CROP BREEDING AND APPLIED BIOTECHNOLOGY

CULTIVAR RELEASE

Hanyou 3015: a water-saving and drought-resistance rice cultivar for dry cultivation in southern China

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Abstract: *Hanyou 3015 is a new Indica hybrid of water-saving and drought-resistance rice (WDR), combining rapid maturation and high yield potential. Whole genome sequencing results show that Hanyou 3015 carries many superior alleles that could explain its drought tolerance, high water use efficiency, rapid maturation, and wide adaptability.*

Keywords: *Rice, breed, water-saving and drought-resistance rice, whole genome sequencing, drought tolerances*

INTRODUCTION

Rice is the main food grain crop in China, and more than one-half of the population eats rice as the staple food. Consequently, providing for security of the rice supply becomes very necessary. However, rice production in China continues to face many challenges, such as drought and flooding (Luo et al. 2019). The water shortage in agriculture in China is about 30 billion cubic meters, and the annual agricultural losses caused by water shortage are more than 150 billion yuan. The first aspect of these challenges is that rice growing is the largest user of agricultural water, representing about 70% of the total water used in agriculture in China and consuming about 50% of all the water used in the country (Luo et al. 2019, Zhang et al. 2022). Drought is the most significant environmental stress in rice production. The occurrence of drought periods throughout the growth period of rice often leads to direct reductions in yield, especially during the reproductive growth period. Drought affects about 42 million hectares of rice in rainfed fields around the world annually, with yield reductions of up to 35% (Lanceras et al. 2004, Yue et al. 2006). Secondly, the excessive use of irrigation water and the use of chemical fertilizers and pesticides in rice production have resulted in significant agricultural pollution and massive emissions of greenhouse gases, such as methane. For that reason, our aim is to breed water-saving and drought-resistance rice (WDR), which facilitates a new low-carbon, energy-saving, and environmentally friendly rice production system, mitigating the contradiction between supply and demand of water resources in China (Yu et al. 2016).

The Shanghai Agrobiological Gene Center has taken WDR from concept to theory and practice and has successively bred Huhan 15, Huyou 2, Hanyou

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73, Hanyou 113, Huhan 549, and other WDRs (Luo et al. 2019). These WDR varieties have been widely cultivated in southern China (Yu et al. 2016, Liu et al. 2022). Yet, WDR currently faces many challenges, for example, excessive plant height, a long whole growth period, and high seed production costs. Therefore, we developed the new WDR hybrid combination Hanyou 3015, which was registered and released for commercial production by the national crop variety appraisal committee in 2020. Hanyou 3015, derived from the CMS (cytoplasmic male sterility) line Huhan 7A and the restorer line Hanhui 3015, has the advantages of the water-saving trait, drought tolerance, early maturity, high yield, and wide adaptability. Its traits, breeding program, and crop management technology are presented in this article.

MATERIAL AND BREEDING METHOD

Hanyou 3015, derived from the CMS line Huhan 7A and the restorer line Hanhui 3015, is a new *Indica* hybrid rice combination developed by Shanghai Agrobiological Gene Center. The overall breeding scheme of Hanyou 3015 is shown in Figure 1.

Huhan 7A, derived from the testcross of an elite single plant selected from the progeny of the cross between the water-saving and drought-resistance maintainer line Huhan 1B and the fragrant maintainer line 'WuxiangB/NeixiangB' to Huhan 1A, followed by successive backcrosses, is a high-quality CMS line in rice. It has water-saving and drought-resistance traits, stable male sterility, fine grain quality, high outcrossing rate, and strong lodging resistance.

Hanhui 3015, derived from the cross between Hanhui 3 and Huanghuazhan, is an elite *Indica* CMS rice restorer line. The parent of Hanhui 3 is water saving and drought-resistance and has strong lodging resistance, whereas the other

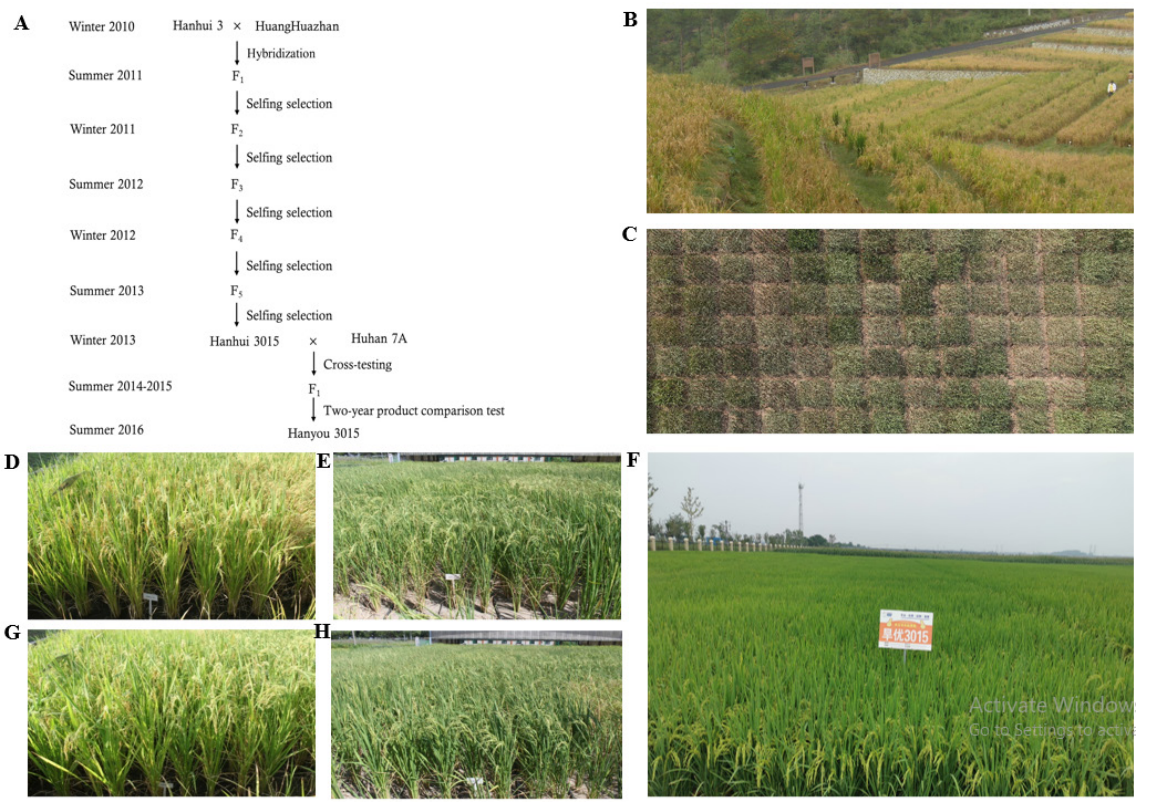

Figure 1. Breeding process and identification of drought tolerance of Hanyou 3015. A. The breeding pedigree. B. Screening for drought tolerance in the mountainous area of Zhejiang Province in F_3 populations, and single plants with good drought tolerance were selected. C. Screening for drought tolerance during the dry season of Hainan in F_4 strains, and the strains with good drought tolerance were selected. D. Hanyou 3015 – water treatment photo of the field. E. Hanyou 3015 – drought treatment photo of the field. F. The large-scale dry cultivation of Hanyou 3015 in Anhui Province, 2021. G. Hanyou 73 – water treatment photo of the field. H. Hanyou 73 – drought treatment photo of the field.

parent, Huanghuazhan, is well adapted to the ecosystems of southern China and exhibits high yield with good grain quality. The F_1 generation was cultivated in the 2011 summer season, while F_2 seeds were harvested in the 2011 winter season. The F_3 generation was directly seeded in the upland area to screen for drought-resistance in the 2012 summer season, and single plants with good drought-resistance were selected. In 2012, the F_4 strains were cultivated during the dry season of Hainan to continue screening for drought tolerance, and the strains with good drought tolerance were selected. The seeds gave rise to the F_6 generation, grown in the 2013 cycle. Through crossing with CMS lines and identification of hybrid advantages, one of the elite F_6 inbred lines was selected and designated as Hanhui 3015 (Figure 1).

Table 1. Agronomic and morphological traits of the WDR cultivar Hanyou 3015 according to the distinctness, uniformity, and stability (DUS) tests performed during the 2018 and 2019 crop cycles in Shanghai

Descriptor	Phenotype
Flag leaf	Semi-erect
Leaf blade pubescence	Absent
Leaf ligule length	Medium
Leaf sheath color	Green
Culm length (cm)	Medium to long (98.17)
Panicle length (cm)	Medium to long (25.83)
Presence of awns	Absent
Spikelet stigma color	White
Spikelet glume color	Pale yellow
Length of hulled grains (mm)	8.39
Number of grains per spike	230.5
Seed setting rate (%)	93.33
Thousand seed weight (g)	26.9

The hybrid combination of Hanyou 3015, derived from Huhan 7A and Hanhui 3015, was evaluated in the national yield test network: regional trials (2018 and 2019) and the value for cultivation and production trail (2019) in southern China. In addition, distinctness, uniformity, and stability testing (DUS) of Hanyou 3015 were performed at Ministry of Agriculture and Rural Affairs Shanghai Sub-Center for Plant New Variety Tests in the 2018 and 2019 seasons so as to better understand the traits of the cultivar. Table 1 illustrates some of the signatures identified in the DUS assay. Hanyou 3015 was upright and compact, with green leaf sheath color; stem diameter was medium; stem length was 98.17 cm; and the basal leaf sheath was green. The flag leaf was semi-erect.

For identification and evaluation of new water-saving and drought-resistance rice varieties in southern China in terms of drought tolerance, high yield, yield stability, adaptability, tolerance, rice quality, and other important traits, the varieties were tested for two consecutive years in 13 district trials in nine provinces (cities). There were 13 district trials in 9 provinces (cities) of Hunan, Hubei, Shanghai, and others. The regional trials were arranged in completely randomized groups, with three replications and a plot area of 13.34 m^2. The yield test was conducted in a randomized arrangement with no replications and a plot area of 333.5 m^2.

PERFORMANCE OF HANYOU 3015

Hanyou 3015 can be grown in southern China as a one-season rice. Its performance for major agronomic traits in two-year regional trials is as follows: The line was sown in mid to late May up to early June, grown in medium fertility soil, with the effective number of spikes of 3,180,000 ha^{-1} in underwater direct seeding and under drought treatment; and the whole growth period was 111.7 d. Plant height was 106.8 cm, and spike length was 23.2 cm; the total number of grains per spike was 146.2; the seed setting rate was 85.8%; and 1,000 seed weight was 26.2 g (Table 2).

The drought-resistance index of Hanyou 3015 is referred to technical specification for identification of water-saving and drought-resistance rice varieties (NY/T2863-2015 of China). For drought-resistance, Figure 1 showed that Hanyou 3015 performs well under drought field conditions (Supplementary Table 1). The drought-resistance index of Hanyou 3015 was 0.984 and 0.805 in two consecutive years; the drought tolerance degree was 2 and 2; and the synthetic evaluation of drought-resistance indicated a drought-tolerant level.

Table 2. Yield traits of Hanyou 3015 at the National regional trial

Variety	Year	Yield (kg ha^{-1})	Effective panicles (1000 ha^{-1})	Plant height (cm)	Number of seeds per spike	Seed setting rate (%)	Thousand seed weight (g)
Hanyou 3015	2018	8,227.4	2910	106.9	150.3	86.7	26.8
	2019	8,251.2	3465	106.6	142.1	85.0	25.6
Hanyou 73	2018	7,924.5	2490	114.3	178.8	86.8	29.4
	2019	7,850.3	2790	112.8	161.9	87.2	28.7

Table 3. Rice quality traits of Hanyou 3015 at the National regional trial

Variety	Year	Brown rice percentage (%)	Head rice percentage (%)	Chalkiness degree %)	Translucency	Alkali spreading value	Gel consistency (mm)	Amylose content (%)	Ratio of length and width
Hanyou 3015	2018	70.5	53.6	6.3	2	4.5	67	14.9	3.3
	2019	80.0	51.1	4.8	2	6.5	53	16.6	3.4
Hanyou 73	2018	72.3	55.9	3.5	2	5.0	75	14.6	3.2
	2019	80.6	40.8	3.8	1	6.5	65	14.3	3.4

The results of comprehensive evaluation of resistance to major diseases and pests in the national WDR regional trial in 2018-2019 indicated that the combined index of rice blast of Hanyou 3015 was 4.75 and 4.22 in the two years, and the bacterial blight resistance index was at level 5 and 7. Hanyou 3015 had an index of resistance to brown planthopper at level 7 and 9 in the two years, and the overall evaluation showed susceptibility to rice blast, susceptibility to bacterial blight, and high susceptibility to brown planthopper. As tested by the Quality Supervision and Testing Center for Rice and Products of the Ministry of Agriculture and Rural Affairs (Wuhan), the main quality indices of Hanyou 3015 were as follows: head rice percentage of 51.1%, length to width ratio of 3.4, chalkiness degree of 4.8%, Grade 2 translucency, alkali spreading value of 6.5, gel consistency of 53 mm, and amylose content of 16.6%. The comprehensive evaluation of rice quality of Hanyou 3015 was within the ordinary standard of the ministry (Table 3).

FUNCTIONAL GENE ANALYSIS OF HANYOU 3015

The respective SNPs of Huhan 7A and Hanhui 3015 were compared with those of Hanyou 3015, and the SNP loci consistent with Hanyou 3015 were ascertained. Next, the SNPs obtained in comparison of the two varieties were observed throughout the entire genome, and the differences in SNPs on different chromosome segments between the two parental species were also demonstrated. With the aim of analyzing functional genes in Hanyou 3015, Hanhui 3015, and Huhan 7A, genome-wide variation in the QTN loci was determined, and those loci associated with variation in functional traits were identified. The variation of the QTN loci in the whole genome was assessed, and gene loci leading to functional trait variation were found. Through the RiceNavi analysis (Wei et al. 2021, Li et al. 2023), we found that Hanyou 3015 inherited the dominant alleles and co-dominant alleles of its parents Hanhui 3015 and Huhan 7A (Figure 2, Supplementary Table 2). Hanyou 3015 pyramided the superior alleles from Hanhui 3015, such as *Rf3, NOG1, Hwi2ORF4, CYP78A13, GW8, Rf4, Rf1b, Hd17, D2, OsBRI1, APO1, TAC1, OsUGT706D1, Bph3, BPH29,* and *Xa3*, as well as the superior alleles from Huhan 7A, namely, *SaF, Sam, Rf2, GW7, Xa1,* and *OsTPP7*.

In examining the reasons for the excellent disease resistance and insect resistance of Hanyou 3015, the results showed that the brown planthopper resistance genes, such as *Bph3* and *BPH29*, were inherited from Hanhui 3015. The genome of Hanyou 3015 retained the *Xa1* mutant genotypes from Huhan 7A and the *Xa3* mutant genotypes from Hanhui 3015. The rice blast resistance genes, *RGA4* mutant genotypes from Huhan 7A, and the excellent disease resistance genes *PiPR1, Pid2, Pid3, OsCERK1*, and *LHCB5* common to were inherited by Hanyou 3015; but most of these genes played an adjunct role of signal transmission in resisting the invasion of the pathogenic fungus. These genes contribute to rice blast resistance in Hanyou 3015.

For high rice quality and yield, Hanyou 3015 inherited superior genes from both parents, such as *Badh2*, a gene controlling rice fragrance, and *GW7*, a gene controlling grain length that are in Huhan 7A, and it retained *CYP78A13* and *GW8* that are in the genotype of Hanhui 3015. The activation of the *CYP78A13* gene effectively adds value to the cells, and it has the potential to increase seed yield. *GW8*, also known as *OsSPL16*, increases both the length and width of the grain and improves its quality. Furthermore, the excellent genotypes *OsLG3*, *GS3*, *GL3.2*, and *OsSPL13*, shared by the two parents, have the effect of increasing the length and width of the grain. The gene *NRT1.1B* improves the nitrogen use efficiency of rice, and there is also the gene *Gn1a,* which increases the number of spikes on a single plant and thus improves rice yield.

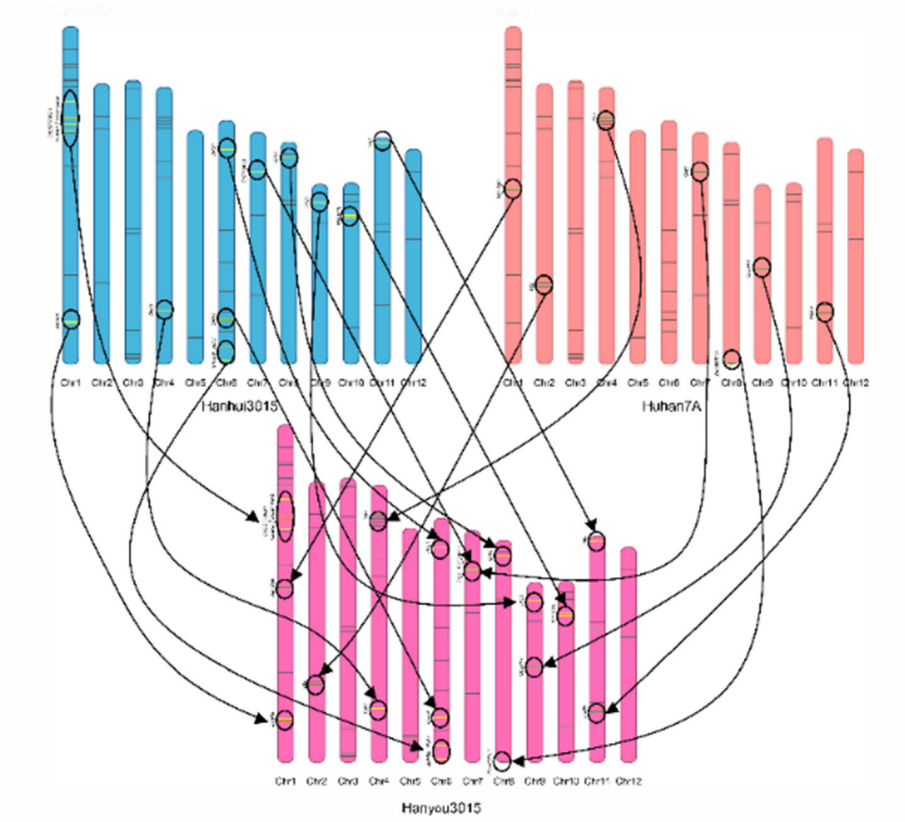

Figure 2. Superior genes of Hanyou 3015 derived from Hanhui 3015 and Huhan 7A. The unique genes in Hanhui 3015 and Huhan7A are represented by yellow and green bars, respectively. The brown bar indicates the superior genes simultaneously contained in the two parent lines, and each bar represents a superior gene.

CULTIVATION TECHNIQUES

In southern China, Hanyou 3015 could be grown in one rice season. In conformity with local ecological conditions, the number of seeds used for direct sowing in water is 15-22.5 kg ha^{-1}, and the number of seed used for direct sowing on dry land is 30-37.5 kg ha^{-1}. Recommendations are to mix the seeds with Thiacloprid, and then to sow sparingly and evenly. The level of fertilizer demand is medium, generally 150 kg ha^{-1} of pure nitrogen, and a nitrogen, phosphorus, potassium dosage ratio of 1:0.5:0.7, making heavy application of bottom fertilization and timely application of tiller fertilization, and avoiding the late biased application of nitrogen fertilizer. Field water management in Hanyou 3015 is based on natural rainfall, and it doesn't require establishment of a water layer; but when severe drought is encountered, especially during the seedling emergence stage, panicle differentiation stage, and filling stage, timely irrigation should be used to replenish water. Controlling weeds is a critical step in direct seeding of rice under dry conditions. First, 40% Benazolam closed weed control is sprayed in direct sowing in paddy fields, whereas pendimethalin and butachlor are applied in direct sowing in dry fields for closed weed control. Secondly, weeds are controlled by direct spraying of 50% quinclorac; and 10% cyhalofop-butyl is applied at the 3-leaf stage. Thirdly, in the late stage, noxious weeds are manually removed.

CONCLUSION

Hanyou 3105 is an early-maturing and high-yielding indica hybrid rice combination with the water-saving trait, drought tolerance, early maturity, short stem, excellent agronomic traits, and wide adaptability. It is suitable for planting in Southern China as a single crop season rice and could also be planted in direct seeding in wheat stubble.

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