PHYSIOLOGICAL AND BIOTECHNOLOGICAL STUDIES ON WHEAT PLANT UNDER SALINITY STRESS CONDITIONS

By

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B.Sc. Agric. Sci. (Plant Pathology), Fac. Agric., Cairo Univ., Egypt, 2000

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

In

Agricultural Sciences

(Plant Physiology)

Department of Agricultural Botany
Faculty of Agriculture
Cairo University
EGYPT

2009

APPROVAL SHEET

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Title of Thesis: Physiological and biotechnological studies on wheat plant under salinity stress conditions

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ABSTRACT

In vitro experiments were performed to determine responses of wheat calli to ascorbic acid (AsA) concentrations (0, 250, 500, 1000 and 2000 ppm) under different levels of sea water (0, 15, 30 and 45%) and to determine suitable concentrations of AsA for exogenous treatments to enhance wheat tolerance to salinity. Results of this study indicated that AsA of (500, 1000 and 2000 ppm) concentrations improved tolerance of wheat calli to salinity. Two pot experiments were conducted during the two successive seasons 2004-2005 and 2005-2006 to determine the effect of exogenous AsA of 0, 500, 1000 and 2000 ppm concentrations at 45 and 75 days after sowing on growth, chemical composition and yield of wheat plants cv. Giza 168 (salt sensitive) irrigated with different levels of sea water (0, 15, 30 and 45%) in comparison with plants of cv. Sids 1 (partially salt-tolerant). The obtained results in this study clearly revealed that exogenous AsA enhanced plant growth, chemical composition and consequently the productivity of wheat plants under salinity stress conditions. These effects may be attributed to the protective role of AsA in plant cells from the oxidative stress induced by salinity. It could be concluded that exogenous AsA of 1000 ppm concentration at 45 and 75 days after sowing is the most effective treatment to increase wheat tolerance to salinity.

Key words: Wheat, salinity, ascorbic acid, *in vitro*, calli.

ACKNOWLEDGEMENT

I wish to express my sincere thanks, deepest gratitude and appreciation to my supervisor **Dr. Farghal Abdelhafiez Zeid,** *Professor of Plant Physiology at the Faculty of Agriculture, Cairo University for suggesting the problem, supervision, permanent support and his valuable guidance through the course of study.*

My deepest gratitude and appreciation to my supervisor **Dr. Osama Mohamed El Shihy** *Professor of Plant Physiology and Biotechnology in the same faculty for his sincere guidance, permanent support, kind encouragement, helpful suggestions and his critical review of my thesis.*

I am deeply indebted and grateful to my supervisor **Dr. Abd El Rahman Morsy Ghallab** *Professor of Plant Physiology in the same faculty for his valuable guidance, support, and his generous efforts during the preparation of the manuscript of this thesis.*

My sincere thanks and appreciation to **Dr. Farouk Mohamed Gadallah** *Professor of Plant Physiology, at the Faculty of Agriculture, Fayoum University and* **Dr. Ahmed Hussien Hanafy Ahmed** *Professor of Plant Physiology, at the Faculty of Agriculture, Cairo University for their evaluating and constructive criticism of this work .*

Grateful appreciation is also extended to all staff members of Plant Physiology Division, Department of Agricultural Botany, Faculty of Agriculture, Cairo University.

CONTENTS

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important growing cereal crop in Egypt. Although wheat production per unit area in Egypt has significantly increased during the past years, wheat production supplies about 40% of its annual domestic demand. Therefore, it is very essential to increase wheat productivity. Extending wheat growing outside the Nile Valley is the first effort toward overcoming wheat problems.

However, most of the area outside the Nile Valley suffers from salinity or depends on water sources that are affected by salinity. Therefore, there is a need to find means for enhancing the ability of wheat to tolerate salinity and consequently increasing its productivity under salinity stress conditions. Salinity stress is a severe environmental factor limiting the growth and productivity of agricultural crops (Boyer 1982). As saline soils and saline waters are common around the world, great efforts have been devoted to understanding physiological aspects of tolerance to salinity in plants, as a basis for plant breeders to develop salinity-tolerant genotypes. Salinity imposes both ionic and osmotic stresses on plants (Munns *et al.* 2006).

Therefore, salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. For instance, reductions in plant growth due to salt stress are often associated with decreases in photosynthetic activities, such as the electron transport (Greenway and Munns 1980). In addition, several factors associated

with salinity stress can lead to an increase in reactive oxygen species (ROS), Asada (1999). Free radical scavenging systems such as superoxide dismutase (SOD) can be a critical component of salinity tolerance (Bohnert and Jensen 1996) because of their protection of chloroplast function under high salinity (Orcutt and Nilsen 2000).

Salinity causes a significant decrease in SOD activity (Dionisio-Sese and Tobita 1998). The predominant peroxidase enzyme is ascorbate peroxidase (APX), which catalyses oxidation of ascorbate (ascorbic acid; AsA) by H_2O_2, generating dehydroascorbate radicals (Hideg 1999).

In chloroplast, the enzyme primarily occurs in stroma thylakoid, where superoxide and H_2O_2 are produced (Asada 2006). Lin and Kao (2000) reported a significant increase in APX activity in salt-treated rice seedlings and concluded that this could be due to the effect of AsA in controlling H_2O_2 under stress. The functioning of this enzyme is supported by a large (10–300 mM) AsA pool, which constitutes the largest pool of antioxidants found in plants (Chen and Gallie 2004). However, this pool would be exhausted within a few minutes without the high-capacity regenerating system consisting of the monodehydroascorbate reductase and dehydroascorbate reductase enzymes (Pignocchi and Foyer 2003).

AsA is an important primary metabolite in plants that functions as an antioxidant, an enzyme cofactor and a cell signalling modulator in a wide array of crucial physiological processes, including biosynthesis of the cell wall, secondary metabolites and phytohormones, stress tolerance, photoprotection, cell division and growth (Wolucka *et al.*

2005). Besides, it is also important for the regeneration of membrane-bound antioxidants (Hideg 1999).

Therefore, two parts of this study were carried out as follows:

1. The First part (*In vitro*): the objective of this part was to determine the effective concentration of ascorbic acid (AsA) which might alleviate the harmful effects of salinity on wheat calli.
2. Th second part (*In vivo*): the objective of this part was to investigate the effectiveness of AsA concentrations which had obtained from the first part in improving the salt tolerance of wheat plants of the salt-sensitive Giza 168 cultivar grown under different levels of salinity (0, 15, 30 and 45% sea water).

REVIEW OF LITERATURE

Wheat is a member of the family Poaceae, tribe Hordeae and placed in the genus *Triticum*. It is an annual, long day and self-pollinated plant. Wheat, the world's most important food crop, covers more cultivated land at the global level than any other crop (Anon 2000). According to Martin and Leonard (1963) there are several species of *Triticum*. These species fall into three distinct groups, such as Diploids, Tetraploids and Hexaploids with 14, 28, and 42 chromosomes respectively.

Salinity is one of the major factors affecting agricultural productivity worldwide. Excess amount of salt in the soil adversely affects plant growth and development. Nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity (Zhu 2001). In the arid and semiarid areas, it could be caused by (1) poor irrigation water which contains considerable amounts of salts, (2) accumulation of salts in the top layer of the soil due to over-irrigation, (3) proximity to the sea, and (4) the capillarity rise of salts from underground water into the root zone due to excessive evaporation. Also, low rainfall, high evaporation rate and poor water management could cause salinity related problems in these areas. Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes (Munns 1993 and 2002). Processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set

are adversely affected by high salt concentration, ultimately causing diminished economic yield and also quality of produce.

Plants are classified as glycophytes or halophytes according to their capacity to grow on high salt medium. Most plants are glycophytes and cannot tolerate salt-stress. Salinity imposes both ionic and osmotic stresses on plants (Munns *et al.* 2006, Munns and Tester 2008). Plants growing under saline conditions are stressed basically in three ways; (1) reduced water potential in the root zone causing water deficit, (2) phytotoxicity of ions such as Na^+ and Cl^-, and (3) nutrient imbalance by depression in uptake and/or shoot transport (Munns and Termaat 1986; Läuchli 1986; Marschner 1995). This is attributed to the fact that Na^+ competes with K^+ for binding sites essential for cellular function (Tester and Davenport 2003). This role makes K^+ an important element as more than 50 enzymes are activated by K^+, and Na^+ cannot substitute in this role (Bhandal and Malik 1988). On one hand, the latter implication of these two macronutrients in salinity is thought be to one of the factors responsible for reduction in the biomass and yield components. One other hand, however, the reduction in growth is generally the consequences of several physiological responses including modification of ion balance, water status, mineral nutrition, stomatal behavior, photosynthetic efficiency and carbon allocation, and utilization (Flowers and Teo 1981; Greenway and Munns 1980; Munns and Termaat 1986). In salt-sensitive plant, shoot, and to lesser extent, root growth is permanently reduced within hours of salt tress and this does not appear to depend on Na^+ concentrations in the growing tissues, but rather is a response to the osmolatity of the external solution

(Munns *et al.* 2000a; Munns 2002). Although reduction in biomass, photosynthetic capacity changes in leaf water potential and leaf turgor have been reported to have a cumulative effect attributed to salinity stress (Wignarajah 1990; Monneveux and Belhassen 1996 and Tourneux and Peltier 1995), it is also clear that several soil and other environmental factors do influence plant growth under salinity conditions.

The mechanisms of salinity tolerance fall into three categories; (1) Tolerance to osmotic stress: The osmotic stress immediately reduces cell expansion in root tips and young leaves, and causes stomatal closure. A reduced response to the osmotic stress would result in greater leaf growth and stomatal conductance, but the resulting increased leaf area would benefit only plants that have sufficient soil water. Greater leaf area expansion would be productive when a supply of water is ensured such as in irrigated food production systems, but could be undesirable in water-limited systems, and cause the soil water to be used up before the grain is fully matured. (2) Na^+ exclusion from leaf blades: Na^+ exclusion by roots ensures that Na does not accumulate to toxic oncentrations within leaves. A failure in Na^+ exclusion manifests its toxic effect after days or weeks, depending on the species, and causes premature death of older leaves. (3) Tissue tolerance, i.e., tolerance of tissue to accumulated $Na^{+,}$ or in some species, to Cl^-.Tolerance requires compartmentalization of Na^+ and Cl^- at the cellular and intracellular level to avoid toxic concentrations within the cytoplasm, especially in mesophyll cells in the leaf. Toxicity occurs

with time, after leaf Na^+ increases to high concentrations in the older leaves (Munns and Tester 2008).

1. Effect of salinity stress on plants

a. Germination

It is well documented that, the performance of crops under saline conditions depends upon seed germination and establishment and also tolerance at the later stages of growth. The capacity of seed germination relates to the extent of imbibition of solution by seeds and the resultant activity of the embryo. Thus, the water absorption phase plays a key role. When seeds were sown under saline conditions, both the rate and percentage of germination were decreased. The process of early germination depends upon the balance between the expansive force (osmotic potential) of the protoplast of the embryo and the restrictive force of the cell wall. The reduction in germination under saline conditions could be attributed to increased osmotic pressures of the soil solutions, which diminish the water absorption rate, leading to moisture stress in the seeds, and mobilization of food reserves. The imbibition of reserve mobilization could be due to the effect of salts on the enzymes responsible for hydrolysis or on the translocation of reserve hydrolysis products from the storage organ to the embryo axis (Lyenger and Reddy 1994). Dhindwal *et al.* (1992) stated that, in a pot experiment in greenhouse, barley cv. Odesski-100 was sown in clay loam soil (pH 7.4) and salinized to EC values of 1.02,1.97,2.83,3.75,4.51 or 5.32 ds/m or not salinized (0.8 ds/m). Low levels of salinity stimulated germination which peaked at 82.8% at 2.83 ds/m compared with the control of 69%. Kord and Khalil (1995) reported that, wheat and pea

seeds were germinated in distilled water or saline solutions containing 10000 ppm NaCl. Seed germination decreased with increasing salinity and did not occur at 10 ppm NaCl in both species; germination was slightly higher in wheat than peas. Seed water uptake was decreased by salinity and it was higher in peas than wheat.

b. Growth

The decreased rate of leaf growth after an increase in soil salinity is primarily due to the osmotic effect of the salt around the roots (Munns and Tester 2008). A sudden increase in soil salinity causes leaf cells to lose water, but this loss of cell volume and turgor is transient. Within hours, cells regain their original volume and turgor owing to osmotic adjustment, but despite this, cell elongation rates are reduced (Cramer 2002; Fricke and Peters 2002; Passioura and Munns 2000 and Yeo *et al.* 1991). Over days, reductions in cell elongation and also cell division lead to slower leaf appearance and smaller final size. Cell dimensions change, with more reduction in area than depth, so leaves are smaller and thicker. For a moderate salinity stress, an inhibition of lateral shoot development becomes apparent over weeks, and over months there are effects on reproductive development, such as early flowering or a reduced number of florets. During this time, a number of older leaves may die. However, production of younger leaves continues. All these changes in plant growth are responses to the osmotic effect of the salt, and are similar to drought responses. The reduction in leaf development is due to the salt outside the roots. That this reduction is largely due to the osmotic effect of the salt is supported by experiments using mixed salts such as concentrated

Hoagland's solution (Termaat and Munns 1986), other single salts such as KCl (Yeo *et al.* 1991) and nonionic solutes such as mannitol or polyethylene glycol (PEG) (Sümer *et al.* 2004 and Yeo *et al.* 1991). These different osmotica all have a similar qualitative effect as NaCl on leaf expansion. However, the salt outside the roots may affect plant growth not only through its effect on osmotic pressure. Sümer *et al.* (2004) found evidence for Na^+ but not Cl^- toxicity during the first phase of salt stress in maize in innovative experiments with different salts and PEG, via the use of additional PEG to adjust the equimolar solutions to equivalent osmotic pressures. Further, Cramer (1992) found evidence for the effect of supplemental Ca^{2+} in the rooting solution affecting rapid responses of leaf elongation rate from working with two maize cultivars of different salinity tolerance. El-Nabarawy (1994), Sakr (1996), Hasegawa *et al.* (2000) and Ozdemir *et al.* (2004) indicated that salinity suppressed both cell division and cell enlargement proportionally in wheat plants. The reduction in plant growth under salinization may be also due to regulation between the endogenous Phytohormones present in the seedlings. Moreover, Khadr *et al.* (1994) noticed that the decrease in growth due to decrease in water absorption, metabolic processes, meristematic activity and/or cell enlargement. In addition, the inhibitory effect of salinity on growth may be attributed to an increase in respiration rate resulting from high energy requirements. Sakr (1996) reported that salt stress reduced plant growth and yield characters. Salinity may decrease biomass production due to low/medium water potential, specific ion toxicity or ion