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Research article

Metabolic indicators of drought stress tolerance in wheat: Glutamine synthetase isoenzymes and Rubisco

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ABSTRACT

Drought stress has a considerable impact on the ecosystem and agriculture. Continuous water deficit induces early leaf senescence in plants. During this process, chloroplasts are degraded and photosynthesis drastically drops. The objective of this investigation was to look into the regulation of nitrogen and carbon metabolism during water deficit. Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase; EC 4.1.1.39) and the total protein contents inform us of the sink-source relation in plants. Glutamine synthetase (GS, EC 6.3.1.2) isoenzymes are good markers of plastid status (GS2) and the nitrogen metabolism (GS1).

Tolerant and sensitive wheat (*Triticum aestivum* L.) genotypes were tested, which are widely used in agriculture. The amount of protein, Rubisco and GS isoforms in leaves were measured during the grain filling period, as indicative traits that ultimately determine the onset and stage of senescence. The symptoms of senescence first appeared on the oldest and finally on the youngest leaves. Drought stress disrupted the sequentiality of senescence in the sensitive varieties. An untimely senescence appeared in flag leaves, earlier than in the older leaves. Total protein and Rubisco contents decreased and the GS2 isoenzyme declined considerably in the youngest leaves. In the tolerant varieties, however, these physiological parameters did not change under drought, only the sequential senescence of leaf levels accelerated in some cases compared to the control, well-watered plants. Our results revealed that GS is a good indicator of drought stress, which can be applied for the characterization of wheat cultivars in terms of drought stress tolerance.

1. Introduction

Drought stress can have a considerable impact on the ecosystem and agriculture. Improving yield under drought is a major goal of plant breeding. In many studies the identification of tolerant and susceptible cultivars is based on a few physiological measures related to drought response. Drought induced leaf senescence can inhibit photosynthesis and the supply of assimilates to grains [1]. Yield is the most important economic trait of wheat plants, and grain production is the main selection criteria for drought resistance. There are several physiological traits related to water stress, and scientists make considerable effort to find direct correlations between these parameters and grain yield in order to facilitate the screening and selection of cultivars for drought tolerance. But besides the sensitivity of the physiological parameters of vegetative organs, the sensitivity of generative organs should also be taken into consideration, because it is the responses of the whole plant that finally determines crop production. Despite its importance, very few studies observed the changes in different physiological parameters in different organs with the aim to provide a more complete and accurate explanation of the responses of the plants [2].

Water stress during the grain filling period reduces photosynthesis, induces early senescence and shortens the grain filling period [3]. Some plants show a sequential type of leaf senescence. During growth of the whole plant, new young leaves are successively formed at the top, while lower and older leaves develop gradually toward the phase of senescence. In the course of this process, leaves from the base to the top pass different developmental stages, from maturation up to the last phase of senescence including cell death [4]. In monocarpic plants/cereals, developing grains represent the most important sink for carbon and nitrogen and other nutrients after anthesis [5], and the onset of grain

Abbreviations: DPA, day post anthesis; FW, fresh weight; GMH, γ-glutamyl monohydroxamate; GS, glutamine synthetase; PVDF, polyvinylidene difluoride; RWC, relative water content; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase.

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development is the most important factor initiating the process of senescence [6]. The role of N in agricultural production is intimately connected with photosynthesis. N determines the synthesis of amino acids thus proteins (for example Rubisco) and, ultimately, of all cellular components [7].

Leaf senescence is the final stage of leaf development. Biochemical, physiological, morphological and ultrastructural analyzes indicate that enhanced degradation of chloroplast components leading to a reduction in photosynthetic capacity is an integral part of the senescence process in green tissue. The amount and activity of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) decreases during senescence [8]. Chloroplasts are one of the earliest sites of catabolism in leaf senescence and mitochondria appear to remain intact until a very late stage of senescence [9]. Senescence is regarded as a nitrogen mobilization process, since glutamine contents increase in leaves by late senescence, while total amino acid content is slightly reduced. Ammonium content was also reduced by leaf senescence, similarly to cotyledon senescence [10].

Early studies have showed that glutamine synthetase (GS, EC 6.3.1.2) is widely distributed in the plant and occurs in two major forms, one in the chloroplast (GS2) and one in the cytosol (GS1). GS plays a central role in nitrogen metabolism [11,12] and there are multiple regulatory controls at the gene and protein level to modify its activity. The expression of GS1 is enhanced in later stages of flag leaf development, which may facilitate the recovery of N during senescence and also signal to the plant that the conditions are right for successful seed filling and maturation [13]. During the vegetative stage, GS2 is the pre-dominant isoenzyme in the leaf mesophyll cells, where it assimilates ammonia originating from nitrate reduction and photorespiration. GS1 is responsible for the generation of glutamine in the remobilization of nitrogen via the phloem. Because of the severe reduction in biomass production during the vegetative stage, there is a shortage of nitrogen and carbon assimilates in senescing source organs, which causes a limitation of remobilization into sink organs. Thus, severe reduction in grain filling could occur in the knockout mutants. Immunolocalisation studies in rice have shown that cytosolic GS has multiple metabolic functions such as assimilating ammonia into glutamine for transport and distribution throughout the plant [14]. Anthesis triggers the start of global changes in wheat leaf metabolism characterized by the co-ordinated and gradual decline of RNA, soluble proteins, chlorophyll, Rubisco subunits and GS2. There is a correlation between the amount of leaf chloroplastic GS and Rubisco protein during senescence, which confirms their co-ordinated regulation during grain development and filling in wheat. Studies on natural and induced senescence in leaves have identified a co-ordinated sequence of biochemical and structural events in chloroplast degradation as well [15].

Wheat (*Triticum aestivum* L.) is one of the main crops consumed by humans and it is cultivated in different environments. In this study we observed four tolerant (MV Emese, Plainsman V, Kharchia, Kobomugi) and two sensitive (GK Élet, Cappelle Desprez) genotypes, which are widely used in agriculture. We examined protein, Rubisco and GS amounts and activities in leaves during the grain filling period in search for the traits that ultimately determine the sequential senescence and the stay-green stage.

2. Results

2.1. Changes in total protein content under drought stress

The results of the determination of total protein contents are presented in Figs. 1 and 2. The senescence process results in a decrease in total protein content in the aging leaves.

The total protein content of the leaves, measured at the 9th DPA, decreased with the age of leaves in well-watered plants. The lower leaves of the sensitive genotype contained more protein than the leaves in the same position of the tolerant one. The same tendency was observed in the protein content of the two upper (younger) leaves, (W(−1) and W(flag)) (Fig. 2) of the other genotypes. In these samples the protein content of the older leaf (W(−1)) was also less than the younger flag leaf.

Drought stress changed the protein content gradient in the sensitive or less tolerant cultivars: protein content was lower in the flag leaf than in the older (S(−1)) leaf. However, the protein contents measured in leaves of the tolerant genotype (Plainsman V) followed the same tendency as well-watered plants, only the slope of the curve was slightly steeper. Other wheat cultivars showed similar results (Fig. 2): in tolerant ones the protein contents were lower in the older leaves, while in the sensitive breeds the flag leaf had less protein than the older leaf tissues.

The values of total protein contents were lower in the two landrace breeds (Kharchia and Kobomugi) relative to the fresh weight than in the other genotypes.

2.2. Rubisco content

The proteins of leaf samples were separated with non-denaturing polyacrylamide gel electrophoresis. After Coomassie staining Rubisco appeared as the largest, dominant band as visible in Figs. 3 and 4. The changes in Rubisco content were in accordance with the changes in total protein content. In the older leaves of well-watered plants the Rubisco content decreased with age in all genotypes (Fig. 4). Under drought stress the Rubisco content of the flag leaves of sensitive breeds was lower than that of the older leaves. In the tolerant genotypes high amounts of Rubisco were found in the flag leaf and less in the older leaves.

2.3. GS activity

Total glutamine synthetase (GS) activity was determined from the leaf samples of well-watered and drought stressed wheat cultivars. The results of these colorimetric assays are presented in Figs. 1 and 2. The enzyme activities were calculated on fresh weight basis. Calculation based on protein content would be misleading in stressed samples because of the dramatic changes in the amount of the major protein, Rubisco, which may give the 50% of the total protein content of leaf cells. The senescence process resulted in decreased enzyme activity in the leaves.

The activity of GS isoenzymes is an appropriate parameter to reveal the degradation and finally the collapse of the assimilation processes in the leaves during senescence. The changes in total GS activity were similar to the changes in total protein content. In well-watered plants the enzyme activities in the older leaves of plants was lower than in the younger flag leaf in the sensitive and tolerant breeds as well (Fig. 1).

Fig. 1 also shows the responses of GS to the drought stress. In the sensitive genotype, the GS activity declined considerably, in fact it was even lower than the values of the first (−1) and second (−2) older leaf. However, in the tolerant genotype, the GS activity in the flag leaf (S(flag)) remained the highest under the drought stress too. In different wheat cultivars the results showed the same tendency (Fig. 2): in tolerant genotypes the GS activities were lower in the older leaves, but in the flag leaf (S(flag)) of the sensitive breeds the GS activity was less than the leaf below it (S(−1)). The values of enzymes activities were extremely low in Kobomugi.