



Autoimmunity In Plants; A Powerful Weapon in Kingdom Plantae to Combat Stresses

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ABSTRACT

Plants possess two immunity systems, such as PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). PTI is the basic defense possessed by plants that is evaded by pathogens because of specific effectors which are not recognized by plants' basal defense system. However, plants have nucleotide-binding leucine-rich repeat proteins (NLR) in their genome that recognize the evaded pathogenic attack, which in turn activates the NLR proteins, and the hypersensitive response (HR) is activated to ingress the pathogenicity. Based on a similar mechanism, lesion mimic mutants (LMMs) have been identified and intentionally developed to induce HR in the absence of pathogens. These LMMs are not only present in a single crop but are present throughout the kingdom *plantae*. Researchers have produced many LMMs in plants by treating the germplasm with EMS or other sources such as genome editing tools, gamma rays, and X-rays. Owing to the simple Mendelian inheritance of these LMMs, conventional and novel plant breeding techniques can be exploited to introduce the causative genes in plants to keep them safe against biotic and abiotic stresses.

Key words: Autoimmunity in Plants, Disease lesion mimic mutants, NLR proteins, Biotic and Abiotic stresses.

INTRODUCTION

Unlike humans, plants can't move for remedy purposes. However, nature has given two kinds of general immunity: constitutive defense components and induced immune responses (Haroon et al., 2022a). The former relies on preferred defense barriers such as waxy cuticles, and physical and chemical compounds like antipathogenic

compounds. However, induced immune responses are also broadly categorized into two kinds of responses; extracellular and intracellular immune responses which are termed PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI), respectively (Jones and Dangl, 2006)(Spoel and Dong, 2012). PTI is an extracellular immunity response that plants show as PRR proteins on the plants' surface and recognizes pathogens and start the

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defense response (Haroon et al., 2022a). While ETI is induced by pathogen effectors transported into plant cells, as discussed by (Freh et al., 2022). In comparison to PTI, ETI is a more complex immune response that begins as a pathogen releases specialized effectors inside the plant's cells to evade the plant's basal defense. However, plants also have the R proteins, which don't permit them to pass effectors without recognizing (directly or indirectly) and fighting. This whole defense scene results in the activation of NLR (R genes encodes) proteins, which ultimately activate the hypersensitive response (HR)(Yuan et al., 2021) (Figure 1).

Since the mid-20th century, plant scientists have been developing types of plants that mimic disease-like symptoms such as lesions and activate the HR in the absence of pathogens, called lesion mimic mutants (LMMs) (Johal, 2007) (Figure 2). These LMMs have been observed across various plant species such as Arabidopsis, rice, maize, wheat, cotton, soybean, etc (Kang et al., 2021). Except for those LMMs which are produced as a result of a mutation in development-regulated genes, others result from the autoactivation of NLR proteins that are encoded by R genes (Freh et al., 2022). In their original form, these NLRs are not activated because of intra- and intermolecular interactions of NLR proteins(Freh et al., 2022). However, as the interaction is broken down because of mutation in NLR encoded genes, these proteins become activated which results in elevated levels of SA (Salicylic acid) and other defense-related genes such as Rp1 in Maize and WRKY (Johal, 2007)(Freh et al., 2022)(Ngou et al., 2021).

For the past few years, in different crops, these LMMs have been developed that carry varying levels of lesions severity. Few are not even capable of maturing and producing seeds, whereas others have lesions, but such plants still get fully mature (Johal, 2007). In the Maize crop, for the first-time, various kinds of LMMs were developed by Neuffer, whose severity was dependent on several other factors such as light, temperature, and genetic background(Hoisington et al., 1982).

In the past two decades, various LMMs responsible genes have been cloned, which increase the broad spectrum disease resistance and also specifically against pathogens (Johal, 2007). Li and his team identified a woody lesion mimic mutant (*lmd*), which was produced as a result of t-DNA insertion in *BpEIL1*. This transgenic plant showed resistance against *Alternaria alternate* in birch. Similarly, they have also been identified in other plant species, as reviewed by (Kang et al., 2021)(Xiaobo et al., 2020). Most LMMs are controlled by dominant genes and fewer are recessive mutations. However, owing to Mendelian inheritance, this trait can be introduced, especially in those elite lines that don't have resistance against specific pathogens.

In this mini-review, we have enlightened the molecular mechanisms behind the formation of lesion-like symptoms in plants. Also, we have put some information about the recently cloned genes that showed disease resistance against pathogens. Further, we have explained how the autoimmunity of NLR proteins can be harnessed to develop new lines.

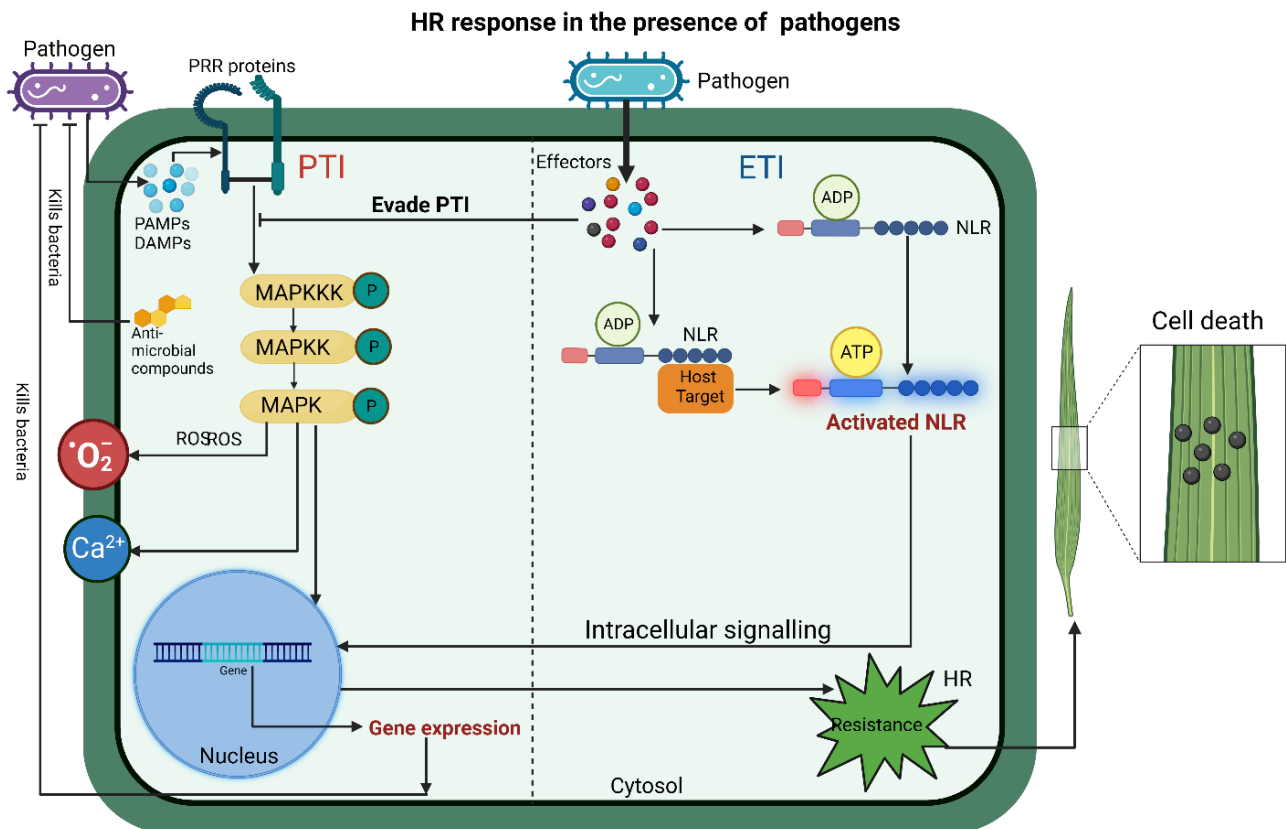


Fig. 1: This figure shows the crosstalk between PTI and ETI. Further, it shows pathogen attack and basal defense tries to contain the pathogen. However, if pathogen release specific effectors which evade the PTI then R gene recognizes these effectors and in return NLR proteins which are auto-active are activated which results in the HR activation and impedes the pathogenicity. At the site of HR activation, plants cells die to contain the pathogens.

Molecular Mechanism of Autoimmunity

Plants have several NLR proteins that have core pathogen recognition domains: Nucleotide-binding (NB) and leucine-rich repeat (LRR) domains to induce the ETI response (Cesari et al., 2022). According to the available information, these NLRs have three main domains, including CC, NBS, and LRR. However, based on the N region, NLRs are divided into three main categories, such as coiled-coil type NLRs (CNLs), Toll/interleukin receptor type NLR (TNLs), and RESISTANCE TO POWDERY MILDEW 8-like CC (CCR) (Saile and El Kasmi, 2023)(Freh et al., 2022). Except for individual NLRs, some other NLRs monitor (guard) the host proteins (guardees). Any kind of manipulation in “guardees” or other direct NLR activates the associated NLR which in turn activates the HR even in the absence of pathogens which results in the appearance of lesions on the attacked site. Similarly, individual NLRs also behave in a similar fashion(Khan et al., 2016)(Ngou et al., 2021). In the past, it was thought that these NLRs could work independently, but they also required other helper NLRs to activate the defense mechanism in a much stronger way, as reviewed by (Freh et al., 2022).

Any mutation in these NLRs encoded R genes makes the plant autoimmune (Figure 2). These NLRs are abundantly present in different crops such as Arabidopsis (150-200) (Saile and El Kasmi, 2023), hexaploid wheat (3400) (Huang et al., 2022), Maize (144)(Ma et al., 2022), *Oryza sativa* (438-440) (Zhang et al., 2020)(Ma et al., 2022) and *Hordeum vulgare* (224)(Ma et al., 2022). Much progress is being made to further understand how more

than one NLRs work with each other to activate the HR and how LMMs can be produced by making changes in these NLRs. Complete detail of these NLRs and their activation mechanism for inducing immunity and the complex association has been explained here(Cesari et al., 2022)(Ngou et al., 2021)(Saile and El Kasmi, 2023).

Recently Cloned LMMs Genes and Their Role in Disease Resistance

LMMs are ubiquitous in almost all crops (Johal, 2007). In the kingdom *Plantae*, the first LMM was observed in Maize by R. A. Emerson in the 1920s. Later, they were identified in other crops as well(Lu et al., 2012). Thanks to molecular biologists, these LMMs can be characterized at molecular levels. For example, in Maize around 50 loci have already been identified, that are responsible for the LMMs (Johal, 2007). Similarly, around 22 LMMs have also been studied in Rice (Kang et al., 2021).

There are still several unreported LMMs present in kingdom plantae that have not been cloned or molecularly characterized. However, for the past two decades, a few gene cloning studies have also been published which show the role of these LMMs against different diseases caused by pathogens.

In the Maize crop, Johal and his team worked on a *les23* mutant plant that was developed in Va35 background and mapped the *les23* on chromosome no. 2. They also crossed this *les23* mutant plant with Mo20w maize inbred line, which suppressed the autoimmunity, and the plant didn't show any lesions. This suppressor, *slm1*, was also mapped on the same chromosome (Johal, 2007). Later,

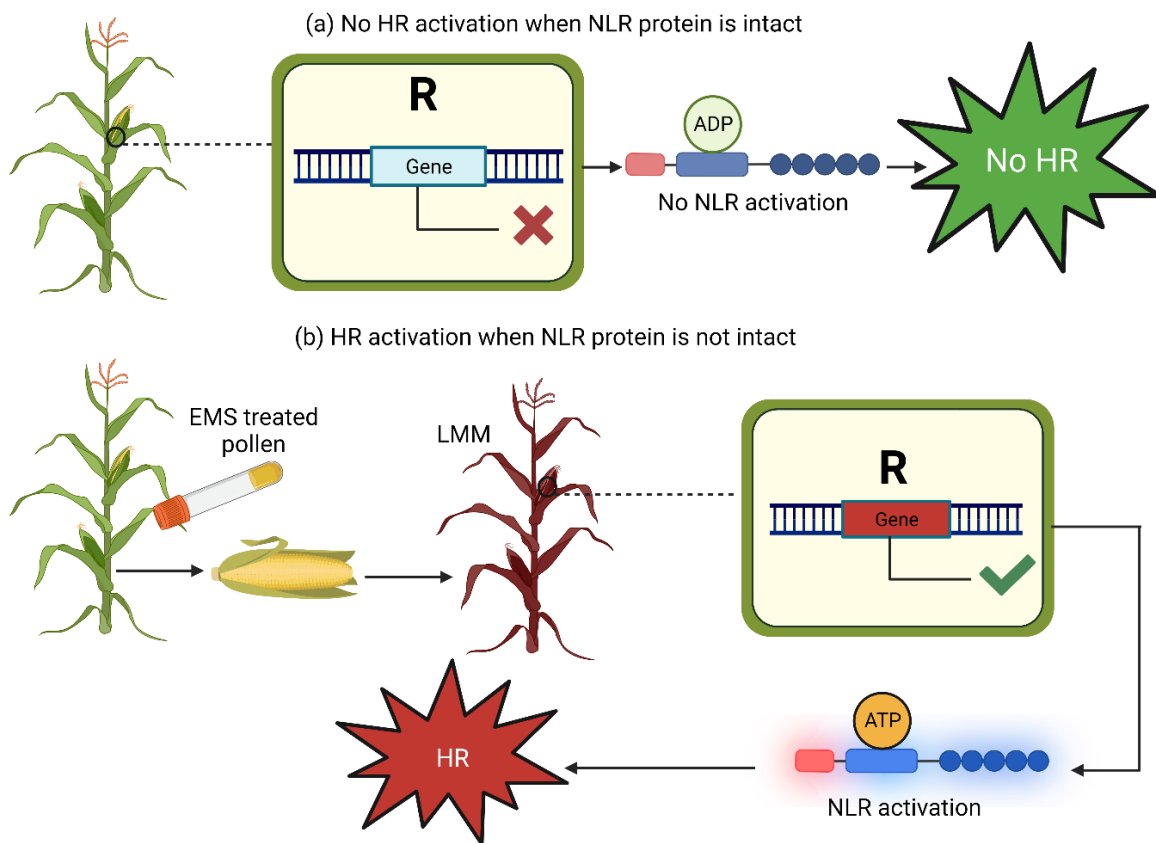


Fig 2: (a) depicts that no HR activation happens when NLR protein is intact or when there is no mutation in R gene, (b) shows when plant is treated with EMS, the next progeny has LMM in which NLR protein is autoactivated which were autoinhibited in (a) and now plants induce the HR and cell death occurs.

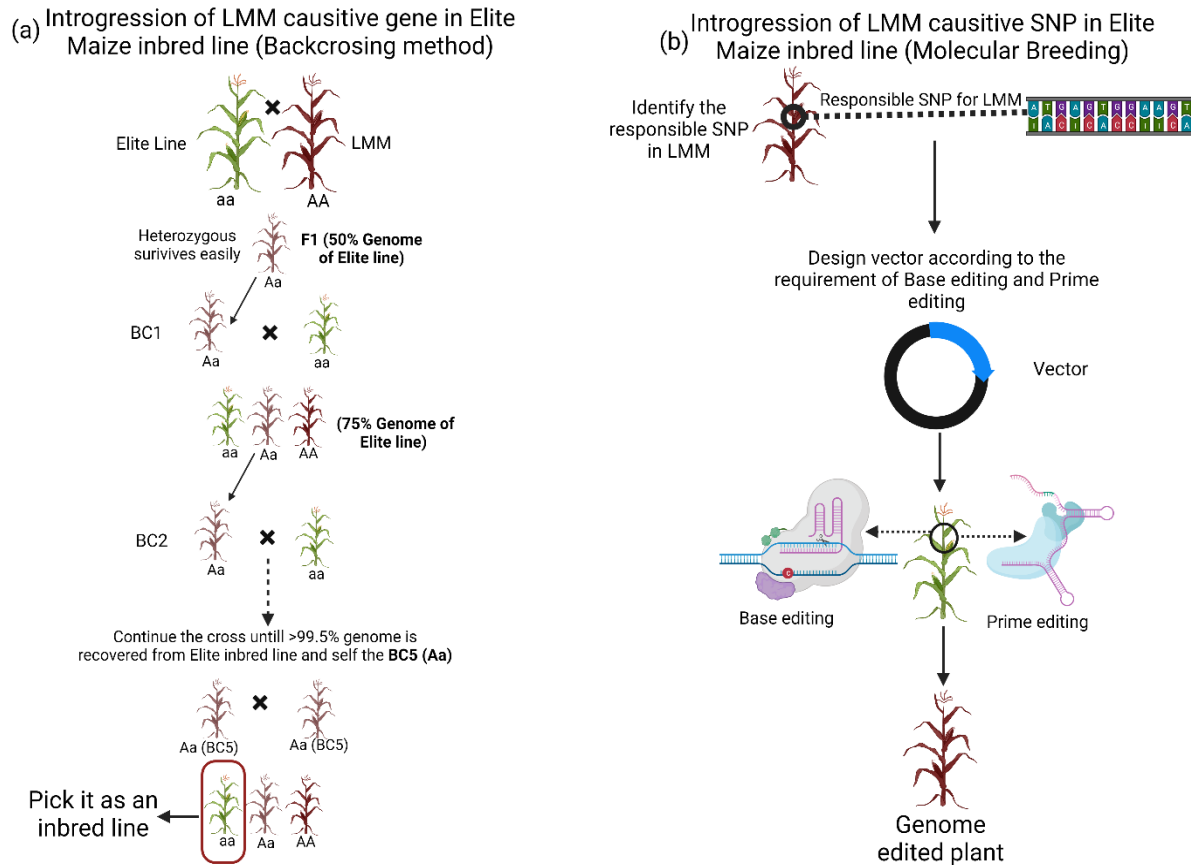


Fig. 3: Use of backcrossing and novel genome editing tools such as base editing and prime editing to change the nucleotide sequence in essence to produce the LMM. (a) shows the backcrossing method, while (b) shows the use of Base editing and Prime editing.

Zhan who was a student of Johal at Purdue University, did the cloning of *les23* and *slm1*. This study helped them to understand that *Les23* and *Slm1* both encode for homologs in Arabidopsis RIN4 and an uncharacterized NLR resistance gene, respectively. They also transiently expressed *Les23* and *Slm1* in *Nicotiana benthamiana*, and it was known that *SLM1* is an auto-active NLR which is autoinhibited by *LES23*, and this model works similarly as RIN4/RPS2 in Arabidopsis (Zhan, 2017). Similarly, other LMM causative genes have been cloned in Rice, Arabidopsis, barley, wheat, and other crops (Bruggeman et al., 2015) (Kang et al., 2021) (Yuchun et al., 2021) (Takahashi et al., 1999) (McGrann et al., 2015) (Liu et al., 2021) (Rosignoli et al., 2022) (Mbaraka et al., 2017) (Singh et al., 2020).

LMMs are not just plants but a source of new germplasm that has resistance against different plant pathogens across plant species. For example, Yuchun and his colleagues performed a study on rice (*Oryza sativa*) *spotted leaf 36* (*spl36*) mutant, which encodes Receptor-like Protein Kinase and was produced as a result of EMS treatment and led to a 1 bp change in *SPL36*. This mutant showed resistance against bacterial pathogens and also negatively regulated the salt stress response (Yuchun et al., 2021). Similarly, *Imm9150/aba2* (Os03g59610) increased the resistance to bacterial blight in rice. Few of the well-studied LMMs and their role against diseases have been discussed here (Freh et al., 2022).

Harnessing LMMs Trait in Plant Breeding

By studying the genetics of these LMMs, we came to know that most of the LMMs follow the Mendelian inheritance pattern. Dominant mutations were found abundantly in many LMMs, which were so easy to transfer to other elite lines. Even a single allele of a gene is enough to induce autoimmunity as it was stated well (Zhan, 2017).

2 However, care should be taken as some of the lines have suppressors too, which don't allow the auto-active NLRs to induce autoimmunity (Zhan, 2017). Johal and his team at Purdue University, USA, are trying to make different crosses between LMM plants and other inbred lines to see the inheritance pattern and further understanding the suppressors (Johal, 2007). In rice and maize, different LMMs have already been reported that follow the above-stated inheritance pattern as reviewed by (Kang et al., 2021) (Johal, 2007).

Most of the LMMs were obtained after treatment with EMS, which makes only one bp change from G to A. These single bp changes can be introduced in other elite lines by using the novel genome editing tools such as Prime editing and Base editing which work efficiently for making changes for single nucleotides mentioned by (Haroon et al., 2019) (Chattha et al., 2022) (Haroon et al., 2022b) (Haroon et al., 2020). Apart from all this, conventional breeding methods such as backcrossing can also be used to introduce the LMMs causative gene in elite crop lines (Haroon et al., 2022b) (Figure 3). However, not all LMMs are beneficial,

which means they are also associated with negative traits such as low yield and disease increment in specific pathogens. Therefore, care must be taken to assess the LMMs and their role against different commonly available pathogens in various crops to make sure they may not cause trouble in the future.

Conclusion and Future Prospects

Over the past two decades, because of the importance of LMMs in elevating gene expression, the level of defense-related genes and other immunity hormones such as SA and JA, much interest has been given to them. Further, according to the changing environmental conditions and different diseases, yield is reduced, which ultimately requires harnessing the LMM characteristics in elite crop lines to activate the autoimmunity in advance before plants face biotic and abiotic stresses. After reading this mini-review, our readers have understood this all depends upon the activation of NLR proteins. So, it's the dire need of the hour to activate those NLRs that are autoinhibited and not suppressed by other suppressor genes. In the past, it has been observed that both JA and SA behave differently when a pathogen attacks. Therefore, it's also necessary to evaluate the lines against biotrophic and necrotrophic pathogens.

Till this century, scientists have modified several conventional breeding methods, such as speed breeding, along with other novel genome editing tools that can be utilized to introduce the LMMs causative mutations in other elite lines to make them resistant to specific pathogens. Based on the simple mendelian nature of these LMMs, we believe these LMMs will be the choice of breeders in the 21st century to improve the already available crop lines that were cultivated in the past but left their use today because of disease susceptibility to newly emerged pathogens.

REFERENCES

- Bruggeman, Q., Raynaud, C., Benhamed, M., Delarue, M., 2015. To die or not to die? Lessons from lesion mimic mutants. *Front. Plant Sci.* 6, 24.
- Cesari, S., Xi, Y., Declerck, N., Chalvon, V., Mammri, L., Pugnière, M., Henriquet, C., De Guillen, K., Chochois, V., Padilla, A., 2022. New recognition specificity in a plant immune receptor by molecular engineering of its integrated domain. *Nat. Commun.* 13, 1524.
- Chattha, M.S., Ali, Q., Haroon, M., Afzal, M.J., Javed, T., Hussain, S., Mahmood, T., Solanki, M.K., Umar, A., Abbas, W., 2022. Enhancement of nitrogen use efficiency through agronomic and molecular based approaches in cotton. *Front. Plant Sci.* 13.
- Freh, M., Gao, J., Petersen, M., Panstruga, R., 2022. Plant autoimmunity—fresh insights into an old phenomenon. *Plant Physiol.* 188, 1419–1434. <https://doi.org/10.1093/plphys/kiab590>
- Haroon, M., Afzal, R., Idrees, F., Sunny, A., Khan, A.S., 2019. Genome Editing and Speed Breeding; Game Changers to Boost the Crop Production. *Int. J. Biol. Res.* 2, 295–300.
- Haroon, M., Afzal, R., Zafar, M.M., Zhang, H., Li, L., 2022a. Ribonomics Approaches to Identify RBPome in Plants and Other Eukaryotes: Current Progress and Future Prospects. *Int. J. Mol. Sci.* 23. <https://doi.org/10.3390/ijms23115923>
- Haroon, M., Wang, X., Afzal, R., Zafar, M.M., Idrees, F., Batool, M., Khan, A.S., Imran, M., 2022b. Novel Plant Breeding Techniques Shake Hands with Cereals to Increase Production. *Plants* 11. <https://doi.org/10.3390/plants11081052>
- Haroon, M., Zafar, M.M., Farooq, M.A., Afzal, R., Batool, M., Idrees, F., Babar, U., Khan, A.S., Mo, H., Li, L., 2020. Conventional Breeding, Molecular Breeding and Speed Breeding; Brave Approaches to Revamp the Production of Cereal Crops. Preprint.
- Hoisington, D.A., Neuffer, M.G., Walbot, V., 1982. Disease lesion mimics in maize: I. Effect of genetic background, temperature, developmental age, and wounding on necrotic spot formation with Les1. *Dev. Biol.* 93, 381–388.
- Huang, Z., Qiao, F., Yang, B., Liu, J., Liu, Y., Wulff, B.B.H., Hu, P., Lv, Z., Zhang, R., Chen, P., Xing, L., Cao, A., 2022. Genome-wide identification of the NLR gene family in *Haynaldia villosa* by SMRT-RenSeq. *BMC Genomics* 23, 118. <https://doi.org/10.1186/s12864-022-08334-w>
- Johal, G.S., 2007. Disease lesion mimic mutants of maize.
- Jones, J.D.G., Dangl, J.L., 2006. The plant immune system. *Nature*. <https://doi.org/10.1038/nature05286>
- Kang, S.G., Lee, K.E., Singh, M., Kumar, P., Matin, M.N., 2021. Rice Lesion Mimic Mutants (LMM): The Current Understanding of Genetic Mutations in the Failure of ROS Scavenging during Lesion Formation. *Plants (Basel, Switzerland)* 10. <https://doi.org/10.3390/plants10081598>
- Khan, M., Subramaniam, R., Desveaux, D., 2016. Of guards, decoys, baits and traps: pathogen perception in plants by type III effector sensors. *Curr. Opin. Microbiol.* 29, 49–55.
- Liu, R., Lu, J., Zheng, S., Du, M., Zhang, C., Wang, M., Li, Y., Xing, J., Wu, Y., Zhang, L., 2021. Molecular mapping of a novel lesion mimic gene (lm4) associated with enhanced resistance to stripe rust in bread wheat. *BMC Genomic Data* 22, 1–9.
- Lu, X.-M., Hu, X.-J., Zhao, Y.-Z., Song, W.-B., Zhang, M., Chen, Z.-L., Chen, W., Dong, Y.-B., Wang, Z.-H., Lai, J.-S., 2012. Map-based cloning of zb7 encoding an IPP and DMAPP synthase in the MEP pathway of maize. *Mol. Plant* 5, 1100–1112.
- Ma, W., Gao, X., Han, T., Mohammed, M.T., Yang, J., Ding, J., Zhao, W., Peng, Y.-L., Bhaduria, V., 2022. Molecular Genetics of Anthracnose Resistance in Maize. *J. Fungi* 8, 540.
- Mbaraka, R.S., Yamagata, Y., Yoshimura, A., Yasui, H., 2017. Genetic mapping and characterization of lethal necrotic mutants in rice (*Oryza sativa* L.). *Am. J. Plant Sci.* 8, 3350–3376.
- McGrann, G.R.D., Steed, A., Burt, C., Nicholson, P., Brown, J.K.M., 2015. Differential effects of lesion mimic mutants in barley on disease development by facultative pathogens. *J. Exp. Bot.* 66, 3417–3428. <https://doi.org/10.1093/jxb/erv154>
- Ngou, B.P.M., Ahn, H.-K., Ding, P., Jones, J.D.G., 2021. Mutual potentiation of plant immunity by cell-surface and intracellular receptors. *Nature* 592, 110–115.
- Rosignoli, S., Cosenza, F., Moscou, M.J., Civolani, L., Musiani, F., Forestan, C., Milner, S.G., Savojarado, C., Tuberosa, R., Salvi, S., 2022. Cloning the barley nec3 disease lesion mimic mutant using complementation by sequencing. *Plant Genome* 15, e20187.
- Saile, S.C., El Kasmi, F., 2023. Small family, big impact: RNL helper NLRs and their importance in plant innate immunity. *Plos Pathog.* 19, e1011315.
- Singh, S., Mishra, V.K., Kharwar, R.N., Budhlakoti, N., Ahirwar, R.N., Mishra, D.C., Kumar, Sundee, Chand, R., Kumar, U., Kumar, Suneel, 2020. Genetic characterization for lesion mimic and other traits in relation to spot blotch resistance in spring wheat. *PLoS One* 15, e0240029.
- Spoel, S.H., Dong, X., 2012. How do plants achieve immunity? Defence without specialized immune cells. *Nat. Rev. Immunol.* 12, 89–100.

- Takahashi, A., Kawasaki, T., Henmi, K., Shii, K., Kodama, O., Satoh, H., Shimamoto, K., 1999. Lesion mimic mutants of rice with alterations in early signaling events of defense. *Plant J.* 17, 535–545.
- Xiaobo, Z.H.U., Mu, Z.E., Mawsheng, C., Xuwei, C., Jing, W., 2020. Deciphering rice lesion mimic mutants to understand molecular network governing plant immunity and growth. *Rice Sci.* 27, 278–288.
- Yuan, M., Ngou, B.P.M., Ding, P., Xin, X.-F., 2021. PTI-ETI crosstalk: an integrative view of plant immunity. *Curr. Opin. Plant Biol.* 62, 102030.
- Yuchun, R.A.O., Ran, J., Sheng, W., Xianmei, W.U., Hanfei, Y.E., Chenyang, P.A.N., Sanfeng, L.I., Dedong, X., Weiyong, Z., Gaoxing, D.A.I., 2021. SPL36 encodes a receptor-like protein kinase that regulates programmed cell death and defense responses in rice. *Rice* 14, 1–14.
- Zhan, R., 2017. Cloning and Characterization of the Maize Lesion Mimic Mutant Les23 and Its Suppressor S1m1. Purdue University.
- Zhang, B., Zhang, H., Li, F., Ouyang, Y., Yuan, M., Li, X., Xiao, J., Wang, S., 2020. Multiple alleles encoding atypical NLRs with unique central tandem repeats in rice confer resistance to *Xanthomonas oryzae* pv. *oryzae*. *Plant Commun.* 1, 100088.