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Advances in Biological Water-saving Research: Challenge and Perspectives

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Abstract: Increasing the efficiency of water use by crops continues to escalate as a topic of concern because drought is a restrictive environmental factor for crop productivity worldwide. Greater yield per unit rainfall is one of the most important challenges in water-saving agriculture. Besides water-saving by irrigation engineering and conservation tillage, a good understanding of factors limiting and/or regulating yield now provides us with an opportunity to identify and then precisely select for physiological and breeding traits that increase the efficiency of water use and drought tolerance under water-limited conditions, biological water-saving is one means of achieving this goal. A definition of biological water-saving measures is proposed which embraces improvements in water-use efficiency (WUE) and drought tolerance, by genetic improvement and physiological regulation. The preponderance of biological water-saving measures is discussed and strategies identified for working within natural resource constraints. The technology and future perspectives of biological water saving could provide not only new water-saving techniques but also a scientific base for application of water-saving irrigation and conservation tillage.

Keywords: biological water saving, water-use efficiency, drought tolerance, physiological regulation, water saving breeding

Biological water saving in agricultural watering

The first ecological problem faced by human beings is the deficiency of water. The total water resources in China are around 2.8 trillion m^3, which ranks in the front positions in the world. However, its volume per capita and per mu is only 1/4 and 1/2 of that in the world respectively, and its distribution is unbalanced regionally. In the north region of Yangtze River, arable land occupies 65% of China's total arable source; however, water resources only hold 19% of that in China. Currently, there is a water deficiency of 40 billion m^3 in ordinary years in China. And within this number, 30 billion m^3 are for agricultural use. It is more serious that water pollution situations need more and more considerations now. In the supervision report in early 2005, 53.3% of China's top 7 rivers were polluted especially in the drainage area of Yellow River, Huai River, and Hai River of north China, where is the most water-deficient and polluted region in China. Because agricultural water is the biggest host of water usage, it occupies 70% of water resources in China; so, in order to face the issues coming from water deficiency, to establish a water-saving atmosphere and to especially develop water-saving agriculture are only choices to the Chinese government.

Water-saving agriculture is feasible to any agricultural region. It is especially applicable in arid, semi-arid and semi-wet regions where high-efficient irrigation can be utilized accompanied with natural precipitation. As keeping the stable process for agricultural escalation, in order to maintain regional balance of water resources, how to fully use of natural precipitation and decrease mass irrigation emerges out to catch high social consideration.

Water-saving agriculture is a systems engineering, it includes: spatio-temporal regulation of water resources, best use of natural precipitation, high efficient irrigation, and promotion of water use efficiency (WUE) of plants etc. All in all, its objective can be summarized as to promote the ratio and efficiency of water resource usage. When it is put in practice, the following ratios need to be escalated: water capacity of soil/precipitation (irrigation), water consumption/water capacity of soil, transpiration/water consumption, biomass/transpiration, and economic yield/biomass. So, we need to select comprehensive technological measures combining engineering, agriculture, and biology. Within these measures, water-saving technology can be tightly jointed with manufacturing process; furthermore, it holds the virtues of low investment with quick popularization, and is feasible both in irrigated field and dry land. Although, engineering water-saving measures are expensive to be put in practice, they are standardized, and their functionalization is obvious. So they were just chosen majorly in the beginning for the promotion of water-saving agriculture in various nations. These measures in biological water saving were constructed based on the rules of biological water demand. Their direct function is to increase transpiration efficiency. And they also provide basis for the corresponding engineering and farming water-saving measures. It is even more important to escalate water usage in plants and their drought resistant capability especially when water loss, evaporation and leaking can be effectively controlled, namely, the spatio-temporal regulation of water is sufficiently utilized. This could be considered as the potential to promote water-saving production, and the research area with multiple unknowns in water-saving agriculture.

The concepts and significance of biological water saving

As a terminology, the word "water-saving" or "water- saving agriculture" was put in practice and popularized by Chinese researchers. It holds the similar meanings with "water efficient" or "efficient water use", and is substituteble with each other in recent international scientific exchanges.

The word of "biological water-saving" was first used in 1991 in Chinese scientific document, and was explained by special papers in consequence [1, 2]. Biological water saving means high efficient water usage in plant. It is fulfilled by escalating plant WUE, and influenced by drought resistant capability of a plant. There existed issues on relations between WUE and drought resistant capability of a plant in the planting history. And in the current viewpoint, these two factors are joined together but not identical. In the 4th international crop science conference, Dr. J. Passioura suggested that [3], in farming, meanings of "drought resistance" or "drought tolerance" are similar to that of "water productivity", and the translation from "drought tolerance" to "water productivity" is a progress, because the latter can be quantified [3]. Recently, Chinese researchers were also emphasized on the significance to strengthen their research in WUE, and recognized that this research should be located at the interface linking fundamental research and application. Some of them also proposed ideas on the development of high water-efficient agriculture [4]. Israel's achievement in water-saving agriculture was recognized globally. They accredited their achievements to the substantial increase in crop transpiration/evaporation volume by the utilization of channel transporting and advanced irrigation technologies. However, when irrigation water use ratio generally approaches to 0.9, the potential in this is limited. So, they realized that the productivity of transpiration must be improved to further decrease farming water use [5]. Based on above, water saving mainly focused on the promotion of plant WUE. WUE can be defined on different levels such as on a single leaf, a plant, or community. According to physiological concepts, the definition of WUE is the assimilation of per unit consumption of water. Traditionally, it is expressed as the ratio of net photosynthetic rate (P_n) to transpiration rate (P_n/T). On the other hand, WUE can be expressed as the ratio of accumulated biomass to transpiration volume during growth period. Recently, based on the theory that the ratio of $^{13}C/^{12}C$ has quantitative relations with WUE and CO_2 concentration between living cells, the technology (put forward by Dr. G.D. Farquher) to use carbon isotope discrimination as the tool to rapidly measure WUE within C_3 plant was put in practice [6]. The extensive meaning of WUE is expressed on the community level by the ratio of dry matter production to evapotranspiration (DW (yd)/ET). And this is measured by a water balancing method. A more general definition, "Rainfall use efficiency of crop" [7], which was expressed by the ratio of yield in unit area to precipitation in this production cycle, was appeared in recent years. Apparently, in order to escalate precipitation use efficiency, we must effectively control evaporation as well as water loss and soil erosion. And this regulation belongs to the research in systems engineering. An even more extensive concept was published in the book —"Water Use Efficiency in Plant Biology" (edited by M.A. Bacon in 2004). In the preface of this book, the concept of WUE was expressed on various levels as molecule, cell, plant, and community. Some chapters of this book were involved in watershed ecosystem [8]. From this, we can find that the definition of WUE on these levels has instinct consistency. It can be digitalized, but is involved in extensive areas, and its related properties are complicated. The relation between WUE and drought resistant capability sometimes was positively correlated, or sometimes negatively correlated. Normally, WUE is positively correlated with yield. The future task is to realize effective combinations of these three elements. Generally, there are two research topics in the relations between WUE and yield: the first is to decrease transpiration as well to maintain productivity; the other is to increase productivity as well to maintain transpiration. Apparently, the latter can be conveniently realized in multiple pathways.

Biological water saving—the theories of high plant WUE

Water deficiency cannot be avoided in the growth process of crop. The longtermly existent question is that, in order to gain high crop yield, how much of deficiency can be sustainable. Historically, there were two different standpoints on the relations between water conditions and crop yield: some researchers insisted that any kind of water deficiency would result in the reduction of crop yield any time, so, in order to gain a high crop yield, we should supply plenty water during crop growth period. This is the theoretical pivot for sufficient irrigation, which is still in its advantagous position nowadays. The other is sufficient water supply interlaced with appropriate water control. The latter is more helpful to escalate crop yield as well as to save water. This has been proven by special researches and farming practices since the 80s of the 20th century. In the following, we summarized recent progresses in this aspect:

1. The influences of water deficiency on physiological processes

Water deficiency has different influences on varieties of physiological processes that have relations to crop yield, and these influences come in different orders. Among them, crop growth is most sensitive to drought conditions, but carbohydrate transportation process within a crop is blunt to its outside drought. It is more interesting that a non-serious drought could even promote the transportation. In a mild drought condition, leaf growth is inhibited, however, photosynthesis process still runs normally, and it can be accelerated by somewhat rewatering. Under a moderate drought condition, the utilizations of photosynthetic assimilation of wheat seeds before and after blossom are all higher than that with normal water supply. The order of drought influences on different physiological processes in cereal crops is: cell growth->stomatal movement->transpiration->photosynthesis-> assimilation transportation. The above theory could be utilized in water saving agriculture.

2. Sensitivity to water deficiency in different stages of crop growth

The sensitivity to water deficiency in different stages of crop growth is different because there exists critical period of water demand, and water supply. To most cereal crops, they are sensitive to water deficiency during the period from the formation of pollen mother cell to pollination, and need not always plenty watering during other periods. The research results showed that: from seedling stage to before jointing stage, rewatering supply after mild and moderate water stress could benefit to physiological characteristics, growth, and yield of cereal crops. For example, in our research, assimilation of the millet and accumulation rate of dry matter all exceeded those in the circumstances of sufficient water supply, and the augment of photosynthetic rate apparently exceeded that of transpiration rate, so, WUE was promoted; for sorghum, before jointing stage, the yield and WUE increased when it was in the moderate drought conditions before rewatering instead of it was in consistent plenty water supply; and the relative higher water potential as well as relative lower osmotic potential were maintained, the photosynthetic rate and the stomatal conductance all surpassed those in consistent plenty water supply. The research in corn also got the similar results. Based on above, controlling water supply during the period of water insensitivity can gain great benefits. However, the formation mechanisms and circum- stances of insensitive period and the relations between the critical period of water demand and the critical period of water supply still need further intensive study.

3. Reactions of the species and varieties of different crops to water deficiency

Reactions to water deficiency are clearly different between different crops and species. These distinctions are majorly expressed by drought resistant capability and WUE. Our research showed that: the differences of WUE in different species were distinctive, normally with a 2-5 times variation; the distinctions of WUE also existed in different varieties of crops, our resent research showed that: in the wheat evolution stage from 2n→6n, WUE had the tendency to increase; the maximum value of WUE had 50% difference with the lowest value with today's wheat culturing technology, and this difference could be expressed by different varieties of wheat as: irrigated land>both irrigated and dry land>dry land. This explained that to breed high WUE species is feasible in consistence with evolutionary rules. Our research further proved that: different crop adaptabilities to farming water variations were distinctive. In an alternative drought and wet circumstance that irrigation amount is only half of plenty water supply, the yield between wheat and potato has no distinctive difference compared with that in the circumstance of consistent plenty water supply. However, the yield of corn is seriously declined, and is only 1/3 of that in the circumstance of consistent plenty water supply.

4. Relations between Yield, water consumption (ET), and WUE

WUE could have a 10 times jump in same crop with different field conditions. The maximum value of WUE is normally gotten in the circumstance of insufficient water supply instead of consistent plenty water supply. This tells that limited water supply is feasible. An experiment in the semiarid area in Ningxia Hui autonomous region showed that: when ET increased to 481mm, the maximum yield was 5.3t/hm^2; however, the further increase in ET would course abrupt decline of yield. It was also shown that when ET reached 76.2% of water demand in maximum crop yield, WUE had its maximum value, however, the crop yield in this circumstance was only 86.5% of the maximum yield. Fortunately, ET was saved by 115mm. Therefore, to save water in semiarid regions as well escalate crop yield and WUE is feasible, however, the reactions of maximum yield and WUE to ET are not in accordance, and are needed to be further studied [9].

Based on the summary of above research results, drought is not always to decline crop yield. Appropriate water deficiency in special growth period could benefit production increment and water saving. This mechanism could be explained as: the influence of water deficiency on crop is a process from adaptation to injuriousness; water deficiency that does not surpass the adaptive scope will cause compensatory effect on crop physiological characteristics, growth and yield after rewatering supply. The crop yield is not influenced in the same time of saving water.

In order to further clarify the compensatory effect, we need to deeply study high efficient water usage and physiological as well as molecular mechanisms of drought tolerance. We already made a lot of explorations in these aspects. Some of the micro specifics have been fundamentally clarified; the current issues are located in: 1) the relative importance to different drought resistant mechanisms, the inner relations between WUE and drought resistant capability [8], and the interconnections between water signals and chemical signals under water stress (these problems are not clear and need integrated research [10]); 2) the compensatory effect is obtained in the circumstance of water variation, the evaluation for drought resistant capability of different crop species or varieties depends not only on tolerant sustainability but also on the recovery ability after water supply; however, current researches on the drought tolerant mechanisms were implemented under the circumstances of water stress, and had not paid high considerations to the diversity and importance of environmental situations. So, we need to integratively study adaptive mechanisms of plant to drought environment, and explore key drought tolerant mechanisms in special circumstances; at the same time, we need to develop our experimentation in diverse circumstances with water deficiency to connect mechanical research with real drought environment.

Water-saving technologies and perspectives

In summary, water-saving technologies include the following three aspects: genetic modification, physiological regulation and community adaptation (or crop complementation). Among them, genetic modification aims at the improvement and cultivation of new drought tolerant and water-saving species or varieties, and is the core technology ever used.

1. Community adaptation

By modification of seeding time, regulation of see- ding density and cropping system, improvement of crop rotation patterns, and intercropping or mix-cropping etc., we can decrease crop transpiration and augment the ratio of transpiration to evaporation to save water in field and region. In biological view, any of above measures can be classified as "community adaptation" or "crop complementation" and be similar to the water-saving agricultural measure according to biological water-saving theories. The basis of these measures is to optimize the deployment of agricultural water resources and establish water-saving breeding system by utilization of water-demand characteristics and water consumption rules in diverse crops. Currently, this is a water-saving agriculture strategy, which can be effective in an extensive scope. In order to realize this strategy, measures as transforming rice from lowland to upland, decreasing corn cultivation and transforming to sorghum were taken by some nations currently. In this respect, we can reference the articles written by N. Van Duivenbooden and N.C. Turner [7, 11]. They emphasized on the importance to promote precipitation utilization efficiency (PUE) in field and watershed area. And through the analysis, since 80s of last century, in the dryland farming of wheat in semiarid regions, the escalation of production and PUE accredited 2/3 to farming measures as well as 1/3 to improvement of species. The experiments in semiarid area of southern Ningxia province showed that: the order of WUE variations should be: pea>proso millet>millet>spring wheat; the value of ET of lucerne was highest and approached 379mm, with an average of 42% percentage higher than other crops; it was better to employ crop-bean rotation to save water and improve soil fertility. However, the promotion of crop-weed rotation should be seriously considered [12].

2. Physiological regulation

(1) Limited irrigation

Based on previous theory that moderate water deficiency could induce compensatory effect, to establish limited irrigation system is the definite choice to face future development. In practice, there emerged many instances of obvious escalation of yield by driblet complementary irrigation. However, there were still existent issues such as: how to popularize this strategy to large scale, and how to make it an extensive utilization. It is needed to point out that the popularization of limited irrigation is not riskless, and need special conditions. Because, in complicated farming circumstances, the benefit or harmfulness of certain water condition to crop varies rapidly, if uncontrollable, benefit could transform into harmfulness. There was an example in the farming history of former Soviet Union that they got mass production loss because they reduced irrigation times for spring wheat according to the results from a special experiment that they maintained photosynthesis rate with a certain drought condition [13]. So, in order to realize limited irrigation, on one hand, we can use normal irrigation technologies and methods based on the existent knowledge and experiences; on the other hand, we can employ new technologies, and gradually progress to precise irrigation. We can consider precise irrigation as one part of precise agriculture. This strategy supplies "limited" water to farming field according to crop demand. To implement precise irrigation, the following conditions should be held in hand: 1) reliable and specific crop water-demand information; 2) advanced information technologies, such as remote sensing, computer automatic controlling and monitoring technologies; 3) technological indexes to connect the above two conditions, especially those indexes to describe crop water deficiency; further more we need to turn these indexes into remote sensing markers and models; 4) adopt appropriate advanced irrigation methods. Above conditions are prerequisites to popularize scientific water usage and reasonable irrigation. This is a cross-disciplinary approach.

(2) Rational fertilization

Through appropriate fertilization, to regulate the relations between water, nutrition and yield is one of effective pathways to escalate WUE and ET of crop. In the decade from 1980 to 1990, the crop yield was escalated one more time in the dry land region of northern China, and 50% of that accredited to the utilization of chemical fertilizer. The functionality of fertilization could be summarized as: 1) in the low yield case, because of prevalent shortage in water and nutrition, crop growth was stressed. With appropriate fertilization, the stress on crop growth was unlashed, and the community close canopy was increased, so, finally, water transpiration/evaporation increased. An experiment with 400-450mm annul precipitation in Ningxia province showed that: in the field with yield under 200kg/mu, when we increased fertilization appropriately, the yield per mu increased by 57%, WUE increased by 49%, however, ET only increased by 8%; 2) the functionality of inorganic nutrition to promote photosynthesis was higher than water transpiration, so, the WUE value of a single leaf, a plant or a community was apparently escalated; 3) fertilization promoted extension of root·system; under certain conditions, it could increase the effectiveness for utilization of deep soil water reservoir to realize "regulating water by fertilizer"; 4) appropriately increasing fertilizer N, P, and K could in- crease physiological drought resistant capability of plant; especially P nutrition, it could promote not only drought resistant capability but also drought tolerant capability, and was shown by: it could escalate root-shoot ratio, stomatal conductance and root system hydraulic conductance, strengthen cell membrane stability, protect enzyme activity and osmotic regulation capability, and promote saccharobiose output through photosynthesis organ etc. [14,15] The problem of water-fertilizer relation in water-saving irrigation and dry land farming is a long-term research topic. We will further study water-fertilizer coupling relations and their quantifications focusing on water saving and drought resistant capabilities.

(3) Chemical regulation

According to previous documentation records, till today, there already developed hundreds of chemical reagents on drought resistant and water saving capabilities; however, only several of them were utilized for farming. In China, several reagents such as Fulvic Acid (FA) were studied systematically and applied specifically. The function of FA is located not only on closing stomata and decreasing transpiration appropriately, but also on promoting the growth of root system; and under certain circumstances, it apparently promoted drought resistant capability and increment of crop yield [16]. $CaCl_2$ presoaking to strengthen drought resistant capability was firstly put into practice in the 50s of the 20^{th} century. This technology had been demonstrated extensively in several provinces in China for a long time, and had certain effect on the increment of crop yield. Because of those special functions of Ca to protect the structural integrity of cytoplasma membrane and chloroplast membrane, and to anti-dehydrate, in addition with the gradually deepened study in Ca^{2+} signaling inner cell, the research in calcium promoted stress biology in plant attracted many scholar's eyeballs. Our research group used $CaCl_2$ and GA together to presoak seeds. We proved in the experiment that these two reagents had mutual compensatory and superimposing effects on physiological metabolism. This further cooperated plant physiological activity with its drought resistant capability. The WUE of a single leaf, a plant or a community of wheat was increased by 9%, 11%, and 15% respectively, and averagely increased by 8%-15% [17]. In the selection of water-saving and drought resistant reagents, original research was focused on anti-transpiration reagents, our suggestion is that, based on the future elaborated physiological mechanisms of drought resistant and water- saving capabilities, we need to select chemical reagents through multiple pathways targeting on decreasing transpiration, strengthening absorption, promoting sustainability and inductiveness etc.

3. Breeding new water-saving and drought resistant species

WUE is a genetic hereditable trait. High WUE is an important factor of plant to describe its adaptivity to water- deficiency and its favorability to high productivity. High WUE oriented breeding has its genetic basis and in accordance with revolutionary rules, so, it sounds feasible. However, to clarify physiological traits and main patterns to control crop WUE is very difficult because WUE is a complicated over-all trait. Australia researchers explored and studied majorly in conventional breeding (they tried new species, such as that from premature seedling to increase transpiration/evaporation and from high carbohydrate transportation after latter stage of growth to increase economic coefficient, to promote effective usage of natural precipitation; and tried once to selectively breed species that had small root vessel diameter to decrease water consumption in the earlier stage of growth), and gained limited achievement, but strictly speaking, there still does not exist a feasible high WUE species breeding method. The drought resistant capability of a crop is not only regulated by multiple genes, but also realized through multiple pathways. As a complex trait, drought tolerance greatly conflicts with yield increase. And in conventional breeding, drought tolerance of current key foodgrain and economic crops is scarcely the definitive factor for drought adaptation. So, we need to elaborate physiological mechanisms and functions of high drought resistant capability and high WUE of a crop at molecular level, and then use gene recombination methods to develop new species with better water-saving and drought resistant traits.

Cloning of around one hundred genes that influenced plant drought resistant capability was once reported [10]. They also produced many drought resistant transgenic plants in their work. In the International Agriculture Science Congress held in Beijing, Dr. Jiankang Zhu reported that, currently, there was no drought resistant economic species bred by biological technological breakthrough. And many researchers emphasized that, single gene had limited functionality, so, multiple genes should be transformed and jointed together for systematical studies [18]. In transgene researches targeting on high WUE, recently, there emerged several instances that the high WUE related genes were located on chromosome; however, the research on directly cloned high WUE genes was scarce. So, in 2001, NSF launched genome research projects targeting on plant (for example, Arabidopsis, rice and tomato) WUE. E. Sivamani reported their achievement such that, by transmitting barley LEA HVAI gene into wheat, the WUE of its descendants was improved (22% higher than before) [19]; the research work of Dr. J. Masle from the Australian National University, was published in 2005 by *Nature* magazine as a paper titled "The ERECTA gene regulates plant transpiretion efficiency in Arabidopsis", and this was a break- through on stress tolerance gene research to decrease plant water usage, and held prevalent significance [20].

To utilize transgene technology to breed new species with water-saving and drought resistant capabilities is the key objective of agricultural development in water deficient regions, and it obviously has prosperous future. However, most researchers recognized that its realization and utilizetion in farming had a long way to go. So, considering China's basic condition, we put forward the following suggestions and ideas:

1. To dig germ plasma resources with water saving and drought resistant capabilities is the prerequisite to improve plant drought resistant capability and WUE genetic modification. Targeting on the circumstances of arid and semiarid regions, we need to highly pay attention to high WUE, dehydration tolerant species with mediocre yield, instead of high water uptaking and consumption species with high yield.

2. We need to emphasize on interrelations and relatively independent functions between various levels of water-saving and drought resistant breeding, promote multiple disciplinary cooperations, and combine conventional breeding with genetic engineering breeding. Genetic

engineering breeding is a convenient way to step over species boundaries and clone independent drought resistant traits, and plays an important role in stress tolerance breeding. However, its practical function and effectiveness need to be further gradually clarified.

3. It is more feasible to locate our central research point of water-saving and drought resistant transgenic plant on forest and grass, because by comparing with annual crop, the conflict between increased yield and drought tolerant capability of forest and grass is relatively smaller, and their ecological effectiveness is better, which means: once survived, they could have chance to realize their protection and output objectives.

4. To explore key effective drought tolerant genes, we need to: start our research both in water-saving techniques and mechanisms, and strengthen the fundamental studies in biological water saving; furthermore, deepen the research in plant drought resistant mechanisms, and clarify different functions of drought tolerant mechanisms to increase the integrative drought resistant capability of a plant. Moreover, we need to expand our research of WUE in aspects of water saving and yield increasing; and study mechanisms and strategies to promote WUE in multiple pathways, to further clarify the relations between WUE, drought tolerance, and yield, and to explore effects on the relations between environmental factors, WUE, and genetic drought tolerant factors. All of above will be helpful to establish breeding strategies and methods, and to develop new research topics.

Biological water saving, or research in high WUE of plant, is a new hot spot in contemporary international biology and its related areas. This trend was shown in a congress, which was held in October 2003 and titled “International Conference on Water-Saving Agriculture and Sustainable Use of Water and Land Resources”. The congress attracted many global well-known experts in the area of plant-water relations. In China, the research and application in water saving not only attracted eyeballs from scientific world but also activated positive responses from industrial world and related national governmental departments. This could be shown in different conferences, plans, as well as projects. However, research in water saving is still waiting for breakthroughs. A forward long way is in the front of us to gain the best effectiveness of water saving.

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Research Progress on Artificial Cultivation of Trhicoloma Matsutake and Theory of the Shiro in Changbai Mountains

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Abstract: Study on artificial cultivation of Tricholoma matsutake carried through by the present research group in Changbai Mountains in recent years was briefly reviewed in the paper, and then, the signify-cance of cultivation, mechanism of formation, hiberar-chy, growth cycle and suitable living environment, were summarized.

Keywords: tricholoma matsutake, artificial breeding, artificial environment. artificial cultivation

Tricholoma matsutake is a typical trophobiotic ectomycorrhizal fungus, which is reputed as "king of mushroom" for its delicious taste and high price. It's very hard to cultivate *T. matsutake* in artificial environment for difficultly simulating nutritional condition and ecological environment of the root system of its symbiont. Since 1993, three funds endowing the study on artificial cultivation of *T. matsutake* in Changbai Mountains Area from NSFC were obtained by our research group, and therefore several progresses were made in this field, and theories of shiro of the fungus were established.

1. Progress in the domestication of T. matsutake in Changbai Mountains

(1) Distributive and ecological characteristics of *T. matsutake* in Changbai Mountains Area were investigated in detail in our work, furthermore, two new growth areas of fungus, Jiaohe (Jilin Province) and Fengcheng (Liaoning Province), were found; the existence of *T. bakamatsutake*, a related species of *T. matsutake*, in Changbai Mountains was confirmed; and then, our research clarifies that the distribu- tion of *T. matsutake* is in mountainous areas close to water system.

(2) DNA Identification based on RAPD analysis was carried through in order to isolates *T. matsutake* from different samples; some parameters of the isolates, e.g. survival rate, growth speed and biomass, were distinctly raised by means of improving formula of culturing medium and culturing conditions.

(3) Natural characteristics, protectable