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## Identification of perennial ryegrass varieties by ultrathin-layer isoelectric focusing (UTLIEF) of seed storage protein

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**Abstract** In this work , seed prolamin variation between tested perennial ryegrass varieties was analyzed by ultrathin-layer isoelectric focusing electrophoresis (UTLIEF) for variety identification . The results showed that UTLIEF is a suitable method for *Lolium* variety identification .

**Key words** : perennial ryegrass , variety identification , UTLIEF

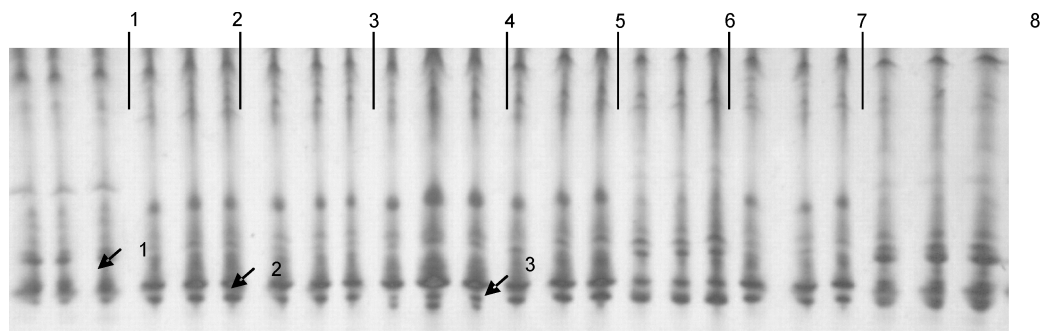
**Introduction** Ultrathin-layer isoelectric focusing is a new method for variety identification and hybrid purity testing , which has lots of advantages including high resolving power , good repetition and rapid identification progress etc . This method has been listed in the International Seed Testing Association rules as a standard method for maize's and sunflower's variety verification , but little work was done for *Lolium* variety identification . The aim of this research is to discuss the potential of UTLIEF for reliable variety identification of perennial ryegrass .

### Materials and methods

**Material** Seed samples of 8 varieties were obtained from various seed company .

**Method** The prolamin was extracted with 8ml glycol from the meal of a bulked seed sample of 200 seeds . UTLIEF gel preparation , the condition of focusing and staining of gel were done according to the method of HAHN (1999) .

**Results and discussion** The results obtained after UTLIEF showed there were genetic differences in seed storage protein composition in perennial ryegrass varieties (Figure 1) . From the 8 perennial ryegrasses we obtained 14 distinctive bands and 7 of these bands were polymorphic . Using these polymorphic bands the tested varieties can be separated . Ultrathin-layer isoelectric focusing(UTLIEF) is a convenient , quick , cheap , and reliable laboratory method and has been included in the international seed testing association rules for variety verification (ISTA ,1999) . The result showed that this method can be used in ryegrass variety identification , and is quicker and cheaper than SDS-PAGE . Further research is needed for standardization of the method of .



**Figure 1** Electrophoregram of seed protein from 8 perennial ryegrass varieties (1 : primier ; 2 : plonecle ; 3 : thunder4 ; 4 : band ; 5 : wintergreen ; 6 : esquire ; 7 : accent ; 8 : toya) .

### References

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