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SECONDARY ASSOCIATIONS IN 'HIMALAYAN PINK' (*Dianthus angulatus* Royle ex Benth., Caryophyllaceae) FROM COLD DESERTS OF LAHAUL-SPITI

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In current investigation we studied the male meiosis and details of secondary chromosomal associations or pairing recorded for the first time in *Dianthus angulatus* Royle ex Benth., from the cold deserts of Lahaul-Spiti (Himachal Pradesh, India). All the presently studied individuals of the species existed at 2*x* level (*x* = 15). The present chromosome count of *n* = 15 in the species is in conformity with the previous counts from India and outside of India. Secondary associations are defined as the affinity of bivalents to be positioned in pairs having gentle connections. The secondary chromosomal associations in the species existed among bivalents/chromosomes were observed in the meiocytes at metaphase-I and continued till the metaphase-II. The bivalents positioned side by side and end to end to form secondary pairing. The difference in the number of bivalents/chromosomes involved in the secondary associations has also been witnessed. A secondary association between bivalents is considered to be of immense importance as it is being taken as a gauge of ploidy in plants. The incidence of such secondary associations of bivalents/chromosomes in *D. angulatus* which existed at 2*x* level indicated the secondary polyploid nature of the species.

Keywords: bivalents; chromosomes; *Dianthus angulatus*; Lahaul-Spiti, pollen mother cells

INTRODUCTION

The genus *Dianthus* belongs to the tribe Silenoideae, of the family Caryophyllaceae. About 333 *Dianthus* species have been identified in Europe, Asia, North America, North Africa (http://www.theplantlist.org/browse/A/Caryophyllaceae/Dianthus/, 2015 GROSHKOVA, 1970; DAVIS, 1965-85; PUNT and HOEN, 1995), of which chromosome numbers for only 146 taxa in this

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genus have been reported (TROPICOS.ORG. MISSOURI BOTANICAL GARDEN, 2015). Five different chromosome number had been reported in the genus i.e. 2*n*=30, 45, 60, 75, 90, 120, 180 (DARLINGTON and WYLIE, 1955; FEDOROV, 1969; GOLDBLATT, 1981, 1984, 1985, 1988; GOLDBLATT and JOHNSON, 1990, 1991, 1994, 1996, 1998, 2000, 2003, 2006; KUMAR and SUBRAMANIAN, 1986; BALAO *et al*., 2009; KUMAR *et al*., 2012; TROPICOS.ORG. MISSOURI BOTANICAL GARDEN, 2015). Based on the basic chromosome number of *x*=15 suggested for the genus (DARLINGTON and WYLIE, 1955), the aforementioned chromosome numbers would correspond to diploid (2*n*=2*x*=30), triploid (2*n*=3*x*=45) tetraploid (2*n*=4*x*=60), pentaploid (2*n*=5*x*=75), hexaploid (2*n*=6*x*=90), octoploid (2*n*=8*x*=120) and dodecaploid (2*n*=5*x*=180) respectively. Polyploidy is a common phenomenon in *Dianthus*. While studying the genus *Dianthus*, CAROLIN (1957) found that 33 % species were polyploids. WEISS *et al*. (2002) and BALAO *et al*. (2009) discussed mode of origin of polyploids in the diploid species of the genus. In the previous communication (KUMAR *et al*., 2012) from this laboratory, authors suggested the role of intraspecific hybridization and cytomixis in the origin of polyploidy in the presently studied species, *Dianthus angulatus.* However, it would be an exceedingly early conclusion to draw on polyploid and evolutionary status of the genus before getting chromosomal information for rest of the 56.16 % (187/333) species in the genus. Here in this paper we have studied the chromosome number, meiotic behaviour and chromosome pairing with the aim to increase the chromosomal knowledge of this species.

MATERIALS AND METHODS

Material for chromosomal studies was collected from high altitude areas of Lahaul-Spiti (Udaipur, 2,700 m, Himachal Pradesh, India) in July 2007. The young unopened floral buds of appropriate sizes from healthy plants were fixed in freshly prepared Carnoy's fixative (6 absolute alcohol: 3 chloroform: 1 glacial acetic acid, v: v: v) for 24 hours and subsequently transfer to 70% ethanol and preserved in a refrigerator until analyzed. Anthers from fixed floral buds were squashed with the help of tapping rod in 1% acetocarmine and glass slide with meiotic preparations were studied for chromosome counts, and detailed meiotic behaviour in pollen mother cells (PMCs) at different meiotic stages. Pollen fertility was estimated through stainability tests for which anthers of mature flowers were squashed in glycerol-acetocarmine mixture (1:1). Well-filled pollen grains with uniformly stained cytoplasm were scored as fertile/viable while shrivelled one with unstained/poorly stained cytoplasm were counted as sterile/unviable. Photomicrographs from the temporary as well as permanent slides were taken using a Nikon Eclipse 80*i* microscope.

RESULTS AND DISCUSSION

D. angulatus had not been studied chromosomally extensively from India and other parts of the world except for few reports (GENTSCHEFF, 1937; KUMAR *et al*., 2012). Intraspecific chromosomal variation exist within the species in the form of two intraspecific cytotypes (diploid, 2*n*=30; hexaploid, 2*n*=90) on the basic chromosome number of *x*=15 known in the species. The course of meiosis was normal with 15 bivalents at metaphase-I (Fig. 1a) and normal segregation of chromosomes /chromatids at anaphases. Sporad formation was also regular (Fig. 1). Pollen fertility is nearly cent percent (Fig. 1). The presently studied wild plants unvaryingly shared the same diploid chromosome number of 2*n*=30 based on *x*=15. So the present meiotic chromosome count of *n*=15 is in conformity with the previous report of 2n=30 by KUMAR *et al*.,

(2012) from India. Present chromosome count of 2*n*=30 is the second report of the diploid cytotypes on worldwide basis.

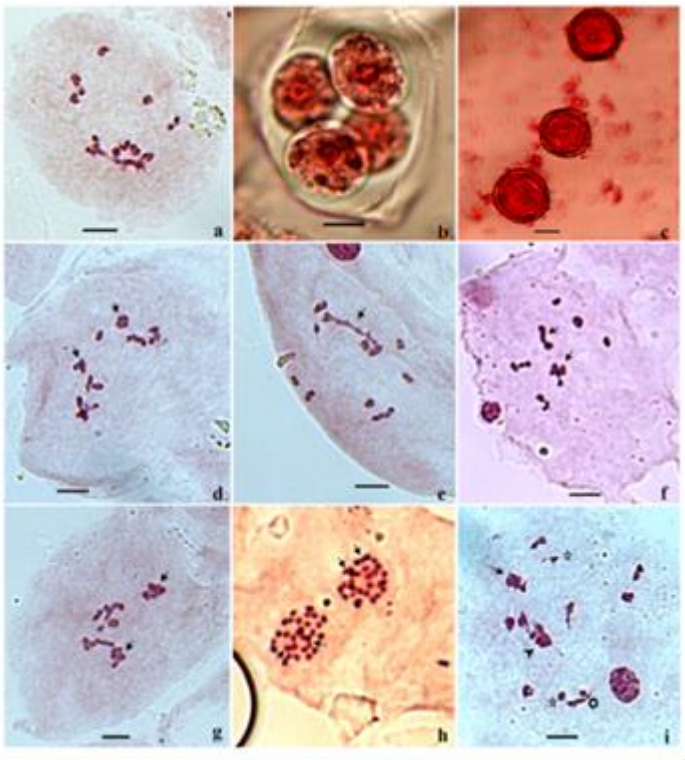

Fig. 1 (a-i). PMCs (pollen mother cells) showing meiotic chromosome numbers, sporad, pollen grains and secondary associations; a) metaphase-I, *n*=15. b) a sporad. c) stained fertile pollen grains. d) a PMC showing bivalents lie side by side (*arrows*). e) a PMC showing end to end attached bivalents (*arrows*). f) a PMC showing both side by side and end to end (*arrow*). g) two groups of bivalents showing secondary paring (*arrows*). h) a PMC showing secondary paring among chromosomes at metaphase-II (*arrows*). i) a PMC showing configuration $1_{(4\ \text{bivalents}}$ marked with *arrow head*) $+1_{(2\ \text{bivalents}+\ 1\ \text{univalent},}$ marked with *arrow*) $+1_{(1\text{bivalent}\ +\ 1\ \text{univalent}}$ marked with *circle*) + $6_{\ (\text{bivalents})}$ + 2 ($_{\text{univalents}}$ marked with *stars*) at metaphase-I. Scale bar =10μm

The scrutiny of chromosome coupling in the species could offer more information on basic number and ploidy level and undeniably meiosis of the species studied here discloses some interesting characteristics. In all the cells analysed at metaphase-I, most of the bivalents are seen to be in very close proximity in groups of two, three and, sometime four, with the groups widely spaced from each other (Figs. 1b, c, d, e). The number and kinds of association in 150 metaphase plates are given in Table 1. Polar views show a discernible secondary association of the bivalents into groups of two, three, and four. Anaphase-I is normal but secondary association is maintained

and at the metaphase-II only very few of the chromosomes were associated (Fig. 1f). Secondary pairing by the bivalents/chromosomes has been observed in two patterns - end to end (Fig. 1) and side by side (Fig. 1). Association of chromosomes in the group of two was observed to be highest with 25.80 %. Chromosomal associations in the group of three and four were 16.27 % and 10.67 %, respectively. Univalents were also observed but with a low percentage of 2.36. Interestingly, univalent were also noticed to secondarily associated with bivalents forming configuration of $1_{(4\ \text{bivalents})} +1_{(2\ \text{bivalents}+\ 1\ \text{univalent})} +1_{(1\text{bivalent}\ +\ 1\ \text{univalent})} +\ 6\ _{(\text{bivalents})}$ (Fig.), where $1_{(1\text{bivalent}\ +\ 1\ \text{univalent})}$ seemed to be a trivalent, however, in such type of associations, bivalents and univalents were loosely attached to each other without any formation of chiasma (Figure). The number of groups in a PMC ranges from 7-13. The most frequently met configuration was $1_{(4\ \text{bivalents})} +1_{(2\ \text{bivalents})} + 9\ _{(1\ \text{bivalents})}$ (Fig.) in 10.67 % of the observed PMCs, while the $1_{(4\ \text{bivalents})} +1_{(2\ \text{bivalents})} + 2\ _{(\text{univalents})}$ (Fig.) was the least observed configuration (3.33 %).

Table 1. Secondary chromosomal associations at metaphase-I (MI) in Dianthus angulatus

		Secondary associations among bivalents at MI					
	PMCs (% age)	Group of 4 bivalents	Group of 3 bivalents	Group of 2 bivalents	Bivalents (II)	Univalents (I)	No. of groups per PMC
	07 (04.67)	-	02	02	05	-	09
	09 (06)	-	01	03	04	04	12
	11 (07.33)	-	01	01	10	-	12
	05 (03.33)	01	-	01	08	02	12
	07 (04.67)	-	01	02	08	-	11
	11 (07.33)	-	-	03	09	-	12
	08 (05.33)	01	-	05	01	-	07
	13 (08.67)	-	03	-	06	-	09
	09 (06)	-	-	03	08	02	13
	12 (08)	-	01	04	03	02	10
	08 (05.33)	01	02	01	03	-	07
	11 (07.33)	-	-	02	11	-	13
	16 (10.67)	01	-	01	09	-	11
	09 (06)	01	-	$01_{II}+01_{I}$	$6+ 1_{II}+1_{I} =07+1_{I}$	02	11
	14 (09.34)	01	01	01	06	-	09
A	150						
B		0-1	0-3	0-5	01-11	0-4	07-13
C	2250	240	366	576+09	1006+09	106	
D	4500	480	732	1161	2021	106	
E		10.67	16.27	25.80	44.90	2.36	

A= Total no. of PMCs analyzed; B= Range of groups; C= Total no. of bivalents/ univalents; D= Total no. of chromosomes involved in association; E=% age of chromosomes involved in association in different groups

Meiosis is a crucial event of high impact which aims to precisely reduce the chromosome number and ensures the viability of gametes. Meiosis is so well devised that even a small change can lead to devastating phenotypic expression. So, the study of the meiotic course

is fundamental for proficient development of breeding programmes. Anything depicted during the study of course of meiosis may suggest evolutionary phenomenon or changes in that particular species. Secondary associations are defined as the close proximity of bivalents or chromosomes of equal or unequal sizes in pairs having diffused connections during meiosis (DARLINGTON, 1965). The phenomenon is not only confined to the bivalents but had also been reported to occur among univalents at metaphase-I, whenever univalents fail to pair owing to weak homology (RICHHARIA, 1936). Secondary associations presently observed have been used one of the criteria in past to construe polyploidy in a particular species where numerical considerations are not available or fail to shed light on it (MATSURA, 1935; AGARWAL, 1983). More than century ago the phenomenon of secondary associations was discovered by TAHARA (1909) in *Morus* (1909). Subsequently, this phenomenon had been reported by many other workers (KUWADA, 1910; ISHIKAWA, 1911; MARCHAL, 1912; DARLINGTON, 1928; LAWRENCE, 1931; HEILBORN, 1936; JACOB, 1957; GUPTA and ROY, 1973; AGARWAL, 1983; ARGIMYN *et al*., 1999; KUMAR *et al*., 2013; KUMAR and CHAUDHARY, 2014). Different views applied by different authors to explain the basis of secondary associations include, the fusion between heterochromatic regions of the involved bivalents (THOMAS and REVELL, 1946), homology of paired bivalents, and artefact induced due to squash technique (HEILBORN 1936; BROWN, 1950) or fixation (PROPACH, 1937). There has been a serious debate on the involvement of homologous chromosomes in the process of secondary pairing. HIRAYOSHI (1957) were of the opinion that secondary association may be a phenomenon operating under bio- and physico-chemical reactions.

The incidence of such secondary associations of bivalents/chromosomes in *D. angulatus* which existed at 2*x* level pointed towards the secondary polyploid nature of the species. The species might have under gone cytological diploidization in the track of evolution to accomplish its near diploid like meiotic behaviour as has been suggested earlier for *Ocimum* (MUKHERJEE and DATTA, 2006) and *Uraria picta* (BHATTACHARYA and DATTA, 2010). Owing to variation in the number of secondary associations at metaphase-I nothing can be said regarding the basic chromosome number in the genus or family. Based on the criterion that haploid number n =14 or more denotes polyploidy (GRANT, 1963, 1981), and also according to the method adopted by GOLDBLATT (1980) that species with n= 11 or above as polyploids. Therefore, considering the above two criterion to determine polyploid in the *Dianthus angulatus*, species with presently reported chromosome number supposed to be polyploid. So the existence of secondary associations somehow seems to indicate towards the secondary polyploid nature of the species. Furthermore the basic chromosome number of x=15 could also be secondary base number however, so far this is the only lowest haploid number reported in the genus. GRANT (1982a, 1982b), were of the opinion that adequate data concerning to chromosome numbers is a precondition for calculating the basic chromosome numbers of a genus. More population based cytological studies are required to get clear picture of polyploid in the genus.

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REFERENCES

AGARWAL, P.K. (1983): Secondary Association of bivalents in *Cissus discolor* Blume, Cytologia, *48*: 577-580.

ARGIMYN, S., A.F. WULFF, C.C.XIFREDA (1999): Chromosome association and basic number in *Anredera krapovickasii* (Basellaceae). Caryologia, *52*: 203-206.

BALAO, F., R. CASIMIRO-SORIGUER, M. TALAVERA, J. HERRERA, S. TALAVERA (2009): Distribution and diversity of cytotypes in *Dianthus broteri* as evidenced by genome size variations. Ann. Bot., *104*:965-973.

BHATTACHARYA, A., A.K. DATTA (2010): Secondary chromosome associations in *Uraria picta* (Jacq.) DC. (Family: Leguminosae), Cytologia, *75*: 37-40.

BROWN, W.S. (1950): Spurious secondary association and asymmetric spindles in *Luzula*. Cytologia, *15*: 259-268.

CAROLIN, R.C. (1957): Cytological and hybridization studies in the genus *Dianthus.* New Phytol., *56*:81-97.

DARLINGTON, C.D. (1928): Studies in *Prunus*. I and II. J. Genet., *19*: 213-256.

DARLINGTON, C.D. (1965): Cytology, London: Churchill.

DARLINGTON, C.D., A.P. WYILE (1955): Chromosome altas of flowering plants. George Allen and Unwin Ltd, London.

DAVIS, P.H. (1965-1985): Flora of Turkey. Edinburgh University Press, England.

FEDOROV, ANA. (1969): Chromosome numbers of flowering plants. Academy of Sciences of the USSR. Komarov Botanical Institute, Leningrad (Reprint 1974).

GENTSCHEFF, G. (1937): Experimental and caryological investigation of the relationship among the species of the genus Dianthus L. PhD. Thesis, University of Sofia, Sofia.

GOLDBLATT, P. (1980): Polyploidy in angiosperms: Monocotyledons. Pp. 219–239 *in* W. H. Lewis (editor), Polyploidy:Biological Relevance. Plenum Press, New York.

GOLDBLATT, P. (ed) (1981, 1984, 1985, 1988): Index to plant chromosome numbers 1975–1978, 1979–1981, 1982–1983, 1984–1985. Monogr Syst Bot Missouri Bot Gard, vols. *5*, 8, *13*, *23*.

GOLDBLATT, P., D.E. JOHNSON, (eds) (1990, 1991, 1994, 1996, 1998, 2000, 2003, 2006): Index to plant chromosome numbers 1986–1987,1988–1989, 1990–1991, 1992–1993, 1994–1995, 1996– 1997, 1998–2000, 2001–2003. Monogr Syst Bot Missouri Bot Gard, vols. *30*, *40*, *51*, *58*, *69*, *81*, *94*, *106*.

GORSHKOVA, S.G. (1970): Centrospermane, In: Flora of the USSR, Illin, M.M., O.E. Knorring, O. I. Kuzeneva, O.A. Muraveva, A.I. Tolmavhev, B.K. Shishkin, E.I. Shteinberg and I.T. Vasil-Chenko (Eds.). Vol. 6. SSSR, Moskva, pp: 447-528.

GRANT, V. (1963): The origin of adaptations. Columbia Univ. Press, New York.

GRANT, V. (1981): Plant speciation. Columbia Univ. Press, New York.

GRANT, V. (1982a): Periodicities in the chromosome numbers of the Angiosperms. Bot Gaz., *143*:379-389.

GRANT, V. (1982b): Chromosome number patterns in primitive Angiosperms. Bot Gaz., *143*:390–394.

GUPTA, P.P., S.K. ROY (1973): Primary and secondary chromosome association in *Euryale ferox* Salisb. Cytologia, *38*: 645-649.

HEILBORN, O. (1936): The mechanism of so called secondary association between chromosomes. Hereditas, *22*: 168-188.

HIRAYOSHI, I. (1957): The chromosomal relationships in Oryzeae and Zizanieae. Cytologia Suppl. Vol. Proc. Int. Genet. Symposia, 1956: 293-297.

ISHIKAWA, M. (1911): Cytologische studien von dahlien. Bot. Mag., *25*: 1-8.

JACOB, K.M. (1957): Secondary association in the castor oil plant. Cytologia, *22*: 380-392.

KUMAR, G., N. CHAUDHARY (2014) Secondary chromosomal association in kidney bean (*Phaseolus vulgaris* L.) Jordan J. Biol. Sci., *7*: 71-74.

KUMAR, P., V.K. SINGHAL, D. KAUR (2012): Impaired male meiosis due to irregular synapsis coupled with cytomixis in a new diploid cytotype of *Dianthus angulatus* (Caryophyllaceae) from Indian Cold Deserts. Folia Geobotanica, *47*:59–68

KUMARA, P., V.K. SINGHAL (2013): Chromosome number and secondary chromosomal associations in wild populations of *Geranium pratense* L. from the Cold Deserts of Lahaul-Spiti (India). Cytol. Genet., *47*: 107-114.

KUMAR, V., B. SUBRAMANIAN (1986): Chromosome atlas of flowering plants of the Indian Sub-Continent. vol. I. Dicotyledons. BSI, Calcutta, India.

KUWADA, Y. (1910): A cytological study of *Oryza sativa* L. Bot. Mag., *24*: 267-280.

LAWRENCE, W.J.C. (1931): The secondary association of chromosomes. Cytologia, *2*: 352-384.

MARCHAL, E. (1912): Recherches cytologiques sur le genre *Amblystegium*. Bull. Soc. R. Bot. Belg., *51*: 189–200.

MATSURA, H. (1935): On the secondary association of meiotic chromosomes in *Tricyrtis latifolia* and *Dicentra spectabilis* Miq. J Fac Sci Hokkaido Imp University, Series V, *3*: 251-260.

MUKHERJEE, M. and A.K DATTA (2006): Secondary chromosome associations in *Ocimum* spp., Cytologia, *71*:149-152.

PROPACH, H. (1937): Cytogenetische untersuchungen in der gattung *Solanum*, Sect. *Tuberarium*. I. die sekundarpaarung. Zeitschrift fur Inductive Abstummungs-und Vererbungslehre, *72*: 555-563.

PUNT, W., P.P. HOEN (1995): The Northwest European pollen flora, 56. Caryophyllaceae. Rev. Palaeobot. Palynol, *88*: 83-272.

RICHHARIA, R.H. (1936): The phenomenon of secondary association. Curr. Sci., *5-6*: 290-291.

TAHARA, M. (1909): On the chromosome of *Morus indica* (Preliminary note). Bot. Mag., *23*: 343-353.

THOMAS, P.T., S.H. REVELL (1946): Secondary association and heterochromatic attraction. I. *Cicer arietinum*. Ann Bot., *10*: 159-164.

THE PLANT LIST, 2015. http://www.theplantlist.org/browse/A/Caryophyllaceae/Dianthus/.

TROPICOS.ORG. MISSOURI BOTANICAL GARDEN, 2015. http://www.tropicos.org/NameSearch.aspx?projectid=9.

WEISS, H., C. DOBEŠ, G.M. SCHNEEWEISS, J. GREIMLER (2002): Occurrence of tetraploid and hexaploid cytotypes between and within populations in *Dianthus* sect. *Plumaria* (Caryophyllaceae). New Phytol., *156*:85-94.

SEKUNDARNO GRUPISANJE HROMOZOMA KOD 'HIMALAYAN PINK' (*Dianthus angulatus* Royle ex Benth., Caryophyllaceae) IZ HLADNIH PUSTINJA LAHAUL-SPITI

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Izvod

U radu su proučavane mejoza muških ćelija i sekundarno hromozomsko grupisanje ili sparivanje, evidentirano po prvi put kod *Dianthus angulatus* Royle ex Benth., iz hladnih pustinja Lahaul-Spiti (Himachal Pradesh, Indija). Sve proučavane individue ove vrste su sa oblikom $2x$ ($x = 15$). Broj hromozoma od $n = 15$ je u saglasnosti sa podacima unutar i van Indije. Sekundarno grupisanje je definisano kao afinitet bivalenata da se pozicioniraju u parovima pomoću slabih veza. Ovo grupisanje između bivalenata/hromozoma je zapaženo kod mejocita u metafazi-I i nastavljeno je do metafaze-II. Bivalenti se pozicioniraju jedni uz druge i formiraju sekundarne parove. Takođe je zapažena razlika u broju bivalenata/hromozoma kod sekundarnog grupisanja. Značaj ovog načina grupisanja je veliki, jer se uzima kao merilo ploidije kod biljaka. Pojava sekundarnog grupisanja kod *D. angulatus* postoji u obliku $2x$ i ukazuje na sekundarnu poliploidnu prirodu ove vrste.

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