

# The HLP mutation confers enhanced resistance to leaf-rust in different wheat genetic backgrounds

—Lesion-mimic mutation confers resistance in wheat

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## ABSTRACT

In several plant species, lesion-mimic mutants simulate the disease-resistance response in the absence of pathogens. Interestingly, some of these mutants confer broad-spectrum resistance to diverse pathogens. We previously demonstrated that the HLP (hypersensitive-like phenotype) mutant of bread wheat (*Triticum aestivum* L.) exhibited spontaneous hypersensitive response (HR) in the absence of any pathogen input. However, when HLP plants showing HR phenotype were challenged with leaf-rust (*Puccinia triticina*) they were more resistant than plants of the mother-line of comparable developmental stage that did not show spontaneous HR, suggesting that the HLP mutation may confer enhanced resistance to the fungus. In this paper we validate the aforementioned finding in several wheat genetic backgrounds. Two-way crosses were performed among the HLP mutant and eight wheat commercial stocks, and third backcross progenies with and without spontaneous HR were challenged with leaf-rust to investigate the response to the fungus. Backcrosses to cv. Sinvalocho M.A., the mother-line, and cv. Purplestraw, highly susceptible to leaf-rust attack, were used as controls. Third backcross progenies of cvs. Sinvalocho M.A., Purplestraw, Buck Guaraní and Pro INTA Imperial bearing spontaneous HR phenotype were more resistant to the fungal pathogen than third backcross progenies that did not carry the HLP mutation. Other four wheat stocks were as healthy as the HLP mutant. As expected, backcross to the mother-line demonstrated that the HLP mutation conferred an additional resistance to the already healthy performance displayed by

the mother-line at adult plant stage. The introgression of the HLP mutation conferred heightened leaf-rust resistance and caused no kernel weight reduction on the backcrossed progenies. Taken together, these data validate the direct use of this type of mutations in disease-resistance breeding.

**Keywords:** Hypersensitive Response; Lesion-Mimic Mutant; Puccinia triticina; Wheat Commercial Stocks

## 1. INTRODUCTION

Plants have evolved several defense mechanisms to overcome pathogen attack. One of the most common and effective defense responses in plants is the hypersensitive response (HR), which results in localized cell death at the site of pathogen infection [1]. Mutants have been identified in several plant species that spontaneously form localized areas of dead tissue resembling those seen in the HR. In these class of mutants, hereafter referred to as lesion-mimic mutants, disease symptoms or HR cell death occur in the absence of pathogens.

Several lesion-mimic mutants have been identified and characterized in Arabidopsis: *acd* [2-4]; *dll* [5]; *hlm* [6]; *len* [7]; *lsd* [8]; *ssi* [9,10]. The *LSD1* gene coding for a novel zinc finger protein functions as a negative regulator of plant cell death. Interestingly, the *lsd1* mutants were resistant to the bacterial pathogen *Pseudomonas syringae* and the oomycete *Peronospora parasitica* [11]. The *HLM1* gene encodes a cyclic nucleotide-gated channel that is permeable to  $K^+$  and  $Na^+$  ions and is activated by cGMP and cAMP. The *hlm1* mutants also exhibited increased resistance to a virulent strain of *Pseudomonas syringae* pv *tomato* [6].

An underlying agronomic feature of some gramina-

ceous lesion-mimic mutants is that they confer broad-spectrum resistance to different pathogens. Mutations of the barley *Mlo* gene conferred expression of a lesion-mimic phenotype which provided resistance to all known races of the fungus responsible for powdery mildew (*Blumeria graminis* f.sp. *hordei*). However, barley seedlings carrying the *mlo* mutation showed enhanced susceptibility to the rice blast fungus *Magnaporthe grisea* [12], and resistance genes ml-o5, ml-o6, and ml-10 were responsible for grain yield reduction in chromosome-doubled haploid lines of barley tested in a disease-free field trial [13]. In rice, some lesion-mimic mutants displayed enhanced resistance to pathogens: *cdr1*, *cdr2* and *cdr3* exhibited heightened resistance to *M. grisea* [14], *spl4*, *spl5-1*, *spl5-2*, *spl7*, *spl10*, *spl11*, *spl12*, *spl13*, *spl14* and *Spl15* conferred non-race-specific resistance to rice blast and bacterial blight [15,16] and *ebr3* showed increased resistance to *M. grisea* and the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* [17]. Co-segregation analyses of blast and bacterial blight resistance and lesion-mimic phenotypes in segregating populations of *spl17* and *Spl26* demonstrated that enhanced resistance to the two diseases was conferred by mutations in the lesion-mimic genes [18]. The wheat mutant M66 showed enhanced resistance to powdery mildew and yellow and brown rusts although its yield was 50% that of the control, (cv. Guardian) [19-21]. Conversely, the HLP mutant of wheat that exhibited spontaneous HR lesions showed also heightened resistance to leaf-rust (*Puccinia triticina*) and similar yield to that of its mother line (cv. Sinvalocho M.A.) [22]. Because enhanced resistance coincided with the presence of spontaneous HR, the HLP mutant was backcrossed to different wheat genetic stocks and challenged with leaf-rust to genetically validate the phenomenon.

We report in this paper that the introgression of the HLP mutation into different wheat genetic stocks confers heightened levels of resistance to leaf-rust and does not affect kernel weight.

## 2. MATERIALS AND METHODS

### 2.1. Plant Material and Backcross Program

A backcross program was performed among the HLP mutant of bread wheat (*Triticum aestivum* L.) and eight wheat cultivars to incorporate the mutation induced in HLP into the eight different genetic backgrounds. Cultivar Sinvalocho M.A., mother-line of HLP, and cv. Purplestraw, highly susceptible to *Puccinia triticina*, were selected because they have been used for many years in Argentina as differential hosts in genetic studies involving leaf rust [23-25]. The remaining six wheat cultivars

(Pro INTA Don Alberto, Pro INTA Isla Verde, Granero INTA, Pro INTA Imperial, Buck Guaraní, and Klein Cacique) were selected from the large public (INTA) and private (Buck and Klein) breeding programs because the average genetic diversity within each of these programs was very similar to the total genetic diversity present in the complete Argentine germplasm [26].

Since the mutation induced in HLP controls the expression of visible and spontaneous HR lesions, its introgression into the wheat genetic stocks was traced, by the naked eye, simply by selfing the plant receptor and selecting the HR phenotypes in the plant receptor progeny.

### 2.2. Pathogen Infection Study and Kernel Weight Evaluation

A spontaneous leaf-rust infection study was carried out in the experimental field at the Agricultural Experimental Station of Concepción del Uruguay, Entre Ríos, Argentina. There were two sowing dates, 30 May 2006 and 23 May 2007, each with two repetitions. Seeds of third backcross progenies corresponding to the aforementioned crosses were sown in the field in 1.2-m rows separated by 0.2-m. For each cross, natural leaf-rust infection was evaluated by scoring the number of pustules on the flag leaf of 10 plants that carried the HLP mutation and 10 plants that did not. Evaluated plants were chosen at random. Results were the means of two independent experiments.

The type of infection was also recorded according to the classification described by Mains and Jackson [27].

The evaluation of the kernel weight trait was performed by using the same experimental design described for the spontaneous leaf-rust study. Results were the means of two independent experiments.

### 2.3. Statistical Analyses

The Mann-Whitney test was used to statistically analyze the leaf-rust infection response and the kernel weight trait in third backcross progenies with and without spontaneous HR.

## 3. RESULTS

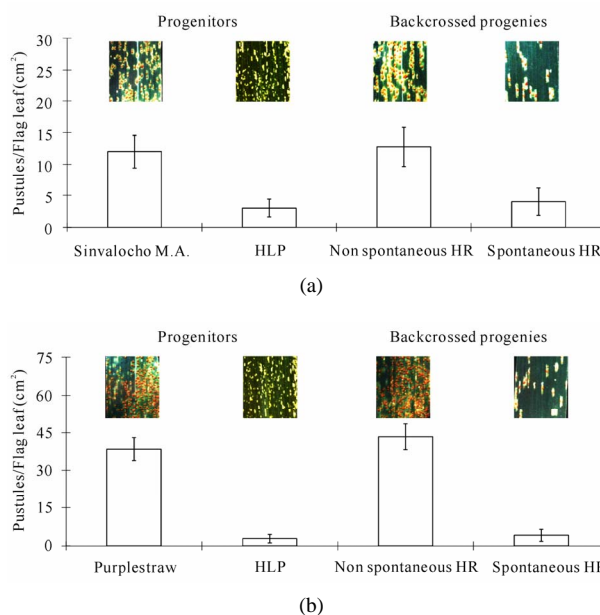
### 3.1. Expression of Resistance of HLP Mutation to Leaf Rust in Different Wheat Stocks

Preferentially, lesion mimic mutations have been characterized only in the genetic background where they were isolated. Even though it is highly desirable to know as well if the candidate mutation is functional in other genetic stocks different from the original one, knowl-

edge about this matter appears to be scarce. Moreover, this information is mandatory to decide whether the mutation is a useful genetic factor to be considered in plant breeding programs.

In a previous investigation we demonstrated that adult HLP plants that exhibited spontaneous HR lesions were more resistant to leaf-rust attack than Sinvalocho M.A. plants of a comparable developmental stage, indicating that the HLP mutation, traced by the naked-eye by the presence of spontaneous HR, was phenotypically associated with fungal resistance.

In an effort to validate at the genetic level, whether the HLP mutation also conferred pathogen resistance in other genetic backgrounds, the HLP mutant was backcrossed to two wheat controls and six commercial cultivars, and field-grown plants of progenitors and third backcrossed progenies were challenged with natural leaf-rust infections. Two-way backcrosses to cv. Sinvalocho M.A., the mother-line, and cv. Purplestraw, highly susceptible to leaf-rust attack, demonstrated that adult plants of progenitor HLP exhibiting spontaneous HR accumulated lower numbers of leaf-rust pustules per cm<sup>2</sup> of flag leaf ( $3.00 \pm 0.89$ ) that in general terms were also smaller (infection type 1) than those observed on control plants of progenitors Sinvalocho M.A. ( $12.00 \pm 2.58$ ;  $U = 1$ ;  $P < 0.001$ ; infection type 2–2++) (**Figure 1(a)**) and Purplestraw ( $38.50 \pm 4.58$ ;  $U = 0$ ;  $P < 0.001$ ; infection type 2–3) (**Figure 1(b)**), of a comparable developmental stage (**Table 1, Figure 1**). Third backcrossed progenies derived from HLP × Sinvalocho M.A. cross demonstrated that adult plants carrying the HLP mutation were more resistant ( $4.00 \pm 2.16$  pustules/flag leaf cm<sup>2</sup>) to the fungal pathogen than adult plants that did not carry the mutation ( $12.75 \pm 3.09$  pustules/flag leaf cm<sup>2</sup>;  $U = 1$ ;  $P < 0.001$ ) and therefore they did not exhibit spontaneous HR lesions (**Figure 1**). Accordingly, third backcrossed progenies derived from HLP × Purplestraw cross showed that adult plants bearing spontaneous HR phenotype were more resistant ( $4.10 \pm 2.30$  pustules/flag leaf cm<sup>2</sup>) to leaf-rust than adult plants that did not exhibit spontaneous HR lesion ( $43.40 \pm 5.15$  pustules/flag leaf cm<sup>2</sup>;  $U = 0$ ;  $P < 0.001$ ) (**Figure 1**). On average, the accumulation of rust pustules/flag leaf area allowed the classification of naturally infected plants in two groups. In one group the number of pustules ranged from 33.92 to 48.55 and it was composed of plants of cv. Purplestraw and plants of third backcrossed progenies that exhibited no spontaneous HR. The other group was composed of plants of the HLP mutant and plants of third backcrossed progenies that showed spontaneous HR, and the accumulation of pustules ranged from 1.80 to 6.40. Interestingly, to 6.40. Interestingly, not only the latter group accumulated a small number of rust pustules com-



**Figure 1.** Differential response of HLP, Sinvalocho M.A. (a), Purplestraw (b), and their corresponding backcrossed progenies to leaf rust attack. Note that field-grown plants from HLP and backcrossed progenies that carry the HLP mutation accumulated less rust pustules than plants of Sinvalocho M.A., Purplestraw, and backcrossed progenies that did not carry the mutation.

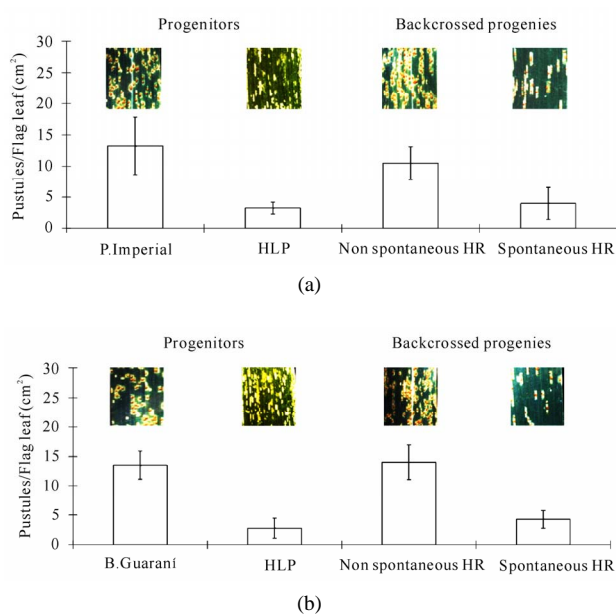
pared to the first one, but it also showed a common feature, *i.e.*, all their members carried the HLP mutation. Since an elevated number of leaf-rust pustules were detected in the susceptible wheat control, this meant that the experiments were performed under conditions of good spontaneous leaf-rust infection (**Table 1, Figure 1(b)**).

Adult plants of four commercial cultivars (Klein Cacicque, Pro INTA Don Alberto, Pro INTA Isla Verde, and Granero INTA) out of the six cultivars analyzed did not differ from the response displayed by the HLP mutant to leaf-rust attack (**Table 1**); however, flag leaves of cvs. Pro INTA Imperial ( $U = 1$ ;  $P < 0.001$ ; infection type 2–2++) (**Figure 2(a)**) and Buck Guaraní ( $U = 1$ ;  $P < 0.001$ ; infection type 2–2++) (**Figure 2(b)**) accumulated higher numbers of leaf-rust pustules compared to HLP (**Table 1, Figure 2**). Third backcrossed progenies derived from both HLP × P. Imperial and HLP × B. Guaraní crosses showed that adult plants that carried the HLP mutation were more resistant ( $4.00 \pm 2.58$  pustules/flag leaf cm<sup>2</sup>, and  $4.25 \pm 1.50$  pustules/flag leaf cm<sup>2</sup>, respectively) to leaf-rust than adult plants that did not carry the mutation ( $10.50 \pm 2.64$  pustules/flag leaf cm<sup>2</sup>;  $U = 1$ ;  $P < 0.001$ , and  $14.00 \pm 2.94$  pustules/flag leaf cm<sup>2</sup>;  $U = 1$ ;  $P < 0.001$ , respectively), and thus they did not exhibit spontaneous HR phenotypes (**Figure 2**).

**Table 1.** Natural leaf-rust infection levels in field-grown plants of several wheat genetic stocks.

Progenitors	Pustules/Flag leaf (Number cm <sup>2</sup> )
Mutant	
HLP	3.00 ± 0.89
Controls	
Sinvalocho M.A.	12.00 ± 2.58
Purplestraw	38.50 ± 4.58
Commercial cultivars	
Pro INTA Imperial	13.25 ± 4.57
Buck Guaraní	13.50 ± 2.38
Klein Cacique	3.10 ± 1.43
Pro INTA Don Alberto	2.80 ± 1.14
Pro INTA Isla Verde	2.15 ± 0.93
Granero INTA	2.35 ± 0.90

Notes: Four commercial cultivars were as healthy as the HLP mutant. Data represent the average of two independent experiments with repetitions.



**Figure 2.** Differential response of HLP, Pro INTA Imperial (a), Buck Guaraní (b), and their corresponding backcrossed progenies to natural leaf-rust infection. Note that field-grown plants from the HLP mutant and from backcrossed progenies that carry the HLP mutation showed less rust pustules than developmentally comparable plants of P. Imperial, B. Guaraní, and backcrossed progenies that did not carry the mutation.

### 3.2. Kernel Weight Evaluation

Because wheat is both nutritionally and economically an important crop worldwide, it is desirable that the mutation induced in the HLP mutant causes no detrimental pleiotropic effects in agronomic traits that are components of the cereal yield. Field-grown plants that were naturally infected with leaf-rust demonstrated, for each cross, that no kernel weight differences ( $P > 0.05$ ) were detected between plants of third backcrossed progenies that showed spontaneous HR and plants that did not (**Table 2**), indicating that the HLP mutation, traced by the naked-eye by the presence of spontaneous HR lesions, did not have a substantial effect on the kernel weight of the different genetic backgrounds examined.

## 4. DISCUSSION

The fact that most lesion-mimic mutations have been solely studied in the genetic background of the wild-type indicates that any putative association between the lesion-mimic mutation and a genetic trait of agronomic

**Table 2.** Kernel weight values in progenitors and backcross progenies carrying and not carrying the HLP mutation.

Plant material	100 Kernel weight (g)
Progenitors	
HLP	3.74 ± 0.16
Sinvalocho M.A.	3.72 ± 0.19
Purplestraw	3.72 ± 0.21
Buck Guaraní	3.82 ± 0.26
Pro INTA Imperial	3.49 ± 0.59
Backcrosses	
HLP × Sinvalocho M.A. (spontaneous HR)	3.75 ± 0.15
HLP × Sinvalocho M.A. (without HR)	3.71 ± 0.18
Purplestraw × HLP (spontaneous HR)	3.70 ± 0.24
Purplestraw × HLP (without HR)	3.70 ± 0.23
Buck Guaraní × HLP (spontaneous HR)	3.84 ± 0.32
Buck Guaraní × HLP (without HR)	3.48 ± 0.29
HLP × Pro INTA Imperial (spontaneous HR)	4.15 ± 0.22
HLP × Pro INTA Imperial (without HR)	3.42 ± 0.35

Notes: The introgression of the HLP mutation, traced by the observation of spontaneous HR lesions, caused no kernel weight reduction. Data represent the average of two independent experiments with repetitions.

interest represents a phenotypic correlation that should be validated at the genetic level by crossing the genotype that carries the lesion mimic mutation to other genetic backgrounds and verifying if the genetic correlation between the mutation and the agronomic trait exists. This validation is deemed important to consider the direct use of this type of mutations in breeding programs.

We previously demonstrated that adult plants of HLP showing spontaneous HR were more resistant to leaf-rust attack compared with plants of the mother-line of comparable developmental stage that did not show spontaneous HR. This finding indicated that the mutation induced in HLP was phenotypically associated with fungal resistance [22].

In this study we have genetically validated the aforementioned finding by challenging with leaf-rust infections the backcross-mediated introgression of the HLP mutation into several wheat genetic backgrounds besides the mother-line. We have demonstrated that adult plants of third backcrossed progenies bearing spontaneous HR phenotype were more resistant to leaf-rust attack than adult plants of third backcrossed progenies that did not carry the HLP mutation (**Figures 1 and 2**). The introgression of the mutation induced in HLP conferred similar levels of resistance to the fungal pathogen in different wheat genetic backgrounds. Interestingly, similarity coefficient among cultivars used as progenitors ranged from 0.7784 (P. Imperial and P. Don Alberto) to 0.6296 (Granero INTA and P. Isla Verde), and it was 0.6625 in the specific case of P. Imperial and B. Guaraní (Manifesto, pers. comm.), indicating that the HLP mutation proved to be functionally efficient against the fungal pathogen in genetically diverse wheat germplasm.

Even though the mother-line of the HLP mutant carries at least two leaf-rust (*Lr*) genes [25] that may explain the durable resistance that Sinvalocho M.A. has shown for decades, the possibility that those genes confer the enhanced resistance observed in plants of third backcross progenies that showed HR phenotype is fairly low.

Diseases are a leading cause of crop losses, primarily leaf-rust that may reduce yield greatly [28]. Currently, the introgression of disease resistance genes into wheat breeding programs requires DNA-molecular-marker-assisted selection to trace those genes in the plant breeding materials. However, because the mutation induced in HLP controls the expression of visible and spontaneous HR lesions, its introgression into wheat genetic backgrounds has the advantage that it can be traced, by the naked eye, simply by selfing the plant receptor and selecting the HR phenotypes in the plant receptor progeny. Indeed, this visual method represents a simpler and more economic way of tracing the mutation, compared to DNA-

molecular-marker-assisted selection method.

On the other hand, several investigations have demonstrated that resistance mediated by lesion-mimic mutations is accompanied, in most plants, by lower yield, stunted growth or other abnormal characteristics that prevent the direct use of these mutations in disease-resistance breeding. Yield of the wheat mutant M66 was 50% lower than that of the control [19]. Mutagen induced resistance genes *ml-o5*, *ml-o6*, and *ml-10* conferred a four per cent reduction in grain yield caused mainly by lower thousand grain weight in a population of 198 chromosome-doubled haploid lines of spring barley tested in a disease-free field trial [13]. In contrast to the *suppressor of SA insensitivity1 (ssi1)*, *accelerated cell death6 (acd6)* and *lsd6* mutations of *Arabidopsis*, and to most *spl* and *cdr* mutations of rice, which reduced final plant height [3,9,15,29] and shorter life cycle [14] and resulted in a lower yield [16], the mutation induced in HLP caused no detrimental pleiotropic effects that affected the agronomic performance of the plant [22] and did not affect kernel weight when it was backcrossed-incorporated in several genetic stocks (**Table 2**). The latter finding, coupled with the fact that backcross-mediated introgression of the HLP mutation in several wheat genetic stocks heightened leaf-rust resistance validates the direct use of this mutation in disease-resistance breeding.

## 5. CONCLUSIONS

The introgression of the HLP mutation into different wheat genetic stocks confers heightened levels of resistance to leaf-rust, similar to the reported for the introgression of the mutation into the genetic background of the mother-line. To our knowledge this is the first report of a lesion-mimic mutation that enhances pathogen resistance without affecting kernel weight in genetically diverse wheat germplasm. Taken together, both findings validate the direct use of this mutation in disease-resistance breeding.

## 6. ACKNOWLEDGEMENTS

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