Chapter 3

MATERIALS AND METHODS

3.1. Field experiment

3.1.1 Site, treatments and sowing

The experiment was carried out at the experimental station of Multiple Cropping Center, Chiang Mai University (19°N, 99°E) during the cool season (November to February). The soil is a sandy loam (San Sai series), pH 5.7-5.9, and contains 0.05-0.06% total N, 34-57 mg Pkg^{-1} soil and 91-124 mg Kkg^{-1} soil, respectively (Preidsripipat, 1988).

The treatments were factorial combination of four levels of soil B, which had been treated in previous years (0.09, 0.12, 0.16 and 0.24 mg Bkg^{-1} soil), and 10 wheat genotypes being investigated for tolerant or sensitive to B deficiency. The experimental lay-out was a split-plot design. Four levels of B were in main plots, each with duplicate. In each main plot, there were 10 subplots, each subplot contained one of the 10 genotypes (Table 2).

Table 2. Ranks of genotypes in response to B deficiency. (Based on Rerkasem, 1989)

Genotypes	Response to Boron Deficiency	Source/Location Identified
SW 41	Susceptible (Bds)	CMU
BL 1022	Susceptible (Bds)	Nepal-Lumle
CMU 285	Susceptible (Bds)	CMU
SW 23	Moderately susceptible (Bdm)	CMU
Sonalika	Moderately susceptible (Bdm)	BARI
Kanchan	Moderately susceptible (Bdm)	BARI
Sonora 64	Tolerant (Bdt)	CIMMYT
KUHR 12	Tolerant (Bdt)	CMU
CMU 26	Tolerant (Bdt)	CMU
NL 460	Tolerant (Bdt)	Nepal-Lumle

The seeds of 10 genotypes were treated with a fungicide (Vitavax) and an insecticide (Marshal - Carbofuran) and sown into moist soil on November 16, 1990. Each subplot consisted of 8 rows, 2.5 m long with 0.25 cm space between rows.

Fertilizers, 100 kgha^{-1} arrophos [$Ca(H_2PO_4)_2$,60% P_2O_5], 200 kgha^{-1} Urea (45% N), and 100 kgha^{-1} K_2SO_4 (50% K_2O), were applied to each plot before sowing. The crop was to get the optimum general management, ie. for land preparation, irrigation, pests and weed control, as normally applied to other wheat nurseries.

3.1.2 Sampling

3.1.2.1 Tissue boron analysis

Of all 8 rows of plants, samples only from 2nd and 7th rows in each plot were cut for tissue analysis, which were dried in ventilated oven at 80^{o}C for 48 hours.

i) Whole tops of twenty plants were cut about 1 cm above soil surface at double ridge stage (Zadoks 3.0).

ii) The flag leaf and developing ear were collected at booting stage (Zadoks 4.5). Twenty tillers which were just in "boot" (Zadoks 4.5) were cut. The ear was still completely enclosed in the leaf sheath and any ears that had begun to emerge were rejected, ie. leaf sheath splitting, in order to overcome the effect of transpiration rate on distribution of B. The developing ear was removed from the leaf sheath.

3.1.2.2 Anther and pollen

Sample were collected at heading stage (Zadoks 6). The length of 15 anthers from five flowering ears was measured. Then, under the microscope at X100 magnification, pollen was observed while it was reacted in KI/I_2 solution. This pollen observation was conducted within the 5 randomly chosen fields. Pollen was recorded as aborted if pollen did not stain dark in KI/I_2 solution.

3.1.2.3 Sterility counts

Floret sterility caused by B deficiency should not be confused with the incomplete, also termed sterile, florets (Bonnett, 1966) commonly found at the tip of each spikelet. In practice, the distinction is often far from obvious. To overcome this difficulty, it is proposed that grain set failure be assessed by an index of basal floret fertility, which is the average number of grains set in the two basal florets 1 and 2 (F1+2) of 10 central spikelets of the wheat ears (Rerkasem *et al.*, 1990).

Twenty ears were collected at early ripening stage (Zadoks 9.1). The number of spikelets per ear, grains per basal florets (F1+2), grains per ear and the percentage of fertile florets (calculated as the ratio of florets with developed grains to the total florets) was recorded.

3.1.2.4 Grain yield

At mature stage (Zadoks 9.4), plants from one meter of the 4 central rows (row 3 to 6, total sample size 1 m^2) was harvested; and, recorded grain yield and 1000 grain weight.

3.2 Pot experiment

3.2.1 Site, treatments and sowing

A pot study was conducted at Multiple Cropping Center, Chiang Mai University. The sowing dates were October 24, 1991 and January 15, 1992.

The experiment involved two wheat genotypes, SW 41 and Sonora 64, which were grown in a sand culture with 4 levels of B: 0, 0.001, 0.005 and 0.02 mg Bl^{-1}. For each treatment, there were 12 pots containing 0.015 m^3 of washed river sand. Seeds were sown 12 per pot and seedling thinned to 6 plants per pot at beginning of tillering stage (Zadoks 2.0). The plants were watered twice daily with 1 L of a complete nutrient solution (Table 3) with various B levels for each treatment.

The seeds were treated with a fungicide (Vitavax) and an insecticide (Marshal-Carbofuran). The crop was to get the optimum management.

Table 3. Composition of basal nutrients (Broughton and Dillworth, 1970)

Stock Solution	Element	µM	Form	MW	gl^{-1}	M
1	N	9000	NH_4NO_3	80.04	40.02	1.0
2	Ca	1000	$CaCl_2.2H_2O$	147.03	294.1	2.0
3	P	500	KH_2PO_4	136.09	136.1	1.0
4	Fe	10	Fe-citrate	334.85	6.7	0.02
	Mg	250	$MgSO_4.7H_2O$	246.5	123.3	0.5
	K	250	K_2SO_4	174.06	87.0	0.5
	Mn	1	$MnSO_4.H_2O$	169.02	0.338	0.002
5	Zn	0.5	$ZnSO_4.7H_2O$	287.56	0.288	0.001
	Cu	0.2	$CuSO_4.5H_2O$	249.69	0.100	0.0004
	Co	0.1	$CoSO_4.7H_2O$	281.12	0.056	0.0002
	Mo	0.1	$Na_2MoO_2.2H_2O$	241.98	0.048	0.0002

3.2.2 Sampling

3.2.2.1 Tissue boron analysis

The plant samples were dried in ventilated oven at 80°C for 48 hours.

i). Whole tops of twenty plants were randomly collected from 12 pots at the beginning of tillering stage (Zadoks 2.0)(thinned seedling), which were cut about 1 cm above sand surface.

ii). Whole top of six plants were collected from 1 pot at double ridge stage (Zadoks 3.0), which were cut about 1 cm above sand surface.

iii). At booting stage, twelve tillers were cut from 1 pot (selected from main ear and 1st tiller ear), being just in "boot" (Zadoks 4.5). The developing ear and flag leaf were collected.

3.2.2.2 Anther and pollen

i) Anther length and the number of pollen (Zadoks 6)

Ear was divided into 3 parts (top, middle and bottom). Six anthers for each part were taken before pollen dehiscence from each treatment. Anther length was measured under the microscope. Then, anther was splitted in 1 ml KI/I_2 solution under the microscope.

A drop (1/24.5 ml) of pollen solution was placed upon the grid paper. The process was observed under the microscope at X35 magnification. There were 10 drops of pollen solution. Total number of pollen in 1 ml suspension was calculated. Aborted pollen, unstained pollen, was also be recorded.

ii) Pollen germination and tube growth (Zadoks 6)

As soon as possible after anthers were extruded from glumes, pollen was shaken directly into a 5 cm diameter glass dish of 0.7% agar medium containing 0.75 M raffinose and 300 mgl^{-1} $CaCl_2.2H_2O$ with various B supply: 0, 10, 15, 20 and 100 mgl^{-1} H_3BO_3 at field temperature (around 25°C) and 30°C. Pollen was observed under the microscope at X100 magnification and was recorded as germinated if the pollen tube was longer than half the diameter of the grain. Burst pollen and grains intact but ungerminated were also scored. The germinated grains were taken as a percentage of the total of germinated, ungerminated and burst grains. The lengths of pollen tubes were recorded after drawing germinated pollen from randomly selected fields (to a total of 30 tubes for each treatment), using an Olympus drawing apparatus (BH 2-DA). Tube length was then calculated by using a piece of damp cotton thread to measure the drawing, and the length calculated from measured value.

3.2.2.3 Sterility counts and 1000 grain weight

Twenty ears were collected at ripening stage (Zadoks 9.1). The number of spikelets per ear, grains per basal floret 1 and 2 (F1+2), the percentage of fertile florets, grains per ear and 1000 grain weight.

3.3 Boron analysis

Dry-ashing technique was used to extract B from plant sample. The azomethind-H method was used to determine B content in extracted solution (Basson *et al.*, 1969).

3.4 Data analysis

Statistix software was adapted to analyze the variance of observed data and to regress pollen germination. Harvard Graphic software was applied to draw figures.