Review Article

# Role of Molecular markers in identification and characterization of Chickpea variety

## Running title: Molecular markers in identification of Chickpea variety.

## Abstract

Chickpea is the second most significant legume grain, growing in 14.80 mhs regions all over the world. It contributes roughly 14.24 million people to the global food basket each year. Desi and Kabuli chickpeas are widely grown in India, and both cultivars have distinct physiochemical characteristics such as seed size and shape, hydration capacity, coat thickness, and protein content. The genetic basis of chickpea cultivars has been limited as a result of agricultural techniques. Molecular markers, on the other hand, have proven to be useful tools for accurate quantification and characterisation within plant species. Markers can be an excellent way to improve chickpeas quantitatively because they are a high source of protein and constitute 80% of total dry weight. Markers such as RAPD (Random amplified polymeric DNA), SSR (Single sequence repeat), AFLP (amplified fragment length polymorphism) and ISSR (Inter simple sequence repeat) have aided in improving breeding efficiency and thus promoting the generation of new species, as well as increasing yield, stress tolerance, and disease resistance. The goal of this review article is to understand the role of various molecular markers in identification, characterization and to know the role of molecular marker how to improve the chickpea variety.


**Keywords:** Chickpea, Molecular marker, Polymerase chain reaction, RAPD, SSR, ISSR.

**Abbreviations:** AFLP- Amplified fragment length polymorphism; DNA- Deoxyribonucleic acid; ISSR- Inter simple sequence repeat; PCR- Polymerase chain reaction; RE- Restriction Endonuclease; RAPD- Random amplified polymeric DNA; RFLP- Restriction fragment length polymorphism; SNP- Single nucleotide polymorphisms; SSR- Single sequence repeat; STMS- Sequence-tagged microsatellite.

## Chickpea: An overview

Cultivated chickpea (Scientific name-Cicer arietinum L.) is a self-pollinated, diploid (2n=16) annual pulse crop with a genomic size of 740 Mbp. Chickpea has grown worldwide and is the second most important legume grain, which is grown ever 14.80 mhs areas. In the global food basket, it contributes about 14.24 million annually (Jha, 2018). The bulk of chickpeas are grown in dry areas of the Indian subcontinent, and India is the leading producer, accounting for 90% of the crop in this region. Despite the fact that chickpeas are cultivated and consumed locally, India is the world's largest importer, accounting for roughly 20% of worldwide imports. Two types of chickpea cultivars- Desi and Kabuli are cultivated in India and both exhibit variations in their physiochemical characters like seed size and shape, hydration capacity, coat thickness and protein content (Rizvi et al., 2014). As chickpea is a good source of protein and containing 80% of total dry weight, so markers can be a good approach to improve chickpeas quantitatively (Vural and Akcin, 2010).

Chickpeas have a small deep tap root structure that increases their ability to survive drought. It thrives in climates with limited rain and a generally chilly climate. The aerial section is densely branched, erect or spreading, and grows to a height of 1 m, with glandular pubescence, dark green coloration. Leaves are imparipinnate, glandular-pubescent, and have 3-8 pairs of leaflets with a terminal leaflet on the rachis. The inflorescence is made up of solitary blooms that are white, pink, purple, or blue in colour. The seeds are housed in a rhomboid ellipsoid, inflated, glandular-pubescent pod with 1-2 or maximum 3 seeds. The colour of the seed might be white, yellow, brown, black, or green. Desi chickpeas, which account for the majority of Indian output, feature a pigmented (usually brown), angular-shaped seed with a noticeable, distinctive "beak" that contains the embryonic axis. Seeds typically weigh between 0.1 and 0.3 g, depending on genotype. Kabuli chickpeas differ from desi in that they are white or cream coloured, have a rounder form with a less obvious beak, and are usually

larger and heavier (0.2–0.6 g) and the seed coat is thinner (Wood et al., 2011). The basic structure of chickpea plant, flower, pod and seed is shown in **figure 1**.

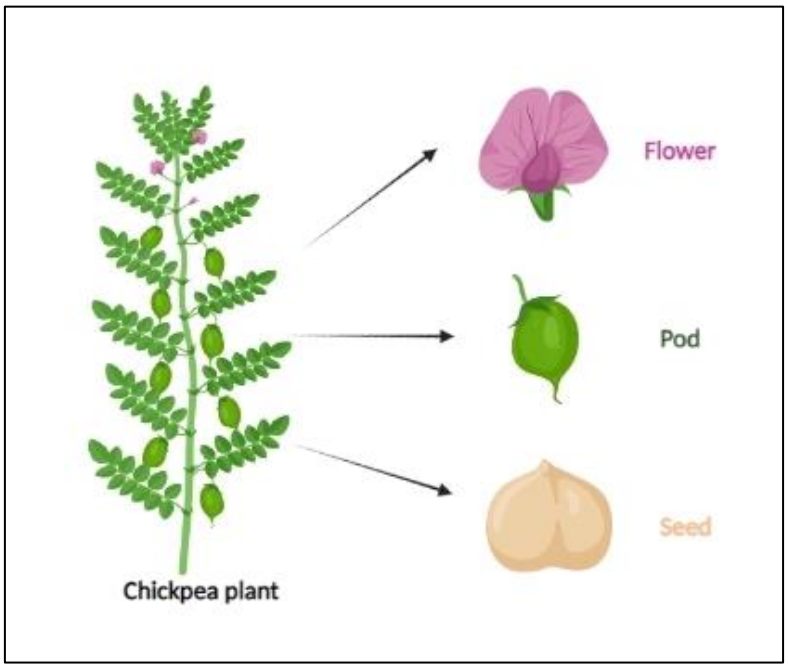


**Figure 1: A general schematic representation of chickpea plant and its different parts.**

Huge variety of chickpea is found in all over India. Recent release variety of chickpea is listed in **table 1** representing its origin and agronomic features.

**Table1: A list of new variety of chickpea release in last few years (Project Coordinator's Report, Annual Group Meet on Chickpea – Aug. 2017, AICRP, ICAR, IIPR, Kanpur).**

| S. No | Variety | Year of release | Originating centre | Agronomic features |
|---|---|---|---|---|
| 1 | Ganguar (GNG 1581) | 2007 (CVRC) | ARS, Sri Ganga Nagar | Medium plant height, semi-erect |
| 2 | Arpan (RSG 902) | 2007 (SVRC) | Durgapur | Late sown condition but also perform well under normal sown as well as rainfed conditions. |
| 3 | Ankur (CSJ 140) | 2008 (SVRC) | Durgapur | Suitable for irrigated situation and have good resistant to wilt and DRR |
| 4 | PKV Kabuli 4 | 2009 (CVRC) | Akola | Semi-sreading, broad leaves, ivory white extralarge seed |
| 5 | Jawahar Gram JG 6 | 2008 (SVRC) | Jabalpur | Resistant to Fusarium wilt and moderately resist. |

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|---|---|---|---|---|
| 6 | Kripa (Phule G 0517) | 2009 (CVRC) | Rahuri | Semi-sreading, broad leaves, ivory white extralarge seed |
| 7 | Pank Kabuli Chana 1 | 2010 (SVRC) | Pantnagar | Semi-sreading, medium height, large seed with prominent beak, late maturing, tolerant to BGM |
| 8 | Raj Vijay Kabuli Gram 101 (RVKG 201) | 2011 (SVRC) | Sehore | Large seeded kabuli, early maturing, moderately resistant to wilt |
| 9 | Gujarat Junagarh gram 3 | 2011 (SVRC) | Junagarh | Moderate high, semi-erect, yellow large seeded, resistant to wilt and stunt |
| 10 | GLK 26155 (L 555) | 2012 (CVRC) | Ludhiana | Suitable for timely planting under irrigated condition, it has light yellow seed colour with 27.8g /100 seeds weight |
| 11 | GNG 1958 | 2013 (CVRC) | Sriganganagar | Suitable for normal sown in irrigated condition. It has brown seed with 25.4g average 100 seed weight |
| 12 | Vallabh Kabuli Chana (WCGK 2000-16) | 2015 (CVRC) | Modipuram | Large seeds (27.5g/100 seeds), white colour, 4 moderately resistant to Fusarium wilt |
| 13 | Nandyal Gram (NBeG 49) | 2016 (CVRC) | Nandyal | Large seeded (38.8g/100 seeds) kabuli variety |
| 14 | Indira Chana 1 | 2017 (SVRC) | IGKVV, Raipur | Erect plant type with more primary branches, resistant to wilt |
| 15 | Pant gram 4 (PG 065) | 2017 (SVRC) | GBPAUT (Pantnagar, UT) | SEMI erect plant type, tolerant to wilt. BGM and dry root rot |

## Molecular markers

The detection and analysis of genetic variation in plants can aid our insight into the molecular basis of a variety of biological processes. Due to the limits of phenotype-based genetic markers, molecular markers were developed, which are more broad and effective direct DNA-based markers. A molecular marker is a piece of DNA that represents variations at the genome level. A trait's phenotypic expression may or may not be correlated with molecular markers. Molecular markers have a number of advantages over traditional phenotype-based alternatives, including the fact that they are stable and detectable in all tissues regardless of the cell's growth,

differentiation, development, or defence status, and are unaffected by the environment, pleiotropic, or epistatic effects (Agarwal et al., 2008). The following are the characteristics of an optimal genetic markers technique: (1) being highly polymorphic and equally distributed throughout the genome, (2) resolve genetic differences adequately, and (3) produce many, independent, and consistent markers (4) easy, rapid, and economical and easy to use system; (5) only requires a little number of tissue and DNA samples; (6) has phenotypic linkage, and (7) There is no need to know anything about a plant genome beforehand (Garrido-Cardenas et al., 2018).

There are various molecular markers has been discovered basically divided into two category- PCR (polymerase chain reaction) dependent and PCR independent. A general classification of molecular markers is shown in **Figure 2**.

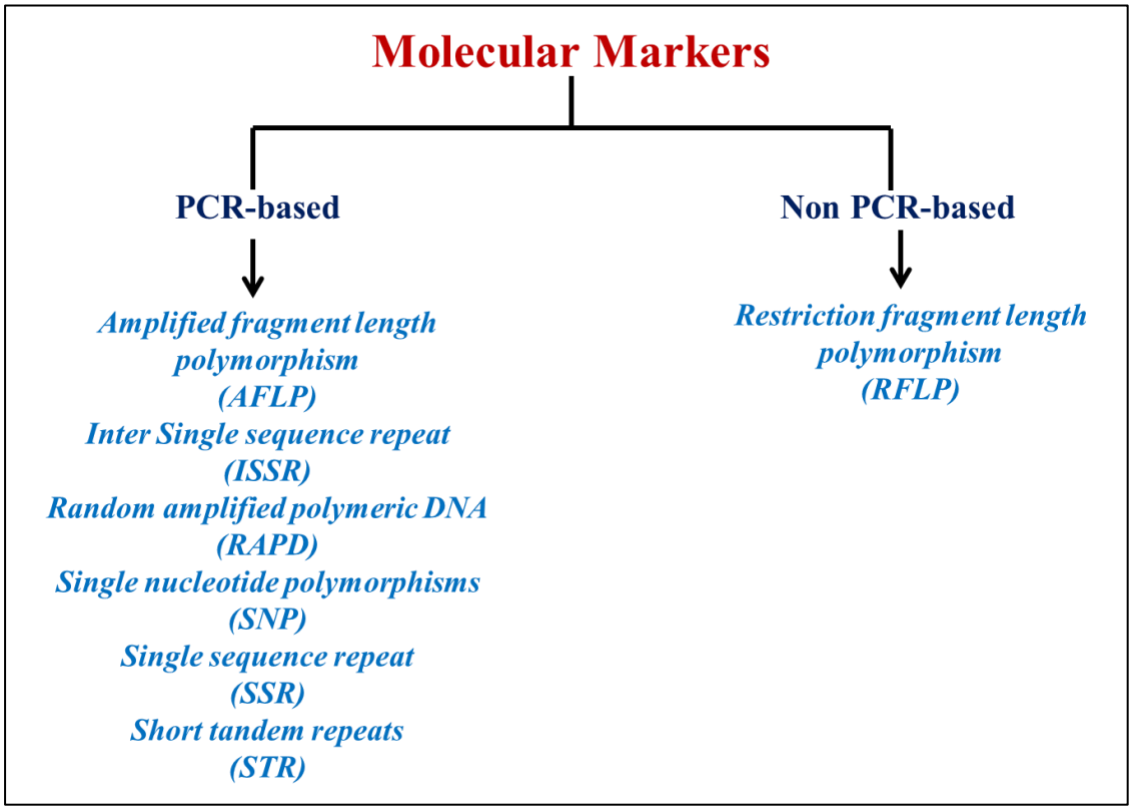


**Figure 2: A Basic classification of molecular marker on the basis of PCR.**

The discovery of PCR makes the assessment of genetic study very easy. With the help of PCR thousands numbers of copy of a particular gene can be synthesize in few hours. The basic mechanism of PCR is same as in-vivo replication where a daughter DNA synthesize from the parental DNA. PCR have three basic steps- (i) denaturation of parental DNA, (ii) annealing of primer on a specific site of DNA/ gene and finally (iii) extension of daughter DNA. The basic mechanism of PCR is shown in **Figure 3**.

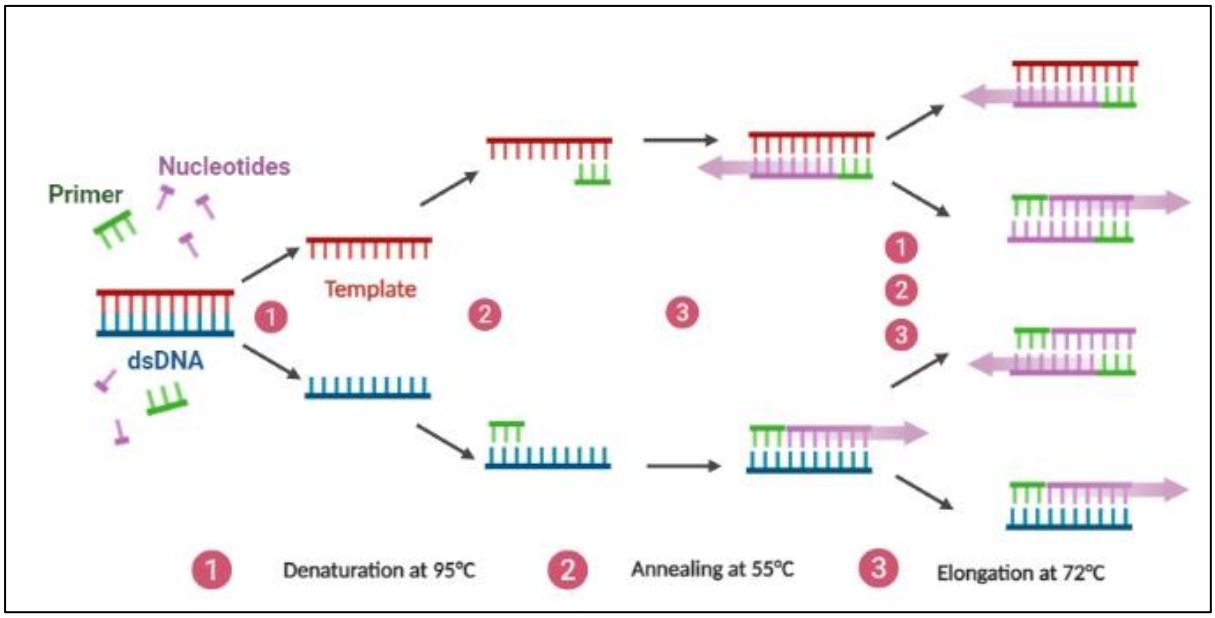


**Figure 3: A basic presentation of polymerase chain reaction mechanism.**

Restriction enzyme (RE) play important role in molecular studies. Digestion of a DNA with a given restriction enzyme or combination of restriction enzymes will produce fragments of different lengths that are directly related to the DNA sequence (Sluss and Hayes, 2016). Most of the present molecular marker detection is depends on RE such as RFLP and AFLP. In RE based molecular marker studies, first the DNA sample is extracted from the plant material (leaf, seed, flower or any other part). Isolated DNA was restricted with a combination of restriction enzyme. Restricted fragments run on agarose gel and after complete run DAN bands transfer on membrane, and hybridized with specific radioactive probe. After hybridization the

membrane is washed and fragments are analysed or compared with other sample of chickpea variety. A general procedure of RE based molecular marker study is shown in **Figure 4**.

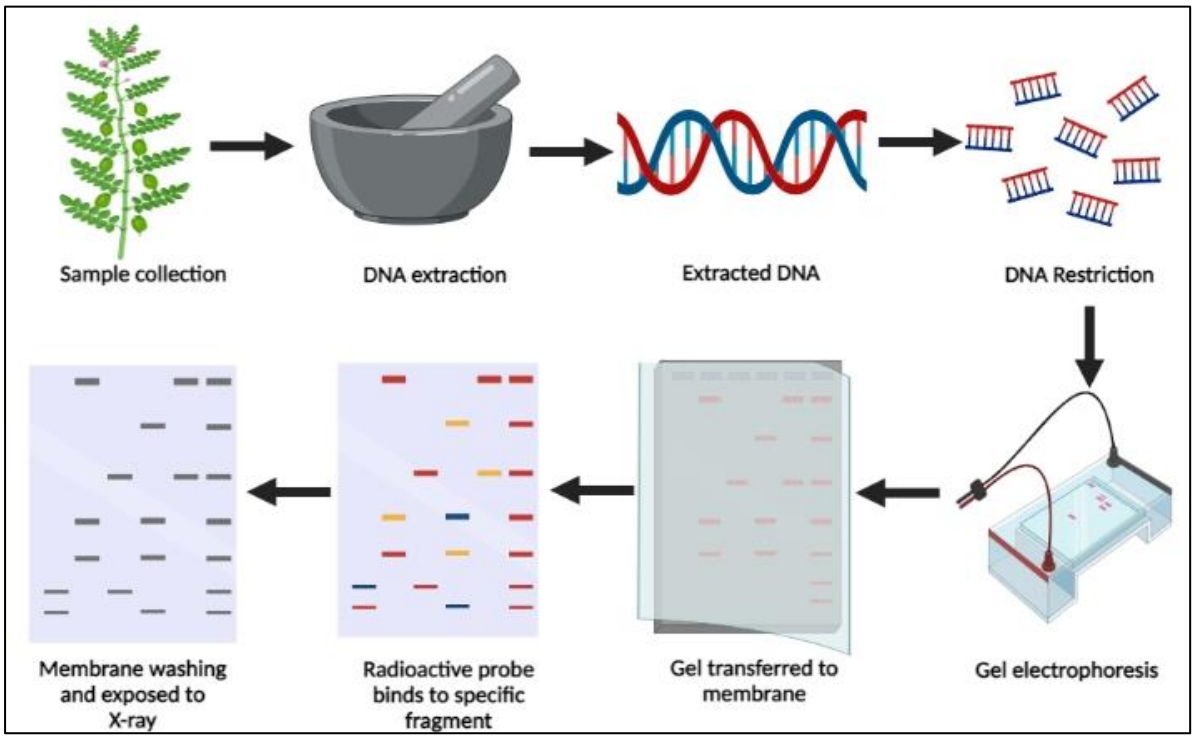


**Figure 4: Flow chart of RE based molecular marker study in chickpea plant.**

## Role of molecular markers in the identification and characterization of chickpea variety:-

The agriculture practices have narrowed down the genetic basis of chickpea cultivars. However, molecular markers have proved to be valuable tools in effective quantification and characterization within plant species. A list of various molecular marker used in different studies of chickpea is listed in **table 2**.

### Role of RAPD in chickpea analysis-

RAPD serves to improve breeding efficiency and thus promote the formation of new species by detecting nucleotide sequence variation in DNA using a single primer of randomised

nucleotide sequence Marker like RAPD. It also helps to boost yield, provide stress tolerance, and disease resistance (Datta and Lal, 2011). In an experiment, morphological features were used to genotypically define 58 different chickpea genotypes, including desi and kabuli (Gediya et al., 2018). DNA markers have also opened up new avenues in genome analysis, allowing researchers to better understand germplasm, genetic diseases, and phylogenetic relationships. In an experimental study, 58 diverse chickpea genotypes including desi and kabuli was characterized genotypically on the basis of morphological traits (Sachdeva et al., 2017). Because chickpeas are self-pollinated, RFLP and RAPD markers revealed relatively little genetic diversity between cultivars. Sant et al., (1999) used RAPD and oligonucleotide probes to discover genetic diversity between 29 elite Indian chickpea cultivars. Only 10 of the 35 RAPD primers tested displayed polymorphic patterns between the cultivars, indicating that the genetic bases of cultivated chickpeas are limited (Sant et al., 1999).

**Role of ISSR and SSR in chickpea analysis -**

ISSR detection involves PCR amplification using SSR motifs between adjacent or inversely oriented microsatellites (Singh et al., 2014). The ultimate goal of this research is to study diversity in chickpeas on genetic level and to provide better understanding of chickpea collection for development of superior chickpea genotypes. However, the ability of SSRs of detecting intra-specific variations in chickpea has been very well determined. Sethy et al., (2006) assessed 25 markers to analyse intra-specific genetic diversity within 36 geographically diverse chickpea accession. Experiment was performed on basis of cloning and sequencing of size between alleles which revealed the variable number of Aa repeats within alleles and were the major source of polymorphism (Sethy et al., 2006). ISSR and SSR PCR-based markers are well-known in plants for their uses, since they allow for the examination of polymorphism in microsatellite and inter-microsatellite without having a complete understanding of the DNA

sequence. Because SSR primers have a 5' or 3' extension of one or more bases, including anchor sequences, the analysis was made easier (Yadav et al., 2015).

**Role of Isozyme in Chickpea analysis-**

Some Biotic and Abiotic factors reduced chickpea production worldwide so continuous search of finest genotype was carried out employing several Biochemical and Molecular markers (Singh et al., 2008a). Biochemical markers include various biochemicals which include bio-molecules like carbohydrate, proteins and lipids. These markers are intermediate between morphological and DNA markers and the most widely used biochemical markers are isozymes also known as 'allozymes' (Feuven, 2002). Isozyme is considered to be a widespread biochemical marker that used in diversity analysis of different plants. Isozyme have been used in genetic diversity analysis of different plants including chickpea, as its electrophoresis could provide rapid and easier access to be used as reliable biochemical marker and only small amount of tissue is required but its electrophoresis had revealed insufficient polymorphism specially in cultivated chickpea species, consequently, narrow genetic variability had observed (Belete, 2018).

**Role of STMS marker in Chickpea analysis-**

In India, there is a few researches on chickpea biodiversity; yet, knowing about genetic diversity for any crop is the first step in developing a viable chickpea through diverse breeding programmes. STMS are found across the genome and have a high degree of polymorphism. Rizvi et al. (2014) used sequence tagged microsatellite site (STMS) makers to examine the Kabuli and Desi types of Indian chickpea cultivars in 2014. He used 51 STMS primer pairs for 68 chickpea cultivars (9 kabuli and 59 desi kinds) in his study, of which 32 STMS primers were polymorphic (Rizvi et al., 2014). In a separate experiment, twenty-four STMS primer

pairs were used to fingerprint 21 chickpea cultivars. Six of the primer pairs were monomorphic, whereas the other eighteen were polymorphic (Winter et al., 1999). Present research evaluates the degree to which STMS markers are capable to analyse the variations in the genetic link between Kabuli and Desi varieties of grown chickpea.

**Role of ASR in Chickpea analysis-**

Chickpeas have been discovered to have nutritional and economic value in vegetarian societies, but their productivity has been steadily declining due to a lack of genetic variety and other environmental pressures. Chickpea is particularly vulnerable to stressors such as drought; a significant decrease in chickpea productivity as a result of drought has a significant impact on the plant's growth processes and yield (Silva et al., 2017). Abscisic acid is one of several transcription factors involved in signalling drought stress, and its tolerance can be influenced by ABA-dependent pathways. The most commonly reported drought tolerance response gene is ASR (Hong et al., 2002). Sachdeva and Bhardwaj (2020) used bioinformatic methods to characterise the Abscisic acid & ripening gene and explain its involvement in chickpea drought tolerance, paving the way for the development of functional markers for chickpea improvement (Sachdeva et al., 2020). Therefore, characterizations of different traits through biochemical and molecular markers for their genetic analysis would be a great approach for plant breeders to utilize them in improvement of Chickpea.

## Conclusion

Chickpea is considered as most nutritional among all the legumes. India is the biggest exporter of chickpea variety at global level. Identification and characterization of various variety of chickpea is difficult by morphological trait however molecular markers play important role in assessment of a variety. Molecular markers including AFLP, RAPD, SSR, ISSR and RFLP are

commonly used for identification of a specific trait as well as in breeding programme for enhancement of nutritional value of chickpea seed.

**Table 2: A list of molecular marker based studies in chickpea cultivars.**

| Types of markers | Application in chickpea | Output | Reference |
|---|---|---|---|
| ISSR | ISSR marker is easier to use in genetic analysis of chickpea due to its quicker electrophoresis | With the use of ISSR marker, it is convenient to analyse the genetic diversity | (Belete, 2018) |
| RAPD | RAPD marker has been to detect genetic variations in chickpea cultivars | It has revealed good polymorphism among chickpea cultivars | (Singh et al., 2014) |
| STMS & AFLP | They were applied to evaluate the genetic diversity among chickpea cultivars | With the help of these markers, genetic relationship among chickpea cultivars can be determined | (Singh et al., 2008b) |
| SSR | SSR markers have been employed to analyse genetic diversity & relationships between crops | It improved the productivity of chickpea & can detect genetic relatedness among species | (Amina et al., 2020) |
| ASR | ASR gene has been used as a putative candidate for tolerance of drought among chickpea varieties and many plants like rice | It improved the drought tolerance in chickpea and enhanced yield & other metabolic processes | (Sachdeva et al., 2020) |
| RFLP | RFLP Marker have been effectively employed for genetic diversity analysis of various chickpea cultivars | These PCR based markers permits detection of polymorphism | (Sharifi et al., 2018) |

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