Proc. Indian Acad. Sci. (Plant Sci.), Vol. 95, No. 3, October 1985, pp. 203–211.

Phenology and biochemical changes in male and female shrubs of Jojoba [*Simmondsia chinensis* (Link) Schneider] during different seasons

VUTUKURI V PRASAD* and E R R IYENGAR

Central Salt and Marine Chemicals Research Institute, Bhavnagar 364 002, India

*Present address: Sree Rama Krishna Drug Stores, Chirala 523 155, India

MS received 6 March 1985; revised 16 October 1985

Abstract. The phenological and biochemical characteristics of male and female plants of Jojoba were studied during different seasons. The phenological studies indicated that the flowering and fruiting behaviour of this species in the semi-arid conditions of India change considerably with continuous flower formation and two fruiting cycles in a year. Marked differences in the carbohydrates, proteins and nucleic acids were observed between sexes and during different seasons. The pistillate plants had greater metabolic activities with more DNA and RNA than the staminate shrubs.

Keywords. Jojoba; phenology; carbohydrates; amylase proteins; amino acids; DNA; RNA; sex differentiation.

1. Introduction

Sex expression in higher plants is important particularly when the species is dioecious. The role of auxins, gibberellins and kinetins have received considerable attention in the work on sex expression (Heslop-Harrison 1956, 1972; Amruthavalli 1980; Mohan Ram 1980) but very little information is available on the physiological processes and associated biochemical changes in the studies on sex expression and also the effect of environment on such changes (Sivtsev and Sizov 1971; Slonov 1974; Jaiswal and Kumar 1978; Jindal and Singh 1975; Raj Kumar and Gopala Rao 1980a, b; Nazeem and Nair 1981). The dioecious species Jojoba [*Simmondsia chinensis* (Link) Schneider] belonging to the family Buxaceae, has its natural population males out-numbering the females. This species which is receiving attention as a commercial crop has been undergoing domestication in various countries (Prasad 1984). In India the species has been introduced by our Institute and has been grown successfully on coastal sand dunes. Reports are available on early flowering (18 months) and also late flowering after 5 or 7 years (Yermanos 1983; Prasad 1984). Studies conducted on some of the aspects of associated biochemical changes in the male and female plants revealed certain differences. These observations prompted us to study the seasonal variations in the carbohydrates, proteins and nucleic acid metabolisms and phenology in male and female plants of Jojoba.

2. Materials and methods

As Jojoba exhibits heteromorphism in a given population the field study was confined to the bushy type of plants with larger leaf areas [leaf area in males is 6·67 sq cms

± 0·12 and in females 6·76 sq cms ± 0·15 (leaf area of all the plants were measured by automatic digital Leaf Area Meter, LI-COR, Model LI-3000, USA)].

Leaves of male and female shrubs of Jojoba growing in the sand dunes were collected from the experimental field plots of the Institute. From a large population the plants were marked with metalic tags and each time collections were made only from these plants and the analysis of metabolic constituents were carried out in all the seasons at monthly intervals. Growth characteristics in the staminate and pistillate shrubs during different seasons were recorded.

2.1 *Biochemical analysis*

Mature leaves collected from the selected male and female plants for the analyses of metabolic constituents *viz* soluble sugars, starch, amylase, proteins, amino acids and nucleic acids.

Nucleic acids and proteins were estimated as described earlier (Prasad and Iyengar 1982). Starch was estimated by the method of McCready *et al* (1950). Soluble sugars and amylolytic activity were estimated by the methods of Yemm and Willis (1954) and Bernfeld (1955). The procedure of Slotta and Primosigh (1951) was used for the determination of amino acids. Proline was estimated by the method of Bates *et al* (1973).

3. Results and discussion

3.1 *Phenology*

The data on the phenology of Jojoba shrubs growing on the coastal sand dunes of semi-arid region of India are presented in table 1. Field observation revealed that the male population exceeds the female and the ratio of females to males was 47:53. This was comparatively higher than in the xeric conditions of the native Sonoran Desert (20:80 female to male ratio, Gentry 1958). Even in cultivated conditions in California, Yermanos (1983) observed 40:60 ratio of female to male which was far lesser than the sex ratio of Indian coastal climate. This indicates the environmental influence on sex differentiation in Jojoba. Freeman *et al* (1976) investigating on dioecious plants of *Atriplex*, *Distichis*, *Thalietrum*, *Ephedra* and *Acer* also concluded that for all the species males were abundant in xeric microsites while females over represented in the moisture parts.

The floral bud formation was noticed throughout the year with maximum in monsoon followed by post-monsoon. The rate of formation in both the sexes was retarded in winter. As summer approaches improvement in the floral bud formation was observed in both male and female shrubs. Also the plants showed active growth in all the seasons but less marked in winter. The environmental effects on the plants are fairly well known with the seasonal variations affecting the vegetative growth pattern and flowering. In the shrubs of Jojoba in winter flower budding has not been reported in the native wild stands of Sonoran Desert (Haase 1976; Thomson 1982). But in the Indian coastal climatic conditions the process of flowering occurs throughout the year. The absence of flowering in colder months in the native stands of Mexico and Arizona and its occurrence in winter, although comparatively less than the other seasons in India may be due to coastal climatic conditions. Probably the species receives less

Table 1. Phenology of Jojoba at experimental study site (Bhavnagar).

Monsoon (June–August)	Post-monsoon (September–October)	Winter (Nov–Feb)	Summer (March–May)
Vegetative growth* (peak)	Vegetative growth (moderate)	Vegetative growth (little)	Vegetative growth (moderate)
Flower** budding (profuse)	Flower budding (good)	Flower budding (little)	Flower budding (moderate)
Fruit formation	Fruit maturation	New fruit formation	Fruit maturation

*Vegetative growth in Jojoba during different seasons.

	Monsoon	Post-monsoon	Winter	Summer
Number of lateral branch production	24·00 ± 1·27	15·66 ± 1·66	4·66 ± 0·64	11·00 ± 2·10
Growth increase in height	17·33 ± 2·12	12·33 ± 1·95	5·33 ± 0·76	10·00 ± 2·40

± Standard deviation.

**Number of floral bud formation in staminate and pistillate shrubs of Jojoba during different seasons.

Season	Staminate	Pistillate
Monsoon	50·67	47·00
Post-monsoon	39·67	33·67
Winter	11·67	11·00
Summer	23·00	20·33
C.D. at p 0·05	12·11	9·68
p 0·01	18·34	14·67

photoperiod and low temperature and this may supress the floral bud formation during winter in the native stands. In its native of Sonoran region (extending in to Mexico and Arizona) the severe cold conditions (less than $-5°C$) some times lead to frost damage of growing and forcing the plant into dormancy (Yermanos 1983).

Forti (1972) noted that initiation and growth of female flowers are often less conspicuous and last for shorter duration than males although apparent differences in growth behaviour of staminate and pistillate plants were not observed in the mediterranian climate. However, in both the sexes flower formation was initiated after a certain period of vegetative growth, thus indicating the necessity of optimal vegetative growth before induction of floral buds. In the present study vegetative growth was seen

in all the seasons in both the sexes with flowering observed throughout the year. Thus the species tending to behave photoperiodically indifferent.

Further two fruiting cycles were noticed consistently. In the first cycle fruit initiation was observed in June–July and fruit maturation in September to October. In the second cycle fruit initiation began during December to February and maturation of fruits occurred during summer. In the wild populations of Jojoba only one fruiting period in a year has been reported (Haase 1976; NRC 1977). Yermanos (1983) noted only one fruiting period under cultivated conditions in California but in the present study two fruiting periods in a year are noticed consistently which ultimately result in higher yield of Jojoba nuts per bush.

3.2 *Carbohydrates and associated metabolism*

The differential accumulation of starch examined in male and female shrubs during different seasons is presented in table 2. Maximum levels in monsoon, particularly in July with some decrease in post-monsoon were recorded. The minimum content was noticed in winter in both the sexes. However, gradual increase was observed in summer months. The staminate shrubs showed higher levels than pistillate shrubs in all the seasons with monsoon and winter recording highest and lowest differences respectively

Table 2. Carbohydrates and amylolytic activity in staminate and pistillate shrubs of Jojoba during different seasons.

	Starch content (mg/g fresh material)		Soluble carbohydrates (mg/g fresh material)		Amylolytic activity (mg maltose released/g f wt/5 min)	
Season	Staminate	Pistillate	Staminate	Pistillate	Staminate	Pistillate
Monsoon						
June	11·34 ± 0·40	9·12 ± 0·05	16·14 ± 0·54	20·16 ± 0·87	28·90 ± 1·36	37·60 ± 1·21
July	15·92 ± 0·59	13·08 ± 0·51	13·90 ± 0·66	16·88 ± 0·74	24·50 ± 0·71	32·38 ± 1·24
August	12·78 ± 0·56	9·46 ± 0·69	17·50 ± 0·79	22·32 ± 0·56	32·68 ± 1·26	41·90 ± 0·99
	(13·21)*	(10·55)	(15·85)	(19·79)	(28·69)	(37·29)
Post-monsoon						
September	10·78 ± 0·37	9·34 ± 1·01	17·00 ± 0·10	20·00 ± 0·44	26·85 ± 0·55	36·80 ± 0·57
October	11·46 ± 0·63	10·00 ± 0·47	15·20 ± 1·02	20·00 ± 0·32	26·27 ± 1·54	33·00 ± 0·47
	(11·12)	(10·55)	(16·10)	(20·00)	(26·56)	(34·90)
Winter						
November	6·22 ± 0·84	5·58 ± 0·27	12·32 ± 0·78	15·20 ± 0·67	21·50 ± 0·71	25·44 ± 1·21
December	9·38 ± 0·29	7·10 ± 0·52	10·00 ± 0·56	12·46 ± 0·52	17·18 ± 1·03	25·00 ± 0·94
January	9·30 ± 0·33	8·16 ± 0·55	10·67 ± 0·32	12·00 ± 0·18	18·32 ± 1·56	22·46 ± 1·20
February	8·46 ± 0·22	6·78 ± 0·37	11·50 ± 0·27	13·40 ± 0·30	21·24 ± 0·58	27·00 ± 0·47
	(8·34)	(6·91)	(11·13)	(13·27)	(19·56)	(24·98)
Summer						
March	11·76 ± 0·58	8·64 ± 0·30	15·74 ± 0·46	21·36 ± 0·24	24·50 ± 0·71	31·42 ± 0·76
April	10·86 ± 0·40	7·78 ± 0·37	14·30 ± 0·53	17·80 ± 0·82	26·96 ± 0·92	35·80 ± 1·04
May	12·10 ± 0·42	9·44 ± 0·21	12·85 ± 0·28	15·16 ± 0·36	23·12 ± 0·89	30·24 ± 2·29
	(11·57)	(8·62)	(14·30)	(18·11)	(24·86)	(32·49)

± Standard deviation

*Mean values of the season

between male and female plants. The imbalance in the starch content between staminate and pistillate shrubs of Jojoba was probably due to differences in the amylolytic activity. Similar results have been reported for male and female flowers of castor (Gopala Rao and Sastry 1971; Raj Kumar and Gopala Rao 1980a).

The soluble carbohydrates (table 2) showed minimum levels in winter (December and January) while in monsoon and post-monsoon there was maximum accumulation. Higher levels were associated with profuse flower budding (table 1). The pistillate plants had much soluble carbohydrates than staminate shrubs. The optimum periods for soluble carbohydrate accumulation i.e. monsoon and post-monsoon also showed maximum differences between male and female shrubs.

Maximum amylolytic activity (table 2) in both the sexes was observed in monsoon and minimum activity in winter with post-monsoon and summer showing intermediate levels. The highest levels of activity was noticed in females than in males in all the seasons as evidenced by more soluble sugars and less starch content in the former than the latter.

The greater levels of soluble carbohydrates in the leaves of female shrubs of Jojoba may be due to the more amylolytic activity in females than in males. Sivtsev and Sizov (1971) reported that in many dioecious species (hemp, asparagus, dock, willow, boxelder and lombardy poplar) female plants surpassed the males in overall accumulation of soluble carbohydrates during various seasons. They pointed out that the feature was found to be stable under changing weather conditions contending that the female plants acquire greater resistence to adverse natural conditions by accumulating more soluble sugars. The present observations indicate that the carbohydrate levels were low in winter in both male and female shrubs of Jojoba probably because of environmental constraints in photosynthetic activity.

3.3 *Proteins and amino acids*

The protein content of male and female shrubs of Jojoba during different seasons is given in the table 3a. Maximum contents were recorded in the monsoon and post-monsoon with highest levels in August. In winter months the protein levels got reduced to lowest in January while it increased gradually in summer in both the sexes. Irrespective of the seasons lower levels of proteins were observed in the leaves of staminate than those of pistillate shrubs and the physiological status of both the sexes were apparently different. Similar conclusions were also drawn by Malaviya (1965) and Slonov (1974) in male and female plants of hemp.

The amino acid composition (table 3b) from male and female shrubs of Jojoba showed a total of 17 free amino acids in both the sexes. The amino acids asparagine, glutamine, aspartic acid, serine, glycine and glutamic acid were more in females while cystine, threonine, alanine, methionine, valine, phenylalanine, proline were higher in male shrubs. The other amino acids lysine, isoleucine and leucine did not show marked difference between sexes. Dzhaparidze and Mikeladze (1969) reported greater values for aspartic acid, threonine and tyrosine which are characteristic feature of male plants of *Morus*, *Pisticia*, *Humulus*, *Dioscorea*, *Diospyros*, *Rhamnus*, *Bryonia*, *Ailanthus* and *Populus* while cystine was predominant in females. But in Jojoba the adult plants show higher levels of cystine in males while aspartic acid was more in females. However, the amides both asparagine and glutamine were more in female shrubs while the aromatic amino acids as tyrosine and phenylalanine were more in males. Contrary to this Raj

Table 3a. Protein content (mg/g fresh material) in staminate and pistillate shrubs of Jojoba during different seasons.

Season	Staminate	Pistillate
Monsoon		
June	87·00 ± 1·47	98·00 ± 1·78
July	90·84 ± 1·54	105·46 ± 0·72
August	98·60 ± 1·80	114·62 ± 1·28
	(92·15)*	(106·03)
Post-monsoon		
September	91·48 ± 1·66	103·20 ± 1·20
October	86·80 ± 2·96	98·15 ± 1·18
	(89·14)	(100·68)
Winter		
November	72·46 ± 1·15	81·25 ± 1·29
December	71·40 ± 1·53	79·00 ± 1·34
January	66·70 ± 1·68	73·50 ± 0·89
February	73·14 ± 1·43	85·66 ± 1·25
	(70·91)	(79·85)
Summer		
March	79·90 ± 1·81	91·84 ± 0·86
April	90·72 ± 2·22	100·82 ± 1·02
May	84·00 ± 0·89	93·26 ± 1·47
	(84·89)	(95·31)

± Standard deviation.
* Mean values of the season.

Table 3b. Free amino acid content (μg/g fresh material) in male and female shrubs of Jojoba during monsoon.

Amino acid	Male	Female
Cystine	31·38 ± 0·79	26·35 ± 1·58
Lysine	53·80 ± 0·46	54·17 ± 0·53
Asparagine	28·10 ± 1·10	39·47 ± 0·84
Glutamine	80·64 ± 0·65	92·26 ± 0·37
Aspartic acid	86·14 ± 1·28	98·75 ± 1·16
Serine	33·61 ± 1·76	40·26 ± 1·84
Glycine	72·93 ± 0·83	82·42 ± 0·69
Glutamic acid	45·65 ± 0·92	56·52 ± 0·88
Threonine	201·30 ± 1·26	193·64 ± 0·99
Alanine	50·97 ± 2·06	39·90 ± 2·54
Methionine	84·12 ± 0·98	77·50 ± 1·08
Valine	65·14 ± 1·10	59·22 ± 1·24
Tyrosine	29·66 ± 1·03	20·90 ± 0·79
Phenylalanine	134·98 ± 2·10	118·45 ± 2·36
Isoleucine	39·58 ± 0·58	40·30 ± 0·46
Leucine	49·82 ± 1·02	48·80 ± 0·96
Proline	39·18 ± 1·56	30·95 ± 1·08

± Standard deviation.

Kumar and Gopala Rao (1980b) reported that the amide asparagine was high in male flowers of castor while the other amide glutamine was high in female flowers.

3.4 *Nucleic acids*

The nucleic acid content estimated in the leaves of staminate and pistillate shrubs during different seasons is presented in table 4. DNA content was more in pistillate plants than in staminates in all the seasons. Maximum content was observed in monsoon in both the sexes in synchrony with profuse vegetative growth and flower bud formation (table 1). Significantly less accumulation of DNA during post-monsoon in both the sexes was observed with correspondingly less profuse vegetative and reproductive growth (table 1). In winter the vegetative growth was less with low floral bud formation showing sharp fall in DNA content in both the sexes. In summer there was improvement in the growth of the plants and floral bud formation concomitantly showing considerable increase in DNA content. Thus increase could be seen in the DNA content and flower bud formation which in turn depends on the season when the environmental conditions are favourable.

The RNA content was much higher than DNA and it almost showed similar trend as that of DNA. The female plants during all the seasons had higher RNA contents than the staminate plants.

In the earlier studies higher quantities of protein are reported in female plants than in males and it is contended that a direct proximal relationship possibly existed between

Table 4. Nucleic acid contents in staminate and pistillate shrubs of Jojoba during different seasons.

	DNA content (μg/g fresh material)		RNA content (mg/g fresh material)	
Season	Staminate	Pistillate	Staminate	Pistillate
Moonsoon				
June	1273·58 ± 9·44	1501·71 ± 5·43	35·14 ± 1·12	47·98 ± 1·20
July	1380·29 ± 10·03	1592·37 ± 11·48	40·52 ± 2·43	53·60 ± 0·66
August	1546·86 ± 8·79	1806·56 ± 33·08	44·50 ± 2·10	58·34 ± 0·32
	(1400·24)	(1633·51)	(40·05)	(53·31)
Post-monsoon				
September	1305·41 ± 2·55	1650·32 ± 4·80	39·16 ± 0·98	50·92 ± 0·54
October	1182·54 ± 10·54	1303·33 ± 6·28	37·78 ± 1·05	48·08 ± 0·99
	(1243·98)	(1476·83)	(38·47)	(49·50)
Winter				
November	569·00 ± 3·91	680·00 ± 4·62	21·12 ± 0·98	28·85 ± 1·69
December	704·48 ± 4·54	847·66 ± 11·02	26·20 ± 1·05	30·46 ± 0·92
January	678·58 ± 4·42	819·80 ± 4·72	24·86 ± 0·34	30·28 ± 1·09
February	886·60 ± 5·42	980·24 ± 3·31	29·40 ± 0·89	36·12 ± 0·88
	(709·67)	(831·93)	(25·40)	(31·45)
Summer				
March	1083·40 ± 6·26	1241·24 ± 2·10	33·04 ± 0·92	44·36 ± 1·30
April	1336·35 ± 3·47	1549·28 ± 6·26	40·24 ± 0·70	50·10 ± 1·59
May	1124·54 ± 3·91	1411·00 ± 7·68	32·48 ± 0·75	41·60 ± 0·44
	(1181·43)	(1400·51)	(35·25)	(45·35)

± Standard deviation
*Mean values of the season

the synthesis of RNA, DNA and proteins (Prasad and Iyengar 1982). Contrary to Slonov (1974) the results of the present investigation revealed that during budding and flowering the DNA and RNA levels were invariably higher in females.

Minimum levels of nucleic acids during winter and maximum levels in monsoon in the male and female shrubs of Jojoba may be due to the prevailing unfavourable or favourable environmental conditions as also observed for spruce (Sovershaev and Barabin 1972).

The observations on the male and female inflorescences in monoecious plants such as cucurbits (Vlasenko 1969) and corn (Kubarev 1965) showed that the total nucleic acids were reliable criteria for sex determination. In the case of Jojoba the DNA levels demarcate the sexes in the adult plants. This clearly signifies the role of DNA in sex expression in this exotic species.

4. Conclusions

The field investigations carried out on bushy type of plants revealed the seasonal variations in metabolic activity between male and female plants. The pistillate plants had higher metabolic activity with more nucleic acids than the staminates. The winter seems to be dormant period of growth for the species with increasing growth and metabolic activities from summer until next winter. Under the semi-arid climate of

India and in dune sand conditions flowering pattern is continuous with two defined fruiting cycles in a year. It has been possible from the present study to conclude that the female plants of Jojoba have higher DNA content and metabolic activities and this may help in differentiation of sex at early seedling stages of the species.

Acknowledgement

The authors are thankful to the Director for facilities and encouragement.

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