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Introduction

Doubled haploids are completely homozygous so their progeny are expected to be genetically homogeneous and, except for rare mutations, should show no genetic diversity. Even so, over 50 years ago George Sprague and his associates demonstrated that heritable variation in quantitative traits quickly emerged among the progeny of doubled haploid maize. Sprague demonstrated that the rate of variation was greater than the rate of mutations that resulted in discrete polymorphisms, but he did not have the means to determine the source of that variation. We believe that, with new technologies, the means now exist.

We are in the process of re-establishing heritable polymorphic lines that have descended from a single doubled-haploid plant. A heritable polymorphism for any particular trait is indicated if there is no significant difference between two generations of a lineage but the lineage is significantly different from other lineages.

Materials and Methods



Figure 1: Double haploid kernels from a monoploid plant. A monoploid kernel was generated by crossing B73 with pollen from a haploid inducer line (Stock 6). The monoploid kernel was germinated and the resulting seedling was treated with nitrous oxide (N₂O) by Akio Kato in the laboratory of Jim Birchler and was selfed. One kernel from this ear was used as a source germplasm for this project.

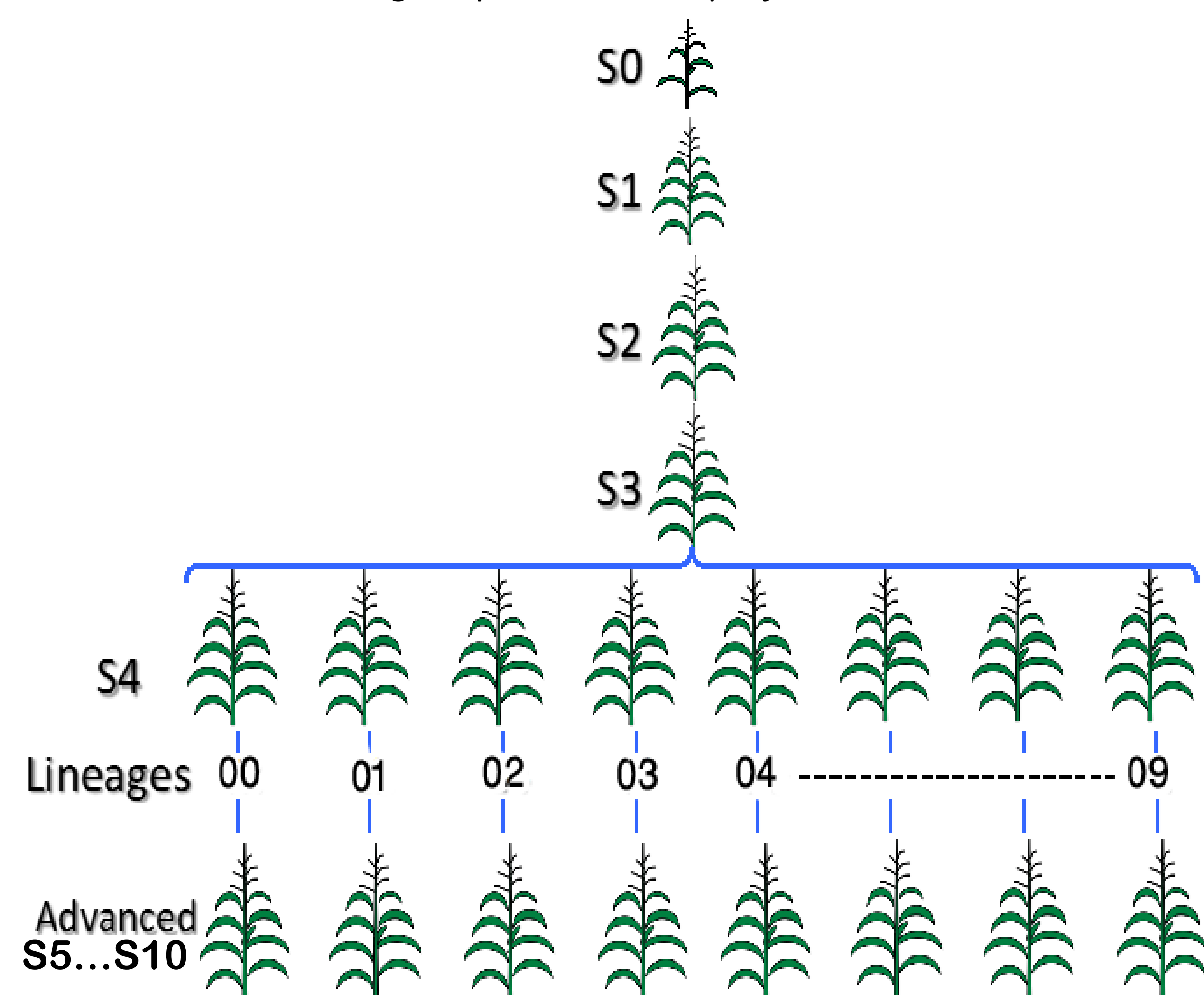


Figure 2: Scheme for generation of double haploid lines that descended from a single double haploid plant.

Materials and Methods

- In the summer of 2014, B73 doubled haploid seeds were planted from ten lineages, each with two or more generations at SDSU.
- These were planted in triplicate in a Randomized Complete Block Design (RCBD).
- The resulting plants were evaluated for 14 quantitative traits: Plant height, number of tassel branches, leaf length, leaf width, number of kernel per ear, etc. The results were analyzed with R Studio version 3.0.1.
- In the summer of 2015, partial replication of the experiment was done using seeds obtained from self progeny in the 2014 generation in order to confirm heritable polymorphism.

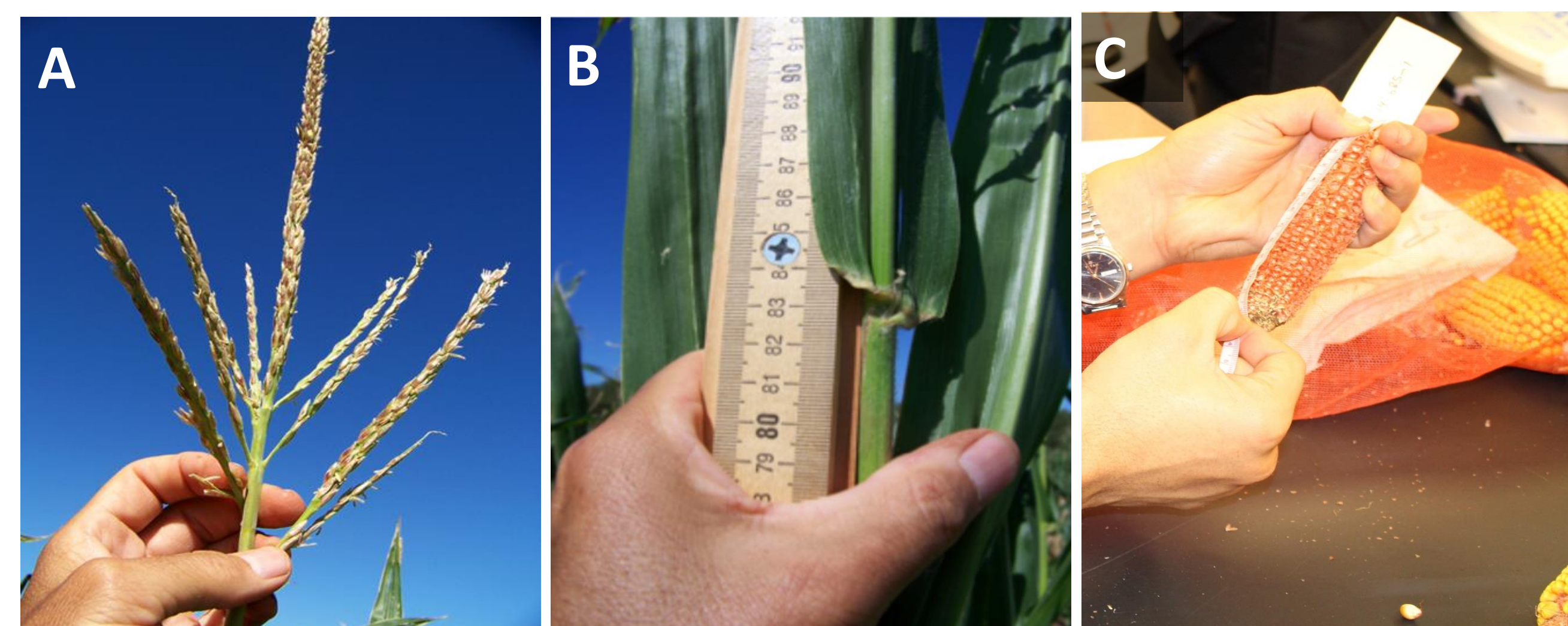


Figure 3: Examples of measured traits. A: Counting tassel branch number; B: Measuring Plant height; C: Measuring Ear length.

Results

Table 1: An analysis of variance (ANOVA) shows that eight out of 14 quantitative traits are significantly different among lineages.

Traits	p-value
No. of tassel branch	0.0004***
Total no. of kernel per ear	0.0071**
Plant height	0.0512
Position of ear	0.0501
Leaflength	0.0401*
No. of nodes	0.0216*
Days to pollen shed	0.0535
Days to silk emergence	0.0246*

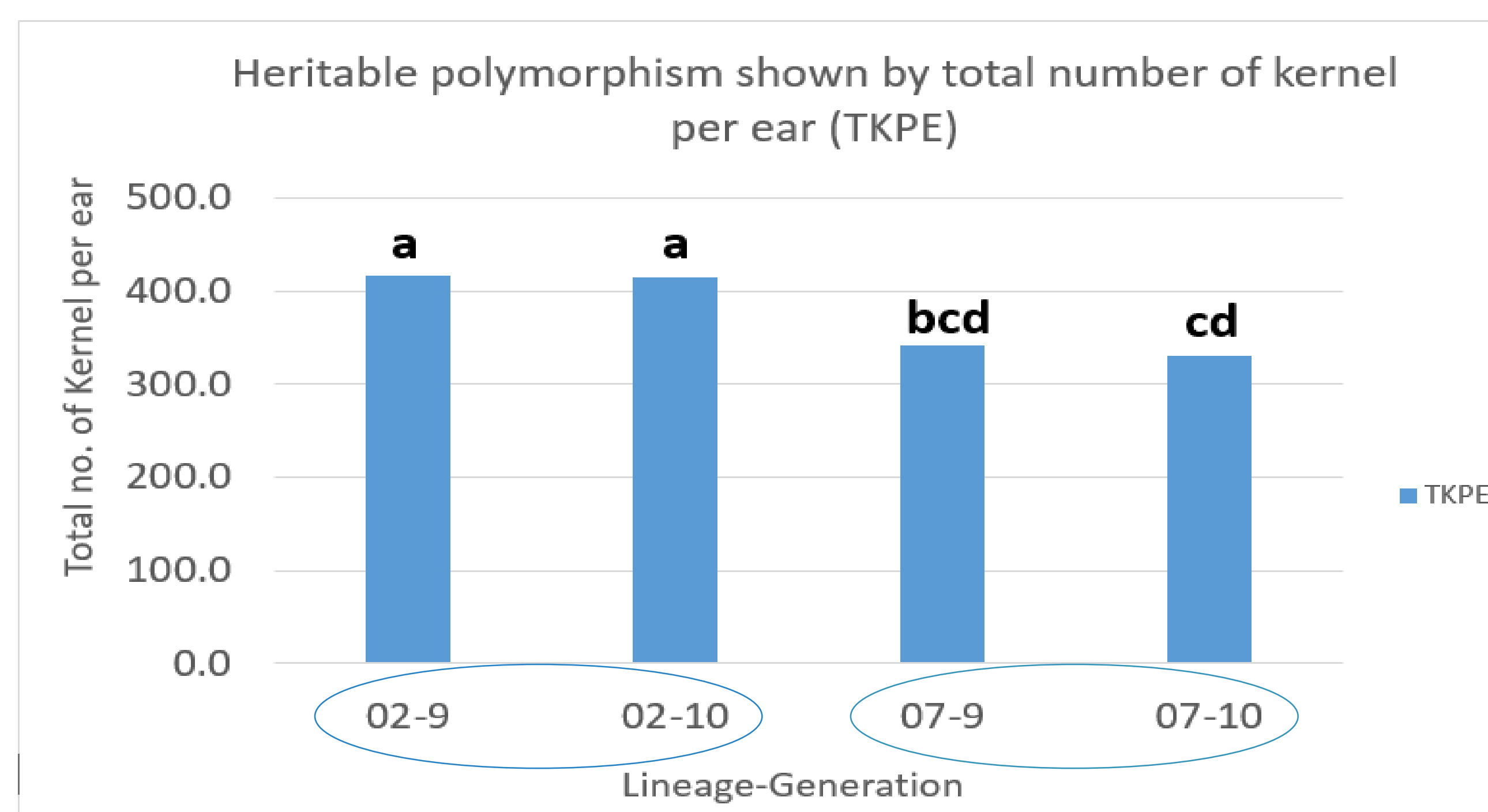


Figure 4: Heritable polymorphism demonstrated by total number of kernel per ear. Shared letters indicate no significant difference whereas different letters indicates significant differences. Data from summer 2014.

Results

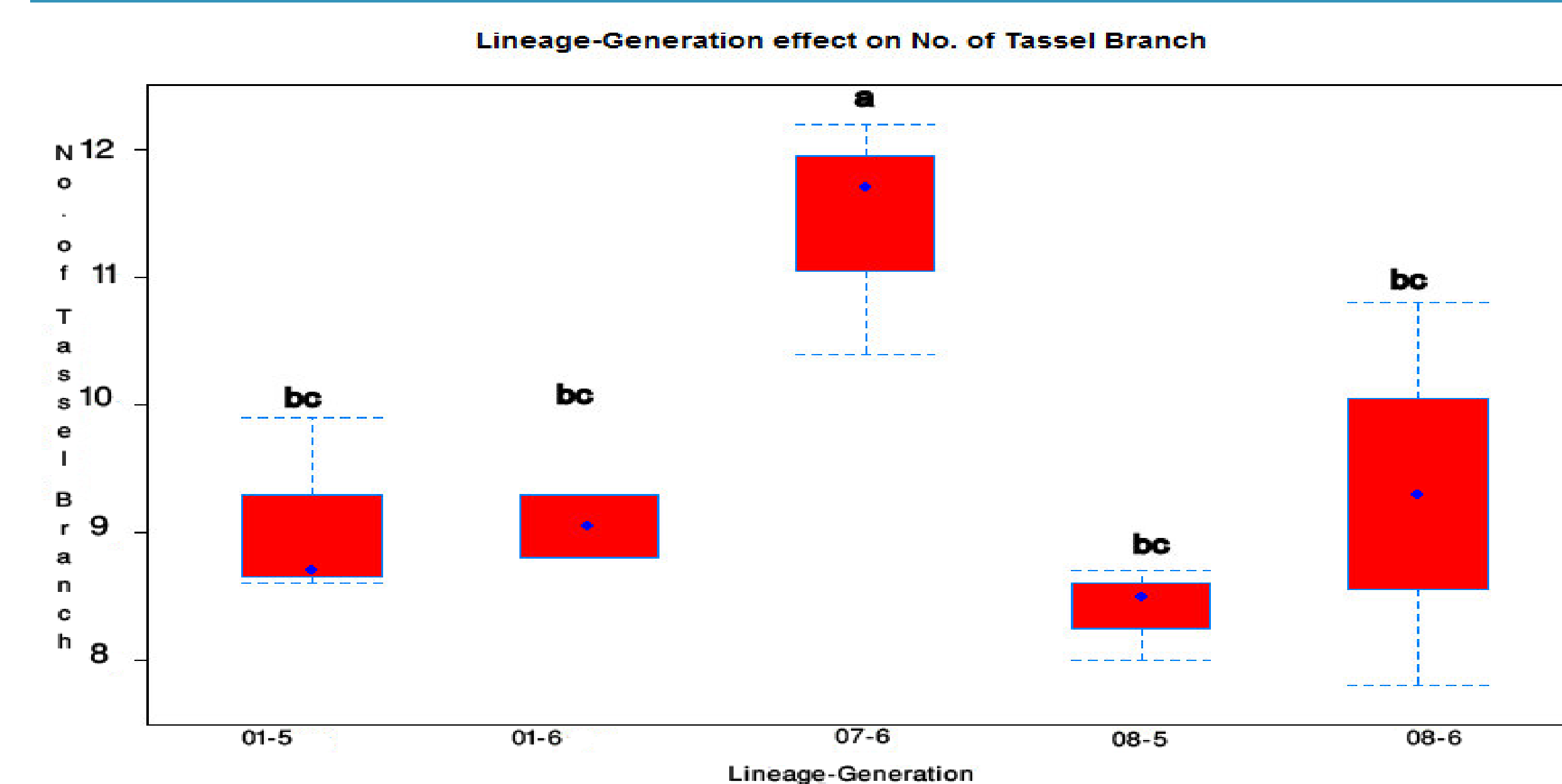


Figure 5: Box and whisker plot showing lineage 07-6 was significantly higher for tassel branch number. Data from summer 2014.

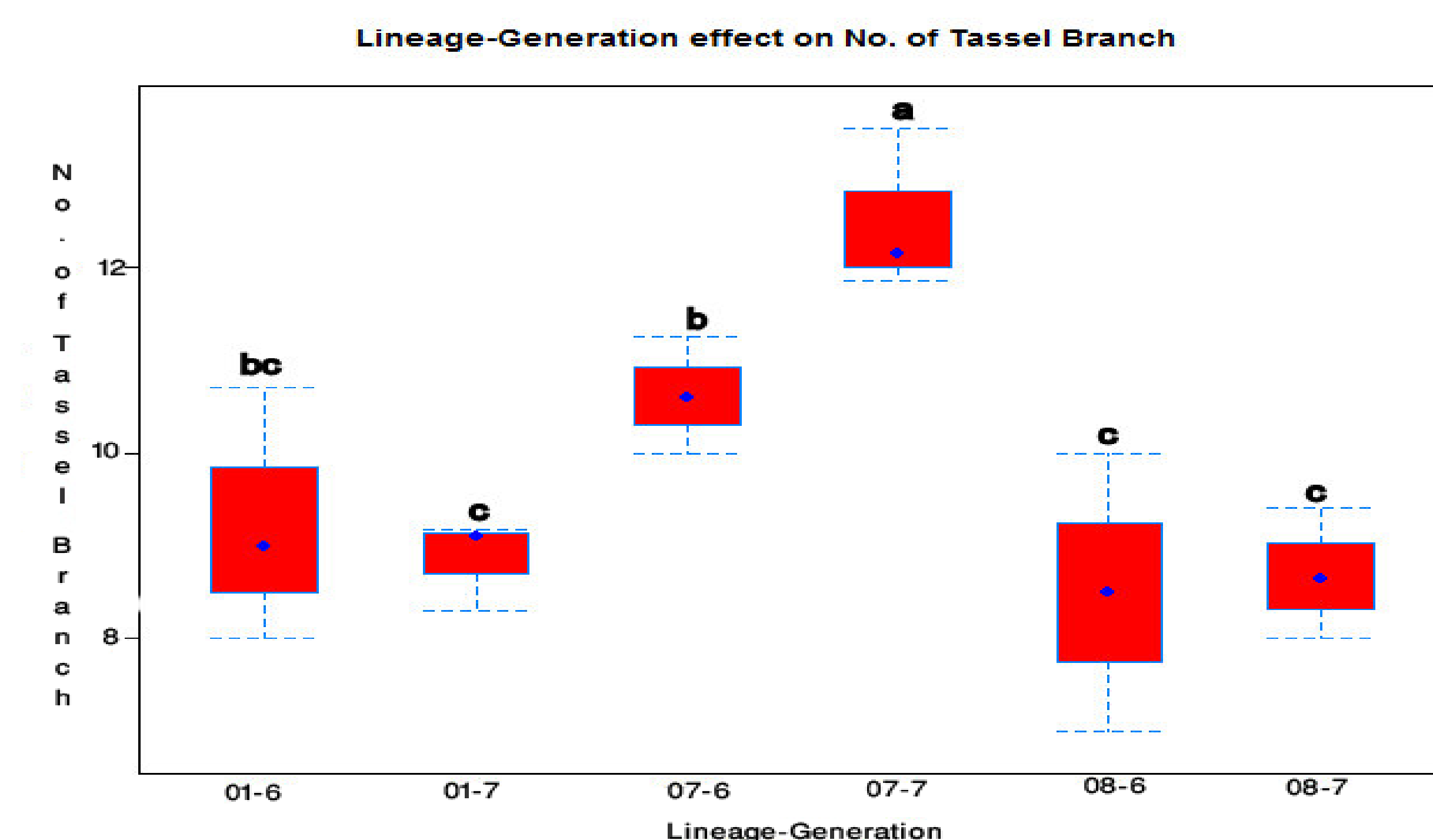


Figure 6: Box and whisker plot shows that 07-7 had significantly higher number of tassel branches in the summer of 2015. These data indicate that this polymorphism is heritable in this lineage.

Conclusion

- Heritable polymorphisms were demonstrated by two traits: number of tassel branches and total number of kernel per year.
- Polymorphisms were also seen among different generations of same lineage (data not shown). This instability of phenotype may implicate epigenetics as a potential source variation.

Future work

- Complete analysis of 2014 and 2015 data is still ongoing.
- Lineages with heritable polymorphisms will be advanced to test stability of phenotype.
- The demonstration of heritable polymorphisms of quantitative traits has encouraged us to pursue molecular analysis.

Acknowledgement



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