Evidence for hexasomic inheritance in *Chrysanthemum morifolium* Ramat. based on analysis of EST-SSR markers

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Evidence for hexasomic inheritance in *Chrysanthemum morifolium* Ramat. based on analysis of EST-SSR markers

Min Fan · Yike Gao* · Zhiping Wu · Saba Haider · Qixiang Zhang

Beijing Key Laboratory of Ornamental Plants Germplasm Innovation and Molecular Breeding, National Engineering Research Centre for Floriculture, College of Landscape Architecture, Beijing Forestry University, Beijing, China

Correspondence:

Yike Gao

gaoyk@bjfu.edu.cn

Abstract

Chrysanthemums (*Chrysanthemum morifolium* Ramat.) are ornamental flowers that are famous worldwide. The mode of inheritance has great implications for the genetic analysis of polyploid species. However, genetic analysis in chrysanthemum has been hampered because of its controversial inheritance mode (disomic or hexasomic). To classify the inheritance mode of chrysanthemums, an analysis of three approaches was carried out in an F₁ progeny of 192 offspring using 223 expressed sequence tag-simple sequence repeat (EST-SSR) markers. The analysis included segregation analysis, the ratio of simplex marker alleles linked in coupling to repulsion, as well as the transmission and segregation patterns of EST-SSR marker alleles. After segregation analysis, 204 marker alleles fit hexasomic inheritance and 150 marker alleles fit disomic inheritance, showing that marker alleles were inherited predominantly in a hexasomic manner. Furthermore, the results of the analysis of allele configuration and segregation behavior of five EST-SSR markers also suggested random pairing of chromosomes. Additionally, the ratio of simplex marker alleles

linked in coupling to repulsion was 1:0, further supporting hexasomic inheritance. Therefore, it could be inferred that chrysanthemum is a complete or near-complete hexasome.

Keywords *Chrysanthemum morifolium* · EST-SSR · allele dosage · hexasomic · disomic

Introduction

Chrysanthemums (*Chrysanthemum morifolium* Ramat.) are one of the most economically important ornamental crops and are cultivated as cut flowers as well as potted plants. The cultivated chrysanthemum is hexaploid and aneuploid with the most frequent somatic chromosome number of 54 ($2n=6x=54$) and an extremely large genome size of approximately 9.4 Gb (<http://data.kew.org/cvalues/>). The origin of cultivated chrysanthemums remains controversial, and it is generally believed to be a hybrid complex derived from natural hybridization involving several species, including *C. vestitum*, *C. indicum*, *C. makinoi*, *C. japonense*, *C. ornatum*, *C. lavandulifolium*, and *C. zawadskii* (Dowrick 1952; Dai 1998; Yang et al. 2006).

Polyploids are generally subdivided into autopolyploids and allopolyploids. Harlan and De Wet defined autopolyploids as species originating from genomic duplication within a single species, and allopolyploids as a product of genomic duplication between species (Harlan and DeWet 1975). In general, the inheritance mode is related to the origin of ploidy, where autopolyploids exhibit polysomic inheritance and allopolyploids exhibit disomic inheritance. Stebbins (1947) referred to “segmental allopolyploid” as species that have arisen from hybridization between very closely related species or those which are the product of partially diploidized autopolyploidy (Soltis et al. 2016); these species exhibit intermediate modes of inheritance. However, researchers have found

43 that autopolyploids do not always exhibit polysomic inheritance, and allopolyploids do not always
44 exhibit disomic inheritance (Doyle and Sherman Broyles 2017). For example, citrus, being
45 allotetraploid, exhibits a diverse mode of inheritance, including disomic, tetrasomic, and
46 intermediate modes (Xie et al. 2015). The mode of inheritance has a large effect on the genetic
47 analysis of polyploid species. For allopolyploids, tools developed for diploids can be used directly,
48 whereas studying autopolyploids requires specialized methods and tools (Bourke et al. 2018). As
49 the definitions of allo- and autopolyploidy do not address the inheritance mode, herein we focus on
50 discussing whether the inheritance mode of chrysanthemum is disomic or hexasomic.

51 The mode of inheritance of chrysanthemum remains uncertain. Based on its heterozygous
52 origin as well as bivalents observed in meiosis, chrysanthemums are generally classified as
53 allohexaploids that exhibit disomic inheritance (Li et al. 2011; Abd El-Twab and Kondo 2012).
54 However, evidence of polysomic inheritance has been found. Watanabe (1977) reported
55 multivalents in meiosis in hybrids of *C. boreale* ($2n=2x=18$) and several polyploids. Moreover,
56 Langton (1989) found that the segregation ratios for carotenoid pigmentation in chrysanthemum
57 indicated hexasomic inheritance. Li et al. (2011) observed univalent, bivalent, trivalent, quadrivalent,
58 and occasionally pentavalent or hexavalent in pollen mother cells of three edible chrysanthemum
59 species. In addition to cytological analyses, hexasomic inheritance is supported by molecular marker
60 evidence in chrysanthemum. Klie et al. (2014) observed that the ratio of single-dose markers (mainly
61 AFLPs) in the coupling phase to those in the repulsion phase was 1:0, which supports hexasomic
62 inheritance in chrysanthemums. Later, Park et al. (2015) extended the research in a 94 F_1 -pseudo
63 progeny using 49 EST-SSR markers and found that the ratio of non-simplex to simplex markers

sustained hexasomic inheritance. More recently, based on the segregation of duplex $\times$ nulliplex single nucleotide polymorphism (SNP) markers in support of hexasomic inheritance and the absence of markers in the repulsion phase in a large population using a 183 k SNP array, van Geest et al. (2017) regarded the inheritance mode of chrysanthemums as near-complete hexasomic mode. However, conflicting results remains. For example, Zhang et al. (2011) found that fewer markers than expected fit the expected ratio of hexasomic inheritance.

SSR markers are hyper-variable, co-dominant, and multi-allelic, and have been used for inheritance mode analysis in several polyploids, such as dog-rose (Nybom et al. 2004; Nybom et al. 2006), *Rosa hybrid* (Koning-Boucoiran et al. 2012), and citrus (Xie et al. 2015). An analysis of three approaches was carried out in an F_1 progeny of 192 genotypes in chrysanthemum using 223 EST-SSR markers, including segregation analysis, the ratio of simplex markers linked in coupling to repulsion and the banding patterns of the EST-SSR markers. The segregation ratio of EST-SSR markers provided information about inheritance mode, which has been used in garden dahlias, for example, to show that the marker alleles were predominantly of polysomic inheritance (Schie et al. 2014).

Another approach was proposed by Wu et al. (1992), where the ratio of markers linked in coupling to repulsion provided a measurement of the inheritance mode. In allopolyploids exhibiting disomic inheritance, the ratio of markers linked in coupling and repulsion is diploid-like and should appear at the same frequency. In autopolyploids exhibiting hexasomic inheritance, repulsion linkage is difficult to detect because there is little chance of chromosome pairing between two homologous chromosomes with contrasting alleles at two loci. Therefore, a 1:0 ratio of simplex markers linked

in coupling to repulsion indicated polysomic inheritance, and a 1:1 ratio indicated disomic inheritance (Wu et al. 1992); this method has been applied in sweetpotato (Kriegner et al. 2003) and *Saccharum officinarum* (Aitken et al. 2007). The transmission and segregation pattern analysis of SSR markers is considered a reliable approach for the analysis of inheritance mode and can provide a direct evidence of preferential pairing (disomic inheritance) or random pairing (hexasomic inheritance) (Park et al. 2015).

Materials and methods

Plant material and EST-SSR protocol

The F₁ progeny comprised 192 individuals bred from two highly heterozygous chrysanthemum cultivars (Fan et al. 2020). A total of 223 EST-SSR markers were analyzed, as described previously (Fan et al. 2019; Fan et al. 2020).

Segregation analysis

Each marker allele from specific EST-SSR primer pairs was scored independently as a dominant markers (1 for presence and 0 for absence) based on product size. According to the double pseudo-testcross mapping strategy (Grattapaglia 1994), the marker alleles were subdivided with respect to their presence in the paternal parent, maternal parent, and both. The dosage of each marker allele was determined by analyzing the segregation ratios of the marker allele in the F₁ progeny, according to the expected segregation ratios under the two inheritance modes (hexasomic vs. disomic) (Langton 1989; Fan et al. 2020). The χ^2 test ($\alpha=0.01$) was performed to test the goodness-of-fit of

the expected segregation ratios for all marker alleles. EST-SSR marker allele configuration was assigned using the Microsatellite Allele Dose and Configuration Establishment (MADCE) procedure with modifications (Park et al. 2015; van Dijk et al. 2012).

Analysis of marker linkage

Wu et al. (1992) suggested that the ratio of markers linked in the coupling to the repulsion phase would be 1:0 for autopolyploid and 1:1 for allopolyploid. The ratio was estimated using simplex markers of the progeny of each parent. The paternal and maternal linkage map was constructed using simplex markers in JoinMap 4.0 (Biometris, Wageningen, the Netherlands, <http://www.joinmap.nl>) with a logarithm of odds (LOD) score > 6.0, respectively. Subsequently, the presence (1) and absence (0) values of the marker data matrix were inverted. Later, the inverted dataset was added to the original dataset, and the paternal and maternal linkage maps were reconstructed. The marker alleles linked in the original dataset were in the coupling phase, and those linked between the original and inverted datasets were in the repulsion phase (Wu et al. 1992). A χ^2 test ($\alpha = 0.05$) was performed to test for goodness-of-fit to the expected ratios.

Results

Segregation analysis

The classification of marker alleles according to the expected segregation ratios under disomic and hexasomic inheritance was summarized by Fan et al. (2020). As a result, 204 (87 duplex, 38 triplex, and 79 duplex-simplex) marker alleles fit the expected segregation ratios under the hypothesis of

123 hexasomic inheritance, and 150 (56 duplex, 49 triplex, and 45 duplex-simplex) fit the expected
 124 segregation ratios under the hypothesis of disomic inheritance (Fan et al. 2020).

125 **Transmission and segregation patterns of EST-SSR marker alleles**

126 The dosage of each marker allele in EST-SSR locus was speculated through segregation analysis.
 127 Five marker alleles were scored (A_1 to A_5) for marker 124-c75428 (LG 2) (Fan et al. 2020), including
 128 one paternal (A_2), two maternal (A_3 and A_4), and two bi-parental marker alleles (A_1 and A_5) (Fig.1).
 129 Among these, two uniparental marker alleles (A_3 and A_4) segregated in a 1:1 ratio and were
 130 considered simplex, whereas one uniparental marker allele (A_2) fit both 4:1 for hexasomic
 131 inheritance and 3:1 for disomic inheritance and were considered duplex. Two bi-parental marker
 132 alleles (A_1 and A_5) fit both 9:1 for hexasomic inheritance and 7:1 for disomic inheritance and were
 133 considered duplex-simplex (Table 1).

134 Based on analyzing the observed segregation ratio of each peak in 192 offspring and the RV,
 135 the marker allele configuration of the parents and offspring in EST-SSR locus was speculated
 136 through MADCE analysis (Supplemental material 1). The allele configurations of maternal and
 137 paternal parents were considered as $A_1A_1A_2A_2A_5A_5$ and $A_1A_3A_4A_5A_{un}A_{un}$ in 124-c75428 locus,
 138 respectively (Table 1 and Fig. 1). The segregation behavior of the five marker alleles was studied in
 139 the F_1 progeny. The allele configurations of 'Offspring-001' and 'Offspring-106' were considered
 140 as $A_1A_2A_3A_5A_{un}A_{un}$ and $A_1A_2A_3A_5A_5A_5$, respectively. Given that chromosomes would
 141 preferentially be paired with their homologs during meiosis, it could be inferred that the A_3 marker
 142 allele would pair with the A_4 marker allele, A_1 with A_{un} , and A_5 with A_{un} in the maternal parent (Fig.

2). However, the allele configuration of ‘Offspring-100’ was $A_1A_1A_2A_5A_5A_{un}$. Under disomic inheritance conditions, the allele configuration is impossible, as homologous chromosomes would segregate from each other during meiosis (Fig. 2). Therefore, we can conclude that all five marker alleles were randomly assigned to the 192 offspring at the 124-c75428 locus.

In addition, we analyzed three other EST-SSR markers: 64-c71464 (LG 3) (Supplemental material 2, Table S1 and Fig. S1), 81-c72865 (LG 1) (Supplemental material 2, Table S2 and Fig. S2), and 115-c75008 (LG 3) (Supplemental material 2, Table S3 and Fig. S3) (Fan et al. 2020). All of them exhibited random assortment in the progeny.

Analysis of marker linkage

To further test the inheritance mode of chrysanthemum, a framework map for simplex markers linked to coupling and repulsion was analyzed. Of the 556 uniparental marker alleles, 326 were polymorphic simplex (161 for the paternal parent and 165 for the maternal parent) (Fan et al. 2020), and were used to identify markers linked to the coupling or repulsion phase. As a result, 234 marker alleles (115 for the paternal parent amplified from 97 EST-SSR markers and 119 for the maternal parent amplified from 81 EST-SSR markers) were linked in the coupling phase at $LOD > 6.0$, whereas the repulsion phase was devoid of any associated markers. The ratio of markers linked in the coupling phase to those in the repulsion phase was 1:0 (Table 2), which is indicative of hexasomic inheritance.

Discussion

The mode of inheritance plays a significant role in the genetic analysis of polyploid species. In general, the inheritance mode can be divided into three patterns: disomic, polysomic, or a combination of those (Stebbins 1947; Allendorf and Danzmann 1997; Stift et al. 2008; Leal-Bertioli et al. 2015; Bourke et al. 2018). Researchers have questioned whether it is convincing to define chrysanthemums as allohexaploids based on their heterozygous origins and bivalent meiotic observations (Klie et al. 2014). In polyploids, chromosomes can pair in bivalent or multivalent chromosomes; the absence of multivalents does not necessarily indicate disomic inheritance because bivalents can result from random pairing of homologous chromosomes. Conversely, the presence of multivalents indicates polysomic inheritance (Stebbins 1947). Therefore, although the prevalence of bivalents in chrysanthemum was found, the presence of multivalents (Chen et al. 2009; Watanabe 1983; Li et al. 2011) suggests polysomic inheritance in some chromosome regions.

With the development of molecular markers, Wu et al. (1992) and Silva et al. (1993) developed methods to distinguish allopolyploids from autopolyploids. Contrary to cytological analyses, studies on molecular markers provide evidence for hexasomic inheritance in chrysanthemum (Langton 1989; Klie et al. 2014; Park et al. 2015; van Geest et al. 2017). However, due to the limited number of markers and offspring, most studies can exclude disomic inheritance, but are unable to exclude a mixture of different inheritance modes. As a result, some researchers have considered chrysanthemums as segmental allopolyploids (Klie et al. 2014). Recent studies have found new evidence that chrysanthemums have near-complete hexasomic inheritance using SNP markers (van Geest et al. 2017). Correspondingly, our study showed conclusive evidence for hexasomic

inheritance using multi-allelic SSR markers using three approaches. First, after segregation analysis, more marker alleles (204 fit hexasomic inheritance and 150 fit disomic inheritance) fit hexasomic inheritance, which is consistent with previous studies (Klie et al. 2014; Park et al. 2015). The dosage of some marker alleles cannot be determined because of their similar segregation ratios, which may require a larger number of offspring. Second and third, the absence of markers linked in the repulsion phase and random pairing in five EST-SSR markers fully supported hexasomic inheritance, which is consistent with the outcomes of Klie et al. (2014) and Park et al. (2015). Moreover, EST-SSR markers are well defined and easily transferable to other populations (Fan et al. 2019), allowing researchers to use these markers to study the inheritance mode in a broader germplasm of chrysanthemums.

In summary, we presented a conclusive analysis of a large number of multi-allelic EST-SSR markers in chrysanthemums. Evidence for hexasomic inheritance was provided in different background progeny. Knowledge of inheritance mode changes will be useful for chrysanthemum breeding. By adopting these findings, some suitable genomic tools could be developed for genetic analysis in chrysanthemum, such as genetic linkage mapping and QTL detection.

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Compliance with ethical standards

Conflicts of Interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human subjects or animals performed by any of the

above authors.

Supplementary Materials:

Table S1. Analysis of genotypic segregation ratio of each allele in 64-c71464, and χ^2 goodness of fit test ($\alpha=0.05$) using the expected segregation ratios of hexasomic and disomic inheritance.

Figure S1. Genotype analysis of the paternal and maternal parents and their three progenies, ‘Offspring-080’, ‘Offspring-082 and ‘Offspring-111’ in 64-c71464 (A) and diagram of possible chromosome pairing of the paternal parent on the assumption of disomic mode of inheritance (B).

Table S2. Analysis of genotypic segregation ratio of each allele in 81-c72865, and χ^2 goodness of fit test ($\alpha=0.05$) using the expected segregation ratios of hexasomic and disomic inheritance.

Figure S2. Genotype analysis of the paternal and maternal parents and their three progenies, ‘Offspring-039’, ‘Offspring-045 and ‘Offspring-057’ in 81-c72865 (A) and diagram of possible chromosome pairing of the paternal parent on the assumption of disomic mode of inheritance (B).

Table S3. Analysis of genotypic segregation ratio of each allele in 115-c75008, and χ^2 goodness of fit test ($\alpha=0.05$) using the expected segregation ratios of hexasomic and disomic inheritance.

Figure S3. Genotype analysis of the paternal and maternal parents and their three progenies, ‘Offspring-140’, ‘Offspring-143’ and ‘Offspring-152’ in 115-c75008 (A) and diagram of possible chromosome pairing of the paternal parent on the assumption of disomic mode of inheritance (B).

References

Abd El-Twab, M.H., Kondo, K. 2012. Physical mapping of 5S and 45S rDNA in Chrysanthemum and related genera of the Anthemideae by FISH, and species relationships. J Genet. 91(2):245-249. doi: 10.1007/s12041-012-0177-z

- 225 Aitken, K.S., Jackson, P.A., McIntyre, C.L. 2007. Construction of a genetic linkage map for *Saccharum*
 226 *officinatum* incorporating both simplex and duplex markers to increase genome coverage. *Genome*
 227 50(8): 742-756. doi: 10.1139/G07-056
- 228 Allendorf, F.W., Danzmann, R.G. 1997. Secondary tetrasomic segregation of MDH-B and preferential
 229 pairing of homeologs in rainbow trout. *Genetics* 145:1083-1092.
- 230 Bourke, P.M., Voorrips, R.E., Visser, R.G.F., Maliepaard, C. 2018. Tools for Genetic Studies in
 231 Experimental Populations of Polyploids. *Front Plant Sci.* 9. doi: 10.3389/fpls.2018.00513
- 232 Chen, F., Li, F., Chen, S., Guan, Z., Fang, W. 2009 Meiosis and pollen germinability in small-flowered
 233 anemone type chrysanthemum cultivars. *Plant Syst Evol.* 280:143-151. doi: 10.1007/s00606-009-
 234 0173-9
- 235 Dai, S., Chen, J., and Li, W. 1998. Application of RAPD analysis in the study on the origin of Chinese
 236 cultivated chrysanthemum. *J Integr Plant Biol.* 40(11):1053-1059.
- 237 Dowrick, G.J. 1952. The chromosomes of Chrysanthemum, I: The species. *Heredity.* 6(3):365-375. doi:
 238 doi: 10.1038/hdy.1953.5.
- 239 Doyle, J.J., Sherman Broyles, S. 2017 Double trouble: taxonomy and definitions of polyploidy. *New*
 240 *phytol.* 213(2):487-493. doi: 10.1111/nph.14276
- 241 Fan, M., Gao, Y., Gao, Y., Wu, Z., Liu, H., Zhang, Q. 2019. Characterization and development of EST-
 242 SSR markers from transcriptome sequences of chrysanthemum (*Chrysanthemum × morifolium*
 243 Ramat.). *Hortscience.* 54(5):772-778. doi: 10.21273/HORTSCI113694-18
- 244 Fan, M., Gao, Y., Wu, Z., Zhang, Q. 2020. Linkage map development by EST-SSR Markers and QTL
 245 analysis for inflorescence and leaf traits in chrysanthemum (*Chrysanthemum morifolium* Ramat.).

- 246 Plants. 9(10): 1342. doi: 10.3390/plants9101342
- 247 Grattapaglia, D., Sederoff, R. 1994. Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus*
 248 *urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics. 137:1121-1137.
- 249 Harlan, J.R., DeWet, J.M.J. 1975. On Ö. Winge and a Prayer: The origins of polyploidy. Bot Rev.
 250 41(4):361-390. doi: 10.1007/BF02860830
- 251 Klie, M., Schie, S., Linde, M., Debener, T. 2014. The type of ploidy of chrysanthemum is not black or
 252 white: a comparison of a molecular approach to published cytological methods. Front Plant Sci. 5.
 253 doi: 10.3389/fpls.2014.00479
- 254 Koning-Boucoiran, C.F.S., Gitonga, V.W., Yan, Z., Dolstra, O., van der Linden, C.G., van der Schoot,
 255 J., Uenk, G.E., Verlinden, K., Smulders, M.J.M., Krens, F.A., Maliepaard, C. 2012. The mode of
 256 inheritance in tetraploid cut roses. Thero Appl Genet. 125(3):591-607. doi: 10.1007/s00122-012-
 257 1855-1
- 258 Kriegner, A., Cervantes, J.C., Burg, K., Mwanga, R.O.M., Zhang, D. 2003. A genetic linkage map of
 259 sweetpotato [*Ipomoea batatas* (L.) Lam.] based on AFLP markers. Mol Breeding. 11(3):169-185.
 260 doi: 10.1023/A:1022870917230
- 261 Langton, F.A. 1989. Inheritance in *Chrysanthemum morifolium* Ramat. Heredity. 62(3):419-423.
- 262 Leal-Bertioli, S., Shirasawa, K., Abernathy, B., Moretzsohn, M., Chavarro, C., Clevenger, J., Ozias-
 263 Akins, P., Jackson, S., Bertioli, D. 2015. Tetrasomic Recombination Is Surprisingly Frequent in
 264 Allotetraploid *Arachis*. Genetics. 199(4):1092-1093. doi: 10.1534/genetics.115.174607
- 265 Li, C., Chen, S., Chen, F., Li, J., Fang, W. 2011. Cytogenetic study of three edible chrysanthemum
 266 cultivars. Genetika. 47(2):199-205. doi: 10.1134/S1022795411010054

- 267 Liu, H., Zhang, Q.X., Sun, M., Pan, H.T., Kong, Z.X. 2015. Development of expressed sequence tag-
268 simple sequence repeat markers for *Chrysanthemum morifolium* and closely related species. Genet
269 Mol Res. 14(3):7578-7586. doi: 10.4238/2015.July.13.1
- 270 Nybom, H., Esselink, G.D., Werlemark, G., Leus, L., Vosman, B. 2006. Unique genomic configuration
271 revealed by microsatellite DNA in polyploid dogroses, *Rosa* sect. *Caninae*. J Evolution Biol.
272 19:635-648. doi: 10.1111/j.1420-9101.2005.01010.x
- 273 Nybom, H., Esselink, G.D., Werlemark, G., Vosman, B. 2004. Microsatellite DNA marker inheritance
274 indicates preferential pairing between two highly homologous genomes in polyploid and hemisexual
275 dog-roses, *Rosa* L. Sect. *Caninae* DC. Heredity. 92:139-150. doi: 10.1038/sj.hdy.6800332
- 276 Park, S.K., Arens, P., Esselink, D., Lim, J.H., Shin, H.K. 2015. Analysis of inheritance mode in
277 chrysanthemum using EST-derived SSR markers. Sci Hortic. 192:80-88. doi:
278 10.1016/j.scienta.2015.05.009
- 279 Schie, S., Chaudhary, R., Debener, T. 2014. Analysis of a complex polyploid plant genome using
280 molecular markers: strong evidence for segmental allooctoploidy in garden dahlias. The Plant
281 Genome. 7(3). doi: 10.3835/plantgenome2014.01.0002
- 282 Soltis, D.E., Visger, C.J., Marchant, D.B., Soltis, P.S. 2016. Polyploidy: Pitfalls and paths to a paradigm.
283 Am J Bot. 103(7):1146-1166. doi: 10.3732/ajb.1500501
- 284 Stebbins, G.L. 1947. Types of Polyploids: Their Classification and Significance. Adv Genet. 1:403-429.
- 285 Stift, M., Berenos, C., Kuperus, P., van Tienderen, P.H. 2008. Segregation Models for Disomic,
286 Tetrasomic and Intermediate Inheritance in Tetraploids: A General Procedure Applied to *Rorippa*
287 (Yellow Cress) Microsatellite Data. Genetics. 179(4):2113-2123. doi: 10.1534/genetics.107.085027

- 288 van Dijk, T., Noordijk, Y., Dubos, T., Bink, M.C., Meulenbroek, B.J., Visser, R.G., van de Weg, E. 2012.
289 Microsatellite allele dose and configuration establishment (MADCE): an integrated approach for
290 genetic studies in allopolyploids. BMC Plant Biol. 12:25. doi : 10.1186/1471-2229-12-25
- 291 van Geest, G., Voorrips, R.E., Esselink, D., Post, A., Visser, R.G., Arens, P. 2017. Conclusive evidence
292 for hexasomic inheritance in chrysanthemum based on analysis of a 183 k SNP array. BMC
293 Genomics. 18(1):585.doi: 10.1186/s12864-017-4003-0
- 294 Watanabe, K. 1977. The control of diploid-like meiosis in polyploid taxa of *Chrysanthemum*
295 (Compositae). Japan J Genetics. 52(2):125-131. doi: 10.1266/jjg.52.125
- 296 Watanabe, K. 1983. Studies on the control of diploid-like meiosis in polyploid taxa of *Chrysanthemum*:
297 4. Colchipooids and the process of cytogenetical diploidization. Theor Appl Genet. 66(1):9-14. doi:
298 10.1007/bf00281840
- 299 Wu, K.K., Burnquist, W., Sorrells, M.E., Tew, T.L., Moore, P.H., Tanksley, S.D. 1992. The detection
300 and estimation of linkage in polyploids using single-dose restriction fragments. Thero Appl Genet.
301 83(3):294-300. doi: 10.1007/BF00224274
- 302 Xie, K., Xia, Q., Wang, X., Liang, W., Wu, X., Grosser, J.W., Guo, W. 2015. Cytogenetic and SSR-
303 marker evidence of mixed disomic, tetrasomic, and intermediate inheritance in a citrus allotetraploid
304 somatic hybrid between‘Nova’tangelo and‘HB’pummelo. Tree Genet Genomes. 11(6):112. doi:
305 10.1007/s11295-015-0940-4
- 306 Yang, W., Glover, B.J., Rao, G.Y., Yang, J. 2006. Molecular evidence for multiple polyploidization and
307 lineage recombination in the *Chrysanthemum indicum* polyploid complex (Asteraceae). New phytol.
308 171(4):875-886. doi: 10.1111/j.1469-8137.2006.01779.x

Zhang, F., Chen, S., Chen, F., Fang, W., Chen, Y., Li, F. 2011. SRAP-based mapping and QTL detection for inflorescence-related traits in chrysanthemum (*Dendranthema morifolium*). Molecular Breeding. 27(1): 11-23. doi: 10.1007/s11032-010-9409-1

Table 1 Allele dosage and segregation ratio of each marker allele in 124-c75428, and χ^2 goodness of fit test ($\alpha=0.05$) using the expected segregation ratios of hexasomic and disomic inheritance

Table 2 The observed proportion of uniparental simplex markers linked in coupling phase to those in repulsion phase and χ^2 goodness of fit test ($\alpha=0.05$, $df=1$) using the expected segregation ratios of hexasomic and disomic inheritance in the F_1 progeny

Figure 1 Genotype analysis of maternal parent, paternal parent and their three progenies, 'Offspring-001', 'Offspring-100' and 'Offspring-106', in 124-c75428

Figure 2 Diagram of possible chromosome pairing of the paternal and maternal parent on the assumption of disomic mode of inheritance in 124-c75428 locus

Null hypothesis: If chrysanthemum is allohexaploidy,

In case of 124-c75428:

Paternal parent ($A_1A_1A_2A_2A_5A_5$)

Maternal parent ($A_1A_3A_4A_5A_{un}A_{un}$)

However, Offspring-100 ($A_1A_1A_2A_5A_5A_{un}$):

$A_1A_2A_5$ from Paternal parent
 $A_1A_5A_{un}$ from Maternal parent

→ **Reject the null hypothesis**

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330

Draft

1 **Table 1** Allele dosage and segregation ratio of each marker allele in 124-c75428, and χ^2 goodness of fit test ($\alpha=$
2 0.05) using the expected segregation ratios of hexasomic and disomic inheritance

Allele type	Observed number of individuals						Expected allele dose of		Expected ratio		Chi-square value
	Paternal parent	Maternal parent	No. present	of	No. absent	of	Paternal parent	Maternal parent			
124-c75428_A ₁	+	+	172		19		2	1	hexasomic	9:1	0.01
									disomic	7:1	0.92
124-c75428_A ₂	+	-	142		49		2	0	hexasomic	4:1	3.47
									disomic	3:1	0.02
124-c75428_A ₃	-	+	83		108		0	1	hexasomic	1:1	3.02
									disomic	1:1	3.02
124-c75428_A ₄	-	+	103		88		0	1	hexasomic	1:1	1.03
									disomic	1:1	1.03
124-c75428_A ₅	+	+	168		23		2	1	hexasomic	9:1	0.67
									disomic	7:1	0.01

3
4
5 **Table 2** The observed proportion of uniparental simplex markers linked in coupling phase to those in repulsion phase
6 and χ^2 goodness of fit test ($\alpha= 0.05$, $df = 1$) using the expected segregation ratios of hexasomic and disomic
7 inheritance in the F₁ progeny

	Allopolyploid		Autopolyploid	
	Coupling	Repulsion	Coupling	Repulsion
Observed	234	0	234	0
Expected	117	117	234	0
p-value ($\alpha= 0.05$)	7.999e ⁻⁵³		1	

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10
11

Figure 1 Genotype analysis of maternal parent, paternal parent and their three progenies, 'Offspring-001', 'Offspring-100' and 'Offspring-106', in 124-c75428

336x334mm (150 x 150 DPI)

Null hypothesis: If chrysanthemum is allohexaploidy,

In case of 124-c75428:

Paternal parent ($A_1A_1A_2A_2A_5A_5$)

Maternal parent ($A_1A_3A_4A_5A_{un}A_{un}$)

However, Offspring-100 ($A_1A_1A_2A_2A_5A_5A_{un}$):

- $A_1A_2A_5$ from Paternal parent
- $A_1A_5A_{un}$ from Maternal parent

→ **Reject the null hypothesis**

Figure 2 Diagram of possible chromosome pairing of the paternal and maternal parent on the assumption of disomic mode of inheritance in 124-c75428 locus

287x138mm (150 x 150 DPI)