Aneuploid offspring indicates imbalanced separation of homologous chromosomes during meiosis in triploid *Hydrangea macrophylla* cv. 'Blaumeise'

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Aneuploid offspring indicates imbalanced separation of homologous chromosomes during meiosis in triploid *Hydrangea macrophylla* cv. 'Blaumeise'

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1. Introduction, Knowledge, Objectives

The ornamental crop *Hydrangea macrophylla* (THUNB.) SER. has a basic chromosome set of 1x = 18 and comprises diploid and triploid varieties. Triploid hydrangeas often develop larger organs and floral structures than diploids (Alexander, 2017). In addition, polyploids are considered to be more robust against abiotic and biotic stresses than their diploid relatives. Hence, the development of polyploid hydrangeas is desired.

Flow cytometric analyses of Zonneveld (2004) and Jones et al. (2007) suggest that nearly 50 % of *H. macrophylla* cultivars are triploids. Recently, Alexander (2017) showed that triploid hydrangeas can be developed by crossings using diploid parents that develop a high proportion of unreduced gametes. However, it is unclear, whether triploids can also be obtained from interploid crosses between diploids and triploids. Triploid crossing partners might be sterile as observed for citrus, banana or watermelon (reviewed by Wang et al., 2016) or they produce aneuploid gametes, which result in aneuploid offspring as observed in maize (McClintock, 1929). In contrast to maize, cross experiments of triploid and tetraploid rose plants indicated that triploid roses were able to produce haploid and diploid male and female gametes (van Huylenbroeck et al., 2005).

In order to study the gamete production and crossing behavior of triploid *H. macrophylla*, we performed a cross between diploid cultivars and a reciprocal cross between a triploid and a diploid cultivar. Subsequently, we analyzed the F_1 populations by flow cytometry.

2. Material and Methods

<u>Plant material:</u> Three F_1 populations were produced by crossing the *H. macrophylla* ssp. *macrophylla* cultivars 'Sweet Dreams' x 'Bläuling', 'Sweet Dreams' x 'Blaumeise' and 'Blaumeise' x 'Sweet Dreams'. F_1 plants were cultivated in pots filled with Einheitserde® CL Hortensien blau on tables in a frost-free greenhouse of the Leibniz Institute of Vegetable and Ornamental Crops in Erfurt, Germany, without additional light supply. Plants were fertilized with Universol® blue 0.1 % (Everris International BV) and irrigated as necessary.

Chromosome counting: Chromosomes of macerated root tips were prepared as described by Tränkner et al. (2018). Chromosome counts were made from 5 metaphase cells per cultivar.

Flow cytometry: Flow cytometry was performed on a Partec CyFlow Space analyzer with a 488 nm blue solid state laser. *Pisum sativum* L. 'Ctirad' (2C = 9.09 pg) or *Secale cereale* L. 'Daňkovské' (2C = 17.05 pg according to *P. sativum* L. 'Ctirad') were used as internal standards. Samples were prepared in Galbraith's buffer supplemented with 50 µg/ml propidium iodide according to Dolezel et al. (2007). For each sample-standard-mixture, about 10,000 nuclei were analyzed. High quality peaks were determined at CV < 4 %. The 2C DNA content of each sample was calculated as follows: $2C_{sample}$ = mean fluorescence value of sample * 2C DNA content of the corresponding internal standard [pg] / mean fluorescence value of the corresponding internal standard.

3. Results

'Sweet Dreams' and 'Bläuling' are diploid and contain 2n = 2x = 36 chromosomes, whereas 'Blaumeise' is triploid and has 2n = 3x = 54 chromosomes (Figure 1). The 2C DNA content of 'Sweet Dreams' and 'Bläuling' is 4.4 ± 0.05 pg and 4.5 ± 0.04 pg, respectively, and 6.5 ± 0.01 pg for 'Blaumeise'. All 3 cultivars produced viable pollen (data not shown).

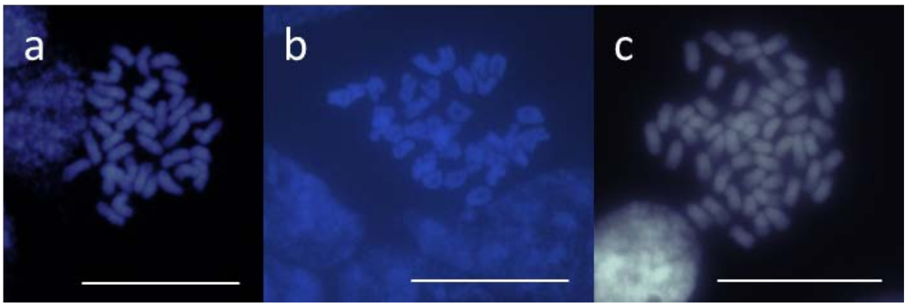

Figure 1. Chromosomes at metaphase of a) 'Sweet Dreams', b) 'Bläuling' and c) 'Blaumeise'. Bar = 20 µm.

The cross between the diploids 'Sweet Dreams' and 'Bläuling' was performed as control and resulted in 70 F_1 plants. The 2C DNA content of 69 F_1 plants ranged between 4.44 and 4.67 pg, on average 4.53 ± 0.04 pg (Figure 2). However, one F_1 plants showed a 2C DNA content of 6.76 pg. These 2C values suggest a diploid ploidy level for 99 % and a triploid level for 1 % in this population. The interploid crosses 'Sweet Dreams' x 'Blaumeise' and 'Blaumeise' x 'Sweet Dreams' produced 14 and 24 F_1 plants, respectively. As presented in Figure 2, F_1 plants of 'Sweet Dreams' x 'Blaumeise' showed 2C DNA contents between 3.87 and 7.72 pg, on average 4.90 ± 1.05 pg. F_1 plants of the reciprocal cross showed 2C DNA contents from 4.60 to 7.39 pg, on average 5.25 ± 0.77 pg. These data suggest that diploid as well as aneuploid F_1 plants were derived from both interploid crosses.

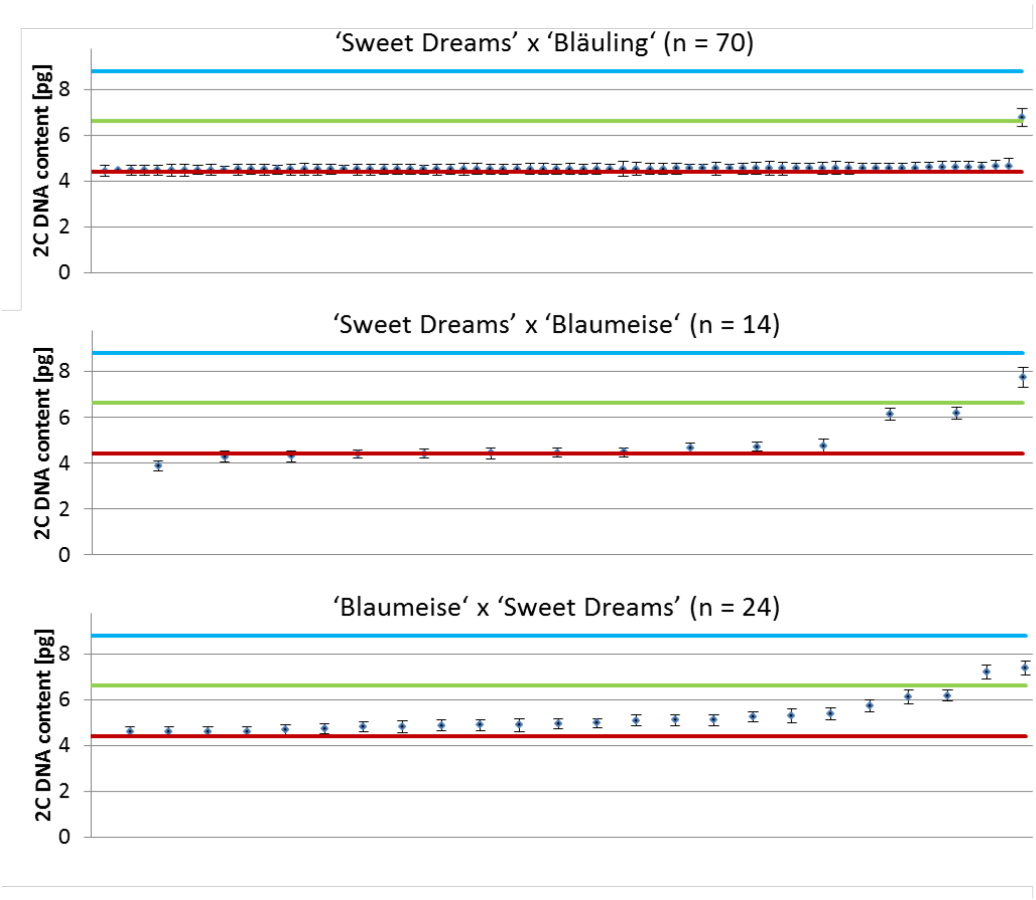

Figure 2. 2C DNA content of F_1 plants derived from crosses between diploid 'Sweet Dreams', diploid 'Bläuling' and triploid 'Blaumeise'.

F_1 plants of the diploid control cross developed normally, whereas the F_1 plants of both interploid crosses were vital but showed often abnormal phenotypes (Figure 3).

Figure 3. Two-year-old F_1 plants from the interploid cross 'Sweet Dreams' x 'Blaumeise' (2C DNA content from left to right: 6.13 pg, 4.44 pg, 4.28 pg, and 4.30 pg). A 2C DNA content of 4.4 pg might indicate diploidy.

4. Discussion

Crossings between diploids and between diploid and triploid hydrangeas were successful and resulted in viable offspring. This observation suggests that the diploid as well as the triploid parent had produced viable and fertile ovules as well as viable and fertile pollen, which was able to perform double fertilization.

Flow cytometric analysis revealed that the cross between diploid 'Sweet Dreams' and diploid 'Bläuling' generated diploid and 1 % triploid F_1 plants. Based on these data, we conclude that both of these parents perform a regular meiosis and form haploid gametes. However, one of these parents may additionally produce unreduced gametes, which results in the occasional generation of triploids. The partial production of unreduced gametes is a widespread phenomenon in angiosperms. It has been found in many species and its occurrence and extent varies among species, populations and individuals (Kreiner et al., 2017). Also in *H. macrophylla*, spontaneous sexual autopolyploidy has been observed. Crossings between the diploid cultivars 'Zaunkönig' x 'Princess Juliana' and 'Princess Juliana' x 'Trophee' produced diploid and 4 to 94 % triploid offspring, probably due to the development of unreduced pollen (Alexander, 2017).

While the cross between 'Sweet Dreams' and 'Bläuling' resulted in diploid and triploid offspring, the direct and the reciprocal cross between diploid 'Sweet Dreams' and triploid 'Blaumeise', produced also aneuploid offspring according to the corresponding 2C DNA content of F_1 plants. The 2C DNA content of most aneuploid F_1 plants ranged between the diploid and triploid DNA level. This observation suggests that plants of the triploid cultivar 'Blaumeise' perform maternally and paternally meiosis based on triploid megasporocytes and microsporocytes, resulting in reduced, but aneuploid gametes. As shown in other species such as maize, Canna or Datura, triploid species form trivalents in the prophase and metaphase of meiosis I and pass randomly one and two chromosomes to either pole (Belling, 1921; McClintock, 1928). This random separation of homologous chromosomes results in gametes with various chromosome numbers. In combination with haploid gametes from the diploid crossing partner, aneuploid offspring is generated showing disomy or trisomy of single chromosomes. However, we also detected F_1 plants, whose 2C DNA content was lower than the diploid or higher than the triploid DNA level. 'Sweet Dreams' might produce unreduced gametes, which fuse with aneuploid gametes of 'Blaumeise', resulting in F_1 plants with DNA contents above triploid DNA level. More likely, the separation of homologous chromosomes during meiosis is more imbalanced in triploid hydrangeas and various gametes with 0 up to 3 homologous chromosomes might be produced. The combination of these aneuploid gametes with haploid gametes will generate F_1 plants with partial monosomy, disomy, trisomy or even tetrasomy of single chromosomes. Most F_1 plants derived from these interploid crosses showed an abnormal phenotype compared to the diploid and triploid parent and the euploid halfsiblings of the diploid control cross, which may be attributed to aneuploidy as observed in maize (McClintock, 1929).

5. Conclusions

Crossings between diploid and triploid *H. macrophylla* result in euploid and aneuploid progenies. This finding suggests that triploid hydrangeas perform meiosis and produce viable gametes. However, these gametes are aneuploid with varying chromosome numbers, most likely due to imbalanced separation of homologous chromosomes during meiosis. Hence, interploid crosses are possible, but the offspring is to a large extent aneuploid and shows aberrant and unattractive phenotypes.

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