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Genetic Diversity of Stock (*Matthiola incana* L.) Cultivars Based on Cytogenetic Characteristics

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ABSTRACT: Twenty cultivars of *Matthiola incana* growing in various areas with different percentages of double flowers were chromosomally investigated, using fixation and staining of root tip cells. The somatic chromosome number (2n = 2x = 14) in mitotic metaphase cells and quantitative karyotypic parameters were evaluated. Aneuploidy was observed in two cultivars with high percentage of double flowers. An extra chromosome was observed as a rod univalent or chain trivalent in PMCs at diakinesis stage in two cultivars. The satellites were found in four cultivars (mostly locating on short arms) and were differed in size (0.62 to 0.83 µm). Two chromosomal types (metacentric, sub-metacentric) formed six different karyotype formulas. The degree of karyotype asymmetry was studied using qualitative and quantitative methods. The most of stock cultivars were placed in 1A classes of Stebbins' karyotype asymmetry. Based on Ward's method, the cultivars separated into three clusters. The results of PCA showed the first three principal components accounted for 95.50% of the total variability.

Keywords: Aneuploidy; Diakinesis; Double flower; Extra chromosome; Idiogram; Karyotype asymmetry and Satellite.

INTRODUCTION: *Matthiola incana*, commonly named stock flower, is an ornamental plant which belongs to Brassicaceae family[1]. Stock flower is commercially cultivated all over the world from Turkey to Egypt with more than 100 cultivars having pleasant scent and attractive colors[2]. Full of alpha-linoleic acid is found in the seed oil of *M. incana*, which plays a key role in human health[3]. Two forms of flowers are identified in *M. incana* that the first form called "Single flower" has reproductive organs and the second form called "Double flower" is infertile[4&5]. Since the reproductive organs are replaced by the petals, the second form due to its more beautiful appearance is popular in the cut flower[6]. Four types of single flower have been identified based on the percent of double production: type I (Single) does not produce doubles, type II produces 25%, type III (Ever sporting) produces 46-54 percent and type IV (High double) produces above 80% double flower[7]. Since the double-flowered type is obtained from single-flowered seeds, the ratio of double to single progenies is economically important in this plant[8]. Some studies demonstrated the relationship of cytogenetic characteristics and the percentage of double-flowered population in this plant[9 & 10]. According to Philip and Huskins's study[4], seven pairs (2n=2x=14) of chromosomes were identified in *M. incana.* Frost and Lesley[10 & 13] recognized aneuploidy cultivar with high percent of double flower (90%). Although morphological diversity has been found in this species[11], no cytogenetic study has been performed on different cultivars of stock flower. In addition, cytogenetic studies on this plant go back to 50 years ago[12 & 13]. Therefore, chromosomal analysis has played a considerable role in the evaluation of genetic variation and breeding research in *Matthiola incana*[14]. Cytogenetic information such as the number of somatic chromosomes, karyotypic asymmetry is used in the investigating genetic diversity, somatic hybridization, ploidy manipulation[15]. In this study, different cultivars of stock flower with different double-flowered states in terms of the number, somatic chromosome characteristics, karyotypic formula and the degree of affinity are investigated. Also, the present work was done to study the karyotype asymmetry/ symmetry among the stock cultivars. It is believed that symmetrical karyotypes have more preliminary evolutional degree than asymmetrical karyotypes[16]. For this purpose, categorization for karyotypic symmetry indices was considered and the symmetry indices were divided into three classes: 1-centromeric symmetry, 2-chromosome size symmetry 3-karyotype symmetry indices[17]. Therefore, accuracy and sensitivity of indices have been used to evaluate karyotypes and draw the relationship between stock cultivars.

MATERIALS AND METHODS:

Plant Materials: In order to study the karyotypic characteristics of twenty stock cultivars, seeds were collected from seed companies and breeding institutions. After sterilizing (2% sodium hypochlorite for 10

min) and washing seeds, they were transferred into glass petri dishes on humid filter paper and grown in the seed germinator at 20°C. The apical meristem (1-1.2 cm long root tip) was used for cytogenetic analysis of somatic chromosomes. Chromosomal study was conducted in four steps:

A) Pretreatment step: Sample roots were placed in 0.002 M of 8-hydroxyquinoline for 3h, at room temperature in darkness.

B) Fixation step: After washing three times with distilled water, fixation stage was performed by Carnoy's fixative (glacial acetic acid: ethanol; 3:1) for 24 h at 4°C. Samples stored in 70% (v/v) ethanol at 4°C[18].

C) Hydrolysis step: Hydrolysis procedure was performed by 1% hydrochloric acid at 60°C for 10 min.

D) Staining step: Materials finally was stained with 2% (w/v) aceto-orcein for 30 min at room temperature. After staining, slides were prepared by squash method in a driblet of 45% (v/v) acetic acid[19].

For meiotic chromosome studies, immature flower buds were fixed in the new Carnoy's fixative solution for 24 hours and anthers were stained and squashed in 2% aceto-orcein. At least thirty pollen mother cells (PMCs) from each plant (6 plants per replicate of each cultivar) were observed to confirm the results of chromosome number at diakinesis. A total of 200 cells at the diakinesis stage of per cultivar were examined.

Chromosome Measurement: On average, ten well-spread mitotic metaphases of each cultivar were photographed and analyzed. The images were magnified 10x100 which obtained using an OLYMPUS CX21 microscope (Olympus optical Co., Ltd., Tokyo, Japan). Measuring the long (L) and short (S) arm length, chromosome length, centromere index (CI), arm ratio index (AR) and relative chromosome length (RL%) was done by Micro measure version 3.3 software[20].

Karyotype Analysis: The classification of chromosomes was done using nomenclature proposed by Levan[21] and karyotypic symmetry was determined using two-sided table of Stebbins[16]. The qualitative method (Stebbins classification) seemed to be less sensible than quantitative methods. Therefore, the degree asymmetries of chromosomes were studied using quantitative parameters. The factors of size and shape of chromosomes and centromere position are very vital in investigating karyotype asymmetry/symmetry[22]. Karyotype asymmetry indices have been employed to evaluate the variation in centromeric position/chromosome size in chromosome complement[23]:

A) Centromere Position Variation: (1) Total form percentage[24]; TF%, (5) mean centromeric asymmetry[17]; M_{CA}, (6) index of karyotype symmetry[25]; Syi, (7) percentage karyotype asymmetry index[26]; As.K%, (11) intra-chromosomal asymmetry index[27]; A_1, (14) coefficient of variation of centromere index[28]; CV_{CI}.

B) Chromosome Size Variation: (4) Chromosome size resemblance index[25]; Rec, (12) inter-chromosomal asymmetry index[27]; A_2.

C) General Karyotype Asymmetry: (2) differences in the relative length of chromosome[24]; DRL, (8) dispersion index[29]; DI, (9) Centromeric gradient[29]; CG%, (10) asymmetry index[30]; AI, (13) the relative length of the shortest chromosome; S%. Some parameters were calculated according to the following formula[23]:

$$TF\% = [\sum S/\sum TL] \times 100 \quad (1)$$
$$DRL = RL\%(Max)-RL\%(Min) \quad (2)$$
$$RL\% = [TL_X/\sum TL] \times 100 \quad (3)$$
$$Rec = [(\sum CL_i)/(\sum LC)]/n \times 100 \quad (4)$$
$$M_{CA} = [\sum (L-S)/(L+S)]/n \times 100 \quad (5)$$
$$Syi = X_S/X_L \times 100 \quad (6)$$
$$As.K\% = [(\sum L)/(\sum TL)] \times 100 \quad (7)$$
$$DI = [CG \times CV_{TL}]/100 \quad (8)$$
$$CG\% = [(Median\ S)/(Medan\ TL)] \times 100 \quad (9)$$
$$AI = [CV_{CL} \times CV_{CI}]/100 \quad (10)$$
$$A_1 = 1-[(\sum S_x/L_x)/n] \quad (11)$$
$$A_2 = SD_{CL}/X_{CL} \quad (12)$$
$$S\% = [TL_{Min}/\sum TL] \times 100 \quad (13)$$
$$CV_{CI} = [SD_{CI}/X_{CI}] \times 100 \quad (14)$$

(1-14) Where S: short arm length, L: long arm length, TL: total chromosome length, n: number of analyzed chromosome, RL: relative length of chromosome, CL: length of each chromosome, LC: longest chromosome, X: mean, SD: standard deviation.

Data Analysis: Data analysis was carried out by SPSS ver.16.0 Software. Pearson's correlation coefficients were computed to investigate the relationships between karyotype asymmetry indices. After measuring chromosomal statistics, chromosome ideogram of each cultivar was drown in Excel 2010. Cultivars were grouped with the Ward's method. Scatter diagrams of correlations among the symmetry indices were generated by using Stat Graphics Software.

RESULTS AND DISCUSSION:

Chromosome Count: The metaphase chromosomes of studied cultivars are presented in Figure 1. The results of chromosomes count indicated that the somatic chromosomal number was 2n = 2x = 14 in eighteen studied cultivars and they were diploid. This chromosome number is consistent to the previous reports[12 & 31]. While metaphase cells of root tips of two

cultivars NB, GD indicated the chromosomal number 15, for making sure of this result, more metaphase samples of these two cultivars were studied.

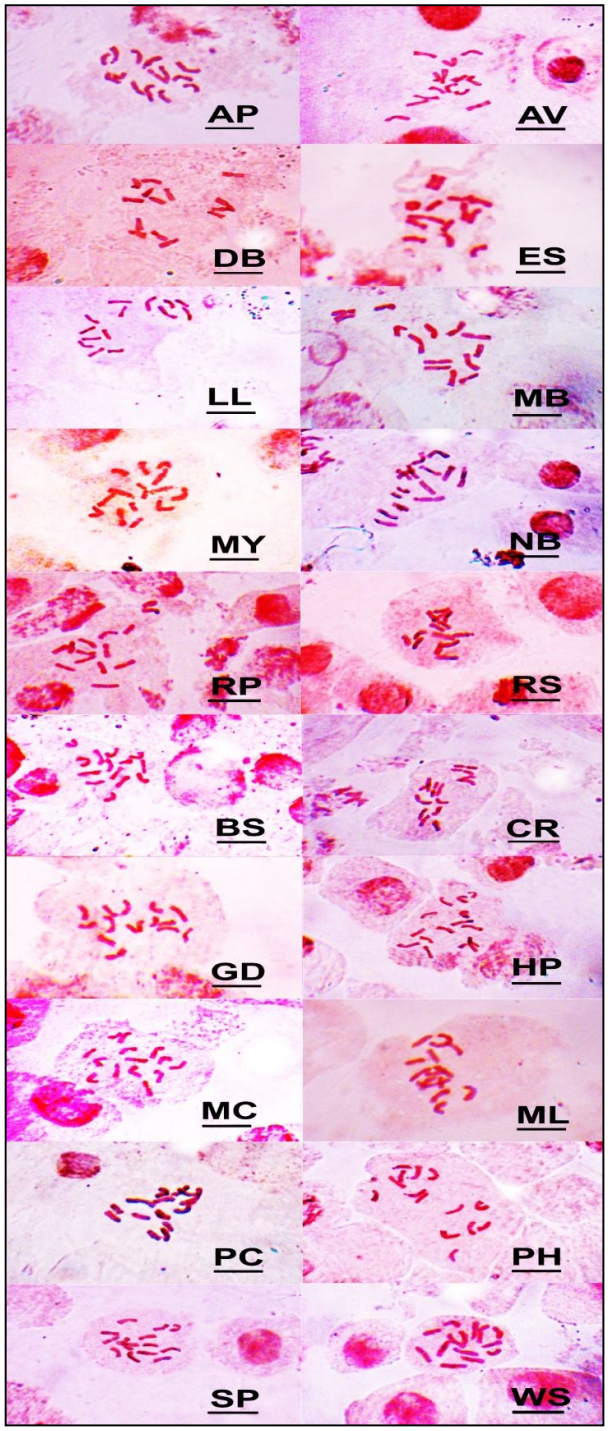

Figure 1: Somatic chromosomes of twenty cultivars of *Matthiola incana* (Scale bar =10µm).

It was noted that meiotic studies performed on these cultivars confirmed the presence of additional chromosome in these two cultivars. Aneuploidy has been reported in this plant previously[13 & 32]. In two cultivars (NB and GD), PMCs (pollen mother cells) at diakinesis stage with 7 normal pairs in the form of bivalents and quadrivalents and an additional chromosome as a rod univalent or chain trivalent are shown in Figure 2. The percent of aneuploidy, a state in which cells have an additional chromosome, observed in two cultivars, GD & NB in diakinesis cells of mother pollen is 13.30% and 36.70% respectively. Therefore, these two cultivars have the potential to produce unreduced gametes. The basic chromosome number of other cultivars was n = x = 7. In these cultivars, meiotic cells showed 7II (bivalent) or 5 II + 1 IV (quadrivalent) or 3 II + 2 IV during diakinesis. Univalent was observed in several cultivars (CR, NB, GD and LL).

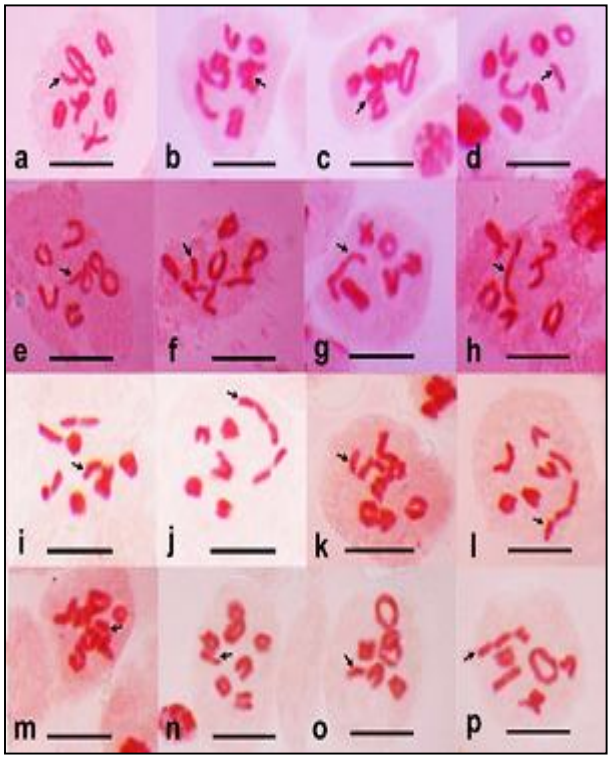

Figure 2: Pollen mother cells (PMC) with 8 chromosome paired at diakinesis stage in two cultivars of *M. incana.* In NB cultivar: (a, c, e) A PMC showing 5 II + 1I (the extra chromosome as a rod univalent, arrowed) +1 IV. (b, d, f) A PMC showing 7 II+ 1I (a rod univalent formed by an additional chromosome, arrowed). (g) A PMC showing 4 II +1 III (chain, arrowed) + 1 IV (zigzag ring). (h) 6 II + 1 III (chain, arrowed). In GD cultivar: (i, k, m) A PMC showing 7II + 1I (the extra chromosome as a rod univalent, arrowed). (n, o) A PMC showing 5 II + 1I (the extra chromosome as a rod univalent, arrowed) +1 IV. (j, l) A PMC showing 6 II + 1 III (chain, arrowed). (p) A PMC showing 4 II + 1 III (chain, arrowed) + 1 IV (typical ring), Bar = 10µm.

Morphological Features of Chromosomes: The analysis of karyotypic data variance indicated that these cultivars have significant difference ($P < 0.001$) in terms of chromosome length, length of long arm and short arm, centromere index, arm ratio index and relative chromosome length (data not shown). Idiograms of twenty stock cultivars are shown in Figure 3.

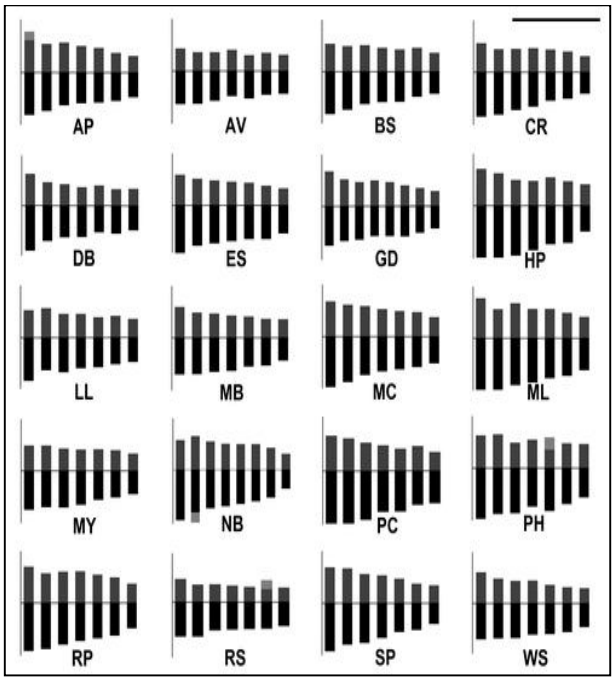

Figure 3: Idiograms of twenty stock cultivars of *Matthiola incana* (Bar = 10µm).

Using chromosomal data for each cultivar, pairs of chromosome were arranged from the largest to the smallest length. Mean comparisons of karyotypic features indicated that RS cultivar has lower mean value in terms of the length of chromosome (2.41 µm), long arm length (1.79 µm) and short arm length (0.62 µm) than other cultivars. The mean value of total length of chromosome was obtained as 3.75 µm varying from 2.41 µm (RS) to 5.25 µm (ML). Additionally, the results implied that NB cultivar (1.35 µm) and ML cultivar (7.52 µm) had the shortest and longest chromosome respectively. The shortest chromosome was found in trisomic cultivar that this may refer to the theory of Frost[13] concerning the breakage of the long chromosome.

Chromosome Structure and Diversity: Karyotypic formula of the cultivars indicated that all cultivars have two types of chromosomes called metacentric and sub-metacentric; this finding is consistent to the report of Allen[33]. Six different karyotypic formulas were observed in these cultivars (Table 1). The highest number of metacentric chromosomes related to GD & NB cultivars with KF = 8m formula, while the lowest count of metacentric chromosomes found in PC cultivar with KF = 2m + 5sm formula. In present study, the satellite was observed on four cultivars on one pair of satellite chromosome that differed in terms of size (0.62 - 0.83µm) and location. In three cultivars PH, AP and RS, the satellite was on the short arm and in NB cultivar, it was found on the long arm which is in agreement with the former study of Philp and Huskins[4].

Karyotype Asymmetry Indices: Categorization based on Stebbins method indicated that except for three cultivars NB, PC and SP which are in 1B class, other cultivars have karyotypic asymmetry Stebbins class[16] of 1A, 2A. Placement of the most cultivars in A class implies high karyotypic symmetry among studied cultivars and the chromosomes are relatively symmetrical and their karyotypic formula confirms this claim. The results of variance analysis (ANOVA) of karyotypic asymmetry indices indicated that there are some differences among the cultivars in terms of the rate and degree of these indices ($P < 0.01$). The TF% value was considered to be close to 50% in the more the number of metacentric chromosomes and karyotype symmetric. The highest and lowest value of TF% was related to SP (40.42%; the most symmetric) and RS (25.63%; the most asymmetric) respectively (Table 2). In general, A_1 and TF% parameters are considered as intra-chromosomal symmetry factors that are inversely correlated. CV_{CI}% index is the most important and effective centromeric symmetry index. This index produced different results. The highest and the lowest values of CV_{CI}% were identified on RS (26.39%; the most asymmetric) and MC (5.85%; the most symmetric) cultivars, respectively. This result is close to the some other centromeric symmetry indices such as TF%, Syi, As. K%, A_1 and M_{CA}. According to Zuo and Yuan study[34], CV_{CI}% is weak to exactly evaluate intrachromosomal asymmetry. Moreover, the more the relative length of the shortest chromosome (S% index) means the more the difference among the chromosomes and consequently the more the karyotype asymmetry. RS cultivar (10.06%) has the most asymmetric karyotype in this regard. The less the DRL means the more karyotype symmetry. The lowest value of DRL was observed in AV cultivar (9.04%). The result of DRL was different from other indicators. The DI index is more reliable than other symmetry indices because the three traits including size variations of chromosomes, the relative size of chromosomes and centromere position used in its calculation. The highest and lowest values of DI were distinguished in SP (16.39%, the most asymmetric) and AV (6.98%; the most symmetric) cultivars, respectively.

Table 1: Data related to karyotype of twenty cultivars of *Matthiola incana*.

Cultivar	Code	Loc[1]	Somatic Ch. NO.	Stebbins classes	HC[2] (µm)	Karyotypic formulae [3]	Sat[4]	L(±SE)[6] (µm)	S(±SE)[7] (µm)	CL(±SE)[8] (µm)
Cinderella red	CR	JPN	2n=14	1A	25.16	KF=6m+1sm	NO[5]	2.34±0.84	1.25±0.38	3.59±1.19
Nobel white	NB	JPN	2n+1=15	1B	32.63	KF=8m	1 Sat	2.34±0.90	1.49±0.50	3.83±1.37
Pacific crimson	PC	USA	2n=14	1B	33.82	KF=2m+5sm	NO	3.30±1.11	1.53±0.53	4.83±1.62
Rose pink	RP	USA	2n=14	1A	30.59	KF=7m	NO	2.65±0.75	1.72±0.50	4.37±1.22
Purple heart	PH	USA	2n=14	2A	32.27	KF=5m+2sm	1 Sat	2.98±0.70	1.63±0.52	4.61±1.05
Lavender lilac	LL	USA	2n=14	1A	22.96	KF=7m	NO	2.05±0.58	1.23±0.35	3.28±0.88
Column apricot	AP	USA	2n=14	1A	24.76	KF=7m	1 Sat	2.15±0.57	1.39±0.53	3.54±1.07
Miracle blue mid	MB	USA	2n=14	1A	22.42	KF=7m	NO	2.02±0.48	1.18±0.40	3.20±0.86
Miracle crimson	MC	USA	2n=14	1A	31.12	KF=7m	NO	2.72±0.91	1.72±0.51	4.44±1.40
Miracle yellow	MY	USA	2n=14	1A	22.53	KF=7m	NO	2.09±0.56	1.13±0.27	3.22±0.81
Avalanche white	AV	USA	2n=14	1A	18.08	KF=5m+2sm	NO	1.76±0.41	0.82±0.23	2.58±0.56
Miracle lavender	ML	NED	2n=14	1A	36.79	KF=6m+1sm	NO	3.39±0.93	1.86±0.58	5.25±1.45
White goddess	GD	NED	2n+1=15	1A	28.15	KF=8m	NO	1.98±0.50	1.29±0.58	3.27±1.07
Salmon pink	SP	NED	2n=14	1B	26.99	KF=7m	NO	2.30±0.90	1.56±0.66	3.86±1.85
Column blue stock	BS	IRN	2n=14	1A	23.48	KF=7m	NO	2.06±0.70	1.29±0.28	3.35±0.93
Hot cakes purple	HP	IRN	2n=14	1A	32.70	KF=5m+2sm	NO	3.06±0.91	1.61±0.50	4.67±1.33
Column white	WS	IRN	2n=14	1A	22.94	KF=5m+2sm	NO	2.16±0.42	1.12±0.55	3.28±0.95
Deep blue stock	DB	IRN	2n=14	1A	23.30	KF=5m+2sm	NO	2.20±0.68	1.13±0.53	3.33±1.20
Column red stock	RS	IRN	2n=14	2A	16.85	KF=4m+3sm	1 Sat	1.79±0.39	0.62±0.31	2.41±0.67
Esfahan purple	ES	IRN	2n=14	1A	28.20	KF=6m+1sm	NO	2.68±0.62	1.34±0.45	4.03±1.07

[1]Loc: Location, JPN: Japan, NED: Netherland, IRN: Iran,[2]HC: Haploid complement, [3] m: metacentric, sm: sub-metacentric, [4] Sat: satellite, [5]NO: not observed, [6]L: mean length of long arm, [7]S: mean length of short arm, [8]CL: mean length of chromosome

Table 2: Quantitative parameters used for the classifications of twenty stock cultivars.

Code	Centromere position variation						Ch. size variation		Karyotype asymmetry				
	A_1	TF%	M_{CA}	CV_{CI}	Syi	As. K	A_2	Rec	CG%	DI	DRL	S %	AI
CR	0.45	34.89	29.31	11.24	53.59	65.10	0.33	68.00	33.77	11.21	14.12	6.88	3.73
NB	0.35	38.82	21.59	7.69	63.46	61.18	0.36	67.79	38.51	13.81	14.04	**4.40**	2.76
PC	0.53	31.66	36.87	11.12	46.32	68.34	0.34	66.30	33.05	11.12	13.17	8.38	3.74
RP	0.35	39.31	21.61	7.30	64.77	60.69	0.28	72.96	38.57	10.80	12.25	7.33	2.04
PH	0.44	35.29	29.61	21.35	54.54	64.71	0.23	75.08	33.18	7.56	9.14	9.89	4.87
LL	0.39	37.60	24.79	11.43	60.27	62.39	0.27	69.41	39.08	10.45	11.34	9.24	3.06
AP	0.37	39.29	23.59	16.25	64.72	60.71	0.30	69.12	**43.24**	13.09	12.77	7.90	4.92
MB	0.42	36.93	26.91	9.86	58.54	63.07	0.27	71.99	33.60	9.04	11.19	8.65	2.65
MC	0.36	38.71	22.37	**5.85**	63.16	61.29	0.31	66.05	40.80	12.86	13.31	8.31	**1.43**
MY	0.45	35.14	29.25	8.06	54.18	64.86	0.25	**76.52**	32.85	8.30	10.18	8.49	2.04
AV	0.52	31.95	36.14	18.44	46.94	68.05	**0.22**	74.88	31.92	**6.98**	**9.04**	10.03	4.03
ML	0.45	35.41	29.48	10.90	54.83	64.58	0.27	69.91	35.14	9.68	11.81	8.62	3.00
GD	0.37	39.54	23.91	15.71	65.41	60.45	0.33	63.31	40.49	13.24	13.58	6.16	5.14
SP	**0.33**	**40.42**	**19.82**	7.68	**67.83**	**59.58**	**0.40**	64.37	40.78	**16.39**	**15.85**	6.34	3.08
BS	0.35	38.51	21.63	11.50	62.64	61.48	0.28	70.54	39.41	10.93	12.04	8.20	3.19
HP	0.46	34.52	30.22	14.68	52.72	65.48	0.28	73.05	31.66	8.99	11.99	7.56	4.17
WS	0.50	34.07	35.27	22.87	51.68	65.93	0.29	69.98	35.88	10.39	12.23	8.18	6.62
DB	0.49	33.92	33.82	14.41	51.34	66.07	0.36	**58.26**	34.37	12.37	15.22	9.30	5.19
RS	**0.67**	**25.63**	**50.91**	**26.39**	**34.47**	**74.36**	0.28	67.81	**26.56**	7.39	11.01	**10.06**	**7.34**
ES	0.51	33.40	34.38	10.16	50.16	66.59	0.26	68.89	34.05	9.02	11.85	8.88	2.69
Min	**SP**	**RS**	**SP**	**MC**	**RS**	**SP**	**AV**	**DB**	**RS**	**AV**	**AV**	**NB**	**MC**
Max	**RS**	**SP**	**RS**	**RS**	**SP**	**RS**	**SP**	**MY**	**AP**	**SP**	**SP**	**RS**	**RS**

Ch.: Chromosome, A_1: intra-chromosomal index, TF%: total form percentage, S%: symmetry index, DRL: differences in the relative length of chromosome, Syi and As.K: karyotype symmetry indices, A_2: inter-chromosomal index, Rec: chromosome size resemblance index, CG%: centromeric gradient, DI: Dispersion index, CV_{CI}: coefficient of variation of centromeric index, M_{CA}: mean centromeric asymmetry, AI: asymmetry index.

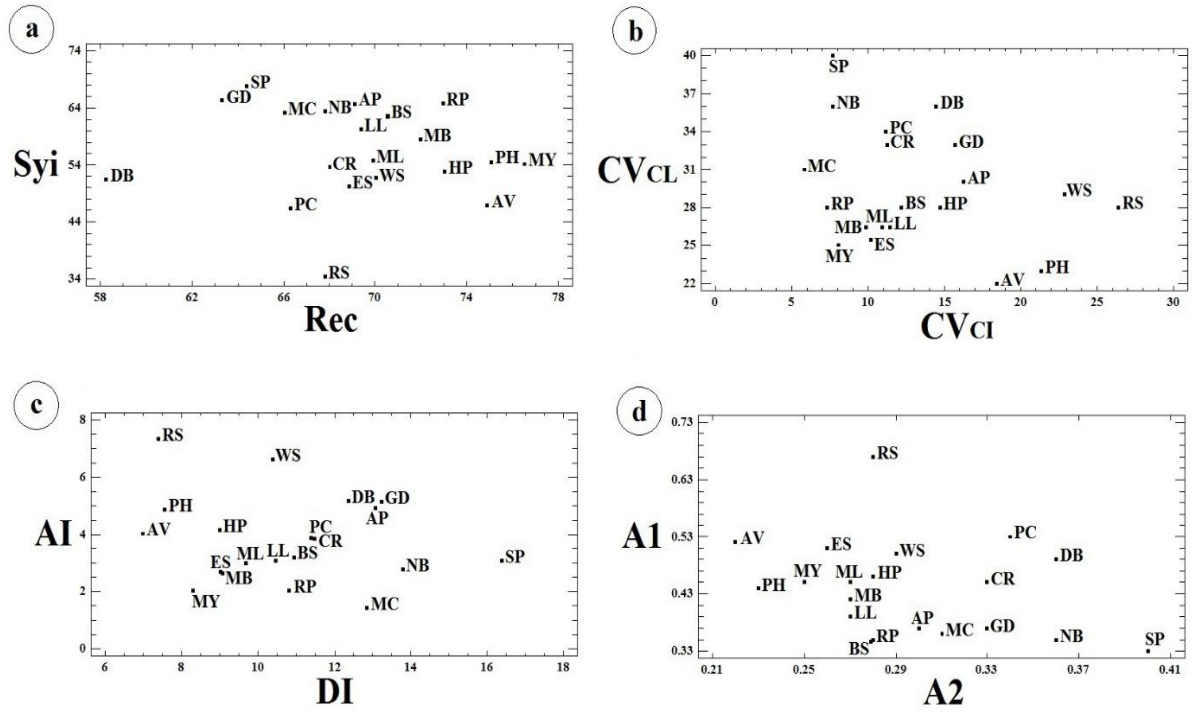

Figure 4: Scatter diagrams for *M. incana* cultivars: (a) the Syi against the Rec index, (b) the CV_{CI} % against the CV_{CL} % parameter, (c) the AI against the DI index, (d) the A_1 against the A_2 index.

Table 3: Pearson correlation for asymmetry indices in twenty *M. incana* cultivars.

	A_1	TF %	M_{CA}	Syi	As.K%	A_2	Rec	CG%	DI	DRL	S%	AI
A_1	1											
TF%	-0.99^{**}	1										
M_{CA}	1.00^{**}	-0.98^{**}	1									
Syi	-0.99^{**}	1.00^{**}	-0.98^{**}	1								
As.K%	0.99^{**}	-1.00^{**}	0.98^{**}	-1.00^{**}	1							
A2	-0.31^{ns}	0.32^{ns}	-0.28^{ns}	0.34^{ns}	-0.32^{ns}	1						
Rec	0.04^{ns}	-0.09^{ns}	0.01^{ns}	-0.11^{ns}	0.09^{ns}	0.81^{**}	1					
CG%	-0.87^{**}	0.90^{**}	-0.85^{**}	0.91^{**}	-0.90^{**}	0.40^{ns}	-0.29^{ns}	1				
DI	-0.65^{**}	0.68^{**}	-0.63^{**}	0.70^{**}	-0.68^{**}	0.88^{**}	-0.68^{**}	0.77^{**}	1			
DRL	-0.36^{ns}	0.39^{ns}	-0.35^{ns}	0.40^{ns}	-0.39^{ns}	0.96^{**}	-0.83^{**}	0.47^{*}	0.89^{**}	1		
S%	0.54^{*}	-0.55^{*}	0.53^{*}	-0.57^{**}	0.55^{*}	-0.86^{**}	0.57^{**}	-0.55^{*}	-0.87^{**}	-0.87^{**}	1	
AI	0.59^{**}	-0.54^{*}	0.63^{**}	-0.52^{*}	0.54^{*}	0.01^{ns}	-0.22^{ns}	-0.35^{ns}	-0.18^{ns}	-0.05^{ns}	0.18^{ns}	1

**, **Correlation is significant at P < 0.05 and P < 0.01 respectively, ^{ns} Non significant differences P > 0.05*

Considering the six asymmetry estimators based on centromere position (A_1, TF%, Syi, As. K%, M_{CA} and CV_{CI}), the SP cultivar had the most symmetrical and RS cultivar had asymmetrical chromosomes. Three pairs of sub-metacentric chromosomes were identified in RS. The A_2 and Rec indices, based on chromosome length in a complement, showed different results. Although SP cultivar had the lowest value of intra-asymmetry chromosomal index (A_1; 0.33), the highest value of A_2 (0.40; inter-chromosomal asymmetry index) was observed in this cultivar. This shows that the SP cultivar, which showed karyotype with a high quantity of metacentric chromosomes (KF = 7m), is the cultivar with asymmetrical karyotype. Considering the three karyotype asymmetry parameters (A_2, DRL and DI), the AV cultivar had the most symmetrical karyotype. Scattered diagrams based on AI-DI, A_1-A_2, Syi-Rec and CV_{CL}-CV_{CI} displayed relationship among cultivars with respect to karyotype asymmetry (Figure 4).

Principal Component Analysis: One of methods in investigating the diversity of plant varieties is cytogenetic and karyotypic studies. For categorizing the cultivars, the number of chromosomes should be counted and also chromosomal similarities should be observed. Decomposition of principal components

based on chromosomal parameters indicated that three components generally explain more than 95.50% of total variability. The first component covers 63.12% and the second one covers 28.83% of total variance. This decomposition is indeed an indicator of the contribution of each component of the total variance. The results indicated that the index of karyotype symmetry (Syi) is the most important one in the formation of the first component. In explaining the second component, chromosome size resemblance index (Rec) and A_2 played more significant role than other traits. The results indicated that the coefficient of variation of centromere index (CV_{CI}) showed stronger association with third component. Hence, we can conclude that these traits have the highest impact on the diversity. Cultivars' distribution was observed using the first two components in a 2-dimensional graphic.

Cluster analysis and Relationship among Cultivars: Cluster analysis was performed using Ward's method. The clustering results indicated that obtained dendrogram by this method yields an acceptable picture of real distances between the cultivars and confirmed the results got from the PCA analysis. Cultivars were categorized based on their cytogenetic similarity into three distinct groups. The first and third groups divided into two sub clusters. In general, eleven cultivars were placed in the first group and one cultivar and eight cultivars were placed in the second and third groups respectively. The least Euclidean distance was observed between LL and BS cultivars. The trisomic cultivars clustered within one group but located in two separate sub cluster. The two cultivars SP-NB were located in one group (Figure 5). The aim of the present study is investigating the karyotype and the chromosome characteristics of different cultivars of stock (*Matthiola incana*) flower for the first time. Based on the results, cultivars were diploid (2n = 2x = 14) and in two cultivars, we observed aneuploidy phenomenon of trisomic type (2n + 1) and the number of studied metaphase chromosomes was 15. Trisomic individuals were observed for the first time in the progenies of a stock flower called "Snowflake"[13 & 14]. Based on Frost's studies, the most of trisomic disorders occurs among long chromosomes and the long arm of one of chromosomes is broken during meiosis division in trisomic mutant stocks. Frost[13] reported the extra chromosome alters the percent of double flower progenies of Snowflake given the special sequence on this separated segment of chromosome. Two trisomic cultivars were very similar to Frost's "Snowflake" in terms of some morphological characteristics such as white flower color, stem height and leaf shape. Although the reason of trisomic status of individuals in these two cultivars is unknown, the shortest chromosome (1.35 µm) was observed in NB cultivar and this may refer to the Frost's theory. To determine whether the presence of additional chromosome leads to the high percent of double-flower status in the progenies of these trisomic cultivars requires more and precise investigations concerning genome sequences on the additional chromosome.

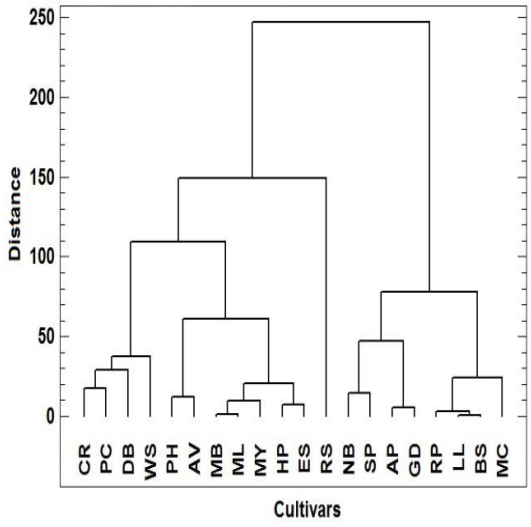

Figure 5: Dendrogram based on asymmetry indices for *M. incana* cultivars (Ward's method).

The chromosomes in stock cultivars were mainly metacentric and sub metacentric. Telocentric chromosome was not found, hence this finding is consistent to the results obtained concerning karyotype's symmetrical structure; this implies that this species (*M. incana*) is in its primary developmental stage. The mean length of chromosomes in the present research was between 2.41µm to 5.25µm. Chromosomes were classified into three Stebbins classes (1A, 2A and 1B). In general, the highest values of karyotype asymmetry indices in terms of centromere location belonged to SP cultivar. Pearson correlation analysis showed a significant negative correlation between A1 and each of the four indices: TF%, Syi, CG% and DI. Also, the intrachromosomal asymmetry index (A_1) had a positive correlation with As. K%, AI and M_{CA} (Table 3). A_2 also had a perfect positive correlation with Rec, DRL and DI. TF% showed perfect negative correlation with As.K% and M_{CA}, while this index had a perfect positive correlation with three indices: DI, Syi and CG% (Pearson coefficient <0.001). The results of correlation analysis displayed a positive relationship between Syi and CG% (Pearson coefficient <0.001). The numerator of both indices is related to the length of the short arm. The outcomes obtained from the scattered plotted diagrams; AI-DI, A_1-A_2, Syi-Rec and CV_{CL}-CV_{CI} have shown that SP (KF = 7m) is comparatively asymmetric than NB and GD cultivars with KF = 8m formula. By analyzing the scatter plot of A_1 ×

A_2, GD was found to have more symmetrical karyotype than NB cultivar. Based on the scatter plot of $CV_{CI} \times CV_{CL}$, MY cultivar had the lowest variations in the centromere position and chromosome length. Differences in karyotype symmetry among the cultivars indicate the presence of different chromosomes from different parents. Data obtained from this research can be used to evaluate the progenies of crosses among cultivars.

CONCLUSION: The results of this research distinguish the somatic chromosome numbers, karyotype parameters and asymmetry indices, idiograms of twenty cultivars of *Matthiola incana*. These results collectively show great genetic variability between *M. incana* cultivars in terms of cytological features and chromosome number that can be considered as a significant gene pool for stock breeding programs. The cultivars with additional chromosomes can lead to genetic diversity in M. *incana* species. Because they can create new chromosome numbers and differentiation of cultivars. This study can help flower growers and breeders choosing different parents for heterosis breeding programs aimed at cultivar improvement.

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