

A15 Identification of *Ldh* Isozyme Locus in Rice (*Oryza sativa* complex)

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Objectives

1. To find out lactate dehydrogenase (*Ldh*, E.C. 1.1.1.27) isozyme locus for genetic characterization and classification of *Oryza sativa* complex.
2. To describe the required conditions for detection of *Ldh* isozyme locus in *Oryza sativa* complex.

Materials and Methods

Plant materials

1. *Oryza sativa*, which consisted of 36 paddy rice strains including indica and japonica; and 19 upland strains collected from worldwide.
2. *Oryza rufipogon*, which consisted of 35 strains collected in Asian countries.
3. *Oryza barthii*, which consisted of 2 strains originated in Africa.

Isozyme detection

Ldh locus was assayed by starch-gel (system H, Tris-citric pH 7.6) methods (Glaszmann et al. 1988; IRRI, 1990). Enzyme extracts were prepared from 1-5 shoots after 2-4 days of germination at 32°C in incubator. The gel staining was performed using the modified procedure described by Wang (1996), in which stain formulation consisted of 50ml 0.05mol pH 8.0 Tris-HCl, 100mg lactic acid (lithium salt), 1ml NAD (1%), 1ml NBT (1%), 1ml MTT (1%), and 0.25ml PMS (1%). The numeral nomenclature systems (Morishima and Glaszmann, 1986) were employed for designating the alleles of *Ldh*.

Results and Discussions

1. Four distinct patterns including a null were observed, suggesting that the locus of *Ldh* has four alleles corresponding to media, slow and fast moving bands, and a null (Figure 1). Thus, four alleles of *Ldh* here were named as *Ldh*¹, *Ldh*², *Ldh*³, and *Ldh*⁰.

2. Only one strain Basmati 385 possessed the allele of *Ldh1*³, and only the strain Lu-tao possessed the allele of null, *Ldh1*⁰.
3. Polymorphisms for three alleles (*Ldh1*¹, *Ldh1*², and *Ldh1*³) were not presented within group of paddy rice but observed in upland rice. Moreover the genetic polymorphism patterns revealed with *Ldh1* were as same as that of alcohol dehydrogenase (*Adh1*, Figure 2) in *Oryza sativa* complex.
4. For detection of *Ldh1*, activity of enzyme extracts was found sensitive to low temperature preservation, it thus should be aware of that to prepare the sample just prior to loading and gel running.

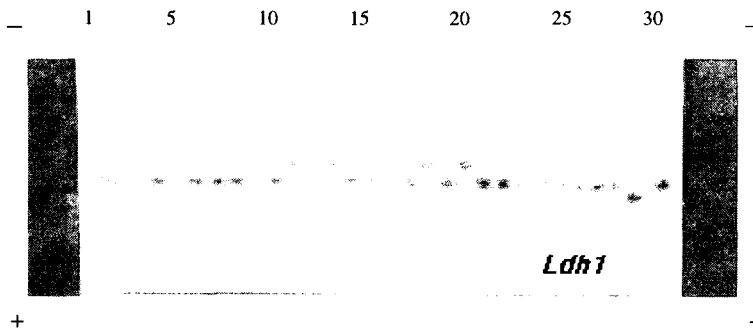


Fig 1. Starch-gel zymogram and interpretation of lactate dehydrogenase (*Ldh1*).

Ldh1 four alleles: *Ldh1*¹, media moving band (Lane 25 etc.)

*Ldh1*², slow moving band (Lane 20 etc.)

*Ldh1*³, fast moving band (Lane 29)

*Ldh1*⁰, null (Lane 3)

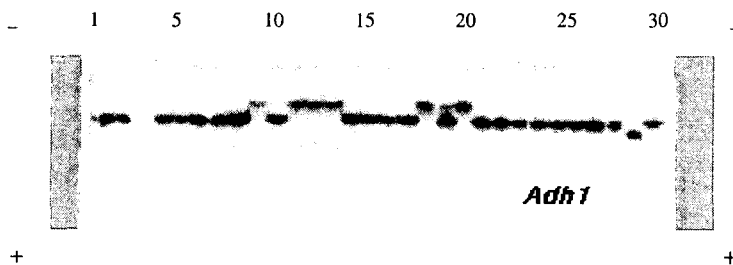


Fig 2. Starch-gel zymogram of alcohol dehydrogenase (*Adh1*).

Adh1 four alleles: *Adh1*¹, media moving band (Lane 25 etc.)

*Adh1*², slow moving band (Lane 20 etc.)

*Adh1*³, fast moving band (Lane 29)

*Adh1*⁰, null (Lane 3)