A Karyotype analysis in *Ledebouria revoluta* (Hyacinthaceae) with a new Cytotype

Priyanka T. Giranje¹, Mayur D. Nandikar²* and Shirrang R. Yadav³

¹,² Naoroji Godrej Centre for Plant Research, 431, Lawkim Campus, Shindewadi, Shirwal, Dist. Satara, Maharashtra, India 412 801.
³Professor and BSR Faculty Fellow, Department of Botany, Shivaji University, Kolhapur, Maharashtra, India 416 004.

*Author for correspondence (mmandikar@gmail.com)
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**ABSTRACT:** *Ledebouria* Roth is a complex genus and it shows a wide range of chromosome counts. Present paper reports chromosome number 2n = 6X = 90 in *L. revoluta* (L.f.) Jessop a number that is not known so far in the entire genus *Ledebouria* which exhibits highly asymmetrical (2C) karyotype. The karyotype symmetry of the chromosomes and extreme level of polyploidy indicates that the *L. revoluta* is actively evolving in varied climatic and geographical regions.

**KEYWORDS:** Karyotype, *Ledebouria*, Hyacinthaceae, new cytotype, India

The genus *Ledebouria* Roth (Hyacinthaceae) consists of ca. 61 species and 2 infraspecific species in the world (WCSP 2018). Although, *Ledebouria* was described as early as 1821 by Roth, it has been treated as a synonym of *Scilla* in most Indian literature (Giranje and Nandikar 1989; Jehan 1989). The genus has very few qualitative characters of diagnostic value in determination of the species. Although, the characters such as blotches on the leaf, size, shape and orientation of the leaves (erect, sub-erect or prostrate), inflorescence size, flower number, etc have been used for identification of the species, however, these characters are varies greatly with reference to their climatic and geographical conditions.

In India the genus is represented by *Ledebouria revoluta* Roth and other three ill-defined species viz. *L. viridis* S. Dutta and P. Harvey ex M. R. Almeida, *L. karnatakensis* Punekar and Lakshmin., and *L. hyderabadensis* M. V. Ramana, Prasanna and Venu. *Ledebouria revoluta* is widely spread from Africa to peninsular India and shows great diversity both in morphological and chromosome number in different habitats. Leaf tip bulbils is a predominant mode of vegetative reproduction in majority of the populations which is subjected to cytological variations (Jessop 1973; Dixit et al. 1989; Jehan et al. 2014). The chromosome counts reported so far in *Ledebouria revoluta* shows 10 cytotypes (2n = 28, 30, 44, 45, 46, 58, 60, 64, 68, and 70: Table 1) indicating active cytological differentiation. The present report (2n = 90) confirms hexa–ploidy (6X) nature of populations, which is first time recorded not only in *Ledebouria revoluta* but also in entire genus *Ledebouria*.

**MATERIALS AND METHODS**

*Plant material:* Plant material of *Ledebouria revoluta* (= described as *L. junnarensis* by Rahangdale and Rahangdale 2016) were collected from the hill slopes of Junnar, Pune district, Maharashtra (Fig. 1A). The plants for the cytological study were grown in Naoroji Godrej Centre for Plant Research (NGCPR) Botanical garden, Shirwal, Satara, Maharashtra. The voucher specimens (M.D. Nandikar 1403, M.D. Nandikar 1418 and P.T. Giranje 1434) have been deposited at NGCPR.

**Sampling of karyotype:** The bulbs were kept for rooting. Water grown healthy root tips (0.5 cm long) were pre–treated with a saturated solution of para–dichlorobenzene (pDB) at 10 ±2°C for 3–4 hrs. Root tips were hydrolyzed in 1 N HCL and then stained and squashed with 2 % aceto–orcein.

The well separated somatic plates were photographed by OPTSCOPES IS–300 camera under LEICA DM 500 microscope at 100x magnification. Five well separated somatic chromosome plates were selected for karyotype analysis. Chromosomes were sorted into different categories on the basis of type of chromosome and arm ratio by following Levan *et al.* (1964). Evaluation of Karyotype symmetry has been determined by using categories of Stebbins (1971).

**RESULTS**

The karyotype analysis of *Ledebouria revoluta* has been given in Table 2. Different karyotypic parameters such as total chromosome length of haploid compliment (THCL), total chromosome length percent (TCL %), total from percent (TF %), symmetric index (SI), Gradient Index (GI) Coefficient of variation indices (CVcl and CVci) (Paszko 2006), and asymmetry indices (Ai, A1, A2) (Zarco 1986) were examined for *L. revoluta*, and are tabulated in Table 3.
Table 1. A detailed account of previous chromosome counts in *Ledebouria revoluta* (L.f.) Jessop

| Reported as taxa | n   | (2n) | Studied by                     | Country/Provision                                                                 |
|-----------------|-----|------|-------------------------------|----------------------------------------------------------------------------------|
| *Ledebouria revoluta* (L.f.) Jessop | 15  | 30   | Haque and Ghosh (2015)        | India (West Bengal, Kolkata, Rahara)                                             |
| *Ledebouria revoluta* (L.f.) Jessop (=*Scilla indica* (Wight) Baker) | 64, 68 |     | Sheeba and Vijayavalli (1998) |                                                                                  |
| *Ledebouria revoluta* (L.f.) Jessop (=*Scilla revoluta* (L.) Baker) | 22  |      | Johnson and Brandham (1997)   | South Africa                                                                     |
| *Ledebouria revoluta* (L.f.) Jessop | 28  |      | Stedje (1996)                 | East Africa, Kenya, Machakos, between Koma Rocks                                |
| *Ledebouria revoluta* (L.f.) Jessop (=*Scilla hyacinthina* (Roth) J. F. Macbr) | 30  | 45, 60 | Dixit *et al.* (1989)         | India (Maharashtra, Mumbai, Kanheri caves)                                      |
|                                                   |      |       |                               | India (Maharashtra, Satara, Kas plateau)                                        |
|                                                   |      |       |                               | India (Maharashtra, Pune, Dive ghat)                                            |
| *Ledebouria revoluta* (L.f.) Jessop (=*Scilla hyacinthina* (Roth) J. F. Macbr.) | 30  | 60   | Jha and Sen (1980),           | India (Maharashtra, Satara, Mahabaleshwar)                                     |
|                                                   |      |       | Nair (1989)                   | India (Maharashtra, Mumbai)                                                     |
| *Ledebouria revoluta* (L.f.) Jessop (=*Scilla indica* Roxb.) | 45  |      | Sheriff and Rao (1981)        | India (Tamil Nadu, Chennai, Adyar & Saidapet)                                   |
| *Ledebouria revoluta* (L.f.) Jessop (=*Scilla indica* Baker) | 20,22, 44,45, 46,58 | | Subramanian (1981)              |                                                                                  |
| *Ledebouria revoluta* (L.f.) Jessop (=*Scilla hyacinthina* (Roth) J. F. Macbr.) | 10  |      | Sarkar *et al.* (1980)        | India (Maharashtra, Uklapani)                                                  |
| *Ledebouria revoluta* (L.f.) Jessop (=*Scilla indica* (Wight) Baker) | 30  |      | Sen (1973), Chakravarty and Sen (1992) | Western Coastal belts of India |
| *Ledebouria revoluta* (L.f.) Jessop | 9   | 10, 11, 12, 13, 15, 16, 17, 22 | Jessop (1973) | South Africa (Rhodes University garden; Henderson's Farm; 34 miles west of Potgietersrust; 5 miles west of Colenso; Kroomie, Fort Beaufort; 2 miles north-west of Grahamstown; Willowmore; SWAZILAND; 34 miles west of Potgietersrust). |
Table 1.  Continued

| Reported as taxa             | n   | (2n)         | Studied by                        | Country/Provision                                                                 |
|------------------------------|-----|--------------|-----------------------------------|----------------------------------------------------------------------------------|
| *Ledebouria revoluta* (L.f.) Jessop | 15  |              | Jessop (1970)                     | South Africa (Eastern Cape, Grahamstown 20 miles N. of Pretoria Rhodes Botany Dept. Garden) |
| *Ledebouria revoluta* (L.f.) Jessop (=*Scilla indica* (Wight) Baker ) | 30  | 44, 45, 46, 58 | Rao (1956)                       | India (Maharashtra, Mumbai; Karnataka, Dharwad; Mysore; Tamil Nadu, Tiruchirapalli, Chennai; Andhra Pradesh, Krishna, Machilipatnam; Madhya Pradesh, Sagar; Maharashtra, Nagpur) |
| *Ledebouria revoluta* (L.f.) Jessop (=*Scilla indica* (Wight) Baker ) | 44, 45, 46, 58 |              | Darlington and Wylie (1955)      |                                                                                  |
| *Ledebouria revoluta* (L.f.) Jessop (=*Scilla indica* (Wight) Baker ) | 30, 44, 45, 46, 58 |              | Rao (1953)                       |                                                                                  |
| *Ledebouria revoluta* (L.f.) Jessop (=*Scilla indica* (Wight) Baker ) | 30  |              | Sheriff and Murthy (1946)        | India (Karnataka, Kolar hills)                                                  |
| *Ledebouria revoluta* (L.f.) Jessop (=*Scilla indica* Roxb.)  | 44  | 46, 45       | Raghavan and Venkatasubban (1939) | India (Tamil Nadu, Chennai, Adyar)                                               |
|                              |     |              |                                   | India (Tamil Nadu, Chennai, Saidapet)                                           |
|                              |     |              |                                   | India (Tamil Nadu, Chennai, Soundarya Nursery)                                   |
Fig. 1: *Ledebouria revoluta* A. habitat, B. habit, C. chromosome count ($2n = 90$), D. idiogram.
The karyotype analysis of *Ledebouria revoluta* can be summarized as follows: haploid chromosome number \( n = 15 \); hexaploid chromosome number \( 2n (6x) = 90 \) (Fig. 1C); total haploid chromosome length (THCL) = 90.72 \( \mu m \). Based on the centromeric position (Table 2) somatic chromosome complement can be broadly classified into 3 distinct chromosome groups: 1) 17 pairs of median chromosome, 2) 23 pairs of sub–median chromosome, 3)
5 pairs of sub–terminal chromosome. The karyotype formula can be expressed as 17m ± 23sm ± 5st and idogram is given in Figure 1D. The karyotype is moderately asymmetrical and falls into "2C" category of Stebbins (1971). The chromosome length ranged from 6.33 to 0.86 µm. The intra–chromosomal difference in long arm and short arm (d value) ranged from 0.16 to 2.27. The arm ratio (r value) ranged from 1.16 to 21.91. The Centromere index (I value) ranged from 4.37 to 46.22. The somatic chromosome count 2n = 90 with total haploid chromatin length =90.72 µm, symmetric index (SI) =54.95, and TF% is 35.47 shows highly asymmetrical gradient index (GI) =13.56.

**DISCUSSION**

The superfluous and illegitimate name *Ledebouria junnaresis* Rahangdale and Rahangdale (2016) refers to *L. revoluta* (Giranje and Nandikar 2016) and there comparison with *Ledebouria viridis* is erroneous. *Ledebouria viridis* (Blatt. and Hallb.) S. Dutta and P. Harvey ex M. R. Almeida (2009) was described as a distinct species based on its hysteranthous habit, less than 40 flowered racemes and flowers pinkish–purple to green perianth, whereas *L. junnaresis* is synanthous and typically agrees with *L. revoluta*. We studied the cytology of *L. junnaresis* from its type locality which revealed diploid chromosome number 2n= 90, which does not match with the number (i.e., 2n= 70) previously reported by Rahangdale and Rahangdale (2016). The present report confirms hexaploid (6X) nature of populations, which being first time recorded in the genus *Ledebouria*.

In asymmetrical karyotype centromere position shifts towards the median to subterminal, or their relative size has too differences to make karyotype more heterogeneous (Stebbins 1971). The previous studies on morphology and cytology in *L. revoluta* have revealed that the species is highly polymorphic with three cytopotypes (2n=30, 45 and 60: Dixit *et al.* 1989), the present reports adds one more cytopotype (2n=90). These records also evidenced by Dixit *et al.* (1989) that adaptation of the taxa to the different eco–climatic regions: diploids (2n=30) are usually found in plains and reproduce sexually, triploids (2n=45) and present hexaploids (2n=90) are so far known from higher altitudes in the Western Ghats adapted to high rainfall zone and usually reproduce vegetatively by formation of bulbils at leaf tip. In addition to these, some of the triploids also shares the habitat with tetraploids in drier parts of peninsular India. However, all above cytopotypes when grown under humid conditions, have tendency to produce bulbils at leaf tip.

Polyploidy played an important role in evolution of angiosperm. Cytological data provides important evidences to evaluate the impact of genetic divergence among species and population, and it has been estimated that more than 15% flowering plants have impacted by polyploidy (Guerra 2008; Wood *et al.* 2009). In many instances polyploidy causes changes in shape and texture of plant organs, like the leaves and petals are usually thicker and firmer, lowering of fertility and seed production, which helps in the process of stabilization and establishment of new habitats (Stebbins 1971). The present studied population of *L. revoluta* shows morphological variations such as linear to linear–lanceolate, faintly blotched leaves with fleshy, bulbils at apices, axillary, solitary flowering scape with 25–30 flowers, 10–15 mm long pedicels, and mauve perianth (Fig. 1B) as compared to the other adjoin populations of *L. revoluta*. Our study indicates that there is much evolutionary advancements in *L. revoluta* is possibly favoured due to the polyploidy and highly asymmetrical karyotype. Therefore, such populations should be

| Parameters | Result |
|------------|--------|
| THCL       | 90.72  |
| TF%        | 35.47  |
| SI         | 54.95  |
| GI         | 13.56  |
| CVcl       | 60.03  |
| CVci       | 26.73  |
| AI         | 16.05  |
| A1         | 0.45   |
| A2         | 0.60   |
| Karyotype formula (K) | 17m ± 23sm ± 5st |
| Classification as per Stebbins (1971) | 2C |
considered as one of the races instead of a new taxon, as it merely trying to establish or adapt to the new habitat.

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