Physiological studies on flowering in coffee under South Indian conditions. V. Growth-substance content during flower bud enlargement and anthesis*

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COMPENDIO

Se estudiaron los cambios progresivos en el contenido de sustancias promotoras del crecimiento y sustancias inhibidoras del crecimiento en fracciones ácidas, neutrales y acuosas de yemas maduras de flores (antes de riego de aspersión), durante su renovado crecimiento (después del tratamiento con agua) y en flores plenamente abiertas, usando plantas de 11 años de Coffea arabica *L. cv 'S.795' que crecían en el campo. Se discute el papel fisiológico de estos contenidos de sustancias de crecimiento (promotoras e inhibidores) y su balance (contenido total de tres fracciones) durante el crecimiento de las yemas y la floración, a la luz del conocimiento existente sobre la influencia de hormonas endógenas de crecimiento específicas así como también de su balance en los mecanismos florales de las plantas de café* — Los autores

Introduction

FLOWERING phenomena in coffee is still one of the great mysteries of coffee technology. Even though coffee is a short-day plant (11), it is often stated that in regions close to the Equator flower bud initiation takes place at any period of the year where the day length remains practically the same throughout the year (26). However, even in Equatorial areas, coffee flowering exhibits a yearly periodicity. This appears to be due to well defined wet and dry seasons. In India, though flower buds are formed continuously starting from about September to March, the blossom is usually completed in one flush after the receipt of blossom showers. Thus, in India, the flowering habit falls under the latter category.

In many glass-house grown coffee varieties, it was found that flower buds are formed under short-day conditions (25, 27, 33). However, this cannot be safely inferred in view of the recent observations on photoperiodic response of mature trees of arabica coffee at Ruiru, Kenya (8). Also, the plants need not be grown continuously under short-day conditions until they produce flower buds which is practically impossible under natural ecoclimate. Rather it appears to be sufficient, if the plants get an inductive stimulus through short- photoperiods for the initiation of floral cells in axillary buds which ultimately leads to the formation of flower buds when factors other than photoperiods are favourable (14). The initiation of floral cells in axillary buds appears to be caused by endogenous hormonal balance (13), and this favourable concentration of hormone(s) might "set in" by the inductive stimulus of short-photoperiods.

Brief review of literature

Many aspects related to floral physiology are not yet clearly understood. Therefore, a series of investigations on flowering in coffee were initiated at Central Coffee Research Institute during 1970. The results obtained so far on different aspects of floral phenomena were reported (15, 22, 14, 19, 17, 20, 16, 18).

Under normal South Indian eco-climatic conditions and in the areas influenced by South-West monsoon

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rains, flower buds are usually visible on arabica coffee from September onwards, but in some years from October-November or even December flower buds continue to form in succession up to January-February (14), or even March, in the event of delayed blossom showers. After attaining a specific size, the mature "flower buds" stop growing into "open flowers" due to insufficient water in the soil around the plants, as also inside the plant including the flower buds at that period. Only after the fall of adequate quantity of rains (blossom showers) the flower buds resume their growth, and usually blossom in about 10 days (14). This pattern in coffee flowering is also common in Peru, Indonesia, Hawaii and Costa Rica (19).

The cessation of normal visible growth in mature flower buds, and after a small spell of drought their renewed enlargement and anthesis, with the advent of blossom showers or overhead irrigation in arabica coffee plants were attributed to diverse reasons which were recently reviewed (6, 7, 14). However, the preceding soil-moisture stress (1), or temperature shifts (26, 33), or rapid fall in ambient temperature also termed as chilling effect (9, 1, 6), do not appear to be the conclusive pre-requisites for the renewed growth and anthesis of flower buds after the cessation of their normal visible growth. Synchrony between the blossom of plants grown in glass-houses and under field conditions (12, 5) appears to be a rare phenomena, because the authors observed for over a number of years that the soil moisture around the plants raised in big earthenware crocks or around the plants grown in field under natural eco-climatic conditions when maintained approximately at field capacity, almost one hundred per cent blossom occurred (18). However, the flowering was slightly delayed or obtained earlier in these plants when compared to the blossom in the plants growing in pot cultures or in field which were not under experiment and responded to natural blossom showers.

Gopal and Vasudeva (14) described in detail the influence of many factors other than endogenous hormones, particularly soil moisture and water content of plants including flower buds on flowering of arabica coffee. It was stressed that opening of mature flower buds into flowers is, perhaps, controlled by a combination of factors, both external and internal, but not by a single factor (19). These authors also pointed out that the water content of mature flower buds is primarily responsible for the renewed growth after their normal visible growth was ceased, because of the fact that a) the quantitative balance of endogenous growth regulatory substances or other compounds and b) the rate of energy releasing metabolic processes is controlled to a great extent by the amount of water present in the whole plant and particularly in the mature flower buds (14, 13, 19).

Present status and object of the study

Recently, Browning (6, 7) described the internal mechanism with particular reference to the role of endogenous hormones abscisic acid, gibberellin and cytokinins on opening of mature flower buds into flowers in arabica coffee cvs 'SL 34' and 'SL 28', after a three week drought period. To find out the balance of total growth regulatory substances (promoters and inhibitors) and their progressive changes during renewed growth of mature flower buds (after their normal visible growth was ceased) and until anthesis, a detailed study has been carried out using arabica coffee plants.

Materials and methods

Three plants of *Coffea arabica* L. cv 'S 795' plants (11 years old) grown under natural shade at "M block" of Central Coffee Research Institute, were selected at random. The plants were free from soil-moisture stress (even no incipient wilting), nutritional disorders, pests and diseases. The situation, aspect and typical example of macro-climatological data of the farm area were previously given (14, 31). On each plant, five tertiary branches were tagged. On 19-3-1973, before blossom showers, mature flower buds at the fourth node from the tip of one branch from each of the three plants were collected. The plants were subjected to sprinkler irrigation with 80 litres of water per plant on 24-3-1973. Starting from two days after irrigation, the samples of flower buds (during enlargement) and flowers (on the first day of anthesis) were collected as was done for the first sample (Table 1).

Extraction of growth-substance content

At each time of sampling, 2 g of flower buds or flowers were extracted with cold 80 per cent methanol for 24 h at-15°C (30). The extracts were filtered through Whatman Nº 2 filter paper, and evaporated to dryness at 30°C. The residue was separated into acid, neutral and aqueous fractions (32). The extracts of the three fractions were evaporated at 30°C, and the residue was dissolved in 1.5 ml of 90 per cent ethyl alcohol.

Chromatography

Measured alcohol extracts of the three fractions were spotted on Whatman Nº 1 chromatographic paper and developed at laboratory temperature, by ascending technique, using the solvent isopropanol-NH_3-H_2O (by volume 8:1:1).

Bioassay

The developed and air dried chromatograms (unsprayed with any location reagents) were cut transversely into ten equal strips. Each strip was eluted in 2 ml of 2 per cent sucrose solution overnight, in dark, before assay. The biological activity of the eluates was determined by rice coleoptile straight growth technique (10).

Results

The data on the total growth-substance content (in terms of biological activity as indicated by growth in

Table 1.—Growth-promoting (GP) and inhibiting (GI) substances in mature flower buds, during flower bud enlargement and anthesis in arabica coffee 'S.795' plants (in terms of rice coleoptile growth, mm, and mean of six replications).

Days after sprinkler irrigation	Fraction	GP	GI	GP/GI
0*	Acid	0.4	6.2	0.06
(19-3-1973)	Neutral	0	6.9	0
	Aqueous	0.3	4.3	0.07
	Total	0.7	17.4	0.04
2	Acid	2.9	0	—
(26-3-1973)	Neutral	0.3	1.9	0.16
	Aqueous	2.0	0.5	4.00
	Total	5.2	2.4	2.16
4	Acid	3.9	0	—
(28-3-1973)	Neutral	8.8	0	—
	Aqueous	11.9	0	—
	Total	24.6	0	—
6	Acid	1.9	0	—
(30-3-1973)	Neutral	10.2	0	—
	Aqueous	11.9	0	—
	Total	24.0	0	—
8**	Acid	1.9	0	—
(1-4-1973)	Neutral	4.1	0	—
	Aqueous	6.6	0	—
	Total	12.6	0	—

* Before sprinkler irrigation (mature flower buds)

** Fully opened flowers on the first day of blossom

length, mm, of the rice coleoptiles) in the acid, neutral and aqueous fractions of the mature flower buds (before sprinkler irrigation) and during different stages of bud enlargement and of flowers (after irrigation) are presented in Table 1. Before sprinkler irrigation, in the mature flower buds, the total (total of the three fractions) content of growth inhibiting (GI) substance was 25 times more than that of growth-promoting (GP) substance (Table 1). At this stage, out of the three fractions, the GI substance was highest in the neutral fraction, and the GP substance was completely absent in this fraction. Even the other two fractions contained only very small amounts of GP substance. The ratio of GP/GI substance was very much below one, indicating a very high GI substance in the mature flower buds.

Two days after sprinkler irrigation, when the flower buds resumed their enlargement, the total content of GI substance of all the three fractions was considerably decreased, while the GP substance was remarkably increased. By four days after irrigation, the total content of GI substance was completely disappeared in all the three fractions, whereas the GP substance was further increased. The total content of GP substance at six days after irrigation was maintained at a higher level, except in acid fraction, and GI substance was continued to be absent. On the first day of blossom (eight days after irrigation), the total content of GP substance decreased by about 50 per cent of the previous amount, and there was no GI substance in any of the three fractions (Table 1).

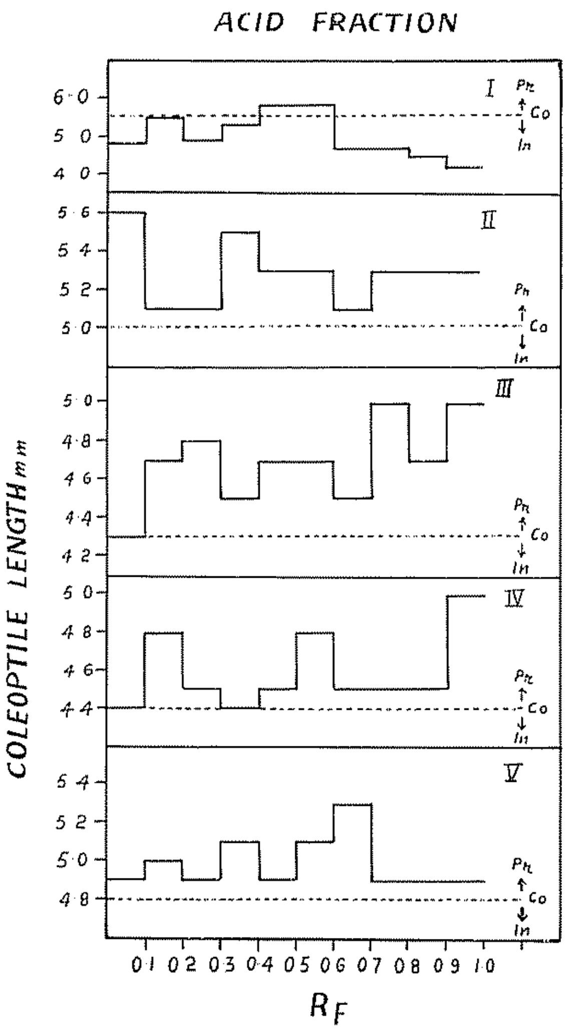

Fig. 1.—*Growth-substance content (in terms of rice coleoptile growth, mm, mean of six replications) of mature flower buds, during bud enlargement and anthesis in S.795 arabica coffee plants. I. Before sprinkler irrigation (mature flower buds); II: 2 days after sprinkler irrigation; III. 4 days after sprinkler irrigation; IV. 6 days after sprinkler irrigation; V. 8 days after sprinkler irrigation (fully opened flowers on the first day of blossom). Co: Control; In: Inhibitor; Pr: Promoter.*

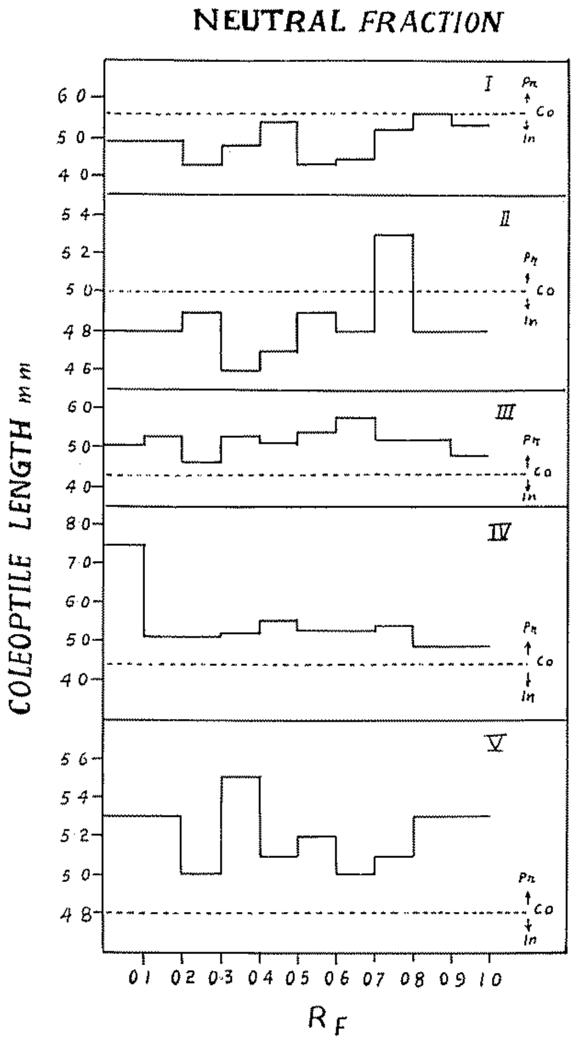

Fig 2.—*Same as described for Figure 1.*

The GP substance of aqueous fraction was the highest at all the stages of flower bud enlargement including the flowers (except in mature flower buds) than in the other two fractions, whereas the GI substance was maximum in the neutral fraction out of the three fractions in the mature flower buds (before sprinkler irrigation) and also at two days after irrigation.

In the mature flower buds before sprinkler irrigation, the GI activity was maximum at the Rf value of 1.0, 0.3 (also at 0.6) and 0.5, in the acid, neutral and aqueous fractions respectively (Fig 1, 2 and 3). Two days after irrigation, and with the renewed growth of flower buds, the GI activity was highest at the Rf value of 0.4 and 0.9 in the neutral and aqueous fractions, whereas the GP activity was maximum at the Rf value of 0.1, 0.8 and 0.1, in acid, neutral and aqueous fractions. Except in the acid fraction, the highest GP activity in the other two fractions was more or less obtained at the same Rf values, at four days after irrigation. On the first day of blossom (eight days after irrigation), the GP activity was maximum at the Rf value of 0.7, 0.4 and 0.1 (also at 0.4) in the three fractions (Fig 1, 2 and 3)

Discussion

The investigations described above revealed that the flower buds of arabica coffee cv 'S.975' plants mature by the second fortnight of February at this elevation (about 823 m), and respond well to blossom by sprinkler irrigation. The flowering occurred on the ninth day after water supply. However, under South Indian conditions, due to natural eco-climate, there is a coincidence between the mature flower bud stage and low moisture status in the soil around the feeder root zone (the portion of the root system having the maximum water absorption capacity). This condition may or may not induce wilting in plants due to this low soil moisture, which depends on the intensity of permanent and temporary shade, soil organic matter and mulch around the plants. The plants used in this study did not exhibit any signs of wilting due to low soil moisture at the

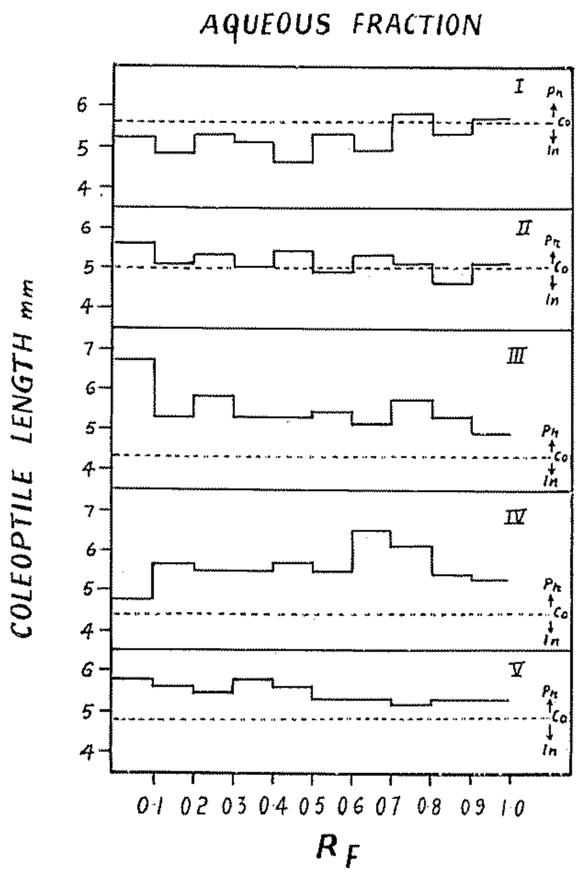

Fig. 3.—*Same as described for Figure 1*

time of sprinkler irrigation treatment. But, still the plants responded for resumption of flower bud enlargement and blossom with sufficient water supply. These findings once again showed that preceding soil-moisture stress to the point of leaf wilting is not necessarily a pre-requisite for renewed growth of flower buds and flowering in this coffee cultivar.

However, during the above stage of flower buds (which coincides with drought period) and before the receipt of blossom showers, it was observed that the water content of mature flower buds was less. The decrease in water content of flower buds from February to March, was found to vary from 1.04 to 6.93 per cent during different years, depending on the amount of rainfall from January until the receipt of blossom showers (4, 14). The rate of metabolic activities was also low in these plants due to low soil-moisture than in the plants with a soil moisture of three fourths of the field capacity or around field capacity (2, 3, 4, 19). Gopal and Vasudeva (14) and Gopal *et al.* (19) discussed in detail about this lower water content of mature flower buds during March (before blossom showers) and its impact on the further growth of flower buds and their opening into flowers. They also enunciated the decisive role of hydric balance between the plant as a whole and the flower buds, and its implications on the growth of flower buds during the drough period. Therefore, water is the primary factor responsible for the resumption of flower bud enlargement and ultimate blossom (after their visible growth was ceased). The associated changes in chemical energy released during the metabolic processes as well as hormonal balance after the intake of sufficient water by the plants also appears to be of vital importance for further reproductive changes in the mature flower buds, after sprinkler irrigation was given or with the advent of natural blossom showers (14, 13, 19).

With sufficient water available to the plants there were changes not only in the various metabolic activities including respiration (2, 3, 4, 13, 19), but also in the growth-substance content as obtained in the present investigations. Recently, Browning (6) postulated that resumption of enlargement of mature flower buds after their growth was ceased, appears to be operated by the absolute quantities of endogenous abscisic acid and gibberellin in the flower buds. Later, Browning (7) reported that simultaneous changes in endogenous quantities of cytokinins along with abscisic acid and gibberellin during flower bud enlargement were also responsible for the resumption of growth in mature flower buds and their ultimate blossom. However, in both these studies, the changes in these endogenous growth hormones occurred only after the over-head sprinkler irrigation was given to the plants, and before the water treatment the hormonal balance was quite different.

In the present study, it was observed that before sprinkler irrigation, the content of total endogenous growth-inhibiting substances was very high in the mature flower buds. After the water treatment and during the enlargement of flower buds the total content of growth-inhibiting substances was drastically reduced with simultaneous increase in the content of total growth-promoting substances (by four days after irrigation). Finally on the day of first blossom, the fully opened flowers contained only growth promoters without any inhibitors of growth (Table 1). Thus, these results indicate that after the plants were irrigated with sufficient water, the shift in the changes of total growth-substance content towards more promoters and finally the complete disappearance of inhibitors appear to have played a beneficial role in the resumption of flower bud enlargement and opening of flower buds into flowers. These changes in mature flower buds might have been induced by endogenous total hormonal balance comprising of total content of growth regulatory substances, but not by any one single endogenous growth hormone. Even here, the changes in the balance of total growth-substance content (promotors and inhibitors) occurred only after the supply of sufficient water to the plants.

The role of endogenous growth regulatory substances on flowering mechanism in perennial woody plants and in other plants in general has been documented in detail (28, 29, 21, 24). It is extremely difficult to decide whether or not a particular endogenous hormone influences a given physiological process like flowering; rather its control is, perhaps, best enunciated by "hormonal balance" with a combination of all the growth regulatory substances in the plants. This point has to be considered in view of the fact that the growth inhibitory effects of certain phenolic fractions and abscisic acid possess some common features, and like-wise between different growth-promoting substances (23). The above facts points out for a cautious approach while explaining the role of a specific endogenous growth hormone in a complex reproductive process like flowering of coffee.

Summary

The progressive changes in the content of growth-promoting and growth-inhibiting substances in acid, neutral and aqueous fractions of mature flower buds (before sprinkler irrigation), during their renewed growth (after water treatment) and in fully opened flowers were studied using 11 year old *Coffea arabica* L. cv 'S 795' plants growing in field. The physiological role of these growth-substance contents (promoters and inhibitors) and their balance (total content of three fractions) during the flower bud enlargement and blossom is discussed in the light of existing knowledge on the influence of specific endogenous growth hormones as well as their balance in flowering mechanism of coffee plants.

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