

ALCOHOL DEHYDROGENASE INDICATING OXYGEN DEFICIENCY IN CHRYSANTHEMUM GROWN IN MINERAL MEDIA

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Abstract

In order to evaluate growth differences of chrysanthemum cv. 'Improved Reagan' in mineral growing media, root porosity, activity of the enzyme alcohol dehydrogenase (ADH) in the roots and leaf mineral concentrations were determined in a number of experiments. In the first three experiments the growth of chrysanthemum on sand (volumetric air content 7-18 %) and two perlite fractions (volumetric air content 16-31% and 60-63%, respectively) was compared at two fertigation frequencies. In the first two experiments (carried out in september through january) using drip fertigation no effects of oxygen deficiency on shoot growth were found. Shoot fresh weight, ADH activity and root porosity were lower and percentage dry weight was higher in the coarse perlite treatments, suggesting water/nutrient stress. ADH activity decreased during growth. In the third experiment (february-march) the same media and pumice (vol. air content 39%) in combination with two fertigation frequencies were used with ebb and flow fertigation. Shoot fresh weight was both lower in sand (oxygen stress) and in coarse perlite and pumice treatments (water/nutrient stress). Besides a higher ADH activity in the sand treatments no clear differences (root porosity, leaf mineral concentrations, percentage dry weight) were found between the sand and the fine perlite treatments. It is suggested that oxygen stress in the roots of chrysanthemum may occur at volumetric air contents below ca. 35%. However, oxygen stress resulting in a yield decrease probably depends on climatic conditions.

1. Introduction

Research in soilless culture is concerned with the suitability of media in the cultivation of e.g. cut flowers. Inadequate growth on mineral media may be related with water, nutrient and/or oxygen deficiency. Water and nutrient supply can - at least partly - be regulated with the composition of the nutrient solution in combination with the fertigation system and fertigation frequency. Oxygen supply however is considered to be mainly determined by volumetric air content, since it can directly be related with oxygen diffusion rates (Gislerod 1982, Bunt 1991). Although it is relatively easy to determine (Wever 1991, Verhagen 1993), volumetric air content may change during cultivation due to degradation and shrinking/settling of the material and/or root growth. Moreover, whether oxygen stress occurs in plant roots depends not only on oxygen supply, but also on its demand, i.e. respiration. Root respiration per liter medium depends on e.g. the crop involved, growth rate (Poorter et al 1990), root temperature (Jackson 1980) and root density. If demand exceeds supply, oxygen stress may induce a number of non-specific physiological reactions such as a reduction in transpiration, nutrient uptake and

growth (Baas 1991, Buwalda et al 1994, Everard and Drew 1989). More or less specific physiological responses of oxygen stress may be found in the roots e.g. increased root porosity due to ethylene accumulation (Buwalda et al 1994, Pezeshki et al 1993), and increased activity of alcohol dehydrogenase (ADH) (Crawford and McManmon 1968, Laan 1990, Wignarajah and Greenway 1976).

The purpose of our experiments was to investigate whether these effects of oxygen deficiency in chrysanthemum could be found in mineral media differing in volumetric air content, and whether they could be used as an indicator for oxygen stress.

2. Materials and methods

2.1 Experiment 1. The experiment had a two factorial design with 3 mineral media and 2 different fertigation frequencies as independent variables. The experiment was carried out in a 150 m² greenhouse in which twelve 550 l tanks were positioned. Each tank supplied two plots of 4 m², giving a total of 24 plots per greenhouse. Each plot consisted of two gullies (length 6 m, width 10 cm, depth 10 cm). The gullies were filled with 40 l (height ca. 5 cm) of the mineral media sand (river sand), perlite 0-1 mm (Agra-perlite 1) and perlite 1-7 mm (Agra-perlite 3).

Cuttings of chrysanthemum (*Dendranthema indicum* cv. 'Improved Reagan'), rooted in small jute plugs, were planted in the medium in week 38 1993 (49 cuttings/gully). Fertigation was provided using trickle tubes which supplied 1.2 liter/minute per gully. Fertigation frequency was either 2*1 minute per day or 6 times 2 minutes per day throughout the experiment.

Once every week the EC and pH (5.5-6.0) of the recirculating nutrient solutions were measured and corrected. Every two weeks (major elements), or 4 weeks (minor elements), nutrient solutions were analyzed after topping up, and corrected if necessary using rain water and stock solution. The average EC of the nutrient solution was 1.9 mS/cm and the composition of the major elements was (mM): NO₃⁻, 11.5; NH₄⁺, 0.2; H₂PO₄²⁻, 0.8; K⁺, 7.0; Ca²⁺, 2.5; Mg²⁺, 0.9; SO₄²⁻, 1.5. Minor elements were supplied as (µM): Fe (as Fe-EDDHA), 50; Mn, 5; Zn, 4; B, 25; Cu, 1.5; Mo, 1.

In week 42 leaves were analyzed for total N, P, K, Ca and Mg. Air-filled porosity of the lower part of the root system was determined in week 46 by means of vacuum-infiltration (Van Noordwijk and Brouwer 1989).

In week 47 shoot fresh weight and dry weight (after drying 48 h 70°C) was determined of 12 plants per replicate .

In situ volume fraction water (f_w) and bulk density (P_d in kg/m³) of the mineral media was determined in weeks 42, 45 and 47 by determining fresh and dry weight of the samples using 100 ml rings (height 5 cm). Porosity (f_p) was calculated from the bulk density as: $f_p = 1 - (P_d/2650)$. Volume fraction air (f_a) was calculated as:
 $f_a = f_p - f_w$.

Determination of enzymatic root alcoholdehydrogenase activity was a modified procedure as described by Bergmeyer (1974) and Laan (1990). In weeks 40, 41, 42, 44, 45, 46 and 47 six plants per replicate were harvested, and the lower parts of the root system were pooled. The roots were immediately frozen in liquid N₂ and stored at -20°C until further handling. In an ice-cold mortar 0.5-1.0 g roots was ground in 6 ml

extraction buffer (50 mM HEPES-buffer (pH 7.5) plus 5 mM DTT), 5% (w:w) PVPP and ca. 100 mg quartz-sand. The homogenate was centrifuged (20 min, 13.500 rpm at 4°C). The supernatant was used for determination of ADH activity.

In a 4.5 ml cuvette 2 ml 50 mM MES buffer (pH 6.5), 300 µl BSA (0.5 mg/(ml 0.1% (w:v) NaCl)), 150 µl 2.2 mM NADH and 200 µl root extract (supernatant) were added together, and after gently stirring, the reaction was started by addition of 200 µl 50 mM acetaldehyde. In vitro activity of ADH was determined according to Bergmeyer (1974) by recording the linear decrease in extinction (340 nm) during 45 seconds with a spectrophotometer at 340 nm. Total soluble protein in the supernatant was determined using a BIORAD protein assay (Bradford 1976). ADH activity then was calculated as µmol NADH used per g protein per minute.

2.2 Experiment 2. In week 49 1993 new chrysanthemum cuttings were planted in the same mineral media as in experiment 1. The experiment was identical to experiment 1 and lasted until week 6 1994. In week 1 1994 leaves were analyzed for total-N, P, K, Ca, and Mg. Shoot fresh and dry weight weight was determined in week 6.

ADH activity was determined in week 50 1993 and weeks 1 and 6 1994 as in exp. 1.

Bulk density and water content were determined in week 51 1993, and weeks 1, 3 and 5 1994.

2.3 Experiment 3. Non-rooted cuttings of chrysanthemum were planted directly in 0.75 liter (height 10 cm) pots filled with the same media as in experiments 1 and 2 or Iceland pumice (2-6 mm) in week 5 1994. After rooting the pots were placed on ebb and flow benches 20 cm apart. Fertigation was provided once or ten times per day during the daytime with a maximum height of 3 cm during 5 minutes. There were four replicates per treatment. In week 11 shoot fresh and dry weight were determined of 4 plants per replicate. In the dried samples total-N, P, K, Ca, and Mg were determined. Root porosity was determined in week 13 and root ADH activity was determined in weeks 8 and 9 1994 as in exp. 1. Bulk density and water content of the mineral media were determined in week 11 1994.

2.4 Experiment 4. Cuttings of chrysanthemum (as in exp. 1 and 2) were planted in pots as in experiment 3, or in holes in plastic covers in week 16 1994. In the latter case, the cuttings received nutrient solution by means of ebb-and flow every 20 minutes. More details of the ebb-and flow cultivation system are given by Buwalda et al (1994). There were twelve replicates. After two weeks, shoot fresh weight and root ADH activity was determined as in exp. 1 of 6 plants per treatment.

3. Results

No significant effects of medium or fertigation frequency were found on shoot FW in experiment 1 (Table 1). There was a trend for lower shoot FW in the perlite 1-7 treatment at the low frequency. In experiment 2 this treatment indeed showed lower shoot FW (Table 2). Root porosity was lower in the perlite 1-7 treatments (Table 1).

With respect to nutrient concentrations, no clear consistent effects were found in experiments 1 and 2. In the perlite 1-7 treatment at low frequency, a higher percentage dry matter and lower concentrations total-N, P and K were found in experiment 2 (Table 2).

ADH activity in the roots decreased in time (Fig. 1). Lower activities were generally found in the perlite 1-7 treatments, whereas no differences were found between the sand and perlite 0-1 treatments. Similar results were found in experiment 2 (Fig. 2).

Bulk density had increased and porosity and volumetric air content had decreased in the sand and perlite 0-1 treatments in experiment 2 compared to experiment 1. This had no apparent effect on shoot fresh weight.

However, in experiment 3 production in the perlite 0-1 treatments was higher than in the sand, the perlite 1-7 and pumice treatments (Table 3). This higher production compared to the sand treatments could not be related with nutrient concentrations, percentage dry matter in the shoot or root porosity, but could be related to a lower root ADH activity (Fig. 3).

When volumetric air content of the medium was plotted against root ADH activity, an increase was found when volumetric air content of the corresponding medium was below ca. 35% (Fig. 4).

In experiment 4, root ADH activity in the perlite 0-1 treatment was higher than in the ebb/flow treatment, although shoot weight was lower (Fig. 5).

4. Discussion

Despite low volumetric air contents, no effects of oxygen deficiency on shoot growth were found in the first two experiments (Tables 1 and 2). Growth was only decreased in the perlite 1-7 treatments, which could be due to a water and/or nutrient stress. The lower N, P and K tissue concentrations (Table 2) suggest that nutrient concentrations were limiting in these treatments. However, the results from experiment 3, in which the perlite 1-7 treatments also lagged behind, do not confirm this.

Only in experiment 3 there was a significant lower shoot FW production in sand than in perlite 0-1 (Table 3), despite higher (average) volumetric air contents in exp. 3 compared to experiments 1 and 2. The lower production in sand was not related to lower concentrations of major elements in the leaf tissue. Possibly water stress, induced by oxygen deficiency (Everard and Drew 1989), is the most likely cause for the yield decrease in exp. 3 compared with exp. 1 and 2, as was suggested for carnation (Baas 1991). If so, lower transpiration in experiments 1 and 2 (in autumn/winter) could explain the absence of water stress under conditions of oxygen stress. However, there were indications that oxygen stress occurred in sand and perlite 0-1 treatments in experiments 1 and 2 as well. For instance, root porosity (Table 1), and root ADH activity (Fig. 1, 2) in sand and perlite 0-1 generally were higher than in perlite 1-7. These results indicate that oxygen stress only results in decreased production under conditions of high transpiration.

The enzyme ADH facilitates the conversion of acetaldehyde in ethanol, the last step in the ethanol fermentation process, which is initiated under anaerobic conditions. Under these conditions, the cytochrome chain in the root cells does not function any more, leading to an accumulation of acetaldehyde, the first end-product of anaerobic glycolysis, which in turn induces the synthesis of ADH. Compared with the other mineral media, root ADH activities were highest in the sand treatments in experiments 1 and 3 (Fig. 1 and 3), which corresponds with the presumed higher oxygen stress in these

treatments. However, as root ADH activity was higher in the perlite 0-1 treatments compared to perlite 1-7 (Fig. 1,2) and pumice (Fig. 3) treatments as well, this would also indicate that even in perlite 0-1 oxygen stress in the roots would occur. When ADH from exp. 3 is plotted against volumetric air content of the mineral media (Fig. 4), it can be seen that ADH increases below a vol. air content of ca. 35%, suggesting oxygen stress. However, as mentioned before, yield was decreased at vol. air content higher than 35%, probably due to water stress. Therefore, root ADH activity was also determined in plants grown in perlite 0-1, simultaneous with plants grown in ebb-and flow soilless culture in exp.4. In such a soilless culture, oxygen and water/nutrient availability is expected to be near to optimal (Buwalda et al. 1994). Root ADH activities as determined two weeks after growth (week 17 1994) indeed were significantly higher in the perlite 0-1 treatment, compared to the soilless culture, whereas shoot fresh weight was lower (Fig. 5). These results therefore confirm that in perlite 0-1 mm oxygen stress may occur.

Whether a potential yield decrease at a volumetric air content below 35% in other crops might also be expected probably not only depends on potential transpiration as suggested above, but also on the root respiration. Compared to other crops such as carnation, Gerbera and Aster, respiration rates of chrysanthemum proved to be relatively high, in the range of 7-9 mg O₂ g⁻¹ root DW h⁻¹ (Baas and Warmenhoven, unpublished results). It is therefore reasonable to assume that for these crops lower vol. air contents can be tolerated.

In order to avoid the risk of yield decrease due to oxygen stress in chrysanthemums completely, presumably either systems without mineral media could be used such as soilless ebb/flow (Buwalda et al. 1994), or systems containing mineral media with high (>35%) volumetric air content. However, in order to avoid water and/or nutrient limitations in these systems, high fertigation frequencies (. 1x/hour) are probably necessary.

5. References

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Table 1. Properties of chrysanthemum grown in 5 cm sand and two perlite fractions in experiment 1 using drip fertigation, and average (n=3) *in situ* medium characteristics.

medium fertigation (min/day)	sand		perlite 0-1		perlite 1-7		LSD
	2*1	6*2	2*1	6*2	2*1	6*2	
shoot FW (g)	71.5	75.5	69.5	73.2	55.3	70.3	-
dry matter (%) 8.8	8.7	9.0	9.4	10.8	9.0	0.5	
root porosity (%)	1.9	2.3	2.0	2.4	1.3	1.3	0.5
Ntot (mmol/kg DW)	3750	3797	3675	3670	3665	3800	-
P (mmol/kg DW)	191	187	193	198	181	203	-
K (mmol/kg DW)	2101	2147	2093	2070	2041	2234	-
Mg (mmol/kg DW)	110	97	103	87	119	101	11
Ca (mmol/kg DW)	178	215	179	138	136	132	-
bulk density (kg/m ³)	1446	1573	97	99	98	104	20
porosity (%)	45	41	96	96	96	96	1
air (vol.%)	16	7	24	19	63	61	3

Table 2. Properties of chrysanthemum grown in 5 cm sand and perlite in experiment 2 using drip fertigation, and average (n=4) *in situ* medium characteristics.

medium fertigation (min/day)	sand		perlite 0-1		perlite 1-7		LSD
	2*1	6*2	2*1	6*2	2*1	6*2	
shoot FW (g)	23.7	24.9	21.2	22.8	14.2	15.8	6
dry matter (%) 8.1	7.6	7.9	7.8	9.7	8.9	0.5	
Ntot(mmol/kg DW)	3270	3117	3259	3334	2612	3024	376
P (mmol/kg DW)	160	176	164	192	126	161	15
K (mmol/kg DW)	2257	2193	2280	2340	1964	2054	146
Mg (mmol/kg DW)	160	140	165	152	169	157	-
Ca (mmol/kg DW)	307	319	310	331	243	287	-
bulk density(kg/m3)	1544	1549	111	108	94	103	16
porosity (%)	42	42	96	96	96	96	1
air (vol. %)	9	7	20	16	62	60	2

Table 3. Properties of chrysanthemum grown in different mineral media in experiment 3 using ebb and flow fertigation, and *in situ* medium characteristics.

medium fertigation (min/day)	sand		perlite 0-1		perlite 1-7		pumice 2-6		LSD
	1*5	10*5	1*5	10*5	1*5	10*5	1*5	10*5	
shoot FW (g)	27.6	28.4	32.7	31.3	23.1	28.6	25.9	26.8	3.9
dry matter (%) 9.8	9.8	9.8	9.9	10.7	9.8	10.0	9.4	0.1	
root porosity (%)	1.9	1.5	2.1	1.6	2.3	2.3	1.3	1.7	0.5
Ntot (mmol/kg DW)	3160	3014	3276	2946	3108	3210	2776	3439	221
P (mmol/kg DW)	145	145	147	146	153	186	146	181	15
K (mmol/kg DW)	1880	1749	1955	1915	1769	1923	1787	2020	76
Mg (mmol/kg DW)	110	102	113	95	110	95	124	122	12
Ca (mmol/kg DW)	306	308	268	273	284	233	268	322	-
bulk density(kg/m3)	1438	1350	84	85	123	123	436	440	30
porosity (%)	46	49	97	97	95	95	84	83	1
air (vol. %)	18	18	31	27	62	60	41	37	3

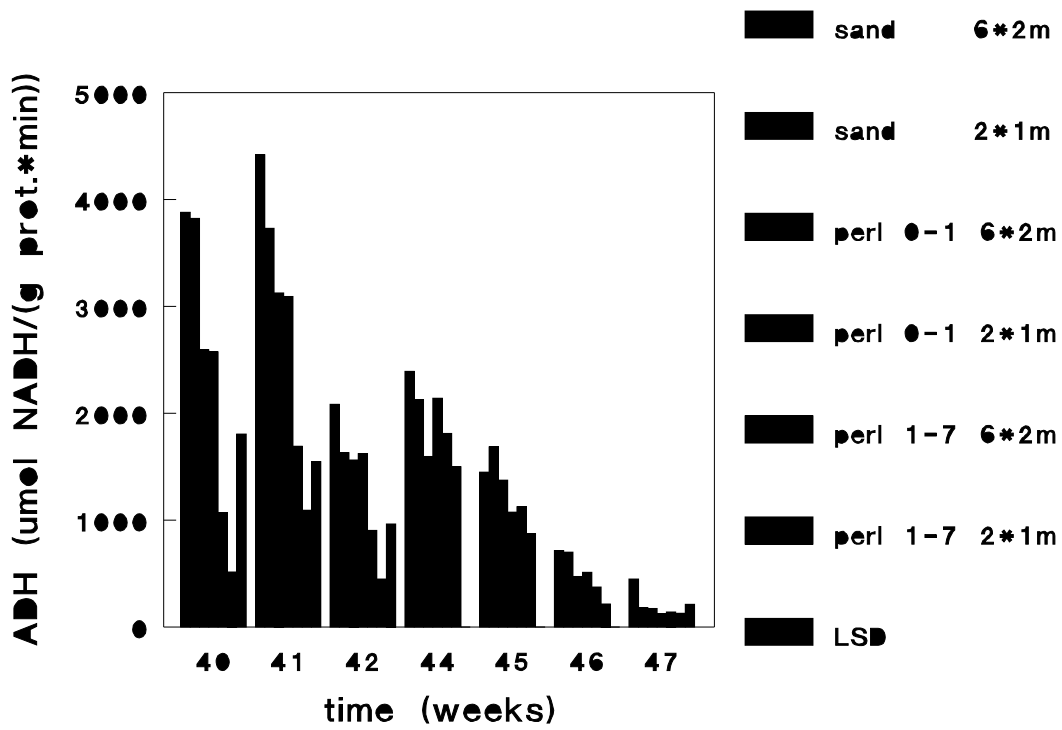


Fig. 1 Root ADH activity in chrysanthemum during experiment 1.

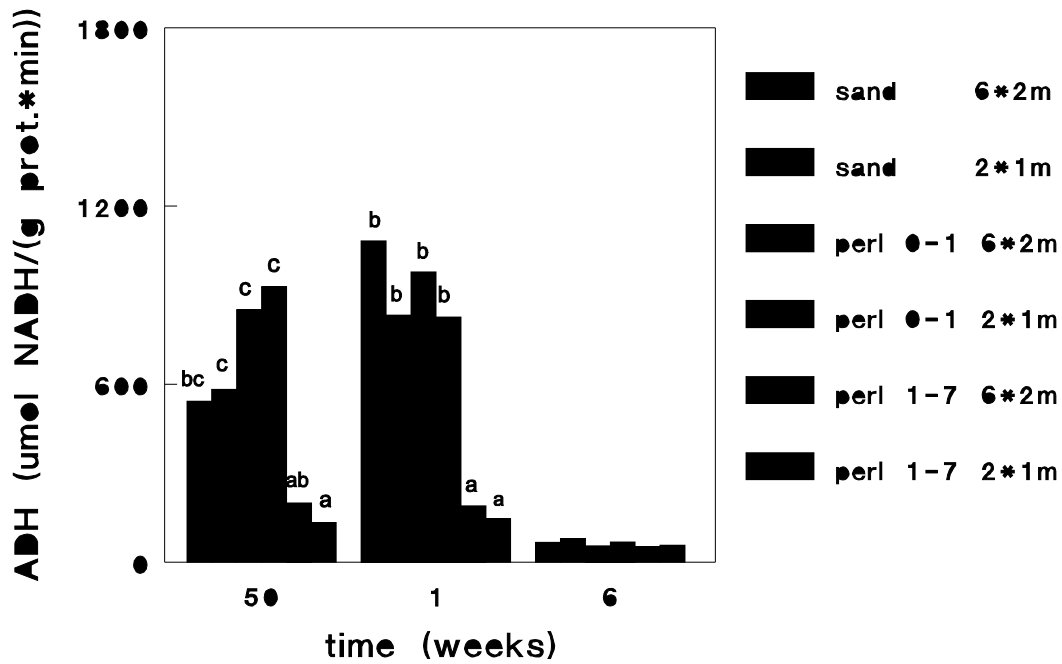


Fig. 2 Root ADH activity during experiment 2. Different letters within one week denote significant differences (Snedden & Hart 1995).

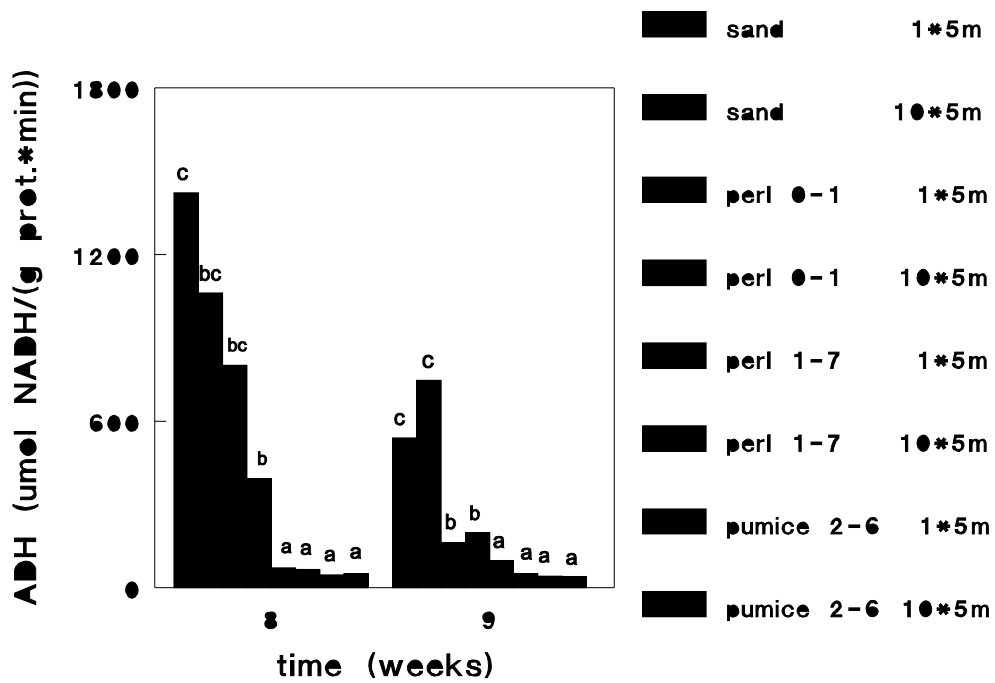
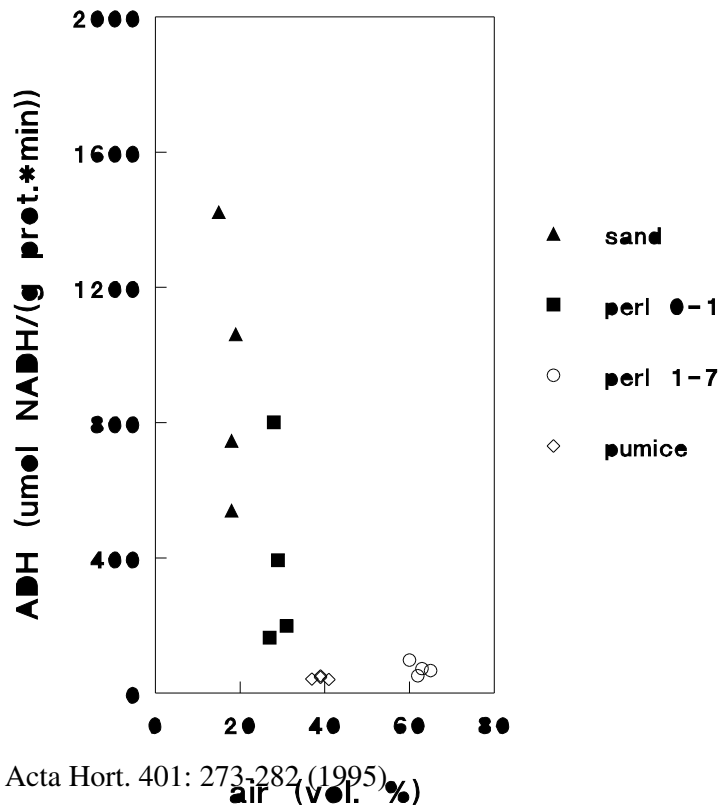


Fig. 3 Root ADH activity during experiment 3. Different letters within one week denote significant differences.



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Fig. 4 Relation between *in situ* volumetric air content of different mineral media and root ADH activity of chrysanthemum grown in these media in experiment 3.

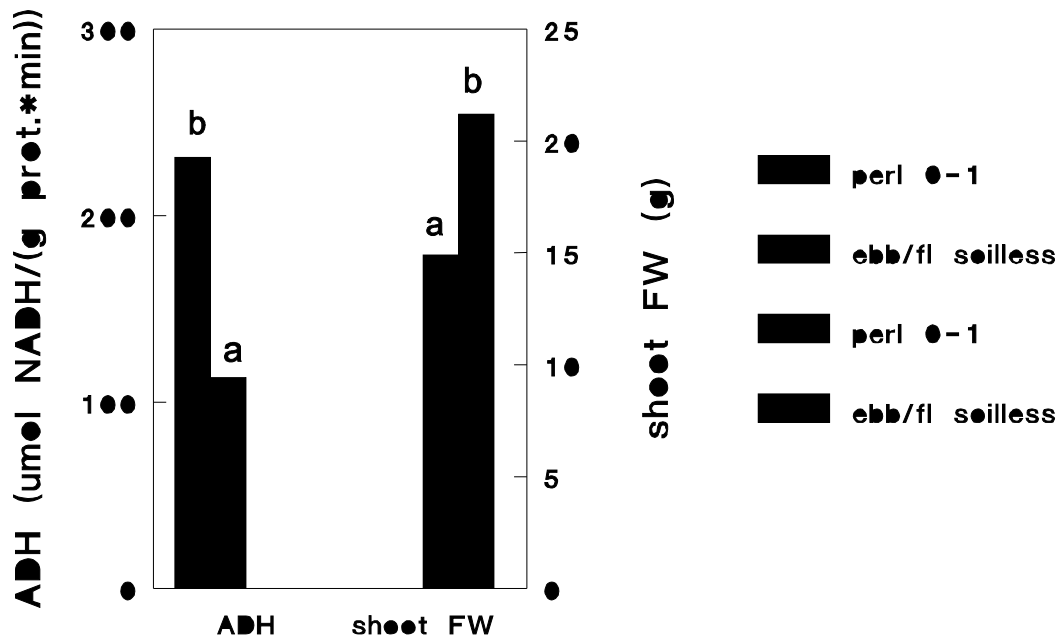


Fig. 5 Root ADH activity and shoot fresh weight in experiment 4. Different letters denote significant differences.

