EXPERIMENT II.

Seed Growth and Seed Malt Quality of Barley Genotypes in Response to Transient Waterlogging

Objectives

1. To study dry matter accumulation, total soluble sugar and total nitrogen content which partitioned to the seeds of three barley genotypes under transient waterlogging.
2. To evaluate seed malt quality of differing barley genotype responses to transient waterlogging.

Materials and Methods

Experimental conditions

Pot experiment was conducted at Lampang Agricultural Research and Training Center, Lampang, Thailand from November 1999 to March 2000. A split plot in completely randomized design with 3 replications was used. Three water regimes of each irrigation interval throughout the crop season as the main plots were;

1. Sufficient irrigation throughout crop season (WW),
2. 1 day of flooding and then drainage (1DWL),
3. 3 days of flooding and then drainage (3DWL).

Three barley genotypes were selected based on previous experiment; SMG1 (tolerant genotype), FNBLS#140 (moderately tolerant genotype) and BRBRF9629 (susceptible genotype) were used as subplots. Plants were grown in a 30 cm diameter and 30 cm high earthen pots and filled up with the rainfed lowland soil (sandy clay loam texture). The soil properties in this experiment were shown in Appendix 2. The basal inorganic fertilizer with 50 kg / ha of each N, P, and K was mixed with the soil and lime at the rate of 250 kg / ha before seeding. Only the vigor barley seeds (over 40 g / 1,000 seed) were seeded in the pots. At the first leave emergence, plants were thinned to be eight vigor plants in a pot.

The irrigations for uniform crop emergence and seedling establishment were applied until 3-4 leaves stage. Then it was irrigated every day after day for W1 treatment. Each transient flooding in W2 and W3 treatments were applied above soil surface when soil moisture remained 75% of field capacity. The soil moisture content in the flooded soil pots were measured everyday after day to check the level of 75% of field capacity before flooding as described by Kibread and Ananboontarick (1980).

Plant measurements

All recorded data were determined during grain filling period, except plant growth dynamics recording throughout the crop season.

1. Dry matter accumulation rates of each plant parts and their partitioning were measured as described in the Experiment I. At each sampling date, eight plants / replication was taken.

2. Photosynthetic efficiency was measured as described in the Experiment I. Four youngest fully expanded leaves samples of each treatments were measured at 10.00-12.00 a.m.

Leaf chlorophyll fluorescence from Photo system II of photosynthesis process was recorded at 650 nm on four youngest fully expanded leaves on the barley plants by using the time-resolving portable fluorimeter (Plant Efficiency Analyser, PEA, Hansatech Instrument Ltd., UK). All measurements were carried out as recommended by Angelopoulos *et al.*,(1996).

3. Total nitrogen content in the shoots and roots were analysed by the methods of Supakhumnert (1998) and Janjalernsuk *et al.* (1998) which detected the color of the ammonium nitrogen (NH_4^+-N) of extracted solution by Kjeldahl method (Appendix 14).

4. Total soluble sugar in the barley leaves, stems and seeds including total starch in the seeds were analysed by the method of Yoshida *et al.* (1976).

5. The percentage of seed protein was investigated at harvesting time. Three sub-samples / replication of each treatments were milled like flour and analysed for the total nitrogen content. Seed protein content was obtained by multiplying the total nitrogen content by 6.25.

The percentage of seed germination within 3 days was evaluated by seed germination testing with wet papers in the closed plastic boxes. The germinated seeds (100 seeds/ subsample) were recorded and calculated. Four sub-samples of each treatment were determined.

Results and Discussion

Plant growth dynamics

Different water regimes affecting plant growth dynamics of barley genotypes were presented in Table 8. All barley genotypes under WW had high leaf growth rates (LGR) than under transient waterlogging (1DWL and 3DWL). The ranges for LGR was 0.62-0.75 g / plants /day under all water regime treatments. The highest LGR was 1.02 g /plants /day for FNBLS#140 grown under 3DWL as compared to BRBRF9629 which had the lowest of 0.5 g /plants / day. It was reported that the waterlogged tolerant genotypes could maintain their leaf growth rate under the severe waterlogging (Jackson and Drew, 1984; Huang *et al.*, 1994b).

SMG1 genotype consistently maintained their stem growth rate (StGR) under different water regimes. StGR for FNBLS#140 grown under 3DWL was 3.66 g /plants /day which was higher than the other genotypes. Although BRBRF9629 genotype had a higher StGR than SMG1 under WW and 1DWL but it decreased to the rate of 1.81 g /plants /day under 3DWL. Thus it is therefore clear that the vegetative growth of susceptible genotype was affected by the severe waterlogging.

The root growth rates (RGR) of barley genotypes under three water treatments were not different. However, under transient waterlogging, FNBLS#140 had a higher in RGR than the other genotypes. It was possibly due to high shoot growth of FNBLS#140 under transient waterlogging which partitioned a large amount of dry matter to the roots. Huang *et al.*(1994b) supported that the shoot growth under waterlogging could maintain the root adsorption efficiency under waterlogging.

SMG1 and FNBLS#140 had high grain growth rates (GGR) under transient waterlogging, especially 3DWL. The highest GGR was 2.1 g /plants /day for SMG1 grown under 3DWL. Whereas FNBLS#140 genotype had the highest GGR under WW and 1DWL as compared with the other genotypes. These results might be the ability of waterlogged tolerant genotypes which can produce high yield under flooding condition (Krizek,1982). Moreover, these results indicated that SMG1 was adapted to severe waterlogging better than FNBLS#140 genotype.

For overall crop growth dynamic, FNBLS#140 and SMG1 genotypes had high crop growth rate (CGR) under 3DWL, i.e. 7.77 and 5.63 g /plants /day, respectively. It was noted that FNBLS#140 had the highest CGR in all water treatments associated with high leaf and stem growth rate. Adaptation of the shoot plant to waterlogging depends on highly food reserved (Vartapetian and Jackson, 1997). Nevertheless, Trought and Drew (1980) commended that dry matter accumulation in the shoots did not indicated transient waterlogged tolerant trait.

There were not markedly difference in dry matter partitioning to seed of barley genotypes between the effect of WW and 1DWL. The 1DWL treatment seemed to be more effected on barley seed growth than the effect of 3DWL treatment. It may possibly associated with low ability of waterlogged adaptation under short and several times of flooding throughout the crop season as compared to 3DWL treatment. However, SMG1 had highest dry matter partitioning to the seeds under 3DWL. Whereas the partitioning coefficient of FNBLS#140 grown under 3DWL markedly decreased as compared to under WW and 1DWL. But this decreasing value did not differed from BRBRF9629 genotype.

Table 8 The leaf, stem, spike, grain and crop growth rates and partitioning coefficients of barley genotypes under different water treatments in the pot experiment.

Barley varieties	Water treatments	Leaf growth rate (g /plants /day)	r^2	Stem growth rate (g /plants /day)	r^2	Root growth rate (g / plants /day)	r^2
SMG1	WW	0.75	0.902**	1.42	0.929**	0.36	0.921**
	1DWL	0.62	0.900**	1.58	0.931**	0.50	0.951**
	3DWL	0.70	0.886*	1.95	0.897**	0.42	0.952**
FNBLS#140	WW	1.09	0.931**	1.95	0.922**	0.53	0.973**
	1DWL	0.70	0.894**	2.63	0.973**	0.71	0.984**
	3DWL	1.02	0.912**	3.66	0.947**	0.49	0.882*
BRBRF9629	WW	1.00	0.989**	2.92	0.973**	0.40	0.91**
	1DWL	0.69	0.970**	2.49	0.919**	0.30	0.907**
	3DWL	0.50	0.978**	1.81	0.913**	0.30	0.962**

Barley varieties	Water treatments	Grain growth rate (g / plants /day)	r^2	Crop growth rate (g / plants /day)	r^2	Partitioning coefficients (%)
SMG1	WW	1.34	0.983**	4.24	0.949**	31.59
	1DWL	1.28	0.910**	4.62	0.969**	27.66
	3DWL	2.10	0.921**	5.63	0.969**	37.20
FNBLS#140	WW	1.53	0.913**	5.22	0.970**	29.39
	1DWL	1.67	0.916**	5.85	0.982**	28.59
	3DWL	1.85	0.998**	7.77	0.978**	23.83
BRBRF9629	WW	1.20	0.901**	5.90	0.980**	20.26
	1DWL	1.11	0.882*	4.98	0.898*	22.29
	3DWL	1.02	0.913**	4.24	0.912**	24.06

Note: Water treatments in each irrigation intervals; WW = To watering everyday after day

1DAWL = 1 day of flooding and then drainage; 3DAWL = 3 days of flooding and then drainage.

$$\text{Partitioning coefficients (\%)} = \left(\frac{\text{grain growth rate}}{\text{crop growth rate}}\right) \times 100$$

*,** Significant at 0.05 and 0.01 probability levels, respectively.

Biochemical substances changes during grain filling of each barley genotypes

SMG1; tolerant genotype :

The photosynthetic rate of SMG1 under transient waterlogging especially 3DWL increased at early grain filling period (Figure 1). It was found that SMG1 had high leaf chlorophyll fluorescence for maintaining its photosynthetic rate (Figure 3). This result suggested that it has a mechanism of plant adaptation after imposing to waterlogging (Jiang, 1995). Increasing photosynthetic rate of tolerant genotype may rapidly recover and increase the efficiency of root adsorption after waterlogging (Drew, 1983; Drew and Stolzy, 1991). As the photosynthetic rate increased, the total soluble sugar in the leaves and stems of SMG1 under transient waterlogging also increased at the early stage and decreased before the end of maturity (Figure 2). The consistently transpiration rate under transient waterlogging may be associated with high total soluble sugar content in the leaves (Figure 1 and 2). This increased the efficiency of root adsorption (Huang et al.,1994b). However, SMG had low photosynthetic efficiency under 3DWL which evidently showed the high stomatal resistance (Figure 1).

It was found that total soluble sugar content in the leaves was greater than the amount of sugar in the stems during grain filling period (Figure 2). Thus, total soluble sugar in the seeds was mainly transported from the leaves more than from the stems. This evidently indicated that the amount of total soluble sugar in the seeds was associated with the total soluble sugar in the leaves (Figure 2). Total starch content in the seeds under transient waterlogging especially 3DWL, increased rapidly at the early grain filling period and then decreased (Figure 5) whereas total soluble sugar still accumulated in the seeds until maturity (Figure 2). This result indicated that SMG1, tolerant genotype, was even sensitive to waterlogging at early maturity stage.

For nitrogen assimilation under transient waterlogging, it was found that SMG1 had high NR activity in the leaves at the early grain filling period, but was however lower value than under WW. This enzyme activity was still high until maturity especially under 3DWL (Figure 4). It indicated that the SMG1 roots still functioned to uptake nitrate and had nitrate reduction in leaves by NR activity (Losada, 1976). In consequnce, it was reported that some particular protein substances were produced for adaptation to waterlogging (Champigny and Foyer,1992). During this period, the rate of photosynthesis and transpiration were consistently as compared with the control (WW) treatment (Figure 1).

SMG1 had high total nitrogen accumulation in the leaves and was more transferred to the seeds than transferring from the stems (Figure 6). The total nitrogen content in the SMG1 shoots under 3DWL was partitioned to the seeds more than under 1DWL (Figure 6). A similar observation was reported by Pate and Layzell (1981). It was found that the total nitrogen content in the seeds under 3DWL, was higher than under WW (Figure 6). Huang *et al.*, (1994b) reported that the increasing in nutrient supply to the waterlogged root medium improved shoot nitrogen status for wheat genotypes and could contribute to the better growth and produce high yield of waterlogged plants. Meechoui and Khoachaimaha (1987); Jackson (1996); and Vartapetian and Jackson (1997), reported that the chlorophyll content in the plant leaf was reduced and degradation into the form of nitrogen which can translocate into the seeds by waterlogging condition.

FNBLS#140; moderate tolerant genotype :

Photosynthetic and transpiration rates of FNBLS#140 under 1DWL significantly increased greater than under 3DWL and was the same rate as under WW during the end of grain filling period (Figure 1). It was found that the photosynthetic rate increased as the increase of leaf chlorophyll fluorescence under transient waterlogging (Figure 3). It was suspected that the stomatal resistance significantly increased in the middle of grain filling period whereas photosynthetic rate and transpiration rate did not differed from the WW treatment (Figure 1). This is attributed to the waterlogging adaptation of the plants by ABA activity in the leaves (Hwang and VanToai, 1991; Drew and Stolzy, 1991). The total soluble sugar accumulation and partitioning of FNBLS#140 was the same as SMG1. Total soluble sugar in the leaves and stems under 3DWL mainly decreased during the early maturity whereas total soluble sugar in seeds did

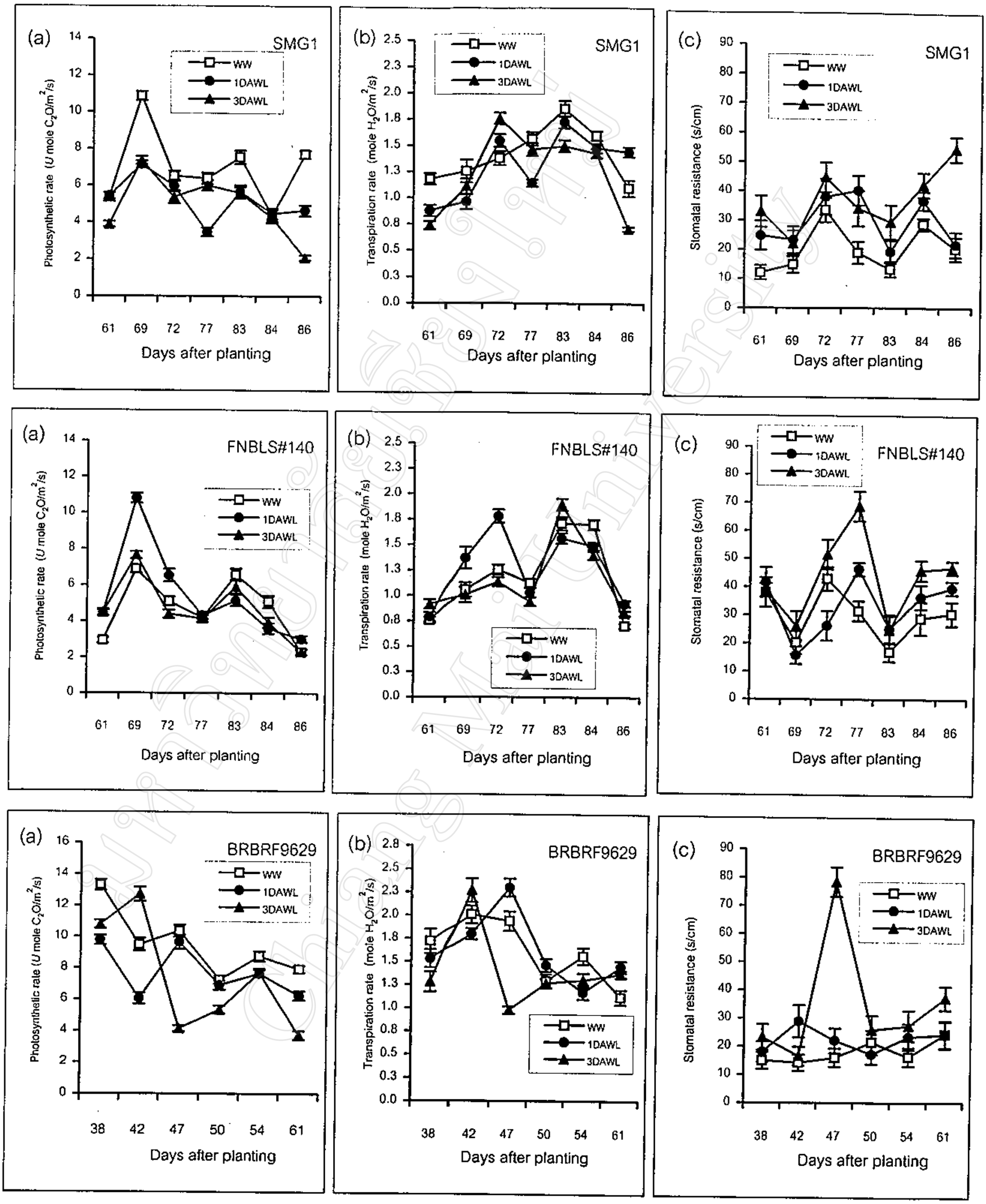

Figure 1 Photosynthetic rate(a),transpiration rate(b) and stomatal resistance(c) of barley genotypes during grain filling period under different water treatments. Data are means of four samples ± SE.

Note : Water treatments in each irrigation; WW= To watering everyday after day ; 1 DAWL and 3 DAWL= 1 day and 3 days of flooding and then drainage, respectively.

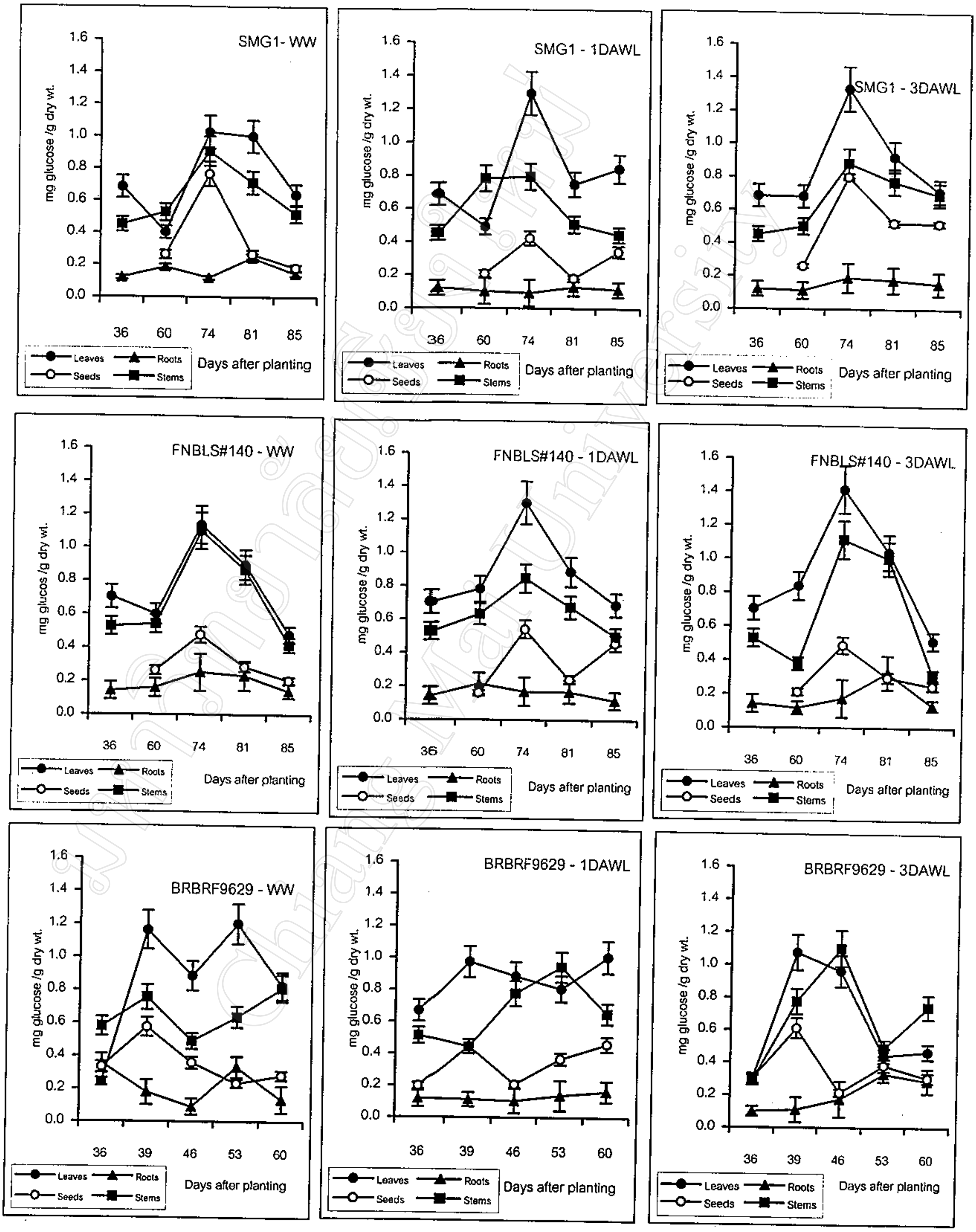

Figure 2 Total soluble sugar in leaves, stems, roots and seeds of barley genotypes during grain filling period under water treatments. Data are means of four samples ± SE.

Note: Water treatments in each irrigation: WW = to watering everyday after day, 1DAWL=1 day of flooding and the drainage; and 3DAWL = 3 days of flooding and then drainage.

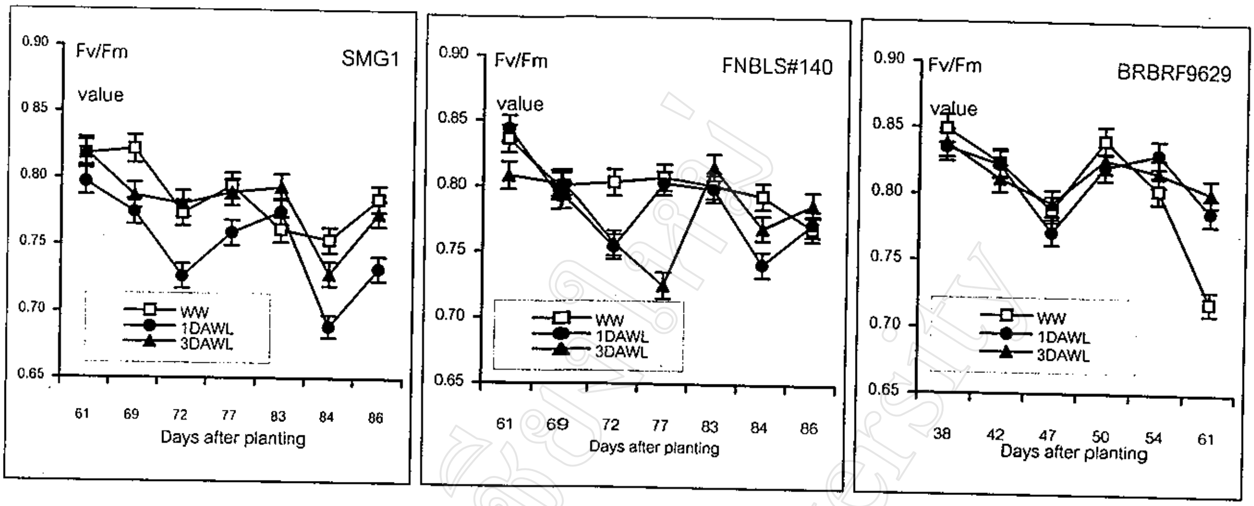

Figure 3 Leaf chlorophyll fluorescence of barley genotypes during grain filling under different water treatments.

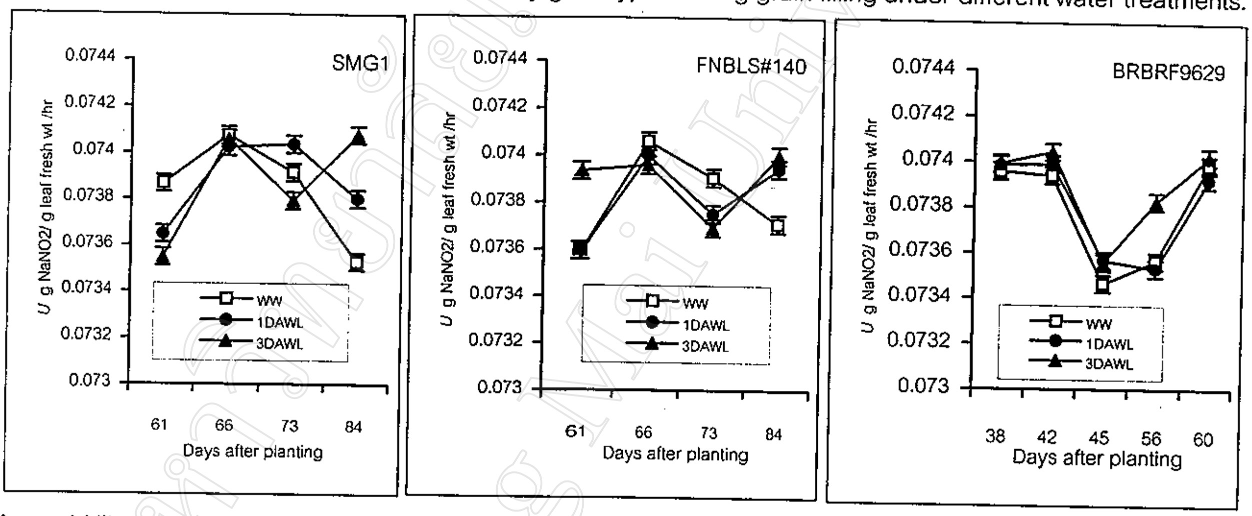

Figure 4 Nitrate reductase activity in leaves of barley genotypes during grain filling under different water treatment

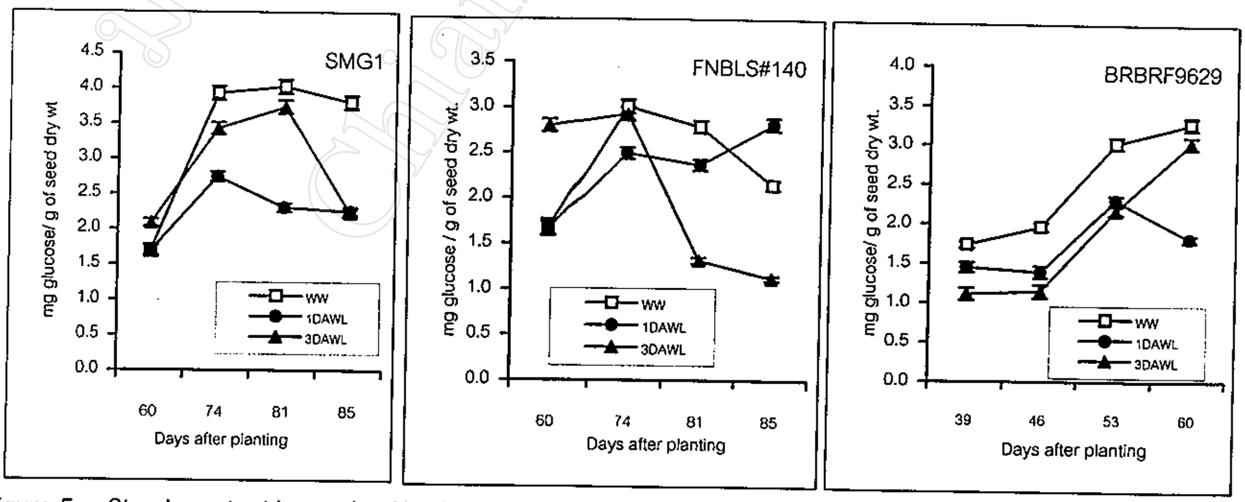

Figure 5 Starch content in seeds of barley genotypes during grain filling period under different water treatments

Note : Water treatments in each irrigation; WW = To watering everyday after day, 1 DAWL = 1 day of flooding and then drainage; and 3DAWL = 3 days of flooding and then drainage. Data are means of four samples ± SE.

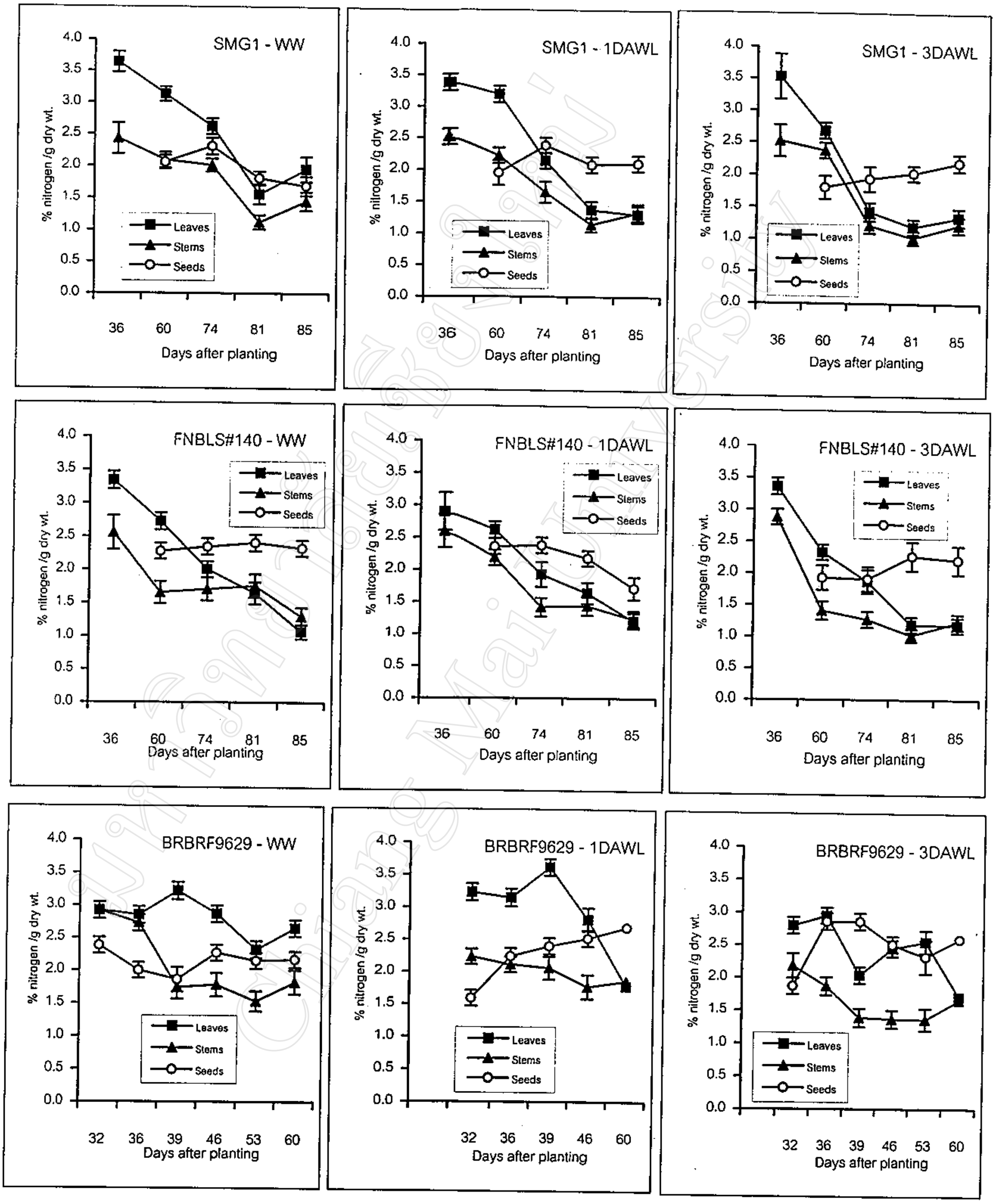

Figure 6 Total nitrogen in leaves, stems and seeds of barley genotypes during grain filling period under different water treatments. Data are means of four samples ± SE.

Note : Water treatments on each irrigation: WW=to watering everday after day, 1DAWL=1 day of flooding and then drainage; and 3DAWL = 3 days of flooding and then drainage.

not changed. (Figure 2). Plants might transport the sugar from the shoots to the roots for survival under waterlogging (Drew, 1983; Jackson and Drew, 1984). The total soluble sugar in the seeds under transient waterlogging, especially 1DWL was still higher than under WW. This result may be associated with the transferring of total soluble sugar from the newer tillers which formed at maturity (Fisher, 1984). However, total starch in the seeds of FNBLS#140 still increased under 1DWL and decreased under 3DWL at maturity (Figure 5).

Total nitrogen content in the FNBLS#140 leaves mostly transported to the seeds (Figure 6). FNBLS#140 grown under 3DWL could maintain the photosynthetic rate by increasing the leaf chlorophyll fluorescence. High NR activity at this period may be associated with accelerating leaf chlorophyll fluorescence (Figure 1, 3 and 4). Losada (1976) and Krizek (1982) confirmed the same as this result. Moreover, it may consequently cause to compensate the loss of dry matter (Jiang, 1995) and contribute the root adsorption efficiency (Everard and Drew, 1987; Krizek, 1982). FNBLS#140 under 3DWL had high total nitrogen accumulation in the seeds (Figure 6) whereas total soluble sugar and starch in seed decreased (Figure 2 and 6). This result may be the effect of leaf chlorophyll degradation (Jackson, 1996; Vartapetian and Jackson, 1997). In the other hand, the rate of photosynthesis for 1DWL was the same rate as WW treatment (Figure 1) which caused high total soluble sugar in the seeds (Figure 2). This result possibly decreased the amount of nitrogen content in the seeds (Figure 6).

BRBRF9629, susceptible genotype:

The photosynthetic and transpiration rate of BRBRF9629 under 1DWL slightly differed from WW treatment (Figure 1). Therefore, BRBRF9629 had consistent total soluble sugar content in the leaves under 1DWL (Figure 2). For the newly tiller production of BRBRF9629 at maturity, it might be the effect of the translocation of photosynthate from the new tillers at early physiological maturity and cause to interfere the analysis of total sugar in the leaves. Fisher (1984) also found the same result in wheat. Under transient waterlogging, total soluble sugar in the stems markedly increased at early maturity as compared to the sugar accumulation in the leaves (Figure 2). It was possibly transported to the roots for survival (Drew, 1983).

BRBRF9629 grown under 3DWL had high photosynthetic and transpiration rate at the early grain filling period due to the increase of the leaf chlorophyll fluorescence as the responses of the other genotypes (Figure 1 and 3). Then both rates significantly decreased until maturity stage. This result increased the total soluble sugar which partitioning to the seeds under WW at early grain filling period. However, total soluble sugar in the seeds of BRBRF9629 under 3DWL significantly increased at maturity (Figure 2). This result was possibly associated with the translocation of photosynthate from the new tillers to the seeds. Total soluble sugar content in the seeds under 3DWL decreased at the middle of grain filling period due to the reduction of photosynthetic rate and had high stomatal resistance (Figure 1 and 2). This may be due to high ambient temperature during those period (Appendix 6).

Under transient waterlogging, total nitrogen content in the leaves and stems of BRBRF9629 increased (Figure 6), whereas NR activity was reduced by waterlogging (Figure 4). It may be the effect of chlorophyll degradation which caused to decrease the photosynthetic rate, especially under 3DWL (Figure 1). However, NR activity at the early maturity under transient waterlogging, especially 3DWL, markedly increased (Figure 4) which possibly affected photosynthetic rate by increasing leaf chlorophyll fluorescence (Figure 1 and 3). Jiang (1995) also supported this result. In consequence, it was found that total starch in the seeds slightly increased at early maturity (Figure 5), whereas the increase of total nitrogen in the seeds was also found under 3DWL treatment (Figure 6).

Seed malt quality

All barley genotypes were significantly difference in seed malt quality. The 1,000 grain weight and the percent of seed protein content were used as the indicators for malt quality (Lersrutaiyotin *et al.*, 1995b; Toojinda *et al.*, 1992b). In this study, the 1,000 grain weight was significantly difference among barley genotypes. There was no interaction effect between water treatments and barley genotypes on the 1,000 grain weight (Appendix 7). This result indicated that it depended on genetics more than environmental effect (Lersrutaiyotin *et al.*,1995a). All barley genotypes grown under three water treatments had the 1,000 grain weight over than 40 g / 1,000 seeds (Table 9) as the standard of barley malting quality (Brummer, 1980; Lersrutaiyotin *et al.*, 1995b). BRBRF9629 genotype had the highest 1,000 grain weight of 46.92 g, as compared to SMG1 and FNBLS#140 which produced only 41.75 and 41.38, respectively.

The percentage of seed protein content of each barley genotype was significantly affected by water treatments (Appendix 7). The leaf photosynthetic rate possibly affected the increments of carbon and nitrogen in the dry matter of plant parts and assessments of photosynthetic gains (Pate, 1980; Kaiser and Foster, 1989; Yoshida, 1981). Only SMG1 under WW and FNBLS#140 under 1DWL, were met the standard of seed protein content (i.e. 10.56% and 10.75%, respectively). All barley genotypes grown under the other treatments had high seed protein content (Table 9). High total nitrogen accumulation and low starch in the seed during grain filling period was detected under transient waterlogging especially 3DWL (Figure 5 and 6). This result was associated with the leaf chlorophyll degradation, high NR activity and low photosynthetic rate (Figure 1 and 3). It was reported that the water deficit and /or hot climate increased the seed protein content, and decreased 1,000 grain weight of wheat genotypes (Lersrutaiyotin *et al.*, 1995a; Toojinda *et al.*, 1992a; Meechoui and Khaochaimaha, 1987).

The percentage of seed germination within 3 days of each barley genotype significantly differed under water treatments ($P<0.01$) (Appendix 7). All barley genotypes under 3DWL had high seed germination within 3 days as compared with the other treatments. SMG1 had the highest percentage of seed germination (96.5%) (Table 9) and met the standard for malting quality (Brummer, 1990). BRBRF9629 grown under transient waterlogging had high 1,000 grain weight and was not met the malting standard for seed germination. The percentage of seed germination for the

Table 9 Average 1,000 grain weight, the percent of germination within 3 days and the percent of protein in seeds of barley genotypes under different water treatments in the pot experiment.

Treatment	Barley genotypes				Barley genotypes			
	SMG1	FNBLS#140	BRBRF9629	mean	SMG1	FNBLS#140	BRBRF9629	mean
	1,000 grain weight (g)				Germination (%) within 3 days			
WW	39.98	41.23	47.40	42.87	60.0	56.5	50.5	55.7
1DAWL	41.63	41.30	46.30	43.08	62.5	61.0	76.3	66.6
3DAWL	43.65	41.60	47.05	44.10	96.5	66.0	85.8	82.8
mean	41.75	41.38	46.92		73.0	61.2	70.8	
LSD at 05 of (G) =		1.79				-		
LSD at 0.05 of (WxG) =		-				8.00		

	% protein in seeds			mean
WW	10.56	14.56	13.56	12.90
1DAWL	13.13	10.75	16.81	13.56
3DAWL	13.69	13.81	16.19	14.56
mean	12.46	13.04	15.52	
LSD at 0.05 of (WxG)	= 0.05			

Note : Water treatments in each irrigation intervals: WW = To watering everyday after day, 1DAWL = 1 day of flooding and then drainage; 3DAWL = 3 days of flooding and then drainage. W = Water treatments, G = Barley genotypes.

other treatments except for SMG1 under 3DWL were low values (Table 9). This result may be related to the seed dormancy. Excess water in the soil reduced seed germination of most plant species. It was believed that lower germination is due to a few amount of oxygen supply in waterlogged soils (Drew, 1991).

Although SMG1 under 3DWL treatment had the 1,000 grain weight and the percentage of seed germination within 3 days which were met the standardization for malting quality, but it was high seed protein content. Whereas, FNBLS#140 genotype grown under 1DWL had also the 1,000 grain weight and seed protein content within the standard of malting quality, but it had low seed germination within 3 days (Table 9). These results affected the percentage of malt yield (Lersrutaiyotin *et al.*, 1995b). Therefore, the seed malt quality could not evaluate in this study.

However SMG1 genotype showed the ability of waterlogged adaptation due to high growth rates, high dry matter partitioning to the seeds, and had good seed malt quality except seed protein content. From this result suggested that some physiological changes such as leaf chlorophyll degradation and high NR activity which affected seed malting quality during grain filling period of SMG1 genotype, should be further investigated.

Results obtained from the Experiment 1 and 2 revealed that waterlogging reduces shoot and root growth, dry matter accumulation and final grain yield. One characteristic identifying the waterlogged tolerant genotypes is the partitioning of dry matter accumulation between the shoot and the root. Krizek (1982) stated that the waterlogged tolerant plant is determined not only by its capacity to undergo morphological-anatomical adaptations, but also by the ability to recover from transient waterlogging of the root system. However, genotypic variation to ethylene and also the acclimatic adaptation of barley genotypes have not been well studied. Therefore, in the next two experiments were designed to investigate whether differential responses to hypoxia and ethylene exist in root characteristics, alcohol dehydrogenase activity and acclimatic adaptation of three barley genotypes differing in waterlogged tolerant.