.92 nmol/g (RVSVTD-2019- 4,RVSVTD-2019- 10)with a mean value of 1.44nmol/g.

Table 4.4: Statistical analysis of biochemical traits of Chickpea genotypes

	SOD	LP	PP	TS
Mean	12.99	1.44	2.16	20.36
Std. Error of Mean	.483	.045	054	.277
Std. Deviation	3.056	.283	.342	1.753
Variance	9.338	.080	.117	3.072

SOD= Superoxide Dismutase, LP = Lipid Peroxide, PP= Proline, TS= Total Sugar

4.8 Correlation coefficient analysis:

Lipid peroxidase is significant and positively correlated with Superoxide Dismutase (r =0.346) at 5% level of significance. (Table 4.5)

Table.4.5: Correlation coefficient among biochemical traits of chickpea genotypes.

	SOD	LP	PP	TS
SOD	1	0.346*	-0.020	0.162
LP		1	0.162	0.181
PP			1	-0.205
TS				1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

SOD= Superoxide Dismutase, LP = Lipid Peroxide, PP= Proline, TS= Total Sugar

4.9 Dendrogram analysis based on biochemical parameters:

Cluster analysis and grouping of 40 Chickpea genotypes was done to observe correlation and similarity between these genotypes based on proline, total sugar, SOD, Lipid Peroxidation. Total 2 Clusters are formed A and B which are further divided into sub-clusters. Group A contains 1 genotype and group B contains 39 genotypes. Group B is further sub clustered in 2 groups B1 and B2 containing 1 and 38 genotypes respectively. B2 is further classified into 2 sub-groups B2*and B2** (13) and (25) genotypes respectively. Then group B2* is also sub-clustered into 2 sub-groups B2*′ and B2*″ containing 11 and 2 genotypes respectively. B2** is further sub grouped into b2, and b2,, having (18) and (07) genotypes respectively.

4.10 Validation of gene based markers for *Fusarium* wilt in chickpea

For validation in check varieties of chickpea for *Fusarium* wilt, a total of 14 reported gene based markers were used. RVG203 was used as a resistant check variety for marker validation. Out of 14 primers from different categories (RAPD, DAF, ISSR, and STMS), 09 markers were not amplified. Other 05 primers, which were showing polymorphism between resistant and sensitive genotypes, were used for amplification of all the 40 genotypes of chickpea.

4.11 Analysis of Genomic DNA of chickpea:

The proportion of genomic DNA obtained from the 40 chickpea genotypes ranged from 100 to 2000 ng/ul. The majority of the DNA samples in Nano-drop exhibited 260/280 and 260/230 values around 2.0, signifying good quality DNA. Genomic DNA which was having values less than 1.7 was sheared and isolated again.

4.12 Screening of chickpea genotypes using gene based marker

The 40 chickpea germplasm samples were screened for wilt disease using a set of 05 gene-based markers. For PCR amplification, high-quality genomic DNA was examined. Among the genotypes, all primers produced polymorphic bands (Fig 4.8). The scoring was done using a standard size ladder and a banding

pattern.The data sheet was produced to run in population structure, and allele pattern A/A was used if the band was on the upper side, and pattern B/B was used if the band was on the lower side, in heterozygous condition banding pattern was A/B, and -/- was used if no amplification occurred.

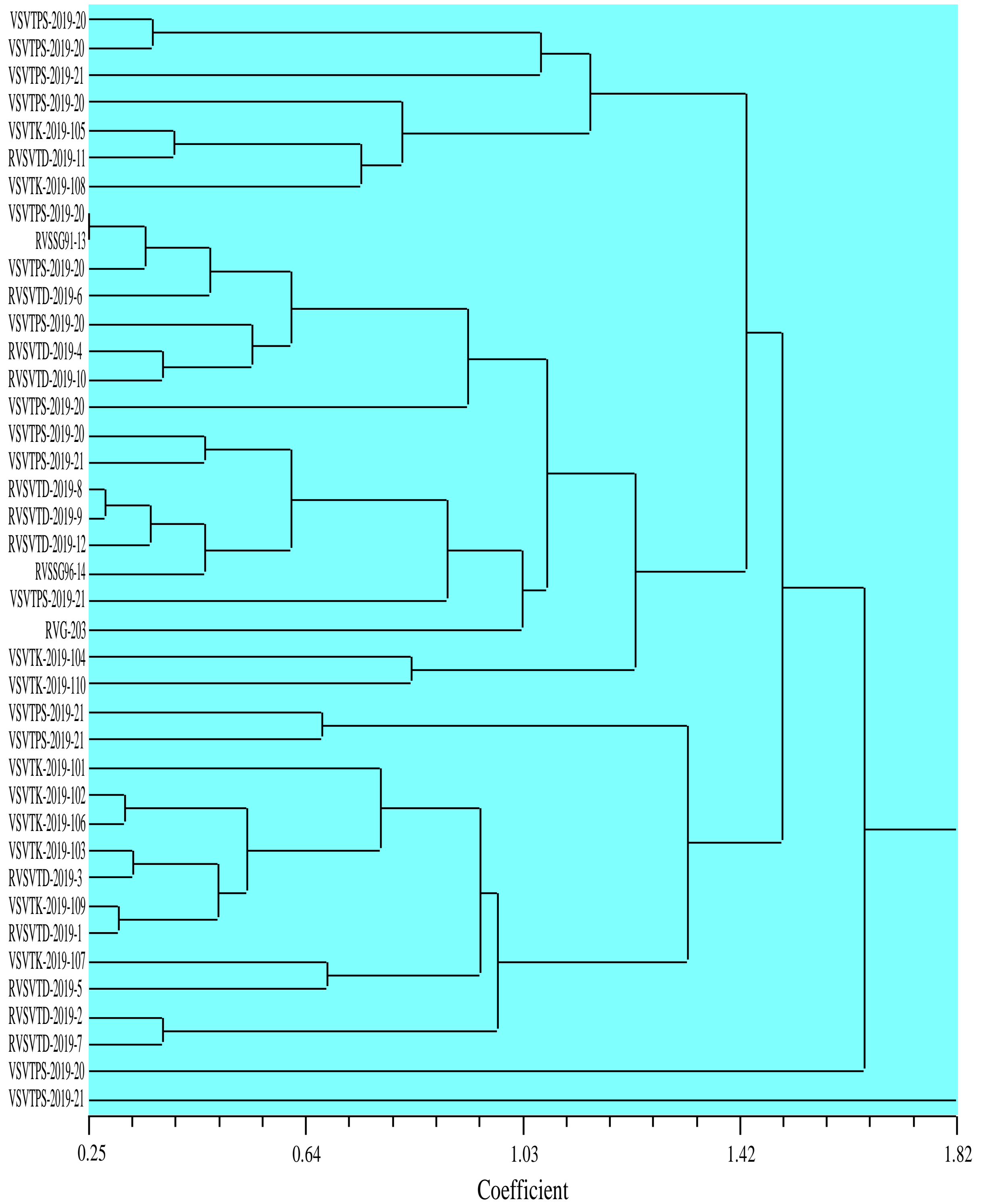


Fig. 4.5:Dendrogram of chickpea genotypes based on biochemical parameters

Fig. 4.6 & Fig.4.7:Two Dimensional Plot showing clustering and association of genotypes based on Superoxide Dismutase and Total Sugar.

Table 4.6: Total sugar, proline, lipid peroxidation and Superoxide Dismutase values of chickpea genotypes

S.NO	Genotypes	SOD (nmol/g)	LIPID PEROXIDATION (nmol/g)	PROLINE (µmol/g)	TOTAL SUGAR (mg/g)
1	RVSVT PS-2019-201	12.6	1.56	2.63	16.8
2	RVSVT PS-2019-202	8.8	1.58	2.24	17.2
3	RVSVT PS-2019-203	11.2	1.57	2.82	16.9
4	RVSVT PS-2019-204	16.5	1.62	2.11	21.9
5	RVSVT PS-2019-205	20.1	1.49	2.13	19.3
6	RVSVT PS-2019-206	18.7	1.58	2.82	19.8
7	RVSVT PS-2019-207	18.2	1.69	2.19	21.1
8	RVSVT PS-2019-208	13.4	1.56	2.25	20.6
9	RVSVT PS-2019-209	15.4	1.74	2.18	20.4
10	RVSVT PS-2019-210	13.1	1.64	2.47	21.6
11	RVSVT PS-2019-211	8.2	1.07	2.44	20.4
12	RVSVT PS-2019-212	8.4	1.61	2.52	21.8
13	RVSVT PS-2019-213	12.1	1.54	2.91	20.4
14	RVSVT PS-2019-214	9.7	1.07	2.20	24.9
15	RVSVT PS-2019-215	9.6	1.06	2.84	19.6
16	RVSVTK-2019-101	9.6	1.04	2.261	18.2

17	RVSVTK-2019-102	11.2	1.07	1.75	19.4
18	RVSVTK-2019- 103	11.7	1.10	1.98	19.5
19	RVSVTK-2019- 104	12.8	1.59	1.49	19.4
20	RVSVTK -2019- 105	10.9	1.74	2.34	18.7
21	RVSVTK-2019- 106	9.7	1.17	1.72	19.2
22	RVSVTK-2019- 107	11.8	1.12	1.53	21.5
23	RVSVTK-2019- 108	9.8	1.51	1.97	19.7
24	RVSVTK-2019- 109	11.3	1.06	1.85	18.2
25	RVSVTK-2019- 110	10.8	1.77	1.69	21.6
26	RVSVTD-2019- 1	13.1	1.06	1.81	18.2
27	RVSVTD-2019- 2	16.3	1.08	2.15	19.4
28	RVSVTD-2019- 3	13.3	1.05	2.07	19.1
29	RVSVTD-2019- 4	17.2	1.92	2.27	20.8
30	RVSVTD-2019- 5	12.2	1.11	1.99	21.7
31	RVSVTD-2019- 6	16.2	1.58	1.89	21.6
32	RVSVTD-2019- 7	15.9	1.06	2.13	20.7
33	RVSVTD-2019- 8	11.5	1.54	2.04	21.9
34	RVSVTD-2019- 9	11.5	1.56	2.21	21.5
35	RVSVTD-2019- 10	17.4	1.92	2.10	21.8
36	RVSVTD-2019- 11	11.9	1.81	2.21	19.7
37	RVSVTD-2019- 12	12.3	1.53	2.19	22.7
38	RVSSG 91-13	17.5	1.55	2.16	21.5
39	RVSSG 96-14	13.2	1.48	1.92	22.1
40	RVG-203	14.6	1.68	1.76	23.5

4.13 Phylogenetic cluster analysis and PIC information

A total of 28 alleles were identified with an average of 5.60 alleles per locus for different markers (Table 4.7). The gene diversity (0.6250 to 0.7900) TR-19 and TR-29 with average of 0.7065 and Polymorphic Information Content (PIC) values varied between 0.566 to 0.7591 (TR-19 to TR-29) with an average of 0.6603 respectively (Table 4.8). The primer which showed highest gene diversity and PIC values was TR29 while the lowest gene diversity and PIC values was observed for the primer TR-19. The major allele frequency varied between 0.2750 (TR29) to 0.5250 (TR-19) with a mean value of 0.4100.

4.14 Cluster analysis

The genetic relationships among chickpea genotypes are presented in molecular based UPGMA tree. All the genotypes were grouped into 4 clusters and among them cluster 3 is grouped with resistance for *Fusarium* wilt disease.

CLUSTER1- (Includes 9 genotypes) RVSVTD-2019-1, RVSVTD-2019-2, RVSVTD-2019-4, RVSVTD-2019-5, RVSVTD-2019-6, RVSVTD-2019-9, RVSVT PS 2019-203, RVSSG 96-14, RVSVTD-2019-10. The above mentioned genotypes are found to be resembling to each other at molecular level.

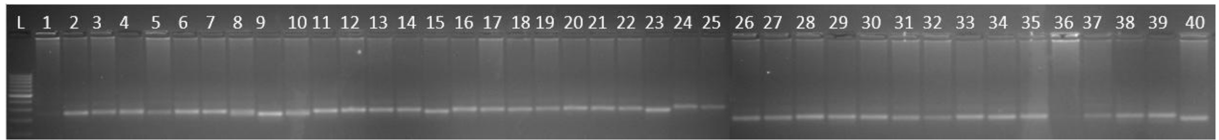
CLUSTER 2- (Includes 9 genotypes) RVSVTK-2019-103, RVSVT PS -2019-214, RVSVT PS- 2019-213, RVSVTK- 2019- 101, RVSVT PS -2019-215, RVSVT PS -2019-104, RVG-203(check variety for *Fusarium* Wilt resistance), RVSVT PS-2019-212, RVSVTK- 2019-105. All the mentioned chickpea genotypes are found similar at molecular level to RVG-203, the check variety used that shows resistance to the *Fusarium* wilt disease.

CLUSTER3- (Includes 11 genotypes) RVSVTK-2019-109, RVSVTK-2019-108, RVSVT PS-2019-205, RVSVT PS-2019-210, RVSVT PS- 2019-204, RVSVTK-2019-102, RVSVT PS- 2019-201, RVSVT PS- 2019-202, RVSVTK-2019-110, RVSVTK-2019-106, RVSVTK-2019-107. All the genotypes shows resemblance to each other at molecular level.

CLUSTER 4 – (Includes 11 genotypes) RVSVT PS-2019-208, RVSVT PS-2019-209, RVSVT PS-2019-207, RVSVTD -2019-12, RVSSG 91-13, RVSVTD-2019-11, RVSVT PS-2019-206, RVSVTD-2019-07, RVSVTD-2019-08, RVSVT PS-2019-211, RVSTVD-2019-03.

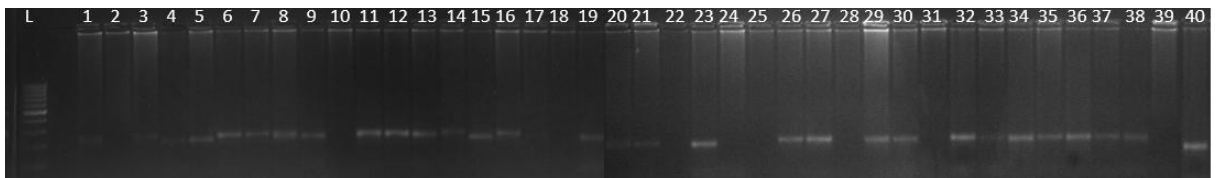
Table 4.7: Allele specific STMS markers presenting Major allele frequency, number of alleles, gene diversity and Polymorphic Information Content (PIC) in Chickpea.

Marker	Major Allele Frequency	Genotype No.	Allele No.	Gene Diversity	PIC
TA-59	0.4000	6.0000	6.0000	0.7525	0.7195
TA-96	0.5000	5.0000	5.0000	0.6313	0.5690
TR-19	0.5250	5.0000	5.0000	0.6250	0.5666
TA-194	0.3500	5.0000	5.0000	0.7338	0.6874
TR-29	0.2750	7.0000	7.0000	0.7900	0.7591
Mean	**0.4100**	**5.6000**	**5.6000**	**0.7065**	**0.6603**

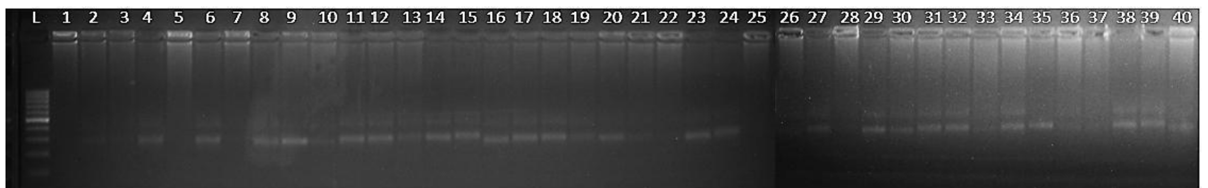


STMS MARKER TA-59

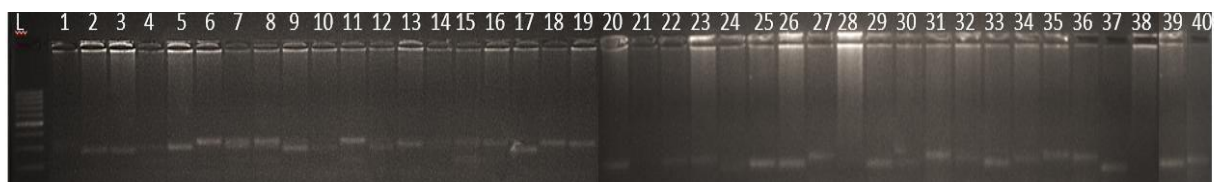
STMS MARKER TR-19



STMS MARKER TR-29



STMS MARKER TA-96



STMS MARKER TA-194

Fig 4.8: Allelic variation using markers showing polymorphism among chickpea genotypes

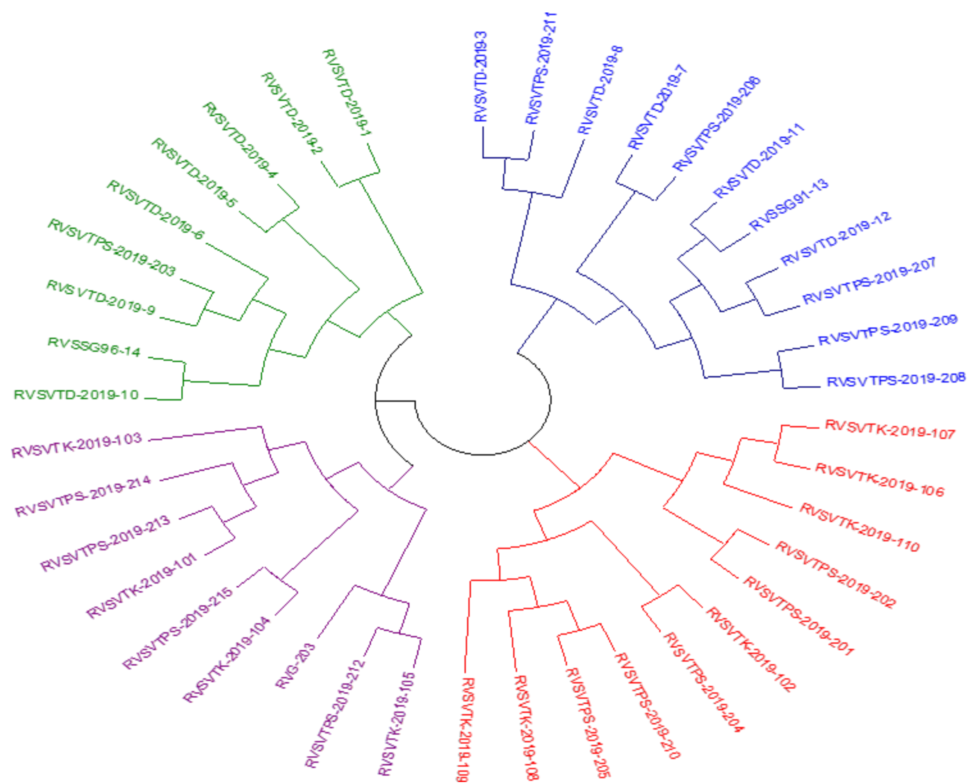


Fig. 4.9: Dendrogram of 40 Chickpea genotypes showing clusters based on similarity using UPGMA relationship.

CHAPTER V

DISCUSSION

The chickpea (*Cicer arietinum* L.) is an important annual legume crop that has been produced in India since ancient times. It's also grown in a lot of places across the world. The crop has been hampered by a variety of biotic and abiotic factors. Diseases, caused by a variety of pathogens, are one of the biotic constraints that crops face.

Fusarium oxysporumf. sp. ciceris causes damage to chickpea (*Cicer arietinum* L.), one of the most important legume crops. In India, this disease was first recorded in 1918.*Fusarium* wilt is the most significant disease threat (Khan, 1980), especially in low-rainfall areas where disease growth is aided by the weather.Many research attempts have been made in India to understand more about the molecular processes that contribute to the development of resistance to chickpea diseases.

Significant progress has been achieved in crop improvement by phenotypic selections for agronomically relevant characteristics, but this approach is fraught with challenges, largely owing to genotype-environment interactions. Molecular breeding, on the other hand, entails the use of molecular marker data to improve the efficacy of different breeding operations, such as breeding programme design and execution, data interpretation, and selection efficiency. Marker aided selection (MAS) is a term used by Beckmann and Soller(1983). to describe the process of selecting a desired allele of a gene/QTL based on molecular markers related to it rather than the phenotype created by this allele. DNA-based markers may be utilised for two fundamental objectives in the MAS process: tracking beneficial allele(s) (dominant or recessive) across generations and finding the most suited individual(s) among segregating progeny. In plant breeding, molecular marker data can be used for a variety of purposes, including germplasm characterization, diversity analysis, parent selection for hybridization, testing for genetic purity, gene introgression, gene pyramiding, segregating population analysis, marker assisted backcrossing, and foreground and background selection.

With the development of modern tools and techniques, such as markers and resistant cultivars, we can reduce the impact of infections. The present investigation was focused on morphological, biochemical and molecular characterization of 40 chickpea genotypes.

Morphological Characterization of chickpea germplasms:

A total of 40 varieties of chickpea were taken under study using 06 morphological traits i.e. Days to 50% flowering, days to maturity, seed yield per plant, 100 seed weight, biological yield per plant and harvest index per cent.

High Phenotypic Coefficient of Variation PCV% (>20) and GCV (%) was observed for characters like seed yield per plant (gm) and biological yield (gm).The moderate Phenotypic Coefficient of Variation (10-20 percent) was observed for trait 100 seed weight while low phenotypic coefficient of variation (<10 percent) was recorded for days to 50% flowering, days to maturity and harvest index. The high estimates of PCV and GCV for these traits suggested the possibility of yield improvement through selection of these traits. The results obtained in the present study are similar to the results obtained by Biru Alemu *et al.,* (2017), Uday *et al.,* (2012), Muhammad Mohibullah *et al.,(*2020), Amir Sohail (2018),Mushtaq, M.A.*et al.,* (2013), Priyanka Joshi *et al.,(*2018),Usmani *et al.,* (2005).

In this study the characters with a high heritability coupled with high genetic advance (>20%) as a percentage of the mean are seed yield per plant(gm), 100 seed weight, biological yield. The above results are in accordance with the studies previously done by Biru Alemu *et al.,(*2017), Sanjay Kumar *et al.,* (2019), Priyanka Joshi *et al.,* (2018), Mathur and Mathur (1996), Sidramappa (2008), Honnappa (2018), R. N. Arora (2019), Sudhandhu Jain et al., (2013). This result indicates that these characters were highly heritable and hence were less affected by the environment. The plant breeder therefore may use these characters for selection on the basis of phenotypic expression.

Days to maturity is significant and positively correlated with days to 50% flowering. Seed yield per plant is significant and positively correlated with Biological yield. Seed yield per plant is significant and positively correlated with

Harvest index. These results obtained are in agreement with the results obtained by Sanjay Kumar *et al.,* (2019), Anita Babbar *et al.,* (2012), M.D. Vaghela *et al.,(*2009), Sudhanshu Jain et al., (2013).

Biochemical analysis of chickpea genotypes

All the forty four genotypes of chickpea were used for analysis of Total sugar, Proline, Superoxide Dismutase and Lipid Peroxidase.

In our study the Total sugar ranged from 16.8 mg/g (RVSVT PS-2019-201) to 24.9 mg/g (RVSVT PS-2019- 214) with a mean value of 20.36mg/g. According to Sanchez-Mata *et al.,* (1999) the total soluble sugar content in chickpea varieties ranged from 5.89 to 8.21 mg/g. Similarly, Veenakumari V.*et al.,* (2017) found that the total sugars content varied from 4.50 mg/100g to 11.10 mg/g. Karamveer Kaur*et al.,* (2019) obtained results for total soluble sugars in these genotypes were 38.08,(desi genotypes), 43.75(kabuli genotypes) and 33.20 mg/g (wild genotypes).Vinod Kumar Sahu *et al.,* (2020) found the values for total sugar (23.6-37.4 mg/g).

Proline is the most important organic solute accumulate in higher plants under drought conditions (Sumera and Asghari 2010). In the present study, Proline varied from 1.49 µmol/g (RVSVTK-2019- 104) to 2.91 µmol/g (RVSVT PS-2019-213)with a mean value of 2.16 µmol/g. Sameer S. Bhagyawant et al.,(2015)The proline content was highest in IPC-12-82 (19.4 mg) while lowest in IPC-12-20 (12.2 mg).Vinod Kumar Sahu *et al.,* (2020) estimated the proline content and found the values ranging from 1.19 to 3.92 µ mol/g.

In our study the value for Superoxide Dismutase (SOD) ranged from 8.2nmol/g (RVSVT PS-2019- 211) to 20.1nmol/g (RVSVT PS-2019- 205) with a mean value 12.99nmol/g. Vaishali Sharma *et al.,*(2017) observed highest activity in tolerant genotype ILC3279 (351.42 unit enzyme/g FW), whereas lowest activity was noticed in GNG 2171 (275.60 unit enzyme/g FW)

In the present study, value of Lipid Peroxidation varied from 1.04nmol/g (RVSVTK-2019-101) to 1.92nmol/g (RVSVTD-2019- 4, RVSVTD-2019- 10)with a mean value of 1.44nmol/g. Similarly, Pradeep kumar Patel *et al.,(*2012) found the value of Lipid Peroxidation 1.19nmol/g to 3.23nmol/g in chickpea

genotypes. Vinod Kumar Sahu *et al.,* (2020) estimated lipid peroxidation and the range observed was 1.1 -3.67 nmol/g.

Lipid peroxidation is significant and positively correlated with Superoxide Dismutase(r =0.346) at 5% level of significance. The results obtained in the present study are in agreement with the following earlier studies of Vinod Kumar Sahu *et al.,* (*2020) investigated* biochemical traits of 29 desi and 15 kabuli chickpea genotypes and found the values for proline (1.19-3.92 μ mol/g), sugar (23.6-37.4 mg/g), malondialdehyde (MDA) (1.1 -3.67 nmol/g) and hydrogen peroxide (H2O2) (10.4-21.5 μ mol/g) in seeds of these genotypes grown under normal field conditions.

Work done for molecular characterization using STMS markers in Chickpea

In our study 14 gene-based markers were used for screening all the 40- chickpea genotypes for *Fusarium* Wilt disease. Among the used markers five STMS markers namely, TA-59, TA-96, TR-19,TA-194,TR-29 showed polymorphism in the chickpea genotypes and amplified alleles associated with resistance and susceptibility.

The results found in our present study are in agreement with the following earlier studies. Vinod Kumar Sahu *et al.,*(2020) investigated chickpea genotypes and used gene-based molecular markers to screen them for *Fusarium* wilt disease. Amadabade *et al.,* (2016) investigated six chickpea genotypes, each with a distinct *Fusarium* wilt response, using DNA-based genetic markers associated with disease resistance/susceptibility. R. V. Padaliya *et al.,* (2013) studied using seven molecular markers formerly linked to disease resistance/susceptibility, a marker-assisted characterisation of six chickpea genotypes varying for *Fusarium* wilt response was carried out.

CHAPTER- VI

SUMMARY, CONCLUSION and SUGGESTION FOR FURTHER WORK

Chickpea (*Cicer arietinum* L.) is a popular food legume that is also a valuable commercial crop. It is divided into two types: the small dark seeded Desi type from India and the large light seeded Kabuli type from the Mediterranean. In addition to numerous environmental restrictions, one of the limiting factors which directly impact the production and causing 10–90 percent loss to the crop, is the fungal disease caused by *Fusarium oxysporum sp. ciceris* (Schlechtends) that causes chickpea wilting. The resistance breeding intended to produce disease resistance variety and Marker Assisted Selection can pump up traditional breeding techniques.

In present experimental work a total of 40 chickpea genotypes received from RAK College of Agriculture Sehore, were screened for *Fusarium* wilt disease. Morphological characterization was done at field condition in two replication with randomly blocked design and observations were recorded for Days to 50% flowering, days to maturity, seed yield per plant, 100 seed weight, Biological yield per plant and Harvest index. Screening of these genotypes for wilt was done using a set of 14 gene based molecular marker i.e., CS-27, CS-27A, OPU17- UBC-825 UBC-170 TA-59, TA-96, TR-19, TA-194 , TR-29 ,TR-31, UBC-811,
UBC841, UBC-864. Some of the important biochemical parameters i.e., Total sugar, Lipid Peroxidation, Proline and Superoxide Dismutase content were also estimated and analyzed.

Among all the morphological traits Days to maturity is significant and positively correlated with days to 50% flowering (r=0.703) at 1% level significance. Seed yield per plant is significant and positively correlated with Biological yield (r
=0.808) at 1% level of significance. Seed yield per plant is significant and positively correlated with Harvest index (r = 0.620) at 1% level of significance. No correlation was found in hundred seed weight with days to 50% flowering and days to maturity. Similarly no correlation was seen in biological yield with days to 50% flowering, days to maturity and hundred seed weight. No correlation was observed in harvest index with days to 50% flowering, days to maturity, hundred seed weight and biological yield. In the same manner there was no correlation

observed in seed yield per plant with days to 50% flowering, days to maturity and hundred seed weight.

Among all the biochemical parameters Lipid peroxidase is found to be significant and positively correlated with Superoxide Dismutase(r =0.346) at 5% level of significance. No correlation was found in proline with SOD and Lipid Peroxidase. Similarly no correlation was observed in total sugar with Lipid Peroxidation, SOD and proline.

High quality DNA was taken from 40 genotypes for validation of a *Fusarium* wilt marker in chickpea genotypes, and 14 primers comprising RAPD, SCAR, DAF, STMS, and ISSR were screened in selected genotypes. The scoring was done based on banding pattern using standard size ladder .The gene diversity and Polymorphic Information Content values varied between 62% to 79% and 56% to 75% respectively. Total 28 alleles were amplified having average of amplified alleles was 5.60, and major allele frequency was giving mean value 0.41 represented good allelic variation among all the selected genotypes. By using the Unweighted Pair Group Method for Arithmetic Average (UPGMA) tree with Power Marker v3.25 software, the observations for major allele frequency, polymorphism information content (PIC), and genetic distance were recorded, and a dendrogram was generated using MEGA 6.0 software.

Conclusion

1. Among the polymorphic STMS markers the highest genetic diversity (0.7900) was observed with TR-29, while lowest genetic diversity (0.6250) was observed with TR-19. Among all polymorphic markers used for screening against *Fusarium* Wilt disease.
2. Cluster analysis of all 40 the genotypes studied formed 4 major groups, as per expectation group 2 that had RVG-203 (Resistant to *Fusarium* Wilt) was representing most of the resistant varieties included in the study.
3. For validation of marker for *Fusarium* wilt in chickpea genotypes high quality DNA was extracted from 40 genotypes and 14 primers including RAPD, SCAR, DAF, STMS, ISSR were screened in selected genotypes out of which only five (STMS) markers revealed to be polymorphic and gave the amplification.

4. The Total sugar ranged from 16.8 mg/g (RVSVT PS-2019- 201) to 24.9 mg/g (RVSVT PS-2019- 214) with a mean value of 20.36mg/g, Proline varied from 1.49 µmol/g (RVSVTK-2019- 104) to 2.91 µmol/g (RVSVT PS-2019- 213)with a mean value of 2.16 µmol/g. Superoxide Dismutase (SOD) ranged from 8.2 nmol/g (RVSVT PS-2019- 211) to 20.1nmol/g (RVSVT PS-2019- 205) with a mean value 12.99 nmol/g. and Lipid Peroxide varied from 1.04 nmol/g (RVSVTK-2019-101) to 1.92 nmol/g (RVSVTD-2019-4,RVSVTD-2019- 10)with a mean value of 1.44nmol/g.
5. Highly resistant varieties i.e., RVSVTK-2019-203, RVSVT PS -2019-214, RVSVT PS -2019-213, RVSVTK-2019-101, RVSVT PS -2019-215,RVSVTK-2019-104, RVSVT PS -2019-212, RVSVTK-2019-105 were validated at genomic level using molecular markers which could be used in hybridization program for chickpea improvement.

Further Work

Future prospects of current study entitled "Screening of Chickpea (*Cicer arietinum* L.) genotypes using gene based markers for *Fusarium* Wilt Disease and study on important Qualitative and Quantitative characters" are given below:

1. Markers with a greater PIC value like TR-29 shall be assessed in more chickpea genotypes to get more informative and reliable findings.
2. Genotype(s) that obtained resistance /tolerance against *Fusarium* wilt disease, can be used further in molecular breeding programmes to develop resistant/tolerant varieties.
3. Highly resistant varieties i.e., RVSVTK-2019-203, RVSVT PS -2019-214, RVSVT PS -2019-213, RVSVTK-2019-101, RVSVT PS -2019-215, RVSVTK-2019-104, RVSVT PS -2019-212, RVSVTK-2019-105could be used in hybridization programme.

CHAPTER VI

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Mean values for all the observed morphological traits

	Days to 50% flowering	Days to maturity	100 seed weight	Biological yield(gm)	Harvest index (%)	seed yield/plant (gm)
RVSVT PS-2019- 201	72	118	25.5	31.5	47.165	14.855
RVSVT PS-2019-202	69.5	115.5	27	35.845	49.43	17.635
RVSVT PS-2019- 203	70.5	117.5	26.5	41.305	54.585	22.56
RVSVT PS-2019- 204	73	120	28	45.72	43.45	19.86
RVSVT PS-2019- 205	73.5	121	27.5	46.53	57.79	27.355
RVSVT PS-2019- 206	74.5	122.5	29.5	35.05	64.82	22.54
RVSVT PS-2019- 207	77	125.5	30.5	4.41	52.395	21.175
RVSVT PS-2019- 208	77.5	116.5	30	27.97	45.535	12.7
RVSVT PS-2019- 209	68	119	29	48.23	43.37	19.995
RVSVT PS-2019- 210	74	120	29.5	32.225	47.475	15.26
RVSVT PS-2019- 211	72	118.5	27	40.805	45.44	18.53
RVSVT PS-2019- 212	70.5	118.5	27	30.13	41.82	12.585
RVSVT PS-2019- 213	72	119	26	43.3	49.595	21.425
RVSVT PS-2019- 214	75	125	30	53.65	58.42	31.25
RVSVT PS-2019- 215	74.5	120	35.5	63.495	50.39	31.935
RVSVTK-2019-101	73.5	122.5	32.5	33.415	46.465	15.46
RVSVTK-2019-102	73	120.5	37	24.735	44.975	11.12
RVSVTK-2019- 103	74	120	35	46.48	47.665	22.15
RVSVTK-2019- 104	73	122	38	44.47	50.665	22.5
RVSVTK -2019- 105	76	122.5	38.5	45.27	47.33	21.4
RVSVTK-2019- 106	77	125	36.5	27.75	46.165	12.82
RVSVTK-2019- 107	76.5	122	38.5	62.63	56.16	35.19
RVSVTK-2019- 108	75.5	120.5	39.5	45.295	50.85	23.05
RVSVTK-2019- 109	72.5	119	36.5	17.27	46.89	8.09
RVSVTK-2019- 110	77	120.5	37.5	33.755	50.19	16.925

RVSVTD-2019- 1	75	118.5	26.5	29.18	48.445	14.135
RVSVTD-2019- 2	77	122.5	25	26.915	50.035	13.465
RVSVTD-2019- 3	77.5	123.5	28	36.44	49.225	17.94
RVSVTD-2019- 4	76.5	126.5	25.5	35.955	47.27	17.005
RVSVTD-2019- 5	77	125.5	25.5	39.295	48.585	19.1
RVSVTD-2019- 6	71	118.5	27.5	40.44	46.895	18.945
RVSVTD-2019- 7	74.5	120	25.5	27.105	45.03	12.15
RVSVTD-2019- 8	73.5	118.5	26.5	42.75	49.56	21.135
RVSVTD-2019- 9	77	124	30	45.975	50.07	22.9
RVSVTD-2019- 10	73.5	121.5	27	22.25	48.55	10.815
RVSVTD-2019- 11	77.5	125.5	27.5	33.86	48.16	16.305
RVSVTD-2019- 12	73	119.5	25	37.23	47.94	17.84
RVSSG 91-13	71.5	118	33.5	40.285	49.425	19.95
RVSSG 96-14	75.5	120.5	17	27.24	48.25	13.1
RVG-203	69.5	116.5	27.5	34.02	47.355	16..105

Mean values for all the biochemical parameters

S.NO	Genotypes	SOD (nmol/g)	LIPIDPEROXIDASE (nmol/g)	PROLINE (µmol/g)	TOTAL SUGAR (mg/g)
1	RVSVT PS-2019- 201	12.6	1.56	2.63	16.8
2	RVSVT PS-2019-202	8.8	1.58	2.24	17.2
3	RVSVT PS-2019- 203	11.2	1.57	2.82	16.9
4	RVSVT PS-2019- 204	16.5	1.62	2.11	21.9
5	RVSVT PS-2019- 205	20.1	1.49	2.13	19.3
6	RVSVT PS-2019-206	18.7	1.58	2.82	19.8
7	RVSVT PS-2019- 207	18.2	1.69	2.19	21.1
8	RVSVT PS-2019- 208	13.4	1.56	2.25	20.6
9	RVSVT PS-2019- 209	15.4	1.74	2.18	20.4
10	RVSVT PS-2019- 210	13.1	1.64	2.47	21.6
11	RVSVT PS-2019- 211	8.2	1.07	2.44	20.4
12	RVSVT PS-2019-212	8.4	1.61	2.52	21.8
13	RVSVT PS-2019- 213	12.1	1.54	2.91	20.4
14	RVSVT PS-2019- 214	9.7	1.07	2.2	24.9
15	RVSVT PS-2019- 215	9.6	1.06	2.84	19.6
16	RVSVTK-2019-101	9.6	1.04	2.261	18.2
17	RVSVTK-2019-102	11.2	1.07	1.75	19.4
18	RVSVTK-2019- 103	11.7	1.1	1.98	19.5
19	RVSVTK-2019- 104	12.8	1.59	1.49	19.4
20	RVSVTK -2019- 105	10.9	1.74	2.34	18.7

21	RVSVTK-2019- 106	9.7	1.17	1.72	19.2
22	RVSVTK-2019- 107	11.8	1.12	1.53	21.5
23	RVSVTK-2019- 108	9.8	1.51	1.97	19.7
24	RVSVTK-2019- 109	11.3	1.06	1.85	18.2
25	RVSVTK-2019- 110	10.8	1.77	1.69	21.6
26	RVSVTD-2019- 1	13.1	1.06	1.81	18.2
27	RVSVTD-2019- 2	16.3	1.08	2.15	19.4
28	RVSVTD-2019- 3	13.3	1.05	2.07	19.1
29	RVSVTD-2019- 4	17.2	1.92	2.27	20.8
30	RVSVTD-2019- 5	12.2	1.11	1.99	21.7
31	RVSVTD-2019- 6	16.2	1.58	1.89	21.6
32	RVSVTD-2019- 7	15.9	1.06	2.13	20.7
33	RVSVTD-2019- 8	11.5	1.54	2.04	21.9
34	RVSVTD-2019- 9	11.5	1.56	2.21	21.5
35	RVSVTD-2019- 10	17.4	1.92	2.1	21.8
36	RVSVTD-2019- 11	11.9	1.81	2.21	19.7
37	RVSVTD-2019- 12	12.3	1.53	2.19	22.7
38	RVSSG 91-13	17.5	1.55	2.16	21.5
39	RVSSG 96-14	13.2	1.48	1.92	22.1
40	RVG-203	14.6	1.68	1.76	23.5

THESIS ABSTRACT

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ABSTRACT

The present investigation entitled, "Screening of Chickpea (*Cicer arietinum* L.) genotypes using gene based markers for *Fusarium* Wilt Disease and study on important Qualitative and Quantitative characters" was conducted during *Rabi* season of 2020-21 at Research Farm, RVSKVV, College of Agriculture; Gwalior (M.P.). Forty genotypes of chickpea (*Cicer arietinum* L.) were grown during Rabi 2020-21 in RBD with 2 replications and the observations were recorded on the characters viz., days to 50% flowering, days to maturity, seed yield per plant for studying the variability, heritability and genetic advance.The analysis of variance(ANOVA) showed highly significant differences for all the characters. The high estimate of phenotypic and genotypic coefficient of variation was recorded for grain yield per plant and biological yield. High estimates of heritability in broad sense were recorded for days to maturity followed by, Days to 50% flowering seed yield per plant, 100 seed weight, biological yield and harvest index. Based on high heritability coupled with high genetic advance as percent of mean it was inferred that additive gene action was in operation for control of seed yield per plant, 100 seed weight and biological yield indicating good chances of improvement of these traits through simple selection. Days to maturity is significant and positively correlated with days to 50% flowering at 1% level significance. Seed yield per plant is significant and positively correlated with Biological yield at 1% level of significance. Seed yield per plant is significant and positively correlated with Harvest index at 1% level of significance. Main constraints in Chickpea productions is the *Fusarium* wilt diseases. Biochemical parameters related to stresses were also analyzed for proline (1.49-2.91 μmol/g), total sugar (16.8 -
24.9 mg/g), Lipid Peroxidation (1.04 -1.92 nmol/g) and Superoxide Dismutase (8.2 -20.1 nmol/g) in seeds of these genotypes grown under normal field conditions. Molecular screening was done by using15 gene-based markers. The genotypes were screened for molecular characterization using 14 gene based marker, out of which 05 STMS markers showed polymorphism and amplified the alleles associated to resistance and susceptibility of *Fusarium* Wilt disease in chickpea genotypes. The highest PIC value is obtained by STMS Marker TR-29 and the least PIC value is obtained by STMS Marker TR-19. On

the basis of molecular characterization RVSVTK-2019-203, RVSVT PS -2019-214, RVSVT PS -2019-213, RVSVTK-2019-101, RVSVT PS -2019-215, RVSVTK-2019-104, RVSVT PS -2019-212, RVSVTK-2019-105 genotypes were found to be similar to RVG -203 (check variety) used in the present study programme.

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