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Seasonal Variation in Bufadienolide Content in Diploid and Tetraploid *Urginea indica* (Indian squill)

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The proscillaridin A and scillaren A contents in diploid and tetraploid reached a maximum at the peak of vegetative phase. However, another peak was observed at the end of dormant phase in the tetraploid bulbs. The content of scilliphaseoside, present only in tetraploid bulbs, reached a maximum value at the end of dormancy and showed a second peak during vegetative phase.

Key Words: *Urginea indica*, **Liliaceae, Seasonal variation, Bufadienolides, Diploid, Tetraploid**

Introduction

The active principles of plants do not remain constant throughout the year. There are several reports on the qualitative and quantitative variation of active principles with growth and development (Buchkova & Gerasimenko 1973, Adamki & Kodym 1978, Fluck 1963, Gritsaeva et al. 1966, McCarthy et al. 1966, Cucu & Paun 1968, Shimuzu 1969, Han et al. 1975). The cardiac glycoside content in *Urginea maritima*—the squill growing in Egypt has been reported (Elkiey et al. 1964) to vary with season. However, there is no report on such variation in the content of principal bufadienolide in *Urginea indica*, the Indian squill, the substitute of *U. maritima* in commerce. Recently, it was reported (Jha & Sen 1981) that the diploid *U. indica* differs from the tetraploid by absence of scilliphaeoside. An attempt has been made in the present study to compare the yield of cardiac glycosides in diploids and tetraploids at different seasons.

The bulbs of *U. indica* are inconspicuous (dormant) during winter but with advent of spring, the inflorescence axis i.e., the scape comes out of the underground stem. Flowering is complete by late March-April and with the premonsoon shower, leaf initials are visible. Leaf formation continues throughout summer till August after which the bulbs enter the dormant phase i.e., there is no vegetative growth of the plant and the portion above the ground dies and withers.

Materials and Methods

The bulbs of *Urginea indica* Kunth were collected from Jodhpur and Pune. The bulbs were grown in the experimental garden under identical conditions of habitat. Cytological investigations of root-tips were carried through scheduled procedure (Sen 1973) after pretreatment in paradichlorobenzene: 0.25% colchicine (1:1) for 3 hours. From each root, 10 to 12 metaphase were studied and bulbs were examined individually. The bulbs from Jodhpur were found to be diploid (2n=20; x=2) and those from Pune tetraploid (2n=40; x=4). Bulbs of almost equal size (diam. 3 ± 0.5 cms and fresh weight 18–20g/bulb) were collected in the first week of every month beginning with May 1, 1978 and continuing through April 28, 1979 (i.e. 13 sampling periods). Four samples were examined per month, each sample consisting of 20–25 bulbs. The bulbs were washed, sliced and sun dried.

Extraction, isolation and identification of bufadienolides: The dried plant material (40–50g) was defatted with petroleum ether (b.p. 60–80°) and the defatted marc was extracted with 90% ethanol. The extract was concentrated, purified (Wartburg 1966) and the glycosides were extracted following the methods adopted by earlier authors (Krishna Rao & Rangaswami 1967, Wartburg 1966). All necessary precautions to prevent hydrolysis during extraction were taken (Trim 1955). The isolation and identification of Scillaren A, proscillaridin A and scilliphaeoside were done as reported earlier (Jha & Sen 1981).

Spectrophotometric estimation of bufadienolides: Quantitative estimation of bufadienolides was carried out modifying the method adopted by earlier authors for determination of glycosides of *U. maritima* (Bombardelli 1965, Kaczmarck & Zygmunt 1974, Karawya et al. 1973).

Conventional thin-layer chromatography was used after extraction of the separated bufadienolides from the adsorbent layer. Liebermann-Burchard reagent (Neher 1969) had earlier been used as a detection reagent for scilladienolides (Bombardelli 1965).

Aliquots of known concentration of scillaren A and proscillaridin A (authentic samples) dissolved in methanol were applied as spots with the help of micropipettes on activated silica gel G thin-layer chromatograms (Neher 1969) with parallel blank and a reference chromatogram as well. The plates were developed in benzene: 95% ethanol: 7:3 (Johnston & Jacobs 1966). Detection was done by spraying reference chromatogram with Liebermann's reagent. The spots of proscillaridin A and scillaren A were marked and silica gel of corresponding area of each spot was scrapped off and extracted with 10ml methanol and centrifuged at 1000g for 15 mins taking all necessary precautions (Ganshirt 1969). From each sample, 9ml of aliquot was taken in a test tube and evaporated to dryness on a water bath. Each sample was dissolved in 1ml of glacial acetic acid (Stoll & Jucker 1955) and colour reaction was performed by adding 0.5ml of acetic anhydride and 0.1ml of conc. sulfuric acid. The colour was found to be stable after 50 mins of adding reagent to substrate. The absorbance of each of the sample was measured on a spectrophotometer (Carl Zeiss, Jena) set at 675nm (Bombardelli 1965) for proscillaridin A and scillaren A and at 580nm for scilliphaeoside against the blank and computed into curves which followed the Beer's law. Extensive recovery studies in the range 20–30μg/ml have been performed. The results showed

an overall recovery of 90% with a relative standard deviation of 0.64%.

For determination of concentration of proscillaridin A and scillaren A in plant materials, the glycoside extract dissolved in methanol was subjected likewise to preparative thin-layer chromatography (Ganshirt 1969).

The solvent system benzene: 95% ethanol : : 7:3 was adequate for separation of proscillaridin A (R_f 0.54) and scillaren A (R_f 0.37) in diploid bulbs. However, a different solvent system was used in case of tetraploid bulbs which contain scilliphaeoside (Jha & Sen 1981) with R_f value in between pro-scillaridin A and scillaren A. The solvent system chloroform : methanol : water : : 80:18:2 (Alfermann et al. 1977) has been found to be satisfactory in separation and extraction of proscillaridin A (R_f 0.66) and scillaren A (R_f 0.30) with minimum interference of scilliphaeoside (0.40). The procedure for extraction of bufadienolides from the adsorbent layer was the same as for preparation of standard curve and the Liebermann reaction was performed after dissolving residue in 1ml of glacial acetic acid. The absorbance of each of the sample of unknown concentration was determined in a similar manner and their concentrations determined by comparing with that of the standard curve. For each sample twelve such replicas were examined and relative standard deviation of the mean ratio was calculated (S_{rel}=0.60–0.81%).

Results

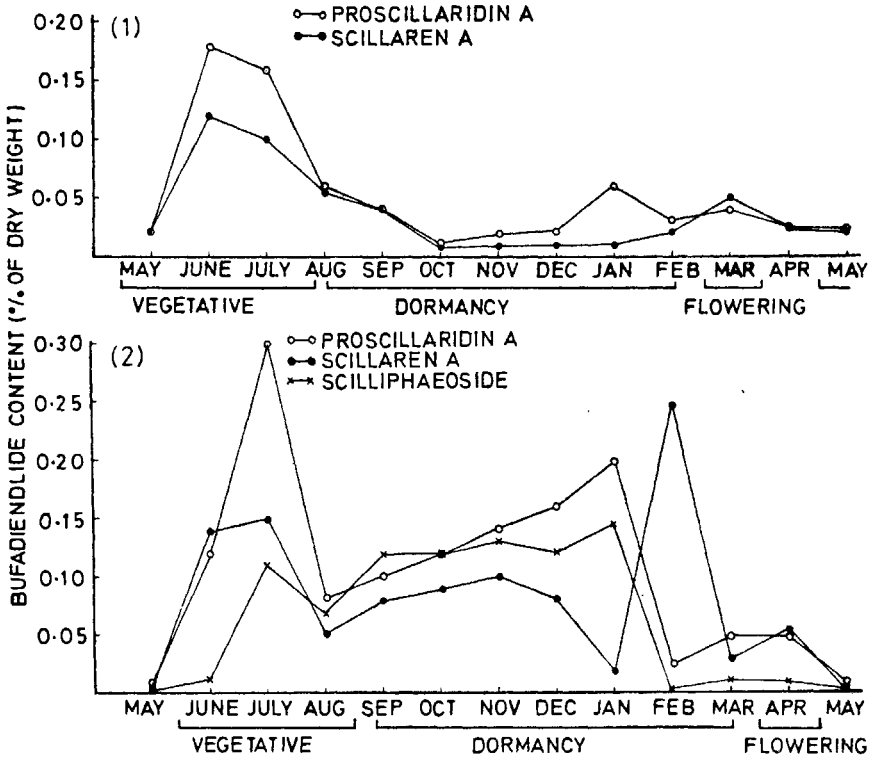
From the figures 1 and 2 and table 1 it is evident that bufadienolide content in bulbs of both the races of *U. indica* showed considerable variation with seasonal changes.

Proscillaridin A content in diploid and tetraploid bulbs: At the beginning of vegetative phase, proscillaridin A content was appreciably low in both the races, it reached its maximum value both in diploid (0.18%) and tetraploid (0.30%) at the peak of vegetative growth, sharply declining thereafter (figures 1 & 2).

In diploid, the content remained low (0.02%) throughout winter and at the end of dormancy there was a slight increase (0.06%) after which it continues to decline with the beginning of flowering (table 1).

Table 1 *Seasonal variation in bufadienolide content (% of dry weight) in bulbs of* U. indica *(Average values of four samples analyzed* ± S.E. 0.03–0.07%)

Month	Diploid		Tetraploid		
	Proscillaridin A	Scillaren A	Proscillaridin A	Scillaren A	Scilliphaeoside
January	0.06	0.01	0.20	0.02	0.15
February	0.03	0.02	0.025	0.25	trace
March	0.04	0.05	0.05	0.03	0.01
April	0.025	0.025	0.05	0.055	0.01
May	0.024	0.024	0.01	trace	trace
June	0.18	0.12	0.12	0.14	0.01
July	0.16	0.10	0.30	0.15	0.11
August	0.06	0.056	0.08	0.05	0.07
September	0.04	0.04	0.10	0.08	0.12
October	0.015	0.01	0.12	0.09	0.12
November	0.02	0.01	0.14	0.10	0.13
December	0.02	0.01	0.16	0.08	0.12



Figures 1–2 Seasonal variation in bufadienolide content in diploid and tetraploid bulbs respectively. (Each point represents average of four analysis)

In tetraploid, however, almost throughout the dormancy, the proscillaridin A content increases every month reaching a high amount (0.20%) in January sharply declining thereafter during transition from dormancy to flowering (figure 2). Flowering is late in tetraploids as compared to diploids and proscillaridin A content slightly increases during flowering.

Scillaren A content in diploid and tetraploid bulbs: From an initially low amount at the beginning of vegetative phase, the scillaren A content increased sharply in both the races and reaching its maximum value (0.12% in diploid and 0.15% in tetraploid) at the peak of vegetative growth. In diploids, it continued to decline to a low value which remained constant (0.01%) throughout the period of dormancy (figure 1). In tetraploid, however, the content sharply increased at the beginning of dormancy and remained almost constant at the same level (figure 2). But it declined sharply to a very low value (0.02%) in January and reached the maximum level at the end of dormancy—the content being higher (0.25%) than at the peak of vegetative growth in tetraploids (table 1).

The content of scillaren A was low (0.02–0.03%) during pre-flowering phase (February–March) in both the races with a slight increase during flowering,

declining thereafter to the lowest value (trace—0.01%) at the beginning of vegetative phase.

Scilliphaeoside content in tetraploid bulbs: At the onset of vegetative phase scilliphaeoside was present only in trace amounts. It sharply increased (0.11%) at the peak of vegetative growth, declining (0.07%) thereafter. Again, the content increased (0.12%) at the onset of dormancy, remaining high and at almost constant level till December. In January, the highest value (0.15%) was recorded. Thereafter, it sharply declined to trace amounts and remained constant till the beginning of vegetative phase (figure 2). The presence of trace amount of scilliphaeoside in tetraploid bulbs prior, during and after flowering period is interesting. This glycoside is absent in the diploid race (Jha & Sen 1981).

Discussion

There is one fundamental similarity in the seasonal variation of scillaren A and proscillaridin A content in diploids in that both increase from a low value at the onset of vegetative growth and reach a maximum at the peak of this phase and decline thereafter. Thus with season and with phasic change of the bulb, the content as well as the relative proportion of the two principal bufadienolides vary but the concentration of proscillaridin A is always either more or equal to the concentration of scillaren A.

The proscillaridin A and scillaren A content in tetraploid bulbs show two peaks of maximal accumulation but this period of maximal accumulation of the two glycosides are different. While the content of proscillaridin A is the highest in vegetative phase (0.30%), the scillaren A content is highest at the end of dormancy (0.25%). The second highest peak of the former glycoside is in January (0.20%) and that of the latter is in July (0.15%). Similar to diploids, the relative proportion of the two glycosides vary in tetraploids, and the content of scillaren A is higher than that of proscillaridin A prior to flowering. In species with tropane alkaloids, a correlation does exist between the onset of flowering and the maximum content of secondary plant product (Fluck 1963). Such two periods of maximal accumulation of active principles have been reported in other medicinal plants (Buchkova & Gerasimenko 1973, Strezlecka 1968) as well.

The significant variation in scilliphaeoside content in tetraploid bulbs cannot be attributed to translocation to other organs (Fluck 1963). No scilliphaeoside could be traced in roots and leaves at any stage (Jha & Sen 1981). The heavy decrease in its content prior, during and after flowering may be an indication of its gradual utilization.

The bufadienolide content in *U. maritima*, the European squill has been reported (Elkiey et al. 1964) to be maximum in summer, gradually decreasing in autumn, winter and spring. The tetraploid Indian squill is, therefore, very different in its response to seasonal variations from the European squill on the one hand and the diploid Indian squill on the other.

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