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Physical and biochemical parameters associated with resistance to *Callosobruchus chinensis* Linn. In chickpea

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Abstract
The performance of 15 chickpea varieties/genotypes to *Callosobruchus chinensis* Linn. was assessed under laboratory conditions based on the development of the insect, seed damage and the various morphological/biochemical parameters of the test varieties. Significant variation was observed among the varieties with respect to pest development and seed damage. Among the various biochemical analyzed, low growth index was observed in the varieties JG 218, JAKI 9218, JG 11, KAK 2, GJG 3, KPG 59, Pusa 391 and JG 315, which recorded < 2.35 growth index were categorized as list susceptible while five varieties *viz*., RSG44, JGK 1, RSG 888, Vishal and JG 63 exhibited moderate susceptible (2.35-3.00) and the remaining 2 varieties/genotypes *viz*., ICCV 2 and ICCV 10 showed highly susceptibility (> 3.0 growth index) to bruchid infestation. Among the morphological parameters, seed colour, seed texture and seed size played a significant role on fecundity, developmental period, adult emergence and seed infestation of the test insect. The biochemical parameters like high phenol, flavonoids, tannin and percent protease inhibitor contents of test varieties were detrimental to the growth and development of test insect while protein content of the test varieties favoured the successful development of bruchids and high infestation. The results of study showed that the chickpea varieties ICCV 2 and ICCV 10 were most suitable for ovipositional preference of the pulse beetle and were not related to suitability of morphological characters of seeds for further development.

Keywords: Pulse beetle, chickpea, screening, growth index

Introduction
Chickpea (*Cicer arietinum* L.) is one of the most important leguminous crops and is extensively cultivated in dry and rainfed areas of the world. India is the largest producer of chickpea with 67 percent of the global production. During 2013-14, area under chickpea production was significantly increased (9.96 million ha) which was the highest in last 10 years. For successful cultivation of chickpea crop, storage of chickpea seed is prerequisite. Almost all pulse growers store the required quantity of pulse seeds in their houses for growing next year. Unfortunately, seeds of pulses are heavily damaged by pulse beetle in storage and chickpea is no exception. In India over 200 species of insects have been recorded infesting various pulses. Out of five known species of *Callosobruchus, Callosobruchus chinensis, C. maculatus* and *C. analis* are most common species of pulse beetle found in India to infest stored legumes **(**Raina, 1970**)**. The pulse beetle, *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae) is one of the three species that cause significant damage to the stored legumes causing up to 55.7 percent of damage in severe infestation (Chaubey, 2008) [6]. Although control of the pest during the storage is possible using various methods, the most eco-friendly and reliable method is the use of resistant sources (Sarwar *et al.*, 2009) [24]. The chickpea intensification programmes can be achieved by producing high yielding varieties with inherent pest resistance characteristics during storage. The use of chickpea resistance in breeding programme to produce the resistant varieties against pulse beetle is a distinct possibility provided, the factors responsible for resistance are identified. Various biological parameters of the bruchid are affected by seed attributes physically or chemically. In different pulses, seed surface, seed coat thickness and seed size have been linked with mechanism of resistance. In addition, the bruchids seemed to be guided in their ovipositional preference by seed surface, colour, texture, volume and nutritional value of seed (Singh *et al.*, 1980) [28].

Though considerable work was done on the varietal preference of pulse beetle to chickpea, cowpea and mungbean (Khokhar and Singh 1987, Dasbak *et al*., 2009) [14, 8], very little work is done on preference of chickpea varieties to *C. chinensis* and the mechanism associated with resistance. With this view the present studies under taken to screen 15 popular chickpea varieties differing morphological and biochemical parameters against C. *chinensis* under laboratory conditions.

Materials and Methods

The test insect, *C. chinensis* was reared on chickpea seeds at 30±02 °C and 70±5% relative humidity in the B.O.D. incubator in the laboratory. A total of 15 varieties of chickpea (JG 11, GJG 3, JG 315, JAKI 9218, RSG 888, KPG 59, ICCV 2, KAK 2, Pusa 391, RSG 44, JGK 1, JG 218, Vishal, ICCV 10 and JG 63 were screened during 2018 for their reaction to *C. chinensis* under no choice artificial infestation conditions as given by Giga, 1995. Different varieties of chickpea were evaluated for their physical parameters, *viz.* seed texture, seed coat thickness, seed colour and shape. Seed texture was examined under the stereo-binocular microscope and seed coat thickness was measured using Vernier calipers (in mm). Hundred weighed seeds of eleven varieties were kept separately in half litre plastic jars and five pairs of one day old adults (5 males and 5 females) of *C. chinensis* were released in the each jar. The mouth of the plastic jar was covered with double folded muslin cloth fastened with rubber band. The jars were placed in incubator at a temperature of 30±02 °C and 70±5% relative humidity. The released pulse beetles were removed after 72 hours with the expectation of maximum oviposition during this period and numbers of eggs laid on seeds of each variety were recorded. The experiment was continued for 45 more days, to observe the adult emergence and the number of eggs oviposited on each seed counted using a magnifying glass. The seeds were examined daily for successful hatching, larval penetration into the seed and emerging adults. The growth index of different chickpea varieties to pulse beetle was calculated on the basis of percent adult emergence divided by developmental period (days) following formula was used as suggested by Singal (1987) [27].

$$\text{Growth Index} = \frac{\text{Percentage adult emergence}}{\text{Developmen tal period (days)}}$$

Following methods had been used for biochemical studies

Crude extraction of seed sample: Two grams powdered sample from each variety were mixed with 15 ml of 80% methanol and ground thoroughly in pestle and mortar individually. The ground material was centrifuged at 10000 rpm for 20 min. The extracts prepared were used for the estimation of total protein, total phenol, total flavonoids, tannins and trypsin inhibitor.

Estimation of total protein

Protein content in the seed extracts were estimated by the method of (Bradford, 1976) [5] using bovine serum albumin as a standard. For each variety protein sample (40µL) was taken in the test tubes and the volume was made up to 300µL with extraction buffer. After addition of 3 ml dye the mixture was incubated for 5 minutes at room temperature. The absorbance was recorded at 595 nm wavelength in the spectrophotometer. The concentration of protein was calculated by the standard curve.

Estimation of total phenol: Phenol from seed extracts were estimated using Folin-Ciocalteu reagent method (Singleton and Rossi, 1965) [29]. The aliquot (100µl) was mixed with 0.1 ml of Folin-Ciocalteu reagent allowed to stand at room temperature for 5 minutes; then 2ml of saturated sodium bicarbonate solution were added to the mixture. After 10 min, absorbance was measured at λ 690 nm wavelength in the spectrophotometer. Results were expressed as gallic acid equivalents. Standard curve for gallic acid were prepared by taking different concentration of gallic acid (10-100µg). The calculation of total phenol**,** from the standard curve, concentrations of' total phenols in terms of mg phenols/gms plant material were estimated.

Estimation of total flavonoids: For estimation of flavonoids, 1ml of methanol extract was added to 10% aluminium chloride solution with some modification (Quettier *et al*., 2000). The test tubes were shaken vigorously for some time and the test tubes were incubated at ambient temperature for 5 minutes. The pink color was developed. The absorbance was measured at λ 510nm wavelength in the spectrophotometer. A calibration curve was prepared with quercetin and the results were expressed as mg quercetin equivalent/g (QE/g) sample. A standard curve of quercetin concentrations from 10 to 100µg were used to prepare for the calibration of flavonoids.

Estimation of total tannin: For the estimation of tannin, folin-ciocalteu reagent method of the procedure (Attarde *et al.,* 2010) [3] was followed. One ml of methanol extract was added to 1ml of folin-ciocalteu reagent; 4 ml of sodium carbonate solution was added. The test tubes were shaken vigorously for some time and the test tubes were incubated at ambient temperature for 5 minutes. The absorbance was measured at λ 740nm wavelength in the spectrophotometer. A calibration curve was prepared with tannic acid and the total tannin amounts were expressed in mg/g of sample. A standard curve of tannic acid concentrations from 10 to 100 µg were used to prepare for the calibration of tannins.

The activity of trypsin inhibitor was assayed by determining the residual trypsin activity following the method of Kakade *et al.* (1969) with slight modifications using BApNA as the substrate and bovine trypsin as the standard enzyme. Assays were run in triplicates with appropriate blanks. The reaction mixture contained 0.1 ml diluted crude trypsin inhibitor (seed extract), 0.2 ml trypsin (2 mg in 40 ml 0.001M HCl) and 1 ml of BApNA (30 mg dissolved inminimum volume of DMSO and adjusting its final volume to 100 ml with 0.05 M Tris-HCl, pH 8.2, containing 0.03 MCaCl2) in a final volume of 1.3 ml. The final concentration of BApNA in the reaction mixture was 0.54 mM and the number of trypsin units was 180. After incubating the mixture at 37ºC for 30min, the reaction was stopped by adding 0.3 ml of 30% (v/v) glacial acetic acid. The absorbance was recorded at λ 410 nm wavelength in the spectrophotometer against the blank. The TI calculated by using standard curve of *p*NA (paranitroanilinide) curve (10µl-100µl). Percent inhibition was calculated by using the following formula:

$$\text{\% Inhibition} = \frac{\text{O.D. in the presence of inhibitor} - \text{O.D. in the absence of inhibitor X 100}}{\text{O.D. in the absence of inhibitor}}$$

Results and Discussion

Physical characteristics of seed: The physical characteristics of seed parameters (colour, shape, texture, seed coat hardness and 100 seed weight) of chickpea varieties are given in Table 1. The colour of chickpea varieties seeds varied from brown, beige and light brown. Maximum six varieties had light brown (JG 11, KAK 2, JGK 1, Vishal, JAKI 9518 and KPG 59) followed by brown (JG 315, ICCV 10, RSG 888 and RSG 44) and three varieties (GJG 3, JG 218 and JG 63) had yellowish brown colour. Based on the shape, these were categorized into two groups *viz*., angular and owl's head. Observations showed that maximum eleven varieties (JG 11, GJG 3, JG 315, JAKI 9218, RSG 888, KPG 59, Pusa 391, RSG 44, JGK 1, ICCV 10 and JG 63) had angular shape followed by owl's head (KAK 2, ICCV 2, JG 218 and Vishal). Seed texture of varieties ranged from smooth and rough. Maximum eight varieties possessed smooth texture (JG 11, JAKI 9218, ICCV 2, KAK 2, JGK 1, JG 218, Vishal, ICCV 10 and), followed by rough (Pusa 391, GJG 3, JG 315, JG 6, RSG 888, RSG 44, and KPG 59). Similarly, seed size, these were categorized into three groups *viz*., small, medium and large. Observations showed that seven varieties (ICCV 2, KAK 2, Pusa 391, JGK 1, JG 315, Vishal, and JAKI 9218) had large in size followed by medium (JG 11, GJG 3, JG 218, JG 63, ICCV 10, RSG 44 and KPG 59) and RSG 888 small in size.

Maximum weight of 100 seed was recorded in JGK 1 (40.04g) followed by KAK 2 (36.11g) and minimum in RSG 888 (14.00g). The seed coat thickness and 100 seed weight varied significantly among the chickpea varieties. Seed coat thickness ranged from 0.06 to 0.19 mm with maximum in JG 218 (0.19 mm) followed by Pusa 391 (0.17 mm) and minimum in Vishal (0.06 mm). The present results are corroboration with the findings of Shaheen *et al.,* (2006) [26] who showed that cultivars with hard, rough, wrinkled and thick seed coat proved to be more resistant when compared with those having smooth, soft and thin seed coat. Divya *et al.* (2013) [9] also reported that presence of thick seed coat in the horse gram accessions might have led to reduction in biological parameters of pulse beetle, it served as a barrier for the entry of bruchids.

The results showed that all the 15 varieties of chickpea were preferred by the *C. chinensis* for oviposition, however, the number of eggs laid by the beetle on the seeds of different varieties varied significantly. The results revealed that the varieties JG 218 (101.26 eggs), KAK 2 (107.17 eggs), JAKI 9218 (109.12 eggs), JG 11 (111.16 eggs), GJG 3 (111.50eggs), Pusa 391 (114.17 eggs), KPG 59 (114.46 eggs) and JG 315 (115.34) were least preferred by the beetle for oviposition while ICCV 2 (138.42 eggs) and ICCV 2 (141.84 eggs) were most preferred for oviposition. The results are in agreement with the findings of Khokhar and Singh (1987) [14]. They reported that the number of eggs laid by the beetle on pigeonpea varieties varied from 34.7 to as high as 238.0 eggs and stated that ICPL-143, ICPL- 148 and H-79-4 were least preferred by the bruchid. Wadnerkar *et al.,* (1978) [33] also observed differential response of oviposition by bruchid to different varieties of pigeonpea. The oviposition of the test insect was found to be more influenced by the physical parameters like 100 grain weight, seed size and seed texture showed negative effect on fecundity of *C. chinensis.* The mean number of adults that emerged from chickpea varieties varied from lowest (10.86) in JG 218 to highest (22.56) in ICCV 10.

The least preferred varieties, JG 218, JG 11, GJG 3 and KAK 2 were not suitable for the development of the bruchid and resulted in prolonged developmental period (33.33, 32.33, 32.33 and 31.33 days, respectively) while the preferred varieties which recorded more number of eggs i.e ICCV 2 (138.42) followed by ICCV 10 (141.84) were found to be highly suitable for the development of insect and they took relatively less time to complete the development resulting in high adult emergence. Singh *et al.* (2001) studied the oviposition and larval development of pulse beetle on chickpea. It was concluded that PG-5 was the most resistant, with maximum growth index of 1.358 and the longest grub development period of 28.33 days.

ICCV 10 was the most susceptible with maximum growth index of (3.10) and the minimum development period of 28.67 days. In the present study among the varieties, JG 218 recorded 2.09 growth index. Significantly highest growth index was observed in ICCV 10 (3.10), ICCV 2 (3.01) and JG 3 (2.76) and these were on par with each other. The growth index of the rest of the varieties varied from 2.12 to 2.56.

The biochemical basis of resistance to *C. chinensis*, parameters such as protein content, phenol, flavonoids, tannin and trypsin inhibitor content were studied which differed significantly among the chickpea varieties (Table 3). The phenol contents of the different varieties lengthen the developmental period of *C. chinensis*. Phenol content varied from 0.045 (JGK 1) to 0.851 mg/gm (Pusa 391) ICCV 2 and ICCV 10 with less phenol content had more growth index as compared to moderate resistance varieties RSG 44, RSG 888, Vishai, JG 63 and JGK 1. (Ghosal *et al*., 2004; Patel, 2002) [10, 17] also reported similar result in stored legume seeds. The bruchid resistance might be due to the antinutritional factor present in the seeds that inhibits the development of larvae. The flavonoids content of different chickpea varieties ranged from 0.047 (JGK 1) to 0.0107 mg/ gm (JG 218). All the varieties of chickpea showed relative behavior to *C. chinensis*, but the varieties JGK 1, GJG 3, JG 315 and JG 63 with less flavonoids content had high growth index as compared to moderately susceptible varieties (PG 4, PG 186 and PKG 2) and moderately resistance varieties (RSG 44, RSG 888, Vishai, JG 63 and JGK 1) which showed less growth index because of the presence of high flavonoids which affect their metabolic activity and inhibit their growth. (Patil *et al.*, 2016; Southgate, 1979) [18, 30]. The tannins are secondary metabolites which inhibit the digestive enzymes and therefore, lower the digestibility of important nutrients especially proteins and starch. Tannin content in eleven chickpea varieties varied from 0.393 to 0.738 (mg/g seed) with lowest tannin content was drowned in varieties, ICCV2 (0.715) followed by ICCV 10 (0.740) and JG 63(0.755 mg/g seed) and highest in varieties JG 218 (1.215 mg/g seed) followed by Vishal (1.095 mg/g seed), JGK 1 (1.072 mg/g seed) and KAK (1.005 mg/g seed). Of the chickpea varieties with high tannin content, JG 218 and JAKI 9218 had low growth index as compared to low tannin content varieties ICCV 2, ICCV 10, and GJ 3 **(**Khattab and Arntfield, 2009) [13].

The highest percent trypsin inhibitor activity was found in variety RSG 888 (18.05%) (Table 3) and rated as moderately resistant followed by GJG 3 (17.48%), JAKI 9218 (16.47%) and Vishal (16.37%) and the lowest in ICCV 2 (7.42%) and ICCV 10 (8.44%). ICCV 2 and ICCV 10 were rated as highly susceptible, indicating the major role of trypsin inhibitor in protein resistance to *C. chinensis*. The highest activity of protease inhibition acts as antimetabolites to *C. chinensis*, which inhibit the feeding of grubs as result higher trypsin content varieties showed relative resistance. Gatehouse and Boutler (1979) reported that high content of trypsin inhibitor might be related to the mechanism of resistance to Bruchid beetle. The trypsin inhibitor functions by blocking digestive proteinases of insect larvae, thereby limiting release of amino acids from food proteins. (Raghuwanshi *et al.*, 2016; Broadway, 1986) [20, 5]. Among the biochemical constituents, significantly low protein content was observed in less susceptible varieties, while in moderately susceptible varieties, Vishal, RSG 888 and RSG 44 these contents were found in the reverse order. The results were in agreement with the findings of Sandhya Rani (1998) [23] and Ramanamurthy (1981) [22] who reported that the chickpea varieties with less protein content were not preferred for development of *C. chinensis*.

The overall results obtained from varietal preference studies revealed that the varietal resistance of *C. chinensis* is influenced both by the morphological as well as biochemical parameters. Morphological parameters like weight of 100 seeds and seed size negatively influenced the pest infestation and development while inter granular space favoured the pest infestation. Among the biochemical parameters, high phenol, flavonoids and tannin offered resistance to the pest attack while protein content favoured high adult emergence in susceptible varieties. Study of these factors would help in better understanding of the resistance mechanisms and subsequent development of resistant varieties to the bruchid infestation. This information can be used to select better parent and crossed to raise more resistant recombinants for the development of resistant chickpea varieties.

Table 1: Mean number of eggs and physical characters of seeds of different chickpea varieties/genotypes

Varieties	Physical characteristics of chickpea seeds					
	100-seed Weight (g)	Thickness of seed coat (mm)	Seed texture	Seed colour	Seed shape	Seed size
JG 11	23.11^{b}	0.15^{c*}	Smooth	Light brown	Angular	Medium
ICCV 2	24.09^{b}	0.07^{a}	Smooth	Brown beige	Owl's head	Large
KAK 2	36.11^{c}	0.07^{a}	Smooth	Light brown	Owl's head	Large
Pusa 391	25.34^{b}	0.17^{c}	Rough	Dark brown	Angular	Large
JGK 1	40.04^{c}	0.11^{b}	Smooth	Light brown	Angular	Large
GJG 3	20.25^{b}	0.15^{c}	Rough	Yellowish Brown	Angular	Medium
JG 315	16.29^{a}	0.11^{b}	Rough	brown	Angular	Large
JG 218	18.12^{a}	0.19^{d}	Smooth	Yellowish Brown	Owl's head	Medium
JG 63	15.37^{a}	0.17^{c}	Rough	Yellowish Brown	Angular	Medium
ICCV 10	17.00^{a}	0.16^{c}	Smooth	Brown	Angular	Medium
Vishal	17.00^{a}	0.06^{a}	Smooth	Light brown	Owl's head	Large
RSG 888	14.00^{a}	0.09^{a}	Rough	Brown	Angular	Small
RSG 44	16.00^{a}	0.11^{b}	Rough	Brown	Angular	Medium
JAKI 9218	18.27^{a}	0.07^{a}	Smooth	Light brown	Angular	Large
KPG 59	19.00^{a}	0.13^{b}	Rough	Light brown	Angular	Medium
S.Em±	1.75	0.01				
CD at 5%	5.25	0.03				

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level

Table 2: Percent of adult emergence mean developmental period, percent weight loss and relative growth index of *Callosobruchus chinensis* (L.) on 15 different varieties/genotypes of chickpea seeds in a no-choice experiment

Chickpea varieties	Mean number of eggs laid (100 seeds)	Adults emergence (%)	Mean developmental period (days)	Weight loss (%)	Classification of evaluated chickpea varieties for *C. chinensis* resistance using relative growth index	
					Growth index	Category
JG 11	111.16^{b}	13.49^{b}	32.33^{d}	15.10^{b}	2.14^{a*}	Less susceptible
ICCV 2	138.42^{e}	20.95^{d}	28.67^{a}	35.99^{f}	3.01^{b}	Highly susceptible
KAK 2	107.17^{a}	13.06^{b}	31.33^{c}	17.51^{c}	2.17^{a}	Less susceptible
Pusa 391	114.63^{b}	12.21^{a}	31.33^{c}	24.50^{d}	2.26^{a}	Less susceptible
JGK 1	131.25^{d}	12.95^{b}	31.00^{b}	22.44^{d}	2.48^{a}	Moderately susceptible
GJG 3	111.50^{b}	13.45^{b}	32.33^{d}	19.75^{c}	2.19^{a}	Less susceptible
JG 315	115.34^{b}	12.13^{a}	31.33^{c}	19.02^{c}	2.34^{a}	Less susceptible
JG 218	101.26^{a}	10.86^{a}	33.33^{e}	5.51^{a}	2.09^{a}	Less susceptible
JG 63	135.12^{d}	17.76^{c}	30.33^{a}	28.63^{e}	2.76^{b}	Moderately susceptible
ICCV 10	141.84^{e}	22.56^{e}	29.00^{a}	37.78^{f}	3.10^{b}	Highly susceptible
Vishal	134.53^{d}	17.09^{c}	30.33^{b}	25.33^{e}	2.56^{a}	Moderately susceptible
RSG 888	129.15^{d}	16.26^{c}	30.33^{b}	24.00^{d}	2.51^{a}	Moderately susceptible
RSG 44	118.39^{c}	14.35^{b}	30.00^{b}	21.33^{d}	2.46^{a}	Moderately susceptible
JAKI 9218	109.12^{b}	10.96^{a}	31.67^{c}	13.15^{b}	2.12^{a}	Less susceptible
KPG 59	114.46^{b}	13.97^{b}	31.33^{c}	17.28^{c}	2.26^{a}	Less susceptible
S.Em±	2.22	0.53	0.34	1.09	0.19	
CD at 5%	6.68	1.59	1.02	3.27	0.58	

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level

Table 3: Effect of biochemical factors on *Callosobruchus chinensis* resistance in chickpea varieties/genotypes

Genotype	Growth index	Phenol (mg/g)	Flavonoids (mg/g)	Tannin (mg/g)	Protease inhibitor (%)	Protein (g/100g)
JG 11	2.14[a]	0.188±0.203[d]	0.078±0.016[b]	0.980±0.156[c]	14.10±3.209[c]	14.00±1.563[c*]
ICCV 2	3.01[b]	0.045±0.103[a]	0.060±0.011[a]	0.715±0.278[a]	7.42±3.330[a]	17.10±1.597[g]
KAK 2	2.17[a]	0.198±0.217[d]	0.080±0.013[b]	1.005±0.286[c]	11.20±2.827[b]	16.65±1.545[f]
Pusa 391	2.26[a]	0.852±0.227[f]	0.081±0.013[b]	1.072±0.294[d]	15.08±2.750[c]	13.33±1.516[b]
JGK 1	2.48[a]	0.094±0.211[b]	0.047±0.012[a]	1.072±0.294[d]	15.23±2.883[c]	15.14±1.520[d]
GJG 3	2.19[a]	0.087±0.125[a]	0.050±0.012[a]	0.715±0.303[a]	17.48±3.037[d]	14.33±1.600[c]
JG 315	2.34[a]	0.115±0.105[b]	0.057±0.011[a]	0.835±0.318[b]	11.20±3.071[b]	15.25±1.686[d]
JG 218	2.09[a]	0.210±0.111[d]	0.107±0.016[c]	1.215±0.337[e]	14.27±3.991[c]	11.50±1.797[a]
JG 63	2.76[b]	0.092±0.118[b]	0.060±0.011[a]	0.755±0.322[a]	14.10±3.215[c]	16.12±1.269[e]
ICCV 10	3.10[b]	0.074±0.103[a]	0.063±0.010[a]	0.740±0.248[a]	8.44±3.488[a]	17.21±1.339[g]
Vishal	2.56[a]	0.425±0.131[e]	0.090±0.010[c]	1.095±0.381[d]	16.37±1.101[d]	15.05±1.023[d]
RSG 888	2.51[a]	0.110±0.015[b]	0.062±0.007[a]	0.830±0.385[b]	18.05±1.260[e]	15.17±1.172[d]
RSG 44	2.46[a]	0.137±0.003[c]	0.071±0.004[b]	0.850±0.436[b]	15.08±1.081[c]	16.10±1.395[e]
JAKI 9218	2.12[a]	0.140±0.002[c]	0.072±0.004[b]	0.865±0.512[b]	16.47±0.523[d]	13.33±0.778[b]
KPG 59	2.26[a]	0.143±0.072[c]	0.078±0.016[b]	0.930±0.649[c]	17.21±3.067[d]	14.43±1.430[c]
S.Em ±	0.19	0.014	0.005	0.028	0.411	0.151
CD at 5%	0.58	0.042	0.017	0.081	1.24	0.43

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level

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