



Impact of Seawater Salinity on Morpho-Biochemical, Photosynthesis, Ultrastructure of Chloroplasts and Oleosomes in Relation to Fat Metabolism in Flag Leaf of Two Wheat Cultivars During Grain-Filling

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Abstract

To cope with the scarcity of fresh water for the sustainable development of agriculture, there is increasing awareness among agricultural scientists and planners in the utilization of seawater (at least diluted) for irrigation of crops. Different responses of two wheat (*Triticum aestivum* L.) cultivars (Gemmieza-9 and Sids-1) subjected to seawater at a percentage of 10% or 25 % were investigated. Gemmieza-9 would be classified as a species susceptible to seawater salinity, because its growth and physiological parameters, such as dry and fresh weight at the whole plant level, leaf area, specific leaf area, shoot to root ratio on the basis of length, some water relations (i.e. relative water content, saturation water deficit, degree of leaf succulence and degree of leaf sclerophylly) and leaf protein and nucleic acids (DNA and RNA) contents were severely affected by seawater stress in comparison to Sids-1. Genotype Sids-1 appears to be more tolerant to seawater salinity than Gemmieza-9 since it able to maintain less amounts of Na⁺ and Cl⁻ and higher amounts of organic solutes (TSS, TSN, proline, organic acids, glycerol and inorganic ions (K⁺, Ca²⁺, Mg²⁺ and K⁺/Na⁺ ratio) in its flag leaf. In addition, this review provides scarce evidence supporting the hypothesis that osmotic adjustment (OA) plays a preponderant role in the resistance to seawater stress in both wheat cultivars. It was clear that osmotic adjustment degree also affected by the rate of stress intensity. Furthermore, osmotic adjustment OA could be a part of the salt tolerance mechanisms developed by wheat and could be exploited in breeding programs for improved salt stress tolerance. A noticeable decline in chloroplast numbers in flag leaves of both wheat cultivars treated with sea water. This reduction could be attributed to the fact that salt stress often induces premature senescence. Furthermore, the observed decrease in the oleosome volume and fatty acids un saturation level together with the increase in lipase activity and glycerol content in the flag leaves is correlated well with the wheat plant tolerance to seawater salinity. Seawater-stress reduced bio-membrane stability through inducing lipid peroxidation resulting in an increase in membrane leakage. Considering all our results antioxidative response is well correlated with growth sensitivity and tolerance of cultivars to seawater-salinity and we can conclude that Sids-1 that could induce more efficiently antioxidative enzyme system is more tolerant towards seawater irrigation than the cultivar Gemmieza-9. The present review suggests that markers for oxidative stress, particularly malondialdehyde content, membrane leakage, and membrane stability index as well as antioxidant enzyme activities can be used as simple, rapid and cost-effective, as potential indicators for in wheat genotypes.

Keywords: Growth vigor; Osmotic adjustment; oxidative damage; Oleosomes, Photosynthesis; wheat; Sea water

Abbreviations: MDA: Malondialdehyde; ML: Membrane Leakage; MSI: Membrane Stability Index; MUFA: Mono-Unsaturated Fatty Acids; PUFA: Poly-Unsaturated Fattyacids; SW: Sea Water; TSFA: Total Saturated Fatty Acids

Introduction

Abiotic pressures like salt stress can impose limitations on crop productivity and also limit land available for farming, often in regions that can ill afford such constraints, thus highlighting a greater need for understanding how plants respond to adverse conditions with the hope of improving tolerance of plants to environmental stress [1]. Water is imperative for plant growth and

development. The scarcity of fresh water and soil salinity are the two most important abiotic stresses facing today's agriculture [2].

The controlled use of alternative water resources, like brackish or seawater could be a valid tool to face drought in the Mediterranean regions. The efficient application of seawater depends on the convenient dilution and use of suitable plant genotypes and

growing techniques [3]. Plants respond in many ways to salinity and at a number of levels. In order to define salt stress tolerance or sensitivity of both cultivars, growth parameters like lengths, dry and fresh weights of roots and shoots as well as flag leaf were tested under the effect of 10 and 25% seawater treatments during grain filling.

Plants have developed various combating mechanisms to survive with the deleterious effects of salt stress. Among these, osmotic adjustment (OA) is one of the strategies that have been a potential defense toward salt stress [4]. This phenomenon is considered to be an important component of salinity tolerance mechanisms in plants [5] and also necessary to maintain water uptake from a saline soil [6]. Hence, OA allows water uptake, cell enlargement and plant growth during water stress associated with partial stomata opening allowing the CO₂ assimilation at low water potentials that are otherwise inhibitory [7].

Salinity is a major environmental constraint that renders fields unproductive and limits plant growth and productivity [8]. Photosynthesis is one of the primary processes most affected by abiotic stresses [9]. Additionally, plant pigments are known to serve a variety of purposes, and are thus critical to the function and health of plants, though the relative concentrations of these pigments can vary significantly depending not only on the species but also on the surrounding environmental factors [10]. It is well documented that salt stress exerts its deleterious effects on plant growth and development through its induction to the reduction in Chl a, Chl b, carotenoids and total pigments [11]. In addition, Aldesuquy & Gabe [12] found that seawater at 25% induced massive reduction in soluble, insoluble and total photosynthates as well as ratio of soluble/insoluble photosynthates during growth and development of *Vicia faba* plants.

Salinity also has been known to affect the ultrastructure of plants, both glycophytes and halophytes. The swelling of organelles such as chloroplasts, Golgi bodies, mitochondria, and nuclei is probably the most obvious phenomenon found in plants growing in high salinities, which was suggested as a response to the changing internal environment of plant tissues [13]. The effects of hyper salinity on leaf ultrastructure and physiology in the mangrove, *Avicennia marina*, were investigated by comparing leaves of adult trees growing naturally in the field under seawater and hyper salinity conditions in Richards Bay, South Africa [14]. Interestingly, oleosomes of wheat leaves have many features in common with those isolated from oil seeds, for example, in unfixed leaf material they are spherical and refractive and appeared in electron microscopic photographs as bodies consist of an evenly-stained matrix bounded by an osmiophilic layer similar in appearance to the proteinaceous coat surrounding mustard seed oleosomes [15].

The extent of damage by ROS depends on the balance between its creation and its removal by antioxidant scavenging systems [16]. Antioxidant defense system comprises both enzymatic and non-enzymatic components [17]. Enzymatic antioxidants include catalase (CAT), peroxidase (POD) and ascorbic acid oxidase (AAO).

Lipid peroxidation rate was found to increase with increase of salt stress especially in sensitive cultivars [18]. In connection, Joshi et al. [19] stated that with increasing level of salinity stress, the malondialdehyde (MDA) content increased in four Brassica juncea varieties. Nichols et al. [20] found that in *Shewanellagelidimarina*, under hyper-osmotic and hypo-osmotic stress conditions, an increase in the proportion of saturated fatty acids was accompanied with increasing salinity level. Moreover, Xu and Beardall [21] revealed that in a green microalga, the proportion of total, saturated and mono unsaturated fatty acids increased, while total polyunsaturated fatty acids decreased. In this connection, Ivanova et al. [22] cleared that plant leaves improve their resistance to salt stress by decreasing the lipid membrane permeability through increasing the amount of saturated fatty acids. The shortage of fresh water is compelling researchers to investigate the use of saline water for irrigation [23]. Thus, the present review was put forward to add more information on the impact of seawater salinity on ultrastructure of chloroplasts and oleosomes in relation to fat metabolism in flag leaves of two wheat cultivars during grain-filling.

Impact of Seawater Salinity

Growth vigor, water relations, protein and nucleic acids

In general, sea water at 10% and 25% caused noticeable reduction in root/shoot ratio, root density, root distribution, flag leaf area and specific leaf area as well as in the degree of succulence and sclerophylly during grain filling. Furthermore, seawater at 10% and 25% caused noticeable reduction in almost all growth criteria that was consistent with the progressive alteration in water relations (RWC & SWD), protein and nucleic acids (DNA and RNA) content of both varieties during grain filling. The magnitude of reduction was more obvious at higher salinity levels than the lower one particularly in Gemmieza-9. Furthermore, reduction was more pronounced at the higher salinity levels as compared to the lower one, particularly in Gemmieza-9.

Osmotic adjustment an osmolytes

Osmotic pressure (OP), osmotic adjustment (OA) and solutes accumulation (TSS, TSN, proline, organic acids, glycerol and inorganic ions (Na⁺, K⁺, Ca²⁺, Mg²⁺ and Cl⁻) were quantified in flag leaf during grain-filling (14 and 21 days post-anthesis). Seawater salinity induced significant increase in osmotic pressure and the magnitude of increase was higher in Sids-1 than in Gemmieza-9. Furthermore, seawater concentrations caused noticeable increase in osmotic adjustment, organic solutes (TSS, TSN, proline, organic acids and glycerol) and inorganic ions (Na⁺, K⁺, Ca²⁺, Mg²⁺ and Cl⁻). On the other hand, clear reduction in K⁺/Na⁺ ratio in the flag leaves of both cultivars was observed. The capacity of osmotic adjustment was greater in younger leaves than in older ones particularly with higher concentration (25%) in both cultivars. Moreover, the production of both organic and inorganic ions tended to be higher in Sids-1 than in Gemmieza-9. Gemmieza-9 appeared to be more sensitive than Sids-1. Osmotic pressure of flag leaf sap appeared to depend mainly on proline, TSN, TSS, organic acids, glycerol and

ions content, where there is a positive correlation between osmotic pressure and all of them.

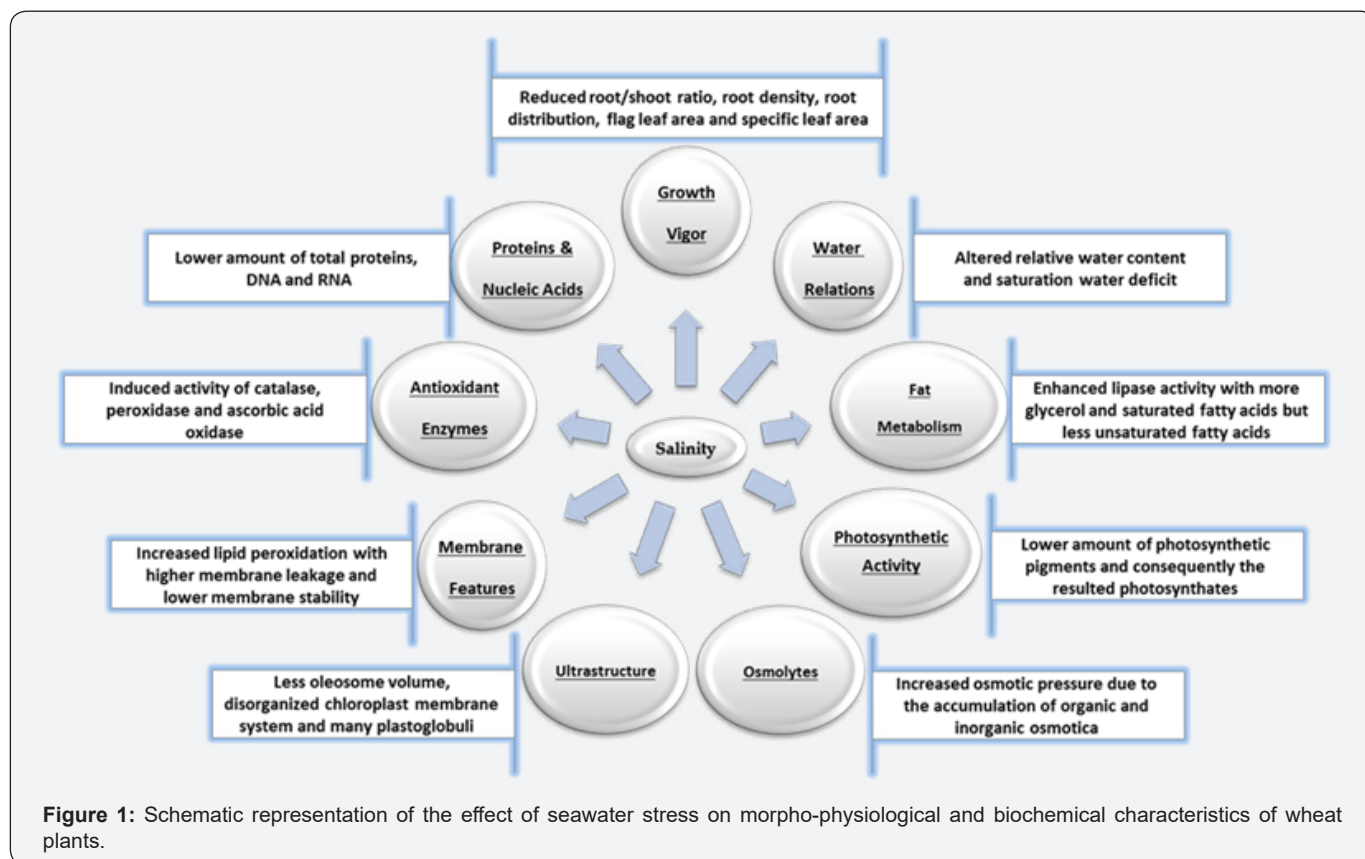
Photosynthesis and Ultrastructure Characteristics

Chloroplasts ultra structure, chloroplasts number, pigment content and photosynthetic activity were quantified during grain-filling (14 and 21 days post-anthesis) of two wheat cultivars (salt sensitive cultivar, Gemmieza-9 and salt tolerant cultivar, Sids-1) subjected to different seawater at percentage 10% and 25%. The results showed that there were slight differences between the two cultivars in response to seawater at 10% and 14 days post-anthesis in terms of chloroplasts ultrastructure. The most obvious changes were observed with the treatment with 25% seawater at 21 days post-anthesis. Moreover, disorganized membrane system was identified with swollen thylakoids and many plastoglobuli were recognized in the chloroplasts in comparing to control plants. Number of chloroplasts was subsequently decreased with increasing seawater concentrations in both cultivars and the reduction was higher in Gemmieza-9 than in Sids-1. Furthermore, the applied concentrations of seawater induced noticeable reduction in pigments content (i.e. Chl a, Chl b, Chl (a+b), Chl (a/b), carotenoids and anthocyanin) as well as in photosynthetic activity (i.e. soluble, insoluble, total photosynthates and ratio of soluble/insoluble photosynthates in both cultivars and this reduction tended to be higher in Gemmieza-9 than in Sids-1. The changes in pigments content and photosynthetic activity of flag leaf appeared to depend mainly on chloroplasts ultrastructure and its numbers,

where there is a positive correlation between chloroplasts number and pigments content. Furthermore, numerous spherical oleosomes were observed as free in the vacuole of flag leaf cells of both untreated and seawater treated plants. Oleosomes appeared to have a sharply-defined osmiophilic interface and apparently lack a limiting membrane. Furthermore, there was a noticeable decrease in oleosomes volume in seawater-stressed flag leaves of both wheat cultivars from 14 to 21 days post-anthesis. Seawater irrigation induced a progressive increase in lipase activity and glycerol content in flag leaf of both cultivars during grain-filling. The tolerant cultivar accumulated more glycerol than sensitive one under salt-stress.

Membrane Characteristics and Antioxidant Enzymes

Seawater increased the malondialdehyde content in both wheat cultivars as compared to control plants, the increase being significantly higher in Gemmieza-9 than in Sids-1. The lower level of lipid peroxidation in Sids-1 probably indicated better protection against oxidative damage caused by seawater. Concomitant, the two applied concentrations of seawater reduced membranes stability resulting in an increased membrane leakage. Gemmieza-9 was characterized by the highest degree in membrane leakage and the lowest value of the membrane stability index. The activity of the antioxidant enzymes catalase, peroxidase and ascorbic acid oxidase increased under seawater irrigation in both cultivars with a higher increase in Sids-1 than in Gemmieza-9. Therefore, Sids-1 (resistant cultivar) had a higher potential to withstand seawater stress than Gemmieza-9 (sensitive cultivar) (Figure 1).



Conclusion

This review discusses impact of seawater salinity on morpho-biochemical, photosynthesis, ultrastructure of chloroplasts and oleosomes in relation to fat metabolism in flag leaf of two wheat cultivars during grain-filling. In conclusion, it is clear from this investigation that the impact of seawater irrigation at 10% or 25% on both wheat cultivars particularly sensitive one had a negative effect on growth vigor of root and shoot, leaf area expansion, pigments content, membrane stability, relative water content, protein content, DNA, RNA as well as ultrastructure of chloroplasts and oleosomes of flag leaf during grain filling. On the other hand, seawater stress resulted in accumulation of inorganic ions, organic solutes, glycerol and saturated fatty acids which in turn involved with cell protection and osmotic adjustment. In addition salinity induced plant defense machinery with varying degrees in both wheat cultivars by enhancing the activity of antioxidant enzymes [24-32].

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