

Meiotic behaviour and morpho-phenological variation in cut stock (*Matthiola incana* L.) flower

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ABSTRACT

Morpho-phenological and meiotic studies were performed in twelve cultivars of *Matthiola incana*. All of the cultivars were diploid ($2n = 2x = 14$) with basic chromosome number $x = 7$. A number of aneuploid PMCs ($n + 1$) were observed in plants of two cultivars, named 'Nobel' (NB) and 'Goddess' (GD), at the diakinesis stage. Trisomic individuals with the frequency of 20% and 5% and ($2n + 1 = 15$) somatic chromosomes were observed in seeds obtained from single-flowered plants of the cultivars NB and GD, respectively. An additional chromosome was mostly observed in the form of a chain trivalent or a rod univalent. Various meiotic abnormalities were found in all the cultivars to different degrees. In these cultivars, the percentage of cells with meiotic abnormalities was higher in anaphase I. Cytomixis was observed for the first time in *Matthiola incana*. ANOVA tests revealed significant differences in morpho-phenological characteristics. 'Nobel' differs from the others in all of the vegetative features investigated in this study. All the cultivars studied except 'Nobel' and 'Pacific Crimson' possessed high pollen fertility ($> 90\%$). Five groups of the cultivars based on morpho-phenological features disagree with the clustering of cultivars based on meiotic traits. It is thought that the various morpho-phenological features observed among the cultivars could be due to their different genetic background and not only to meiotic anomalies.

Key words: aneuploidy, chiasma frequency, chromosome pairing, pollen fertility, vegetative and floral traits

INTRODUCTION

The stock flower (*Matthiola incana* L.) is a species of the Brassicaceae family. It is regarded as an important ornamental plant due to its pleasant aroma and beautiful form (Sanchez et al. 2005). This plant exists in two forms: single-flowered and double-flowered. The double-flowered stock plant is popular on the market and that makes it an important export commodity worldwide (Winge 1931, Eid et al. 2009). Seeds of the stock flower contain oil rich in linolenic acid (55-65%) that is of medicinal importance (Yaniv et al. 1999). In double-flowered

plants, it has been observed that mutations in the AGAMOUS (AG) gene cause deactivation of the gene. So, in an AGAMOUS mutant there are petals instead of the stamen and carpel (Meyerowitz et al. 1989). Production, however, is problematic because of the genetic disorder in the double-flowered plant, which lacks the stamen and carpel. The double-flowered form is sterile and can only be produced from seeds obtained from single-flowered plants (Johnson 1953, Mizukami and Ma 1997). The seeds of the single-flowered plant produce various percentages of double-flowered plants, depending on the cultivar. According to reports there are

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three types of single-flowered stock plant used as a production base for double-flowered plants. These are the “Single” type that, when self-pollinated, do not produce double-flowered plants; the second type (“High double”) can produce 25% double-flowered plants, and the “Eversporting” type produces 40-50% double-flowered plants (Emsweller et al. 1937). Some reports suggest that the effect of chromosomal abnormality could be connected with the appearance of double-flowered plants, as well as other morphological properties (Frost and Lesley 1954). Most studies on chromosomes related to this plant date back 50 years. Based on these studies, the basic chromosomal number ($n = 7$, $2n = 2x = 14$) was reported for this plant (Allen 1924, Frost and Lesley 1927). Frost (1931) identified aneuploidy in a plant called “Snowflake” with 86% double-flowered progenies: cytological investigation determined additional chromosomes in this type. Philp and Huskins (1931) reported that this additional chromosome had a V shape that was eliminated during meiotic division but remained in mitosis. Frost and Lesley (1959) reported that an extra chromosome occurred as a consequence of chromosome breakage at the first meiotic anaphase. This presented the possibility of more than one additional fragment of chromosome in the process (Armstrong and Huskins 1934). Chromosomal research plays an important role in studies relating to systematics and determinations of genetic diversity (Ortega et al. 1977). There is high diversity among stock flower cultivars in terms of morphological properties, such that the appearance of double-flowered progenies is variable (Tatsuzawa et al. 2012). However, no systematic study has been done to date to determine the chromosomal characteristics of stock cultivars.

In this study, we investigated: i) cytological traits such as chiasma frequency and chromosome pairing, ii) morphological (vegetative and reproductive) traits, iii) phenological characteristics of three types of stock cultivars: “Eversporting” cultivars, named ‘Cinderella Red’ (CR), ‘Goddess White’ (GD), ‘Pacific Crimson’ (PC), ‘Rose Pink’ (RP), ‘Lavender Lilac’ (LL), ‘Miracle Mid Blue’ (MB), ‘Avalanche White’ (AV), ‘Hot Cakes Purple’ (HP), ‘White Stock’ (WS) and ‘Deep Blue Stock’ (DB), a “High double” cultivar named ‘Nobel White’ (NB) with 80% production of double-flowered plants, and the “Single” type cultivar ‘Esfahan Purple’ (ES), which produces single-flowered plants. Finally, our intention was to categorize cultivars according to morphological

and cytological properties. Double-flowered plants are sterile, so their meiotic properties cannot be studied and no relationship has been determined between cytogenetic and morpho-phenological properties in double-flowered plants. Therefore, in this study, double-flowered plants were excluded from the beginning of the experiment and were counted only for the purpose of investigating the double-flowered plant rate of each cultivar.

MATERIAL AND METHODS

Plant material

The meiotic and morpho-phenological study was performed in twelve cultivars of *M. incana* listed in Table 1. One hundred seeds of each cultivar were sown in seedling trays, and at the four-leaf stage, the plants were transferred to small plastic pots, then elimination their requirement for chilling, the seedlings were transferred to 20-cm-diameter pots for better development and put in the greenhouse under the same conditions. The average daily temperature in the greenhouse was 15-18°C, and the minimum temperature was 10°C. All the plants of each cultivar were labelled and after recording their morpho-phenological properties and the time of full opening of the inflorescence (flower), collecting buds of the plants was conducted.

Analysis of morpho-phenological characteristics

In general, 14 morpho-phenological traits related to vegetative and reproductive growth of each plant were measured. This experiment was performed in a completely randomized design (CRD) with 20 replicates (single-flowered plants) per cultivar.

Separation of single- from double-flowered plants

According to Ecker (1993), seedlings with pale green cotyledons were considered double-flowered plants. There are two types of cultivars in this study:

- i). *Non-selectable cultivars*. In cultivars with no selectivity potential (the cultivars NB and ES), the separation of single-flowered plants (Fig. 1e) from double-flowered plants (Fig. 1f) was not possible until the emergence of inflorescence.
- ii). *Selectable cultivars*. They (all the cultivars, except NB, ES) have the potential to be distinguished (double-flowered from single-flowered plants) at the four-leaf stage by the colour of the cotyledon. So, individuals with a pale green cotyledon (double-flowered plants) were counted and removed.

Table1. Data showing twelve stock cultivars with locality, type, meiotic chromosome number, chromosome association, pollen fertility, percentage of double-flowered plants

Cultivar	Code	Source ^a	No. of diakinesis cells	n ^b	Chromosome associations at diakinesis ^c	PF ^d (%)	PD ^e (%)	Type of stock
Cinderella Red	CR	JPN	378	7	[7 ^{II}], [5 ^{II} +1 ^{IV}], [6 ^{II} +2 ^I], [5 ^{II} +4 ^I]	94.7	45	Eversporting
Nobel White	NB	JPN	510	7	[7 ^{II}], [7 ^{II} +1 ^I], [5 ^{II} +1 ^{IV}], [6 ^{II} +1 ^{III}], [4 ^{II} +1 ^{III} +1 ^{IV}], [6 ^{II} +2 ^I], [3 ^{II} +2 ^{IV}]	82.1	80	High double
Goddess White	GD	NED	491	7	[7 ^{II}], [7 ^{II} +1 ^I], [6 ^{II} +2 ^I], [5 ^{II} +1 ^{IV}], [4 ^{II} +1 ^{III} +1 ^{IV}], [6 ^{II} +1 ^{III}]	96.4	70	Eversporting
Pacific Crimson	PC	USA	569	7	[7 ^{II}], [5 ^{II} +1 ^{IV}]	89.8	60	Eversporting
Rose Pink	RP	USA	248	7	[7 ^{II}], [5 ^{II} +1 ^{IV}], [3 ^{II} +2 ^{IV}]	93.9	50	Eversporting
Lavender Lilac	LL	USA	339	7	[7 ^{II}], [5 ^{II} +1 ^{IV}], [6 ^{II} +2 ^I]	97.5	45	Eversporting
Miracle Mid Blue	MB	USA	277	7	[7 ^{II}], [5 ^{II} +1 ^{IV}]	95.4	50	Eversporting
Avalanche White	AV	USA	225	7	[7 ^{II}], [5 ^{II} +1 ^{IV}], [3 ^{II} +2 ^{IV}]	98.5	60	Eversporting
Hot Cakes Purple	HP	IRN	338	7	[7 ^{II}], [5 ^{II} +1 ^{IV}]	95.3	30	Eversporting
White Stock	WS	IRN	458	7	[7 ^{II}], [5 ^{II} +1 ^{IV}], [3 ^{II} +2 ^{IV}]	98.6	40	Eversporting
Deep Blue Stock	DB	IRN	340	7	[7 ^{II}], [5 ^{II} +1 ^{IV}], [3 ^{II} +2 ^{IV}]	98.9	35	Eversporting
Esfahan Purple	ES	IRN	297	7	[7 ^{II}], [5 ^{II} +1 ^{IV}]	97.4	00	Single

^aJPN – Japan, NED – Netherlands, IRN – Iran; ^bn – haploid number; ^cchromosome associations were observed at diakinesis (italics – extra chromosome was observed in the form of a trivalent or univalent), II – bivalent, IV – quadrivalent, I – univalent; ^dPF – pollen fertility; ^ePD – percentage of double-flowered plants produced by each cultivar (number of plants studied of each cultivar was 20)

Cytogenetic study

a). Meiotic study

To study meiosis, young buds of terminal racemes of each plant in different developmental stages (various sizes) were collected from early April to late May. The samples were transferred to a fresh Carnoy's fixative solution (glacial acetic acid: ethanol; 1:3) and stabilized at room temperature for 24 hours. After washing with distilled water, they were stored in 70% ethanol at 4°C. Finally, we used 2% aceto-orcin for staining the chromosomes. After squashing, the divided cells were investigated under an OLYMPUS CX21 microscope. Thirty to fifty pollen mother cells (PMCs) of each cultivar were studied for chiasma frequency and chromosome pairing at diakinesis, while about 800 to 1000 PMCs of each cultivar were evaluated for abnormalities in meiotic behaviour. In order to determine pollen fertility by the classical method, at least 1000 pollen grains were stained using a mixture of 2% aceto-orcin and glycerine in the volume ratio 1:1. In this method, shrunken and colourless pollen grains were considered sterile and the well-stained pollen grains were scored as fertile (Sheidai and Jalilian 2006).

b). Mitotic study

For counting somatic chromosomes, we collected 100 seeds per plant and at least 50 metaphase plates

from each seed were counted. The root tips were pretreated with 0.002 M of 8-hydroxyquinoline at room temperature for 3h in darkness and fixed with Carnoy's fixative. After hydrolysis with 1% hydrochloric acid at 60°C for 10 minutes, the staining step was performed with 2% (w/v) aceto-orcin for 30 minutes at room temperature (Sheng et al. 2008).

Statistical analysis

Analysis of variance (ANOVA) was performed to distinguish significant differences in diakinesis traits, morpho-phenological characteristics, and compared with Duncan's test ($p < 0.05$) using SPSS version 11.0 software. Cluster analysis was conducted using Ward's method. Diagrams were drawn using STATGRAPHICS version 16.1 software. Pearson correlation analysis (PCA) was conducted to find the relationship between chromosomal and morpho-phenological traits in twelve cultivars. The first principal component showed the greatest amount of variance. From the first principal component to the last, each component explained less variance.

RESULTS AND DISCUSSION

The percentage of double-flowered plants (PD) in the studied cultivars ranged from 0% to 80%. The highest value of PD was observed in the cultivars NB (Figs 1e and f) and GD (Figs 1a and b) with

aneuploid cells. Frost and Lesley (1954) suggest that chromosomal fragments and trisomy could be correlated with the high proportion of double-flowered plants (86%). To verify the finding made

by Frost and Lesley, we performed an extensive cytogenetic study:

Cytogenetic study

1 - Counting chromosomes in pollen mother cells

The results for the meiotic chromosome number and pollen fertility for the twelve cultivars are listed in Table 1. Aneuploidy ($n + 1$) was observed in two cultivars (NB, GD), which is in agreement with the former study (Frost and Lesley 1959). All single-flowered plants of the cultivars NB and GD showed a percentage of aneuploidy. Diakinesis cells with an extra chromosome ($n + 1$) and normal cells (n) of each plant were counted and the ratio indicated that the average percentage of aneuploid cells in each single-flowered plant of the GD cultivar was 13.30% and 36.70% in the NB cultivar. In other words, it was observed that there was a total of 187 cells of NB (from 510 PMCs at the diakinesis stage) and a total of 64 cells of GD (from 491 PMCs at the diakinesis stage) in which the presence of an extra chromosome in the form of chromosomal combination of [trivalent + bivalent, quadrivalent] or [univalent + bivalent, quadrivalent] was confirmed. Thus, there was a lower frequency of aneuploid PMCs at diakinesis in GD than in NB. An additional chromosome most often appears in the form of a trivalent and the trivalent shape was observed mostly in the form of a chain in trisomic PMCs of the NB cultivar (Fig. 2_20). In the studied trisomic PMCs of the GD cultivar, an additional chromosome appeared almost at equal frequency as a chain trivalent or as a rod univalent. Anther wall somatic cells showed $2n = 2x = 14$ chromosomes, suggesting diploid plants in all the studied single-flowered individuals of both cultivars (NB, GD).

2 - Aneuploid individuals in the progenies

Seeds obtained from single-flowered plants of two cultivars with aneuploid PMCs were investigated in terms of counting the number of somatic chromosomes. The results showed that there were trisomic individuals ($2n + 1$; Figs 1c and g) with the frequency of 20% and 5%, and 15 somatic chromosomes in the cultivars NB and GD, respectively (Irani et al. 2016). Thus, the plants' progenies were observed as diploid (Figs 1d and h) and aneuploid individuals. The mitotic investigation confirmed the presence of an extra chromosome. The important issue is that a high rate of double-flowered plants ($> 70\%$) was obtained from the seeds of these plants. Aneuploid individuals were not observed in the progenies of the other cultivars.

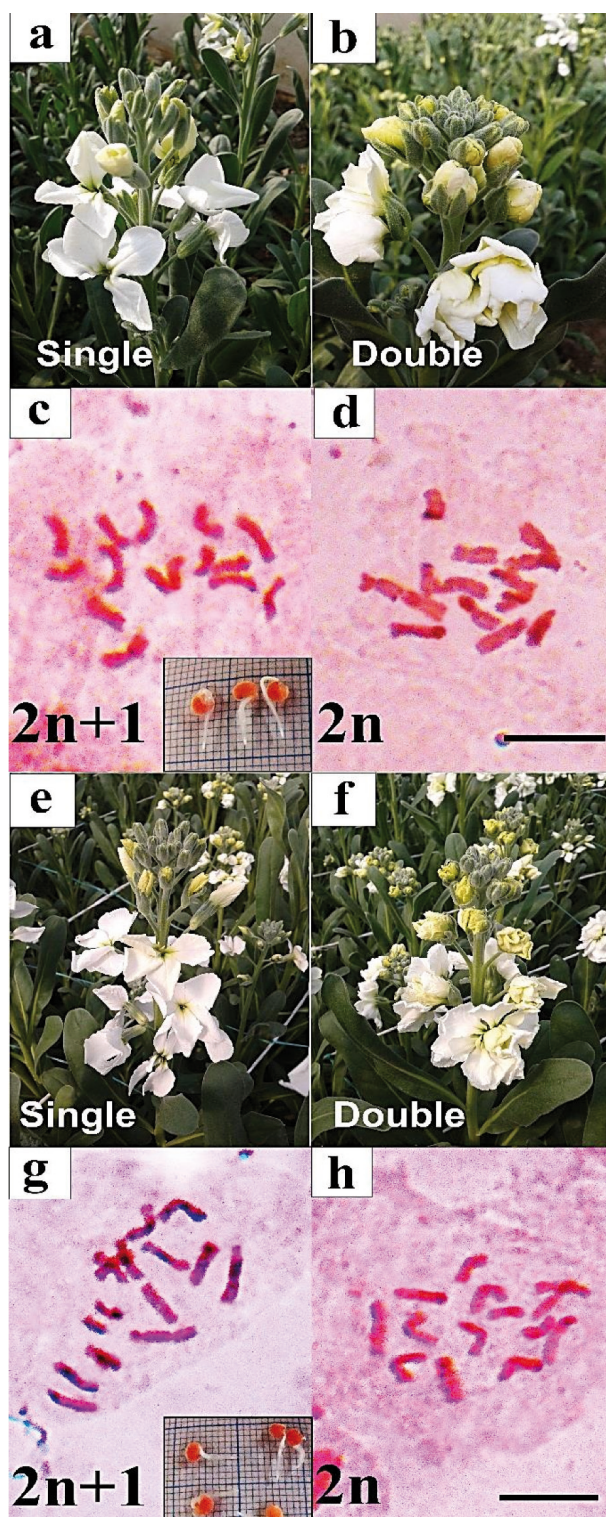


Figure 1. The flowers start opening in single and double flowers of GD cultivar (a, b) and NB cultivar (e, f). The root tip cells in metaphase stage showed a karyotype of the series with 14, 15 chromosomes in seeds obtained from single-flowered plants of GD cultivar (c, d) and NB cultivar (g, h)

3 - Chiasma frequency and chromosome pairing

Two forms of bivalents were observed: ring bivalents that are usually developed by metacentric and sub-metacentric chromosomes, and rod-like bivalents. In this study a higher number of ring bivalents were observed than rod-like bivalents. Analysis of variance (ANOVA) indicated significant differences between the cultivars ($p < 0.001$) in 10 features related to chromosome pairing and chiasma frequency (Tab. 2), and no significant differences in 3 features; middle chiasmata/bivalent, ring bivalents/cell and rod bivalents/cell. The number of bivalents and quadrivalents in the studied cultivars ranged from 3 to 7 and from 0 to 3 per cell with means of 6.19 and 0.40, respectively. The average chiasma frequency ranged from 9.96-12.76 per cell. The highest terminal chiasma was observed in the LL cultivar and the highest number of middle chiasmata was found in the GD cultivar. Univalents were observed only in four cultivars (NB, GD, LL and CR; Fig. 2_19) with their respective means as follows: 0.40, 0.26, 0.15 and 0.06. Despite the diploid nature of this species, quadrivalents were observed in all the studied cultivars (Fig. 2_1). The highest and lowest numbers of quadrivalents were observed in the cultivars NB and CR, respectively. In the cultivars GD and NB, the number of quadrivalents per cell ranged from 0 to 3, with average frequencies of 0.70 and 1.16, respectively. It is thought that the high quadrivalent formation in the NB cultivar led to asymmetric segregation of chromosomes. Pearson correlation analysis indicated a perfect negative relationship between the mean of terminal chiasmata

and the mean of middle chiasmata in these cultivars ($r = -0.80$). A significant positive relationship was observed between the number of bivalents and the percentage of pollen fertility ($r = 0.59$).

4 - Meiotic behaviour

Chromosomal behaviour was studied in twelve cultivars of *Matthiola incana* and the results are presented in Table 3. Many studies have shown a wide variety of meiotic abnormalities such as fragmented chromosomes, bridges, sticky chromosomes, laggards, asynchronous nucleus, triad formation and cytomixis in pollen mother cells (PMCs) of the Brassicaceae family.

a. Cytomixis

In the present study, the cytomixis phenomenon was observed in telophase II stage in the GD cultivar with 0.51% distribution, in the PC cultivar with 0.09% distribution (Fig. 2-16) and in HP with 0.34% distribution.

b. Anaphase bridges

The greatest level of anaphase bridges with 2.77% distribution was observed in the NB cultivar at anaphase I stage (Fig. 2_9). The cultivars LL and MB did not show any bridges in either of the two meiotic phases. Multiple anaphase bridges were observed in the cultivars HP (2.55%) and GD (1.79%) at anaphase II stage (Figs 2_10 and 2_14).

c. Laggard chromosome

Laggard chromosomes during anaphase I and II are shown in Figures 2_3 and 2_15. Chromosomes that

Table 2. Analysis of chromosome pairing at the late stage of diakinesis in twelve cultivars of *Matthiola incana*

CV ^a	Chiasma ^b			II ^c			IV ^d		I ^e	TB ^f	MB ^g	CB ^h	RI ⁱ	RO ^k
	Terminal	Middle	Total	Ring	Rod	Total	Range	Mean						
CR	10.20	1.53	11.73	4.40	2.33	6.73	0-1	0.10	0.15	1.51	0.23	1.74	0.65	0.35
NB	9.33	1.60	10.93	3.23	1.40	4.63	0-3	1.16	0.40	2.01	0.34	2.36	0.70	0.30
GD	9.20	1.83	11.03	3.10	2.53	5.63	0-3	0.70	0.26	1.63	0.32	1.96	0.55	0.45
PC	8.56	1.40	9.96	2.93	3.66	6.60	0-1	0.20	0.00	1.29	0.21	1.51	0.44	0.56
RP	10.03	1.40	11.43	4.00	2.26	6.26	0-2	0.36	0.00	1.60	0.22	1.83	0.64	0.36
LL	11.96	0.80	12.76	5.73	0.96	6.70	0-1	0.13	0.06	1.78	0.12	1.90	0.86	0.14
MB	10.03	1.36	11.40	4.30	2.10	6.40	0-1	0.30	0.00	1.57	0.21	1.78	0.67	0.33
AV	10.03	1.53	11.56	4.33	1.66	6.00	0-2	0.53	0.00	1.67	0.25	1.93	0.72	0.28
HP	10.53	1.43	11.96	4.93	1.73	6.66	0-1	0.16	0.00	1.58	0.21	1.80	0.74	0.26
WS	10.33	1.33	11.66	4.43	1.56	6.00	0-2	0.50	0.00	1.72	0.22	1.94	0.74	0.26
DB	10.26	1.46	11.73	4.56	1.63	6.20	0-2	0.40	0.00	1.65	0.23	1.89	0.74	0.26
ES	11.46	0.80	12.26	5.10	1.43	6.53	0-1	0.23	0.00	1.75	0.12	1.88	0.78	0.22

^aCV – cultivar; ^bchiasma – mean of different types of chiasma; ^cII – mean of bivalents; ^dIV – mean of quadrivalents; ^eI – mean of univalents; ^fTB – terminal chiasmata/bivalent; ^gMB – middle chiasmata/bivalent; ^hCB – total chiasmata/bivalent; ⁱRI – ring bivalents/cell; ^kRO – rod bivalents/cell

Table 3. Percentage of abnormal pollen mother cells during meiosis in twelve cultivars of *Matthiola incana*

CV	E1	S1	L1	B1	A1	M1	E2	L2	B2	A2	M2	Triad	UM	Cyt
CR	2.32	4.65	17.67	0.93	2.32	3.72	8.83	1.39	0.46	0.34	0.96	-	1.57	-
NB	5.34	8.24	23.80	2.77	-	-	0.75	5.04	-	0.14	-	11.30	9.44	-
GD	1.22	6.14	16.81	1.79	1.02	-	-	-	1.79	2.81	-	2.60	5.40	0.51
PC	1.18	4.91	4.37	1.55	-	-	0.36	4.95	2.09	0.18	2.47	0.27	7.14	0.09
RP	3.25	0.65	-	1.65	-	-	-	0.23	0.86	-	-	-	0.61	-
LL	1.02	6.04	5.14	-	-	-	-	0.88	-	-	-	-	1.53	-
MB	1.34	5.86	4.68	-	-	-	-	0.68	-	-	-	-	1.05	-
AV	3.46	2.66	-	0.21	-	-	-	-	1.54	-	-	0.18	0.78	-
HP	2.78	1.56	1.95	0.51	-	-	3.02	1.06	2.55	-	-	-	3.84	0.34
WS	2.80	7.56	10.04	-	-	-	-	1.58	-	-	-	-	0.92	-
DB	1.96	4.76	12.35	1.07	-	-	-	-	-	0.58	-	-	2.95	-
ES	2.89	8.08	8.52	0.35	-	-	-	1.40	-	-	-	-	1.64	-

CV – cultivar, E1 – metaphase-I early segregation, S1 – anaphase-I stickiness, L1 – anaphase-I laggard, B1 – anaphase-I chromosome bridges, A1 – telophase-I asynchronous nuclei, M1 – telophase-I micronucleus, E2 – metaphase-II early segregation, L2 – anaphase-II laggard, B2 – anaphase-II chromosome bridges, A2 – telophase-II asynchronous nuclei, M2 – telophase-II micronucleus, UM – unbalanced microspore, Cyt – cytomixis

had lagged during anaphase division were observed in the NB cultivar with the highest distribution (23.80%). CR and GD showed 17.67% and 16.81% laggard chromosomes in PMCs during anaphase I, respectively.

d. Chromosome stickiness

The highest adhesion rate (8.24%) was observed in NB at anaphase I. Then, the cultivars ES and WS (Fig. 2_17) showed the highest stickiness at anaphase I stage (8.08% and 7.56%, respectively).

e. Micronuclei

The development of univalent and laggard chromosomes is a factor that leads to micronuclei

development in telophase II of meiosis; it was observed with 2.47% and 0.96% distribution in the cultivars PC (Fig. 2_12) and CR (Figs 2_4 and 2_6), respectively.

f. Asynchronous nuclei

Asynchronous nuclei were observed in the cultivars GD (Fig. 2_11; 2.81%) and NB (Fig. 2_13; 0.14%) at telophase II, and the cultivars CR and GD (Fig. 2_5) had asynchronous nuclei at telophase I.

g. Formation of triads

Toward the end of telophase II in the NB cultivar, nearly 11.30% of tetrads were observed in the triad form, and this meiotic behaviour may be an

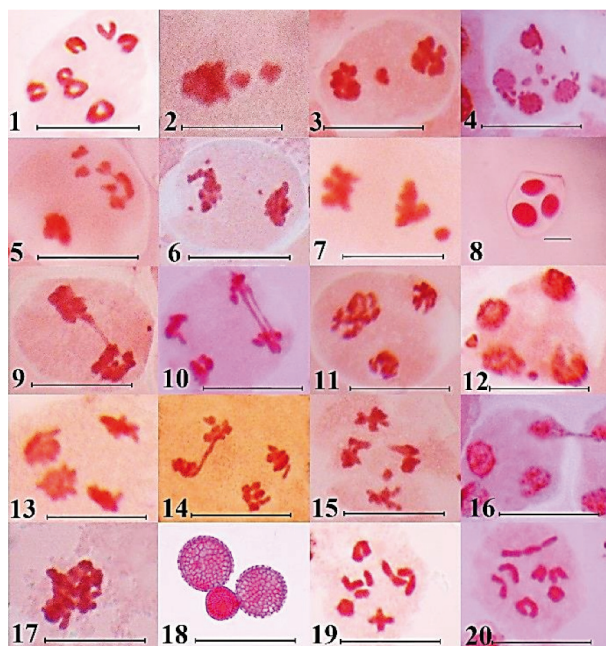


Figure 2. Representative PMCs in studied cultivars of *M. incana*. A PMC showing: (1) 5II + 1IV at diakinesis in DB, (2) Unoriented bivalents at metaphase I in RP, (3) Laggard at anaphase I in LL, (4) Telophase II with micronucleus in CR, (5) Asynchronous nuclei at telophase I in GD, (6) Micronuclei at telophase I in CR, (7) Metaphase II with early segregation in NB, (8) Triad in GD, (9) Anaphase I with a chromosome bridge in NB, (10, 14) Anaphase II with multiple chromosome bridges in HP and GD respectively, (11) Telophase II with asynchronous nuclei in GD, (12) Telophase II with micronuclei in PC, (13) Telophase-II with asynchronous nuclei in NB, (15) Laggard at anaphase-II in ES, (16) Cytomixis at telophase II in PC, (17) Sticky metaphase I in WS, (18) Unbalanced microspore in NB, (19) Univalents at diakinesis in CR, (20) Extra chromosome was shown at diakinesis as a trivalent (chain) in NB (Bar 10 µm)

indicator of non-reduced cell development. Also, triads were observed in 3 cultivars (AV, PC and GD; Fig. 2_8). Koduru and Rao (1981) presents a meiotic index for determining stability in meiosis. In order to determine meiotic indices, the tetrads having four equal natural cells are considered. The meiotic index was 83.25% (lower than 90%) in NB, so this cultivar had inadequate stability.

h. Unbalanced microspore

Some abnormally small-sized pollen was observed in all the studied cultivars. Higher proportions of this pollen were observed in NB (9.44%, Fig. 2_18), PC (7.14%) and GD (5.40%).

i. Early segregation

In the NB cultivar, 5.34% of metaphase cells showed early segregation of chromosomes (Fig. 2_7), while the lowest early segregation was observed in the LL cultivar (1.02%) at the metaphase I stage.

5 - Pollen fertility

The percentage of fertile pollen grains was quite high (> 80%) (Tab. 1). The pollen viability means determined in plants of each cultivar ranged from 82.1% (NB) to 98.9% (DB). In single-flowered plants of the NB cultivar, the lowest and highest percentages of pollen fertility were 72.6% and 91.6% (on average 82.1%), respectively. In single-flowered plants of the GD cultivar, the lowest and highest percentages of pollen fertility were 94.5% and 98.3% (on average 96.4%), respectively. Although meiotic abnormalities can cause pollen sterility, it is thought that pollen viability in the NB and GD cultivars had no correlation with meiotic irregularities. Investigations by Frost and Lesley (1959) showed a link between singleness allele (S) and pollen lethal allele that caused pollen carrying an extra chromosome to become sterile so that the extra chromosome affected pollen viability. Our observations are inconsistent with Frost's study.

Morphological features

1 - Vegetative traits

The ANOVA statistic indicated significant differences in all traits at the 1% level ($p < 0.001$). There was no morphological difference among individual plants within a cultivar, but very significant ($p < 0.001$) differences were found between cultivars. Based on the results obtained from investigating the vegetative features determined in single-flowered plants of each cultivar, the highest values for the height of stem, stem diameter, and the length and width of leaves

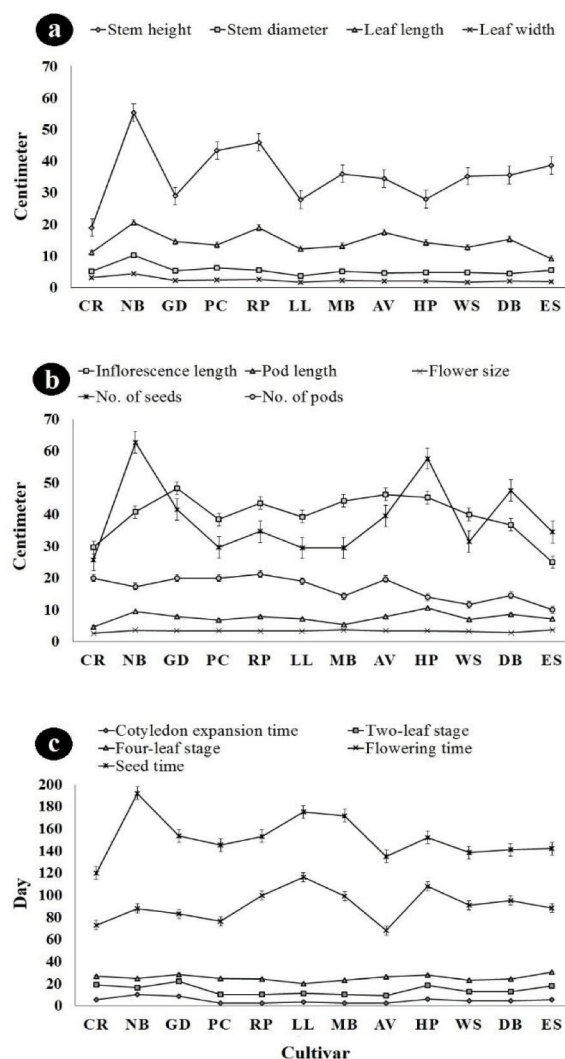


Figure 3. Mean values of (a) vegetative traits (stem height: from ground to the point of beginning of the inflorescence, stem diameter: at a height of 20 cm above soil surface, leaf length and width: average length and width of leaves). (b) floral traits (inflorescence length: from the top of stem to the inflorescence apex, pod length: average length of pods, No. of seeds: average seed number per pod, No. of pods: average pod number, flower size: average size of flowers on an inflorescence). (c) phenological traits (cotyledon expansion time: sowing to emergence of cotyledon, two- or four-leaf stage: sowing to emergence of two or four primary leaves, seed time: sowing to seed harvest, flowering time: sowing to appearance of the first open flower) in adult plants of twelve stock cultivars

were recorded in the NB cultivar (Fig. 3a). In terms of morphological features impacting the quality of cut flowers, the best morphological features were identified in the following order; the best was the NB cultivar, followed by the PC cultivar, and the GD cultivar in third place. The NB cultivar had a thicker flower stem than the other cultivars. A strong stem prevents the inflorescence of a cut stock flower from bending.

2 - Reproductive traits

We recorded longer lengths of inflorescence in the GD cultivar than that in the NB cultivar (Fig. 3b). Frost and Mann (1924) had reported that the morphological marker of long and wide leaves was a marker of a cultivar with a high double stock production rate. Previous investigations on the stock flower had shown changes of morphological characteristics in stock plants that give an especially high proportion of double-flowered progenies (Frost and Lesley 1927, Emsweller et al. 1937). According to Frost and Lesley's study (1927), unstable plants are characterized by slender stems, long and narrow leaves and drooping inflorescence. In 1936 a researcher named Hospers (Prakken 1942) observed seven mutated stock flowers with distinct morphological traits in her research garden. She found that mutant plants had stout, fleshy stems with many leaf-scars. In the present study, such morphological features were not observed among any single-flowered individuals of either the NB or the GD cultivar. The highest evaluation for the seed number per pod was obtained from single-flowered plants of the NB cultivar (62.7; Fig. 3b). The maximum value of pod length was recorded in the HP cultivar.

Phenological features

Significant variation ($p < 0.001$) was found in five phenological traits recorded during plant growth of the stock cultivars. The longest time for germination and emergence of a cotyledon was observed in the NB cultivar. In terms of emergence of two primary leaves (two-leaf stage), the AV cultivar takes

less time to enter into this developmental stage. This feature is important for selectable cultivars. Following the appearance of primary leaves (2-4 main leaves), the colour of the cotyledons remains pale green in the double-flowered plants and dark green in the single-flowered plants; identification of these stages is important for making distinctions between double-flowered and single-flowered plants (Kappert 1937, Ecker et al. 1994). The results of this study for flowering time showed that the inflorescence emerged faster in the AV cultivar than it did in other cultivars, while in the LL cultivar, the inflorescence was observed later than in the other cultivars (Fig. 3c). Seed pods of the CR cultivar formed faster than in the other cultivars, since its inflorescence and seed pod were formed faster than in the other cultivars.

Relationships among meiotic and morphological traits

Pearson correlation analysis was performed for all the studied characteristics (important data is presented in Table 4). The results indicate a positive relationship of the vegetative characteristics of: stem height, stem diameter, leaf length, leaf width and internode distance with the average of quadrivalents ($p < 0.05$), while the total bivalents had a significant negative relationship with the vegetative characteristics and the number of seeds per pod. A positive relationship was observed between the percentage of double-flowered plants and the number of quadrivalents. It can be concluded that the aneuploidy in the NB cultivar may change the ratio of double-flowered plants

Table 4. Coefficient of correlation between morphological and meiotic traits in *Matthiola incana* cultivars

	TC	MC	TOC	RI	RO	TOB	AQ	TB	MB	CB	PF
SH	-0.38 ^{ns}	0.05 ^{ns}	-0.48 ^{ns}	-0.47 ^{ns}	0.05 ^{ns}	-0.60*	0.61*	0.33 ^{ns}	0.29 ^{ns}	0.40 ^{ns}	-0.66*
SD	-0.53 ^{ns}	0.34 ^{ns}	-0.55 ^{ns}	-0.63*	0.11 ^{ns}	-0.76**	0.74**	0.39 ^{ns}	0.61*	0.55 ^{ns}	-0.93**
LL	-0.47 ^{ns}	0.55 ^{ns}	-0.38 ^{ns}	-0.48 ^{ns}	-0.02 ^{ns}	-0.71**	0.70*	0.33 ^{ns}	0.68*	0.53 ^{ns}	-0.52 ^{ns}
LW	-0.48 ^{ns}	0.43 ^{ns}	-0.45 ^{ns}	-0.55 ^{ns}	0.12 ^{ns}	-0.64*	0.60*	0.31 ^{ns}	0.62*	0.48 ^{ns}	-0.89**
ID	-0.53 ^{ns}	0.22 ^{ns}	-0.60*	-0.62*	0.16 ^{ns}	-0.68*	0.68*	0.28 ^{ns}	0.45 ^{ns}	0.41 ^{ns}	-0.73**
IL	-0.39 ^{ns}	0.55 ^{ns}	-0.28 ^{ns}	-0.33 ^{ns}	0.11 ^{ns}	-0.34 ^{ns}	0.36 ^{ns}	-0.02 ^{ns}	0.51 ^{ns}	0.15 ^{ns}	-0.03 ^{ns}
PL	-0.04 ^{ns}	0.17 ^{ns}	0.02 ^{ns}	-0.03 ^{ns}	-0.31 ^{ns}	-0.42 ^{ns}	0.42 ^{ns}	0.40 ^{ns}	0.29 ^{ns}	0.46 ^{ns}	-0.22 ^{ns}
NS	-0.18 ^{ns}	0.37 ^{ns}	-0.08 ^{ns}	-0.18 ^{ns}	-0.31 ^{ns}	-0.63*	0.61*	0.50 ^{ns}	0.54 ^{ns}	0.63*	-0.43 ^{ns}
NP	-0.44 ^{ns}	0.42 ^{ns}	-0.40 ^{ns}	-0.43 ^{ns}	0.48 ^{ns}	-0.05 ^{ns}	0.04 ^{ns}	-0.33 ^{ns}	0.34 ^{ns}	-0.18 ^{ns}	-0.25 ^{ns}
PD	-0.64*	0.65*	-0.57 ^{ns}	-0.69*	0.22 ^{ns}	-0.70*	0.70*	0.19 ^{ns}	0.79**	0.43 ^{ns}	-0.58*

**Correlation is significant at $p < 0.01$. *Correlation is significant at $p < 0.05$. ^{ns} non-significant at $p > 0.05$.

SH – stem height, SD – stem diameter, LL – leaf length, LW – leaf width, ID – internode distance, IL – inflorescence length, PL – pod length, NS – no. of seeds per pod, NP – no. of pods, PD – percentage of double-flowered plants, TC – terminal chiasmata, MC – middle chiasmata, TOC – total chiasmata, RI – ring bivalents, RO – rod bivalents, TOB – total bivalents, AQ – average of quadrivalents, TB – terminal chiasmata/bivalent, MB – middle chiasmata/bivalent, CB – total chiasmata/bivalent, PF – pollen fertility

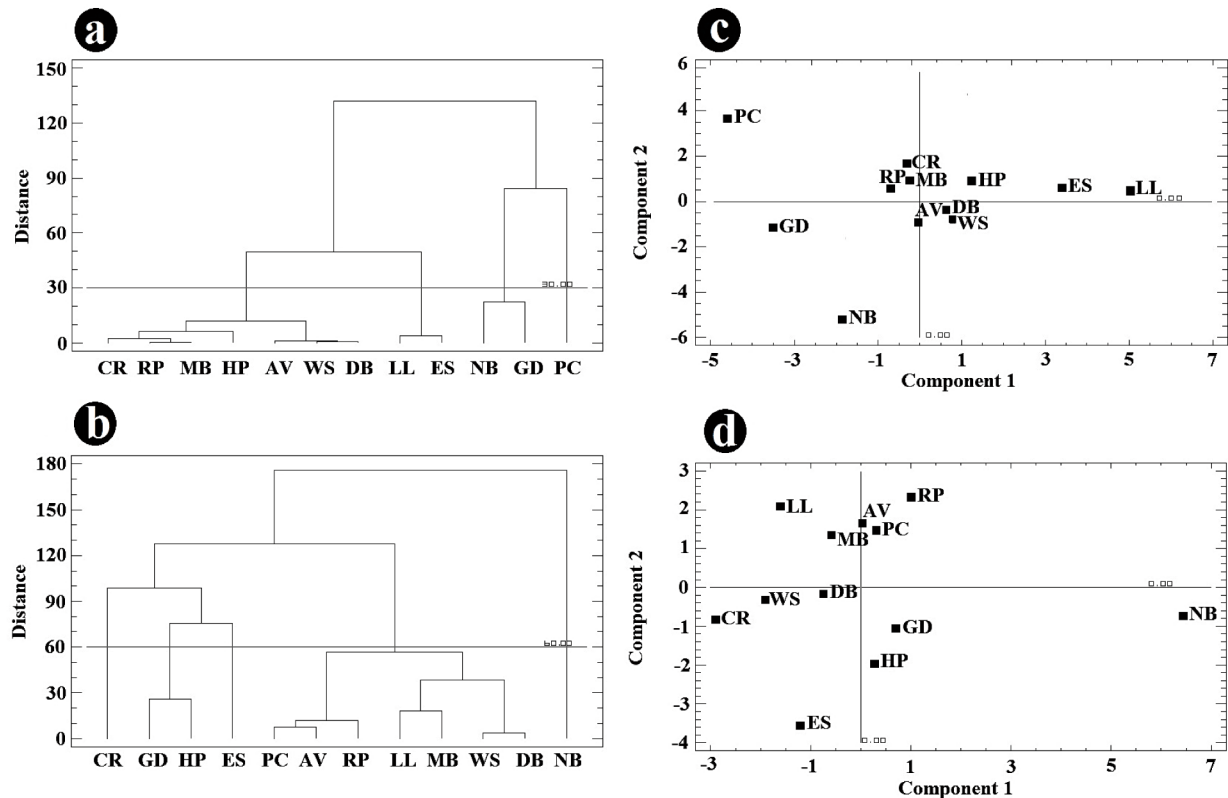


Figure 4. (a) Ward's clustering based on meiotic data in twelve cultivars of *Matthiola incana*, (b) Dendrogram obtained from cluster analysis for morpho-phenological traits in stock cultivars, (c) Diagram resulting from principal components analysis (PCA) based on meiotic data, (d) Diagram obtained from PCA based on morpho-phenological data

(80%). It seems that a strong relationship exists between the occurrence of change in chromosome number and the percentage of double-flowered plants in the cultivars NB and GD.

Relationships among cultivars

Principal component analysis (PCA) was applied to characteristics to identify the most variable ones. The result of PCA analysis based on 14 morpho-phenological traits indicated that the first five principal components described approximately 89% of cumulative variance (Fig. 4d). The result indicated that the parameters of stem diameter, two-leaf stage time, flowering time, inflorescence length and four-leaf stage time were strongly connected with the principal components, from the first to the fifth, respectively. Also, a PCA was performed involving twelve diakinesis characteristics. The first two principal components (mean of terminal chiasma, total bivalents) described approximately 95% of cumulative variance (Fig. 4c). The dendrogram based on the morpho-phenological traits (using Ward's method) indicated five groups of cultivars in the study at a Euclidean distance of 35% (Fig. 4b). The highest Euclidean distance was observed for the cultivars CR and NB.

The most similarity was observed between WS with DB (the lowest Euclidean distance). The clustering of meiotic traits demonstrated four groups at a Euclidean distance of 20% (Fig. 4a). A comparison of the two dendrograms indicated that the cultivars WS and DB were located in the same group in both groupings. The cultivars NB and GD were placed in one group in the meiotic dendrogram, while NB was placed in a separate group in the morpho-phenological cluster. Although aneuploid PMCs at the diakinesis stage were observed in two cultivars (NB and GD) with similar chromosomal features, their morphological and phenological traits were significantly different. According to the low number of distinct groups, the cluster analysis showed lower performance of meiotic traits in cultivar separation compared with morpho-phenological features.

CONCLUSIONS

1. The meiotic study showed that the irregular separation of chromosomes during meiosis I and II could be due to the formation of non-oriented bivalents or univalents. Therefore, some neuploid cells were observed in two cultivars, in

which a number of trisomy seeds were produced in plants. The production of aneuploid PMCs could be a main factor leading to spontaneous variation within the species of *Matthiola incana*.

2. All of the cultivars in our study had few meiotic abnormalities and, as a consequence, high pollen fertility (> 80%). We suggest that the differential high production of double-flowered plants observed among the cultivars is due to genetic control and not only to meiotic abnormalities.
3. Collectively, these results display large genetic variability between stock cultivars in terms of morpho-phenological traits, such that they can be considered a noteworthy gene pool for stock breeding programmes.

FUNDING

We would like to express our gratitude to the Tehran University for providing the required facilities and equipment. Moreover, our thanks go to Sabz Parvar Co. as the stock flower growers for their kind assistance in seed collection.

AUTHOR CONTRIBUTIONS

S.F.I. and M.A. – contributed equally to this work.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Received July 9, 2016; accepted March 16, 2017