CHAPTER–I

REVIEW OF LITERATURE

Review of Literature on *Agave* species.

1. Introduction

Agave is one of the major fibre crops. The Leaves of various *Agave* species viz., *Agave americana*, *A. sisalana*, *A. cantala*, *A. veracruz*, *A. fourcroydes*, *A. amaniensis*, *A. angustifolia*, etc., yield the fibre. This fibre is popular in the market under the name of sisal fibre, though true sisal fibre denotes only *Agave sisalana*.

Agave can be cultivated under wide agro-climatic conditions. The most important merit of this hardy crop is that it can be grown profitably in such areas which are unsuitable for other crops. Unlike other crops *Agave* is perennial in nature with a life span of 10-12 years. The plant is monocarpic i.e. flowering is followed by death. The plant multiplies mainly by bulbils and suckers.

Agave fibre is among the hard fibres. The fibre is long, bold; creamy white and strong. These characteristics benefit it for making various useful materials. The most important uses of the fibre are the manufacture of marine and industrial ropes, agricultural and commercial twines, other forms of cordage and cables in addition to bags, sacs, carpets, fishing nets, rugs, various types of brushes and brooms, etc. Sisal was obtained from sisal pulp is used for shoe and car polishes and

manufacture of carbon paper. Further, the juice of leavesis an important source of hecogenin.

Thus it is clear that Agave is economically very important plant and further it can be cultivated in environments not suitable for most of the economically important species. Hence it is very essential to have brief knowledge of various aspects of this plant species.

2. History of Agave

The Agaves are natives of South and Central America from where these were introduced in large numbers to other couhtries (Chakravarty and Biswas, 1986). Agave growing main countries are Tanzania, Kenya, Mozambique, Brazil, Angola, Madagascar, Haiti. Prior to 1941 Indonesia was a major exporter of sisal but since its industry has declined, It is now virtually nothing (Lock, 1969). According to him minor sisal growing countries are South Africa, Comoro Islands,Uganda and Malawi in addition to Venezuela, Jamaica, Dominica, and China. Similarly experimental planting of sisal has been tried in Moroccos, Israel, Rhodesia, Congo Republic, Ghana, the Ivory Coast Republic, Trinidad, Mauritius, Papua and New Guinea. Sisal also exists in Libya, India, Burma, Malasiya,Queensland, Western Australia, Figi and Hawaii.

The Agaves were introduced to India in the fifteenth century by the Portuguese and have since been so acclimatised

and naturalised to the Indian soil that they can be observed growing scattered (and as hedge plants) in different soil and under diverse climatic conditions(Chakravarty and Biswas,1986).

3. Taxonomic Position :

Formerly agaves were included in family Liliaceae.Lateron the agaves were used to be included among the Amaryllidaceae. But more recently agaves have been included in to a new family Agavaceae (dewit, 1965). However, the validity of this classification, and particularly the delimitation of the individual families, continues to be a subject of controversy among taxonomists.

The members of Amaryllidaceae have umbellate inflorescences, subtended by an involucre of two or more (rarely only one), usually membranous bracts. But agaves differ from these not only in having rosettes with fleshy leaves but also in the structure of their inflorescences : agaves have their flowers in tall panicles (dewit, 1965).

Some of the important species of genus Agave are as follows (Usher, 1971) :

(i) Agave americana L (century plant) : originated in Mexico, now widely cultivated as an ornamental plant in N. America, Africa and Europe.

(ii) Ag atrovirens Karw syn.A latissima Jocobi:from Mexico.

(iii) A. cantala (Haw.) Roxb.(Cantula) : Cultivated in India. The fibre is used for cordage and is called Bombay Aloe fibre, Bombay Hemp, Cantala fibre.

(iv) A. complicata Trel : from Mexico.

(v) A. deserti Engelm.: Cultivated in S.W.United States of America and N. Mexico.

(vi) A. falcata Engelm : Mexico.

(vii) A. fourcroydes Lemaire (Henequen Agave) : Mexico

(viii) A. funkiana Koch & Bouche : Mexico.

(ix) A. gracilispina Engelm : Mexico.

(x) A. heteracantha Zucc. Syn. A.lechuguilla Torr.: United States of America, Mexico.

(xi) A. kircheneriana Berger.

(xii) A. lespinassei Trel = A.sapupe. Trel. (Zapupe Azul, Zapupe Estopier): Mexico.

(xiii) A. letonae F.W. Taylor (letona): C. America.

(xiv) A. lophantha Schiede : Mexico.

(xv) A. mapisaga Trel. : Mexico.

(xvi) A. melliflua Trel. Mexico.

(xvii) A. palmaris Trel syn. A. tequilana. Weber (Chino, Azul, Mescal) : Mexico.

(xviii) A.pesmulae Trel. Syn A. tequilana. Weber syn. A. pseudo-tequilana Trel

(xix) A. quiotifera Trel. (Maguey ceniso) : Mexico.

(xx) A. schotti Engelm. (Amole) : Arizona.

(xxi) A. sisalana Perrine Syn. A. rigida Mill (Sisal Agave) : Mexico.

(xxii) A. utahensis Engelm. : S.W. United States of America.

(xxiii) A. victoriae-reginae Moore : S.E.United States of America.

(xxiv) A. virginica L. (False Aloe) : E. United States of America.

(xxv) A. weberi Celo. (Maguaey liso) : Mexico.

4. Distribution in India :

Agaves were introduced in to India in the 15th century by Portuguese and now they are completely naturalised throughout the country (C.S.I.R.,1948; Chakravarty and Biswas,1986). In India the species Agave-americana; A. amaniensis, A. angustifolia, A. cantala, A. fourcroydes, A. sisalana, A. veracruz and A. wightii are found presently (Chakravarty and Biswas, 1986). According to them out of these species A. sisalana, A. cantala and A. veracruz were found to have established commercially in the country. But A. sisalana became more popular because of its superior fibre quality and higher fibre productivity hence found to spread throughout the country. A. veracruz is mainly grown in the drier belts of Andhra Pradesh and Maharashtra, as this species is highly tolerant to moisture stress condition. A cantala is found in West Bengal, Bihar, Maharashtra and Orissa.

At present there are about 5,000 hectares under sisal plantation in the country. The statewise areas are more or less as follows (Chakravarty and Biswas, 1986) :

State	Area (in hectares)
Orissa	2,505
Bihar	280
Andhra Pradesh	752
West Bengal	560
Maharashtra	726
Madya Pradesh	147
Karnataka	84
Total	5,054

Presently, the country's annual production of Agave fibre is only around 3,000 tonnes from an area of about 5,000 hectares spread over mainly in Orissa, Bihar, Andhra Pradesh, West Bengal, Maharashtra, Madhya Pradesh and Karnataka, though the requirement of country is 16,000 tonnes as assessed by different committees (Chakravarty and Biswas, 1986). In fact, the actual yield is less because proper attention is not still paid to the crop.

5. Morphology :

Agave is a large 'genus' of short stemmed, half woody plants, bearing a rosette of leaves (Fig. 1). Most agaves are devoid of stems and bear their leaf rosettes close to the

Fig.1 Plants of Agave cantala Roxb.

ground. Thus the agaves are the plants which appear to consist of nothing more than a cluster or whorl of leaves springing from ground level like a rosette. The leaves are, however, crowded densely around a thick sappy stem, which is short, single and unbranched and is completely hidden by the leaves (Aiyer, 1966).

The leaves are thick, fleshy, long, narrow with a spiny tip and often a more or less spiny margin. The leaves are flat or with a deep central groove running on the upper surface from base to apex. The leaves are very strong, and are from about 90-150 cm. or even 180 cm long and about 10-15 cm wide. The width is more or less uniform throughout and narrows only towards the pointed tip. The leaves are smooth and glaucous, green or bluish with an ashy grey bloom on the surface (Aiyer, 1966).

The agave plant flowers only once in its life time and dies, not after 100 years, as its popular name of "century plant" implies; but after only 10-15 years (i.e. monocorpic). Once the inflorescence appears, the rosettes stop growing, to wither completely after fruit formation. However, many agaves form secondary rosettes at the base or on runners (dewit, 1965).

Aiyer (1966) stated that the flowering stem arises from the centre of the plant in the form of a thick pole, which rapidly grows to a height of 6-9 m. The flowers are

borne on a pole like scape in fascicles. The perianth is 6-lobed and funnel shaped, the stamens are 6 in number, the stigma is 3-lobed and the ovary is 3-celled and inferior. Fruits are normally formed and contain numerous black flattened seeds. Frequently, however, floral buds get developed into bulbils or plantlets.

6. Propagation and Planting

(a) Propagation : The agaves are propagated by means of (1) suckers, (2) Bulbils and (3) Seedlings.

(1) Suckers : The well grown up plants produce root suckers from about their third year and continue to do so for 2 to 3 years thereafter, each plant yielding some 20 suckers in this period (Aiyer, 1966).

(2) Bulbils : The old plants when they are about; ten to twelve years old begin to flower. The flower stalk called 'pole' is stout and mast like structure measuring about 12-15 cm in diameter at the base and about 6-9 m, in height. The pole produces lateral branches developing bunches of "bulbils" or "small plantlets" which can be removed and used as planting material (Aiyer, 1966).

After the "poling" the parent plant begins to die down but suckers are sent out from the root, which also provide planting material.

(3) Seedlings : Rarely the agaves are propagated by means of seeds producing seedlings. Establishment of seedlings of Agave deserti in the Sonoran desert is a rare event. Only one seed in 1.2 million apparently leads to a mature plant and young seedlings are difficult to find (Gentry, 1972; Nobel, 1977). According to Jordan and Nobel (1979) survival of seedlings of A. deserti, required unusually wet years and the protection afforded by nurse plants or other shelters and water stress in the seedling stage may be the most important factor affecting establishment.

(b) Planting : A large number of agaves are cultivated as indoor ornaments, including A. americana which is now completely naturalised in the Mediterranean region. A.cantala,A.sisalana and A. veracruz occur in India. They are planted along railway embankments and road sides and are suitable for hedging and fencing. They may also be planted for checking soil erosion.

The suckers and "bulbils" are generally planted in a nursery and reared therein for a year before they are put down in the plantation. According to Lock (1969) "bulbils" are preferred to suckers for planting because the plants obtained from "bulbils" give comparatively more yield than that of suckers.

The "bulbils" are very small; being only about 5-15 cm in height have to be grown in nursery till they are at least 30 cm in height before transplanting. When bulbils are planted

directly into a field, they grow very unevenly and a big proportion die. Therefore bulbil nurseries are indispensable to obtain best planting material, and good transplants are required to establish satisfactory sisal fields. The soil should be a well-aerated, sandy for a nursery (Lock,1969).

Bulbils are collected by shaking the pole and gathering them after they have fallen to the ground. After collection, bulbils should be left in small heaps under shade to avoid spoiling in sunlight caused by sweating in the heap. Large bulbils are selected while those smaller than 10 cm. are discarded. Rows are marked out for planting. A tagged chain can be used for planting up the rows and a short dibbing-stick can be used for placing the bulbils in the ground. Nurseries should be prepared well ahead of the main rains. Late planted nurseries are never a success, and they frequently become droughted after the rains have ended. The spacing should be wide enough. The spacing used should be 50 X 25 cm and the population of plants should not exceed 80,000 per hectare. The wide spacing is much beneficial which aids in yield (Lock, 1969).

After one year the plants are put in their permanent places during transplantation in the pits. The pits are about 30 cm square and 30 cm in depth. The pits are made at distances of 3 m to 3.5 m each way. Distances of about 2 m each way have been adopted in certain plantings, but this has been found later on invariably too small for inter-cultivation and

convenient handling. The wider planting is found the best in the long run. Exceptionally when planted as hedge plants they are planted on raised ridges or mounds of earth at distances of about 1 m from each other in a line or even closer to check erosion (Lock, 1969).

Transplanting is done in the months of August and September in Africa when the ground is soft enough to dig after the rains of south-west monsoon and the young plants have the advantage of the later or north-east monsoon rains to become sufficiently well established to withstand the following hot weather. After transplanting the interspaces have to be kept weeded or cleared of the larger bushgrowth. In large plantations where the plants are put in wide, at intervals of 3.5 m, the interspaces are also ploughed and minor crops of groundnut, cowpeas and so on, raised during the first two years (Aiyer, 1966).

During the fourth year, the plants are old enough to yield leaves which can be cut. A large number of root suckers also come up and these have to be removed systematically, if this is neglected the interspaces may become covered with suckers coming from all around the parent plants. About 20-30 leaves can be cut in the first year of harvest from each plant and this number will increase as the plants grow older. The lowest leaves should be cut when these leaves are slanting more than 75^{o} from the vertical. Those at right

angles and those droop are more mature. The harvesting of leaves usually begins in the month of January and goes on till March or April (Aiyer, 1966).

The agaves go on yielding for about 10-12 years under normal conditions and then begin to flower or "pole" after which they die down. However, many causes bring about premature "poling" and such plants become useless long before their time. Following are some of the causes (Aiyer, 1966; Lock, 1969).

(a) The cutting of a large number of leaves at a young age and consequent weakening of the plants is considered one of the causes.

(b) The non-removal of root suckers for a long time after they have appeared is also stated to accelerate "poling."

(c) It is also believed that the plants raised from bulbils are more likely to "pole" prematurely than plants raised from root suckers.

(d) Lack of sufficient time, wet conditions and want of aeration in the soil are also considered causes.

(e) Peculiar conditions of rain fall and weather are probably more responsible, as the "poling" by a few plants is often accompanied by a somewhat widespread "poling" making it appear the result of wether conditions. The subject is certainly obscure.

Ecological factors affecting growth :

(a) Rain fall : As agaves can withstand droughts when most plants would perish, this thing sometimes gives rise to misconception that agaves need low rainfall. Actually, the xerophytic nature of agaves is totally unconnected with the way they grow. Sisal responds to favourable growing conditions like most plants.

The optimum rainfall for sisal is between 1,200 to 1,800 mm (50 to 70 inch). Sisal is also grown in many places where the rainfall is less than 760 mm (30 inch), or may be erratic, but its growth is affected reducing annual fibre yields thereby. If the precipitation exceeds 2,000 mm (80 inch), it results in waterlogging of soils which prevents proper root formation so that the plants are stunted.

(b) Temperature : Maximum temperatures should preferably range between 27°C to 32°C. While minimum temperatures should not fall below 16°C. The diurnal range should not be more than 7° to 10°C. Agaves can withstand high and low atmospheric temperature but their growth is slowing down; as a result the life cycle of the agaves is usually prolonged.

(c) Light : Agaves need plenty of light and they prefer strong sunshine. Shade is conducive to flaccid waxless leaves with a poor fibre content; the fibres are also fine and weak. Day length is not considered to have much effect upon sisal.

(d) Soils : Agaves are tolerant of a wide variety of soils. Soils should be friable, freely draining and not too acid or low in nutrients. Both waterlogging and salinity are fatal to agaves. According to El-Gamassy et al. (1974) the increase in salinity levels of soils reduce the fresh and dry weights of the shoot in Agave sisalana.

7. Pests and Diseases

The agaves are not subject to any serious pests. The shoot is sometimes attacked and bored through by a beetle resembling the Rhinocers beetle. When the shoot grows and the leaves unfold, the beetles are found to be cut through by holes about an inch in diameter.

Leaf spots are found, due to a fungus disease. These spots or patches widen gradually, become black in colour, dry up and form a hole on the leaf. This disease can be controlled by spraying with Bordeaux mixture.

8. Economic Importance

A) Fibres : Botanically speaking fibres refer to wood fibres. Wood fibre is the mass of wood cells, in tapering form having very thick walls and simple pits in the secondary wall (Schery, 1954). According to him fibres are composed of mainly cellulose, with lignins, hemicelluse, and occasionally other substances from chemical point of view.

The fibres of agaves are obtained from leaves. The

agave fibre is one of the important hard fibres, e.g.,sisal hemp, which is obtained from the leaves of Agave sisalana (dewit, 1965). The other different species of Agave yielding fibres, reported by Usher (1971) are Agave americana; A. cantala, A. falcata, A. fourcroydes, A. funkiana, A. gracilispina, A. lechuguilla, A. kircheneriana, A. letonae, A. lophantha, A. victoriae-reginae, A. zapupe, etc.

I) Extraction of Fibres :

The fibres of agave are extracted from the leaves by two methods (Aiyer, 1966), dry and the wet. The aim in both methods is to separate the long strands of the fibre from the soft pulpy tissue in which it is embeded.

(i) Dry Method

Dry method is purely mechanical. In this method after cutting the leaves; the tip and the thorny margins of the leaves are removed, the leaves are passed between a hard board and the blunt blade of a knife which is pressed on the leaf surface to the degree required. This process is repeated until the fibres are completely separated from the adhering tissue. The fibres are washed to get the fibres free from further adhering matter.

(ii) Wet Method :

In this method after cutting the leaves are put up in the bundles either whole or split in to long strips and kept

immersed in water, being at the same time weighed down with a heavy stone. Thus the leaves are allowed to ret in water. The retting of leaves helps to disintegrate soft tissues and gummy matters by chemical and bacterial action; as a result, the fibres are separated easily. After twenty days the leaves are ready to be taken out.

Then the leaves are beaten on a stone, scrapped and washed and the fibres separated from both the soft tissue and the tough epidermal layer. This method is very much slower but at the same time it is much less costly.

The colour of the fibre is dull and is much inferior to the fibre made by the dry process, which yields a white and brilliant fibre. The fibre yielded by dry method is about 3 to 3.5% of the green weight of the leaf, while that by wet method is about 4 to 6 % (Aiyer, 1960).

II) Properties and Uses of fibres :

Aiyer (1966) has given following properties and uses of agave fibres.

a) Properties :

1) The fibres of agave is very white and brilliant in colour when prepared properly by the dry method.

2) The fibres are from 90 to 150 cm in length ordinary, but when the plants are not well grown may go down to 60 cm.

3) The fibre made by retting in water is somewhat dull in colour, but is generally longer than in the dry method.

4) Incontrast to other fibres, the agave is clean and free from soft fibres and tow to a remarkable extent.

5) The cordage made from agave fibres can not stand usage under sea-water, nor any prolonged usage under even fresh-water.

b) Uses :

1) The most important use of sisal fibre is manufacturing of binder twine, which is used in binding the sheaves in the self-binder harvesting machines which hot only cut the grain crop but also deliver it tied up in to sheaves of uniform size, as the machine moves along the field.

2) The ropes made from agave fibres are strong and also last a long time and are in great demand for many, agricultural purposes, marine and industrial ropes.

3) Agave fibres are used for the making of door rugs, bags, sacks, soles for sandles, cordage, cables, carpets, fishing nets, hammocks, and various types of brushes and brooms.

4) The fibre can also be used in the manufacture of flag and corrugated polyester sheets reinforced which is stronger and cheaper than the comparable sheets of other materials.

B) Waxes : Sisal wax recovered from sisal pulp can replace carnoba wax used for shoe and car polishes and manufacture of

carbon paper (Chakravarty and Biswas, 1986).

C) Food Stuffs : The inner stem of agaves is edible. During the times of famine the poor people boil and eat it. It is favourite food for pigs also (Aiyer, 1966). Usher (1971) noted that the leaf bases of Agave deserti and A. utahensis are roasted and eaten by local Indians, similarly the flower stalks are chewed like sugarcane.

D) Alcoholic drinks :

(i) Pulque : It is a fermented beverage made from the juice of leaves of various species. Agave atrovirens (Hill, 1952); A. lehmannii (dewit, 1965); A. complicata (dewit,1965; Usher, 1971); and A.gracilispina, A. mapisaga etc.(Usher,1971).

(ii) Aquamiel : It is also fermented beverage made from the juice of flower stalks of various species such A. complicata, A. weberi and A. quiotifera (Usher,1971).

(iii) Mescal : It is distilled from the fermented stems and leaf bases of various species. According to Usher (1971) these species are Agave atrovirens, A. kircheneriana, A. tequilana etc.

(iv) Tequila : It is alcoholic beverage obtained from Agave tequilana (Usher, 1971 and Kluge & Tinge, 1978).

E) Medicinal Importance :

It has been noted by Usher (1971) that an extract of

the roots of Agave virginica was used by the Indians to cure stomach complaints.

F) Chemical Importance :

i) According to Usher (1971) Agave schotti can be used as a soap substitute.

ii) Usher (1971) noted that the drug 'mecogenin' occurs in the leaves of several species and it can be converted into cortisone.

iii) According to Chakravary and Biswas (1986) the juice of leaves is an important source of 'hecogenin' which is the starting material for the production of corticosteroids which are used as highly valued anti-inflammatory drugs having a world wide market. Hecogenin is obtained only from the juice of Agave veracruz leaves because the juice of this leaves contains hecogenin in an almost pure form.

iv) Lin (1984) observed that the leaves of sisal (Agave sisalana) and its fibre residue contain 5-9% crude protein.

9. Cytology :

Cytological work by Doughty (1936) has established that the basic chromosome or haploid number of the genus Agave is n = 30. However, there are some species that have more than two sets of chromosomes and these are known as polyploids.

Doughty found that Agave sisalana has about 138 chromosomes, or approximately five times the basic number of chromosomes for the genus, and therefore it ranks as a pentaploid (5n). This is responsible for the very wide variation in the characters of sisal plants raised from seed which exceeds that for the diploid (2n) species of Agave. Other Agave species are triploids (3n), or tetraploids (4n). Besides differing in number, the chromosomes may be either long or short. Examination of the main species of Agave carried out by Doughty (1936) resulted in counts of long and short chromosomes as shown in table :

Chromosome Number and Form

Species	Number 2n	Form		Polyploidy
		long	short	
A. sisalana	138	24	114	5n
A. fourcroydes	140	24	116	5n
A. cantala	90	15	75	3n
A. amaniensis	60	10	50	2n
A. angustifolia	60	10	50	2n

10. Embryological Studies :

Schlimbach (1924) has described the embryosac formation in Agave chloracantha and A. attenuata which is of polygonum

type. Cantalano (1930) has given the account of megasporogenesis in A. sisalana. Grove (1941) and Regan (1941) studied the megagametophyte development in A. lechuguilla and A.virginica respectively. Lloyd (1970) has studied megagametophyte in A. parryi. Shirke and Mahabale (1978) have studied embryology of A. wightii and A. cantala.

Shirke (1984) studied microsporogenesis and megasporogenesis of A. angustifolia. According to him the anther is tetrasporangiate; endothecium is 2-3 layered; the pollen grains are smooth, monoporate, 60% of them being sterile. His studies also indicated that ovules are anatropous, bitegmic and crassinucellate. The chalazal megaspore cell is functional.

Shirke and Mahabale (1985) studied in detail embryology of Agave wightii. Their observations on microsporogenesis, megasporogenesis and structure of mature embryo of A. wightii suggest that the anther is tetrasporangiate, endothecium develops fibrous thickenings, the tapetum is of secretory type, pollen grains are smooth surfaced, monoporate and 70% of them are sterile, the chromosome number being n = 90. Their studies also indicate that ovules are anatropous, bitegmic and crassinucellate. The chalzal megaspore cell is functional. Development of female gametophyte conforms to the monosporic, eight-nucleate Polygonum type.

11. Anatomical Studies

Gopal et al. (1981) studied epidermal structure and

histochemistry of 5 succulent monocot plants (Aloe vera, Sansevieria roxburghiana, Agave veracruz, Furecraea gigantea and Tradescantia sp.). They observed that above all plant except Tradescantia sp. show similarities in several epidermal characters i.e. presence of a thick cuticle, sunken stomata, low stomatal frequency and mostly closed stomata during the day. Most of the epidermal cell types have a preponderance of lipids over starch protein and insoluble polysaccharides.

Critical study of fibre development of the genus Agave is not found in records. Existence of two types of fibres (long and short) in Agave has been recorded by Fahan (1967). Datta (1971) studied karyological anatomy of fibre development in the leaves of Agave americana L. var. marginata alba Trel. From a study of 100 metaphase plates in root-tip squashes and 75 fibre-cells in leafbase squashes, it is found that the chromosome number; although having an irregular type of variation commonly tend to occur as multiples of 15, the halloid number. In developing fibre-cells the tendency of complete endomitosis (resulting in 2n, 4n and 8n numbers) is stronger than the partial endomitosis (resulting in 5n, 6n, 7n, etc.). Nuclear volumes of fibre-cells also roughly correspond to 2C, 4C, 8C. He concluded that the development and differentiation of fibres involve complete and partial endomitosis, accompanied by somatic non-disjunction phenomena.

A study of the fibre development in leaf of A. americana

var. marginata alba by Datta (1973) revealed two loci of first formation of tracheary elements, one continuous with the older axial bundles and the other slightly above the leafbase.Phloem development seemed to be related to xylem. Fibres developed from the protophloem as well as from the ground tissue. There were many evidences of apical intrusive growth of long fibres. Short crystalliferons fibres showed no such growth.Wattendorff (1976) has studied the leaf anatomy and found six sided raphides with laminated sheath in Agave americana.

12. Physiological Studies

(i) Growth

Agave belongs to a category of Crassulacean Acid Metabolism (CAM) plants that have relatively low growth rate, which is probably due to the fact that CAM is more a survival mechanism. It has been observed by Black (1973) that the maximum growth rate (g dry wt. dm^{-2} d^{-1}) in CAM plants is 0.015 to 0.018. According to Kluge and Ting (1978) among CAM plants like pineapple, has maximum growth rate e.g. the yield varied from 8.94 to 0.81 tons ha^{-1} $month^{-1}$ (Bartholomew and Kadzimin, 1977). These workers further compared that Agave under desert rainfall conditions has only 8% of growth rates of pineapple.

(ii) Crassulacean Acid Metabolism:

Perhaps the most studied physiological aspect in Agave

species is Crassulacean Acid Metabolism (CAM). Since first report of occurrence of dark carbon dioxide fixation in Aloe by De Saussure in 1804; considerable advances have been made regarding the operation and significance of this metabolic pathway. It is now very well established that these plants have an ability to close the stomata during day time and to fix carbon dioxide during night hours; thus checking the rate of transpiration. The key enzyme of carboxylation in these plants is Phosphoenolpyruvate Carboxylase (PEP-Carboxylase) and the malate is the first stable product of carbon dioxide fixation during dark. The malic acid accumulates in the vacuoles during dark hours. During day time malic acid is decarboxylated and CO_2 enters the reductive pentose phosphate cycle. As a result of this peculiar type of metabolic hebaviour, well defined diurnal fluctuations in organic acid and carbohydrate contents, occur in CAM plants. The CAM is now recognised as a special kind of physiological adaptation to cope with arid environment. Agave is a plant growing luxuriantly in desert areas due to the presence of CAM in this plant.

The stomatal behaviour is an important feature of CAM plants and the first report about opening of stomata at night in Agave was made independently by Neales et al., (1968) and Ehrler (1969). Nobel (1976) extended these findings by investigating the water relations of Agave deserti under field conditions. The shallow root system of A. deserti (the mean root depth for the numerous fibrous roots was only

8 cm for medium sized plants with an average of 29 leaves) enabled a relatively rapid response to rainfall, and the succulent nature of the leaves allowed stomatal opening to continue for up to 8 days after the plants became unable to extract water from a drying soil. The water-use efficiency (mass of carbon dioxide fixed/mass of water transpired) had the extremely high values of 0.056 for a winter day and 0.040 for an entire year for Agave deserti, compared with 0.019 to 0.029 for other CAM plants and 0.002 to 0.005 for C_3 and C_4 plants with day-time carbon dioxide uptake (Neales et al., 1968; Ehrler, 1969; Ting and Szarek, 1975; Nobel, 1976).

Besides these two species (Agave americana and A. deserti) CAM has been shown to operate in other species of Agave also namely A. lechuguilla (Eickmeier, 1976); A. utahensis (Nobel and Hartsock, 1981); A. virginica (Martin et al., 1982); and A. fourcroydes (Nobel, 1985). According to Neales (1975) there can be regarded two categories of CAM plants, weak CAM plants and full CAM plants or super CAM plants. He further explains that the type of gas exchange patterns i.e. most typical of CAM plants where dark assimilation of carbon dioxide contributes significantly to the carbon balance of plants. According to him the full CAM pattern is shown quite typically in A. americana plant.

Isotope discrimination ratios (δC^{13}) have provided an useful technique for the separation of C_4, C_3 and CAM species on the basis of their carboxylation reactions though

variation in $\delta\ C^{13}$ values can occur within plants and in aquatic environments (Troughton, 1971 and 1979). This ratio arises from the fractionation of carbon isotopes during carboxylation and is caused by preferential utilization of $^{12}CO_2$ and partial exclusion of $^{13}CO_2$ by the plant. A greater discrimination in C_3 plants results in lower ratios than for C_4 plants, while the variability in ^{13}C ratios in CAM plants is evidence for a shift between C_4 and C_3 photosynthesis according to environmental conditions. Thus in C_3 plants $\delta\ C^{13}$ values range from -22 to -34, in C_4 plants -11 to -19 while in CAM plants they are in the range of -13 to -34. Neales (1975) reported that in Agave americana $\delta\ C^{13}$ value ranges from -14.2 to -14.5. In case of A. virginica (Martin et al., 1982) it has been reported that the $\delta\ C^{13}$ value was -16.

Kluge and Ting (1978) have shown that the succulent plants have fewer stomata distributed over exposed surfaces than most mesophytic non-succulent plants. This is quite understandable in view of drought resistance potential of these plants. They observed that the number of stomata/cm^2 on abaxial leaf surfaces of A. americana and A. deserti were 2,100 and 2,000 respectively. The number of stomata/cm^2 on adaxial leaf surface of A. americana and A. deserti were 2,100 and 1,800 respectively.

Generally in most of the CAM plants the product of dark carbon assimilation is malic acid. Besides malic acid in some

other CAM species the organic acids like isocitric and citric acid also accumulate (Pucher et al., 1949; Vickery, 1954 a and b). An interesting observation has been made by Nordal and Ogner (1964) and Bernatek et al. (1963) in Agave americana this respect. In A. americana, they identified piscidic acid (p-hydroxybenzyl-tartaric acid, a dicarboxylic aromatic acid) which is also known in the Leguminosae and Liliaceae (Nordal and Ogner, 1964). They estimated that piscidic acid is composed of approximately half of the accumulated organic acids in Agave. However, no further attempts have been made to study the biosynthesis of this organic acid.

The enzymological studies in CAM species have been mainly performed on the species such as Kalanchoe and Sedum and there have been very few attempts to study enzymes of CAM in Agave species. Sanada and Nishida (1982) have demonstrated the occurrence of enzyme pyruvate orthophosphate dikinase in 14 CAM species. Their work indicated that the leaves of A. americana contain appreciable activity of this enzyme which is responsible for conversion of pyruvate to phosphoenol pyruvate (PEP).

The levels of pyruvate pi dikinase, NADP malic enzyme, NAD malic enzyme and phosphoenolpyruvate carboxykinase have been estimated in 10 different CAM plants including A.americana (Reddy, et al., 1982). According to them the species utilizing NADP malic enzyme and NAD malic enzyme to decarboxylate malate

possessed more pyruvate pi dikinase and this enzyme was minimal in species utilising PEP carboxykinase for decarboxylation.

CAM behaviour in Agave is influenced by number of factors; the temperature has been recognised to be one of the most important factors in this respect. Neales (1973) reported that the accumulation of titrable acidity in the leaves of Agave americana is decreased by high night temperature and favoured by low night temperature. He further observed that the high night temperatures decrease or eliminate the nocturnal assimilation of CO_2. Thus Agave americana responds to high night temperature by nocturnal stomatal closing and some day opening, whereas the usual pattern with cool nights and warm days is night opening and day closing. Eickmeier and Adams (1978) observed that high night temperature (35°C) eliminated hight CO_2 uptake, reduced net 24 hour uptake, was associated with minimal night acidification and dealta acidity values, and increased both night and 24 hour transpiration, in A. lechuguilla.

Nobel (1985) studied the influence of temperature on CAM behaviour in A. fourcroydes. He observed that net CO_2 uptake over a 24 hour period was maximal near day/night air temperatures of 30°C/20°C. As the day/night air temperatures were raised or lowered, the daily net CO_2 uptake decreased, e.g., it decreased 34% at 14°C/4°C and 97% at 46°C/36°C. As the day/night air temperatures were lowered. daytime CO_2

uptake increased and also became proportionally more important, e.g., daytime CO_2 uptake contributed 16% to total daily uptake at 30°C/20°C and 33% at 14°C/4°C. The work of Nobel and Hartsock (1981) indicated that there exists an inter-specific difference in Agave in this response. They observed that when the day/night air temperatures were raised from 10°C/10°C to 30°C/30°C, the optimal temperature for nocturnal CO_2 uptake by six species of cacti and three species of agaves shifted from an average of 12°C to an average of 28°C for Agave americana, the net CO_2 uptake was 66% higher for day/night air temperatures of 30°C/30°C than for 10°C/10°C, but it was 43% lower for A. deserti at the higher temperature and 65% lower for A. utahensis. The shift in optimal temperature was about 3°C for A. deserti, 7°C for A. americana and 9°C for A. utahensis. Nobel and Hartsock (1981) have earlier studied shifts in the optimal temperature for nocturnal CO_2 uptake caused by changes in growth temperature for agaves. The optimal temperatures for the three species of Agave have been reported by them as follows :

	Species	Optimal temperature for net CO_2 uptake (°C)	
		10°C/10°C	30°C/30°C
(i)	Agave americana	11.6	18.6
(ii)	A. deserti	15.2	17.8
(iii)	A. utahensis	10.4	19.8

They further concluded that A. utahensis had much lower nocturnal CO_2 uptake rates at day/night air temperatures of $30^{o}C/30^{o}C$ than $10^{o}C/10^{o}C$, while A. americana did much better at the higher temperatures and A. deserti had an intermediate pattern.

Kristen (1969) studied the relationship between CO_2 gas exchange and the air passage in Agave americana. He observed that the diurnal course of the air passage within leaves of A. americana as well as the CO_2 gas exchange in these plants are largely controlled by changes of temperature. He concluded that in Agave dropping and rising temperatures have not effect on air passage. There are few reports describing the influence of soil water status on CAM behaviour in Agave americana. The day time was exchange of A. americana (Neales et al., 1968) is greatly reduced in plants deprived of water. The nocturnal phase of gas exchange is much less affected, thus the positive carbon balance of the plant is maintained and water loss is greatly reduced. Under drought, 98% of the total net carbon dioxide assimilation of an A. americana plant (in a 16 hour/8 hour light/dark regime) takes place in the 8 hour dark period, when the plant approaches the 'super CAM' condition.

In case of A. fourcroydes, Nobel (1985) observed that when water was withheld for various periods, appreciable CO_2 uptake began later in the night and the maximum uptake rate was not as great. The total net daily CO_2 uptake,

determined by integrating the curves over the entire 24 hour period, was 325 mmol m^{-2} under well-watered conditions, decreasing to 71 mmol m^{-2} after 11 days of drought and to 2 mmol m^{-2} after 30 days of drought. The daytime CO_2 uptake was more sensitive to drought than was the considerably greater CO_2 uptake occurring at night. Three days after watering the plant that had been droughted for 30 days, its maximal CO_2 uptake rate was 48% of that under well-watered conditions, and 7 days after returning to well-watered conditions it was 91%. Hartsock and Nobel (1976) earlier reported that in A. deserti irrigation abolishes dark CO_2 fixation completely, which indicates that the irrigation has profound influence on the CAM behaviour.

The atmospheric level of CO_2 has been steadily increasing since the beginning of the Industrial Revolution, in large measure reflecting the accelerating consumption of fossil fuels. Specifically, the ambient CO_2 level is estimated to have been about 270 $\mu l L^{-1}$, and is predicted to reach 650 $\mu l L^{-1}$ before 2100. Very little work has been so far done on the response of CAM plants to elevated CO_2 level. Nobel and Hartsock (1986) studied the short term and long term responses of A. deserti to elevated CO_2. They found that increasing the ambient CO_2 level from 350 microliters per litre to 650 microlitres per litre immediately increased daytime net CO_2 uptake about 31% leaving hightime net CO_2 uptake approximately unchanged.

(iii) Water relations

The process of transpiration is of utmost importance in CAM plants like Agave because the evolution of CAM is mainly for the purpose of maintaining water status and checking transpirational water loss. Transpiration ratios(TR) have been estimated for a variety of CAM plants including Agave species. CAM plants have low TR values with minima often in the range of 50 to 55 (Black 1973) but higher TR values have been reported in A. americana like 47 and 70 (Neales, et al., 1968 and Ehrler, 1969). The TR of A.deserti was estimated to be as low as 18 for winter days, and 25 as an average for the entire year (Nobel, 1976). The mean TR for A. lechuguilla was generally comparable to the TR of A. americana and A. deserti for similar experimental conditions. Only dew point temperature significantly influenced day time TR. The mean TR was 171 at high dew point and 238 at low dew point (Eickmeier and Adams, 1978). These TR values indicate that in terms of water-use efficiency, CAM plants are having low TR values. This data supports the theory that CAM plants are well adapted to survival in arid lands.

The transpiration of A. lechuguilla was studied by Eickmeier and Adams (1978) under laboratory conditions. They found that daytime water loss was largely controlled by chamber dew point. Low dew points increased transpiration due to an increased water vapour gradient between the leaf

and the chamber atmosphere. This indicated that stomatal closure was not complete and was varified by the daytime CO_2 uptake values. Infrequent watering reduced transpiration by about 20% and increased average daytime by about 14%. They further noted that night transpiration was significantly controlled by night temperature. Night transpiration exceeded average day values at 35°C, but was much reduced at 15°C. Similarly, low chamber dew points were associated with high rates of transpiration, while high dew points substantially reduced these rates. Pretreatment watering regime did not affect nighttime water loss as it did for daytime transpiration. They have also observed that total 24 hour transpiration response was similar to night transpiration. Only night temperature and dew point were significant, therefore both high night temperature and low dew point stimulated high transpiration rates.

Nobel (1976) studied the gas exchange of Agave deserti under field conditions in the Colorado Desert. He showed that during the winter Agave had the CAM CO_2 exchange pattern and the inverse rhythm of stomata of CAM plants, i.e., there was high stomatal resistance during the day and low resistance during the night. He made an interesting observation that the resistance for water vapour diffusion was correlated with the soil water potential. He did not observe natural stomatal opening during the drought season but at the same time he noted that it could be induced by artificial irrigation of the plant.

Nobel reported that in consequence of a shallow root system, A. deserti responds quickly to the infrequent rains, and because of the water-storing capacity of the succulent leaves, the CAM type pattern of stomatal opening was maintained for about 8 days after the water potential of the soil became more negative than that of the plant. This suggests clearly the importance of cool nights and plant water potential for CAM of A. deserti responds quickly even to a single period of precipitation by means of rapid development of shallow roots.

Transpiration rates and water potentials of a CAM succulent Agave deserti were further analysed by Nobel and Jordan (1983) for the leaves, stems and roots. They reported that the water storage capability of the organs of A. deserti was considerably high. The diurnal changes in water storage could support maximum transpiration rate of A. deserti for 16 hour. They further observed that the time constant for equilibration of water from storage to the xylem was 52 minutes for leaves of A. deserti. Resistances for such movement were relatively low for the succulent leaves of A. deserti.

Anatomical and kinetic aspects of water storage were investigated for the leaves of A. deserti by Smith, et al. (1986). They found linear relationship between the number of vascular bundles and leaf surface area, both for leaves of

different sizes and also along the length of a single leaf. They observed that rehydration kinetics of partially dehydrated leaf segments were resolved into three phases :(1) a relatively rapid movement into vascular tissue, (2) water movement into storage in the ground tissue and (3) water movement into the intercellular air spaces.

Nobel (1977) investigated the water budget during flowering for the leaves, inflorescence and lateral floral branches of Agave deserti in the Western Colorado desert. According to him during the 159 days from the emergence of the inflorescence until the fruit could be removed from the plant without affecting seed viability, approximately 68 leaves on a plant decreased 24.9 Kg in fresh weight (7.1 Kg attributable to leaf transpiration) and 1.84 Kg in dry weight. He also observed that the decrease of water in the leaves, which accompanied their death, was balanced by water storage in the inflorescence (3.1 Kg), transpirational water loss from its surface (4.3 Kg) and water loss from the lateral floral branches (10.8 Kg), most of which came from the fruit.

El-Gamassy et al. (1974) have studied the vegatative patterns and water economy of A. sisalana in saline soil. They have found that the increase in salinity level resulted decrease in the relative turgidity and rate of transpiration but on the contrary salinity increased the osmotic pressure of the sap of leaves.

(iv) Temperature Tolerance :

Temperature tolerance has been also investigated by some research workers in Agave. Nobel and Smith (1983) investigated the potential influence of tissue tolerances to extreme temperatures on distribution limits for 14 species of agaves from the south western United States and northern Mexico. They observed high and low temperature acclimation in response to changing day/night air temperatures in all 14 species. They have pointed out that agaves as a group are able to survive both the high temperature stress and the low temperature stress. As a group, the agaves exhibited a moderate low temperature tolerance of -11^{o}C (based on a 50% inhibition in the number of mesophyll cells taking up a stain, neutral red). However, nearly all the species were able to tolerate extremely high tissue temperatures of over 60^{o}C. They have observed that leaf morphology of individual species can reflect the temperature regime of their native habitats. According to them the species occupying the coldest habitats, such as A. utahensis and Agave schottii, are having small rosette size and thin leaves, which are more suitable for survival in cold habitats; while the species from the hottest habitats, such as A. deserti and A. lechuguilla, are having reduced shortwave leaf absorptances, which are more suitable for survival in open desert habitats. Species with substantial high temperature tolerances and acclimation ability tend to be not as tolerant of low temperatures and

vice versa. However, according to them certain species such as A. deserti and A. lechuguilla, can be exposed to both high and low temperature stress over the course of a year, are well adapted to both. Thus morphology and tissue tolerances to stressful temperatures reflect the temperature extremes of a plant's native habitat, although low temperature tolerance appears to limit distribution of agaves more than high temperature tolerance.

To understand the tolerances of desert succulents to extremely high temperatures (above 60°C), the effect of growth temperatures on fatty acid composition of various membrane fractions has been investigated by Chuan and Nobel (1985) from Agave deserti. They observed that when day/night air temperatures of 30°C/20°C were maintained, chlorenchyma fatty acid compositions were similar to those of mesophytic leaves, except that desert succulents (A. deserti) had appreciably less linolenic acid (18:3) and more oleic acid (18:1) and hence greater fatty acid saturation in the succulents, particularly in the chloroplast fraction. For A. deserti, increasing the day/night air temperatures did not result in increased fatty acid saturation, although its high temperature tolerances increase, as the air temperature is increased. Thus, according to them, high temperature acclimation in desert succulents need not be directly correlated with major changes in fatty acid composition or the saturation of the membrane lipids.

(v) Mineral Nutrition :

Although agaves are of commercial importance and of ecological interest, their nutrient responses have not been studied in detail under controlled conditions.

Nobel and Berry (1985) have studied seedling responses of A. deserti to high concentrations of various elements for monitoring both 12-day growth in hydroponic solution and 6-month growth in sand culture. They selected a species Agave deserti because its reponses to rainfall patterns, temperature regimes, and photosynthetically active radiation have been investigated in more detail than those of any other agaves. They observed that nocturnal acid accumulation by adult plants of six Agave species (A. americana, A.deserti, A. fourcroydes, A. lechuguilla, A. salmiana and A.utahensis) was related to element levels in their chlorenchyma. According to them the nocturnal acid accumulation by adult plants of all these species was positively correlated with levels of 10 elements in the chlorenchyma, especially N(r^2 = 0.70), B (r^2 = 0.51) and Ca (r^2 = 0.46). More over, they found a low Na and High Ca level compared with agronomic crops in the chlorenchyma of all these species. In contrast, nocturnal acid accumulation was weakly and negatively correlated with chlorenchyma Na(r^2 = 0.13), consistent with the deleterious effects of salinity on the growth of seedlings. They further found that with comparison to common agronomic crops, seed-

lings of Agave deserti were quite sensitive to salinity, with 50 mM NaCl greatly reducing root elongation in hydroponic solution and watering with 25 mM NaCl preventing growth in sand culture. The seedlings were rather sensitive to Ca concentrations from 0.2 to 5 mM and pH from pH 5 to 8. They also tolerated high levels of B and of the heavy metals Cu and Zn.

Nobel and Hartsock (1986) have examined nutrient responses of seedlings and adult plants of A. deserti, with particular emphasis on nitrogen. They observed that growth of seedlings in hydroponics was enhanced by increasing potassium, phosphate, and especially nitrate. Seedling growth in sand culture was also enhanced by adding nitrate, leading to just over 2% N by dry weight in the leaves. Seedlings had optimal growth in soil having about 0.1 % N by dry weight and a pH between 6 and 8. According to them in going from irrigation with no added nutrients to full-strength hoagland solution for mature plants in soil, leaf unfolding (a non-destructive measure of productivity) approximately was doubled. The rate of leaf unfolding in the field was also doubled by adding 100 Kg N hectare^{-1}, higher levels providing inhibitory. This study suggests the nitrogen application levels that could maximize productivity for other Agave species also.

(vi) Productivity Studies :

The productivity of various Agave species has been determined by different workers. Their observations can be summarised as follows :

Sr.No.	Name of species	Productivity
1.	Agave deserti	0.1 Kg m^{-2} $year^{-1}$ (Noble, 1984)
2.	A. fourcroydes	0.60 Kg m^{-2} $year^{-1}$ (Nobel, 1985)
3.	A. salmiana	0.05 Kg m^{-2} $year^{-1}$ (Nobel and Meyer, 1985)
4.	A. lechuguilla	0.38 Kg m^{-2} $year^{-1}$ (Nobel and Quero, 1986).

According to Nobel (1985) the annual productivity of A. fourcroydes is apparently the highest annual productivity reported for a CAM plant and is slightly lower than that of most C_3 and C_4 agricultural crops.

(vii) Other Biochemical Studies

Citrate Synthase is one of the important enzymes of TCA cycle which catalyses the condensation of acetyl CoA and Oxalo acetic acid. Alejandre, et al. (1979) have studied characteristics and activity of citrate synthase from different enzyme preparations from A. americana leaves. They

found a similar specific activity in the supernetants of centrifugation to 4000, 7000, 10,000, and 48,000 g. Zafra, et al. (1981) have studied citrate synthase for the first time in peroxisomes and mitochondria from leaves of A. americana. They isolated cell organelles from leaves and after characterisation they have observed the presence of glycoxylate cycle enzyme (citrate synthase) and a glycolate pathway enzyme (catalase) in the same organelles. They have reported the absence of another glyoxylate cycle enzyme (malate synthase) for the first time, suggesting that peri-oxisomal and glyoxysomal proteins are synthesized at the same time and stored in the same organelles.

Glutamate dehydrogenase is one of the key enzymes involved in ammonia assimilation. Ramirez et al. (1977) studied the activity of glutamate dehydrogenase from leaves of A. americana and determined some properties of it. They used cell-free extracts and acetone powder extracts from medulla and cortex tissues. Ramirez et al. (1978) further have studied differential effect of thiol binding reagents on the glutamate dehydrogenase activity from Agave americana. Glutamate dehydrogenase NAD-linked from cell-free extracts was found to be sensitive to thiol binding reagents.

Bhatia and Nandra (1979) conducted studies on fructosyl transferase from A. americana. They investigated the possible role of fructosyl transferase in the biosynthesis of fructosans

in A. americana. This enzyme was extracted from stem of A. americana and it was purified by salt fractionation and DEAE-cellulose chromatography. Bhatia and Nandra (1980) further reported the in vivo biosynthesis of glucofructosans in A. americana by the action of fructosyl transferase on sucrose. Demonstration of Mevalonate kinase activity and its properties, in acetone powder extracts from A. americana leaves, flowers and scape was given by Suarez, et al. (1977).

Du Toit, et al. (1978 a,b) have isolated a new enzyme aminopeptidase from A. americana and it was characterised physically. They also studied chemical properties of aminopeptidase which was obtained from same plant. Further purified Agave aminopeptidase was characterised with respect to the thermodynamic properties of the reaction catalysed by the enzyme.

Sane, et al. (1979) described a technique for the resolution of functionally similar enzymes that hydrolyse phosphate esters i.e. 3'-nucleotidase, pyrophosphatase, phosphatase and RNase from Agave cantala were resolved.

Cyclic-AMP (Adenosine 3':5'-cyclic monophosphate) has been considered as one of the most important hormone messengers in animal kingdom. Ashton and Gideon (1977) have isolated and characterised this compound from Kalanchoe daigremontiana and Agave americana.

Manetas, et al. (1986) studied magnesium-regulation of 4-carbon pathway as well as CAM adenylate kinase. They observed positive cooperation with Mg^{2+} in adenylate kinase from some C_4 plants as well as from CAM plants like Kalanchoe tubiflora and A. americana. It is suggested that the enzyme might be regulated through the diurnal changes in stromal Mg^{2+} concentration.

Golvano (1971) studied the action of IAA on flavo-protein enzymes localized in the soluble cytoplasmic fraction of leaves of Agave americana var. variegata and spadix of Arum italicum. He found that IAA increased the reduction of cytochromes b, c and c_1. It also stimulated oxygen uptake.

(viii) Phytochemical Studies :

Various organic compounds have been isolated and purified from different species of Agave in last few years.

1. Glycosides : Subramanian and Nair (1970) reported the occurrence of chlorogenin and kaempferol glycosides from the flowers of Agave americana. They isolated chlorogenin in a yield of 0.5% from the fresh flowers. They identified flavanol glycosides as kaempferol-3-glycoside and kaempferol-3-rutinoside.

Kintia, et al. (1975) isolated nine steroid glycosides, Agave saponins A-I, by column chromatography, composing 0.2% of fresh leaf substance.

Bobeiko, et al. (1975) reported the occurrence of ten glycosides of the spiroketal and urostannol series from the leaves of A. americana.

Lazur' Evskii, et al. (1975) isolated ten steroid glycosides of spirostanol and furostanol series from the leaves of A. americana.

Pant, et al. (1986a, b) isolated and characterised three spirostanol glycosides (cantalasaponins-2, -3 and -4) from methanolic extract of the rhizomes of A. cantala.

2. Saponins :

Wilkomirski, et al. (1975, 1976) isolated new steroidal saponins (agavasaponin E and agavasaponin H) from leaves of Agave americana and they used thin layer chromatography (TLC) to achieve separation of these saponins.

Yan and Huang (1976) made studies on the isolation and identification of steroidal sapogenins from some species of Agave like A. americana, A. sisalana, A. cantala and A. angustifolia, in order to search for more raw materials for the industrial production of steroid drugs.

Higgins (1976) analysed benzoate esters of sapogenins isolated from Agave species by reversed phase, a high performance liquid chromatography.