agronomy

MDPI

Article

Evaluating, Screening and Selecting Yardlong Bean [*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.] for Resistance to Common Cutworm (*Spodoptera litura* Fabricius)

Tianchi Yao [1,2], Yufei Xu [1], Huiyan Jiang [1], Xinxin Chen [1], Xiaofeng Liu [1], Huatao Chen [3], Hongmei Zhang [3,*] and Guangnan Xing [1,*]

[1] Soybean Research Institute, MARA National Center for Soybean Improvement, MARA Key Laboratory of Biology and Genetic Improvement of Soybean, National Key Laboratory for Crop Genetics & Germplasm Enhancement and Utilization, Jiangsu Collaborative Innovation Center for Modern Crop Production, Nanjing Agricultural University, Nanjing 210095, China
[2] College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China
[3] Institute of Industrial Crops, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China
* Correspondence: xinggn@njau.edu.cn (G.X.); zhm@jaas.ac.cn (H.Z.)

Abstract: The yardlong bean [*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.] is an important vegetable crop, but it is prone to pest infestation. Therefore, breeding insect-resistant varieties is essential to reduce pesticide applications and to increase bean quality and yield. In the present study, 64 yardlong bean varieties were screened for their resistance to the common cutworm (*Spodoptera litura* Fabricius). In the greenhouse, leaves, pods, and seeds of yardlong beans were harmed by naturally occurring common cutworms. Seventeen insect-resistant and four insect-susceptible yardlong bean varieties were identified based on the weight of the nine-day-old larvae and 72 h weight increases of 4th instar larvae through feeding newly hatched and 4th instar larvae, respectively. Subsequent verification feeding experiments with newly hatched larvae showed that Zhuzaidou and Pingtangjiangdou's insect resistance are the weakest and Jiangdou No.5, j-1, Zhijiangtezao No. 30, and Changcaidou have the strongest insect resistance. In 21 yardlong bean varieties, starch content and larval weight showed negative correlation and there was positive correlation between crude protein and larval weight, but almost neither of them reached significant levels. Through organ antibiotic and antixenotic experiments, it was concluded that common cutworms preferred feeding on yardlong bean leaves, and the weight increase of common cutworms feeding on leaves was higher than that of pods and seeds. These insect-resistant yardlong bean varieties warrant further investigation in basic antibiosis mechanism research in yardlong beans and can serve as germplasm resources for breeding programs engaged in reducing pesticide usage.

Keywords: yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis*); common cutworm (*Spodoptera litura*); insect resistance; evaluating; germplasm screening

Citation: Yao, T.; Xu, Y.; Jiang, H.; Chen, X.; Liu, X.; Chen, H.; Zhang, H.; Xing, G. Evaluating, Screening and Selecting Yardlong Bean [*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.] for Resistance to Common Cutworm (*Spodoptera litura* Fabricius). *Agronomy* **2023**, *13*, 502. https://doi.org/10.3390/agronomy13020502

Academic Editor: C. Eduardo Vallejos

Received: 13 November 2022
Revised: 20 December 2022
Accepted: 3 January 2023
Published: 9 February 2023

1. Introduction

Cowpea, *Vigna unguiculata* (L.) Walp., is an annual legume known for its resistance to high temperature and drought conditions [1,2]. The genus *Vigna* contains about 150 species and southern Africa has been suggested as the center of origin for the wild cowpea [2]. Cowpea varieties are generally divided into three cultivated subspecies, including the short cowpea [*V. unguiculata* subsp. *cylindrica* (L.) Estt.], the common cowpea [*V. unguiculata* subsp. *unguiculata* (L.) Walp.], and the yardlong bean [*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.] [1–3]. Pods of the short cowpea are typically 7–12 cm, while the common cowpea pods do not often exceed 30 cm, and the pods of yardlong bean range from 30–100 cm long [3]. These elongated pods are tender and commonly eaten as fresh vegetables, rather than harvested to produce dry beans [4]. Yardlong beans are nutrient rich and can provide

31% of daily nutritional requirements for vitamin C, 17% of vitamin A requirements, and contain 25% of the recommended daily allowance of vegetable protein [3,5]. In many Asian, African, and Latin American developing nations, the yardlong bean continues to serve as an important dietary component [6–8]. In China, the yardlong bean is widely cultivated in summer and autumn as a commercially and culturally important crop [9] in all provinces except Qinghai and Tibet, accounting for 330,000 hm^2 of planting area [1].

Although the yardlong bean shows resistance to some abiotic stresses, there are numerous insect pests that feed on this crop, negatively impacting yield and bean quality, including the cowpea aphid (*Aphis craccivora* Koch), the bean borer (*Maruca vitrata* Geyer), the common cutworm (*Spodoptera litura* Fabricius), the vegetable leaf miner (*Liriomyza sativae* Blanchard), the carmine spider mite (*Tetranychus cinnabarinus* Boisduval), and the legume bud thrip (*Megalurothrips sjostedti* Tryb), etc. [10–12]. Cowpea aphids and legume bud thrips are commonly found in Africa, Asia, and South America, whereas bean borers and common cutworms are found worldwide, affecting all yardlong bean production regions [10]. During the spring growing season, thrips, mites, aphids, and bean borers are responsible for the most damage in the yardlong bean; by contrast, bean borers, mites, thrips, aphids, common cutworms, and vegetable leaf miners incur the largest losses in summer sowing fields [11]. A large infestation with common cutworms can seriously reduce yields in yardlong bean production. From late July to early August, the summer and autumn yardlong bean is in the seedling stage. During this period, common cutworm outbreaks are prevalent [13]. In addition, 4th instar common cutworm larvae can consume all the leaves of a yardlong bean plant within a few days, causing greater harm to leaves than pods [13].

In order to minimize damage from insect infestation, plants have evolved mechanisms to resist some insects, which can be categorized as antibiosis, antixenosis, and tolerance [14,15]. Antibiosis refers to the ability of plants to not provide all of the nutrients required by insects or to contain substances that are toxic to insects, resulting in stunted growth, reduced fertility, or even death after insects consume them [16]. Thus, plant resistance to insects is evaluated by quantifying antibiosis indicators such as weight change, survival rate, and delayed growth and the development of insects after feeding on a potentially resistant host [17,18]. For example, individual leaves can be isolated in a petri dish with test insects, and phenotypic comparisons with control insects reared on defined media or known susceptible varieties can illustrate resistant plant phenotype [19]. In the 3rd instar common cutworm, food intake, insect weight gain, and fecal volume are all used as indicators of antibiosis [20].

The cowpea trypsin inhibitor (CpTI), which has a broad-spectrum toxicity towards insects and is widely used in insect resistance genetic experiments or breeding, is largely found in cowpea seeds [21]. The transgenic expression of CpTI in cabbage can greatly reduce feeding by cabbage worms [22]. The bean borer is the most common pest of the cowpea in Africa and South America [10]. The screens of accessions revealed a strong resistance to the bean borer in wild cowpea [*Vigna vexillata* (L.) A. Rich] [10]. In other laboratory assays for antibiosis in legumes, the newly hatched bean borer larvae were transferred to the flower buds of the yardlong bean [*V. unguiculata* subsp. *sesquipedalis* (L.) Verdc.], the haricot bean (*Dolichos lablab* L.), and the kidney bean (*Phaseolus vulgaris* L.) [23]. Then larval survival rates, pupation time, and pupa emergence were recorded, and the 5th instar larvae and the recently molted pupae were weighed, which together indicated that the cowpea is the most suitable host for the bean borer, while kidney beans were unsuitable for the growth of the bean borer [23]. Similarly, in another study, the weight of 13-day-old larvae, pupal weight, and changes in the fall armyworm [*Spodoptera frugiperda* (J.E.Smith)] development or life history were observed to evaluate resistance in cowpea cultivars, ultimately identifying Juti and Nioaque landraces as highly resistant varieties [24]. Distant hybridization of the rice bean [*V. umbellata* (Thunb.) Ohwi & Ohashi] with the yardlong bean, followed by evaluation of pod traits and insect resistance in field trials, showed that leaves of the hybrids exhibited broad resistance to numerous major pests such as the cotton leafworm (*Prodenia litura* Fabricius) and beet armyworm (*Spodoptera exigua* Hübner), while pod traits remained similar to that of the yardlong bean lines [25].

Toxicity from cowpea vegetables due to pesticide residues was responsible for the sickening and death of people in Hainan, 2010 [26]. This event was likely due to the use of excessive or banned pesticides to control pests and increase yields [26]. To avert such public health and safety threats in food production, while providing economic and ecological benefits, pesticide use can be reduced through the deployment of insect-resistant varieties [27,28]. However, few studies [25] to date have examined the resistance to foliar pests in the yardlong bean. The present study used different methods to evaluate the resistance of different yardlong beans to the common cutworm, in order to screen out insect-resistant varieties. These assays identified a subset of resistant varieties which could be used as a source germplasm to improve resistance in elite cultivars, ultimately reducing pesticide application while improving the yield and quality of yardlong bean.

2. Materials and Methods

2.1. Plant Materials

The 64 tested varieties of yardlong bean [*V. unguiculata* subsp. *sesquipedalis* (L.) Verdc.] are from 15 provinces including Fujian, Guangdong, Guizhou, Hebei, Jiangsu, Sichuan, Hubei, Shanghai, Henan, Heilongjiang, Liaoning, Ningxia, Shanxi, Xinjiang, and Jiling, all provided by the Jiangsu Academy of Agricultural Sciences. On 23 March 2021, 64 varieties of yardlong beans were sown in the greenhouse of Liuhe Animal Base, at the Jiangsu Academy of Agricultural Sciences (32°28′ N, 118°37′ E, ASL: 14 m). On 28 June 2021, 10 July 2021, and 29 June 2022, according to the preliminary evaluation results of the resistance of yardlong bean varieties to common cutworm (*Spodoptera litura* Fabricius), 21 yardlong bean varieties including 17 resistant and 4 sensitive varieties were sown in the greenhouse of the Jiangsu Academy of Agricultural Sciences (32°01′ N, 118°52′ E, ASL: 9 m).

2.2. Common Cutworm Rearing

Common cutworms were raised by the National Center for Soybean Improvement of China with artificial feed for successive generations in a standard insectarium. The insectarium was sterilized once per generation. The insectarium was kept at a constant 27 ± 1 °C, with a relative humidity of 60% to 70% and the light cycle was 14 h of light and 10 h of darkness [29].

2.3. Investigation on the Damage of the Common Cutworm to the Yardlong Bean in the Greenhouse

The yardlong beans planted on 19 August 2021 were affected by naturally occurring common cutworms during the podding stage. We collected 50 pods from late, medium, and early maturity varieties, respectively, measured the length of the pods and wormholes on them, counted the wormholes on the pods, and calculated the average wormhole length and the ratio of wormhole length to total pod length.

2.4. Evaluation of Yardlong Bean Varieties for Resistance to the Common Cutworm

The yardlong beans were sown on 23 March 2021, and when they reached the podding stage, whole leaves of each leaf position were picked, put in ziplock bags, and kept in an ice box to keep them from wilting. A disposable plastic petri dish with a diameter of 9 cm was used, and moistened filter paper was placed on the bottom of the petri dish to replenish leaf moisture.

Method 1: On 10 June 2021, put one compound leaf of yardlong bean in each petri dish and put five newly hatched larvae on it. Each yardlong bean variety is repeated three times, the leaves are replaced every two days, and the larvae are weighed on the 9th day. Larvae are weighed using a 1/10,000 analytical balance (accuracy: 0.0001 g; range: 0.01–65 g; OHAUS Instruments Co., Ltd., Shanghai, China).

Method 2: On 17 June 2021, put one compound leaf of yardlong bean in each petri dish and put two 4th instar larvae (similar weight) on it. Each yardlong bean variety is repeated three times, with random block design. The leaves are replaced every 12 h, and the larvae are weighed after 72 h. Larvae are weighed using a 1/10,000 analytical balance (accuracy: 0.0001 g; range: 0.01–65 g; OHAUS Instruments Co., Ltd., Shanghai, China).

2.5. Selection of Yardlong Bean Germplasm Resources Resistant to Common Cutworm

On 16 August 2021, 22 August 2021 and 6 August 2022, method 1 was used to validate 4 insect-susceptible varieties and 17 insect-resistant varieties which were sown on 28 June 2021, 10 July 2021, and 29 June 2022, separately, based on preliminary screening results. The experiment was repeated three times; larvae were weighed on the 6th, 9th, and 12th day.

2.6. Determination of Crude Protein Content and Starch Content in Yardlong Bean Leaves

Twenty-one varieties of yardlong bean sown on 29 June 2022 were selected, and whole leaves were collected on 15 August for starch content and crude protein content detection. The starch detection method uses the anthrone sulfuric acid method [30]; the Kjeldahl method was used to detect crude protein content [31]. The collected leaves were dried for 48 h, and starch content and crude protein content detection were repeated three times.

2.7. Resistance for Common Cutworm of Different Organs of Yardlong Bean

Organ antibiotic resistance experiment: Two susceptible varieties Zhuzaidou and Pingtangjiangdou, and two resistant varieties, Jiangdou No.5 and Zhijiangtezao No.30, were used for antibiotic resistance analysis. A moistened filter paper was placed on a plastic petri dish with a diameter of 9 cm. To compare the insect resistance of three different organs of these four yardlong bean varieties, one compound leaf or one 5 cm-long de-seeded pod or three seeds were put in each petri dish, respectively, receive three 3rd instar larvae with a similar weight and size. Each variety was repeated three times, and the larvae were weighed at 24 h, 48 h and 72 h, respectively. Larvae were weighed using a 1/10,000 analytical balance (accuracy: 0.0001 g; range: 0.01–65 g; OHAUS Instruments Co., Ltd., Shanghai, China).

Organ antixenotic resistance experiment: Two susceptible varieties Zhuzaidou and Pingtangjiangdou, and two resistant varieties, Jiangdou No.5 and Zhijiangtezao No.30, were used for antixenotic resistance analysis. A moistened filter paper was placed on the bottom of a glass petri dish with a diameter of 15 cm. In each petri dish, three pieces of yardlong bean compound leaves, two 5 cm de-seeded pods and four seeds from the same variety were placed on the outer circle of petri dish. The organs were weighed with a 1/10,000 analytical balance (accuracy: 0.0001 g; range: 0.01–65 g; OHAUS Instruments Co., Ltd., Shanghai, China). Six 4th instar larvae of similar weight and size were released in the center of petri dish. Each variety was repeated three times. The number of common cutworms on different organs was recorded at 8 h, 16 h and 24 h, and the remaining organs were weighed after 24 h.

2.8. Data Analysis

The experimental data were integrated with Excel 2016 (Microsoft, Redmond, WA, USA). SAS 9.4 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. PROC GLM was used for variance of analysis, Person correlation coefficient was used for correlation analysis and means were separated with Duncan's multiple range test at the 5% level.

3. Results

3.1. Common Cutworm Damage to the Yardlong Bean in a Greenhouse

A detailed examination of naturally infested yardlong bean plants showed damage to leaves, pods, and beans (Figure 1). The pods were nicked, perforated, and necrotic after common cutworms had damaged them. The calculation of the average number and the average wormhole length in pods showed that the wormhole number and length were both highest in medium-ripening pods, and the lowest in late-ripening pods (3.1 vs. 1.2 holes per pod; 1.4 vs. 1.3 cm in medium vs. late pods; Figure 2; Table S1). The proportion of wormhole size to total pod length was roughly equivalent among three kinds of pod maturity (~3.0%).

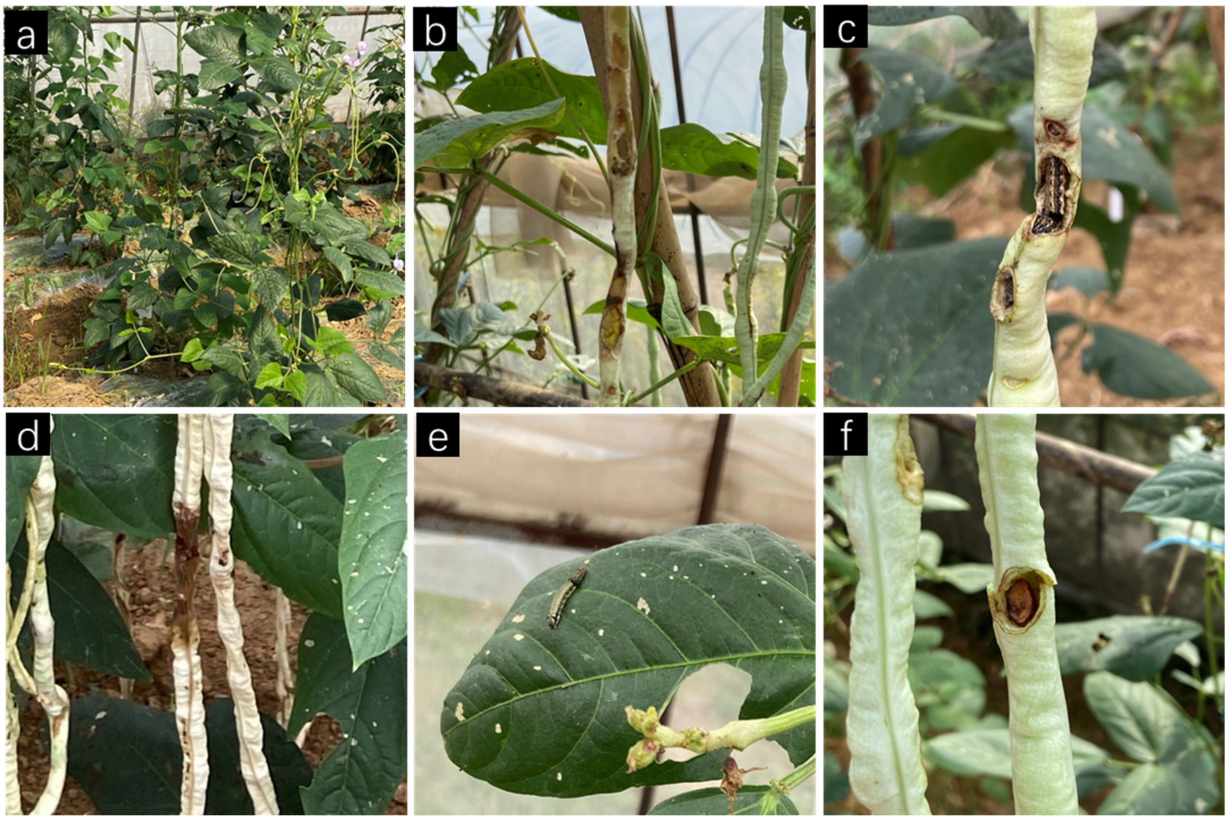

Figure 1. The damage of yardlong bean caused by the common cutworm. (**a**) Growth of the yardlong bean in a greenhouse; (**b**) The pod is nicked by the common cutworm; (**c**) The pod is perforated by the common cutworm which hid in the pod; (**d**) The pod is necrotic after the common cutworm feeds; (**e**) Common cutworm on the leaflet; (**f**) The pod and the seed are injured by the common cutworm.

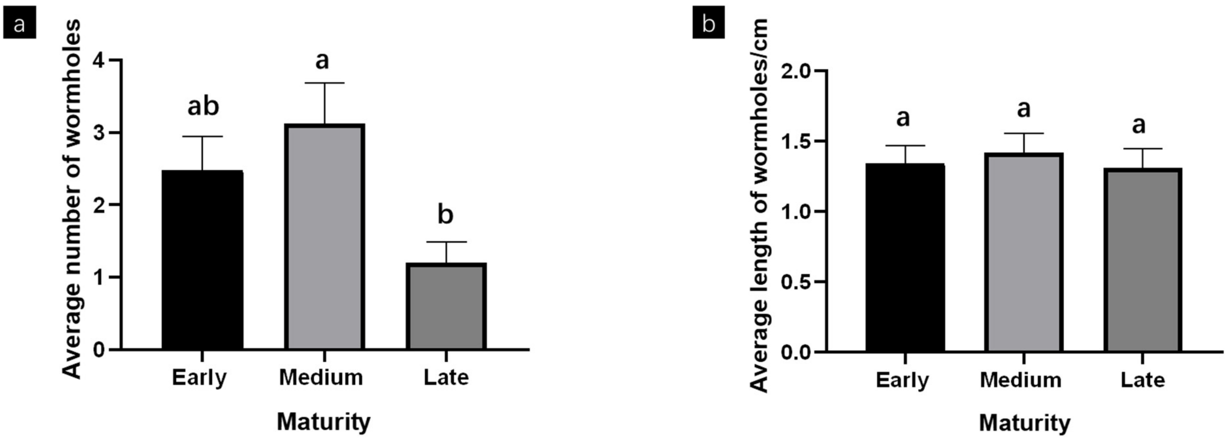

Figure 2. The average wormhole number and length in pods of three maturity types. (**a**) The average wormhole number in pods of three maturity types; (**b**) The average wormhole length in pods of three maturity types. Error line represents standard error. The same lowercase letter means no significant difference.

3.2. Evaluation of Yardlong Bean Varieties for Resistance to the Common Cutworm

To test whether feeding by the common cutworm varies among different varieties of yardlong bean, that is, to determine whether some varieties exhibit resistance to common cutworm feeding, we next compared the weight of nine-day-old larvae (LW9) and 4th instar larvae weight increase reared on leaves of 64 yardlong bean varieties (Figure 3; Table S2).

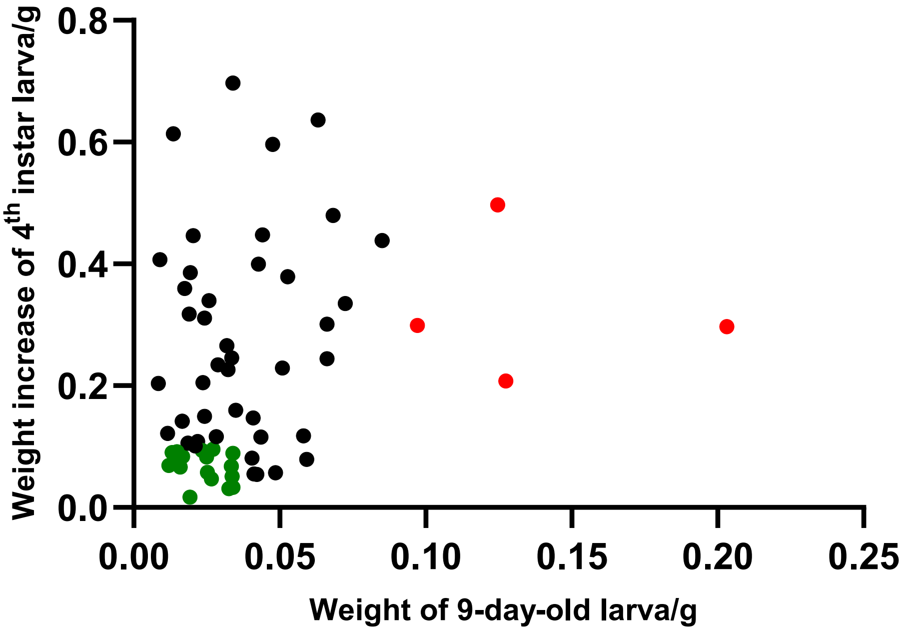

Figure 3. Scatter plot of weight increase of 4th instar larva after feeding on leaves for 72 h and weight of 9-day-old larva. The red dots in the figure represent the sensitive varieties, and the green dots represent the resistant varieties.

The results showed that the average LW9 was highest on the Zhuzaidou variety, reaching 0.203 g, and the lowest in Sujiang52, only 0.008 g (Table S2), indicating highly significant differences in consumption among varieties ($F = 4.91$, $p < 0.001$ for LW6 and $F = 4.09$, $p < 0.001$ for LW9).

In 4th instar larvae, the 72 h fresh weight increase (4thLW) also significantly differed from yardlong bean varieties ($F = 6.43$, $p < 0.001$), with larvae fed with JDMS009 leaves showing the greatest increase at 72 h (0.697 g) and those fed on Zhijiangtezao No.30 having the least increase in weight (0.017 g, Table S2).

Correlation analysis between the 72 h fresh weight gain and LW9 further revealed a significant correlation between these measurements ($r = 0.26654$, $p = 0.0347$). Examination of a pairwise scatter plot of LW9 and 4thLW (Figure 3) thus identified 156fanjiang, Changtingbaopihongjiangdou, Zhuzaidou and Suijiao101 as susceptible varieties that led to differentially greater increases in cutworm weight, based on an LW9 threshold of >0.09 g and 4thLW of >0.2 g. Conversely, using an LW9 cut-off of <0.04 g and a 4thLW cut-off value of <0.1 g, 17 common cutworm-resistant varieties were screened out, including Baijiangdou, Changcaidou, Jiangdou No.5, Zhijiangtezao No.30, SS-97, Suzi41, Sujiang1419, j-5, Sujiang12, Dabaitiaojiangdou, Paojiangdou, Gaochan No.4, Pingtangjiangdou, j-1, Feicui, Sujiang No.1 and Jiangdou2045 (Table 1), suggesting that some varieties could indeed show greater resistance to common cutworm feeding. In the sensitive yardlong bean varieties, the average nine-day-old larval weight was 0.123 g and the average 72 h fresh weight increase of 4th instar larvae was 0.349 g. Conversely, the average nine-day-old larval weight was only 0.024 g and the average 72 h fresh weight increase of 4th instar larvae was 0.067 g in the resistant yardlong bean varieties.

Table 1. Insect-sensitive or resistant yardlong bean varieties screened by evaluation of newly hatched larvae and 4th instar larvae weight increase.

Varieties Code	Resistance Type	Variety	Origin	4thLW/g	LW9/g
1	sensitive	Changtingbaopihongjiangdou	Changting, Fujian	0.497	0.125
2	sensitive	Zhuzaidou	Guangzhou, Guangdong	0.297	0.203
3	sensitive	Suijiao101	Guangzhou, Guangdong	0.301	0.066
4	sensitive	156fanjiang	Wuhan, Hubei	0.299	0.097
		S-MEAN		0.349	0.123
5	resistant	Baijiangdou	Danzhai, Guizhou	0.067	0.016
6	resistant	Changcaidou	Zanhuang, Hebei	0.067	0.033
7	resistant	Jiangdou No.5	Wuhan, Hubei	0.031	0.033
8	resistant	Zhijiangtezao No.30	Wuhan, Hubei	0.017	0.019
9	resistant	SS-97	Nanjing, Jiangsu	0.095	0.027
10	resistant	Suzi41	Nanjing, Jiangsu	0.051	0.034
11	resistant	Sujiang1419	Nanjing, Jiangsu	0.033	0.034
12	resistant	j-5	Nanjing, Jiangsu	0.057	0.025
13	resistant	Sujiang12	Nanjing, Jiangsu	0.083	0.017
14	resistant	Dabaitiaojiangdou	Shenyang, Liaoning	0.077	0.015
15	resistant	Paojiangdou-2	Yaan, Sichuan	0.069	0.012
16	resistant	Gaochan No.4	Shantou, Guangdong	0.090	0.013
17	resistant	Pingtangjiangdou	Pingtang, Guizhou	0.047	0.027
18	resistant	j-1	Jiangsu Academy of Agricultural Sciences	0.091	0.015
19	resistant	Feicui	Guangdong Academy of Agricultural Sciences	0.089	0.034
20	resistant	Sujiang No.1	Jiangsu Academy of Agricultural Sciences	0.094	0.023
21	resistant	Jiangdou2045	Wuhan, Hubei	0.081	0.025
		R-MEAN		0.067	0.024

4th LW means the 72 h fresh weight increase of the 4th instar larvae. LW9 means the nine-day-old larval weight. S-MEAN and R-MEAN mean the average larval weight of common cutworms feeding on sensitive or resistant yardlong bean varieties, respectively.

3.3. Selection of Yardlong Bean Germplasm Resources Resistant to Common Cutworm

In order to determine whether the resistance observed in this subset of varieties was affected by the stage of larval development and different growth periods of the yardlong bean, we next verified larval weight in three independent feeding experiments with the 21 varieties of the yardlong bean evaluated by the previous experiment.

Yardlong bean varieties showed significant differences in the weight of six-day-old larvae ($F = 5.64$, $p < 0.0001$) and the weight of twelve-day-old larvae ($F = 1.92$, $p = 0.014$) (Table 2). Three kinds of larval weight ($F = 315.40$, $p < 0.0001$ in LW6; $F = 22.97$, $p < 0.0001$ in LW9; $F = 32.55$, $p < 0.0001$ in LW12) showed extremely significant differences among three different batches of experiments, therefore it was necessary to repeat the verification experiments. Varieties and batches showed no obvious interaction in the weight of nine-day-old larvae and the weight of twelve-day-old larvae, indicating that the insect resistance of the yardlong bean was similar at different growth stages or in different environments (Table 2).

In the first verification experiment on 16 August 2021 when the yardlong beans were in the flowering period, the weight of six-day-old larvae ($F = 3.87$, $p = 0.0001$), the weight of nine-day-old larvae ($F = 11.11$, $p < 0.0001$), and the weight of twelve-day-old larvae ($F = 10.49$, $p < 0.0001$) showed extremely significant differences (Table 3). In the second verification experiment on 22 August 2021 when the yardlong beans were at the podding period, the weight of six-day-old larvae ($F = 9.51$, $p < 0.0001$), the weight of nine-day-old larvae ($F = 19.23$, $p < 0.0001$), and the weight of twelve-day-old larvae ($F = 10.18$, $p < 0.0001$) showed extremely significant differences (Table 3). In the third verification experiment on 6 August 2022 when the yardlong beans were at the pre-flowering period, the weight of twelve-day-old larvae ($F = 2.30$, $p = 0.0122$) also showed significant differences (Table 3).

Table 2. The joint ANOVA of larval weight of common cutworm feeding on four sensitive and seventeen resistant yardlong bean varieties in three verification experiments.

Source of Variation		LW6			LW9			LW12	
	DF	*F*	*p* (>*F*)	*DF*	*F*	*p* (>*F*)	*DF*	*F*	*p* (>*F*)
Variety	20	5.64	<0.0001	20	1.46	0.0992	20	1.92	0.014
Batch	2	315.40	<0.0001	2	22.97	<0.0001	2	32.55	<0.0001
Variety × Batch	40	5.74	<0.0001	40	0.47	0.9947	40	0.85	0.7264
Block	2	1.89	0.1550	2	1.55	0.2163	2	2.92	0.0581
Error	119			110			108		

LW6 means the six-day-old larval weight. LW9 means the nine-day-old larval weight. LW12 means the twelve-day-old larval weight. *DF*: degree of freedom; *F*: *F* value. *p*: *p* value.

Table 3. The ANOVA of larval weight of common cutworm feeding on four sensitive and seventeen resistant yardlong bean varieties in three verification experiments.

Indicator		Variety				Block			Error
	DF	*MS*	*F*	*p* (>*F*)	*DF*	*MS*	*F*	*p* (>*F*)	*MS*
LW6-1	20	0.00003	3.87	0.0001	2	0.00007	10.32	0.0003	0.00001
LW6-2	20	0.00017	9.51	<0.0001	2	0.00002	1.04	0.3629	0.00002
LW6-3	20	0.00005	1.24	0.2723	2	0.00037	9.85	0.0003	0.00004
LW9-1	20	0.00985	11.11	<0.0001	2	0.00224	2.53	0.0930	0.00089
LW9-2	20	0.03681	19.23	<0.0001	2	0.00760	3.97	0.0276	0.00191
LW9-3	20	0.00305	0.92	0.5680	2	0.00690	2.08	0.1379	0.00332
LW12-1	20	0.05880	10.49	<0.0001	2	0.01310	2.34	0.1104	0.00560
LW12-2	20	0.13736	10.18	<0.0001	2	0.00749	0.56	0.5789	0.01350
LW12-3	20	0.06898	2.30	0.0122	2	0.09932	3.32	0.0465	1.19794

LW6-1, LW6-2, LW6-3 means the six-day-old larval weight of the first, second and third verification experiments, respectively. LW6-1, LW9-1, LW12-1 means the six-day-old larval weight, the nine-day-old larval weight, the twelve-day-old larval weight of the first verification experiment, respectively. *DF*: degree of freedom; *MS*: mean square; *F*: *F* value. *p*: *p* value.

Correlation analysis was carried out among three verification experiments, and it was found that $r = 0.55$, $p = 0.0347$ between LW9 of the first and the third verification experiment; $r = 0.59$, $p = 0.005$ between LW12 of the first and the second verification experiment; $r = 0.64$, $p = 0.0347$ between LW12 of the first and the third verification experiment (Table 4). Only a handful of correlations are significant in different batches. There are often strong positive correlations among different observation times in the same experiment batch (Table 4). The correlation coefficients of larval weight in the first verification experiment were 0.60~0.95, which all reached extremely significant levels (Table 4). The correlation coefficients of larval weight in the second verification experiment were 0.76~0.95, which also all reached extremely significant levels (Table 4). Except for LW6-3 and LW12-3, the correlation coefficients of larval weight in the third verification experiment were 0.65~0.69, which also reached extremely significant levels (Table 4). It shows that the stability of different observation times in the same batch is higher than that of different batches, and there should be multiple batches for insect resistance evaluation experiments.

The differences between the insect-resistant and susceptible varieties were also evident from the larval size (Figure 4). In the sensitive yardlong bean varieties, the average six-day-old larval weight was 0.009~0.025 g, the average nine-day-old larval weight was 0.135~0.249 g and the average twelve-day-old larval weight was 0.318~0.630 g (Table 5). While in the resistant yardlong bean varieties, the average six-day-old larval weight was 0.006~0.024 g, the average nine-day-old larval weight was 0.081~0.202 g and the average twelve-day-old larval weight was 0.196~0.493 g (Table 5). This result is relatively consistent with the preliminary screening results in Table 1, which means the insect resistance identification of yardlong beans is stable. However, Pingtangjiangdou was identified as resistant variety in preliminary experiment, but was identified as sensitive variety in the verification experiments. Therefore, the insect resistance identification of yardlong bean needs to be repeated, otherwise the results will be unstable. The average twelve-day-old weight of Pingtangjiangdou was the highest, reaching 0.660 g; the average twelve-day-old weight of Jiangdou No.5 was the lightest, only 0.167 g. Through the combined analysis of three experiments, the insect-susceptible varieties Pingtangjiangdou and Zhuzaidou; the insect-resistant varieties Jiangdou No.5, Changcaidou, Zhijiangtezao No.30 and j-1 were screened out (Figure 4; Table 5).

Table 4. Correlation analysis of insect weight index and physiological index of sensitive and resistant yardlong bean varieties in three verification experiments.

Correlation	LW6-1	LW6-2	LW6-3	LW9-1	LW9-2	LW9-3	LW12-1	LW12-2	LW12-3	Starch
LW6-2	0.41									
LW 6-3	0.02	−0.19								
LW 9-1	0.67 **	0.65 **	−0.14							
LW 9-2	0.34	0.81 **	−0.11	0.55 *						
LW 9-3	0.24	0.18	0.65 **	0.33	0.27					
LW 12-1	0.60 **	0.61 **	−0.11	0.95 **	0.59 **	0.37				
LW 12-2	0.30	0.76 **	−0.21	0.54 *	0.95 **	0.18	0.59 **			
LW 12-3	0.40	0.19	0.18	0.58 **	0.29	0.69 **	0.64 **	0.23		
Starch	0.09	0.14	0.14	−0.27	−0.04	−0.02	−0.28	−0.03	−0.47 *	
Crude protein	−0.15	0.10	−0.24	0.16	0.29	0.12	0.24	0.32	0.20	−0.26

LW6-1, LW6-2, LW6-3 means the six-day-old larval weight of the first, second and third verification experiments, respectively. LW6-1, LW9-1, LW12-1 means the six-day-old larval weight, the nine-day-old larval weight, the twelve-day-old larval weight of the first verification experiment, respectively. *: correlation is significant, $p < 0.05$. **: correlation is extremely significant, $p < 0.01$.

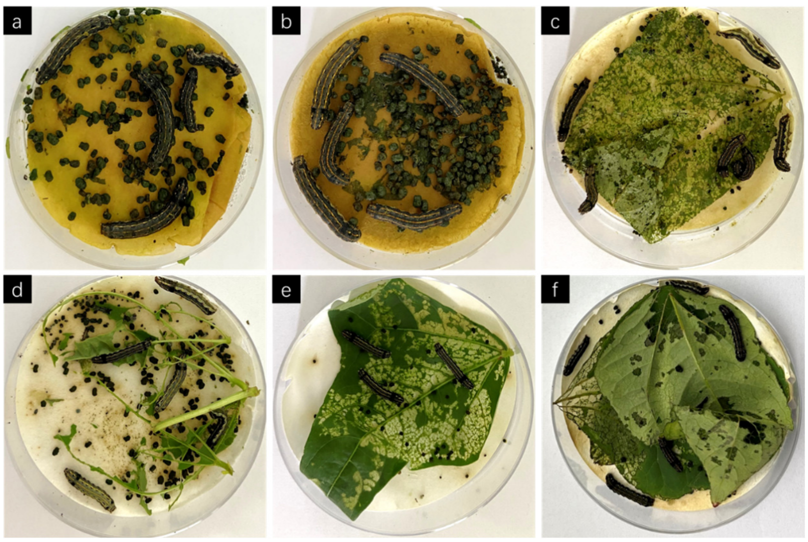

Figure 4. Twelfth-day-old larvae fed on leaves of different varieties of yardlong bean. Sensitive variety: (**a**) Zhuzaidou; (**b**) Pingtangjiangdou. Resistant variety: (**c**) Jiangdou No.5; (**d**) j-1; (**e**) Zhijiangtezao No.30; (**f**) Changcaidou.

3.4. Determination of Crude Protein Content and Starch Content in Yardlong Bean Leaves

In order to study whether starch content and crude protein content in yardlong bean leaves affect insect resistance, we detected leaves of 21 yardlong bean varieties sown on 29 June 2022. There are significant differences in starch content ($F = 5.67$, $p < 0.001$) and crude protein content ($F = 4151$, $p < 0.001$) in the leaves of different varieties of yardlong bean. However, it seems that the crude protein content and the starch content were not significantly correlated with larval weight except the starch content with the twelve-day-old larval weight of the third verification experiment (Table 4). Maybe there are other factors affecting the resistance of yardlong bean to common cutworm.

Table 5. The larval weight and leaf nutrient content of the preliminarily screened insect-resistant and insect-sensitive varieties in verification experiments.

Varieties	LW6-1 /g	LW6-2 /g	LW6-3 /g	LW9-1 /g	LW9-2 /g	LW9-3 /g	LW12-1 /g	LW12-2 /g	LW12-3 /g	Starch mg/g	Crude Protein g/kg
Changtingbaopihongjiangdou	0.006	0.014	0.031	0.107	0.337	0.238	0.260	0.690	0.730	55.6	325
Zhuzaidou	*0.007*	*0.015*	*0.020*	*0.168*	*0.274*	*0.219*	*0.430*	*0.740*	*0.600*	*52.3*	*308*
Suijiao101	0.013	0.006	0.029	0.152	0.145	0.188	0.340	0.450	0.480	72.1	304
156fanjiang	0.010	0.016	0.019	0.112	0.240	0.214	0.240	0.510	0.710	66.8	316
S-MEAN	0.009	0.013	0.025	0.135	0.249	0.215	0.318	0.598	0.630	61.7	313
Baijiangdou	0.006	0.021	0.021	0.167	0.282	0.220	0.390	0.640	0.580	55.0	385
Changcaidou	**0.003**	**0.002**	**0.013**	**0.036**	**0.098**	**0.109**	**0.060**	**0.280**	**0.180**	**54.8**	**317**
Jiangdou No.5	**0.004**	**0.001**	**0.020**	**0.021**	**0.046**	**0.147**	**0.050**	**0.190**	**0.260**	**58.3**	**321**
Zhijiangtezao No.30	**0.005**	**0.002**	**0.028**	**0.018**	**0.049**	**0.207**	**0.020**	**0.220**	**0.380**	**81.4**	**326**
SS-97	0.005	0.003	0.022	0.039	0.082	0.198	0.110	0.290	0.450	93.3	288
Suzi41	0.011	0.024	0.028	0.096	0.259	0.184	0.230	0.520	0.270	99.3	266
Sujiang1419	0.010	0.015	0.020	0.159	0.350	0.213	0.390	0.680	0.600	60.9	342
j-5	0.003	0.009	0.021	0.010	0.170	0.159	0.010	0.410	0.330	75.2	331
Sujiang12	0.003	0.004	0.031	0.062	0.155	0.242	0.290	0.420	0.560	74.0	340
Dabaitiaojiangdou	0.004	0.011	0.023	0.051	0.253	0.185	0.110	0.760	0.380	.	.
Paojiangdou-2	0.005	0.012	0.026	0.069	0.157	0.236	0.170	0.370	0.510	82.7	327
Gaochan No.4	0.007	0.002	0.037	0.091	0.101	0.281	0.180	0.190	0.620	57.1	310
Pingtangjiangdou	*0.007*	*0.022*	*0.023*	*0.162*	*0.414*	*0.239*	*0.430*	*0.830*	*0.720*	*52.0*	*313*
j-1	**0.002**	**0.001**	**0.034**	**0.010**	**0.041**	**0.202**	**0.020**	**0.110**	**0.460**	**47.9**	**278**
Feicui	0.009	0.004	0.026	0.098	0.064	0.261	0.210	0.210	0.740	59.7	304
Sujiang No.1	0.008	0.001	0.020	0.104	0.042	0.175	0.310	0.200	0.810	38.0	321
Jiangdou2045	0.006	0.017	0.020	0.183	0.080	0.180	0.360	0.290	0.530	63.2	295
R-MEAN	0.006	0.009	0.024	0.081	0.155	0.202	0.196	0.389	0.493	65.8	317

LW6-1, LW6-2, LW6-3 means the six-day-old larval weight of the first, second and third verification experiments, respectively. LW6-1, LW9-1, LW12-1 means the six-day-old larval weight, the nine-day-old larval weight, the twelve-day-old larval weight of the first verification experiment, respectively. Bold fonts indicate the most possible resistant varieties. Italic fonts indicate the most possible sensitive varieties.

3.5. Antibiotic and Antixenotic Experiments in Different Organs of Yardlong Beans

To explore whether resistance to cutworm feeding was equal across yardlong bean organs, we next compared differences in 3rd instar larval weight gain over 72 h of feeding on leaves, pods, or seeds. In the organ antibiotic experiment, there were significant differences among different organs (F = 4.16, p = 0.0281) (Table 6). The average weight increase of larvae feeding on yardlong bean leaves in 72 h is 0.14 g, higher than that of seeds (0.10 g) and significantly higher than that of pods (0.08 g). Among the common cutworm larvae fed with leaves, the gain of larval weight of Pingtangjiangdou at 72 h is 0.2062 g, which is about twice that of Jiangdou No.5 and Zhijiangtezao No.30. Among the common cutworm larvae fed with pods, the increase of larval weight of Pingtangjiangdou at 72 h was 0.1006 g, and Zhuzaidou was 0.09391 g, which were both higher than 0.07888 g of Jiangdou No.5 and 0.06084 g of Zhijiangtezao No.30. Among common cutworm larvae fed with seeds, the increase of larval weight of Jiangdou No.5 at 72 h was 0.1306 g higher than 0.1218 g of Pingtangjiangdou, 0.08547 g of Zhijiangtezao No.30 and 0.07743 g of Zhuzaidou (Figure 5).

Table 6. The joint ANOVA of the 72h larval weight gain in different varieties and different organs.

Source of Variation	*DF*	*SS*	*MS*	*F*	*p* (>*F*)
Variety	3	0.0171	0.0057	2.46	0.0868
Organ	2	0.0192	0.0096	4.16	0.0281
Variety × Organ	6	0.0125	0.0021	0.90	0.5107
Error	24	0.0554	0.0023		

DF: degree of freedom; *SS*: sum of squares; *MS*: mean square; *F*: *F* value. *p*: *p* value.

In the organ antixenotic resistance experiment, analysis of variance was performed on the number of larvae on different organs at 8 h, 16 h and 24 h. It was concluded that the relationship between the location of larvae and different varieties was not significant (F = 0.15, p = 0.9281, Table S3), the relationship with observation time was not significant (F= 0.31, p = 0.7343, Table S3), and the relationship with different organs was extremely significant (F = 203.54, p < 0.001, Table S3). The number of larvae on leaves was the highest, with an average of 4.54, accounting for 80.78% of the total; the second was in pods, with an average of 0.83, accounting for 14.77% of the total; and the lowest was in seeds, with an average of 0.25, accounting for 4.45% of the total (Figure 6). According to the 24 h organ reduction values of different varieties, it was found that the common cutworm had the least amount of food on yardlong bean seeds, indicating that it had the lowest selectivity to yardlong bean seeds (Figure 6).

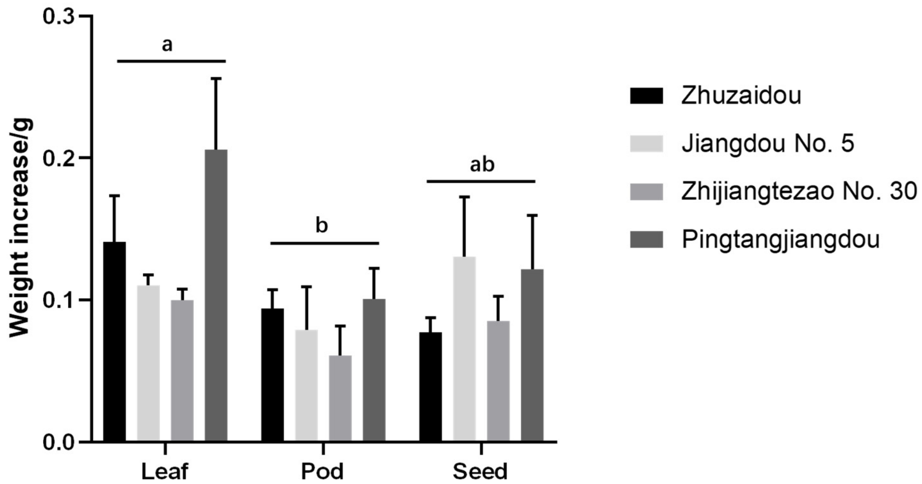

Figure 5. 72 h weight increase of the 3rd instar larvae fed with different organs and different varieties of yardlong bean in organ antibiotic resistance experiment. Zhuzaidou and Pingtangjiangdou are insect-sensitive varieties. Jiangdou No.5 and Zhijiangtezao No.30 are insect-resistant varieties. The error line represents a standard error. The same lowercase letter among different organs has no significant difference.

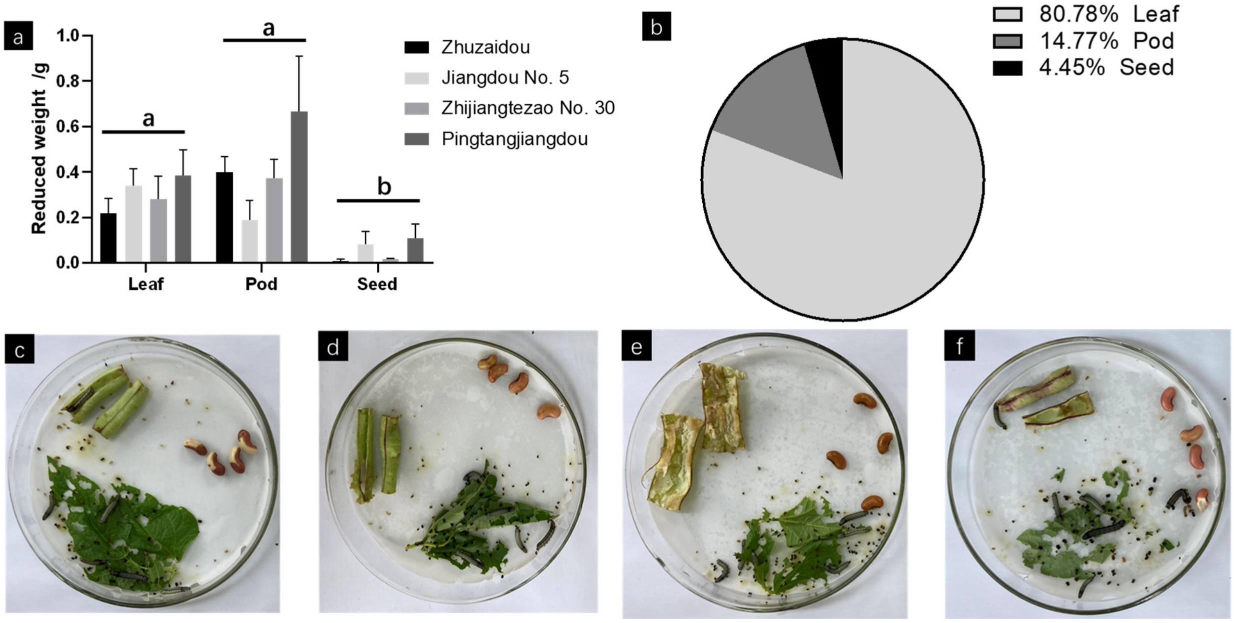

Figure 6. Common cutworm 4th larvae feeding preference experiments between leaf, pod, and seed tissue. (**a**) The bar graph of the weight reduction of yardlong bean organs at 24 h. The same lowercase letter means no significant difference. (**b**) The pie chart of the average number of common cutworm on different yardlong bean organs. (**c**–**f**). Feeding of different organs of Zhuzaidou, Jiangdou No.5, Zhijiangtezao No.30, Pingtangjiangdou varieties by common cutworm. Zhuzaidou and Pingtangjiangdou are insect-sensitive varieties. Jiangdou No.5 and Zhijiangtezao No.30 are insect-resistant varieties.

4. Discussion

4.1. Insect Resistance Evaluation Method of Yardlong Bean

Previous work using four soybean recombinant inbred line populations identified quantitative trait loci (QTL) associated with a resistance to the bean pyralid (*Lamprosema indicate* Fabricius) by screening the percentage of rolled leaflets under field infestation conditions [32]. In light of that approach, we therefore investigated the number of wormholes, length of wormholes, and the proportion of total pods with wormholes in a yardlong bean production greenhouse infested with the common cutworm to determine differences in insect resistance between pod maturity stages, which preliminarily judged the feasibility of field test and the difference of damage among different yardlong bean varieties.

In laboratory experiments, differential foliar resistance to common cutworm feeding among yardlong bean varieties was quantified using newly hatched larvae and 3rd or 4th instar larvae. The correlation between 72 h fresh weight gain and LW9 of 64 yardlong bean varieties is significant but weak. Furthermore, the best resistant varieties are not the same in newly hatched larvae and in 4th instar larvae methods. This might imply that the plant uses a different mechanism to confer resistance and such observations are not rare [19,20]. Larvae at late growth stage can often resist the resistance response of plants [33]. In our experiments, larvae at late growth stage and early growth stage were used to screen insect-resistant varieties, which makes the results more reliable. Previous research has shown that common cutworms enter a 'gluttony period' in the 4th instar, characterized by substantially increased food intake and rapid growth in body size [34]. Changes in the weight of newly hatched larvae can reflect the effects of yardlong bean leaves on common cutworm growth during early developmental stages, while changes in insect weight in 3rd or 4th instar larvae mainly reflect the effects of excessive consumption of yardlong bean. The leaves and pods of yardlong bean are generally tender, and thus prone to insect damage. The common cutworm damages not only the leaves but also pods and seeds. Thus, the 4th instar larvae were selected to investigate differential insect resistance among yardlong bean varieties as well as among organs. These assays showed that larvae significantly prefer leaves to pods and seeds. Using 3rd instar larvae to compare differences in feeding among specific organs of different varieties revealed differences in the resistance of organs within the same variety.

Insect resistance is affected by field temperature, humidity, host growth stages and other factors, and varies among insect individuals [32,35], which led us to perform three independent verification experiments in different growing seasons to capture the full variability of resistant varieties and thus identify stable insect resistance. Since growth periods differ among early, medium, and late maturing yardlong bean varieties, selecting a suitable period for experiments can pose a challenge. Thus, in the current study, leaf resistance was a more reliable indicator of insect resistance and enabled us to screen out 17 resistant varieties and four insect-susceptible varieties first, then screened out Jiangdou No.5, j-1, Zhijiangtezao No.30, and Changcaidou as resistant varieties, and Zhuzaidou and Pingtangjiangdou as insect-susceptible varieties.

4.2. Reasons for Antibiosis

This study shows that different varieties of the yardlong bean can influence the common cutworm growth and development to varying extents. Rearing on Jiangdou No.5, j-1, Zhijiangtezao No. 30, and Changcaidou varieties results in a significantly lower insect weight than other varieties. Plants can modulate nutrient composition, regulate phytohormone production, and synthesize secondary metabolites and defense enzymes to deter insect feeding [16,36]. Excluding the effects of the growing season and pest species, the combination of two traits, the higher C:N and the higher latex tannin content, result in an elevated resistance to insects in banyan trees (*Ficus microcarpa* Linn.) [37]. In addition, soluble sugar content, antioxidant enzyme (SOD, CAT, PPO) activity, and plant hormone contents (e.g., ethylene, jasmonic acid (JA) and abscisic acid) all significantly increased in soybean leaves after feeding by bean pyralid (*Lamprosema indicata* Fabricius) [38]. In most plants, the jasmonic acid signaling pathway regulates the response to insect feeding, especially in the early stages of plant growth in which JA levels increase following insect feeding, triggering a defense response [39,40]. Specifically, vacuolar benzoxazinoids (BXs), stored as glucosides, are hydrolyzed by glucosidases to release toxic aglycones following an insect attack [41]. By contrast, in lepidopteran insects, the responses of allelopathic receptors to sucrose, glucose and other stimuli are inhibited by plant sesquiterpenoids, consequently reducing feeding damage by insects [42]. Furthermore, soybean plants produce isoflavones which cannot be metabolized by the common cutworm and can significantly inhibit larval growth and development [43]. This diversity of resistance mechanisms suggests that insect resistance warrants considerably deeper study with regard to the yardlong bean.

4.3. Meaning of Experiments and Future Directions

Yardlong bean leaves are primarily responsible for photosynthesis, and thus excessive pest damage to leaves can result in severe yield loss. The quality and purity of agricultural products are essential for food safety, and pesticide residues are closely linked to produce quality. The 'poisonous cowpea incident' should thus serve as a cautionary tale to emphasize the value of reduced pesticide application and promote the use of insect-resistant varieties and responsible crop management [44]. Chemical methods can reduce the frequency of pest infestations or outbreaks, but overuse can pose a danger to public safety. Well-informed guidance for farmers on correct pesticides also requires the availability of elite, high-yield, pest-resistant varieties. In the present study, we only evaluated the insect resistance of 64 yardlong bean varieties by in vitro antibiosis assays, but there are many cowpea germplasms and many breeding lines, so it is necessary to establish high-throughput resistance evaluation methods. A high throughput antixenotic evaluation method for the common cutworm has been established in soybeans [35], which can be used to evaluate the antixenotic of the yardlong bean to the common cutworm, to screen more varieties. The greenhouse experiment of the yardlong bean in the present study is a prelude to proper field experiments which will determine the stability of the resistance/tolerance of any given variety and how field conditions might affect the trait. Tolerance is different from resistance, which means that plants can still have strong proliferation and recovery ability after being affected by pests, and can significantly reduce the damage [15,16]. Field experiments in multiple locations can provide an assessment of genotype-by-environment interactions, can determine the tolerance of yardlong bean varieties, and see if insect-resistant varieties can overcome low and moderate infections and keep a high harvest index. Future research will need to verify the insect resistance described here in large scale field trials, while also investigating the genetic and biochemical mechanisms and metabolites responsible for deterring cutworm feeding [45–47]. The differentially expressed genes under high salt conditions were analyzed by transcriptomics, and some transcription factors were found to distinguish salt-tolerant and salt-sensitive yardlong bean varieties [48]. We can use transcriptome analysis to find the differentially expressed genes of the yardlong bean under the stress of the common cutworm and find the transcription factors that are significantly up-regulated or down-regulated, to find the reasons for the insect-resistant mechanism. The insect-resistant yardlong bean varieties screened in the present study can be used as a germplasm for breeding programs, and the eventual detection of the underlying genes will lead to their deployment in elite lines, ultimately reducing the use of chemical pesticides and promoting agricultural sustainability.

5. Conclusions

Yardlong beans in greenhouse were often harmed by naturally occurring common cutworms, and the nicked, perforated, and necrotic pods reduced their commercial value. Yardlong bean varieties have different levels of resistance to common cutworms. Seventeen insect-resistant varieties and four insect-susceptible varieties were screened out from 64 yardlong bean varieties by larval weight of two stages of common cutworm. Two susceptible varieties, Zhuzaidou and Pingtangjiangdou, and four resistant varieties, Jiangdou No.5, j-1, Zhijiangtezao No. 30, and Changcaidou, were screened out from 21 yardlong bean varieties through three larval experiments. Starch content and larval weight had negative correlation and there was a positive correlation between crude protein and larval weight, but almost neither of them reached significant levels. Common cutworms preferred to feed on yardlong bean leaves, and the weight increase of those feeding on leaves was higher than that of pods and seeds. These findings will help to improve the evaluation of insect resistance of yardlong beans in the future and provide an excellent germplasm for the breeding of insectresistant yardlong beans varieties.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13020502/s1, Table S1: Analysis of the wormhole number and length in pods of three maturity types of yardlong bean varieties. Table S2: Sixty-four yardlong bean varieties screened by evaluation of newly hatched larvae and 4th instar larvae weight increase. Table S3: The joint ANOVA of the number of common cutworms on different organs and varieties at different observation times.

Author Contributions: Conceptualization, G.X., T.Y. and H.Z.; methodology, G.X., T.Y. and H.Z.; formal analysis, T.Y. and G.X.; investigation, T.Y., H.Z., G.X., Y.X., H.J., X.C. and X.L.; data curation, T.Y. and G.X.; resource, H.Z. and H.C.; writing, reviewing, and editing, T.Y., G.X., H.Z. and Y.X. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Key R&D Program of China (2021YFD1201604), the Jiangsu Agricultural Science and Technology Innovation Fund (CX(20)3161), the Agriculture Research System of China (CARS-09), the Natural Science Foundation of China (31571694), and the Jiangsu JCICMCP Program.

Data Availability Statement: Data presented in this study are available on fair request to the corresponding author.

Acknowledgments: The authors thank the reviewers for their constructive comments and suggestions.

Conflicts of Interest: All other authors declare no conflict of interest.

References

1. Pan, L.; Li, Y.; Yu, X.; Li, J.; Chen, C. Research progress of molecular genetics in cowpea (*Vigna unguiculata*). *J. Changjiang Veg.* **2014**, *24*, 1–13. [CrossRef]
2. Smýkal, P.; Coyne, C.; Ambrose, M.; Maxted, N.; Schaefer, H.; Blair, M.; Berger, J.; Greene, S.; Nelson, M.; Besharat, N.; et al. Legume crops phylogeny and genetic diversity for science and breeding. *Crit. Rev. Plant Sci.* **2015**, *34*, 43–103. [CrossRef]
3. Wang, S. Origin classification and genetic resources of cowpea. *China Veg.* **1989**, *6*, 49–52.
4. Wang, B.; Wu, X.; Li, S.; Chen, X.; Li, Y.; Wang, Y.; Lu, Z.; Wu, X.; Li, G. Evaluation of cowpea germplasm accessions collected from Zhejiang province. *J. Plant Genet. Resour.* **2021**, *2*, 380–389. [CrossRef]
5. Coker, C.; Ely, M.; Freeman, T. Evaluation of yardlong bean as a potential new crop for growers in the Southeastern United States. *HortTechnology* **2007**, *17*, 592–594. [CrossRef]
6. Phillips, R.D.; McWatters, K.H.; Chinnan, M.S.; Hung, Y.-C.; Beuchat, L.R.; Sefa-Dedeh, S.; Sakyi-Dawson, E.; Ngoddy, P.; Nnanyelugo, D.; Enwere, J.; et al. Utilization of cowpeas for human food. *Field Crop. Res.* **2003**, *82*, 193–213. [CrossRef]
7. Rabé, M.M.; Baoua, I.B.; Baributsa, D. Farmers' referred genotype traits and socio-economic factors influencing the adoption of improved cowpea varieties in South-Central Niger. *Agronomy* **2022**, *12*, 2668. [CrossRef]
8. Bell, L.W.; James, A.T.; Augustin, M.A.; Rombenso, A.; Blyth, D.; Simon, C.; Higgins, T.J.V.; Barrero, J.M. A niche for cowpea in sub-tropical Australia? *Agronomy* **2021**, *11*, 1654. [CrossRef]
9. Zhang, W.; Wang, Y.; Deng, Q. Inheritance and breeding of important traits in cowpea. *China Veg.* **1992**, *1*, 50–53.
10. Jackai, L.E.N. Integrated pest management of borers of cowpea and beans. *Int. J. Trop. Insect Sci.* **1995**, *16*, 237–250. [CrossRef]
11. Pan, Y.F.; Luo, F.; Lei, C. The niche of important pests and natural enemies in the cowpea field ecosystem. *Chin. J. Appl. Entomol.* **2005**, *42*, 404–408.
12. Nyarko, J.; Asare, A.T.; Mensah, B.A.; Adjei, F. Assessment of the response of fifteen cowpea [*Vigna unguiculata* L. (Walp.)] genotypes to infestation by *Callosobruchus maculatus* Fab. (Coloeptera: Bruchidae). *Cogent Food Agric.* **2022**, *8*, 2095713. [CrossRef]
13. Huang, K.; Wen, L.; Zhang, X. Investigation on population dynamics of *Prodenia litura* for cowpea and its control measures. *Anhui Agric. Sci. Bull.* **2008**, *9*, 163. [CrossRef]
14. Painter, R.H. Insect resistance in crop plants. *Soil Sci.* **1951**, *72*, 481. [CrossRef]
15. Roy, D.; Chakraborty, G.; Biswas, A.; Sarkar, P.K. Antixenosis, tolerance and genetic analysis of some rice landraces for resistance to *Nilaparvata lugens* (Stål.). *J. Asia Pac. Entomol.* **2021**, *24*, 448–460. [CrossRef]

16. Smith, C.M. Antibiosis: Adverse effects of resistance on arthropod biology. In *Plant Resistance to Arthropods: Molecular and Conventional Approaches*; Smith, C.M., Ed.; Springer: Dordrecht, The Netherlands, 2005; pp. 65–99.
17. Eduardo, W.I.; Júnior, A.L.B.; Moraes, R.F.D.O.; de Souza, B.H.S.; Louvandini, H.; Barbosa, J.C. Protocol for assessing soybean antixenosis to *Heliothis virescens*. *Èntomol. Exp. Appl.* **2020**, *168*, 911–927. [CrossRef]
18. Bruna, M.F.; Alessandra, R.B.; Luís, A.F. Biology and reproductive capacity of *Spodoptera eridania* (Cramer) (Lepidoptera, Noctuidae) in different soybean cultivars. *Rev. Bras. Entomol.* **2015**, *59*, 89–95. [CrossRef]
19. Yang, Y.; Xing, G.; Gai, J. Evaluation of antibiosis to common cutworm (*Spodoptera litura*) and screening for resistance sources among wild soybeans (*Glycine soja*) in China. *Soybean Sci.* **2016**, *35*, 448–454.
20. Hu, Z.; Xu, X.; Pan, L.; Li, M.; Zeng, J.; Razzaq, M.K.; Xing, G.; Gai, J. Resistance analyses of soybean organs to common cutworm (*Spodoptera litura*) at different reproductive stages. *Soybean Sci.* **2020**, *39*, 932–939.
21. Xu, H.; Zhai, H.; Wang, F.; Piao, J.; Yang, X.; Zhu, Z. Cowpea trypsin inhibitor gene (cpti) and its application in insect resistance transgenic plants. *J. Agric. Sci. Technol.* **2008**, *10*, 18–27.
22. Ma, X.; Zhu, Z.; Li, Y.; Yang, G.; Pei, Y. Expressing a modified cowpea trypsin inhibitor gene to increase insect tolerance against *Pieris rapae* in Chinese cabbage. *Hortic. Environ. Biotechnol.* **2017**, *58*, 195–202. [CrossRef]
23. Zhou, X.; Pan, Y.; Wang, X.; Li, H.; Lei, C. Effects of food-plants on the survival, development and fecundity of *Maruca vitrata* (Fabricius). *J. Huazhong Agric. Univ.* **2008**, *3*, 367–369.
24. Costa, E.N.; Evangelista, B.M.D.; Fernandes, M. Antibiosis levels to *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in cowpea commercial cultivars and landrace varieties. *J. Econ. Èntomol.* **2019**, *112*, 1941–1945. [CrossRef] [PubMed]
25. Shao, J.; Pan, L.; Wan, H.; Guo, R.; Chen, G.; Xie, Q.; Ren, S.; Lu, S.; Yan, M.; Chen, C. Germplasm innovation of pest-resistant asparagus bean by interspecific crosses. *Acta Hortic. Sin.* **2020**, *47*, 562–570. [CrossRef]
26. Lu, W.; Wei, Y.; Tan, D.; Zuo, F. The enlightenment of the poisonous cowpea incident in Hainan to plant protection in Guangxi. *J. Guangxi Agric.* **2010**, *25*, 86–87, 96.
27. Enders, L.; Begcy, K. Unconventional routes to developing insect-resistant crops. *Mol. Plant* **2021**, *14*, 1439–1453. [CrossRef] [PubMed]
28. Eker, T.; Erler, F.; Sari, H.; Sari, D.; Berger, J.; Toker, C. Deployment of *Cicer echinospermum* P.H. Davis for resistance to *Callosobruchus chinensis* L. *J. Plant Dis. Prot.* **2022**, *129*, 843–851. [CrossRef]
29. Tu, Y.; Zeng, J. A method for artificial rearing of common cutworm (*Spodoptera litura*). *Acta Agric. Jiangxi* **2010**, *22*, 87–88.
30. Laurentin, A.; Edwards, C.A. A microtiter modification of the anthrone-sulfuric acid colorimetric assay for glucose-based carbohydrates. *Anal. Biochem.* **2003**, *315*, 143–145. [CrossRef]
31. Zhao, F.; Qian, J.; Liu, H.; Wang, C.; Wang, X.; Wu, W.; Wang, D.; Cai, C.; Lin, Y. Quantification, identification and comparison of oligopeptides on five tea categories with different fermentation degree by Kjeldahl method and ultra-high performance liquid chromatography coupled with quadrupole-orbitrap ultra-high resolution mass spectrometry. *Food Chem.* **2022**, *378*, 132130. [CrossRef]
32. Xing, G.; Zhou, B.; Wang, Y.; Zhao, T.; Yu, D.; Chen, S.; Gai, J. Genetic components and major QTL confer resistance to bean pyralid (*Lamprosema indicata* Fabricius) under multiple environments in four RIL populations of soybean. *Theor. Appl. Genet.* **2012**, *125*, 859–875. [CrossRef]
33. Cong, S.; Xu, D.; Yang, N.; Wang, L.; Wang, J.; Liu, W.; Wan, P. Effects of stacked genetically modified cotton on the feeding behavior and nutrient metabolism of *Spodoptera litura* larvae. *Plant Prot.* **2022**, *48*, 104–110. [CrossRef]
34. Yao, W. Biology characteristies of *Prodenia litura*. *J. Biosaf.* **2005**, *2*, 122–127.
35. Xing, G.; Liu, K.; Gai, J. A high-throughput phenotyping procedure for evaluation of antixenosis against common cutworm at early seedling stage in soybean. *Plant Methods* **2017**, *13*, 66. [CrossRef]
36. Yang, J.; Xiao, G. The insect-resistance physiology of plants: A review. *Chin. Agric. Sci. Bull.* **2021**, *37*, 130–136. [CrossRef]
37. Zhao, J.; Segar, S.T.; McKey, D.; Chen, J. Macroevolution of defense syndromes in *Ficus* (Moraceae). *Ecol. Monogr.* **2021**, *91*, e01428. [CrossRef]
38. Zeng, W.; Cai, Z.; Zhang, Z.; Chen, H.; Yang, S.; Tang, X.; Lai, Z.; Sun, Z. Physiological and biochemical characteristics of *Lamprosema indicate* (Fabricius)-resistant soybean. *J. S. Agric.* **2015**, *46*, 2112–2116. [CrossRef]
39. Gaquerel, E.; Stitz, M. Insect resistance: An emerging molecular framework linking plant age and JA ignaling. *Mol. Plant* **2017**, *10*, 537–539. [CrossRef]
40. Diezel, C.; Allmann, S.; Baldwin, I.T. Mechanisms of optimal defense patterns in *Nicotiana attenuata*: Flowering attenuates herbivory-elicited ethylene and jasmonate signaling. *J. Integr. Plant Biol.* **2011**, *53*, 971–983. [CrossRef] [PubMed]
41. Malook, S.U.; Qi, J.; Hettenhausen, C.; Xu, Y.; Zhang, C.; Zhang, J.; Lu, C.; Li, J.; Wang, L.; Wu, J. The oriental armyworm (*Mythimna separata*) feeding induces systemic defence responses within and between maize leaves. *Philos. Trans. R. Soc. B Biol. Sci.* **2019**, *374*, 20180307. [CrossRef]
42. Gershenzon, J.; Dudareva, N. The function of terpene natural products in the natural world. *Nat. Chem. Biol.* **2007**, *3*, 408–414. [CrossRef] [PubMed]
43. Zhou, Y.; Luo, S.; Yi, Q.; Li, C.; Luo, Q.; Hua, J.; Liu, Y.; Li, S. Secondary metabolites from *Glycine soja* and their growth inhibitory effect against *Spodoptera litura*. *J. Agric. Food Chem.* **2011**, *59*, 6004–6010. [CrossRef] [PubMed]
44. Abbas, A.; Ullah, F.; Hafeez, M.; Han, X.; Dara, M.Z.N.; Gul, H.; Zhao, C.R. Biological control of fall armyworm, *Spodoptera frugiperda*. *Agronomy* **2022**, *12*, 2704. [CrossRef]

45. Koch, K.G.; Chapman, K.; Louis, J.; Heng-Moss, T.; Sarath, G. Plant tolerance: Aunique approach to control hemipteran pests. *Front. Plant Sci.* **2016**, *7*, 1363. [CrossRef] [PubMed]
46. Amin, M.R.; Roy, M.C.; Rahman, M.M.; Miah, M.G.; Kwon, Y.J.; Suh, S.J. Foraging and growth responses of cotton armyworm *Spodoptera litura* to the biophysical characteristics of five cotton varieties. *Entomol. Res.* **2015**, *45*, 286–293. [CrossRef]
47. Gomes, A.M.F.; Draper, D.; Nhantumbo, N.; Massinga, R.; Ramalho, J.C.; Marques, I.; Ribeiro-Barros, A.I. Diversity of cowpea [*Vigna unguiculata* (L.) *Walp*] landraces in mozambique: New opportunities for crop improvement and future breeding programs. *Agronomy* **2021**, *11*, 991. [CrossRef]
48. Zhang, H.; Xu, W.; Chen, H.; Chen, J.; Liu, X.; Chen, X.; Yang, S. Transcriptomic analysis of salt tolerance-associated genes and diversity analysis using indel markers in yardlong bean (*Vigna unguiculata* ssp. sesquipedialis). *BMC Genom. Data* **2021**, *22*, 34. [CrossRef]