Breeding to Enhance Osmotic Adjustment

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Biological stress refers to adverse effects on organisms due to environmental factors that inhibit normal growth and function. In plants, stress arises from conditions like drought, salinity, extreme temperatures, and air pollution. These stresses can limit dry matter production and hinder physiological processes. Any change in environmental conditions that negatively affects plant growth qualifies as stress.

Plants lack specific osmoregulatory organs, relying instead on stomata and vacuoles to regulate water balance. Stomata control water loss through evapotranspiration, while vacuoles help maintain solute concentrations in the cytoplasm. Environmental factors such as strong winds, low humidity, and high temperatures increase water loss. Abscisic acid, a crucial hormone, helps plants conserve water by closing stomata and stimulating root growth to enhance water absorption. Unlike animals, plants require water loss to facilitate nutrient transport from the soil.

Water stress primarily reduces turgor pressure, which plants counteract by accumulating solutes like amino acids, sugars, and amines. Under high salinity, plants alter nitrogen and carbon metabolism, affecting enzyme function. The accumulation of organic solutes plays a dual role: maintaining osmotic balance when electrolyte concentrations fluctuate.

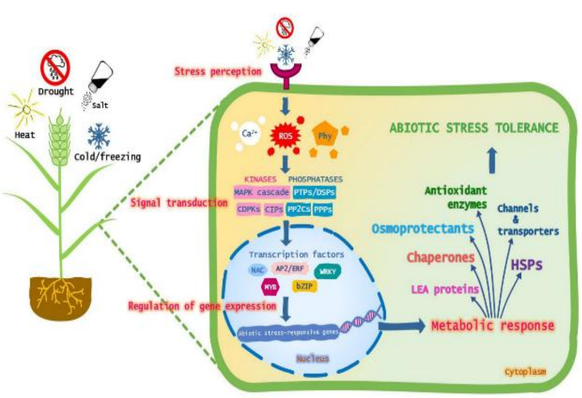

Fig. 1. Schematic representation of the signalling pathway leading to the plant response to abiotic stresses (Trono and Pecchioni, 2022)

Osmotic adjustment

Osmotic adjustment, in particular, plays a key role in plant resistance to high salt and drought stress conditions, especially when causing severe osmotic stress, through the accumulation of low molecular weight solutes and inorganic ions that reduce the osmotic potential of the tissues and therefore minimize water loss. The following is a diagrammatic representation of different types of stresses and the pathway for osmotic adjustment.

The mechanism of osmotic adjustment

1. Organic solutes

Many plants are in response to environmental stresses of salinity and drought by accumulating organic solutes, which are known as compatible solutes and reported to be nontoxic even in relatively high concentrations. Generally, they protect plants from stress through different courses, including contribution to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of enzymes or proteins.

Table 1: Main organic osmolytes in osmotic adjustment on different plants

Species	Stress	Organic osmolytes
Beta vulgaris L. (Sugar beet)	Drought	Glycinebetaine
Medicago truncatula and *Phaseolus vulgaris* (Legumes)	Salt	Trehalose
Nicotiana tabacum L. (Tobacco)	Drought	Proline
Oryza sativa L. (Rice)	Salt	Total soluble sugar(Glucose, Fructose and Sucrose)
Triticum aestivum L. (Durum wheat)	Drought	Proline

2. **Role of enzymes**

In osmotic adjustment, some enzymes play main roles in the synthesis of osmolytes to alleviate or eliminate saline and drought environmental stresses. Most important enzymes include- betaine aldehyde dehydrogenase (BADH), pyrroline-5- carboxylate reductase (P5CR), ornithine -d- aminotransferase (OAT)

Glycine betaine

Glycine betaine is synthesized by several plant families in response to saline or drought stress, whose primary effect on plant cells is to balance the osmotic potential of intracellular and extracellular ions to keep water and reduce salinity toxicity, and also function as a compatible solute to stabilize the structure of proteins to protect the major enzymes. In plants, glycine betaine is synthesized in chloroplasts.

Proline

Proline is an important amino acid for plant resistance to osmotic stress. Pyrroline-5-carboxylate synthetase (P5CS) and pyrroline - 5- carboxylate reductase (P5CR) are key enzymes in proline biosynthetic pathway. In plants, proline accumulation could mediate osmotic adjustment, stabilize subcellular structures. In durum wheat (*Triticum aestivum* L.), for example, a positive correlation was observed between proline level and osmotic potential, and it was concluded that proline is an important osmolyte in osmotic adjustment under salinity stress.

Glycerol

In some plants, glycerol is the main osmolyte, which is synthesized from glucose. glycerol-3-phosphate dehydrogenase (G3PDH) plays a major role in glycerol biosynthesis. Glycerol may be an effective osmotic element at high salinities. First, the high solubility of glycerol cannot be matched by most other compatible solutes. Second, glycerol is chemically inert and therefore non-toxic. Third, glycerol is an end-product metabolite, and therefore its accumulation is unlikely to offset major metabolic pathways. Fourth, the energetic cost of glycerol synthesis from glucose is relatively low and it does not depend on the availability of nitrogen.

3. **Inorganic ions**

The effect of inorganic ions in osmotic adjustment Sodium ion, K^+, and Ca^{2+} are the main inorganic ions in some halophytic plants under osmotic stress, and these ions prevent plants from harm caused by drought and salinity stresses by absorbing water into cells. For example, three species of cassava were grown in greenhouse conditions and subjected to water deficit treatments, and it was found that the concentration of K^+ increased in response to water stress, which was positively correlated with the extent of osmotic adjustment. In the presence of Ca^{2+}, for example, wheat (*Triticum aestivum*) showed significantly more accumulation of osmolytes in response to water stress by osmotic adjustment.

Table 2: Main inorganic ions in osmotic adjustment on different plants

Species	Stress	Inorganic ions
Arabidopsis thaliana	Salt	Na^+
Manihot esculenta (Cassava)	Drought	K^+
Oryza sativa L. (Rice)	Salt	Cl^-
Triticum aestivum L. (Wheat)	Drought	Na^+
Vicia faba L. (Bean)	Salt, Drought	Ca^{2+}

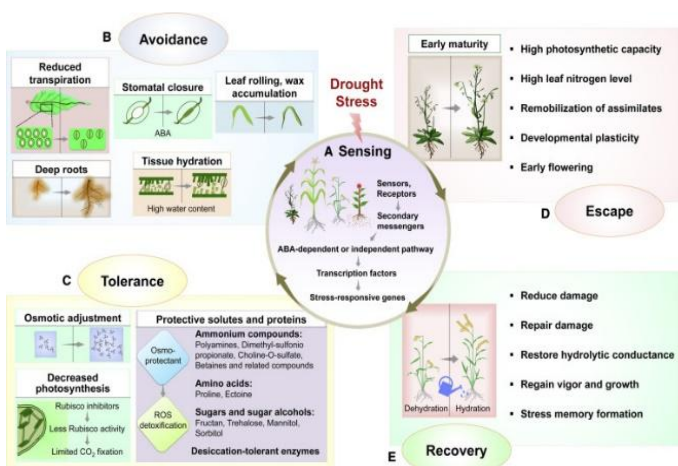

Fig. 2. Identifying different traits in plants in response to stress

(A–E) Four critical processes involved in plant stress tolerance after sensing the stress **(A)** are depicted, along with major changes used by plants in each of the processes, which include avoidance **(B)**, tolerance **(C)**, escape **(D)**, and recovery **(E)**. Specific plant species may respond to stress differently, and plants use more than one type of process for growth and survival (Shelake *et al*., 2022).

Osmotic adjustment occurs in saline and dehydrating soils. Osmotic adjustment results from solute accumulating faster than it is used. Growth is inhibited first, decreasing solute use, but remaining growth is more rapid than in absence of osmotic adjustment. Solute may be obtained from inorganic salts in soil and from products of photosynthesis. Solute accumulates in vacuole and cytosol. Osmotic adjustment maintains ability to absorb water from environment thus maintaining water volume and Turgor.

Breeding to enhance osmotic adjustment

Here are some steps that could be involved in breeding to enhance osmotic adjustment:

1. **Identifying Traits:** Researchers would first need to identify traits associated with osmotic adjustment. This could involve studying the physiological responses of plants to water stress and identifying genetic markers associated with these traits.
2. **Genetic Variation:** Assessing the genetic variation present in plant populatio osmotic adjustment traits is crucial. This might involve screening diverse germplasm collections or conducting genetic studies to identify genes associated with osmotic adjustment.
3. **Selective Breeding:** Once traits and genetic markers associated with osmotic adjustment are identified, breeders can use selective breeding techniques to develop plant varieties with enhanced osmotic adjustment. This involves crossing plants with desirable traits and selecting offspring that exhibit improved osmotic adjustment in successive generations.
4. **Genetic Modification (Optional):** In some cases, genetic modification techniques such as gene editing may be used to introduce specific genes associated with osmotic adjustment into plants. This allows for more precise manipulation of the plant's genetic makeup to enhance desired traits.
5. **Field Testing:** Developed plant varieties with enhanced osmotic adjustment would undergo rigorous field testing to evaluate their performance under different environmental conditions, particularly under water stress. This helps ensure that the breeding efforts have been successful in improving osmotic adjustment without compromising other important traits such as yield and disease resistance.
6. **Release and Adoption:** Once proven successful, new plant varieties with enhanced osmotic adjustment can be released to farmers for cultivation. Extension services and agricultural agencies play a crucial role in promoting the adoption of these new varieties among farmers.
7. **Continuous Improvement:** Breeding for enhanced osmotic adjustment is an ongoing process. Researchers continually work to improve breeding techniques, identify new genetic markers, and develop varieties with even better osmotic adjustment and other desirable traits.

Breeding for enhanced osmotic adjustment can help improve the resilience of crops to water stress, ultimately contributing to increased agricultural productivity and food security, especially in regions prone to drought or erratic rainfall patterns.

Osmotic adjustment complexity and future challenges

Bacterial, fungal, and plant cells share common mechanisms for osmotic and ionic stress tolerance, including ion transport, osmoprotectant synthesis, oxidative stress protection, and metabolic adjustments. Advances in molecular genetics have identified numerous components in abiotic stress signaling pathways, making them key targets for engineering stress-resistant crops. Overexpression of stress-regulated transcription factors, protein kinases, and phosphatases has improved plant tolerance to freezing, drought, and salinity. "Regulon" engineering, focusing on regulatory networks rather than single genes, is a more effective strategy. Genomics, through gene expression profiling and mutational analysis, will enhance understanding and enable better crop protection strategies for the future.

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