Advances in Crop Science and Technology

Kumar and Sparjanbab, Adv Crop Sci Tech 2013, 1:4
http://dx.doi.org/10.4172/2329-8863.1000113

Review Open Access

Haploid Breeding in Palms-A Brief Review

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Abstract

Haploids are sporophytic plants with gametophytic chromosome number, and they originate from a single gamete. The chromosome number of haploid could be doubled spontaneously or artificially to obtain doubled haploids carrying two sets of chromosomes from a single parent (homozygous). The important use of haploids is based on the fact that marked improvements in the economics of plant breeding can be achieved through doubled haploid production, since selection and other procedural efficiencies can be markedly improved through the provision of elite true-breeding (homozygous) progenies. Haploids have value in allowing the isolation of mutants, which may be masked in a diploid, particularly where the mutant allele is non-functional. Haploids also have value in transformation programmes. If haploids are transformed directly, then true breeding diploid transgenic plants can be produced in one step, following doubling of chromosomes. Using an integrated approach, including biotechnological tools and conventional methods, it is possible to achieve main goals of crop improvement in short time. Hence, efforts were made to exploit the potential of haploids in breeding of perennial monocots such as palms. The present paper discusses various developments in haploid breeding with respect to economically important palm species.

Keywords: Haploids; Oil Palm, Date Palm, Coconut, Breeding

Introduction

The life cycle of higher plants proceeds *via* alternating stages of sporophyte and gametophyte. The free living sporophyte is the most dominant and obvious life form in these plants. The sporophyte is formed by the fertilization of male and female gametes, and contains a set of chromosomes from each parent and the genomic composition is 2n. Sporophytes with n number of chromosomes could be produced in higher plants which are termed as haploids. In other words, haploids are sporophytic plants with gametophytic chromosome number and they originate from a single gamete. The chromosome number of haploid could be doubled spontaneously or artificially to obtain doubled haploids carrying two sets of chromosomes from a single parent (homozygous).

History of haploids

Dorothy Bergner was credited with describing the first natural haploid in *Datura stramonium* [1]. This was followed by reports of natural haploids in tobacco, as well as wheat, and subsequently, in many other species [1,2]. The breakthrough in haploid research came when Guha and Maheswari [3,4] reported the development of haploid embryos from *in vitro* culture of immature anthers of Datura *anoxia*. This was the first report of inducing a change in normal gametophyte development into sporophyte development and embryo with haploid chromosome number can be obtained. Subsequently, a lot of work was carried out for development of haploid and doubled haploids in higher plants [5,6], through artificial methods. Of late, various researchers directed their attention to perennials including palms.

Use of haploids

Classical breeding and cross pollinating procedures are unpredictable with respect to obtaining recombinants of desirable traits, and also time consuming. Against this deficiency of classical methods, production of haploids is considered as the most prolific and desirable approach of new plant varieties. Hence, the important use of haploids is based on the fact that marked improvements in the economics of plant breeding can be achieved *via* doubled haploid production. This is achieved by markedly improving selection and other procedural efficiencies through the provision of elite true-breeding (homozygous) progenies [7-13]. With doubled haploid production systems, homozygosity is achieved in one generation. Thus, the breeder can eliminate the numerous cycles of inbreeding that is usually necessary to achieve practical levels of homozygosity by conventional methods. Indeed, absolute homozygosity for all traits is not achievable by conventional breeding methods. Consequently, an efficient doubled haploid technology would enable breeders to reduce the time and the cost of cultivar development relative to conventional breeding practices. With respect to merits of haploidy, firstly, such haploids upon chromosome doubling (DHs), are having value in their own right as potential new varieties. Secondly, homozygous plants also have utility for the generation of F_1 hybrid plants, where crosses are made between selected homozygous males and females. These F_1 plants often exhibit hybrid vigour (heterosis), a characteristic often associated with dramatic increases in yield compared with either of the parents, and was first described by Shull [14]. Furthermore, the production of F1 hybrids allows the breeder to produce large quantities of seed comprising of a single genotype from homozygous parental lines. This property will have many advantages over a genetically heterogeneous mix of genotypes because of the potential to select single elite genotypes that produce high yields, and/or posses other desirable characteristics. There is also potential to achieve higher yields by selecting genotypes for adaptation to specific environments, and to optimize agronomic and management practices. In many crops, the only realistic alternative to producing a single genotype in commercial quantities is by asexual cloning. There are well-developed methods of vegetative propagation, using suckers, cuttings or grafts to produce clones for some crops (for example, rubber, cocoa and coffee), but not all crops (for example, oil palm and coconuts).

As in case of self-pollinators, the application of haploidy in cross-pollinated diploid crops is based on the use of DH-lines (Doubled

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Received July 09, 2013; **Accepted** September 25, 2013; **Published** September 27, 2013

Citation: Kumar SK, Sparjanbabu DS(2013) Haploid Breeding in Palms-A Brief Review. Adv Crop Sci Tech 1: 113. doi:10.4172/2329-8863.1000113

Haploid lines). However, owing to inbreeding depression (note: homozygous individuals of a normally out crossing species typically exhibit reduced vigour, and this is known as inbreeding depression), these lines cannot be used directly, but only as parental inbred lines for the production of hybrid varieties. When inbred lines are being developed *via* haploids [7], all barriers to repeated selfing, which are characteristics of natural cross-pollinators, are bypassed, e.g. dioecy, self incompatibility and long juvenile periods. The time saving is particularly apparent in biennial crops, and in crops with a long juvenile period. Inbred lines can be developed in these crops only *via* haploidy [9].

Haploids have value in allowing the isolation of mutants, which may be masked in a diploid, particularly where the mutant allele is non-functional. Haploids also have value in transformation programmes. If haploids are transformed directly, then true breeding diploid transgenic plants can be produced in one step, following doubling of chromosomes. As such, haploid plants (and doubled haploids) reveal all their genetic information, or in other words, their genotype is completely displayed by their phenotype. Doubled haploid plants (transgenic plants) produce viable seed and the desired trait is passed on to successive generations. Some of the genetically determined traits can be introduced into plants by a single gene or possible a small cluster of genes, including insecticidal activity, protection against viroid infection, resistance to herbicides, delay of senescence, tolerance to environmental stresses, improved nutritional quality of plant products and self incompatibility. Resistance to pest and diseases or unfavorable external factors (drought, salinity, heavy metal toxicity etc) can thus be directly recognized and selected. Haploid plants allow the detection of mutants that are unable to pass through the embryonic phases. For similar reasons, haploid plant tissue make ideal vehicles for genetic transformation, by whatever gene manipulation techniques are relevant, to give genetically modified material that upon doubling give homozygous versions of the introduced gene or genes.

The agricultural applications for haploids centers around their capacity for the rapid generation of homozygous genotypes after chromosome doubling, and are listed below.

- Reduce time for variety development, e.g. in seasonal crops, the duration reduced from 10 to 6 years or even less, and the reduction will be drastic in case of perennial crops
- Homozygous recombinant lines can be developed in one generation, instead of after numerous backcross generations; and
- Selection for recessive traits in recombinant lines is more efficient because recessive alleles are not 'masked' by the effects of dominant alleles.
- Introgression of desirable traits/genes speeded by allowing homozygotes to be developed readily.

Using an integrated approach, including biotechnological tools and conventional breeding, it is possible to achieve main goals of crop improvement in short time.

Routes of haploidy

Spontaneous or in vivo haploids and induced or *in vitro* haploid production are the routes of development of haploid plants.

Spontaneous haploids may occur in many species of plants, albeit at low frequencies. For tropical perennial crop species of commercial importance, the following summary is relevant-coffee (reports of spontaneous haploids) [15], cotton (many examples of spontaneous haploids), cacao (spontaneous haploids reported) [16]. In other words, they are rare in occurrence. For this reason, emphasis has turned to alternative means of generating haploids and doubled haploids.

Besides the two important techniques to obtain haploids (androgenesis and gynogenesis pathway) [17], wide hybridization is one of the technique to achieve them [18]. Haploids also can be obtained naturally or spontaneously, but they exist in low frequency. They were few important haploids in plants like barley, wheat, triticale, maize, and rice and rape seed. Doubled haploids or haploids advantages include usefulness in fixing traits combinations that fulfill industrial needs, facilitate hybrid or wide breeding, applied in molecular marker research, and increase the development of mapping populations and trait load, produced better hybrid and improved parental performance in hybrid combinations [19]. Wide hybridization is crossing or hybridizing a crop cultivar with another plant, that is outside its gene pool, and not sexually compatible [20]. This technology has been applied to several Brassicas, barely, wheat, rice, melon, pepper, and tobacco [21,22]. Wan Ibrahim Wan Hassan et al. [23] reported pollen of *Cocos nucifera* have been crossed with *E. guineensis* (Dura) in the efforts to obtain haploids. For screening culled oil palm, germinated seeds were characterized according to their abnormalities, prior to being planted and followed by flow cytometry analysis 12 weeks after planting. Flow cytometry analysis has been used to estimate genome size and ploidy levels, and it is simple, accurate, rapid and convenient method [24,25].

Screening seedlings grown from culled germinated seeds from this study showed 1.36% chances of obtaining potential haploids. However, the seedlings died, which may be due to their own weakness of having half genome size or due to planting conditions. Wide hybridization of *E. guineensis* (dura) with *C. nucifera* has resulted in 10 plantlets, which embryos have been rescued from 15 weeks old fruits, and they are currently being sub cultured in rooting media.

There are now four methods [26-30], generally applicable to the production of haploids in plants at frequencies useful for a breeding programme, and a recent monograph detailed the protocols applicable to haploid and doubled haploid production in a number of species. These methods are:

1. Androgenesis, where cultured anthers or isolated microspores undergo embryogenesis/organogenesis, directly or through intermediate callus.

2. Gynogenesis, where cultured unfertilized isolated ovules, ovaries of flower buds, develop embryos from cells of the embryo sac.

3. Wide hybridization crosses, followed by chromosome elimination from one parent of a cross, usually the pollinating parent.

4. Parthenogenesis, where there is development of an embryo by pseudogamy, semigamy or apogamy.

The general lack of progress towards haploid and doubled haploid production in woody species [31-34] is due mainly to the present emphasis on production methods, involving an *in vitro* phase. There are numerous problems associated with the general intransigence of woody species to growth under such conditions.

Detection of haploids

Haploids of higher plants can be distinguished from their diploid equivalent in many ways. Most obviously from the perspective of

phenotype, they are usually smaller in appearance, partly because of their smaller cell size; in general terms, cell volume in plants is positively correlated to ploidy level. Several methods [35] for the provisional assignment of the haploid status to a plant do exploit this relationship. The most widely used of these phenotypic methods is the measurement of stomatal guard cell length and chloroplast content in these cells, although none of the phenotypic predictors of haploidy is absolutely reliable. Methods providing direct measurements of genome size provide a far more reliable diagnosis of haploid status. These include direct measurement of the chromosome number, using conventional chromosome counting techniques and measurement of the DNA content using micro densitometry [36], or more especially, flow cytometry [24,25,37-39]. The latter technique has also been applied to characterize the cell cycle stages in various tissues in case of oil palm material, although not for the detection of haploid plants or tissues [40]. It is also possible to exploit the absolute absence of heterozygosity in haploids and doubled haploids to detect such plants using various co-dominantly inherited molecular marker methods [41].

Achievements in Palms of Economic Importance

The literature relating to haploid research in palms is limited to date palm, oil palm, and to some extent, coconut and such works are briefly reviewed in this section.

Date palm

Although, studies dedicated to anther and ovule culture recovery are scarce for date palm, attempts under various conditions have led to achieving cell division and to the formation of globular embryos from immature micro spores. Some of the successful attempts with cold treatment, combined with the use of two auxins and one cytokinin, have been proven to be the key elements to generate embryoids [42,43], that unfortunately were unable to develop. Investigations of different treatments and various exogenous factors were successful, when there was a formation of the weak calli surviving only during a short period of time. The main difficulties encountered in such studies were related to the short time flowering period that does not allow, usually having enough fresh anthers with uninucleate microspores. Furthermore, date palm male anthers typically turned brown and died a few weeks after their culture.

Some haploid recovery attempts were made with unfertilized ovules also. Due to the small size of these ovules, browning and necrosis were the main limits encountered by such cultures. Although the carpals enlarged and became quite prominent when cultured, the use of activated charcoal is required to ensure them a much longer survival and roots and callus formation [43]. As on date, the best results ever obtained were from flowers taken from closed spathes, and in which the embryo sacs were formed that contained undifferentiated cells. There is a claim that treatment of unpollinated female date palm inflorescences with gibberellic acid that induced doubled haploid "apomictic" progeny [44].

Oil palm

Oil palm is a perennial monocotyledon, with a long generation period. Hence, breeding of the crop is a very slow process; generally taking approximately 20 years to develop and progeny test a new generation of palms for commercial seed production. There are no reports of breeders producing inbred lines by inbreeding (i.e., eight generations of selfing), because this would take a biological minimum of 40 years to achieve, which is as follows; the time required to make crosses (6 months), process seed (3 months), grow seedlings in nursery (12 months), field plant seedlings (male and female inflorescences will develop after 18-24 months), collect pollen and self pollinate and harvest bunch (24-30 months). Currently, genetic improvement of oil palm is mainly performed by conventional means. Compared to other oil producing crops, which are predominantly annuals, the introduction of novel traits into oil palm is an extremely protracted process; it may require between 12 to 14 years to improve or to introduce a trait into oil palm. In addition to the long generation period, breeding of perennials, such as oil palm, require large areas for breeding trials and an extensive series of time-consuming backcrosses.

Some studies on the development of chromosome doubling techniques in oil palm have already been reported, and whilst these data relate to the doubling of diploid material (to give polyploids), the protocol described will also have utility for haploid doubling [45,46].

The crossing of two homozygous elite lines (like one that can be produced by doubling haploids) can generate genetically uniform, highly heterozygous 'hybrid' varieties. However, to date, there has been no corresponding progress with the highest yielding of all oilseed crops, i.e. oil palm, although oil yields of 4.8-7 t/ha is 3-8 times greater than other oil seed crops [47]. Overall, oil palm is the world's leading source of vegetable oils and fats, on a par with soybean [48], but has nevertheless yet to benefit from the release of hybrid varieties.

The slow or rather lack of progress towards the generation of hybrid varieties for oil palm could be mainly because the breeding system of the crop precludes the simple production of inbred lines. Oil palm is essentially an out breeding species, but unlike corn, in which a male and a female flower are produced on the same plant at the same time, each oil palm plant produces either male or female flowers at any one time, and therefore, a palm can only readily be self-pollinated by methods of controlled pollination using stored pollen. To date, however, there is no published example of any haploid, or homozygous diploid oil palm plant. Nevertheless, there has been extensive breeding [23] cell culture [48-50], and transformation studies (US Application 20030159175) geared towards the genetic improvement of the oil palm crop. Two main species of oil palm plant commercially grown are: *Elaeis oleifera* Kunth and *Elaeis guineensis* Jacq. The latter has three sub-types: Dura, Tenera, and Pisifera. Most cultivars or planted stands are Tenera, which produces fruit with higher oil content.

All oil palm seeds currently used for commercial plantings are produced from parents selected from genetically heterogeneous populations of non-homozygous palms. Variation in the level of parental heterozygosity and in the genetic divergence between parental lines means that there is extensive genetic segregation amongst the resulting seed offspring. Thus, the seeds produced from palm crosses are, therefore, not genetically uniform. This genetic variation impedes the oil palm industry from selecting specific genotypes for high yield or other desirable traits.

Mention should be made of the apparent claim by Maluszynski et al. [51] that Texeira et al. [50] have already published a protocol for doubled haploid production in oil palm. This claim is erroneous. In fact, the latter publication describes somatic embryogenesis from diploid floral tissue, and does not describe the culture of anthers or other reproductive tissue, to produce haploid embryos and plants.

Other palms

The only related work on other palm species include, unsuccessful attempts to produce haploids of coconut *(Cocos nucifera) via* anther culture [52-64] and date palm (*Phoenix dactylifera)* [42,43]. There

is one report of attempt at ovule culture in coconut [38]. However, no haploid plants were produced from any of these *in vitro* studies. Nevertheless, there is a single example of a haploid coconut plantlet isolated from a twin seedling [65], and cytological evidence of a haploid chromosome number (n=16) observed from a single embryo from the same species.

However, none of these publications describe an effective method to produce and select spontaneous haploids or doubled haploids, or provide teaching relevant to the production of haploid or homozygous material of palms.

Conclusion

Haploid research has emerged in the recent as study dealing with fascinating developmental phenomenon in the field of plant breeding. Recent technological innovations, greater understanding of underlying control mechanisms, and an expansion of end user applications has brought about a resurgence of interest in haploids in higher plants. This is evidenced by the publications being brought out recently, whereas in palms, it has met with less success because of its perennial nature. In the future, haploid research will have an enormous impact on traditional plant breeding programmes, including palms, because it can significantly reduce the time taken to develop a new variety against traditional plant breeding techniques. Further, it will also be used to create plants with novel characteristics.

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