Identification and genetic analysis of EMS-mutagenized wheat mutants conferring lesion-mimic premature aging

Weiwei Kong, Liming Wang, Pei Cao, Xingfeng Li, Jingjing Ji, Puhui Dong, Xuefang Yan, Chunping Wang, Honggang Wang, Jiaqiang Sun*.

1 Agronomy College, Henan University of Science and Technology, Luoyang 471023, China;
2 Institute of Botany, Chinese Academy of Sciences, Beijing, 10093, China
3 State Key Laboratory of Crop Biology/Agronomy College, Shandong Agricultural University, Taian, 271018, China
4 Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, 10081, China

# These authors contributed equally to this work.
* To whom correspondence may be addressed. E-mail address: lmwang1@163.com ; sunjiaqiang@caas.cn.
Abstract

**Background:** Lesion-mimic and premature aging (*lmpa*) mutant *lmpa1* was identified from the ethyl methane sulfonate (EMS) mutant library in the bread wheat variety Keda 527 (KD527) background. To reveal the genetic basis of *lmpa1* mutant, phenotypic observations and analyses of chlorophyll content and photosynthesis were carried out in *lmpa1*, KD527 and their F₁ and F₂ derivatives. Further, bulked segregation analysis (BSA) in combination with a 660K SNP Chip were conducted on the F₂ segregation population of *lmpa1*/Chinese spring (CS) to locate the *lmpa1* gene.

**Results:** Most agronomic traits of *lmpa1* were similar to those of KD527 before lesion-like spots appeared. Genetic analysis indicated that the F₁ plants from the crossing of *lmpa1* and KD527 exhibited the *lmpa1* phenotype and the F₂ progenies showed a segregation of normal (wild type, WT) and *lmpa*, with the ratios of *lmpa*:WT=124:36 ($\chi^2=1.008<3.841$), indicating that *lmpa1* is a dominant mutation. The combination of BSA and the SNP Chip analysis of CS, *lmpa1* and *lmpa1*/CS F₂ WT pool (50 plants) and *lmpa* pool (50 plants) showed that polymorphic SNPs were enriched on chromosome 5A, within a region of 30-40 Mb, indicating that the wheat premature aging gene *Lmpa1* was probably located on the short arm of chromosome 5A.

**Conclusions:** EMS-mutagenized mutant *lmpa1* deriving from elite wheat line KD527 conferred *lmpa*. *lmpa* phenotype of *lmpa1* mutant is controlled by a single dominant allele designated as *Lmpa1*, which affected wheat growth and development and reduced the thousand grain weight (tGW) of single plant in wheat. The gene *Lmpa1* was tentatively located within the region of 30-40 Mb near to the short arm of chromosome 5A.

**Keywords:** Wheat, *lmpa1*, Mutant, Chromosomal location

**Backgroud**

Lesion-like mutants (*llm*) can spontaneously form spots on leaves, sheaths, or whole plants without significant damage, stress, or external pathogen infection [1]. The phenotype of *llm* is very similar to the hypersensitivity response (programmed cell death, PCD) after infection with pathogens [2]. Lesion-like spots (*lls*) formation is controlled by specific genes and/or affected by certain environmental conditions. They may be mostly caused by cell death and partially be correlated with pigment accumulation [3]. Previous researches [4] indicated that the mechanism of...
the lesion formation is very complicated because they may be controlled by genes related to
disease resistance, regulation of death, and basic metabolic enzymes. Both signal molecules in
plant defense to diseases and in environmental responses also play important role on the formation
of *lls*.

In recent years, ethyl methane sulfonate (EMS) has been widely used to induce mutants with
different agronomic traits in crops because it has the advantages of higher point mutation, fewer
chromosomal aberrations, and easier screening of mutants over other methods [5-8]. EMS is a
useful tool for improving particular agronomic traits, breeding new varieties, and screening elite
germlasms [9]. Mutant germplasms induced by EMS can be effectively used to mine new genes,
promote functional genomics studies, and accelerate breeding program [10].

To date, *llm* have been reported in corn [11], Arabidopsis [12], barley [13], and rice [14]. In
recent years, wheat *lls* have been gradually found. For example, Geng [15] mapped a new wheat
spot-like mutation gene *lm3*. Li et al. [16] found that wheat white spot mutation *I30* was controlled
by a pair of recessive nuclear genes which were located on wheat chromosome 6D by using of
BSA method and 660K gene chip technology. Yao et al. [17] obtained a LLM from the crossing
between normal parents Yanzhan 1 and Zaosui 30 and the LLM was controlled by two recessive
genes named *lm1* and *lm2*.

Senescence is the final stage of plant development and an active process of extracting
nutrients from old tissues. Premature aging can shorten the growth stage of crops, cause premature
senility of functional organs earlier before grain filling [18], thus affecting crop yield and quality
[19]. Many reports and in-depth studies on premature senescence in rice have been documented to
date [20]. The gene of leaf premature senescence mutant *wss1* was located within 1200 kb near the
centromere region of the long arm of chromosome 11 in rice [21]. Signs of senescence began to
appear in the rice premature senescence mutant *es4* in about 60 days, due to the loss of function of
the calcium-dependent protein kinase *OsCPK12* [22]. The 3-bp deletion in the gene of *WLS5* also
leads to premature senescence in rice [23]. The early senescence mutations *esl2* [24], *esl3[25]*,
*esl4[26], esl5[27]*, and *esl6* [28] selected by the Rice Research Institute of Southwest University
by EMS mutation were controlled by mononuclear genes. Xiao et al. [29] located the mutant gene
of premature aging mutant *zs* in the 600 kb region on the short arm of rice chromosome 12. The
rice premature senescence gene *PLS2* was preliminarily determined to encode a
glycosyltransferase by Wang et al [30]. In recent years, wheat premature senescence has also been reported. Two additive QTLs on chromosomes 3A and 3B detected by Wei [31] were related to wheat early senescence indicators and six physiological traits related to premature senescence. An additive QTL controlling the flag leaf senescence was located between markers gwm526 and gwm382 on the long arm of chromosome 2A [32]. The leaf senescence gene els1 was located on the chromosome of 2BS by bulked segregant RNA sequencing (BSR-Seq) method in common wheat [33].

This study reports the isolation of wheat lesion-like premature senescence mutants by EMS mutagenesis, and the genetic analysis of these mutants. The chromosomal localization of the premature aging gene was performed by the analysis of the segregating populations. This study generated germplasm resource for future cloning of new genes related to early senescence and exploring the regulatory mechanism of early senescence in wheat. It also laid a foundation for breeding new wheat varieties conferring resistance to premature senescence.

Results

Generation and identification of Impa mutant

Multi-year agronomy comprehensive identification was used to select the Impa mutant from the M6 generation induced by EMS in KD527 (Figure 1A, 1C), which was named Impa1 (Figure 1B, 1D). The agronomic identifications were conducted in KD527 and Impa1 and shown in Table 1. As can be seen from Table 1 and Figure 1, the agronomic characters of Impa1 is similar to KD527 in ph, flag leaf length (fll), ear length (el), number of grains per ear (ngpe) and other agronomic traits. The process of the formation of Impa in Impa1 was observed throughout its whole growth period. Impa1 grows normally at seedling stage. After flag leaf picking, it appeared lls before senescence. Its leaves present some brown-yellow round disease spots which can gradually enlarge and expand. After heading, the disease spots quickly spread to the leaf sheath, stem and spike of Impa1. With the extension of growth period, Impa1 emerges more and more disease spots over the whole plant and dried up even died during the filling stage (Figure 2). In addition, the number of mutant individuals increases obviously after rain during grain filling and they get worse than before rain. The reason is unclear yet.

Genetic analysis of Impa mutant

In order to clarify the inheritance and genetic effects of the Impa traits, the single plant Impa
traits and other agronomic traits of *Impa*, KD527 and CS crosses F₁ and F₂ were investigated. The results were statistically shown in Table 1 and 2. As can be seen from Table 1 and Figure 3, the KD527 plants behaved normally, *Impa* suffered from plaque-like premature aging. The plants of the constructed hybrid F₁ population all showed lesion-like premature senescence characters. The plants from the F₂ population showed two types of premature senescence plants and normal plants. Chi-square test showed that *Impa* gene is dominant and conforms to the separation ratio of single gene 3:1. *Impa* had shorter el and lower npge than that of KD527 (Table 2). The tgw and ypp in F₂ were significantly higher than those in *Impa* plants, indicating that the mutant's early-like traits could significantly reduce wheat yield. However, the reduction extent to which it causes wheat yield and whether it has other disease resistance still needs further identification. **Photosynthetic assay of Impa mutants** In order to further understand the effects of mutants on wheat photosynthetic physiology, *SPAD-502 Plus* and *LI-6400 XT* were used to measure chlorophyll content (SPAD), stomatal conductance (Cond), and transpiration rate (Tr) of KD527, *Impa*, and their hybrids in the field (Figure 4).

Physiological indicators comprising SPAD, Cond and Tr of *Impa*/KD527 F₁ are higher than that of *Impa*. However, these indicators in normal plants from F₂ population were not significantly different from that of KD527 and were significantly higher than those of *Impa* plants. It indicated that the *Impa* mutant had a significant effect on wheat photosynthetic physiological process. As a result, *Impa* affected wheat growth and development so seriously that the plant cannot age normally and premature senescence occurs, which may also be one of the reasons for reducing the thousand grain weight of single plant in wheat. **Chromosomal location of Impa gene** DNA samples from CS, *Impa*, and mixed samples of normal plants (50) and premature senescent plants (50) in the F₂ population of combination *Impa*/CS was used to construct a BSA pool for 660K SNP chip analysis. As a result, 170 polymorphic SNP loci distributed on chromosomes 1A, 2A, 3B, 4B, 5A, 5B respectively were found (Figure 5) and 164 SNP loci were located on chromosome 5A. It is presumed that the *Impa* gene is located on the 5A chromosome.
of wheat. Based on physical positions of the polymorphic SNPs in Chinese Spring (IWGSCv2.0), a genetic linkage map of SNPs linked with *Lmpa1* genes on chromosome 5A were constructed by MapChart (Figure 6). The results showed that most of the polymorphic SNPs are enriched within a 30-40Mb region near to the short arm of chromosome 5A, indicating that the *LMPA1* gene is highly possible within this region.

**Screening of candidate genes related to Lmpa1**

Based on the results of the 660K SNP chip, we used the website of JBrowse (http://202.194.139.32/jbrowse-1.12.3-release/?data=Chinese_Spring) to screen related genes in the 30-40Mb segment of the short arm of wheat 5A chromosome. A total of 120 genes were found within the 30-40Mb region of chromosome 5A. And 13 genes related to plant growth and development may be the candidate genes associated with *Lmpa1* (Table 3).

**Discussions**

Premature senescence is a phenomenon that aging of plants physiological and biochemical process in their growth period takes place earlier than that of normal plants. Premature senescence in cereal crops such as wheat, rice and corn, will affect the production of photosynthetic products and their transportation and accumulation into grains and in turn decrease grain yield. Premature aging mutants can be regarded as an important tool to understand premature senescence and benefit elucidating the PCD in plants. Precious researches on premature senescence mainly focus on rice premature senescence mutants and their gene mapping. There are few reports on the creation of wheat premature senescence mutants. This study reports the *lmpa1* mutant deriving from the EMS-induced mutant library in the KD527 background. The mutant with the characteristics of both lesion-like spots and premature senescence, will enrich the wheat premature senescence mutant library and lay the germplasm foundation for further research on the traits related to early senescence in wheat.

In this study, we characterized the mutants *lmpa1* and analyzed its photosynthetic physiology. We found that lesion-like spots and premature senescence can significantly affect *el*, seed setting rate(*ssr*), *tgw* and other agronomic traits in wheat. They can reduce the expression of chlorophyll, cause the physiological dysfunction of leaves and decrease the ability of photosynthetic assimilation. As a result, the grain filling time was shortened, the dry matter accumulation of the grain was reduced, the *ssr* and the *tgw* were affected, and the yield and quality were damaged. In
order to better understand the physiological and biochemical mechanisms of premature senescence, we will refer to Wang Beifang's [34] methods on premature aging mutants in rice. It is planned to use cell histochemical staining, determination of net photosynthetic rate and photosynthetic pigment content and determination of enzyme activity to find physiological and biochemical indicators related to senescence. In the meantime, cell morphology of mutants will be observed by transmission electron microscope. And the expression of gene related to senescence and hormone content in mutants will be analyzed. The differences in physiological and biochemical, hormone, and cell morphology between premature aging mutants and normal plants will be discussed. It has been reported that rice lesions-like mutant *spl41* [35] can enhance resistance to rice bacterial leaf blight. Therefore, disease resistance of *lmpa1* should be identified in the future.

In this study, the *Lmpa1* was located within 30-40 Mb region on chromosome 5A by using of SNP chip sequencing and BSA analysis. Up to date, there is no report of premature aging gene on the chromosome 5A in wheat. Among the 13 candidate genes, the candidate gene *TraesCS5A01G040300.1* encoding a zinc finger protein is similar to the zinc finger transcription factor found in wheat leaf premature senescence mutant *m68* [36] and may be associated with premature aging. It is important to screen premature aging genes and explore the causes and mechanisms of premature aging. It was found [37] that water deficit during grain filling period could cause premature senescence of flag leaves, but the senescence process could be delayed by changing hormone concentration of plants. There are many reasons for rice premature aging, for example, the effect of NAC transcription factors on abscisic acid (ABA) [19], the functional impairment of calcium-dependent protein kinase *OsCPK12* [22], the deletion of gene fragment [23], the response and regulation of genes related to antioxidant and carbohydrate metabolism [38], and so on. Based on the research experience of rice early senescence, further work should be focused on the cloning and functional verification of candidate gene for premature aging. The effects of premature aging on protein expression, hormone signaling pathways, and gene expressing related to metabolism, will be emphasized in order to further reveal the molecular mechanism of wheat premature senility.

**Conclusions**

We identified an EMS-mutagenized mutant *lmpa1*, which derived from elite wheat line KD527 and conferred *lmpa*. Genetic analysis indicated that the *lmpa* phenotype of *lmpa1* mutant
is controlled by a single dominant allele designated as Lmpa1, which affected wheat growth and development and reduced the tgw of single plant in wheat. By applying BSA method and 660K SNP Chip sequencing, the gene Lmpa1 was tentatively located within the region of 30-40 Mb near to the short arm of chromosome 5A.

**Methods and materials**

**Plant materials**

The materials in this study included a new wheat line KD527 bred in our laboratory, the mutant lmpa1 isolated from the EMS mutant library in the KD527 background, the hybrid populations of F1 and F2 between lmpa1 with KD527, and the hybrid F1 and F2 populations from the crossing of lmpa1 with CS. All materials from the Wheat Germplasm Innovation Group of the Henan University of Science and Technology are maintained and planted in Luoyang City, Henan Province, China.

**EMS mutagenesis**

Seeds were soaked with distilled, deionized water at room temperature for 16~20h until seeds completely absorb water and fully swell. Seeds were then treated with 0.3% EMS in phosphate buffer, pH 7 at room temperature for 4~6h. The treated seeds were then rinsed in tap water for 12h, dried for 30mins, and immediately sown in the field.

**Screening of lmpa mutants**

EMS-mutagenized KD527 seeds were grown with row spacing of 20 cm and plant spacing of 5 cm. Individual plants with lesion-like spots were identified from the M0 population materials and harvested as M1. In the second year, M1 seeds were grown in the field, evaluated for their agronomic traits during growth, and harvested as a single plant as M2. M2 were planted and evaluated on the stability of mutant traits during their growth. From M3 generation on, field investigation was conducted every 7 days. Ten plants were randomly selected from typical mutant lines and were evaluated on the agronomic traits including plant height (ph), plant panicle number (ppn), panicle length (pl), panicle grain number (pgn) and other traits. All individuals from stable mutant lines were harvested and threshed to survey ear length (el), thousand-grain weight (tgw), yield per plant (ypp) and other seed traits. The agronomy identification and stability evaluation
were carried out continuously in M4 and M5 generations. Finally, the stable mutant lmpa1 was bred in M6 generation.

**Construction of segregating population of premature senescence mutants**

lmpa1 was first crossed with KD527. The F1 seeds were harvested on a single plant basis and were planted in the field to investigate the lesion-like spot premature senescence trait and other agronomic traits during the growing period. They were harvested as F2 seeds. The lesion-like spot premature senescence trait and other agronomic traits of the individual plants in F2 population were also investigated to determine the genetic mode and genetic effect of the LMPA gene.

lmpa1 was also crossed with CS as described above. Based on the lesion-like spot premature senescence trait of the F2 population, the leaf DNA samples of typical Lmpa and normal individual plants were extracted and combined as a BSA pool for the 660K SNP chip sequencing analysis to locate LMPA gene to specific chromosome. DNA extraction from leaves was performed according to the methods described by Wang et al. [39].

**Measurement of chlorophyll content and photosynthesis activities**

After wheat heading, the chlorophyll content and photosynthesis activities of lmpa1, KD527 and their hybrid F1 and F2 populations were measured by using chlorophyll meter SPAD-502Plu (Konica Minolta, Japan) and portable photosynthesis meter LI-6400 XT (LI-COR, American) on May 1st and May 18th, 2019, respectively. The measurement methods were strictly in accordance with the operation manuals.

**Chromosomal location analysis**

Fifty typical lmpa plants and 50 normal plants selected from the lmpa1 x CS F2 population were combined respectively into two DNA BSA pools marked lmpa pool(LP) and normal pool (WT). The 660K SNP chip analysis was conducted by Zhongyujin Label Biotechnology Co., Ltd. in Beijing, China. Using the DNA pools of CS and lmpa1 as controls, the candidate chromosome segments were estimated by screening significant differences in allele frequencies (AF) of polymorphic sites (SNPs) between the two BSA pools of LP and WT respectively.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.
Availability of data and materials

The data sets supporting the results of this article are included in this manuscript.

Competing of interests

The authors declare no competing or financial interests.

Funding

This work was financially supported by the National Natural Science Foundation of China-Henan Joint fund (U1304318, U1904108) and Scientific and technological projects of Henan Province of China (202102110022).

Author’ contributions

Liming Wang and Jiaqiang Sun designed the research project; Weiwei Kong and Liming Wang performed the experiments and wrote the manuscript; Jingjing Ji, Xuefang Yan and Puhui Dong performed the experiments and managed experimental materials in the field; Weiwei Kong, Xingfeng Li and Chunping Wang analyzed the data; Liming Wang and Jiaqiang Sun edit paper; Liming Wang, Pei Cao and Honggang Wang provide funding. All authors have read and approved the final manuscript.

Acknowledgments

We thank Dr. Fa Cui from Ludong University for his guidance and help in genetic mapping and thank Dr. Zhongguo Xiong from Arizona University of the United States of America for providing language help of writing manuscript.

Author information

Agronomy College, Henan University of Science and Technology, Luoyang 471023, Henan, China;

Institute of Botany, Chinese Academy of Sciences, Beijing, 10093, Beijing, China

State Key Laboratory of Crop Biology/Agronomy College, Shandong Agricultural University, Taian, 271018, Shandong, China

Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, 10081, Beijing, China

References

1. G.S.Johal, S.H.Hulbert, S.P.Briggs. Disease lesion mimics of maize: a model for cell death in plants. Bioessays 2010: 685-692.
2. J.L. Dangl, R.A. Dietrich, M.H. Richberg. Death don't have no mercy: cell death programs in plant-microbe interactions. Plant Cell 1996: 1793-1807.

3. Z.Q. Zhong, W.L. Luo, Y.Z. Liu, H. Wang, Z.Q. Chen, T. Guo. Characterization of a novel spotted leaf mutant spl32 and mapping of spl32(t) gene in rice (Oryza sativa). Acta Agronomica Sinica 2015: 861-871.

4. G.Q. Xiao, Y.F. Zhang, B.N. Yang, B.C. Liu, J.H. Zhou, H.W. Zhang. Research progress of plant lesion mimic mutants. Molecular Plant Breeding 2017: 300-309.

5. H.P. Ma, H.P. Zhao, G.Y. Yang. Application of induced mutation technology for crop breeding. Hereditas (Beijing) 1998: 50-52.

6. B.G. Zhu, Z.X. Lu, Y.X. Geng, X.D. Deng, A.Q. Gu. Effects of peanut character variations induced by EMS and breeding of high yielding mutant strains. Scientia Agricultura Sinica 1997: 87-89.

7. Z.C. Zhang, S. Dai, D.G. Cheng, Q. Peng, Y.X. Xing, J.M. Song. Effect of EMS mutagenesis on physico-chemical properties of wheat starch. Journal of Southern Agriculture 2011: 479-482.

8. D.C. Amberg, D.J. Burke, J.N. Strathern. Ethyl methane sulfonate (EMS) mutagenesis. Csh Protocols 2006: 4180.

9. J.C. Joanna, T. Bradley J. Chemical mutagenesis of seed and vegetatively propagated plants using EMS. Current Protocols in Plant Biology 2016: 617-635.

10. Y.L. Qian. Identification of mutant traits in wheat induced by EMS. Shandong Agricultural University 2017.

11. J. Gray, P.S. Close, S.P. Briggs, G.S. Johal. A novel suppressor of cell death in plants encoded by the Lls1 gene of maize. Cell 1997: 25-30.

12. C.Y. Guo, G.H. Wu, J. Xing, W.Q. Li, D.Z. Tang, B.M. Cui. A mutation in a coproporphyrinogen III oxidase gene confers growth inhibition, enhanced powdery mildew resistance and powdery mildew-induced cell death in arabidopsis. Plant Cell Reports 2013: 687-702.

13. X.Q. Zhang, B. Tian, Y.X. Fang, T. Tong, J.J. Zheng, D.W. Xue. Proteome analysis and
phenotypic characterization of the lesion mimic mutant bspl in barley. Plant Growth Regulation 2019: 329-339.

14. R. Jiao, N.Xu, J.Hu, Z.L.Song, J.Q.Hu, Y.C.RAO, Y.X.Wang. Advances in traits of lesion mimic mutants and its molecular mechanisms in rice. Chinese Journal of Rice Science 2018: 285-295.

15. J.F. Geng, Fine mapping of wheat disease-like spot mutation gene lm3. Northwest A & F University 2018.

16. Q.Q. Li, Q.S. Zhao, H.B.Jiang, J.F.Geng, L.X.Liu, X.Y.Zhang, Y.Z.Xie, C.S.Wang. Characteristics and genetic analysis of wheat mutant I30 with white stripe pattern. Journal of Triticeae Crops 2017: 871-879.

17. Q.Yao, R.H.Zhou, T.H.Fu, W.R.Wu, Z.D.Zhu, A.L.Li, J.Z.Jia. Characterization and mapping of complementary lesion-mimic genes lm1 and lm2 in common wheat. Theoretical and Applied Genetics 2009: 1005-1012.

18. C.R.Chen, Y.J.Wu, Z.L.Wang, Y.P.Wang, S.D.Liu, X.B.Wang. Characteristics of stay-green and grain-filling and the evaluation of anti-senescence properties of winter wheat. Acta Botanica Boreali-occidentalia Sinica 2011: 715-723.

19. C.J. Mao, S.C. Lu, B.Lv, B.Zhang, J.B.Shen, J.M.He, L.Q.Luo, D.D.Xi, X.Chen, F.Ming. A rice NAC transcription factor promotes leaf senescence via ABA biosynthesis. Plant Physiology 2017: 1747-1763.

20. Y.He, L.J.Li, Z.H.Zhang, J.L.Wu. Identification and comparative analysis of premature senescence leaf mutants in rice (Oryza sativa L.). International Journal of Molecular Sciences 2018: 140-157.

21. F.F. Xu, Z.Y.Ji, J.M.Xu, F.J.Wang, Y.C.Tang, K.l.Zheng, C.L.Wang, KJ.Zhao. Identification and molecular mapping of water-soaked spot leaf early senescence mutant wss1 in rice. Journal of Plant Genetic Resources 2019: 144-151.

22. B.F.Wang, Y.X.Zhang, Z.Z.Bi, Q.N.Liu, T.T.Xu, N.Yu, Y.R.Cao, A.K.Zhu, W.X.Wu,
X.D.Zhan, G.B.Anis, P.Yu, D.B.Chen, S.H.Cheng, L.Y.Cao. Impaired function of the calcium-dependent protein kinase, OsCPK12, leads to early senescence in rice (Oryza sativa L.). Frontiers in Plant Science 2019:1-12.

23. C.Y.Zhao, C.L.Liu, Y.Zhang, Y.T.Cui, H.T.Hu, N.Jahan, Y.Lv, Q.Qian, L.B.Guo. A 3-bp deletion of WLS5, gene leads to weak growth and early leaf senescence in rice. Rice 2019:1-13.

24. F.F.Xu, X.C.Sang, D.Y.Ren, Y.Q.Tang, H.W.Hu, Z.G.Yang, F.M.Zhao, G.H.He. Genetic analysis and gene mapping of early senescence leaf mutant esl2 in rice. Acta Agronomica Sinica 2012:1347-1353.

25. R.L.Miao, Y.D.Jiang, H.X.Liao, F.F.Xu, G.H.He, Z.G.Yang, F.M.Zhao, X.C.Sang. Identification and gene mapping of rice early senescent leaf (esl3) mutant. Acta Agronomica Sinica 2013:862-867.

26. S.Guo, T.Q.Zhang, Y.D.Xing, X.Y.Zhu, X.C.Sang, Y.H.Ling, N.Wang, G.H.He. Identification and gene mapping of an early senescence leaf 4 mutant of rice. Crop Science. 2014:2713-2723.

27. X.C.Sang, F.F.Xu, X.Y.Zhu, Y.D.Xing, P.L.He, C.W.Zhang, Z.L.Yang, G.H.He. Identification and gene fine mapping of early senescent leaf mutant esl5 in oryza sativa. Acta Agronomica Sinica 2014: 1182-1189.

28. B.Yang, M.Xin, X.B.Zhang, X.W.Wang, X.Y.Zhu, P.L.He, G.H.He, X.C.Sang. Identification and gene mapping of an early senescent leaf mutant esl6 in oryza sativa L.. Acta Agronomica Sinica 2016: 976-983.

29. L.J.Xiao, J.Huang, P.H.Cao, C.l.Mou, Thanhliem Nguyen, S.J.Liu, L.M.Chen, L.Jiang. Analysis and gene mapping of rice premature senescence mutant zs. Journal of Nanjing Agricultural University 2018:793-800.

30. M.Wang, T.Zhang, H.Peng, S.Luo, J.J.Tan, K.F.Jiang, Y.Q.Heng, X.Zhang, X.P.Guo, J.K.Zheng, Z.J.Cheng. Rice premature leaf senescence 2, encoding a glycosyltransferase (GT), is involved in leaf senescence. Frontiers in Plant Science 2018:560-573.
31. X.Y. Wei, S.S.Li, F.S.Jiang, Y.Guo, R.J.Li. QTL mapping for premature senescence and related physiological traits in wheat. Acta Botanica Boreali-occidentalia Sinica 2007: 485-489.

32. H.Q.Wu, T.X.Liu, T.T.Li, P.Zhao, C.L.Li, Z.H.Wang, L.Quan. QTL mapping for early aging of flag leaf in wheat. Acta Botanica Boreali-occidentalia Sinica 2016: 1962-1967.

33. M.M.Li, B.B.Li, G.H.Guo, Y.X.Chen, J.Z.Xie, P.Lu, Q.H.Wu, D.Y.Zhang, H.Z. Zhang, J.Y.Yang, P.P.Zhang, Y.Zhang, Z.Y.Liu. Mapping a leaf senescence gene els1 by BSR-Seq in common wheat. Crop Journal 2018: 236-243

34. B.F.Wang, Y.Y.Chen, Y.X.Zhang, Q.E.Liu, B.Sun, X.J.Xiang, Y.R.Cao, S.H.Cheng, L.Y.Cao. Identification and fine mapping of an early senescent leaf mutant es5 in oryza sativa L.. Scientia Agricultura Sinica 2018:613-625.

35. Y.J.Ren, B.G.Zhu, J.Tao, C.Z.He, X.L.Niu. Phenotypic and physiological analysis of a rice lesion mimic mutant spl4l. Molecular Plant Breeding 2020:1-14.

36. Q.Zhang, C.Xia, L.C.Zhang, C.H.Dong, X.Liu, X.Y.Kong. Transcriptome analysis of a premature leaf senescence mutant of common wheat (Triticum aestivum L.), International Journal of Molecular Sciences 2018:782-800.

37. Y.L.Luo, D.W.Pang, M.Jin, J.Chen, X.Kong, W.Q.Li, Y.L.Chang, Y.Li, Z.L.Wang. Identification of plant hormones and candidate hub genes regulating flag leaf senescence in wheat response to water deficit stress at the grain-filling stage. Plant Direct 2019:1-23.

38. Z.W.Li, X.F.Pan, X.D.Guo, K.Fan, W.X.Lin. Physiological and transcriptome analyses of early leaf senescence for ospls1 mutant rice (Oryza sativa L.) during the grain-filling stage. International Journal of Molecular Sciences 2019:1-22.

39. Y.Wang, H.Z.Zhang, J.Z.Xie, B.M.Guo, Y.X.Chen, H.Y.Zhang, P.Lu, Q.H.Wu, M.M.Li, D.Y.Zhang, G.H.Guo, J.Yang, P.P.Zhang, Y.Zhang, X.C.Wang, H.Zhao, T.J.Cao, Z.Y.Liu. Mapping stripe rust resistance genes by BSR-Seq:YrMM58 and YrHY1 on chromosome 2AS in Chinese wheat lines Mengmai 58 and Huaiyang 1 are Yr17. Crop Journal 2018:91-98.
FIGURE LEGENDS

Figure 1 Phenotypes of the WT KD527 (A, C) and the mutants lmpa1 (B, D) during late heading (A, B) and mid-late filling stage (C, D), respectively.

**Note:** A: WT KD527 grows normally during late heading stage; B: A small amount of brown spots can be found on the leaves of mutant lmpa1 at the late heading stage; C: KD527 grows normally at mid-late filling stage; D: Lls expand quickly to the leaves, stems and even spikes of lmpa1 and premature aging appears during mid-late filling stage.

Figure 2 Formation, expansion and spread of lmpa of lmpa1 mutant at different growth stages after heading in 2019.

**Note:** A: lmpa1 single plants have not been found lls on 23\textsuperscript{th} April; B: lmpa1 leaves have a small amount of brown spots on 24\textsuperscript{th} April; C: lmpa1 leaves have a significant increase in brown spots on 26\textsuperscript{th} April; D: A large number of brown-yellow spots spread on the stem of lmpa1 on 30\textsuperscript{th} April; E: lls on the leaf spread to the leaf sheath, and a few brown-yellow spots appeared on the stem of lmpa1 on 4\textsuperscript{th} May; F: The brown and yellow spots on the stems continued to increase, and a few brown and yellow spots appeared on the spikes of lmpa1 on 16\textsuperscript{th} May; G: lmpa1 leaves began to dry, and the brown and yellow spots on the spikes continued to increase on 20\textsuperscript{th} May; H: lmpa1 leaves and stems were withered, part spikes were drying up on 24\textsuperscript{th} May.

Figure 3 Phenotype of spikes (a) and flag leaves (b) of KD527(A) and lmpa1(E) and the F\textsubscript{1} (B) and F\textsubscript{2} (C, D) offspring of lmpa1/KD527 (bar = 2 cm)

**Note:** A: KD527, WT; B: lmpa1/KD527 F\textsubscript{1}, lmpa; C: lmpa1/KD527 F\textsubscript{2} lmpa; D: lmpa1/KD527 F\textsubscript{2}, WT; E: lmpa1, lmpa.
Figure 4 Determination of photosynthetic physiological indexes of KD527 and *lmpa1* and their hybrids

**Note:** A: relative chlorophyll content (SPAD); B: stomatal conductance (mmol m\(^{-2}\) s\(^{-1}\)); C: transpiration rate (g m\(^{-2}\) h\(^{-1}\)); a,b,c: significant difference at 0.05 level

Figure 5 Distribution of polymorphic SNPs on each chromosome

Figure 6 Genetic linkage map of SNPs related to premature aging gene on chromosome 5A

**Notes:** The red segment indicates the estimated centromeric region. The rectangle in green on the right of the chromosome indicates the estimated chromosomal region of the gene *Lmpa1*.

SUPPLEMENTARY FILES

**Table S1** Statistical analysis of *lmpa* traits in mutants and their hybrid progenies

**Table S2** Agronomic traits of KD527, *lmpa1* and their hybrids

**Table S3** List of Candidate Genes Related to Wheat Early Aging
### Table 1 Statistical analysis of *Impa* traits in mutants and their hybrid progenies

| Material/Generations | Phenotype | Separation | Theoretical | X² | P |
|----------------------|-----------|------------|-------------|----|---|
| KD527                | /         | WT         | All         | /  | / |
| *Impa1* F₁           | /         | All        | /           | /  | / |
| *Impa1*/KD527 F₂     | 10        | 0          | 10          | 3.44 | 120:40 | 1.008 | 0.465 |

Note: df=1; $X^2_{0.05}=3.841$, $X^2_{0.01}=6.635$

### Table 2 Agronomic traits of KD527, *Impa1* and their hybrids

| Materials | *ph* (cm) | *fl* (cm) | *el* (cm) | *ngpe* | *tgw* (g) | *hpp* (g) |
|-----------|-----------|-----------|-----------|--------|-----------|-----------|
| KD527     | 58.3±2.9 a| 17.4±0.4 a| 9.7±0.5 a | 62.7 a | 54.45 a   | 27.31 a   |
| *Impa1*   | 50.3±3.3 c| 17.2±0.6 ab| 8.8±0.6 c | 52.5 c | 42.17 c   | 17.71 c   |
| F₁        | 55.9±4.5 ab| 16.8±0.7 b | 9.5±0.5 ab | 57 b   | 44.67 bc  | 20.37 bc  |
| *Impa1*/F₂| *Impa*  | 52.6±2.1 bc| 16±0.5 c  | 9.1±0.7 bc | 57.51 b | 43.50 c | 20.01 bc |
| KD527     | F₂       | WT        | 54.6±2.2 b| 16.6±0.2 b| 9.4±0.4 ab | 59.3 ab   | 49.17 b   | 23.33 b |

Note: a,b,c: significant difference at 0.05 level
Table 3 List of Candidate Genes Related to Wheat Early Aging

| Gene name                            | Gene annotation                        | Gene length (bp) | Protein length (aa) |
|--------------------------------------|----------------------------------------|------------------|---------------------|
| TraesCS5A01G034300.1                 | Protein kinase superfamily protein     | 1194             | 397                 |
| TraesCS5A01G035200.1                 | Protein kinase family proteins         | 2346             | 781                 |
| TraesCS5A01G037100.1                 | Kinase family proteins                 | 1831             | 477                 |
| TraesCS5A01G043600.1                 | Protein kinase family proteins         | 2148             | 715                 |
| TraesCS5A01G038300.2                 | Auxin response factor                  | 4008             | 899                 |
| TraesCS5A01G039100.1                 | peroxidase                             | 1250             | 340                 |
| TraesCS5A01G039400.1                 | peroxidase                             | 1129             | 277                 |
| TraesCS5A01G039500.1                 | peroxidase                             | 1400             | 340                 |
| TraesCS5A01G040300.1                 | Zinc finger protein                    | 1246             | 287                 |
| TraesCS5A01G040500.1                 | Remorin                                | 1800             | 449                 |
| TraesCS5A01G041500.1                 | Myb-related transcription factor       | 390              | 309                 |
|                                      | Pentapeptide repeat superfamily protein| 1248             | 266                 |
| TraesCS5A01G041600.2                 | protein                                | 1660             | 446                 |
| TraesCS5A01G042500.1                 | Protein TRIGALACTOSYLDIACYLGLY CEROL 2  |                  |                     |
Figure 1

A

B

C

D
Figure 2
Figure 3

(b)
Figure 4

(A)

(B)
Figure 5
Figure 6