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Drought-induced enhancement of protease activity during monocarpic senescence in wheat

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The effect of water stress on flag leaf senescence and protease activity during grain development was examined in a wheat variety. Total chlorophyll content and soluble protein content were used as markers for monocarpic senescence. Endopeptidase activities and exopeptidase activities were assayed using ribulose-1,5-bisphosphate carboxylase (RuBisCo) as a physiological substrate at acidic (pH 4.8), neutral (pH 7.0) and alkaline (pH 8.5) values at three stages during grain-filling period. Water stress enhanced the rate of monocarpic senescence and concomitantly increased the endopeptidase and exopeptidase activities at all pHs tested in the leaves. The above observations showed that different proteolytic enzymes may come into play under water stress which are independent of the reproductive sink effect.

MONOCARPIC senescence is a genetically-programmed decline in physiological functions during which there is a process of protein turnover with the greater effect on protein degradation rather than protein synthesis¹, thereby leading to death of the plant following a single reproductive phase². There have been a large number of studies concerning the role of proteases during monocarpic senescence^{3,4}. Leaf senescence, however, is subjected to regulation by many environmental factors such as drought, besides autonomous factors such as reproductive development⁵. There are some reports on the individual effects of drought on proteolytic activities^{1,6,7}. However, there have been very few studies on the combined effect of drought and grain development on the proteases activity. In the present study, the effect of drought and monocarpic senescence on proteolytic activities in the flag leaf of wheat has been examined using RuBisCo as the physiological substrate at acidic, neutral and alkaline pH to see if there is any specific increase or a general enhancement of all proteases.

For the present study *Triticum aestivum* var. HD 2329 was field grown in the loamy soils of Water Technology Centre, IARI during rabi season (November–April 1996–97). The crop was sown on 15 November 1996. Cultivation and water stress treatment were carried out as described earlier⁸. Total rainfall was 5.62 cm during the crop season. Total water availability in well watered and stressed region were 43.92 cm and 28.92 cm, respectively. Flag leaf was sampled for water relations and biochemical analyses at anthesis, 15 days after anthesis

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(DAA), and 20 DAA, respectively. Three replicates were used for all the measurements. Leaves were cut into small pieces after measuring their fresh weight. All the operations for biochemical analyses were carried out at 4°C, unless otherwise stated.

Water potential ψ of the fully-expanded flag leaf was measured using the pressure chamber (Model 3005, Soil Moisture Equipment Corporation, USA)⁹. All determinations of water potential were carried out between 1000 and 1100 h. Relative water content (RWC) was measured¹⁰ and chlorophyll was extracted in 80% (V/V) acetone, and the content determined¹¹. For total soluble proteins (TSP), the leaves were ground to a fine powder (3 ml/0.5 g fresh weight)¹², and the content determined¹³. Protease activity was measured following the modified method⁴. Different assay buffers with varying pH were used¹⁴. Endopeptidases were measured as TCA-soluble peptides generated during the reaction at A_{340} of the supernatants. Exopeptidases were measured as TCA-soluble amino nitrogen in the supernatant by the ninhydrin procedure¹⁵. Glycine was used as the standard.

The wheat crop received only 0.8 cm of rainfall before flowering, while it received 3.7 cm of rainfall near

maturity. The irrigated, and water-stressed treatments were begun in the vegetative stage with the application of the first-line source irrigation at 39 DAS. The plants under water stress treatment did not receive any irrigated water and, hence, continued growth only on the stored soil moisture. Anthesis was observed 106 DAS and 103 DAS in irrigated and water-stressed plants respectively. At anthesis, the irrigated plants were taller, had more leaf area and flowered later than the water-stressed plants.

Flag leaf water potential was lower by 0.27 MPa and 0.45 MPa in the water-stressed plants compared to the irrigated plants during grain development (Table 1). The RWC of the flag leaf in water-stressed plants at anthesis was lower and exhibited a faster decline during grain development than the irrigated plants. Flag leaf senescence exhibited a faster rate in the water-stressed leaves compared to the irrigated plants and exhibited maximum loss of both the total chlorophyll and soluble protein content.

Protein hydrolysis, *in vitro* can be carried out by a wide range of peptide hydrolase enzymes¹⁶, but, to date, there is no consensus as to which particular enzyme or

Table 1. Effect of water stress on water potential (ψ), relative water content (RWC), chlorophyll content and total soluble proteins (TSP) during grain development expressed as mean \pm SE ($n=3$) in flag leaf of wheat

	Irrigated			Water-stress		
	A	A + 15	A + 20	A	A + 15	A + 20
Leaf ψ (MPa)	-2.12 \pm 0.18	-1.71 \pm 0.01	-2.21 \pm 0.07	-2.37 \pm 0.13	-2.15 \pm 0.07	2.51 \pm 0.1
RWC (%)	80.77 \pm 3.39	71.8 \pm 1.56	75.17 \pm 2.51	74.8 \pm 0.92	63.93 \pm 1.6	52.59 \pm 4.91
Chlorophyll (mg gDW ⁻¹)	10.21 \pm 0.02	10.4 \pm 0.27	6.29 \pm 0.09	7.45 \pm 0.04	8.22 \pm 0.02	1.98 \pm 0.02
TSP (mg gDW ⁻¹)	81.37 \pm 0.43	55.49 \pm 0.14	41.05 \pm 0.17	60.92 \pm 0.07	53.6 \pm 0.03	6.06 \pm 0.21

A, anthesis; A + 15, 15 days after anthesis; A + 20, 20 days after anthesis.

Table 2. Effect of water stress on endopeptidase activity (activity-mg protein⁻¹) in flag leaf of wheat during grain development expressed as mean \pm SE ($n=3$)

pH	Irrigated			Water-stress		
	A	A + 15	A + 20	A	A + 15	A + 20
4.8	14.04 \pm 0.07	39.03 \pm 0.23	29.59 \pm 0.14	22.17 \pm 0.14	132.74 \pm 0.09	608.52 \pm 2.62
7.0	12.34 \pm 0.02	39.26 \pm 0.18	46.81 \pm 0.25	24.55 \pm 0.03	47.33 \pm 0.09	388.2 \pm 5.25
8.5	17.26 \pm 0.06	60.7 \pm 0.45	39.73 \pm 0.28	22.31 \pm 0.08	68.61 \pm 0.09	710.82 \pm 1.75

A, anthesis; A + 15, 15 days after anthesis; A + 20, 20 days after anthesis.

Table 3. Effect of water stress on exopeptidase activity (μ mol-aminoN-mg protein⁻¹) in flag leaf of wheat during grain development expressed as mean \pm SE ($n=3$)

pH	Irrigated			Water-stress		
	A	A + 15	A + 20	A	A + 15	A + 20
4.8	1.51 \pm 0.01	2.67 \pm 0.02	3.58 \pm 0.02	1.6 \pm 0.03	5.69 \pm 0.04	32.48 \pm 0.22
7.0	1.27 \pm 0.01	4.06 \pm 0.03	3.85 \pm 0.01	2.14 \pm 0.03	5.83 \pm 0.04	43.5 \pm 0.87
8.5	1.21 \pm 0.02	3.31 \pm 0.02	3.3 \pm 0.07	1.76 \pm 0.01	6.46 \pm 0.05	46.73 \pm 0.22

A, anthesis; A + 15, 15 days after anthesis; A + 20, 20 days after anthesis.

even class of enzymes plays a predominant role. In our results, both endo- and exopeptidase activities increased during grain development in the irrigated plants at all pHs examined (Tables 2 and 3). The endopeptidase activities were maximum at alkaline pH and exopeptidase activities maximum at neutral pH. It has been reported in the literature that during monocarpic senescence, RuBisCo is degraded and nitrogen is mobilized to the developing grains^{3,4}. The breakdown of RuBisCo has usually been studied with a pH optimum of 4 to 5 (refs 17–19), while a pH optimum of 6.5 has also been reported²⁰. The highest rates of degradation have been reported at pH 8.0 for RuBisCo and at pH 6.0 for small subunit (SSU) alone²¹. Differences in the proteolytic properties of SSU may be due to different conformations of the substrate proteins and the true pH of the enzyme can thus be concealed. It has been observed that the highest cleavage of the large subunit of endogenous RuBisCo is at pH 4.0 but maximal degradation of external [¹⁴C]-methylated RuBisCo is observed at pH 9.0 (ref. 22). In oat leaf segments, different degrees of degradation and differences in degradation products were demonstrated for both subunits of RuBisCo at pH 4.5, 7.0 and 8.0 and it was suggested that proteases have different pH optima in senescent leaves²³.

Water deficit increased both endo- and exo-proteolytic activities (endopeptidases and exopeptidases) almost ten fold (Tables 2 and 3). In water-stressed plants, the acidic endoprotease activity is maximum till 15 DAA followed by an increase at alkaline pH at 20 DAA, whereas protease activities were more or less same at all pH values tested with an increase at alkaline pH being slightly more than at neutral pH at 20 DAA (Table 3). Water deficit induced an increase in proteolytic activities which could be responsible for the decrease in leaf protein content (Table 1) (ref. 7). Proteolytic activities were assayed using azocasein and water deficit increased the activities at different pH values – 6.0, 8.5, 9.0 and 10 in *Vigna* and *Phaseolus* cultivars⁶.

In conclusion, it can be said that drought-induced enhancement of monocarpic senescence in flag leaf of wheat during grain development was concomitant with the enhancement of both exopeptidase activity and endopeptidase activity against RuBisCo. Both the endopeptidase activity and exopeptidase activity was enhanced at acidic, neutral and alkaline pH.

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Identification of blocks in chlorophyll biosynthetic pathway in chlorophyll-deficient mutants of *Pennisetum glaucum* (L.) R. Br.

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Blocks in chlorophyll biosynthetic pathway among different chlorophyll-deficient phenotypes of *Pennisetum glaucum* (L.) R. Br. were investigated employing 77 K fluorescence spectroscopy. Green seedlings from yellow/white stripe mutants and an *albino* mutant showed emission maxima at 657, 685 and 733 nm corresponding to protochlorophyllide (pchlide), photosystem II and photosystem I respectively. Yellow seedlings showed peaks at 657 nm and 678 nm corresponding to pchlide and chlorophyllide (chl) respectively, indicating a block in the reduction of chl, whereas the white seedlings and *albino* accumulated pchlide, suggesting an impairment in the photo-reduction of pchlide. Of the four loci identified²⁰, *vi₃* and *vi₄* blocks the conversion of pchlide to chl, whereas the conversion of chl to chlorophyll is under the control of *vi₁* and *vi₂* loci.

STEPS in chlorophyll biosynthesis are subject to modulation by both light and cell type^{1,2}. Information on

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