SHORT COMMUNICATION

Glutamate Dehydrogenase Isozyme Pattern As a Unique Marker for Identifying Cold-Tolerant Wheat Genotypes

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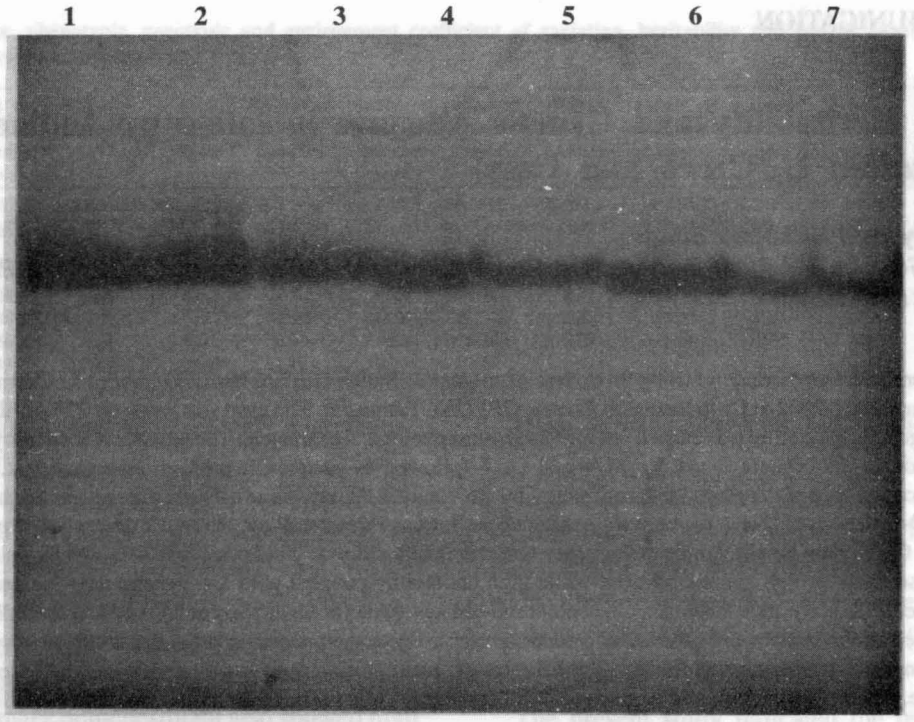
Identification of wheat genotypes that are tolerant to cold by using glutamate dehydrogenase isozyme pattern has been investigated. Glutamate dehydrogenase isozyme analysis of cold-tolerant wheat genotypes (VWFW1350 and HS240) and cold-sensitive wheat genotypes (VL404 and HD 1949) under cold-acclimated and non-acclimated conditions have shown that one unique glutamate dehydrogenase isozyme form was expressed in the cold tolerant genotypes under cold acclimated condition. This particular isozymic form has the potential to serve as a biochemical marker for selecting cold tolerant genotypes.

Key Words: Cold Tolerance, Glutamate Dehydrogenase, Isozyme, Marker, Wheat

The term isozyme refers to the multiple molecular forms of an enzyme which catalyze the same biochemical reaction (Scandalios, 1974). These multiple forms may occur in a single organism or in different members of the same species (Harris and Hopkinson, 1976). Isozymes can vary in size, structure, isoelectric point, amino acid composition, temperature and pH optima (Thorp and Duke, 1992). Isozymes serve as unique biochemical markers in a wide range of plant species. Biochemical markers in the form of isozymes have been correlated with stress adaptation and stress tolerance in different plant systems from time to time (Gangopadhyay and Basu, 2000). However, the study of isozyme patterns in identification of cold tolerance and cold-induced changes in isozyme profile are relatively few. We have established a way of identifying cold-tolerant wheat genotypes using the glutamate dehydrogenase isozyme pattern.

Wheat (*Triticum aestivum* L.) seedlings of four different genotypes that differ in cold tolerance were used as experimental material. Of the four genotypes, two genotypes (VWFW 1350, HS 240) were cold-tolerant and two other genotypes (VL 404, HD 1949) were cold-sensitive. All the four genotypes were obtained from Vivekananda Parvatiya Krishi Anushandhan Shala, Almora, India. The seedlings were grown in growth chamber with 22°C/18°C day/night temperature and with 12h photoperiod. Seven-day-old seedlings were cold acclimated for a period of 15 days with 4°C/2°C day/night temperature while another set of seedlings were grown at normal temperature. Cold-acclimated and non-acclimated seedlings were used for glutamate dehydrogenase (GDH) isozyme profiling. For glutamate dehydrogenase isozyme analysis the enzyme was extracted with 25 mM Tris buffer (pH 7.5), having 2.5 mM cysteine hydrochloride, 4.0 mM $MgSO_4$. $7H_2O$ and 0.25 mM EDTA in ice cold environment, and the enzyme extract was taken for acrylamide gel electrophoresis (PAGE) electrophoresis under non-denaturing condition. After electrophoresis, the gels were incubated in mixture containing 20 mg $NADP^+$, 30 mg nitroblue tetrazolium, 2 mg phenazine methosulfate, 25 ml of 0.5 M phosphate buffer (pH 8.0), 5 ml of 1 M sodium glutamate (pH 7.0) and 70 ml of water for 2h, and the bands were visualized.

Comparing the GDH isozyme bands of all the genotypes under both cold-acclimated and non-acclimated conditions reveal the presence of clear and distinct pattern associated with cold-acclimated tolerant genotypes. The tolerant genotypes VWFW-1350 and HS-240 under cold-acclimated conditions (Fig. 1 lane no. 1 and 2) produce a distinct GDH isozyme band which is not present in the cold-acclimated sensitive genotypes, and all non-acclimated genotypes. The density of the bottom most bands also differed between cold-tolerant and cold-sensitive genotypes and also between non-acclimated and cold-acclimated conditions. Among the cold-acclimated genotypes, the tolerant types had thicker GDH isozyme band than the sensitive genotypes. All the cold-acclimated genotypes had thicker band when compared to their non-acclimated condition. Among the non-acclimated genotypes, the



Lane No.1 Cold-acclimated tolerant genotype (VWFW1350)
Lane No.2 Cold-acclimated tolerant genotype (HS240)
Lane No.3 Cold-acclimated sensitive genotype (VL404)
Lane No.4 Cold-acclimated tolerant genotype (HD1949)

Lane No.5 Non-acclimated tolerant genotype (VWFW1350)
Lane No.6 Non-acclimated tolerant genotype (HS240)
Lane No.7 Non-acclimated sensitive genotype (VL404)
Lane No.8 Non-acclimated sensitive genotype (HD1949)

Fig. 1. Glutamate dehydrogenase isozyme pattern of cold tolerant and sensitive wheat genotypes under cold-acclimated and non-acclimated conditions.

tolerant genotypes had thicker GDH isozyme band when compared to the sensitive genotypes.

GDH isozyme analysis for cold-acclimated and non-acclimated tolerant and sensitive genotypes have shown the presence of a distinct GDH isozyme form in the cold-acclimated tolerant wheat genotypes. This unique isozyme band was not present in the non-acclimated tolerant genotypes and also in acclimated and non-acclimated sensitive genotypes. Also, the intensity of GDH bands that are common to cold-tolerant and sensitive genotypes was higher in the cold-acclimated genotypes. This increase correlated well with that of the GDH activity. The presence of unique isozyme band only in the cold-acclimated tolerant genotypes has the potential of using GDH isozyme pattern for the identification of cold-tolerant genotypes.

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