EXPERIMENT III.

Influence of Hypoxia on Alcohol Dehydrogenase Activity, Internal Ethylene Production and Aerenchyma Formation in Barley Roots

Objective

To study the relationships among alcohol dehydrogenase activity, internal ethylene production and aerenchyma formation in the nodal roots of barley genotypes differing in waterlogged tolerant under hypoxic condition.

Materials and Methods

Experimental conditions

This experiment was conducted in 1998 at the Central Laboratory of LARTC, Lampang, Thailand. Three barley genotypes selected from the first experiment which differed in waterlogged tolerant; SMG1 (tolerant), FNBLS#140 (moderately tolerant) and BRBRF9629 (susceptible), were grown in the aerated nutrient solution (as control) and grown in the stagnant agar nutrient solution (as hypoxia, which was not more than 0.5 ppm oxygen concentration and caused oxygen deficiency for root respiration). The nutrient solution preparation and plant culture in this experiment were done as described by Wiengweera *et al.* (1997).

When the first nodal root was emerged at least 5 cm long from the base of the shoots, 150 vigor plants of each barley genotypes was transferred to the stagnant agar nutrient solution with full strength nutrient solution. The other 150 vigor plants were grown in the aerated nutrient solution. Both treatments were studied in the growth chamber throughout the experiment. The oxygen concentration and temperature in

each nutrient solution were illustrated in Appendix 8a. The experimental data were determined after 5 days of transferring to the different nutrient solutions. Four replications of each treatment was set.

Plant culture conditions

The vigor seeds were germinated in the dark at 20 $^{\circ}$ C for 3 hours on the plastic mesh over the deionised water solution of 0.5 mol m^{-3} $CaSO_4$. After the first leaf emergence, all barley seedlings were transferred to 10% of strength nutrient solution which filled in 50 liter of polyethylene containers. On day 7, they were transferred to the full strength aerated nutrient solution with 240-270 ml /min rate of air pumping and grown in the plant growth chambers (Fitotron™, SANYO Gallenkamp PLC, SGC970 Model) until the first nodal root emergence. The plant growth chambers were controlled diurnal cyclic and photoperiod by computerized system. Light intensities (PAR) varied between 250-1400 μmole m^{-2} s^{-1} under natural light, day length was about 12 hours. Daily maximum air temperature varied in 24 hour cycles was 20 $^{\circ}$ C in the mid day and controlled to 15 $^{\circ}$ C during the night. The ambient CO_2 concentration was about 330-400 ppm. All climatic data in the growth chambers was shown in Appendix 8b.

Plant measurements

1. Aerenchyma formation (the intercellular air filled spaces) in the nodal roots was measured by the methods as described by Greenway (1993). Four replications of 10-15 cm length hypoxic and aerated roots were free-hand cross sectioned at 5 cm from the root tips. The root cross sections were stained with 0.1% Safranin-O dye and were sampled to study with the Olympus compound microscope at 10X. The percentage of the cortex area that comprised of aerenchyma was determined with haemacytometer slide and divided by the total area of root cross section.

$$\% \text{ Aerenchyma} = \frac{\text{(The area of root cortex by haemacytometer)}}{\text{Total cross section area of the root}} \times 100$$

In the previous observation in laboratory was found that all three barley genotypes started arenchyma formation in the nodal roots after 5 days of hypoxia. This experiment was determined at 0, 3, 5, 6, 7, 8, and 9 days after hypoxia, especially every 3 hours of the fifth to the sixth days of hypoxia and were determined every day at 0900 a.m. for four days.

2. Alcohol dehydrogenase activity (ADH) in the nodal roots (EC. 1.1.1.1) (unit: nanomole ADH / g root fresh weight / min) was measured. Three nodal root samples of 10-15 cm length of each barley genotypes (approximately 1.5–2 g of root fresh weight) were analysed by bioassay method of Greenway (1993). Each barley genotypes were investigated at 0, 1, 2, 3, 4, 5, 7 and 9 days after hypoxia.

3. Internal ethylene production in the nodal roots (unit: ppm / g root fresh weight) were investigated at the same times as the aerenchyma measurements by gas chromatography using the method of Drew *et al.* (1989) and the gas samples in the nodal roots were collected as described by He *et al.*(1996).

Three nodal root samples of 10-15 cm length of each barley genotypes were taken about 1.5-2.0 g of root fresh weight and placed in 10-mL tubes. They were incubated in the deionised water under the dark at 20 $^{\circ}$C. Each tube was seal with a rubber septum cap. The ethylene content of incubation sample was sucked with a 25 mL hydrodermic glass syringe and collected about 3 ml of the gas withdrawn in 7 ml vaccutainer tube by replacing the deionised water in the tube. Gas sample was estimated 1 ml of a 10 mL hydrodermic needle and syringe and injected to the gas chromatography for ethylene analysis.

The gas chromatography was flame ionization detector (Shimaszu GC-14A) with SUS Packed Column (4mm.OD x 3mm.ID.x 1M long, Porapak N 80/100 mesh). The analysed conditions were the sensitivity range = 10^3; the column and inject temperature = 90 $^{\circ}$C; the detector temperature = 100 $^{\circ}$C; the dry air flow rate = 100 ml /min; and using nitrogen gas as gas carrier. The standard ethylene concentration were preliminary test for GC analysis were presented in Appendix 9. In addition, the

standard ethylene concentration with 1 ppm was used to check before and after sample gas analysis.

Results and Discussion

Alcohol dehydrogenase activity

Plant injury resulting from O_2 deficiency stress is due to anaerobic respiration leading to toxin accumulation, stress tolerance can conceivably be due to prevention of toxin accumulation or tolerant of toxin accumulation (Wignarajah *et al.*,1976). If the toxin is ethanol, its accumulation could be prevented by the energy ADH. Prolonged flooding resulted in larger increases in activity of ADH in the roots of flooding tolerant than in those of flooding intolerant varieties (Drew and Stolzy,1991). In general, ADH activity in the roots was not induced under oxygen sufficiency but in this experiment, I found the activity of ADH of barley grown under aerated nutrient solution (Figure 7a). It can describe that the oxygen concentration in the solution generally diffuses less than $1x10^4$ fold of diffusion in the air. In consequence, it takes a long time to diffuse into the cortex of the roots. Therefore, the lack of oxygen for respiration in the core cells of the roots are occurred and caused to induce ADH activity for anaerobic respiration (Drew 1983, Jackson and Drew 1984; Thomson and Greenway, 1991). However, the ADH activity of hypoxic barley roots had much greater than aerated roots (Figure 7a).

ADH activity of BRBRF9629, susceptible genotype was immediately occurred after transferring to hypoxic condition. Whereas SMG1 and FNBLS#140 (tolerant and moderately tolerant genotypes, respectively) had ADH activity for 1 day after hypoxia. BRBRF9629 had the highest ADH activity, followed by SMG1 and FNBLS#140, respectively (Figure 7a). The ADH activity of FNBLS#140 significantly increased about two fold on the 2^{nd} days of hypoxia and then decreased. ADH activity of BRBRF9629 also decreased after the 2^{nd} days of hypoxia (Figure 7a). It indicated that the root cells might have enough the intercellular air space production (aerenchyma formation) to diffuse oxygen for aerobic respiration (Figure 7c). Moreover, it was found that ADH

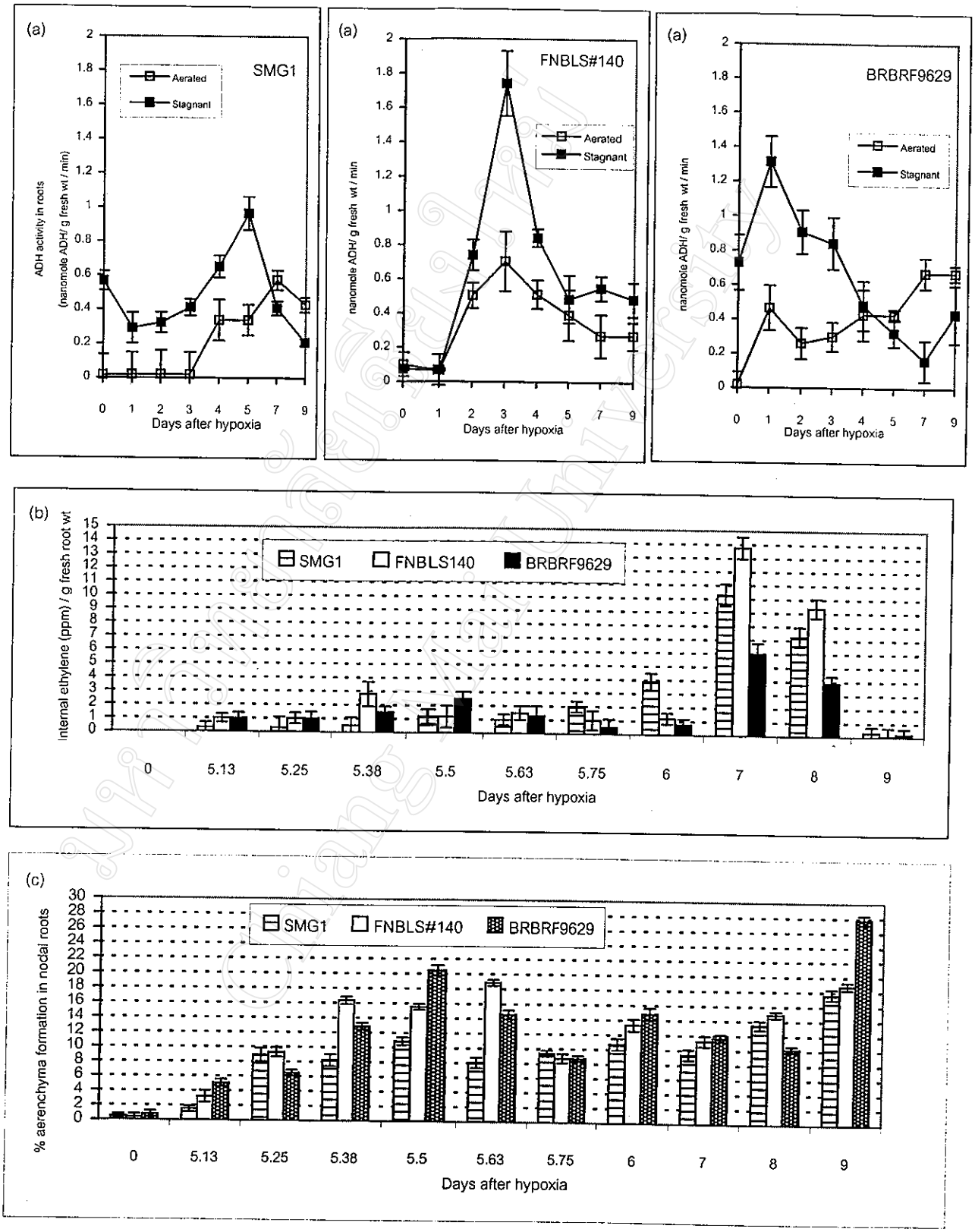

Figure 7 Alcohol dehydrogenase activity in nodal roots (a), internal ethylene concentration (ppm) (b), and the percent of aerenchyma formation in nodal roots (c) of barley genotypes in nutrient solutions. Data are means of four samples ± SE.

activity for SMG1 slowly increased and had lower activity compared with the other genotypes (Figure 7a). This might be some morphological adaptation to prevent oxygen loss from the roots by having less aerenchyma formation (Vartapetian and Jackson,1997).

The relationship between internal ethylene production and aerenchyma formation

ADH activity of barley roots was induced after hypoxia while internal ethylene production and aerenchyma formation did not occurred until the fifth days of hypoxic treatment (Figure 7b and 7c). The waterlogged adaptation of the plant root is based on aerenchyma development and internal aeration pathways as reported by Arikado (1955). Drew (1997) reported the same result and concluded that the root adaptation to hypoxia was a sequence system which firstly produced the related enzyme followed by internal ethylene production and aerenchyma formation in the roots.

In this experiment, no internal ethylene of the barley roots was detected under the aerated condition. It has been reported that only hypoxia condition increases the activity of ACC synthase which relates to induce internal ethylene formation in the roots (Adams and Yang, 1979; He *et al.* ,1996; Jackson *et al.*, 1985; Vartapetian and Jackson, 1997). In addition, the oxygen concentration is primarily triggered to increase in ethylene synthesis (Vartapetian and Jackson, 1997).

All barley genotypes tended to increase aerenchyma formation in the roots along with the time period of hypoxia (Figure 7b and 7c). He *et al.*,(1996) reported that adventitious rooting relates to ethylene formation as aerenchyma, non-reversible mechanism in a few days. In this experiment, the barley roots produced internal ethylene at 0900 a.m. of the fifth days of hypoxic treatment and formed the aerenchyma at 0300 p.m. of the same day (Figure 7b and 7c). The internal ethylene in barley roots varied from genotypes and the time period of hypoxia occurrence. It was found that the internal ethylene in barley roots increased during 7-8 days after hypoxia whereas the aerenchyma formation in the roots was extended in later (Figure 7b and 7c). It might explained that it takes time for internal ethylene to induce cellulase

enzyme and then this enzyme will catalyse root cell wall to be aerenchyma formation in later (He *et al.*1996; Huang *et al.*, 1994a; Kawase, 1979). However, the higher ethylene production, the more aerenchyma formation occurred (Figure 7b and 7c).

FNBLS#140 genotype produced the highest internal ethylene during hypoxia, followed by SMG1 and BRBRF9629, respectively (Figure 7b). Roots of BRBRF9629 genotype developed more extensive aerenchyma during 7-9 days after hypoxia compared with the other genotypes. It was indicated that BRBRF9629, susceptible genotype adapted to waterlogging by developing aerenchyma in the roots. Whereas SMG1 and FNBLS#140 had high internal ethylene production, but less aerenchyma formation (Figure 7c). SMG1 genotype extended slowly aerenchyma formation for oxygen diffused from the shoots. These might be the amount of lignin accumulation in the cell wall can prevent the radial oxygen loss from the root surface and may associated with some other waterlogged adaptation of the plant roots (Drew and Stolzy, 1991). Moreover, the tolerance root with lignified cell wall could also support the shoot plant under waterlogged condition.

However, little is known about the effect of internal ethylene associated and lignified cell wall of the hypoxic root (Vartapetian and Jackson, 1997). In this study showed that SMG1 and FNBLS#140 had less aerenchyma formation but increased the internal ethylene production as compared with BRBRF9629. Both genotypes might have the other hypoxic adaptation affected by ethylene formation such as adventitious root production which was not necessary to extend aerenchyma, non-reversible mechanism in a few days (He *et al.*, 1996). But this mechanisms by which flooding promotes adventitious rooting are not clear and differ depended on plant species and ambient temperature (Armstrong and Backett, 1987; Vartapetian and Jackson, 1997). Therefore, it sould be further studied on the physiological responses including acclimatic adaptation to hypoxia and high temperature which generally occurred.