

Heterochromatin Distribution Pattern in Aid of Understanding Plant Species Inter-Relationships

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Commentary

Abstract

Direct staining using Chromomycin A3 (CMA) and DAPI (DAPI) fluorochromes has been widely adopted for studying the heterochromatin in various plant taxa. CMA and DAPI show preferential binding for GC- and AT-rich heterochromatin regions respectively and can divulge the amount, type, location of heterochromatin regions and thus revealing the relative AT- and/or GC-rich fractions in the chromatin. This short commentary documents the efficacy of CMA and DAPI binding pattern in comparative analysis of relative AT- and/or GC-rich repetitive heterochromatin fraction(s), thus giving an insight about species diversification and evolutionary pattern in many plant taxa besides helping in assessment of genetic fidelity in micropropagated plants.

1. Introduction

Cytogenetical information is considered to be informative and powerful tool in deducing species relationships and evolutionary pattern in many plant taxa, even at lower taxonomic levels. Heterochromatin comprises of highly repetitive DNA which is enriched at the centromeric and telomeric regions of the chromosomes. Heterochromatin plays a number of important biological as well as cellular functions and is subjected to rapid evolutionary changes, probably contributing to species diversification due to chromosomal rearrangement and segregation [1,2]. Analysis of the composition and type of heterochromatin can also help in providing clear information on genome constitution such as the repetitive sequence families in different plant species [3]. With the advent of new cytogenetical methods and use of base-specific fluorochromes (CMA and DAPI) have enhanced our knowledge about heterochromatin and euchromatin distribution pattern in the genome. Realizing its multifacet potential, the Plant Biotechnology Laboratory, North-Eastern Hill University, Shillong has been actively involved in studying heterochromatin constitution and distribution pattern in some horticultural, medicinal and insectivorous plants and a few papers have already been published (4 in numbers). In the next few sections, we briefly discuss these aspects with focus on understanding the species relationships in these taxa based on our published data.

In Vigna spp., karyo-morphological studies confirmed 2n=22 as the somatic chromosome number of various species and the overall karyotype symmetry among them, signify that the diversification at inter- and intra-specific levels owing to structural alterations rather than numerical changes in the chromosomes [4]. Fluorochrome binding pattern using CMA and DAPI revealed the presence of constitutive blocks of heterochromatin either in the terminal or interstitial region of the chromosomes exhibiting high chromosomal variability with characteristic binding pattern in all the taxa studied [5]. Based on our study, high divergence was recorded in a few taxa viz. Vigna glabrescens, Vigna khandalensis and Vigna mungo advocating the involvement of GC-rich repetitive heterochromatin regions in the process of divergent evolution. The presence of GC- and/or ATrich heterochromatic regions facilitated chromosomal rearrangement and might have contributed significantly to the diversification of the genus Vigna.

Fluorochrome binding pattern in Abelmoschus spp. showed the presence of constitutive CMA+, DAPI+ and CMA+/DAPI+ heterochromatin [6]. Quantum amount of polymorphism was observed with regard to heterochromatin distribution pattern. It was clearly evident that AT-rich are comparatively more than GCrich heterochromatic regions in all the taxa analysed. A noteworthy feature in the genus Abelmoschus is that the number of CMA+ and DAPI+ binding sites increase with the chromosome numbers, and an increase in the DNA, though often, but not always, can be accounted to increase in heterochromatin. Therefore, polyploidy and repetitive heterochromatin rich fraction(s) might have played an important role in the speciation and diversification of the genus Abelmoschus.

In yet another study, we have used CMA and DAPI fluorochromes binding pattern to determine the genetic integrity of micropropagated plantlets of

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N. khasiana [7]. Assessment of genetic fidelity in tissue-culture-raised Nepenthes khasiana of three consecutive regenerations was successfully carried out using chromosome counts and heterochromatin distribution pattern. The chromosome number as well as the AT-fractions increased with successive generations, whereas the GC-rich fraction decreased. Our observations indicated that the variability in the amount and distribution of the AT- and GC-rich repetitive sequences might have resulted in the rearrangements and numerical as well as structural changes in chromosome thus resulting in genetic instability of tissue-culture-raised N. khasiana.

Recently we reported fluorochrome banding pattern in fifteen Curcuma species and showed that constitutive heterochromatin differed in their amount, type and distribution pattern in various species [3]. The study provided a clear insight on genomic components such as the repetitive (GC, AT and GC/AT-rich) and non-repetitive DNA fraction(s) which reflected higher molecular organization of the genome and evolutionary status of the genus Curcuma.

2. Conclusion

Thus the above studies clearly demonstrated the utility of CMA and DAPI fluorochromes in differentiating the amount, type and distribution pattern of heterochromatin-rich repetitive DNA sequences in the genome of a given plant species. It suggests that heterochromatin plays a crucial functional role in chromosomal rearrangements which have led to speciation and diversification of many plant taxa. Comparative analysis of heterochromatin binding pattern can also be utilized for studying genetic fidelity of *in vitro* raised plantlets confirming clonal

uniformity of grown plantlets. Such efforts and more focused strategies gave in depth understanding about genome constitutions at both intra- and interspecific levels of various plant species and its role in speciation and diversification of related plant species.

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