

Development And Utilization Of Chromosome Substitution Lines (Csls)

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Abstract: *Deportation of one set of chromosomes from a strain into the other distinct strain is defined as chromosome substitution. In order to investigate the effects of individual chromosomes they are transferred from a variety to another variety sharing common genetic history. Alternatively, a chromosome carrying a desirable gene or a group of genes may be transferred into another variety lacking those genes. Chromosome substitution is most widely used in plant breeding to developing a resistant variety by advanced mapping populations, including CSSLs, ILs, BILs & NILs. Furthermore, for achieving the objective of enhancing the cultivated species through ingression of novel genes isolated from wild relatives, production of chromosome substitution lines with the help of marker assisted selection acts as the most promising tool. The wild and weedy cultivars of the major crops are sometimes used as germplasm sources in breeding programs. Unwanted effects are more exhibited by alien substitutions rather than alien additions due to which they are not directly used in agriculture.*

Key words: *Chromosome substitution, mapping populations, introgression, cultivars, germplasm*

1. INTRODUCTION

Historically, breeders have encountered many difficulties in their attempts to interspecific crosses and introgression efforts due to cytological abnormalities, infertility, and deform segregation in F₂ and later generations (Beasley and Brown 1942; McKenzie 1970; Saha *et al.*, 2004). Generally, total genome level work carried out by breeders, during introgression on targeted genes undesirable Deoxyribose nucleic acid also comes along with that leads to improvement in on character reduced by another character. One of the economic important considerations is correlation between characters (Saha *et al.*, 2004). Breeders have identified the more restoration of back cross parent types in early BC generations of introgression efforts. Qualitative morphological characters and molecular markers components reports are strongly distorted by segregation; it has been verified (Reinisch *et al.*, 1994; Mei *et al.*, 2004). Unfortunately, genetic “noise” is diluted by the conventional BC & BC inbreeds development it helps in screen the interest of gene in offset by gradually non-random genome sampling appear through repeating better transmission against sexual generation (Saha *et al.*, 2004;

Yamamoto *et al.*, 2009; ChitraMani & Kumar, P. (2020); Sharma, M., & Kumar, P. (2020); Chand, J., & Kumar, P. (2020); Naik, M., & Kumar, P. (2020); Kumar, P., & Naik, M. (2020); Kumar, P., & Dwivedi, P. (2020), Devi, P., & Kumar, P. (2020); Kumari, P., & Kumar, P. (2020); Kaur, S., & Kumar, P. (2020); Devi, P., & Kumar, P. (2020); Sharma, K., & Kumar, P. (2020); Kumar, S. B. P. (2020); Devi, P., & Kumar, P. (2020); Chand, J., & Kumar, P. (2020).

Usually, breeder uses very rarely interspecific crosses in crop improvement, damaging of agriculture effect, affecting production and profit occurred due to the specific alien genes potential value is normally compromised by closely co-inheritance of linked genes (He *et al.* 2008). Specific breeding strategies are extremely difficult and several generations is required by breeding to improve the beneficial and undesirable alien gene. Therefore in wide range of cross combination for improving of new population mapping compulsory requires mapping of genetics and cloning of QTLs on map-based. Alien substitution lines are used for the interspecific introgression based on population or conventional pedigree expanding the recombination with only chromosome segment (Jenkins *et al.*, 2006, 2011, 2007; Kumar, P. (2019); Kumar, D., Rameshwar, S. D., & Kumar, P. (2019); Dey, S. R., & Kumar, P. (2019); Kumar *et al.* (2019); Dey, S. R., & Kumar, P. (2019); Kumar, P., & Pathak, S. (2018); Kumar, P., & Dwivedi, P. (2018); Kumar, P., & Pathak, S. (2018); Kumar *et al.*, 2018; Kumar, P., & Hemantaranjan, A. (2017); Dwivedi, P. & Prasann, K. (2016). Kumar, P. (2014); Kumar, P. (2013); Kumar *et al.* (2013); Prasann, K. (2012); Kumar *et al.* (2011); Kumar *et al.* (2014), therefore highly decreasing probability of unwanted linkages. Researchers were developed new mapping populations as chromosome substitution lines (CSLs) in wheat (Knott 1987; Campbell *et al.*, 2003; Campbell *et al.*, 2004) and cotton (Ren *et al.*, 2002;), as ILs in tomato (Eshed and Zamir 1995) and Rapeseed (Howell *et al.*, 1996), as CSSs in Arabidopsis (Koumproglou *et al.*, 2002), as CSSLs or ILs in rice (Doi *et al.*, 1997, Kubo *et al.*, 2002), recently as RCSLs in *Hordeum vulgare* (Matushita *et al.*, 2003).

There are several crops where alien genes from closely related species as well as from more distant relatives have been successfully transferred to crop species through introgression and chromosome manipulation. These transfers are possible at:

- (i) Genome substitution for the production of amphidiploids (*Raphanobrassica*, Triticale, etc). Cytoplasm from alien species are magnificent source of CMS (Virmani *et al.*, 1983). Cytoplasmic male sterility lines are formed by crosses between female × male, here alien species is female and cultivar is male. Proceeded by a backcross method of recurrent to remove the unwanted characters contribute by the female. Alloplasmic line is modified copy of the male. It is very tough to produce alloplasmic lines.
- (ii) Chromosome substitution lines (CSLs): - CS is the replacing of one or more chromosomes by others (totally or partially homologous) from another source (either a different strain of the same species or a related species that allows hybridization) by a suitable crossing programme. Chromosome of another species replace the own chromosome of a species is known as alien substitution. It is a powerful complement to existing resources for studying multigenic traits; one of the major application of cytogenetics to plant breeding is the ability to transfer alien genes (wild species) to cultivated species through wide hybridization and chromosome manipulations. There are several crops in which chromosome substitution lines were used for developing new varieties resistant to disease, pest, early maturity, etc., viz., Cotton, *Beta vulgaris*, *Brassica rapa*, Rice, Wheat, Barley, Oats, Tobacco and Tomato etc.
- (iii) The level of chromosomal segment was replaced in the producing of terminal and intercalary locations.

Abbreviations

ILs: - Introgression lines
CSSs: - chromosome substitution strains
CSSLs: - chromosome segment substitution lines
MSA: - Marker assisted selection
BC: - Back cross
RCSLs: - Recombinant chromosome substitution lines
CMS: - Cytoplasmic male sterility
BAC: - Bioinformatics analysis uses the published sequence.
PAC: - P1-derived artificial chromosome
BILs: - Backcross inbred lines
NILs: - Near-isogenic lines

2. METHODOLOGY

A normal procedure outlined for Development of chromosome substitution lines.

1. **Selection of host and donor parents:** A plant material has been chosen for chromosome substitution.
2. **Crossing and construction of CSLs:** Particular breeding scheme should be followed to obtain CSLs (Fig 1).
3. **The choice of polymorphism markers:** SSR markers were selected and used as polymorphic markers and they further used for MAS in the procedure of developing CSLs and genotyping of the CSL population.
4. **DNA isolation, PCR amplification and marker detection:** Tender leaf tissue is taken from 2 parent line and every individual from BC. According to the CTAB method kept these samples at -80° C. By the using nucleic acid detector set the concentration of Deoxyribose nucleic acid and diluted to $5\text{ng } \mu\text{l}^{-1}$ for Polymeric chain reaction. Further polymeric chain reaction has to be run for DNA amplification and marker detection.
5. **Estimation of CSL length:** “Graphical genotype” method is used to judge the value of Substituted chromosome segment length estimation in CSLs (Young et al., 1989) (Figure 2). ‘A’ letter represents the back cross parent genetic constitution and letter ‘B’ for contributor(donor) genetic constitution. “L” letter was represents the length of CSL extent. Chromosome maximum and minimum length indicated by L_{max} & L_{min} . The segment of chromosome driven by 2 genetic markers of contributor genetic constitution (BB) are treating as hundred percent contributor variety, The segment of chromosome driven with 2 molecular markers of back cross parent genetic constitution (AA) are treating as zero percent contributor variety. The segment of chromosome driven by one molecular markers of contributor genetic constitution and the segment of chromosome driven by one molecular markers of back cross parent genetic constitution (AB) are treating as fifty percent contributor variety and $L = (L_{\text{max}} + L_{\text{min}})/2$ this formula is used to compute it (Fig 2).

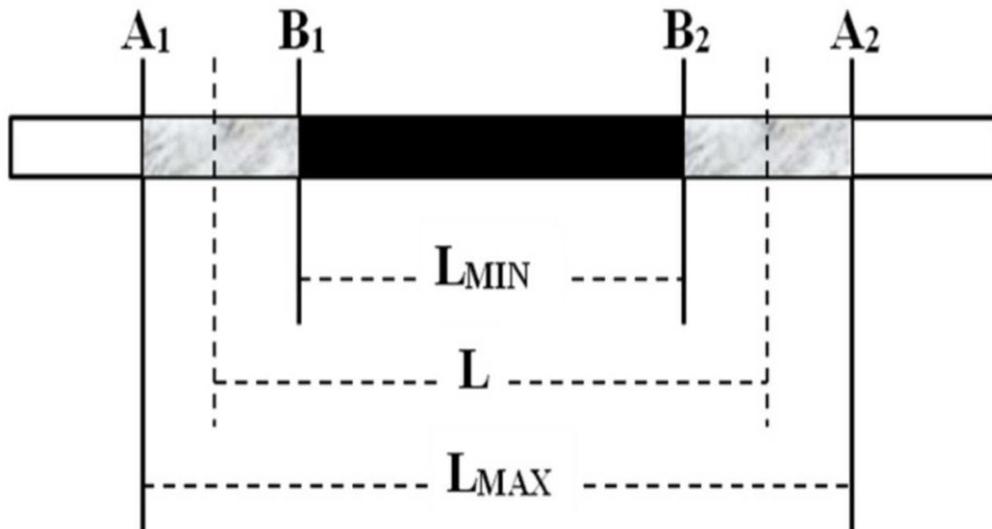


Fig. ii CSL length computing.

6. **Agronomic traits measurement:** Data were collected for donor and recurrent parent for all of the agronomic characters of CSL line and data were analysed for mean, additive and dominance gene effects, and combining ability studies.
7. **Identification and substituting mapping of QTLs:** T- test is helps in the calculating the mean value and quantitative trait locus are recognised by the important variation between the every CSL and parent.
8. **Target gene development based on physical map:** - The substituted segmental genes were developed by BCA & PAC clones used to draw the physical map.

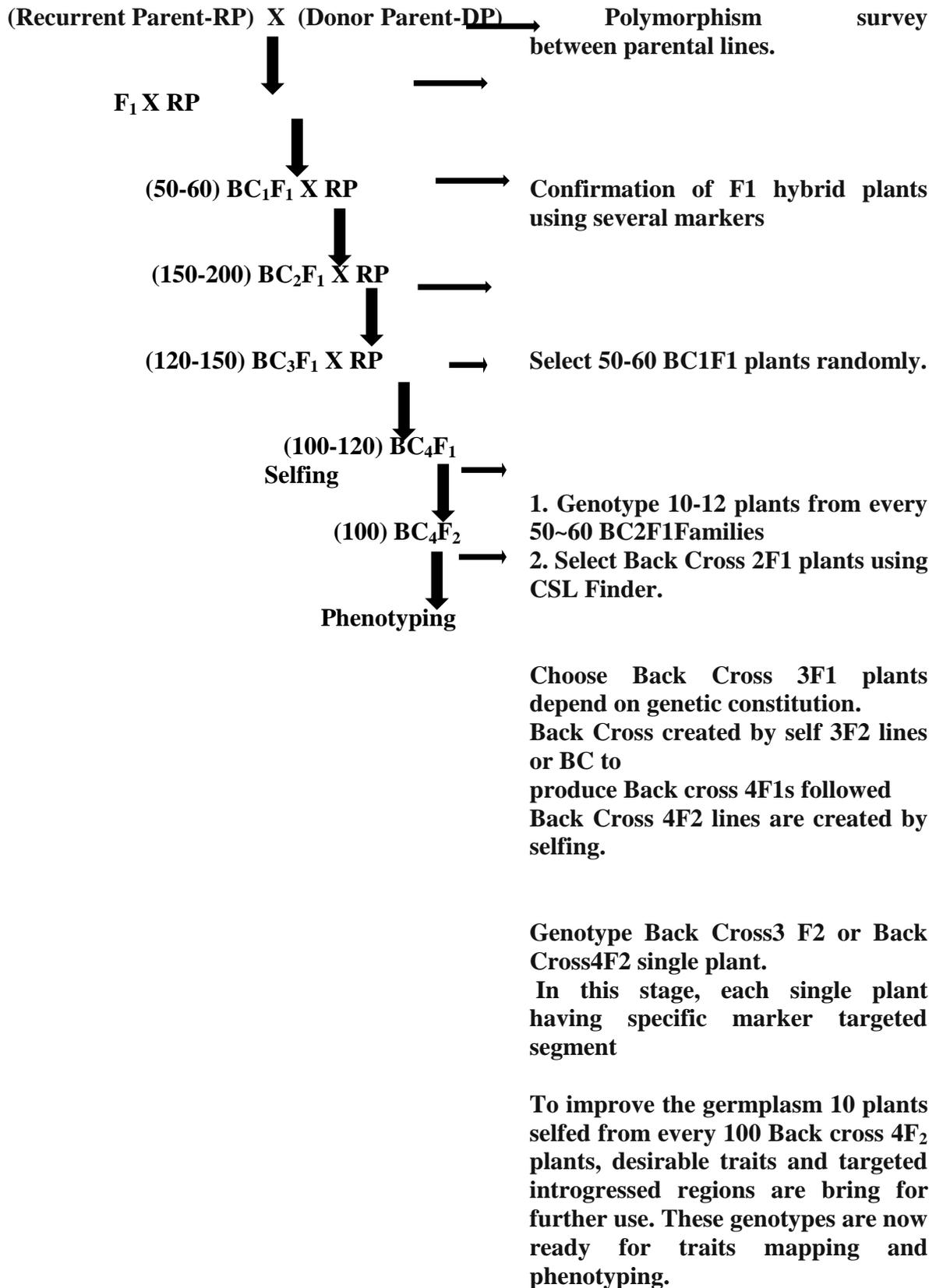


Fig. 1. BC scheme for developing CSL, selecting of every generation of progeny by using the Molecular markers

Development of chromosome substitution lines: example in Cotton crop

By the cytogenetic analyses formerly displayed tetraploid cultivated species like *Gossypium hirsutum* & *Gossypium barbadense* having chromosome no-51 and it is constitution of AADD disomic tetraploid. *Gossypium hirsutum* chromosomes having sub genome-A and sub genome-D designated from 1-13 & 14-26 respectively. If one quantitative trait influenced by one gene of substituted chromosome the possible no. of genetic constitution values observed of 8 traits and thirteen CSLs will be $[8 \times 13] 104$. Over the thirteen CSLs and fifty-six significant values for genotypic effects fibre observed by chromosomal substitution. Effect of 5 chromosomes substitute to the sub genome A and 8 chromosomes substitute to the sub genome D of the chromosomes substitution -B lines reported by (Saha *et al.*, 2004). Sub genome A&D genotypic values are significantly 32.5% (13 of 40) & 71.6% (43 of 64) of the time. Chromosome substitution lines completely reported by (Stelly *et al.*, 2005). Four specific stages involved in CSLs the each alien species:

1. Texas marker-1 line (TM-1) like hypo-aneuploidy stock development.
2. Stock of cytogenetic used as back cross parent with repeated BC programme for producing F₁ substitution stock of monosomic.
3. Euploid disomic substitution lines recover by the inbreeding with Texas marker-1 line like hypoaneuploid stock.
4. Chromosome specific SSR markers and cytological analysis helps in the genotype and cytogenetic of the disomic lines conformation.

At present Cytogenetic stocks collection in cotton consequently closely having the same genetic makeup of Texas marker-1 line. In developing locating of specific chromosome markers monosomic stocks are highly used (White *et al.*, 1965, Guo *et al.*, 2008 & Gutierrez *et al.*, 2009). Translocations of chromosomes identified by the using of monosomic stocks. In specific arm of chromosome for mapping of disease between marker locus and centromere monotelodisomic plants are used (Endrizzi *et al.*, 1985). In cotton conventional methods of introgression interspecific obstructions are many:

- Important characters of complex antagonistic relationship.
- Difference in ploidy level cause the cytogenetics changes in species, Inversion and translocation are structural change of chromosome caused by meiotic
- “linkage drag effects” primarily leads to agronomic qualities sufferin
- Reduction in recombination
- Further generations losses the alien genetic material
- Sterility of hybrid
- Muller-Dobzhansky like complexes genetic interactions
- Deformed segregation (Endrizzi *et al.*, 1985).

Saha *et al.*, 2012 reported the Chromosome substitution lines prospectives:

- i. In upland cotton developing the genetic diversity is one of the important characters.
- ii. To detect the new alleles from other tetraploid species and their potential availability,
- iii. To figure out the composite of agronomic and fibre characters as well as reaction of epistasis,
- iv. To diagnose the location of chromosomal for essential characters like fibre and agronomic.

CSSLs (Chromosome segment substitution lines)

CSSLs used of coding of genetic material describes total genome of any genetic constitution capacity of a cultivar as alternative subdivision. Chromosome segment substitution lines are entire study of ILs with gene compartment generally a wide genetic constitution is modified history and importance of germplasm for essential and enforced

research on development of compound characters. Normally, each chromosome segment substitution line have only one gene compartment from the contributor parent with a maximum back cross parent genetic material improve in the capacity produced by using MAS. Especially, Chromosome segment substitution lines (CSSLs) can be using whereas diagnosing of quantitative trait loci's and fine mapping. CSSL development program need wide or distinct BC breeding and genomic wide MAS followed by selfing before last backcross. Every line of chromosomal segment substitution lines study has a particular marker-defined with broad contributor segment. Chromosomal segment substitution lines are estimates for any addressed phenotype are significantly distinct from their parent genetic constitution. These chromosomal segment substitution lines are used to map QTLs. In mapping of Quantitative trait loci CSSLs are used. Quantitative trait loci mapping helps in the gene interaction and their functions in crop improvement analysis. Wild and distant related species genetic diversity and current varieties broad genetic base occurred due to the pre breeding implements like chromosomal segment substitution lines.

The improvement programme of chromosomal segment substitution lines (Doi et al., 1997 & Kubo et al., 2002), Agronomical traits in plants detected and well explained by the quantitative trait loci mapping (Li et al., 2015). So far, several chromosomal segment substitution lines in *Oryza sativa*, cotton, *Brassica*, etc., For detecting the economical and biological interest there are many improved quantitative trait loci's are present (Takai et al., 2007, Zhu et al., 2009 & Chen et al., 2014). The main example is *Triticale* i.e., W X R substitutions are spontaneous origin, routinely found in nature, here W denotes wheat and R denotes the rye and several extensively grown in European wheat varieties contain mainly the 1RS (1BL) substitution or the 1R chromosome is involved in translocations (1RS. 1BL; 1RS. 1AL) with wheat genomes (Metten et al., 1973; Zeller and Fuchs 1983). These achievements have doubtless where, increasing the understanding of complex characters and developing plant genomic studies (Yang et al., 2016).

Example of CSSLs in *Brassica*: Marker assisted back crossing procedure in 2 *Brassica rapa* sub species (Li et al., 2015). "Chiifu" (*pekinensis* species), "A" genome designating line used by the contributor in *Brassica*. "49 caixin" (*parachinensis* species) receiver is the non-heading cultivar and F₁ derived from back crossing for population improvement and obtained sixty three Chromosome segment substitution lines (Fig 3).

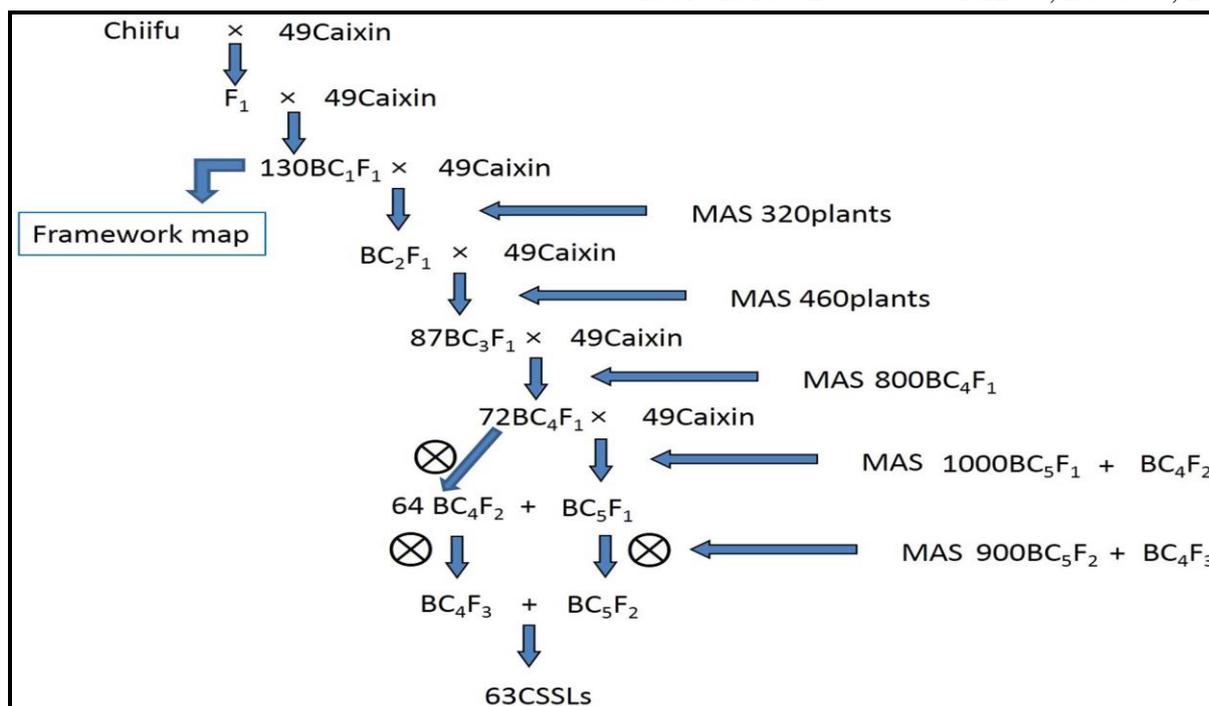


Fig. 3. Breeding procedure of constructing CSSL population

The sixty three CSSLs enclosed total chromosome region of *Brassica rapa* variety “Chiifu” hereditary back ground of “49 caixin,” and provides chance in addition to carry out the action of gene, “Chiifu” allele consequence. The desirable characters determine by the quantitative trait loci’s chromosomal region present in the Chromosome segment substitution lines could allow association mapping approach in natural population to release natural allelic variation. In various environments CSSLs allow us to regulate new QTLs for other composite quantitative traits.

Applications

1. In cotton, transferred gene for black arm resistance from *G. barbadense* (Egyptian cotton) to *G. hirsutum* (American upland cotton), the interspecific hybrid is fertile, and upon backcrossing to *G. hirsutum* produced resistant to black arm.
2. In tobacco, the transfer of necrotic type of mosaic resistance from *N. glutinosa* (n=12) to *N. tabacum* (n = 24) and the amphiploid was crossed with *N. tabacum* for resistance in successive backcrosses, leading to development of a resistant variety ‘Samsoun’.
3. In oats, a chromosome from *A. barbata* carrying gene for resistance against powdery mildew, was substituted for each of four different chromosomes of *A. sativa* (cultivated oats).
4. In wheat x rye translocation is 1B. 1R, chromosome which carries disease resistance and is present in several wheat cultivars around the world.
5. In rice, Disease and insect resistant genes are transferred from *Oryza officinalis*, *O. longistaminata* to *O. sativa*.
6. In soybean, 1st time produced for the inter sub generic fertile plants of hybrids by crossing with *Glycine max* wild perennial species *G. tomentella*.
7. In humans, chromosome substitution has been used to study the genetic control of an extraordinary resistance to testicular cancer, sex determination, anxiety, metastasis, pubertal mating and mammary tumour progression.

Achievements

Receiver	Contributor	Course of action	Heredity Transferred	Writer
Oat	Wild oat	Translocation	Resistance of Mildew	Aung and Thomas (1978)
Rapeseed	Black mustard	Substitution	Blackleg	Chevre et al., (1996)
Asian rice	Longstamen/red rice	Homoeologous pairing	Bacterial blight	Brar and Khush (1997)
Asian rice	<u>Oryza officinalis</u>	Homoeologous pairing	Brown plant hopper	Brar and Khush (1997)
Bread wheat	Rye	1B(1R) Substitutions and translocations	Resistance of Powdery mildew	Mettin et al.,(1973); Zeller (1973)
Bread wheat	Tall wheat grass	Substitution	Wheat streak mosaic virus	Larson and Atkinson (1973)

3. CONCLUSION

Chromosome substitution lines helps in making of superior cultivars by using the genes in broad level genomes and ILs through distance genotypes helps in the genetic base improvement in the commercial and staple crops extensively. CSLs with narrow homozygous marker defined region with high contributor introgressions developed homozygous sub divisions of MSA. The purpose of CSLs are discovery of genes and altering of BC and selfing. The significant lines helps in the discovery of genes and their mapping. CSL helps in improve cultivars from phenotypically important CSLs which evaluated from multiplication traits for new variety released in this way these are developing the agriculture. In breeding programmes pre breeding and CSLs developing are important components. ILs have long term expanding collection and utility of genotypes in crop improvements. The role of CSLs and ILs very clear in development of agriculture.

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