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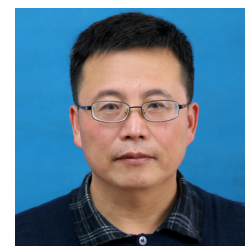
Applications of DNA Technologies in Agriculture



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Abstract: With the development of molecular biology, some DNA-based technologies have showed great potentiality in promoting the efficiency of crop breeding program, protecting germplasm resources, improving the quality and outputs of agricultural products, and protecting the eco-environment etc., making their roles in modern agriculture more and more important. To better understand the application of DNA technologies in agriculture, and achieve the goals to promote their utilities in modern agriculture, this paper describes, in some different way, the applications of molecular markers, transgenic engineering and gene's information in agriculture. Some corresponding anticipations for their development prospects are also made.



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1. INTRODUCTION

DNA is the main genetic materials of all cellular organisms; preserving DNA itself is one way of preserving germplasm resources [1]. The development of biotechnology and molecular biology make it possible for us to regulate or even control the plant traits, by using DNA sequence information, such as the structure, function and mechanism etc. DNA technologies based on DNA molecular markers, transgenic technology and gene expression have been widely used in agricultural production which have showed great potential in improving agricultural yields and quality, reducing the loss that various biotic and abiotic stress caused, promoting the utilization of germplasm resource, improving breeding efficiency and strengthening the regulation of plant growth [2-4]. These modern DNA technologies with high feasibility and necessity are important measures to guarantee the sustainable development of agricultural. Despite the agriculture including plant and animal production, DNA technologies in these fields share the same technical purpose and type. Therefore, in this study, we will review the agricultural applications of DNA technologies by introducing the utilization of DNA technologies in plant production.

There were many reports about the application of various DNA technologies in agricultural production till now; and the DNA technologies have been constantly developed and updated, which make it difficult to introduce every DNA technique. Therefore, we will mainly introduce the agricultural applications of DNA molecular marker and transgenic technology in this paper.

2. THE APPLICATION OF DNA MARKERS IN AGRICULTURE

Since the advance of the first generation molecular marker technology restricted fragment length polymorphisms (RFLP)

and the Polymerase Chain Reaction (PCR) in 1980 [5], various DNA markers were developed and used in biology research, such as DNA random amplified polymorphic DNA (RAPD), single nucleotide polymorphism (SNP), amplified fragment length polymorphism, (AFLP) and sequence-related amplified polymorphism (SRAP) etc. Compared with the traditional morphological markers, cytological markers, and biochemistry markers, DNA markers have much more information and higher polymorphism, and can work without the influence of plant organs, developmental stages and various environmental factors, which have been widely used in identification of crop varieties, conservation and evaluation of plant germplasm resource, analysis of genetic adversity and evolution, construction of genetic maps, cloning of important agronomic trait genes, and molecular mark assisted breeding.

2.1. Cultivar Identification and Analysis of Seed Purity

For a long time, plant cultivar identification and seed purity test were mainly based on phenotypic traits. However, the rapidly increasing crop cultivars and the close evolutionary relationship among some cultivars and the confuse of the varietal names and their local names, in addition, the phenomena of 'homonym' and 'synonym' have been making it more and more difficult to collect, classify and conserve the plant resources. The incompetence of the traditional plant cultivar identification techniques calls for a more stable, simple and accurate technique. DNA molecular marker has high specificity, selectivity, simplicity, accuracy and genetic stability can reflects the differences in DNA level without environmental impacts, and thus has great advantages in seed purity identification [6]. The DNA molecular marker technology has been used in the identification of cultivar and seed purity of rice (*Oryza sativa*), wheat (*Triticum aestivum*), maize (*Zea mays*), cotton (*Gossypium hirsutum*) and grape (*Vitis Vinifera*) etc. [6, 7].

In order to better apply the DNA markers to cultivar identification, the conversion of DNA fingerprint to the

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available and convenient information which can be directly used in the cultivar identification practice is crucial. However, in the early days, the lack of available technique or measure make it difficult to convert the DNA marker fingerprint to the intuitive and easy access information, which lead to the research findings on cultivar identification were barely used in the agriculture production. In the former study, the DNA marker identification results were mainly analyzed by electrophoresis band database, binary table and cluster analysis, but these information were too abstract and difficult to be used in the ordinary cultivar identification practice. Recently, with the development and application of the manual cultivar identification diagram (MCID) (Fig. 1) [6, 7]; the cultivar identification by DNA marker has achieved the goal to serve for the agriculture directly. In MCID, the information of DNA marker primer and the polymorphic bands were marked to the cultivar identification diagram (CID) through artificial drawing, by which the cultivar identification results can be used or referred in the cultivar identification practice. Zhang *et al.*, (2012) [7], Zhao *et al.*, (2011) [8], Leng *et al.*, (2012) [9] and Mu *et al.*, (2012) [10] applied the RAPD molecular marker to the cultivar identification of grapevine, pomegranate, loose-skin mandarin and lemon. The CID they have drawn can provide the useful information of primer and polymorphic bands which can be used to identify the cultivar of grape and pomegranate. The combination of DNA marker and the MCID make it possible to exert the advantages of DNA marker in cultivar identification sufficiently.

2.2. Evaluation of Germplasm Resource

Preservation and evaluation of plant germplasm resources are very important for keeping the rich diversity of breeding parents and genetic base and the rational using the

germplasm resource efficiently and rationally. DNA molecular marker technology provides an important technical mean for preservation, identification, evaluation, mining and innovation of plant germplasm resources. Ram *et al.* (2007) [11] and Moncada *et al.* (2006) [12] analyzed the genetic diversity of field crops such as rice and grapevine by SSR marker, and this results provided important theoretical basis for the utilization of these crops. DNA molecular marker technology has become efficient technology to evaluate the germplasm resources of different crops. By using DNA molecular marker, not only can we protect the genetic integrity of germplasm resources, maintain minimum breeding population and seed amount, screen important germplasm, and preserve large amount of germplasm resources, but also we can study the genetic diversity and evolutionary relation of germplasm resources. The information about their DNA level diversity and their origin and evolution relationship will greatly help us to make better use of the excellent germplasm resources of crop and provide an important scientific basis for the protection of these germplasm resources.

2.3. Heterosis Prediction

Heterosis prediction can greatly reduce the blindness and increase the pertinence of breeding, it is important to improve breeding efficiency and shorten the breeding process. By DNA marker technology, we can detect the positive markers or positive locus related to heterosis in any period or organ, and then predict the heterosis based on the heterozygosity of these locus. This technique overcome the deviation of genetic distance-based heterosis prediction (In some case, genetic distance has no relationship with heterosis). In addition, DNA marker overcome the shortcoming of isozyme-based heterosis prediction, in which it has too much restriction to be widely used. In recent years, the use of molecular

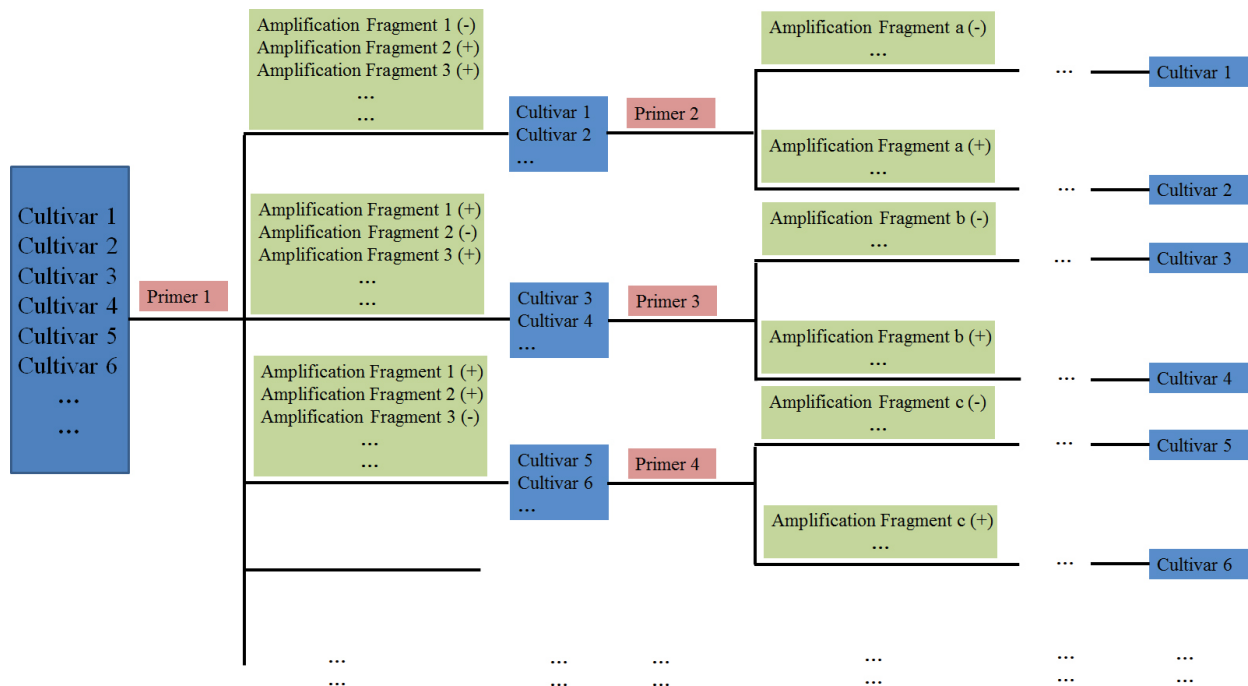


Fig. (1). The schematic of manual cultivar identification diagram (MCID). The plus sign (+) indicates the presence of the amplification fragment, the minus sign (-) indicates the absence of the amplification fragment, ellipsis (...) indicates that the lists of cultivars/primer/ amplification fragment are not intended to be complete.

markers has made some progress in the prediction of heterosis in maize, rice, cotton, rape and other crops. For example, Percy *et al.* (2006) [13] found that the heterosis of boll number and weight of single cotton were significantly related to the genetic distance of the molecular marker; Solomon *et al.* (2007) [14] reported that the genetic distance of the molecular marker has significantly positive correlation with the heterosis of grain weight.

2.4. Construction of Genetic Map

The genetic map is the chromosome linear linkage map that uses the chromosome recombination and exchange rate as the relative length and genetic markers as the main body. It has great theoretical and practical significance in fields such as quantitative trait loci (QTL) mapping, marker-assisted selection, gene map-based cloning and comparative genomics etc. The genetic map constructed by DNA molecular marker technique give us chance to analyze the linkage relation of the genetic loci at the molecular level directly. The genetic loci we can get from the genetic map are far more than that we can get from the traditional genetic markers methods constructed based on morphology, physiology, or biochemistry. In addition, the construction cycle is shorter, and the density of markers is higher compared with the traditional ways. In recent years, with the rapid development of molecular marker technology, the genetic maps for most crops have been drawn. For example, Cui *et al.* (2012) [15] constructed the high-density genetic linkage map of wheat (average genetic distance is 4.42) using DArT markers, and Blenda *et al.* (2012) [16] constructed the high-density genetic linkage map of cotton (average genetic distance is 4.3) using SSR marker. Most of the field and economic crops have finished the construction of high density and even saturated genetic linkage maps, and this will be of great help to understand the information of genomic composition and structure, and will finally benefit to the breeding practice.

2.5. Gene Mapping, Cloning and Marker-assisted Breeding

Molecular marker can link to the target phenotypic traits loci, by which we can locate the important agronomic traits related genes, provide the convenience for marker-assisted breeding and map-based cloning of these genes. On the basis of precise location of the target gene by using DNA molecular markers, breeders can take advantage of the molecular markers which closely linked to or co-separated with the target genes, by which they can identify the existence of the target gene in breeding offspring, and determine whether they obtain the desired individuals. Molecular-assisted breeding makes up for the shortcomings of the traditional field selective breeding method which is time consuming, low deficiency and inaccurate. At present, molecular-assisted breeding technologies has been widely used in crop breeding and has developed a number of new crop cultivars and lines. Jena and Mackill (2008) [17] and Shi *et al.* (2009) [18] applied the molecular marker assisted breeding techniques to select the excellent crop quality traits, and succeed to select the blast resistance trait of rice and resistance to soybean mosaic virus of soybean. Gene map-based cloning technology is a technology suitable for gene cloning, which is de-

veloped on the basis of DNA molecular markers and marker genetic linkage map. Based on accurate location of the target gene by molecular markers, and use the molecular markers that are closely linked to the target gene as probes to screen genomic library, by which we can clone the large fragments of the target gene. Map-based cloning techniques has great advantages for novel gene cloning, and has been successfully used in the separation and cloning of excellent agronomic, growth and development and resistance related genes in rice and maize. For example, Tamura *et al.* (2014) [19] and Gao and Lin (2013) [20] successfully cloned salt- and insect-resistant genes in rice by map-based cloning technique, Lu *et al.* (2012) [21] cloned the key enzymes genes of terpenoid metabolic pathway in maize.

3. APPLICATION OF GENE EXPRESSION INFORMATION IN AGRICULTURAL PRODUCTION

Traditionally, the growth and development condition and the phenological period of crops are the main reference basis for the agricultural production. These information are mainly collected through observing and recording the phenotypic trait of crops. This method has played important roles in the traditional agricultural production that is intuitive, simple and effective. However, the appearance of phenotypic traits always lags, and cannot feedback the real growth and development situation of crops timely and accurately. What's worse, it always too late to take remedial measures when the negative phenotype appears. Every traits of plant is genetically controlled by gene, and the gene information can accurately reflect the real growth, development and metabolic status. Furthermore, the expression of gene is always earlier than the appearance of phenotype, so monitor and diagnose the growth and development status of crops at gene level makes it possible to predict the unfavorable change or harm before it happens, and take remedial measures in advance (Fig. 2). The development of modern molecular biology technique provides extremely rich and even redundant gene information to the researchers. Even though gene expression profile has been widely used to address the relationship between ecologically influenced or disease phenotypes and the cellular expression patterns, the information is mainly limited to laboratory experience, it is necessary to apply the crop growth and development related gene information to agricultural production, so the techniques or methods that are able to convert these gene information to convenient and easy access information are urgent.

In the era of molecular biology, there are high necessity and feasibility to apply the gene information to agricultural production. People can use genetic information to observe or monitor the growth status of crops and provide guidelines for the field management, by which we can improve the efficiency of agricultural measures such as fertilization and irrigation, and regulate the maturity and growth habits and other important growth process of crop.

3.1. Gene Information Describing the Crop Phenology

Plant phenology is the appear time of various iconic morphological characteristics such as seed germination, leaf, blossom, fruit growth and development status in plant life cycle, is the reaction of growth, development and activity of plant response to climate. By observing phenological events,

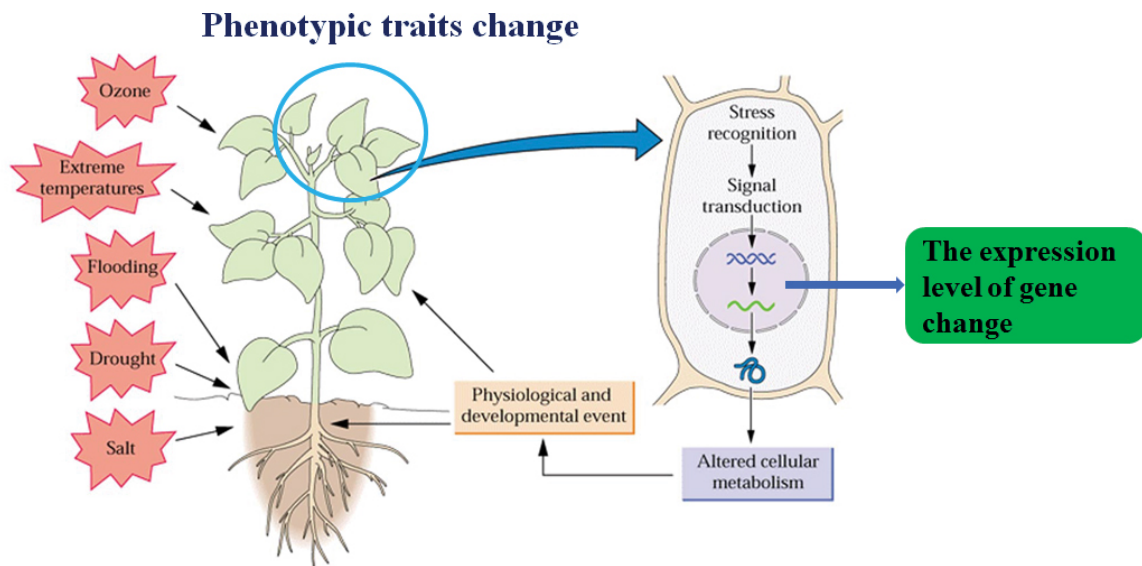


Fig. (2). The model of gene expression information for depiction of plant growth status.

we can grasp the seasonal variation of crop, guide crop planting, cultivation management and disease-pest control. However, observing and recording the phenological period need to spend a lot of time, work force and financial resources, and the data obtained is limited by the cultivar, climate, geographical environment and agricultural operations and other factors. Thus phenology and phenological period is different in different years, last year record for reference for the following year is not precise enough. Therefore, the current situation, i.e., phenological events cannot be used to accurately depict phenophases, calls for the advent of a more accurate means of depicting phenology. The expression of genes involved in plant growth and development is affected by the common regulation of plant growth and environmental conditions, so the researchers can more accurately describe the phenological period of crop growth by using the gene information, i.e., gene phenology, and then according to gene phenology we can early elaborated plans and employed appropriate management.

When the changes of external environment caused the advance or delay of phenology, the expression of related genes at the molecular level invariably precedes the emergence of phenological phenomenon, therefore, we can timely and reasonable judge and implement farming operation. Wang *et al.*, (2014) [2] found changes in expression information of flower development related gene APETALA1 and APETALA3 in grape can be used to describe the phenology of grapes, and can guide the precise fertilization in grape production, restrain flower and fruit drop and promote the development of flower and fruit.

As more and more genes related to crop growth and development genes have been excavated, the researchers were able to describe different phenological events in different crops at different developmental stages systematically, precisely and quickly [2]. This will enable the use of gene information to describe phenology and take appropriate management measures become simpler, more efficient and cost less. This strategy promotes the practice of using gene information in agricultural production.

3.2. Prediction of Crop Growth Status Under Stress

Gene expression information can also be used to predict the effects of environmental stress on the growth and development of crops, which is convenient for people to take appropriate management measures (Fig. 2). Crops sometimes suffer various biotic or abiotic stresses in agricultural production, resulting in reduction in agronomical yield, falling off in quality, and will affect agricultural output.

In the past, people grasp the growth state under stress through the phenotypic traits of crop. Nevertheless, these phenotypic traits require a certain amount of time, and the effect of some stress on crop is difficult to observe from the outside. More often, when people have observed the phenotypic traits of crop under stress and then take measures, it is often difficult to bring the plant back to normal state. Therefore, it is not the suitable way and measure to guide the remedial or recovery of crops under stress through the phenotypic observation of the growth state of crop under adversity.

There are many genes of crop involved in transcriptional response to stress such as drought, high salinity or low temperature, the expression level of these genes increased or decreased affected by the stress condition. Therefore, through the expression information of these resistance related genes, researchers will be able to know whether the crop encounter unfavorable growth conditions, and take timely remedial measures.

Krasensky and Jonak (2012) [22] studied the expression patterns of stress-inducible genes in drought, extreme temperature and salinity stress. The beginning, end and changes in the expression of genes related to plant signaling events involved in responses to environmental stresses such as salicylic acid, jasmonic acid and abscisic acid and calcium ion, reflect the growth state of crop under stress. Researchs about the expression information of gene in different tissues and different developmental stages under different environmental conditions will provide support for the prediction of growth state of crop under stress at the molecular level [23]. These researches not only contribute to complementing understand-

ing of metabolic adjustments of plant under stress, but also play an important role in increasing agricultural production, reducing the consumption of resources.

3.3. Evaluation of the Effect of Fertilizers

In the past, determining fertilization time, fertilization types, and the amount of fertilizers in agricultural production are general implemented based on crop growth and phenology, generally is experience operation, and would be greatly affected by geographical and environmental conditions, thus it is undoubtedly difficult to accurately guide the effect of fertilization. These practices not only failed to achieve the best benefits of fertilizer application, but also resulted in the waste of fertilizer and environmental pollution. Therefore, how to invest a reasonable input of nutrient, at the same time guarantee the quality and yield of crops, and how to choose the more effective fertilizers and more scientific fertilizing methods, are issues of concern.

Modern DNA technology makes it possible for people to understand the effect of fertilization promoting plant growth and development at the molecular level. Chu *et al.*, (2012; 2013) [24, 25] found we can determine the effect of fertilization by the expression of some genes related to nitrogen metabolism in grape. Through the analysis of expression level of some gene such as glutamine synthetase (GS) and glutamate dehydrogenase (GDH), the time of these gene reached the highest expression level was 6h earlier after foliar fertilization compared with soil fertilization, and gene expression levels were higher, these results partly revealed the reason why foliar fertilization take effect quickly. The use of genetic information not only can accurately clarify the influence of conventional fertilization on crop growth and development; also can more accurately assess the use efficiency of fertilizer, the work speed of different fertilizing methods such as foliar and soil fertilizer, the fertility of different fertilizer and so on. Gene expression information can reflect the metabolism and transport of fertilizer in crop, so through analysis of spatio-temporal expression of related gene, we can obtain the gene information on the response of crops in different kinds and concentration of fertilizer in different growth periods [26]. On this basis, combined with the status of crop growth and development, we can establish more scientific and reasonable fertilization measures in different growth stages, such as the period of fertilization, the type of fertilization and the amount of fertilizer. These measures will greatly save fertilizer dosage, reduced investment in agriculture, increased the income of farmers, and reduced the environmental pollution.

Crop cultivation along with many farming operations, each farming operation will cause the corresponding changes in gene expression at the DNA level, the usage of DNA technology to detect such changes at the molecular level can help us to understand regulation mechanism of farming operation on crop growth and development [27]. Now there were more and more studies about the influence of various cultivation operations on the expression level of related genes. The expression level of some genes of grape increased and expressed early because of treatment with abscisic acid [28], similarly, branch girdling makes the high expression level of citrus genes about 1 week in advance [29].

According to soil characteristics and the needs of crop growth development, we can use gene information to manage every growth stage of crop, and decide the consumption of various agricultural material (fertilizer, herbicide, pesticide, and hormone etc.), and which cultivation measures (girdling, bagging etc.) to use. Thus, we can make full use of the potential of soil and crop, archive the best results from field management technology, meet the needs of crop growth, and reduce agricultural materials inputs, thereby reduced the material consumption, increased commercial profits, protected the ecological environment, and realized the sustainable development of agriculture.

In total, plant growth and development are controlled by the programmed (by time, tissue and abundance) expression of suites of genes in response to exogeneous or endogeneous queues. Hence, there is a need to generate genome-wide expression data from a range of tissues/ developmental stages in order to understand and relate phenotypic traits and gene expression profiles. And data at the transcriptome and epigenome level can contribute greatly to the application of gene information in agriculture [30].

4. APPLICATIONS OF TRANSGENIC TECHNOLOGY IN AGRICULTURE

Transgenic technology can improve accurately crop quality and yield, compared with traditional crop, the all traits like yield, stress resistance (including disease-resistant, insect-resistant, cold-resistant and herbicide-resistant) and nutritional quality of genetically modified crops will be significantly heighten. The commercialization of genetically modified crops dramatically lowering the cost of agriculture, and bring continuously a great deal of benefits of economic, environmental and social in the world.

4.1. Prevention and Control of Agricultural Disease and Pest

Transgenic technology is widely used to control the agricultural disease and insect-pest, reduce the cost of agricultural production, improve the effect of disease and insect control, reduce pesticide pollution, and increase crop production, thus brought new vigor and hope in the prevention and control of crop diseases and pest.

The research and promotion of transgenic herbicide-resistant crop has been dominant in the transgenic crop, to date there are many herbicide-resistant cultivars were created by transgenic technology in cotton, maize, soybeans and rape [31]. Transgenic insect-resistant crop is one of the most important kinds of transgenic crops, in the past 10 years, transgenic insect-resistant crop such as cotton, maize and potato have achieved fruitful results [32, 33]. In recent years, plant disease-resistance genetic engineering has achieved many breakthroughs, and has bred many kinds of transgenic disease-resistant crop such as the Fusarium wilt- and Verticillium wilt-resistance transgenic cotton [34].

Currently, insect resistance, disease resistance, herbicide-resistant transgenic maize, cotton, potato and soybean have entered the commercialization stage, and have a larger cultivation area [4, 35].

4.2. Quality Improvement

Transgenic technique can be applied to improve the qualities of crop [36], including protein content, amino acid composition, starch composition, polysaccharide compounds and lipid in seeds and other storage organs (tubers, tuberous root, bulbs, etc.) [37, 38], finally improve food quality, increase nutrition and ameliorate health function and processing performance etc.

4.3. Enhanced Resistance

Transgenic technology is also widely applied in breeding new cultivars possessed high drought-, salt- and cold-resistance [39]. The rice which introduced into c-repeat binding factor (CBF) possess significantly resistance to abiotic stress [40, 41]. The offspring of rice introduced into *Arabidopsis thaliana* dehydration responsive element binding (DREB) gene exhibited better growth status than non-transgenic plants under drought stress [42]. The tobacco introduced into S-adenosylmethionine decarboxylase (SAMDC) gene grew well under abiotic stress [43]. A bacterial mannitol-1-phosphate dehydrogenase (mtlD) gene was transferred into tomato which showed a significant resistance to the low temperature stress [44].

Transgenic crops have been extended into many aspects, such as biotic and abiotic stress-tolerant, fertility, yield and quality improvement [45]; furthermore, some techniques have been or are being applied in crop breeding program. Golden rice and drought-tolerant maize created early by transgenic technique could be used in actual production cultivation.

Transgenic cotton is among the first transgenic plants commercially adopted around the world. Transgenic cotton has offered many important environmental, social, and economic benefits, including reduced usage of pesticides, indirect increase of yield, minimizing environmental pollution, and reducing labor and cost. Although transgenic cotton plants with disease-resistance, abiotic stress tolerance, and improved fiber quality have been developed in the past decades, insect-resistant and herbicide-tolerant cotton are the two dominant transgenic cottons in the transgenic cotton market. Thus in the future, the development of transgenic crops no doubt will focus on fields like stress resistance, quality improvement, yield increased, functional components, this will bring opportunities and challenges for the development of world agriculture.

4.4. Molecular Design Breeding

In recent years, molecular design breeding developed on basis of the combination of the DNA technology and cultivar improvement has gradually attracted people's attention. Before the field experiments, molecular design breeding would integrate the information related breeding program on the computer, and simulate the implementation plan, thus considered more factors, more comprehensively, and put forward the best strategies of selection of parental and progeny, to enhance predictability in the breeding process, thereby substantially improve breeding efficiency [46].

Molecular design breeding will greatly improve the theory and technology of plant breeding, and make the traditional breeding technology more efficiently and targeted. At

present, molecular design breeding have achieved certain effects in wheat, rice, soybean and rape [46]. Molecular design breeding is a new and highly integrated research field; we need to use multiple DNA techniques such as mapping and analysis of agronomic QTL, the mapping and cloning of important trait related genes, construction of genetic linkage map and prediction of heterosis. Among them, molecular marker-assisted breeding and gene genetic transformation techniques are two of the most basic and most important technologies in molecular design breeding, which are also key factors to restrict the development of molecular design breeding. With the innovation and breakthrough of both technologies in the further, the development of crop molecular design breeding will be more rapid.

In the development of future, molecular design breeding is bound to become an integrated breeding method that would combine multiple disciplines and would be constantly improved. Molecular design breeding will in the future have a profound influence on the development of theory and technology of crop breeding, and DNA technology is playing an irreplaceable role in this process.

5. PROBLEM AND PROSPECT

Now agricultural production has relied on hormones and chemical fertilizers on long-term steroids, and especially the abuse of pesticides increased the dosage and residual of pesticides, pollution are becoming much severer, the quality of agricultural products continue to decline. Agricultural production is unable to meet the growing demand. With the rapid development of molecular biology today, it is necessary for scientist to use the technology based on the molecular level to do better on the agricultural industry. DNA techniques can be applied to the analysis of genetic background of crop germplasm, cultivar identification, crop genetic improvement as well as the analysis of growth state, and has shown very good results. There are a lot of work to be done in some areas, such as techniques improvement, utilization of chromosome engineering in the innovation of crop germplasm, further increase of crop quality and yields and bio-safety prevention, is the goal or task that relevant experts pay attention and efforts on.

Currently, the world is facing the crisis of soaring population and food shortage, agricultural production has become a very important issue in the construction of national economy. With modern DNA technology becomes more and more widely used in agriculture, the current major issues like food, population, pollution and other problems that our world facing would get better solution. Therefore, the development of modernization of agriculture based on new DNA technology is an important choice.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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REFERENCES

- [1] Gepts, P. Plant genetic resources conservation and utilization. *Crop Sci.*, **2006**, *46*(5), 2278-2292.
- [2] Wang, C.; Han, J.; Shangguan, L.; Yang, G.; Kayesh, E.; Zhang, Y.; Leng, X.; Fang, J. Depiction of grapevine phenology by gene expression information and a test of its workability in guiding fertilization. *Plant Mol. Biol. Rep.*, **2014**, *32*(5), 1070-1084.
- [3] Agarwal, M.; Shrivastava, N.; Padh, H. Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Rep.*, **2008**, *27*(4), 617-631.
- [4] Garcia, M. A.; Altieri, M. A. *Bulletin of Science, Technology & Society*, SAGE Publications: USA, **2005**.
- [5] Saiki, R. K.; Bugawan, T. L.; Horn, G. T.; Mullis, K. B.; Erlich, H. A. Analysis of enzymatically amplified β -globin and HLA-DQ α DNA with allele-specific oligonucleotide probes. *Nature*, **1986**, *324*(6093), 163-166.
- [6] Nicholas, K. K.; Han, J.; Shangguan, L.; Wang, C.; Kayesh, E.; Zhang, Y.; Fang, J. G. Plant variety and cultivar identification: Advances and prospects. *Crit. Rev. Biotechnol.*, **2012**, *33*(2), 111-125.
- [7] Zhang, Y. P.; Tan, H. H.; Cao, S. Y.; Wang, X. C.; Yang, G.; Fang, J. G. A novel strategy for identification of 47 pomegranate (*Punica granatum*) cultivars using RAPD markers. *Genet. Mol. Res.*, **2012**, *11*(3), 3032-3041.
- [8] Zhao, M. Z.; Zhang, Y. P.; Wu, W. M.; Wang, C.; Qian, Y. M.; Yang, G.; Fang, J. G. A new strategy for complete identification of 69 grapevine cultivars using random amplified polymorphic DNA (RAPD) markers. *Afr. J. Plant Sci.*, **2011**, *5*(4), 273-280.
- [9] Leng, X. P.; Li, H. R.; Zhong, G. Y.; Song, C. N.; Sun, X.; Fang, J. G. Employment of a new strategy for identification of loose-skin mandarin (*Citrus reticulata* Blanco) cultivars using RAPD markers. *Rom. Biotechnol. Lett.*, **2012**, *17*(2), 7073-7083.
- [10] Mu, Q.; Sun, X.; Zhong, G.; Wang, X. C.; Song, C. N.; Fang, J. G. Employment of a new strategy for identification of lemon (*Citrus limon* L.) cultivars using RAPD markers. *Afr. J. Agric. Res.*, **2012**, *7*(14), 2075-2082.
- [11] Ram, S. G.; Thiruvengadam, V.; Vinod, K. K. Genetic diversity among cultivars, landraces and wild relatives of rice as revealed by microsatellite markers. *J. Appl. Genet.*, **2007**, *48*(4), 337-345.
- [12] Moncada, X.; Pelsy, F.; Merdinoglu, D.; Hinrichsen, P. Genetic diversity and geographical dispersal in grapevine clones revealed by microsatellite markers. *Genome*, **2006**, *49*(11), 1459-1472.
- [13] Percy, R. G.; Cantrell, R. G.; Zhang, J. Genetic variation for agronomic and fiber properties in an introgressed recombinant inbred population of cotton. *Crop Sci.* **2006**, *46*(3), 1311-1317.
- [14] Solomon, K. F.; Labuschagne, M. T.; Viljoen, C. D. Estimates of heterosis and association of genetic distance with heterosis in durum wheat under different moisture regimes. *J. Agric. Sci.*, **2007**, *145*(03), 239-248.
- [15] Cui, F.; Ding, A.; Li, J.; Zhao, C.; Wang, L.; Wang, X.; Qi, X.; Li, X.; Li, G.; Gao, J.; Wang, H. QTL detection of seven spikerelated traits and their genetic correlations in wheat using two related RIL populations. *Euphytica*, **2012**, *186*(1), 177-192.
- [16] Blenda, A.; Fang, D. D.; Rami, J. F.; Garsmeur, O.; Luo, F.; Lape, J. M. A high density consensus genetic map of tetraploid cotton that integrates multiple component maps through molecular marker redundancy check. *PLoS One*, **2012**, *7*(9), e45739.
- [17] Jena, K. K.; Mackill, D. J. Molecular markers and their use in marker-assisted selection in rice. *Crop Sci.*, **2008**, *48*(4), 1266-1276.
- [18] Shi, A.; Chen, P.; Li, D.; Zheng, C.; Zhang, B.; Hou, A. Pyramiding multiple genes for resistance to soybean mosaic virus in soybean using molecular markers. *Mol. Breed.*, **2009**, *23*(1), 113-124.
- [19] Tamura, Y.; Hattori, M.; Yoshioka, H.; Yoshioka, M.; Takahashi, A.; Wu, J.; Sentoku, N.; Yasui, H. Map-based cloning and characterization of a brown planthopper resistance gene *BPH26* from *Oryza sativa* L. ssp. *indica* cultivar ADR52. *Sci. Rep.*, **2014**, *4*.
- [20] Gao, J. P.; Lin, H. X. *Methods in Molecular Biology*, Humana Press: New York, **2013**.
- [21] Lu, X. M.; Hu, X. J.; Zhao, Y. Z.; Song, W. B.; Zhang, M.; Chen, Z. L.; Chen, W.; Dong, Y. B.; Wang, Z. H.; Lai, J. S. Map-based cloning of *zb7* encoding an IPP and DMAPP synthase in the MEP pathway of maize. *Mol. Plant*, **2012**, *5*(5), 1100-1112.
- [22] Krasensky, J.; Jonak, C. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *J. Exp. Bot.*, **2012**, *63*(4), 1593-1608.
- [23] Reddy, A. S.; Ali, G. S.; Celesnik, H.; Day, I. S. Coping with stresses: roles of calcium and calcium/calmodulin-regulated gene expression. *Plant Cell*, **2011**, *23*(6), 2010-2032.
- [24] Chu, J. Q.; Wang, W. Y.; Fang, J. G.; Zhang, C. H.; Zhang, Y. P.; Song, C. N. Effects of foliar applied urea on expression of genes related to nitrogen metabolism in Fujiminori grapevine. *J. Plant Nutr. Fertil. Sci.*, **2012**, *18*(2), 405-416.
- [25] Chu, J. Q.; Yue, L. X.; Fang, J. G.; Liu, H.; Song, C. N.; Zhang, A. Effects of fertilizer application on expression of genes related to nitrogen metabolism in Fujiminori grapevine. *Acta Hort. Sinica*, **2013**, *40*(2), 221-230.
- [26] Yang, X. S.; Wu, J.; Ziegler, T. E.; Yang, X.; Zayed, A.; Rajani, M. S.; Zhou, D.; Basra, A. S.; Schachtman, D. P.; Peng, M.; Armstrong, C. L.; Caldo, R. A.; Morrell, J. A.; Lacy, M. Staub, J. M. Gene expression biomarkers provide sensitive indicators of in planta nitrogen status in maize. *Plant Physiol*, **2011**, *157*(4), 1841-1852.
- [27] Tenea, G. N.; Cordeiro R. F.; Maquet, A. Comparative transcriptome profiling in winter wheat grown under different agricultural practices. *J. Agric. Food Chem.*, **2012**, *60*(44), 10970-10978.
- [28] Xiao, H.; Siddiqua, M.; Braybrook, S.; Nassuth, A. Three grape CBF/DREB1 genes respond to low temperature, drought and abscisic acid. *Plant, cell Environ.*, **2006**, *29*(7), 1410-1421.
- [29] Yang, X. Y.; Wang, F. F.; Teixeira da Silva, J. A.; Zhong, J.; Liu, Y. Z.; Peng, S. A. Branch girdling at fruit green mature stage affects fruit ascorbic acid contents and expression of genes involved in l-galactose pathway in citrus. *N. Z. J. Crop Hort. Sci.*, **2013**, *41*(1), 23-31.
- [30] Varshney, R. K.; Kudapa, H.; Pazhamala, L.; Chitkineni, A.; Thudi, M.; Bohra, A.; Gaur, M. P.; Janila, P.; Fikre, A.; Kimurto, P.; Ellis, N. Translational genomics in agriculture: some examples in grain legumes. *CRC Crit. Rev. Plant Sci.*, **2015**, *34*(1), 169-194.
- [31] Green, J. M.; Owen, M. D. K. Herbicide-resistant crops: Utilities and limitations for herbicide-resistant weed management. *J. Agric. Food Chem.*, **2010**, *59*(11), 5819-5829.
- [32] Bates, S. L.; Zhao, J. Z.; Roush, R. T.; Shelton, A. M. Insect resistance management in GM crops: past, present and future. *Nat. Biotechnol.*, **2005**, *23*(1), 57-62.
- [33] Ferry, N.; Edwards, M. G.; Gatehouse, J.; Capell, T.; Christou, P. A. M. R.; Gatehouse, A. M. R. Transgenic plants for insect pest control: a forward looking scientific perspective. *Transgenic Res.*, **2006**, *15*(1), 13-19.
- [34] Cheng, H. M.; Jian, G. L.; Ni, W. C.; Yang, H. H.; Wang, Z. X.; Sun, W. J.; Zhang, B. L.; Wang, X. F.; Ma, C.; Jia, S. R. Increase of *Fusarium*- and *Verticillium*-resistance by transferring chitinase and glucanase gene into cotton. *Sci. Agri. Sinica.*, **2005**, *6*, 13.
- [35] Raney T. Economic impact of transgenic crops in developing countries. *Curr. Opin. Biotechnol.*, **2006**, *17*(2), 174-178.
- [36] Tester, M.; Langridge, P. Breeding technologies to increase crop production in a changing world. *Science*, **2010**, *327*(5967), 818-822.
- [37] Shewry, P. R. Improving the protein content and composition of cereal grain. *J. Cereal Sci.*, **2007**, *46*(3), 239-250.
- [38] Stupak, M.; Vanderschuren, H.; Gruissem, W.; Zhang, P. Biotechnological approaches to cassava protein improvement. *Trends Food Sci. Technol.*, **2006**, *17*(12), 634-641.
- [39] Bhatnagar-Mathur, P.; Vadez, V.; Sharma, K. K. Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. *Plant Cell Rep.*, **2008**, *27*(3), 411-424.
- [40] Oh, S. J.; Song, S. I.; Kim, Y. S.; Jang, H. J.; Kim, S. Y.; Kim, M.; Kim, Y. K.; Nahm, B. H.; Kim, J. K. Arabidopsis CBF3/DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. *Plant Physiol.*, **2005**, *138*(1), 341-351.
- [41] Oh, S. J.; Kwon, C. W.; Choi, D. W.; Song, S. I.; Kim, J. K. Expression of barley HvCBF4 enhances tolerance to abiotic stress in transgenic rice. *Plant Biotechnol. J.*, **2007**, *5*(5), 646-656.
- [42] Takuma, I.; Kyonoshin, M.; Mitsuhiro, O.; Fukutani, A. Expression of *Arabidopsis DREB1C* improves survival, growth, and yield of upland new rice for Africa (NERICA) under drought. *Mol. Breed.*, **2012**, *31*(2), 255-264.

- [43] Wi, S. J.; Kim, W. T.; Park, K. Y. Overexpression of carnation S-adenosylmethionine decarboxylase gene generates a broad-spectrum tolerance to abiotic stresses in transgenic tobacco plants. *Plant Cell Rep.*, **2006**, 25(10), 1111-1121.
- [44] Khare, N.; Goyary, D.; Singh, N. K.; Shah, P.; Rathore, M.; Anandhan, S.; Sharma, D.; Arif, M.; Ahmed, Z. Transgenic tomato cv. Pusa Uphar expressing a bacterial mannitol-1-phosphate dehydrogenase gene confers abiotic stress tolerance. *Plant Cell Tissue Organ Cult.*, **2010**, 103(2), 267-277.
- [45] Vasil, I. K. Molecular genetic improvement of cereals: transgenic wheat (*Triticum aestivum* L.). *Plant Cell Rep.*, **2007**, 26(8), 1133-1154.
- [46] Moose, S. P.; Mumm, R. H. Molecular plant breeding as the foundation for 21st century crop improvement. *Plant Physiol.*, **2008**, 147(3), 969-977.