ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 4 (4): 358-364, 2005
ISSN 1682-296X

Activities of Some Enzymes During Seed Germination of Some Leafy Vegetables under Saline Drainage Water

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Abstract: Seeds of five leafy vegetable crops were germinated to determine the effect of saline agriculture drainage water used as a resource to planting high value horticultural crops. Some glycolytic enzymes (Embeden-Meyerhof-Parnas: EMP) and Pentose Phosphate Pathways (PPP) enzymes [namely, Pyruvate Kinase (PK), lactate dehydrogenase (LDH), glucose 6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) were investigated in addition to alcohol dehydrogenase (ADH). The leafy vegetables included 'Red Giant' mustard greens [*Brassica juncea* L. (Czerniak)], 'Vitamen Green' salad greens [*Brassica rapa* L., (Narinosa Group)], pac choi [*Brassica rapa* L., (Chinensis Group)], 'Winterbor' kale [*Brassica oleracea* L., (Acephala Group)] and tatsoi [*Brassica rapa* L. (Narinosa Group)].. The enzyme activity levels differ with various varieties in response to different saline concentrations. However, in mustard greens PK and 6PGDH were inhibited while LDH, ADH and G6PDH were activated in response to salinity stress. In salad greens, salinity inhibited both ADH and 6PGDH whereas LDH activity was activated up to 15 dS m^{-1}. In addition, in salad greens PK and G6PDH were inhibited with low and activated with high saline concentrations. In tatsoi, salinity activated PK and ADH but inhibited LDH, G6PDH and 6PGDH. ADH and 6PGDH were activated in pac choi seedlings, while LDH was inhibited in response to salinity. PK and G6PDH in pac choi were inhibited at low and moderate (7, 11 and 15 dS m^{-1}) saline concentrations, but were stimulated at higher concentrations (19 and 23 dS m^{-1}). In kale, the response of the enzyme activities to salinity differed where PK and LDH from (EMP) and G6DH from (PP) pathways were enhanced with the increasing saline concentration, whereas ADH and 6PGDH (PPP) pathways were inhibited in response to the irrigation saline water. In conclusion, the enzymes of PP pathway and those of glycolysis expressed different response to salinity regardless the plant cultiver. The induction of some tested enzymes by salinity could be of a crucial importance in protecting the living cell.

Key words: Alcohol dehydrogenase, glucose 6-phosphate dehydrogenase, lactate dehydrogenase, 6-phosphogluconate dehydrogenase and pyruvate kinase, salinity, 'red giant' mustard greens [*Brassica juncea* L. (Czerniak)], 'Vitamen green' salad greens [*Brassica rapa* L., (Narinosa group)], pac choi [*Brassica rapa* L., (Chinensis group)], 'Winterbor' kale [*Brassica oleracea* L., (Acephala group)] and tatsoi [*Brassica rapa* L. (Narinosa group)]

INTRODUCTION

Many environmental factors play a role in quality success or failure of the crops in both irrigated and rained agriculture. In arid and semiarid region the supplies of good quality water are decreasing. One of the few on-farm water management options available to growers is the reuse of agriculture drainage effluents. This strategy is particularly attractive because significant amounts of good quality water are preserved. Also because the volumes of drainage water that require ultimate disposal are substantially reduced, pollutants that are attached to the soil particles (salts, nutrients, pesticides) can be transported with drainage effluent which often composed of salts with Na^+, SO_4^{2-},Cl^-, Mg^{2+} and Ca^{2+} predominating in that order. At each step in the sequence, the drainage water becomes progressively more salinized. Some high value leafy vegetable species belong to plant family *Brassicaceae*, whose relatives grow vigorously in saline environments. Shannon *et al.*[1] reported that nine species of vegetables from these taxa, are potentially useful in the drainage water reuse-system where only moderate salt tolerance required. Leafy vegetables are the primary source of mineral nutrients for human diets[2].

The availability, uptake and partitioning of mineral ions within the plant are controlled by numerous

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environmental factors, including the concentration and composition of solutes in the soil solution. Under saline conditions, mineral ion interactions in the external media may affect the internal requirements of essential minerals required for plant growth and development[3].

Carbohydrate on the one hand, acts in the capacity of an osmotic agent while on the other, it provides energy (ATP), reducing power (NADPH) and carbon skeletons for biosynthesis. The two pathways for the oxidation of carbohydrate in the tissues of higher plants are the Embden-Meyerhof-Parnas (EMP) and pentose phosphate pathway (PPP)[4]. Since enzymes controlling these two pathways they appear to occur in the cytoplasm[5].

The regulatory movement of glucose-6-phosphate into and through the two pathways is achieved by modulating enzyme levels and the concentrations of cofactors in the respective pathways. The two pathways have been shown to be operative simultaneously in many tissues and plant organs. The increased activity of PP pathway relative to EMP pathway has been implicated in germination of seeds[6,7], during shoot differentiation in cultured cells[8], as well as with increasing nitrate assimilation[9].

Salinity is reported to affect the enzymes of EMP and PP pathways differently[10,11].

Thus, the present study reports the effect of saline drainage water irrigation on the carbohydrate metabolism during seed germination and early seedling growth of the five vegetable species.

MATERIALS AND METHODS

Seeds of the following leafy vegetable crops were imported from Johnny's Selected Seeds, Albion, Maine: 'Red Giant' mustard greens [*Brassica juncea* L. (Czerniak)], 'Vitamen Green' salad greens [*Brassica rapa* L., (Narinosa Group)], pac choi [*Brassica rapa* L., (Chinensis Group)], 'Winterbor' kale [*Brassica oleracea* L., (Acephala Group)] and tatsoi [*Brassica rapa* L. (Narinosa Group)]. Seeds were surface sterilized and germinated aseptically. The seeds were washed and treated for one min with 95% ethanol, followed by rinsing in water several times before transferring to plastic Petri plates contain one standard blue germinating plotter prepared by rinsing in nutrient solution consisting of (mM): 3.5 Ca^{2+}, 2.5 Ng^{2+}, 21.5 Na^{+}, 6.0 K^{+}, 10.9 SO_4^{2-}, 7.0 Cl^{-}, 5.0 NO_3^{-}, 0.17 KH_2PO_4, 0.050 Fe (as sodium ferric diethylenetriamine pentaacetate), 0.023 H_3BO_3, 0.005 $MnSO_4$, 0.0004 $ZnSO_4$, 0.0002 $CuSO_4$ and 0.0001 H_2MoO_4. This solution with an Electrical Conductivity (Ec_i) of 3 dS m^{-1}, served as the control treatment. Irrigation waters (EC_i = 3, 7, 11, 15, 19 and 23 dS m^{-1}) were prepared to stimulate the high-sulfate, high-sodium saline drainage waters (Table 1). Seedlings were harvested after 15 days to further studies of salinity effects on enzyme activities.

Enzyme extraction: The crude enzyme extract was prepared by homogenizing 500 mg of the tissue in 5ml of 0.1 M Tris-HCl buffer (pH 7.5) at 4°C. The homogenate was centrifuged at 18,000 g for 30 min. The resulting supernatant was used as the crude enzyme preparation.

Enzyme assays: All enzyme activities were monitored at 30°C and expressed as μ mol of NADH or NADPH (oxidized or formed). The enzyme activity was expressed as μ mol g^{-1} fw min^{-1}.

Pyruvate kinase (EC 2.7-1.40; PK) was assayed after the method of De Luca and Dennis with modification of Misra and Dwivedi[12].

Lactate dehydrogenase (EC 1.1-1.27; LDH) was assayed as described by Srivastava *et al.*[13] with the modification of Misra and Dwivedi[12].

Alcohol dehydrogenase (EC 1.1-1.71; ADH) activity was determined as described by Srivastava *et al.*[13] with the modification of Misra and Dwivedi[12].

Glucose 6-phosphate dehydrogenase (EC 1.1-1.49; G6PDH) activity was determined according to the method of Simcox *et al.*[14] by monitoring NAD^{+} reduction at 340 nm.

6-Phosphogluconate dehydrogenase (EC 1.1-1.44; 6PGDH) activity was determined by the method of Simcox *et al.*[14] with the modification of Misra and Dwivedi[12].

The variation in the treatments in relation to plant species was assessed using the one-way analysis of variance (ANOVA). All the values are mean of five replicates±SE.

RESULTS

PK activity in mustard greens was variably inhibited in response to the salinity (Fig. 1). The presence of low levels of salinity (7, 11 and 15 dS m^{-1}) has resulted in a

Table 1: Salinizing ions concentration in solutions used to irrigate the germinated seeds

ECi (dS m^{-1})	Ca^{2+} (mol m^{-3})	Mg^{2+} (mol m^{-3})	Na^{+} (mol m^{-3})	SO_4^{2-} (mol m^{-3})	Cl^{-} (mol m^{-3})
3	3.5	2.5	21.5	10.5	7.0
7	7.3	5.7	50.9	25.9	24.7
11	10.1	9.8	87.0	42.0	42.2
15	13.0	13.9	123.0	58.2	59.6
19	13.5	18.9	168.0	75.2	81.3
23	13.6	24.3	215.6	93.5	98.4

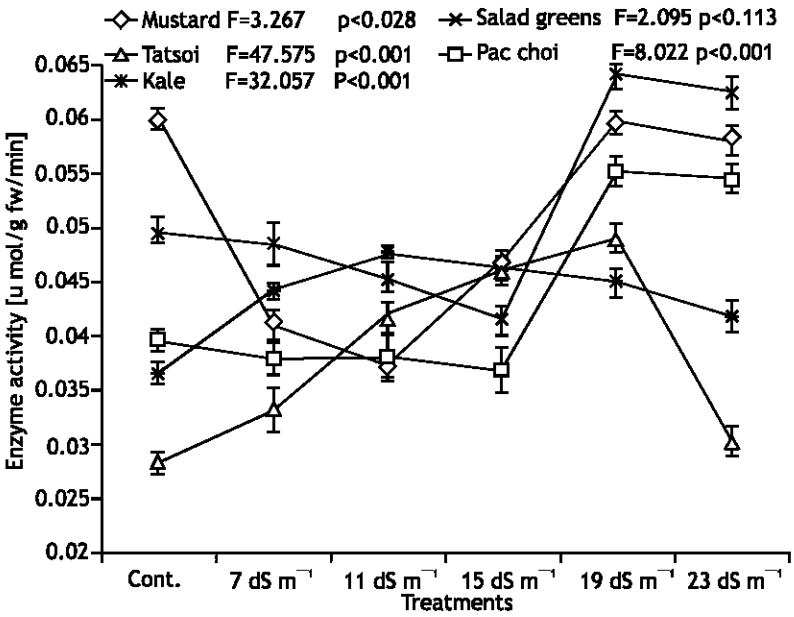

Fig. 1: Effect of increasing salinity on the activities of pyruvate kinase enzyme involved in carbohydrate metabolism of selected species of *Brassicaceae*. Values are means of 5 replicates

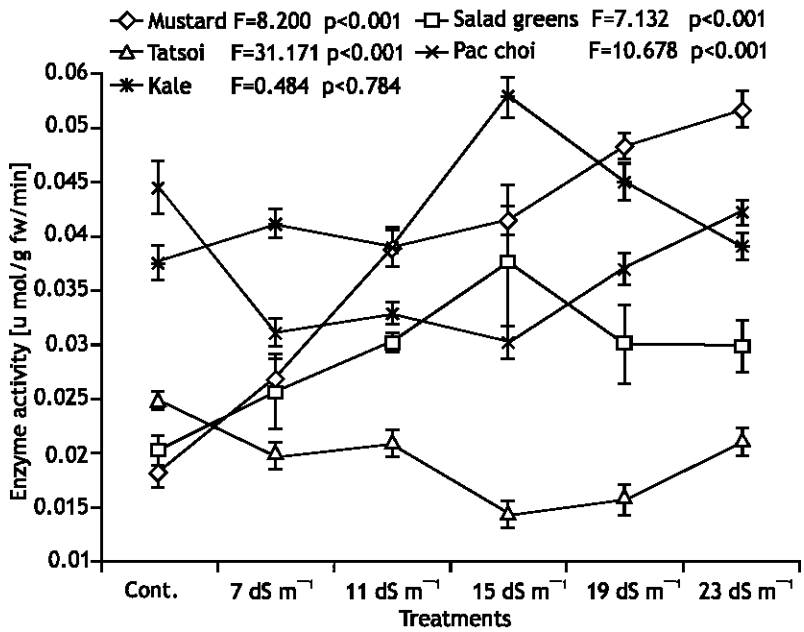

Fig. 2: Effect of increasing salinity on the activities of lactate dehydrogenase enzyme involved in carbohydrate metabolism of selected species of *Brassicaceae*. Values are means of 5 replicates

30% decrease in the enzyme activity, while the high level of salinity (19 and 23 dS m^{-1}) reduced the activity with about 3%. In contrast, the PK activity in tatsoi was increased up to 17.03, 48.75 and 62.81% with 7, 11 and 15 dS m^{-1}, respectively. PK activity has reached its maximum increase 73.3% with 19 dS m^{-1} then dropped to 6.58% with 23 dS m^{-1} salinity. In kale PK activity reached its maximum increase at 11 dS m^{-1} then decreased

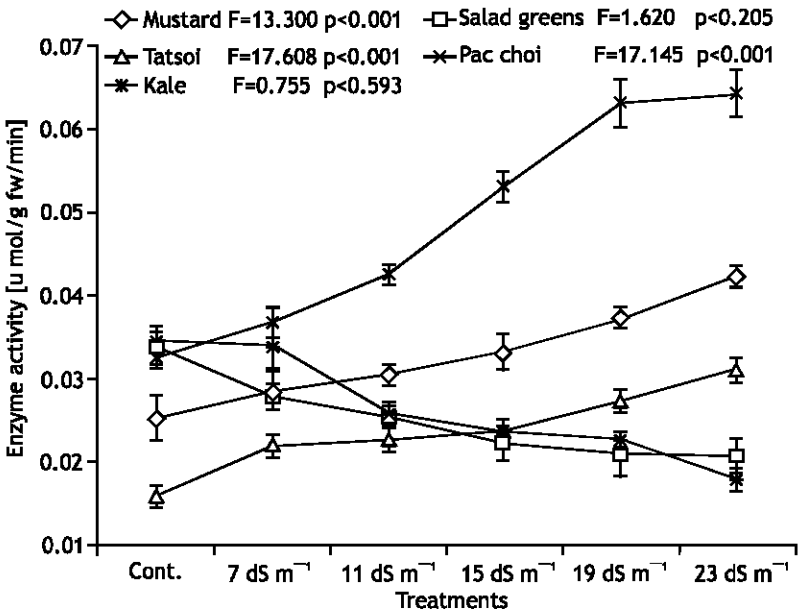

Fig. 3: Effect of increasing salinity on the activities of *Brassicaceae*. Values are means of 5 replicates

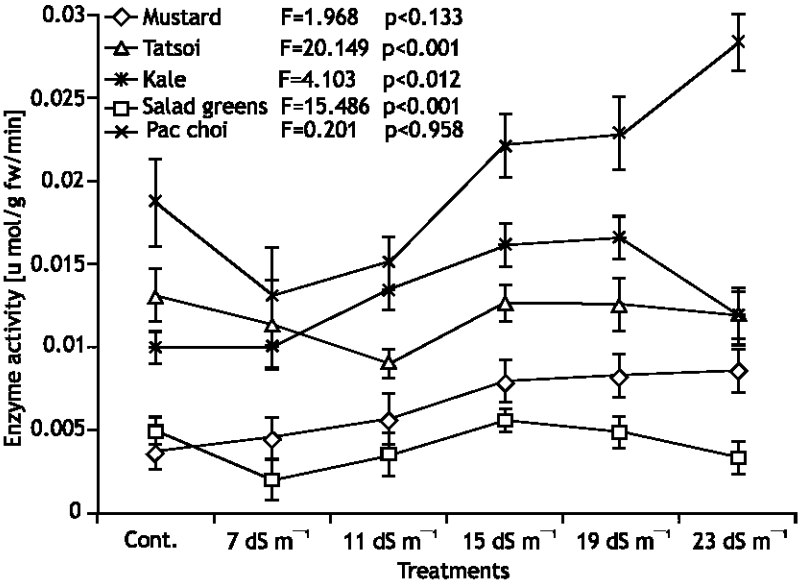

Fig. 4: Effect of increasing salinity on the activities of glucose 6-phosphate dehydrogenase enzyme involved in carbohydrate metabolism of selected species of *Brassicaceae*. Values are means of 5 replicates

gradually to its minimum with 23 dS m^{-1}. In salad greens and pac choi the lower salinity levels (7, 11 and 15 dS m^{-1}) slightly decreased the PK activity. However, the activity was increased in response to the higher salinity levels 19 and 23 dS m^{-1}.

In mustard, LDH activity (Fig. 2) was increased gradually with the increase from 49.071 to 189.306% with 7 and 23 dS m^{-1}, respectively. In salad greens and kale LDH activity was increased and reached its maximum at 15 dS m^{-1} followed by a decline to 3.717%, particularly at 23 dS m^{-1} in kale. In contrast, in tatsoi and pac choi the activity increased up to 15 dS m^{-1} then enhanced slightly at 19 and 23 dS m^{-1}.

ADH activity in mustard greens, tatsoi and pac choi was increased gradually with the increase in saline concentrations until it reached its maximum activity at

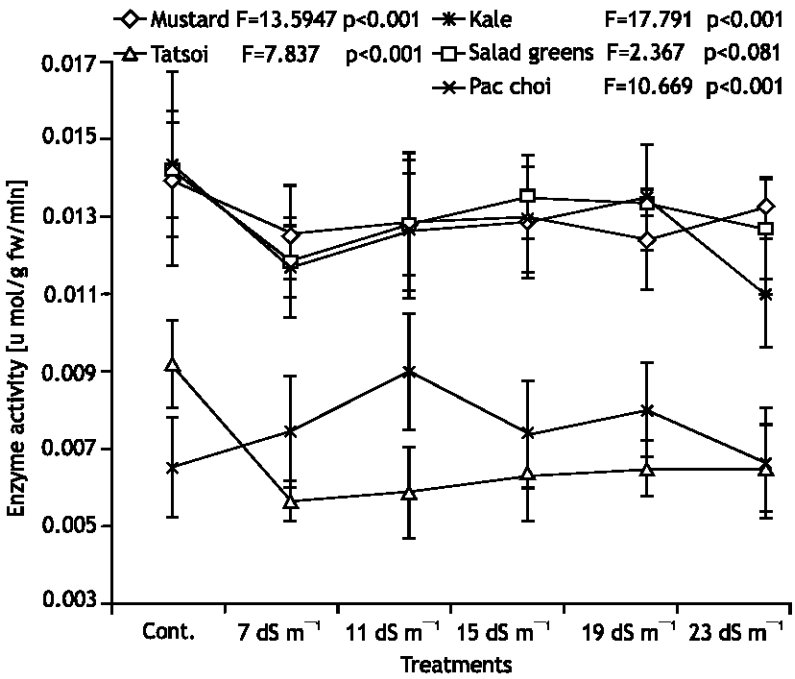

Fig. 5: Effect of increasing salinity on the activities of 6-phosphogluconate dehydrogenase enzyme involved in carbohydrate metabolism of selected species of *Brassicaceae*. Values are means of 5 replicates

23 dS m^{-1} (Fig. 3). On the other hand, ADH in kale and salad greens behaved similarly throughout the various tested concentrations, where the activity decreased gradually with increasing salinity. However, ADH activity at 23 dS m^{-1} was higher in salad greens than in kale.

However, in mustard greens the activity of G6PDH was increased with increasing salt concentration until it reached its maximum at 23 dS m^{-1} (Fig. 4). Moreover, in kale and salad greens G6PDH behaved in a similar way to that in mustard greens but its activity was decreased at 23 dS m^{-1}. On the other hand, G6PDH of tatsoi have fluctuated between a decrease and an increase depending on saline levels. In contrast, the enzyme activity in pac choi seedlings was inhibited at both 7 and 11 dS m^{-1} then increased progressively and reached its maximum at 23 dS m^{-1}.

In contrast, 6PGDH activity showed a similar pattern in seedling of studied plants varieties (mustard greens, salad greens and tatsoi) within salinity concentration between 7 and 19 dS m^{-1} (Fig. 5). However, at 23 dS m^{-1} the activity of 6PGDH has declined in both salad greens and kale, while increased in mustard greens. On the other hand, 6PGDH in pac choi behaved differently where its activity fluctuated between the increase and decrease depending on salinity levels. In contrast, the 6PGDH activity was decreased in tatsoi in the response to salinity stress.

DISCUSSION

The present investigation monitored some changes in the activity of some enzyme caused by salt stress. Nevertheless, very little information is available regarding the relative salt tolerance of plants at different stages of development[15]. The results obtained to date are not clear, even though plant response to salinity has been one of the most widely researched subjects[16]. The present results show that the activity of the tested enzymes differed in response to different levels of salinity. Not only activity was dependent on the salinity level but also on the variety. In this regard, Poljakoff- Mayber *et al.*[11] has reported that salinity affected the activities of enzymes involved in EMP and PP pathways. Different responses to salinity have been reported between germinating and growing seedlings of a number of halophytes[17]. Ungar[18] observed that seeds of *Atriplex patula* were less affected by salinity than the growing plants.

The sensitivity of plants to salinity may depend on their developmental stage[19]. Olmos and Hellin[20] suggested that calli of *Pisum sativum* adaptation to NaCl might depend on the modification of the osmotic adjustment through activation of enzymes responsible for reducing sugars, together with modification in physiological and biochemical parameters.

In the present investigation G6PDH was activated by salinity in mustard and kale. Also, LDH was also activated in salad greens, mustard and kale under the effect of salinity. Such increase in LDH is in harmony with those of Misra and Dwivedi[12] who showed that salinity induced the activity of LDH and ADH in *Phaseolus aureus*. Similar results were reported for alcohol dehydrogenase from *Spartina sp* and bacteroid cytoso[21,22]. Furthermore, LDH and ADH activities were increased in roots of wheat exposed to salinity[23].

In contrast, both *in vivo* and *in vitro*, NaCl inhibited ADH and RUBP carboxylase. Cl^- inhibited ADH and was competitive towards NAD^+ and NADH and ethanol as substrate. Cl^- was a mixed function inhibitor (competitive/noncompetitive) towards acetaldehyde and the inhibition constant for ADH was approx. 10-21> mM. The bending site was possibly the central Zn^{2+} atom present in the ADH active site[24]. G6PDH activity was decreased with salinity in various cultivars of rice[25,26] and salinity has reduced G6PDH activity in two members of Cyamobacteria *Phormidium* and *Nostoc*[27].

The reduction of some examined enzymes in the present investigation by saline water could be explained on the basis that excess Na^+ and Cl^- in plant tissues are toxic since these ions can disrupt the structure and function of enzymes. Furthermore, high soil Na^+ can inhibit uptake of K^+ and high Na^+: K^+ ratio in tissue further inhibits enzyme function[28].

As a general observation, each of G6PDH and 6PGDH (PPP enzymes) have exhibited the lowest activity compared to the other examined enzymes. Also, PK activity was the highest one compared to the other investigated enzymes in most of the tested plants.

Moreover, the increase in the activity of some examined enzymes by salinity stress can be due to *de novo* synthesis of enzyme protein and/or modulating the activity of some existing enzymes. It is possible that the presence of moderate concentration of the salt changes the tertiary and quaternary structure of the enzyme protein so as to give a more active form. This implies that the binding of the substrate to its enzyme is affected by NaCl and cooperatively increases. Also, if the salt is present in supraoptimal concentration it may cause hystresis and consequently inactivation of the enzyme and a decrease in its activity[29]. Therefore, the induction of enzyme synthesis by salinity stress may be crucial in protecting the living cells.

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