Cultivation Techniques, Morphology and Enzymatic Properties of *Crocus sativus* L.

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Keywords: corms, cultivation media, enzymes, salinity, roots

Abstract

Traditional cultivation techniques and empirical knowledge about best soil composition and most suitable corms have been transmitted from generation to generation of *Crocus sativus* L. (saffron) farmers. The range of soil conditions is vast, with great possible variations in acidity, water availability, air supply, salts concentration and organic compounds. Thus delineating accurately the nutritional requirements of the saffron plant is difficult to achieve in field studies. In order to conduct controlled studies of the plant development, we have been cultivating corms in the laboratory either 1) in pots, using field cultivation soil, or 2) in semi-liquid agar medium, or 3) in liquid medium. We selected cultivation either in potted soil or, mostly, in liquid medium that appeared to be the most convenient and controllable technique. Using it, we have studied the effect of a wide range of experimental conditions such as changes in the medium nutritional content, salinity and oxidative stress, etc. Roots and shoots growth was used as criteria to determine gross alterations in plant morphology. Concomitantly, we studied the enzymatic properties of the saffron plant, with emphasis on the saffron corm. Saffron is a triploid plant, thus its beautiful purple-violet flowers cannot produce any seed. The plant propagation is possible only via corms. Any improvement in crops quality and quantity must emerge from the corm quality. Even though resistance to certain undesirable environmental stress can be achieved by gene transfer, understanding the role of nutritional factors as well as studying their effect on the metabolic and enzymatic properties of the corm might lead to desired flowering quality as well as quantity in this post-genomic era. Moreover, perfection in the liquid cultivation medium and development to large-scale liquid system may provide for a method additional to farm culturing.

INTRODUCTION

Plants require a variety of mineral nutrients. However, the mineral composition of a given soil or cultivation medium may produce an optimum growth or may be detrimental to plant growth. Plant-soil interactions are very complex. The range of soil conditions is vast, with great possible variations in acidity, water availability, air supply, and salts concentrations. In addition, soil contains both living and non-living components. The living components range from bacteria, fungi, yeasts to various nematodes that might either be beneficial or detrimental to plants. On the other hand, the chemical composition and physical structure of soils differ dramatically from place to place, even when considering different area of a single farm. Consequently, delineating accurately the nutritional requirements of a given plant, e.g. *Crocus sativus* L. (hereafter named "saffron plant"), is difficult to perform in such a situation. To achieve controlled growth conditions, one must transfer living organisms to the laboratory and cultivate them in media of strictly known composition containing essential elements (non-mineral and mineral). Moreover, such culturing media enable scientists to evaluate the biosynthesis of various components, their inhibitors, inducers, etc.

Even though plants require essential elements for their growth and development, excess of these elements has a deleterious effect on their development. For example,

excess of metals such as iron, copper and others, lead to the production of free radicals and oxidative stress (Halliwell and Gutteridge, 1986, 1990; Halliwell, 1996). In general, various abiotic stresses produce oxidative stress and the release of free radicals in plants (Hernández et al., 1993, 2000, 2001; Gueta-Dahan et al., 1997; Schutzendubel and Polle, 2002; Garratt et al., 2002). The most important defenses of plants as well as animals against oxidative stress are a series of enzymes that includes superoxide dismutases, catalases and peroxidases (Gaspar et al., 1991; Castillo, 1992; Dalton, 1995; Tsay et al., 2000; Acevedo et al., 2001). Thus the objective of our research is to evaluate the effect of cultivation media such as soil or liquid media of various compositions on the morphology of saffron plant and on enzymatic activities detectable in the plant corm. Emphasis has been put on enzymes involved in the plant defense against oxidative stress and on two other enzymes, namely L-lactate dehydrogenase and alcohol dehydrogenase, involved, respectively, in cellular respiration (Capeillere-Blandin et al., 1975; Capeillere-Blandin, 1982) and carbohydrate metabolism (Kimmerer, 1987; Voet et al., 1999).

MATERIALS AND METHODS

Corms and Cultivation Media

Saffron plant corms were obtained from the University of Tehran farm located in Karaj, near Tehran. Unearthed corms were depleted from their sheathing leaves, cleaned from any dirt particle and placed in a cultivation medium. The various cultivation media are described below.

1. Potted Soil. Soil similar to that in the farm was placed in plastic pots measuring 24 cm in diameter and 20 cm in depth. 6 to 7 corms prepared as described above were planted per pot and were cultivated for up to 30 days.

2. Liquid Media. For cultivation in liquid media, glass jars measuring 6.5 cm in diameter and 10 cm in depth were used. One corm was placed per jar and 50 ml medium was added. Corms were cultivated for up to 110 days in some media. Care was taken to maintain the liquid level in each jar throughout cultivation.

a. *Distilled Water.* This medium consisted of simply distilled water.

b. *A Medium.* This medium was prepared in distilled water and contained the following minerals: $(NH_4)_2SO_4$ 15.0 mM, $MgCl_2$ 9.5 mM, KH_2PO_4 7.5 mM, NaCl 1.5 mM, $CaCl_2$ 0.9 mM, Na_2MoO_4 0.97 μM, $FeSO_4$ 6.5 μM, $MnSO_4$ 3.25 μM, $ZnSO_4$ 3.0μM, $NiSO_4$ 0.65μM, $CuSO_4$ 0.25 μM, KI 0.25 μM. A medium was prepared in full concentration as well as in 1/4, 1/20 and 1/100 dilutions.

c. *B Medium*. This medium was prepared in distilled water and contained the following minerals: $(NH_4)_2SO_4$ 15.0 mM, $MgCl_2$ 9.5 mM, KH_2PO_4 7.5 mM, NaCl 1.5 mM, $CaCl_2$ 0.9 mM, Na_2MoO_4 0.97 μM, B medium was prepared in full concentration as well as in 1/4, 1/20 and 1/100 dilutions.

d. *Saline media (NaCl).* Each medium was prepared in distilled water with NaCl in one of the following concentrations: 0.005 M, 0.01 M, 0.025 M, 0.05 M, 0.1 M, 0.2 M, 0.5 M, 1 M, 2 M.

e. *Saline media (KCl).* Preparation of these media was as described in *d*, except that NaCl was replaced by KCl.

Enzymatic Activity Assays

All assays were carried out at room temperature (~ 22-25ºC). The specific procedure followed for each enzyme assayed is described below. Results were averages of at least three assays.

1. Alcohol Dehydrogenase. Alcohol dehydrogenase activity was determined by following spectrophotometrically the rate of reduction of NAD^+ at 340 nm. An extinction coefficient of 6.22 mM^{-1} cm^{-1} was used to calculate the amount of NADH produced. The reaction, conducted in phosphate buffer 0.1 M, pH 8.5, with ethanol and NAD^+ as substrates, was initiated by the addition of corm extract. One unit was defined as the amount of enzyme needed to reduce 1 μmol NAD^+ per minute.

For the reverse reaction, the same procedure as that described above was followed except that acetaldehyde and NADH were used as substrates and that, this time; the amount of NADH consumed was measured at 340 nm.

2. Peroxidases. Peroxidase activity was determined by following the oxidation of o-dianisidine at 460 nm, guaiacol at 470 nm, pyrogallol at 430 nm and ascorbate at 290 nm with extinction coefficients 11.3 $mM^{-1}cm^{-1}$, 26.6 $mM^{-1}cm^{-1}$, 2.47 $mM^{-1}cm^{-1}$ and 2.8 $mM^{-1}cm^{-1}$, respectively. One unit was defined as the amount of enzyme needed for the oxidation of 1 μmol of substrate per minute. Assays were carried out at room temperature (≈ 22-25ºC), in 0.01 M citrate-phosphate-borate buffer, at given pH, with 0.3 mM H_2O_2, using Aminco DW2 and Milton Roy spectrophotometers, as described in Keyhani et al. (2000).

3. Lactate Dehydrogenase. L-Lactate dehydrogenase activity was determined as described in Keyhani and Sattarahmady (2002) by following the reduction of potassium ferricyanide at 420 nm with extinction coefficient 1040 $M^{-1}cm^{-1}$. One unit was defined as the amount of enzyme reducing 1 μmol ferricyanide per minute. Assays were carried out at room temperature (~ 22-25°C), in 0.1 M phosphate buffer, pH 8.4, in the presence of 1 mM EDTA, 50 mM L-lithium lactate and 0.25 mM potassium ferricyanide, using Aminco DW2 and Milton Roy spectrophotometers.

4. Catalase. Catalase activity was measured as described in Keyhani et al. (2002) by following the dismutation of H_2O_2 spectrophotometrically using an extinction coefficient for H_2O_2 at 240 nm of 27 $M^{-1}cm^{-1}$. One unit was defined as the amount of enzyme decomposing 1 μmol H_2O_2 per minute. All assays were done at room temperature (~ 22-25°C) with an Aminco DW-2 spectrophotometer; the reaction mixture consisted of 0.01 M citrate-phosphate-borate buffer, at given pH, 10 mM H_2O_2, and corm extract.

5. Superoxide Dismutase. The superoxide dismutase activity present in corm extract was assayed by measuring the extract ability to inhibit the reduction of nitro-blue tetrazalium (NBT) by superoxide. The assay was conducted in 0.067 M phosphate buffer, pH 7.8, 0.007M EDTA, 0.002 mM cyanide, 0.1 mM NBT. After incubation of the reaction mixture with and without corm extract in a light box for 6 minutes, riboflavin was added at timed interval. OD at 560 nm was measured after further incubation for 6 and 12 minutes. One unit was defined as that amount of enzyme causing half the maximum inhibition of NBT reduction.

RESULTS AND DISCUSSION

As described in the introduction, our basic concern was to bring saffron plant cultivation under laboratory conditions in which all of the nutritional requirements could be strictly controlled so that subsequently the effect of various stresses could be assessed. Although such an approach can lead to the detection and studies of various metabolic pathways in corm under experimental conditions, it does not substitute for studies of corm cultivated in field conditions or in soil.

The best criterion to study the effect of cultivation conditions on the saffron plant is the rooting system. In the study of stress conditions, rooting showed to behave as an early warning.

Root Number

Figure 1 shows the variation of the average root number per corm after 6 days cultivation in distilled water, A medium, B medium, various concentrations of NaCl and KCl and potted soil. The highest number of roots was found in distilled water. The same number was found in A and B media and in potted soil. However, both NaCl and KCl caused a decrease in root number that was noticeable from 0.05 M NaCl and KCl. As the salt concentration increased, the root number decreased to reach zero in 1 M NaCl or KCl. In concentrations between 0.05 and 0.5 M, the number of roots was 20% lower in NaCl than in KCl.

Root Elongation

Figure 2 shows the length of saffron plant roots in various cultivation conditions. In potted soil the length of roots was about 4 times that in distilled water (80 mm after 10 days in potted soil compared to 25 mm after 10 days in distilled water). Corms cultivated in minerals-supplemented water (A medium) showed a marked inhibition of root elongation compared to the other two conditions (only 10 mm after 10 days in A medium.

Figures 3-6 show a comparison between rooting in distilled water and rooting in various dilutions of A and B media. No difference was observed between rooting in A medium and rooting in B medium. Furthermore, there was no significant difference between the length of roots of corm cultivated in undiluted and 4-20 times diluted A and B media. In contrast, rooting in a 100 times dilution of A or B medium resulted in roots of a length intermediary between that observed in undiluted A or B medium and that observed in distilled water.

The effect of salinity (NaCl or KCl alone) on root growth is shown in Figures 7 and 8. With increasing concentration of NaCl or KCl, root growth was severely inhibited and in 1 or 2 M NaCl or KCl, no root growth was observed. Moreover, even low amounts of salt (0.005 M NaCl or KCl) did not enhance root length compared to distilled water.

Plant Morphology

Figs. 9 to 20 show the effect of environmental modifications on the gross anatomical changes in saffron plant. Corm cultivated in A medium gave rise to flower even though the roots aspect was necrotic (Figure 9). Such results were obtained even for corm rooted in diluted A medium. As seen in Figure 10, even rooting in A/100 or B/100 resulted in some root growth inhibition.

For corm grown in medium containing only KCl, flowering occurred from 0.005 M to 0.025 M (Figure 11); in higher concentrations of KCl, from 0.1 to 2 M, flowering was not observed.

When corms were grown in NaCl medium from 0.05 M to 0.1 M, rooting was observed after 3 (Figure 12) or 6 days (Figures 13 and 14). However these roots never grew more than 5 mm and all were necrotic after 6 days of growth. In NaCl 0.005 M the corm showed flower after 40 days of rooting. But for NaCl concentrations of 0.01 or 0.025 M, even though shoot was formed and root formation was seen (Figure 15) there was a delay in flowering compared to control (Figure 15).

Whenever roots and shoots formation was observed, even when flowers were not seen, new corm formation occurred under all experimental conditions (Figures 16-18). Even in media producing necrotic roots, new corm formation was observed that was comparable to that of the control in distilled water as shown in the pictures of Figures 19 and 20 obtained after 50 days culturing.

Enzymatic Activities

The amount of superoxide dismutase, catalase, ascorbate peroxidase and alcohol dehydrogenase activity was measured for corms cultivated in potted soil and standard medium that was distilled water. Superoxide dismutase activity (Figure 21) did not show any significant change for corms cultivated in water from 3 to 30 days. In contrast, the amount of superoxide dismutase increased 4-fold from unrooted to rooted corms in potted soil (from roughly 0.7 u/mg protein in corms before rooting to 2.5 u/mg protein in corms that rooted for 30 days). Catalase activity (Figure 22) did not change noticeably in corms rooted in potted soil while some variations were observed for corms rooted in distilled water. The amount of ascorbate peroxidase activity (Figure 23) detected in corms rooted in potted soil as well as in corms rooted in distilled water showed some fluctuation from day 0 to day 20, while at day 30 (around flowering time), the amount of ascorbate peroxidase became close to zero under both experimental conditions. Alcohol dehydrogenase activity (Figure 24) was not detected in corms rooted for 20 or 30 days in potted soil. In contrast, for corms rooted in distilled water, activity was highest at 20 days; at 30 days it dropped dramatically but was still well detectable. Interestingly, a surge in

activity was observed under both experimental conditions (at 10 days in potted soil and at 20 days in distilled water) before a dramatic drop. Results for corms cultivated in A or B medium were similar to those obtained for corms cultivated in soil.

All morphological and biochemical studies reported so far indicated that the growth of corms in distilled water was the most reliable and easy method to study the biochemistry and molecular biology of saffron plant. Thus our subsequent effort was only oriented to study this system and the effect of various stresses in these culturing conditions, e.g. salinity.

Among the enzymatic activities studied in corm rooted for 6 days in distilled water containing increasing concentrations of NaCl, ascorbate peroxidase (Figure 28) and the peroxidases using o-dianisidine as substrate at pH 4.0 and 6.5 (Figure 30) did not show an appreciable change in activity even after rooting in 1 M NaCl. In contrast, alcohol dehydrogenase activity (Figure 26) progressively decreased from roughly 0.014 u/mg protein for rooting in distilled water to 0.001 u/mg protein for rooting in 1 M NaCl. Guaiacol peroxidase activity at pH 4.0 showed a two-fold increase in corms rooted in 1M NaCl compared to corms rooted in distilled water and in 0.05 M, 0.2M, 0.5 M NaCl. On the other hand, compared to the activity found in corms rooted in distilled water, guaiacol peroxidase activity at pH 6.5 almost doubled for corms rooted in 0.05 M, 0.2 M and 0.5 M NaCl, but dropped to 75% of the control activity for corms rooted in 1 M NaCl. Lactate dehydrogenases activity (Figure 25) assayed at pH 5.5 and 9.5 showed that at pH 5.5, the activity decreased progressively as corms rooted in increasing NaCl concentrations; it became close to zero for corms rooted in 1 M NaCl. The enzymatic activity measured at pH 9.5 showed a progressive decrease as rooting was taking place in NaCl concentrations up to 0.5 M; however, in corms rooted in 1 M NaCl, the activity had increased back to the value found in corms rooted in distilled water. Catalase activity was assessed for all three isoenzymes previously detected in dormant corms (Keyhani et al., 2002); all three isoenzymes decreased progressively compared to the control as NaCl concentration increased, and the lowest values were for corms rooted in 1 M NaCl. Enzymatic pattern was roughly the same for different KCl concentrations in water.

CONCLUSIONS

Saffron plant corms were cultivated under various environmental conditions. Root elongation was four times more in potted soil than in distilled water. However, while the pattern of some enzymes differed between the two experimental conditions, that of others did not.

Superoxide dismutase activity was consistently higher in corms rooted in potted soil compared to corms rooted in distilled water. Catalase and ascorbate peroxidase activity evolved roughly in the same manner under both experimental conditions. Alcohol dehydrogenase showed similar pattern up to 10 days culture under both experimental conditions. Then, the alcohol dehydrogenase activity became undetectable in corms rooted for 20 or 30 days in potted soil, but peaked in corms rooted in distilled water for 20 days then decreased for corms that rooted for 30 days in distilled water.

The enzymatic activity in corms rooted for 6 days in NaCl stress showed that the isoenzymes of L-lactate dehydrogenase would behave differently in response to increase NaCl concentrations in the cultivation medium. For the isoenzyme of L-lactate dehydrogenase most active at pH 5.5, the activity decreased progressively as the NaCl concentration increased from 0.05 M to 0.5 M; no activity was detectable in corms rooted in 1 or 2 M NaCl. The isoenzyme most active at pH 9.5 showed a decrease in activity as NaCl concentration in the cultivation medium increased from 0.05 to 0.5, but the activity was back at control level in corms rooted in 1 M NaCl. Alcohol dehydrogenase activity decreased progressively, becoming barely detectable in corms rooted in 1 M NaCl. The three isozymes of catalase (pH 6, pH 7, pH 9) decreased progressively so that in corms rooted in 1 M NaCl, the activity was four to six times less than in the control. Ascorbate peroxidase showed a surprising resistance to increasing NaCl concentrations in the cultivation medium. After an initial increase in activity for corms rooted in NaCl 0.05 to

0.2 M, the activity level became close to the control value for corms rooted in 0.5 and 1 M NaCl. Guaiacol peroxidase assayed at pH 4 exhibited a slight increase in activity as the NaCl concentration in the cultivation medium increased; when assayed at pH 6.5 the activity increased in corms rooted in 0.05 to 0.5 M NaCl but dropped by more than half in corms rooted in 1 M NaCl. Peroxidases assayed by o-dianisidine at pH 4 and 6.5 did not show any appreciable change in activity as a function of NaCl concentration in the cultivation medium.

Morphologically, any combination of cultivation medium led to the formation of roots and to flowering as long as the initial rooting was morphologically normal for the first three days. NaCl and KCl inhibited rooting and flowering at high concentrations (0.05 M and higher), but did not affect rooting and flowering at lower concentrations.

Results suggested the ability of the corm to fine-tune its metabolism according to environmental conditions. Furthermore they showed the corm ability to survive and to allow for the formation of a full plant with flower and new corms without the supply of any element. Our experiments did not go beyond 110 days to obtain mature new corms.

ACKNOWLEDGEMENTS

This work was supported in part by the University of Tehran (Interuniversities Grant # 31303371), Tehran, Iran, and in part by the J. and E. Research Foundation, Tehran, Iran.

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Figures

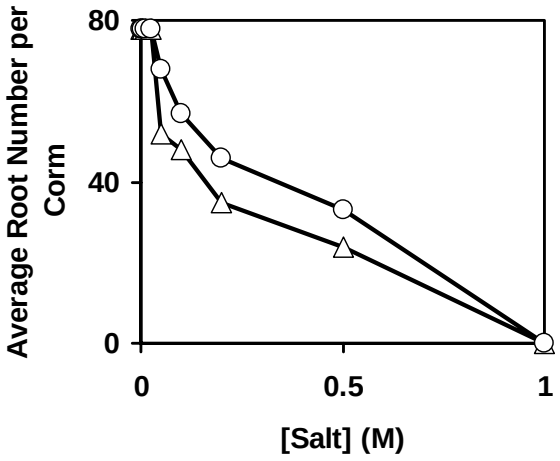

Fig. 1. Average root number for corms rooted for 6 days in various NaCl (△) or KCl (○) concentrations. Root number was the same in distilled water and all other cultivation media.

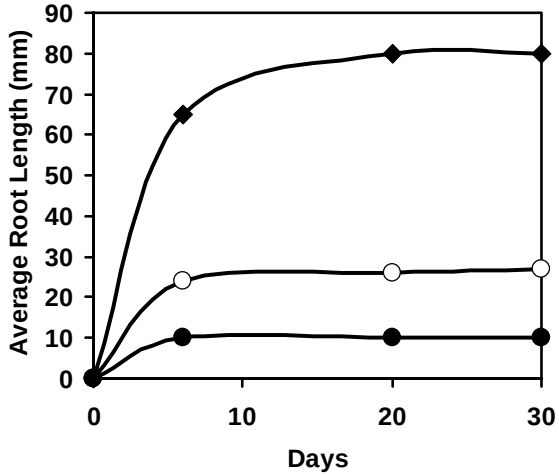

Fig. 2. Average root length for corms rooted in potted soil (♦), in distilled water (○), or in A medium (●).

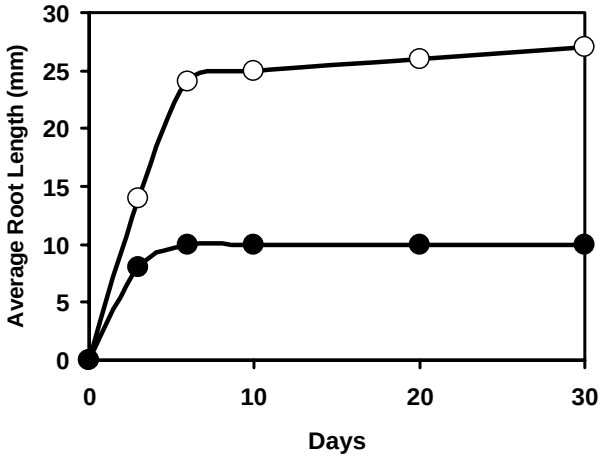

Fig. 3. Average root length for corms rooted in distilled water (○) or in A or B media (●).

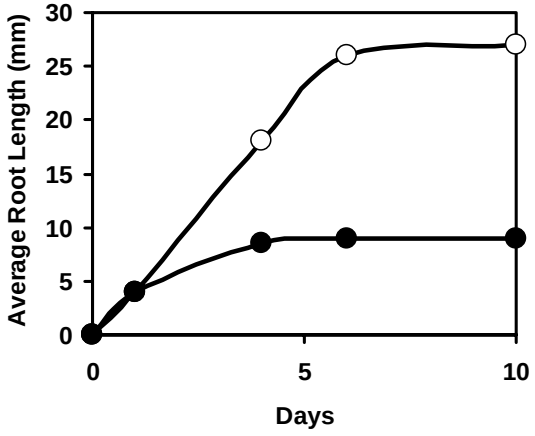

Fig. 4. Average root length for corms rooted in distilled water (○) or in A or B media diluted 4 times (●).

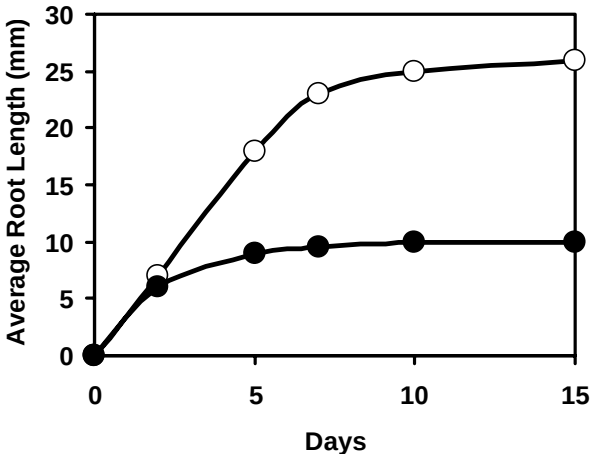

Fig. 5. Average root length for corms rooted in distilled water (○) or in A or B media diluted 20 times (●).

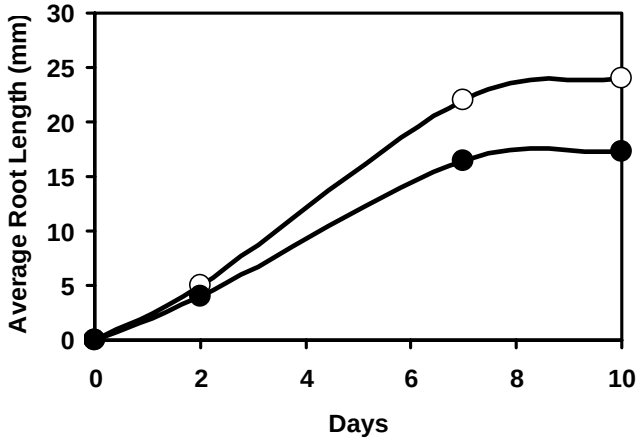

Fig. 6. Average root length for corms rooted in distilled water (○) or in A or B media diluted 100 times (●).

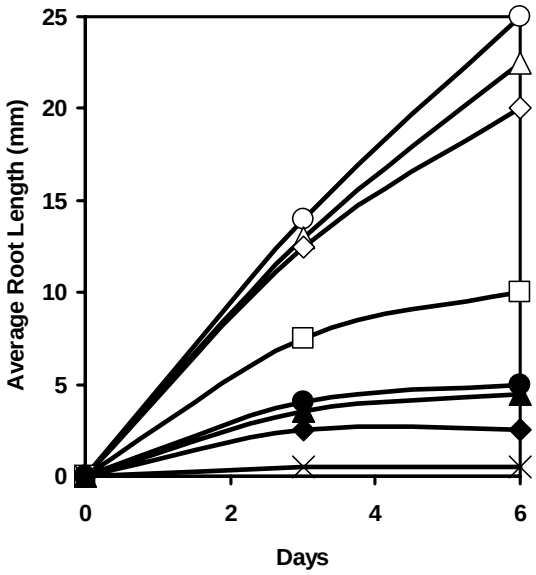

Fig. 7. Average root length for corms rooted in various NaCl concentrations. (○) Control; NaCl 0.005 M (△), 0.01 M (◇), 0.025 M (□), 0.05 M (●), 0.1 M (▲), 0.2 M (♦), 0.5 M (X). No roots were produced in 1 M or 2 M NaCl.

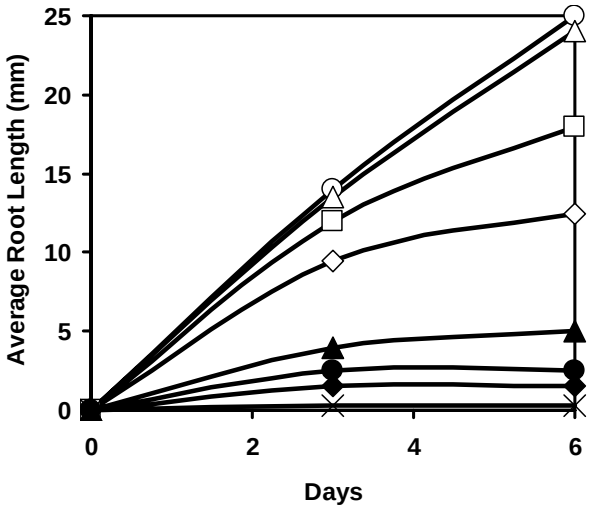

Fig. 8. Average root length for corms rooted in various KCl concentrations. (○) Control; KCl 0.005 M (△), 0.01 M (□), 0.025 M (◇), 0.05 M (▲), 0.1 M (●), 0.2 M (♦), 0.5 M (X). No roots were produced in 1 M or 2 M KCl.

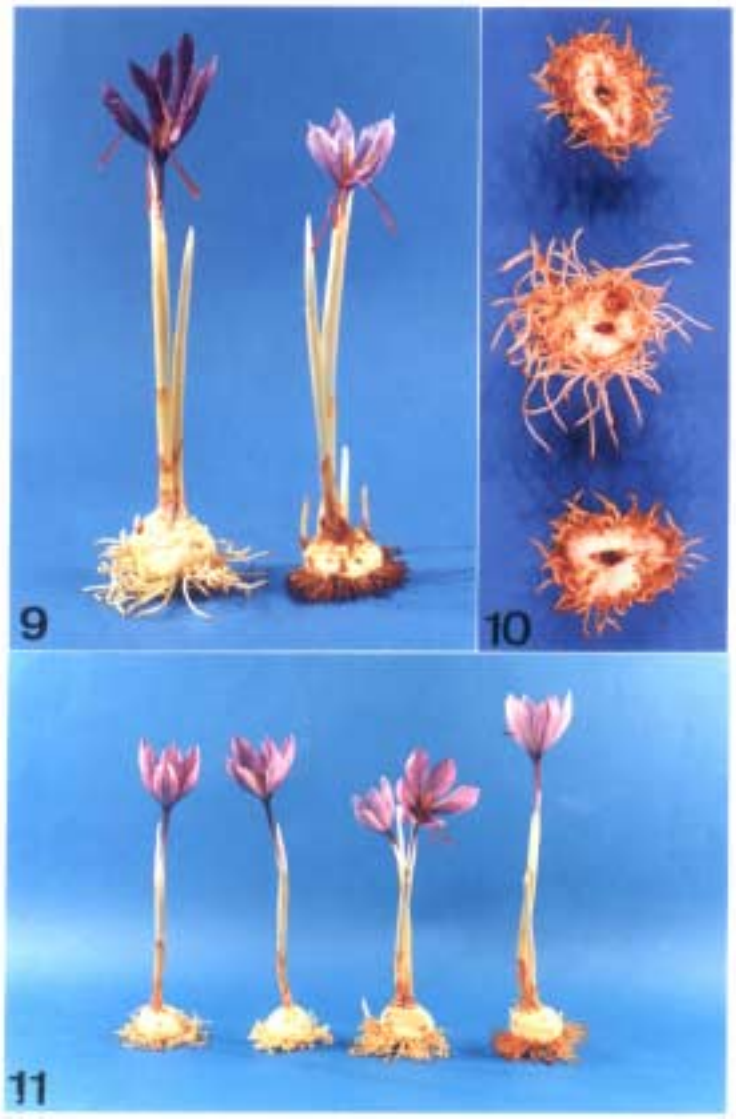

Fig. 9. Comparison between cultivation for 30 days in distilled water (left corm) and in A medium (see text) (right corm). Note that flowering was observed for both corms even though cultivation in medium A led to reddish and necrotic roots.

Fig. 10. Rooting aspect of corm cultivated for 22 days in distilled water (middle corm), A/100 medium (upper corm) and B/100 medium (lower corm).

Fig. 11. From left to right: corm cultivated in distilled water, 0.005 M KCl, 0.01M KCl, 0.025 M KCl. Note the reddish aspect of roots for corm cultivated in 0.025 M KCl.

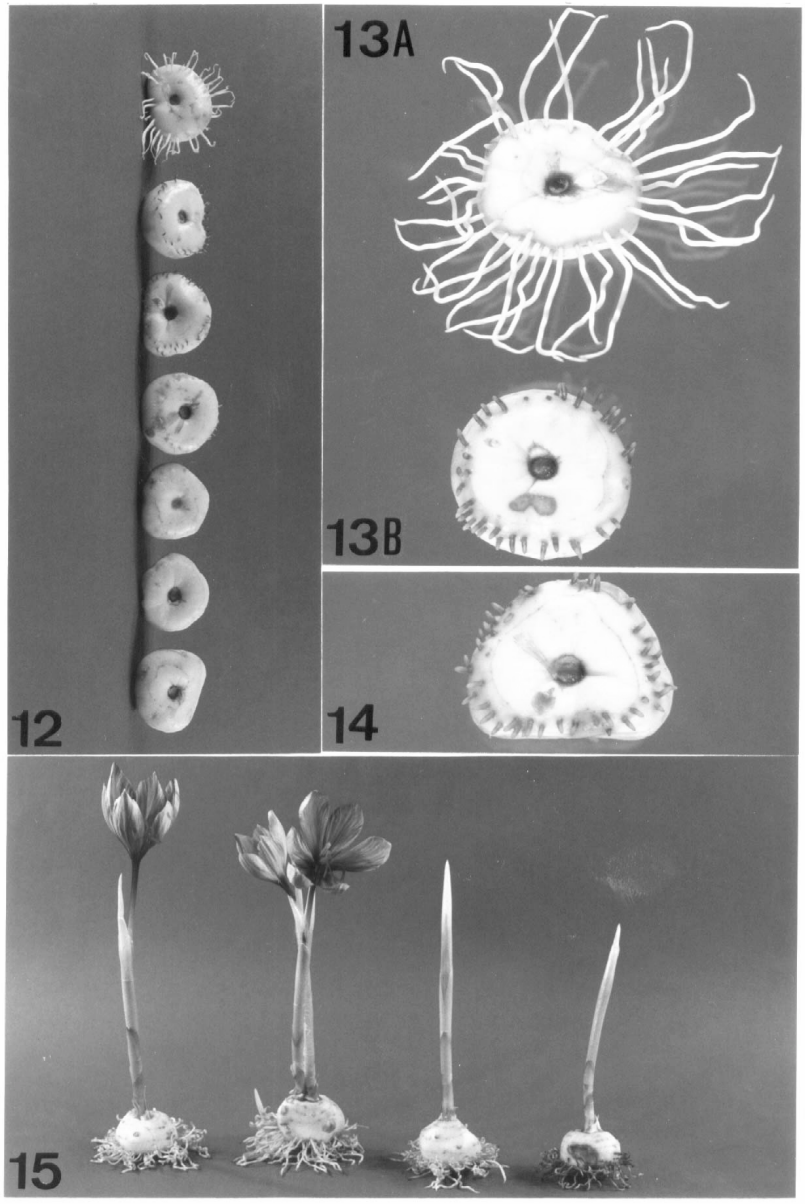

Fig. 12. Cultivation for 3 days in increasing NaCl concentrations. From top to bottom: corm cultivated in distilled water, 0.05 M, 0.1 M, 0.2 M, 0.5 M, 1 M and 2 M NaCl.

Fig. 13 and 14. Rooting after 6 days cultivation in distilled water (Fig. 13A), 0.05 M NaCl (Fig. 13B), 0.1 M NaCl (Fig. 14).

Fig. 15. Cultivation for 40 days in lower NaCl concentrations. From left to right: corm cultivated in distilled water, 0.005 M, 0.01 M and 0.025 M NaCl. Note that in spite of the necrotic aspect of the roots, flowers were visible in the shoots produced by the corms in 0.01 M and 0.025 M NaCl.

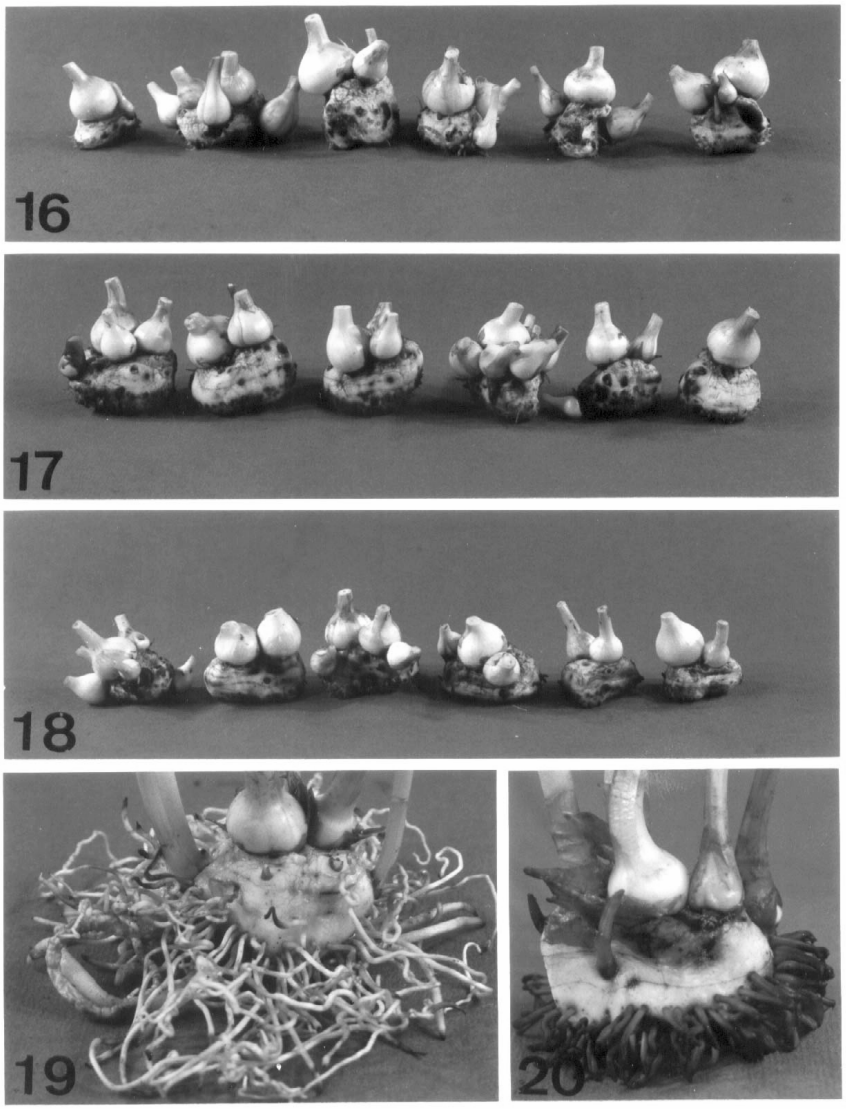

Fig. 16-18. New corm production from corms cultivated in distilled water (Fig.16), A/4 medium (Fig. 17), A/20 medium (Fig. 18). In each figure, corms shown were collected after 110 days (first two, left), 75 days (two in the middle), 60 days (last two, right).

Fig. 19. New corm production and root development in corm cultivated in distilled water for 50 days.

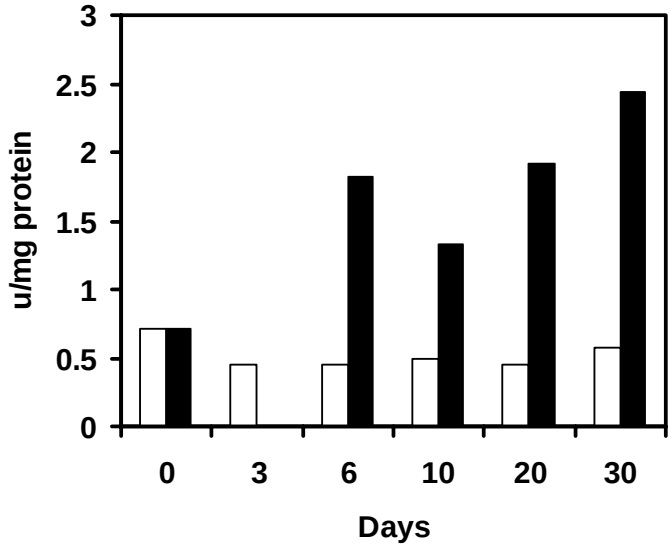

Fig. 20. New corm production and root development in corm cultivated in B medium for 50 days.

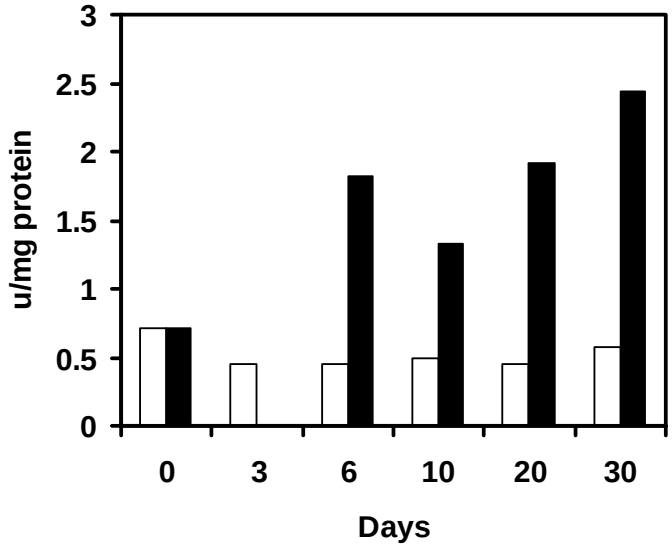

Fig. 21. Superoxide dismutase activity in corms at various times during rooting in distilled water (□) or in potted soil (■). Enzymatic activity was assayed at pH 7.8, with nitroblue tetrazolium 50 μM and riboflavin 2 μM.

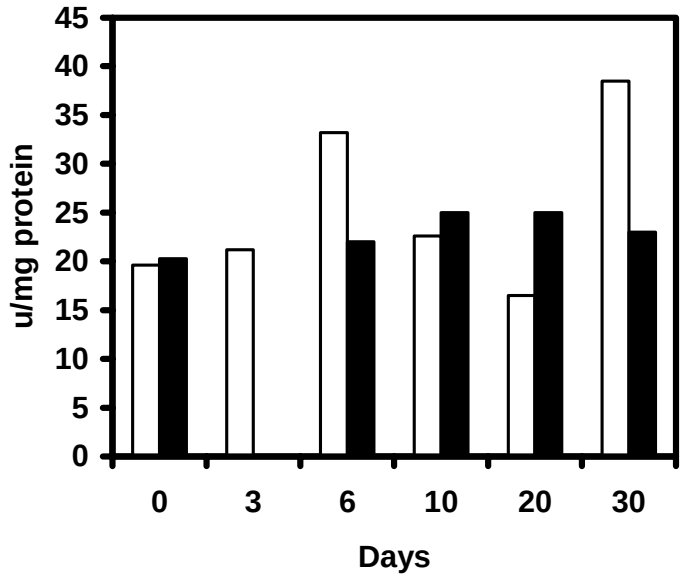

Fig. 22. Catalase activity in corms at various times during rooting in distilled water (□) or in potted soil (■). Enzymatic activity was assayed at pH 7.0 in the presence of 25 mM H_2O_2.

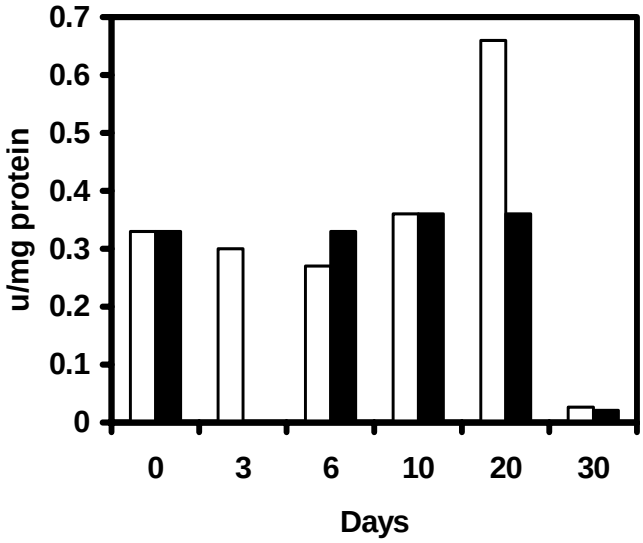

Fig. 23. Ascorbate peroxidase activity in corms at various times during rooting in distilled water (□) or in potted soil (■). Enzymatic activity was assayed at pH 8.0, with 1.6 mM H_2O_2 and 0.3 mM ascorbate.

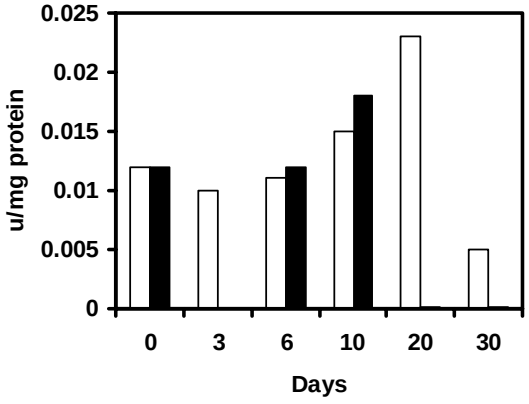

Fig. 24. Alcohol dehydrogenase activity in corms at various times of rooting in distilled water (□) or potted soil (■). Enzymatic activity assayed at pH 8.0, NAD^+ 3 mM, ethanol 0.35 mM. No activity detected after 20 or 30 days rooting in potted soil.

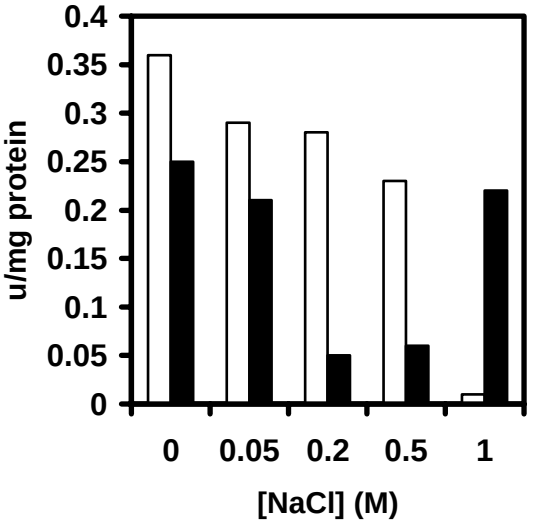

Fig. 25. Lactate dehydrogenase activity in corms rooted for 6 days in various NaCl concentrations. Enzymatic activity was assayed at pH 5.5 (□) and pH 9.5 (■) with 50 mM L-lactate and 0.5 mM $K_3Fe(CN)_6$.

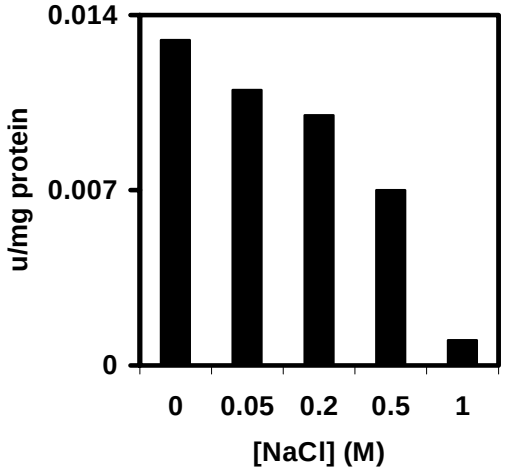

Fig. 26. Alcohol dehydrogenase activity in corms rooted for 6 days in various NaCl concentrations. Enzymatic activity was assayed at pH 8.0 with NAD^+ 3 mM and ethanol 0.35 M.

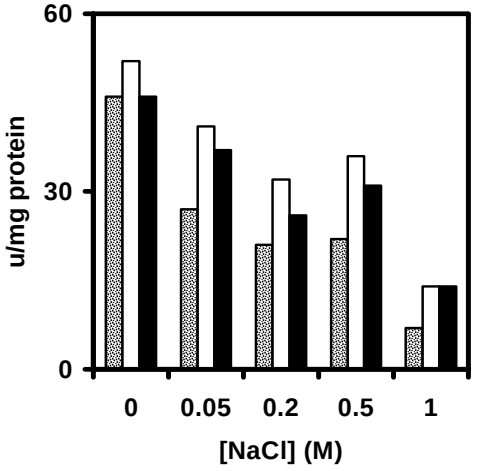

Fig. 27. Catalase activity in corms rooted for 6 days in various NaCl concentrations. Enzymatic activity was assayed at pH 6.0 (▒), pH 7.0 (□) and pH 9.0 (■) with 0.01 M H_2O_2.

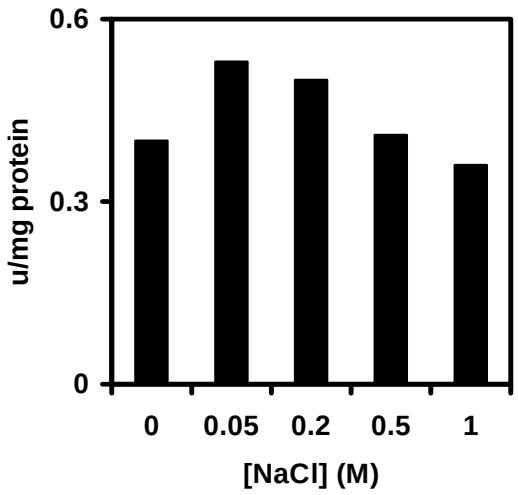

Fig. 28. Ascorbate peroxidase activity in corms rooted for 6 days in various NaCl concentrations. Enzymatic activity was assayed at pH 8.0 with ascorbate 0.3 mM and H_2O_2 1.5 mM.

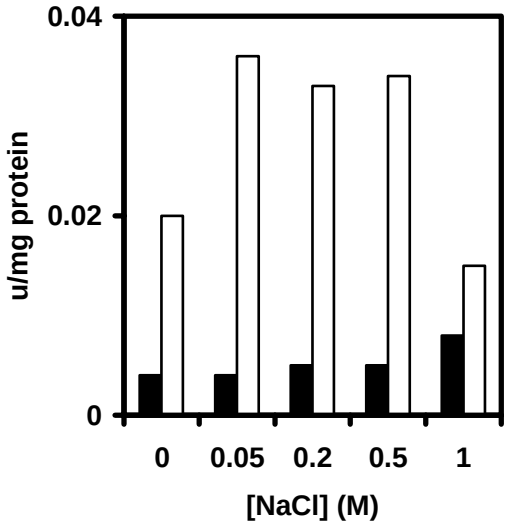

Fig. 29. Guaiacol peroxidases activity in corms rooted for 6 days in various NaCl concentrations. Enzymatic activity was assayed at pH 4.0 (■) and pH 6.5 (□) with 8.7 mM guaiacol and 0.27 mM H_2O_2.

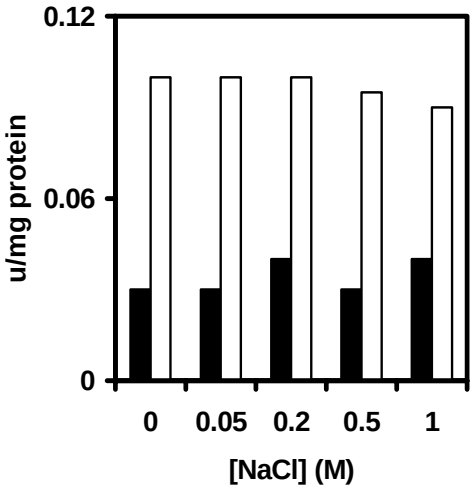

Fig. 30. Peroxidases (o-dianisidine) activity in corms rooted for 6 days in various NaCl concentrations. Enzymatic activity was assayed at pH 4.0 (■) and pH 6.5 (□) with 0.32 mM o-dianisidine and 0.27 mM H_2O_2.