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综　述

Chromosome Number Manipulation as Part of Potato Pre-breeding Programs

Kear Philip J, Lu Wenhe*

(College of Agronomy, Northeast Agricultural University, Harbin, Heilongjiang 150030, China)

Abstract: The cultivated potato (Solanum tuberosum L.) is a tetraploid (2n = 4x = 48) and can be improved with the incorporation of desirable traits from other Solanum species. Often the transfer of these traits is hindered by complex genetics and breeding barriers within potato. Parthenogenesis and microsporogenesis are used in chromosome number manipulation allowing breeders to reduce the potato's chromosome number to dihaploid (2n = 2x = 24) [diploid] or monohaploid (2n = x = 12) from which a predictable transfer of traits can be made, in accordance with the endosperm balance number theory (EBN). Furthermore, the reproductive processes of first division restitution (FDR) and second division restitution (SDR) are utilized in order to increase the chromosome number for incorporation into the cultivated potato.

Key Words: Solanum tuberosum L; haploid; polyploidy; EBN; 2n gamete

The cultivated potato (Solanum tuberosum L.) is an autotetraploid (2n = 4x = 48) with tetrasomic inheritance. This makes the genetics and breeding of new cultivars more complicated than breeding at a diploid (2n = 4x = 24) level[1]. The difficulty in analyzing species with tetrasomic inheritance also makes it difficult to analyze even simple inherited characteristics. Furthermore the shortage of pure lines adds difficulty to this genetic analysis[2]. Most cultivars and genetic stock genotypes are genetically heterozygous. However, developments in chromosome number (or ploidy level) manipulation, have lead to important advancements being made.

It was Chase[3], who first suggested the method of 'analytic breeding', for the cultivated potato. According to Chase's method tetraploid cultivars are reduced to a diploid level and the resultant 'dihaploids' are further utilized for breeding potato at the diploid level using diploid wild species[4]. These advancements have enabled breeders to utilize desirable traits from other diploid potato plants[2]. Furthermore, as part of a (pre-) breeding program, manipulation of the chromosome number can make efforts to analyze the genetics of the potato much easier[1].

1 Chromosome Number Manipulation

1.1 Endosperm balance number (EBN)

Although the endosperm balance number (EBN) is considered very important in potato breeding today, prior to proposing the EBN hypothesis things were not so straight forward. Breeders observed that the occurrence of natural hybrids was relatively frequent, most species belonging to the same series cross with little hindrance and meiotic paring in hybrids generally occurs at the same frequency as the original parents. In addition, when crossing took place between different series a marked reduction in recombination frequency was noted[5]. Furthermore, Hermsen, et al.[6] noted a complete male sterility when crossing between the taxonomically separate groups of Estolonifera and Potatoe,

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Biography: Kear Philip J. (1976-), male, PhD candidate. Research area: potato breeding and genetics.

*Corresponding author: whlu@mail.neau.edu.cn

illustrating 'internal incompatibility mechanisms'[7], which appeared to operate between distantly related species, but not between the same species or adjacent species.

Prior to the existence of the EBN theory, it was unclear why some species failed to produce hybrids, even when they were reduced to diploid. However, Den-Nijs, et al.[8] made the first hypothesis on EBN, later to be given more development by other authors[9-10], whom helped to explain why closely related species with apparently homologous genomes failed to produce progeny when crossed at the diploid level. The concept of an endosperm balance number was borne, and today we are able to determine whether or not the ploidy of the embryo and endosperm are compatible, prior to starting a breeding program. In the hypothesis a species is given a value in the endosperm, which may be different for species of the same ploidy. The EBN determines the effective ploidy in the endosperm, which must be in a ratio of 2 maternal: 1 paternal.

Thus, diploid species would have an EBN of 1 or 2[11]. Species with the same EBN number should readily sexually hybridize, but exceptions do exist. In addition, with the production of unreduced gametes in diploids, it becomes possible to make successful crosses between a tetraploid of 4EBN and a diploid of 2EBN tuber bearing species, consequently producing 2n gametes (4x-2x), or alternatively, tetraploids with a 2EBN crossed with a diploid of 1EBN, which will also produce 2n gametes (4x-2x)[12].

1.2 Reduction of ploidy number from tetraploids

Tetraploid S. tuberosum can be induced to form haploids by the use of either male or female organs. These haploids would represent a diploid tubersosum. This can be brought about by means of a pollination mechanism which allows fertilization within the central cell, but not in the egg, allowing the egg to develop by maternal parthenogenesis[13]. The most common method of applying maternal parthenogenesis is by the use of a "pollinator" to hybridize with cultivars, such as a genotype from S. phureja Juz. et Buk. ($2n=2x=24$). These maternal haploids are obtained by the union of two chromosome sets of S. phureja with the polar nuclei of tetraploid S. tuberosum, but lack fertilization of the egg[1]. Thus the pollinator does not contribute any chromosomes to the haploid embryo, and for this reason studies have been undertaken to understand the influence that the 'pollinator' has upon haploid frequency. Peloquin, et al.[14] undertook research into the 'pollinator effect' after having noted a high haploid frequency of 20~40 haploids per 100 fruit. Peloquin, et al.[14] furthermore concluded that evidence from their research supported the hypothesis of two haploid gametes unite with one polar nuclei as the basis of the 'pollinator effect'. The data from this research also supports the endosperm balance number (EBN) hypothesis stating that 2 maternal: 1 paternal EBNs are necessary in the endosperm for normal seed development. It is also possible to assure easy visual selection of haploids by using genetic markers that produce an embryo spot after a 4x-2x cross. The haploid cross is absent of the spot.

It is also possible to have the formation of a paternal haploid through the formation of a microspore. After microsporogenesis has occurred, the most widely applied method to induce microspore embryogenesis is anther culture as detailed below. In the Solanaceous family the process of microspore embryogenesis has been carried out in potato, tomato and pepper. Anther culture can have a key role in the production of paternal haploids by inducing androgenesis, which changes the microspores' process of development, leading to the formation of two identical nuclei in the cells that develop into embryos or calli[2]. Anther culture was first used in the 1980s as a method to obtain paternal haploids via androgenesis. However, now it is widely used as a precursor to the production of dihaploids, diploids and further increasing to polyploids.

1.3 Further reduction to monohaploid

It is possible to further reduce the ploidy level to that of monohaploid or monoploid ($2n = x = 12$)[13]. Uijtewaal, et al.[15] used a diploid S. tuberosum and S. tuberosum × S. phureja Juz et Buk to select for monohaploids. It was argued that because of potatoes' high level of heterozygosity many cultivars "comprise [of] deleterious alleles at many loci", which are fully expressed in monohaploid potato, even if the alleles are dominant or recessive. Thus of surviving monohaploids an opportunity presents itself to select for viable, vigorous plants with good gene combinations. It seems though the weight of scientific thinking would suggest that androgenesis be preferable for the production of monohaploids, with much research being taken out at the Max Planck Institute for Breeding Research in Cologne, Germany in the 1970s. Nevertheless, Uijtewaal, et al.[15] again, suggested that gynogenesis, as apposed to androgenesis also had favor in the production of monohaploids.

The reduction of ploidy allows greater ease when incorporating specific traits with desirable qualities. However, after the reduction of the potatoes' ploidy level to haploid or even monohaploid, it is necessary to increase the ploidy before the plant can potentially be used for potato tuber production.

1.4 Increasing the ploidy number from haploids sexually

Haploids that have been extracted from S. tuberosum can be crossed with other diploid species ($2n = 2x = 24$) producing progeny that are diploid hybrids. Gametes produced from these hybrids are occasionally 2n gametes. Tetraploids are usually produced by either unilateral sexual polyploidization (4x-2x, or 2x-4x) or bilateral sexual polyploidization (2x-2x) crossing. The two most common mechanisms by which 2n gametes are formed in potato are first division restitution (FDR) and second division restitution (SDR) [2]. This mechanism was first observed after trying to obtain triploids from a 4x-2x mating. In most cases instead of the expected triploid being formed, tetraploids were formed. This is referred to as the 'triploid block'.

FDR and SDR are the processes by which most 2n gametes can be formed. Both mechanisms have a valuable place in the improvement of potato, but the ease in which they are used and the amount of general heterozygosity that is transmitted, influence the geneticists' choice. For instance, even though 2n eggs and 2n pollen can generally occur in potato, normally 2n pollen would be more widely observed in some taxa than 2n eggs[16].

Moreover the selection process for pollen is easier than that for 2n eggs. 2n pollen occurrence varies from less than 5% to 80% depending on the genotype[16]. FDR is equivalent to the occurrence of genetic restitution at first division of meiosis and SDR is equivalent to genetic restitution at second division meiosis. During FDR "parallel/ fused spindles" are formed during anaphase II of meiosis, preventing cell division and two 2n microspores are formed. Also, SDR occurs when a "premature cytokinesis", following the first division during meiosis, causes the formation of a dyad of two 2n microspores[17].

Estimates suggest that 80% (FDR) and 40% (SDR) of the heterozygosity is transmitted by a 2n gamete[18]. Thus numerically unreduced gametes (2n) from FDR are considered superior to 2n-gametes from SDR, because they transfer more of the total parent heterozygosity and epistasis intact to the tetraploid progeny[19].

Synaptic mutants provide a very good opportunity for potato breeding. Okwuagwu, et al.[20] proposed the use of synaptic mutant combined with parallel spindles (ps) during sexual hybridization. During microsporogenesis, synaptic mutants affect synapsis, resulting in mainly univalents at metaphase I and high male sterility. Fertile 2n pollen is produced when a synaptic mutant is combined with a ps mutant, increasing the heterozygosity that can be transmitted to the offspring of the diploids. If the synaptic mutant has no pairing and no crossovers (asynaptic), a great opportunity presents itself, to transfer 100% of the heterozygosity and epistasis to the progeny[21].

1.5 Increasing the ploidy number from haploids asexually

Prior to the discovery of the triploid block and subsequent ability to use diploids for sexual chromosome manipulation the more common form of increasing the ploidy number was via asexual manipulation.

Colchicine is used for doubling of the chromosome set in young plants. Chromosome doubling is effective in increasing the homozygosity of tetraploid parents[2].

Somatic (protoplast) fusion has some degree of importance, but its applications can be limited in breeding programs; however, fused protoplasts can be used for rapid propagation and possibly its greatest would be for obtaining hybrids from S. tuberosum and sexually incompatible wild species[5]. The techniques of somatic fusion are normally undertaken by one of two methods; chemically (polycation polyethyleneglycol: PEG) or by electro-fusion. Electro-fusion is more popular due to it being less damaging to the plant cells[5]. Fish, et al.[22] describes the frequent failure of a chemical fusion because the cells cannot tolerate the fusogen. Also, Gilissen et al. [23] reported on the 'production [of] asymmetric hybrids due to preferential elimination of chromosomes in one of the fusion partners', which is found in mammalian fusion experiments. This phenomenon does not frequently occur in plants, however if it were to be used it would be a useful method by which specific chromosomes could be transferred to a new hybrid.

2 Potato Pre-breeding Programs for Germplasm Enhancement

Tetraploid progenies are gained by sexual polyploidization of the parents that form unreduced 2n gametes[2]. By utilizing the aforementioned techniques of chromosome number manipulation it is now possible to incorporate the traits that are required for a specific breeding program.

Furthermore, genes can be selected and incorporated directly into the potato's DNA with the use of molecular biology and comparative genomic techniques, which can also allow the incorporation of specific chromosomal regions for selection of genes of interest. Molecular genetic markers may provide the key by which the potato can be better analyzed combined with a more extensive use of such techniques in breeding programs[24]. The methods of mapping will not be discussed here, but the author refers the reader to Watanabe[25] and Celebi-Toprak, et al.[24] that give more detailed information on techniques and theory of molecular genetics and the usage of molecular markers.

3 Discussion

Although, in terms of actual improvement of the species for cultivation, a huge amount of change has occurred, but when these changes are placed on a time scale, they are very infrequent. This change, initially being due to the short-day/long-day differences between the southern and northern hemispheres, but later due to potato's tetraploidy and tetrasomic inheritance making it difficult to improve the cultivated potato. Thus today with the advent of ploidy manipulation, which was only introduced some 4 decades ago[3], we have a very real tool with which to potentially address the challenges to potato cultivation. Alongside molecular advances which have been taking place, ploidy manipulation can allow breeders to look positively to the future because of the potential that exists. Late blight (Phytophthora infestans) for example, is a fungus which has adapted to avoid continued breeding schemes to produce resistance for nearly a centaury. This type of difficulty in improving the potato has very much delayed potato's progress compared to other food crops. The continued improvement of the potato crop depends on continued improvement in the breeding techniques undertaken by breeders. The use of improved initial generations in a pre-breeding program allows breeders to access a wide range of advanced lines for development of the tetraploid potato. These lines could have resistance to pests such as potato cyst nematode (PCN) or root-knot nematode (RKN); resistance to diseases such as, late blight (LB) or early dying disease (EDD); improving nutritional content such as, starch content or minor element content.

With the continued need to advance the potato for the needs of mankind, increasing the general heterozygosity of potato should be a key area to pursue. Using the reproductive biology of potato, it should be possible to be done with success. Heterozygosity in the potato will give hybrid vigor and more access to possible avenues for further research in the future and also the continued improvement of the techniques by which de-polyploidization and re-polyploidization can be undertaken. Furthermore, a deeper understanding of the areas affecting compatibility between species is necessary. At present this area is covered by the EBN hypothesis, which appears to explain the compatibility between species, but doesn't explain for the exceptions which do occur[26]. Understanding the exceptions may also give better understanding of the reproductive biology of potato.

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