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Effect of water deficit on proline accumulation, protein and chlorophyll content during flowering and seed formation in winter rape ⟨*Brassica napus* L. var. *oleifera*⟩

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Abstract

Water deficit affecting winter rape plants during flowering and seed formation caused metabolic responses characteristic for drought. Proline accumulation took place in the leaves, the inflorescences and in the siliques. Protein content during flowering and seed formation was reduced in all rape organs except leaves in the latter stage. The decrease of chlorophyll content in the leaves was greater during the period of seed formation than during flowering.

INTRODUCTION

The water content in plants undergoes dynamic changes in dependence on the water conditions in the soil, atmosphere and on the developmental stage of the plant ⟨H s i a o, 1973⟩. A frequently used measure of water content in plants is the relative water content and the percentage of water in fresh weight ⟨B o t h a and B o t h a, 1979; F u k u t o k u and Y a m a d a, 1981; W e a t h e r l e y, 1950⟩.

Differential resistance to drought is an important determinat of distribution of plants and productivity of crop plants. This property may be conditioned by the existence of regulatory mechanisms producing adaptive modifications in metabolism ⟨H a n s o n and H i t z, 1982⟩. Parameters of metabolic changes easy to determine may serve as indices of hydration of the crop or for selection of resistant plants ⟨B a t e s et al., 1973⟩. Proline accumulation and a depressed protein content are frequently considered as adaptive reactions of plants to drought which can be detected much earlier than external symptoms ⟨B o t h a and B o t h a, 1979; A s p i n a l l and P a l e g, 1981⟩.

The influence of osmotic stress on proline accumulation and protein content in winter rape plants have been the object of earlier investigations ⟨R o g o-

ziński and Flasiński, 1983). The present paper is their continuation and concerns the effect of soil drought on proline accumulation, protein and chlorophyll content in the critical periods of development of rape plants.

MATERIAL AND METHODS

Winter rape plants (*Brassica napus* L. var. *oleifera* cv. 'Skrzeszowicki') were brought from an experimental plot (April 15, 1982) for pot culture. Three plants were set in each Wagner pot filled with cultivable soil and placed in the vegetation house. Water was supplemented every day to 60 per cent of the soil full water capacity (FWC). Water stress was provoked in the course of two development stages of rape by discontinuing water supply in the pots for 8, 6, 4 and 2 days. Thereafter material was taken for analysis (May 13, 1982 – flowering stage, June 7, 1982 – seed forming stage), and in the particular combinations the percentage of the full water capacity of the soil was determined. The control consisted of plants not subjected to stress. The experiments are shown schematically in Figure 1. For analyses leaves of the 5th pair were taken and segments of shoots between these leaves, flowers from the central part of the inflorescences, siliques, unripe seeds from the central part of infructescences and roots.

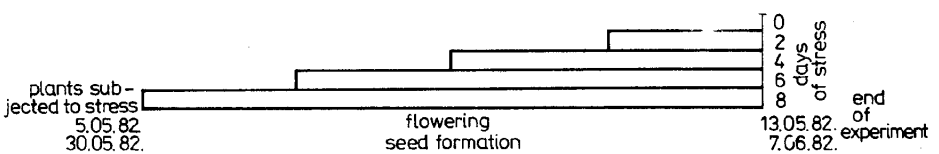

Fig. 1. Scheme of stress induction in winter rape plants beeing in the stage of flowering and seed formation

Proline content in leaves, inflorescences and siliques was determined by the method of Bates (Bates et al., 1973). Total soluble protein was extracted by the method given by Botha and Botha (1980). Tissue samples were homogenized in 50 mM Tris-HCl buffer, pH 7.5 containing 1 mM EDTA. After centrifugation at 20 000 g, the sediment was once more extracted with buffer containing additionally 0.1 per cent Triton X-100. The protein contained in the supernatants referred to as total soluble protein was determined by Lowry's method (Lowry et al., 1951), using bovine serum albumin as the standard. Chlorophyll from leaves and siliques was extracted with 80 per cent acetone and determined by Arnon's method (Arnon, 1949) by measuring absorption at 645 and 663 nm.

Relative water content in leaves ⟨RWC⟩ was determined by the method of W e a t h e r l e y ⟨1950⟩ and calculated from the following equation:

$$RWC = [\langle FWt - DWt\rangle : \langle TM - DWt\rangle]\ 100,$$

where FWt – fresh weight, DWt – dry weight, TM – matter in state of full turgor ⟨after 24 h under conditions of full saturation of atmosphere with water vapour⟩.

All analyses were performed in three replications and are given as arithmetic means. For proline the statistically significant differences and for protein and chlorophyll standard deviations are given.

RESULTS

Rape plants examined in the flowering or seed formation stage are susceptible to a reduced water content in the soil ⟨Table 1⟩. Visible symptoms of water deficit appeared as early as after two days and became more pronounced with prolonged stress. The plants lost their turgor and shed older leaves. Especially in the period of seed formation the plants reacted very strongly.

Table 1

Changes in full water capacity of the soil during flowering and seed formation of winter rape due to the days of stress

Days of stress	Full water capacity of the soil (%)	
	flowering	seed formation
0	60.0	60.0
2	31.5	23.0
4	29.0	19.8
6	22.4	18.3
8	20.4	15.4

As seen from the data in Table 2, the relative water content in leaves in the period of rape flowering was depressed as the water content in the soil decreased. In plants subjected to 8-day stress RWC was 41.6 per cent. After resumed hydration most leaf blades recovered their turgor. In the period of seed formation the considerable decrease in the full water capacity of the soil on the second day of stress was accompanied by a marked decrease of RWC. Prolonged stress caused gradual dying of the leaves. This was also due to the higher temperature and lower relative moisture in this period as compared with the period of flowering.

Table 2

Effect of soil drought on RWC of leaves and water content in different organs of winter rape

Days of stress	Flowering					Seed formation					
	% of water					% of water					
	RWC %	leaves	stems	roots	inflor-escences	RWC %	leaves	stems	roots	siliques	seeds
0	78.7	86.0	84.7	82.3	83.6	85.6	88.5	75.2	68.6	79.6	84.4
2	65.6	85.2	84.0	80.6	81.7	46.6	72.5	69.5	58.8	75.3	83.2
4	65.5	84.0	83.7	79.5	81.4	34.9	50.0	60.4	58.4	71.1	81.8
6	62.0	82.8	82.4	75.0	79.9	–	29.8	60.9	47.9	70.9	81.8
8	41.6	76.7	79.3	71.9	78.3	–	13.6	58.2	39.6	67.7	81.0

The percentual water content in the particular rape organs is shown in Table 2. Leaves and roots lost larger water quantities, especially in the stage of seed formation, whereas the difference was smaller in the shoots, inflorescences and siliques, and the decrease in seeds was slight.

Changes in water content in the plants and soil were associated with certain metabolic reactions characteristic for drought. One of these reactions is the accumulation of proline by plants (H s i a o, 1973; S i n g h et al., 1973a, b), and it was also noted in rape. Under normal water conditions the proline level in rape leaves was low, both during flowering (0.57 mg/g DWt) and in the period of seed formation (1.0 mg/g DWt). A much higher level was noted in inflorescences and siliques (mean 6.6 mg/g DWt). Under conditions of drought in the period of flowering, accumulation of proline in leaves and inflorescences took place (Fig. 2). This accumulation rapidly increased when the water content in the soil fell to about 29-22 per cent FWC. In plants subjected for 8 days to water stress the proline level in the leaves (with RWC equal to 41.6%) increased by about 45 times, and in the inflorescences by about 7 times.

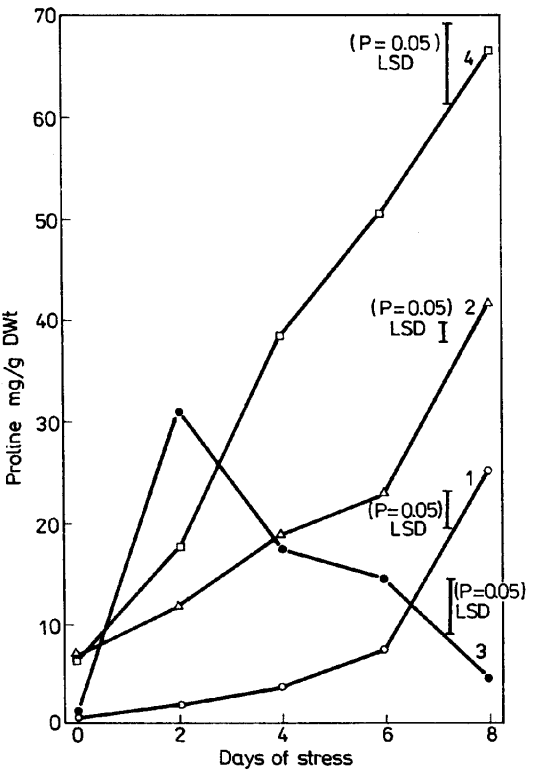

Fig. 2. Effect of water deficit on proline accumulation in the leaves, inflorescences and siliques of winter rape during flowering and seed formation. Flowering: 1 – leaves, 2 – inflorescences; seed formation: 3 – leaves, 4 – siliques

In the period of seed formation accumulation of proline in leaves also occurred very rapidly. As the result of strong dehydration of the leaves the proline level rose as early as the second day of stress by about 30 times. This may have been also caused by inhibition of nitrogen translocation to other plant organs. As the stress was prolonged, the proline content in the leaves decreased, however. This occurred at a soil water content lower than 20 per cent FWC and a decrease of water content in the leaves to about 50 per cent of fresh weight. Such drastic stresses occur, however, but seldom in field conditions. Proline accumulation in the siliques was proportional to the duration of drought. In plants subjected to 8-day stress, when the water content in the siliques diminished to about 12 per cent, the level of this amino acid increased about 10 times.

Water stress also caused a depression of the soluble protein content ⟨Table 3⟩. In the period of rape flowering, beginning with the 6th day of stress, there occurred, after the initial increase, a slight fall of the protein level in the leaves. In the shoots and roots, characterized by a much lower protein level, water stress also reduced their content amounting on the 8th day of stress to about 33 and 37 per cent, respectively. This decrease in protein content occurred in the inflorescences after 4 days, and in plants subjected to 6- and 8-day stress it amounted to about 20 per cent.

In the period of seed formation wide variations were noted in protein content in the leaves of plants subjected to water deficit. There occurred in the shoots and roots under the influence of stress a decrease in protein content to a level lower in this period than at the time of flowering. A marked depression of the protein level in conditions of water deficit appeared in seeds and, symilarly as in the inflorescences, amounted to about 20 per cent. In the siliques the protein content was rather constant and was not affected by stress.

Water deficit also caused a characteristic decrease of the chlorophyll content in leaves in both the developmental stage of rape ⟨Table 4⟩. The chlorophyll level diminished singnificantly in the period of flowering in plants subjected to stress for 4 days. In leaves under 8-day stress in which the relative water content decreased about two times the chlorophyll content reached 57 per cent of that in control leaves.

The decrease in chlorophyll content under the influence of drought in the period of seed formation was more pronounced than during flowering. After as short a time as two days of stress the chlorophyll content in the leaves diminished by about 2.6 times. RWC in these leaves was close to that in leaves of plants under 8-day stress in the period of flowering. The leaves of plants subjected to 6- and 8-day stress exhibited complete chlorosis and chlorophyll could be revealed in them. Drought caused in the siliques a depression of the chlorophyll value only in the end period of stress.

The here described metabolic responses in rape plants strictly depended upon the water content of the soil. They were noticeable as early as in the second day of stress. With prolongation of the latter these modifications were aggravated.

Table 3

Effect of water deficit on soluble protein content in winter rape organs

Days of stress	Flowering				Seed formation				
	protein (% DWt)				protein (% DWt)				
	leaves	stems	roots	inflor-escences	leaves	stems	roots	siliques	seeds
0	31.9 ± 0.9	6.1 ± 0.4	5.5 ± 0.6	24.1 ± 3.5	28.1 ± 1.3	4.3 ± 0.5	2.4 ± 0.2	6.6 ± 0.1	15.2 ± 0.3
2	35.4 ± 0.5	6.6 ± 0.2	5.2 ± 0.5	22.9 ± 1.6	22.7 ± 1.5	3.2 ± 0.2	2.5 ± 0.1	6.8 ± 0.2	14.5 ± 0.4
4	31.4 ± 2.1	5.9 ± 0.2	4.8 ± 0.5	20.1 ± 0.9	35.1 ± 0.4	2.5 ± 0.1	2.9 ± 0.2	6.4 ± 0.2	13.5 ± 0.9
6	29.1 ± 2.1	4.5 ± 0.1	2.6 ± 0.1	19.3 ± 0.6	34.1 ± 1.2	2.9 ± 0.2	2.3 ± 0.1	6.2 ± 0.2	12.5 ± 0.2
8	29.4 ± 0.2	4.0 ± 0.2	3.5 ± 0.2	19.5 ± 0.8	29.9 ± 0.6	3.1 ± 0.1	1.8 ± 0.1	6.2 ± 0.2	13.1 ± 0.3

Table 4

Effect of water deficit on chlorophyll content in leaves and siliques of winter rape

Days of stress	Chlorophyll mg/gDWt		
	flowering	seed formation	
	leaves	leaves	siliques
0	14.77 ± 0.43	14.55 ± 1.60	1.36 ± 0.12
2	13.92 ± 0.62	5.49 ± 1.41	1.33 ± 0.06
4	9.06 ± 1.21	2.27 ± 1.26	1.22 ± 0.09
6	9.59 ± 0.85	0	1.31 ± 0.06
8	8.46 ± 0.53	0	1.11 ± 0.03

Distinct reactions of plants could be observed with moderate deficit, when the water content fell to about 30 per cent FWC and the RWC in the leaves decreased by about 15 per cent. The leaves began to die when the water content fell to 20 per cent FWC and RWC to about 41-35 per cent. Thus the investigations demonstrated that in rape, like in other crop plants, water deficit causes proline accumulation, changes in the protein level and a decrease in chlorophyll content.

DISCUSSION

A characteristic biochemical reaction of mesophytes to drought is accumulation of proline and other low molecular weight amine compounds ⟨H s i a o, 1973; S i n g h et al., 1973a⟩. Proline accumulation is due to the inhibition of protein synthesis, their hydrolysis and de novo synthesis from glutamic acid ⟨A s p i n a l l and P a l e g, 1981⟩. The course of the process of proline accumulation is dependent on light, the accessibility of carbohydrates and other factors ⟨S i n g h et al., 1973b; H a n s o n and H i t z 1982; T a n a b e et al., 1982⟩.

As demonstrated in the present study, the process of proline accumulation in rape plants growing under conditions of water deficit occurred with different intensity in the two tested development stages. Proline accumulation was noted in leaves, inflorescences and siliques, the latter containing the highest amounts, up to 6.6 per cent of dry weight. A higher proline content reaching as much as 10-20 per cent of dry weight was found only in halophytes ⟨S t e w a r t and L e e, 1974⟩. In the leaves of other crop plants, as for instance soya, an about threefold lower proline level was found on the 10th day of stress than in rape leaves and it was demonstrated that this accumulation may be the consequence of protein hydrolysis ⟨F u k u t o k u and Y a m a d a, 1981⟩. As reported by S i n g h et al. ⟨1973a⟩, however, accumulation of proline takes place in barley

even when the protein level is not depressed. Sano and Kawashima ⟨1982⟩ ascertained that proline accumulation in tobacco leaves is not associated with protein metabolism, and as shown by further investigations is rather dependent on the accessibility of carbohydrates. In etiolated tobacco leaves proline accumulation was lower than in green ones ⟨Tanabe et al., 1982⟩. The slight changes in proteins content, revealed in rape leaves under the influence of stress, may suggest that the process of intensive proline accumulation is not directly related to protein metabolism. A more important depression of protein content was found in maize leaves ⟨Botha and Botha, 1980⟩. These authors further demonstrated that the decrease in the rate of protein synthesis was correlated with the lower chlorophyll content ⟨Botha and Botha, 1979⟩. Other investigations indicate that water stress inhibits chlorophyll synthesis ⟨Duysen and Freeman, 1974⟩ and causes marked structural changes in the chloroplasts and chlorophyll-protein complexes ⟨Vapaavuori and Nurmi, 1982⟩.

The intensity of proline accumulation caused by stress depends both on the plant organ and on the chlorophyll content. This relation was demonstrated when comparing various barley organs ⟨Singh et al., 1973b⟩. In leaf blades with a high chlorophyll content the highest proline accumulation was noted; in the leaf sheaths which contain less chlorophyll proline accumulation was less intensive. In the growth apexes of the shoots and roots deprived of chlorophyll proline accumulation was very low or there was none.

Similar relations were demonstrated for rape plants. Accumulation of proline was found to be higher under stress in leaves than in stems and roots ⟨Rogozińska and Flasiński, 1983⟩. The present study showed that only in the inflorescences and siliques of rape, to which physiologically active substances and metabolites flow in the course of seed development from the mother plants, the proline level was higher than in the leaves.

Analysis in detail of the influence of the duration time of water stress on proline accumulation and chlorophyll content in rape leaves in the period of flowering, indicates that proline accumulation is associated with a decrease of the chlorophyll content. In the period of seed formation, however, proline accumulation was noted only up to the second day of stress. At the same time the chlorophyll level decreased down to complete disappearance. It is possible that with the loss of the physiological function of the leaves, proline migrated from the dying leaves to other organs. As reported by Hanson and Hitz ⟨1982⟩, nitrogen translocation from tissues which have ended growth to meristematic tissues may occur as the result of protein degradation caused by stress, this attenuating the nitrogen deficit in zones of growth. These processes indicate the positive adaptive functions of the changes in proline and protein content.

In the here described studies of metabolic reactions of rape to water stress the critical stages were taken into account ⟨Dębiński, 1975⟩. The revealed

reactions precede the appearance of the symptoms of drought and may serve as basis for further investigations concerning the relation between sensitivity to drought of various rape genotypes and proline accumulation.

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REFERENCES

Arnon D. I., 1949. Copper enzyme in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24: 1-15.

Aspinall D., Paleg L. G., 1981. Proline accumulation: physiological aspect. [In:] Physiology and Biochemistry of Drought Resistance, ed. Paleg L. G., Aspinall D., Sydney, Acad. Press pp. 205-241.

Bates L. S., Waldren R. P., Teare I. D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39: 205-207.

Botha F. C., Botha P. J., 1979. The effect of water stress on nitrogen metabolism of two maize lines: II. Effect on the rate of protein synthesis and chlorophyll content. Z. Pflanzenphysiol. 94: 179-183.

Botha F. C., Botha P. J., 1980. The effect of water stress on nitrogen metabolism of two maize lines: I. Effect on protein content and RNAse activity. J. S. Afr. Bot. 1: 45-52.

Dębiński F., 1975. Rośliny oleiste. PWRiL, Warszawa.

Duysen M. E., Freeman T. P., 1974. Effect of moderate water deficit ⟨stress⟩ on wheat seedling growth and plastid pigment development. Physiol. Plant. 31: 262-266.

Fukutoku Y., Yamada Y., 1981. Sources of proline-nitrogen in waterstressed soybean ⟨*Glycine max* L.⟩. I. Protein metabolism and proline accumulation. Plant Cell Physiol. 22: 1397-1404.

Hanson A. D., Hitz W. D., 1982. Metabolic responses of mesophytes to plant water deficits. Ann. Rev. Plant Physiol. 33: 163-203.

Hsiao T. C., 1973. Plant responses to water stress. Ann. Rev. Plant Physiol. 24: 519-570.

Lowry O. H., Rosebrough N. J., Farr R. J., Randall R. J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Rogozińska J., Flasiński S., 1983. Wpływ stresu osmotycznego na akumulację proliny u rzepaku. Zesz. Nauk. ART w Bydgoszczy, Rolnictwo 16: 5-9.

Sano M., Kawashima N., 1982. Water stress induced proline accumulation at different stalk position and growth stages of detached tobacco leaves. Agric. Biol. Chem. 46: 647-653.

Singh T. N., Paleg L. G., Aspinall D., 1973a. Stress metabolism. I. Nitrogen metabolism and growth in the barley plant during water stress. Aust. J. Biol. Sci. 26: 45-56.

Singh T. N., Aspinall D., Paleg L. G., Boggess S. F., 1973b. Stress metabolism. II. Changes in proline concentration in excised plant tissues. Aust. J. Biol. Sci. 26: 57-63.

Stewart G. R., Lee J. A., 1974. The role of proline accumulation in halophytes. Planta 120: 279-289.

Tanabe Y., Sano., Kawashima N., 1982. Changes in free amino acid in white and green tissues of variegated tobacco leaves during water stress. Plant Cell Physiol. 23: 1229-1235.

Vapaavuori E., Nurmi A., 1982. Chlorophyll-protein complexes in *Salix* sp. 'Aquatica gigantea' under strong and weak light. II. Effect of water stress on chlorophyll-protein complexes and chloroplast ultrastructure. Plant Cell Physiol. 23: 791-801.

Weatherley P. E., 1950. Studies in the water relation of the cotton plant. I. The field measurment of water deficits in leaves. New Phytol. 49: 81-97.

Wpływ deficytu wody na akumulację proliny, zawartość białka i chlorofilu w okresie kwitnienia i formowania nasion rzepaku ozimego ⟨*Brassica napus* L. var. *oleifera*⟩

Streszczenie

Deficyt wody wywołany w okresie kwitnienia i formowania nasion rzepaku powodował charakterystyczne reakcje metaboliczne. Następowała akumulacja proliny w liściach, kwiatostanach i łuszczynach. Zawartość białek ulegała obniżeniu we wszystkich organach rzepaku, z wyjątkiem liści roślin będących w okresie formowania nasion. Deficyt wody powodował również obniżenie zawartości chlorofilu w liściach, które było większe w okresie formowania nasion niż w okresie kwitnienia.