

Reconfiguring the wild-rice *RBA1* locus breaks the trade-off between chilling tolerance and blast resistance

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Dear Editor,

Rice adaptation is constrained by opposing selection on biotic and abiotic stress responses across agroecological environments¹⁻³. Chilling stress limits seedling establishment and adaptation to different growing regions, particularly at high latitudes, during early-season cultivation, and in areas prone to spring cold spells. Rice blast also poses a major threat to yield stability, especially under warm and humid conditions⁴⁻⁷. Although substantial progress has been made in identifying and deploying loci for chilling tolerance^{8,9} and blast resistance¹⁰⁻¹², improving both traits simultaneously remains challenging because of the uneven distribution of favorable alleles among rice subspecies, linkage drag, and complex genetic interactions¹³⁻¹⁵. Here, we identified *RESISTANCE TO BIOTIC AND ABIOTIC STRESS 1* (*RBA1*) from wild rice as a key locus underlying the trade-off between chilling tolerance and blast resistance. Rather than representing a conventional single gene, *RBA1* is a supergene-like region defined by structural variation, and its alternative haplotypes have been differentially maintained during rice domestication and ecological adaptation. This genetic architecture provides a framework for understanding why simultaneous improvement of chilling tolerance and blast resistance has been difficult in breeding, and it also offers a clear target for the rational dissection and redesign of this trade-off.

Through screening of wild rice germplasm resources, we identified a wild rice accession GX2 (accession number YD2-0636) with significantly enhanced chilling tolerance at the seedling stage (Fig. 1a, b). Further genetic analysis revealed a major chilling-tolerance QTL on chromosome 11, named *RBA1* (Supplementary Fig. S1). Fine mapping together with high-quality genome assembly showed that the critical variation at this locus is not a conventional small sequence polymorphism, but a presence-absence variant in which chilling-sensitive *indica* rice variety PL (PERUBAK LUEY) carries an additional 25.7-kb insertion absent from GX2 (Fig. 1c). This insertion contains three predicted genes, *ORF1*, *ORF2*, and *ORF3*, all of which encode atypical NLR-related proteins (Supplementary Figs. S2, S3). Functional analyses demon-

strated that these three genes jointly account for the chilling-sensitive phenotype associated with the insertion haplotype. Knocking out any one of them in the PL background significantly improved seedling survival after chilling treatment, whereas introducing or overexpressing them in a *japonica* background (ZH11) reduced chilling tolerance (Fig. 1d-g; Supplementary Figs. S4, S5). These results indicate that the core genetic effect of *RBA1* is defined by the insertion itself, with the deletion haplotype being favorable for chilling tolerance and the insertion haplotype being unfavorable for seedling performance under low temperature. In this sense, *RBA1* is best understood not as an acquired determinant of chilling tolerance, but as a structural module that suppresses low-temperature adaptation in specific genetic backgrounds, such that its absence becomes the favorable state for cold adaptation.

Importantly, the effect of *RBA1* on blast resistance is not parallel to its effect on chilling tolerance. Blast inoculation assays showed that materials carrying the 25.7-kb insertion displayed stronger disease resistance (Supplementary Fig. S6). Overexpression of *ORF1* enhanced blast resistance in a *japonica* background, whereas loss of *ORF1* increased susceptibility, while *ORF2* and *ORF3* made little detectable contribution to blast resistance (Fig. 1h, i). Thus, the insertion haplotype contains a functionally asymmetric set of linked genes in which *ORF1* is beneficial for blast resistance, while *ORF1*, *ORF2*, and *ORF3* together reduce chilling tolerance. The long-recognized negative association between chilling tolerance and blast resistance therefore reflects not a diffuse physiological correlation, but a discrete and genetically tractable architecture encoded by a structurally defined haplotype. Together with the observed differences in reactive oxygen species accumulation and peroxidase activity under chilling stress and pathogen infection (Supplementary Figs. S6, S7), these results suggest that *RBA1* may influence stress-associated redox responses under chilling stress and pathogen infection, and that the three *ORFs* at this locus function in a shared genetic pathway or convergent regulatory process, whereas the downstream molecular mechanisms remain to

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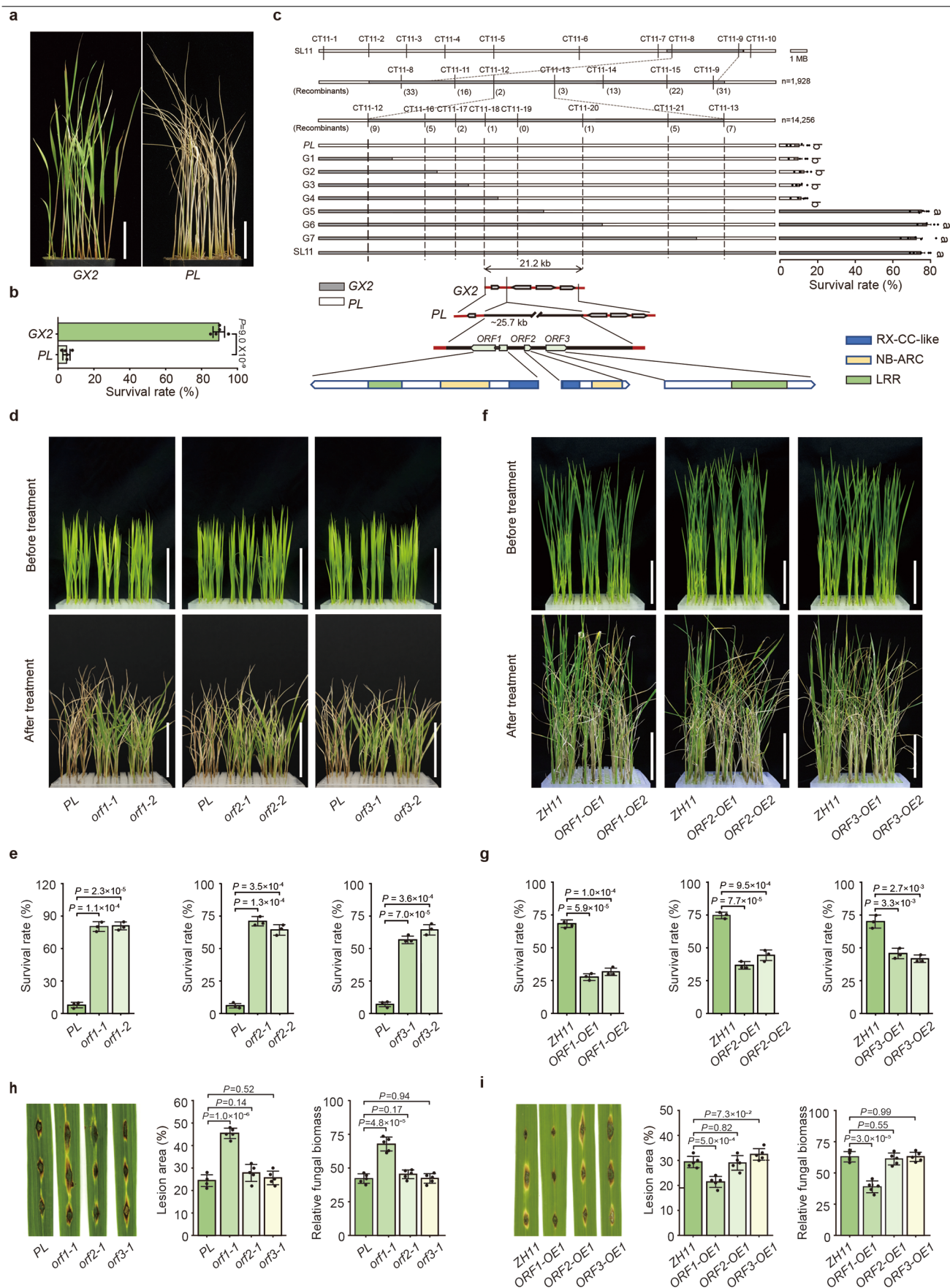


Fig. 1 The *RBA1* locus underlies antagonistic effects on chilling tolerance and blast resistance. **a** Chilling phenotypes of GX2 and PL seedlings after 60 h at 4 °C followed by 7 d of recovery. Scale bars, 3 cm. **b** Survival rates of GX2 and PL seedlings shown in **a**. **c** Fine mapping of *RBA1* using two randomly segregating populations ($n = 1,928$ and 14,256). Survival rates of representative recombinants are shown on the

right. The candidate interval was delimited to a 21.2-kb region, and comparison between GX2 and PL revealed an approximately 25.7-kb insertion in PL containing three predicted genes, *ORF1*, *ORF2* and *ORF3*. Domain structures of the encoded proteins are shown below. Different letters above the bars indicate significant differences ($P < 0.01$, one-way ANOVA followed by Tukey's HSD test). **d** Chilling phenotypes of PL and the indicated knockout lines (*orf1*, *orf2* and *orf3*) before treatment and after 60 h at 4 °C followed by 7 d of recovery. Scale bars, 6 cm. **e** Survival rates of the seedlings shown in **d**. **f** Chilling phenotypes of ZH11 and the indicated overexpression lines (*ORF1*-OE, *ORF2*-OE and *ORF3*-OE) before treatment and after 96 h at 4 °C. Scale bars, 6 cm. **g** Survival rates of the seedlings shown in **f**. **h** Blast resistance of PL and representative knockout lines (*orf1*-1, *orf2*-1 and *orf3*-1) at 7 d after punch inoculation with the *M. oryzae* isolate Guy11, with representative lesion images, lesion area quantification and relative fungal biomass shown. **i** Blast resistance of ZH11 and representative overexpression lines (*ORF1*-OE1, *ORF2*-OE1 and *ORF3*-OE1) at 7 d after punch inoculation with the *M. oryzae* isolate Guy11, with representative lesion images, lesion area quantification and relative fungal biomass shown. In **b**, **e** and **g**–**i**, values are means $\pm$ SD ($n = 3$ (**b**, **e** and **g**) or 5 (**h** and **i**) biological replicates). P values were calculated by two-sided Student's t -test against the corresponding control.

be resolved.

The contrasting population distributions of the two *RBA1* haplotypes suggest that their relative adaptive values may differ among agroecological contexts (Fig. 2a, b; Supplementary Fig. S8). The insertion-bearing haplotype contains a component that contributes to blast resistance, whereas the deletion haplotype is associated with enhanced chilling tolerance. These contrasting effects may have influenced the distribution of the two haplotypes across both rice populations and cultivation regions. However, the current population data are correlative and do not directly demonstrate opposing natural selection or trait-driven maintenance and fixation during rice domestication. *RBA1* is therefore better viewed as a structurally defined trade-off locus with context-dependent value, whose linked components have distinct effects on chilling tolerance and blast resistance.

From a breeding perspective, the greatest value of *RBA1* lies in its separability and reconfigurability, and more specifically in the fact that it offers three distinct solutions for three different ecological settings. In warm and humid *japonica*-growing regions where blast pressure is high but chilling stress is relatively limited, *ORF1* can be introduced as a favorable disease-resistance module into elite local cultivars to strengthen blast resistance. This strategy was validated in the elite *japonica* cultivar Nan-Jing 9108 (NJ9108), in which introduction of *ORF1* with its native promoter significantly enhanced blast resistance, demonstrating that the beneficial disease-resistance component of *RBA1* can be extracted and deployed independently to address the common problem of strong chilling tolerance but insufficient blast resistance in temperate-adapted *japonica* germplasm (Fig. 2c; Supplementary Fig. S9).

In contrast, in southern double-cropping systems or *indica*-growing regions characterized by high temperature and humidity but also by transient low-temperature episodes during early spring seedling establishment, *ORF2* and potentially *ORF3* can be knocked out by targeted editing to improve chilling tolerance while retaining the blast-resistance benefit conferred by *ORF1*. This strategy was demonstrated in the elite early-season *indica* cultivar Mei-Xiang-Zhan 2 (MXZ2), in which knockout of *ORF2* significantly improved seedling chilling tolerance without an obvious penalty in blast resistance or agronomic performance, suggesting that this approach may be well suited for environments requiring both disease resistance and safe establishment under episodic chilling (Fig. 2d; Supplementary Fig. S9 and Table S4). A third strategy applies to regions where low-temperature adaptation is the primary breeding objective and the stability of hybrid rice parents and combinations under cold-prone conditions must be

enhanced. In this case, the favorable *RBA1*^{GX2} allele can be introduced to replace or reconstruct the *RBA1* region, thereby improving cold adaptation. Introgression of the *RBA1*^{GX2} allele into PA64s enhanced chilling tolerance in the sterile line, and the resulting hybrid combination also exhibited stronger low-temperature adaptation while maintaining stable yield-related traits (Fig. 2e; Supplementary Fig. S9 and Table S4). This result shows that *RBA1* is useful not only for improving varieties but also for optimizing hybrid rice parents and combinations, particularly for higher-latitude rice-growing areas or regions exposed to low-temperature risk. Taken together, these results establish three breeding routes centered on *RBA1*: introducing *ORF1* improves blast resistance in *japonica* regions prone to disease; editing *ORF2* balances blast resistance and chilling tolerance in southern early-season *indica* systems; and substituting the *RBA1*^{GX2} allele enhances chilling tolerance in hybrid rice, though this particular approach is not designed to confer blast resistance. This ecology-oriented deployment of different genetic components highlights *RBA1* not merely as a locus explaining natural variation and domestication history, but as a practical breeding target that can be configured to address production constraints (Fig. 2f). However, broader validation across additional germplasm and field environments will be required before general breeding recommendations can be made.

Overall, *RBA1* reveals how a structurally defined supergene-like locus can simultaneously shape low-temperature adaptation and disease resistance in rice, and explains why it is difficult to improve these traits together through conventional breeding. More importantly, our results show that this trade-off is not immutable. Once the internal functional components of the locus are resolved, unfavorable linkage can be converted into a designable breeding target. Favorable wild rice alleles are underutilized in breeding, not because they lack value, but because their beneficial effects are genetically linked to undesirable components. *RBA1* illustrates that when the genetic composition, functional partitioning, and background-dependent utility of such loci are clearly defined, targeted improvement can be achieved through introgression, allele substitution, or genome editing. *RBA1* therefore provides not only a new genetic resource for the coordinated improvement of chilling tolerance and blast resistance, but also a broader framework for the molecular design of complex adaptive traits in crop breeding. More generally, this study suggests that many trait combinations long regarded as difficult to reconcile may similarly be determined by structural variants or tightly linked modules, and that once these modules are accurately identified and functionally dissected, long-standing breeding barriers can be overcome for preci-

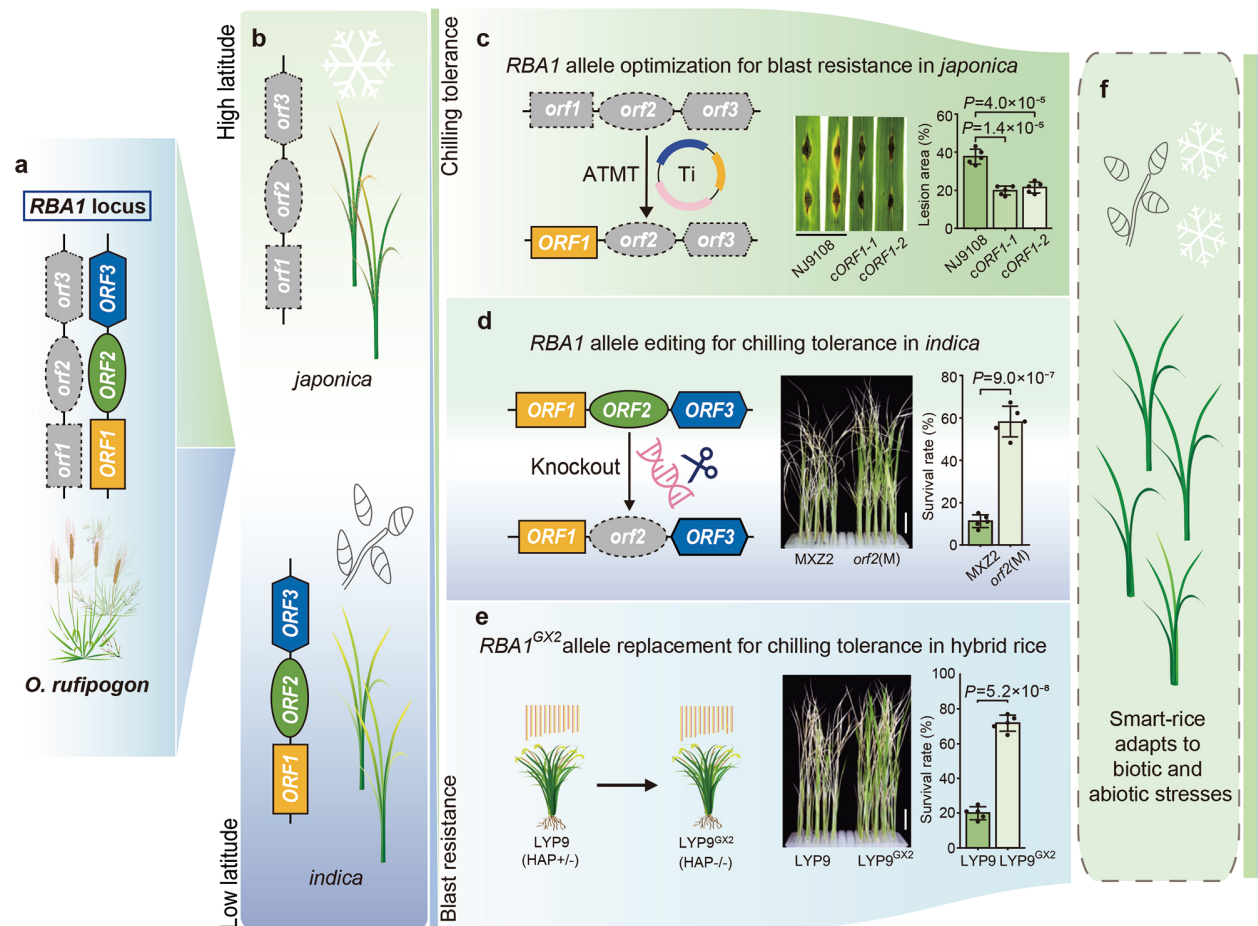


Fig. 2 Stepwise reconfiguration of *RBA1* enables ecology-specific improvement of chilling tolerance and blast resistance in rice. **a** Schematic of the *RBA1* locus from *O. rufipogon*. **b** Model of ecological differentiation of alternative *RBA1* haplotypes, showing that the deletion haplotype (Hap⁻) is associated with chilling tolerance in *japonica*, whereas the insertion haplotype (Hap⁺) is retained in *indica* and contributes to blast resistance. **c** Strategy for improving blast resistance in *japonica* by introducing *ORF1* into the elite cultivar NJ9108 through *Agrobacterium*-mediated transformation (ATMT). Representative blast lesions and lesion area quantification are shown for NJ9108 and the *ORF1*-introduced lines cORF1-1 and cORF1-2 at 7 d after punch inoculation with the *M. oryzae* isolate Guy11. **d** Strategy for improving chilling tolerance in *indica* by targeted editing of the *RBA1* insertion haplotype. Representative seedling phenotypes and survival rates are shown for MXZ2 and the *orf2*(M) line after 60 h at 4 °C followed by 5 d of recovery. *orf2*(M), *ORF2* knockout mutant in the MXZ2 background. Scale bar, 6 cm. **e** Strategy for improving chilling tolerance in hybrid rice by replacing the *RBA1* region with the *RBA1*^{GX2} allele. Representative seedling phenotypes and survival rates are shown for LYP9 and LYP9^{GX2} after 60 h at 4 °C followed by 5 d of recovery. Scale bar, 6 cm. **f** Conceptual summary showing that stepwise dissection and reconfiguration of *RBA1* enables the design of rice adapted to both biotic and abiotic stresses. In **c–e**, values are means $\pm$ SD ($n = 5$ biological replicates). P values were calculated by two-sided Student's *t*-test against the corresponding control.

sion improvement.

(2021YFA1300703).

DATA AVAILABILITY

The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. The genomic information of PL has been uploaded to the National Genomics Data Center (BioProject accession number: PRJCA069795, ngdc.cnpc.ac.cn). The gene accession numbers for *ORF1*, *ORF2*, and *ORF3* are PL_Os11gG020480, PL_Os11gG020490, and PL_Os11gG020500, respectively.

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AUTHOR CONTRIBUTIONS

Q.Q., X.Z. and L.S. conceived and designed the experiments. B.Z. and Y.P. created genetic populations and materials. B.Z., J.X., W.L., M.G., Z.G., J.H., G.G. and Y.J. performed the experiments. L.S. and Z.Z. performed genome annotation. B.Z. drafted the manuscript. G.X., K.C., Q.Q. and X.Z. reviewed and revised the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at <https://doi.org/10.15302/vita.2026.09.0073>.

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