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Effect of shoot-tip pruning dates on yield and yield components of various brown-seeded soybean lines under shade stress

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Abstract. This study aimed to determine the effect of shoot pruning dates on yield and yield components of some brown-seeded soybean genotypes under shade stress. The experiment was conducted under a shading net house in Sekarbela, Mataram, which was according to Completely Randomized Design, with three replicates and two treatment factors, i.e. brown-seeded soybean genotypes consisting of 7 lines (KH 7B, KH 7C, KH 7D, KH 9, KH 14, KH 50B and KH 35), and shoot pruning dates consisting 3 treatments (P0= without pruning, P1= pruning at 4 weeks after planting (4 WAP), P2= 5 WAP and P3= 6 WAP). The results indicated that there were significant differences in performance of those genotypes of brown-seeded soybeans and significant effects of shoot pruning, especially on grain yield, which was highest (10.99 g/plant) under without pruning but significantly reduced by different pruning dates. Among the brown-seeded soybean lines tested under shade stress, KH35 (G7) and KH50B (G6) lines performed the best as indicated by the highest 100 grain weight, grain number, and grain yield per plant. However, there was a significant interaction between the treatment factors on plant height and weight of 100 grains, in which plant height was highest n G4 without pruning (193 cm) and lowest on G5 pruned at 4 WAP (36.5 cm), while 100-grain weight was highest on G6 pruned at 6 WAP (8.59 g) and lowest on G4 pruned at 6 WAP (6.30 g), so delaying pruning date could increase weight of 100 grains, especially on G6.

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1. INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is a legume crop that produces grains containing the important nutrition for human health. Every 100 g of soybean grains contains 331.0 kcal; 34.9 g protein; 18.1 g fat; 34.8 g carbohydrates; 4.2 g fiber; 227.0 mg calcium; 585.0 mg phosphorus; 8.0 mg iron and 1.0 mg vitamin B1. Soybean is widely consumed in the forms of tofu, "tempe", soy sauce, "tauco", soy milk, and various kinds of snacks. In addition to its uses as food ingredients, soybean is also used as industrial ingredients and animal feed (Milani et al., 2013). However, among the various types of soybean, the essential materials containing high antioxidant (high anthocyanin content) used by soy-sauce ("kecap") industries are dark color-seeded soybean such as black-seeded and brown-seeded soybean (Zanetta et al., 2014; Kisman et al., 2015, 2019). Unfortunately, production of brown and black-seeded soybean in Indonesia is still very low, which is able to meet only 30% of the needs and the rest is imported (Ministry of Agriculture, 2009).

Increasing production of black soybeans can be pursued through the expansion of planting areas and increased productivity (Zanetta et al., 2014). To increase the planting and harvesting area, soybean can be planted in several land typologies, such as in dry land, irrigated land, in intercropping systems with other food crops, or even under estate plantation and agroforestry systems. However, the biggest problems in growing soybean in an intercropping system or under estate crop plantations are lodging (Wei-guo et al., 2019) due to etiolation-like growth (shade avoidance) (Gong et al., 2015). In conditions of shade stress, the plant height can reach twice as the plant height under normal conditions, causing the plants to collapse.

Under shade stress, soybean shoot death during the early growth caused by shoot topborer, *Melanagromyza dolichostigma* De Meijere, often occurs. About 30% of soybean yield loss occurred due to the attack of these pests in Bangladesh (Biswas, 2013). In Indonesia, the shoot topborer is one of the important insect pests on soybean plants that widespread in both the lowland and highland areas. The pest attacks about 28% on the first trifoliate leaf, 27% on the second trifoliate leaf, 25% on the third trifoliate leaf (Van der Goot, 1984; Atmajaya, 2017).

From the physiological point of view, the soybean shoot deaths will suppress the growth of apical shoots but it will trigger and maximize the growth of lateral shoots that have the potential to increase the number of pods and increase yields. The effect of the shoot deaths is similar to the effect of shoot tip pruning. According to Devens (2017) and Anggarsari et al. (2017), the way to encourage the growth of lateral shoots is pruning. Pruning will suppress the growth of apical shoots and maximize the growth of lateral shoots, so that the formation of new branches will be balanced, and this will have an impact on improving crop productivity. According to Wade and Westerfield (2020), pruning is an invigorating process by removing the apex, destroying the apical dominance (growing point at the tip of main stem) and stimulating the growth of lateral buds into shoots.

Kisman et al. (2021) reported that in the Research Center for Genetic Resource Management of legumes, tubers, and horticulture crops of the Faculty of Agriculture, University of Mataram more than thirty advanced lines of brown-seeded soybeans have been developed. They need to be tested for their ability to grow well and to produce high grain yield under various growing conditions. Since there are some difficulties to find lands for growing soybean crops in irrigated lands due to the farmers' preference for growing rice instead of soybean, growing soybean under additive intercropping systems will be a good choice. There are only few numbers of soybean varieties that are capable of growing under shade in an intercropping system. One of them is Dena-1 variety that is suitable for growing in an additive intercropping system with red rice crops grown on raised-beds under aerobic irrigation systems, and additive intercropping of this soybean variety relay-planted between double-rows of red rice significantly increased yield of red rice (Wangiyana et al., 2019; 2021).

Growing soybean in the dryland areas in Indonesia also faces similar problems such as shading by canopies of perennial crops such as coconut trees and other fruit crops, or in intercropping with maize or sorghum, due to competition for land availability. Therefore, it is essential to develop soybean varieties capable of growing and producing high grain yield under shaded environments. Shading has been reported by many researchers to have some detrimental effects on soybean growth. Wu et al. (2017a) reported that shade-treated soybean increased stem and petiole length, and decreased stem diameters, shoot biomass and its partition to leaves compared with soybean plants under full sunlight. Khalid et al. (2019) also reported that soybean stem diameter significantly decreased while plant height increased as the intensity of shading by maize canopy increased from 25, 50 to 75%. Pod number and grain number per plant also decreased as the shading intensity increased, but grain yield per plant was not significantly different between the treatments of 25% and 0% shading.

From the experimental results of intercropping soybean and tea plants reported by Sedaghathoor and Janatpoor (2012), there was a significant interaction between soybean cultivar and its planting density on soybean height and grain yield, which means that different cultivars of soybean showed different yield responses to intercropping systems with tea plants. In addition, Wu et al. (2017b) reported some adaptive responses of different soybean genotypes to shading in relay intercropping systems, and suggested a need for genotype screening in order to find suitable soybean genotypes for cultivation in intercropping systems. Those advanced lines of brown-seeded soybean developed in the Faculty of Agriculture, University of Mataram also need to be screened for adaptation to shade conditions in combination with other treatments. Therefore, this study aimed to examine the effect of shoot-tip pruning dates on yield and yield components of various brown-seeded soybean lines under shade stress.

2. MATERIALS AND METHODS

2.1. Design of the experiments

The study applied the experimental method, and the experiment was conducted by using polybags under a net house located at Tanjung Karang village, Subdistrict of Sekarbela, Mataram, Lombok, Indonesia. The genetic materials used in this study were seven brown-seeded lines of soybean (G1= KH 7B, G2= KH 7C, G3= KH 7D, G4= KH 9, G5= KH 14, G6= KH 50B dan G7= KH 35). Some other chemical materials such as pesticides (Mipsin, Furadan 3G, Dhitane M 45 and Movento), fertilizers (organic, NPK Phonska fertilizers), and some tools such as net house, polybag, ruler, analytical scale, scissors, sticky label and other stationeries were also used during the implementation of the experiment.

The polybags containing soil and planted with the seeds of the soybean lines tested as the experimental units were placed under a black net house with a 35% reduction of sun light intensity. The experiment was designed according to the factorial Completely Randomized Design (CRD) with two treatment factor. The first factor was brown-seeded lines (G) of soybean, consisting of G_1= KH7B, G_2= KH7C, G_3= KH7D, G_4= KH9, G_5= KH14, G_6= KH50B and G_7= KH35, and the second factor was pruning dates (P) of the soybean shoot-tips of the main stem, consisting of P_0 = control (without pruning), P_1 = pruning at 4 weeks after planting (WAP), P_2 = pruning at 5 WAP, P_3 = pruning at 6 WAP. All of the 28 treatment combinations were made in three replicates.

2.2. Implementation of the experiment

The net house construction was build up with the size of 3.7 meters height, 16 meters length, and 6 meters width, and was covered using a black shading net with an ability to reduce the intensity of sun light by 35%. The planting medium was the soil taken from the rice fields that have been air-dried, sieved using a 2 mm opening sieve, and mixed with the organic fertilizer with the ratio of 2:1. Each polybag was filled with 7 kg of the growing mixture, and each polybag was planted with 3 brown-seeded soybean seeds at 2-3 cm depth, then was applied 3 g of Furadan 3G. The polybags were laid at the distance of 25 cm x 40 cm. Watering the plants was done every day since the seedling age was one week after planting (WAP). The Phonska (NPK 15-15-15) fertilizer dosage of 50 kg/ha (or 3 g per polybag) was applied two times, i.e. before planting and when the plant age was 30 DAP (days after planting). Pest control was carried out using Mipsin and Dithane M-45 with the concentration of 28 ml/liter and 2 g/liter of water respectively. The pruning process was done by cutting the third node of the shoot tip of the main stem at the ages of 4, 5, and 6 weeks after planting (WAP) in accordance with the treatments.

2.3. Observation variables and data analysis

The observation variables consisting of growth, yield and yield components of soybean in this study were plant height, number of productive branches, days to flowering, days to harvest, number of filled pods, number of unfilled pods, number of grains per pod, number of grains per plant, weight of 100 grains, and weight of total grains per plant. The data were analyzed using the statistical program CoStat for Windows ver. 6.303 for the analysis of variance (ANOVA) and the Tukey's HSD test at 5% level of significance. Correlation analysis was done using Minitab for Windows Rel. 13.

3. RESULTS AND DISCUSSION

As shown in the Table 1, there were significant effect of the interaction between the brown-seeded soybean lines and pruning dates on plant height and the weight of 100 grains. The brown-seeded soybean lines had different effect on all observation variables except on the number of unfilled pods. The different pruning dates had a significant effect on plant height, days to harvest, number of productive branches, number of filled pods, number of grains per plant and grain yield per plant. As reported by Adie (2013), grain yield is a very important quantitative character in soybean breeding depending on genetic potential, growing conditions, and cultivation management. The maximum genetic potential of the yield of soybean varieties will be expressed under the perfect environmental conditions.

Based on the interaction effect shown in Fig. 1, the highest plant height (193 cm) under shade condition was shown by the KH9 line with no pruning (G_4P_0) and the lowest one (36.5 cm) was on the KH14 line with pruning at 4 WAP (G_5P_1). Similar to those reported by Nursamsidar et al. (2019) that KH9 brown-seeded soybean line showed the highest plant height in the condition of shade stress compared to KH4, KH1, Dena-1, and Anjasmoro varieties. Brown-seeded soybean lines grown in shade conditions and without shoot pruning such as the KH9 line (G_4P_0) showed lengthening growth of the stems (etiolation-like). According to Gong et al. (2015), the variety exhibiting clear preference of shade avoidance was those showing a tall main stem, hypocotyl elongation, and biomass allocation towards the stem. Our results showed that the KH14 line pruned at 4 WAP (G_5P_1) had the lowest plant height under shade stress condition. This was about the same as those reported by Ohyama and Harper (1991) that cutting shoots at initial growth can shorten the height of soybean plants and multiply lateral branches.

Table 1. Analysis of variance of the effect of pruning date on yield and yield components of some brown-seeded soybean lines under shade stress

Variables	Treatments		
	Brown-seeded lines (G)	**Pruning Dates (P)**	**Interaction of soybean line x pruning (G*P)**
Plant height	s	s	s
Number of productive branches	s	s	ns
Days to flowering	s	ns	ns
Days to harvest	s	s	ns
Number of filled pods	s	s	ns
Number of unfilled pods	ns	ns	ns
Number of grains per plant	s	s	ns
Weight of 100 grains	s	ns	s
Grain yield per plant	s	s	ns

Remarks: s = significant effects; ns = non-significant effects

In relation to the interaction effect on the weight of 100 grains (Fig. 2), the highest average weight of 100 grains, on the other hand, was shown by the soybean line pruned at 6 WAP, i.e. on the KH50B line (G_6P_3 = 8.59 g) or on the KH7C line (G_2P_3 = 8.51 g) and the lowest one was on the KH9 line pruned at 6 WAP (G_4P_3 = 6.30 g). The results of this study are similar to those reported by Faozi et al. (2020) that pruning the tips of soybean shoots during the initial vegetative stages up to around the flowering date effectively affects branch growth of soybean varieties. The growth of plant heights, number of branches, number of productive nodes, and soybean grain yields vary among soybean varieties used.

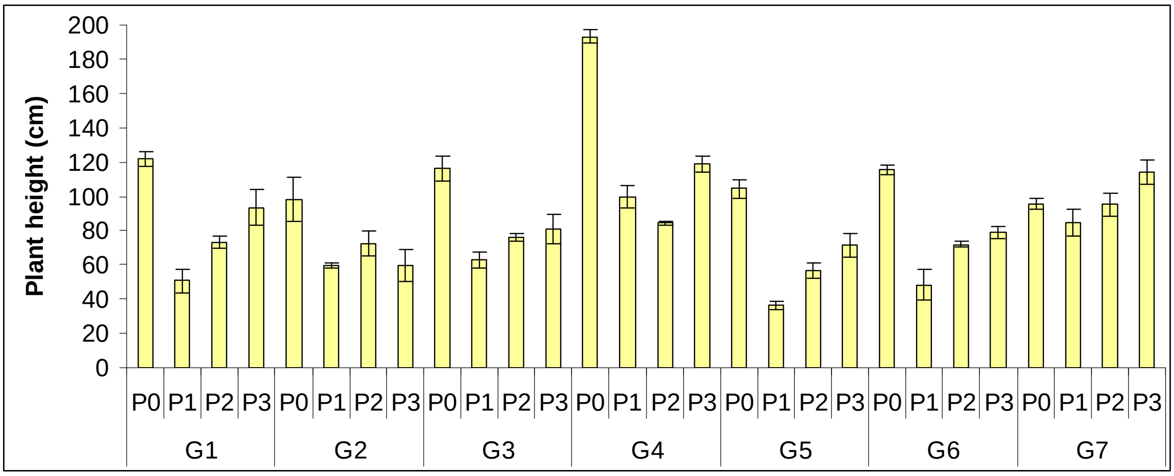

Fig. 1. Average (Mean ± SE) plant height (cm) as affected by interaction effects between soybean lines and pruning treatments

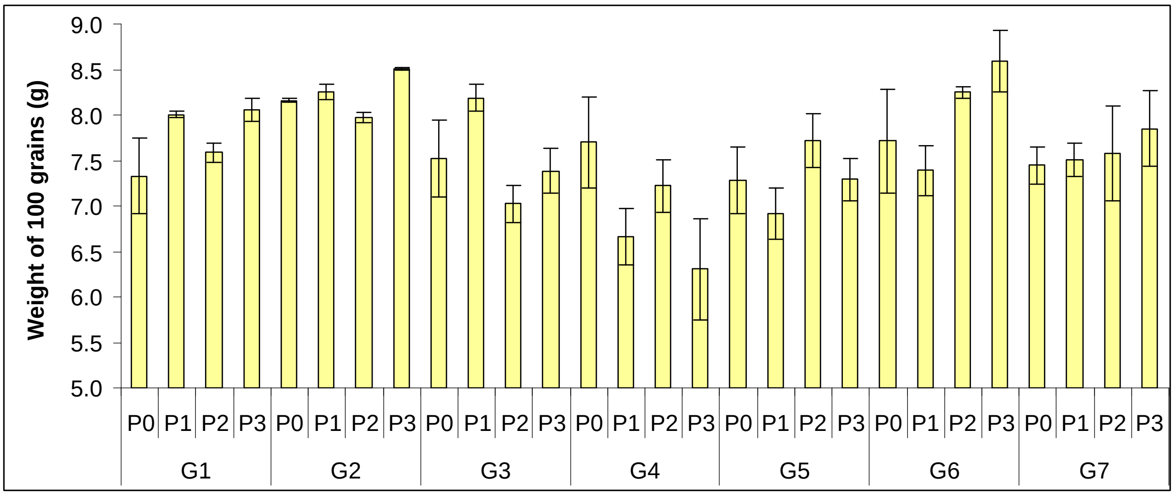

Fig. 2. Average (Mean ± SE) weight of 100 grains (gram) as affected by interaction effects between soybean lines and pruning treatments

As shown in the Table 2, the highest plant height was on KH9 (123.83 cm) and the lowest was on KH14 (67.12 cm). The highest number of productive branches was on KH50B (6.33 branches) and the lowest was on KH35 (4.08 branches). KH9 also showed the latest flowering date (42.75 DAP) and harvest date (96.16 DAP) and the KH7B line showed the earliest flowering date (39.41 DAP) and harvesting date (86.91 DAP). KH35 showed the highest number of filled pods per plant (70.83 pods) while the lowest was on KH7C (47.08 pods). KH35 also showed the highest number of grains per plant (147.33 seed) and the highest grain yield per plant (11.11 g). KH9 also showed the lowest weight of 100 grains (6.97 g). KH14 had the lowest number of grains per plant (111.33 seed) and the lowest grain yield per plant (8.11 g). These were different from those reported by Nursamsidar et al. (2019) that the KH14 line had better growth characters compared to the KH9, KH1 and yellow seeded Dena-1 and Anjasmoro varieties.

Table 2. Effect of brown-seeded soybean lines on the yield and yield components under shade stress

Genotype	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
G1	84.71 bc	5.33 ab	39.41 b	86.91 c	51.33 bc	0.50	112.25 b	7.74 abc	8.61 b
G2	72.33 cd	4.41 b	40.33 b	88.00 bc	47.08 c	0.08	101.25 b	8.22 a	8.37 b
G3	84.04 bc	4.83 b	39.83 b	87.41 bc	57.58 abc	1.16	126.66 ab	7.53 abc	9.57 ab
G4	123.83 a	4.41 b	42.75 a	96.16 a	61.83 ab	0.00	126.00 ab	6.97 c	8.87 ab
G5	67.12 d	5.33 ab	39.75 b	88.58 b	52.91 bc	0.50	111.33 b	7.30 bc	8.11 b
G6	78.58 cd	6.33 a	39.66 b	87.50 bc	55.75 bc	0.41	118.50 ab	7.98 ab	9.49 ab
G7	97.33 b	4.08 b	42.16 a	96.00 a	70.83 a	0.50	147.33 a	7.78 ab	11.11 a
HSD5%	13.35	1.62	1.05	1.34	13.97	-	29.64	0.77	2.48

Remarks: Figures followed by the same letter indicated non significant difference at HSD 5%.
G_1= KH7B, G_2= KH7C, G_3= KH7D, G_4= KH9, G_5= KH14, G_6= KH50B and G_7= KH35
1 = plant height, 2 = number of productive branches, 3 = days to flowering, 4 = days to harvest, 5 = number of filled pods, 6 = number of unfilled pods, 7 = number of grains per plant, 8 = weight of 100 grains, and 9 = grain yield (g/plant)

As shown in the Table 3, shoot pruning significantly reduced the plant height, the number of filled pods, the number of grains per plant, and grain yield per plant. Pruning at 4 WAP resulted in the lowest plant height (63.14 cm), i.e. about 50% reduction from the normal plant height, the lowest number of pods per plant (47.85 pods), number of grains per plant (101.04 seed), and grain yield per plant (7.59 g) but the highest days to harvest (90.42 DAP). Results of this study were mainly similar to research findings reported by Ohyama and Harper (1991) that shoot pruning interrupt the carbohydrate supply from shoot due to rapid decline in N_2 fixation, thus disrupting the growth of plants including the plant height, the number of filled pods, the number of grains per plant, and also grain yield per plant. This can be compared to soybeans without pruning which indicates that the brown-seeded soybean performed the highest plant height (120.64 cm), number of filled pods (67.66 pods), number of grains per plant (144.52 seed), grain yield per plant (10.99 g), and also the earliest harvest date (89.52 DAP).

Table 3. Effect of pruning dates on the yield and yield components of brown-seeded soybean lines under shade stress

Pruning	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
P0	120.64 a	4.90 ab	40.52	89.52 b	67.66 a	0.47	144.52 a	7.59	10.99 a
P1	63.14 d	4.85 ab	40.76	90.42 a	47.85 c	0.28	101.04 c	7.56	7.59 c
P2	75.57 c	5.57 a	40.28	90.19 ab	58.42 b	0.52	123.57 b	7.62	9.38 ab
P3	88.05 b	4.52 b	40.66	90.19 ab	53.09 bc	0.52	112.76 bc	7.82	8.69 bc
HSD5%	8.87	1.06	-	0.87	9.15	-	19.42	-	1.62

Remarks: Figures followed by the same letter indicated non significant difference at HSD 5%.

P_0 = control (no pruning), P_1 = pruning at 4 WAP, P_2 = pruning at 5 WAP, P_3 = pruning at 6 WAP

1 = plant height, 2 = number of productive branches, 3 = days to flowering, 4 = days to harvest, 5 = number of filled pods, 6 = number of unfilled pods, 7 = number of grains per plant, 8 = weight of 100 grains, and 9 = grain yield (g/plant)

According to Wade and Westerfield (2020), pruning is an invigorating process by which removing the apex, destroying the apical dominance (growing point at the tip of main stem) and stimulating the growth of lateral buds into shoots. In conditions of shade stress, the plant height can reach twice the plant height under normal conditions. This causes the plants to collapse, which at the end can interfere with the growth and yield of soybean plants. As reported by Wu et al. (2017b), shade treated plants showed significant increase in stem length and petiole length, and decrease in stem diameters, and shoot biomass.

Shoot tip pruning of soybean in general will limit the high growth of plants, especially those done at the beginning of vegetative growth, namely at the age of 2 and 3 weeks after planting. By removing the apex, pruning temporarily destroys apical dominance and stimulates the growth of lateral buds into shoots. Pruning at 5 WAP promoted the highest number of productive branches (5.57 branch). This was the same as those reported by Singh et al. (2020) that pruning on the green gram (*Vigna radiata* L.) significantly increased the growth characters such as dry matter accumulation (g/plant), trifoliate leaves, branches per plant and root nodules per plant as well as yield and yield components such as pod number per plant, number of grains per pod, 100-grain weight and grain yield (kg/ha).

When correlation analysis between observation variables was run in each pruning treatment (P0, P1, P2 & P3), the results showed different set of significant correlation, especially between grain yield per plant and other observation variables, as it can be seen from Table 4.

Table 4. Correlation between observation variables and its p-value

Observation variables	Height	DTF	DTH	PBN	FPN	GNPP	GYPP
P0= Winthout pruning:							
Days to flowering (DTF)	0.404						
p-value	0.069						
Days to harvest (DTH)	0.397	0.839					
p-value	0.074	**0.000**					
Productive branch number (PBN)	-0.175	-0.458	-0.406				
p-value	0.448	**0.037**	0.068				
Filled pod number (FPN)	0.169	0.157	0.264	0.324			
p-value	0.465	0.496	0.247	0.152			
Grain number per plant (GNPP)	0.057	0.000	0.073	0.432	0.922		
p-value	0.807	0.999	0.753	0.051	**0.000**		
Grain yield per plant (GYPP)	0.106	0.065	0.143	0.239	0.872	0.920	
p-value	0.649	0.780	0.536	0.297	**0.000**	**0.000**	
Weight of 100 grains (W100)	-0.010	0.088	0.140	-0.363	-0.023	-0.051	0.307
p-value	0.966	0.703	0.545	0.105	0.921	0.825	0.176
P1= Pruning at 4 WAP:							
Days to flowering (DTF)	0.703						
p-value	**0.000**						
Days to harvest (DTH)	0.772	0.856					
p-value	**0.000**	**0.000**					
Productive branch number (PBN)	-0.518	-0.474	-0.637				
p-value	**0.016**	**0.030**	**0.002**				
Filled pod number (FPN)	0.535	0.284	0.472	-0.162			
p-value	**0.013**	0.213	**0.031**	0.483			
Grain number per plant (GNPP)	0.439	0.198	0.426	-0.194	0.947		
p-value	**0.047**	0.389	0.054	0.399	**0.000**		
Grain yield per plant (GYPP)	0.329	0.038	0.235	-0.129	0.851	0.928	
p-value	0.145	0.870	0.304	0.579	**0.000**	**0.000**	
Weight of 100 grains (W100)	-0.234	-0.406	-0.489	0.183	-0.208	-0.162	0.208
p-value	0.307	0.068	**0.025**	0.427	0.366	0.484	0.365
P2= Pruning at 5 WAP:							
Days to flowering (DTF)	0.692						
p-value	**0.000**						
Days to harvest (DTH)	0.700	0.882					
p-value	**0.000**	**0.000**					
Productive branch number (PBN)	-0.567	-0.618	-0.556				
p-value	**0.005**	**0.002**	**0.006**				
Filled pod number (FPN)	0.327	0.400	0.377	-0.173			
p-value	0.128	0.059	0.076	0.429			
Grain number per plant (GNPP)	0.239	0.373	0.322	-0.136	0.943		
p-value	0.272	0.080	0.134	0.535	**0.000**		
Grain yield per plant (GYPP)	0.232	0.283	0.281	-0.055	0.917	0.931	
p-value	0.287	0.191	0.194	0.805	**0.000**	**0.000**	
Weight of 100 grains (W100)	-0.163	-0.339	-0.195	0.296	-0.313	-0.406	-0.069
p-value	0.457	0.113	0.374	0.170	0.146	0.055	0.756
P3= Pruning at 6 WAP:							
Days to flowering (DTF)	0.551						
p-value	**0.014**						
Days to harvest (DTH)	0.679	0.729					

p-value	**0.001**	**0.000**					
Productive branch number (PBN)	0.433	0.111	0.446				
p-value	0.064	0.651	0.056				
Filled pod number (FPN)	0.673	0.422	0.704	0.410			
p-value	**0.002**	0.072	**0.001**	0.082			
Grain number per plant (GNPP)	0.688	0.347	0.639	0.442	0.939		
p-value	**0.001**	0.145	**0.003**	0.058	**0.000**		
Grain yield per plant (GYPP)	0.234	0.121	0.249	0.207	0.644	0.766	
p-value	0.336	0.622	0.303	0.395	**0.003**	**0.000**	
Weight of 100 grains (W100)	-0.352	-0.233	-0.474	-0.251	-0.166	-0.042	0.548
p-value	0.140	0.338	**0.040**	0.299	0.498	0.864	**0.015**

As it can be seen from Table 4, pruning of shoot tips increased the number of observation variables showing significant correlation with other observation variables. The most notable changes were the correlation between days to flowering and the number of productive branches per plant. Without tip pruning or pruning at 4 or 5 WAP, days to flowering showed a significant negative correlation with the number of productive branches per plant, while tip pruning at 6 WAP actually increased the number of productive branches per plant. Although between treatments of pruning dates there was no significant differences in days to flowering, it was significantly different among the genotypes of the brown-seeded soybean lines tested. This could mean that for the late flowering genotypes, pruning at 6 WAP could be the best for increasing the number of productive branches per plant. In addition, the number of productive branches per plant showed a positive correlation (r = +0.410, p-value = 0.082) with the number of filled pods per plant, which coefficient was the highest under tip pruning at 6 WAP, while the number of filled pods per plant showed a significant positive correlation with number of grains per plant and grain yield per plant (Table 4). Further studies need to be carried out in relation to this pruning treatment under a real condition in the field, i.e. under shading by canopies of other crops in an intercropping system.

4. CONCLUSION

The significant interaction effect on plant height and 100-grain weight indicates different responses between the soybean lines to pruning under shade stress in terms of plant height and 100-grain weight, but delaying pruning date could increase weight of 100 grains, especially on G6. However, among the brown-seeded soybean lines, KH35 (G7) and KH50B (G6) lines performed the best under shade stress, as indicated by the highest 100 grain weight, grain number, and grain yield per plant, especially the KH50B genotype, which might be potential for developing shade tolerant varieties.

AUTHORS' CONTRIBUTIONS

All authors contributed in the development of the manuscript. Author1 is the principal author, who designed and organized the experiment and prepared the manuscript draft; Author2 prepared references, data analysis, and discussion; Author3 prepared the data, and references; and Author4 proof-read the entire manuscript draft, rewrote and expanded the introduction, run the correlation and interaction analysis and produced the graph, and finished the manuscript until ready to submit.

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