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The influence of temperature on plant development in a vernalization-requiring winter wheat: A 2-DE based proteomic investigation

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ABSTRACT

In this work, proteomics was used to study the influence of both optimal and low temperatures on growth and development in a vernalization-requiring winter wheat (*Triticum aestivum* L. cv Cheyenne) after prolonged times of treatment. For this purpose, plants were grown at optimal temperature (20 °C) for 14 days (zero point) after which half were transferred to conditioned chambers kept at 4 °C for a period of 63 days. Cold tolerance, as estimated from lethal temperatures (LT_{50}), and phenological development, as measured by final leaf number (FLN) and shoot apex dissection, were determined. Proteomic analysis indicated a down-accumulation of several photosynthesis-related proteins and a concomitant increase in abundance of some Calvin cycle enzymes. A cold-induced accretion of soluble sugars and proline was observed as well. In parallel, an increase of proteolysis accomplished by an up-modulation of TCA cycle enzymes was also noticed, probably suggesting an efficient recycling of amino acids as energy source. Proteomic analysis of plants grown at optimal temperature allowed to specifically discriminate cold-induced proteins and highlight molecular processes driven by vernalization. Among identified proteins typically involved in vernalization responses and floral transition we observed a marked increase of wrab17, wcor18 and glycine-rich RNA-binding proteins.

1. Introduction

Although the influence of temperature varies during wheat developmental phases [1], its action remains crucial throughout the whole plant life cycle [2]. A temperature between 17 and 23 °C is generally recognized as the optimum range for wheat vegetative growth, whereas 0 and 37 °C are considered the minimum and maximum tolerable limits, respectively. On the other hand, the plant ability in adjusting to extreme temperatures has been widely recognized [3]. *Triticum aestivum* L.,

Abbreviations: CNN, Cheyenne winter wheat; COR, cold-related; FBA, fructose-1,6-bisphosphate aldolase; FLC, flowering locus C; FLN, final leaf number; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GR-RBP, glycine-rich RNA binding protein; LEA, late embryogenesis abundant; LT, low temperature; LT_{50}, lethal temperature at which 50% of plants died; OEE, oxygen evolving enhancer protein; PGK, phosphoglycerate kinase; PMSR, peptide methionine sulfoxide reductase; PPFD, photosynthetic photon flux density; PRK, phosphoribulokinase; RT, room temperature; RuBisCO, ribulose-1,5-bisphosphate carboxylase oxygenase.

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commonly termed bread wheat, is among the most resistant crops able to grow under severe and diverse environmental conditions [4]. This successful fitness is achieved through strict control of the flowering process, in other words getting the flowering time right is crucial to the plant survival [4]. Vernalization represents a perfect example of such a plant strategy. This term is used to define a period of exposure to low, non-freezing temperatures, which certain plants require in order to be able to flower in spring [5]. This fine mechanism implies plant maintenance in the vegetative stage during the cold season and the entrance in the more sensitive reproductive phase when both long day photoperiod and mild climate occur. Spring cereals do not have a vernalization requirement, normally developing rapidly into their reproductive phase when grown under long day photoperiod [6,7]. On the contrary, in winter wheat varieties, not only this process fortifies the plant permitting to survive winter, but is also an unavoidable step for the plant entrance in the reproductive phase. The chilling requirement is mandatory and programmed, thus is positively faced by these wheat varieties. At the molecular level, this is the result of a fine tuning of carbon fluxes which respond to temperature regulating their switching point in anabolic/catabolic direction (for reviews elucidating the molecular basis of vernalization see Refs [8,9]).

The requirement for vernalization is linked to a plant capacity to cold acclimate and develop freezing tolerance. A study on crop plants has revealed that low temperature (LT) tolerance usually depends on a complex network of inducible genes, which are fully expressed during the vegetative stage; in contrast, this control appears to be switched off during reproductive stage when the plant has limited ability to adapt to cold [10]. Genes involved in development, such as those involved in the photoperiod and vernalization processes, determine the duration of expression of LT-tolerance genes [6,11]. It has been postulated that developmental genes are mainly responsible for the duration of expression of LT-induced structural genes; in contrast, the rate of acquisition of LT tolerance is determined at the genetic level by differences in cold-hardiness potential [6,12]. Limin and Fowler, through studies on wheat (*T. aestivum* L.) and rye (*Secale cereale* L.) cultivars, reported a very close association between the point of vernalization saturation and a decline in cold tolerance [13]. Vernalization-requiring plants go through a process of reducing their final leaf number (FLN) up to the point of vernalization saturation [6]. After this point, cold tolerance is gradually reduced, as it has been observed in many studies on over wintering cereals [6,9,10].

Despite some apparent limitations when dealing with low abundance and membrane proteins, proteomics proved to be a powerful approach to identify plant response to stresses and a valid tool to discover key proteins for the biotechnological improvement of crop productivity [14]. In fact, the application of proteomics in crop breeding is usually initiated by detection of stress responsive proteins thought comparison between stressed and control plants, and the identification of these expressional candidate proteins may then reveal that some of them have functions clearly consistent with the stress tolerance trait [14]. However, in the field of cold stress, this approach has been applied so far to mainly study plant responsiveness to short-term cold treatments [15–21]. In contrast, only few proteomic studies focused on vernalization [22,23].

In our work, two temperature regimes (one favoring cold acclimation and the other letting plant to optimally growth), were chosen to identify proteomic changes in vernalization-treated plants when the key steps in controlling the flowering time occur. For this purpose, we used a winter-habit wheat cultivar (named Cheyenne) with a long vernalization requirement and excellent cold tolerance [12]. In our experimental set up plants were grown at optimal environmental conditions for 14 days, then transferred into conditioned chambers kept at 4 °C and 20 °C, respectively, both for a period of 63 days. The results indicated *sustained* high-level *expressions* of typically LT-associated polypeptides (such as wcor18 and wrab17 proteins) after vernalization fulfillment. This was confirmed at both protein and transcript level. Moreover, proteins so far associated to vernalization such as VER2 [24], were demonstrated to be also expressed during normal plant growth at optimal temperatures suggesting multiple roles in development and flowering processes. Furthermore, the most important metabolic pathways involved both in plant vegetative growth and vernalization, and their different tuning upon these two experimental conditions, are outlined.

2. Materials and methods

2.1. *Sample preparation*

A winter wheat cultivar (*Triticum aestivum* L. cv Cheyenne) was planted at the green house and cold room of Seed and Plant Improvement Institute (SPII) in Karaj (Iran). Seeds were placed on moist filter paper in Petri dishes and imbibed in the dark at 4 °C for 48 h and then germinated at a constant temperature of 20 °C in the dark for 24 h. Actively germinating seeds were grown at 20 °C under 12 h day lengths at a light intensity (mixture of fluorescent and incandescent lamps) of 350 μmol m^{-2} s^{-1}. After 14 days (experimental day 0), plants were subjected to 4 °C acclimation for 63 days (cold treatment) or they were maintained at 20 °C (vegetative growth). Different pots for each sampling date were grown side by side in the chamber in four replications. Each pot contained 15 plants. A random sample of young leaves (2–4 leaves above the crown on main stem samples) from plants in a single pot was therefore collected for subsequent analyses.

2.2. *Determination of cold tolerance and phenological development stage*

The procedure outlined by Limin and Fowler [25] was used to determine the LT_{50} of Cheyenne cultivar at the 0-d (14-d establishment with no exposure to cold), 63-d acclimation and vernalization treatment at 4 °C and 63-d at 20 °C (no exposure to cold). At the end of each treatment plant crowns were covered in moist sand in aluminum weighing cans and placed in a programmable freezer that was held at −3 °C for 12 h. After 12 h, they were cooled at a rate of 2 °C/h down to −17 °C, then cooled at 8 °C/h. Five crowns were removed at 4 °C intervals for each of eight test temperatures in each replications. Samples were then thawed overnight at 4 °C. Thawed