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THE STUDY OF CHROMOSOMES AND SOLUBLE PROTEINS IN FOUR SPECIES OF *VINCA (V. ROSEA, V. MAJOR, V. MINOR, V. HERBACEA)* GROWING IN IRAN

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Abstract

Indole alkaloids of *Vinca* species are valued greatly in medical sciences, one reason being for their use as powerful anticancer agents. There are four species of *Vinca* in Iran: *V. rosea, V. major, V. minor* and *V. herbacea.* In previous investigations, we found that these plants have considerable alkaloid similarity (58.8-75%). Therefore, chromosomic and electrophoretic studies followed after investigation. We tried to provide exact chromosome numbers and karyotypes of *Vinca* plants by using acetocarmine and Feulgen staining procedures. Diploid numbers of *V. rosea, V. major, V. minor* and *V. herbacea* were 16, 64, 46, 46, respectively. The observed number for *V. major*, with one exception, was different from existing reports. In fact, we had a new cytotype of this species in Iran. *Vinca* plants were also compared with each other electrophoretically. The SDS-PAGE of total proteins was provided and their dendrogram was drawn. The results showed that *V. rosea, V. herbacea* and *V. minor* resemble one another closely. In general, it can be concluded that *V. major* on the one hand and *V. minor* and *V. herbacea* on the other may have originated from *V. rosea* species.

Introduction

A great number of indole alkaloids produced by the *Vinca* species have been identified. Several of these have been found to be valuable agents in the treatment of hypertension and a number of neoplastic ailments [15].

The species of *Vinca* which grow in Iran are *V. rosea, V. major, V. minor* and *V. herbacea* . *V. rosea* is an annual plant [14], while the others are perennial and herbaceous [1]. Of the several *Vinca* species, *V. herbacea* sub. sp *herbacea* grows naturally in some regions, specially in the north and north east of Iran; the others are ornamental.

In our previous study [11], we found alkaloid similarit of these species to be about 58.8 to 75 percent. Whethe they have great similarity in other taxonomic characteristic is a question which requires the study of chromosomes an soluble protein profiles to find the answer.

Although in reports 2n =8,16 in *V. rosea*, 2n = 16, 46 92 in *V. major*, 2n = 32, 46 in *V. minor* and 2n = 46, 92 i *V. herbacea* were identified, there is no detailed report o the study of *Vinca* protein profiles [3, 4, 7, 10, 12, 13, 16]

Materials and Methods

We used *V. rosea* samples which had been cultivate

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in pots. *V. major* and *V. minor* were obtained from the Forest and Rangeland Institute and *V. herbacea* from the north of Iran (13 kilometers from Siahbisheh to Kandavan). Chromosome preparation was made by the following squash method: Root tips were stained in 1% acetocarmine for 2-3 weeks in a cold room after pretreatment with 0.002 M 8-hydroxychinoline for 3 h and fixation in Carnoy solution for 24 h at about 4°C [8].

In Feulgen staining, root tips were stained in Feulgen for 3 h after pretreatment with 0.002 M 8-hydroxychinoline for 3 h, using Peanear solution as fixator and hydrolysis in HCl 1 M for 10 min at 60°C [8].

For electrophoresis of *Vinca* soluble proteins, the vertical SDS-PAGE method was used. One gram of fresh leaf tissue was carefully weighed and placed in small prechilled dishes in a cold room. To each leaf sample, 2.5 ml refrigerated tris-boric buffer solution, pH=8.4 (composed of 0.09 M tris, 0.08 M boric acid, 0.93 g/l Na_2 EDTA) was added [6]. The samples were ground. The density of the samples was increased by adding 2.5 ml of a 40 percent sucrose solution to prevent diffusion of the sample into the reservoir buffer. To protect the proteins and prevent oxidation of products, 20 mg of L-ascorbic acid and 20 mg of polyvinylpyrrolidine (PVP) were added to each sample. Samples were then centrifuged for 40 min at 27000 g and the supernatants were used for electrophoresis. To estimate the approximate protein loading of the samples used in electrophoresis, protein assays were conducted in accordance with the Lowry method [5]. For resolving gel, 12.5 percent acrylamide was chosen for better resolution. The samples were heated for 3 min at 100°C after adding sample buffer (1: 1V/V) [2]. To determine the injection volume, we used the following proportion:

$$\text{The desired injection volume} = \frac{\text{Max injection volume x min. protein concentration}}{\text{Desired protein concentration}}$$

The gel was charged at a constant amperage of 15 milliampers in the stacking gel and 13 milliampers in the resolving gel, with the surrounding buffer temperature maintained at about 6°C. After fixation for 30 min, the gels were stained in coomasie blue for 48 h [2]. Following staining, the gels were destained for three days, then placed in conservative solution [2]. For a more precise comparison of polypeptide bands, the densitometry of gels was achieved at 560 nm after staining.

Results and Discussion

Because of the large number and specially because of the small size of chromosomes among the species of

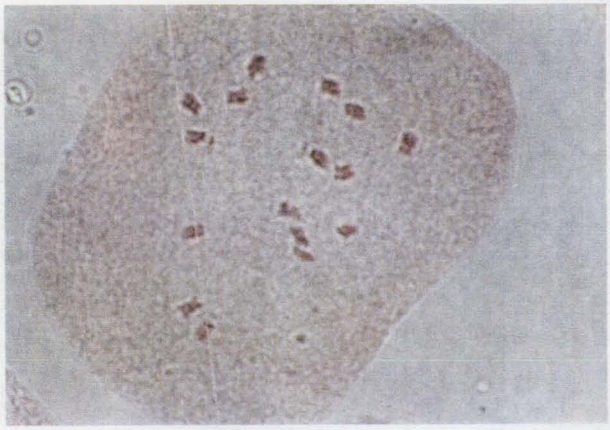

Figure 1. Mitotic metaphase of *Vinca rosea* (2n=16)

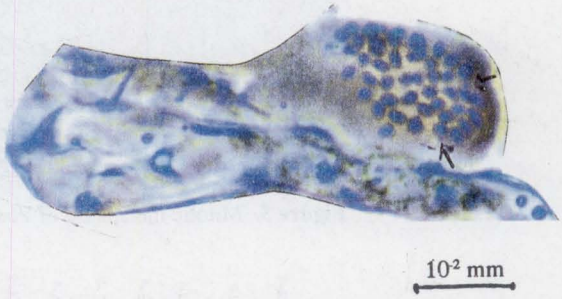

Figure 3. Mitotic metaphase of *Vinca minor* (2n=46)

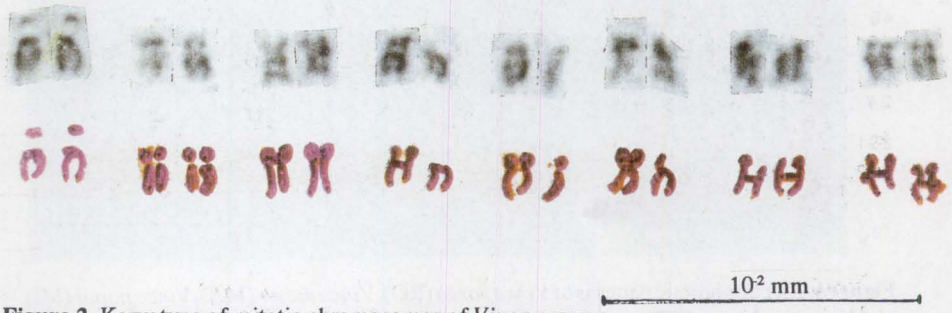

Figure 2. Karyotype of mitotic chromosomes of *Vinca rosea*

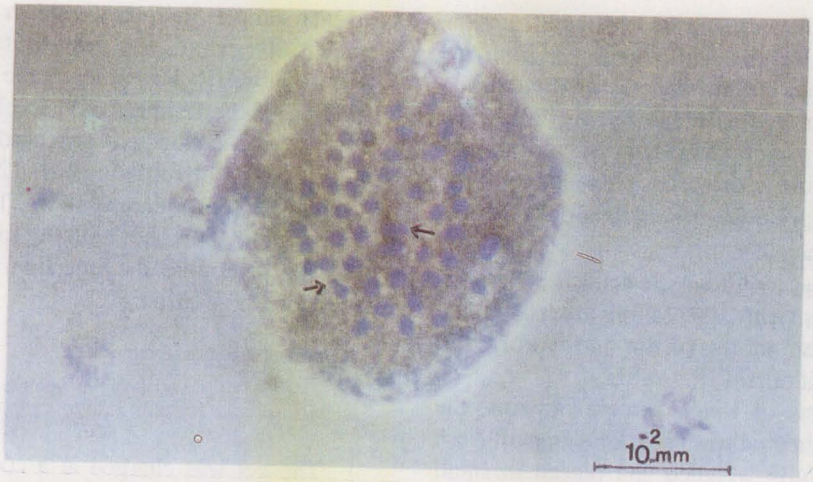

Figure 4. Mitotic metaphase of *Vinca herbacea* (2n=46)

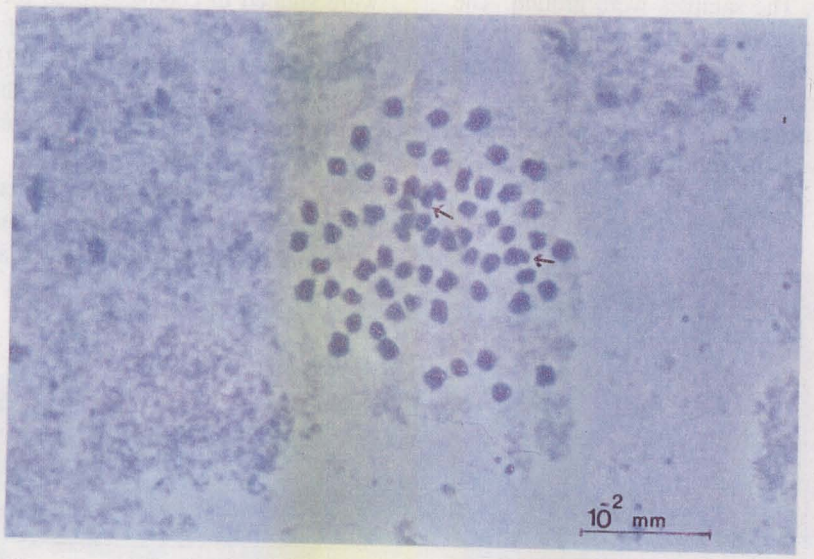

Figure 5. Mitotic metaphase of *Vinca major* (2n=64)

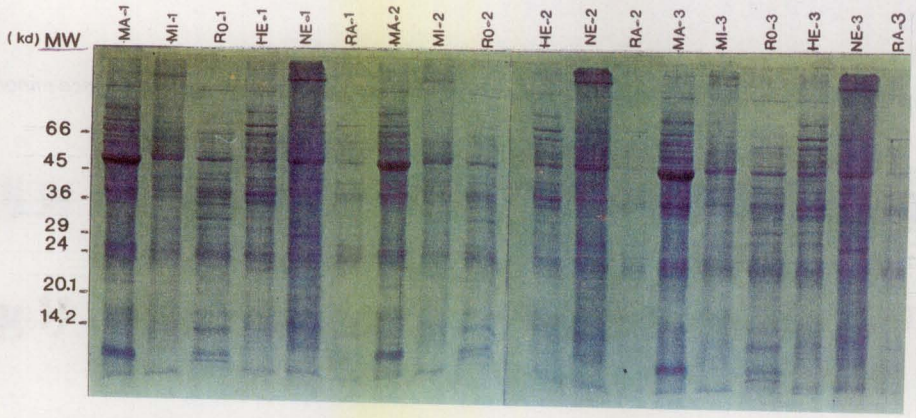

Figure 6. Electrophoretic patterns of *Vinca rosea* (RO), *Vinca major* (MA), *Vinca minor* (MI) and *Vinca herbacea* (HE) proteins in three repeats (1,2,3) after SDS-PAGE.

inca, V. rosea was the only sample whose karyotype was epared (Figs. 1,2). The latter had fewer and relatively gger chromosomes than other *Vinca* species. We found at in *V. rosea*, 2n = 2x= 16 and one pair of chromosomes id satellites. Furthermore, one pair of satellited romosomes was also observed in the other species. The itotic chromosomes of *V. major*, *V. minor* and *V. erbacea* are shown in Figures 3-5 respectively. The sults obtained for *V. rosea* are consistent with those rived at by Kramers [4] and Segawa [13]. The number of itotic chromosomes of *V. minor* was 46 (2n = 2x= 46) hich confirms the reports of Rossitto [10] and Hill [3]. *V. erbacea* had 46 chromosomes (2n = 2x= 46) in mitosis ivision which is in agreement with Loon [7] and Vachova [6]. The number of chromosomes in *V. major* was 64 (2n 64). This is the second report for this species. This basic hromosome number of *V. major* is consistent only with ie findings of Schurhoff [12], thus, it appears that we are ıced with new cytotypes of *V. major*. If the basic number ere 8, the *V. major* used in this investigation would be ctaploid. Since the frequency of polyploid in perennial is igher than in annual plants [17], octaploidy of *V. major* oes not seem unusual.

As the results presented in Figures 1 to 5 show, *V. rosea* hromosomes are fewer (16 in comparison with 46 and 64) nd longer than those of the other three *Vinca* species. This s especially important with regard to the chemotaxonomic ifferences of these species. Based on chemotaxonomic tudies, the differences between *V. rosea* and the other *Vinca* species, with regard to the former's alkaloid type, re greater [9]. In addition, comparing the chromosome umbers and their general features, it may be mentioned hat in this group, *V. major* is placed between *V. rosea* on he one hand and *V. minor* and *V. herbacea* on the other, ecause the general feature of *V. major* chromosomes is unctual the same as *V. minor* and *V. herbacea*, whereas its asic number is consistent with *V. rosea*.

To study the electrophoretic profiles, three different olumes 40, 50, 60 µlit were considered as maximum njection volume. Figure 6 shows the electrophoregram btained. Following gel densitometry (Fig. 7), Rms were alculated and similarity indices of species were determined 5].

$$\text{Similarity index} = \frac{\text{No. of pairs of similar bands} \times 100}{\text{No. of different bands + no. of pairs of similar bands}}$$

By using these indices, similarity percents were letermined and a dendrogram was drawn (Fig. 8). As the electrophoregram and dendrogram indicate the similarities etween the *V. rosea*, *V. herbacea* and *V. minor* profiles are greater than that of *V. major*.

However, according to the comparison of this dendrogram with the alkaloidic dendrogram obtained in our previous study (Fig. 9), as well as cytogenetic results, it could be emphasized that the alkaloidic and chromosomic similarity between *V. minor* and *V. herbacea* is higher than

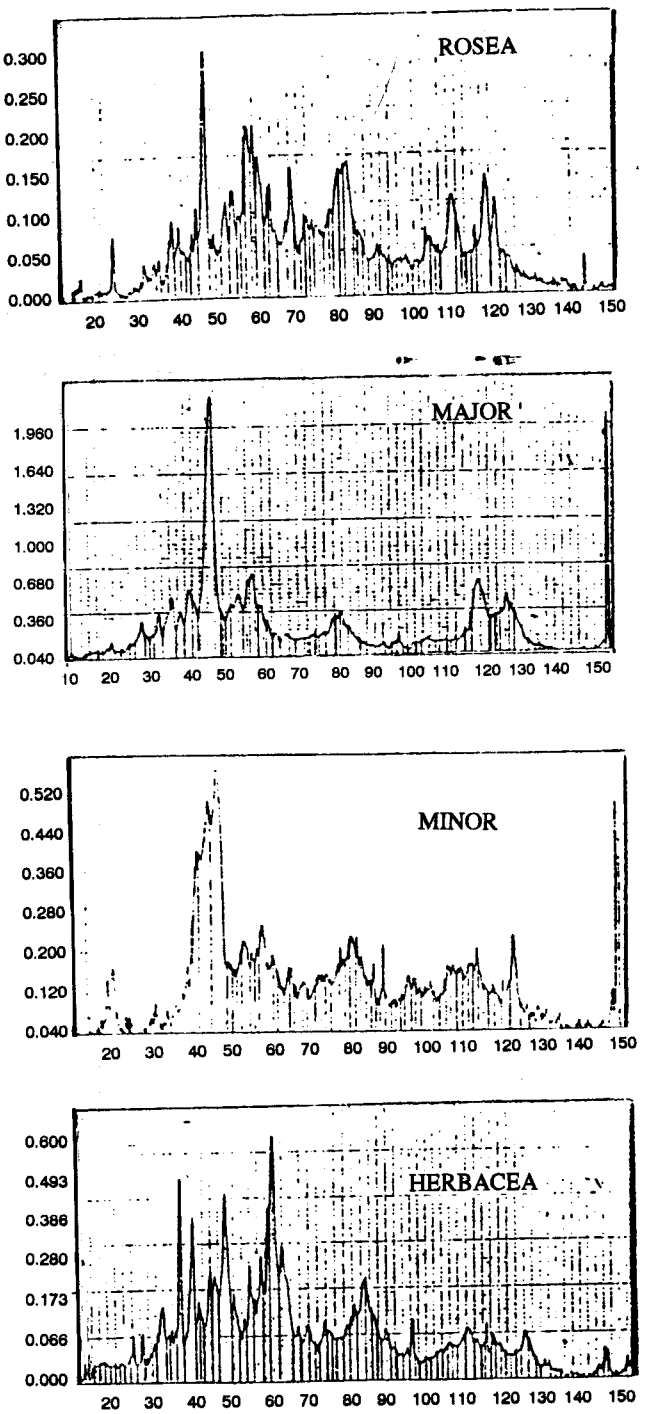

Figure 7. Polypeptide bands densitometry of *Vinca rosea*, *Vinca major*, *Vinca minor* and *Vinca herbacea* in 560 nm

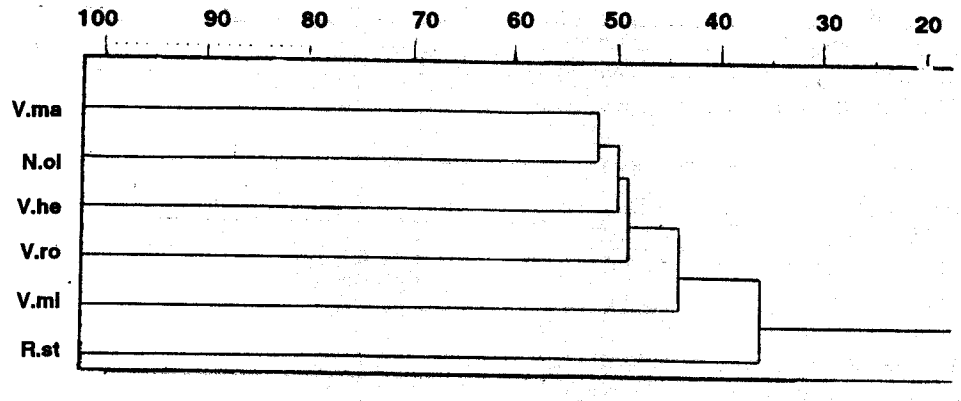

Figure 8. Dendrogram of four species of *Vinca*, *V. rosea* (V.RO), *V. major* (V.MA), *V. minor* (V. MI) and *V. herbacea* (V. HE) on the basis of protein similarity indices. N.OI and R.ST represent the two other genera of the Apocynaceae family.

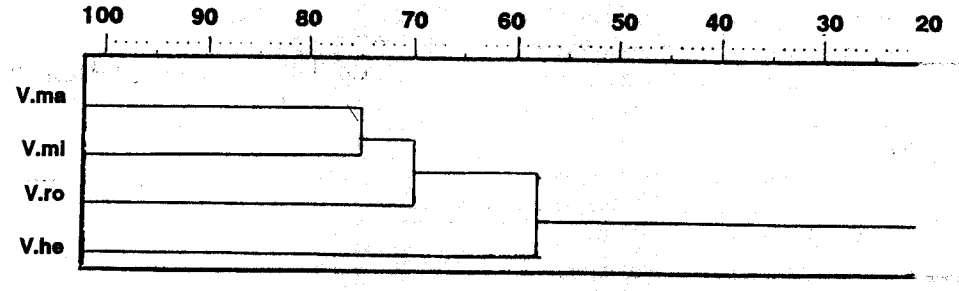

Figure 9. Dendrogram of four species of *Vinca*, *V. rosea* (V.RO), *V. major* (V.MA), *V. minor* (V. MI) and *V. herbacea* (V. HE) on the basis of alkaloid similarity indices.

between the other species. *V. major* is placed between these two species and *V. rosea* in terms of its alkaloids and chromosomes and is different from them in terms of its proteins. *V. rosea* is seen to be closely related to *V. major* in terms of proteins yet is quite different from it in terms of alkaloids and chromosomes.

From an evolutionary point of view, it could be suggested that *V. major* on the one hand and *V. minor* and *V. herbacea* on the other may have originated from *V. rosea* in two different forms.

Acknowledgements

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