PLANT RESISTANCE

Resistance of Faba Bean and Pea Germplasm to Callosobruchus chinensis (Coleoptera: Bruchidae) and Its Relationship With Quality Components

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ABSTRACT In total, 339 faba bean (Vicia faba L.) and 100 pea (Pisum sativum L.) accessions were screened for their ability to resist Callosobruchus chinensis L. in free choice laboratory tests. Four, 15, and 43 faba bean varieties were highly resistant, resistant, and moderately resistant to C. chinensis, respectively. Three immune, three highly resistant, and six resistant accessions were discovered among the pea germplasm. The faba bean and pea varieties presented a hundred-kernel weight reduction varied from 0.18 to 35.36% for faba bean varieties and 0 to 56.53% for pea varieties. Varieties with brown and black seed color had significantly fewer wormholes and higher C. chinensis resistance than varieties with light-color seeds. Resistance to C. chinensis showed a significant, positive correlation with catechin, total polyphenol, and γ-aminobutyric acid contents, but a significant, negative correlation with oligosaccharide content. Correlation coefficients (r) between infestation rate of faba bean and total phenol, catechin, and oligosaccharide contents were −0.9723, −0.8071, and 0.7631, respectively. The values of r for pea resistance and total phenol, catechin, and oligosaccharide content were −0.8846, −0.7666, and 0.8308, respectively. The results suggest that quality components in faba bean and pea have a great role in resistance against C. chinensis.

KEY WORDS Vicia faba, Pisum sativum, Callosobruchus chinensis, resistance screening, quality component

Faba bean (Vicia faba L.) and pea (Pisum sativum L.) are two traditionally significant human foods, and are important economic crops of peasants in mountain areas. China accounts for the largest planted area (42.7%) and highest total yield (46.9%) of faba bean worldwide (Food and Agriculture Organization of the United Nations [FAO] 2009). Faba bean can adapt to cold weather and different soil conditions, and is designated as the “king of biological nitrogen fixation” (Shukla et al. 1989, Zheng et al. 1997). Faba bean is rich in amino acids, can be digested easily, and provides added value after deep processing to extract procyanidines and protease inhibitors (Merghem et al. 2004). It is also an important intercropping and soil improving crop for structural adjustment in planting (Zheng et al. 1997). Pea is the fourth most important food legume worldwide, possessing good drought resistance and strong adaptation to different soil conditions (Zheng et al. 1997, FAO 2009). China has >2,000 yr of planting history and accounts for 15.2% of the total planting area and 13.8% of the total yield of dried pea worldwide, next to Canada (Zong et al. 2005). Moreover, China contributes 23.1% of the total planting area and 30.4% of the total yield of green pea worldwide, next to India (FAO 2009). Pea is an important fodder and medically edible crop, rich in nutrients (Zheng et al. 1997, Smyk et al. 2012) and is an important economic crop in China (Zong et al. 2010).

Callosobruchus chinensis L. (Coleoptera: Bruchidae) is one of the most threatening pests of leguminous stored seeds in Asia (Sonita et al. 2008). The larvae of C. chinensis use a variety of dried legume seeds as their hosts, such as mungbean (Vigna radiata L.; Shinoda et al. 1991), cowpea (Vigna unguiculata L.; Tomooka et al. 2000), adzuki bean (Vigna angular Olwi & Ohashi; Tomooka et al. 2000), faba bean (Podoler and Applebaltm 1968), pea (Bhagwat et al. 1995), chickpea (Cicer arietinum L.; Duan et al. 2014), soybean (Glycine max L. Merrill; Wang et al. 2010b), kidney bean (Phaseolus vulgaris L.; Li and Zhu 2009), pigeonpea (Cajanus cajan (L.) Millspaugh; Nahdy et al. 1998), peanut (Arachis hypogaea L.; Li and Zhu 2009), and lotus seed (Nelumbo nucifera Gaertn.; Li and Zhu 2009). As is typical of the life history of the Bruchidae, larvae bore into legume seeds on hatching and feed on the seed and consume the cotyledons (Tuda et al. 2004). They pulate and metamorphose into adults within the seed and emerge to seek mates and new hosts, which causes secondary infestation and often causing considerable damage to stored food le-
gumes (Tuda et al. 2004). Usually, the afflicted legumes show an infestation percentage of 30 to 64%, or even as high as 80 to 100%, thereby significantly decreasing or even completely losing the thousand-kernel weight, nutritive value, and germination rate, which makes them unfit for human consumption or for agricultural and commercial use (Umrao and Verma 2002, Chauvey 2008, Duan et al. 2014).

A national survey of the types and damage degree of bean weevil to stored edible beans was implemented by authors through random sampling and field sampling during 2012–2013. Findings indicated that aside from broad bean weevil (Bruchus rufimanus Boheman) and pea weevil (Bruchus pisorum L.), C. chinensis was also a major threat to the storage of faba bean and pea in Southwest China, such as in Yunnan, Guizhou, Sichuan, and Chongqing. The rate of damaged seeds ranged from 20 to 70%, causing great losses to local peasants and related seed enterprises (C.-X.D., Z.-D.Z., and X.-M.W., unpublished data). One approach for safe, economic, and effective control of C. chinensis is the use of resistant varieties of the crops. Mungbean varieties with high C. chinensis resistance have been developed (Kaga and Ishimoto 1998, Lee et al. 2000, Somta et al. 2007, Duan et al. 2013a). However, faba bean and pea resistance to C. chinensis has not been reported to date.

Phytophagous insects acquire necessary nutrients from host plants and their growth and development depends largely on the type and content of nutrient components (Makino et al. 1983, Qin 1987). Beyond that, different types of secondary compounds (e.g., tannin, total polyphenols, γ-aminobutyric acid) may influence the development of insects (Ding et al. 2000, Cipollini et al. 2005). Therefore, insect growth is vulnerable to the type and content of nutrient components and other biochemical substances in host crops (Qin 1987). Varieties (lines) from different regions or derived from different parents may differ largely in types and contents of nutrients and secondary metabolites, which is likely to affect feeding, oviposition, growth and development of pests, and different levels of insect resistance (Ciepiela et al. 1999, Chen et al. 2011, Zheng 2013).

Therefore, we evaluated C. chinensis for resistance to faba bean and pea in a laboratory assay and identified several resistant accessions. Furthermore, selected faba bean and pea accessions with different degree of resistance were assessed for quality and the relationships between C. chinensis resistance and quality were analyzed. This study will contribute to understanding resistance against C. chinensis in faba bean and pea, as well as future research on C. chinensis-resistance breeding.

### Materials and Methods

**Plant Materials.** In total, 339 faba bean accessions were provided by the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China; Institute of Grain Crops, Yunnan Academy of Agricultural Sciences, Kunming, China; Qujing Institute of Agricultural Sciences, Qujing, China; and Sichuan Academy of Agricultural Sciences, Chengdu, China. In total, 100 pea accessions were provided by the Institute of Grain Crops, Yunnan Academy of Agricultural Sciences, Kunming, China; Gansu Academy of Agricultural Sciences, Lanzhou, China; Qujing Institute of Agricultural Sciences, Qujing, China; Nantong Institute of Agricultural Sciences, Nantong, China; and Anhui Academy of Agricultural Sciences, Hefei, China.

**C. chinensis.** The pests were collected from mungbean seeds stored in Haidian District, Beijing, China, and were fed with mungbean seeds in the greenhouse at the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China. Test insects were available all year round.

**Identification and Evaluation of Faba Bean and Pea Resistance to C. chinensis.** Faba bean and pea resistance to C. chinensis was evaluated using modified protocol of Liu’s method of indoor artificial infestation and free choice test (Liu et al. 1998). In total, 30 healthy faba bean and 30 healthy pea seeds were selected from each variety and placed into a small (6 cm by 15 cm) petri dish (uncovered). This procedure was done in triplicate for each variety. A large plastic box (66 by 44 by 18 cm) was filled at random with dishes containing the seed of 60 accessions and two susceptible control varieties (H5086 for faba bean, Yunwan 4 for pea). Approximately 310 pairs of adults (1–3 d after eclosion) were placed in each big plastic box, i.e., five pairs for each test variety on average. The boxes were covered with a black cloth and placed in a dark insectary (28 ± 2°C and 75% relative humidity [RH]). All adults were removed after the control varieties had more than five C. chinensis eggs on the average. These eggs completely emerged ≈30 d after infestation, and the amount of afflicted kernels and wormholes in seed of each test accession was investigated. Resistance was evaluated according to the following scale: 0% seed damage = 0 (immune); 0.1–10.0% damage = 1 (highly resistant); 10.1–30.0% damage = 3 (resistant); 30.1–60.0% damage = 5 (moderately resistant); 60.1–90.0% damage = 7 (susceptible); 90.1–100% damage = 9 (highly susceptible) (Table 1).

In addition, the number of wormholes is another measurement index of infestation. More wormholes often cause larger losses of hundred-kernel weight. Therefore, the mean number of wormholes in faba bean and pea accessions was surveyed after identification.

<table>
<thead>
<tr>
<th>Rate of damaged seeds (%)</th>
<th>Scale</th>
<th>Evaluation of resistance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>I</td>
</tr>
<tr>
<td>0.1–10.0</td>
<td>1</td>
<td>HR</td>
</tr>
<tr>
<td>10.1–30.0</td>
<td>3</td>
<td>R</td>
</tr>
<tr>
<td>30.1–60.0</td>
<td>5</td>
<td>MR</td>
</tr>
<tr>
<td>60.1–90.0</td>
<td>7</td>
<td>S</td>
</tr>
<tr>
<td>90.1–100</td>
<td>9</td>
<td>HS</td>
</tr>
</tbody>
</table>

* I, immune; HR, highly resistant; R, resistant; MR, moderately resistant; S, susceptible; HS, highly susceptible.
we calculated with Microsoft Excel 2010.

used for means separation. Correlation coefficients when treatment effects were statistically significant when treatment effects were statistically significant.

Xizang 1 0 0 0 1 0 1.75
Guangxi 6 0 0 0 3 3 1.56
Yunnan 44 1 0 5 20 18 1.49
Anhui 2 0 0 0 1 1 1.30
Sichuan 144 0 7 17 95 25 1.26
Inner Mongolia 27 0 0 3 13 2 1.19
Hebei 30 0 0 3 25 2 1.19
Shaanxi 3 0 0 0 2 1 1
Gansu 9 0 0 1 8 0 0.97
Qinghai 51 2 5 13 24 7 0.87
France 4 0 0 0 2 0 0.78
Total 339 4 15 43 215 62

Number of worm holes are means ± SD. The values with the same lowercase letter in the column are not significantly different (P < 0.05) according to Duncan’s multiple range test.

Crude Protein Extraction. Crude protein was extracted and determined with the semimicro Kjeldahl method (Fawcett 1954).

Crude Fat Extraction. Crude fat was extracted from faba bean and pea and determined with diethyl ether by the Randall method (Thiex et al. 2003).

Raw Starch Extraction. Raw starch was determined according to the national standard of “Method for the determination of raw starch in cereals seeds” (GB5006-85; Meng 1985).

Oligosaccharide Extraction. Oligosaccharides were extracted and tested using HPLC method of Wang et al. (2010a).

Catechin Extraction. Catechin was extracted using the method of Liu (2010).

γ-Aminobutyric Acid Extraction. γ-Aminobutyric acid was tested using the colorimetric method of Zhao et al. (2009).

Total Polyphenol Content Extraction. Total polyphenol content was tested through the method of Yao et al. (2012).

Statistical Analysis. Data for the number of worm holes, number of damaged seeds, and quality components in faba bean and pea were subjected to analysis of variance (ANOVA) with SPSS 10.0 (SPSS Inc., Chicago, IL). Results were expressed as mean ± SD, and when treatment effects were statistically significant (P < 0.05), Duncan multiple range test (DMRT) was used for means separation. Correlation coefficients (r) between damage and quality component contents were calculated with Microsoft Excel 2010.

Results

Faba Bean Resistance to C. chinensis. Among the 339 faba bean accessions, Yuncan 82, Tongcan 5, H5067, and H5631, with resistance scale of 1 and rate of damaged seeds of 5.5, 7.7, 10.0, and 10.0%, respectively, from Yunnan, Jiangsu, and Qinghai were identified with high C. chinensis resistance. Another 15 varieties with resistance scale of 3 and rate of damaged seeds varying from 12.2 to 28.9% mainly from Qinghai and Sichuan were screened for resistance to C. chinensis and 43 with resistance scale of 5 and rate of damaged seeds ranging from 33.3 to 60.0% mainly from Sichuan, Qinghai, and Yunnan were identified with moderate resistance to C. chinensis. In total, 215 varieties with resistance scale of 7 were susceptible and 62 varieties with rate of damaged seeds varying from 92.2 to 100% were highly susceptible. Moreover, there was no faba bean immune to C. chinensis. Among these test varieties, 5.60% were highly resistant and resistant, whereas 81.71% were identified as susceptible and highly susceptible varieties (Table 2). The mean rate of damaged seeds for highly resistant, resistant, moderately resistant, susceptible, and highly susceptible faba bean accessions was 0.10, 0.28, 0.55, 0.83, and 0.97, respectively. The difference in rate of damaged seeds was significant in faba bean varieties with different resistance scales (Table 3).

The average wormholes of these 339 test faba bean varieties ranged from 0.09 per seed to 12.5 per seed. H5086 contributed the highest infestation percentage, with average wormholes of 12.5 per seed (up to 16 per seed) and hundred-kernel weight reduction of 35.36%, thereby losing its commercial quality completely. Tongcan 5 and Yuncan 82 were identified with the highest C. chinensis resistance, with average wormholes at 0.09 per seed and 0.10 per seed (maximum 1 wormhole per seed), respectively. Their hundred-kernel weight reduction was only 0.18%. In the whole, the average number of worm holes for highly resistant,
resistant, moderately resistant, susceptible, and highly susceptible accessions was 0.10, 0.29, 0.67, 1.19, and 1.55, respectively. There was significant difference in number of worm holes resistant and susceptible faba beans (Table 3).

**Pea Resistance to C. chinensis.** In total, 100 pea varieties were screened for resistance to *C. chinensis*. Three varieties (Woyaowandou, Macaiwandou, and Haimenbaihua) with resistance scale of 0 from Anhui, Chongqing, and Jiangsu were immune to *C. chinensis*. Zihuaxiaowandou, HL-10, and L0368 with rate of damaged seeds of 2.7, 3.2, and 10.0%, respectively, were identified to have high resistance. A total of six varieties such as Huangwandou, Shuoshadabaiwan, Qijingbahuawandou, Xinilanshuanghua, Xiaomawan, and Madouzi with resistance scale of 3 and rate of damaged seeds ranging from 23.6 to 53.3% were resistant. In addition, there were six moderately resistant accessions with rate of damaged seeds varying from 43.9 to 56.7%. Among these 100 test varieties, 12% were resistant to *C. chinensis*, and 82% were susceptible and highly susceptible accessions (Table 4). The average rate of damaged seeds for immune, highly resistant, resistant, moderately resistant, susceptible, and highly susceptible accessions was 0.06, 0.34, 0.84, 2.45, and 4.19, respectively, which suggest significant difference in number of worm holes on resistant and susceptible faba beans (Table 5).

**Relationship Between Seed Color and *C. chinensis* Resistance.** The 339 faba bean accessions were divided into four seed color groups: brown & black, green, yellow, and reddish violet. Resistance to *C. chinensis* was correlated with seed color. Dark colored seed had significantly fewer wormholes and seed damage than the three lighter colored seed groups. Among the identified four highly resistant varieties and 15 resistant accessions, most of them, especially Yuncan 82, H5067, H5631, H5032, H4956, and H5629 were dark-colored varieties (e.g., dark brown and light brown) and suffered fewer damage from *C. chinensis*. These varieties manifested fewer *C. chinensis* eggs on their surfaces, lower eclosion rates, and fewer wormholes. The green group presented higher infestation percentage than the yellow and reddish violet groups (Table 6).
The 100 test pea varieties were divided into three seed color groups: green, yellow, and brown. Similar to faba bean, dark colored peas showed significantly higher *C. chinensis* resistance compared with yellow and green ones, as manifested by lower average wormholes and infestation percentage. In this study, two pea varieties (*Woyawondou* and *Macawiandou*) immune to *C. chinensis* were semiwild varieties with brown seeds. Yellow and green accessions presented significant difference in mean number of wormholes, but exhibited no significant difference in rate of damaged seeds (Table 7; DMRT, *P* < 0.05).

**Correlation Between *C. chinensis* Resistance and Quality Components.** *C. chinensis* damage (number of wormholes, percent infestation) to faba bean was significantly and negatively correlated with catechin, polyphenol, and γ-aminobutyric acid content, and significantly and positively correlated with oligosaccharide content (Table 8). For faba bean, the correlation coefficients (*r*) of the number of wormholes with catechin, total polyphenol, γ-aminobutyric acid, and oligosaccharide were −0.7862, −0.9428, −0.4628, and 0.6652, respectively. The *r* values of the infestation percentage with catechin, total polyphenol, γ-aminobutyric acid, and oligosaccharide were −0.8071, −0.9723, −0.4352, and 0.7631, respectively. However, no evident correlation was found between faba bean resistance to *C. chinensis* and crude protein, raw starch, and crude fat contents (Table 8).

Similar to faba bean, *C. chinensis*-induced damage of pea showed significantly negative correlation with catechin and total polyphenol contents, but significantly positive correlation with oligosaccharide content. The *r* values of the number of wormholes with catechin, total polyphenol, and oligosaccharide were −0.5750, −0.7180, and 0.7541, respectively. The *r* values of infestation percentage with catechin, total polyphenol, and oligosaccharide were −0.7666, −0.8846, and 0.8308, respectively (Table 8). Furthermore, crude protein, raw starch, and crude fat were uncorrelated with pea resistance to *C. chinensis*. Overall, faba bean and pea with higher catechin and total polyphenol contents but lower oligosaccharide content had higher resistance to *C. chinensis*, whereas those with lower catechin and total polyphenol contents but higher oligosaccharide content were susceptible to *C. chinensis*. In addition, γ-aminobutyric acid was positively correlated with resistance to *C. chinensis*. However, there is no clear correlation between crude protein, raw starch, and crude fat contents and resistance to *C. chinensis* in faba bean and pea.

**Discussion**

In this article, *C. chinensis* resistance of 339 faba bean and 100 pea varieties was evaluated for the first time through indoor artificial inoculation and free choice tests. Up to 5.6% of the faba bean varieties and 12% of pea varieties were highly resistant or resistant to *C. chinensis*. The mean number of *C. chinensis* eggs on seed of the accessions varied from 4 to 20 eggs per seed, indicating all seeds were exposed to damage by larvae. However, the difference in damage degree was significant in tested varieties with different resistance scales (Tables 3 and 5). Therefore, this method was applicable to the identification and evaluation of *C. chinensis* resistance of faba bean and pea in this article, which will contribute to mass screening of faba bean and pea germplasm resources resistant to *C. chinensis*. Faba bean and pea varieties with dark seed color had significantly more resistance to *C. chinensis* (significantly lower average infestation percentage and number of wormholes) than varieties with yellow or green colored seed. This resistance may be caused by their different degrees of hardness, nutrient contents, and biochemical substances (Sauphanor 1988, Zhang and Deng 1993, Gan et al. 2012).

Insect resistance of plants includes physical resistance, chemical (biochemical) resistance, and ecological resistance (Qin 1987). Chemical resistance is the most basic, which includes secondary substances and nutrient substances. Plant protein, soluble sugar, and starch are the most basic nutrients for insect growth and hence they may affect insect resistance of plants directly (Makino et al. 1983, Nagata et al. 1998, Duan et al. 2013b). A significantly positive correlation was reported between rice plant soluble sugar, protein, starch, and total free amino acid with survival and feeding preference of *Laodelphax striatellus* Fallén nymphs (Liu et al. 2007, Zheng et al. 2009). The soluble sugar and amino acid contents in *Pinus massoniana* Lambert leaf were negatively correlated with its resistance to *Hemiberlesia pityosophila* Takagi and Den-
The growth of bean (2.65%) and pea (3.53%), which may inhibit charose, rafñnose, and lupeose. Our results showed a
Oligosaccharide (soluble saccharide) includes sac-
and starch for
or susceptible to
Aminobutyric acid content of tea trees resistant to the
phenol and catechin in wheat are important secondary substances
after an insect attack (Sandanayaka et al. 2005, Eyles et al. 2007, Carmona et al. 2013). However, our results indicate that protein,
starch, and fat are merely slightly correlated (the values of r between infestation rate and protein, starch, and fat for faba bean and pea are close to zero) with C. chinensis resistance in faba bean and pea (Table 9). Faba bean and pea are rich in protein, amino acids, and starch (Zheng et al. 1997, Smykal et al. 2012, Tables 6 and 7). All of the accessions tested, whether resistant or susceptible to C. chinensis, contain adequate protein and starch for C. chinensis growth and development. Oligosaccharide (soluble saccharide) includes saccharose, rafñnose, and lupeose. Our results showed a relatively low mean oligosaccharide content in faba bean (2.65%) and pea (3.53%), which may inhibit growth of C. chinensis. This result agrees with previous research results indicating that oligosaccharide content is negatively correlated with insect resistance.

γ-Aminobutyric acid, a nonprotein amino acid, is an important secondary substance in plants and has an important function in the chemical resistance of higher plants to phytophagous insects (Ciepiela et al. 1996). γ-Aminobutyric acid content in wheat plant has been reported to restrict the growth of Macrosiphum avenae F., and M. avenae-resistant wheat grain contains higher γ-aminobutyric acid compared with M. avenae-sensitive wheat (Liu et al. 1993, Ciepiela et al. 1999). γ-Aminobutyric acid in rice plants affects the survival and growth of brown planthopper (Nilaparvata lugens Stål). Rice varieties resistant to N. lugens contain far higher γ-aminobutyric acid than susceptible varieties (Sogawa and Futhak 1970, Peng et al. 1979). γ-Aminobutyric acid content of tea trees resistant to the leafhopper Empoasca vitis Göthe, a serious pest of tea, is negatively correlated with E. vitis oviposition (Jin 2012).

Catechin and total polyphenol are important defensive substances of plants which may contribute to the growth and reproduction of phytophagous insects (Etzler 1985, Zhou et al. 2013). All of these results are in good accordance with our results.

Although catechin and total polyphenol contents of faba bean and pea are positively correlated with resistance to C. chinensis, the pea varieties immune and strongly resistant to C. chinensis have lower catechin and total polyphenol contents than the faba bean varieties susceptible to C. chinensis (Table 9). Consequently, we infer that other substances mediate C. chinensis resistance in these pea varieties. Moreover, tannin, alkaid, and terpene in secondary metabolites of plants are important defensive substances, and lectin and amylase inhibitors are two common anti-nutritional factors in faba bean and pea. P-lec and α-amylase inhibitor (α-AI) in pea are more active than those in faba bean (Gatehouse and Gatehouse 1998, Cheng et al. 2005, Ji et al. 2007). Although P-lec in pea is less toxic to human beings, it can inhibit the growth of some coleopteran. α-AI is toxic to some coleopteran and can lower their protein digestibility, thereby inhibiting their growth and development (Etzler 1985, Liu et al. 1995). This finding further confirms the contributions of multiple compounds to C. chinensis resistance in faba bean and pea.

All in all, a batch of faba bean and pea germplasm resources were effectively identified and evaluated for resistance to C. chinensis through indoor artificial inoculation and free choice tests and some resistant accessions were discovered. The resistance to C. chinensis in faba bean and pea accessions showed a significant, positive correlation with catechin, total polyphenol, and γ-aminobutyric acid contents, but a negative correlation with oligosaccharide content.

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**Table 9. The comparison of quality component contents between in faba bean and pea**

<table>
<thead>
<tr>
<th>Crop</th>
<th>Catechin content (mg/g)</th>
<th>γ-Aminobutyric acid content (mg/g)</th>
<th>Total phenol content (mg/g)</th>
<th>Oligosaccharide content (%)</th>
<th>Crude protein content (%)</th>
<th>Raw starch content (%)</th>
<th>Crude fat content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faba bean</td>
<td>2.366 ± 0.0723a</td>
<td>23.243 ± 0.5000a</td>
<td>8.205 ± 0.4112a</td>
<td>2.65 ± 0.260b</td>
<td>24.56 ± 0.208a</td>
<td>42.11 ± 0.258b</td>
<td>0.92 ± 0.013b</td>
</tr>
<tr>
<td>Pea</td>
<td>1.023 ± 0.0546b</td>
<td>22.352 ± 0.6984a</td>
<td>2.631 ± 0.1729b</td>
<td>3.53 ± 0.272a</td>
<td>24.99 ± 0.218a</td>
<td>45.95 ± 0.351a</td>
<td>1.25 ± 0.018a</td>
</tr>
</tbody>
</table>

Values are means ± SD. The values with the same lowercase letter in the column are not significantly different (P < 0.05) according to Duncan’s multiple range test.
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