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Review on Tea Germplasm and Utilisation in Indian Perspective

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Origin of tea is mainly believed to had been taken place in south and southeast Asian countries like China, India and Cambodia. More than 50% of world tea production is contributed by China and India only. Taxonomically there are three major cultivating species of tea. First one is known as *camellia sinensis* (L.) O.Kuntze indigenous to China. Second one is *camellia assamica* (Masters) indigenous to Assam, India. Lastly *Camellia assamica* ssp. *Lasiocalyx* (Watt) indigenous to Cambod. *C. sinensis* also known as China variety, *C. assamica* known as Assam variety and *C. assamica* ssp. *Lasiocalyx* is commonly known as Cambod variety. These species shows a large number of variations in different growing areas. These species grows or cultivated side by side and free crossing over among themselves highly heterogenous early population can be observed. Tocklai Tea Research Association have largest collection of tea germplasm around the world. In a survey Dr. I.D. Singh (Sr. Plant Breeder) showed that diversity of tea maintained at the research station. Following table shows the number of collected germplasm

Till date 35 TV (Tocklai vegetative) clones has been released for the tea planters. More than 150 number of garden series clones also have been released by Tea Research Association authority. These clones either comes through breeding programmes or through conservation prograrammes (*in-situ* and *ex-situ*). Both programmes done by selection in tea estates having old seed sections. *In-situ* conservation done by developing seed bari where selected plants left to grow till they become seed bearer plant. From these plants seeds were collected and trials were established to have variety with desirable phenotypic characters. *Ex-situ* conservation is done through clonal selection where plant material from the selected plants were collected and preserved in a different place outside of their natural habitat. Nowadays selection has become very selective where presence of essential trait is checked through molecular marker. These markers mainly used for checking of characters like yield, drought tolerance, quality etc.

Planters favour clonal plants than seed plants these days as they shows fast growth in nursery and in the field. But because of this genetic diversity is becoming homogenous and need more conservation strategies for sustanibility of the industry.

Morphological characterization of tea can be summerised as, PVP or plant variety protection is a form of intellectual property rights to new varieties of plants and their motto is innovations of plant varieties. Before 1990 it was mainly used by developed countries and after that it started slowly to spread worldwide. Indian government validated the legislation on "Protection of Plant varieties (PPV) and Farmers Right (FR)" in 2001 and "protection of Plant Varieties and Farmers rights Rules in 2003 (Anonymous, 2004). These acts will enable new plant varieties to get registered if it fulfil criteria of distinctiveness, uniformity and stability. New recommended variety should be clearly distinguishable than any other present variety as per the rule. Varietal characteristics should be uniform in all of the nursery and field plants of same variety and characters should be stable throughout of its life cycle as any variation will indicate environmental effect on the plant and eliminate possibility of genetical variation. Morphological and agronomic characters should be described accurately in the form of DUS as per guidelines of PVP (Smith and Smith, 1989). Test Guidelines' Procedures (TGP) documents, into detailed practical guidance for the harmonized examination of DUS and, in particular, to identify appropriate characteristics for the examination of DUS and production of harmonized variety descriptions (TG/1/3, Chapter 1.3, UPOV 2002). In DUS characterization just like Test Guidelines of different crops, tea plant guidelines also contain ten chapters (TG/Tea (proj.5), UPOV 2007).

Time, place, quantity and quality of the required plant material for DUS testing are decided by the authority (PPV and FRA). According to legislations for testing a plant material, applicant has to supply minimum 75 plants. Plant material has to be nine months to one year old. Plants has to be healthy (*i.e.* without any pest and disease) and without any chemical treatment. If treated, full details of application, applicant has to provide full details of application. DUS testing has to be multi-locational (at least two places) and duration should be minimum of two years. Plantation should be of single hedge with 75 x 75 cm in south India, 110 x 75 cm in plains of north India and 90 x 45 cm in hills of Darjeeling. Plantation should be of two replications with 20 number of plants in each replication.

As per the guideline all total of 20 number of characters should be studied and documented during evaluation.

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Guideline for the experiment was developed by Tea Research Association, Jorhat, Assam under the Chairmanship of Director, TRA, Jorhat and Director, UPASI. Tocklai Tea Research Association is the nodal DUS testing centre along with UPASI Tea Research Foundation is co-nodal DUS testing centre.

DUS evaluation is not profitable for the plant breeders as it will be easy to differentiation a variety from the others elimination maximum chances for environmental effect but also effective for planters as they can select planting material according their needs.

Tea is a sessile organism which constantly attacked by various pests and diseases. More than 20% loss of world tea production is due to pest and diseases (Agarwal, 2011). Plant protect themselves by launching different direct and indirect defence mechanisms. Plants use hairs, trichomes, waxy cuticles as their physical barrier against herbivores or chewing pests where as plants use secondary metabolites like phenolics, alkaloids, terpenoids against sucking type of pests. (Wu and Baldwin, 2010). Tea is a perennial crop which may stay in the field for more than 50 years. It can cause susceptibility of the particular plant to certain disease or pest favoured by particular agroclimatic zone. It requires a strong equilibrium with the agroclimatic to stay healthy and productive at a particular location (Fuchs, 1989) . Economic loss *i.e.* loss in production of tea is greater due to diseases than animal pests. Unfavourable equilibrium can cause different leaf, shoot or root disease. Common occurring leaf, shoot and root diseases given below in the table:

Plants defend themselves through systemic acquired resistance and (SAR) and Induced systemic resistance (ISR). Infection of parasitic pathogen activate salicylic acid signalling pathway through systemic acquired resistance. Induced systemic resistance caused by PGR (plant growth promoting) bacteria through jasmonic acid and ethylene signalling pathway (Naruska *et. al,* 2008; Oniki & Ando, 1985). There has been report that flavonoid compounds of the plant act as passive or inducible barriers against different microbial pathogens. Content of the flavonoids changes with severity of the infection (Miranda *et al.* 2007; Carlsen *et al.* 2008). In response to infection of M. medusae induction of the genes encoding pathway from phenylalanine and malonyl-CoA to proanthocyanidin synthesis was also reported by M. Miranda in 2007. In a study it was reported that Cs4CL, which is a gene involved in synthesis of flavoinoids was induced by the infection of blister blight in tea (Nisha *et.al.,* 2018). Same reports on defense mechanism also published in bean by Cramer *et. al.* in 1985. POX and APX enzymes which are the anti oxidative enzymes increase during any pathogen attack. However it was also reported that induction of these enzymes are more in susceptible varieties than tolerant ones (Nisha *et.al.*, 2018). Though content of enzymes increase during infection but these mechanism doesn't help in resistance of the infection (Senthilkumar *et al.* 2012). 4CL (4- coumarate: CoA ligase) is a key enzyme of phenylpropanoid metabolism with flavonoid and lignin branch pathways play a major role in plant defense mechanism. Increased CsCHI, CsF3H and CsANS induction also reported by the tolerant varieties of tea than the susceptible ones.

Beside disease pests are also reason of concern for the tea industry. Some major pests causing destruction of world tea production are enlisted below in the table:

Insect injury can reduce plant photosynthates by defoliating leaves. Which bring the plant in the blink of dying. Economic injury level (EIL) is an important component of modern pest management strategy. Though EILs are developed for a given crop, not clones (Hammond, *et.al.,* 1979) tolerant variety show less EIL than the susceptible one.

Hyposidra is a Lepidopteran pest which threat to the tea industry of Northeastern (NE) India. H. talaca is the most abundantly found insect pest among all other major pests of tea. It has suppressed *Buzura suppressaria Guen*. (Lepidoptera: Geometridae), which was a historically important pest of tea (Das, 1965; Hazarika *et al.,* 2009) since 1903. Since *Hyposidra talaca* is a polyphagous species, it extended its host range to include tea from nearby Sal-dominated forests (*Shorea robusta* Roth. [Dipterocarpaceae]).

Helopeltis theivora commonly known as tea mosquito bug is also a serious pest of tea for plains and hills. Yearly it reduces 7-15% crop yield accordance of favourable agroclimatic conditions. It is a sucking pest and infested leaves dark brown spots and punctures. Not only it reduce yield, manufactured tea from infested leaves also shows less quality.

Polyphagous insects encounter numerous toxins during their life cycle produced by plants as their defence mechanism. These toxins also known as secondary metabolites. Plants produces alkaloids, flavonoids, terpenoids which act as substance of their defence mechanism. Many acaricide, pesticides are also in use to reduce severity of pest infestation. But use of excessive usage of pesticide brings harmful effect on plant and the environment. Enzymes like eneral esterases (GEs), glutathione Stransferases (GSTs) and cytochrome P450-mediated monooxygenases (CYPs) which act as metabolic detoxification enzymes of pesticides / insecticides also present in some pest which help them to survive, even in the application of pesticides. Some pests shows also shows behavioural changes which made them insecticide or pesticide resistant. This kind of behavioural changes are visible in *H. thiovera* where they show different egg laying pattern (Roy and Mukhopadhayay, 2011) .

Nowadays selection of tolerant plants to pest and diseases is an important criteria for plant breeders. For selection different techniques are applied. Analyzing anatomical, biochemical behaviour of the plant helps in selection. Molecular approaches are also available for selection. Different methods like introduction of natural predators are also reported by scientists to reduce pest infestation like red spider mite and looper caterpillar on tea crops.

Plant phenotyping is a technique which identifies and record different traits of interest the plant. It is becoming difficult to make balance with growing food demand because of adverse environment as emission of the green house gases causing global warming (Yang *et.al.,* 2015). Here modern techniques of management, cultivation and plant breeding will help in increase in crop production with less environmental effect. Development of stress tolerant crop variety with increase yield and also automated systems of plant health monitoring system is a solution to combat issues like this. Tools of high throughput plant phenotyping are necessary in these situations. Marker assisted selection is a popular technique nowadays among plant breeders for selection of plant varieties with desirable traits. Linkage analysis and genome wide association study (GWAS) are the methods for identification of such markers. Analysis of gene expression , linkage and GWAS can be integrated for identification of potential candidate genes in a locus identified with linkage analysis or GWAS. For identification of root morphology of *Arabidopsis* this technique has been followed earlier. Specific techniques for identification and recording of desirable traits is still underdeveloped (Mahlein, 2016; Minervini *et.al.,*) and therefore require improved selection and breeding programme and also for reduction of quantitative and qualitative losses accelerating genetic gains is needed for automated monitoring of plant health status. Integration of Integration pest management along with plant phenotyping can fulfil demands of consumers and producers with increasing yield and quality with minimum effect on the environment. (Chawade *et.al.*, 2018).

Requirement of plant phenotyping is not same among the farmers and breeders. Though naowadays plant phenotyping mainly used for weed detection and fertilizer application, it has significant importance in identification of crop varieties with desirable traits. Through the technique detection of plant pathogen and pests can be made early. Plant phenotyping technique can differ based on the crop, their development and available resources. With the help of developed phenotyping tools and methods for proximal and remote sensing can accelerate screening of a large number of germplasm with reduce costs. High throughput plant phenotyping also helps to evaluate traits imposible to visualize with naked eyes which enable these traits being undertaken into breeding programmes. Some field high throughput phenotyping techniques are satellite imaging, UAV'S and proximal phenotyping. Satellite imaging is a useful technique technique to evaluate germplasm in moderate to large size plots. It is available with having multispectral spatial resolution ranging from 1.24 m WorldView-4) to 260 m (CBERS-2). In 2016 Tattaris *et.al.* reported evaluation of wheat germplasm breeding trial using Digital Globe WorldView-2 satellite having a multispectral spatial resolution of 1.84 m ground sample distance (GSD). In the evaluation procedure he used a plot of 8.4m at 2.4m in size which is suitable and big enough for his study where as another evaluated plot 2m at 0.8m could not be analyzed because of smaller area. Applications can measure plant health evaluated earlier with the help of satellite imaging. These application can measure can measure crop damage due to different biotic and abiotic stresses (Yang, 2018; Seelan *et.al,,* 2003). Negative side of satellite imaging is first it is not always cost effective and is not applicable for all of the farmers. Second because it is not available 24x7, any delay in irrigation, plants suffering from nutrient deficiency or detection of disease outbreak can lead to serious crop damage. Lastly imaging in a sunny day will be always different than a cloudy day. So it will be very difficult to get always a clear image. By which UAV and proximal phenotyping has an upper hand over satellite imaging. UAV or unnamed aerial vehicles has as distict advantage over satellite imaging as it has higher spatial resolution and a lower ground sampling distance compared to satellites that are at an altitude of approximately 700 km. Parachutes, blimps, rotocopters and fixed wing systems are four separate groups of UAV. UAV was extensively used in different crops in different times (Sankaran *et.al.*, 2015). Wheat was evaluated by Tattaris *et.al.* by using octocopter mounted with multispectral and thermal sensors. Octocopter with an RGB camera was used to evaluate to cotton by Xu *et.al.* at 2018. Maize grain yield was evaluated with vegetation index maps and 3D crop surface models using a hexacopter with an RGB camera from 50 m altitude. Plant phenotyping with ground based vehicles and sensors known as proximal phenotyping. With the help of phenotyping sensors like vehicles, cable suspensions or stationary towers sensors are handled (Derry *et.al.*, 2014). In maize handheld sensors were used to evaluate traits like plant height, canopy tempereture, leaf area, plant chlorophyll fluroscence, nitrogen content etc. Handheld thermometers with infrared facility were used to evaluated drought tolerance of maize varieties (Kenner *et.al,* 1983). Main goal of plant phenotyping is to find out the plant varieties more suitable to the environment that is currently under use. Within a given time increase of performance via artificial selection is known as genetic gain (Xu et.al., 2017). **Genetic gain from selection, or simply "genetic gain,"** is defined as the improvement in average genetic value in a population or the improvement in average phenotypic value due to selection within a population over cycles of breeding (Hazel and Lush, 1942).In breeding programmes following formula is used to determine genetic gain:

$$R_t = (i\ r\ \square_A\ ) \div Y$$

Here R_t stands for genetic gain over time, i denotes selection intensity, r indicates selection accuracy, $\square_A$ stands for genetic variance and Y signifies the number of years required per cycle.

Assessing tea germplasm with genome sequencing and different molecular markers becoming a practice day by day. Leaf yield is the main important agronomic trait in tea as leaf itself is the crop. Because of different biotic and abiotic stresses crop yield can reduce dramatically. Tea plants can be morphologically or phenotypically characterized though DUS characterization or characterization for distinctiveness, uniqueness and stability of the tea varieties. Different morphological traits indicative for bush heath, their tolerance level to disease and pest and quality of the particular variety can be assessed through the study. Characterization process continued for multiple years in different agro-climate to eliminate any environmental effect. Later these targeted traits can be assessed through different molecular markers. As nowadays drought is becoming a serious concern for tea planters there are many examples for selection of drought tolerant varieties with the help of drought tolerant markers. Similarly quality characters like content of flavonoids and anti-oxidants also can be assessed with the help of genome assisted selection.

Harnessing gene pool of any crop species or variety benefits in crop improvement by delivering superior plant performance in agriculture, food, energy and biomaterial production (Morrell *et. al.,* 2011). Through integration of crop performance *i.e.* environment, plant phenotype and management practices with genomic information agriculture can become a predictable science. Collection of a large data in different agroclimatic condition helps in understanding about the nature of the plant i.e. phenotype through integration of plant genotype. First multicellular plant organism to be sequenced in 2000 was Arabidopsis thaliana (AGI initiative). Sequencing was done through overlapping Bacterial Artificial Chromosome method (BAC). After two year first crop plant *i.e.* rice was sequenced using same method (Yu *et. al.,* 2002; Goff *et. al,* 2002). Next poplar or cottonwood plant was sequenced through a different technique where whole genome was randomly broken down in smaller pieces and then sequenced and assembled through whole genome shotgun strategy (Tuskan *et. al ,* 2006). In 2007 two different research team sequenced grapevine production where a team was focused on inbred lines (Jaillon *et. al,* 2006), others were on heterozygous genotype (Velasco *et. al.,* 2007) in the aim of production of better wine. Sequencing grapevine factors like grape development or genomes helps in production of better wine was understood. Then came the NGS or Next generation sequencing technique where first thechique was 454, based on pyrosequencing but it was suited for only bacterial sized genome. Later another sequencing technology offerd by illumina was approached by which greater read count count was improved dramatically and it was also less expensive than WGS method. Though many crops were sequenced using the technology, first plant was woodland strawberry to be sequenced using only next generation seguencing (Shulaev et.al, 2011). Genomes of crops like Potato, cabbage, banana, orange, chickpea, pigeon pea, watermelon, spruce was also sequenced using this methodology (Wang *et. al.,*2011; Xu *et. al.*, 2011; Xu *et.al,* 2013; D'Hont *et. al,* 2012; Varashney *et. al,* 2012, 2013, ; Guo *et. al,* 2013; Nystedt *et. al.*, 2013).

One of the major advantages of having techniques of genome sequencing is availability of molecular markers which is nowadays extensively in use for mapping of agronomically usable traits. These high density markers helps to identify regions of interest within the genome. It can be widely visible in in the increased use of genomes for QTL mapping (Paux *et. al.*, 2012). After characterization traits can be bred into elite varieties (Cabezas *et. al,* 2011). In 2012 estarese production responsible gene was idenfied in tomato which helped in understanding of differences in volatile ester content in different tomato species. Similarly genes responsible for uniformly ripening of the fruit was also identified with the help of genome sequencing which turned out to be Golden -2 like transcription factor (Goulet *et. al.*, 2012). Rice genome sequencing helped in identifying QTL for rice grain production, called as cytokinin oxidase and a transcription factor controls expression of the gene known as DST (Drought and Salt tolerance). This factor helps in tolerance of stress like drought and salinity of rice (Huang *et. al,* 2009).

During the last few decades, the use of *molecular markers*, revealing polymorphism at the DNA level, has been playing an increasing part in plant biotechnology and their genetics studies. There are different types of markers *viz.* morphological, biochemical and DNA based molecular markers. These DNA based markers are differentiates in two types first non PCR based (RFLP) and second is PCR based markers (RAPD, AFLP, SSR, SNP etc.), amongst others, the microsatellite DNA marker has been the most widely used, due to its easy use by simple PCR, followed by a denaturing gel electrophoresis for allele size determination, and to the high degree of information provided by its large number of alleles per locus. Despite this, a new marker type, named SNP, for Single Nucleotide Polymorphism, is now on the scene and has gained high popularity, even though it is only a bi-allelic type of marker. Day by day development of such new and specific types of markers makes their importance in understanding the genomic variability and the diversity between the same as well as different species of the plants. In this review, we will discuss about the biochemical and molecular markers their advantages, disadvantages and the applications of the marker in comparison with other marker types.

Currently, depending on the technique of execution and the characterized part of the genome, there are a large number of different DNA markers: RFLP, AFLP, RAPD, □APS, SSR, SCAR, SNP, *et al.* Repeating SINE and LINE sequences can be such a genetic marker. They are retrotransposons or mobile genetic elements that are widely represented in the genomes of the entire eukaryotic kingdom and make up a large portion of genomic DNA (up to half of the plant genome). One of the earlier methods of DNAmarkers getting is Restriction Fragment Length Polymorphism, also named as RFLP. One of the earlier methods of DNA-markers getting is Restriction Fragment Length Polymorphism, also named as RFLP. The principle of this method founded on detection of specific nucleotide sequences in genomic DNA, with help of "blothybridization". This method of analysis is in demand in our days, but in identification and differentiation of plants it has changed by methods, which depending on PCR-amplification of DNA with using of random selected primers, or primers, that compliment to known plots of genomics. (Hugo *et. al.*,1998)

Table 1 : *Camellia* spp. germplasm maintained at Tocklai Tea Research Association at 1994

Species	Source of collection	No. of Accessions
Camellia assarnica	Assam, Manipur, Sri Lanka, South India	2337
Camellia sinensis	China, Darjeeling of West Bengal	35
C. assamica ssp *lasiocalyx*	Indo China, Myanmar, Assam	60
C. kissi (drupifera)	Meghalaya	50
C. caudafa	Assam	-
Eurya japanica	North East India	7
E. acuminata	North East India	2
Gordonia excelsa	North East India	2
G. imbricata	Sri Lanka	2
C. japonica	U.S.A., Japan	-
C. sasanqua	U.S.A., Japan	-
C. irrawadiensis	Upper Myanmar	2
C. japanica var. Kyoniski	Japan	1
C. rosiflora	Sri Lanka	1
TOTAL		2507

Table 2 : Test guidelines for conduction of the test are prescribed below:

Chapter	Contents
1	Subject of these Test Guidelines
2	Material Required
3	Method of Examination
4	Assessment of Distinctness, Uniformity, and Stability
5	Grouping of Varieties and Organization of the Growing Trial
6	Introduction to the Table of Characteristics
7	Table of Characteristics
8	Explanations on the Table of Characteristics
9	Literature
10	Technical Questionnaire

Table 3 : Test guidelines for conduction of the test are prescribed below:

Sl No.	Trait name	Sl No.	Trait name
1	Growth Habbit	11	Leaf blade attitute
2	Young Leaf colour	12	Leaf pubescence: Density
3	Mature leaf colour	13	Position of style in relation to stamen
4	Petiole: Anthocyanin pigmentation	14	Position of style splitting
5	Leaf Shape	15	Flower colour of inner petals
6	Leaf size (Length & Breadth ratio)	16	Flower diameter
7	Leaf upper surface bullation	17	Flushing behaviour
8	Leaf apex	18	Leaf blade shape in cross section
9	Leaf base	19	Branch zigzaging
10	Leaf margin	20	Leaf blade undulation of leaf edges

Table 4 : Major diseases and their symptoms in Tea (*Camellia sinensis*)

SL No.	Disease	Infected part	Causal organism	Symptom
1	Blister blight	Leaf	*Exobasidium vexans*	First, pale yellow translucent spots, then circular blisters on leaf underside. Then white velvety and later circular brown spot
2	Dieback	From node towards 1st leaf	*Fusarium solani*	White mycelia growth at nodal region, die off of twig
3	Red rust	Leaf and Stem	*Cephaleurus parasiticus* and *C. virescens*	Red film covering leaf and stem effecting photosynthetic abilities
4	Thorny stem blight	Stem	*Tunstallia aculeata*	Branches die off. Projection of fruiting bodies from bark of the dead branches.
5	Charcoal stump rot	Root	*Ustulina deusta*	Fan like patches at the underside of root
6	Black root rot	Root and stem base	*Rosellinia arcuata*, *R. bunodes*	Rot of stem and root base, discolouration of black wood, white color mycelia on roots, stem ringing
7	Seedling disease	Root	*Cylindrocladium ilicicola*	Seedling wilt and dieback

Table 5 : Major pests of Tea (*Camellia sinensis*)

Sl No.	Pest name	Pest type
1	Looper caterpillar (*Hyposidra talaca*)	Chewing pest
2	Red slug caterpillar (*Eterusia magnifica*)	Chewing pest
3	Tea Mosquito bug (*Helopeltis theivora*)	Sucking pest
4	Red spider mite (*Oligonychus coffeae*)	Sucking pest
5	Jassid (*Empoascaf lavescence*)	Sucking pest
6	Thrips (*Scirtothrips dorsalis*)	Sucking pest

Table 6 : Different molecular markers used in modern plant breeding.

Parameters	RFLP	RAPD	AFLP	SSR	SNP
Genomic coverage	Low copy coding region	Whole genome	Whole genome	Whole genome	Whole genome
Amount of DNA required	10-50 µg	1-100 ng	1-100 ng	50-120 ng	Atleast 50 ng
Quality of DNA required	High	Medium	High	Medium	High
Type of polymorphism	Single base pair change	Single base pair change	Single base pair change	Change in lengths of repeats	Single base pair change
Degree of polymorphism	Medium	High	High	High	High
Effective multiplex ratio	Low	High	High	High	High
Inheritance / alleles	Co-dominant	Dominant	Dominant/ co-dominant	Co-dominant	Co-dominant
Types of probes/ markers	Low copy DNA or cDNA	Decamer or dodecamer, random DNA bp	Specific sequence	Specific sequence	Allele-specific sequence
Technical requirements	High	Low	Medium	Low	High
Radioactive detection	Yes	No	Yes	Yes	No
Time requirement	High	Low	Medium	Low	Low
Reproducibility	High	Medium	High	High	High
Time demanding	High	Low	Medium	Low	Low
Automation	High	Medium	High	High	High
Suitability	High	High	High	High	High

The most popular type of markers in mapping of plants genomics is RAPD (Random Amplified Polymorphic DNA). The method is in amplification of DNA of object during PCR with random primer, which has length of 10- 11 nucleotides. Method of detection of RAPD-markers is easy by technic execute. If the protocol has optimized, RAPD is convenient, easy and suitable for analyze of plants genomics. Another very important but technically different method is usage of AFLP (Amplified Restriction Fragment Length Polymorphism) markers; According to this DNA are processing with combination of 2 restriction enzymes (Vos *et. al.,*1995; Sivolap *et.al.,* 1997). Specific adapters are lysing with "sticky" ends of restrictive fragments. After this fragment are amplify with using primers that complimentary to sequence of adapter and to site of restriction, and additionally bearing one or more randomly selected bases at their 3'-ends.

The CAPS (Cleaved Amplified Polymorphic Sequences) methodology, as well as SCAR, belongs to the STS (Sequence Tagged Site) group, since it is based on the amplification of strictly defined fragments of the genome with a known sequence. The principle of the method is as follows: genomic DNA is amplified using a pair of highly specific primers, then the resulting fragment is processed with a restriction endonuclease; differences between genomes manifest themselves in the form of different numbers and lengths of restriction fragments during agarose gel electrophoresis (Arcade *et. al.,* 2000).

The molecular markers namely random amplified polymorphic DNA, amplified fragment length polymorphism, sequence-characterized amplified region, inter simple sequence repeats, simple sequence repeats and single-nucleotide polymorphism, etc. have been successfully used for genetic diversity studies in J. curcas. The assessment of genetic variations among Jatropha germplasms using molecular markers show the presence of high genetic diversity for the Central and South American regions and insignificant genetic variation from Asia and Africa. The use of molecular markers for the assessment of phylogenetic relationships among different Jatropha species has been restricted only to the well adapted and acclimatized species in India. The significant genetic variability in the genus Jatropha for vegetative and floral traits for seed oil content, productivity, toxicity (phorbol esters, curcin), fatty acid profiles, etc. has been studied in the past decade using molecular markers. Genetic enhancement of Jatropha through conventional breeding and interspecific gene transfer can be attempted by exploiting the diverse genetic resource form J. curcas and their wild species. This review focuses on the importance and use of molecular markers towards studying diversity analysis in J. curcas growing at different areas (Robero *et. al*., 2015).

Approaches towards Genome sequencing of Tea includes Varietal improvement of any perennial plant is very slow. Because of self- incompatible reproductive system, genome of the tea plant is heterogenous in nature (Zhang *et. al.*, 2016). Therefore through classical breeding methods, crop improvement of tea plant is quite stressful (Mondal *et al.,* 2004). Therefore, approaches like marker assisted breeding, transgenic, genetical manipulation through CRISPR/CAS-9 is very important nowadays. Tea genome consists 2n=30 chromosomes having 4GB genome size. From 2017 to 2019 two successful attempts were done at China with two different tea varieties: *C. sinensis* var. assamica and *C. sinensis* var. sinensis. (Xia *et al.,* 2017; Wei *et al.,* 2018, Xia *et al.* 2019). Over the years through construction of cDNA library, cDNA microassay, sequencing and annonation of EST (expressed sequence tags), gene expression profiling, identification of EST SSR marker and specific trait linked genes, significant progress has been made on tea genome sequencing (Chen *et al.* 2009a, b). Till now EST sequencing is an important technique to gather information on genetic data of tea. Till October, 2016 GenBank dbEST database had 49,760 EST sequences and 3,70,886 sequence was present at NCBI (National Center for Biotechnology Information) . Since chalcone (aromatic ketone) synthase gene was isolated from a Japanese tea cultivar "Yabukita" many work has been done to characterize, cloning, and expression of trait-specific gene (Liu and Han 2010; Borchetia *et al.* 2011; Paul *et al.* 2012a, b; Deng *et al.* 2013). In India first genotype decoding was done on TV1 (Tocklai vegetative 1) which was the first attempt of genome encoding on Indian tea plant (*Camellia asamica*). TV1 genotype was decoded using NGS (next generation sequencing) data including Illumina, PacBio and Hi-C long reads. Mitochondrial and chloroplast genome of clone TV1 was assembled using Illumina & PacBio reads where exhibited mitochondrial genome result was 707,441bp long and chloroplast genome was 157,353bp long. This was the first try to decode mitochondrial genome of any *Camellia* species and first attempt of decoding chloroplast genome of *C.assamica*.

For marker based selection, derived markers from functionally characterized sequences are advantageous over randomly selected markers for having linkage with exact trait loci. These linkage property helps in agriculture crop selection. RAPD markers were the first to use in preparation of genetical map of tea plant. RAPD markers were used to determine anine content, anthracnose resistance, time of bud sprouting and cold tolerance capability (Tanaka, 1996). Later other dominant markers *i.e.* RAPD, AFLP, ISSR were also used to prepare tea genetical maps. Exploiting these markers QTLs were recognized for yield and drought tolerance in tea (Kamunya *et al.,* 2009; Kamunya *et al*., 2010). Co-dominant SSR markers were also used to create high density reference map of tea. Limitation of DNA base molecular markers in tea is till genetic diversity, relationship and molecular identification analysis (Mondal, 2002; Chen *et al.,* 2005; Das *et al.,* 2016). In 2011 Shi *et.al.* reported as sequencing technology getting advanced large scale RNA sequencing is becoming a fast and cost effective approach. Transcriptome sequencing also made quite progress in *C.Sinensis* within short period of time. Recently in 2017 Xia *et.al.* reported sequencing data of Yunkgkang 10 (*C. sinensis* var. *assamica*) through WGS methodology where ~29.7 Gb of

RNA data was sequenced from developmental stages like tender shoot, flower bud, young shoot, root, seedling etc.

Different genes were identified and decoded responsible for biotic and abiotic stresses in tea. In 2017 Pan *et.al.* identified and cloned 14 CsGAox genes (GA20ox, GA3ox and GA2ox) responsible for biosynthesis and deactivation of gibberellins. In 2016 Wang *et.al.* identified and cloned CsNAC genes CsNAC17 and CsNAC30, highly responsive in drought, high tempereture, salinity like abiotic stresses. Genes like CsZfp, CsGPX2, CsDHN1 , CsDHN2, CsTUA, CsICE1, CsCBF1, CsSMT also identified responsive against different types of abiotic stresses in tea. Wang *et.al.* also reported on decoding of CsAOC gene which helps plant in their defense mechanism against different pests and pathogens via transcriptional and metabolic changes. Like that many genes like CsCAD, CsCYS, CsPR, CsNAM, CsGS were identified and decoded activates during different biotic stresses like pest & diseases.

With the help of advance genetic sequence technologies and molecular markers, selection of tolerant tea clones to different abiotic and biotic stresses are very much possible which may become beneficial in aid of tea industry for improvement of tea yield and quality.

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