DNA Marker of *Tilletia controversa* Kühn, a Causal Agent of Wheat Dwarf Bunt

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Abstract: Dwarf bunt of wheat, caused by *Tilletia controversa* Kühn (TCK), is a destructive disease on wheat, as well as an important international quarantine disease. The traditional methods for diagnosis and detection of the disease were mostly based on the morphological characteristics, germination test of teliospores, and the symptom of disease in the field, resulting in a long procedure and low accuracy. So, it is imperative to develop a molecular assay for rapid identification and accurate detection of TCK. Seventeen physiological races and twelve geographical isolates of *Tilletia controversa* Kühn, the causal agent of dwarf bunt of wheat, and several related species were collected from the United States and China, respectively. Genomic DNA of fungal teliospores (1 mg or so) was extracted after high moisture keeping in mortars for twenty-four hours using a modified cetyltrimethylammonium bromide (CTAB). A total of 92 primer combinations were tested to characterize DNA polymorphism between *Tilletia controversa* Kühn and its related species with amplified fragment length polymorphism (AFLP) technique. Out of those 92 only the primer combination E08/M02 generated a polymorphic pattern displaying a 451bp DNA fragment specific for the *Tilletia controversa* Kühn. This product was present in all of seventeen physiological races and twelve geographical isolates of *Tilletia controversa* Kühn, but was not present in the *Tilletia laevis* Kühn (syn. *Tilletia foetida* (Wallr.) Liro), *Tilletia caries* (DC.) Tul (syn.*Tilletia tritici* (Bjerk.) Wint), *Tilletia indica* Mitra and *Sphacelotheca reiliana* (Kühn) Clint. The polymorphic AFLP product was successfully cloned and sequenced. Based on the sequence of the polymorphic DNA fragment, one pair of specific primer ($SC\text{-}01_{49}$/$SC\text{-}02_{415}$) was designed with the software of Primer Premier 5.0, and a conversion of the AFLP marker to a sequence characterized amplified regions (SCAR) was completed. The results of detection using the designed primers in the fungi tested indicated an unique DNA fragment was amplified in all of the TCK isolates, and no any DNA fragments were obtained in the other alien fungus species. The genomic DNA from the different number of TCK teliospores was amplified using the specific SCAR primer, displaying an accuracy of three teliospores and reliability of 100%. Based on the 62 AFLP fingerprinting of 12 geographical isolates of TCK, clustering analysis was conducted by the SAS software, indicating the molecular polymorphism of the dwarf bunt fungus was closely related to the original areas, but irrelevant to wheat cultivars. By comparison of the virulence spectra and AFLP fingerprinting of 17 TCK races, it was found that DNA polymorphism was not obviously relevant to virulence polymorphism (r = 0.26).

Keywords: dwarf bunt of wheat; *Tilletia controversa* Kühn; AFLP; SCAR marker; genetic diversity