staminate, simple or branched inflorescence, fasciculate-glomarate; fascicles short, stipitate; 2-3 flowered perianth rotate, .5-2 mm broad; male fascicles 15-30 cm long, fertile stamens, 6; in female flowers stigma bifid, Tubers are white, large and deep-rooted.

This Central American species grows well in Peninsular India. This plant does well at Bangalore and in the Union Territory of Goa, Jammu and Jorehat. It is also doing well in the Anaimalai Hills and in Darjeeling Hills.

The rhizomes, commonly known as yams, are the main sources of diosgenin. Diosgenin occurs as rhamno-rhamno glocuside (dioscin) in the root tubers. Diosgenin is the base for several steroidal hormones including sex hormones (testosterone, estradiol and progesteron); Corticosteroids (Cortisone and Prednisolone) and also, an active ingredient in the oral contraceptive pill containing esterogen (ethynyl estradiol and menstranol) and Progesteron.

Experimental Methods

Selected crown portions of tubers weighed 60 gms each (for both the species) were taken and planted in experimental nurseries. After sprouting, the tubers were directly sown in experimental plots during 1st week of May, at spacing

2' x 2' between the rows and 2' X 2' between the plants.

A. Systematic growth data were taken in following lines :-

a) Extension growth : The linear growth of the plants was measured at intervals of 15 days upto the senile stage and data of each plant were collected separately and mean per plant was calculated. Rate of extension growth was calculated as percentage increase or decrease over control during different stages of development.

b) Rate of leaf formation : Number of leaves were counted per plants separately at intervals of 15 days, till new leaves ceased to appear. Data of each plant were collected separately and mean value per plant was calculated. Rate of leaf formation was calculated as percentage increase or decrease over control during different developmental stages.

c) Flowering and Fruiting time : Days required for flower initiation (Flower bud formation) and fruit initiation were recorded seperately for each plant and mean time (days) per plant was calculated.

d) Total number of panicle and fruits : Total number of panicle and fruits were separately recorded regularly at intervals of 15 days for each plant and mean was calculated. Rates of formation of panicle and fruits was calculated as

percentage increase or decrease over control during different stages of development.

e) Dry matter accumulation : Dry matter accumulation of leaves of experimental plants was calculated after random sampling. In each case, 10 leaf discs of 1-cm diameter were taken at intervals of 15 days; dried at 80°C and expressed in mg. Rate of formation of dry wt. was calculated as percentage increase or decrease over control during different stage of development.

The experimental data per plant as described above were recorded systematically at intervals of 15 days and the patterns of their manifestation during different developmental stages viz. vegetative, reproductive and post-reproductive have been expressed in terms of percentage increase or decrease over control during the course of a particular developmental stage; the stages being distinguishable on the basis of absence or presence of different reproductive units. The duration of different developmental stages of two species of Dioscoreas and the relative occurrence of buds/flowers/ fruits during such developmental stages have been tabulated below:

Table 1

Species	Duration of stages (Age-days)					
	Vegetative		Reproductive		Post-reproductive	
	Early	Late	Early	Late	Early	Late
D.prazeri	20-25		65-80	90-110	125-140	155-170
D.composita	20-55	65-80	95-110	125-140	155-170	185-205

Table II

Species	Reproductive units	% occurance of different Reproductive units					
		Vegetative Early	Late	Reproductive Early	Late	Post-reproductive Early	Late
	Buds		X	10	35	x	x
D.prazeri	Flowers		x	90	60	60	5
	Fruits		x	x	5	40	95
	Buds	x	x	10	35	x	x
D.composita	Flowers	x	x	90	65	75	30
	Fruits	x	x	x	x	25	70

B. The following biochemical parameters were estimated :-

a) Chlorophyll level : 100 mg fresh tissues were taken for chlorophyll estimation. The tissues were crushed using pestle morter with acetone and centrifuged. The supernatant was taken and the residue was again centrifuged with the same solvent, volume being made upto 10 ml. The optical density of the acetone extract was measured at 650 mm in a Hilger pattern Biochem-absorptiometer. The amount of chlorophyll was determined following Arnon's[5] formula at intervals of 15-days and later calculated as /ug/mg DW. Chlorophyll content was also expressed on the basis of percentage increase or decrease over control during different developmental stages.

b) Total Nitrogen and Soluble Nitrogen : The total nitrogen (TN) of leaves was measured colorimetrically following the method of Vogel[105] after suitable modifications. 15 gm of dry leaf tissues were used in each estimation and dried tissue-material was digested with 2 ml of cone H_2SO_4 for one hour. 2 ml of H_2O_2 was added after cooling the material to room temperature to bleach the colour. After bleaching, the volume was made upto suitable dilutions, depending on the colour reaction with Nesseler's reagent. 5 ml of alkaline Nesseler's reagent and 1 ml of 10% NaoH and 10% Na_2SiO_3 solution (1:1) mixture were added to a 1 ml of aliquot. Intensity of the colour developed was measured (410 nm) using Hilger pattern Biochem absorptiometer after allowing the mixture to react for 10 minutes. The content of total nitrogen was determined by comparing with standard curve and was expressed in /ug/mg DW.

Soluble nitrogen (SN) was seperately estimated in 100 mg of fresh leaf tissue. 100 mg fresh material was crushed in a morter with 10 ml of distilled water using neutral sand. The crushed material was centrifuged and to the decant, 1 ml of 50% trichloroacetic acid (TCA) was added to precipitate the colloids. It was again centrifuged after allowing to stand overnight in low temperature (4°C) and later decanted. The total decant was digested with 1 ml concentrated H_2SO_4 for one hour. While estimating the soluble nitrogen, the method

described earlier for total nitrogen was followed and SN content was expressed as % dry weight basis (/ug/mg DW). The SN and TN contents were also calculated on the basis of percentage increase or decrease over control during different developmental stages of plants. The SN and TN estimation was done at interval of 15 days.

Preparation of Nesseler's reagent : 125 gm of KI and 110 gm of I_2 were put into one-litre flask. 140 gm metallic Hg was added and was shaken vigorously until the colour of I_2 nearly disappeared and greenish colour of the filtrate was made upto 2 litres. 75 ml of above solution was mixed with 350 ml of 10% NaoH solution.

c) Extraction and estimation of diosgenin : The method of Selvaraj[92] for extraction and estimation of diosgenin was followed after suitable modifications. The tubers were washed with water after chopping the roots and the excess adhering water was removed by drying this material under shade. The tubers made into small pieces and powdered in grinder machine. 20 gms of dried and powdered material was taken in a 500 ml flask with B₇24 joint and 50 ml water was added and shaken for 10 minutes. 150 ml of water was later added and 52.6 ml of conc Hcl was added to maintain the required acid concentration (2.5 Hcl). Hydrolysis of the sample was carried out for three hours and the slurry after hydrolysis was

allowed to attain the room temperature, and filtered. The residue was repeatedly washed with distilled water, till the filtrate was free from acid, The filtered residue was dried in an oven at 100°C for 6 hours. It was extracted with petroleum ether (bp 40° - 60°C) in a Soxhlet, for 8 hours. The extract was concentrated to 25 ml; chilled in ice and filtered. The residual mother liquor, after filtering and washing the diosgenin, was added to the first crop and the whole diosgenin extracted was weighed after drying it in an oven for 2 hours at 100°C. The diosgenin content was estimated regularly at intervals of 15-days and was expressed as % dry weight basis. Presence of diosgenin was also calculated on basis of percentage increase or decrease over control during different developmental stages of plants.

C. Four different sets of studies with D.prazeri and D.composita were conducted :-

a) Experiments with phenoxy compounds.
b) Experiments with different periods of exposure of light and dark hours.
c) Agronomic experiments with N:P:K fertilizers.
d) Experiments on reproductive manipulation.

a) Experiments with phenoxy acids : In order to study the effects of different phenoxy acetic acids viz. Phenoxy

acetic acid (PAA), 4-chlorophenoxy acetic acid (4-Cl), 2,5-dichlorophenoxy acetic acid (2,5-D) and 2,4,6-trichlorophenoxy acetic acid (2,4,6-T), (possessing auxin, weak-auxin and non-auxin properties) on growth, development and diosgenin of active principles, the experimental plants were treated with different growth substances in the following manner :

20 ml of each of PAA, 4-Cl, 2,5-D and 2,4,6-T in two concentrations (10mgl^{-1} and 100 mgl^{-1}) were fed very carefully through leaves when the plants attained six-leaved stage. The treated plants were kept covered with transparent polythene-bag for 6 hours and every day 4 ml of the solution was fed and this process was repeated 5 times, covering a total period of 5 days. The growth and biochemical data and diosgenin estimation of two species were recorded carefully at intervals of 15-days.

b) Experiments with light and dark hours : Experimental seedlings (6-leaved stage) from sprouting tuber pieces of D.prazeri and D.composita were exposed to following different light and dark treatments :

1) Normal control : Experimental seedlings were allowed to grow in normal day length conditions, (i.e. 12 hrs light exposure followed by 12 hrs dark) and the plants were kept till the senescence.

2) Plants with additional light exposures : In these experiments 60 and 120 additional hours of light were administered to normal plants within a total period of 30 days (2 and 4 hours additional light every day) from artificial light source of 100 fc. The positions of pots containing experimental plants were placed under light and interchanged everyday during treatment hours to expose all the plants equally to illumination. After the light treatments were complete, the plants were returned to normal day length conditions and maintained till senescence.

3) Plants with additional dark exposures : In these experiments, additional dark periods were made available to the plants every day for 30 days; one set receiving additional 2 hours and another set receiving additional 4 hours of darkness. After the treatments were complete, the plants were returned to natural day-length conditions and maintained till senescence.

Growth and biochemical data and estimation of diosgenin were done systematically at intervals of 15-days.

c) Agronomic experiments with N:P:K fertilizers : In these experiments nitrogen was applied in the form of ammonium

sulphate (containing 20% N_2); phosphorus in the form of super phosphate (containing 16% P) and potassium in the form of Murate of potash (containing 62% K). Three nitrogen levels (0, 150 and 300 kgs/ha) three phosphorus levels (0, 150 and 300 kgs/ha) and three potassium levels (0, 150 and 300 kgs/ha) were applied in D.prazeri and D.composita. NPK was applied in two equal splits; one split applied as basal dose and the other split applied after 15 days of transplanting as top-dressing. Growth, biochemical and diosgenin data were collected systematically, at intervals of 15 days.

d) Experiments on reproductive manipulation : In order to study the effects of removal of reproductive parts on growth, development and biogenesis of active principles, the plants were undergone systematic debudding, deflowering and defruiting operations. Three sets of experimental treatments were undertaken as follows :

Set I : Debudding : In these experiments, the buds were systematically removed during their initiation. Twenty plants were subjected to each treatment.

Set II: Deflowering : In these experments, plants were allowed to develop buds and systematic removal of flowers (panicles) were done. This operation was continued till the new panicle ceased to appear. Twenty plants were subjected to each treatment.

Set III : Defruiting : In these experiments, fruits were systematically removed. Defruiting operation was followed every alternate day till new fruits ceased to appear. Twenty plants were subjected to each treatment.

Growth and biochemical data and estimation of active principle were done systematically at intervals of 15-days.

All the four categories of studies mentioned earlier involved different experiments which modified the general growth and developmental behaviour of treated plants; thus resulting varying duration of successive developmental stages of experimental plants. Following tables describe these durations :-

Table III

Duration of different developmental stages of D.composita undergoing varying treatments

Treatments	Initiation of flowers (Age-days)	Duration of the active stages (Age-days)					
		Vegetative Early	Vegetative Late	Reproductive Early	Reproductive Late	Post-reproductive Early	Post-reproductive Late
Study I							
Control	100	20-55	65-80	95-100	125-140	155-170	185-205
4-Cl ($10\ mgl^{-1}$)	100	20-55	65-80	95-110	125-140	155-170	185-205

Table III (contd.)

Study I (contd.)							
2,5-D ($10\ mgl^{-1}$)	100	20-55	65-80	95-110	125-140	155-170	185-205
2,4,6-T ($10\ mgl^{-1}$)	100	20-55	65-80	95-110	125-140	155-170	185-205
PAA ($10\ mgl^{-1}$)	100	20-55	65-80	95-110	125-140	155-170	185-205
4-Cl ($100\ mgl^{-1}$)	102	20-57	67-82	97-112	127-142	157-172	187-207
2,5-D ($100\ mgl^{-1}$)	100	20-55	65-80	95-110	125-140	155-170	187-205
2,4,6-T ($100\ mgl^{-1}$)	100	20-55	65-80	95-110	125-140	155-170	187-205
PAA ($100\ mgl^{-1}$)	100	20-55	65-80	95-110	125-140	155-170	187-205
Study II							
60 Hrs.Addl. light	72	20-35	45-55	65-80	95-110	125-140	155-175
120 Hrs.Addl. light	70	23-33	43-53	63-78	93-108	123-138	153-173
60 Hrs.Addl. dark	130	20-55	75-105	120-135	150-165	180-195	210-230
120 Hrs.Addl. dark	100	20-50	65-80	95-110	125-140	155-170	185-200
Study III							
$N_{300}P_{150}K_{300}$	72	20-30	40-50	65-80	95-110	125-140	155-175
$N_{150}P_{150}K_{150}$	90	20-40	50-70	80-100	115-130	145-160	175-195
$N_{300}P_{150}K_{150}$	72	20-30	40-50	65-80	95-110	125-140	155-175
$N_{300}P_{0}K_{300}$	72	20-30	40-50	65-80	95-110	125-140	155-175

Table III (Contd.)

Study IV							
Debudded	100	20-55	65-80	95-110	125-140	155-170	185-205
Deflowered	100	20-55	65-80	95-110	125-140	155-170	185-205
Defruited	100	20-55	65-80	95-110	125-140	155-170	185-205

Table IV

Duration of different developmental stages of D.prazeri undergoing varying treatments

Treatments	Initiation of flowers (Age-days)	Duration of the active stages (Age-days)				
		Vegetative	Reproductive Early	Reproductive Late	Post-reproductive Early	Post-reproductive Late
Study I						
Control	70	20-50	65-80	95-110	125-140	155-170
4-Cl ($10\ mgl^{-1}$)	70	20-50	65-80	95-110	125-140	155-170
2,5-D ($10\ mgl^{-1}$)	69	20-49	64-79	94-109	124-139	154-170
2,4,6-T ($10\ mgl^{-1}$)	70	20-50	65-80	95-110	125-140	155-170
PAA ($10\ mgl^{-1}$)	70	20-50	65-80	95-110	125-140	155-170
4-Cl ($100\ mgl^{-1}$)	70	20-50	65-80	95-110	125-140	155-170
2,5-D ($100\ mgl^{-1}$)	70	20-50	65-80	95-110	125-140	155-170
2,4,6-T ($100\ mgl^{-1}$)	70	20-50	65-80	95-110	125-140	155-170
PAA ($100\ mgl^{-1}$)	70	20-50	65-80	95-110	125-140	155-170

Table IV (Contd.)

Study II						
60Hrs. Addl light	60	20-40	55-70	85-100	115-130	145-165
120 Hrs.Addl. light	58	20-38	53-68	83-98	113-198	143-163
60 Hrs.Addl. dark	100	20-80	95-110	125-140	155-170	185.205
120 Hrs.Addl. dark	103	23-83	98-113	128-143	158-173	188-208
Study III						
$N_{300}P_0K_{300}$	70	20-50	65-80	95-110	125-140	155-175
$N_{150}P_{150}K_{300}$	90	20-65	80-95	110-125	140-155	170-190
$N_{150}P_{150}K_{150}$	80	20-60	75-90	105-120	135-150	165-185
$N_{300}P_{150}K_{300}$	68	20-48	63-78	93-108	123-138	153-193
Study IV						
Debudded	70	20-50	65-80	95-110	125-140	155-170
Deflowered	70	20-50	65-80	95-110	125-140	155-170
Defruited	70	20-50	65-80	95-110	125-140	155-170

The results of our experiments were evaluated statistically. Statistical significance was calculated at 1% and 5% level and multiple correlation of various parameters viz. extension growth, radial growth (leaf formation) SN, TN and dry weight alongwith diosgenin was determined following Snedecor[98] multiple correlation formula.