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Soil Available Phosphorus Deficiency Reduces Boll Biomass and Lint Yield by Affecting Sucrose Metabolism in Cotton-Boll Subtending Leaves

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Abstract: Soil available phosphorus (AP) deficiency and shortage of phosphate rocks limit cotton production in China. Therefore, pool-culture experiments were conducted in 2019 and 2020 using two cotton cultivars (CCRI-79, low-P tolerant; SCRC-28, low-P sensitive) under three soil AP levels (P_0: 3 ± 0.5, P_1: 6 ± 0.5, and P_2 (control): 15 ± 0.5 mg kg^{-1}) to ascertain the effect of soil AP on boll biomass and lint yield. P_0 and P_1 decreased the P concentration and net photosynthetic rate (Pn) of subtending leaves, thus, reducing boll biomass and lint yield. Additionally, soil AP deficiency decreased boll wall:boll, lint:boll, and lint:seed, and increased seed:boll ratio. Upper fruiting branch positions (FB_{9-12}) had higher lint:seed ratio and proportion of the total lint yield under low soil AP. Moreover, soil AP deficiency also reduced the sucrose transformation rate (Tr) and activities of sucrose-metabolizing enzymes, such as ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), sucrose phosphate synthase (SPS), and sucrose synthase (SuSy), while increased carbohydrate levels (soluble sugar, sucrose, and starch) and the activity of cytosolic fructose-1,6-bisphosphatase (cy-FBPase) in the subtending leaves. The sucrose and starch contents, cy-FBPase, and SPS activities of SCRC-28 were more sensitive to low soil AP than CCRI-79. Higher Tr and activities of initial Rubisco and SuSy in the subtending leaves improved boll biomass and lint yield.

Keywords: cotton; soil available phosphorus; sucrose metabolism; fruiting branch position; boll biomass; lint yield

1. Introduction

Cotton is an essential cash crop grown in approximately 76 countries [1]. In China, cotton production increased from 444 thousand tons in 1949 to 6096 thousand tons in 2018, with the highest-ranking worldwide output between 1983 and 2015 [2], and reached 5731 thousand tons in 2021 [3]. However, an insufficient supply of phosphorus (P) fertilizer during the 1980s limited cotton production in the Yellow River Valley. A similar trend was observed in the middle- and low-yield areas of Shandong and Henan provinces that highly depend on P fertilizer. However, the P fertilizer effect on cotton yield declined gradually in the Yangtze River valley [4]. Numerous studies have documented how P fertilizer affects cotton yield and fiber quality [5–8], but the results differ. For example, the boll number reduced by 8–40% and yield by 10–40% under P deficiency conditions [9,10]. Dorahy et al. [10] also reported that in 17 experiments of P application in eastern Australia,

only three experiments indicated a significant increment in lint yield. Similarly, prior studies unraveled varied effects of the intensity and nature of P fertilization on fiber quality [5–7,11]. Several factors, including the P status of the soil, or other external factors, such as water [10] and nitrogen (N) fertilizer [12], probably determine the effects of P fertilizer on cotton production.

Phosphorus is required for crop development and growth, as it promotes cell division and stimulates flower bud and boll formation in cotton plants [13]. In contrast, P deficiency seriously limits cotton growth [14] by decreasing the net photosynthetic rate (*Pn*) [15], resulting in low yield [6]. Thus, P fertilizers are applied to complement soil available P. Unfortunately, metallic elements in soils, such as aluminum, iron, calcium, and magnesium, fix most of the soil P, making it unavailable to plants [16,17]. The range of total P content in the world was 200–5000 mg kg^{-1}, and that in China was 200–1100 mg kg^{-1} [18]. Globally, 5700 million hectares have insufficient soil available P (AP) [19,20], and approximately 30% of the cultivated land in China has merely 3–5 mg kg^{-1} of soil AP [21] using the Olsen-P method [22].

Phosphorus deficiency reduced leaf *Pn* but caused massive starch accumulation in rice shoots [23]. Meanwhile, low-P levels increased the amounts of soluble sugar, sucrose, and starch in bean [24], tomato [25], and cotton [26] leaves. Hermans et al. [27] showed that P deficient arabidopsis accumulates more carbohydrates in its leaves. However, low-P decreased the soluble sugar content of wheat flag leaves [28]. These contrasting reports might be related to the materials and treatments used per study. Nonetheless, the increase/decrease in carbohydrate contents in leaves under low-P ultimately inhibited plant photosynthesis and growth in the different studies [29–31].

Carbohydrates can help cotton plants cope with environmental stress by increasing the osmotic pressure in leaves (capacity of humidity guarantee) and roots (capacity of uptake), especially non-structural carbohydrates (sucrose and starch) [32,33]. Photosynthesis of the leaf subtending the cotton boll produces the carbohydrates necessary for cotton boll development, especially sucrose. Therefore, studying sucrose physiological metabolism in the subtending leaves under low-P is crucial [34].

Rubisco is the key enzyme that determines the carbon assimilation rate during photosynthesis. Sucrose phosphate synthase (SPS) and cytosolic fructose-1,6-bisphosphatase (cy-FBPase) are the key rate-limiting enzymes for sucrose synthesis, and their activities are closely related to the sucrose content in plant leaves [35–37]. Meanwhile, sucrose synthase (SuSy) catalyzes sucrose synthesis and decomposition [38,39]. Coordinated actions of SPS, SuSy, and cy-FBPase in cotton leaves improved the source (subtending leaf)-sink (cotton boll) sucrose concentration gradient and transportation [40]. Low-P affects the activities of sucrose-metabolizing enzymes [41]. Rubisco activity decreased by 76 and 42% in spinach and maize leaves [42,43] under low-P stress, but its activity was unaffected in sugar beet leaves [44]. Moreover, P deficiency increased SPS and cy-FBPase activities by 97 and 58% in sugar beet leaves [45]. In contrast, SPS and cy-FBPase activities decreased by 4 and 44% in maize leaf under low-P treatment [43]. Meanwhile, P deficiency hardly affected the SuSy activity of sugar beet and maize leaves [43,45]. However, low-P caused lower SuSy activity in tobacco shoots (over ten-fold) and roots (eight-fold) than adequate-P supply [46]. Beans had higher SuSy activity (over two-fold) in root tips [33]. Therefore, different crops and organs have different sucrose metabolic responses under low-P. Many studies showed that P deficiency affects carbohydrate accumulation and distribution in cotton leaves and bolls [47,48], but the explanation for this response is lacking.

Subtending leaves are the main carbohydrate donors for cotton bolls, and boll biomass is an important factor in cotton yield [8]. Therefore, the physiological characteristics of subtending leaves directly affect carbohydrate synthesis and transportation for cotton boll development [49]. During growth and development, cotton plants maintain an indeterminate growth pattern; thus, cotton bolls and subtending leaves at different fruiting branch positions (FBPs) have different ecological environments [50,51]. However, most analyses of the influence of P fertilization on cotton yield were based on the total plant [6,8].

In contrast, some studies have demonstrated that cotton bolls at different FBPs respond differently to nutrient stress [52,53]. The above reports suggest that cotton bolls at different FBPs probably respond differently to P fertilization. However, there are no reports on the effects of low soil AP content on boll biomass, lint yield, and the levels of carbohydrate and sucrose metabolic enzyme activities in subtending leaves of different FBPs.

Thus, this study investigated (1) the effect of low soil AP contents on the carbohydrate content and activities of sucrose-metabolizing enzymes in the subtending leaves, boll biomass, and lint yield under different FBPs; (2) the key carbohydrate and sucrose metabolic enzymes available under low soil AP contents; and (3) the relationship between source indices (carbohydrate content and sucrose-metabolizing enzyme activities) and sink indices (boll biomass and lint yield) under low soil AP. These results will inform cotton production under soil AP deficiency.

2. Materials and Methods

2.1. Description of Experimental Site

Pool-culture experiments were performed at the Institute of Cotton Research, Chinese Academy of Agricultural Sciences (Anyang, Henan Province, China; 36°06′ N and 114°21′ E) in 2019 and 2020. The soil type is classified as Inceptisols (USDA Soil Taxonomy). The experimental soil was clay loam [2], and the soil nutrient profile within 0–20 cm of the soil layer before sowing is presented (Table 1). Figure 1 shows the weather data (including sunshine duration, average temperature, and precipitation) during the 2019–2020 cotton-growing periods.
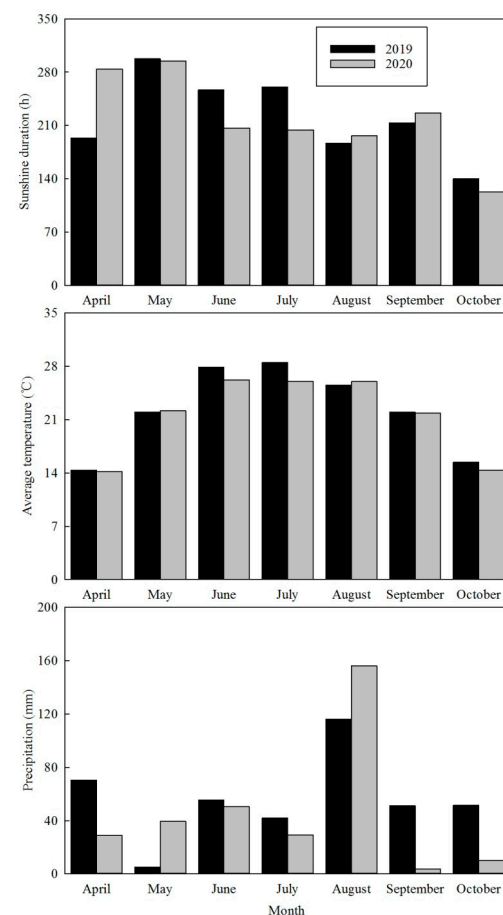

Figure 1. Monthly meteorological data during the 2019–2020 cotton-growing periods at the test station in Anyang. The meteorological data were obtained from an automatic weather station located ~4 km from the test station.

Table 1. Nutrient properties of the top 0–20 cm soil from the experimental site during the 2019–2020 cotton-growing periods.

Year	OM [1]	TN	AN	AK	pH
	(g kg^{-1})		(mg kg^{-1})		
2019	12.9	0.86	64.4	163.6	7.7
2020	13.1	0.85	63.3	180.4	7.6

[1] OM: organic matter; TN: total nitrogen; AN: available nitrogen; AK: available potassium.

2.2. Experimental Design and Field Management

Two cotton cultivars with different responses to low-P, CCRI-79 (low-P tolerant) and SCRC-28 (low-P sensitive) were used in this study based on our previous study [26]. The experiment was conducted in a completely random block design at three soil AP levels [26]. The three soil AP levels included: 3 ± 0.5 mg kg^{-1} (P$_0$, severe soil AP deficiency), 6 ± 0.5 mg kg^{-1} (P$_1$, mild soil AP deficiency), and 15 ± 0.5 mg kg^{-1} (P$_2$, as a control) using triple superphosphate (44% P$_2$O$_5$) to adjust soil AP content. The rate of P fertilizer was 0, 50.6, and 202.4 g pool^{-1} for P$_0$, P$_1$, and P$_2$ in 2019 and 2020. Each pool contained 225 kg N ha^{-1}, with total nitrogen (N), as urea (46% N). The basal fertilizers included 50% total N, total P, and 150 kg K$_2$O ha^{-1} as potassium sulfate (51% K$_2$O). Moreover, the top application included 50% N fertilizer applied at the beginning of the flowering period [54]. The fertilizers were thoroughly mixed with soils 7 d before planting. After fertilization, each pool was watered sufficiently to dissolve the fertilizer. One month after base manure application, the soil samples were collected, air dried, filtered through a 1 mm sieve, and profiled for AP content using the Olsen-P method [22]. The cotton seeds were sown with a manual drilling machine, leaving 80 cm row spacing. Each pool had five rows with 14.4 m^2 area and 52,500 plants ha^{-1} density. Therefore, the experiments were conducted following the high-yield cotton cultivation management protocol.

2.3. Sampling and Processing

When plants entered the flowering stage, the white flowers of FB$_{2-3}$, FB$_{6-7}$, and FB$_{10-11}$ at the first fruiting position were marked with white plastic tags to record the flowering date [40]. From 10 days post-anthesis (DPA), 8–10 subtending leaves were harvested at 9:00–10:00 a.m. daily for seven days, and the harvested samples were washed in distilled water. Half of the sampled leaves were frozen with liquid nitrogen and stored at -80 °C until enzyme activity determination. In comparison, the other half was dried at 105 °C for 30 min followed by 70 °C until the weight remained unchanged for P concentration and carbohydrate content determination.

When the bolls opened, 20 bolls with boll walls were harvested manually at FB$_{1-4}$, FB$_{5-8}$, and FB$_{9-12}$ [55]. Subsequently, the bolls were divided into boll walls, seeds, and lint. The boll walls and seeds were dried at 70 °C, and the lint at 35 °C to a stable state and weighed. The lint percentage was determined after ginning cotton lint from seeds. The final boll number (according to the FBP) was determined from ten uniformly grown cotton plants. Next, the lint yield at the three FBPs was calculated by the total biomass of lint.

2.4. Leaf Photosynthesis

The net photosynthetic rate (*Pn*) of the subtending leaves at the first fruiting positions of FB$_{2-3}$, FB$_{6-7}$, and FB$_{10-11}$ were determined at 10, 17, 24, 31, 38, and 45 DPA (9:00 to 11:00 a.m.). The LI-6400XT portable photosynthetic apparatus (LI-COR Biosciences, NE; Li-Cor, Inc., Lincoln, NE, USA) determined the leaf *Pn* under the settings: light intensity, 1500 µmol m^{-2} s^{-1}; relative humidity, 65 ± 5%; leaf temperature, 32 ± 2 °C; CO$_2$ concentration, 380 µmol mol^{-1}.

2.5. Leaf Phosphorus Concentration and Carbohydrate Content

The dried leaf samples were grated using an FS-II disintegrator (Zhejiang Top Yunnong Technology Co., Ltd., Hangzhou, Zhejiang, China) with a 0.5 mm sieve. The P concentration was determined using the colorimetric method on an ultraviolet SPECORD 40 spectrophotometer (Analytik Jena GmbH, Jena, Germany).

The dried leaf powder (0.1 g) was put in a 10 mL centrifuge tube containing 80% (*v/v*) ethanol (5 mL) and mixed for 30 min at 80 °C to extract carbohydrates. After centrifugation ($10,000 \times g$, 5 min), the supernatant was poured into a 25 mL volumetric flask. The extraction was performed twice and finally collected and topped to 25 mL with 80% (*v/v*) ethanol. The soluble sugar and sucrose contents were determined following a previous method [56]. The soluble sugar and sucrose extraction residue was mixed with distilled water (2 mL) and incubated in boiling water for 15 min.

The residue was cooled to 25 °C, $HClO_4$ (2 mL, 9.2 M) was added to the centrifugal tube and chilled in an ice bath for 15 min to extract starch. Distilled water (4 mL) was added to the extract, mixed, and centrifuged at $10,000 \times g$ for 10 min, and the supernatant was collected. Next, the procedure was repeated using $HClO_4$ (2 mL, 4.6 M). Finally, all the supernatants were combined and mixed with distilled water to 25 mL. The starch content was determined using the sulfuric acid anthrone colorimetric method at 620 nm, and the standard curve was drawn using different glucose contents [57].

2.6. Analysis of Leaf Enzymes Activities

The frozen leaf tissues (0.2 g) were ground in an ice bath with cold buffer containing Tris HCl (50 mM, pH 7.5), $MgCl_2$ (1 mM), ethylene diaminetetraacetic acid (1 mM), glycerol(12.5%, *v/v*), polyvinylpyrrolidone (10%, *w/v*), and β-Mercaptoethanol (10 mM). The homogenate was centrifuged at $15,000 \times g$, 0–4 °C for 15 min, and the supernatants were reserved for determining the ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco, E.C. 4.1.1.39) activity using the method of Liu et al. [58].

Cy-FBPase (E.C. 3.1.3.11) was extracted from frozen leaf samples (0.3 g) with buffer containing Tris-HCl (100 mM, pH 8.2), glycerine (5%, *v/v*), EDTA (1 mM), and β-mercaptoethanol (15 mM) [59]. Briefly, 1 mL of the reaction solution contained Tris-HCl (100 mM, pH 8.2), $MgCl_2$ (5 mM), EDTA (5 mM), NADP (0.5 mM), phosphoglucoisomerase (2 units), glucose-6-phosphate dehydrogenase (1 unit), and an enzyme solution (50 µL). The reaction was started by fructose-1,6-bisphosphate (60 µL, 60 mM) at 25 °C. The cy-FBPase activity was determined from the per-minute change of light absorption at 340 nm.

However, SPS (E.C. 2.4.1.14) and SuSy (E.C. 2.4.1.13) were extracted using a previously described method [60]. Essentially, each reaction solution (550 µL) contained 50 mM fructose-6-P (for SPS) or fructose (for SuSy in the sucrose synthesis direction), 50 mM of UDP-glucose, 50 mM of extraction buffer, 10 mM of $MgCl_2$, and 200 µL of the supernatant. The mixture was incubated at 30 °C for 30 min, and the reaction terminated using 100 µL 2 mol L^{-1} NaOH and heated to 100 °C for 10 min. Finally, the mixture was cooled to 25 °C and mixed with 0.1% (*w/v*) resorcin (1 mL) in 95% (*v/v*) ethanol and 30% (*w/v*) HCl (3.5 mL) before incubation at 80 °C for 10 min. The activities of SPS and SuSy were measured using the colorimetric method at 480 nm.

2.7. Statistical Analysis

The means, standard errors, and coefficients of variation (CV, %) were determined using MS Excel 2007 (Microsoft corp., Redmond, WA, USA). The means were subjected to 5% variance analysis with the SPSS statistical software Version 18.0 (IBM corp., New York, NY, USA), using the least significant difference (LSD). The Pearson correlation established the specific relevance of individual variables. The sucrose transformation rate (Tr) of subtending leaves to cotton bolls was determined following the Liu et al. [58] protocol as follows:

Tr = (maximum sucrose content − minimum sucrose content)/maximum sucrosecontent × 100%

3. Results

3.1. Phosphorus Concentration in the Subtending Leaves

Soil available phosphorus (AP) deficiency (P_0 and P_1) reduced the P concentration of leaves subtending the cotton bolls (Figure 2). Soil AP Deficiency (P_1 and P_0) reduced the average leaf P concentration across the sampling times by 9.8–10.6% and 24.3–24.9% (CCRI-79), and 14.4–18.9% and 32.0–38.7% (SCRC-28), respectively, for FB_{2-3}, FB_{6-7}, and FB_{10-11} (Figure 2). In SCRC-28, soil AP deficiency decreased the P concentration in the FB_{10-11} subtending leaves compared to FB_{2-3} and FB_{6-7}. In contrast, the effect was negligible among the three FBPs of CCRI-79 (Figure 2). Additionally, the P concentration in the subtending leaves decreased gradually over increasing days post-anthesis (DPA) and fruiting branch positions (FBP) (Figure 2).
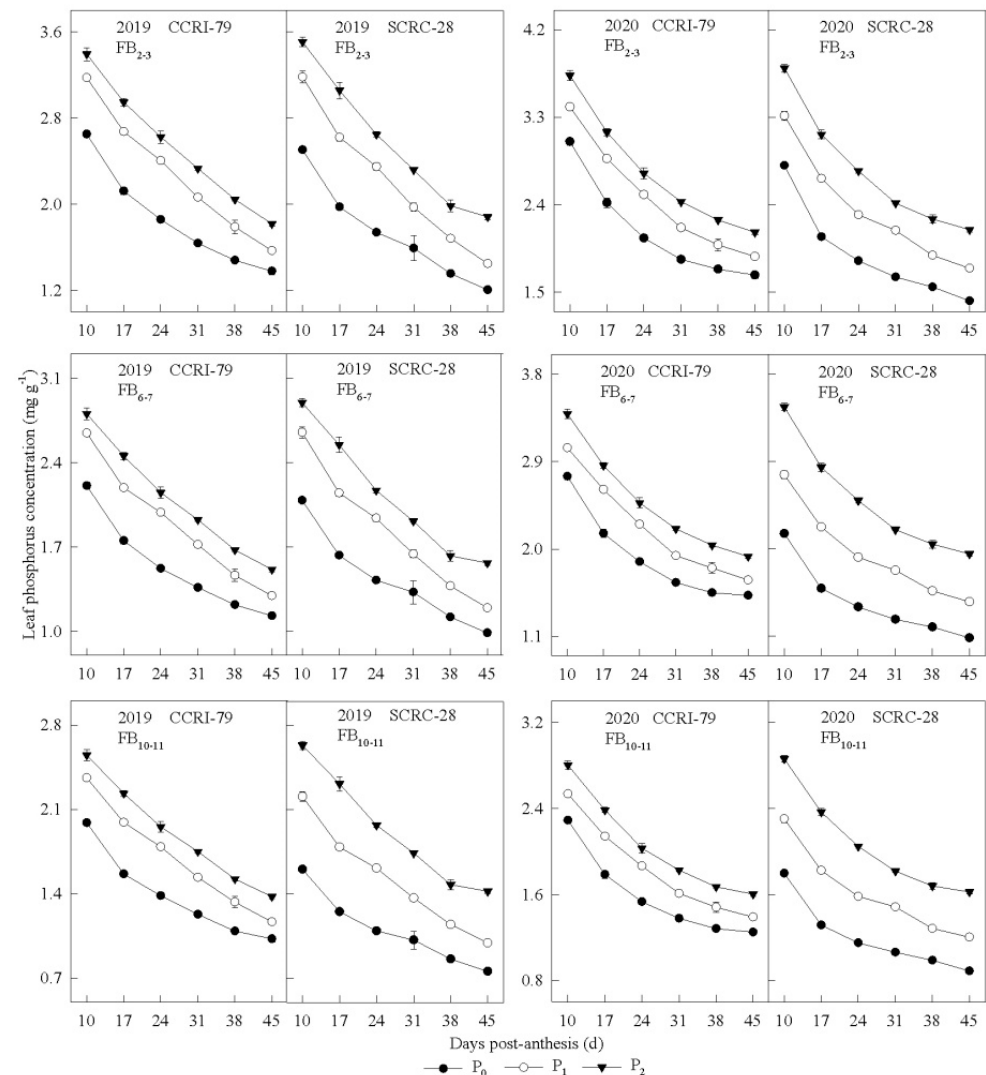

Figure 2. The effect of soil available phosphorus (AP) contents on the phosphorus (P) concentration in the subtending leaves during the 2019–2020 cotton-growing seasons. d: day. FB: fruiting branch. P_0: 3 ± 0.5 mg kg^{-1}. P_1: 6 ± 0.5 mg kg^{-1}. P_2: 15 ± 0.5 mg kg^{-1}.

However, increasing the soil AP content (P_0 to P_2) decreased the decline rate of leaf P at the three FBPs (except for P_1 for both cultivars during the two years). The average concentration of leaf P in CCRI-79 and SCRC-28 was 1.7–2.3 mg g^{-1} and 1.5–2.3 mg g^{-1} for the three soil AP treatments, but the variation was higher in SCRC-28 than CCRI-79. Under P_1 and P_0, the concentrations of leaf P were 8.1 and 18.3% higher in CCRI-79 than SCRC-28 and 1.1% lower than SCRC-28 under P_2 (Figure 2).

3.2. Pn and Carbohydrate Content in the Subtending Leaves

The *Pn* of subtending leaves from the three FBPs declined with increasing DPA (Figure 3). Under P_1 and P_0, the *Pn* at the three FBPs decreased by 2.0–7.5% and 5.0–14.7% in CCRI-79, and by 4.6–12.2% and 9.0–19.9% in SCRC-28 over the two years (Figure 3). In addition, the subtending leaves of FB_{10-11} registered lower *Pn* decline rates than FB_{2-3} and FB_{6-7} from both cultivars in the two years. The *Pn* of CCRI-79 was 6.7 and 8.8% higher than SCRC-28 under P_1 and P_0 treatments, respectively. In contrast, the *Pn* was 3.4% higher in CCRI-79 than SCRC-28 in 2019 and 2020 under P_2 treatment (Figure 3).

Figure 3. The effect of soil available phosphorus (AP) on the net photosynthetic rate (*Pn*) of subtending leaves in 2019 and 2020 cotton-growing seasons. d: day. FB: fruiting branch. P_0: 3 ± 0.5 mg kg^{-1}. P_1: 6 ± 0.5 mg kg^{-1}. P_2: 15 ± 0.5 mg kg^{-1}.

The soluble sugar contents of the subtending leaves increased between 10 and 17 DPA and reduced between 17 and 45 DPA (Figure 4). Essentially, the soluble sugar content increased by 5.1–11.5% and 9.7–22.1% in CCRI-79, and 5.1–10.6% and 10.7–21.0% in SCRC-28 under P_1 and P_0 treatments, respectively, across the FBPs and years.
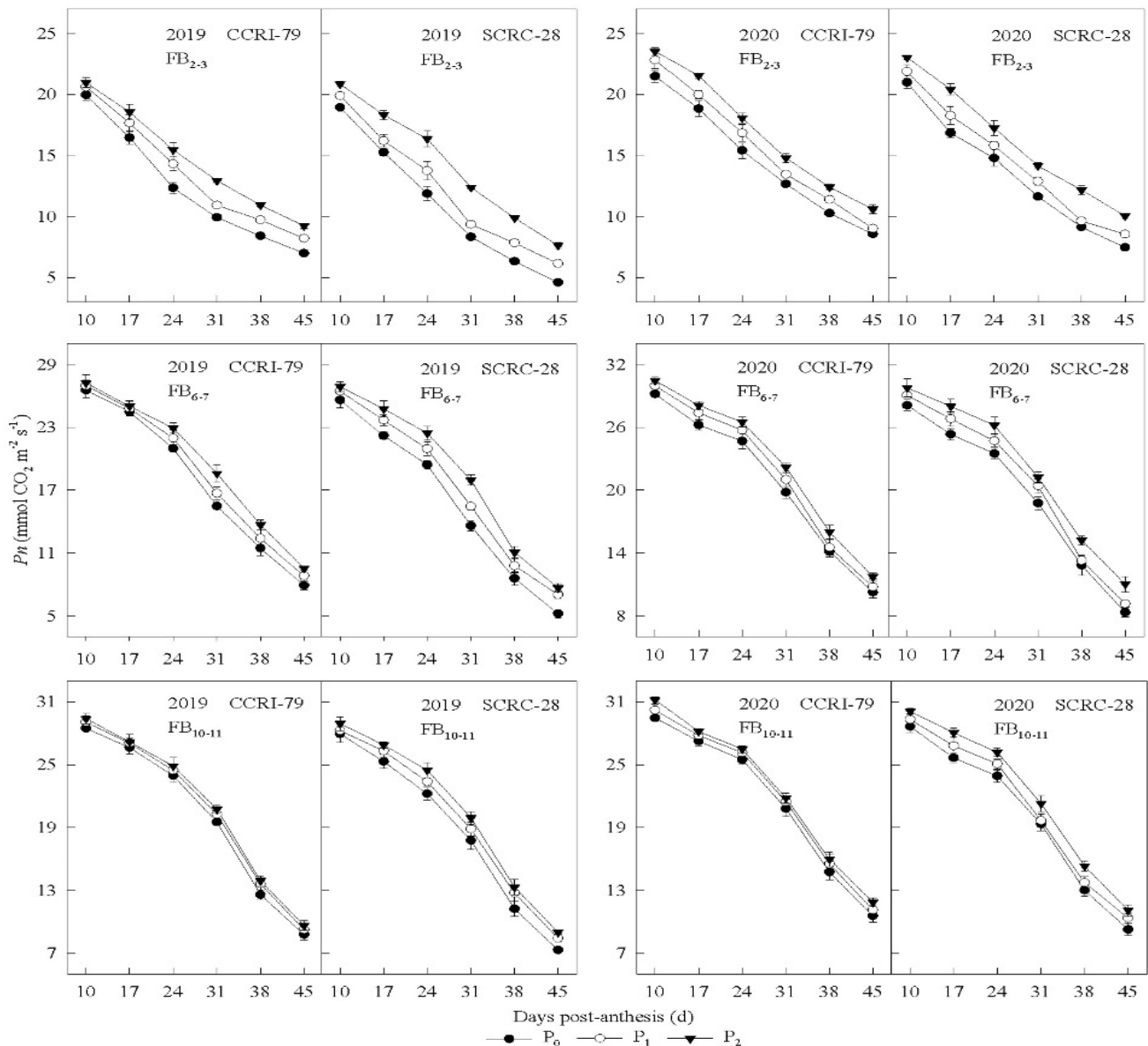
Figure 4. The effect of soil available phosphorus (AP) on the soluble sugar content of subtending leaves in 2019 and 2020 cotton-growing seasons. d: day. FB: fruiting branch. P_0: 3 ± 0.5 mg kg^{-1}. P_1: 6 ± 0.5 mg kg^{-1}. P_2: 15 ± 0.5 mg kg^{-1}.

Nonetheless, the sucrose content in the subtending leaves reduced with increasing DPA, especially during the early (10–17 DPA) than the late stage (17–45 DPA) (Figure 5). In CCRI-79, the sucrose content increased by 10.2–13.8% and 21.6–28.3%, respectively, under P_1 and P_0 compared to P_2 at the three FBPs in 2019 and 2020. A similar trend was recorded in SCRC-28 with 10.1–17.4% and 24.5–29.1% increments under the same condition and growth period (Figure 3). Increasing soil AP (P_0 to P_2) decreased the sucrose contents and maximum/minimum sucrose contents of both cultivars at the three FBPs (Table 2). However, the sucrose transformation rate (Tr) increased with increasing soil AP content.
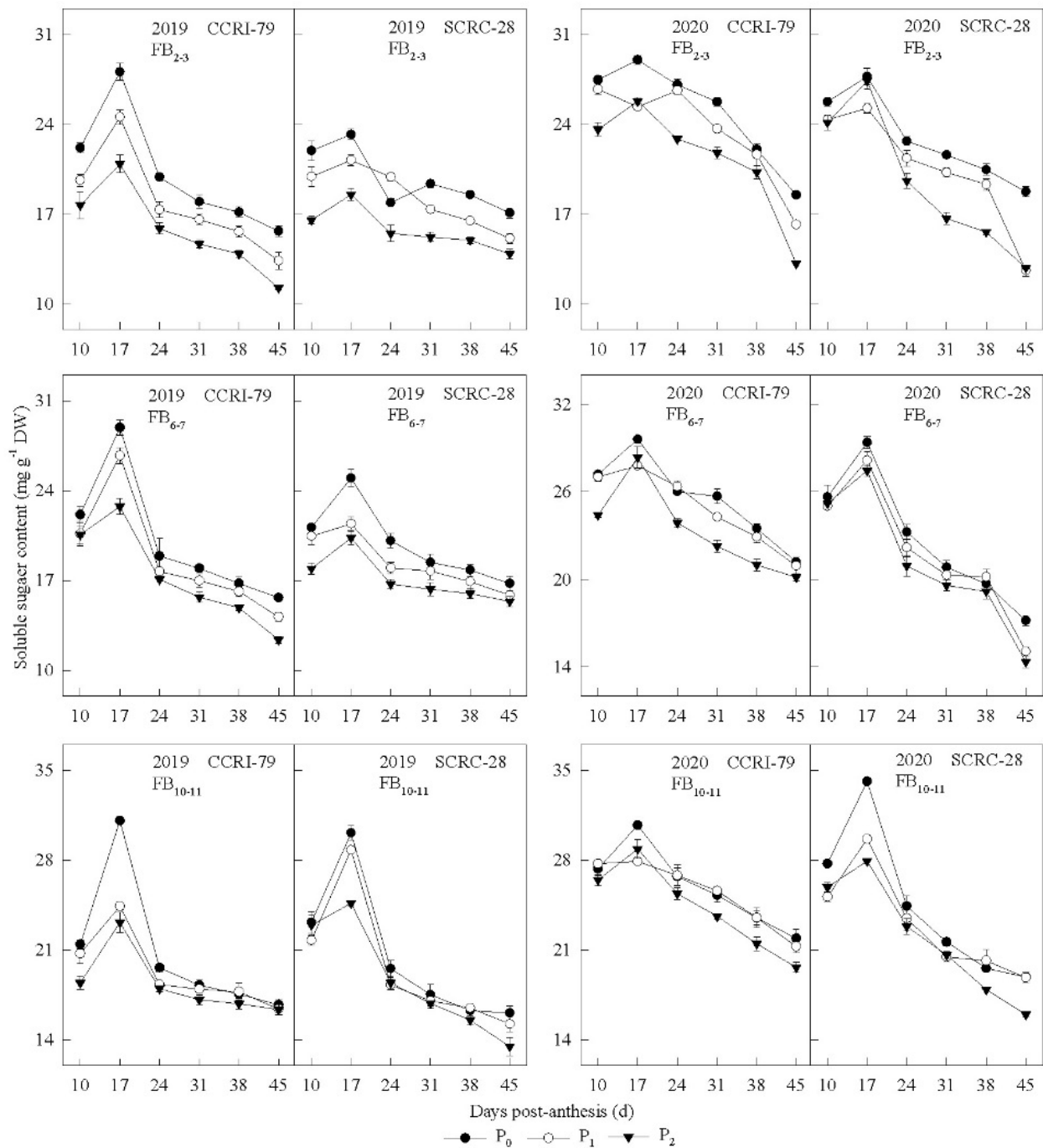
Figure 5. The effect of soil available phosphorus (AP) on the sucrose content of subtending leaves in 2019 and 2020 cotton-growing seasons. d: day. FB: fruiting branch. P_0: 3 ± 0.5 mg kg^{-1}. P_1: 6 ± 0.5 mg kg^{-1}. P_2: 15 ± 0.5 mg kg^{-1}.

In CCRI-79, Tr decreased by 1.9–2.9% and 4.1–5.9%, but SCRC-28 registered 2.3–4.2% and 4.8–8.3% declines under P_1 and P_0 treatments at the three FBPs in both years. However, the Tr was 5.7 and 6.5% higher in CCRI-79 than SCRC-28 under P_1 and P_0, and 4.8% higher in CCRI-79 than SCRC-28 under P_2. In addition, the coefficients of variation (CVs) of the Tr were higher in the SCRC-28 than the CCRI-79 subtending leaves (except for FB$_{10-11}$ in 2020) at the three FBPs (Table 2).

Table 2. The effect of soil available phosphorus (AP) on the maximum and minimum sucrose contents and sucrose transformation rates of subtending leaves in 2019 and 2020.

Year	FBP [1]	AP Treatment	Maximum Sucrose Content (mg g^{-1} DW)		Minimum Sucrose Content (mg g^{-1} DW)		Sucrose Transformation Rate (%)	
			CCRI-79	SCRC-28	CCRI-79	SCRC-28	CCRI-79	SCRC-28
2019	FB$_{2-3}$	P$_0$ [3]	21.7 a [2]	21.7 a	11.1 a	12.4 a	48.9 b	42.9 c
		P$_1$	17.6 b	20.5 b	8.9 b	10.9 b	49.5 b	47.0 b
		P$_2$	15.2 c	18.6 c	7.1 c	9.3 c	53.3 a	49.8 a
		CV (%) [4]	18.2	7.6	22.2	14.0	4.7	7.4
	FB$_{6-7}$	P$_0$	22.9 a	24.8 a	11.4 a	13.8 a	50.1 b	44.2 c
		P$_1$	21.1 b	24.4 a	9.7 b	13.0 b	53.8 a	46.7 b
		P$_2$	17.7 c	22.1 b	8.0 c	11.1 c	54.8 a	50.1 a
		CV (%)	12.8	6.0	17.6	11.3	4.7	6.3
	FB$_{10-11}$	P$_0$	22.2 a	22.7 a	10.9 a	13.2 a	50.9 b	42.1 b
		P$_1$	18.9 b	21.7 b	9.0 b	11.8 b	52.6 ab	45.7 a
		P$_2$	18.7 b	19.9 c	8.6 b	10.5 c	54.1 a	47.1 a
		CV (%)	9.7	6.6	13.0	11.0	3.0	5.7
2020	FB$_{2-3}$	P$_0$	26.3 a	30.1 a	14.1 a	17.3 a	46.3 c	42.3 b
		P$_1$	25.0 b	26.9 b	12.1 b	14.1 b	51.7 b	47.5 a
		P$_2$	22.9 c	24.6 c	10.6 c	11.8 c	53.9 a	52.0 a
		CV (%)	6.9	10.3	14.6	19.4	7.7	10.3
	FB$_{6-7}$	P$_0$	27.0 a	29.8 a	13.0 a	16.3 a	51.9 b	45.5 b
		P$_1$	25.3 b	26.4 b	12.0 b	13.8 b	52.6 b	47.7 b
		P$_2$	24.6 b	24.4 c	11.0 c	11.6 c	55.3 a	52.7 a
		CV (%)	4.8	10.2	8.2	17.0	3.4	7.6
	FB$_{10-11}$	P$_0$	24.7 a	27.6 a	11.3 a	15.4 a	52.0 b	44.1 b
		P$_1$	23.7 b	25.1 b	10.2 b	13.6 b	54.1 ab	45.7 b
		P$_2$	22.2 c	22.6 c	9.3 c	11.6 c	58.0 a	48.9 a
		CV (%)	5.4	9.9	9.7	14.3	5.5	5.2

[1] FBP: fruiting branch position. [2] Different letters in the column represent a significant difference at *p* = 0.05. [3] P$_0$: 3 ± 0.5 mg kg^{-1}. P$_1$: 6 ± 0.5 mg kg^{-1}. P$_2$: 15 ± 0.5 mg kg^{-1}. [4] CV represents the coefficient of variation.

During boll development, the starch contents of the subtending leaves showed a unimodal curve, and peak values appeared 31–38 DPA (Figure 6). In CCRI-79, the starch content increased by 20.2–21.2% and 49.4–51.0% in P$_1$ and P$_0$ compared to P$_2$ across the FBPs and years. Cultivar SCRC-28 had 19.7–24.3% and 46.4–51.3% starch content increments under the same conditions and seasons. Additionally, the subtending leaves of FB$_{6-7}$ and FB$_{10-11}$ delayed attaining the peak starch content (at 38 DPA) compared to those of FB$_{2-3}$ (at 31 DPA). Under the same soil AP treatment, the subtending leaves of FB$_{10-11}$ had higher starch contents than those of FB$_{2-3}$ and FB$_{6-7}$. Meanwhile, the average carbohydrate content (soluble sugar, sucrose, and starch) and Tr over the two years were more affected by soil AP deficiency at FB$_{2-3}$ and FB$_{6-7}$ than FB$_{10-11}$ in both cultivars. The carbohydrate content variation was consistent for both cultivars in 2019 and 2020.
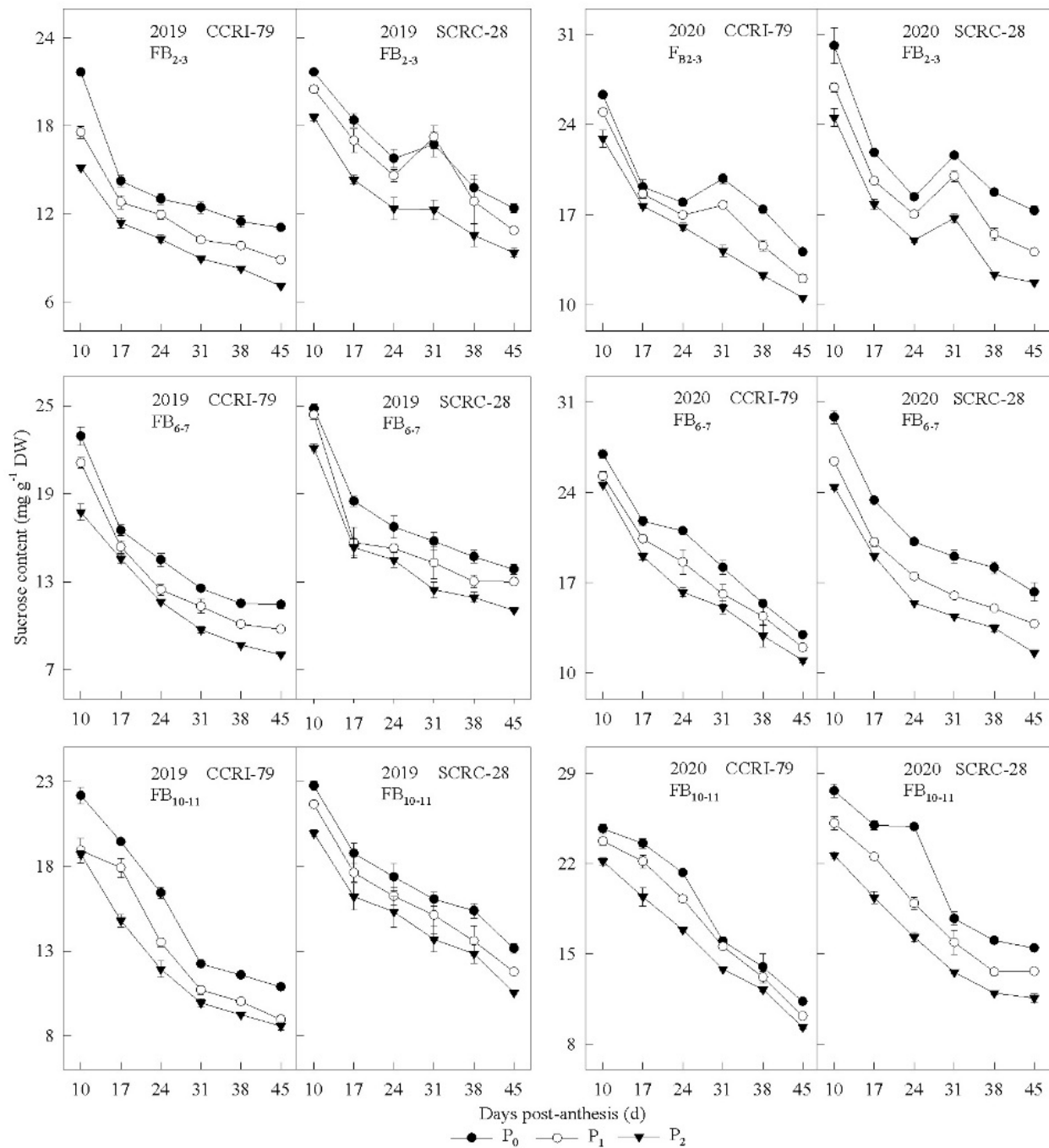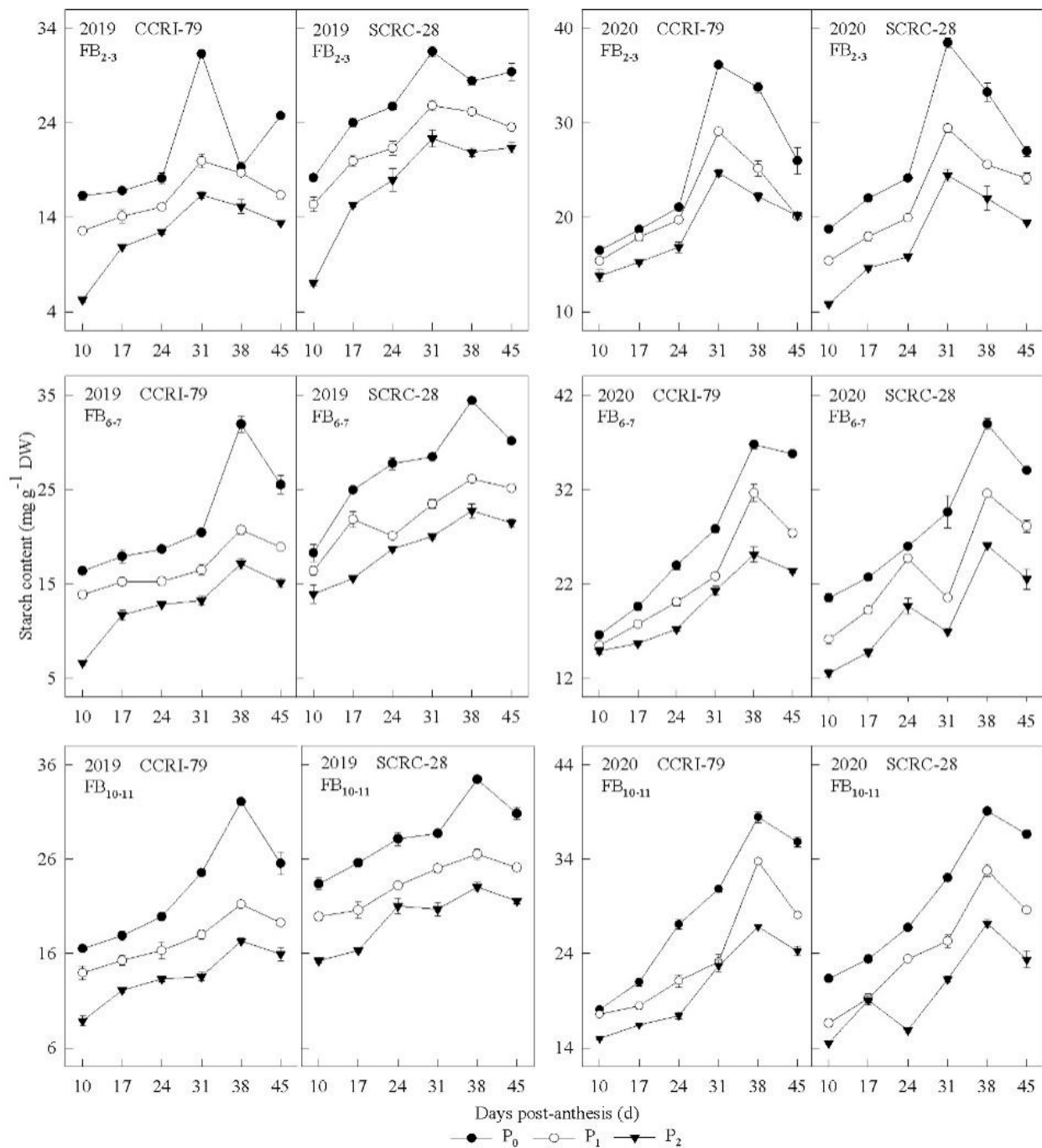
Figure 6. The effect of soil available phosphorus (AP) on the starch content of subtending leaves in 2019 and 2020 cotton-growing seasons. d: day. FB: fruiting branch. P_0: 3 ± 0.5 mg kg^{-1}. P_1: 6 ± 0.5 mg kg^{-1}. P_2: 15 ± 0.5 mg kg^{-1}.

3.3. Correlation of the Carbohydrate Content and Phosphorus Concentration of Subtending Leaves

Carbohydrate content (soluble sugar, sucrose, and starch) is an important index for characterizing the "source" activity of leaves [40]. From 10 to 45 DPA, P concentrations had significant negative correlations with the soluble sugar contents of the subtending leaves of both cultivars, especially at 17 DPA ($p < 0.05$) (Table 3). The P concentration, sucrose content (at 17–24 DAP, ($p < 0.05$)), and starch content (at 10–45 DPA, ($p < 0.05$)) of the subtending leaves were all significantly negative in SCRC-28 but nonsignificant in CCRI-79 (Table 3).

Table 3. Correlation between carbohydrate content and P concentration of the subtending leaves in 2019 and 2020 cotton-growing periods. (n = 18, $R_{0.05}$ = 0.468, $R_{0.01}$ = 0.590).

Cultivar	Comparison with	Days Post-Anthesis (d)						
		10	**17**	**24**	**31**	**38**	**45**	**Mean**
CCRI-79	Soluble sugar	0.071	−0.538 * [1]	−0.002	−0.050	0.077	−0.131	−0.096
	Sucrose	−0.002	−0.314	−0.269	−0.016	0.083	0.066	−0.075
	Starch	−0.312	−0.444	−0.459	−0.146	−0.376	−0.241	−0.330
SCRC-28	Soluble sugar	−0.251	−0.597 **	−0.406	−0.357	−0.201	−0.667 **	−0.413
	Sucrose	−0.218	−0.565 *	−0.715 **	−0.220	−0.451	−0.447	−0.436
	Starch	−0.887 **	−0.872 **	−0.863 **	−0.473 *	−0.773 **	−0.796 **	−0.777

[1] * significantly different at *p* = 0.05. ** significantly different at *p* = 0.01.

3.4. Sucrose-Metabolizing Enzyme Activities in the Subtending Leaves

The activity of the initial Rubisco from the subtending leaves showed a unimodal curve during the boll development stage and peaked at 17 DPA (Figure 7). However, the initial Rubisco activity in CCRI-79 decreased by 4.3–10.4% and 13.6–21.2% under P_1 and P_0 treatments compared to P_2 at all three FBPs for both years. Under the same conditions, the initial Rubisco activity decreased by 5.7–20.3% and 14.0–28.2% in SCRC-28. Low soil AP (P_0 and P_1) had a smaller effect on $FB_{10–11}$ than $FB_{2–3}$ and $FB_{6–7}$ (Figure 7).
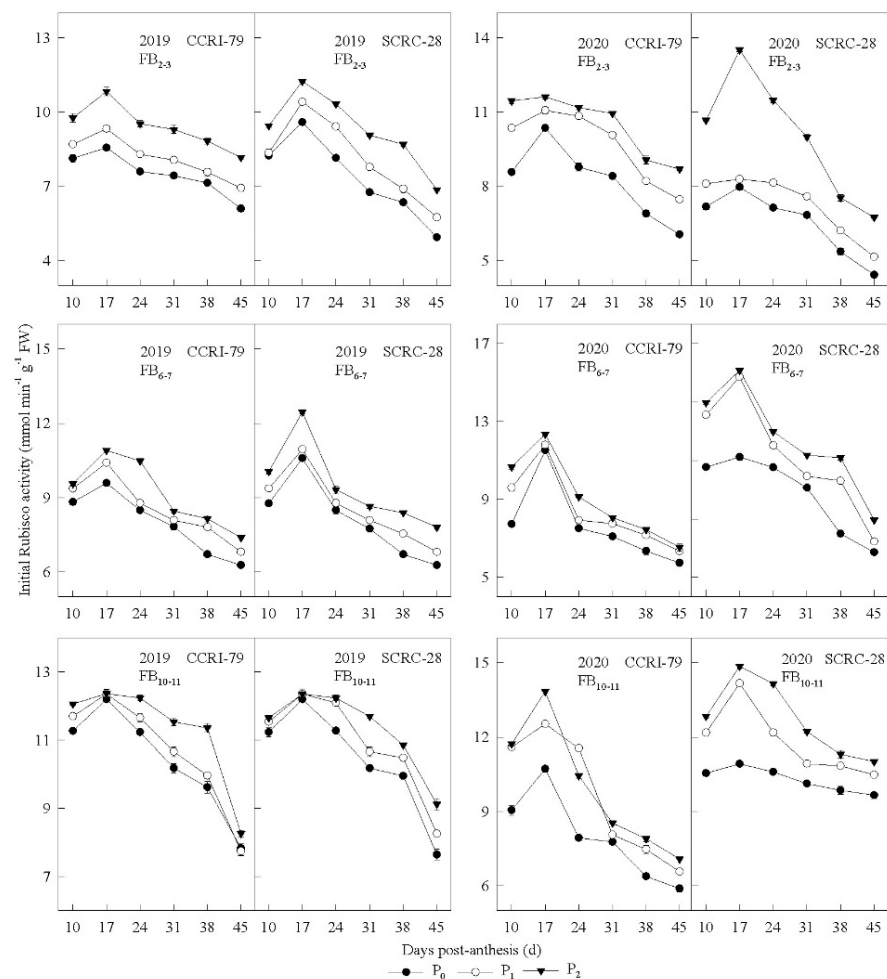

Figure 7. The effect of soil available phosphorus (AP) on the initial ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) activity of subtending leaves in 2019 and 2020 cotton-growing seasons. d: day. FB: fruiting branch. P_0: 3 ± 0.5 mg kg^{-1}. P_1: 6 ± 0.5 mg kg^{-1}. P_2: 15 ± 0.5 mg kg^{-1}.

The cy-FBPase activity of subtending leaves showed similar changes, peaking at 17–24 DPA (Figure 8), but decreased with increasing soil AP (Figure 8). In CCRI-79, the cy-FBPase activity increased by 4.0–5.2% and 8.6–12.7% under P_1 and P_0, and by 9.8–22.0% and 20.5–33.1% in SCRC-28 compared to P_2 at the three FBPs in 2019 and 2020.
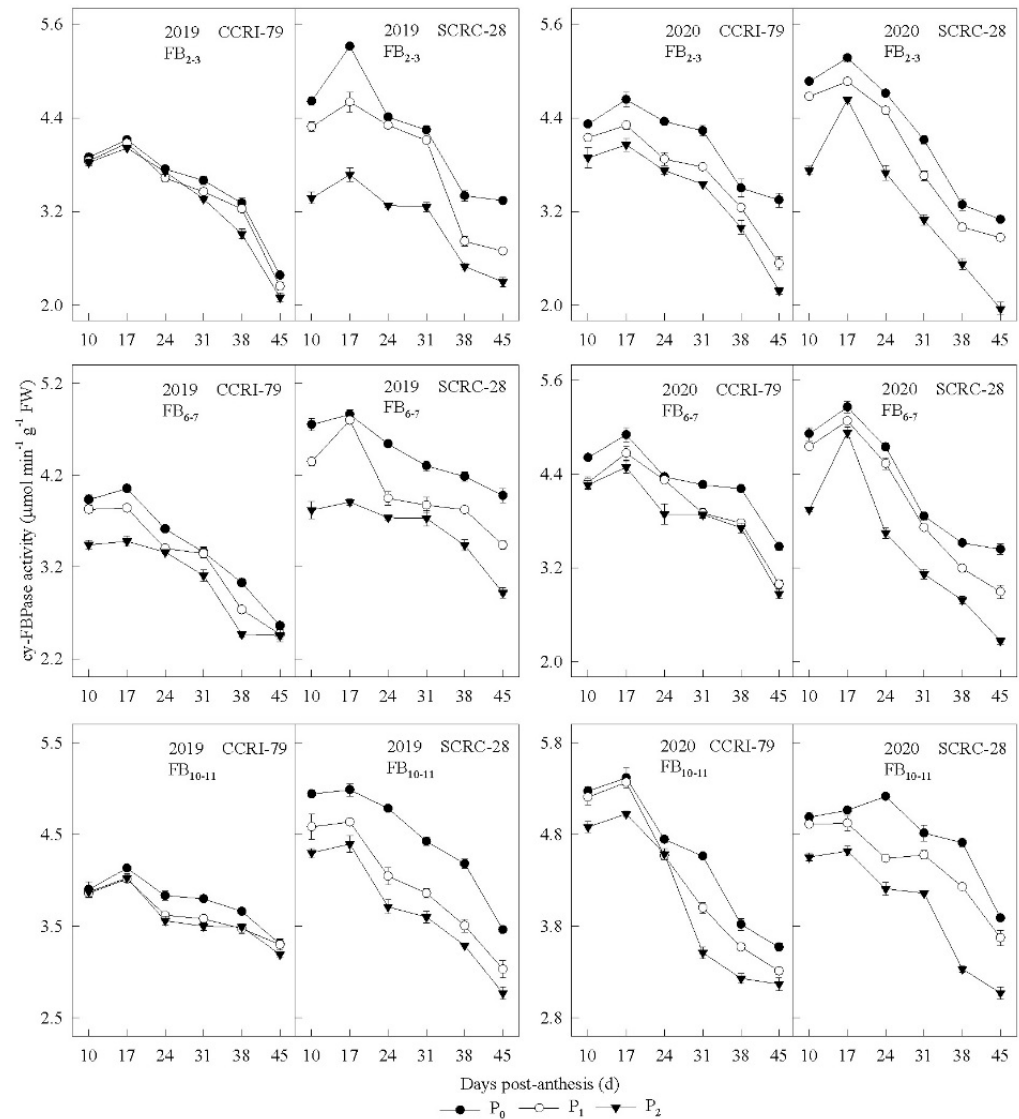

Figure 8. The effect of soil available phosphorus (AP) on the cytosolic fructose-1,6-bisphosphatase (cy-FBPase) activity of subtending leaves in 2019 and 2020 cotton-growing seasons. d: day. FB: fruiting branch. P_0: 3 ± 0.5 mg kg^{-1}. P_1: 6 ± 0.5 mg kg^{-1}. P_2: 15 ± 0.5 mg kg^{-1}.

During the flowering and boll formation stages, the SPS and SuSy activities of the subtending leaves also demonstrated a unimodal curve with maximum values appearing 24–31 DPA (Figures 9 and 10). In addition, the subtending leaves of FB$_{2-3}$ attained maximum SPS and SuSy activity faster than FB$_{6-7}$ and FB$_{10-11}$. In CCRI-79, the SPS activity of subtending leaves reduced by 10.4–15.4% and 23.8–28.2%, 15.2–22.2%, and 7.8–35.6% in SCRC-28 under P_1, and P_0 compared to P_2 treatments across FBPs and seasons. However, the SuSy activity decreased by 12.2–13.9% and 23.6–27.3% (in CCRI-79), 9.1–20.8%, and 17.1–34.0% (in SCRC-28) under P_1 and P_0 compared to P_2 treatments across the FBPs and years. Interestingly, the activities of SPS and SuSy across the three FBPs were consistent with the initial Rubisco activity of both cultivars over the two years.
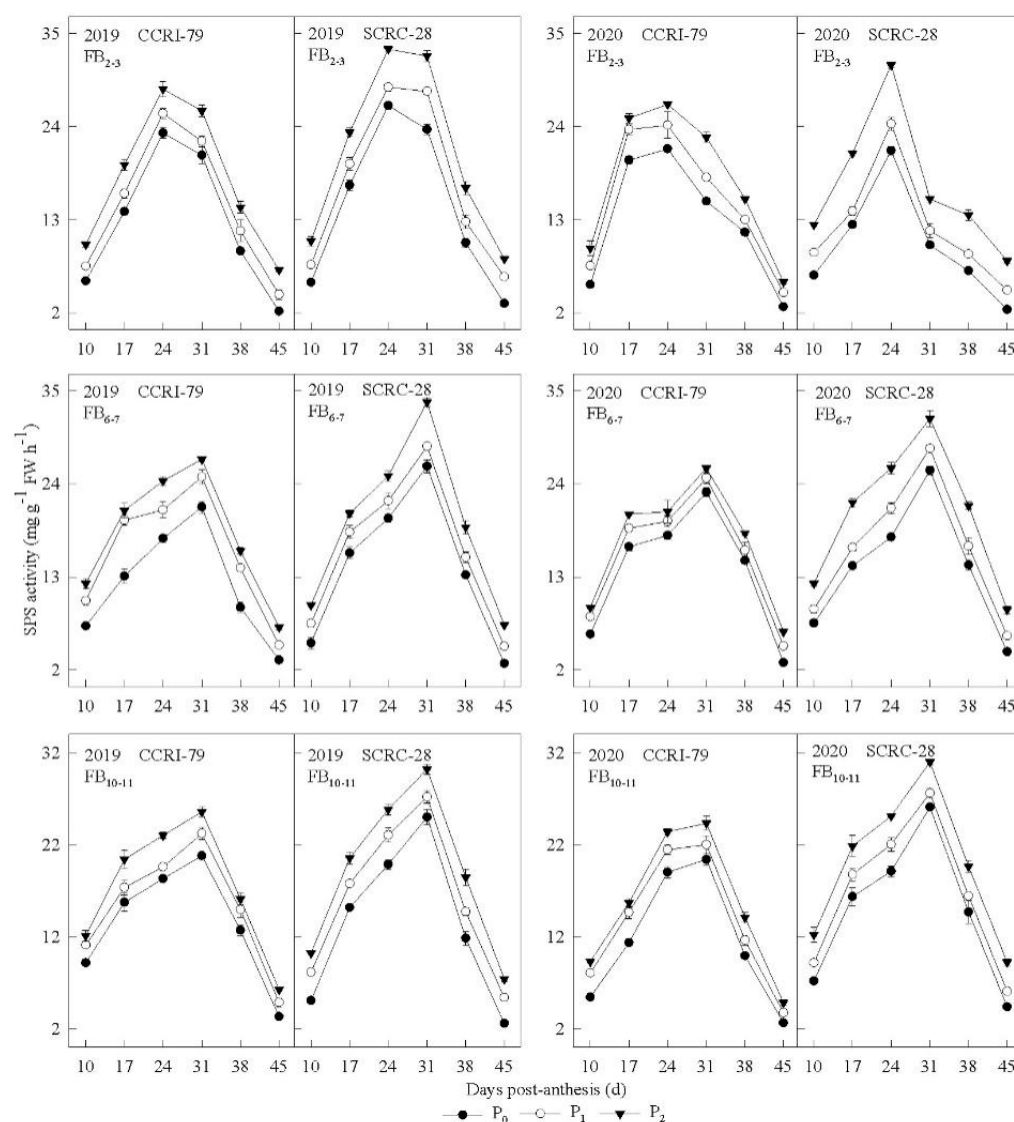
Figure 9. The effect of soil available phosphorus (AP) on the sucrose phosphate synthase (SPS) activity of subtending leaves in 2019 and 2020 cotton-growing seasons. d: day. FB: fruiting branch. P_0: 3 ± 0.5 mg kg^{-1}. P_1: 6 ± 0.5 mg kg^{-1}. P_2: 15 ± 0.5 mg kg^{-1}.
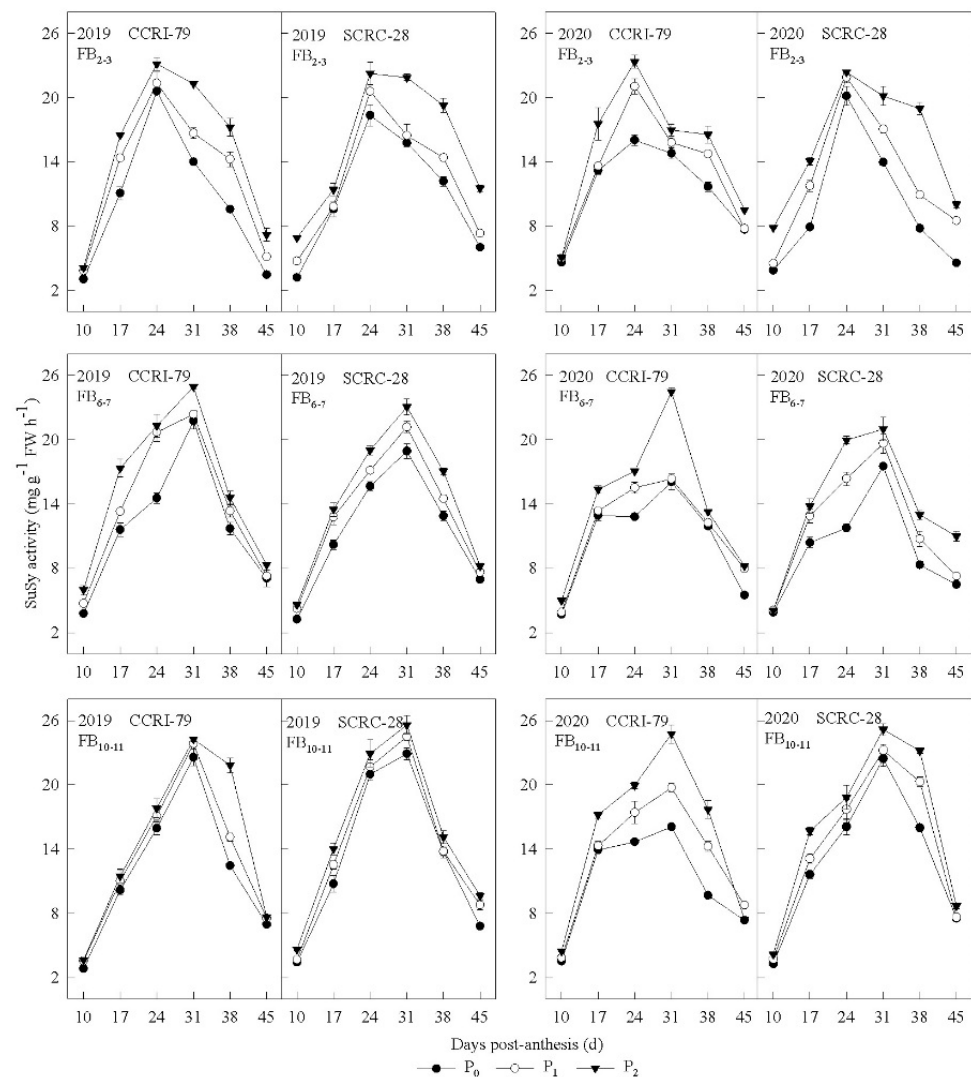
Figure 10. The effect of soil available phosphorus (AP) on the sucrose synthase (SuSy) activity of subtending leaves in 2019 and 2020 cotton-growing seasons. d: day. FB: fruiting branch. P_0: 3 ± 0.5 mg kg^{-1}. P_1: 6 ± 0.5 mg kg^{-1}. P_2: 15 ± 0.5 mg kg^{-1}.

3.5. Correlation of Sucrose-Metabolizing Enzyme Activity to the Phosphorus Concentration of Subtending Leaves

At 10–45 DPA, there was no significant correlation between the P concentration and initial Rubisco activity of the subtending leaves of CCRI-79 and SCRC-28 (Table 4). However, the correlation between P concentration and cy-FBPase activity of the subtending leaves varied for both cultivars. For SCRC-28, the P concentration and cy-FBPase activity at 10–38 DAP were significantly negative ($p < 0.05$) but insignificantly different for CCRI-79 (Table 4). A significant positive correlation was observed between SPS, SuSy activities, and P concentrations of the subtending leaves of both cultivars. A significant positive correlation occurred at 10–24 DPA ($p < 0.05$) (except for SPS activity at 10 DAP (CCRI-79) and SuSy activity at 17 DAP (SCRC-28)) (Table 4).

Table 4. Correlation between sucrose-metabolizing enzyme activity and P concentration in the subtending leaves in 2019 and 2020 cotton-growing periods. (n = 18, $R_{0.05}$ = 0.468, $R_{0.01}$ = 0.590).

Cultivar	Comparison with	Days Post-Anthesis (d)						
		10	**17**	**24**	**31**	**38**	**45**	**Mean**
CCRI-79	Initial Rubisco [1]	−0.014	0.046	0.161	0.199	0.021	0.237	0.108
	cy-FBPase [2]	−0.166	−0.180	−0.212	−0.280	−0.218	−0.423	−0.247
	SPS [3]	0.046	0.760 ** [5]	0.772 **	0.258	0.495 *	0.452	0.464
	SuSy [4]	0.616 **	0.699 **	0.678 **	−0.089	−0.343	0.337	0.316
SCRC-28	Initial Rubisco	−0.053	0.173	0.039	−0.042	−0.101	−0.110	−0.016
	cy-FBPase	−0.771 **	−0.573 *	−0.783 **	−0.811 **	−0.884 **	−0.880	−0.784
	SPS	0.720 **	0.634 **	0.861 **	−0.068	0.208	0.738 **	0.516
	SuSy	0.770 **	0.369	0.553 *	−0.115	0.240	0.641 **	1.410

[1] Rubisco: ribulose-1,5-bisphosphate carboxylase-oxygenase. [2] cy-FBPase: cytosolic fructose-1,6-bisphosphatase. [3] SPS: sucrose phosphate synthase. [4] SuSy: sucrose synthase. [5] * significantly different at p = 0.05. ** significantly different at p = 0.01.

3.6. Lint Yield and Yield Components on Different Fruiting Branches

The boll number, boll biomass, lint percentage, and lint yield decreased significantly with decreasing soil AP (P_2 to P_0) (Table 5). Boll number was the most sensitive (except for CCRI-79 from FB_{9-12} in both years), and lint percentage was the least sensitive to low soil AP.

In CCRI-79, lint yield decreased by 12.0–25.1% and 28.6–57.1% under P_1 and P_0 treatments compared to P_2, mainly because the boll number decreased by 8.2–12.7%, 12.3–39.4% in 2019 and 2020 across the FBPs. For SCRC-28, lint yield decreased by 14.5–25.8% and 39.3–67.1% at P_1 and P_0 compared to P_2 because the boll number decreased by 5.3–21.4% and 22.8–47.2% over the two years at different FBPs. Under P_1 and P_0, the lint yield of CCRI-79 was 27.1 and 58.3% higher than SCRC-28, respectively. In contrast, the lint yield was 19.7% higher in CCRI-79 than SCRC-28 under P_2 treatment during the 2019 and 2020 seasons (Table 5). The cotton bolls on the upper FBP (FB_{9-12}) contributed 26.4, 29.4, and 33.8% (in CCRI-79), and 19.6, 22.5, and 28.2% (in SCRC-28) of the total lint yield under the three soil AP levels (P_2, P_1, and P_0).

The CVs of lint yield and yield components were lower for FB_{9-12} than FB_{1-4} and FB_{5-8} in CCRI-79, and the values were even smaller for SCRC-28. Lint yield was most affected by the interactions of FBP and AP (p < 0.01) (Table 5).

Table 5. The effect of soil available phosphorus (AP) on lint yield and yield components of different fruiting branches in 2019 and 2020.

Cultivar	FBP [1]	AP Treatment	2019				2020			
			Boll Number (Boll Plant^{-1})	Boll Biomass (g Boll^{-1})	Lint Percentage (%)	Lint Yield (kg ha^{-1})	Boll Number (Boll Plant^{-1})	Boll Biomass (g Boll^{-1})	Lint Percentage (%)	Lint Yield (kg ha^{-1})
CCRI-79	FB$_{1-4}$	P$_0$ [2]	4.3 b [3]	5.3 c	38.0 b	349.7 c	4.0 b	5.4 c	37.7 c	342.3 c
		P$_1$	5.5 ab	6.2 b	38.2 b	527.1 b	6.9 a	6.0 b	40.4 b	681.0 b
		P$_2$	6.3 a	6.9 a	41.0 a	727.4 a	7.4 a	7.0 a	42.7 a	885.8 a
		CV (%) [4]	18.8	13.3	4.4	35.3	30.1	12.8	6.1	43.1
	FB$_{5-8}$	P$_0$	3.1 b	5.9 c	40.3 b	295.1 b	3.9 b	6.1 b	40.4 b	384.9 c
		P$_1$	3.4 ab	6.4 b	42.6 a	367.2 b	4.5 ab	6.5 b	42.3 a	496.1 b
		P$_2$	4.2 a	7.1 a	43.7 a	524.1 a	4.8 a	7.1 a	42.9 a	592.2 a
		CV (%)	16.6	9.7	4.0	29.6	10.4	8.1	3.1	21.1
	FB$_{9-12}$	P$_0$	3.4 a	6.2 b	42.5 b	332.5 b	3.0 a	6.5 b	46.9 a	367.0 b
		P$_1$	3.2 a	6.4 b	46.7 a	396.9 ab	3.5 a	7.1 a	47.1 a	464.9 a
		P$_2$	3.7 a	7.3 a	45.6 a	469.1 a	3.6 a	7.7 a	46.8 a	510.3 a
		CV (%)	7.3	8.7	4.9	17.1	9.5	8.5	0.3	16.4
SCRC-28	FB$_{1-4}$	P$_0$	3.2 b	5.0 c	37.6 c	246.0 c	3.5 c	4.7 c	36.1 b	248.1 c
		P$_1$	4.8 a	6.3 b	38.9 b	473.1 b	6.5 b	6.2 b	38.7 ab	641.6 b
		P$_2$	5.6 a	6.7 a	42.9 a	633.6 a	7.1 a	7.2 a	41.5 a	868.5 a
		CV (%)	27.0	14.2	6.9	43.2	33.8	20.3	6.9	53.6
	FB$_{5-8}$	P$_0$	2.2 b	5.4 b	37.7 b	176.8 c	3.2 b	5.5 c	38.4 b	268.8 b
		P$_1$	2.9 b	6.3 a	40.3 a	285.9 b	3.7 ab	6.5 b	41.2 a	388.5 b
		P$_2$	3.8 a	6.7 a	41.0 a	408.7 a	4.6 a	7.5 a	41.3 a	579.6 a
		CV (%)	27.0	11.3	4.4	39.9	18.5	15.3	4.1	38.0
	FB$_{9-12}$	P$_0$	2.5 b	5.3 b	38.2 b	196.2 b	1.9 b	5.5 c	40.3 b	172.6 b
		P$_1$	3.0 ab	6.1 a	39.0 b	277.5 a	2.4 a	5.9 b	42.9 a	241.9 a
		P$_2$	3.3 a	6.5 a	42.3 a	345.3 a	2.4 a	6.5 a	42.7 a	262.1 a
		CV (%)	13.8	10.3	5.5	27.3	12.9	8.9	3.6	20.8
Significance										
Cultivar (C)			NS [5]	**	**	**	**	**	**	**
FBP			**	**	**	**	**	**	**	**
AP			**	**	**	**	**	**	**	**
C × FBP			**	**	**	NS	NS	**	**	**
C × AP			*	**	*	NS	NS	**	NS	NS
FBP × AP			NS	*	*	**	**	**	**	**
C × FBP × AP			NS	NS	**	NS	NS	*	NS	NS

[1] FBP: fruiting branch position. [2] P$_0$: 3 ± 0.5 mg kg^{-1}. P$_1$: 6 ± 0.5 mg kg^{-1}. P$_2$: 15 ± 0.5 mg kg^{-1}. [3] Different letters in the column represent significant differences at p = 0.05. [4] CV means coefficient of variation. [5] NS means nonsignificance at p = 0.05. * represents significance at p = 0.05. ** represents significance at p = 0.01.

3.7. Cotton Boll Biomass Accumulation and Distribution on Different Fruiting Branches

Low soil AP decreased the boll wall, lint, and seed biomass, thus, reducing the cotton boll biomass (Table 6). Boll wall biomass was most affected in CCRI-79, while lint biomass was most affected in SCRC-28. However, low soil AP least affected the seed biomass of both cultivars. Low soil AP decreased the boll wall:boll, lint:boll, and lint:seed ratios but increased the seed:boll ratio.

Table 6. The effect of soil available phosphorus (AP) on the cotton boll biomass partitioning across different fruiting branches in 2019 and 2020.

Cultivar	FBP [1]	AP Treatment	2019				2020			
			Boll Dry Mass (g)			Proportion (%)	Boll Dry Mass (g)			Proportion (%)
			Boll Wall	Lint	Seed	L/S [2]	Boll Wall	Lint	Seed	L/S
CCRI-79	FB_{1-4}	P_0	1.2 b [3]	1.5 c	2.5 b	61.2 b	1.1 c	1.6 c	2.7 c	60.6 c
		P_1	1.4 ab	1.8 b	3.0 a	61.8 b	1.3 b	1.9 b	2.8 b	67.7 b
		P_2	1.6 a	2.2 a	3.2 a	69.6 a	1.6 a	2.3 a	3.1 a	74.4 a
		CV (%) [4]	12.4	17.6	11.2	7.3	16.7	17.0	7.7	10.2
	FB_{5-8}	P_0	1.4 c	1.8 c	2.7 b	67.6 b	1.4 b	1.9 c	2.8 a	67.8 a
		P_1	1.5 b	2.1 b	2.8 b	74.2 a	1.5 b	2.1 b	2.9 a	73.3 a
		P_2	1.7 a	2.3 a	3.0 a	77.5 a	1.7 a	2.4 a	3.1 a	75.1 a
		CV (%)	11.7	13.0	6.4	6.9	9.8	11.1	4.8	5.2
	FB_{9-12}	P_0	1.5 b	2.0 c	2.7 ab	74.0 b	1.5 b	2.3 b	2.7 b	88.3 a
		P_1	1.6 b	2.2 b	2.5 b	87.5 a	1.7 a	2.5 a	2.9 ab	89.0 a
		P_2	2.0 a	2.4 a	2.9 a	84.0 a	1.9 a	2.7 a	3.1 a	88.0 a
		CV (%)	13.9	9.9	6.2	8.6	12.9	7.3	7.9	0.5
SCRC-28	FB_{1-4}	P_0	1.1 c	1.5 c	2.4 b	60.3 b	1.0 c	1.4 c	2.4 b	56.6 b
		P_1	1.5 b	1.9 b	3.0 a	63.6 b	1.4 b	1.9 b	3.0 a	63.3 ab
		P_2	1.6 a	2.2 a	2.9 a	75.1 a	1.7 a	2.3 a	3.3 a	71.0 a
		CV (%)	17.6	19	10.2	11.7	24.4	26.5	15.5	11.3
	FB_{5-8}	P_0	1.3 b	1.5 b	2.5 b	60.5 b	1.3 c	1.6 c	2.6 c	62.5 b
		P_1	1.6 a	1.9 a	2.8 ab	67.5 a	1.6 b	2.0 b	2.9 b	70.2 a
		P_2	1.7 a	2.0 a	3.0 a	69.5 a	1.9 a	2.4 a	3.3 a	70.3 a
		CV (%)	14	14.5	7.7	7.2	17.8	20.0	11.7	6.6
	FB_{9-12}	P_0	1.4 b	1.5 c	2.4 b	61.8 b	1.3 cc	1.7 c	2.5 b	67.4 b
		P_1	1.6 ab	1.8 b	2.8 a	63.8 b	1.5 b	1.9 b	2.6 b	74.7 a
		P_2	1.7 a	2.0 a	2.7 ab	73.3 a	1.7 a	2.1 a	2.8 a	75.2 a
		CV (%)	12.3	14.2	7.2	9.2	13.6	9.2	5.3	6.0
Significance										
Cultivar (C)			NS [5]	**	*	**	*	**	**	**
FBP			**	**	**	**	**	**	**	**
AP			**	**	**	**	**	**	**	**
C × FBP			*	**	NS	**	**	**	**	**
C × AP			NS	NS	**	*	*	*	*	NS
FBP × AP			NS	*	**	**	NS	**	*	**
C × FBP × AP			NS	NS	NS	**	NS	NS	**	NS

[1] FBP: fruiting branch position. [2] L/S: lint:seed ratio. [3] Different letters in the column represent significant difference at $p = 0.05$. [4] CV represents coeffcient of variation. [5] NS represents nonsignificance at $p = 0.05$. * represents significance at $p = 0.05$. ** represents significance at $p = 0.01$.

Decreasing the lint:seed ratio by -2.3–7.3% and 4.8–11.1% across the FBPs and seasons reduced the boll biomass by 9.5–11.9% and 15.2–22.9% in CCRI-79 under P_1 and P_0 treatments compared to P_2 (Table 6 and Figure 11). In SCRC-28, decreasing the lint:seed ratio by 1.1–9.6% and 8.4–14.5% over the seasons and FBPs reduced the boll biomass by -0.9–11.3% and 14.2–30.9% under P_1 and P_0 than P_2 treatment (Table 6 and Figure 11). For P_0, the boll biomass of CCRI-79 was 12.5% higher than SCRC-28 and 8.3% higher than SCRC-28 under P_2 in 2019 and 2020 (Table 6). In addition, the lint:seed ratio of FB_{9-12} was higher than FB_{1-4} and FB_{5-8} (Table 6 and Figure 11).
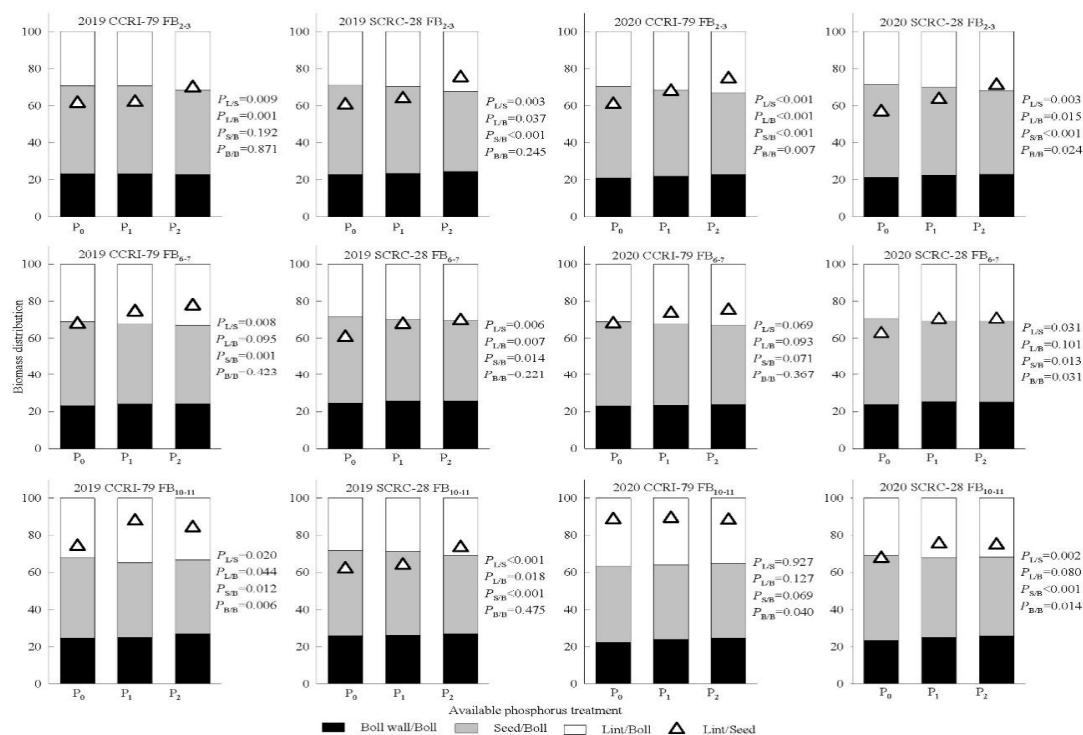
Figure 11. The effect of soil available phosphorus (AP) on cotton boll biomass partitioning in the boll wall, seed, and lint in 2019 and 2020. The subscripts of L/S, L/B, S/B, and B/B represent lint:seed, lint:boll, seed:boll, and boll wall:boll ratios, respectively.

Increasing the FBP decreased the boll biomass CVs (Table 5). Moreover, boll biomass was most affected by the C × FBP and C × AP ($p < 0.01$) interactions. In contrast, the lint:seed ratio was most affected by the C × FBP and FBP × AP ($p < 0.01$) interactions (Tables 5 and 6).

3.8. Correlation of Source Indices to Lint Yield and Yield Components in the Subtending Leaves

This study determined the lint yield and yield components and their relationship with *Pn*, carbohydrate content, and sucrose-metabolizing enzyme activities (Tables 6 and 7). The results show that boll biomass negatively correlated with the sucrose and starch contents of the subtending leaves ($p < 0.05$) but positively correlated with the Tr and SuSy activities ($p < 0.01$) in CCRI-79 and SCRC-28 (Table 7). However, the boll number had a significant negative correlation with the *Pn* and soluble sugar contents of the subtending leaves of both cultivars ($p < 0.05$). The lint:seed ratio was positively correlated with the activities of initial Rubisco and SuSy in the subtending leaves of both cultivars ($p < 0.01$). In addition, there was a significant negative correlation between the lint yield and soluble sugar content of subtending leaves ($p < 0.01$). The lint yield was significantly and positively correlated with SuSy activity ($p < 0.01$) in CCRI-79 and SCRC-28 (Table 7).

Moreover, there was a significant negative correlation between boll biomass and cy-FBPase activity ($p < 0.01$) and positive correlations between boll biomass, lint yield, and SPS activity ($p < 0.01$) in CCRI-79. Lint yield also significantly but negatively correlated with cy-FBPase activity ($p < 0.01$) in SCRC-28. Therefore, higher Tr and activities of initial Rubisco and SuSy increased the boll biomass and lint:seed ratio, eventually increasing the lint yield.

Table 7. Correlation between source indices, lint yield, and yield components in 2019 and 2020. (n = 18, $R_{0.05}$ = 0.468, $R_{0.01}$ = 0.590).

Cultivar	Correlation with	*Pn*[1]	Soluble Sugar	Sucrose	Starch	Tr [2]	Initial Rubisco [3]	cy-FBPase [4]	SPS [5]	SuSy [6]
CCRI-79	Boll biomass	0.466	−0.708 ** [7]	−0.526 *	−0.854 **	0.780 **	0.486 *	−0.670 **	0.728 **	0.804 **
	Boll number	−0.615 **	−0.755 **	−0.047	−0.335	0.095	−0.300	−0.383	0.570*	−0.253
	Lint/seed ratio	0.827 **	0.049	0.103	−0.061	0.065	0.912 **	0.103	0.312	0.602 **
	Lint yield	−0.256	−0.888 **	−0.035	−0.467	0.196	0.083	−0.443	0.831 **	0.633 **
SCRC-28	Boll biomass	0.470 *	−0.129	−0.837 **	−0.603 **	0.723 **	0.389	−0.276	0.442	0.715 **
	Boll number	−0.546 *	−0.772 **	−0.127	−0.643 **	0.204	−0.053	−0.815 **	0.149	0.325
	Lint/seed ratio	0.182	−0.781 **	−0.602 **	−0.686 **	0.192	0.684 **	−0.526 *	0.746 **	0.771 **
	Lint yield	−0.357	−0.814 **	−0.324	−0.757 **	0.313	0.161	−0.869 **	0.377	0.499 *

[1] *Pn*: net photosynthetic rate. [2] Tr: sucrose transformation rate. [3] Rubisco: ribulose-1,5-bisphosphate carboxylase-oxygenase. [4] cy-FBPase: cytosolic fructose-1,6-bisphosphatase. [5] SPS: sucrose phosphate synthase. [6] SuSy: sucrose synthase. [7] * represents significant difference at *p* = 0.05. ** represents significant difference at *p* = 0.01.

4. Discussion

Numerous studies have focused on the effects of P fertilizer on P uptake and distribution [8], yield, and fiber quality of the whole cotton plant [6,61]. However, P is easily fixed under natural conditions; thus, this study determined the effects of low-P on cotton growth using the soil AP content [26]. Phosphorus deficiency affects leaf carbohydrate metabolism [62] and the distribution of cotton boll biomass [26], but the relationship between the two is unclear. Previous studies showed that leaves subtending cotton bolls impact cotton boll growth and development, providing 60–87% carbon [63–65]. Therefore, this study investigated the effect of soil AP on sucrose metabolism in subtending leaves from three fruiting branch positions ($FB_{2–3}$, $FB_{6–7}$, and $FB_{10–11}$) and the relationship between boll biomass and lint yield. Table 1 shows the negligible pre-experimentation differences between the organic matter, total nitrogen, available nitrogen, and available potassium contents of the soils, indicating that soil AP caused the observed experimental differences between treatments.

Subtending leaf photosynthesis synthesizes the required carbohydrates, mainly transported as sucrose to the sink organs [66]. However, the level of sucrose in the leaves depended on the coordination of *Pn*, sucrose metabolism, and transport [58,67]. Phosphorus is important for crop growth and development, and its nutritional status regulates P concentration and sucrose metabolism in leaves [26]. In this experiment, the contents of soluble sugar, sucrose, and starch were negatively correlated with the P concentration in the subtending leaves from 17 to 31 DPA. This correlation suggests that soil AP deficiency increased the contents of soluble sugar, sucrose, and starch (Table 3), as reported in previous studies [27,68,69]. This may indicate that the carbon distribution between sucrose and other non-structural carbohydrates has changed, so that more fixed carbon was retained in the P deficiency subtending leaves. Therefore, soluble sugar, sucrose, and starch accumulated excessively in the opposite leaves of cotton boll, which hindered the transportation of sucrose to cotton boll under P_0 and P_1 (Table 2). These results might help to explain why low phosphorus increases boll biomass and lint yield (Table 5). The soluble sugar and sucrose increments improved the plant water holding capacity, which positively correlated with the resistance to low-P [68,70]. For this study, the starch contents of subtending leaves were 21.2 and 49.5% higher in P_1 and P_0 than in P_2 (Figure 6), indicating that soil AP deficiency blocked sucrose transportation from leaves to bolls (Table 2), thus, affecting cotton boll development (Tables 5 and 6). Previous observations confirmed similar results with rice [23] and soybean leaves [71]. This trend occurred possibly because mesophyll cells lacked P, hampering triose phosphate transportation from the chloroplast to the cytoplasm for sucrose synthesis; thus, the synthesized starch accumulates in the chloroplast [27].

Moreover, the Tr of subtending leaves from $FB_{2–3}$, $FB_{6–7}$, and $FB_{10–11}$ were lower under P_0 and P_1 than P_2 (Table 2), confirming that low soil AP restricted photosynthate transport to cotton bolls. Excessive sugar retention (soluble sugar, sucrose, and starch) (Figures 4–6) in the leaf reduces initial Rubisco activity, possibly affecting the *Pn* (Figures 3 and 7), as reported in previous studies [26,72]. The vacuole phosphorus pool was exhausted during boll development, and more triose phosphate was involved in starch synthesis. Therefore, the starch content increased (10–31 DAP) (Figure 6), but the soluble sugar (17–45 DAP) and sucrose contents (10–45 DAP) of subtending leaves decreased (Figures 4–6). Natr et al. [73] suggested that enhanced starch synthesis under low-P promotes phosphate group conversion to inorganic phosphorus. Most likely, starch is converted to sucrose and exported to the boll only when the sucrose content is seriously low [74]. Nonetheless, there were significant positive correlations between Tr and SuSy activity of the subtending leaves and boll biomass (Table 7). These correlations indicate that soil AP deficiency reduces boll biomass by altering sucrose synthesis and distribution (Table 2), as confirmed in previous reports [26].

Low soil AP content altered cotton boll biomass composition by reducing boll wall:boll, lint:boll, and lint:seed and increasing the seed:boll ratios (Table 6). These results suggest that low soil AP limits carbohydrate transport from the seed to the fiber inhibiting fiber development. However, a higher seed:boll ratio improved cotton breeding, positively

responding to low-P stress [75]. In addition, the lint:seed positively correlated with the activities of initial Rubisco and SuSy in the subtending leaves (Table 7). The correlations were consistent with cotton boll biomass accumulation (Table 7), indicating that high soil AP was beneficial for lint biomass accumulation and further improved lint yield (Tables 5 and 6).

Phosphorus deficiency disordered sucrose metabolism because of the changes in key sucrose-metabolizing enzyme activities [23,27]. Previous studies showed that low-P increased sucrose and starch contents in sugar beet [45] and maize [76], positively correlating with cy-FBPase activity. The results confirm these observations since the cy-FBPase activity of the subtending leaves increased by 11.7 and 21.4% under P_1 and P_0, respectively (Figure 8). In this research, SPS and SuSy activities were opposite to the cy-FBPase activity of the subtending leaves under low soil AP, similar to the observations in citrus [77]. Excessive sucrose (Figure 5) also inhibited the activity of SPS and SuSy (Figures 9 and 10), as reported in a previous study [78]. In this study, low soil AP had a higher impact on the activities of initial Rubisco, SPS, and SuSy in FB_{2-3} than FB_{10-11} (Figures 7, 9 and 10). The varied impact is probably because the subtending leaves from FB_{2-3} and FB_{10-11} had different durations of low-P exposure. Moreover, older leaves translocate P to young leaves [79], and young leaves generally have stronger stress resistance than old leaves [80]. The SuSy activity, boll biomass, and lint yield was significantly and positively correlated (Table 7), suggesting SuSy as a key enzyme affecting boll biomass and lint yield under low soil AP. However, Liu et al. [26] showed that SPS is a key enzyme in the functional leaves of cotton plants during low-P. These reports may explain the different responses to low-P stress in subtending and functional cotton leaves. Moreover, low soil AP had a higher impact on boll biomass and lint yields of FB_{1-4} and FB_{5-8} than FB_{9-12} (Tables 5 and 7). The main reason for the varied impact is that the CVs of the Tr and activities of initial Rubisco and SuSy were smaller at FB_{9-12} than at FB_{1-4} and FB_{5-8} (Table 2 and Figures 7 and 10). This variation explains the higher lint:seed ratio and proportion of total lint yield on the upper FBP (FB_{9-12}) under soil AP deficiency (Tables 5 and 6).

Different cotton varieties are differentially sensitive to low-P [81,82]. In this study, the effects of low soil AP on SCRC-28 and CCRI-79 caused different subtending leaf Tr (Table 2), boll biomass, and lint yield (Table 5). SCRC-28 was more sensitive to low soil AP than CCRI-79. These results suggest that different cultivars present different physiological responses under soil AP deficiency, as reported in cotton seedlings [83]. The average correlation coefficient revealed the strongest correlation between the leaf P concentration and starch content of both cultivars. Moreover, starch, sucrose, and leaf P contents differed between CCRI-79 and SCRC-28. These results may explain why SCRC-28 was more sensitive to low soil AP than CCRI-79 (Table 3). Therefore, achieving higher Tr in the subtending leaves might be critical for boll biomass and lint yield [58].

In this study, the cy-FBPase activity of SCRC-28 subtending leaves was more sensitive to low P concentration than CCRI-79 (Table 4). Meanwhile, the relationship between cy-FBPase activity, boll biomass, and lint yield varied between CCRI-79 and SCRC-28 (Table 7). Cy-FBPase activity increased by 4.0–12.7% in CCRI-79 and 9.8–33.1% in SCRC-28 under P_1 and P_0 across the three FBPs. Furthermore, the SPS activity in the subtending leaves, boll biomass, and lint yield was significantly and positively correlated ($p < 0.01$) in CCRI-79 but nonsignificant in SCRC-28. Under P_1 and P_0 treatments, the SPS activity in the subtending leaves of CCRI-79 decreased by 10.4–28.2% and 15.2–35.6% in SCRC-28 compared with P_2 over the FBPs and years. These results show that cy-FBPase and SPS activities in SCRC-28 are more sensitive to soil AP content, possibly explaining the different sensitivities of both cultivars to low soil AP.

5. Conclusions

The research performed in 2019 and 2020 suggests that: (1) Soil AP deficiency (P_0 and P_1) decreases the P concentration, Tr, and activities of initial Rubisco and SuSy in the subtending leaves; thus, decreasing boll biomass and lint yield. Soil AP deficiency decreased

the boll wall:boll, lint:boll, and lint:seed, and increased seed:boll ratio. (2) The decline of Tr and activities of initial Rubisco and SuSy was less on FB_{10-11} than FB_{2-3} and FB_{6-7} under low soil AP. These results suggest that higher lint:seed and the proportion of total lint yield on upper FBP (FB_{9-12}) are positive responses to soil AP deficiency. (3) Soil AP deficiency reduced *Pn* and sucrose-metabolizing enzyme (initial Rubisco, SPS, and SuSy) activities in the subtending leaves but increased carbohydrate (soluble sugar, sucrose, and starch) contents, reducing the Tr. However, the same conditions were unconducive for boll biomass accumulation and lint yield. (4) The P concentration of SCRC-28 subtending leaves strongly affected sucrose and starch contents, which may explain the higher SCRC-28 sensitivity to low soil AP than CCRI-79. The activities of cy-FBPase and SPS were significantly different between CCRI-79 and SCRC-28, further explaining the different sensitivities to low soil AP.

The results could improve the understanding of the effects of low soil AP on sucrose metabolism in subtending leaves during boll development, providing reliable data and theoretical guidelines for cultivating low-P tolerant varieties.

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