Study on the Determination and Selection of Physiological Indexes of Agricultural Products Cultivated in Saline-alkali Soil

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Abstract. Soil salinization has become one of the most serious environmental problems in the world, which seriously affects the yield and quality of agricultural products. The physiological indexes of agricultural products grown in saline-alkali soil could show the ability of resisting saline-alkali. Therefore, the relationship between some important physiological indexes (photosynthesis, chlorophyll, osmotic regulation substances, phytohormones, antioxidant enzyme system, membrane permeability etc.) and salt tolerance of agricultural products was analyzed comprehensively. There are many kinds of physiological indexes, but a single physiological index could not be used to evaluate its salt tolerance.

Introduction

Soil salinization is not only a global resource problem, but also an environmental problem which is threatening human survival and ecological balance. According to the statistics of United Nations Educational, Scientific and Cultural Organization and Food and Agriculture Organization of the United Nations, the global salinized soil is about 954×10⁸ hm², covering more than 100 countries and regions. In addition, the salinization degree of agricultural land is still increasing. The types of saline-alkali soil are miscellaneous and widely distributed in China. The total area of saline-alkali soil is about 9913×10⁵ hm², accounting for about 10.3% of China's land area. What more serious is that, there is 1733×10⁵ hm² potential saline-alkali soil at present in China. The research on soil salinization in China could be traced back to the response of crop yield to saline-alkali soil by Xu and Zhang in 1951. After more than 60 years of research, a large number of data and experience had been accumulated on the improvement and utilization of salinized soil [1-4]. However, the area of soil salinization is still increasing. The increase of soil area and the decrease of soil quality caused by salinization directly affect the yield and quality of crops which affected the development of agricultural production and the improvement of people's living standards.

Agricultural products include crops, cash crops and other types, such as food crops, Chinese herbal medicine, fruit trees, vegetables and so on. Different agricultural products showed different tolerance characteristics when they grew in saline-alkali soil. The agricultural products with strong tolerance could be adapt to the soil conditions with high salt and alkali content. The research on salt tolerant plant resources is an important applied basic study, and its results have obvious ecological and economic benefits [1-3,5-9]. The ability of different agricultural products to resist salt and alkali could be obtained by measuring physiological and ecological indexes. Ecological indicators were often more intuitive and could be easily judged and observed to evaluate the salt and alkali resistance of agricultural products. Therefore, some important physiological indicators of agricultural products would be summarized and discussed here, in order to provide the selection suggestions and data.
support for the planting, screening and suitability research of agricultural products in saline-alkali soil.

Analysis of Various Physiological Indexes

For different agricultural products, their ability to resist salt and alkali could be obtained mainly by measuring the following physiological indexes.

Photosynthesis

Photosynthesis provides material and energy for the growth and development of plants, which can best reflect the response of plants to habitat changes. Under salt and alkali stress, the excessive concentration of salt ions in plants would produce salt ion toxicity, which could reduce the water potential of cells, and then lead to the decrease of photosynthesis [1,6-7]. Zheng et al. [8] found that salt stress could significantly affect photosynthesis of plants through different salt concentration treatment of *Lycium barbarum*. When the salt concentration was less than 0.60%, the stomatal diameter of leaves decreased, which lead to the chain reaction of the decrease of the intercellular CO₂ concentration (Cᵢ), the increase of stomatal limitation (Lₛ) and the decrease of photosynthesis rate (Pₙ). Under this condition, stomata was the main limiting factor. When the concentration of salt was more than 0.60%, the accumulation of Cl⁻ in plant decreased the activity of many enzymes, and the main limiting factor leading to the decline of Pₙ is the non-stomatal limiting factor.

Chlorophyll

Chlorophyll (Chl) participates in plant photosynthesis, and reflects plant growing. Strogonov et al. found that the concentration of ions in leaves determined the binding degree of chloroplast protein and Chl [10]. However, the increase of ion concentration in the high salt-alkali environment lead to the decrease of binding degree above mentioned, which lead to the dissociation of Chl and affects the photosynthesis of plants. When rice is under short-term salt stress, the content of Chl increases first and then decreases [11].

Osmotic Regulation Substance

Osmoregulation refers to the accumulation of a large number of inorganic salt ions and some organic matters to regulate osmotic pressure in the saline-alkali soil, so as to reduce the damage of stress on cell structure and enhance the salt-alkali resistance of plants. Therefore, osmotic regulators such as proline (Pro), betaine, soluble sugar, soluble protein and inorganic ions are important physiological indexes to study salt and alkali tolerance of plants.

Organic Osmotic Substances

**Proline.** Pro is a kind of amino acid which exists in cells in free form, and it’s also a soluble organic osmotic regulator. Under salt and alkali stress, protein synthesis is blocked and protein decomposition is accelerated, then amino acid content would increase, especially Pro. Pro content was positively correlated with the degree of salt-alkali stress [3,9].

In order to maintain the normal growth, plants accumulate Pro to maintain osmotic pressure, so as to prevent cell dehydration and protect cell structure under salt-alkali stress [9,12]. In addition, Pro also has the functions to keep the activity of photosynthesis and enzyme, and to clear the accumulated active oxygen, etc. It was found that the survival rate of transgenic *Arabidopsis* plants with altered levels of Pro dehydrogenase by sense increased by 30% when exogenous Pro was added [9,12-13].

**Betaine.** The content of betaine did not change significantly under alkali stress, but was more sensitive under salt stress. Betaine could protect the structure of biomembrane and avoid the damage of the function of biomembrane by improving the stability of some compound proteins in cells, which were beneficial to improve the osmotic regulation ability of plants [14]. Exogenous application of betaine could improve the photosynthetic characteristics and biomass accumulation of plants, and then effectively improved the stress resistance of plants.
**Soluble Sugar.** As the material basis of organic synthesis, soluble sugar is the main osmotic regulator of vegetation in saline-alkali soil, which is conducive to the stability of protoplast colloid and plasma membrane, and to protect the enzyme activity. The content of soluble sugar in cytoplasm increased with the increased degree of salt-alkali stress [3,15], especially under alkali stress [16-17].

In addition, some studies showed that the soluble sugar content increased with short time salt stress, but decreased with the increased stress time. With the increase of salt stress time, $P_n$ decreased, and the respiration rate increased to keep their own needs, which led to the decrease of soluble sugar content [17].

**Soluble Protein.** Salt and alkali stress broke the protein balance in plants. Many studies showed that plants could enhance water holding capacity by increasing protein synthesis rate, so that plants could adapt to saline-alkali environment [18]. Yan et al. found that the content of soluble protein in the leaves of 4 jujube species increased significantly compared with the control, and was positively correlated with the intensity of salt and alkali stress [18].

**Inorganic Osmotic Substances**

**K$^+$ and Na$^+$.** In the natural saline-alkali soil, K$^+$ and Na$^+$ are the main soluble cations. The concentration and ratio would affect the physiological and biochemical activities of plant cells. Therefore, plants adapt to the high saline-alkali environment by changing their inorganic ion concentration and ratio.

According to the experiment of 19 kinds of plants in saline-alkali soil, the concentration of K$^+$ and Na$^+$ is lower in the plants with weak salt and alkali tolerance, but higher with strong tolerance [19-20].

In addition, K$^+$/Na$^+$ ratio is an important physiological index to study the stress resistance of plants, especially crops and cash crops. Accumulation of excessive Na$^+$ occurs in plant cells, the absorption of K$^+$ will be affected, resulting in the decrease of K$^+$ content in plants. In the study of *Leymus chinensis* growing in saline-alkali soil, the ratio of K$^+$/Na$^+$ decreased significantly with the increase of salt stress [20]. The imbalance of K$^+$/Na$^+$ ratio caused ion toxicity, which seriously affected the normal growth of plants.

**Ca$^{2+}$.** Ca$^{2+}$ participates in the growth, development and senescence of plants, improves photosynthesis and promotes protein stability. In addition, Ca$^{2+}$ could protect the structure and function of membrane and maintain the stability of membrane by inhibiting the generation of active oxygen, so as to improve the water holding capacity and promote the absorption of nutrients of plants [21].

Under Na$^+$ stress, Ca$^{2+}$ bounded by the plasma membrane might be replaced by Na$^+$, resulting in the shedding of enzymes bound to the plasma membrane and the destruction of the structure of the plasma membrane. Ca$^{2+}$ could also bind with some calcium-binding proteins, regulate gene expression, thus affect the cell metabolism and enhance saline-alkali tolerance of plants [22].

However, the content of Ca$^{2+}$ in the solute of plant cells is very low. The normal physiological function could only be exerted by keeping the very low level of Ca$^{2+}$ [22]. Studies on tobacco and tomato showed that high salt stress resulted in an increase in the transcription level of endoplasmic reticulum Ca$^{2+}$-ATPase gene, while Ca$^{2+}$ concentration was controlled effectively in plants could prevent the occurrence of ion toxicity [23].

**Phytohormones**

**Abscisic Acid.** Phytohormones play the important roles in plants, among which abscisic acid (ABA) is the most widely studied. Hanson and kavikishor reported that ABA could improve the ability of plant cells to resist saline-alkali stress [24-25]. By releasing a large amount of ABA, the concentration of Na$^+$, Cl$^-$ and the ratio of K$^+$/Na$^+$ were decreased, so as to improve the saline-alkali tolerance of plants. ABA could induce the expression of some genes related to salt tolerance signal pathway, which increased the content of Pro and soluble sugar, and finally enhanced the saline-alkali resistance of plants. The accumulation of ABA caused the decrease of stomatal conductance, stomatal
diameter, $P_n$ and transpiration rate ($T_r$), which could reduce the energy consumption and be good for survival of plants under stress.

**Other Phytohormones.** Gibberellin could increase the activity of $\alpha$-amylase, stimulate the activity of vacuole membrane, and promote seed germination and plant growth [26-27].

Pretreatment of plants with low concentration of cytokinin could reduce the inhibition of salt ions on plant cells, which might be related to the stimulation of $\text{H}_2\text{O}_2$ scavenging enzymes in cells. Cytokinin could break seed dormancy and protect the normal growth of plants in saline-alkali soil [27].

Auxin could improve the activity of enzyme system, reduce membrane damage and improve the survival ability of plants [28-29].

**Antioxidant Enzyme System**

The redox state of cells would be changed under salt and alkali stress, which was easy to produce a large number of active oxygen. The damage of cell structure mentioned above affected the activity of cytoplasmic exosmosis, protein and enzyme. Active oxygen also weakened the fixation ability of carbon, and reduces $P_n$.

The enzymes in antioxidant enzyme system include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), etc. This system is mainly used to eliminate the accumulation of active oxygen in plants caused by salt and alkali environment.

SOD scavenges superoxide anion mainly and disproportionates them to $\text{H}_2\text{O}_2$ and $\text{O}_2$. CAT and POD could prevent the accumulation of peroxides by disproportionating peroxide $\text{H}_2\text{O}_2$ into $\text{O}_2$ and $\text{H}_2\text{O}$ to protect the membrane system, improve the activity of antioxidant enzymes in plants, and finally enhance saline-alkali tolerance [30].

In addition, some peroxidases in plants, such as ascorbic acid peroxidase (APX), could also eliminate active oxygen to maintain the normal growth of plants.

**Membrane Permeability**

In the 1960s, Eridovich et al. proposed the hypothesis of biological radical injury theory. The hypothesis was that the generation and elimination of free radicals in plants were in a dynamic balance under normal conditions, and excessive accumulation of free radicals would cause biological damage, aging and even death. There would be a lot of free radicals in plants under saline-alkali stress. The protein would denature, and then the structure of cell membrane would be destroyed. According to McKay's study, relative conductivity could be used to indicate the degree of membrane damage and membrane permeability. The relative conductivity of plant leaves gradually increased with the increase of salt stress [31-32], resulting in the increase of cell membrane permeability. The higher permeability, the weaker saline-alkali tolerance. At the same time, the product of membrane lipid peroxidation destroyed the plant defense system, thus accelerating membrane lipid peroxidation. Malondialdehyde (MDA) is the product of membrane lipid peroxidation, so the detection of MDA could be used to predict the damaged degree of cell membrane.

**Discussion and conclusion**

Photosynthesis and Chl are more direct reactions to the normal growth and development of crops etc. However, the mechanism of photosynthetic reaction is complex, there are various photosynthetic pathways, which require high technology and complicated steps to determination. As the concentration of salt ion in soil increased, the osmotic pressure of plant changed. The accumulation of a large number of inorganic salt ions and some organic matters to regulate osmotic pressure in the saline-alkali soil, so as to reduce the damage in stress on cell structure and enhance the saline-alkali resistance of plants. However, the current research was not enough due to the variety of osmotic regulators and the complex relationship with salt stress, so it could not be used as a single indicator. There are many kinds of phytohormones, which are sensitive to saline-alkali stress, and can quickly
reflect the change in plants. However, the phytohormone content is very low in plant, so the requirements for the determination methods and operators are very high. The measurement of propylene glycol content is more intuitive for understanding the permeability of cell membrane. Antioxidant enzymes have a great influence on $P_n$, the damage principle of saline-alkali stress could be understood through further study.

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