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The effect of water and salt stresses on the phosphorus content and acid phosphatase activity in oilseed rape

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Abstract

Oilseed rape plants responded to water and salt stresses (−0.5 MPa, PEG 6000 and NaCl) by reduction of the fresh and dry weights of shoots and roots. When PEG was used, the ratio of dry weights of roots:shoots surpassed that of controls. The leaf protein content increased considerably. The phosphorus content decreased only in the roots, most significantly after three days of stress. Immediately after the stresses were induced, an increase in the acid phosphatase (AP) activity was noted. Water and salt stresses caused four- and two-fold increases in AP activity in leaves, respectively. Changes in the enzyme activity were negligible in stems and roots. There are nine forms of AP in young leaves of oilseed rape. In the stressed plants, from No. 5 revealed lower activity and forms Nos 8 and 9, higher activities than in the control. The increase in AP activity was directly accompanied by the decrease in the water potential of the tissues. Oilseed rape is considerably less sensitive to salt stress than to water stress, which is manifested as the lower inhibition of plant growth and also by a smaller increase in acid phosphatase activity.

Key words: oilseed rape, acid phosphatase, water and salt stresses

INTRODUCTION

Water deficit brings about, among others, inhibition of nutrient uptake (Shone and Flood 1983), a fall in the protein and chlorophyll contents, accumulation of proline and an increase in the activity of hydrolytic enzymes

(Hsiao 1973, Hanson and Hitz 1982, Rogozińska and Flasiński 1983, Flasiński and Rogozińska 1985). An increase in the activity of acid phosphatase has been found in several plant species (Vieira-da-Silva 1969, Barrett-Lennard et al. 1982). In spite of the rather common occurrence of acid phosphatase in plants, its physiological role has been only partially elucidated. This enzyme regulates phosphorus metabolism by affecting the uptake and transport of phosphorus and through the hydrolysis of cellular and extracellular phosphate esters (Bieleski 1973, Ferens and Morawiecka 1984).

The activity of acid phosphatase is clearly related to the phosphorus content in the plant (Bieleski 1973). A rise in the activity of this enzyme due to stress caused by phosphorus deficit has also been determined in all oilseed rape organs (Rogozińska and Flasiński 1986), in isolated cotyledons and roots and in whole seedlings as well (Flasiński et al. 1986).

In this study, the effects of water stress caused by polyethylene glycol (PEG 6000) and of salt stress evoked by sodium chloride, on the protein and phosphorus contents and activity of unspecific acid phosphatase in various oilseed rape organs were investigated. The objective was to determine if water and salt stresses directly affect acid phosphatase, or do so indirectly through affecting phosphorus content.

MATERIAL AND METHODS

Winter oilseed rape (*Brassica napus* var. *oleifera* cv. Start) was grown in a phytotron (prototype OBURUCiG Bydgoszcz) under a photoperiod of 12h/12h, 70% humidity, illumination intensity 40 W m^{-2} and temperature of $24 \pm 2°C$. The seeds were germinated on perlite, after which 7-day-old seedlings were transferred to hydroponic cultures on 1:1 Hoagland's medium. Three-week-old plants were subjected to water stress by adding a total of 141 g dm^{-3} polyethylene glycol (PEG 6000) to the medium (47 g dm^{-3} every 4 days), or to salt stress by adding a total of 5.85 g dm^{-3} NaCl (1.71 g dm^{-3} every 2 days). The water potential of the medium fell to about -0.5 MPa (Tal et al. 1979).

After 3, 7, 14 and 21 days of growth of the plants under stress conditions, the contents of water, protein, phosphorus and acid phosphatase activity were determined in the individual organs. Young leaves (well-developed, youngest leaves), older leaves (lying below), stems (the segment between a young leaf and the base of the cotyledon) and roots were taken for analysis. The biochemical analyses were done according to previously described methods (Flasiński et al. 1986). The presented results are the mean values of three

repetitions and have been statistically analysed by the method of variance analysis.

The fractionation of proteins extracted from young leaves with 50 mM Na-acetate buffer, pH 5.0, was accomplished by Disc-PAGE (Laemmli 1970). The separating gel (7.5%) was polymerized in 8 cm long glass rods with an internal diameter of 0.5 cm. A 5% stacking gel, about 0.5 cm high, was layered over the separating gel. A sample containing approx. 100 μg protein was applied to each rod. Electrophoresis was run for the first 30 min at 2 mA per rod, then at 3.5 mA. Acid phosphatase activity was assayed on the gels by incubating them for 1 h in 50 mM Na-acetate buffer, pH 5.0, containing 0.05% 1-naphthyl phosphate and 0.05% fast garnet GBC.

RESULTS

Subjecting oilseed rape plants to water or salt stress (−0.5 MPa) inhibited their growth; PEG was here much more effective than NaCl (Table 1). Water stress also caused a large fall in the shoot water content, and the ratio of root to shoot weight was much greater than in controls. In plants subjected to salt stress, the water content and root to shoot weight ratio were similar to the controls (Table 1).

Table 1

The effects of water and salt stresses on shoot and root fresh and dry weights and concentration of phosphorus in pretreated oilseed rape and in 6-week-old plants after 3 weeks of growth under stress conditions

		Pretreated plant	Control	NaCl	PEG
Fresh weight (g plant^{-1})	shoot	0.51	5.44	4.74	0.57
	root	0.04	0.82	0.65	0.36
Dry weight (g plant^{-1})	shoot	0.036	0.420	0.384	0.120
	root	0.004	0.088	0.080	0.040
Water (% f.w.)	shoot	92.9	92.3	91.1	78.8
	root	90.0	89.3	87.7	88.9
Phosphorus (mg plant^{-1})	shoot	0.28	1.10	1.20	0.39
	root	0.03	0.70	0.44	0.31
Phosphorus (mg g^{-1} d.w.)	shoot	8.06	2.43	3.10	3.33
	root	7.50	7.95	5.46	7.70
Root/shoot d.w. ratio		0.08	0.21	0.21	0.33

The phosphorus content of oilseed rape plants under conditons of water stress was the lowest, which indicates that PEG strongly inhibited the uptake of this element. Under conditions of salt stress, only in the roots was the phosphorus content lower than in the control. The inhibition of phosphorus uptake by PEG was greater than its inhibition of growth, since the phosphorus content increased 2-fold during the studied period, while the dry weight of the plant, 4-fold. When calculated per dry weight, the phosphorus content was similar to that in the controls (Table 1).

The water content during growth of the plants underwent only slight oscillations (88-92%). Water stress caused a decrease in the water content in young and older leaves, especially after 2 weeks of stress (Table 2). Its content in stems and roots was similar to control values. Also, under conditions of salt stress, the water content in all of the organs was similar to the controls.

The phosphorus content depended on the organ, and was similar in leaves and stems, while in the roots it was about 3 times greater (Fig. 1). In leaves and stems its content decreased, especially in the initial stages, but was maintained on a more or less even level in the roots. The lowering of the water potential of the medium by addition of PEG or NaCl affected the phosphorus content of the plants, especially in the roots. In these organs, after 3 days of stress, the

Table 2

The effect of water and salt stresses on water content (%) in oilseed rape organs

	Days of stress	Control	NaCl	PEG
Young leaves	0	91.7		
	3	92.0	91.2	89.9
	7	92.1	92.3	87.6
	14	90.1	91.8	47.8
	21	88.1	88.2	77.1
Older leaves	0	92.6		
	3	91.5	90.4	91.4
	7	93.1	92.2	88.9
	14	92.0	92.5	43.8
	21	90.9	89.3	74.1
Stems	0	94.6		
	3	92.3	90.2	91.8
	7	94.2	93.3	92.9
	14	93.4	93.4	84.1
	21	92.4	91.5	91.6
Roots	0	90.7		
	3	93.2	92.2	88.5
	7	93.6	92.9	90.7
	14	93.7	92.9	90.4
	21	91.9	91.4	90.6

phosphorus content significantly decreased. During the final stages of stress, the phosphorus level in the leaves and stems even exceeded that in the controls.

The protein content of the oilseed rape organs, as in other plants, is the greatest in the leaves, lower in the roots and stems (Fig. 1). It was found that the protein content of the leaves fell considerably as the result of stress evoked by addition of PEG or NaCl to the medium. This occurred after 7 days and was larger in the case of PEG than NaCl. Water and salt stresses did not have any significant effect on the protein content of stems. However, in the roots a significant fall in the protein content took place after 7 days and was maintained during prolongation of stress.

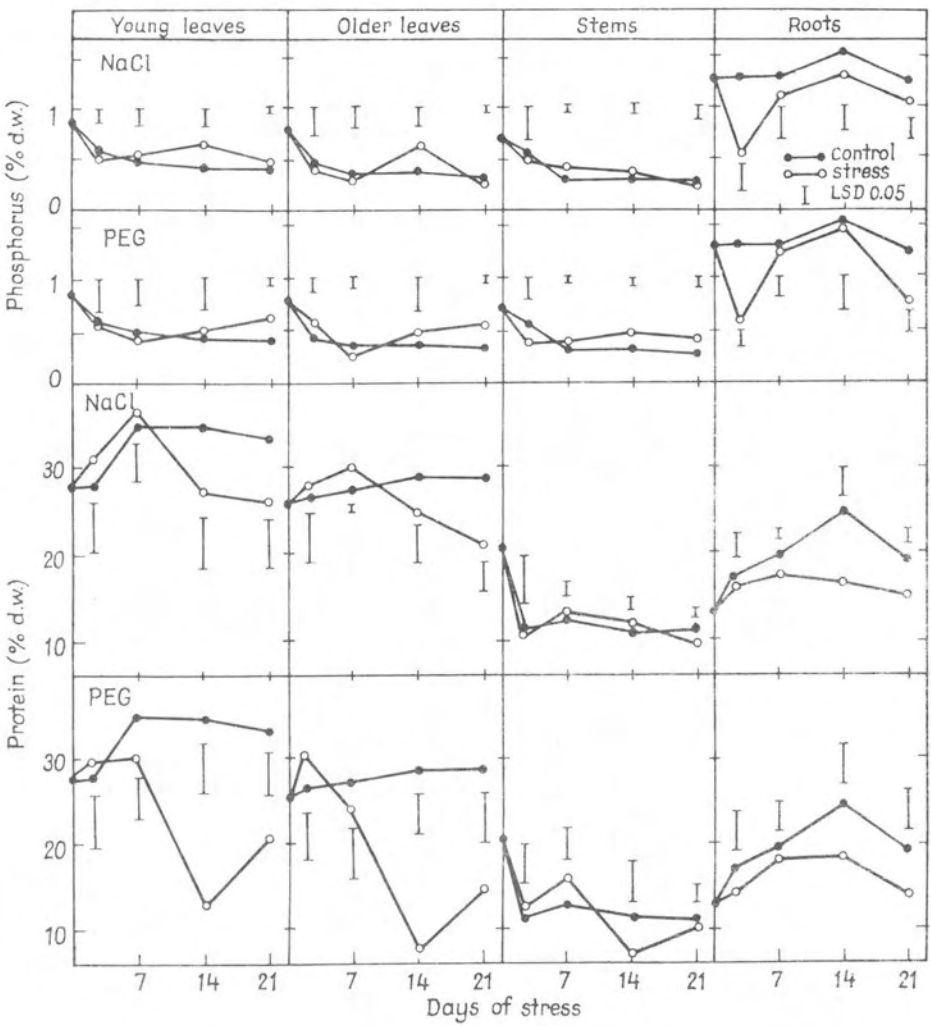

Fig. 1. The effect of water and salt stresses on phosphorus and protein content

The activity of acid phosphatase was determined in relation to plant weight (total activity) and in relation to protein content (specific activity), because water deficit inhibits both weight increase and protein content. The level of activity of phosphatase was rather varied in the particular organs (Fig. 2). The highest total activity was found in the leaves, somewhat smaller in the stems, the smallest in the roots. The specific activity was the greatest in the stems,

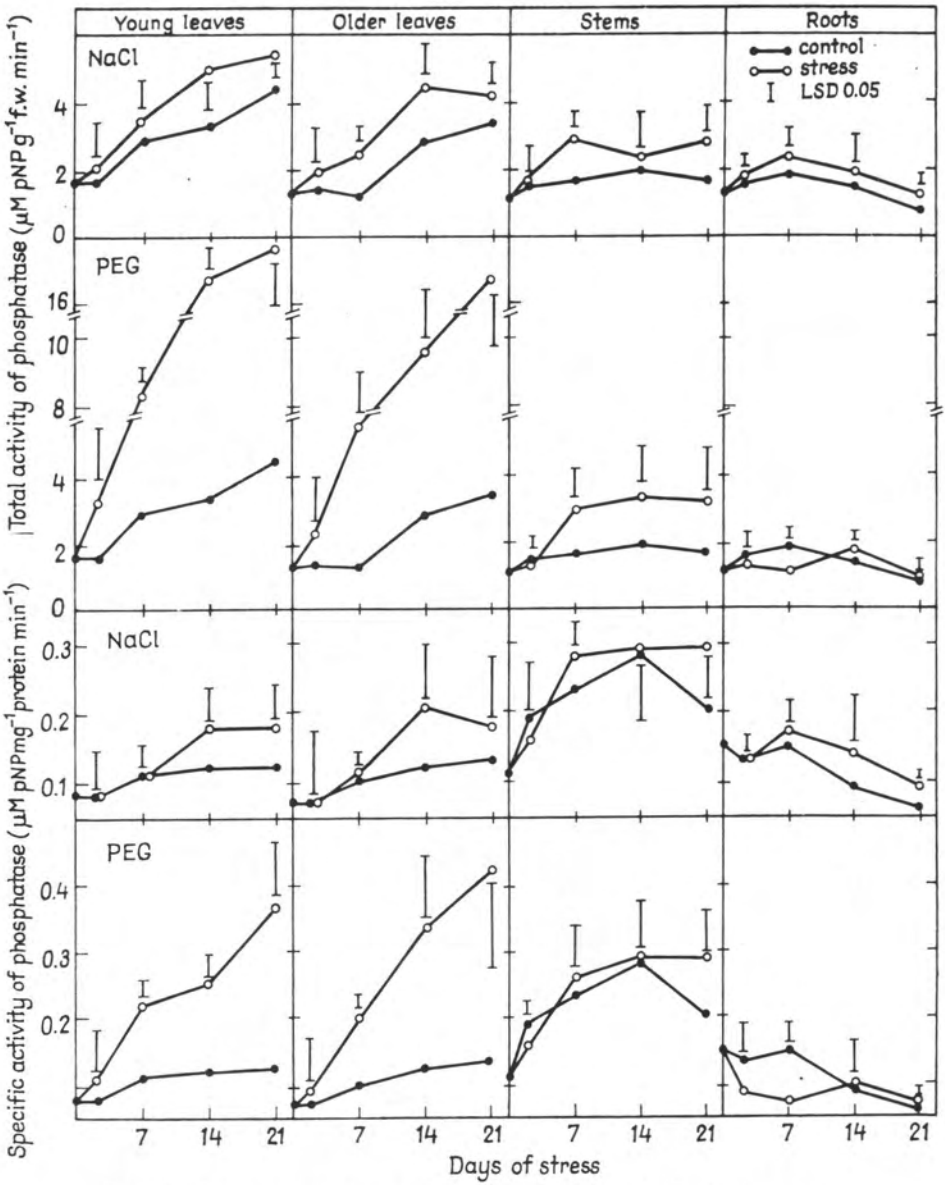

Fig. 2. The effect of water and salt stresses on acid phosphatase activity

smaller in the leaves and roots. In the control plants, both total and specific activities slightly increased with growth. In the stems, total activity was maintained on a rather stable level, while the specific activity increased up to the 5th week of growth of the plants. The increases in the specific activity were related to the falls in protein content in these organs during the studied period. Decreases in the total and specific activities of acid phosphatase occurred in the roots.

The effects of stress conditions on the activity of acid phosphatase were varied depending on the stress factor and on the organ (Fig. 2). In the leaves of oilseed rape plants growing under conditions of salt stress, an approximately 1.5-fold increase in total activity and an approx. 2-fold increase in specific activity occurred. Increases in total and specific activities were also found in stems and roots, however the differences were not always statistically significant. Water stress caused by addition of PEG to the medium brought about significant increases in total and specific activities in leaves after only 3 days of stress. Depending on the duration of the stress, the activity of phosphatase was about 2 to 4 times that in the leaves of control plants. In the stems, the total activity was significantly greater after 7 days, while the specific activity only increased after 21 days. Whereas, in the roots during the initial period of stress, the total and specific activities were lower than in the controls, and after 14 days of stress their values approached those of the controls.

Separation of proteins from young oilseed rape leaves by electrophoresis on polyacrylamide gel demonstrated the presence of at least 9 molecular of acid phosphatase (Fig. 3). The electrophoretic profiles of acid phosphatase obtained from plants growing on PEG- and NaCl-containing mediums were different. Significant quantitative differences were found among the forms

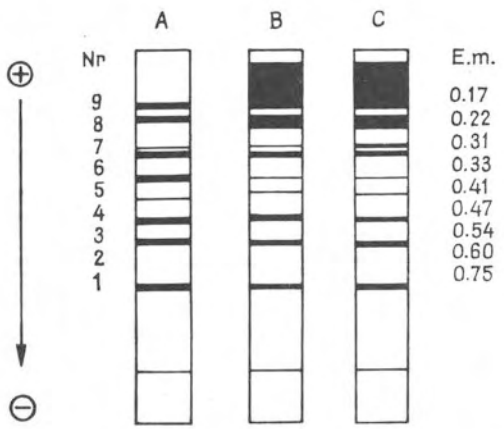

Fig. 3. A schematic diagram of polyacrylamide gel electrophoresis of protein from young leaves of control (A), water-stressed (B) and salt-stressed (C) oilseed rape. Gels were stained using α-naphthylphosphate as the substrate of acid phosphatase. E.m. – electrophoretic mobility

denoted on the diagram as Nos. 5, 8 and 9. Molecular from No. 5 demonstrated a lower activity, while forms Nos. 8 and 9 had decidedly higher activities than in the controls. The activities of the remaining forms did not notably differ.

The presented results show significant increases in the activity of acid phosphatase in the leaves of oilseed rape subjected to water and salt stresses. PEG, which inhibited growth more than did NaCl, also caused a greater increase in the activity of this enzyme. The increase in acid phosphatase activity was not accompanied by a fall in the level of phosphorus in the leaves. In addition to quantitative changes in activity, differences in the electrophoretic patterns of the molecular forms of phosphatase from control and stressed plants were found.

DISCUSSION

PEG-evoked water stress caused greater inhibition of growth of oilseed rape than did salt stress. PEG reduced to a minimum the uptake of nitrogen and phosphorus as well as several other elements, not only by lowering the water potential of the medium, but also by reducing the permeability of membranes to ions (Shone and Flood 1983, Yeo and Flowers 1984).

During the growth of rape under water stress, an increase in the rhizzogenic activity (Balestrini and Vartanian 1983) takes place, which can also be seen from the results of this investigation. A significant increase in root weight in relation to shoot weight was observed. Because water deficit greatly inhibits photosynthesis (Hsiao 1973, Hanson and Hitz 1982), it can be supposed that the development of the roots during water stress takes place at the expense of degradation of leaf organic compounds, which may be indicated by the fall in the protein content of these organs in rape.

Earlier reports have said that a lowering of the soil water content was accompanied by a fall in the phosphorus level in leaves (Wilson and Muchow 1983, Turner 1985). The experiments conducted in this study have shown that a fall in the phosphorus content occurred only in the roots. The plants did not grow almost at all in the presence of PEG. However, plants growing under conditions of salt stress, suffer rather from the deficit of cations, mainly potassium (Levitt 1972, Tal et al. 1979). The elements taken up in the form of anions may be present in sufficient amounts, which is also suggested by the results of this study.

The normal level of unspecific acid phosphatase in plants is about 0.5-4.5 $\mu M\ pNP\ g^{-1}\ min^{-1}$ (Bieleski 1973, Rogozińska and Flasiński 1986). Under conditions of phosphorus deficit, its activity can increase considerably (Bieleski 1973, Ferens and Morawiecka 1984). The deficit of other elements, particularly calcium, also acts to increase the activity of this enzyme,

which was shown in a previous paper (Rogozińska and Flasiński 1986). In this study, a 2- and 4-fold increase in the activity of acid phosphatase in leaves, particularly young ones, was shown in plants growing in the presence of PEG and NaCl, respectively. An increase in the activity of acid phosphatase was also found in the leaves of wheat growing under conditions of soil drought (Barrett-Lennard et al. 1982).

It was shown previously that the activity of acid phosphatase in the cotyledons of rape growing under conditions of water or salt stress was greater than in the controls, while no increase of activity occurred in isolated cotyledons (Flasiński et al. 1986). In the studies presented here it was shown that rape leaves react much more strongly to stress than its cotyledons. The higher activity of phosphatase was not related to a fall in the phosphorus content, but only with a decrease in the water content. Salt stress also caused an increase in the phosphatase activity in the leaves, even though the water content did not change. Under these conditions, however, significant accumulation of ions takes place in the plant, which lowers the water potential of the cells (Tall et al. 1979). This suggests that the changes associated with osmotic adaptation of a plant to conditions of water stress may have a significant effect on the activity of acid phosphatase. It was shown in earlier studies conducted on cotton plants that the activity of acid phosphatase increased proportionally to the dehydration of the leaves (Vieira-da-Silva 1969). In the case of rape, it is shown here that this is not a proportional relationship or dependent on the duration of the stress.

Our study has confirmed earlier reports that an increase in the activity of acid phosphatase under conditions of water deficit is the result of activation of an enzyme already existing in the cell (Berrett-Lennard et al. 1982). No new molecular forms of acid phosphatase were found in rape leaves. It was also demonstrated that the electrophoretic patterns of the molecular forms of acid phosphatase from the leaves of plants growing under water and salt stresses were similar. This suggests that water and salt stresses can initiate similar regulatory mechanisms in a plant. It seems probable that under stress conditions, phosphatase is released from various cellular membrane structures, due to which its activity rises.

Under conditions of moderate water deficit, the rise in phosphatase activity may be a defensive mechanism related to a quicker turnover of phosphorus. Under conditions of severe water deficit, however, such as was the case in the presence of PEG, the observed effect could be the cumulative result of various metabolic modifications related also to degradation of cell structures during aging and dying of leaves. Our present and previous studies indicate that acid phosphatase is under the control of several factors, such as the contents of phosphorus and calcium (Rogozińska and Flasiński 1986) and the availability of phosphorus, the water potential and the osmotic potential of the cell.

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Wpływ stresu wodnego i solnego na zawartość fosforu i aktywność fosfatazy kwaśnej u rzepaku ozimego

Streszczenie

Rośliny rzepaku ozimego reagowały na stres wodny i solny (−0.5 MPa: PEG 6000 lub NaCl) zmniejszeniem zawartości świeżej i suchej masy pędu i korzenia. W przypadku zastosowania PEG stosunek masy korzeni do pędu był większy niż w kontroli. Zawartość białka w liściach ulegała znacznemu zmniejszeniu. Poziom fosforu ulegał obniżeniu tylko w korzeniach, zwłaszcza po trzech dniach stresu. Bezpośrednio po wywołaniu stresu nastąpił wzrost aktywności fosfatazy kwaśnej (AP). Stres wodny i solny powodował, odpowiednio, 4- i 2-krotny wzrost aktywności AP w liściach rzepaku. Zmiany aktywności enzymu w łodygach i korzeniach były znacznie mniejsze. W młodych liściach rzepaku występuje 9 form molekularnych fosfatazy kwaśnej. U roślin poddanych stresowi forma nr 5 wykazywała mniejszą aktywność, a formy nr 8 i 9 wykazywały znacznie większą aktywność niż kontrola. Zwiększenie aktywności AP w liściach roślin było wywołane bezpośrednio przez obniżenie potencjału wody tkanek. Rzepak ozimy jest znacznie mniej wrażliwy na NaCl niż na PEG, co objawia się mniejszym zahamowaniem wzrostu, jak również mniejszym zwiększeniem aktywności fosfatazy kwaśnej.