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Flowering biology and fruiting phenology study in white cross-berry (*Grewia tenax* (Forssk.)) Fiori.

Rahul Dev[1]*, M. Sureshkumar[1], Traloki Singh[2] and Sushil Kumar[1]

[1]*ICAR-Central Arid Zone Research Institute, Regional Research Station, Kukma, Bhuj – 370 105 (Gujarat), India.*
[2]*ICAR-Central Arid Zone Research Institute, Krishi Vigyan Kendra, Kukma, Bhuj – 370 105 (Gujarat), India.*
**E-mail: rahul2iari@gmail.com*

Abstract

Knowledge of flowering biology is key for the conservation and efficient utilization of any species in *Grewia tenax*. This fruit species is now appropriate for commercial production due to the emphasis on food supply, market value and therapeutic utility of underutilized crops in the modern period. Therefore, flowering biology and fruiting phenology studies were carried out on field-established *Grewia tenax* plants in Kachchh, Gujarat during February to March 2018. Flowers are scented and cream-white with yellow stamens and composed of 5 petals and sepals, ovary and a style, 2 anther lobes and many anthers (66.33 ± 7.51). Bud initiation started in the last week of February and anthesis was observed after 6.40 ± 0.18 days of bud initiation. Flower anthesis starts at 1130 h and is completed at 1330 h with peak anthesis during 1215-1230 h in February. On average 15.55±0.98 and 19.55±0.98 days are required for fruit maturity and ripening. The fruit traits *viz.* fruit length ranged from 4.93-6.69 mm, fruit diameter 4.06-5.91 mm, respectively. Given the paucity of information on white cross berries, the results of this study will open the path for more in-depth genetic research on this species.

Key words: *Grewia tenax* (Forssk.) Fiori, flowering biology, fruiting phenology, Kachchh

Introduction

The white-cross berry is a near-wild cousin of the phalsa (*Grewia subinaequalis* DC), a widely domesticated fruit plant in northern India. Under the genus *Grewia*, 321 recognized species of trees and shrubs with potential fodder and fruit usage have been documented (http://www.theplantlist.org) and 40 species of *Grewia* are documented from different parts of India (Chopra *et al.,* 1956; Kirtikar and Basu, 1975). Different species of the genus *Grewia* have a wide distribution across Asia, Africa, and Australia (Bredenkamp, 2000; Whitehouse *et al.,* 2001). Many wild species that are orphaned and ignored by academics are part of the tribal people's food basket. *Grewia tenax* or white-cross berry, is one such species. It is an important shrub species of the Tiliaceae family, native to the arid and semiarid regions of the world. Owing to excellent fodder material, browsing pressure is also high on this species, particularly in the dry season. *G. tenax* is an important source of forage material and nutritious feed for livestock predominantly during the dry, lean seasons in Rajasthan and Gujarat (Sharma and Patni 2012, and BSI, 2014), particularly in western Rajasthan (Venkatesan *et al.,* 2019), Kachchh, Gujarat (Dev *et al.,* 2017). *Grewia tenax* is a multipurpose, multi-stemmed medium to small tree that can reach three meters in height and is used for food, feed, fiber, firewood, timber, and traditional medicines. Stem with ash-grey bark, branches glabrescent, young twigs stellate hairy. The leaves are alternate, broadly ovate, dentate with rounded base, and leaf size is about 2.09 cm long and 2.14 cm wide (Dev *et al.,* 2017). It has pure white flowers that are born single or in pairs of 2-3 flowers. Sepals are long, hairy, and linear. Petals are white and are about 1 cm in length. The ovary is 4-lobed, the style is long, and the stigma is 4-5 lobed. Fruit is drupe usually 1-4 lobed, 8-12 mm wide, the lobes 6-8 mm long, 5–6 mm wide, orange, yellow or reddish tinge, shining, glabarous (Whitehouse *et al.,* 2001; Gebauer *et al.,* 2013). Generally, *G. tenax* flowers two times in a year *i.e.* first flowering in March-April and fruiting occurs in August-September. While additional flowering and Fruiting occurs during October-February. The lifespan of a single flower is only one day and is mainly pollinated by insects (Ghazanfar, 1992 and Sharma and Patni, 2012). Hashmi and Qaiser (1990) reported that bagged flowers develop into fruits, so bagging of flowers did not affect fruit production while emasculated flowers did not produce fruit, indicating the genus is amphimictic and inbreeder. On the contrary, Cruden (1977) found that *G. tenax* is facultative xenogamous, and may be involved in hybridization on a limited scale particularly in the region where the flowering period overlaps with other related species (*G. erythraea*) of the same genus. *G. tenax* flowers emerge with new expanding leaves. Fruits of *G. tenax* are high in nutritional components and essential micronutrients, such as minerals (K, Ca, Mn, Fe, Cu and Zn) and amino acids, and have functional qualities (Aboagarib *et al.,* 2015). *Grewia* species are employed for a variety of uses in both rural and urban areas. The fruits are edible and can be consumed fresh or dried because of their great nutritional content, especially during the dry seasons. The stem is used to make cages, baskets and bows, while the stem and branches are used to make fuel and wood products. Because it is not attacked by termites, it is also used to produce instrument handles, spears and harpoon shafts. The bark is utilized for making braided rope, while bark extracts are used to glue tobacco leaves and the leaves are employed in traditional medicine. During the dry seasons of the year, leaves and twigs provide excellent forage for animals. Livestock eat

the young leaves, which are fairly palatable at the end of the dry season and have a good feed value. Despite the wide diversity and multipurpose uses, still research attempts are scanty, particularly on its domestication, improvement and characterization. This species has a higher level of economic potential, however, one of the things holding it back is the lack of understanding about floral biology and pollination in *G. tenax*. This plant has year-round continuous flowering (*i.e.* three times a year), and harvesting is difficult owing to asynchronous fruit ripening. Understanding reproductive biology is critical for genetic improvement and effective conservation of the genetic diversity of any crop species. Species effectiveness or failure for evolutionary success requires a meticulous understanding of its breeding system (Lattoo *et al.,* 2006; Namroud *et al.,* 2012) because the transmission of genetic information from one generation to the next and the genetic makeup of progenies are determined by the kind of breeding system. Survival, prospering, genetic enhancement and evolution are all also dependent on the reproductive quality of species (Lattoo *et al.,* 2007). Therefore, the present investigation was carried out to describe the flower biology, morphometric features of flowers and fruiting phenology in *G. tenax*. To the best of our knowledge, it is the novel comprehensive description of floral biology and phenological aspects of *G. tenax*. Given the paucity of information on white cross berries, the results of this study will open the path for more in-depth genetic research on this species.

Materials and methods

Study site: The research was done in the Central Arid Zone Research Institute's Regional Research Station in Bhuj, Gujarat (latitude 23°21′19″-23°21′33″ N, longitude 69°79′72″-69°78′78″E). The soil is a mixture of saline/sodic, sandy, and silty loam. The area has a hot, dry climate with summer temperatures reaching 48°C. The average annual rainfall in Kachchh is 326 mm, with evapotranspiration ranging from 1500 to 2000 mm.

Flower biology and phenology study: To study the flowering and fruiting phenology tagging of three branches in a plant and 20 flowers in each branch was done in February. Traits like anthesis time, anther dehiscence, flower longevity, bud initiation, anther dehiscence, pollen viability, fruit set, time to fruit maturity and ripening were recorded. The buds that were ready to open (stage 3rd buds) on the following days were selected for recording anthesis time. About 60 buds were tagged randomly on three branches in three replications and flowers were observed for opening time at hourly intervals followed by a 15-minute interval to record peak anthesis time. Anther dehiscence was examined in the about-to-open flower buds under the microscope (n=10). The longevity of the flower is examined through the examination of the period from the time of flower anthesis till the drying of the stigma.

The morphometric feature of the flower (n=10) was also measured from a fully opened flower with the help of a digital caliper and stereo binocular microscope. The traits like flower bud stage 1 size (mm), bud stage 2 size (mm), pre-anthesis bud size (stage 3) (mm), flower size (mm), style size (mm), stigma lobe size (µm), petal size (mm), sepal size (mm), anther lobe size (µm), nectar cavity size (mm), mature fruit size (mm), number of anther/ flowers, number of style/ flower and number of anther lobe / flower were measured.

Results and discussion

This is the novel report on floral biology and fruit phenology of *G. tenax* in India. The flowers are born singly in a season of old branches along with new leaves. Flowers are scented and cream-white with yellow stamens and composed of 5 petals and sepals, ovary and a style, 2 anther lobes and many anthers (66.33 ± 7.51). Bud initiation started in the last week of February and anthesis was observed after 6.40 ± 0.18 days of bud initiation. Whereas, fruit setting was noted in the second week of March at 5.85 ± 0.73 days of anthesis. On average 15.55±0.98 days are required for fruit maturity. The ripening of fruits starts after 19.55 ±0.98 days of anthesis (Table 1 & Fig. 1).

Pre anthesis bud is divided into 3 stages based on the size. The size of the bud at the first stage was about 4.82 ± 0.76 mm long and 3.34 ± 0.1 mm wide respectively which increased up to 10.63 ± 0.44 mm long and 3.83 ±0.04 mm wide at the second bud stage. Whereas, third stage bud just a day before anthesis was measured about 11.89 ± 0.43 mm long and 4.40 ± 0.09 mm wide. While the size (length × width) of the individual flower was about 14.95±0.97 mm × 29.86 ± 0.24 mm respectively. The size of the petal and sepal was about 16.26 ± 0.71 mm long × 2.61 ± 0.17 mm wide and 11.31 ± 1.10 mm long × 133 ± 0.04 mm wide respectively. Flower style was about 9.86 ± 0.17 mm long and 319 ± 15.51 µm wide and the average length of four stigmatic lobes was about 318.58 ± 12.80 µm and width was 417.67 ± 61.26 µm, respectively. Male parts of the flower *i.e.* anther filaments were about 10.92 ±0.29 mm long and 17.30 ±1.02 µm wide in size while the average size of its lobes (2 lobes) was about 167.56 ± 4.39 µm × 324.20 ± 7.55 µm. Nectar cavity paced in the base of flowers which was about 4.53 ± 0.6 × 4.29 ± 0.02 mm size. Whereas the size of a mature fruit was about 7.58 ± 21 mm long and 6.06 ± 0.14 mm wide (Table 1).

The antithesis of *G. tenax* flowers was between 1130 h and 1330 h with peak anthesis between 1215-1230 h (Fig. 1). Flowers remain open for 24 hours thereafter floral parts like petals, and sepals turn brown and shrink. While stigma becomes brown and style bends downward. Anther dehiscence was taken place on the same day of anthesis between 1400 and 1600 h (Fig. 3 & 4). The maximum fruits ripened in the last week of March and turn red/orange at full ripening. Joshi and Dwivedi (1979) recorded that the flower of *G. tenax* is pentaeyelie and up to 50 stamens are present in a

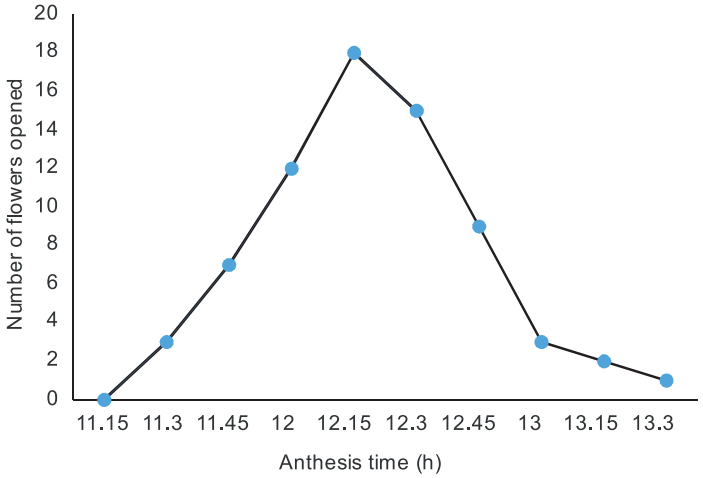

Fig. 1. Time of flower anthesis in *G. tenax*

Table 1. Morphometric characteristics of white cross-berry flower

Character	Mean ± S.E.	Minimum	Maximum	CV (%)
Bud stage 1 (L, mm)	4.82±0.76	3.59	6.21	27.36
Bud stage 1 (W, mm)	3.34±0.16	3.1	3.64	8.19
bud stage 2 (L, mm)	10.63±0.47	9.83	11.46	7.68
Bud stage 2 (W, mm)	3.83±0.04	3.75	3.9	1.99
Pre-anthesis bud (L, mm)	11.89±0.43	11.04	12.33	6.21
Pre-anthesis bud (W, mm)	4.40±0.09	4.21	4.5	3.68
Flower (L, mm)	14.95±0.97	13.51	16.8	11.26
Flower (W, mm)	29.86 ±0.24	22.9	36.04	22.12
Style (L, mm)	9.86±0.17	9.56	10.13	2.90
Style (W, µm)	319.64±15.91	288.4	340.5	8.62
Stigma lobe (L, µm)	318.58±12.80	295.73	340.00	6.96
Stigma lobe (W, µm)	417.67±61.26	313.03	525.18	25.40
Petal (L, mm)	16.26±0.71	14.84	17.03	7.56
Petal (W, mm)	2.61±0.17	2.27	2.81	11.27
Sepal (L, mm)	11.31±1.10	9.6	13.35	16.76
Sepal (W, mm)	1.33 ± 0.04	1.26	1.41	5.63
Anther filament (L, mm)	10.92±0.29	10.36	11.34	4.61
Anther filament (W) µm	17.30±1.02	16.25	19.34	10.20
Anther lobe L (both) µm	167.56±4.39	159.23	174.14	4.54
Anther lobe W (both) µm	324.20±7.55	309.28	333.69	4.04
Nectar cavity (L, mm)	4.53±0.06	4.45	4.65	2.34
Nectar cavity (W, mm)	4.29±0.02	4.25	4.33	0.94
Matured fruit (L, mm)	7.58±0.21	7.21	7.94	4.82
Matured fruit (W, mm)	6.06±0.14	5.85	6.32	3.96
No. of anthers/flower	66.33±7.51	53	79	19.62
No. of style/ flower	1.00 ± 0.00	1	1	0.00
No. of anther lobe/ flower	2.00±0.00	2	2	0.00
C.D.	50.53			
SE(m)	17.76			
SE(d)	25.11			

L=Length, W=Width

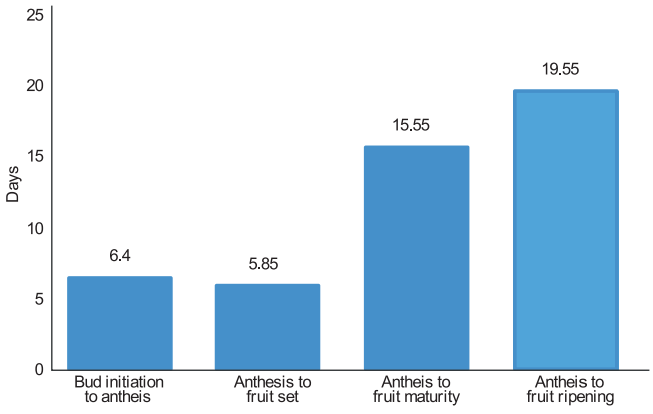

Fig. 2. Days required for different phenophases of *G. tenax*

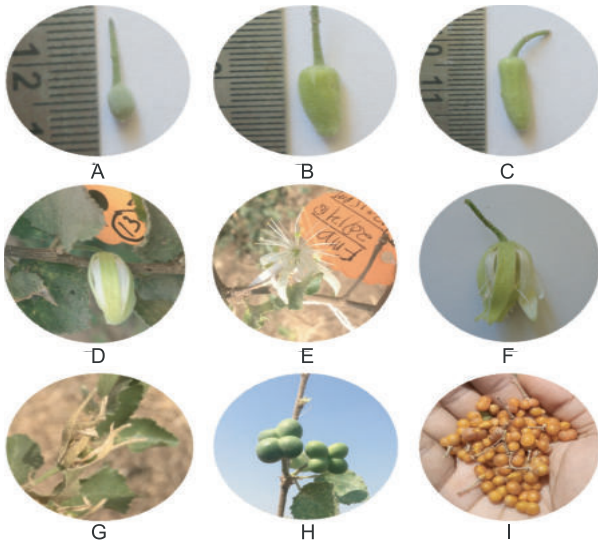

Fig. 3. Flowering phenological stages. A & B: Early floral buds, C: Matured floral bud at anthesis, D: Bud just before anthesis, E: After anthesis, F: Floral senescence, G: Fruit initiation, H: Fruit maturation, and I: Ripened fruit.

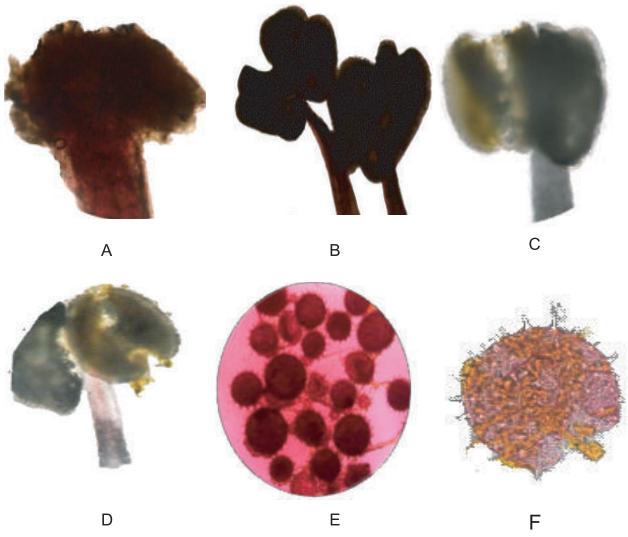

Fig. 4. Macrophotograph of: A- Stigma, B- Anther lobe, C & D- Anther dehiscence, E & F- Pollen

flower. Similar results were recorded on *G. flavescens* that revealed the flower bud initiation in the fourth week of May to prime flowering in fourth week of June. Peak anthesis was in between 1200 and 1230 h. While stigma becomes responsive 6 h after flower opening. Pollen dehiscence was noted 1–2 h before flower opening with maximum pollen viability (80.34 ± 5.16%) (Kumar *et al.,* 2017). Similarly, many studies on *G. asiatica* reported flower bud initiation in the first week of March and peak flowering in the fourth week of March (Randhawa and Dass, 1962; Wani *et al.,* 2015; Dey *et al.,* 2016). Flower durability for five days and pollen dehiscence was recorded immediately after anthesis but maximum stigma receptivity was found on the second day after anthesis (Wani *et al.,* 2015). In the present study, three flowerings were noticed in a year, *i.e.* Hasta Bahar (September-October) and Mrig Bahar (June- July) in addition to regular Ambe Bahar (March-April) flowering. Normally, fruits are gathered when fruit colour changes gradually from green to orange or dark red. The fruit has 2-4 lobes each rounded fleshy mesocarp.

Understanding the floral biology of a species is a key requirement of any breeding program, and it contributes significantly to the genetic improvement of the species. The present investigation revealed bud initiation in *Grewia tenax* in the fourth week of February and anthesis in the first week of March at 6.40 days of bud initiation. Whereas, fruits were set 5.85 days of anthesis. Peak anthesis of *Grewia tenax* flower was recorded between 1215-1230 h. Flower's durability was just a day. Anther dehiscence on the same day of anthesis in between 1400 and 1600 h. To utilize the full potential and further combine the desirable traits, present work may be beneficial for initiating breeding work in this species.

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Conflict of interest The authors proclaim that they have no conflict of interest.

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