Multistress-induced changes in water relations, leaf gas exchange, chlorophyll *a*, ABA content and antioxidant activity in two maize genotypes differing in susceptibility to the soil compaction stress

Maciej T. Grzesiak*, Franciszek Janowiak*, Piotr Szczyrek*, Katarzyna Kaczanowska* Agnieszka Ostrowska*, Grzegorz Rut, Tomasz Hura*, Andrzej Rzepka**, Stanisław Grzesiak***

* The *F. Górski* Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, PL 30-231 Cracow, Poland,

** Department of Plant Physiology, Institute of Biology, Pedagogical University , Podchorążych 2, PL 30-084 Cracow, Poland

ABSTRACT In this study seedlings of two maize hybrids (sensitive and resistant to soil compaction) were grown by 28 days in low, moderate and high soil compaction levels and within 14 days in drought or waterlogging. Soil, root and leaf water potential, membrane injury, chlorophyll content, gas exchange parameters, ABA and antioxidant activity were measured.

In seedlings grown under different soil compaction levels differences between water potential of soil, leaf and roots were observed at noon and in the afternoon. Differences in the responses between resistant and sensitive genotypes were observed in the membrane injury, leaf water potential, chlorophyll content, gas exchange parameters, ABA content and antioxidants activity.

Keywords: abscisic acid (ABA); drought; soil compaction; water relations; waterlogging; maize (*Zea mays* L.) *Abbreviations*: L, M, S – low, moderate and severe soil compaction; C – control; D – drought; W – waterlogging; ψS, ψR, ψL – soil, root and leaf water potential; PN – photosynthesis rate; E – transpiration rate; gS – stomatal conductance; FWC – field water capacity

INTRODUCTION An excessive soil compaction results from natural processes and the use of heavy machinery in cultivation. Most important soil physical factors influencing plant growth in compacted soil are soil water status, aeration and penetration resistance. Characteristic symptoms in the root system structure in compacted soil is decrease root size retarded root penetration ability, smaller root depth and deformations of root cells and tissues.

Similarly, growth in high soil impedance causes changes in plant above-ground parts *e.g.* decrease of stem diameter and plant height and a decrease in leaf number, area, thickness, specific leaf area (SLA), thickness of epidermal cell and cell wall.

In the recent years, many papers have been published on the role of hormones in plant tolerance mechanisms to various stressors. Under high soil compaction shoot and leaves growth and functions may be reduced as an effect of root-to-shoot signaling and due to reduced soil water content under drought or decrease of oxygen content under soil waterlogging.

AIM The aim of this study was to examine the response of sensitive and resistant to soil compaction stress maize hybrids grown in low, moderate and high soil compaction levels with shortage or excess of water in soil. Generally, combined exposure to two or more abiotic stresses causes more harmful effect compared to a single stress. Maize is one of the most important cereals in the world and is cultivated under a wide range of climatic conditions. Determinations of physiological markers (water potential in soil, root and leaf, membrane injury, chlorophyll content, gas exchange parameters, ABA content and antioxidant activity), which may explain how maize manages its growth under environmental multistress.

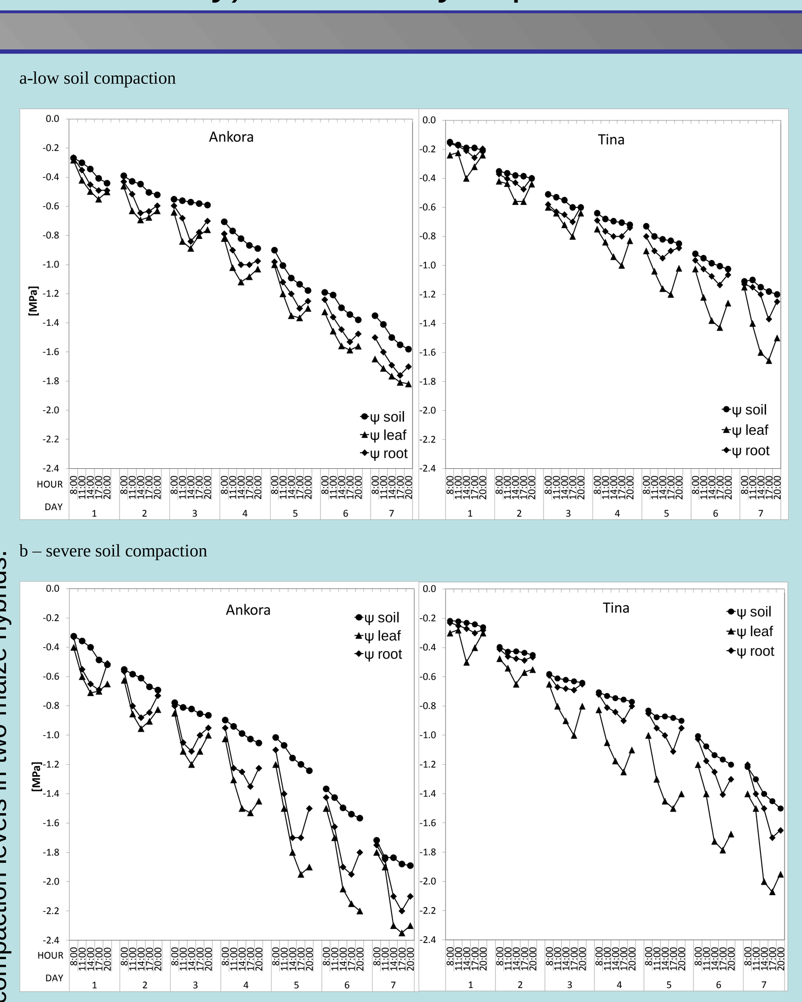


Fig. 1. Diurnal changes of water potential in the soil (ψS), root (ψR) and leaf (ψL) during 7 days without pot watering and (a) under low (L) or (b) severe (S) soil compaction levels in two maize hybrids.

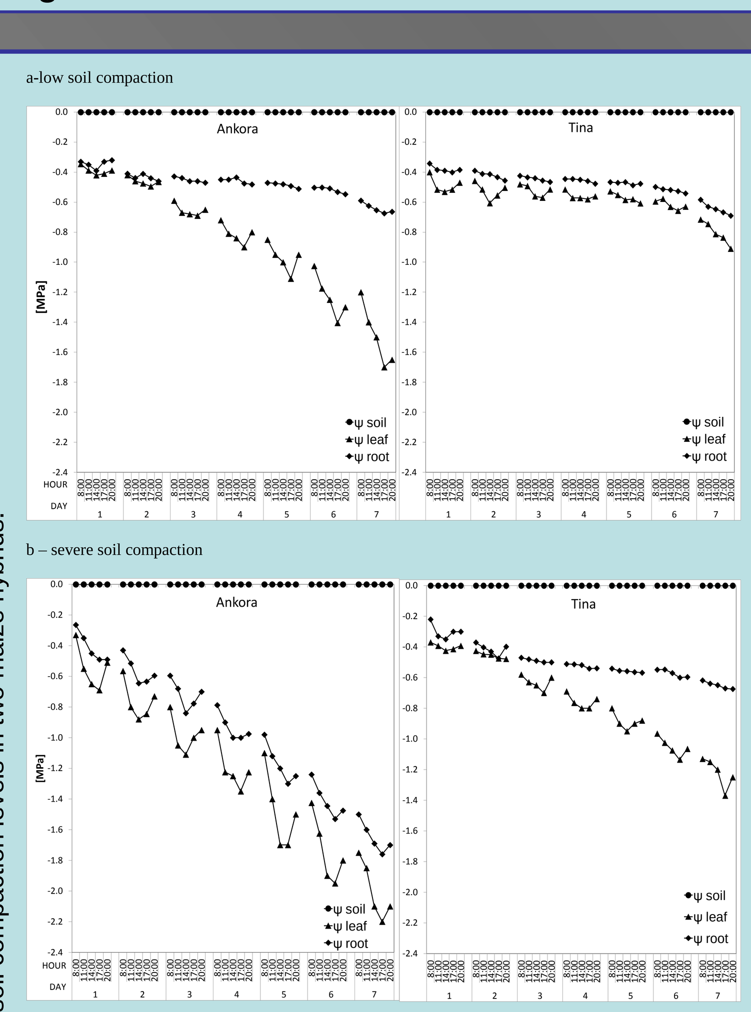
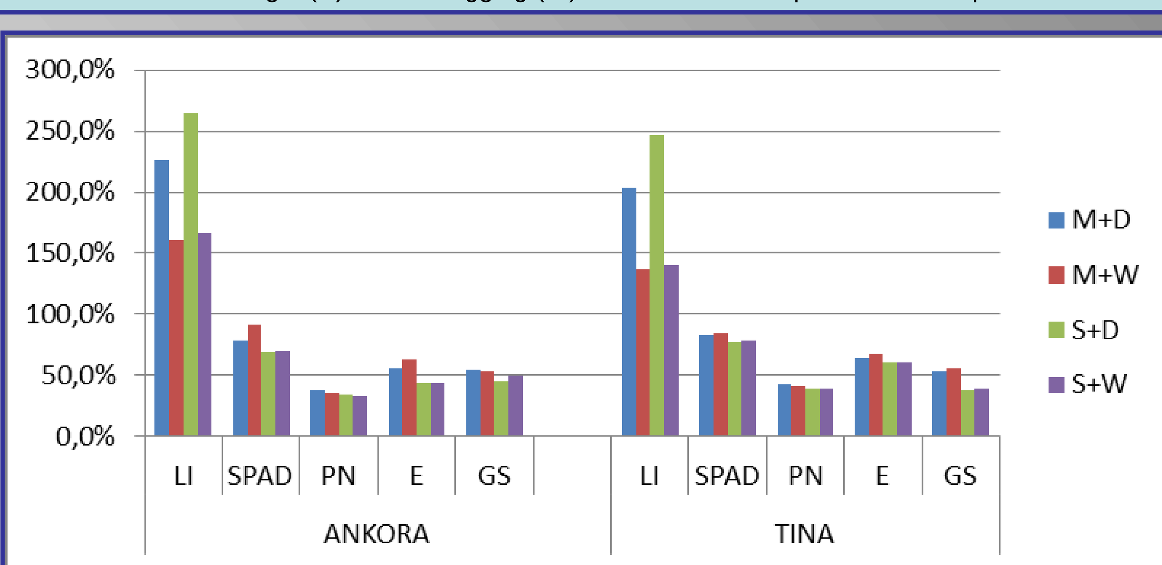


Fig. 2. Diurnal changes of water potential in the soil (ψS), root (ψR) and leaf (ψL) during 7 days of waterlogging conditions and (a) under low (L) or (b) severe (S) soil compaction levels in two maize hybrids.

RESULTS pt.2
Effect of drought (D) *or waterlogging* (W) stresses *on membrane injury* (LI)*, chlorophyll content* (SPAD) *and leaf gas exchange parameters (Pn, E,* gS)

Fig. 3. Changes of membrane injury (LI), chlorophyll content (SPAD) and gas exchange parameters (Pn, E, gS) in maize hybrids sensitive (Ankora) or resistant (Tina) to the soil compaction and grown under moderate (M) and severe (S) soil compaction levels and under drought (D) or waterlogging (W) stress. Result are presented as a percent of control plants.



Results of physiological markers (LI, SPAD, PN, E, gS) indicate that changes in sensitive hybrid (Ankora) were higher than in resistant (Tina). Also harmful effect of this physiological markers in plants subjected to soil drought (D) were higher in comparison to plant subjected to the waterlogging (W). Our results indicate that differences between sensitive and resistant genotypes might result from the fact that stress resistant genotypes possess more efficient mechanisms of protecting membrane structures and tissues water status.

MATERIAL AND METHODS
Plant material: The experiments were carried out on two maize single-cross hybrids (Ankora, Tina) obtained from SEMPOL-Holding Trnava, Slovakia.
Growth and treatment conditions: Plants were grown in an air-conditioned greenhouse. Plants were grown in PCV tubes. Air-dried sand was sieved and mixed with compound fertilizer: N–28mg, P–18mg, K–14mg per 1 kg of soil substrate. Three soil compaction treatments were applied, low (L–1.1 g cm^{-3}), medium (M–1.3 g cm^{-3}), and severe (S–1.6 g cm^{-3}). Field water capacity was determined according to Hillel and van Bavel (1976). For control treatments (L+C, M+C and S+C) soil water content was kept at 65-70% FWC from sowing to 28th day. In drought treatments soil water content at 30-35% FWC, and the pots were not watered for 7 days from 14th to 21st day (L+D, S+D) or for 14 days from the 14th till 28th day (L+D, M+D, S+D). Similarly for waterlogging soil water content was kept at 100% FWC from 14th to 21st day (L+W, S+W) or from 14th to 28th day (L+W, M+W, S+W). In waterlogging conditions PCV tubes were submerged 2cm below the water surface.
Measurements: *Membrane injury index* (LI): Blum and Ebercon (1981); *Water potential:* (ψ) Wesor HR33T (Wescor Inc., Logan, USA); *Chlorophyll content (SPAD)*: SPAD CL 01 (Hansatech, Norfolk, UK); *Gas exchange parameters:* CIRAS-2 (PP System, Amesbury, USA); *Quantification of ABA:* ELISA according to Walker-Simmons and Abrams (1991); *Total antioxidant activity:* DPPH method according to Brand-Williams et al. (1995).

RESULTS pt.1
Diurnal changes of water potential in soil **(**ψS)*, root* (ψR) *and leaf* (ψL)*.*

During 7 days of growth under drought (L+D, S+D) or waterlogging (L+W, S+W), stresses strongly affected diurnal changes of ψR and ψL and also ψS but only in drought stressed plants (Fig. 1). During the successive days of growth without watering, differences between ψS and ψR and between ψS and ψL were observed and were highest around noon and in the afternoon but lower in the morning and evening. In both soil compaction levels and in waterlogging, changes of ψR and ψL were lower in comparison to drought conditions (Fig. 2). Under L+W conditions for Ankora the differences between ψR and ψL were significantly greater compared to L+D conditions, and for Tina they were significantly smaller. The reason for the changes in diurnal fluctuations of ψR and ψL under drought and waterlogging treatments at noon and in the afternoon is that the high rate of transpiration at midday is not counterbalanced completely by the roots water uptake from the soil. In the afternoon the evaporative demand gradually declines because more water enters the plant through the roots than is transpired by the leaves. The tissue again becomes filled with water, and ψL and ψR increase. At the end of the night an almost complete balance is achieved between ψR, ψL and ψS.

Fig. 4. Content of ABA in leaf, stem and root in two maize hybrids grown in low and severe soil compaction levels and combined with optimal (L+C, S+C), drought (L+D, S+D) and waterlogging (L+W, S+W) soil water content.

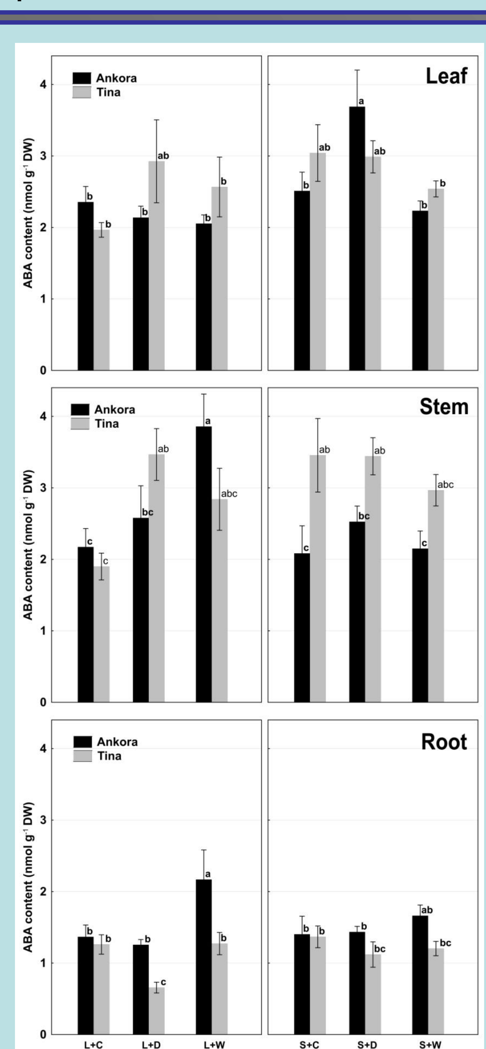
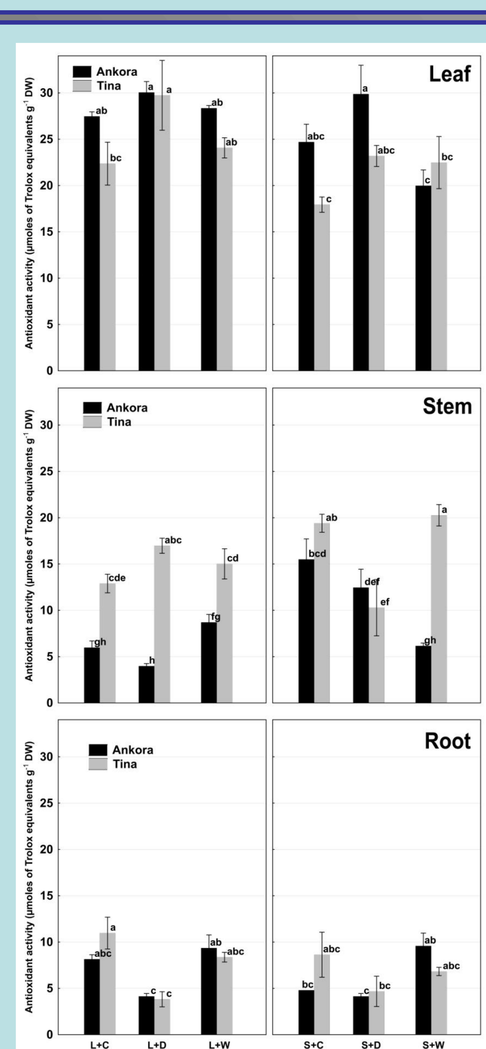


Fig. 5. Antioxidant activity in leaf, stem and root in two maize hybrids grown in low and severe soil compaction levels and combined with optimal (L+C, S+C), drought (L+D, S+D) and waterlogging (L+W, S+W) soil water content.



RESULTS pt.3
ABA content and antioxidants activity: ABA content under control (L+C) conditions ranged from 1.3 (roots) to 2.4 (leaves and stem) nmol g^{-1} DW (Fig. 4). Severe soil compaction or drought as a single stress increased ABA content in the stem and leaves but only in the tolerant hybrid Tina. In case of drought, ABA increase in the stem and leaves was accompanioned with its significant decrease in roots. Multistresses affecting roots (S+D and S+W) did not change their ABA level but they substantially increase ABA level in the stem and leaves of the tolerant hybrid Tina and in the leaves of the sensitive hybrid Ankora. Total antioxidant activity under control (L+C) conditions was slightly higher in leaves than in stem or root and it differed significantly between hybrids only in the stem (Fig. 5). Under severe soil compaction it increased significantly in both hybrids but only in the stem tissue. Multistress S+D substantially decreased antioxidant activity in the roots of both hybrids.

Conclusions: In our study we found that physiological responses of maize hybrids to abiotic stresses are associated with plant water status, which is manifested in the changes of physiological traits such as membrane permeability, chlorophyll content, leaf gas exchange parameters, ABA content and antioxidant activity. Differences between sensitive (Ankora) and resistant (Tina) maize hybrids indicate that resistant hybrids have more efficient protection mechanisms against water loss and physiological cell membrane status. Tolerance to the combination of different stress conditions should be the focus of future research programs aimed at developing new crop genotypes with enhanced tolerance.

Acknowledgements

Authors are grateful to the National Science Centre (NCN) for financial support (Project No. NN 310 782540) and to the SEMPOL-Holding, Trnava, Slovakia for providing the maize hybrids.