**MORPHOMETRICS OF BROWN PLANTHOPPER,**

***Nilaparvata lugens* (Stål) POPULATIONS COLLECTED FROM DIFFERENT LOCATIONS OF INDIA.**

**ABSTRACT**

The experiment was conducted at the ICAR–Indian Institute of Rice Research, Hyderabad. Brown Planthopper (BPH) specimens were collected from farmers' fields located in BPH-prone regions across the states of Telangana, Karnataka, Chhattisgarh, Odisha, and Punjab. Following collection, the specimens were dry-mounted and carefully examined under a stereoscopic binocular microscope to study their morphological characteristics. Morphometric measurements—including total body length, body width, wing length, wing width, head length, interocular distance, and tibial spur length—were recorded for both brachypterous and macropterous forms of adult males and females. Additionally, body length and width were measured for all five nymphal instars. The study included BPH populations from different geographical locations, as well as a glasshouse-maintained BPH culture. All morphometric data were recorded in millimeters. The analysis revealed slight morphological variations among the different BPH populations.

**Key words:** Morphometric, populations, *Nilaparvata lugens,* rice

**Introduction**

The rice brown planthopper (BPH), Nilaparvata lugens (Stål) (Hemiptera: Delphacidae), is recognized as one of the most destructive sucking insect pests affecting rice crops across all major rice-growing regions. It feeds on the phloem sap of rice plants, leading to a condition known as “hopper burn,” which causes the plants to wilt and dry up, resulting in extensive yield losses. Outbreaks of BPH have been reported frequently in different parts of the country, posing a significant threat to food security and the livelihood of rice farmers (Jhansi Lakshmi et al., 2010).

In recent years, there has been a growing interest in understanding the morphological and biological diversity of BPH populations, as such variations can influence pest virulence, adaptability, and resistance to insecticides. Morphometrics -the quantitative analysis of form-serves as a powerful tool in this context. It involves precise measurements of various body parts and is widely used in systematics to study morphogenesis, population structure, and the effects of environmental stress on organisms, especially arthropods (Daly and Gregg, 1985). By applying morphometric analysis to BPH populations collected from different regions, researchers can detect subtle morphological differences that may be indicative of emerging biotypes or ecological adaptations, ultimately aiding in the development of more effective pest management strategies.

**MATERIALS AND METHODS**

Field collection of Brown Planthopper (BPH) specimens was conducted from farmers' fields located in BPH-prone areas across five states: Telangana, Karnataka, Chhattisgarh, Odisha, and Punjab. Insects were collected using aspirators (suction bulb with mouthpiece) and placed into plastic boxes (25 cm × 15 cm) with multiple perforations for ventilation. Rice stems wrapped with moistened cotton swabs were provided as food and to maintain humidity. From each location, 10–15 such boxes were collected, packed into cotton bags, and transported in the evening via buses and trains. During transit, cotton swabs were intermittently re-moistened with water to ensure the insects' survival.

Upon arrival at ICAR–Indian Institute of Rice Research, Rajendranagar, Hyderabad, the BPH specimens were transferred to an isolation chamber and gently released onto potted rice plants. Morphometric investigations were carried out at the Instrumentation Cell, College of Agriculture, PJTSAU, Rajendranagar, during 2018. Measurements recorded included body length and width, wing length and width, head length, interocular distance, and tibial spur length for both brachypterous and macropterous adult males and females. Similarly, body length and width of all five nymphal instars were recorded. One-day-old adults and freshly moulted nymphs were used for measurement, all in millimeters.

BPH populations reared in glasshouse cages were also used. These were placed in plastic containers with rice stems and transported to the instrumentation laboratory, where they were frozen and processed. The specimens were dry-mounted and examined under a Lawrence and Mayo stereoscopic binocular microscope (Model: LM-52-3611 [ZTX-3E] with 2X zoom; Kathirithamby, 1994). From each developmental stage and region, twenty individual insects were sampled. Morphometric data were captured using T Capture Software (version 3.9 build 500). Data were subjected to appropriate transformations and analyzed using Completely Randomized Block Design (CRBD) in Statistix 8 (version 8.1), with treatment means compared using Duncan’s Multiple Range Test (DMRT).

**List 1. Details of the field collection of *N. lugens* carried out during the study period**

|  |  |  |
| --- | --- | --- |
| **Place** | **District** | **State** |
| Kampasagar | Nalgonda | Telangana |
| Gangavathi | Koppal | Karnataka |
| Raipur | Raipur | Chhattisgarh |
| Bargarh | Bargarh | Odisha |
| Ludhiana | Ludhiana | Punjab |

**RESULTS AND DISCUSSIONS**

Body length of brachypterous female BPH from Kampasagar was more *i.e.* 3.356 mm and length of the head of the brachpterous female was more 0.847 mm in Raipur BPH population compared to that of other BPH populations. The body length of the adult macropterous female BPH from Kampasagar was more (3.448 mm) and tibial spur was longest (0.456 mm) in Bargarh macropterous females compared to that from other locations. Maximum length of the head (0.777 mm) of the brachpterous male BPH was observed in Raipur population as compared to the other geographical regions. Interocular distance in adult macropterous male was significantly more in the Kampasagar BPH population (0.810 mm). The tibial spur was the longest in the glasshouse macropterous males (0.320mm).

The body length of the first instar BPH nymphs was more in Gangavathi BPH population (0.716 mm), the body length of second instar nymph was more in Bargarh BPH population (1.153mm). The body length and width of fourth instar BPH nymphs were more in Raipur BPH population (2.447mm and 0.988mm, respectively) and the body length of fifth instar BPH nymphs was more in Bargarh BPH (2.996mm).

The main disadvantage of morphometrics is that the method alone cannot easily distinguish between environmental and genetic contributions to the phenotype; therefore, it cannot directly establish the biological validity of a species, *i.e.,* whether populations are reproductively isolated (Lane, 1994)

The present findings with regard to morphometerics of wings are in agreement with the measurements of length of macropterous male forewings 4.00 (3.80-4.20) mm and width across the compound eyes 1.02 (1.01-1.03) mm by Shashank, (2009). Measurements of length of macropterous male including forewings 3.90 (3.70-4.10) mm and width across the compound eyes 1.01 (1.01-1.02) mm by Brahma, (2010). As reported by Cook *et al.* (1987) the variation in wing length across geographical locations seems to be determined primarily by environmental dissimilarities between the locations. The adult forewing length in *N. lugens* macropterous male 3.0-3.2mm, brachyptrous male 1.0 -1.6mm, macroptereous female 3.0-3.8mm and brachypterous female 1.1- 1.6mm ([Cook](https://www.cabdirect.org/cabdirect/search/?q=au%3a%22Cook%2c+A.+G.%22) and [Perfect](https://www.cabdirect.org/cabdirect/search/?q=au%3a%22Perfect%2c+T.+J.%22), 1982). Similar morphometrical studies were conducted by Bhattacharyya *et al.* (1983) on macropterous forms of Pantnagar and Hyderabad populations. Little microtypic numerical variations recorded on some characters were unable to separate these two populations morphologically.

Studies on different morphological characters between rice and *Leersia*-infesting *N. lugens* from 11 locations revealed the range of length of tibial spur betweeen 0.386 to 0.482mm (Latif *et al*. 2013) and this is in accordance with the present findings. Individuals reared on TN1 were generally somewhat larger than those on resistant varieties. The length of spur length (mm) of *N. lugens* in sequence: biotype l (B l ) on TN l; biotype 2 (B2) on Mudgo; biotype 3 (B3) on ASDT; biotype 2 on TNI; biotype 3 on TNI range of 0.3-0.5, 0.3-0.4, 0.25-0.45, 0.3-0.6 and 0.35-0.50 (Claridge *et al*.1984) which was similar to the present findings where length of spur length (mm) in *N.lugens* recorded 0.30-0.45.

**REFERENCES**

Brahma, K. S. H. 2010. Taxonomic studies on planthopper Fauna (Fulgoroidea: Hemiptera) of AndhraPradesh. *M.Sc. Thesis*. Acharya N.G. Ranga Agricultural University, Hyderabad

[Bhattacharyya, P. R.](https://www.cabdirect.org/cabdirect/search/?q=au%3a%22Bhattacharyya%2c+P.+R.%22),   [Bhattacharya, A. K](https://www.cabdirect.org/cabdirect/search/?q=au%3a%22Bhattacharya%2c+A.+K.%22) and [Verma, S. K. 1983.](https://www.cabdirect.org/cabdirect/search/?q=au%3a%22Verma%2c+S.+K.%22)Morphometrical studies on the Pantnagar and Hyderabad populations of brown planthopper, *Nilaparvata lugens* (Stal).[*Indian Journal of Entomology*](https://www.cabdirect.org/cabdirect/search/?q=do%3a%22Indian+Journal+of+Entomology%22): 45 (2)101-107.

[Claridge, M. F.](https://www.cabdirect.org/cabdirect/search/?q=au%3a%22Claridge%2c+M.+F.%22), [Hollander, J](https://www.cabdirect.org/cabdirect/search/?q=au%3a%22Hollander%2c+J.+den%22)  and  [Haslam, D.](https://www.cabdirect.org/cabdirect/search/?q=au%3a%22Haslam%2c+D.%22) 1984. The significance of morphometric and fecundity differences between the 'biotypes' of *N. lugens.*[*Entomologia Experimentalis et Applicata*](https://www.cabdirect.org/cabdirect/search/?q=do%3a%22Entomologia+Experimentalis+et+Applicata%22). 36 (2).107-114.

[Cook, A. G](https://www.cabdirect.org/cabdirect/search/?q=au%3a%22Cook%2c+A.+G.%22) and [Perfect, T. J.](https://www.cabdirect.org/cabdirect/search/?q=au%3a%22Perfect%2c+T.+J.%22) 1982. Determining the wing-morph of adult *Nilaparvata lugens* and *Sogatella furcifera* from morphometric measurements on the fifth-instar nymphs. [*Entomologia Experimentalis et Applicata*](https://www.cabdirect.org/cabdirect/search/?q=do%3a%22Entomologia+Experimentalis+et+Applicata%22) . 31 (2) 159-164.

Daly, J.C and Gregg P. 1985. Genetic variation in *Heliothis* in Australia: species identification and gene flow in two pest species *H. armigera* (Hubner) and *H.punctigera* Wallengren (Lepidoptera: Noctuidae). *Bulletin of Entomology Research*. 75.169-184.

Jhansi Lakshmi, V., Krishnaiah, N. V., Katti, G.,Pasalu, I. C and Bhanu, K. V. 2010. Development of insecticide resistance in rice brown planthopper and whitebacked planthopper in Godavari delta of Andhra Pradesh. *Indian Journal of Plant Protection.* 38. (1): 35-40.

Kathirithamby, J. 1994. Records and a checklist of Strepsiptera (Insecta) from Sri Lanka. *Journal of South Asian Natural History*. 1(1): 17-25.

[Latif](https://www.cabdirect.org/cabdirect/search/?q=au%3a%22Mohammad+Abdul+Latif%22), M. A., [Omar](https://www.cabdirect.org/cabdirect/search/?q=au%3a%22Mohd+Yosoh+Omar%22), M. Y., [Rafii](https://www.cabdirect.org/cabdirect/search/?q=au%3a%22Mohd+Yusop+Rafii%22), M. Y., [Malek](https://www.cabdirect.org/cabdirect/search/?q=au%3a%22Mohammad+Abdul+Malek%22), M. A and [Guan](https://www.cabdirect.org/cabdirect/search/?q=au%3a%22Tan+SoonGuan%22). T. S. 2013. Evidence of sibling species between two host-associated populations of brown planthopper, *N. lugens* (Stǻl) (Homoptera: Delphacidea) complex based on morphology and host plant relationship studies. *[Comptes Rendus Biologies](https://www.cabdirect.org/cabdirect/search/?q=do%3a%22Comptes+Rendus+Biologies%22)*. 336 (7) 354-363.

Shashank, P. R. 2009. Taxonomic studies on leafhopper and planthopper fauna associated with rice ecosystem and their management. *M.Sc Thesis*. Acharya N.G. Ranga Agricultural University, Hyderabad

**Table 1. Morphometrics of brachypterous brown planthopper females from different locations**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **BPH**  **populations** | **Body length (mm)** | **Body**  **width**  **(mm)** | **Wing length (mm)** | **Wing width (mm)** | **Head length (mm)** | **Interocular distance (mm)** | **Tibial Spur**  **length**  **(mm)** |
| **Glasshouse** | 3.314 ± 0.041 a | 1.457 ± 0.009a | 1.574 ±0.001a | 0.837 ± 0.001b | 0.842 ± 0.002a | 0.277 ± 0.003b | 0.385 ± 0.006bc |
| **Kampasagar** | 3.356 ± 0.037a | 1.249 ± 0.019b | 1.474 ± 0.003b | 0.773 ± 0.014 c | 0.781 ± 0.048c | 0.274 ±  0.005 bc | 0.434 ±  0.001 a |
| **Gangavathi** | 2.920 ± 0.051b | 1.420 ± 0.015 a | 1.539 ± 0.009 ab | 0.778 ± 0.007 c | 0.816 ± 0.045 b | 0.246 ± 0.004c | 0.360 ±  0.024 d |
| **Raipur** | 3.278 ± 0.033 a | 1.410 ± 0.025 a | 1.316 ± 0.004 c | 0.758 ± 0.001 c | 0.847 ± 0.035a | 0.445 ± 0.023a | 0.372 ± 0.013cd |
| **Bargarh** | 2.988± 0.023b | 1.148 ± 0.017 c | 1.274 ± 0.017c | 0.838 ±0.005 b | 0.743 ± 0.010 d | 0.253 ±  0.004 bc | 0.416 ± 0.006ab |
| **Ludhiana** | 3.293 ± 0.028 a | 1.182 ± 0.014c | 1.565 ± 0.032 a | 0.918 ± 0.015 a | 0.788 ± 0.004c | 0.266 ±  0.002 bc | 0.350 ±  0.008 d |
| **S.E (m)** | 0.042 | 0.017 | 0.026 | 0.013 | 0.015 | 0.010 | 0.012 |
| **C.D** | 0.116 | 0.048 | 0.072 | 0.036 | 0.021 | 0.027 | 0.035 |
| **C.V** | 5.8 | 6.0 | 6.4 | 5.0 | 7.2 | 3.9 | 6.3 |

Values in a column followed by the same letter do not differ significantly by DMRT (*P* = 0.05%)

**Table 2. Morphometrics of marcropterous brown planthopper females collected from different locations**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **BPH**  **Populations** | **Body length (mm)** | **Body width (mm)** | **Wing length (mm)** | **Wing width (mm)** | **Head length (mm)** | **Interocular distance (mm)** | **Tibial Spur**  **length (mm)** |
| **Glasshouse** | 3.336 ± 0.032b | 1.210 ± 0.20 c | 3.621 ± 0.009 ab | 1.107 ± 0.003 a | 0.786 ± 0.007a | 0.266 ± 0.003c | 0.437 ± 0.001a |
| **Kampasagar** | 3.448 ± 0.029 a | 1.415 ± 0.018a | 3.679 ± 0.033 a | 0.989 ± 0.002 c | 0.757 ± 0.004 ab | 0.281 ± 0.003b | 0.360 ± 0.002c |
| **Gangavathi** | 3.428 ± 0.009a | 1.294 ± 0.002 b | 3.566 ± 0.044c | 1.115 ± 0.001 a | 0.694 ± 0.002 c | 0.258 ± 0.002 c | 0.324 ±  0.008d |
| **Raipur** | 3.169 ±  0.035c | 1.246 ± 0.001c | 3.532 ± 0.032c | 1.083 ± 0.013 a | 0.755 ± 0.005ab | 0.366 ± 0.003a | 0.393 ±  0.007 b |
| **Bargarh** | 3.199 ±  0.033 c | 1.225 ± 0.010 c | 3.553 ± 0.008bc | 1.023 ± 0.016b | 0.747 ± 0.004ab | 0.290 ± 0.004b | 0.456 ± 0.015a |
| **Ludhiana** | 3.461 ±  0.040 a | 1.392 ± 0.030 a | 3.675 ± 0.003 a | 1.033 ± 0.013b | 0.725 ± 0.005 bc | 0.285 ± 0.003b | 0.373 ± 0.008bc |
| **S.E (m)** | 0.031 | 0.015 | 0.028 | 0.013 | 0.018 | 0.009 | 0.007 |
| **C.D** | 0.087 | 0.043 | 0.079 | 0.036 | 0.049 | 0.014 | 0.021 |
| **C.V** | 4.8 | 3.5 | 7.2 | 4.0 | 6.4 | 4.9 | 5.0 |

Values in a column followed by the same letter do not differ significantly by DMRT (*P* = 0.05%)

**Table 3. Morphometrics of brachypterous brown planthopper males collected from different locations**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **BPH**  **populations** | **Body length (mm)** | **Body width (mm)** | **Wing length (mm)** | **Wing width (mm)** | **Head length (mm)** | **Interocular distance (mm)** | **Tibial Spur**  **length (mm)** |
| **Glasshouse** | 2.286 ± 0.025cd | 0.859 ± 0.007b | 1.286 ± 0.007b | 0.673 ± 0.012 b | 0.652 ± 0.006c | 0.255 ± 0.004c | 0.365 ± 0.001d |
| **Kampasagar** | 2.322 ± 0.034 c | 0.880 ± 0.008a | 1.244 ± 0.021 b | 0.616 ± 0.004d | 0.729 ± 0.002 b | 0.273 ± 0.003b | 0.352 ± 0.010d |
| **Gangavathi** | 2.396 ± 0.015 b | 0.852 ± 0.001bc | 0.965 ± 0.003c | 0.636 ± 0.002c | 0.668 ± 0.003c | 0.263 ± 0.001bc | 0.403 ± 0.001c |
| **Raipur** | 2.454 ± 0.031 b | 0.840 ± 0.001 c | 1.250 ± 0.025b | 0.671 ± 0.001 b | 0.777 ± 0.002a | 0.262 ± 0.001bc | 0.424 ± 0.010b |
| **Bargarh** | 2.247 ± 0.022d | 0.880 ± 0.001 a | 0.993 ± 0.020c | 0.663 ± 0.004b | 0.649 ± 0.021c | 0.293 ± 0.004a | 0.448 ± 0.010a |
| **Ludhiana** | 2.504 ± 0.021a | 0.891 ±0.001a | 1.459 ± 0.033 a | 0.773 ± 0.005a | 0.663 ± 0.004c | 0.273 ± 0.002b | 0.361 ±0.001d |
| **S.E (m)** | 0.024 | 0.025 | 0.019 | 0.009 | 0.011 | 0.009 | 0.003 |
| **C.D** | 0.066 | 0.012 | 0.052 | 0.011 | 0.030 | 0.012 | 0.009 |
| **C.V** | 4.7 | 1.5 | 7.2 | 4.0 | 5.2 | 6.4 | 5.0 |

Values in a column followed by the same letter do not differ significantly by DMRT (*P* = 0.05%)

**Table 4. Morphometrics of macropterous brown planthopper males collected from different locations**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **BPH**  **Populations** | **Body length (mm)** | **Body width (mm)** | **Wing length (mm)** | **Wing width (mm)** | **Head length (mm)** | **Interocular distance (mm)** | **Tibial Spur**  **length (mm)** |
| **Glasshouse** | 2.521 ± 0.045bc | 1.011 ± 0.023 b | 3.064 ± 0.068 bc | 1.022 ± 0.004 a | 0.745 ± 0.007b | 0.248 ± 0.001ab | 0.320 ±  0.008a |
| **Kampasagar** | 2.397 ± 0.011d | 1.123 ± 0.041a | 3.026 ±0.043 c | 0.966 ± 0.019bc | 0.685 ± 0.032c | 0.259 ± 0.008a | 0.306 ±  0.006ab |
| **Gangavathi** | 2.481 ± 0.022c | 1.014 ± 0.007 b | 3.077 ± 0.040c | 0.963 ± 0.004 bc | 0.703 ± 0.006 c | 0.241 ± 0.010bc | 0.309 ±  0.010b |
| **Raipur** | 2.697 ± 0.001a | 1.133 ± 0.001 a | 3.149 ± 0.010 ab | 0.871 ± 0.005 d | 0.825 ± 0.017 a | 0.235 ±  0.003 c | 0.304 ±  0.012ab |
| **Bargarh** | 2.551 ± 0.017b | 1.090 ± 0.019 a | 3.112 ± 0.014bc | 0.944 ± 0.005 c | 0.693 ± 0.003 c | 0.220 ±  0.004 d | 0.278 ±  0.012b |
| **Ludhiana** | 2.496 ± 0.023bc | 1.019 ± 0.001 b | 3.254 ± 0.032a | 0.990 ± 0.005 ab | 0.757 ± 0.004b | 0.239 ±  0.001 bc | 0.301 ±  0.015b |
| **S.E (m)** | 0.025 | 0.018 | 0.039 | 0.012 | 0.008 | 0.007 | 0.011 |
| **C.D** | 0.069 | 0.051 | 0.110 | 0.035 | 0.024 | 0.013 | 0.031 |
| **C.V** | 4.3 | 8.0 | 5.7 | 4.2 | 7.2 | 5.7 | 6.2 |

Values in a column followed by the same letter do not differ significantly by DMRT (*P* = 0.05%)

**Table 5. Morphometrics of brown planthoppers nymphs collected from different locations**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **BPH**  **Populations** | **I instar** | | **II instar** | | **III instar** | | **IV instar** | | **V instar** | |
|  | **Length** | **Width** | **Length** | **Width** | **Length** | **Width** | **Length** | **Width** | **Length** | **Width** |
| **Glasshouse** | 0.785 ± 0.016a | 0.326 ± 0.003a | 1.093 ± 0.003b | 0.461 ± 0.005 cd | 1.768 ± 0.121 ab | 0.865 ± 0.022 a | 2.411 ± 0.051 a | 0.977 ± 0.027 ab | 2.781 ± 0.040 c | 1.090 ± 0.020 b |
| **Kampasagar** | 0.767 ± 0.036 a | 0.297 ± 0.004 c | 1.098 ± 0.002 b | 0.541 ± 0.004 b | 1.809 ± 0.065 ab | 0.909 ± 0.025 a | 2.380 ± 0.042 ab | 0.935 ± 0.022 abc | 2.774 ± 0.04 c | 1.085 ± 0.054 b |
| **Gangavathi** | 0.716 ± 0.004b | 0.255 ± 0.004 d | 1.098 ± 0.024 b | 0.578 ± 0.001 a | 1.842 ±0.034 a | 0.879 ±0.026 a | 2.215 ± 0.060 b | 0.905 ± 0.031 bc | 2.867 ± 0.121 bc | 1.063 ± 0.023 bc |
| **Raipur** | 0.766 ± 0.008a | 0.290 ± 0.003 c | 1.071 ± 0.029 b | 0.451 ± 0.009 d | 1.789 ± 0.059 ab | 0.833 ± 0.041 a | 2.447 ± 0.094 a | 0.988 ± 0.036 a | 2.941 ± 0.049 ab | 1.162 ± 0.020 a |
| **Bargarh** | 0.773 ± 0.007 a | 0.310 ± 0.013 b | 1.153 ± 0.024a | 0.454 ± 0.011 cd | 1.980 ± 0.136 a | 0.829 ± 0.044 a | 2.219 ± 0.057 b | 0.902 ± 0.024 c | 2.996 ±0.090 a | 1.122 ± 0.020 ab |
| **Ludhiana** | 0.752 ± 0.017 a | 0.290 ± 0.007c | 1.090 ± 0.002 b | 0.465 ± 0.006 c | 1.595 ± 0.080 b | 0.850 ± 0.025 a | 2.333 ± 0.074 ab | 0.876 ± 0.005 c | 2.620 ± 0.073 d | 1.012 ± 0.039 c |
| **S.E (m)** | 0.013 | 0.008 | 0.013 | 0.008 | 0.084 | 0.030 | 0.065 | 0.026 | 0.038 | 0.021 |
| **C.D** | 0.036 | 0.009 | 0.036 | 0.013 | 0.237 | 0.085 | 0.184 | 0.073 | 0.107 | 0.060 |
| **C.V** | 7.3 | 7.1 | 5.4 | 4.8 | 6.1 | 3.2 | 8.8 | 3.1 | 4.7 | 8.3 |

Values in a column followed by the same letter do not differ significantly by DMRT (*P* = 0.05%)