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Effect of Temperature on Root and Shoot Development in Wheat Seedlings during Early Growth Stage

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Abstract: The aim of the present study was to follow the early growth of wheat seedlings at different temperatures regimes and to assess the threshold of sensitivity of the wheat seedlings to heat constraints during the early stage of development. Obtained results showed that solely temperatures 15° and 25 °C allow early growth of wheat seedlings, while this growth was inhibited at all other temperatures 5°, 35° and 45 °C. The following of the mineral ionic content in K^+ and Ca^{2+} as function of time showed, after 2, 4 and 6 days of germination at 25°C, a decrease in the amount of potassium and calcium in both roots (R) and aerial parts (AP) or shoots of the young wheat seedlings. The drop in the content of Ca^{2+} is more pronounced, particularly, in the shoots of the seedlings. The temperature 15 °C caused a significant decrease of the ionic contents (K^+ and Ca^{2+}) of roots and shoots of seedlings obtained in the 6th day with regard to those obtained in the 4th day of early growth. After 6 days of growth at 25 °C, we obtained content in Ca^{2+} more important in the roots than that in the shoots, whereas at 15 °C the opposite was observed. The peroxidasic activity in roots and AP (shoots) of wheat seedlings obtained from seeds have germinated either at 15 ° or at 25 °C, decreases as function of time. After 4 days of the early growth at 15 °C, the activity of the total soluble peroxidases of R and AP is higher than that of R and AP of seedlings grown at 25 °C. After 6 days, this enzymatic activity increased solely in the R of seedlings grown at 25 °C if compared to those (R) obtained from seedlings established at 15 °C.

Key words: Peroxidasic activity, wheat, heat constraint, early growth, ionic content

INTRODUCTION

Cereals play a crucial role in the human food and the alimentary security. Therefore, the cereals cultivation occupies an important place in the world agriculture. Several factors of the surrounding environment, such as drought, temperature and salinity (Naheed *et al*., 2007), hinder the production of cereal in arid and semi-arid regions. Naheed *et al*. (2007) have shown a decline in the productivity of rice (*Oryza sativa* L.) under salt stress conditions. The screening of tolerant varieties to environmental stresses, based on physiological parameters and biochemical markers, constitutes a powerful tools contributing to resolve this problem.

In response to both biotic and abiotic stresses, plants synthesize a wide set of proteins including chitinases and peroxidases and up-regulate phenylalanine ammonialyase, PAL (Yun *et al*., 1997). In fact, plants submitted to environment aggressions develop defensive reactions involving an activation of many genes (Collinge and Salusarenko, 1987; Sturm and Chrispeels, 1990) as a response to various stimulations. Germination may constitute a precocious investigation for known the threshold tolerance of plants subjected to heat constraints. Dell'Aquila and Spada (1994) has shown that hard wheat seeds have a good aptitude to germinate and give seedlings when seeds were exposed to optimal thermals conditions (25°C). On the other hand, low and high temperatures modify, at the same time, velocity and capacity of germination (Bewley and Black, 1982). In Tunisia terminal heat stress is a major reason of yield decline in wheat due to delayed germination and planting. Similarly heat stress is a major challenge to wheat productivity in India (Joshi *et al*., 2007). Enzyme activity particularly that of peroxidases considered as biochemical markers of stress (Castillo, 1992; Jbir *et al*., 2001, 2002) varies according to temperature during germination of wheat seeds.

The subject of this work is the evaluation of the effect of heat constraints (5° to 45°C) on the early growth in hard wheat seedlings (6 days-old seedlings). Furthermore, we have studied the activity of total solubles peroxidases, in the different parts of the 6 days-old seedlings of wheat, under tow regimes of temperature (15 and 25°C).

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MATERIALS AND METHODS

Plant material: Present study was carried out using hard wheat (*Triticum durum*, cv. Karim), considered among main cereal in the human food and presents a big economic interest. The variety Karim arises from the crossing: 21563 AA "S" * Fg "s" realized in Mexico and introduced in Tunisia in 1973 (Maamouri *et al*., 1988). It is half premature and very productive (Bradford, 1976) and can support strong nitrogenous manure. This variety seems to be adapted to all the regions of the north of Tunisia and can be recommended in culture with irrigation (Maamouri *et al*., 1988). The studied seeds were collected in last summer and were gifted to us by the National Institute of the Agronomic Researches for Tunis (I.N.R.A.T). This study was carried out in the laboratory of Plant Biology and Biotechnology in Sciences Faculty of Tunis (University of Tunis El Manar, Tunisia), at years 2007-2008.

Determination of the mineral ionic contents: The mineral ionic contents (K^+ and Ca^{2+}) of the different organs of the hard wheat seedlings were determined on the products of acid extraction (HNO_3 0.1 N during a week in the ambient temperature) roots and aerial parts of wheat seedlings obtained after 2, 4 and 6 days of growth at 15 and 25°C. The determination of the K^+ and Ca^{2+} contents was realized by photometer Eppendorf. The device was calibrated by means of the solutions with known concentrations from 20 to 100 mg L^{-1} of K^+ or of Ca^{2+}.

Determination of the activity of total soluble peroxidases
Samples preparation: Roots and shoots of the wheat seedlings obtained from seeds having germinated during 6 days were quickly rinsed with distilled water, dried and then weight of each parts have been taken. The fresh matter was broken in the mortar in a phosphate buffer 0.1 M, pH 7 at 4°C (100 mg of fresh matter in 400 μL of the buffer), then centrifuged in 13000 rpm during 15 min at 4°C. The recuperate supernatant was used in measurements of the activity of the total soluble peroxidases.

Measurements of the activity of total soluble peroxidases: The activity of the total soluble peroxidases was determined at room temperature in the presence of the gaïacol as the substrate. The peroxidase /gaïacol activity was assessed with 20 μL of enzymatic extract in 900 μL of gaïacol (0.1%) dissolved in a phosphate buffer 100 mM, pH 6 and in the presence of H_2O_2 (0.5%). The absorbance was measured with the spectrophotometer (DU640 Beckman), in a wavelength of 470 nm. The results were expressed as ΔDO/min/g MF.

RESULTS AND DISCUSSION

Effect of temperature on the growth evolution of hard wheat seedlings: We obtained seedlings (2, 4 and 6 days) only when the germination was carried out at 15 and 25°C (Fig. 1). The kinetic of the growth of hard wheat seedlings was followed, as function of time, by determining the physiological parameters (weight of fresh and dry matter and ionic contents) of roots and shoots (coleoptiles and first leaf). The obtained results were shown in Fig. 1 and 2. The recorded results show (Fig. 1) that the temperatures 5, 35 and 45°C inhibit the growth. As it was previously demonstrated, this inhibition could be owing to a decrease of the speed of mobilization of the seeds reserves at the temperatures 5°, 35° and 45°C, which are not favourable to the growth and can, damaged the embryos. Mazia (1969) explained a slowing down of the growth of the roots of winter wheat at low temperature, by an extent of the mitotic cycle that insures the multiplication of the zygotic cells. Histograms of Fig. 2 represent the time course of the production of fresh and dry matter of roots and shoots (coleoptiles and first leaf) of wheat seedlings obtained from seeds germinated at 15° and 25°C and show the following results:

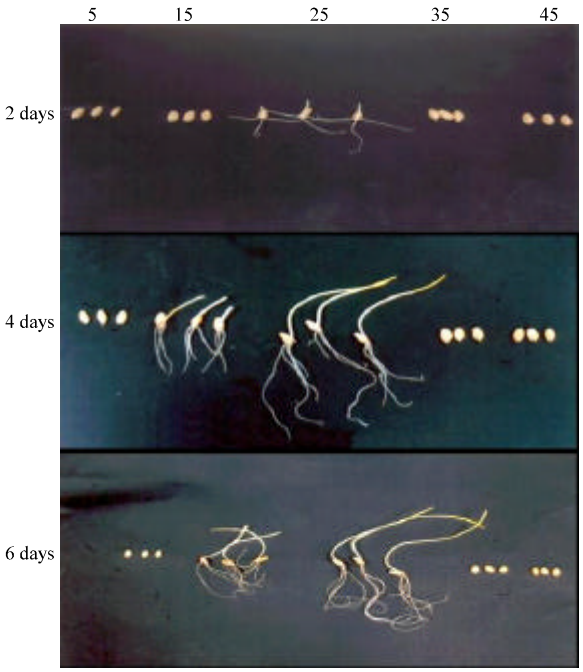

Fig. 1: Time course of the growth (2, 4 and 6 days) of wheat seedlings at different temperatures (5, 15, 25, 35 and 45°C). The growth was followed on Petri dishes added with diluted nutrient solution without thinning the seedlings of cultivar Karim

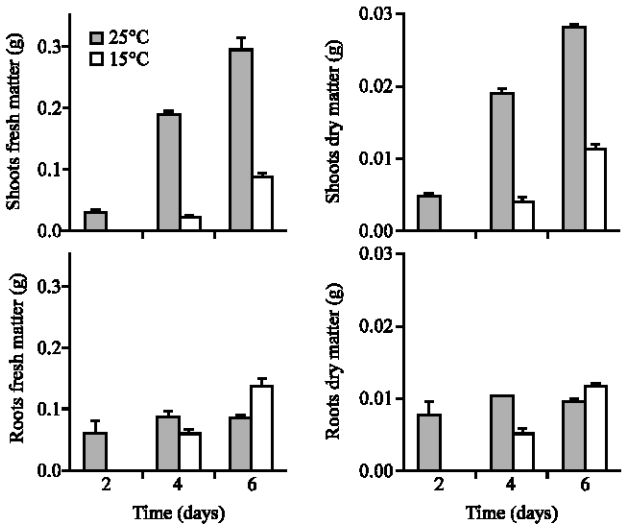

Fig. 2: Production of fresh and dry matter of roots and shoots (coleoptiles and first leaf) after 2, 4 and 6 days of early growth of cultivar Karim at 15° and 25°C. Mean values of 3 replicates and vertical bars indicate the standard error

- **Tow days-old seedlings:** The weight of fresh and dry matter was determined solely at 25°C (no seedlings were obtained at 15°C)
- **Four days-old seedlings:** The weight of fresh and dry matter was determined at 25 and 15°C in roots and shoots and revealed that growth is very important at 25°C than at 15°C
- **Six days-old seedlings:** The weight of fresh and dry matter become very important at 15°C than at 25°C (Fig. 1)

Our findings confirmed the results obtained by Fitter and Hay (1981), in which they demonstrated that the optimal temperature of roots growth in cereal is near 25°C. Perino and Come (1979) were found that the temperature of germination monitoring the future growth of apple plants.

It is expected to find differed results from the previous study because the experimental procedure is no the same. Wheat seedlings not thinned on medium solution but the growth was followed in petri dishes (added with nutrient solution) under similar conditions, such as luminosity and, to those prevailing in the growth room.

Effects of temperature on the ionic content in hard wheat seedlings: Figure 3 depicts the distribution of the major ions between the organs of the wheat seedlings obtained at 15°C and at 25°C. After 2, 4 and 6 days of early growth at 25°C, wheat seedlings present a decrease of the contents of potassium and as well at the level of the roots as at the level of the shoots. However, the decrease of the quantity of the bivalent cation Ca^{2+} is important in the shoot of seedlings obtained from wheat seeds germinated at 25°C. This suggests the use of these mineral elements in the growth of wheat seedlings (Fox *et al.*, 1998). Marme (1985) explained the reduction of the content in calcium by the fact that this cation is implied in several metabolic processes in plants subjected to a stress, such as turgor and cellular signals.

Effect of temperature on the activity of total soluble peroxidases of hard wheat seedlings: Recorded results in Fig. 4 have shown that the peroxidasic activity of the roots and shoots of the hard wheat seedlings (*Triticum durum*, cv. Karim) provided by germinating seeds at 15° and 25°C decreases during the course of early growth. After 4 days of development the activity of the total soluble peroxidases, at the level of roots and shoots of wheat seedlings maintained at the temperature 15°C is higher than that of seedlings maintained at the temperature 25°C. The reverse was observed after 6 days of early growth only for roots. The increase of the activity of peroxidases observed in wheat seedlings at 15°C is to be in relation with the decrease in the growth obtained previously. As it was already indicated that the works (Fielding and Hall, 1978; Requelme and Cardemil, 1993) which showed that peroxidases were involved in the processes of growth and development of plants. As well as Dejaegher *et al.* (1985) and Lefevre *et al.* (1993) were put in evidence the existence. In addition, Dejaegher *et al.* (1985) and Lefevre *et al.* (1993) were shown the presence of a relationship between the peroxidasic activity and the decrease in the growth of

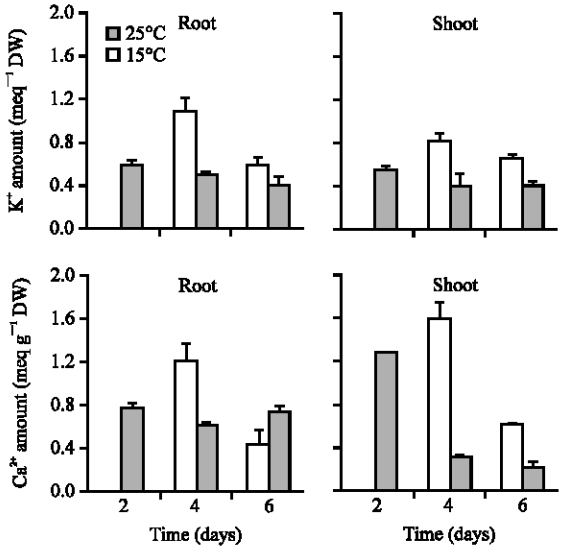

Fig. 3: Evolution of the contents of K^+ and Ca^{2+} (meq g^{-1} DW) in roots and shoots (coleoptiles and first leaf) after 2, 4 and 6 days of early growth of cultivar Karim seedlings at 15° and 25°C. Mean values of 3 replicates and vertical bars indicate the standard error

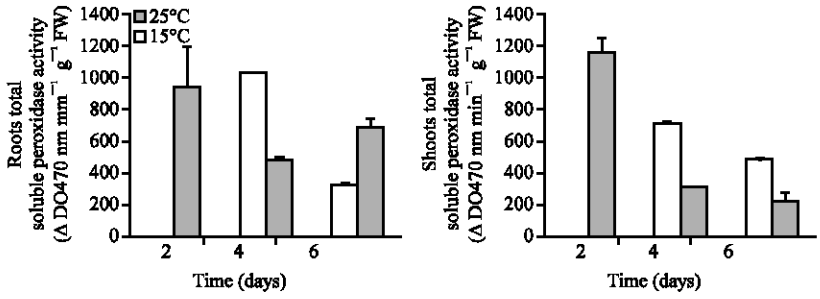

Fig. 4: Activity of the total soluble peroxidases of shoots (coleoptiles and first leaf) and roots of cultivar Karim seedlings after 2, 4 and 6 days of early growth at 15 ° and 25°C. Mean values of 3 replicates and vertical bars indicate the standard error

seedlings exposed to another type of stress. On the other hand, Stevens *et al.* (1978), Lutts and Guerrier (1995) were obtained that the reduction in growth not always accompanied by an increase in the activity of total soluble peroxidases. The latter increased in plants submitted to heat stress. This is in line with other works performed on other types of abiotic stresses (such as; salt, drought and heat).

Therefore, At the low temperature (5°C), the increase of the delay time having a goal as preventing the emergence of the radicle and as tool contrasting the establishment of wheat seedlings which could not continue the lifecycle of development (not adapted). The high temperatures (35 and 45°C) could provoke irreversible embryonic damage. Only the temperatures 15 and 25°C allow the growth of hard wheat seedlings studied herein. The sharing of the mineral ions, between the organs of the seedlings, is sensitive to the temperature of the environment in which was realized the germination and subsequently the early growth. In the 4th day, this peroxidasic activity is more important in the organs of the young plants maintained in 15°C than that of the organs of seedlings maintained in a temperature of 25°C. As well for the roots as for the shoots of seedlings given by seeds geminated at 15 and 25°C, the peroxidasic activity decreases as function of growth.

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