# Isozyme and Protein Analysis of Induced Rust Resistant Wheat Mutants

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*SUMMARY: Isozymes and protein banding patterns of eighteen students of wheat (Triticum aestivum L.) resistant to rust disease caused by Puccinia recondita Rob. ex Desm were investigated. The results showed that the mutants were different in the isozymes GOT, LAP, EST and ACP as well as their proteins. The differences included the number of bands, $R_f$ value and intensity on the gel. Such results suggested a high degree of purity and genetic stability of the test mutants.*

*Key Words: Triticum aestivum L., puccinia recondita rob. ex desm, GOT, LAP, EST, ACP.*

## INTRODUCTION

Production of mutants by ionizing radiation (i.e. induced mutation) became a common practice in many laboratories all over the world. Useful wheat (*Triticum aestivum* L. cv. Sabeer Bag) mutants that are resistant to rust disease caused by puccinia recondita Rob. ex Desm have been generated in our institute, following irradiation with gamma rays or fast neutrons (4). Morphological differences were observed among these mutants. However, no genetical analysis has been conducted to determine their genetic purity and stability. Therefore this study was conducted to determine the differences among the plants in the same mutant based on their protein and isozyme banding pattern using polyacrylamide gel electrophoresis technique.

## MATERIALS AND METHODS

Leaf samples were collected from 4 weeks old seedlings of wheat (*Triticum aestivum L.*) mutants grown in the greenhouse. The samples were individually powdered in liquid nitrogen and their contents of isozymes extracted in a mixture consisting of 0.15 M Tris, 0.001 M EDTA, 0.3 M PEG, 0.18 M sodium thioglycolate, 0.01 M dithioerythritol, 0.05 mM phenylmethylsulponyl fluoride and 12% glycerol (pH=7.9). Following centrifugation (18x1000 rpm at 4°C for 15 min.), the supernatants were loaded into a pre-formed wells in stacking gel (4%) at a rate of 100 μl/per well.

Electrophoresis was allowed to proceed for 4-5 h through 12% polyacrylamide gel at 4°C at a rate of 3 mA per well. The gels were then stained for glutamate oxaloacetate transaminase (GOT), leucine amino peptidase (LAP), esterase (EST) and Acid phosphatase (ACP) according to Al-Jibouri (1).

Protein was extracted from mature seeds. Method of extraction, electrophoresis and staining were performed according to Sekhar and Demason (3).

## RESULTS AND CONCLUSIONS

The results showed that there were differences between the mutants regarding the number of forms (i.e. bands) of each enzyme system under investigation, as well as their location on the gel ($R_f$ value) and intensity. Similar observation may also be stated for protein analysis. The GOT system appeared in 5 molecular forms in all test mutants and had the $R_f$ value (Table 1, Figure 1A) suggesting a possible stability of this enzyme among the mutants and within the same mutant. Other enzyme system showed a slight difference in the number of molecular forms. For example the LAP was detected in 2-3 forms, EST between 9-14 and ACP between 4-6 forms (Table 1, Figures 1B, 1C and 1D). Protein banding pattern was also different among the mutants and the number of bands ranged from 13 bands in IRATOM-2 mutant to 20 bands in SB-77 for example (Table 1).

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Table 1: Number and purity of GOT, LAP, EST, ACP isozymes and protein bands for 18 wheat mutants.

| Mutant varieties | No. analysed plants | GOT | LAP | EST | ACP | Protein | No. variant plants | % purity |
|---|---|---|---|---|---|---|---|---|
| Sabeer Bag*** | 10 | 5 | 2 | 10 | 6 | 16 | - | 100 |
| SB-337*** | 10 | 5 | 3 | 10, 11 | 5 | 15 | 1 | 90 |
| SB-356** | 10 | 5 | 3 | 11 | 5 | 17 | - | 100 |
| SB-335** | 10 | 5 | 3 | 12 | 5 | 17, 18 | 1 | 90 |
| SB-314** | 10 | 5 | 3 | 13 | 4 | 17, 18 | 1 | 90 |
| SB-131** | 10 | 5 | 3 | 11, 13 | 5, 6 | 20 | 2 | 80 |
| SB-126** | 10 | 5 | 3 | 12 | 5 | 18 | - | 100 |
| SB-274** | 10 | 5 | 3 | 11, 13 | 5 | 15, 16 | 2 | 80 |
| SB-77** | 10 | 5 | 3 | 13 | 6 | 20 | - | 100 |
| SB-191** | 10 | 5 | 3 | 13 | 5 | 19 | - | 100 |
| SB-333** | 10 | 5 | 3 | 10, 12 | 5 | 18 | 1 | 90 |
| SB-632** | 10 | 5 | 3 | 11 | 4 | 16 | - | 100 |
| SB-1464** | 10 | 5 | 3 | 10, 11 | 5 | 15 | 1 | 90 |
| SB-1466** | 10 | 5 | 2 | 9, 10 | 5, 6 | 14 | 2 | 80 |
| IRATOM-1** | 10 | 5 | 2 | 13 | 5 | 13 | - | 100 |
| IRATOM-2** | 10 | 5 | 3 | 14 | 5 | 20 | - | 100 |
| SB-2255* | 10 | 5 | 3 | 10 | 4 | 16 | - | 100 |
| SB-3051* | 10 | 5 | 2 | 10 | 5 | 16 | - | 100 |

* : Highly resistant, ** : Moderately resistant, *** : Susceptible

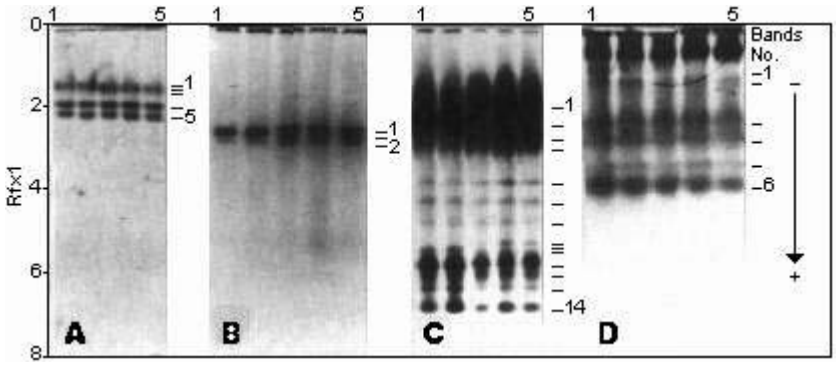


Figure 1: Isozyme banding patterns of GOT (A), LAP (B), EST (C) and ACP (D) of wheat mutants. Numbers 1 trough 5 represent the same mutants in each system.

Variations observed within the individual mutant (i.e. % purity) are shown in Table 1. A high percent of genetic purity was observed in most of the mutants, where it reached 100%. However less purity was associated with some mutants, but never reduced below 80% (Table 1). Furthermore, no identical mutants were observed among the 18 test mutants. Some mutants were identical in some enzyme system, but varied in protein for example. This suggests that these mutants are definitely different genotypes, since the enzymes are gene product (5).

Based on these information regarding genetic stability and high purity, one may conclude that these mutants can be registered as new cultivars. In addition, the usefulness of isozymes as genetic markers, and protein and isozymes analysis by polyacrylamide gel electrophoresis in classification and genetic purity determination is thus justified (2).

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