Chromosomal aberrations and Ac/Ds transposition in Garlic

Rasha kamal Helmey 1,3 and Gehan Mohamed Anwar 2

1 Department of Botany &Microbiology, Faculty of Science, Minia University, Egypt;
2 Department of Genetics, Faculty of Agriculture, Minia University, Egypt
3 Author for correspondence: (rashahelmey@yahoo.com)

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ABSTRACT: Garlic (Allium sativum L.) is a commonly used Allium crop worldwide. It might be expected that garlic would show some intraspecific variations because of its vegetative reproduction. This study shed light on such variations joined with the existence of the transposable element Ac in garlic. The examined cells exhibited different types of chromosomal aberration were recorded as percentages of the total abnormal cells. Microscopic examination of root tip cells of the (Egaseed 2) clone showed a variety of chromosomal aberrations represented as: gap, chromatid deletion, chromatid break, end-to-end association and centromeric attenuation. DNA was isolated from ten individual cloves of Egaseed 2 clone. Using Ac primer, a monomorphic fragment of 100 bp was detected in 10 cloves of the Egyptian clone (Egaseed 2) of garlic. One of the same cloves, no. 7, has an additional fragment of 300 bp. The relationship between Ac element existence and chromosomal changes occurrence was discussed.

KEYWORDS: Garlic, Chromosomal aberration, Ac/Ds element

Garlic is a diploid species (2n = 2X = 16). Broad variation has been observed at the structural level of chromosomes in the garlic karyotype. So far, good evidences have been proven to assure that garlic karyotype is not clearly identified because of apomictic nature of its propagation and the accumulation of particular structural aberration (Ali 1998; El-Mamlouk et al. 2002; Ata 2005; Ata and Osman 2009; Mahmoud et al. 2017). Chromosomal aberrations are common in garlic, due to multiple translocation sometimes involving 8 or 10 chromosomes (Sanai and Davis 1967). Osman et al. (2007) studied the mitotic aberrations of fourteen garlic clones at metaphase and ana-telophase. They recorded abnormalities included chromosomal and chromatid bridges, chromatin stickiness, chromosomal breaks and fragments. Meiotic studies of flowering clone (Egaseed 2) revealed that more than 40 chromosomal associations were found in garlic genome (Anwar and Ata 2017).

The discovery of transposable elements (TEs) was accomplished by the observation of their mutability, which unlike the growing genome size, can often have a phenotypic manifestation. Transpositions into the coding regions of genes are usually deleterious; however, those transpositional events that passed through the sieve of selection can induce a variety of genetic changes, including interruption of the host genes creating different expression forms, changing intron length, and affecting expression levels of adjacent genes (Huang et al. 2008). The significant mutable effect of TEs to be mentioned is the macro-transposition, that is, a transposition involving two physically close, interacting elements, and an intervening chromosomal segment. Such transposon pairs may produce other complex rearrangements, including deletions, inversions, and reshuffling of the inter-transposon segment (Huang and Doneer 2008). Thus macro-transposition can be another contributor to genome divergence and speciation (Civan et al. 2011). In most eukaryotic organisms, centromeres and telomeres contain large numbers of retrotransposons, raising the possibility that retrotransposons have acquired structural or functional roles in these heterochromatic regions (Zoé and Thomas 2014). DNA transposons have been implicated in the induction of large-scale chromosomal rearrangements in plants and animals, mainly because their transposition mechanisms involve multiple double-strand breaks and repair events (Feschotte and Pritham 2007).

The well-known Ac/Ds elements in maize were initially identified by Barbara McClintock through their ability to induce chromosome breakage. In addition, McClintock identified a number of major chromosome rearrangements that were apparently induced by the Ac/Ds system (McClintock 1951; McClintock 1978; Yu et al. 2011). Ac belongs to the eukaryotic hAT transposon superfamily (Kempken and Windhofer 2001).

Studies on different cultivated garlic clones in Egypt (El-Mamlouk et al. 2002; Ata 2005; Anwar and Ata 2017; Mahmoud et al. 2017) indicated the presence of many structural chromosomal changes. Therefore, attempting to clarify the relationship between Ac transposition existence and origin of genome instability with respect to structural chromosomal occurrence in a flowering clone (Egaseed 2) of garlic was the main goal of this study.

MATERIALS AND METHODS

Mitotic preparations Growing root tips in 1-2 cm length were collected from plants of Egaseed 2 clone and pretreated in 0.05% colchicines at room temperature for three hours. Treated roots were directly fixed with Farmer's solution (absolute Ethyl alcohol and Glacial acetic acid 3:1 v/v) for 24h and stored in 70% ethanol at 4°C. Before
cytological examination, roots were hydrolyzed using 1 N HCl at 60ºC for six minutes. Acetocarmín-squashed root tips were examined, then metaphase plates with well-spread chromosomes were chosen. Chromosomal aberrations (gaps, chromatid deletions, chromatid breaks, centromeric attenuation and end-to-end association) were identified and quantified at thousand metaphase cells. Good metaphase spreads were photographed microscopically using SIS computer program (version 4) with CCD camera (Olympus C-4040). The cells exhibited different types of chromosomal aberration were recorded as percentages of the total abnormal cells.

**Extraction of DNA** DNA was isolated from ten individual cloves of Egaseed 2 clone using Cornell extraction buffer (500 mM NaCl; 100 mM Tris-HCl, pH 8.0; 50 mM EDTA and 0.84% SDS, equilibrated to 65ºC, mixed with 0.38 g sodium bisulfite/100 ml buffer, and then pH of the warm buffers was adjusted to 7.8-8.0 with NaOH). Concentration and purity of DNA were spectro-photometrically assessed according to Sambrook et al. (1989).

**PCR conditions** The amplification of DNA was carried out using Ac primer (Ac-1, 5’-GCCTCTACTGGCA AAACAAA-3 and Ac-2, 5’-GCTGCTACTGCCTACACT CTG-3’). PCR reaction was performed in a final volume of 25µl containing 12.5µl 2X master mix [0.05 units/µl Taq DNA polymerase in 2X PCR buffer (4mM MgCl₂ and 0.4mM of each 4dNTPs)], 10 µM of primer and 1ng/µl of DNA template. The final reaction volume was completed to 25 µl using sterilized double distilled water.

DNA amplifications were carried out in a thermal cycler (Thermo Hybaid) programmed for initial preheating period in one step of 5 min at 94º C; subsequent 30 cycles of 3 steps: DNA denaturation at 94º C for 45 sec, followed by primer annealing at 56 ºC for 45 sec and then primer extension at 72º C for 45 sec; subsequent the final cycle in one step of post extension at 72ºC for 10 min. Amplified products were resolved by gel electrophoresis on 2% agarose gel, stained with Ethidium bromide (0.1g Ethidium bromide dissolved in 10 ml 1X TAE buffer) for 30 min, visualized on UV light. Size of DNA fragments were estimated by comparison vs. the standard marker of 100 bp ladder.

**RESULTS**

Various chromosomal aberrations were observed at metaphase in the examined cells of Egaseed 2. These aberrations include: chromatid deletion (Fig.1a. 1), gap (Fig.1a. 2 & Fig.1b), chromatid break (Fig.1c), centromeric attenuation (Fig.1d), duplication (Fig.1e), end-to-end association (Fig. 1f).

Data in Figure 2 showed the frequency of aberrations as percentages. For instance, chromatid gap with 60%, chromatid and chromosome deletions (19%), chromatid breaks (18%) and centromeric attenuation (16%). A slight increase of duplication (8%) was observed while low percentage of end-to-end association (5%) was recorded.
As shown in Figure 3, one monomorphic, common fragment (100 bp) with approximately similar intensity was detected in the ten cloves of Egaseed 2 clone, but it is slightly faint in clove number 9. In addition, one unique band of 300 bp was observed in clove number 7.

**DISCUSSION**

Now, we can ask: Are transposable elements (such as Ac elements) important players in structural chromosomal changes induction of garlic clones? Attempting of tackling this problem may begin from identify the correlation of structural aberrations occurrence and the existence of some TE elements like those of Ac genes. High frequencies of chromosomal gap (non-stained region of chromosome), chromatid deletion, breakage and end to end fusion were observed in the present material. This type of chromosomal aberrations was clearly identified as biomarker of environmental pollutants (Firbas and Amon 2014). On the other hand, one monomorphic band of Ac transposon with 100 base pair was amplified, whereas only one clove showed unique band with 300 bp. The finding that there is additional (unique) 300 bp fragment of Ac element in some tissues might be due to molecular relationship between autonomous and non-autonomous transposition (Ata 1994). These data may reflect the effect of transposition in garlic genome which could be explained as a direct action of transposons as pointed by Zhang and Peterson (1999) in Maize. They suggested that large deletions and inverted duplications could be generated via transposition reactions involving Ac/Ds termini located on sister chromatids [sister-chromatid
transposition (SCT)]. Reinsertion of the excised transposon ends into the chromatid bridge generates structurally altered sister chromatids containing a reciprocal deletion and inverted duplication. Page et al. (2004) identified several large deletions (≥100 kb) in Arabidopsis that were apparently generated during Ac-Ds that induced excision of a simple Ds element (Zhang and Peterson 2005).

Examined cells in this study showed centromeric attenuation which may reveal the effect of transposons on cohesion complexes of mitotic chromosomes. Sister chromatids are held together by multisubunit complexes called cohesions. Disruption of cohesion can lead to genome instability, such as aneuploidy, defects in DNA repair, and chromosomal translocations (Brooker and Berkowiz 2014). Mutations in cohesins have also been shown to result in an increased distance between sister centromeres (Brooker and Berkowiz 2014). Therefore, TE mobility may play an important role in garlic genome instability by means of point mutation and chromosomal changes induction. Thus, accumulation of these minor and major changes throughout apomictic asexual propagation has continuously occurred.

**CONCLUSION**

It could be concluded that one of the very important source of genetic variation in garlic clones may due to the preserved chromosomal aberrations. Transposons, might be considered as the internal genomic elements that autonomously or non-autonomously mobilize to induce structural aberrations. Further studies on garlic are nearby concerning the variation even at individual level. Also, more transposon families should be examined on garlic to clarify the main sources of genetic variations.

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