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*Short communication*

# Effect of temperature shock on the dynamics of abscisic acid and wheat germ agglutinin accumulation in wheat cell culture

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**Abstract**

Abscisic acid (ABA) and lectin content was immunoassayed in wheat cell cultures affected by temperature stress. The elevated temperature (40 °C) resulted in a 7-fold increase in the level of ABA and a 10-fold increase in that of lectin. The increase in the lectin content in cells was preceded by ABA accumulation. It is suggested that this ABA increase induces the synthesis of lectin, which in addition to stress proteins, play an important role in controlling mechanisms of plant adaptation to unfavourable environments.

*Abbreviations:* ABA – abscisic acid; WGA – wheat germ agglutinin

## 1. Introduction

Wheat germ agglutinin (WGA), a typical representative of cereal lectins, is involved in the development of plant resistance to various stresses [2]. Abscisic acid (ABA) is known to participate in the regulation of lectin synthesis. ABA accumulation preceeds lectin accumulation in the case of fungal pathogenesis [5], drought, osmotic shock [1] and salinity [8]. However, there are no data on whether quantitative changes in ABA induced by temperature shock affect lectin levels. Consequently, we studied the effect of high temperature on the dynamics of ABA and WGA contents in wheat callus tissue.

## 2. Materials and methods

### 2.1 *Plant material*

Callus cells were formed from immature embryos of wheat (*Triticum durum* L.) cv. Bezenchukskaya 139, obtained from Chishminsky Crop Production, Bashkortostan, Russia after 10 days of subcultivation on Murashige and Skoog nutrient medium [7].

### 2.2 *Treatment of wheat callus cells*

For temperature stress treatment, tubes with calli were placed in thermostatically-controlled conditions at 40 °C whilst control tubes were kept at 24 °C. After 4, 9, 14 and 18 h of treatment, samples of callus tissue were fixed in liquid nitrogen.

### 2.3 *Quantitative estimation of ABA and WGA content*

Callus tissue was homogenized in 80% ethanol and kept at 4 °C for 16 h to extract ABA (meanwhile lectin remained insoluble [6]). After centrifugation at 10,000 *g* for 10 min. the ethanol extract was evaporated to an aqueous residue from which ABA was partitioned into diethyl ether. The procedure of purification and ABA immunoassay was carried out according to [10]. After removal of ethanol, proteins were extracted from the pellet with 0.05 *N* HCl for 1 h at room temperature. The extract was centrifuged at 15,000 *g* for 10

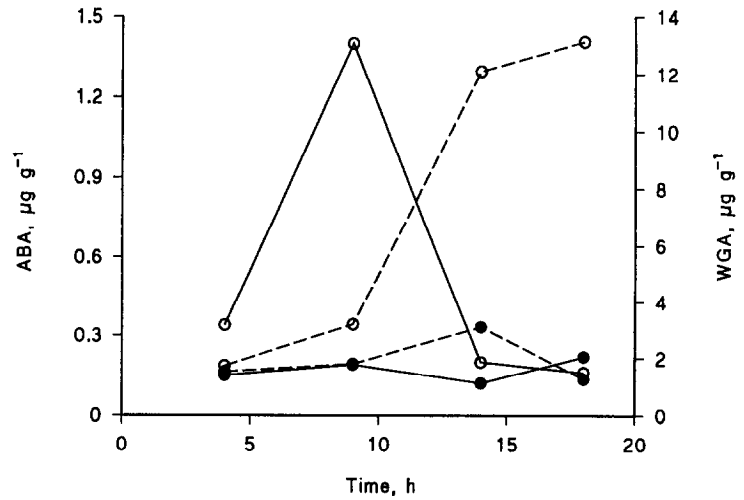


*Figure 1.* Effect of heat shock on the dynamics of ABA and lectin content in wheat callus cells. Solid lines – ABA, broken lines – WGA. ● 24 °C (control), ○ 40 °C. The ratios of standard deviation to average were less than 12%.

min; the supernatant was neutralized with 1 *M* sodium phosphate buffer (pH 7.2) and aliquots of the resulting crude plant extract were tested for WGA content by ELISA using specific rabbit antibodies to WGA and anti-rabbit peroxidase-labelled antibodies as described previously [5].

## 3. Results and discussion

High temperature resulted in a sharp ABA accumulation in cells (Figure 1). Thus, 4 h after callus was exposed to 40 °C the ABA content doubled and after 9 h increased by 7 times, in comparison with controls. Subsequently, ABA content decreased in comparison to the controls. These large ABA changes indicated that cells cultivated at 40 °C suffered from severe stress which caused major changes in their metabolic activity. These results are consistent with the literature reporting increases in ABA level in response to different unfavourable factors in the environment [4, 10]. It has been proposed that ABA acts as a common mediator in defence responses of plants to stresses [4].

In parallel with ABA determinations, we analysed lectin content in the same callus tissues. Figure 1 shows that heat shock resulted in an increase in WGA level nearly 10 times greater than in control cells after 18 h of exposure. The progressive lectin accumulation which followed the sharp increase in ABA level may be the result of a rise in *de novo* lectin synthesis induced by stress. This agrees with the results of Spadoro-Tank and Etzler [9] who showed induction of the synthesis of a lectin-related protein in *Dolichos biflorus* cell suspension culture affected by temperature shock.

Under stress conditions ABA induces the synthesis of stress proteins [4]. This process is observed within a background of suppression of some major proteins present in cells under normal conditions [4]. However, together with stress proteins there are some proteins contained by normal cells [3] which are increased during plant adaptation to stresses. Wheat lectin is probably an example of such proteins, the quantitative increase in lectin level being one of the universal nonspecific mechanisms of plant protection against the influence of unfavourable environmental conditions.

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