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ORIGINAL PAPER

Drought stress effects on Rubisco in wheat: changes in the Rubisco large subunit

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Abstract The leaf protein pattern from drought-tolerant and drought-sensitive wheat varieties subjected to severe soil drought but with the possibility for recover from stress was studied by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). The spots representing Rubisco large subunit (RLS) were identified using polyclonal antibodies against Rubisco and immunoblotting. Some qualitative and quantitative differences in the 2D-PAGE protein map of wheat varieties were revealed under drought conditions. Three days recovery of wheat plants were not enough for restoring RLS quantity to the level of controls after 7 days drought, especially in the drought-sensitive variety Miziya. There are contradictory data in the literature concerning increased or diminished RLS level in drought stressed plants. A comparison of RLS after SDS-PAGE and 2D-PAGE was made. The revealed protein pattern depended on the presence or absence of protease inhibitors in the extraction buffer, on the procedure of extraction, and on the degree of stress.

Keywords 2D-PAGE · Drought stress · *Triticum aestivum* L. · Rubisco LS · SDS-PAGE

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Abbreviations

BSA	Bovine serum albumin
2D-PAGE	2-Dimensional polyacrylamide gel electrophoresis
DTT	1,4-Dithio-DL-threitol
DW	Dry weight
EDTA	Ethylenediaminetetraacetic acid
EL	Electrolyte leakage
FW	Fresh weight
IEF	Isoelectric focusing
2-ME	β-Mercapthoethanol
MM	Molecular mass
PMSF	Phenylmethanesulfonyl fluoride
Rubisco	Ribulose-1,5-bisphosphate carboxylase/oxygenase
RLS	Rubisco large subunit
RSS	Rubisco small subunit
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SL	Shoot length
TCA	Trichloracetic acid
TW	Turgor weight
WD	Water deficit

Introduction

Drought affects morphology, growth, metabolism of plants and limits grain yield in most regions of the world. The susceptibility of plants to drought varies in dependence of stress degree, different accompanying stress factors, plant species, and their developmental stages. Plant responses to drought stress are very complex and include adaptive changes or deleterious effects (Chaves et al. 2002). In spite

of the extended researches on drought, the primary effects of this stress at the cellular, biochemical, and molecular level are still not well understood (Reddy et al. 2004; Bartels and Sunkar 2005; Jaleel et al. 2009).

It is obvious that drought induces some metabolic changes related to protein turnover increasing protein synthesis, aggregation, denaturation, or degradation. An interesting example is the most abundant protein on the Earth—Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)—the key enzyme in CO_2 fixation and oxygenation, which is composed of eight large (RLS) and eight small subunits (RSS) with MM 55–56 and 14–16 kDa, respectively (Ellis 1979). The regulation of Rubisco activity and quantity remains very complex and not yet elucidated (Houtz and Portis 2003; Feller et al. 2008). Under stress conditions, it becomes evident that Rubisco undergoes down-regulation of protein synthesis or degradation (Feller et al. 2008). Its quantity could be restored after recovery of normal conditions. Surprisingly, some fragments derived from RLS were detected by 2-DE analysis under control conditions (Salekdeh et al. 2002a, b; Zhao et al. 2005) and it was explained that some fragmentations occur in vivo as a result of hydroxyl radical production or may occur in vitro during protein solubilization (Salekdeh et al. 2002a, b).

The biggest band after SDS-PAGE of leaf extracts from C_3 plants is RLS and it is not a problem to detect it (Feller et al. 2008). The observations on the effect of different stresses especially drought on RLS level are contradictory in the literature. Pääkkönen et al. (1998) detected an increase in Rubisco content under drought stress in birch (*Betula pendula* Roth.). According to Pelloux et al. (2001), 34 days water stress did not induce any reduction in total Rubisco activity or RLS protein quantity in *Pinus halepensis* M. performing 12.5% SDS-PAGE. On the contrary, Inmaculada et al. (2006) using 2D-PAGE indicated a decrease in RLS after 7 days drought conditions of *Quercus ilex* L. leaves. The question about the mechanism of Rubisco dynamics—its induction or degradation in plants subjected to stress conditions—does not have enough clear responses up to now.

To date it is unarguable that 2D-PAGE gives the possibility for a large scale of investigations with its ability of standardized and reproducible procedures (Thiellement et al. 1999) as well as provides extensive information about polypeptide patterns and their dynamic under different environmental conditions. That is why 2D-PAGE has become an essential field of research in plant biology where the combined approaches provide essential tools to understand the mechanisms underlying plant growth and development. Recently, 2D-PAGE was successfully used to analyze drought-affected plants (Salekdeh et al. 2002a, b).

This study is an attempt to determine RLS level after water deprivation in different wheat varieties, to relate RLS quantity to drought sensitivity and tolerance and to understand better the reason for the discrepancy in the literature regarding changes in Rubisco level under stress conditions. Wheat varieties with different drought resistance were subjected to severe drought stress. The degree of water deprivation stress was determined by analyzing changes in growth parameters of wheat plants, the water deficit and electrolyte leakage. A combination of 12% SDS-PAGE, 2D-PAGE and immunoblotting identification of RLS was used.

Materials and methods

Plant material, growth conditions, and treatment

Four Bulgarian varieties of winter wheat (*Triticum aestivum* L.) with different field drought resistance were used (Katya and Sadovo from South Bulgaria, Zlatitza and Miziya from North Bulgaria). Variety Katya was recognized as drought-tolerant (Kalapos et al. 1996). Variety Sadovo was known as a disease-resistant one. In our preliminary experiments, this variety was more sensitive to drought than Katya (Simova-Stoilova et al. 2006). Variety Zlatitza and Miziya in field conditions showed a drought sensitivity index estimated according to Fisher and Maurer (1978) of 0.657 and 1.282 (unpublished data). Thus, Zlatitza is drought-tolerant variety and Mizyia is drought-sensitive variety.

Plants were grown in pots with a 400 g of leached meadow cinnamonic soil (pH 6.2) optimally fertilized with N, P and K, under day/night temperatures of 25/21°C, 150 μmol m^{-2} s^{-1} photosynthetically active radiation, 16-h photoperiod and 70% relative soil humidity. The four varieties were sown in sections in the same pot (16 plants per pot) in order to obtain uniform growth and stress conditions. For drought experiments, water was withheld at day 8 after sowing for a period of 7 days, followed by 3 days recovery by maintaining optimal water supply. The control plants were watered daily. Leaf samples, collected from the fully developed first leaf of control and stressed plants, were immediately immersed and stored in liquid nitrogen until analyses.

Growth parameters, water deficit, and electrolyte leakage

As common growth parameters shoot length (SL), their fresh weight (FW) and dry weight (DW) were determined measuring 18 individual plants per variety and treatment. Water deficit (WD) of the first leaf was calculated

according to the formula (TW − FW)/TW in percentages (TW, turgor weight). Membrane integrity was estimated as relative electrolyte leakage (EL) from 2 cm leaf segments floating in distilled water for 24 h at 4°C. The initial conductivity of the effusate and the conductivity after boiling the segments for 10 min were measured and the EL was calculated as % of initial to final conductivity.

Electrophoretic analyses

The soluble leaf proteins for SDS-PAGE were extracted in ice cold 100 mM Tris–HCl buffer, pH 8.0 containing 20 mM $MgCl_2$, 10 mM $NaHCO_3$, 1 mM ethylenediaminetetraacetic acid disodium salt (EDTA), 2 mM phenylmethanesulfonyl fluoride (PMSF), 12.5% glycerol (v/v), 20 mM β-mercapthoethanol (2-ME) and 2% Polyclar. In some analyses, the protease inhibitors PMSF and EDTA were not included in the extraction buffer. The proteins were separated by 12% SDS-PAGE in a *Mini Protean II Dual Slab Cell* (Bio-Rad) according to Laemmli (1970). Samples with protein quantity equivalent to the same FW were loaded for all variants. The gels were stained with Coomassie brilliant blue R-250. Protein markers (Dalton Mark VII-L Standard Mix 14–66 kDa) from Sigma were used.

For isoelectric focusing (IEF) and 2D-PAGE leaf sample was ground in liquid nitrogen and was suspended in an ice cold 10% (w/v) trichloracetic acid (TCA) in acetone containing 0.07% 2-ME. The proteins were precipitated at −20°C for 1 h. After centrifugation at 15000*g* for 15 min, the pellets were washed twice with 0.07% of 2-ME in acetone for 1 h at −20°C for 1 h followed again by centrifugation. The pellets were dried for an hour in thermostat at 37°C and were solubilized in sample buffer containing 20 mM Tris–HCl (pH 8.8), 8 M urea, 4% CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate) 30 mM DTT, 1 mM PMSF by sonification on water bath for 15 min (Zorb et al. 2004). The pH gradient on the gel was created by adding Pharmalyte ampholyte solution (Sigma) with pH range 3–11 to the IEF gel rehydration buffer (10% sorbitol, 8 M urea in distillated water). On the strip protein equivalent to the 12.5 mg FW was loaded. The strips were equilibrated before the vertical electrophoresis in 12% SDS-PAGE in equilibration buffer with 50 mM Tris–HCl (pH 8.8), 6 M urea, 30% glycerol, 2% SDS, 0.001% bromphenol blue, with addition of 1% DTT (1,4-dithio-DL-threitol) for 15 min at room temperature followed by the incubation in the equilibration solution containing 4% iodoacetamide instead of DTT. The first dimension by IEF was run onto 3.5% PAAG and the second dimension 12% SDS-PAGE. The gels with protein spots were visualized by kit for silver staining (Amersham). Molecular weight markers (Bio-Rad) with molecular mass (MM) range 207–7.1 kDa were used. Melanie software was used for the 2D-PAGE image analysis of revealed protein spots.

Immunoblot analysis

For immunoblot analysis, the extracted proteins were separated by 12% SDS-PAGE and then transferred into nitrocellulose membrane (Bio-Rad) as described by Mitsuhashi and Feller (1992) using Trans Blot system (Bio-Rad). Prestained SDS Molecular Weight Standard Mixture 31.2–174.6 kDa (Sigma) was used. After electrophoresis the membrane was blocked in TTBS buffer (0.1 M Tris pH 7.9, 0.15 M NaCl, 0.1% Tween 20) containing 1% BSA for 60 min. RLS was identified using rabbit polyclonal antibodies against the corresponding proteins (Demirevska-Kepova and Simova 1989). Second antibody, goat-anti-rabbit-IgG for bridging and peroxidase–anti-peroxidase soluble complex was used additionally. The peroxidase reaction was developed with 4-chloro-alpha-naphthol (Sigma).

The content of total soluble proteins was measured by the method of Bradford (1976) at 595 nm with bovine serum albumin as a standard.

Data processing and statistical analysis

Results were based on at least three replicates from two independent experiments. Values were analyzed by ANOVA at the level of significance in the difference between controls and treatments and among varieties: $P < 0.01$.

The revealed bands after SDS-PAGE and RLS spots after 2D-PAGE or immunoblotting analyses were scanned. Electrophoresis and immunoblotting analyses were repeated three times and the obtained results were similar. A representative picture of the results is given. The stained RLS bands or spots were scanned and processed using ImageJ 1.30v software (National Institutes of Health, NIH, Maryland, USA).

Results

In the previously established experimental design, a gradual diminution of relative soil humidity (from 70% of the maximal soil capacity) was observed after withholding irrigation to reach 50–55% at the seventh day of drought (Simova-Stoilova et al. 2006). Varieties developed up to 60% WD for 7 days of water deprivation, without significant differences among them (Table 1). In the result of drought treatment, growth inhibition of SL and shoot FW approximately 33 and 74%, respectively, was observed for all varieties. Seven days drought had less effect on shoot

Table 1 Shoot length (SL), shoot fresh weight (FW), shoot dry weight (DW), leaf water deficit (WD) and electrolyte leakage (EL) in control plants, drought-treated plants for 7 days, recovered plants and age control of recovery for wheat varieties: Katya, Sadovo, Zlatitza and Miziya

Variety	Treatment	Shoot L (cm)	Shoot FW (mg)	Shoot DW (mg)	WD (%)	EL (%)
Zlatitza	Control	26.03 ± 1.47	260.8 ± 32.02	25.99 ± 3.52	5.45 ± 0.64	5.36 ± 0.28
	Drought	18.03 ± 0.43	80.0 ± 12.55	21.31 ± 2.47	59.65 ± 1.96	17.10 ± 1.57
	Recov. Control	27.88 ± 3.35	421.0 ± 40.15	48.51 ± 1.78	8.88 ± 2.47	5.03 ± 0.19
	Recovery	22.63 ± 2.28	258.4 ± 12.5	33.61 ± 2.85	6.95 ± 0.98	8.18 ± 1.29
Miziya	Control	24.33 ± 1.88	291.2 ± 74.41	31.74 ± 0.7	5.49 ± 0.38	5.21 ± 0.41
	Drought	18.70 ± 0.22	60.0 ± 11.02	22.84 ± 2.77	57.14 ± 1.70	18.32 ± 2.88
	Recov. Control	28.75 ± 1.57	385.2 ± 60.44	51.09 ± 5.01	10.05 ± 0.76	3.61 ± 0.69
	Recovery	24.10 ± 0.73	234.2 ± 32.25	32.14 ± 3.53	8.03 ± 0.25	11.84 ± 0.74
Katya	Control	28.48 ± 2.04	272.6 ± 63.6	23.22 ± 4.35	6.81 ± 0.74	4.27 ± 0.06
	Drought	16.58 ± 1.39	74.6 ± 18.98	17.96 ± 1.51	61.6 ± 0.91	24.61 ± 2.03
	Recov. Control	26.70 ± 0.97	311.2 ± 43.49	33.92 ± 5.24	7.59 ± 0.54	4.57 ± 0.46
	Recovery	23.53 ± 2.34	196.40 ± 33.87	32.05 ± 8.17	7.02 ± 2.25	5.81 ± 0.68
Sadovo	Control	33.23 ± 3.04	327.2 ± 43.89	36.4 ± 0.92	5.66 ± 1.13	3.93 ± 0.79
	Drought	20.30 ± 0.84	76.6 ± 10.5	24.75 ± 4.03	60.95 ± 0.71	23.98 ± 3.33
	Recov. Control	32.67 ± 1.73	491.0 ± 49.84	58.43 ± 2.02	5.92 ± 1.17	4.25 ± 0.43
	Recovery	29.03 ± 4.71	243.2 ± 35.47	33.21 ± 1.4	7.86 ± 2.01	7.11 ± 1.40

Values are mean of at least three replicates. Standard deviations are shown by ± . Significant differences between controls and drought stressed or recovered after stress plants are indicated by asterisks

DW with approximately 33%. If compared changes in DW of different varieties, Miziya showed 11% less and Zlatitza showed 6% more shoot DW.

At day 7 of drought, when plants were under severe WD, the EL sharply increased because of membrane instability. The membrane injury seemed to be reversible after 3 days recovery from the 7-day water deprivation, because it was restored nearly to the level of the control plants, which means that the severe water stress used in our experiments is not lethal. When the stress was prolonged by 1 day, the recovery of plants was not possible (data not shown). Thus, in our study, reversible drought stress conditions were used.

After 2D-PAGE, average 150–200 protein spots were detected covering the p*I* range 3–11 and MM range 7.1–207 kDa (Fig. 1). The image maps of variety Katya and Zlatitza (drought-tolerant varieties) were similar as well as the image maps of varieties Sadovo and Mizyia (drought-susceptible varieties). RLS was significantly altered under stress conditions (Fig. 2a). The results are clearer after using Image J analysis of the spots (see Fig. 2b). The big spot of RLS is composed of one main spot and approximately one to three small spots to the two p*I* directions (one to three more alkaline spots, $pI > 7$ and one more acidic bigger spot, $pI < 7$). In all varieties, the content of RLS with different p*I* values decreased to 50–75% in drought stressed leaves and some of them recovered up to the control, except drought-sensitive variety Mizyia. Some very small spots with different MM below 55 kDa were revealed with anti-Rubisco antibodies on the nitrocellulose sheet but their intensity was quite small (because of low concentration) and they quickly disappeared (data not shown). In the region of 20 kDa, an increase of protein content was observed in all varieties subjected to drought stress, except variety Mizyia (Fig. 1). A very clear difference of RLS with different p*I* values between susceptible and tolerant varieties was at hand.

RLS spots were identified by immunoblotting using polyclonal antibodies against barley Rubisco (Fig. 3). The image of RLS was obtained near the p*I* range 7 as 2 main spots with MM 53–55 kDa (one more acidic bigger spot and one more alkaline smaller spot). Some aggregated forms of RLS were observed with MM 110 kDa. Immunoblots of RLS and their Image J plots from control plants of different wheat varieties used are presented on Fig. 4a and b. We did not receive any immunologically detectable RLS fragments after 2-DE. We tried to detect C- and N-terminus of RLS after 2-DE using specific polyclonal antibodies, but we did not receive visible spots contrary to our previous results after one-dimensional SDS-PAGE (Demirevska et al. 2008) which is with different protocol of protein extraction and sample preparation. Probably the antibodies against C- and N-termini of RLS failed to detect Rubisco LS fragmentation in 2-DE. However, such fragments have been detected using MS analysis in rice after drought and salt stress (see Salekdeh et al. 2002a, b).

We performed immunoblotting analysis with the most sensitive variety Miziya to show changes of RLS spots and

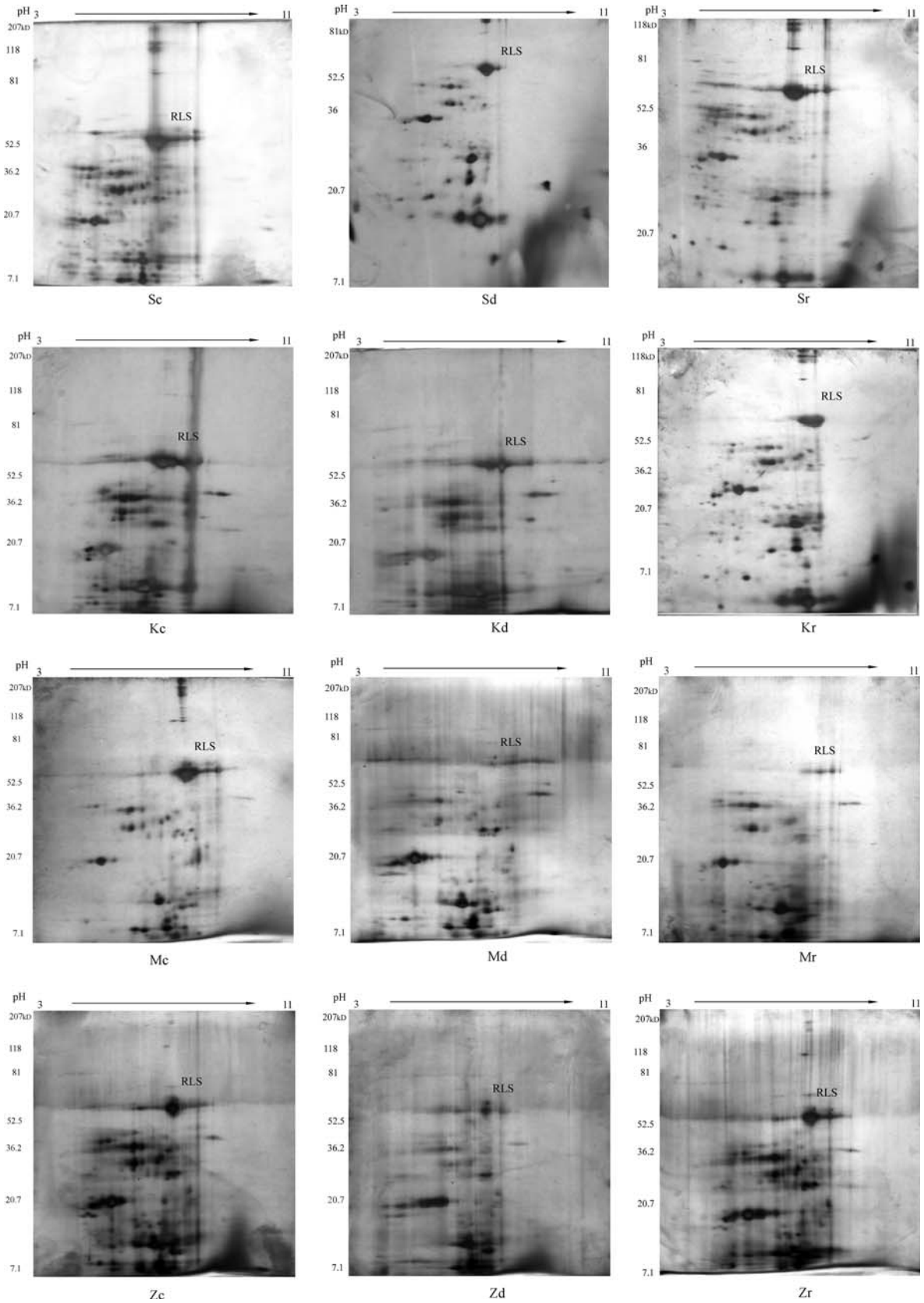

Fig. 1 2D-PAGE analysis of extracts from wheat varieties—Katya (*K*), Sadovo (*S*), Zlatitza (*Z*) and Miziya (*M*) in control conditions (*c*), drought (*d*), recovery after drought (*r*). The position of MM standards is indicated on the *left*. The position of RLS is shown on the figure

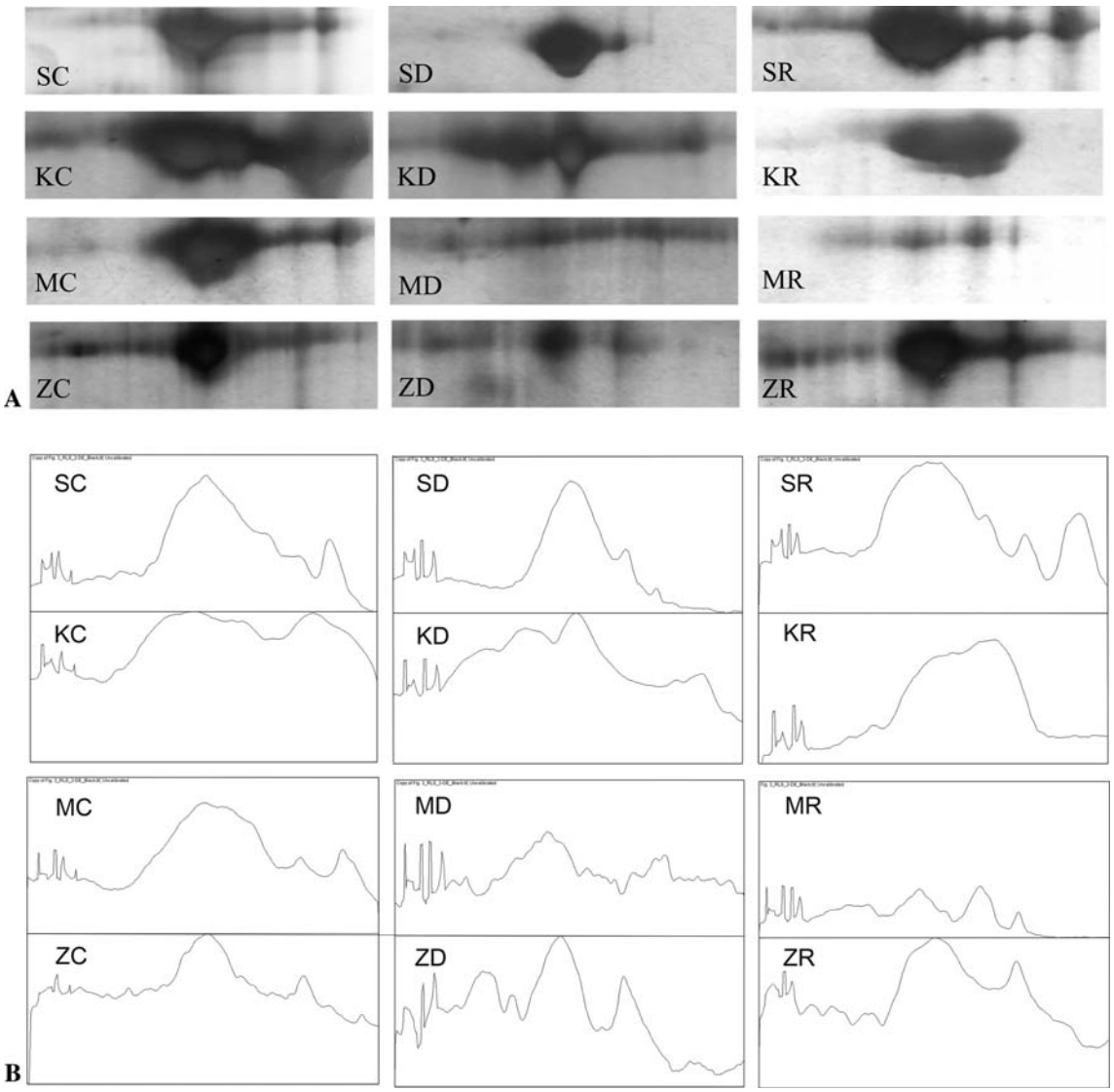

Fig. 2 RLS, revealed after 2D-PAGE of leaf extracts from wheat varieties – Katya (*K*), Sadovo (*S*), Zlatitza (*Z*) and Mizya (*M*) in control conditions (*C*), drought (*D*), recovery after drought (*R*) (**a**) and its plots using Image J analysis (**b**)

their Image J plots after 2-DE provoked by drought stress and its recovery (Fig. 5a, b). The characteristics of RLS spots after drought and recovery were similar after silver staining of 2-DE gels (Fig. 2a, b) comparing with colored immunoblots. Changes of RLS spots in the drought sensitive wheat variety Miziya under drought and recovery are probably connected with more strong stress response.

In our previous study (Demirevska et al. 2008), it was found that under drought conditions, RLS detected as a band with MM around 53–55 kDa, was enhanced when the extracts for SDS-PAGE were loaded on FW basis. RLS showed about 30% increase of the band intensity for all varieties, especially for the drought-tolerant varieties Katya and Zlatitza. The result could be due to the water loss (60% WD) in leaves subjected to drought causing an increase per FW unit. However, there was no increase in the RLS quantity at all when the samples were loaded on a protein basis (Fig. 6a). It is necessary to point out that these two types of experiments were performed with an extraction procedure accompanied with protease inhibitors—2 mM PMSF as a serine-protease inhibitor and 1 mM EDTA (disodium salt) as a metallo-protease inhibitor. But if the soluble protein extraction is prolonged in time with incubation of extracts in the refrigerator for 30–60 min (time dependant modification of Rubisco structure could be induced) or with an absence of protease inhibitors in extraction buffer, the polypeptide picture after SDS-PAGE is different (Fig. 6b). In this case, the severe stress provoked a decrease in RLS quantity for the more susceptible variety Sadovo in comparison with the drought-tolerant

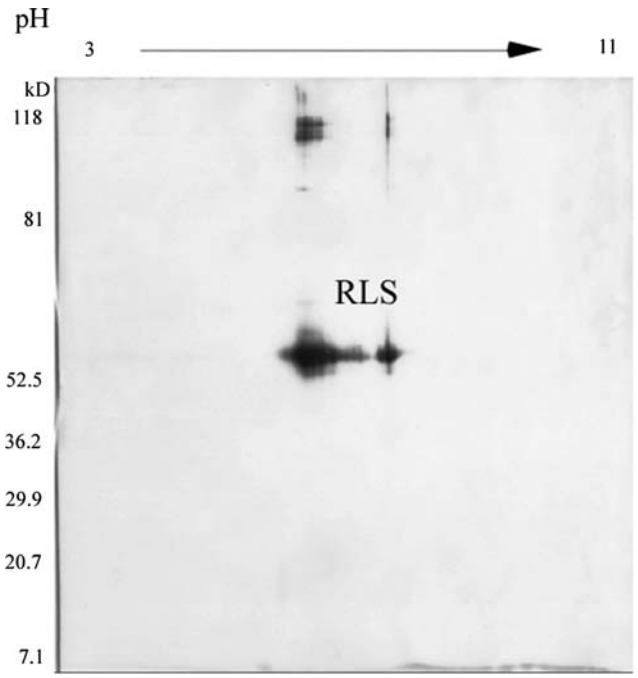

Fig. 3 Immunoblotting after 2D-PAGE of extracts from wheat variety Sadovo, in control conditions, using polyclonal antibodies against RLS. The position of MM standards is indicated on the *left*. The position of RLS is shown on the figure

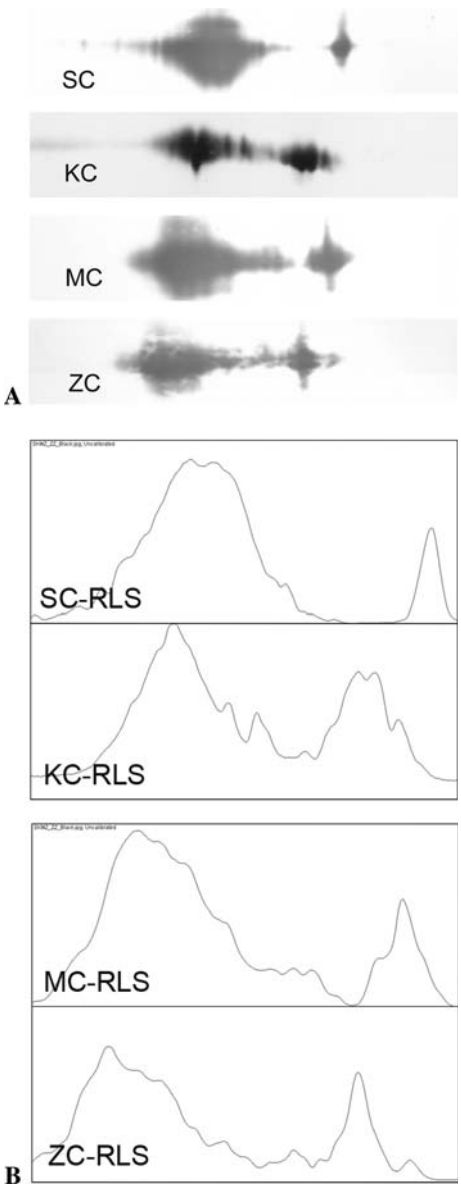

Fig. 4 RLS revealed after immunoblotting of leaf extracts from wheat varieties—Katya (*K*), Sadovo (*S*), Zlatitza (*Z*) and Mizya (*M*) in control conditions (*C*) subjected to 2D-PAGE and using polyclonal antibodies against RLS (**a**) and its plots using Image J analysis (**b**)

variety Katya. In these experiments, the changes in the protein pattern for varieties Katya and Sadovo were compared in dynamics—on the 1st, 3rd, 5th and 7th day of drought treatment. The splitting of the RLS band into two components was evident on the seventh day of drought for variety Katya and earlier on the fifth day of drought for the more sensitive variety Sadovo. The differences in RLS between susceptible and tolerant to drought were more obvious when the extraction procedure gave possibility for proteolytic degradation of some proteins.

Discussion

Water deficit is a very hard stress on plant metabolism, affecting the growth parameters, membrane integrity, total protein quantity, and quality. It was established that water stress decreases the level of some plant proteins, maintains others, and induces the synthesis of some specific ones (Salekdeh et al. 2002a, b). The choice of sample preparation for protein extraction greatly influences the electrophoretic protein separation and the results after immunoblotting in terms of their quality, quantity, and protein species distribution (Islam et al. 2004). Thus, it could be supposed that our opposite results concerning the level of RLS received by SDS-PAGE and 2D-PAGE mostly depend on the extraction procedure and the choice of electrophoretic analysis. Our results also confirm the statement that one of the criteria to evaluate leaf protein extraction is RLS (Wang et al. 2003).

After extraction of soluble proteins with a non-denaturing Tris-buffer in the presence of protease inhibitors, SDS-PAGE and immunoblotting, an increase of RLS at the seventh day of severe but reversible drought was received. Analyses of Riccardi et al. (2004) showed that some of the observed increases of protein relative quantity might be an indirect consequence of the reduction of leaf growth rather than a direct response to drought. Pelloux et al. (2001) also

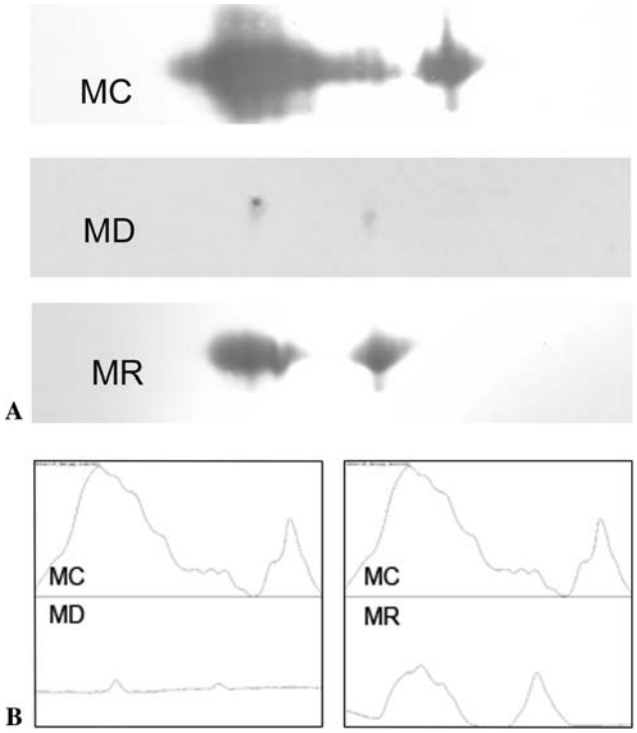

Fig. 5 RLS revealed after immunoblotting of leaf extracts from wheat variety Miziya in control conditions (*C*), drought (*D*), recovery after drought (*R*) subjected to 2D-PAGE and using polyclonal antibodies against RLS (**a**) and its plots using Image J analysis (**b**)

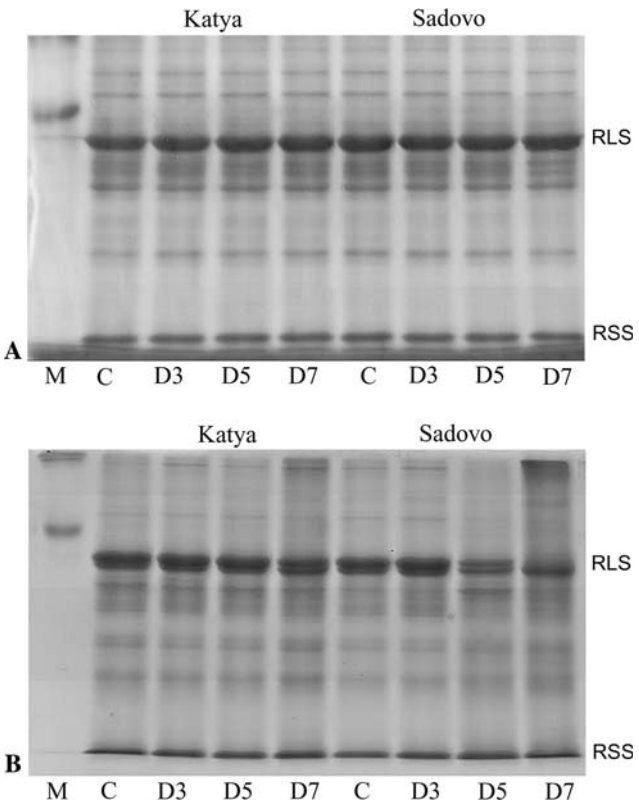

Fig. 6 Protein pattern after SDS-PAGE of leaf extracts from wheat varieties—Katya and Sadovo in drought conditions during 1, 3, 5 and 7 days (D1, D3, D5 and D7). *C* control plants, **a** the extraction buffer with protease inhibitors and sample loading in a protein basis; **b** the extraction buffer without protease inhibitors and sample loading in a FW basis. *M* BSA as a marker. The position of RLS and RSS is indicated on the *right*

noticed increased protein quantities of RLS in *Pinus halepensis* M. subjected to drought stress.

At the opposite of these results and their interpretation, when using extraction Tris-buffer without protease inhibitors, a converse picture to the previous one was received, more probably due to the proteolytic degradation of RLS and its partial level diminution.

2D-PAGE analysis is a powerful tool for separating complex protein mixtures and has been employed to analyze protein alterations in response to the environmental changes. Using wide range of gradients and extended separation distances, the 2D-PAGE gives a chance for optimal resolution and detection of minor components. Our results prove the possibility to easily reveal differences in protein overview between varieties using 2-DE (samples were loaded also in a FW basis). Surprisingly, RLS has another revealing under stress conditions—it diminishes its level in contrary to SDS-PAGE results. There are different possible reasons. First, after TCA/acetone precipitation, a direct precipitation of total proteins occurs (Granier 1988). It is possible some of the proteins to be discarded as an insoluble aggregates. The osmotic stress under water deficit is expected to lead to increased protein aggregation and denaturation (Zang and Komatsu 2007). Finally, the quantity of total soluble proteins could diminish. Probably the sensitive varieties are more vulnerable to protein aggregation under stress. If aggregates were discarded after TCA/acetone precipitation and centrifugation, the real picture of polypeptide composition would change. In sample preparation for SDS-PAGE, more mild conditions were used. Second, the 2D-PAGE avoids accumulation of multiple proteins with similar MM in a spot. It is known that Rubisco could exist as a multifunctional complex (Demirevska-Kepova et al. 1999). Third, the procedures of drying protein sediments at 37°C or the sonification for the full solubilization of samples could also be a reason for receiving some differences in the results. Fourth, the loss of RLS was regarded as a consequence of an oxidative damage and possible degradation (Desimone et al. 1996; Yoshida and Minamikawa 1996; Anderson and Aro 1997) but it is not yet clear whether Rubisco is degraded into oligopeptides and/or amino acids within the chloroplasts (Chiba et al. 2003).

Besides the observation of a big spot of RLS and some other small spots in the range of the same MM, some small degraded fragments with different MM were detected

additionally by immunoblotting before subjecting the plants to stress conditions both on SDS-PAGE (Demirevska et al. 2008) and on 2D-PAGE (data not shown). It was established that Rubisco assembly state is exerted by RLS feedback regulation in higher plants and it could be involved in the chloroplast response to metabolic, environmental, and developmental signals (Wostrikoff and Stern 2007). RLS synthesis is subject to assembly state-dependent regulation. In some cases, RLS synthesis could be subjected to repression under oxidative stress (Cohen et al. 2005, 2006).

Ishida et al. (1997) suggested a proteinase independent mechanism in which RLS could be directly degraded by active oxygen probably by the hydroxyl radical generated in the wheat chloroplasts. Evidence is presented that ozone may induce oxidative modification of Rubisco leading to subsequent proteolysis (Pell et al. 1993). The severe drought is also connected to oxidative stress (Reddy et al. 2004). It could be considered that under some physiological conditions, a fragmentation of the RLS occurs and increases under drought stress. According to Zhao et al. (2005), Rubisco fragments display different expression pattern during growth. Their conclusion is based upon protein identification by mass spectrometry measurements.

It is not enough elucidated how Rubisco synthesis, content, and stability are affected by different environmental stresses including drought. Thus, the choice of methods for studying Rubisco protein induction or degradation under stressed conditions depends on the main objective of the experimental work. If there is a need for clear revealing of variability between different species or varieties, the 2D-PAGE using protease inhibitory cocktail is the best choice (Damerval et al. 2005). After 2D-PAGE the RLS from drought-sensitive variety Miziya was affected in the highest degree compared to the other varieties. It becomes evident that for other purposes, it is necessary to compare several experimental procedures, subsequent results and then to make conclusions. Drought causes a decrease or absence of some proteins in more cases than an increase or a new appearance. A potential identification of these proteins and their response to different kinds of stress awaits further investigations.

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