



Endogenous progesterone and its cellular binding sites in wheat exposed to drought stress



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ARTICLE INFO

Article history:

Received 26 March 2013

Received in revised form 24 July 2013

Accepted 29 July 2013

Keywords:

Plants

Wheat

Progesterone

Putative receptors

Drought stress

ABSTRACT

Progesterone is a basic hormone that regulates the metabolism in mammals. The presence of this compound has also been found in certain plants. It is believed that progesterone can regulate growth processes and resistance to stress, however, its precise role in plants remains unknown. The research conducted in this study was aimed at analyzing the content of endogenous progesterone and its cellular binding sites in the leaves of spring wheat exposed to drought. Changes were studied in two cultivars of wheat – a cultivar sensitive to drought (Katoda) and tolerant cultivar (Monsun). Plants had undergone periodic droughts during the seedling stage or in the phase of heading. The occurrence of free progesterone as well as its conjugated forms was observed in wheat studied. The amount of progesterone ranged from 0.2 to 5.8 pmol g FW⁻¹ and was dependent on the cultivar, age of the plants, stage of development and fluctuated as a result of the exposure to drought. Cv. Katoda responded to a water deficit by lowering the amount of progesterone and cv. Monsun by increasing its level. Progesterone in plants grown in limited water conditions occurred primarily in a free form. While in the optimal watering conditions, some of its pool was found in the form of conjugates. In the spring wheat the occurrence of binding sites for progesterone was detected in cell membranes, cytoplasm and nuclei in the range of 10–36 fmol/mg of protein. The wheat cultivars tested, Monsun and Katoda, differ in their concentration of cellular binding sites for progesterone. This number varied in the individual fractions during different stages of plant development and due to the effect of drought stress. The number of binding sites for progesterone located in the membrane fraction of seedlings and flag leaves increased significantly under drought in the cv. Katoda (35–46%), but did not change in the cv. Monsun. Whereas the number of cytoplasmic progesterone binding sites increased during the drought in the cv. Monsun (about 50%), they did not change in the cv. Katoda. Changes in the amount of progesterone and its binding sites in the cell under the influence of drought were then different depending on whether the cultivar was tolerant or sensitive to drought. The possibility of utilizing these changes as markers of drought resistance is discussed. The results obtained suggest that progesterone is a part of wheat response to stress factors (drought).

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1. Introduction

The presence of typical mammalian steroid hormones in plants have been already postulated in the first half of the twentieth century [1], but only in recent years has the development of analytical techniques allowed for the accurate quantitative determination of some of these compounds in plant tissues. An example of this is progesterone. In mammals, this steroid is the primary regulator of

metabolism, responsible for the reproductive processes including proper pregnancy outcomes, as well as for mediation in the biosynthesis of other steroid hormones. The presence of small quantities of progesterone was also found in plants. Hartman et al. [2] determined the content of progesterone by gas chromatography–mass spectrometry (GC–MS) of potatoes (5.07 µg kg⁻¹), wheat flour (0.60–2.86 µg kg⁻¹), rice (0.38 µg kg⁻¹) and soy (0.3 µg kg⁻¹). Iino et al. [3] assayed progesterone using GC–MS in various parts of plants (*Arabidopsis thaliana* L., *Pisum sativum* L., *Lycopersicon esculentum* Mill., *Alium cepa* L., *Vigna radiata* (L.) R. Wilczek and *Solanum tuberosum* L.), e.g. in the stems, leaves, generative parts etc. and found that it ranged from 6 to 1540 ng kg⁻¹. Ultra-performance

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liquid chromatography-electrospray tandem mass spectrometry, in turn, was used for assaying the progesterone level in leaves of *Nicotiana tabacum* L., *Digitalis purpurea* L. and *Inula helenium* L. [4].

In 2005, there were reports of finding proteins in plant cells that resembled animal receptors binding progesterone [5]. Membrane protein (MSBP1) was isolated from *A. thaliana*, and it was determined that the *MSBP1* gene encodes 220 aa peptide that can bind progesterone with high affinity and several other steroids with low affinity. This protein is important in the process of cell elongation in *A. thaliana* by acting as a negative regulator. These authors postulated that similar proteins can be identified in rice and other plants. Binding sites for progesterone in the cells were also studied in vernalized and non-vernalized winter wheat [6]. They were localized in both the cytoplasm and the cell membranes (microsomal fraction).

Externally administered progesterone stimulates the growth of stems of sunflower seedlings [7] and the generative development of wheat and *A. thaliana* [8,9]. It was also shown that progesterone acts on some of the processes relevant to the resistance to stress factors (salinity and bacterial infection), i.e. stimulation of the activity of antioxidant enzymes, reducing the permeability of cell membranes, improving the efficiency of the photosystem II [10–16].

Even in the 1970s, the studies with radiolabeled compounds showed that progesterone was synthesized in plants from sterols. When applied externally, it was metabolized and biotransformed, and formed a number of steroid derivatives in suspension cultures of *D. purpurea* and *Dioscorea deltoidea* Wall. ex Kunth [17–19].

As already mentioned, the content of progesterone in plants varies depending on the organ [3]. Nevertheless, there is a complete lack of studies on changes in the content of this compound during the growth and development of plants in response to stress factors. Changes in the levels of regulatory compounds (including hormones) are a component of the mechanism of the development of organisms and their response to the surrounding environment. Perception of the regulators in the cells seems to be equally important, which is associated with the presence and abundance of receptor structures, i.e. specific binding sites for ligands. The knowledge about the occurrence and action of the progesterone-binding proteins is chiefly acquired from the studies on *A. thaliana* [5]. This issue was also analyzed to a lesser extent in wheat plants [6]. Taking these facts into account, the current study aimed at obtaining answers to the following questions:

- (1) Is progesterone present in the plants of spring wheat, and in what form, free or bound in the form of conjugates?
- (2) Is the progesterone concentration in wheat plants dependent on the phase of development and does it change as a result of drought?
- (3) Where in the cell (cytosol, nucleus, membrane) are binding sites located for progesterone in spring wheat?
- (4) Is the concentration of cellular binding sites for progesterone dependent on the developmental stage and does it change under drought stress?
- (5) Are changes in the level of progesterone and in the concentration of its binding sites caused by drought stress different among tolerant and sensitive cultivars of spring wheat?

The analyses were performed on optimally-watered wheat plants and plants that were subjected to drought stress at the seedling stage and heading stage (stages of particular vulnerability of plants to drought in field cultivation). Genotypes of cultivars studied differed in the resistance to this stress factor. Drought is one of the most common agricultural problems in the world and the mechanisms of plant resistance to this stress have been extensively studied [20,21]. The participation of steroid compounds in

plant resistance to drought factors is known to a very limited extent, and this knowledge is mainly limited to brassinosteroids [22,23].

2. Materials and methods

2.1. Plant material

Katoda and Monsun wheat cultivars were used in the experiments. 10-day and 16-day-old seedlings of wheat cv. Katoda were used in the pilot studies. 10-day-old seedlings were grown in Petri dishes on filter paper, and were watered daily. 16-day-old seedlings were grown in pot cultures in soil. Seedlings were cultured in greenhouse (temp. 20 °C day/night, natural light condition in September: latitude: 50°03' North, longitude: 19°55' East).

In a further stage, new pot cultures were started for both cultivars Monsun and Katoda in an open vegetation hall during the natural growing season, i.e. from March to July (latitude: 50°03' North, longitude: 19°55' East) to obtain 21-day-old seedlings and fully-grown plants. Prior to the experiments, only general information was available about different drought resistance of the Monsun and Katoda cultivars. Therefore, measurements were carried out (photosystem II efficiency, CO₂ assimilation, chlorophyll content, enzyme activity accompanied by carbohydrate metabolism, content of carbohydrates, growth and yielding of plants) enabling a more accurate description of a response of both cultivars to water deficit occurring in the seedling phase and in the heading stage. The results of these assays create a background for studies on progesterone content and its putative receptors in optimally-watered and drought-stressed plants.

2.2. 21-Day-old wheat seedlings culture

Seeds were sown into pots (40 cm × 15 cm × 15 cm) in the amount of 100 seeds per pot, and then they were watered. The pots were split in two groups and starting from day 11 of the vegetation, only the first group was watered. This group was defined as optimally-watered plants. The second group of plants was grown in conditions of increasing water deficit. This group was defined as drought-stressed plants. The only watering applied in the latter group at that time was to balance the uneven amounts of decreasing water among the different pots. On day 15 of vegetation, plants in the Katoda cultivar wilted, but not the Monsun cultivar. The first symptoms of wilting in the Monsun cultivar were noted on day 16 of vegetation. On day 21 of vegetation, measurements of growing plants were carried out (the length of the aerial part of 10 randomly selected plants, PSII efficiency, net photosynthesis, chlorophyll content) and then the samples were collected (all the aerial parts of the plants) for analysis (activity of sucrose phosphate synthase, content of soluble carbohydrates, content of progesterone, presence of cellular binding sites for progesterone).

2.3. Obtaining wheat plants in the heading stage

The seeds were germinated in water (24 h, 24 °C, in darkness) in Petri dishes and then planted in pots (40 cm × 15 cm × 15 cm) with soil, 10 seedlings per pot. Plants were watered as needed until day 53 of vegetation. On day 54 of vegetation, plants were split in two groups and from that day on, only the control group, defined as optimally-watered plants, was watered. The development of the second group of plants (drought-stressed plants), including flag leaf formation and the time of heading, took place in conditions of increasing water deficit. The only watering applied at that time in this group was to balance the uneven amounts of decreasing water between different pots. On day 58 of vegetation, each pot was watered with 300 ml (deficit watering) to prevent premature and complete drying of plants, and then watering was ceased again.

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