QUANTITATIVE GENETIC STUDIES ON SENNA

(*Cassia sp.*)

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INTRODUCTION

Cassia obovata L. or semma is one of the most important sources of Laxative endigenous to Egypt.

The active ingredients are mainly anthraquinone glycosides.

In addition to the other species of senna (*C. angustifolia* or *C. acutifolia*) that are now being explored as potent addition to the drug resources for the same pharmacodynamic fate, the activities of identification of higher quality yield through genetic manipulation is still a cryptic tool. In this particular instance, the real problem of manufacturing sennosides from *Cassia spp*. is not an extraction problem especially after the high performance liquid chromatographic tools being now available in the pilot system. The real problem is how to develop new satisfactory strains with their distingushed yield of sennosides which may compensate the high costs of extraction system.

This study was conducted, therefore, to generate genetic informations which could be used to design an efficient genetic selection procedures.

(Jamiican senna) is sometimes used as substitute. In cases of chronic constipation these senna species are valuable for their cathartic properties. Meanwhile, C. fistula L. (purging cassia), C. javanica L. (apple blossom senna), C. grandis L. (horse cassia), C. nodosa (Joint wood senna) are all cultivated as showy - flowered, ornamental and shade trees exhibited in the Royal Botanical Gardens in Jamaica. C. fistula L. (Indian Laburnum, cassia stick tree) is also one of the most important drug herbs imported from india; e.g. due to the antimicrobial activity of its root barck water extract, containing rhein, anthraquinones and tannines. Seaforth (1962) reviewed the importance of C. alata (Candle-stick cassia), C. absus (Chaksu), C. occidentalis, C. emarginata L., C. glauca Lam and C. tora L. in folk-lore medicine of West Indies.

Swarup and Singh (1965) reported the possibility of the occurrance of natural hybrids in some ornamental Cassia. They described the morphological characteristics of three naturally occuring interspecific hybrids, one of which is considered outstanding tree and shows the combined characters of C. fistula and Cassia nodosa.

Fairbairn and Shrestha (1967) studied the taxonomic validity of C. acutifolia and C. angustifolia; namely Allexandrian and Tinnevelly (Indian) senna, respectively.

They reported that their investigations indicated that two valid species were represented, which should be called C. senna L. for Allexandrian and C. angustifolia for Tinnevelly. Seymour (1973) identified 11 species of the genus cassia as natives to Nicaragua. They are mainly C. diphylla, C. flexuosa, C. leptocarpa, C. simplex, C. moschata, C. hispidula, C. obtusifolia, C. targera, C. lelophylla, C. siamea and C. xiphoides.

Irwin and Barneby (1977) offered a new revision of genus cassia in Barazil where the number of the section Apoucovita raised from 13 - 15 spp. including C. asplenii-folia, C. eitenorum, C. compitalis and C. boyanii.

Eight Brazilian weedy species of genus cassia, e.g. C. alata, C. bicapsularis L., C. occidentalis L., C. sulcata Dc., C. tora L., C. rotundifolia Pers., C. flexusa L. and C. patellaria Dc., were identified under subfamily Caesalpinioideae on the basis of morphological criteria in their Juvenile stages, Menezes (1978). Sixteen Cassia spp. were studied on basis of chemotaxonomic relationships, with reference to their distribution patern of the different secondary metabolites. The results obtained were expressed in the form of synthetic numerical indices; and polygonal graphs were suggested of close biochemical kinship among the taxa, as well as evidence from collateral disciplines.

The studied species included C. alata L., C. bicapsularis L., C. occidentalis L., C. sulcata Dc., C. tora L., C. rotundifolia Pers., C. flexuosa L. and C. patellaria Dc. Menezes (1978).

II. Phytochemical value of senna spp. to folk-lore remidies:

Prista et al. (1959) investigating the anthraquinones of C. occidentalis L., reported that emodin was found in the roots and seeds, while chrysophanic acid and a compound with the characteristics of 1 : 8 - dihydro anthraquinone were found in the roots, leaves and seeds.

Crellin et al. (1961) isolated five glycosides from Alexandrian senna (C. acutifolia Delile). These compounds were identified as rhein - 8 - glucoside, rhein - 8 - diglucoside, rhein anthrone 8 - glucoside, a primary glycoside having a mol. wt. of about 2000 and aloe - emodin glycoside.

Seaforth (1962) reported that "bush tea" in the West Indies was the aqueous extract of various Cassia spp.; i. e., C. javanica L., C. grandis L., C. nodosa L. and C. fistula L.... etc., which were used in folk - lore medicine to remedy kidney disorders, indigestion, ringworm, constipation, gout, rheumatism and general pains of undefined origin. About 40-50 species currently used in Jamaica have not examined for their physiological active constituents. The auther had reported that root barcks of C. *fistula* and leaves of C. *alata* contain glycosides of a reduction product of cassic acid (rhein) which is 4, 5 dihydroxy-anthraquinone-2 carboxylic acid, found in C. *articulata* L. (Anchel, 1949) and C. *acutifolia* Delile. However, cassilysine and cassilysidine, the antibiotic principles in the root bark of C. *excelsa* schrad, are reported to be alkaloids. The investigations of correlating chemical structure and physiologically active crude resources revealed that C. *occidentalis* L.; indigenous to Spanish Guinea is used for curing haemoglobin disorders. It contains emodin and an active principle. This wild senna is used as coffee substitute "wild coffee". Several *Cassia* spp. such as C. *absus* (chaksu) which contains chaksine alkaloide, act as antibiotic and causes nervous system depression, C. *emarginata* (yellow candlewood), highly toxic, C. *glauca* Lam.; anti diabetic and C. *tora* L. were proposed to justify the phytochemical investigations. Duquenois and Anton (1968) investigated leaves and pods of two African *Cassia* spp.; e.g. C. *nigricans* Valh and C. *podocarpa* Guill. et Perr. The two species contained appreciable quantities of anthracenic derivatives but of different chemical affiliations. C. *nigricans* possessed emodin, anthrone, Leuko anthocyanidin; which make the infusion of leaves avaliable laxative.

Cassia podocarpa contained only derivatives of ˌhenic acid including sennosides A and B. This species is used for its good laxative activity in Senegal, Sierraleone, Liberia and Nigeria. Subramanian (1968) reported that seeds of *Cassia tora* folia contain the closely related anthraquinones; chrysophanol, physcion, obtusifolin, chryso-obstin and aurantio-obstusin.

Kerharo (1969) investigated eight species of cassia used in traditional Senegalese therapy and reported that Sengal senna (*Cassia italiaca*) can be placed in the largest group of anthracenoid drugs. *C. italiaca* produced two interesting types of action; e.g. the purgative action as well as antibiotic, antiparasitic and antidermitis actions attributable to the several anthracene flavonoid-combinations. As a feature of interspecific variability, the species *C. absus* is quite different from the others by its seeds toxicity and alpoloid nature of its active priciples with hypotensive, antistaphylococcus, ganglioplegic, local anesthetic and smooth muscles stimulant.

Matsurra *et al*., (1978) reported that *Cassia tora* contains napthopyrones where the seeds of *Cassia obtusifolia* L. leaves contains triacontant - 1-01, stigmasterol, B-sitosterol-B-D-glucoside, friedelin, palamatic acid, stearic acid, succinic acid, d-tartaric acid, uridine, myoinositol, d-ononitol, kaempferol, quercetien, juglanin, astragalin, quercitrin and isoquercitrin.

The investigation of Cassia absus, showed that its roots contained chrysophanol, aloe-emodin, chaksine and isochaksine, while the leaves contained flavonoides; e.g. quercetin and rutin as well as chaksine and is ochaksine, Rao et al. (1979).

Singh (1979) reported that a large number of senna plants were obtained in the presumed F_2 generation resulting from a natural cross between C. acutifolia and C. angustifolia and showed considerable variation in pod characters. The two species were previously sown adjacently in experimental plots. A study of both species revealed morphological similarities (chromosome number of 14, regular meiosis, 90% pollen fertility and 90% seed germination).

Smit and All (1979) reported that Cassia alata grown in Fiji contained isochrysophanol and physcion-1-glucoside as well as aloe-emodin and rhein.

III. Phytotherapeutic value of senna active ingradients :

III. 1. Reaction to intestinal bacteria :

Kobashi et al., (1980) reviewed some metabolic processes of sennosides, the main effective components of sennae folium, by human intestinal bacteria and accounted for the relation between their defaecation effect and intestinal flora, where the intestinal bacteria is mostly (99%) anaerobic. It was reported that some unknown species of intestinal bacteria participate in the purgative effect, while the purgative activity of sennosides was stronger than that of aglycones when they were given orally (Fairbairn, 1949), the aglycones were more effective than sennosides when applied directly into colon (Okada, 1940). This suggested that sennosides were not absorbed in the intestine and become effective in purgative action due to the intestinal bacteria in the lower part of digestive tract (Fujimura, 1973). Kobashi et al., (1980) through a qualitive study of the ability of 20 species of anaerobes metabolize sennoside A, they included four types : Type (I): Possessing B-glucosidase activity to produce sennidine; Type (II): Isomerizing sennoside A to sennoside B; Type (III): Producing unknown gluconsides and type (IV): Possesing no metabolic activity. For example, type I bacterium uses Clostridium sphenoids reductively to cleave sennoside A and produce 8-glucosyl rhein anthrone (8 GRA) which is hydrolyzed by B-glucosidase to produce rhein anthrone (RA) which is directly oxidized to sennidine.

III. 2. Purgative activity :

Fairbairn and Moss (1970) studied the relative purgative activities of 1, 8 dihydroxy anthracene derivatives of senna pods on mice by repeated oral administration. It was reported that senna pod (as sennosides A and B) exhibited 100 mol relative potency as exactly did the A and B crude glycoside concentrate. Meanwhile, sennoside A and sennidin (as Rhein dianthrone) expressed relative potency of 68 and 53.0 mol but Rhein anthrone and free Rhein gave activities of 7 and 5 mols, respectively. Emodin and Emodin drivatives and chrysofanol showed about zero value activities of 2.0-4.0 mol.

III. 3. Significance of anthraquinone and pigment production :

The active acetate assimilation was studied briefly through the biogensis of isoprenoide by fungi (Lynen and Henning, 1960). Furthermore, it was proved through catalytic reactions that the acetate is one way by which plant species could capture the up-reteneous energy and as a mechanism for unaeobic respiration (Burmmiester and Guttenberg, 1960).

Antibiotic from fungi such as cephalosphorine and grisefulivin was developed and their biological significance through Allelopathy was assessed.

Following the labelling patterns in some natural compounds formed in the presence of ^{14}C labeled acetate, several anthraquinone compounds were biosynthesized by fungi among which are "Alternariol" from Alternaria tenuis, "Grisefulvin" from Penicillium spp., "Emodin" from Cortinarius sanguineus and "Rugulosin" from P. rugulosum (Shibata, 1967). Studies were carried out to establish the biosynthetic origins of anthraquinones characteristic for fungi, such as compounds of helmithosporin, cynodontin and islandicin (Fig. 1), like emodin....... etc., which are formed by the acetate - malonate pathway. Helminthosporin is produced by Helminthosporium granineum, Cynodontin by Pyrenocheate terrestris and Islandicin by Penicillium islandicum (Geissman, 1969).

The antimicrobial acitivity of anthraquinone glucosides (sennosides) and free anthraquinones (sennidines) in cássia senna was therefore, hypothesized in the current scheme of investigation. Acharya and Chatterjee (1975) reported the isolation of chrysophanic acid 9 - anthrone, the major antifungal principle of C. tora. Matsumoto et al., (1985) reported that Rhein which is present in rhubarb and senna inhibits Bacillus fragilis. The antimicrobial activity of rhein was also observed against E. coli, Bacillus subtilis, Micrococcus leteus, Candida albicans, Clostradium perfringens and Fusobacterium varium. Kitanka et al., (1986) isolated Helminthosporin - O - methyl chrysophanol from Cassia obtus. Dimethoxy benzoquinone and Aloe-emodin were isolated from roots and questun and isotora - lactone from the seeds and they showed antimicrobial activities.

Although classical flavonoids such as quercetol kaempferol, luteolin, quercetol-3-B-monogalactosides were reported in leaves of Cassia rogeoni (Haag - Berrurier et al. 1977), kaempferol in leaves of Cassia alata (Rao and Shastry, 1975) anthraquinone pigments were isolated from Cassia renigera seeds (Tiwari and Richards, 1979). Anton and Duguenois (1967) reported the presence four flavonoid compounds in Cassia senna. Wassel and Bagdadi (1979) reported higher flavonoids content in leaves than fruits of C. acutifolia and C. anguslifolia. Tiwari and Singh (1978) isolated three flavonoids from C. laevigata pods. These flavonoids are 3, 5, 3-trihydroxy-7, 4-dimethoxy flavone-3-O-B-D (+) galactopyranoside, 3, 5-O dihydroxy-7, 3, 4 trimethoxy flavone-3-O-B-D (+) galactosyl-O-B-D(+) galactopyranoside and quercetin.

Singh et al., (1980) isolated one flavanol; e.g. quercetin 7-4 dimethyl ether from the roots of Cassia laevigata. A new flavonal glycosides (Kaempferol-3-O-B-D-manno pyranosyl (1-4)-O-B-D-glucopyranoside was isolated