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INTRASPECIFIC POLYPLOIDY IN *VERONICA ANAGALLIS-AQUATICA* L. (PLANTAGINACEAE) FROM NORTH INDIA

Dalvir Kaur [1],Vijay Kumar Singhal [2]

[1]*Department of Botany, Patel Memorial National College, Rajpura, Patiala, Punjab (India)*

[2] *Department of Botany, Punjabi University, Patiala, Punjab (India)*

ABSTRACT

The present study investigates the cytological and morphological analysis, and intraspecific polyploidy in V. anagallis-aquatica L. which sampled from Kinnaur district of Himachal Pradesh in India revealed the existence of diploid (n=9) and tetraploid (n=18) cytotypes based on x=9. The tetraploid chromosome count (2n=36) in the species confirms the earlier chromosome report from India and other parts of the world while the diploid chromosome count in the species is made for the first time from India. Morphologically, diploid plants were found to be taller and much branched than the tetraploid individuals.

Keywords: chromosome count, intraspecific polyploidy, morphology

I. INTRODUCTION

Veronica L. is large genus with about 300 species [1] commonly known as '*Gypsyweed*' found mostly in temperate Northern Hemisphere and some species in Southern Hemisphere. Before molecular analysis, genus *Veronica* considered as members of the Scrophulariaceae, recently reclassified as a member of the Plantaginaceae [2]. *Veronica* species used as an expectorant tea to alleviate bronchial congestion associated with asthma and allergies.

Various species of *Veronica* have been investigated for chromosome counts [3, 4, 5, 6, 7] pollen morphology [8], seed morphology [9] and phylogenetic analysis [10]. The present study covers the chromosome count, meiotic course, morphological analysis and intraspecific polyploidy in *V. anagallis-aquatica* L. which habitats moist and wet conditions, is a rhizomatous perennial herb with decumbent-ascending stem, oppositely arranged leaves and light blue coloured flowers arising from the leaf axils with protruding anthers are present in racemes.

II. MATERIAL AND METHODS

2.1 Sample collection and submission

Study materials were collected from Kinnaur district of Himachal Pradesh (India) and Voucher specimens were deposited in the Herbarium, Department of Botany, Punjabi University Patiala (PUN).

2.2 Cytological Analysis

Young floral buds were fixed in freshly prepared Carnoy's fixative (6 ethanol: 3 chloroform: 1 acetic acid v: v: v) for 24h and preserved in 70% alcohol at 4°C. Squash method was applied to analyze the meiocytes and pollen grains by using acetocarmine (1%) and glycerol-acetocarmine (1:1) respectively.

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2.3 Morphometric Analysis

The morphological analysis includes both macro- and microscopic characters. Stomatal studies were made from the abaxial epidermal peels of the leaves through KOH treatment. Following expression was used to calculate the stomatal index: $SI = S/E+S \times 100$

Where, SI = stomatal index, S = number of stomata per field, E = number of epidermal cells per field.

2.4 Photomicrographs

Photomicrographs of chromosome counts, meiotic abnormalities, sporads, pollen grains, and stomata were made from the freshly prepared slides using Leica Qwin Digital Imaging System (X290, X900 and X2180) and Nikon Eclipse 80*i* microscope (X330, X1340 and X3400).

III. RESULTS AND DISCUSSION

Presently the species has been extensively studied cytologically collected from different localities falling in the valleys of Ganjul, Kalpa, Ropa, Sangla and Tidong of Kinnaur district (Himachal Pradesh) where it commonly grows along water channels. The species revealed the existence of diploid (n=9) and tetraploid (n=18) cytotypes based on x=9 (Table 1). The individuals worked out from Ropa Valley are found to be diploid (n=9) whereas all the other accessions scored from Ganjul, Kalpa, Sangla and Tidong Valley showed a tetraploid chromosome number of n=18. Cytological and morphological analysis has been performed in both the cytotypes.

3.1 Cytological analysis

(i) The diploid (n=9)

Two individuals studied from Ropa (3000m) in Ropa Valley showed the diploid meiotic chromosome count of n=9 which was confirmed from the presence of 9 bivalents at M-I (Fig. 5) and 9:9 chromosomes distribution at A-I (Fig. 6). Further meiotic course in both the individuals were found to be perfectly normal resulting into 100% pollen fertility.

(ii) The tetraploid (n=18)

All the individuals studied from Ganjul, Kalpa, Sangla and Tidong Valley showed a tetraploid meiotic chromosome count of n=18 as confirmed from the presence of 18 bivalents at M-I (Fig. 7) and 18:18 chromosomes distribution at A-I (Fig. 8). Further meiotic course in all the individuals was normal resulting into nearly high pollen fertility (99-100%).

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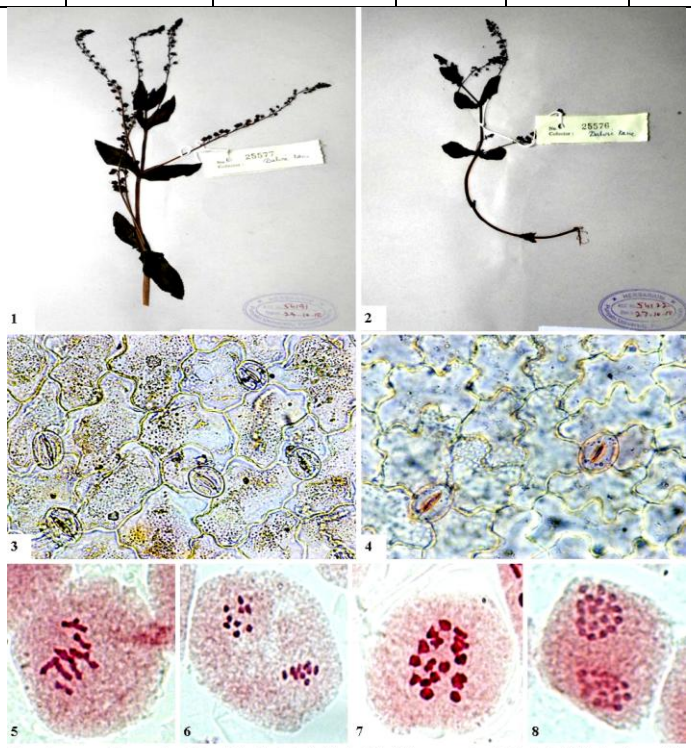
TABLE 1 : Information on locality with altitude, accession number/s (PUN*), meiotic chromosome number, ploidy level, pollen fertility %age, and previous chromosome reports in *Veronica anagallis-aquatica* L.

Cytotype (x=9)	Locality with altitude (m)	Accession number/s (PUN*)	Meiotic chromosome number (n)	Ploidy level	Pollen fertility (%age)	Previous chromosome reports
(i) The diploid	**Ropa valley**					**2n=18** Kliphuis & Wieffering, 1979; **2n=18, 36** Ozturk & Fischer, 1982;
	Ropa, 3000	54123	9	2x	100	
	Ropa, 3000	54191	9	2x	100	
(ii) The tetraploid	**Ganjul Valley**					**2n=36** Ehrenberg, 1945; Löve & Löve, 1956; Gadella & Kliphuis, 1968; Krischner *et al*., 1982; Dmitrieva & Perfenov, 1983; Dalgaard, 1985, 1986; Chuang & Heckard, 1992; Al-Bermani *et al*., 1993; Tanaka, 1995, 1996; Dzhus & Dmitrieva, 1999, 2001; Lövkvist & Hultgård, 1999; Tanaka *et al*., 1999; Shatalova, 2000; **2n=36+2B** Bala & Gupta, 2011b
	Pooh, 2840	54121	18	4x	99	
	Kalpa Valley					
	Reckong Peo, 2670	54122	18	4x	99	
	Sangla Valley					
	Sangla, 2680	54124	18	4x	100	
	Tidong Valley					
	Moorang, 2590	54120	18	4x	100	
	Thangi, 2700	54135	18	4x	100	



Veronica anagallis-aquatica **(Figs. 1-8). 1)** An individual of diploid cytotype (n=9). **2)** An individual of tetraploid cytotype (n=18). **3)** Stomata of diploid cytotype. **4)** Stomata of tetraploid cytotype. **5)** A PMC with 9 bivalents at M-I. **6)** A PMC with 9:9 chromosomes at A-I. **7)** A PMC with 18 bivalents at M-I. **8)** A PMC at A-I showing 18:18 chromosomes distribution.

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3.2 Morphometric analysis

Macroscopic characters include plant height, number of leaves per plant, size, shape, margin and colour of leaves, and number of flowers per inflorescence along with the size and colour of flowers. The diploid plants which measured 36.00-40.00cm in size were taller and much branched than the tetraploid individuals (19.00-27.00cm) (Figs. 1-2) (Table 2). The size of leaves, length of spike, and number and size of flowers per inflorescence, and size of capsule was recorded to be more in the diploid individuals than the tetraploid individuals. The two cytotypes also differ in the stomatal characters (Figs. 3-4).

TABLE 2. Comparison of macro- and microscopic morphological characters of the diploid and tetraploid cytotypes of *Veronica anagallis-aquatica* L.

	Cytotypes	
Characters ↓	**Diploid (n=9)**	**Tetraploid (n=18)**
Occurrence	Less	More
Plant height (cm)	36.00-40.00	19.00-27.00
Leaves		
(i) Number per plant	12	8-10
(ii) Size (cm)	1.40-5.50 × 0.60-2.00	0.40-2.40 × 0.30-1.50
(iii) Shape and margin	Ovate lanceolate; serrate	Ovate; entire to serrate
(iv) Colour of leaves	Green	Green
Length of spike (cm)	More (14-22)	Less (10-12)
Flowers		
(i) Number per spike	40-45	15-25
(ii) Colour	White with bluish tinch	White
(iii) Size (mm)	2.5	2.0
Size of capsule (mm)	3.0-4.0	2.0-2.5
Stomatal size (μm)	26.39-30.16 × 18.85-20.73	22.62-27.14 × 18.85-19.60
Stomatal index	18.51	10.52

It is thus apparent that morphological characters in the intraspecific cytotypes directly correlated with the increase in ploidy level as had been reported earlier in *Tephrosia villosa* [14], *Catharanthus pusillus* [15], *Parasenecio auriculata* [16], a number of woody species [17], *Centaurea stoebe* [18]. It does not mean necessarily that polyploids always showed gigantism in the morphological characters [19]. Presently, the lack of gigantism in the vegetative, floral and fruit characters in *V. anagallis-aquatica* (2x, 4x) has been detected which have also been reported earlier in *Plantago* sp. [20].

In the presently studied species, genetic and morphological variations are generally expected having a wide distribution range which provides the species a way to survive and adapt in different kind of climatic and environmental conditions. The geographical variation has a significant effect on the morphological characteristics of plant [21, 22]. Bradshaw suggested that morphological variations are due to genetic changes influenced by changes in environmental conditions [23].

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IV. CONCLUSION

V. anagallis-aquatica L. examined from high altitude ranges (2500-3000m) and cold arid region of Kinnaur (Himachal Pradesh) depicted with intraspecific polyploidy with diploid (2n=18) and tetraploid (2n= 36) cytotypes. Individuals of both the cytotypes also depicted with morphological variations. This widely distributed species yet to discover for its more genotype and morphotype adapting different climatic and environmental conditions.

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