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Enzyme Activity, and Ethanol Accumulation as Related to Zinc Uptake in Four Varieties of Beans (Phaseolus Vulgaris L.) Under an Aeration Stress.

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Normah Mohamad Noor

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W. W. Adams

Major professor

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ENZYME ACTIVITY AND ETHANOL ACCUMULATION AS RELATED TO ZINC UPTAKE IN FOUR VARIETIES OF BEANS (*PHASEOLUS VULGARIS* L.) UNDER AN AERATION STRESS

By

Normah Mohamad Noor

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ABSTRACT

ENZYME ACTIVITY AND ETHANOL ACCUMULATION
AS RELATED TO ZINC UPTAKE IN FOUR VARIETIES
OF BEANS (PHASEOLUS VULGARIS L.) UNDER
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Relationships between alcohol dehydrogenase and pyruvate decarboxylase activities, ethanol accumulation, and zinc concentration in the roots grown under aerated and anaerobic conditions were studied. A greenhouse experiment was conducted using four varieties of Phaseolus vulgaris L.; Seafarer, MSU 31908, NEP-2, and San Fernando grown at 0 and 0.2 ppm levels of zinc. An anaerobic condition was established by flooding at flowering for thirty-six hours.

Variety Seafarer had the highest ethanol accumulation, the highest alcohol dehydrogenase activity and the highest zinc concentration in the roots. MSU 31908 had the least response to treatments except for greater pyruvate decarboxylase activity. NEP-2 and San Fernando showed intermediate responses. Alcohol dehydrogenase activity and ethanol accumulation were hypothesized to be correlated with zinc concentration in roots. The varietal differences were then explained according to the developed hypothesis.

To my father.

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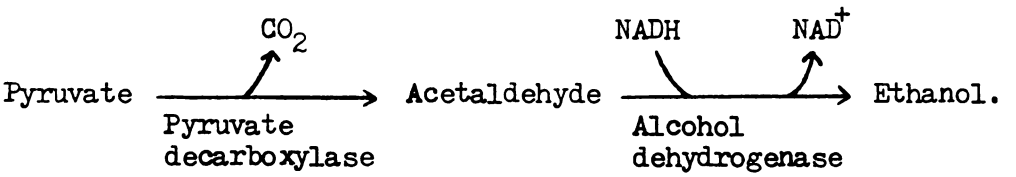
INTRODUCTION

Anaerobic conditions in soil can be caused by a period of heavy rainfall. When a soil is flooded, oxygen concentration in the soil becomes limiting for plant growth. The anaerobic condition is enhanced especially in a compacted soil where poor drainage exists.

Oxygen deficiency for a one day period can have a great influence on growth and on yield of bean plants (Smucker, 1975). Navy beans were found to be susceptible to poor soil drainage and to aeration stress, especially at the pre-blossom stage. Twenty-four and forty-eight hours of flooding significantly reduced bean yield (Smucker et al., 1978).

Navy beans (Phaseolus vulgaris var. Seafarer) also produce a large quantity of ethanol during short periods of oxygen stress to plant roots and with greater accumulation when the stress is imposed at the flowering stage. The production of ethanol is due to the limited oxidation in the mitochondria, thus inducing this anaerobic respiration reaction:



An increase in alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) activity is associated with the production of ethanol.

Alcohol dehydrogenase in turn requires zinc as a component of its active structure, while pyruvate decarboxylase requires Mg^{++} as its cofactor. Since zinc deficiency is one of the major production problems for growing beans in Michigan, and the bean crop is always fertilized with a zinc-containing fertilizer, a correlation of ethanol production and alcohol dehydrogenase with zinc uptake can be postulated.

Thus, the objectives of this research were: (1) To determine the activity of enzymes ADH and PDC in four varieties of field beans (Phaseolus vulgaris L.); i. Seafarer, ii. MSU 31908, iii. NEP-2, iv. San Fernando under normal and aeration stress conditions. (Seafarer and NEP-2 have been found earlier to be susceptible to aeration stress while the other two were more tolerant to the condition (Adams et al., 1977)); (2) To determine the amount of ethanol produced in the xylem exudate; (3) To compare ethanol accumulation and ADH activity with the amount of zinc in the roots; (4) To fit the individual observation into a working hypothesis or a model which is consistent with field observations of other workers and the result of other experiments.

LITERATURE REVIEW

Oxygen is a limiting factor for root and plant growth under water-saturated soil conditions. It has been shown that diffusion of oxygen through liquid is in the order of 10^4 times slower than in air (Steward, 1960). Gas exchange is also reduced as water films around the root becomes larger (Grable, 1966). In a saturated soil the oxygen concentration generally approaches zero twenty-four hours after flooding (Van Doren, 1958; Purvis and Williamson, 1972).

Unger and Danielson (1965) suggested that reduced oxygen supply rather than a build-up of carbon dioxide might be responsible for reduced growth of young corn plants under poorly aerated soil. Williamson (1970) demonstrated that tobacco was permanently injured by flooding and this injury was primarily due to the lack of oxygen in the root zone and not due to excess carbon dioxide. Later, Purvis and Williamson (1972) also showed that the primary cause of injury and reduced growth of flooded corn was due to the lack of oxygen.

Much work has been done on the effects of flooding on plant roots and plant growth. Kramer (1951) showed that twenty-four hours of flooding was sufficient to produce serious injury to the roots of tomatoes, sunflower, and tobacco. Kramer also concluded that flooding causes a rapid decrease in the capacity of roots to absorb and conduct water. Flooding of more than twenty-four hours duration, can result in permanent injury (Kramer and Jackson, 1954). Reduced growth of soybeans,

corn, and sorghum was noted when such crops were grown at a high water-table (Williamson, 1964). Williamson (1970) also showed that pure nitrogen treatment imposed on tobacco for twenty-four hours essentially prevented further growth and after forty-eight hours the roots and shoot were essentially dead.

A reduction in growth can and usually does lead to a reduction in yield. Tomatoes can be severely stunted and the yield reduced if oxygen deficiency occurs early in the life of the plant (Erickson and Van Doren, 1960). They also showed that yield of peas can be reduced by one-third by a one-day oxygen deficient period at the early bloom stage. Oxygen deficiency also decreased corn, sorghum and sugarbeet yields (Williamson, 1964; Erickson and Van Doren, 1960). The magnitude of the reduction in yield, however, depended on the species and variety of the plant and its stage of development as well as on light, temperature, fertility, etc. (Erickson and Van Doren, 1960).

Kramer (1951) suggested that injury of the roots and death of the leaves may be caused at least in part by toxic substances moving up from the dead roots or even from the surrounding soil. Kenefick (1962) found that ethanol accumulated in sugar beet under oxygen stress. Later, Fulton (1963) showed that ethanol appeared in xylem exudates of tomatoes when the soil supplied less than 38×10^{-2} ug oxygen cm^{-2} min^{-1} and the ethanol concentration increased as supply of oxygen was further reduced. Ethanol concentration of xylem exudate samples taken from plants flooded in the light was greater than from plants flooded in the dark (Fulton and Erickson, 1969).

In Crawford's (1967) study, there was an increase in ethanol production in flood-sensitive plants. However, in rice, a flood-tolerant

plant, ethanol was also produced under anaerobic conditions (App and Meiss, 1958; John and Greenway, 1976; Avadhani et al., 1978). Natural anaerobiosis during germination also induces ethanol production (Leblova et al., 1969; Leblova et al., 1974; Avadhani et al., 1978).

Ethanol was shown to be toxic to tomato plants when added to Hoagland water culture media with concentrations corresponding to those observed in the xylem exudates (Fulton, 1963). Kiyosawa (1975) showed in Nitella that alcohol molecules interact with the cell membranes to make equivalent pore radii of the membranes narrower without changing the nature of the water flow, causing a decrease in water permeability. This might be the cause of the reduction of the absorption and translocation of water by bean plants in an anaerobic condition, as shown by Smucker (1975).

App and Meiss (1958) showed that ADH (alcohol dehydrogenase) activity increased in proportion to ethanol production. ADH activity has also been reported to increase during flooding in flood-sensitive plants (Crawford, 1967), in corn seedlings (Hageman and Flesher, 1960), in flood-sensitive subspecies of Trifolium subterraneum (Francis et al., 1974), and in rice (John and Greenway, 1976; Wignarajah et al., 1976). ADH activities were also found in natural anaerobiosis of germinating seeds (Cossins and Turner, 1962; Kolloffel, 1968; Leblova et al., 1969; Leblova et al., 1974).

John and Greenway (1976) also showed that an increase in PDC (pyruvate decarboxylase) activity in rice is maximum after twenty-four hours of anaerobiosis. They also showed that absolute activities of PDC were about fifteen times lower than for ADH and the degree of increase in activity in response to anaerobiosis was smaller for PDC

than for ADH.

An increase in ADH activity was found to be due to de novo synthesis of the enzyme (McManmon and Crawford, 1971; John and Greenway, 1976). App and Meiss (1958) suggested that ethanol concentration in rice shoots might control ADH activity, however, acetaldehyde was found to be a natural inducer of ADH in corn seedlings by Hageman and Flesher (1960); in Senecio intolerant species by Crawford and McManmon (1968), and in germinating seeds by Leblova et al. (1974).

John and Greenway (1976) suggested that PDC activity may be enhanced by an increase in NADH levels and a decrease in NAD^+ levels, which occur during anaerobiosis.

Vallee and Hock (1955) showed that the ADH of yeast is a zinc metalloenzyme containing four moles of zinc firmly bound to one mole of protein. They also showed that the activity of the enzyme is directly dependent on zinc. Zinc atoms are thought to stabilize the quarternary structure of the enzymes through the formation of bridges between monomers to form the enzymatically active tetramer (Kagi and Valle, 1960).

Zinc deficiency occurs mostly on calcareous soils (Thorne, 1957). High pH causes the solubility of Zn^{2+} in soils to decrease and thereby reduces the uptake and availability of zinc to plants (Linsay, 1972). High soil phosphorus levels also induce zinc deficiency by restricting zinc movement within the plant, resulting in accumulation in the roots and deficiency in the tops (Vitosh et al., 1973).

In Michigan, zinc deficiencies have been identified in navy beans, especially in the Sanilac variety (Robertson and Lucas, 1976). The Saginaw variety, however, is less affected by low zinc availability in

the soil (Ellis, 1965; Polson, 1968).

Smucker (1977) later found that greater accumulation of ethanol occurred in the xylem exudates of flooded navy bean plants having greater quantities of tissue zinc than plants with lower concentration of zinc. Thus, the production of ethanol in xylem exudates might be further induced by higher application of zinc.

MATERIALS AND METHOD

Four varieties of field beans (Phaseolus vulgaris L.); Seafarer, MSU 31908, NEP-2 and San Fernando were grown in one gallon plastic milk bottles filled with acid-washed pea-sized gravel. The gravel was washed with distilled water twice to remove the acid.

The plants were grown in a modified Hoagland's solution (Shellenberger, 1970) containing 300 ppm P and Zn concentrations of 0 and 0.2 ppm by formulation. In actuality, the concentrations of Zn at the low level was 0.03 ppm, and at the high level was 0.23 ppm, as determined by analysis of leachate. Phosphorus concentration was increased above Hoagland's to insure zinc deficiency. The micronutrients were according to Hoagland with the exception of the varying Zn concentrations.

Each pot was watered with 300 ml of nutrient solution three to four times a day. The nutrient solution was collected in an acid bottle and reused for two or three days after which it was collected and tested for zinc using a Perkin-Elmer model 303 atomic absorption spectrophotometer.

The plants were grown in a greenhouse under natural light supplemented by artificial light from gro-lux tubes, providing a total intensity of 214 uE m^{-2} sec^{-1}. The photoperiod was 14 hours.

The design of the experiment was a split-plot with plots being completely randomized. The plots were the flooding and zinc levels

while the sub-plots were the varieties.

At flowering plants were flooded with nutrient solution for 0 and 36 hours. The control plants were never under water stress as they were watered normally. There were also no visible symptoms of water stress appeared for both non-flooded and flooded plants.

Flooding was accomplished by stopping drainage from the pots and completely submerging the gravel and roots. After 36 hours xylem exudates were collected from all plants by severing the stem at the first internode from the root and attaching a piece of surgical rubber tubing to the excised stem. When a sufficient sample of exudate accumulated in the tubing, the stem was cut and the open end of the tubing was closed by folding and tying it with a piece of copper wire. The entire sample was held in a labelled test-tube and frozen until ready for ethanol analysis.

Shoots were weighed and dried. Roots were cleaned of gravel and kept in a $5^{o}C$ room until ready for extraction.

Enzyme Extraction and Assay

Root extraction procedure used for alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) was essentially the same as that reported by John and Greenway (1976). Root tissue was homogenized in a high-speed blender. The extraction buffer (5 ml per gram fresh weight) contained 50 mM HEPES (N-2-hydroxyethyl piperazine-N'-2 ethanosulphonic acid), 5 mM $MgCL_2$ (magnesium chloride), 2 mM cysteine hydrochloride and 2% (w/v) PVP-40 (polyvinyl pyrrolidone). TPP (thiamine pyrophosphate) at 0.5 mM was included for extracting PDC. Extraction buffer was made up to pH 8.0 for ADH and pH 6.0 for PDC using KOH (potassium hydroxide).

After filtration through Miracloth (Chicopee Mills, Inc.),

extracts were centrifuged for 20 minutes at 25,000 g. Crude extracts (supernatants) were desalted on Sephadex G25 (coarse) columns, length 2 cm and column width 1 cm. The first 2 ml was collected for assay.

Residues were dried for zinc determination.

Enzymes were assayed at 28°C by spectrophotometric determination of oxidation or reduction of pyridine nucleotides at 340 nm. ADH assay contained 0.48 mM pyrophosphate buffer, pH 8.8, 1 mM ethanol, 0.025 mM NAD (Nicotinamide adenine dinucleotide), and 0.1 ml of enzyme (Worthington, 1978). PDC assay contained 0.186 M citrate buffer, pH 6.0, 30 mM pyruvate, 0.32 mM NADH (reduced nicotinamide adenine dinucleotide), 33 ug/ml of ADH and 0.02 ml of enzyme solution (Bergmeyer, 1974).

A_{340}/minute was calculated from a linear portion of the curve. Enzyme activity was determined by the following calculation:

$$\text{Units/mg protein} = \frac{A_{340}/\text{min}}{6.2 \times \text{mg protein/ml reaction mixture}}$$

Protein Analysis

The method of Lowry et al. (1951) was used for protein analysis. Protein assay consisted of 1 ml of Reagent C (alkaline copper solution which is a mixture of 50 ml of Reagent A (2% Na_2CO_3 in 0.10 NaOH) and 1 ml of Reagent B (0.5% $CuSO_4 \cdot 5H_2O$ in 1% solution tartrate)), 0.10 ml of Reagent E (diluted Folin reagent), and 0.2 ml of protein sample.

Readings were made at 660 nm on a Beckman DU spectrophotometer. Protein values were calculated from a standard curve.

Ethanol Analysis

1 microliter of the xylem exudate was injected in the column of gas chromotograph:

Model: Beckman GC 72-5

Column: 6' x $\frac{1}{4}$"

Packing: PPQ 100/200 mesh

Temperature: 150°C

Flow rate: 60 ml/minute

Detector: Hydrogen flame

The recorder was equipped with an integrator. Ethanol concentration was calculated by counting the number recorded by the integrator for standards and for samples and using the equation:

$$\text{Ethanol ppm} = \frac{\text{unknown}}{\text{standard}} \times \text{ppm of the standard}$$

Zinc Analysis

Root residues were ground in a Wiley mill to pass a 20-mesh screen. Samples of one half to one gram were used. They were dry-ashed in porcelain crucibles at 500°C for four hours. The ash was moistened with deionized water and was taken up in 5 ml of 2N HCl and filtered through a Whatman No. 2 filter paper. Four rinsings of 10 ml each were made with deionized water: the crucible twice, the filter paper once and the funnel once. The final solution volume was made up to 50 ml with deionized water. The resulting solution was then analyzed for zinc with a Perkin-Elmer model 303 atomic absorption spectrophotometer.

Absorption readings were converted to absorbance by a formula, 2- log A. This was done by drawing a standard curve on a one-cycle semi-log paper with the numbering reversed. Concentrations of zinc were then calculated from this standard curve.

Concentration for the amount of tissue used in microgram/gram was calculated by the equation:

$$\frac{\text{Volume of solution}}{\text{g of sample used}} \times \text{concentration of solution} = \text{microgram/gram.}$$

RESULTS AND DISCUSSION

Due to experimental variability not attributable to replications, no statistical analysis was performed on enzyme and ethanol responses.

Results on alcohol dehydrogenase (ADH) activity (Table 1) show that for varieties Seafarer, NEP-2, and San Fernando, there were increases in the activity in the roots of flooded plants grown at high levels of zinc. The degree of increase was highest with Seafarer, followed by NEP-2 and San Fernando, respectively. MSU 31908 did not show any ADH activity at either level of treatment.

ADH activity was also found in flooded plants grown at the low zinc level but the activity was not as much as for those that grew at the high zinc level.

The presence of ADH activity at 0 level of flooding was presumably due to some anaerobic microsites in the pots, thus causing anaerobic respiration in some parts of the roots.

As can be noted in Table 1 and Table 2, there was a discernible relationship between ADH activity and zinc concentration in the roots. Seafarer again contained the most zinc while MSU 31908 contained the least. This agrees with Vallee and Hoch (1955) who reported that activity of ADH in yeast was directly dependent on zinc. The difference in the amount of zinc in the roots for flooded and non-flooded plants was probably due to greater utilization of zinc by plants growing in an aerobic condition than when growing in an anaerobic condition. Also,

Table 1. Alcohol dehydrogenase activity* of Seafarer, MSU 31908, NEP-2, and San Fernando grown at two levels of zinc and subjected to two levels of flooding (Greenhouse experiment, February - May 1979).

Levels of flooding (hours)	Zinc in nutrient solution (ppm)		Varieties			
			Seafarer	MSU 31908	NEP-2	San Fernando
			units/mg protein			
0	0	Mean	0.33	0	0.18	0
		Range	0 - 1.02		0 - 0.8	
	0.2	Mean	0.48	0	0	0.07
		Range	0 - 2.62			0 - 0.42
36	0	Mean	0.47	0	0.60	0.50
		Range	0 - 1.97		0.04 - 1.6	0 - 2.45
	0.2	Mean	12.53	0	2.16	1.74
		Range	0 - 45.62		0.14 - 5.24	0 - 8.39

* Means of six replications.

Table 2. Zinc concentrations* in roots of Seafarer, MSU 31908, NEP-2, and San Fernando grown at two levels of zinc and subjected to two levels of flooding (Greenhouse experiment, February - May 1979).

Levels of flooding (hours)	Zinc in nutrient solution (ppm)		Varieties			
			Seafarer	MSU 31908	NEP-2	San Fernando
			micrograms/g			
0	0	Mean	61.52	25.87	48.67	39.34
		Range	59.0 - 64.0	22.73 - 30.0	46.0 - 51.0	38.0 - 41.5
	0.2	Mean	73.80	43.08	69.65	54.94
		Range	70.0 - 79.55	34.1 - 52.3	65.90 - 72.73	52.25 - 59.20
36	0	Mean	93.29	39.50	95.70	80.04
		Range	87.5 - 102.28	29.55 - 62.50	84.10 - 134.10	76.2 - 86.5
	0.2	Mean	216.77	57.87	142.73	118.79
		Range	190.7 - 233.0	38.0 - 84.55	129.55 - 170.45	101.0 - 135.0

* Means of six replications.

oxygen stress might have reduced translocation of zinc to the top, resulting in greater accumulation in the roots under anaerobiosis.

Analysis of variance of zinc concentrations (Table 3) shows that there are significant varietal differences in the amount of zinc in the roots. There are significant interactions between flooding and variety and also between zinc and variety.

For MSU 31908, which did not show any ADH activity, the differences in the concentration of zinc at different levels of treatment were not as much as for the other varieties which showed an increase in ADH activity.

Table 4 shows ethanol concentrations in xylem exudate of the four varieties. Seafarer had the highest ethanol production under anaerobic conditions. However, the difference in ethanol production at low zinc and at high zinc was not as great as the difference in ADH activities at the same levels. San Fernando at high zinc level produced higher ethanol than NEP-2 at the same level when flooded.

Loss of ethanol through root exudates into the rhizosphere has been reported by Bolton (1966) and Smucker and Erickson (1976). This then might be the cause of the low concentration in the exudate and also might be the cause of the uncorrelated values of ethanol to ADH activities for NEP-2 and San Fernando.

MSU 31908 accumulated some ethanol which was the least among all varieties.

The difference in the ethanol concentrations also might be due to the varietal difference in the flow rate of the exudate. Seafarer had the highest flow rate while MSU 31908 had the least. Ethanol concentration in Seafarer then would be diluted more than that of NEP-2,

Table 3. Analysis of variance* of zinc concentrations in the roots (Greenhouse experiment, February - May 1979).

Source	df	Mean Square	F
Flooding	1	1.756	319.27***
Zinc	1	0.813	147.81***
Flooding X Zinc	1	0.018	3.35 +
Error (a)	20	0.006	
Variety	3	0.719	153.04***
Flooding X Variety	3	0.064	13.62***
Zinc X Variety	3	0.018	3.72 +
Flooding X Zinc X Variety	3	0.021	4.47**
Error	60	0.005	

* Using transformed data (log X)
** Significant at $\alpha = 0.01$
*** Significant at $\alpha = 0.005$
\+ Significant at $\alpha = 0.025$

Table 4. Ethanol concentrations* in xylem exudate of Seafarer, MSU 31908, NEP-2, and San Fernando grown at two levels of zinc and subjected to two levels of flooding (Greenhouse experiment, February - May 1979).

Levels of flooding (hours)	Zinc in nutrient solution (ppm)		Varieties			
			Seafarer	MSU 31908	NEP-2	San Fernando
			ppm/g dry wt			
0	0	Mean	0	0	0	0
	0.2	Mean	0.2	0	0	0
		Range	0 - 1.14			
36	0	Mean	6.04	0.33	2.5	2.33
		Range	0 - 14.71	0 - 2.0	0 - 15.02	0 - 10.51
	0.2	Mean	7.86	0.74	2.31	5.91
		Range	0 - 17.91	0 - 2.97	0 - 13.84	0 - 15.84

* Means of six replications.

San Fernando and MSU 31908, respectively.

Pyruvate decarboxylase (PDC) activities for each variety (Table 5) were found to be higher with flooding. However, there was no general pattern between activities for those plants grown at low zinc and for those grown at high zinc level.

Roots of the variety MSU 31908 had the greatest PDC when flooded. These differences, however, were small. During flooding, the reaction for these varieties might have stopped at acetaldehyde. Acetaldehyde had been shown to induce ADH synthesis (Crawford and McManmon, 1968; John and Greenway, 1976), but McManmon and Crawford (1971) suggested that for tolerant plants acetaldehyde might cause negative feedback and thus stop the reaction from pyruvate to acetaldehyde.

Seafarer, which was reported earlier to be sensitive to flooding, had the highest increase in ADH activity and also had the highest concentration of zinc in the roots, while MSU 31908, which was reported to be a non-ethanol producer, had the least ADH activity and had the least concentration of zinc in the roots. Between NEP-2 and San Fernando, there were small differences in the responses though NEP-2 showed a slightly higher ADH activity and also had a higher zinc concentration than San Fernando. The similar responses of these two varieties are presumably attributable to the fact that they are genetically closely related (NEP-2 is a mutant of San Fernando).

These results show a general pattern or a framework that suggest a relationship of zinc uptake to ADH activity and ethanol production. Consequently, it may be postulated that a plant sensitive to an anaerobic condition could have a glycolytic pathway to pyruvate, and acetaldehyde, with ethanol being the final electron acceptor. ADH

Table 5. Pyruvate decarboxylase activity* of Seafarer, MSU 31908, NEP-2, and San Fernando grown at two levels of zinc and subjected to two levels of flooding (Greenhouse experiment, February - May 1979).

Levels of flooding (hours)	Zinc in nutrient solution (ppm)		Varieties			
			Seafarer	MSU 31908	NEP-2	San Fernando
			units/mg protein			
0	0	Mean	0.11	0	0	0
		Range	0 - 0.21			
	0.2	Mean	0.18	0	0	0
		Range	0 - 0.5			
36	0	Mean	0.48	0	0.94	1.05
		Range	0 - 1.40		0 - 2.1	0 - 6.29
	0.2	Mean	0.44	1.04	0.84	0.16
		Range	0 - 1.10	0 - 5.0	0 - 4.20	0 - 0.59

* Means of six replications.

activity and ethanol production would correlate with the amount of zinc taken up by the roots. A tolerant plant to an aeration stress, however, would accumulate less zinc in the roots, resulting in a limited ADH activity and ethanol production. The metabolic pathway of a tolerant plant also could possibly stop at acetaldehyde which then acts as an end product inhibitor, and diverting pyruvate into organic acids production (McManmon and Crawford, 1971).

Variety Seafarer showed a fully consistent result to the suggested hypothesis. There was a large increase of ADH activity, there was a large amount of ethanol produced during flooding and also there was a high amount of zinc uptake by the roots. For this variety, NADH may have increased due to an increase in glycolysis in order to meet the demand for ATP (adenosine triphosphate). The increase could have enhanced the PDC activity causing the formation of acetaldehyde which in turn might induce ADH activity. ADH which required zinc for its activity then presumably catalyzed the reaction from acetaldehyde to ethanol.

Results with MSU 31908 agree with the suggested model for a tolerant plant, in the sense that it had no or very little ADH activity, it produced small amounts of ethanol during anaerobiosis and it also accumulated small amounts of zinc in the roots. Due to the limited amount of zinc present, the functional metalloenzyme molecule of ADH [(ADH) Zn_4] might not be formed thus leading to no ethanol production.

Another possible reaction to have taken place in a tolerant plant would have involved acetaldehyde end product inhibition. Relatively high PDC activity suggested that there was a reaction from pyruvate to acetaldehyde. Acetaldehyde in this case, presumably acted as an

inhibitor and failed to induce ADH activity. Organic acids such as malate, oxaloacetate, and shikimate might be the products of anaerobic respiration such as reported by Crawford and Tyler (1969) and Tyler and Crawford (1970). These organic acids, particularly malate and pyruvate, were also found to be inhibitors for ADH (Leblova et al., 1977). These organic acids produced are not toxic to plants and can be further metabolized when oxygen is restored.

For NEP-2 and San Fernando, the responses were intermediate between those of Seafarer and MSU 31908. The reaction from pyruvate to ethanol probably occurred but the ADH activity and ethanol production were much less than those of Seafarer. This presumably was due to a lesser amount of zinc in the roots. Acetaldehyde may still induce ADH activity, especially in NEP-2, however, with the amount of zinc accumulated, ADH activity was assumed to be lower than those of Seafarer. High PDC activity in San Fernando may also cause an acetaldehyde negative feedback and this could lead to the production of organic acids such as suggested for MSU 31908. Ethanol produced in these varieties also could have been excreted and also further metabolized such as suggested by Cossins and Turner (1962).

The presence of isoenzymes of ADH such as found in maize (Marshall et al., 1973), can also be suggested for the differences in the response by the above varieties.

SUMMARY AND CONCLUSION

Short-term aeration stress has been implicated to be the cause of yield reductions in beans. Ethanol is produced due to an induction of or activation of alcohol dehydrogenase. This enzyme requires a zinc cofactor for its activity.

In this greenhouse experiment, an attempt was made to develop a hypothesis for the relationship between ethanol production, alcohol dehydrogenase and pyruvate decarboxylase activities, and zinc uptake in four varieties of Phaseolus vulgaris L., namely, Seafarer, MSU 31908, NEP-2, and San Fernando under aerated and anaerobic conditions.

Seafarer had the highest alcohol dehydrogenase activity, the highest ethanol accumulation, and the highest zinc concentration in the roots. MSU 31908 had the lowest values of all the findings except for pyruvate decarboxylase activity. NEP-2 and San Fernando were the intermediates.

It was then hypothesized that zinc had an effect on the response of the four bean varieties under an aeration stress. Under this hypothesis the more zinc in Seafarer lead to a higher alcohol dehydrogenase activity while the lower uptake by NEP-2, San Fernando, and MSU 31908 presumably resulted in a lower activity of the enzyme. Also, acetaldehyde was suggested to be an inhibitor to alcohol dehydrogenase in a tolerant variety and this might lead to another possible pathway, that is, the production of organic acids.

Nevertheless, from the results obtained it can be postulated that the increasing zinc application in fertilizer used in growing beans might promote yield reduction under aeration stress.

APPENDIX

Table 6. ADH and PDC activities, ethanol concentration and zinc concentration in Seafarer (Greenhouse experiment, February - May 1979).

Flooding (hours)	Zinc in nutrient solution (ppm)	Replication	ADH (units/mg protein)	PDC (units/mg protein)	Ethanol (ppm/g dry wt.)	Zinc (micrograms/g)
0	0	1	0	0	0	59.0
		2	0	0.073	0	59.10
		3	0.420	0.212	0	59.50
		4	0.517	0.197	0	63.85
		5	1.015	0.034	0	64.0
		6	0	0.139	0	63.65
	0.2	1	0.256	0.117	1.136	79.45
		2	0	0.303	0	72.73
		3	0	0.149	0	70.60
		4	2.62	0.50	0.667	79.55
		5	0	0	0	70.45
		6	0	0	0	70.0
36	0	1	0	1.40	3.46	90.4
		2	0.034	0.169	0	87.5
		3	0.466	0.269	6.183	95.0
		4	1.965	0.811	0	102.28
		5	0.209	0.126	14.71	93.18
		6	0.131	0.109	11.23	90.9
	2.0	1	1.468	0.126	0	190.9
		2	1.680	0	2.60	200.0
		3	9.77	0.691	3.99	220.4
		4	0	0.737	7.79	231.0
		5	16.67	1.10	14.84	225.3
		6	45.62	0	17.91	233.0

Table 7. ADH and PDC activities, ethanol concentration and zinc concentration in MSU 31908 (Greenhouse experiment, February - May 1979).

Flooding (hours)	Zinc in nutrient solution (ppm)	Replication	ADH (units/mg protein)	PDC (units/mg protein)	Ethanol (ppm/g dry wt.)	Zinc (micrograms/g)
0	0	1	0	0	0	26.0
		2	0	0	0	22.73
		3	0	0	0	25.5
		4	0	0	0	30.0
		5	0	0	0	27.25
		6	0	0	0	23.73
	0.2	1	0	0	0	38.05
		2	0	0	0	37.50
		3	0	0	0	34.1
		4	0	0	0	47.5
		5	0	0	0	52.3
		6	0	0	0	49.0
36	0	1	0	0	1.997	62.50
		2	0	0	0	40.0
		3	0	0	0	40.90
		4	0	0	0	30.0
		5	0	0	0	29.55
		6	0	0	0	34.10
	0.2	1	0	0.205	0	38.0
		2	0	0	0	38.4
		3	0	0	0	75.0
		4	0	1.059	0	72.75
		5	0	5.0	1.44	38.50
		6	0	0	2.97	84.55

Table 8. ADH and PDC activities, ethanol concentration and zinc concentration in NEP-2 (Greenhouse experiment, February - May 1979).

Flooding (hours)	Zinc in nutrient solution (ppm)	Replication	ADH (units/mg protein)	PDC (units/mg protein)	Ethanol (ppm/g dry wt.)	Zinc (micrograms/g)
0	0	1	0	0	0	46.0
		2	0	0	0	47.5
		3	0.262	0	0	50.0
		4	0	0	0	49.5
		5	0	0	0	48.0
		6	0.80	0	0	51.0
	0.2	1	0	0	0	77.28
		2	0	0	0	70.0
		3	0	0	0	72.73
		4	0	0	0	66.0
		5	0	0	0	65.9
		6	0	0	0	66.0
36	0	1	0.50	0.349	0	86.38
		2	1.60	2.097	0	96.59
		3	0.183	0	0	84.1
		4	0.035	0.349	0	86.5
		5	0.259	0.749	15.024	134.1
		6	1.0	2.097	0	86.5
	0.2	1	5.242	0.411	0	170.45
		2	0.142	4.194	0	129.55
		3	2.725	0	0	145.45
		4	1.922	0	26.29	140.9
		5	0.839	0.456	0	130.0
		6	2.097	0	0	140.0

Table 9. ADH and PDC activities, ethanol concentration and zinc concentration in San Fernando (Greenhouse experiment, February - May 1979).

Flooding (hours)	Zinc in nutrient solution (ppm)	Replication	ADH (units/mg protein)	PDC (units/mg protein)	Ethanol (ppm/g dry wt.)	Zinc (micrograms/g)
0	0	1	0	0	0	41.0
		2	0	0	0	41.5
		3	0	0	0	38.65
		4	0	0	0	38.5
		5	0	0	0	38.4
		6	0	0	0	38.0
	0.2	1	0	0	0	56.83
		2	0	0	0	56.83
		3	0.419	0	0	59.2
		4	0	0	0	52.3
		5	0	0	0	52.25
		6	0	0	0	52.25
36	0	1	0.139	0	1.52	80.0
		2	0.419	0	1.92	86.5
		3	0	6.29	0	76.2
		4	0	0	0	76.15
		5	0	0	10.51	77.28
		6	2.446	0	0	84.1
	0.2	1	0	0	0	101.0
		2	8.387	0	7.76	135.0
		3	0	0.362	11.87	125.0
		4	0	0	15.84	122.73
		5	2.097	0.599	0	120.0
		6	0	0	0	109.0

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