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# Induced resistance for yellow rust and leaf blight in barley (*Hordeum vulgare* L.) using chemical mutagen

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## Abstract

**Seeds of two commercial malt barley cultivars, VJM201 and DWR28, were treated with different concentrations of the chemical mutagen, 1, 3-buta diene diopoxide (DEB) to induce mutations for disease resistance. The number of chlorophyll/ morphological mutants and mutation frequency, calculated $1000^{-1}$ $M_2$ plants, was higher in DWR28 compared to VJM201 in $M_2$. Many mutants were isolated with higher degree of resistance to yellow rust, brown rust and leaf blight, whereas some others showed higher degree of susceptibility to these diseases compared to the parent cultivars. Besides the mutants for disease resistance, some morphological mutants were also discovered especially for the quantitative traits like tiller number, spike length, etc. Plants having multiple mutant traits were also observed in both parent cultivars. Practical utility of desirable mutants having resistance to leaf rust, stripe rust and leaf blight has been discussed.**

**Keywords:** Barley, *Hordeum vulgare,* Mutation, Chemical mutagen

## Introduction

The use of physical mutagens, like X-rays, gamma rays and chemical mutagens etc for inducing useful mutants has been used in many crops such as wheat, rice, barley, etc. variation, is well established. Mutations have primarily been used for the improvement of characteristics of well-adapted existing cultivars mostly to serve as new cultivars. It is relatively a quicker method for improvement of crops and help to change many agronomical important traits such resistance to abiotic and biotic stresses..A number of cultivars have been developed in barley (*Hordeum vulgare* L.) using mutation breeding directly or by involving mutants as parents (Ahloowalia et al. 2004). Several morphological and other mutants of economic importance and of theoretical interests have been isolated in barley by various researchers and a system of categorization and symbols for various mutants had been proposed (Gustafsson *et al.* 1969). Among the chemical mutagens, several chemicals have been tried with varying success in different crop plants including barley. DEB (1, 3-Buta diene diopoxide), a chemical mutagen, was found to be very effective for inducing desirable mutants in rice (Wu et al. 2005) but has not probably been used in barley. DEB is known to cause small deletions and point mutations (Recio *et al*. 2001). Barley is an ideal crop for inducing mutation for various traits of economic importance such as plant height, heading date, maturity, disease resistance, and components of yield and malt quality. Leaf rusts, both yellow rust and brown rust as well as leaf blight are the major diseases of barley which effect yield and well as the grain quality. Induced mutations are advantageous for disease resistance particularly for specific end use cultivars such as malt purpose barley. Keeping in view the above facts, the present investigation was planned to induce mutations for resistance to major diseases like yellow rust (*Puccinia striiformis* f.sp. *hordei*), brown rust (*Puccinia hordei*) and leaf spot/blight (*Bipolaris sorokiniana*) in two well-adapted two-rowed malt barley cultivars using chemical mutagen, DEB.

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## Materials and Methods

### *Plant material*

The plant material for the present study comprised two commercial malt barley cultivars, VJM201 (1-89-666/ Clipper//Natasha) and DWR28 (BCU 73/PL 172). VJM201 is a two-rowed, medium tall cultivar having surface wax coating on spike and leaf sheath/stem. DWR28 is a two-rowed, medium tall cultivar, having surface wax coating on leaf sheath and stem but the spikes are waxless. The cultivar VJM201 is released for commercial cultivation for Punjab state, whereas DWR28 released for the North-Western plains of India.

### *Methods*

Seeds of these two commercial malt barley cultivars were treated with three concentrations of the chemical mutagen, (DEB) i.e, 0.1000mM (T1), 1500 mM (T2) and 0.2000 mM (T3). For each treatment, 600 dry healthy seeds of each cultivar were soaked overnight in distilled water and then treated with the chemical mutagen, DEB, for 4 hrs in a shaker at room temperature with gentle shaking followed by 7 hrs washing in running tap water, kept overnight and then sown next morning. For the preparation of the liquid solution of DEB, 22 µl of DEB were added to 198 µl of water to make 220 µl stock-solutions. From the stock-solution, 23.32 µl, 34.80 µl and 46.30 µl quantities were added separately in 300 ml of water to make finally three concentrations of the mutagen, i.e, 0.1000 mM ($T_1$), 0.1500 mM ($T_2$) and 0.2000 mM ($T_3$). The seed lot for mutagenesis was taken from the single plant progenies raised to produce nucleus seed of both the parent cultivars. One set of seeds of each cultivar from the same seed lot was kept as control (without treatment) and soaked in water for the same time period. The treated seeds together with the control were planted in a well-prepared field. The mutants in M3, M4 and M5 generations were screened for their reaction to yellow and brown rusts under

artificial epiphytotic field conditions. The parent cultivars were planted for comparison. The resistant $M_3$ progenies were harvested in bulk and were kept for evaluation in $M_4$ and $M_5$ generations during next seasons for disease reaction. The mutants in $M_3$, $M_4$ and $M_5$ generations were screened for their reaction to yellow and brown rusts under artificial epiphytotic field conditions. For this, a mixture of prevalent races of yellow rust and brown rust was sprayed to create epiphytotic conditions. The disease reaction was recorded as per modified Cobb's scale (Peterson *et al.* 1948). The experimental location is a hot-spot for screening against leaf spot/blight which appeared in epiphytotic conditions during all the 3 years of evaluation. The reaction to leaf spot/blight was recorded in double digit score (Nagarajan, 1998).

## Results and Discussion

Some mutants were discovered with higher degree of resistance, whereas some others were recovered with higher degree of susceptibility to yellow and brown rusts and leaf blight diseases. The parent cultivar VJM201 was susceptible to yellow rust (30S) and highly susceptible to brown rust (80S). Two mutants, VM1-10 and VM1-11, were found completely free from yellow rust, whereas four mutants each showed 5S and 10S reaction (Table 1). With regard to brown rust, one mutant was found completely resistant, while nine other mutants recorded 40S reaction. Fourteen mutants recorded 12 score on a double digit scale to leaf blight and five mutants recorded 24 score compared to 47 score of parent cultivar, VJM201. Three mutants were found highly susceptible to leaf blight with a score of 78-99 (Table 1).

**Table 1.** Reaction of some barley mutants to yellow rust, brown rust and leaf blight diseases (Highest disease score recorded during three years- $M_3$ to $M_5$)

| S. No. | Mutant Identity | Yellow rust | Brown rust | Leaf blight |
|---|---|---|---|---|
| 1 | VM1-1 | 5S | 50S | 88 |
| 2 | VM1-2 | 30S | 60S | 35 |
| 3 | VM1-4 | 40S | 40S | 12 |
| 4 | VM1-5 | 20S | 40S | 35 |
| 5 | VM1-7 | 10S | 40S | 24 |
| 6 | VM1-8 | 20S | F | 99 |
| 7 | VM1-10 | F | 60S | 24 |
| 8 | VM1-11 | F | 50S | 12 |
| 9 | VM1-12 | 30S | 50S | 12 |
| 10 | VM1-13 | 5S | 50S | 57 |
| 11 | VM1-14 | 40S | 40S | 58 |
| 12 | VM1-15 | 20S | 60S | 12 |
| 13 | VM2-2 | 20S | 40S | 35 |
| 14 | VM2-3 | 30S | 40S | 12 |
| 15 | VM2-4 | 10S | 60S | 34 |
| 16 | VM2-6 | 5S | 40S | 46 |
| 17 | VM2-10 | 5S | 40S | 35 |
| 18 | VM2-12 | 40S | 40S | 78 |
| 19 | VM2-15 | 20S | 40S | 12 |
| 20 | VM2-16 | 10S | 60S | 24 |
| 21 | VM2-17 | 20S | 60S | 12 |
| 22 | VM2-18 | 20S | 60S | 12 |
| 23 | VM2-19 | 20S | 80S | 12 |
| 24 | VM2-21 | 20S | 80S | 12 |
| 25 | VM2-22 | 60S | 80S | 12 |
| 26 | VM2-24 | 80S | 80S | 12 |
| 27 | VM2-25 | 30S | 80S | 46 |
| 28 | VM2-27 | 30S | 60S | 12 |
| 29 | VM2-29 | 10S | 80S | 12 |
| 30 | VM2-37 | 20S | 60S | 24 |
| 31 | VM2-38 | 20S | 60S | 24 |
|  | VJM201 © | 30S | 80S | 47 |

| S. No. | Mutant Identity | Yellow rust | Brown rust | Leaf blight |
|---|---|---|---|---|
| 1 | DM1-3 | 20S | 40S | 57 |
| 2 | DM1-4 | 40S | F | 46 |
| 3 | DM1-5 | 5S | F | 24 |
| 4 | DM1-6 | 5S | F | 24 |
| 5 | DM1-9 | F | F | 68 |
| 6 | DM1-10 | 10S | 80S | 57 |
| 7 | DM1-11 | 10S | F | 77 |
| 8 | DM1-12 | 10S | 40S | 46 |
| 9 | DM1-13 | 10S | 80S | 46 |
| 10 | DM1-14 | F | F | 88 |
| 11 | DM1-15 | 30S | F | 78 |
| 12 | DM1-16 | F | 40S | 67 |
| 13 | DM1-17 | F | F | 78 |
| 14 | DM1-19 | 100S | 60S | 57 |
| 15 | DM1-23 | 10S | 60S | 34 |
| 16 | DM1-24 | 10S | F | 78 |
| 17 | DM1-25 | 20S | F | 34 |
| 18 | DM1-26 | 10S | F | 99 |
| 19 | DM1-27 | F | 40S | 78 |
| 20 | DM1-28 | F | 40S | 46 |
| 21 | DM2-1 | F | F | 99 |
| 22 | DM2-2 | F | F | 99 |
| 23 | DM2-3 | F | F | 78 |
| 24 | DM2-4 | F | 30S | 57 |
| 25 | DM2-5 | F | 10S | 34 |
| 26 | DM2-6 | 20S | 5S | 12 |
| 27 | DM2-7 | 10S | 40S | 12 |
| 28 | DM2-9 | 20S | 10S | 35 |
| 29 | DM2-10 | F | 30S | 67 |
| 30 | DM3-1 | 40S | 80S | 57 |
| 31 | DM3-2 | F | F | 12 |
|  | DWR28 (C) | 30S | 30S | 57 |

The parent cultivar DWR28 recorded a reaction of 30S to both rusts. As many as thirteen mutants were completely resistant to yellow rust, whereas two mutants showed 5S, eight showed 10S and four showed 20S reaction to yellow rust. A highly susceptible mutant, DM1-19, with 100S reaction to yellow rust was also observed (Table 1). With respect to brown rust, fifteen mutants were resistant, while one mutant showed 5S and two showed 10S reaction. Five mutants, DM1-10 (80S), DM1-13 (80S), DM1-19 (60S), DM1-23 (60S) and DM3-1 (80S), registered a highly susceptible score to brown rust. Against leaf blight, three mutants recorded a score of 12, two recorded 24, three showed 34 and one had 35 score compared to 57 score of parent cultivar, DWR28. On the other hand, thirteen mutants were found susceptible to highly susceptible to leaf blight with a score of 67-99 (Table 1). These results indicate that occurrence of mutations was in both directions, positive as well as negative. Some of the mutants showed multiple mutant traits. For example, the mutants VM1-1 and VM1-8 had six rowed spikes, broad leaves, early heading, high protein content, and decreased plant height, reduced tillers and spike length and susceptibility to leaf blight. The mutant VM1-11 had early heading, decreased plant height, tillers and a higher degree of resistance to yellow rust and leaf blight. The mutant DM1-5 had increased plant height, tillers, spike length, kernels per spike, 1000-grain weight and grain yield and a higher degree of resistance to yellow rust, brown rust and leaf blight. DM1-8 had higher grain yield, while DM1-9 possessed higher degree of resistance to yellow rust and brown rust.

The mutant DM2-1 can be defined as semi-brachytic as it had shorter plant height, delayed heading, initial prostrate growth pattern, tip sterility and six-rowed spikes and was characterized by resistant to yellow and brown rusts but highly susceptible to leaf blight. The mutant DM2-3 had six-rowed spikes, early heading, and lower 1000-grain weight. and was resistant to yellow and brown rusts but highly susceptible to leaf blight. The mutant DM2-5 had six-rowed spikes, lower plant height, and 1000-grain weight and a higher degree of resistance to yellow and brown rusts. The mutant DM3-1 had six-rowed spikes, a lower number of tillers, shorter spike length and lower 1000-grain weight. It had a higher degree of susceptibility to leaf blight, yellow rust and brown rust.. The results indicate that high degree of resistance can be successfully induced using chemical mutagen, though this kind of forward mutation is very rare. The mutants having high degree of resistance to yellow and brown rusts are a very good source of resistance in agronomically elite background to be used in hybridization to incorporate resistance in elite barley lines. These desirable mutants are being used in the breeding programme for the improvement of malt barley cultivars.

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