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DIPLOID F_1 HYBRID TPS POTATO BREEDING - PIPELINE AND PROSPECTS

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ABSTRACT: Potato is the third most important food crop worldwide. Till date, the potato breeding has been carried out at the tetraploid level, which hampered the genetic studies to decipher the trait genetics. Diploid inbred based hybrid breeding in potatoes provide the opportunity to take advantage of modern breeding methods, tools and genomics resources. The first step in the diploid inbred based hybrid breeding strategy involves identification of elite diploid lines and di-haploids of adapted varieties, followed by introgression of self-compatibility gene (*Sli*) for advancing the lines through selfing for few generations. A number of inbred lines from different lineages with more than 95% homozygosity will be generated through selfing. The best heterotic hybrids will be selected through evaluation of inbred lines for general and specific combining ability for tuber and quality traits. Finally, the best hybrid combinations will be evaluated along with commercial tuber-based tetraploid varieties for comparative analysis. Although, the methodology of F1 diploid hybrid TPS based potato breeding has several advantages over conventional tetraploid tuber based approach, a number of challenges need to be overcome to bring this technology at the forefront in the farmers field.

Keywords: Diploid potato, Inbreds, *Sli*, Heterosis breeding, F_1 hybrid TPS

INTRODUCTION

Potato is propagated clonally through tubers to maintain the purity of cultivars due to high heterozygosity and polyploid genome. The true potato seeds (TPS) i.e. botanical seed could not be used for propagation of varieties due to genetic non-uniformity i.e. each TPS is genetically different. TPS however is easy to maintain, easy to transport, easy to store, amenable to genetic manipulations and is free from diseases and pests inoculums (Sood et al., 2020). Potato breeding through inbred diploid hybrid TPS is a completely new breeding method (Lindhout et al., 2011), being adopted in all potato growing countries including India (Sood et al., 2021). The new diploid hybrid breeding technology works by sexual propagation in each generation like cereal crops. The method is based on identification, evaluation of diploid cultivated species and di-haploids of *Solanum tuberosum* for selection of desired clones with acceptable tuber and other plant traits. Diploid potatoes are naturally self-incompatible. *Sli* is the key gene for self-compatibility in diploid potatoes and now offers a path forward for the inbred-based diploid F1 hybrid potato breeding program (Ma et al., 2021). First identified in *Solanum chacoense* clones 'chc 525-3' by Hosaka and Hanneman (1998), the gene has been recently fine mapped and introduced in diploid tuberosum lines for inbreeding and generation of inbred lines (Eggers et al., 2021). Different sources of self-incompatibility identified in other species also have been found to be regulated by the *Sli* gene (Clot et al., 2020). Recent studies have unraveled the structure and function of the *Sli* gene which has been narrowed down to a region of 12kb on chromosome 12 (Eggers et al., 2021). It was further found that the *Sli* gene encodes an F-box protein which is expressed in the pollen of self-compatible clones only.

The introgression of the *Sli* gene in self-incompatible diploid clones made inbreeding

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possible in hitherto self-incompatible lines. However, the accumulation of deleterious genes in the recessive phase over a hundred years of clonal selection in cultivated tetraploid potatoes is another major hindrance in inbred lines development. High inbreeding depression is evident from decreased fertility and vigour in selfing generations. To overcome the problem of deleterious genes, Zhang et al. (2021) suggested the genome design pipeline for the development of inbred lines where they emphasized to use of the starting material with low heterozygosity and few deleterious mutations. To identify inbred lines with beneficial alleles, the genetic analysis of S_1 inbreeding generation was carried out to identify beneficial alleles and purge large effect deleterious mutations. The diploid inbred lines selection following this breeding pipeline would be highly homozygous and remain true to type on selfing. The inbred lines originating from different lineage could be crossed to develop F_1 hybrids TPS for evaluation. This sexual crossing of the two inbred parent lines results in thousands of true potato seeds per plant instead of only a few potato tubers. So multiplication of a new variety can occur much faster. Since the offspring of a sexual cross is a pristine true seed, these are completely free of diseases and therefore make excellent seed material for potato growers around the world.

Diploid inbred F_1 hybrid methodology

Diploid F_1 hybrid potato breeding, and producing varieties from true potato seed, has been getting a lot of attention worldwide. Since the potatoes have been multiplied from tubers and people are unaware of the botanical seeds-based hybrid system, the basics workflow of the development of diploid inbred lines and hybrid TPS is explained below (Fig. 1).

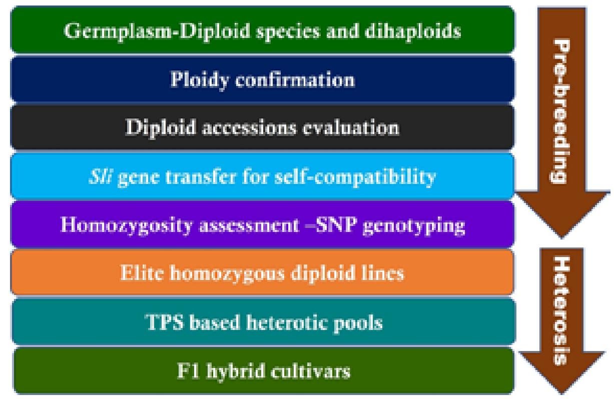

Fig. 1: Work flow of inbred based diploid hybrid potato breeding

Diploid germplasm lines: The first and foremost requirement of diploid F_1 hybrid potato breeding methodology is elite diploid germplasm lines in the cultivated background. To do so the tetraploid cultivated potatoes are crossed with haploid inducer lines (HI) and di-haploids are developed, which originate from the female parent only i.e. we get exactly half the genetic constitution of tetraploid variety. The di-haploids are weak and mostly self-incompatible. The weak plants with deleterious alleles are removed and strong plants will be selected and maintained.

Self-compatibility and inbreeding: The healthy plants will be made self-compatible by introgressing the *Sli* gene from elite *Sli* donor lines to fix the genetic constitution upon selfing in inbred lines. The diploid lines will be selfed for few generations to achieve >95% homozygosity. Robust, vigorous and fertile plants are selected in each selfing generation for advancement to the next generation to accumulate beneficial alleles in inbred lines.

Heterosis: Selfing will generate many inbred lines originating from different di-haploid parents i.e. lineage. The inbred lines from different lineages will be evaluated for general and specific combining ability and hybrid vigour for tuber yield, quality and processing traits. The best hybrid combinations will be evaluated along with commercial tuber-based tetraploid varieties for comparative analysis.

Advantages

- The technology offers numerous advantages over tuber-based potato breeding and cultivation methodology.
- The seed requirement will be drastically reduced due to TPS being the propagation material. Transport will be very easy to far off locations.
- The degeneration of tubers due to virus accumulation will not be a problem anymore. Less than 5% of the pathogen are seed-borne which means the significantly reduced transmission of many of the pathogens causing diseases in potato seeds.
- Genetics will be simple at the diploid level and it will become easy to track and map genes for important traits in potatoes, which at present is difficult to do due to tetraploid inheritance and heterozygosity.
- The trait stacking will be very easy as backcrossing can be used to introduce the desired trait in true breeding lines without altering the genome of the pure lines.
- The genetic gain will be higher over time due to the sexual breeding cycle and accumulation of desired alleles in inbred lines.

Challenges

- The inbred lines produced to date in diploid potatoes are not completely homozygous for all the loci in the genome, which means the F_1 hybrids may lack uniformity for some traits governed by these loci. This could be a major deterrent to the acceptance of technology.
- The approach involves a complete transformation of potato breeding methodology, hence it will require rigorous evaluation in various agro-ecologies to convince the stakeholders on the adoption of this technology. The trials in African countries have shown at par or less tuber yield of F_1 TPS in comparison to tuber raised tetraploid varieties. At par tuber yield and quality to tetraploid varieties is a must for the adoption of diploid F_1 hybrid TPS technology.
- The TPS is the starting material for growing potatoes, which will require additional time and resources to raise the crop in the nursery and transplanting seedlings in the field. The crop duration of TPS based approach needs to match with tuber raised crop to fit in the cropping system in India.
- Both scientists and farmers are accustomed to growing potatoes from tubers i.e. conducting trials or raising crops. It will be important to standardize the agronomy of TPS-based crop as a complete package and practice of the diploid F_1 hybrid TPS.

Preliminary reports of productive diploid F_1 hybrid TPS and its advantages as an inbred-based crop (Jansky et al., 2016) made it one of the most exciting approaches in potato breeding. The inbred diploid potatoes would allow for systematic genetic studies and incorporation of new genes and traits through back-crossing in these fixed inbred lines. Over time, the identification and stacking of beneficial alleles in inbred lines would show substantial yield gains between defined heterotic groups. The possibility of combining complementary traits from the parents, obtaining heterosis from hybridization of inbred parents, avoiding pathogen load of seed tubers, and facilitating the transport of true seeds leaves no doubt that hybrid varieties will attract a lot of interest across the globe including India.

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