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Haploids Induction Mediated Through Genome Editing

Rapid generation of homozygous lines in genetical studies and crop improvement programmes are aimed for the stability to study the expression of traits and selection of superior lines in segregating populations for the development new crop variety. Functional genomics studies involving T DNA tagging, insertional mutations, CRISPR-Cas 9 mediated genome editing is being utilized for identifying the mechanism of gene actions. Homozygous lines will be having stable expressions and opted materials for transcriptomic, metabolomics and structural genomic studies. Rapid development of mapping populations *viz.*, recombinant inbred lines, near isogenic lines and homozygous lines are necessary for structural genomic studies. Homozygous lines respond well to nutrient uptake studies, plant pathogenic interaction studies, and abiotic stress tolerances. In vivo methods like MATL and CenH3 mediated haploid inductions are genotypic independent and easy system to generated larger number of stable DH lines for crop improvement and genetical studies.

Evolution of new varieties through crop improvement programmes follow different methods involving selection of parents, evaluation, identification of traits and bringing the desirable traits into one genetic background followed by screening of segregating populations under specific conditions repeatedly till the attainment of stable homozygosity. After attainment of homozygosity, stable performing lines need to undergo several trials for their performances across the locations, and seasons to be released as new variety. Station trials (Average Yield Trials, AYT to be conducted for consecutively three years or three seasons based on the crop flowering habit), Multi location trials (MLT: Pre-release cultures should be evaluated in different geographical locations for adaptability,

adaptive research trials (ART: should be conducted in farmer's fields by the officials of Dept of Agriculture, and evaluated by following farmers practices and feedback from farmers is taken into consideration), and on farm trials (OFT: Breeders evaluate the pre-release cutures in farmers field under their supervision and scientific interventions) have to be conducted for minimum period of 3-5 years depending upon the life cycle, duration, photo sensitive nature of crops. Economically important pulse crops like red gram, chickpea and horse gram are photo and thermos sensitive. They can be grown only under short day conditions which coincide during November-December in India. Rapid generation advancement by induction of flowering by artificial lighting and temperature requires huge investment cost and most of the times it is a tedious and laborious processes for standardization for artificial flower induction.

Perennial crops like fruit trees and plantation crops are open pollinated and they longer gestation cycle. It is practically impossible to develop true breeding lines by selfing. Open pollinated parents are crossed and the progenies are selected for traits of interest. Upon identification of superior genotypes, they are fixed by vegetative propagations i.e., mango, coconut, tea, coffee and medicinal plants. Hybrid vigour is function of divergent parents and homozygosity, hence it is necessary to develop homozygous lines in any crop for increased yields and precise transfer of traits by combination breeding. Vegetative propagated crops like potato, sugarcane, tapioca and banana are clones selected by crossing with desirable open pollinated parents either through poly cross and parental combinations which always give complexity in genotypes which hinder the selection processes. Homozygosity development is a main objective for crops like sugarcane, coconut, potato, coffee and tea to have true breeding lines to be used in genetical studies and hybridization progammes.

Rapid development of homozygous lines have been routinely used in some crops through culture of gamete cells *viz.,* microspore cultures and ovule cultures and intact reproductive organs like another culture through in vitro techniques. Somatic embryogenesis was induced in anthers and regenerated plantlets undergo doubling chromosomes by artificial treatments or natural doubling. But callus induction from anthers or microspores or ovule is highly genotypic specific in economically important crop plants, regeneration percentage is also not higher and it was not reproducible. Cultural conditions, combinations of auxins and cytokinins, (2,4-D, IAA, NAA, BAP, ABA, GA3) with concentrations of plant growth regulators, genotypes of crop plants and pre and post treatments of explants and callus decide the callus induction, and from embryogenic callus, somatic embryogenesis is induced followed by plant regeneration from somatic embryos. Most of the times, successful regeneration of haploid plants through *in vitro* is not having higher percentages. Higher percentages of haploids regeneration are required for developing highly divergent inbred lines from open pollinated crops and higher rates of haploids regeneration from the crosses involving divergent parents for identification of superior recombinants to be screened for varietal identification. Mere haploids regeneration from crop varieties will not be used in genetical studies and varietal development. Higher percentage of haploids regeneration is required for stability and divergent analysis, especially for mapping populations. Genetic variability should be normal distributed but genetic variability of haploids through in vitro cultures always follows skewed distribution. It affects the precision of mapping also not successfully utilized in varietal development programes.

In vivo haploids induction was successfully utilized in crops *viz.,* barley and maize. *Hordeum bulbosum* technique has been successfully utilized in barley, F_1 crosses are routinely crossed with bulbosum species and during the processes selective elimination of paternal genome takes place and producing haploid progenies of maternal genome.

The first report on in vivo induction of haploids in maize was shown in maize as *intermediate gametophyte 1* (*ig1*) mutant. It was spontaneous mutant identified in the inbred line Wisconsin-23 (W-23) with paternal haploid induction rate of 3%, when *ig1* was used as female parent. Paternal haloids contained cytoplasm of female parent (haploid inducer *ig1* containing line) and genomes of pollen parent. Maternal haploids also induced at lower frequency of 0.1%. The haloid inducing ig1 has been utilized in maize to develop parental haploids to transfer cytoplasm and chromosomes from selected lines (Ren *et al.,* 2017). Mechanism of haploid

induction by *ig1* is still unclear amidst molecular cloning and characterization. Hence it is having very limited application only with maize and could not be reproduced in other crop species.

Spontaneous haploids induction was discovered in a maize line and popularly known as Stock-6 and it has been routinely utilized in inbreds development programmes in corn with stable induction frequency of 2.3-3.2% (Coe, 1959). Further the cross between Stock-6 lines and *ig1* haploid inducer lines showed the haploid induction rate of 6-17% and *ig1* gene responsible for HI and incorporated in haploid inducer lines like RWS, UH400, MHI, and PHI (Hu *et al.,* 2016 and Prigge *et al*., 2012).The haploid induction phenomenon of Stock-6 derived lines was identified as QTL present in chromosome number 1 bin 1.04 and based on the function of this gene, it is named as *gynogenesis inducer 1* (*ggi1*). Mechanism of HI is due to incomplete penetration and segregation distortion of ovules which lead to lower rate of transmission via male gametes (Barret et al. 2008). Further studies revealed that haploid induction by Stock-6 derived lines are regulated by different QTLs were identified and characterized *viz., qhir1* (Prigge et al., 2012), *qhir8* (Liu *et al.* 2015; Prigge et al. 2012). *qhir11* was found to cause segregation distortion and kernel abortion which lead to haploid induction to complete the life cycle, as an adaptive mechanisms of plants(Nair et al. 2017). Further studies revealed that a patatin line phospholipase located in *qhir11* was responsible for HIR in stock-6 derived lines. The phospholipase was named as MATRILINEAL (MTL), PLA1(PHOSPHOLIPASE A1) and NOT LIKE DAD (NLD) by different groups (Gilles et al.2017; Kelliher et al.2017; Liu et al.2017). The genes MTL/PLA1/NDL contain a insertion of four bp with in the fourth exon results in premature truncation of 29 amino acids. *MTL/PLA1/NDL* mutants has the characteristics of segregation distortion and kernel abortion (Gilles et al.2017;Kelliher et al.2017;Liu et al.2017). CRISPR-Cas9 deletion lines of first exon of Stock-6 lines through transcription-activator-like effector nuclease (TALEN)-mediated target mutagenesis created HIR of 4-12% (Kelliher *et al.,* 2017). Phosphorylation of target membranes for kinase activity involved in signal transduction during fertilization is proven. The possible assumptions for haploid induction by MTL/PLA1/NDL i) failure of fertilization while zygote identity is activated in the egg cell resulting in haploid induction via parthenogenesis/ endosperm identity in the central cell resulting in defective endosperm followed by kernel abortion, ii) by post zygotic uni-parental elimination of the parental genome. But exact mechanism of haploid induction phenomenon of stock-6 derived lines and *MTL/PLA1/NDL* mutant lines is not being proven mechanistically and research studies have been initiated to unravel the exact mechanism.

Generation of haploids via induction of parthenogenesis /BBM triggered maternal haploid induction was studied by Ronceret and Vielle Calzada, 2015). Generation of haploids through interspecific hybridization involves uniparental genome elimination due to intergenomic parental conflicts (Riddle and Birchler, 2003). Uniparental elimination of genomes has been identified in more than 100 species combinations including 75 and 26 examples for mono and eudicot species respectively (Ishii et al. 2017). Haploids in wheat can be induced by pollination of wheat with pollens of *H. vulgare, Z. mays, Coix lachrymajobi, Teosinte, Trypsacum dactyloides, Pennisetum glaucum, Imperata cylindrical* or *Sorghum bicolor* (Ishii *et al.,* 2016). The classical *bulbosum* technique followed by embryo rescue in barley resulted in induction of haploids with frequency of 30% (Kumlehn, 2014). Centromere Histone 3 variant of derived from *H. vulgare* and *H. bulbosum* showed uniparental elimination (Sanei *et al.,* 2011). It showed the involvement of defective CENH3 on elimination of uniparental genomes.

In Arabidopsis, *CENH3* mutant was developed through transgenic approach (targeted mutagenesis) and fused with GFP for tracking resultant "GFP tail swap" complements the null protein TILLING mutant. When *CENH3* tailswap was used as maternal parent, the haploid induction rate was 25-45% and when it was used as pollinator, the haploid induction rate was 5%. Haploid induction line should always be a stable maternal parent and the targeted genome of elimination should be male parent to avoid regular genetic modification of F_1s with *CENH3* (Ravi and Chan, 2010). Based on CENH3 mutants, haploids have been routinely developed in other crops. Arabidopsis thaliana with haploid induction percentage of 2.5% by *CENH3* modification through targeted TDNA interruption (Kuppu *et al.,* 2015). In Maize, *CENH3* modification by RNAi induced haploids of 2.4% (WO2017 KEY GENE). In Tomato, K9E induced by EMS produced haploids of 2.3% (WO2017 KEYGENE).

In japonica rice, haploid induction by modifying CENH3 through EMS induced *CENH3* with different lines had the HIR of 0.3, 0.7 and 1.0 % respectively. Recently, series of mutations of OS*MTL* in rice induced haploids of 2-6.0%. It is based on *ZMMTL* approaches where frameshift mutation caused premature termination of proteins leading to kernel abortion and segregation distortion. *MTL* related sequence present in rice was targeted using genome editing approaches (Yao *et al.,* 2018).

ADVANTAGES OF IN VIVO HAPLOID INDUCTION METHODS

1. Haploid inducer lines are stable lines and can be maintained by crossing them with respective non-lethal alleles carrier genotypes.
2. They can be routinely utilized in haploids induction programmes and totally independent from genotypic background.
3. Double haploidy occurs in higher percentages and does not require artificial induction of doubling which is again time consuming.
4. It is a routine crossing procedure; hence it is simple, easy and timely method.

CONCLUSION

Release of new varieties for the cultivation is a continuous process and always subjected to changes. Breeding goals need not be a stable one and it is highly influenced by the climatic changes, consumer preferences and marketing facilities. Breeding efforts need to be intensified to be suited to particular geographical areas for yield maximization and marketing feasibilities. Hence, development of homozygous lines from crosses through induction of haploids by crossing them with haploid inducers will be a cheaper and stable method having wider and huge potentials in near future breeding programmes.

REFERENCES

Kelliher T, Starr D, Richbourg L, Chintamanani S, Delzer B, Nuccio ML, Green J, Chen Z, McCuiston J, Wang W, Liebler T, Bullock P, Martin B (2017) MATRILINEAL, a sperm-specific phospholipase, triggers maize haploid induction. Nature 542:105–109.

Kuppu S, Tan EH, Nguyen H, Rodgers A, Comai L, Chan SW, Britt AB (2015) Point mutations in centromeric histone induce postzygotic incompatibility and uniparental inheritance. PLoS Genet 11:e1005494.

Ravi M, Chan SW (2010) Haploid plants produced by centromeremediated genome elimination. Nature 464:615–618.

Ravi M, Marimuthu MPA, Tan EH, Maheshwari S, Henry IM, MarinRodriguez B, Urtecho G, Tan J, Thornhill K, Zhu F, Panoli A, Sundaresan V, Britt AB, Comai L, Chan SWL (2014) A haploid genetics toolbox for Arabidopsis thaliana. Nat Commun 5:5334. https ://doi.org/10.1038/ncomm s6334.