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Application of Double haploid in vegetable improvement

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Abstract

Crop improvement can help us to meet the food requirement and feed for the growing population. The scientific developments made during this period convinced the scientific community about the potential role and tissue culture and haploidy became one of important areas of interest. Haploidy is a valuable tool for applied as well as basic research for improvement in vegetable crops. The successes of double haploid production depend on various factors like flower parts development stage, culture media, genotype, donor parent growth condition and haploid detection methods. Double haploid help for developing homozygous inbred line, shortening breeding cycle or time, enhanced genetic purity, efficient population for QTLs mapping, marker development and accelerate marker assisted breeding programme. Hence, DH technology has important contribution in accelerating breeding program in vegetable crops and breaking chain for biotic and abiotic constraints and sustainable vegetable production.

Keywords: Double haploid, Generation advancement, Vegetable crops, Crop improvement

Introduction

Particularly, recent advances in biotechnology represent a valuable and powerful tool to enhance the efficiency and shorten the time required to reach the fixed purposes in a breeding programme, as well as to address economic and ecological goals. Among the biotechnological methods, haploid (H) and doubled haploid (DH) technology has long been recognized as a valuable tool to help plant improvem-

-ent. Haploids and doubled haploids are very important in plant breeding, enabling the time needed to produce homozygous lines to be shortened compared with conventional breeding. The first report of the haploid plant was published by Blakeslee *et al*. (1922) in *Datura stramonium*. Subsequently, haploids were reported in many other species. Guha and Maheswari (1964) developed an anther culture technique for the production of haploids in the laboratory. Haploid production by wide crossing was reported in barley and tobacco. Doubled haploid methodologies have now been applied to over 250 species. Further various treatments were employed for induction of haploids. These included high or low temperature shock, growth regulators and other chemicals, delayed or early pollination, and use of foreign and X-ranged pollen. Success was claimed by various laboratories working on crops like wheat, rye, maize, though the number of haploids remained rather small to have any seal impact. These methods were later supplemented by other e.g., the use special pollinators by Chase (1947) in maize and use of alien cytoplasm's developed by chase (1947) in wheat. Doubled haploids can be produced *in vivo* or *in vitro*. There are two method which are most commonly used for haploid production among which gynogenesis (ovary and flower culture) and androgenesis (anther and microspore culture) are most preferred than other. Androgenesis is the most preferred method for haploid production. Regeneration of double haploid are affected by various factor such as genotypes of explant, growth condition (Temperature is very crucial factor influenced on regeneration), stage of pollen development (microspore culture should contain microspore at mid to late uninucleate stage most preferred in Cole crops) and media composition. High sucrose level may play an osmoregulatory role during induction, but it is not necessary or even detrimental during embryo development.

In anther cultures of paprika and eggplants the inclusion of maltose in the induction medium improved the induction of microspore embryogenesis and raised the number of regenerant plants. Plant growth regulator (PGR) and their interaction with the plant's genotype and environmental factors play a crucial role in microspore embryogenesis (ME), controlling microspore-derived embryo differentiation and development as well as haploid/doubled haploid plant regeneration. There are two method, which are most commercially utilized for haploid production are listed below. Haploid induction and their achievement in some vegetable crops are illustrated below table no 1.

Crops	Method	Achievement
Tomato	Anther culture	MS pure lines
Chilli	Anther culture	Haihua
Muskmelon	Anther culture	Double haploid plant
Summer squash	Ovule culture	Haploid plant
Sweet pepper	Anther culture	Double haploid plant
Onion	Anther culture	Double haploid plant
Asparagus	Direct/indirect androgenesis	-
Beta vulgaris	Direct/indirect androgenesis	-
Brassica oleracea	Direct/indirect androgenesis	-
Solanum tuberosum	Direct/indirect androgenesis	-
S. melongena	Direct/indirect androgenesis	-

1. Gynogenesis

The haploid embryo can arise from an egg cell (gynogenesis) or a gametophytic cell other than the egg cell (apogamy) or a male gamete (androgenesis) (Ghuha *et al*., 1966). The observations in *Datura* were soon reproduced for tobacco both in France (Bourgin and Nitsch 1967) and Japan. This was followed quickly by success in the production of androgenic haploids in rice (Niizeki and Oono, 1968). Sipra Guha (later Guha Mukherjee) shifted to anther culture of rice with Dr. M.S. Swaminathan at IARI, Pusa New Delhi (Ghuha *et al*., 1964, 1966). Double haploid is simply and highly attracted by plant breeder because it's simple and one step leads to produced raid homozygous line. Development of homozygous line in cucumber it takes 6-7 generation (4-5 year) instead 1-2 year using Double haploid approaches. The first report of gynogenesis was by San Noem in 1976 in case of barley, but success has been obtained with sugarbeet, potato and onion. Haploid plants generally originate from egg cells in most species (*in vitro* parthenogenesis), but in some cases they may arise from synergids; *Allium tuberosum* and even antipodal cells. Therefore, ovary culture is preferred over anther culture only where anther culture fails e.g., sugar beet and in cases of male sterile lines, ovary culture assumes significance. Kobayashi et al. (1993) proposed ovule culture techniques

for sweet potato and in two of its related species i.e., *I. triloba* and *I. trifida*. The ovules from the former crop could be successfully cultured 5-6 days after pollination, while in the latter two, it was successful in 3-4 days after pollination.

2. Androgenesis

The principle of androgenesis is based on the arresting of the development of the pollen grains (male gametophytes) and forcing them towards a somatic pathway. Anther culture is the main technique for haploid induction in crop improvement (Dutta 2005). The technique has been employed in tomato, potato, onion breeding programs in some countries and some varieties have been developed. Anther culture has been successfully utilized for induction of haploid plant in several vegetable such as Potato, Brinjal, chilli, cabbage, cauliflower and regeneration of super male plant in asparagus plant. One remarkable achievement has been made utilized anther culture one cultivar "Haihua-3" has been developed in chilli.

Genetic of Haploid and Identification

In DH method only two types of genotypes occur for a pair of alleles, A and a, with the frequency of ½ AA and ½ aa, while in diploid method three genotypes occur with the frequency of ¼ AA, ½ Aa, ¼ aa. Thus, if AA is desirable genotype, the probability of obtaining this genotype is higher in haploid method than in diploid method. If n loci are segregating, the probability of getting the desirable genotype is (1/2) n by the haploid method and (1/4) n by the diploid method. Double haploids in potato can be produced from tetraploid genotypes of *Solanum tuberosum* by pollination with the diploid potato sp. *Solanum phureja*. There is various conventional method are utilized for identification of double haploid plant from the mixed population but there is necessary to developed fast and reliable technology for rapid dissection of haploid to maximized breeding efficiency, accelerate genetic gain and faster varietal development. Morphological markers expressed at the embryo, seed or early seedling stages are preferentially used. Recent discovery of molecular marker has been utilized for characterization and identification of haploid breeding line such as AFLP (Amplified Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), SCAR (Sequence Characterized Amplified Regions) or SST (Simple Sequence Repeat), are commonly used for homozygosity testing and assessment of plant origin.

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Double haploid can help for faster genetic gain and rapid genetic advancement for faster varietal development. In conventional plant breeding program, a total of about eight inbreeding (selling) generation is required to get an almost complete homozygous plant (99.2%) with traits of interest. Through double haploid-based breeding, 100% homozygosity for all the traits can be achieved in only one generation. In self-pollinating species, double haploid can reduce 4 generations in a breeding cycle to release a variety (Figure 1). Traditional breeding methods are slow and take 12-15 years for cultivar development. DH line are effectively utilized in cole crops, root crops such as carrot and identification of genetic or mapping of QTLs/genes underlying various targeted traits in vegetable crops and accelerate the development of homozygous inbred lines can be produced in 1-2 years in Cole crops. Most of the economic traits are controlled by genes with small, but cumulative, effects. The double haploids are true-breeding lines, which can be used repeatedly in marker development program and considered best population for QTL mapping. In backcross programme, genes are introgressed from a donor cultivar or related species in to a recipient elite line through repeated backcrossing and hence combination of DH and marker assisted breeding provide a short cut for getting desired genotypes within shorter generation. DH populations are commonly used in bulked segregant analysis, which is a popular method in marker assisted breeding and construction of genetic linkage map, hence this method offers an advantage to use a smaller population for genetic study of quantitative traits. The genetic research in onion has been hampered by large nuclear genome size. In this regard, gynogenic doubled haploids promise several advantages over inbred lines in support of onion breeding programs and genetic studies. Hence DH, has been utilized for development of the stable inbred line with improved quality and yield, quality and resistance to diseases were obtained (Lim et al., 2004). Lofti et al. (2003), suggested an effective method for developing of double haloid line for improved quality and disease resistance. Christensen and Bamford (1943) first reported haploid lines and spontaneous haploid plant in capsicum. On the other side, by using colchicine, haploid plants were produced in cultivars of Capsicum sp. (Toole and Bamford, 1945).

Figure 1. A comparison of a standard time for a cultivar release using traditional and double haploid breeding methods (N.B: The numbers within the bracket is the percentage of homozygosity) (Rahman & de Jimenez, 2016).

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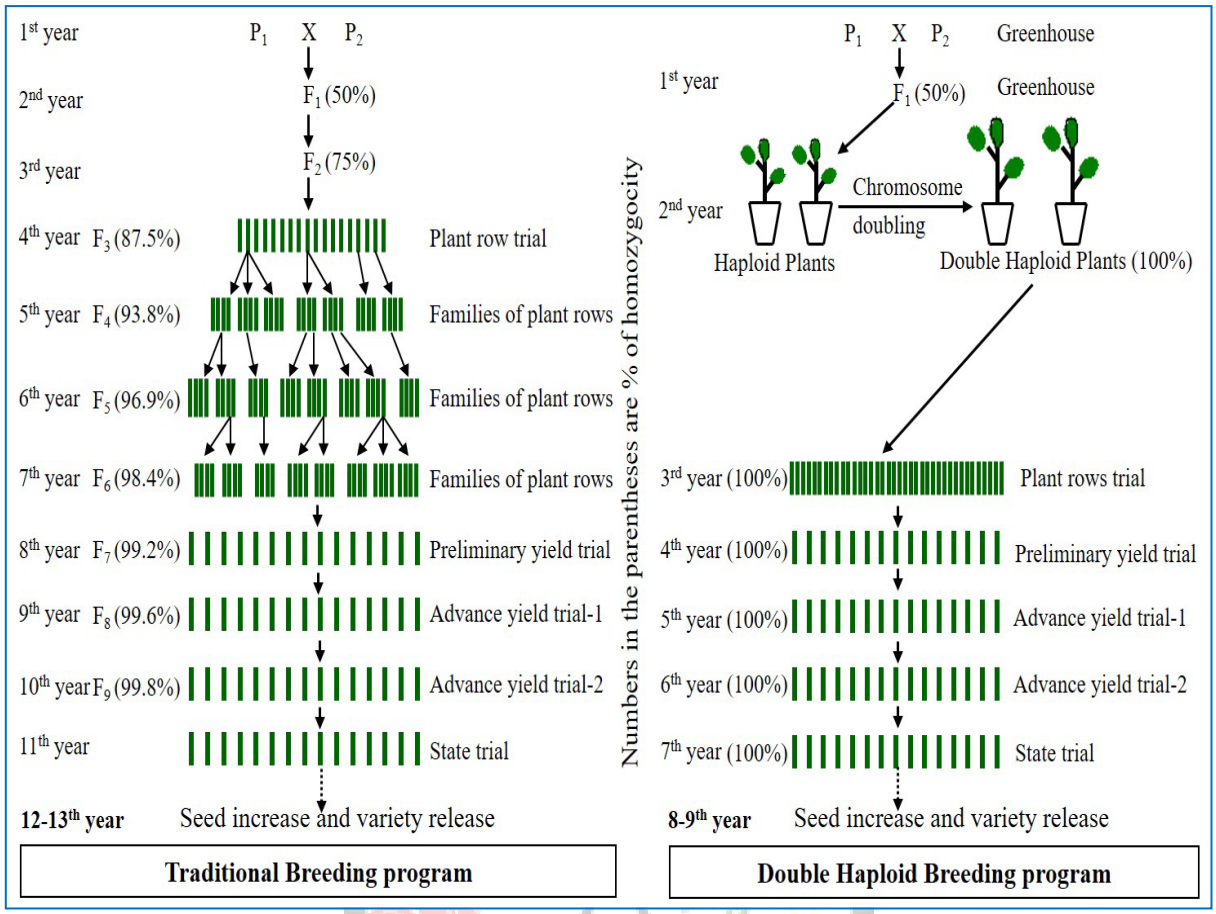

Conclusion

As to conclusion the DH technology plays an important role in the field of plant breeding, genetics and genetic engineering. They help to shorten the generation cycles and production of complete homozygous inbred lines and accelerate various method for crop improvement such as back crossing, genome mapping, QTL mapping, gene identification, gene discovery and transgenic plant development in vegetable crops. Double haploid opens a new way for accelerate breeding programme by creating homozygous inbreed line either by androgenesis in brassicas and pepper, gynogenesis in onion and beetroot, or induced parthenogenesis in vegetables belonging to the family Cucurbitaceae. Androgenesis in brassicas can be induced using both anther and microspore cultures. Hence, therefore necessary to optimized standardized reaeration protocol for generation of DH in several vegetable such as tomato, onion, Cole crop, cucurbitaceous and several other vegetables which have immense economic important to feed for growing population.

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