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Morpho-physiological studies associated with flowering in high-density planted guava (*Psidium guajava*)

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ABSTRACT

The study was carried out to know the mechanism of less or no flowering in well maintained high-density planted (CHDP) guava (*Psidium guajava* L.) orchard (2 m × 2 m) during 2014 and 2015. Biochemical parameters determine flowering and fruiting in high-density planted guava orchard of 15 years old. Maximum length (17.48 cm), diameter (3.68 mm) of shoot and number of leaves pair (5.49) per shoot were found in flowering plants; highest leaf area (32.20 cm^2) was observed in non-flowering plants. With respect to biochemical parameters, total chlorophyll (5.05 mg/g), chlorophyll a (3.02 mg/g), chlorophyll a (2.03 mg/g), total sugars (15949.57 µg/g dry wt.), starch in leaves (2361.50 µg/g dry wt.) and roots (2024.45 µg/g dry wt.) were highest in matured leaves of flowering plants. Whereas, mature leaves of flowering plants of guava showed lowest Chlorophyll a: b ratio (1.58 mg/g). Anthocyanin content was highest (0.052 mg/g) in the immature leaves of flowering plants. Similarly, Chlorophyll b content in leaf was found less (0.72 mg/g) in the non-flowering plant compared to a flowering plant. Allahabad Safeda and L-49 cultivars are better among the four cultivars in such phenomenon of in bearing level of old guava orchard. Relevant information got in the study of Chlorophyll b, which indirectly plays important role in flowering and fruiting. Chlorophyll b increases the range of light, which is used by the plant for producing energy.

Keywords: Anthocyanin, Chlorophyll, Flowering, Guava, High-density planting

Guava (*Psidium guajava* L.) is most important fruit crop of India and fruit is often called "poor man's apple" though fruit is neither poor in nutritive value and nor commercial value (Singh *et al.* 2012). Area under guava in the country during 2018-19 was 265 thousand ha producing 4054 thousand MT with the productivity of 15.3 MT/ha. Guava contributes 3.4% of total fruit area and 3.9 % of total fruit production in India (Anon. 2018). Uttar Pradesh leads in production while Allahabad region of U P produces best quality of guava in the world. Guava is rich source of ascorbic acid, good source of dietary fiber and pectin. It can be processed into number of products like jam, jelly, nectar, juice, guava cake, puree etc. Its roots, bark, leaves, and fruits have great medicinal value. Plant population in case of high-density planted (HDP) guava is 5 to 20 times more than traditional planting system. It is difficult to achieve continuous higher levels of production right from plantation to long-term duration, but in case of HDP in guava that is, meadow orcharding requires canopy management. Due to pruning, penetration of sunlight due to which there is an increase in bearing of plant and also getting the even size and quality fruits. It was noticed that, after 12 to 15 years guava plantation, there is a decrease in bearing level of plants in particular season which are planted by meadow planting technique. HDP involves pruning technique, which is directly related to anabolic and catabolic life processes with respect to flowering and fruiting in a plant (Singh *et al.* 2005). With this approach, there is need to understand the low bearing or no bearing mechanism of 12 to 15 years HDP guava orchard. Keeping in a view the above facts, it is felt to undertake the research work on morpho-physiological studies associated with flowering in HDP cultivated guava as a need-based problem of guava growers in India. The major objective of the present work is to study the phenological pattern of the shoot of pruned guava and the biochemical parameters of leaves and roots of pruned guava.

MATERIALS AND METHODS

Research work was carried out at Plant Physiology Laboratory, Central Institute for Subtropical Horticulture, Lucknow (India) during 2014 and 2015. The climate of this area is semi-arid and subtropical with extremes of weather condition and the soil of the experimental field was light to medium in texture with good drainage within the depth 0.2–0.4 m. The mean maximum temperature ranges from 41–46°C in summer, minimum from 2–7^{0}C in winter and

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an annual rainfall of about 700 mm. The experiment was conducted in 15 years old meadow orchard having of 2 m × 2 m spacing. The treatment includes–Factor A: for varieties that is L 49/Sardar (A_1), Shweta (A_2), Lalit (A_3) and Allahabad Safeda (A_4). Factor B: Plant characteristics that is Flowering (B_1) and Non-flowering (B_2) and experiment was laid out in a factorial randomized block design (FRBD) with eight treatments replicated four times. All the observations were recorded at flowering stage in the ambe-bahar in late winter climatic situation. The aspect of non-flowering or less flowering was taken in the investigation with main focus were given all relevant growth and flowering parameters where the mechanism of the above mentioned problem was studied.

Growth parameters: Growth parameters like length, diameter of shoot were recorded with the help of Vernier caliper. Similarly numbers of pair of leaves per shoot were manually counted and leaf area was recorded with the help of graft paper manually.

Biochemical parameters: The fresh matured leaves of flowering and non-flowering plant were taken for biochemical analysis. The total sugar content was measured by hydrolyzing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharides by Anthrone method (Hedge 1962). Quantity of the carbohydrate was expressed as glucose equivalent as determined from linear regression obtained by plotting absorption against known standard glucose concentration. Reducing sugars were estimated by using Dinitrosalicylic Acid (DNSA) method (Miller 1972). For estimation of chlorophyll, 1 g fresh sample was taken after grinding by addition of 20 ml of 80% acetone and that was centrifuged at 500 rpm for 5 min. the volume was then made up to 100 ml with 80% acetone. The absorbance of the solution was then read at 645 and 652 nm against the solvent (80% acetone) blank. Anthocyanin was extracted with ethanolic HCL and measured calorimetrically by the procedure of Swain and Hillis (1959). With regards to starch content of roots, all sampled roots were oven-dried at 70°C for 48 h. The samples were ground, and 50 mg samples were then extracted with 5 ml of 0.1 M acetate buffer (*p*H 5.0) at 60°C for 40 min and centrifuged. After withdrawal of a 1 ml aliquot, 0.1 ml of thermostable α-amylase was added and the extraction was continued at 90°C for 60 min. Glucose, fructose, sucrose, fructan and soluble starch were determined in the first extract, and the second extract was used for determination of residual starch. Sucrose and fructose were hydrolyzed in 0.037 M sulphuric acid at 80°C for 70 min. The partially hydrolyzed or soluble starch was hydrolyzed to glucose by amylo-glucosidase at 60°C for 60 min. Glucose and fructose were determined by the glucose phosphate dehydrogenase method. Details of the methods are described by Steen and Larsson (1986).

RESULTS AND DISCUSSION

The length and diameter of shoots were found to be significant, the highest shoot length and diameter were found in flowering guava plants (17.48 cm and 3.68 mm) as compared to non-flowering guava plants (17.13 cm and 3.60 mm). Similarly, Allahabad Safeda cultivar was superior over rest of treatments with respect to the length and diameter of a shoot (25.95 cm and 3.80 mm) (Table 1). From the results, it is indicated that there is more length and diameter of shoot recorded in pruned trees of Allahabad Safeda and more diameter of shoot also recorded in Allahabad Safeda in the flowering guava plant as compared to non-flowering guava plants. This might be due to the effective rate of photosynthesis in flowering plants which lead to translocation of metabolites in newly emerged shoots lead to increase in vegetative growth of plants which may be less in case of non-flowering guava plants. Likewise, Dasarathi (1951) and Aravindakshan (1963) had also reported an increase in shoot growth in guava with the increased severity of pruning and pruning causes increasing rate of photosynthesis in the plants.

The leaf area was found to be significant in flowering plants. The maximum leaf area was recorded in flowering guava plants (32.20 cm^2) and less were recorded in non-flowering guava plants (31.42 cm^2) (Table 1). Cultivar L 49 was found greater in leaf area (35.45 cm^2) as compared to rest of varieties (Table 1). Regarding more leaf area of cultivars might be due to the sprouting ability of a genotype to produce more number of leaves per shoot. It also related to photosynthesis process and food supply, which resulted in more leaf area. More or less, a similar result was also reported by Kumar (2012) reported the presence of carbohydrates in the plants causes an increase in sprouting of leaves and increase in leaf area. Chlorophyll plays a relevant role in photosynthesis process in the plant. The Chlorophyll a content was found to be significant due to various varieties of flowering and non-flowering guava plants. The maximum Chlorophyll-a content in leaf was recorded in flowering plants (3.02 mg/g) and in cv. L 49 (4.00 mg/g) as compared to non-flowering guava plant and rest of cultivars (Table 2). Similarly, the highest Chlorophyll b content in mature leaf was recorded in flowering plants (2.03 mg/g) and in cv. L 49 (3.14 mg/g) as compared to non-flowering guava plant and rest of cultivars (Table 2). With above results, it can explain that there is a maximum content of Chlorophyll a as compared to Chlorophyll b. it might be due to the maximum density of plants that is also related to solar radiation and its penetration in the plant canopy. There is a more acropetal movement of stored food material towards new sprouted shoot and there is an increase in the rate of photosynthesis process of flowering guava plants as compared to a non-flowering guava plant. Above results up to some extent are in consonance with Siqueria *et al.* (2011) who reported the levels of pigments in guava, such as chlorophyll (6.8–21.70/g) in the fruit pulp of cultivar Pamula. The total sugar content was found to be significant due varieties in flowering and non-flowering guava plants. The maximum total sugar content was recorded in mature leaves of flowering guava plants (15949.57 µg/g dry wt.) and also maximum was recorded in variety Lalit

Table 1 Length, diameter of shoot, leaf area and Chl. a and b in flowering and non-flowering plants

Treatment	Length of shoot (cm)			Diameter of shoot (cm)			Leaf area (cm^2)			Chl. a (mg/g)			Chl. b (mg/g)		
	B_1	B_2	Mean A	B_1	B_2	Mean A	B_1	B_2	Mean A	B_1	B_2	Mean A	B_1	B_2	Mean A
A1	13.92	14.06	13.99	3.4	3.0	3.2	37.0	33.9	35.45	4.858	3.158	4.008	3.17	3.04	3.10
A_2	14.80	11.72	13.26	3.7	3.6	3.6	32.8	31.1	31.95	2.268	2.178	2.223	1.79	1.51	1.65
A_3	14.24	17.84	16.04	3.8	3.8	3.8	26.5	32.2	29.35	1.888	2.608	2.248	1.79	1.09	1.44
A_4	26.98	24.93	25.955	3.6	4.0	3.8	29.4	31.6	30.5	3.088	1.798	2.443	1.38	1.22	1.30
Mean B	17.485	17.138		3.625	3.6		31.42	32.2		3.025	2.435		2.03	1.71	
Factors	*CD@5%.*	*SE(d) ±*	*SE(m) ±*	*CD@5%*	*SE(d) ±*	*SE(m) ±*	*CD@5%*	*SE(d) ±*	*SE(m) ±*	*CD@5%.*	*SE(d) ±*	*SE(m) ±*	*CD@5%.*	*SE(d) ±*	*SE(m) ±*
Factor (A)	0.011	0.005	0.004	0.002	0.001	0.001	0.01	0.005	0.003	0.005	0.002	0.002	0.002	0.001	0.001
Factor (B)	0.007	0.004	0.003	0.001	0.001	0.000	0.007	0.003	0.002	0.004	0.002	0.001	0.001	0.001	0.000
Factor (A × B)	0.015	0.007	0.005	0.002	0.001	0.001	0.014	0.006	0.005	0.007	0.004	0.002	0.002	0.001	0.001

*A_1- L 49/Sardar, A_2-Shweta, A_3-Lalit and A_4-Allahabad Safeda. B_1-Plant characteristics that is flowering and B_2-Non-flowering.

Table 2 Total sugar, reducing sugar, anthocyanin, starch in leaves and starch in roots in flowering and non-flowering plants

Treatment	Total sugar in shoots (µg/g dry wt.)			Reducing sugar in leaf (µg/g dry wt.)			Anthocyanin (mg/g)			Starch in leaf (µg/g dry wt.)			Starch in root (µg/g dry wt.)		
	B_1	B_2	Mean A	B_1	B_2	Mean A	B_1	B_2	Mean A	B_1	B_2	Mean A	B_1	B_2	Mean A
A_1	13984.9	10790.7	12387.8	4625.4	4118.7	4372.0	0.003	0.007	0.005	1395.7	1000.0	1197.8	1411.4	1339.4	1375.4
A_2	15653.9	13380.6	14517.2	6130.3	4636.9	5383.6	0.005	0.005	0.005	2517.9	1,812.9	2,165.4	2231.4	1123.6	1677.5
A_3	18934.3	14013.6	16474.0	6265.4	5158.7	5712.1	0.002	0.006	0.004	2287.7	1906.4	2097.0	2411.3	1627.1	2019.2
A_4	15225.0	12979.5	14102.3	6679.3	4626.4	5652.8	0.004	0.003	0.003	3244.6	1834.6	2539.6	2043.5	1994.1	2018.8
Mean B	15949.5	12791.1		5925.1	4635.2		0.004	0.005		2361.5	1638.5		2024.45	1521.1	
Factors	*CD@5%.*	*SE(d) ±*	*SE(m) ±*	*CD@5%*	*SE(d) ±*	*SE(m) ±*	*CD@5.*	*SE(d) ±*	*SE(m) ±*	*CD@5%*	*SE(d) ±*	*SE(m) ±*	*CD@5%*	*SE(d) ±*	*SE(m) ±*
Factor (A)	5.762	2.752	1.946	14.23	6.79	4.80	0.000	0.000	0.000	0.537	0.258	0.183	0.287	0.138	0.098
Factor (B)	4.074	1.946	1.376	10.06	4.80	3.39	0.000	0.000	0.000	0.379	0.183	0.129	0.203	0.098	0.069
Factor (A × B)	8.148	3.892	2.752	20.12	9.61	6.79	0.000	0.000	0.000	0.759	0.366	0.258	0.405	0.195	0.138

*A_1- L 49/Sardar, A_2-Shweta, A_3-Lalit and A_4-Allahabad Safeda. B_1-Plant characteristics that is flowering and B_2-Non-flowering.

(16474.03 µg/g dry wt.) which were found to be superior over rest of cultivars (Table 2). As well as, the higher reducing sugar content was recorded in mature leaves of flowering guava plants (5925.12 µg/g dry wt.) and also maximum was recorded in variety Lalit (5712.11 µg/g dry wt.) and in Shweta (3727.32 µg/g dry wt.) (Table 2) which were found to be superior to rest of cultivars.

One important thing with respect to a flowering and non-flowering mechanism of 15 years old pruned guava is time of pruning, temperature and solar radiation. This plot was actually pruned in October; this time is actually a period of winter. There are less temperature and less availability of solar radiation and due to which proper C: N ratio is not maintained, which leads to less flowering or no flowering in plants. Results are less similar to the results obtained by Hossen *et al.* (2009) who observed total sugar (3%) and reducing sugar (2.75%) in the pulp of guava fruit of local cultivar. Tiwari and Lal (2007) reported that maximum number of fruits per branch were recorded in the treatments of two leaf pair pruning as compared to control ones. Singh *et al.* (2001) also reported that May and June pruned trees significantly set a maximum number of fruits per tree than control in guava.

The highest anthocyanin content in mature leaf was recorded in the non-flowering guava plant (0.005 mg/g) and in cultivar Allahabad Safeda (0.005 mg/g) as recorded in an leaf of the non-flowering guava plant as compared to rest of the varieties. There is the maximum content of anthocyanin in leaves as compared to flowering and guava plants it might due to the less content of chlorophyll and inactive photosynthesis process in leaves (Table 2). This finding is less or more related to the work of Siqueria *et al.* (2011) who reported the levels of pigments in guava that is anthocyanin (0.24–0.37 mg/100 g) in cultivar Pamula. The maximum starch content in the shoot was recorded in the flowering plant (2361.50 µg/g dry wt.) and lowest in the non-flowering guava plant (1638.50 µg/g dry wt.). Similarly, in case of starch content in the root of the plant was recorded maximum in the flowering guava plant (2024.45 µg/g dry wt.) and lowest in a non-flowering guava plant (1521.10 µg/g dry wt.). Whereas, in case of variety, the maximum starch content was recorded in a shoot of flowering plants of Allahabad Safeda cultivar (2539.62 µg/g dry wt.) which was more than the starch content in the root of the plant of the same variety. In case of starch content, it was less in the shoot of a flowering guava plant as compared to the root of a flowering plant of guava cultivars, but which are more as compared to non-flowering guava plants shoot and root (Table 2). So, in this case, it can be explained that starch is also very important component and plays important role in flowering and fruiting of a plant, maximum starch content in the root along with strong source functions leads to optimal growth and flowering in plants (Miyanishi and Kellman 1986).

It was concluded that reason of less or no flowering in the well managed high density planted guava is low temperature, less availability of solar radiation, dense canopy and plant density due to which relevant metabolites are not synthesized in sufficient quantity. In relation to that, content of Chlorophyll b was found to be the important parameter with respect to a flowering and non-flowering mechanism of 15 years old pruned guava. The role of Chlorophyll b which is very relevant for increasing the range of radiation and supportive to Chlorophyll a, which leads to photosynthesis process in plant and effective photosynthesis causes flowering and fruiting in the plant.

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