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Effect of Water Deficit on Protein Reserves in Cotyledons of Germinating Cotton Seeds

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ABSTRACT: Germinating cotton seedlings were subjected to desiccation and the effect of low water stress on the protein mobilization in cotyledons was observed by SDS-PAGE on the first, third, fifth, and seventh day after the start of treatment. Qualitative as well as quantitative differences were observed between the control and treated samples from the third day onwards, and these differences became more obvious with the passage of time. The effect of desiccation on protein mobilization in germinating seeds is discussed.

Abbreviations: HSPs-Heat Shock Proteins, SDS-Sodium Dodesyl Sulfate, PAGE- PolyAcrylamide Gel Electrophoresis.

KEYWORDS: *Gossypium hirsutum*, cotyledon proteins, SDS-PAGE, seed desiccation.

Germinating seeds and seedlings are sometimes subjected to environmental stresses like moisture deficit, high temperatures and salinity. These stresses are especially frequent in areas with hot arid climates. After imbibition of seeds, such stresses cause poor seedling development and ultimately reduced crop yield. Burke [1] demonstrated that water stress reduced leaf area, plant size and dry matter accumulation in cotton plants.

Reduced germination has been reported in lentil seeds when exposed to high temperatures, salinity or water stress[2]. Salinity reduces radical and root elongation and mobilization of reserves from the cotyledons of mung bean[3]. Water deficit resulted in an increase in the rate of seed dry mass depletion and seedling dry matter accumulation, and an increase in the soluble sugar content, during germination of wheat seeds[4]. Heat shock proteins are reported to be developmentally regulated during seed formation[5], and in maturing seeds heat shock proteins are expressed in the absence of heat stress[6]. Some of these heat shock proteins respond to elevated temperatures and appear during the onset of desiccation, then disappear several days after germination[6]. Heat shock proteins are also produced in response to low temperatures[7] salinity and drought [9]. This study examines the impact of water deficit on protein depletion and/or production in cotyledons of germinating cotton seeds.

Plant material (*Gossypium hirsutum* L.) comprised a drought tolerant line NIAB-111, and sensitive lines NIAB-200, NIAB-218 and NIAB-999. Uniform size seeds were germinated in the dark on water saturated filter papers at 28°C for 24 hours. Half of the seeds were then transferred to Petri dishes lined with filter papers soaked in a 10% solution of polyethylene glycol (PEG 4000), while the other half was kept on water soaked filter papers as controls. Both treated and control seeds were then kept in the dark at 28°C for another 8 days. Samples (three cotyledons each) were taken on the first, third, fifth, and seventh day after the start of treatment.

Proteins were extracted by grinding cotyledons in the Tris-Cl sample buffer of Laemmli[10] containing 2% SDS, 5% 2-mercaptoethanol, 10% sucrose, and 0.0001% bromophenol blue. Samples were ground in a mortar and pestle, in the presence of sample buffer, to a fine slurry and then put in a boiling water bath for 5 minutes. After centrifugation at 14,000 rpm for 5 minutes, 200 µg of protein from each sample was loaded on to the gels. For electrophoretic separation of proteins, 10% acrylamide gels, with 1 mm thickness, were used with the dissociating discontinuous buffer system of Laemmli[10]. The protein molecular weight marker was from MBI Fermentas (#SM0431).

Figs 1 to 3 show the protein profiles of control and treated plants at the first, third, and seventh day after the start of treatment, respectively. All the studied lines show similar protein banding patterns on the first day after the start of treatment. The cotyledon protein patterns, therefore, cannot be used for the identification of different lines used in this study. Each protein pattern can be divided into high molecular weight (30 to 60 kDa) and low molecular weight (14 to 25 kDa) zones, each of which can further be

distinguished into two darkly stained groups of two to three bands. Two very intense bands at about 50 kDa form the first group (group I), two bands at about 30 kDa the second (Group II), three bands between 18.5-20 kDa the third (Group III), and three bands at about 14 kDa comprise the fourth group (Group IV). The densly stained bands at about 55 kDa and 14 kDa represent the large and small subunits of Rubisco, respectively, while bands in second group at about 28 kDa represent the light harvesting complex (LHC). At the first day (Fig.1), all these groups showed the same pattern in all the control and treated cotyledons. On the third day after treatment, quantitative and/or qualitative differences can be observed between the control and treated cotyledons of all the lines (Fig. 2). In both control and treated cotyledons of line NIAB-111, there was only one band at 50 kDa but the intensity of the band was much less in control plants. Similarly, the number and intensity of bands were also less in group II, III and IV proteins of control samples compared to the treated ones. For NIAB-200, NIAB-218 and NIAB-999, although the number of bands was same for control and treated samples, in group I and II proteins, the intensity of the bands was lower in control samples. In groups III and IV, qualitative differences were also observed for NIAB-200 and NIAB-218, with only two low intensity bands in each group, but NIAB-999 showed only minor quantitative differences. It is clear that on the third day, the high, as well as low molecular weight proteins, were comparatively less or completely absent in control samples. On the seventh day after the start of treatment (Fig. 3), the effect was very obvious and qualitative as well as quantitative differences were observed in all the lines and in all groups of proteins. There was only one major band in control samples of all the lines at 50 kDa (group I) while all the treated samples, except NIAB-111, showed two bands. In group II proteins, only quantitative differences were observed between treated and control samples, and again the difference was more obvious in NIAB-111. For group III and IV proteins three strong bands were observed in treated samples of NIAB-200, NIAB-218 and NIAB-999, but these band were very faint and less in number in control samples of these lines. Group III bands were completely absent in control profiles of NIAB-111, while faint bands were observed in treated samples. NIAB-111 therefore, showed lesser differences between the treated and control samples. There was only one band of almost similar intensity for group I proteins. Group II bands disappeared completely in control, while there was only one band in treated samples. For groups III and IV, there were very faint bands in both treated and control samples. It is evident from these studies that the mobilization of enzymes of photosynthesis and

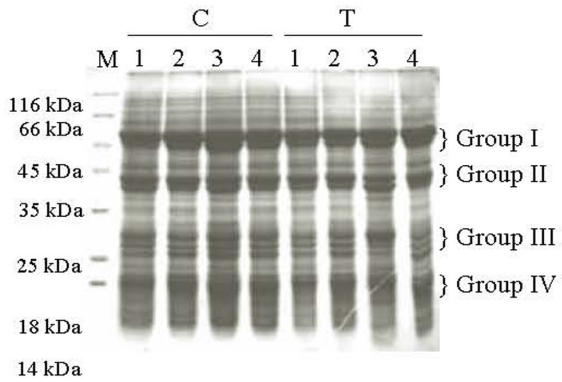

Fig 1. Cotyledon protein profiles of treated (T) and control (C) seeds on the first day after the start of water-deficit treatment. Lane 1 is N-111, lane 2 is N-200, lane 3 is N-999, and lane 4 is N-218. M denotes the protein molecular weight marker.

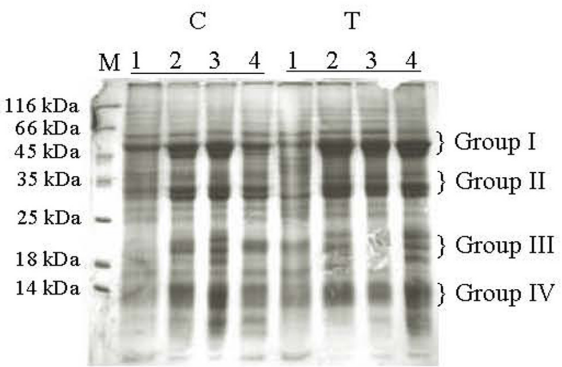

Fig 2. Cotyledon protein profiles of treated (T) and control (C) seeds on the third day after the start of water-deficit treatment. Lane 1 is N111, lane 2 is N-200, lane 3 is N-999, and lane 4 is N-218. M denotes the protein molecular weight marker.

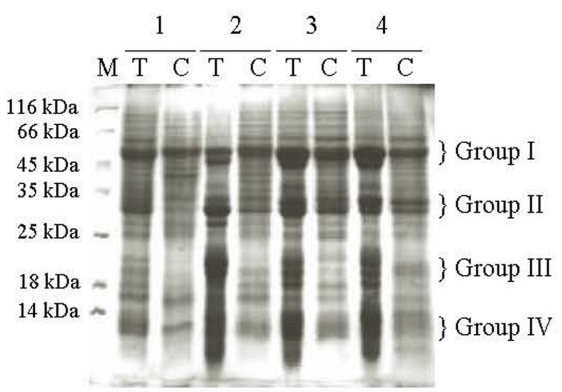

Fig 3. Cotyledon protein profiles of treated (T) and control (C) seeds on the seventh day after the start of water-deficit treatment. Sample 1 is N-111, sample 2 is N-200, sample 3 is N-999, and sample 4 is N-218. M denotes the protein molecular weight marker.

other reserves, including the Rubisco and LHC were greatly effected by water stress.

No new bands were observed in the treated samples although accumulation of HSPs has been reported in response to desiccation [11,12]. A slow rate of mobilization of proteins from cotyledons was apparent, indicating that water deficit affects the metabolism of stored reserves and their mobilization to developing seedlings. The slower rate of hypocotyle growth in treated seedlings is attributed to this observation. The hypocotyle length of stressed seedlings was less than half the length of control seedlings, except NIAB-111, which showed only about one third reduction in length (data not presented). These observations are in accord with Miazek [3] who reported inhibition in the rate of seed dry matter depletion and seedling dry matter accumulation in wheat seedlings when exposed to dehydration. Stresses have been reported to inhibit or reduce enzyme activities and protein reserve degradation and affect protein synthesis in stressed seeds and seedlings [13]. This study suggests that after imbibition the seeds started the normal germination process, which remained unaffected until 24 hours after the induction of desiccation. However, from the third day onwards the normal depletion of protein reserves was affected and became increasingly limiting until the seventh day, where seedling growth was drastically slowed. NIAB-111, a drought tolerant variety, showed less effect in protein depletion from the cotyledons, and the rate of hypocotyl growth was also higher compared to other varieties. Although no HSPs were observed in any of the studied varieties, including the resistant variety (NIAB-111), we have demonstrated that drought tolerant and susceptible varieties can be distinguished based on the mobilization of protein reserves between 3 to 5 days after germination in water-stressed conditions.

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