

## Discussions

Agrotechnology and Chemistry of Medicinal and aromatic plants have been authentically compiled by a number of authors of which earlier ones<sup>55, 18, 33</sup> mainly dealt with morphological description and uses of active principles; and the recent compilations like Atal and Kapoor<sup>6</sup>, Sevendsen et al.<sup>101</sup>, Alfermann and Reinhard<sup>4</sup> and Nandi<sup>67</sup> mainly focus the biogenesis of different active principles in medicinal plants with reference to their cultural and growth habits. The Central Institute of Medicinal and Aromatic Plants (Lucknow) and Regional Research Laboratories of CSIR have also made successful contribution on agrotechnological methods of some commonly growing medicinal and aromatic plants in India.

Analysis of behavioral growth and developmental physiology, particularly in relation to biosynthetic patterns of different active principles, are some of the interesting areas of studies being attempted in the Research Laboratories of Cinchona and Other Medicinal Plants, West Bengal situated in Darjeeling hills. In this matter significant contribution of Nandi<sup>67</sup> Acharyay<sup>2</sup>, Sarma<sup>88</sup>, Panda<sup>76</sup> and also Ghosh<sup>38</sup> are worth mentioning. While Nandi<sup>67</sup> and Ghosh<sup>38</sup> worked out the agrotechnology of some alkaloid/glycoside yielding and essential oil yielding plants to be grown successfully in Bengal plains, Panda<sup>76</sup> has made significant contribution on acclimatisation, developmental physiology and biochemistry of Costus spp. in Darjeeling hills of Bengal.

No comprehensive attempts have so far been made on growth, development and biochemistry of Dioscoreas growing in Darjeeling Hills and the present study is an attempt in this direction. The study encompasses interesting areas of developmental growth analysis and biochemistry of Dioscorea prazeri and Dioscorea composita and in the course of analysis, some generalisation have been projected in the areas of synthesis of glycosides and their modulation by environmental factors like light, developmental factors like reproduction, chemical factors like N, P and K and some auxin like factors, like phenoxy auxin analogues.

The two species of Dioscorea under study, though revealed more or less an identical pattern of developmental growth and diosgenin formation during their normal course of life cycle, certain differences could be sorted out. While D.prazeri is an annual, regularly shedding leaves during winter, plants drying and again sprouting in next summer, D.composita is a perennial climber, having distinct and well defined developmental stages during each successive year. In both the species, vegetative stages of development recorded maximum rate of diosgenin synthesis which in D.prazeri was accompanied by comparatively enhanced rate of radial growth like leaf formation and in D.composita, by extension growth like stem formation. Both the species more or less exhibited an identical pattern of diosgenin formation during later stages of development like reproductive stage; but vegetative stage of D.composita, having a comparatively

prolonged growth period also registered an enhanced rate of diosgenin synthesis during this stage. It may be mentioned in this connection that the post-reproductive stage, accomodating no active growth process (total cessation of linear growth, leaf formation), however, permitted synthesis of diosgenin significantly in both the two species.

While analysing nitrogenous fractions like TN and SN during different developmental stages of two species, an increased rate of SN during vegetative stage could be related with increased rate of diosgenin synthesis during the same stage and such a relationship could be more asserted in D.composita. D.composita also revealed higher rate of dry matter accumulation as compared to D.prazeri. Thus in the course of normal life cycle of the D.composita and D.prazeri growing in Darjeeling hills, some distinction could be traced in manner of participation of their developmental growth, formation of some biochemical macromolecules as well as in the overall picture of diosgenin synthesis.

The use of different phenoxy compounds offer an opportunity for experimentation with some auxin-analogues that posses high, medium and low auxin activities. Such a method has been utilized by Chatterjee and Leopold<sup>27</sup> in determining the auxin sensitivity of growth process. Acharyya Choudhury<sup>1</sup> used phenoxy compounds having varying degrees of auxin activities, to assess auxin control of some growth process in relation to prevailing seasons. Dependence of changing metabolic activities in the course of developmental

process in plants<sup>108, 21, 22</sup> on auxin system has been reported by Gupta<sup>41</sup> while studying the senescence process. Auxin-dependence of synthesis of active principles in medicinal plants have not yet been reported in the literature and in this respect, the present set of experiments with some phenoxy auxin analogues like PAA, 4-Cl, 2,5-D and 2,4,6-T and their effects on developmental biochemistry and diosgenin synthesis in D.prazeri and D.composita is new and interesting. Of these phenoxy compounds, PAA possess weak auxin properties; 4-Cl and 2,5-D possess medium auxin activities and 2,4,6-T have no auxin activities. In this context, analysis of patterns of developmental growth; changes of some biochemical parameters with growth and style of diosgenin synthesis in two species of Dioscoreas will be a novel exercise.

Results show that diosgenin synthesis might be a function of auxin, atleast in part. This will be borne out by the fact that phenoxy analogues like 2,5-D and 4-Cl induce increased diosgenin synthesis during all the developmental stages. That these compounds possess auxin-like properties have been shown by Chatterjee<sup>29</sup> and Rubenstein et al.<sup>86</sup>. This contention is further corroborated by the fact that the inhibition of diosgenin synthesis in both the Dioscorea species takes place in treatments with higher concentrations; reminiscent of typical two-phase action of auxin<sup>57</sup>. Our observation will be further strengthened in the results obtained with 2,5-D (which possess higher auxin activity as compared to 4-Cl) where augmentation of diosgenin synthesis is comparatively high.

Fantastically, if we analyse the growth behaviour and diosgenin synthesis of Dioscorea prazer and Dioscorea composita under treatments with non-auxin analogues like PAA and 2,4,6-T, enhancement of diosgenin synthesis does not take place; rather the same is depressed.

In contrast to observations on growth behaviour of normal plants mentioned earlier, phenoxy auxin-analogue treated plants revealed a positive relation with the phenomenon of augmentation of extension growth with a concomitant enhancement of diosgenin synthesis. Auxin promotes extension growth<sup>57</sup> and in our experiments, parallel effects on growth promotion and on diosgenin synthesis provide further basis of our arguments of auxin-dependence of diosgenin synthesis. It would be interesting to note that non-auxin had not been able to demonstrate such a relationship. Even in 2,4,6-T, rate of diosgenin synthesis vis-a-vis extension growth were poorer as compared to PAA treatments. Incidentally, 2,4,6-T is known to be a weaker auxin as compared to PAA<sup>29</sup>.

Chatterjee et al.<sup>30</sup> reported negative effect of reproduction on diosgenin synthesis in D.prazeri. Nandi<sup>67</sup> could increase alkaloid/glycoside in a number of medicinally important plants by systematic removal of reproductive units. In this study auxin-analogues of low concentration inhibited panicle formation which was accompanied by increased diosgenin synthesis in both D.prazeri and D.composita. The non-auxin, on the other hand, did not affect the panicle formation appreciably and also the patterns

of diosgenin synthesis remained unaltered. It is to be noted, however, that treatments of non-auxins inhibited panicle formation, being not associated with increased diosgenin synthesis; pointing thereby that inhibitory role of reproduction on glycoside synthesis, as reported by earlier authors<sup>30,67</sup> is not simply a correlative effect. In this context it will be further interesting to note that there are certain evidences in our experiments (like decrease in diosgenin synthesis with inhibition of panicle formation in high concentration treatments) which may lead to a speculation of a typical two phase auxin action i.e., promotive in lower concentration and inhibitive in higher concentration, on a biochemical synthetic process, not frequently reported in literature.

Pattern of dry weight accumulation in auxin and non-auxin analogue-treated plants were rather uniformly consistent with their increase remaining associated with increase of diosgenin synthesis. The picture gradually became weak with progress of developmental stages in both the species. Furthermore one has to hesitatingly admit from the results of our study that a simple (?) relationship between increased TN and diosgenin does not always exist in those experiments using auxin and non-auxin homologues. Rather a diffused association between augmentation of SN and diosgenin is tracable; particularly in plants treated with low concentration of auxin analogue and high concentration of non-auxin analogue. That the suggested correlation between some particular growth and biochemical parameters including diosgenin

of D.prazeri and D.composita, treated with phenoxy compounds, is of statistical significance will be borne out by the data presented below :-

D.prazeri

	Dry wt.	Diosgenin
Extension growth	0.60	0.72*
Dry wt.		0.70*

\*Significant at 5% level

D.composita

	Dry wt.	Diosgenin
Extension growth	0.58	0.74*
Dry wt.		0.72*

\*Significant at 5% level

Diosgenin, a steroid precursor having a complex structure (Cyclopentanoperhydrophenanthrene) continuously accumulates in the underground tuber during all stages of development which may be explained on the basis of this molecule being synthesized in leaf<sup>10</sup> and is ultimately mobilized to tuber. The very fact that tuber of Dioscorea<sup>9</sup> and rhizome of Costus<sup>89</sup> lose diosgenin during sprouting also suggests the storing nature of the tuber.

Site of synthesis of diosgenin being leaf, effects of light as well as darkness on its synthesis will provide some interesting areas of analysis. With this objective, effects of exposure of

additional light period and of darkness on growth patterns, changes in nitrogenous fractions and on diosgenin formation were analysed in D.prazeri and D.composita. In general, it could be shown that provision of additional light hours increased diosgenin synthesis more in D.composita and to some extent in D.prazeri, whereas additional provision of dark hours inhibited diosgenin synthesis in both the species with the exception of augmentation of the same in D.prazeri during initial stages of dark experiment.

The enhancement of synthesis of active principles under the influence of long days have been mentioned by authors<sup>48,3</sup>. Nandi and Chatterjee<sup>72</sup> further demonstrated the quantitative effects of photoperiods on enhancement of active principle synthesis in conjunction with augmentation of different growth and biochemical parameters. Only reported literature in identical lines had been the studies of Ghosh<sup>38</sup> who quantified rates of essential oil synthesis in Cymbopogon and Mentha in relation to light cycle. However, effects of some net amount of light period as well as of darkness on diosgenin synthesis vis-a-vis other growth and functional parameters in these two species of Dioscorea, (as reported in the present investigation) is a newer concept in the area of light-dependence of developmental physiology of steroid yielding plants. Light augmentation of diosgenin was clear in our study, which remained identified with increased rate of extension growth, leaf formation, panicle formation and fruit formation. A distinct quantitative relation was also there with lower augmentation of these parameters as well as diosgenin synthesis

in lesser quantity of additional light period.

Light inhibition of extension growth has been shown by Nandi<sup>67</sup>, Parkar et al.<sup>77</sup> and Sale and Vince<sup>87</sup>. Nandi<sup>67</sup> reported that increasing long day cycles reduced extension and leaf growth and increased reproductive growth. Comparable effects of light were also revealed in the present study. Phenomenon of diosgenin synthesis in relation to growth and other functional parameters was thus differentially manifested in plants experiencing changing photoperiod and results of our experiment with additional provision of light period were also confirmatory of such findings.

Findings of light experiments would get support from results of experiments where additional dark hours were administered to two species of Dioscoreas. Dark inhibition of diosgenin synthesis was remarkably evident and in this connection mention may be made to the effects of short days on D. metel<sup>67</sup> where inhibition of active principle was noted. In our studies, the dark effect on diosgenin inhibition remained generally associated with extension growth inhibition, leaf inhibition, panicle inhibition and inhibition of reproductive growth. Dark effects could even be quantified in the sense of a lesser provision of dark hours to Dioscorea plants which was reflected in reduced quantum of individual parameters, including rate of diosgenin synthesis.

A more or less clear relationship between occurrence of different nitrogenous fractions with diosgenin synthesis could be established

in the study. The results uniformly exhibited that increase in SN remained associated with increase in diosgenin and quanta of increase gradually declined with the progress of developmental stages. In D.composita, where a comparatively enhanced synthesis of diosgenin took place, there remained a higher increase of SN, rather uniformly. Treatments of darkness inhibited diosgenin synthesis as well as decreased SN content. Changes in the patterns of TN in relation to diosgenin content, which was revealed in normal plants, could not be traced in light and dark experiments.

Discussion on the effects of light and dark hours on diosgenin synthesis may remain incomplete if particular mention is not made to the fact that additional dark hours augmented diosgenin contents during initial periods of experimentation in D.prazeri. In the later species, there also occurred an enhancement of extension growth, leaf formation, panicle formation as well as content of soluble nitrogen. Such effects may be explained in the context of light-saturation effects, being alleviated by a very restricted period of darkness to facilitate further biosynthesis of diosgenin.

Auxin dependence of diosgenin synthesis at least to some extent, has been mentioned in preceding pages and it is possible that during initial hours of darkness, photooxidation of endogenous auxin remaining suspended, which may be linked with higher diosgenin formation. Thus in both the two species, synthesis of active principle remained associated with the existence of a correlative control of growth, development and endogenous contents

of certain chemical parameters in relation to modifying effects of some environmental factors. However, the nature of relationship between light augmented biosynthesis of a complex compound like diosgenin and light-augmented growth and development need to be elaborated with further details. Statistical correlation of different growth and biochemical parameters of both the species in support of the above description is cited below :-

D. prazeri

	<u>Leaf</u>	<u>Dry wt.</u>	<u>SN</u>	<u>Diosgenin</u>
Extension growth	0.84**	0.60	0.49	0.85**
Leaf formation		0.65*	0.52	0.77*
Dry wt.			0.52	0.87**
SN				0.72*

\*\* Significant at 1% level, \* Significant at 5% level

D. composita

	<u>Leaf</u>	<u>Dry wt.</u>	<u>SN</u>	<u>Diosgenin</u>
Extension growth	0.80**	0.56	0.47	0.83**
Leaf formation		0.69*	0.55	0.73*
Dry wt.			0.60	0.82**
SN				0.65*

\*\* Significant at 1% level, \* Significant at 5% level

Augmenting effects of fertilizer treatments on active principles in different medicinal plants have been reported in literature. Thus mention may be made to the report of Hamidi *et al.*<sup>46</sup> in Datura stramonium; Cruzedo *et al.*<sup>34</sup> in D. composita; Saini and Mukherjee<sup>90</sup>

in Rauvolfia serpentina; Ferguson and Hyres<sup>36</sup> in Dioscorea spp; Sahu<sup>91</sup> in Rauvolfia serpentina; Singh et al.<sup>97</sup> in Atropa belladonna; Nandi and Chatterjee<sup>68</sup> in Rauvolfia serpentina; Chatterjee<sup>28</sup> in Cephaelis ipecacuanha; Nandi<sup>67</sup> in some important alkaloid/glycoside yielding medicinal plants and Panda<sup>76</sup> in Costus.

In contrast to a fertilizer experiments of Nandi<sup>67</sup> on Dioscorea composita where only N & P have been taken into consideration, the present study encompasses a rather comprehensive lay out of the effects of N, P and K (both single & in combination) on diosgenin content of D.prazeri and D.composita. Regulating effects of different fertilizer treatments on diosgenin synthesis are mentioned below :

<u>Species</u>	<u>Treatments</u>	<u>Diosgenin content(%)</u>
<u>D.prazeri</u>	N <sub>0</sub> P <sub>0</sub> K <sub>0</sub> (Control)	1.79
<u>D.composita</u>	N <sub>0</sub> P <sub>0</sub> K <sub>0</sub> (Control)	2.75
Treatment	% decrease(-) or increase (+) of diosgenin over control	
	<u>D.prazeri</u>	<u>D.composita</u>
N <sub>0</sub> P <sub>0</sub> K <sub>150</sub>	+ 0.39	+ 0.47
N <sub>0</sub> P <sub>0</sub> K <sub>300</sub>	+ 0.41	+ 0.47
N <sub>0</sub> P <sub>150</sub> K <sub>0</sub>	+ 0.46	+ 0.44
N <sub>0</sub> P <sub>300</sub> K <sub>0</sub>	+ 0.57	+ 0.55
N <sub>0</sub> P <sub>150</sub> K <sub>150</sub>	+ 0.77	+ 0.91
N <sub>0</sub> P <sub>150</sub> K <sub>300</sub>	+ 0.77	+ 1.02
N <sub>0</sub> P <sub>300</sub> K <sub>300</sub>	+ 0.82	+ 1.14
N <sub>0</sub> P <sub>300</sub> K <sub>150</sub>	+ 0.84	+ 1.19

<u>Treatment</u>	<u>% decrease (-) or increase (+) of diosgenin over control</u>	
	<u>D.prazeri</u>	<u>D.composita</u>
N <sub>150</sub> P <sub>150</sub> K <sub>150</sub>	+ 1.10	+ 3.09
N <sub>150</sub> P <sub>150</sub> K <sub>300</sub>	+ 1.11	+ 2.42
N <sub>150</sub> P <sub>300</sub> K <sub>150</sub>	+ 0.90	+ 2.56
N <sub>150</sub> P <sub>300</sub> K <sub>300</sub>	+ 0.87	+ 2.50
N <sub>150</sub> P <sub>150</sub> K <sub>0</sub>	+ 0.80	+ 2.50
N <sub>150</sub> P <sub>300</sub> K <sub>0</sub>	+ 0.79	+ 2.74
N <sub>150</sub> P <sub>0</sub> K <sub>150</sub>	+ 0.70	+ 2.67
N <sub>150</sub> P <sub>0</sub> K <sub>300</sub>	+ 0.70	+ 2.29
N <sub>150</sub> P <sub>0</sub> K <sub>0</sub>	+ 0.60	+ 1.80
N <sub>300</sub> P <sub>0</sub> K <sub>0</sub>	+ 0.70	+ 2.00
N <sub>300</sub> P <sub>150</sub> K <sub>0</sub>	+ 0.74	+ 2.52
N <sub>300</sub> P <sub>150</sub> K <sub>300</sub>	+ 1.00	+ 3.20
N <sub>300</sub> P <sub>300</sub> K <sub>150</sub>	+ 0.91	+ 2.80
N <sub>300</sub> P <sub>300</sub> K <sub>300</sub>	+ 0.89	+ 2.88
N <sub>300</sub> P <sub>150</sub> K <sub>150</sub>	+ 0.92	+ 3.76
N <sub>300</sub> P <sub>300</sub> K <sub>0</sub>	+ 0.94	+ 2.80
N <sub>300</sub> P <sub>0</sub> K <sub>150</sub>	+ 0.92	+ 2.46
N <sub>300</sub> P <sub>0</sub> K <sub>300</sub>	+ 1.56	+ 3.59

Chatterjee and Chatterjee<sup>31</sup> in their short-term fertilizer studies with D.prazeri established augmenting effects of N & K on diosgenin synthesis of the species; the effect being more relevant in plants undergoing defruiting operation. Nandi<sup>67</sup> established higher effectiveness of P as compared N on the diosgenin yield of D.composita. In our studies the superiority

of N fertilizer and to some extent P fertilizer in both the species of Dioscorea could be demonstrated; thus confirming the findings of Panda<sup>76</sup> in his experiment with Costus speciosus.

Out of the total 27 treatments, our studies could locate four treatments which revealed higher effectiveness of four treatments on diosgenin synthesis in two species of Dioscorea. The treatments were as follows :

D.prazeri

1. N<sub>300</sub>P<sub>0</sub>K<sub>300</sub>
2. N<sub>150</sub>P<sub>150</sub>K<sub>300</sub>
3. N<sub>150</sub>P<sub>150</sub>K<sub>150</sub>
4. N<sub>300</sub>P<sub>150</sub>K<sub>300</sub>

D.composita

1. N<sub>300</sub>P<sub>150</sub>K<sub>150</sub>
2. N<sub>300</sub>P<sub>0</sub>K<sub>300</sub>
3. N<sub>300</sub>P<sub>150</sub>K<sub>300</sub>
4. N<sub>150</sub>P<sub>150</sub>K<sub>150</sub>

Attempts have been made to further analyse the effects of these treatments on patterns of growth and development as well as on patterns of changes of nitrogenous fractions and diosgenin during the course of life history of treated plants. In all the effective treatments with both D.prazeri and D.composita, maximum increase of diosgenin synthesis took place during vegetative stage

of development, the quantum of increase declining later. Increased rate of diosgenin synthesis could be conveniently correlated with increase rate of leaf formation and extension growth particularly in D.composita and such correlative effects remained clear during early stage of development only. It is interesting to note that as a result of fertilizers experiments, rate of leaf formation vis-a-vis diosgenin synthesis had, in general, a greater correlation. Also interesting is the fact that the panicle formation rate could not clearly be linked with rate of diosgenin formation, as in light experiment and in both the species, either a negative or a positive correlation became clear according to treatment variations.

In general dry weight accumulation rate was consistent with diosgenin formation rate in both the species and the effects remained clear till early-reproductive stage of development. It is to be noted, however, in contrast to the behaviour in normal plants, rate of increase of SN remained positively associated with rate of increase of diosgenin synthesis in both the species. This correlation could also be traced till early-reproductive stage. These findings are, in general, confirmatory to the earlier findings of Nandi<sup>67</sup>, Chatterjee et al.<sup>30</sup> and Chatterjee et al.<sup>31</sup>. The above findings will be further corobotated by statistical analysis, where a correlation between some growth and biochemical parameters including diosgenin of both the species of Dioscorea has become evident as follows :-

D.prazeri

	<u>Leaf formation</u>	<u>Dry wt.</u>	<u>SN</u>	<u>Diosgenin</u>
Extension growth	0.77*	0.62	0.53	0.87**
Leaf formation		0.66*	0.60	0.69*
Dry wt.			0.60	0.84**
SN				0.70*

\*\* Significant at 1% level, \* Significant at 5% level

D.composita

	<u>Leaf formation</u>	<u>Dry wt.</u>	<u>SN</u>	<u>Diosgenin</u>
Extension growth	0.83**	0.61	0.48	0.87**
Leaf formation		0.65*	0.60	0.73*
Dry wt.			0.59	0.86**
SN				0.74*

\*\* Significant at 1% level, \* Significant at 5% level

Experiments of Leopold et al.<sup>58</sup> revealed that removal of reproductive units stagger the anabolic events thus delaying the senescence of plants. Gupta<sup>41</sup> indicated that removal of reproductive structures enhanced laminar growth as well as retarded axial growth of medicinally important plant viz. Nicotiana plumbaginifolia where senescence process was sufficiently delayed. Elimination of reproductive phenomenon in medicinal plants like D.prazeri and D.composita and its effect on diosgenin synthesis has not been reported so far and in this respect the present study is a novel one. Systematic removal of reproductive units at different stages

of development and following the patterns of growth behaviour and changes in different functional parameters in two species of Dioscoreas have produced results of interest (described earlier) that merit analytical consideration.

Removal of reproductive structures, which are considered to be as effective mobilizing centres of different metabolites in plants<sup>106</sup>, may be argued to save depletion and likely to modify the developmental patterns and biochemical make-up of experimental plants. It could be seen that early removal of reproductive buds from D.composita and D.prazeri augmented diosgenin synthesis, more pronouncedly in D.composita and associated with this augmentation there occurred an enhancement of extension growth, laminar formation, dry weight accumulation and also SN content. A gradual delay in the time of removal of reproductive units which permitted Dioscorea plants to enter into the reproductive phase rather incompletely, also enhanced diosgenin synthesis but to a lesser extent. The phenomenon also remained concomitantly associated with subdued increase of different functional parameters, described above. In extreme treatments where developing fruits were removed, diosgenin synthesis did increase but to a much lesser degree, which remained associated with increase of SN only and in D.composita, no appreciable effects could be traced. All the above observation would point out to one generalised phenomenon : removal of 'Sink' i.e. developing bud, flowers and fruits would conserve different metabolic events including bioenergetics

leading to diosgenin synthesis, and as a result, both the species exhibited higher synthesis of the complex macromolecule (i.e. diosgenin) that could be related to a well defined patterns of changes of different functional parameters. Statistical analysis of related results will indicate the following :

	<u>D.prazeri</u>		
	<u>Dry wt.</u>	<u>SN</u>	<u>Diosgenin</u>
Extension growth	0.65*	0.49	0.70*
Dry wt.		0.50	0.73*
SN			0.65*

\* Significant at 5% level

	<u>D.composita</u>		
	<u>Dry wt.</u>	<u>SN</u>	<u>Diosgenin</u>
Extension growth	0.60	0.50	0.65*
Dry wt.		0.47	0.70*
SN			0.65*

\* Significant at 5% level

To conclude, experiments with auxin-analogues, light and dark exposures, fertilizers and reproductive manipulation clearly revealed that the effective treatments, yielding higher amount of active principles (i.e. glycosides) remained linked with the correlative control of growth, different developmental phases and endogenous content of certain biochemical parameters. And lastly, the analysis of pattern of developmental growth, changes of

biochemical parameters vis-a-vis style of diosgenin formation did present an intimate relationship between developmental control of diosgenin, in one hand, and an effective biochemical indexing, on the other.