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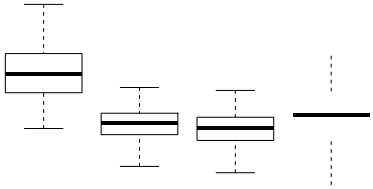
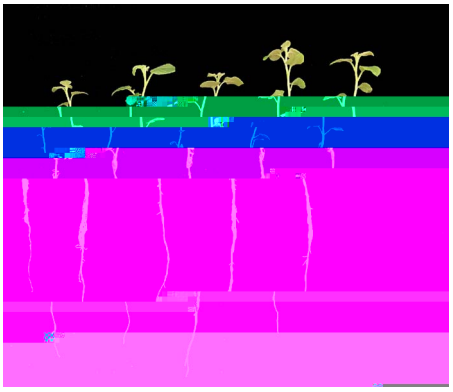
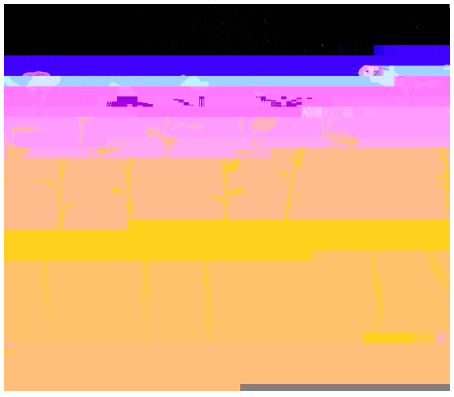






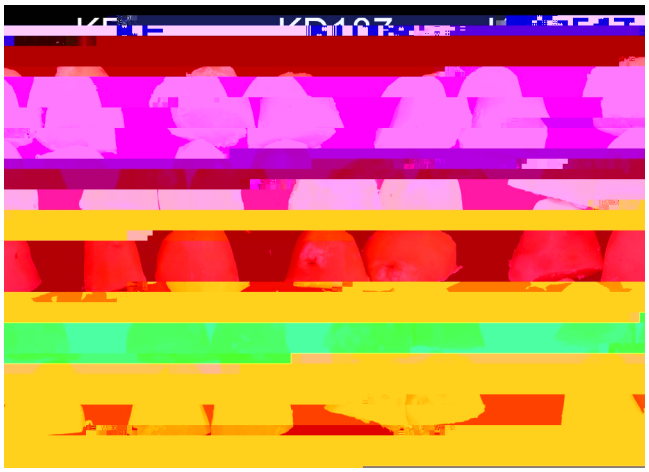
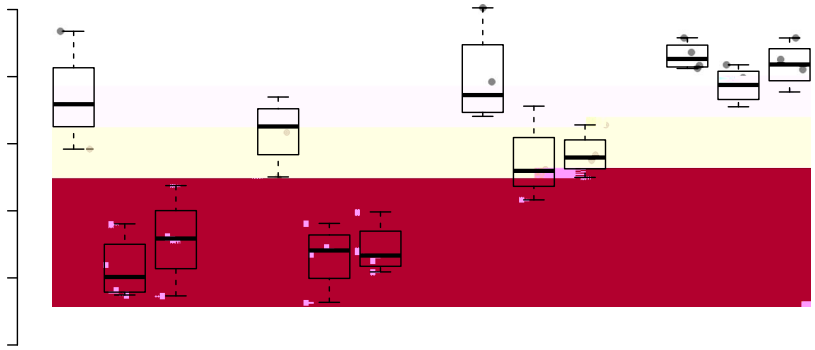


Figure 5. Cooking quality test of five tubers of each line from the field trial in 2023. KE denotes the cultivar King Edward background, and KD denotes the *Sd 6-1* mutant lines.

Data from field trials

Field resistance to late blight

In our previous publication with the *Sd 6-1* mutant lines, we showed increased resistance to *P. infestans* in controlled conditions [24]. In the present study, we have collected four years of field trial data, quantifying the field resistance to complex natural *P. infestans* infestation [40]. The disease was manually scored as percentage of symptomatic foliage twice a week, and the severity was then quantified by the area under the disease progression curve (AUDPC) (Fig. 6), as this is a standard method for pathogen symptom scoring in the field and the method of quantification recommended by the International Potato Center (CIP) when investigating field resistance to the polycyclic disease late blight [41]. During the years 2020, 2021, and 2022, significantly lower disease severity was observed in both mutant lines as compared to KE (background). Generally, the disease progression curves followed a gradual increase starting mid- or end of July during these years, and the mutant lines had a slower increase of disease. In 2023, however, a significant disease reduction was observed only in the line KD187, while there was no significant decrease in disease in the line KD517 (Fig. 6). We speculate that this deviation from the trend of the previous years could be influenced by the special weather and disease pattern of the 2023 growing season. The start of the season was exceptionally dry, while the latter half was exceptionally humid. Disease onset started when the weather changed, around a week into August, and progression was quicker than any of the previous years, reaching complete infection in



Together, these results demonstrate the prospect of *Sd 6-1* mutants as valuable assets in future sustainable potato cultivation, which come without any apparent trade-offs.

Materials and methods

Plant material and propagation

The tetraploid potato cultivar King Edward (KE), the background genotype, along with lines of KE with *Sd 6 1* knocked out using CRISPR/Cas9 described by Kieu *et al.* [24], were maintained in vitro by sub-culturing stem internodes every 3 to 4 weeks. For experimental use, apical shoots with two to three leaves were sub-cultured and left for 7 days to allow root development, before transference to experimental setups in a hydroponic system or soil. All propagation was done onto 90 × 25 mm Petri dishes

position 55.75289, 13.04872), with a rotation scheme of at least four years. The experimental design was as described in Bubolz *et al.* [40], with four randomized blocks. The only change was that the number of plants was increased from 10 to 16 per row (plot) in 2023, while the planting distance remained the same. Tubers were harvested by individual rows in the middle of September each year. Yield was measured in kg per plot, and then normalized to ton ha^{-1} with consideration of number of plants per plot each year. Late blight scoring was done as described by Bubolz *et al.* [40], from mid-June until the end of August. Late blight disease incidence based on area under the disease progress curve (AUDPC) was calculated according to Simko [45]. The whole field was sprayed against aphids with Fibro (paraffin oil) once a week, and

11. Moon KB, Park SJ, Park JS. *et al.* Editing of StSR4 by Cas9-RNPs confers resistance to Phytophthora infestans in potato. *F [illegible] Pla[illegible] Sci*. 2022;13:997888
12. Sun K, Schipper D, Jacobsen E. *et al.* Silencing susceptibility genes in potato hinders primary infection of *Ph. h h a i fe. a* at different stages. *H . ic Re* . 2022;9:uhab058
13. Sun K, Wolters AM, Vossen JH. *et al.* Silencing of six susceptibility genes results in potato late blight resistance. *T a e ic Re* . 2016;25:731–42
14. Peng Y, Yang J, Li X. *et al.* Salicylic acid: biosynthesis and signaling. *A Re Pla Bi l*. 2021;72:761–91
15. Zhang Y, Zhao L, Zhao J. *et al.* S5H/DMR6 encodes a salicylic acid 5-hydroxylase that fine-tunes salicylic acid homeostasis. *Pla Ph i l*. 2017;175:1082–93
16. Hasley JAR, Navet N, Tian M. CRISPR/Cas9-mediated mutagenesis of sweet basil candidate susceptibility gene ObDMR6 enhances downy mildew resistance. *PL S O e*. 2021;16:e0253245
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