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Molecular Marker-Based Screening and Hybrid Combination Evaluation of Drought-Resistant Sweet Potato *Germplasm*

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Abstract Global climate change has intensified the threat of drought to agriculture. Sweet potatoes (*Ipomoea batatas*), which were once regarded as "drought-tolerant and easy-to-grow" crops, have also begun to fail in some areas. To ensure its stable production in the future, it is necessary to find new varieties that can both resist drought and achieve high yields. Starting from this reality, this study sorted out the main ideas of drought-resistant breeding of sweet potatoes and also attempted to build a more practical process. Let's start with the visible phenotypes, such as which sweet potatoes can still have plump leaves, deep root systems and little yield loss during drought. Indicators like survival rate, relative moisture content and leaf temperature, which seem simple, are actually the most direct basis for judging drought resistance. Next, let's return to the genetic level to explore why sweet potatoes are drought-tolerant - their genome is inherently complex and hexaploid. It is not just one or two genes that control drought resistance, but a complete regulatory network. Traditional breeding, which relies on field screening, is often much slower, while molecular marker technology enables us to identify useful gene loci more quickly. We also conducted an association analysis in combination with genomic data, identifying QTLS related to drought resistance from a vast number of markers, and then evaluated the germplasm performance using a comprehensive model to pick out the materials that are truly worthy of being used as parents. Finally, we attempted to use this information to design hybrid combinations and found that the combination guided by molecular markers could indeed significantly improve the breeding efficiency, especially in the early generation when good seedlings could be screened out. Overall, this "phenotypic + molecular" combined approach is faster and more accurate than traditional methods, and it also makes drought-resistant breeding of asexually propagated crops like sweet potatoes more feasible.

Keywords Sweet potato; Drought stress; Molecular marker-assisted selection; Genetic diversity; Hybrid breeding

1 Introduction

Sweet potatoes are actually not a new crop. They are grown in over a hundred countries around the world, and can be found in both staple food and side dishes. It can tolerate poor soil, has a high yield and can also be helpful in times of intensified climate change (Andrade et al., 2016). However, this impression of being "tough" is sometimes exaggerated. When drought strikes, sweet potatoes may not be able to withstand it - especially in those regions of Africa that are perpetually dry and hot, the yield often drops sharply, sometimes to just half. Climate warming is making this situation increasingly common, and water shortage has become one of the biggest threats to sweet potato cultivation. Although sweet potatoes have well-developed root systems and can barely survive a period of drought after survival, the early lack of water is very fatal: if there is no water during the seedling stage and when the tubers are just formed, the vines cannot grow, the leaves are small, and the tubers cannot expand. Worse still, under extreme drought conditions, the differences among various varieties have become less obvious, making it difficult to select the "drought-resistant champion". At present, the main sweet potato production areas urgently need new varieties that can maintain yields in water-scarce environments, which has also promoted the exploration of drought-resistant genetic improvement and new breeding technologies (Low et al., 2020).

The trait of drought resistance is complicated to talk about and even more troublesome to implement. It is not the kind that can be explained by a single gene, but is controlled by many genes together and is also easily influenced by the environment. The traditional field screening relies on the weather, has a long cycle and takes a long time to show results. Later, molecular marker technology emerged, enabling breeders to directly identify drought-resistant genes through DNA fragments, just like installing a navigation system in a genetic map. This way of seed selection is faster and more accurate. Other crops have long reaped the benefits - sorghum and corn. After locating the "stay-green" gene in this way, many drought-resistant varieties have been cultivated. Although sweet potatoes are asexually propagated hybrid crops, they need this molecular approach even more to avoid variety confusion and improve screening efficiency. Applying molecular markers to parental selection can also help determine in advance which combinations are more compatible, which is much more reliable than relying solely on appearance and experience. The ultimate goal is straightforward: to bring together the scattered drought-resistant genes from different parents to make the next generation stronger. In this way, breeding is not only an empirical job but also a data-driven scientific one.

The starting point of this study is actually very realistic - to find ways to ensure the stable yield of sweet potatoes even in fields with little water. To this end, we plan to combine molecular marker screening with conventional hybridization evaluation to establish a more efficient drought-resistant breeding process. The general idea is as follows: First, test the sweet potato materials from different regions under drought conditions to see which ones perform well and which ones do not, and then pick out the key traits. Then, high-density molecular markers were used to genotype these materials to identify QTLS and candidate genes that might be related to drought resistance. Then, the phenotype and genotype are combined for association analysis to identify those markers that are truly stably associated. Based on these results, we then select parents, design hybrid combinations, and screen out plants with outstanding drought resistance in the offspring. Finally, verify the feasibility of this entire system through one or two specific cases to see if it can play a role in actual breeding.

2 Theoretical Basis and Research Progress

2.1 Key phenotypes and evaluation index system of sweet potato drought resistance

When evaluating the drought resistance of sweet potatoes, it is often not advisable to rely on just one indicator (Laurie et al., 2022). Some people will first notice its roots - drought-resistant sweet potatoes usually have deep and thick roots that can "drink water" from deeper soil layers, which can be regarded as a way to escape drought. However, there are exceptions. Some roots, though shallow, can survive longer through water-saving strategies, such as making leaves smaller, curled, or even thicker, by reducing transpiration to "save water". However, excessive water conservation also comes at a cost, such as premature leaf aging or restricted photosynthesis. In the end, one still has to pay for the yield. Apart from their appearance, the "reactions" inside sweet potatoes also speak for themselves. Drought-tolerant plants often maintain a higher water content in their leaves, accumulating water-retaining substances such as proline and sugars, which is like building a "water storage bag" in their bodies. Some varieties experience a rise in leaf temperature and semi-closed stomata during drought, seemingly protecting themselves. Meanwhile, the antioxidant enzymes in the body become active to combat the oxidative stress brought about by drought - a part that is often overlooked but is an important defense line for drought tolerance. Ultimately, output is the most direct criterion. No matter how good the indicators are, ultimately it all depends on whether the potato tubers can be produced stably. Usually, we compare the yield difference between drought and irrigation conditions, or see if the harvest index can still remain at a high level. Some drought-tolerant varieties can still distribute more nutrients to the tubers even when they are short of water. This "distribution priority" ensures that they do not have no harvest at all even in adverse conditions. Overall, the drought resistance of sweet potatoes is the result of the coordinated efforts of multiple aspects - the root system, leaves, physiological regulation and yield maintenance are all indispensable. To make an accurate assessment, it often relies on a comprehensive analysis of multiple indicators rather than a single seemingly attractive value.

2.2 Genetic basis of drought resistance: overview of QTL, candidate genes and regulatory pathways

The drought resistance of sweet potatoes is indeed complex. It is not determined by a single gene, but rather the result of many genes pulling together. Coupled with the interference of the environment, it often becomes unpredictable. The genome of sweet potatoes themselves is quite troublesome - hexaploid, highly heterozygous, and structurally disordered (Zong et al., 2023). Studying it is like piecing together a jigsaw puzzle that is always missing corners. In the past, people attempted to use linkage analysis to identify key genes for drought resistance, but the progress was not rapid. Most of the targeted QTLS were unstable and often failed to adapt to the local conditions when used in breeding. In contrast, there are more QTL studies focusing on yield and quality. However, even if some markers have been found, they have not yet been truly implemented. Often, sites that perform well in experiments "fail" when exposed to different environments or varieties (Liu et al., 2023).

As a result, the research focus gradually shifted from identifying single major genes to exploring candidate genes and signaling pathways that function at multiple levels. Among these genes, transcription factors are often mentioned, such as NAC, bZIP, WRKY, etc., which act like switches to control drought responses. Some genes that can enhance drought resistance have indeed been found in sweet potatoes, such as regulatory factors involved in ABA signaling or GATA genes that regulate stomatal opening and closing and antioxidant responses (Zhao et al., 2022). The common feature of these genes is that they can help plants maintain water balance and reduce damage when they are short of water. There are also some protective proteins, such as LEA proteins, which have been proven to enhance the drought resistance and salt tolerance of sweet potatoes (Cheng et al., 2024). With the advancement of sequencing technology, researchers can now more clearly observe the differences throughout the entire genome - through GWAS or transcriptome analysis, not only can potential functional genes be identified, but also a rough network for drought resistance regulation can be pieced together (Yin et al., 2024). The result is becoming increasingly clear: sweet potatoes' drought resistance does not have a so-called "core gene", but is maintained through the coordinated efforts of an entire system. In the future, perhaps only by integrating these scattered minor genes through gene editing or whole-genome selection can sweet potatoes truly remain stable in arid areas.

2.3 Application progress and implications of molecular marker technology in crop drought-resistant breeding

Molecular marker technology has advanced rapidly over the past few decades, evolving from the earliest random fragment labeling to more precise SSR and SNP, and now to high-throughput sequencing capable of measuring tens of thousands of points at once. The "molecular ruler" in the hands of breeders has become increasingly sophisticated. In fact, these technologies have been widely applied in rice and barley for a long time - some people have used them to stack multiple drought-resistant genes together to cultivate new rice varieties that can save water without reducing production. Some people have also identified stable drought-tolerant sites through marking, laying the foundation for the improvement of multiple traits. The sweet potato side started a bit slower, but it's not completely quiet either. For instance, some studies have screened out markers related to yield and dry matter, and some teams have used AFLP to identify disease-resistant genes, accumulating experience for subsequent stress-resistant breeding (Beyene et al., 2015). Nowadays, international institutions are also working on "sweet potato ID cards". A set of polymorphic SNPS can be used to identify varieties and control quality, which has made the molecular resources of sweet potatoes much richer than before. In drought-resistant breeding, the main tasks that marker technology can do fall into two categories: one is to explore resistance genes and observe the molecular differences between drought-resistant and non-drought-resistant materials; The second is to use markers to track the offspring carrying drought-resistant genes in hybrid populations. The benefits are obvious-there is no need to wait for the crops to grow up or for a drought; seedlings can be selected during the seedling stage or in the greenhouse. However, it is not omnipotent. The key is to have stable marker sites, and sweet potatoes have a relatively weak foundation in this regard. Some people have suggested that it could be used in combination with genome-wide selection, with the former targeting large genes and the latter capturing small effects, to maximize the overall potential for drought resistance. In the future, sweet potato breeding is likely to follow this "dual-track" approach: MAS for precise management and GS for comprehensive management, ensuring that sweet potatoes can grow steadily and yield good harvests even in water-scarce fields.

3 Material Sources and Experimental Design

3.1 Composition and representativeness of germplasm materials

To make breakthroughs in drought-resistant breeding of sweet potatoes, the first step is to lay a solid "material foundation". All kinds of germplasm resources from different sources must be utilized; otherwise, truly promising genes cannot be screened out (Andrade et al., 2016). Local varieties are often grown by farmers in arid fields for decades or even hundreds of years. Their survival is itself a result of screening. Some areas can still achieve stable production under conditions of low input and less irrigation, which indicates that there must be some key drought-resistant factors hidden in their bodies (Low et al., 2020). In contrast, although modern bred varieties are more productive and have better appearances, many of them were selected under good water and fertilizer conditions, and their drought resistance may not be strong. However, including them is still important. At least it can ensure that the hybrid offspring do not lose yield. Wild relatives are a different story. They often have resilience genes that cultivated species do not have. For instance, their leaves close quickly under moderate drought and grow fast after rewatering. However, when encountering extreme drought, most of them cannot survive and may not even grow tubers (Roullier et al., 2013). It's not realistic to plant it directly, but it's a good idea to use it as a "gene donor". Their useful fragments can be gradually introduced through distant hybridization or gene introduction (Yada et al., 2017). When constructing experimental materials, the key lies in having a wide representativeness and a balanced combination. For local varieties, several samples should be selected from different ecological regions. It is best to have both drought-tolerant and drought-sensitive ones to facilitate comparison. For modern varieties, it is necessary to choose those with good fertility and clear genotypes to avoid the trouble of analysis later. For wild materials, it is advisable to choose existing bridge systems or synthetic species as much as possible to avoid incompatibility issues. Suppose we finally determine twenty cultivated varieties and five wild relatives, which can basically cover the mainstream types from local varieties in arid areas to improved varieties. All materials must be re-identified, undergo molecular fingerprint analysis, remove duplicates, and ensure the data is clean. Such a set of combinations, featuring both genetic differences and ecological spans, is sufficient to support subsequent drought resistance comparisons and gene mining.

3.2 Drought stress setup and experimental layout

To more accurately assess the performance of sweet potatoes under water shortage conditions, this experiment did not rely on a single method but combined the field and greenhouse environments (Andrade et al., 2016). In the fields, drought is mainly artificially created by controlling irrigation. When sweet potatoes start to grow tubers, they are divided into two groups: one group is watered as usual, while the other group simply stops watering to allow the soil moisture to naturally decrease. Generally, after four to five weeks, most plants can show differences. We also took into account the uncertainty of the climate and built a rain shelter to prevent rain from causing trouble (Laurie et al., 2022). The experiments were arranged in random blocks, with each variety repeated several times and the positions also shuffled to avoid soil differences interfering with the results. The duration and intensity of the drought will be flexibly adjusted according to the weather, and irrigation will not resume until some sensitive varieties start to wilt. By comparing the yield and growth indicators, one can roughly judge their drought resistance capacity. It's done more meticulously over there in the greenhouse. First, screen the seedlings in pots to select those with obvious reactions. Then, simulate drought with PEG solution and let the root system grow under different osmotic potentials to observe the reactions of the seedlings, such as growth inhibition and leaf changes (Yin et al., 2024). The concentration of PEG is divided into several grades, from light to heavy, making it easy to find the grade that best distinguishes the differences among varieties. All trials were randomized as much as possible, and each genotype was repeated at least three times to ensure the reliability of the data (Liu et al., 2023). Overall, such a design can not only reflect the real situation in the field but also control variables in the greenhouse, making it easier to see the different ways sweet potatoes respond to drought. Some varieties can survive short-term droughts, while others can endure prolonged mild water shortages. The combination of multiple scenarios can more comprehensively identify truly potential drought-resistant materials.

3.3 Phenotypic determination and grading criteria

To determine whether sweet potatoes are drought-resistant or not, it is not enough to just look at the yield. One also needs to start from details such as survival, water content, leaf temperature and root system (Laurie et al., 2022). In this study, after a field drought trial, water was reapplied to see which plants could survive. The proportion of those that survived was the survival rate. Those that could exceed 80% were mostly considered drought-resistant. Meanwhile, we regularly measure the relative water content (RWC) of the leaves. Varieties with slower leaf water loss tend to be more drought-tolerant. Also, the leaf temperature can be measured with an infrared instrument at noon on a sunny day. If it rises too quickly, it usually indicates that the stomatal response is not timely. On the contrary, those that can stably control the temperature usually do a good job in regulating moisture (Figure 1) (Yin et al., 2024). When we dig out the root system during harvest, we can see that those with deeply rooted and widely distributed roots generally have a higher drought resistance potential. In addition, indicators such as the degree of wilting, electrical conductivity, proline, and sugar content were also recorded. Some varieties have leaves that wilt early but recover quickly, which can also be regarded as a kind of "strategy" (Liu et al., 2023). Of course, the output is still the most crucial factor in the end. When comparing the drought treatment with the control output, the one with a smaller gap is more stable. All these indicators are converted into relative values, and then integrated by principal component analysis and fuzzy membership method to calculate a comprehensive drought resistance score. Those with high scores are classified as Level I, medium as Level II, and low as Level III. In simple terms, it is to extract a number that best represents the overall drought resistance level from a bunch of traits. After such classification, it becomes clear which materials are worth keeping as parents and which do not require too much investment.

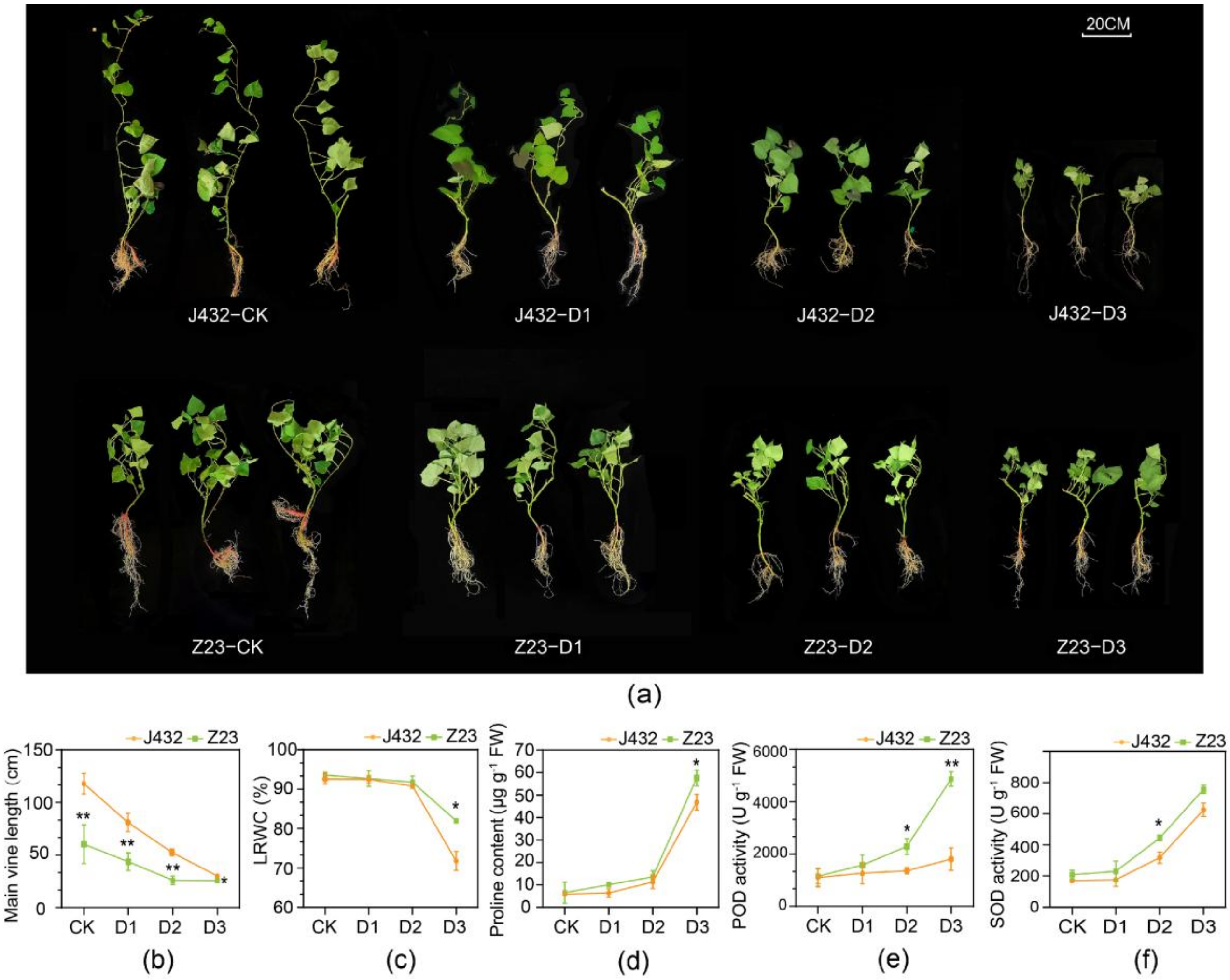

Figure 1 Morphological traits and physiological indices of J432 and Z23 at different drought levels. (a) Morphological traits of J432 and Z23 after the conclusion of the entire drought treatment. (b) Main vine length. (c) Leaf's relative water content. (d) Proline content. (e) POD activity. (f) SOD activity. * and ** indicate significant differences (at $p < 0.05$ and $p < 0.01$, respectively) between J432 and Z23 at each drought level based on Student's t-tes (Adopted from Yin et al., 2024)

4 Molecular Marker Detection And Data Quality Control

4.1 Marker selection strategy: a combination of known drought-resistant site markers and genome-wide SNPS/SSRS

After the initial phenotypic screening, we began to conduct molecular marker detection. To put it bluntly, I just want to see where the differences lie at the genetic level among these sweet potatoes with different drought resistance performances. We did not follow a single approach in the selection of markers, but rather took into account both "targeted" and "comprehensive scanning". Let's start with the former. Some genes related to drought resistance have already been identified, such as those that control transcription, osmotic regulation or antioxidant responses (Zhao et al., 2022). We will select a few key fragments from these genes, design specific markers, and see if there are any key variations in different varieties. The quantity is not large, but the information is quite substantial and can directly verify whether it carries drought-resistant genes. The problem is that these known genes may not be able to explain all the differences. So we used high-density whole-genome markers and ready-made SNP chips for genotyping (Yada et al., 2017). The chip can cover most of the genetic regions of sweet potatoes at one time, with neat data and convenient analysis. If the chip cannot be obtained, we will use the simplified sequencing method. Although it is more troublesome, the results will be more comprehensive (Figure 1) (Zong et al., 2023). In addition, some SSR markers have been added, mainly for the analysis of group structure and breed relationships. Ultimately, we identified approximately two hundred markers: a small number of functional markers targeting drought-resistant genes, dozens of SSRS for quality control, and the rest are SNPS distributed throughout the genome. This combination not only enables precise verification of known genes but also offers the opportunity to identify new drought-resistant sites. It can be regarded as a approach that strikes a balance between cost and information volume.

4.2 Genotyping process: DNA extraction, PCR/ sequencing or chip typing and parameter setting

The step of genotyping actually starts from the most fundamental DNA. First, the DNA in the leaves must be extracted completely; otherwise, all the subsequent PCR tests will be in vain. We usually use CTAB or kit methods. The key is to remove polyphenols and sugars. Especially sweet potatoes are hexaploids with a lot of impurities. Without adding PVP and β-mercaptoethanol, it is almost impossible to extract clear bands (Kant et al., 2022). If the electrophoresis looks neat and the OD value is around 1.8, it is basically considered qualified. After extracting the DNA, PCR amplification should be performed first. Primers should be designed for each SSR site, and the temperature should be adjusted in advance; otherwise, the amplification is prone to blurring. Small fragments can be used for gel running, while large ones or those with multiple alleles can be placed on the fluorescence platform. When encountering SNPS or indels of candidate genes, typing is carried out by KASP or AS-PCR methods, and sometimes sequencing is also required for confirmation (Yada et al., 2017). Anyway, what is ultimately needed is the genotype information of each material at these loci - which allele it is, how many bases are missing, and so on. If a chip is used, it will be even more straightforward. After the DNA is labeled, it is dropped onto the chip, and the machine can scan it once to produce the results of AA, AB, and BB (Beyene et al., 2015). We usually do two hybridizations to prevent the machine from making mistakes. Some people prefer to use GBS, which is fine too. However, the process is longer, involving enzymatic digestion, adding linkers, library construction and sequencing, and the data processing is also troublesome. Given the complexity of the sweet potato genome, we mainly rely on chip data. After getting the results, we still need to screen them. All loci that are too missing or have too few alleles must be removed, and those that are mixed up due to multiple copies should also be deleted. Ultimately, what remains is a clean genotype matrix - including dozens of target loci and tens of thousands of genome-wide SNPS - for subsequent diversity analysis and association studies.

4.3 Data quality control and bias Control: Missing rate, MAF, repeat consistency, population stratification correction

To ensure the accuracy and reliability of molecular marker data and reduce the impact of systematic errors on sweet potato drought-tolerant germplasm screening and hybrid combination evaluation results, this study performed rigorous data quality control and bias correction on the raw molecular marker data before genotype data analysis. The quality control process mainly included steps such as missing rate filtering, minimum allele frequency screening, repeatability consistency testing, and population structure correction.

First, a statistical analysis of missing data in the molecular markers was performed. At the sample level, the genotype missing rate of each sweet potato material was calculated, and materials with a missing rate exceeding 5% were removed to avoid analytical bias caused by poor DNA quality or amplification failure. At the marker level, molecular marker loci with a missing rate higher than 5% were removed, and only markers with high detection stability were retained for subsequent analysis. This missing rate control effectively improved the completeness and reliability of the genotype data. Based on this, the molecular markers that passed the missing rate screening were further filtered using the minor allele frequency (MAF). This study set the MAF threshold to 0.05, removing marker loci with a minor allele frequency below this threshold to reduce random errors caused by low-frequency alleles and improve the statistical power of drought-tolerance-related locus screening and hybrid combination evaluation. To verify the stability and repeatability of the molecular marker detection results, technical replicates were performed on some samples, and the genotype consistency between replicate samples was calculated. The results showed that the consistency of replicate samples at each marker locus remained above 99%, indicating that the molecular marker detection system used in this study has high reliability. Marker loci with low consistency were re-examined or removed to further ensure data quality.

Considering that differences in the genetic background of different sweet potato germplasm materials may cause population structure bias, this study used principal component analysis (PCA) to assess the population structure. Major genetic principal components were extracted based on high-quality molecular marker data and introduced as covariates in subsequent drought tolerance analysis and hybrid combination evaluation models. This effectively corrected potential biases caused by population stratification, improving the accuracy and reliability of the analysis results.

5 Association analysis and Comprehensive Evaluation of drought-resistant germplasm

5.1 Genetic Diversity and Population Structure Analysis (PCA/ Structural Clustering/Kinship)

When conducting molecular marker analysis, data quality often determines half of the outcome. After we obtain the original typing data, the first step is not to rush into analysis but to first "find faults". If too many markers or samples are missing, they should be directly removed. For instance, if a certain marker cannot be detected in more than half of the samples, it is basically useless. For individual samples that are too severely missing, supplementary testing should be conducted if possible; otherwise, they might as well not be involved in the subsequent analysis. For some key sites, if they are only sporadically missing, a cautious filling can be made based on similar materials or genetic relationships. However, the fewer such operations, the better, to avoid artificially distorting the results. After filtering out the missing data, the frequency of alleles still needs to be considered. Those low-frequency variations that only occur in a very small number of materials have little statistical significance. Keeping them will only increase the risk of false positives. We have set a lower frequency limit and retained markers that can represent group differences. Afterwards, to confirm that there were no errors in the experiment, we compared the repeated samples with the standard varieties. The consistency rate of the results was over 99%, indicating that the data itself is reliable. After this round of quality control, the issue is not whether the measurement was correct or not, but whether the analysis will be misled by the group structure. Sweet potato germplasms from different regions and sources all have some degree of kinship. If no correction is made, the model may misjudge family similarities as genetic associations. Therefore, before the association analysis, we added structural and kinship corrections, incorporating both clustering information and kinship coefficients into the model, and striving to ensure that the statistical results only reflect the true genetic signals. Finally, there is the stage of multiple tests. When a large number of markers are tested simultaneously, false positives are inevitable. We have controlled them using methods such as FDR and Bonferroni. Overall, the data loss rate after screening is less than 1%, the allele distribution is balanced, and no excessive linkage was found. It can be used with confidence for subsequent analysis.

5.2 Label-trait association and stability test (GWAS/ multi-environment G×E)

When dealing with the association analysis of drought resistance in sweet potatoes, we did not target genes from the very beginning, but started with a bunch of complex phenotypes. Yield, moisture content, proline accumulation... These indicators are interrelated and each is influenced by the environment, making it difficult to make a one-size-fits-all judgment on which one is the "most drought-resistant". So we used GWAS to match the markers of the entire genome one by one with these traits (Huang and Han, 2014). The analysis results are quite interesting: On chromosome 11, a locus called S11_2578431 stands out particularly. Materials carrying it generally have a more stable yield under drought conditions, approximately 20% higher than those without it.

However, this mark shows almost no reaction under normal irrigation conditions, a typical "environmental picky" effect - it becomes valuable as soon as there is a drought. We suspect that there might be a transcription factor related to drought response nearby, but this is only speculation and functional verification is still needed . In addition to it, a signal related to the decrease in leaf water content also appeared on chromosome 5, although its significance was slightly weaker, but the direction was consistent. What's more interesting is that in the analysis of proline content, we found several small peaks. Although some of them might be false positivity, they also suggest that certain osmotic regulatory genes are at play (Luo et al., 2022). Later, we put the data from multiple environments together for review. Some sites remained stable in all arid environments, while others were "just a gust of wind", and their performance was not significant when changing environments. Overall, locus 11 is the most reliable and has the greatest potential for use in subsequent breeding screening. We also conducted a small-scale verification, testing several varieties that did not participate in the analysis. The results were consistent with the model's prediction, which makes people more confident (Yada et al., 2017). Of course, this is far from the end. The genome of sweet potatoes is too complex. What has been found this time is just the tip of the iceberg. The rest still needs to be explored by larger populations and more environments.

5.3 Construction of comprehensive evaluation model and screening of superior germplasm

After obtaining all the phenotypic data and correlation results, we did not immediately carry out complex calculations but first thought clearly about one thing - what kind of sweet potatoes can be considered "drought-resistant". Different indicators have different opinions. Some look at the yield, some at the moisture content of the leaves, and others believe that "survival" is the key. So we attempted to compare all these indicators on the same ruler and created a comprehensive model by combining principal components and membership functions. The approach is actually quite straightforward: First, standardize all the indicators so that different dimensions can be considered together; Negative indicators like the reduction rate should be calculated in reverse. After analysis, the first three principal components explain most of the differences: one is more about yield, one is more about water metabolism, and the other is related to survival recovery. Interestingly, not all materials are excellent in all three aspects. Some varieties have decent yields but are prone to wilting, while others do not produce potatoes at all but survive tenaciously. That is to say, looking at just one indicator can easily lead to misjudgment. Next, we use the membership function to calculate a "score" for each indicator, and then weighted them by weight to obtain the comprehensive value D. Those with high scores have strong drought resistance, while those with low scores have poor drought resistance. The results are quite clear: several materials with a D value above 0.8 performed outstandingly, with stable yields, no wilting of leaves, and quick recovery after rewatering. However, some high-yield types dropped even more severely, with almost no harvest during droughts. We have designated those with $D \geq 0.8$ as core materials, reserved those with D - 0.8 for improvement, and temporarily disregarded those with D lower than 0.5. This classification is both simple and practical. Later, we found that the results calculated by the model were almost consistent with the on-site scores given by experts, indicating that it was indeed reliable (Sapakhova et al., 2023). Of course, this model is not set in stones. The weights can be adjusted according to the target. For instance, if survival is given more emphasis, more points should be allocated to physiological indicators. Overall, this set of methods has helped us find truly drought-resistant and fertile materials more quickly, and has also made the subsequent breeding direction clearer.

6 Construction of Hybrid Combinations And Evaluation Of Combinations

6.1 Principles for parental selection: Genetic distance, complementary traits, and combination of drought-resistant dominant loci

Choosing a parent is quite simple - just pick the right one. To say it's complex is truly an art. When we were conducting drought-resistant breeding of sweet potatoes, our first reaction was not who was the most drought-resistant, but who was more "connected" when working together. Sometimes, when two drought-resistant varieties are put together, there is not much surprise. Their genes are too similar, and the changes in their offspring are small, making it difficult for new varieties to emerge. On the contrary, it is those that are slightly different and have a somewhat distinct background that become interesting when hybridized. Based on the results of genetic distance and compatibility, we prioritize finding parents with moderate differences that are not too close, such as a water-saving type paired with a high-yielding type, to complement each other's shortcomings (Bernardo, 2016). There are also exceptions. Some varieties may not stand out on their own, but when paired with a specific parent, their offspring perform astonishingly well. Such special combinations should not be overlooked either. When choosing parents, it is also necessary to consider whether their traits complement each other. For instance, if one can "save water" and the other can "grow", when combined, they might be able to balance drought resistance and yield. We also need to be on guard against combinations that share the same illness. When two people are both afraid of the disease, no matter how drought-resistant they are, it won't help. In addition, the molecular marker information makes things clearer. We can see who carries which drought-resistant gene fragments, and then, like a jigsaw puzzle, gather the different beneficial fragments together (Varshney et al., 2021). If the genes carried by two varieties are repetitive, it would be in vain to have them. But if several different drought-resistant sites can be brought together in the same offspring, that would be the kind of "aggregation" we desire. Finally, we determined four core parents-one water-saving type, one high-yield type, one deep-rooted wild variety, and one local disease-resistant variety. They are different yet complementary, and all can flower and bear fruit normally, saving a lot of trouble. Overall, choosing a parent is more like finding a partner. They need to be able to cooperate with each other and withstand drought together. Just having a single champion is not enough.

6.2 Mating design Scheme: NCII/ Partial two-row hybridization/backcrossing and population size planning

After the parents have been determined, the matter of pairing is not merely about simply mixing several varieties together. Sweet potatoes are not prone to self-pollination and are cross-pollinating crops, so some strategies are needed. We plan to conduct a small-scale mutual probing first, picking out four core parents to pair up with each other and see who gets along better with whom. This approach is mainly aimed at understanding the compatibility of each parent and also taking the opportunity to see which combinations' offspring can perform more stably in drought conditions. In the experiment, sufficient F_1 seeds will be prepared. Even if the germination rate is not high, dozens of plants can still be left for testing. When F_1 is manifested, a comparison of yield and drought resistance will make the superiority and inferiority naturally clear. If a combination like A×D stands out prominently, we will focus on promoting it. Other mediocre ones can be put on hold for now. Then, a more systematic mating design is adopted to expand the scale. For instance, the well-performing parents are crossed with several others respectively to create more offspring groups (Bernardo, 2016). This way, more recombinant individuals with superior genes can be obtained, and it is also convenient for the next screening step. Artificial pollination is troublesome, so more seeds for the main combination will be prepared, and a moderate amount of seeds for the secondary combination will follow to avoid missing out on potential good seedlings. When encountering materials with overly strong wild traits, it is also necessary to consider backcrossing, bringing back the useful drought-resistant genes and eliminating the undesirable traits. In terms of group size, we plan to expand the key combinations a bit, starting with over a thousand F_2 plants, and follow up on a small scale for the rest of the combinations. The advantage of a large group is that there are more separations and greater opportunities. Even if the selection rate is only a few percentage points, it is still enough for us to pick out a few truly promising ones. The selected good plants can be directly propagated asexually, eliminating the trouble of subsequent fixation. The issue of uneven pollination periods has also been taken into account in advance. The flowering period will be adjusted to ensure that the parents can match. Overall, this set of group design not only has an exploratory element but also reserves room for adjustment. It selects as it is done and gradually accumulates drought-resistant advantages.

6.3 Combined evaluation method: Selection process of GCA/SCA, heterosis, and MAS embedding

After the hybridization and combination are produced, having just a bunch of offspring is not enough; it is necessary to first figure out which combinations truly have potential. This step is actually quite complicated. It depends on the compatibility and heterosis of the parents, and molecular marker breeding needs to be embedded in the early generation. We will first use the data from double-row hybridization to estimate the general and special compatibility - simply put, it's to see which parent "carries better genes" and which combination "matches ingeniously" (Bernardo, 2016). Some parents are like reliable players, capable of contributing good genes wherever they are placed. Some combinations have a "chemical reaction" and perform beyond expectations. Both situations are important, but the former is suitable for long-term improvement, while the latter may be directly promoted. If it is found that drought resistance mainly depends on additive genes, then multi-parent aggregation should be carried out. But if the heterosis is obvious, it would be more cost-effective to retain F_1 or its offspring. Next, let's look at heterosis. Let's compare F_1 with both parents - it's best if it can be higher than the average high point, but if it exceeds the best parent, it's truly worth it. Especially in crops like sweet potatoes that can be propagated quickly, F_1 can be directly expanded and propagated as soon as it is needed. As for marker assistance, we will conduct DNA testing at an early stage to select plants with drought-resistant gene loci and then verify them in the fields. Marking is not an absolute standard, but it can help us filter out a batch of individuals who are "busy for no reason" first, saving time and space. For the few strains selected later, they will be tested in different regions and different years to see if they are stable even in drought and have high yields even in rain. The last ones left should not only be reliable in drought resistance but also ensure that the output does not decline. After completing the entire set, it's not about seeking to be fancy, but about making seed selection more accurate, faster, and closer to the actual needs of the field.

7 Case Study

7.1 Case background and objectives: regional drought scenario, material selection and verification purpose

To test whether the drought-resistant breeding concept we proposed can hold water in practice, the experimental site we chose this time was a bit "serious" - in the typical dry land of the northern hills, with an annual rainfall of less than 500 millimeters, mostly concentrated in summer. Sweet potatoes are grown in large quantities here, but often due to the lack of rain in the later stage, the tubers do not grow well, and the harvest is good year after year and bad year after year. Although the local old varieties have good adaptability, in drought years, it is common for their yields to drop by 30% to 40% (Low et al., 2020). With the increasing number of extreme weather events in recent years, farmers' expectations for "drought-resistant sweet potatoes" have become more urgent. We selected three varieties: one is the drought-tolerant type A that was screened out, with deep roots, small leaves and low water consumption; One is the local high-yield variety D, which is stable in production but loses its strength when there is a drought. There is another one called Z, which is a commonly used improved variety and is used as a control. The plan is to combine A and D to see if the next generation can balance yield and drought resistance (Makhubu et al., 2024).

7.2 Case Implementation Process

At first, the twenty samples of sweet potato germplasm we received seemed not much different, but as soon as we conducted genotype testing, the situation became clear immediately. Only variety A carries drought-tolerant genes at key sites, while the rest are mostly blank (Yada et al., 2017). The drought resistance of A is indeed outstanding in the field. Although variety D is not drought-tolerant, it grows uniformly, has a high yield and good quality. Rather than saying that we "pick out" A and D, it would be more accurate to say that their respective advantages and disadvantages precisely form a pair. D has a broad genetic background and A has a clear genotype. When combined, they seem like complementary puzzles. Later water control tests also confirmed this judgment-A could withstand the drought and D could produce stably, so they naturally became hybrid parents (Figure 2) (Abrham et al., 2021).

The hybridization process is not all smooth sailing. Sweet potatoes are difficult to pollinate and do not cooperate when the temperature is high. We adjusted the lights and timing just to ensure that A and D could bloom on the same day. Fortunately, all the efforts were not in vain and we reaped several hundred F_1 seeds. The following spring, we planted these F_1 seedlings together with A, D and control Z in the dry field. The conditions in the fields soon showed a clear contrast - some F_1 were as lush and green as A, while others learned D's "laziness", with their leaves wilting early. The best one is called F_1-17. Its yield not only exceeds that of D but also slightly surpasses that of A. It's obvious that the genes of the two have indeed created a "spark".

Later, we conducted a marker test, and the result was as expected: F_1-17 carried the two drought-resistant alleles of A. The subsequent trial planting was even more surprising - in the farmers' fields, it not only maintained stable yields in normal years but also led even further in drought years. With high yield and good shape, farmers fell in love with it at first sight. Ultimately, we named it "Drought-Resistant Sweet Potato No. 1". This entire process won't take many years, while traditional methods take at least two or three generations. To put it bluntly, this case proves that by integrating molecular markers with hybrid breeding, the drought resistance improvement of sweet potatoes can also proceed quickly and steadily.

7.3 Presentation and interpretation of case results

In the case analysis, the performance of several key markers is quite interesting. For instance, the two loci S11 and S5 may not seem particularly special at first, but upon follow-up, it was found that they almost determine the drought resistance level of the offspring (Yada et al., 2017). Parent A carries two favorable sites, while parent D has none. As a result, among the descendants of A×D, as long as they inherited two sites of A, that small group of plants-approximately a quarter - had significantly stronger drought resistance and a much higher yield index. Conversely, the batch that did not inherit any loci is almost the same as the inferior parents. This gap indicates that the combined effect of multiple drought-resistant genes is far stronger than that of a single one. Even better, by using molecular markers for screening, potential plants can be identified at an early stage, saving a great deal of time and effort for field verification. To put it bluntly, it means not to waste resources on those three thirds of ordinary plants.

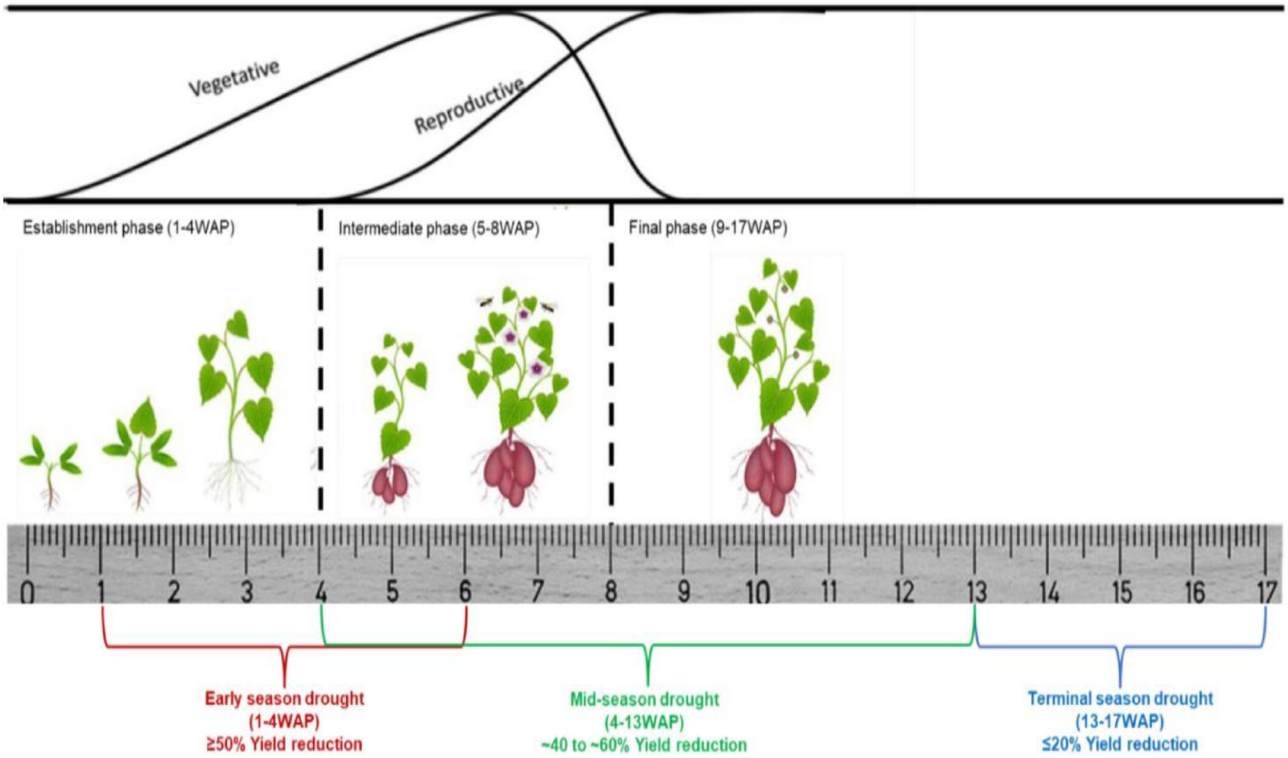

Figure 2 Sweet potato developmental stages and approximated yield losses when subjected to early, mid or terminal season drought stress. WAP represents week after planting (Adopted from Abrham et al., 2021)

The reason why the A×D combination stands out is not only because A itself has more drought-resistant genes, but also because its traits complement those of D. Although D is not drought-resistant, it has a large leaf area and strong photosynthetic capacity. When the two come together, they create a spark. However, for a combination like A×Z, although both parents are "water-saving", unfortunately, they are too similar, and the hybridization has not brought out any advantages. It seems that drought-resistant breeding cannot merely rely on piling up genes; it also requires proper combination. In summary, this case provides several practical tips: When choosing parents, select those with key QTLs and also look for those with significant differences in traits. When combining, try to make the drought-resistant mechanisms complementary as much as possible. Label and screen in the early generation. Don’t wait until the field to verify. Finally, plant a few more seasons to see if it is stable (Wang et al., 2025). The emergence of a variety like F_1-17, which is both drought-resistant and high-yielding, proves that it is entirely possible for sweet potatoes to achieve dual excellence through hybridization. Those views that sweet potato cross-pollination is troublesome and progresses slowly should also be reconsidered.

8 Conclusions and Prospects

The most direct outcome of this research is that we have finally clarified which sweet potato materials are truly "drought-resistant" and how to find them. After multiple experiments, it was found that the materials with deep roots, leaves that do not wilt easily and can still maintain a certain yield in drought years are more stable than we imagined. Varieties like A and D have a tuberous root yield reduction of less than 40%, which is particularly outstanding in arid areas and has also become the germplasm basis for subsequent hybridization. Looking further in, the molecular results revealed the roles of several key genetic loci. The two dots on chromosomes 11 and 5 stand out particularly, as they manage a considerable proportion of the differences in drought resistance. Based on this, we have developed several PCR markers that enable breeders to immediately identify which plants carry "good genes". In fact, this marking system is quite practical. The laboratory can screen out most of the individuals with no potential. Then, we attempted to combine the molecular results with the hybridization design. The results show that a simple "strong alliance" is not necessarily the best. Sometimes, a combination of one drought-tolerant and one high-yield with complementary mechanisms is more stable. For instance, in the group A×D, the water-saving type met the growth type, and the offspring performed well both in drought and normal years. Finally, we verified the value of MAS - if used early and accurately, it can halve the breeding cycle and also increase the selection rate. Overall, this method has screened out reliable parents, effective markers and scalable combination patterns, providing a relatively clear path for drought-resistant breeding of sweet potatoes.

Of course, these results are still some distance away from being conclusive. The most obvious problem is phenotypic fluctuation. The yield and drought resistance of sweet potatoes are easily affected by the environment - the same variety may look good in a dry greenhouse but blend into obscurity in a large field. We try to eliminate errors by using multiple indicators and repeated experiments, but interannual climate, soil depth and disease stress are all difficult to control completely. Some results are thus inconsistent at different points. Furthermore, the genetic effects of drought resistance may not be universally applicable. The QTL we found is significant in this set of materials, but it may not be effective in another group or region. The portability of the markers needs to be verified; otherwise, MAS may fail elsewhere. The complex polyploid genome of sweet potatoes also adds uncertainty - are the associations we observe causal relationships or chain phenomena? It cannot be completely asserted at present. Furthermore, some drought-resistant genes may be associated with traits such as low yield and susceptibility to diseases. Excessive pursuit of drought resistance may instead sacrifice other traits. This kind of "side effect" was detected in our research but has not been analyzed in depth yet. In conclusion, this study is more like a directional verification, proving that the method is feasible, but it still requires a broader range of materials, environments and time for confirmation. Although MAS is powerful, it is not a panacea. Phenotypic verification remains indispensable.

The idea of how to move forward is actually quite clear: first, one needs to "see faster", and second, one needs to "make more accurate choices". At present, the efficiency of manual root and moisture measurement is too low. The next step should be to leave it to machines to do. Drones, infrared cameras and hyperspectral imaging can screen out the differences of thousands of sweet potato strains in the fields within minutes, saving several months of labor. Combined with AI analysis, an objective drought resistance scoring system can be formed. On the other hand, MAS only covers large-effect genes, while the majority of small-effect ones still rely on genomic selection. By integrating MAS and GS and using models to predict the "drought resistance value", it is even possible to assess potential at the seedling stage without having to plant everything in the field. The future breeding process may change to: first test the genotype, then calculate the breeding value, and finally take the top few to the field for verification. At the same time, it is necessary to truly understand the functions of these candidate genes. By conducting gene editing or overexpression experiments to knock out or enhance key genes and observe the plant's response, the target can be determined. A further direction is to conduct large-scale trial planting in real environments, allowing farmers to participate in the screening and observe the performance of new varieties under different climates and management methods. Drought-resistant breeding will eventually have to return to the fields. When this system matures, the drought resistance of sweet potatoes will no longer rely on "fate", but on design. At that time, what we could do was not only to resist drought, but also to take into account heat tolerance, salt tolerance and even disease resistance, truly turning sweet potatoes into the representative of "adversity crops".

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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