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Evening Primrose (*Oenothera biennis* L.): Morphology and Reproductive Biology

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A B S T R A C T

Oenothera biennis L., commonly known as Evening Primrose as flowers open in the evening. Inflorescence is racemose (spike) and flowering continues for about 3 months. Circular floral nectary of translucent colour are present at the point of attachment of petals and hypanthium. Pollen are triporate, of variable sizes held together by viscin threads. Sepals colour change from green to yellowish green, act as indicator of anther dehiscene in the morning in closed conditions and anthesis in the evening. Flower is protandrous. The stigmatic lobes remain compact above the anthers till anthesis. Stigma radiates and turns receptive at the time of anthesis on the same day in the evening and remains so till next day afternoon. Anthesis completes with in 15 minutes i.e. from closed bud to full bloom. The species is both self and cross pollinated but mostly cross pollinated and is carried out by insects during both day and night. It is both cross as well as self-compatible.

Introduction

Oenothera biennis L. (family Onagraceae), a native north American species, commonly known as evening primrose as the flowers open in the evening, king's cureall and night willow herb etc. Evening primrose is important because of its seeds oil i.e. evening primrose oil (EPO) (Hall *et al.*, 1988), which characterized by its content of gamma linolenic acid (7-10%) (GLA), the precursor of prostaglandin E1 and its derivatives (Hudson, 1984 and Yunusova *et al.*, 2007). The oil is used in preparation of medicines, nutrients, health products and cosmetics (Deng *et al.*, 2001). It is used for a wide range of conditions including premenstrual syndrome (PMS), mastalgia, atopic eczema, rheumatoid

arthritis (Riaz *et al.*, 2009; Ratnayake *et al.*, 1989; Yunusova *et al.*, 2007; Ghasemnezhad, 2007; Anonymous, 2009; Horrobin, 1992). In recent years there has been an increased interest in plants that produce GLA. EPO is preferred to other source of GLA because it is most effective among others. Its simple composition, high level of linoleic acid that may facilitate the absorption of GLA, having most biologically active form of GLA (enotherol), easy to produce, high cost of GLA extraction from fungus etc. make it preferable than others (Hudson, 1984; Ghasemnezhad, 2007; Pesche *et al.*, 2007; Riaz *et al.*, 2009). It is available as a nutritional supplement in over 30 countries and is grown commercially in at least 15 countries. It has been reported that during the year 2008, EPO was the 20th top selling botanical dietary supplement (Cavaliere *et al.*, 2009; Gunstone, 1992). Due to increasing scope of the species in various medicinal and pharmaceutical industries, there is need not only to grow this crop on a large scale but also for its improvement to make it economically acceptable to the growers. Therefore, the present study was undertaken to understand its morphological features for correct identification of the plant, reproductive biology to determine the degree of variability expected in a population and understanding of its breeding behaviour which helps the breeder in formulating the hybridization programme and genetic improvement.

Materials and Methods

Morphological and pollination studies were carried out on field grown plants in Dr. Y. S. Parmar University of Horticulture & Forestry, Nauni-Solan campus. All morphological parameters data were collected at full inflorescence stage. Cauline leaves were measured at full inflorescence stage, while radical leaves were measured just before appearance of cauline leaves. Pollen dehiscence stage was determined by opening the closed bud manually at different time interval. Stigma receptivity was determined by placing fresh pollen on stigma and periodically observing for pollen germination under a microscope. Pollen viability was studied by hanging-drop method using 10% sucrose solution, micronutrients and fresh pollen treated with hexane to convert it into dust form. Controlled pollination was done at stigma-receptive stage using fresh pollen. In bagging and controlled selfing, buds that were about to open were covered with butter paper bags in such a way that one flower bloom in one paper bag. While in the former the buds/flowers were left as such till the flower turns the colour to dark orange i.e. next day evening or flower detach from the ovary. In the later i.e. controlled selfing, after flower-opening, the bag was removed, stigma was hand-pollinated with pollen from the same flower or flower from same plant and rebagged. In open cross and controlled cross, buds which are about to turn or turns greenish yellow in colour in the morning were carefully emasculated (in the morning). In the former such buds were left open, while in the later the emasculated buds were bagged and at the time of anthesis were hand-pollinated with pollen from other plants and rebagged. In open pollination, unopened buds were tagged and left for natural pollination without any artificial intervention. Detachment of flower from its ovary, increase in ovary size and its transformation into capsular fruit were taken as signs of fruit set. Seed germination of all the pollination treatments were carried out in the germinator at 25±5°C.

Results and Discussion

Plants of *Oenothera biennis* are erect, annual herb (Fig. 1). The initial phase of growth commencing from nursery up to the emergence of main shoot was characterized by the presence of large rosette, radical leaves. The main shoot that becomes hard at the later

stages, emerged producing cauline leaves, flowers, capsules and seeds. It flowers and bears seed only once in a life time. Qualitative and quantitative data on morphological features of the species were given in Table 1 & 2. Earlier Pullaiah (2006) and Hall *et al.,* (1988) in *O. biennis* and Naik *et al.,* (1999) in *O. lamarckiana* also reported the description of plant.

Floral bud, after appearance, took about 104 days for complete capsule dehiscence. Initially the bud showed slow progression in growth reaching its maximum in about 24 days after bud appearance when flower blooms (Table 4). The flowering occurs from 3rd week of July to 2nd week of October (75% of flowering completes) and fruits harvested from 2nd week of October to last week of December (Table 3). The time of anthesis is evening. The flower remains open till next morning, then wilt by forenoon and evening and fall by next day or day after tomorrow. Similar finding i.e. the flowers open at dusk and wilt in the following morning was reported by Hall *et al.,* (1988). The colour of flower at the full bloom stage and after that when flower wilt & dehisce, changes from yellow to orange (Fig. 1). Fixed number of flowers open in the evening. In one spike 3-5 buds bloom in the evening in one day and whole anthesis completes within 15 minutes. Anthers mature and dehisce, 24 days after flower bud appearance, on the day of anthesis, in the morning from 10:00 a.m. up to 12:00 noon. Pollen were shed while the petals were convolute in the bud i.e. flower is protandrous (Table 5). Hall *et al.,* (1988), also revealed that the pollen is shed while the petals are convolute in the bud. The flowers which is about to open in the evening changes their sepals colour from green to yellowish green and it act as indicator of anther dehiscence and anthesis in the evening. The pollen is held together by viscin threads which makes it a web like structure. When the pollen is shed in the closed condition it does not touch the stigma as stigma is compact vertically and above the range of pollen and not receptive at this stage (Fig. 1). Anthesis occurs on the same day in the evening i.e. 7-9 hrs after anther dehiscence and flower remains open till next day afternoon i.e. 25 days after floral bud initiation. At the anthesis stigma radiates and turns receptive and continues so till next day early noon (Table 5).

The maximum pollen germinability was observed to be 50 per cent at anther dehiscence stage (Fig. 1). When stigma radiates, pollen touches only lower part of stigma. Complete pollination is assisted by insect visitation leading to cross pollination as pollen are sticky in nature and sticks to insects body. Availability of viable pollen over a longer period helps protandrous flowers in cross-pollination. The vectors are rewarded by pollen and nectar. Circular ring shaped translucent floral nectary was observed at the point of attachment of petal and hypanthium. Different insect vectors, bumble bee (*Bombus sp*.), honeybee (*Apis sp*.) were seen visiting flowers in the day time and hawk moth (*Hyles* spp.) in the night (Table 6 and Fig. 1). They are responsible for maximum pollination. Immel (2003) believed, flower to be pollinated by night visiting hawk moths, which feed on their nectar. Immel (2003) reported that humming birds (*Archilochus* spp.) visit the flower to obtain nectar and to eat insects, which was not seen. Another feature that attract the insects in the night is the bright colour which is visible in night by nocturnal insects. This encourages the insect to enter the flower for effective pollination. This is a common feature of flowers of *Oenothera* sp. Dement and Raven (1974) reported that *O. hookeri* spp. Venusta has nectar guides visible by their contrasting UV patterns to insects but not humans.

Except selfing (bagging), all the treatment are at par with each other for fruit set (%) and other parameters.

Table.1 Qualitative morphological features of *O. biennis*

Plant part	Character
Habit	Annual
Stem	Single erect, round, cylindrical, ascending, green to reddish with the presence of red blotches, appressed terminal unicellular dermal hairs
Leaves	
Radical	Narrowly oblanceolate to ovate
Cauline	Alternate, subsessile, simple, pinnate, narrowly oblanceolate to elliptic, pubescent, reticulate venation.
Inflorescence	Racemose type (Spikes in acropetal succession).
Flower	Bracteate, sessile, complete, actinomorphic, tetramerous, pentacyclic, hermaphrodite, epigynous, hypanthium present, yellow.
1. Calyx	Sepals four, polysepalous, valvate, reflexed, greenish yellow sometimes red striped, pubescent, superior with free sepal apex.
2. Corolla	Petals four, polypetalous, twisted aestivation, clawed, superior, yellow.
3. Androecium	Stamens eight, polyandrous, diplostamenous (8 stamen in 2 whorls, outer alternipetalous and inner antipetalous), bithecous anther, dorsifixed, longitudinal dehiscence, superior, yellow. Pollen of variable size, sticky, jumbled in a jelly like substance, held with viscin threads, spherical, triporate, yellow.
4. Gynoecium	Tetracarpellary, syncarpous, tetralocular, axile placentation, inferior, long style runs throughout the hypanthium, stigma tetrafid, initially compact vertically above anthers, split and radiate horizontally with anthesis, pubescent.
5. Fruit	Denticidal capsule, pubescent, narrowly lanceoloid with tapering towards the apex. Green or green with red stripe when fresh and brown when dried, bluntly 4 angled, dehisce nearly throughout the length.
6. Seed	Numerous, 2-3 rows per locule, irregular shape, brown to dark brown (blackish)

Table.2 Quantitative morphological parameters of *O. biennis*

Parameter	Dimension/number
Plant height	160±10 cm
Root	
Number (main roots)	15±4
Length (longest root)	39±7.5 cm
Leaves	
Number of radical leaves	22±5
Number of cauline leaves	186±50
Size of radical leaves	20±10 cm x 5.5±1.0 cm
Size of cauline leaves	15±10 cm x 4.4±2.0 cm
Number of flowering shoot per plant	12±5 cm
Length of inflorescence	35±5 cm
Flower	7.5±0.8 cm x 5.0±0.4 cm
Bracts	3±2.1cm x 1.3±0.6 cm
Calyx	
Number of sepals	4
Length	2.9±0.5 cm x 0.4±1 cm
Corolla	
Number of petals	4
Length	2.5±0.5 cm x 2.5±0.7 cm
Androecium	
Number of stamens	8
Length of stamens	1.8±0.3 cm
Anther length	0.6±0.3 cm
Pollen	
Size	2 (0.08 mm and 0.06 mm)
Gynoecium	
Number of carpel	4
Length of ovary	1.0±0.2 cm
Ovary diameter	0.15±0.2 mm
Length of stigma	0.8±0.2 cm
Fruit (capsule)	
Length	3.0±0.5 cm
Weight (green)	0.8±0.13 g
Seed	
Length	1.6±0.4 mm x 0.9±0.2 mm
1000 seed weight	0.347±0.004 g

Table.3 Corresponding sequence of events in development of *O. biennis*

Event	Date	Time Taken
Sowing of seeds	12th March	6 days
Seed germination	18th March	
Transplanting	20th May	63 days
1st flowering	20th May	61 days
Flowering period	20th July – 10th October (3rd week July–2nd week of Oct.)	83 days
Capsule ripening (Harvesting)	8th October – 28th December (2nd week of Oct.–last week of Dec)	81-82 days
Total growth period	12th March – 28th December	295 days

Table.4 Sequence of events starting from the bud initiation to seed dispersal in *O. biennis*

Number of days*	Events
0-2 Days	Floral buds started appearing with four calyx lobes closely held together
2nd – 25th day	Bud grew in size with increase in size of ovary
24th day morning	Colour of bud changed from green to yellowish green
24th day morning	Anther below the stigma start dehiscing with stigmatic lobes compact vertically
24th evening	Anthesis occur with spliting or radiating stigmatic lobes
25th morning	Flower remain open
25th evening	Flower starts to wilt with the colour changing from yellow to orange
26th and 27th	Flower with its floral parts detach from the ovary
27 – 103 day	Size of capsule increases
104th day	Capsule tip starts opening and start dehiscing

* Number of days from bud initiation

Table.5 Corresponding sequence of events in development of pollen and stigma receptivity

No. of days since bud initiation	Time	Floral stage	Maturation stage	
			Anther	**Stigma**
24th day	10:00 am–12:00 noon	Bud closed, greenish yellow in colour	Anther dehisce	Stigma compact vertically, above anthers and not receptive
24th day	6:00 pm–7: 00 pm	Separation in sepals and petals, anthesis starts, Flower yellow in colour	Anthers dehisced	Stigma splits and radiates, stigma turns receptive
25th day	Morning till 12:00 noon	Flower remain open	Anthers shrivelled	Stigma receptive
25th day	Evening	Flower changes colour to dark orange colour and whole flower wilt		Stigma lobes loose receptivity
26- 27th day		Flower wither and detach from ovary		

Table.6 Floral characters of *O. biennis*

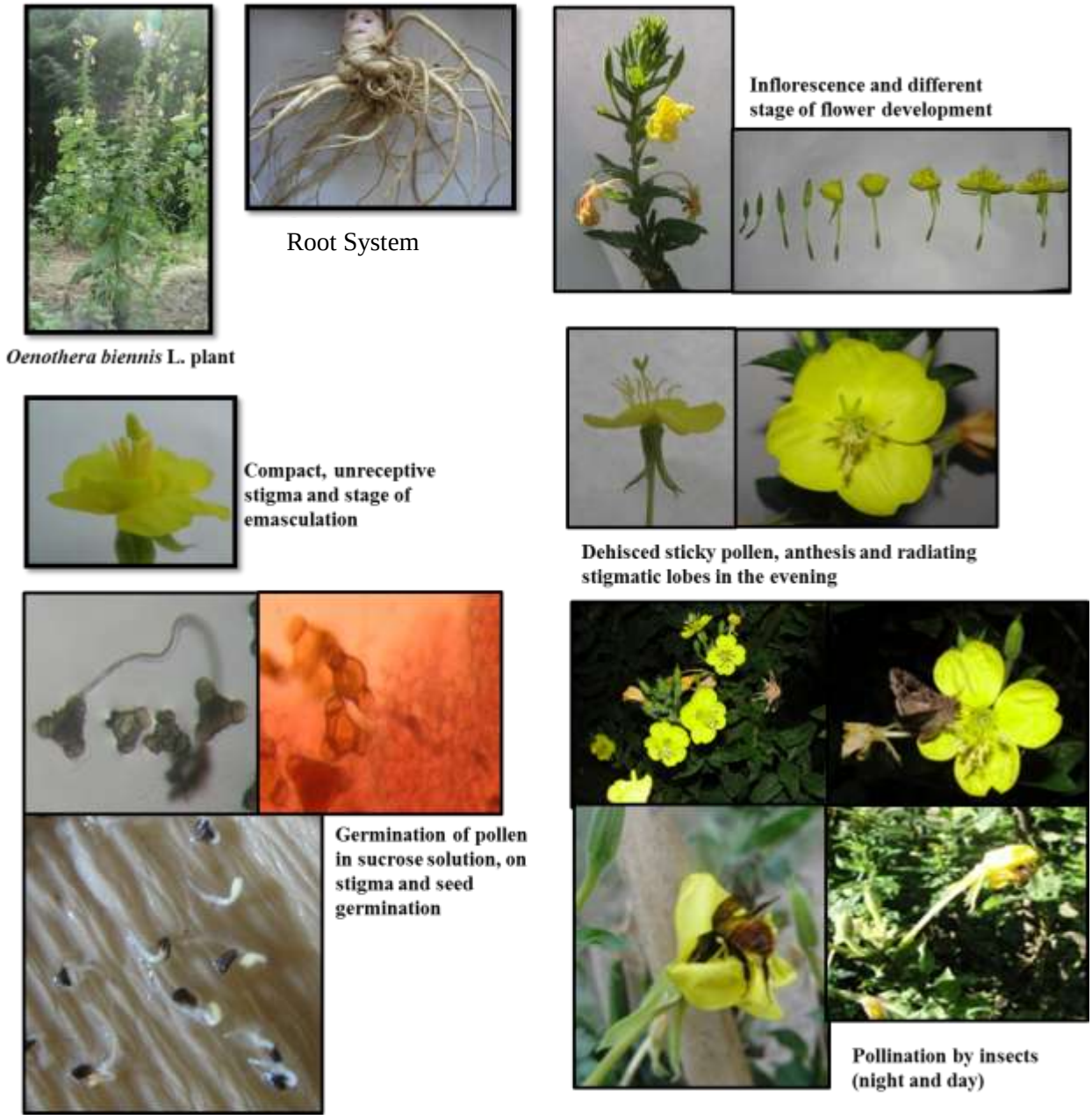
Floral characters	Observations
Flowering period	Mid July to Mid October
Pollination	Either by contact to some extent (selfing) or by insects (entomophily)
Odour	Absent
Nectar	Present (translucent and circular ring)
Flower opening time	Dusk
Anther dehiscence time	10:00 am – 12:00 noon
Anther dehiscence mode	Longitudinal
Type of dichogamy	Protandrous
Pollen shape	Spherical, triporate
Stigma type	Tetrafid above the anther level
Days taken for capsule maturity	80 – 82 days after pollination

Table.7 Effect of different pollination methods in *O. biennis*

Parameters / Treatment	Fruit set (%)	Capsule length (cm)	Capsule weight (g)	Seed yield /capsule (g)	Seed yield /plant (g)	No of Seeds /capsule	No of seeds /plant	Germination (%)
Open pollination	95.00 (80.78)*	2.96	**0.26**	0.13	39.52	325.00 (2.51)**	98730.00 (4.99)**	95.00 (9.75)***
Selfing (bagging)	62.50 (52.28)	1.73	0.05	0.02	3.56	25.28 (1.40)	5057.00 (3.703)	95.50 (9.77)
Open cross (emasculate flower)	85.00 (70.98)	2.84	0.23	0.13	37.76	296.40 (2.47)	87720.00 (4.94)	94.50 (9.72)
Controlled self	**97.50 (85.39)**	2.93	0.25	**0.14**	42.08	357.70 (2.55)	111600.00 (5.05)	94.00 (9.70)
Controlled cross	**97.50 (85.39)**	**2.97**	0.25	**0.14**	**44.48**	**365.30 (2.56)**	**114000.00 (5.06)**	96.50 (9.82)
CD	13.33	0.05	0.01	0.006	3.19	0.01	0.03	NS

*Figures in parentheses are ARC sine transformed values;
** Figures in parentheses are LOG transformed values;
***Figures in parentheses are Square Root Transformed values

Fig.1 Morphological and reproductive parts of *O. biennis*



This reveals species to be both self and cross pollinating but strongly cross pollinating. Both self and cross pollination takes place in this plant in nature but cross pollination is more prevalent as indicated from open cross and open pollination.

This is further strengthened by the observation that the presence of anthers (in open-pollinated flowers) or their absence (due to emasculation in open cross-pollinated flowers) does not make any significant difference for fruit set in open and open cross-pollinated flowers and shows pollination by vector is the best for maximum fruit set in nature while some self-pollination is still possible in nature without vector as indicated by selfing (bagging). Inadequate pollens to fertilize

ovules in selfing due to position of anthers and stigma and absence of vectors could be the reason for low fruit set percentage (Table 7).

High fruit set (%) in selfing is possible with human intervention. While Hall *et al.,* (1988) and Steiner (1956) reported that the flowers are predominantly self-pollinated, but outcrossing does occur. Schooley (1965), Steiner (1961) and Levin *et al.,* (1972) also mentioned the plant as naturally self-pollinating contrast to the present finding.

Insect vector visitation shows crossing is possible. This also supports the cross pollinating nature besides the position of anthers and stigma at and before anthesis. Hoff (1962) reported the relative lengths of anther and style as wel1 as the amount of pollen produce influences the degree of outcrossing. Outcrossing was negatively correlated with the additions of self-pollen applied to the stigma. The germination percent indicated fertile seeds from every pollination treatment which represents cross and self-compatible nature of the species (Table 7). More than 2 morphotypes were observed in the species.

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