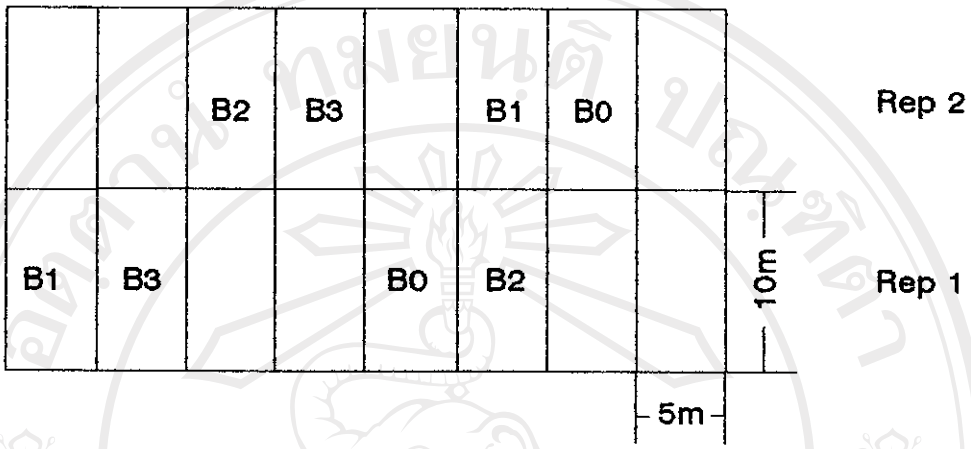
APPENDIX

Appendix A

Layout of experiment design

Appendix A-1. Layout of field experiment.



Rep 1

B0		B1		B2		B3	
4	5	8	5	5	7	1	8
3	6	3	9	6	3	6	5
2	7	4	2	4	8	0	2
1	8	6	0	2	0	7	3
0	9	1	7	1	9	9	4

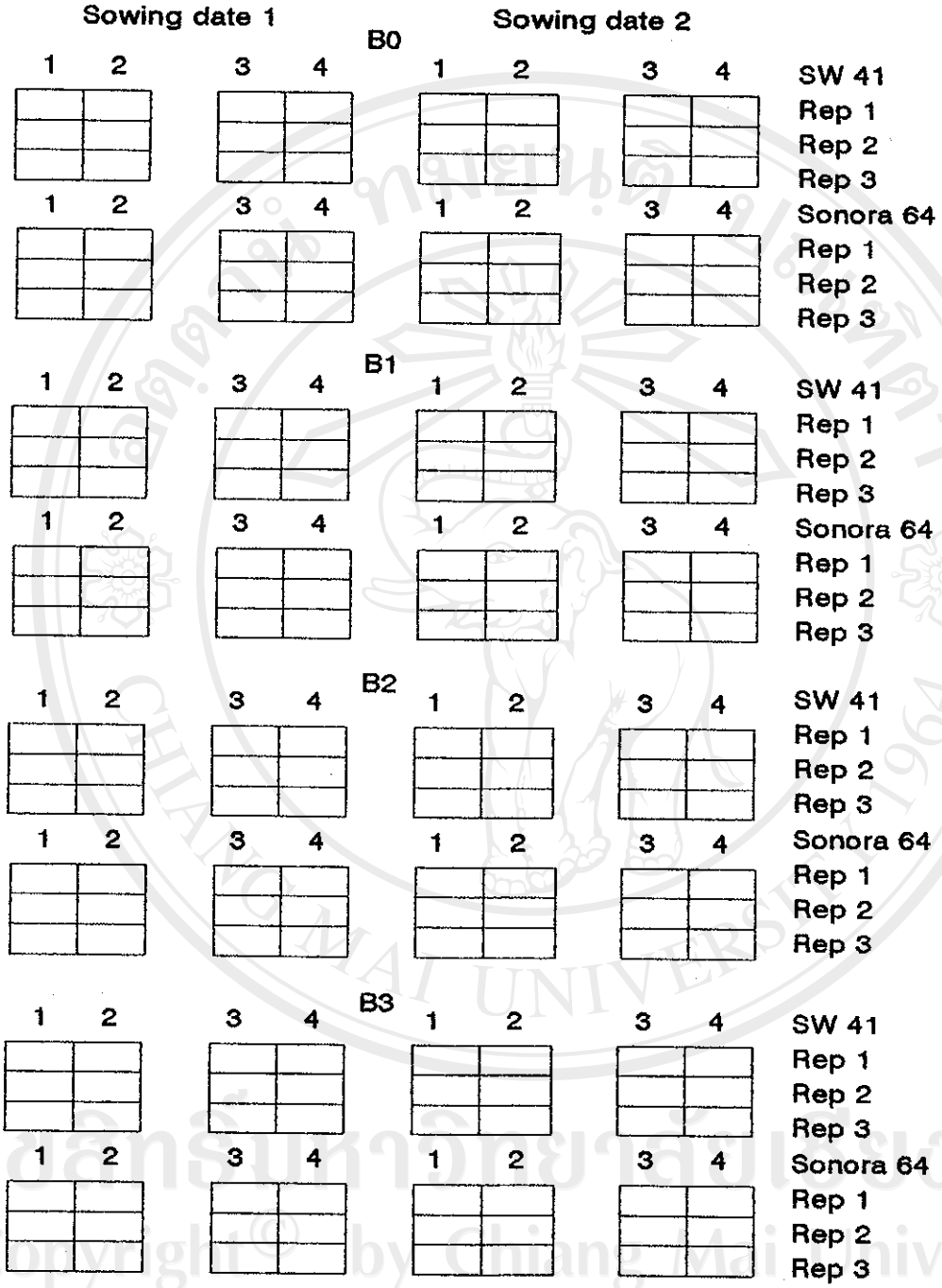
Rep 2

B0		B1		B2		B3	
8	0	5	2	6	9	3	4
1	9	3	6	3	8	7	2
4	6	7	9	1	4	8	5
2	3	8	1	2	7	1	0
5	7	4	0	5	0	9	6

Note:

0 - Sonora 64
1 - Sonalika
2 - NL 460
3 - SW 41
4 - KUHR 12
5 - CUM 285
6 - Kanchan
7 - BL 1022
8 - CMU 26
9 - SW 23

Appendix A-2. Layout of pot experiment.



Note:
No. 1: sample for double ridge stage
No. 2: sample for booting stage
No. 3: sample for pollen viability testing
No. 4: sample for yield component counting

Appendix B

Analysis of variance of field experiment

Appendix B-1. ANOVA for days of reaching double ridge stage of 8 genotypes (excluding Sonora 64 and NL 460).

Source of variation	DF	MS	P
Rep (A)	1	0.81000	0.2132
Boron (B)	3	0.83437	0.2305
A*B	3	0.32625	
Genotype (C)	7	46.03400	0.0000
B*C	21	0.25688	0.6827
A*B*C	28	0.31540	

Appendix B-2. ANOVA of anther length and percentage of positive pollen reaction to iodine of 9 genotypes (excluding NL 460).

Source of variation		Anther length		The percentage of positive pollen reaction to iodine	
	DF	MS	P	MS	P
Rep (A)	1	0.013889	0.4354	8.5422	0.5581
Boron (B)	3	0.10185	0.0892	895.73	0.0054
A*B	3	0.017222		19.794	
Genotype (C)	8	0.30545	0.0000	179.65	0.0000
B*C	24	0.019248	0.3852	91.066	0.0006
A*B*C	32	0.017326		26.464	

Appendix B-3. ANOVA of basal floret fertility and the percentage of fertile florets of 10 genotypes.

Source of variation		Basal floret fertility		Percentage of fertile florets	
	DF	MS	P	MS	P
Rep (A)	1	0.03042	0.1539	49.141	0.3949
Boron (B)	3	0.11343	0.0303	245.07	0.1124
A*B	3	0.008443		50.082	
Genotype (C)	9	0.052589	0.0012	142.93	0.0003
B*C	27	0.01608	0.2684	80.582	0.0029
A*B*C	36	0.012946		29.82	

Appendix B-4. ANOVA of yield and yield components of 10 genotypes.

Source of variation	Spikelets/ear			Grains/ear			1000 grain weight*			Yield/m² *		
	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P
Rep (A)	1	0.82012	0.6521	1	23.762	0.4084	1	32.805	0.4333	1	4269	0.2774
Boron (B)	3	2.8155	0.5499	3	39.412	0.3686	3	5.8998	0.9255	3	6053.3	0.2372
A*B	3	3.2951		3	25.843		3	40.273		3	2436.6	
Genotype (C)	9	8.9239	0.0000	9	198.6	0.0000	8	85.182	0.0000	8	2563.8	0.2175
B*C .	27	0.39157	0.9564	27	17.771	0.287	24	4.362	0.7517	24	1943.9	0.4012
A*B*C	36	0.74387		36	14.595		32	5.7208		32	1778	

* Variation of 9 genotypes (excluding NL 460)

Appendix B-5. ANOVA of [B] in tissue of 10 genotypes.

Source of variation	Double ridge*			Flag leaf			Developing ear		
	DF	MS	P	DF	MS	P	DF	MS	P
Rep (A)	1	1.8753	0.6022	1	0.078125	0.8125	1	0.041861	0.8966
Boron (B)	3	33.37	0.0877	3	18.21	0.0246	3	11.022	0.1031
A*B	3	5.5632		3	1.1667		3	2.0961	
Genotype (C)	8	12.135	0.0035	9	2.8303	0.0001	9	1.9291	0.0012
B*C	24	2.8203	0.6396	27	1.7762	0.0003	27	0.86465	0.0490
A*B*C	32	3.2613		36	0.51349		36	0.47914	

* Variation of 9 genotypes (excluding NL 460)

Appendix B-6. Matrix of correlation among various characters in B0 plants of 9 wheat genotypes.

	A	B	C	D	E	F	G	H	I	J	K	L
A	1.0000											
B	0.3923	1.0000										
C	0.1796	0.0281	1.0000									
D	0.1676	-0.2221	0.8486	1.0000								
E	-0.0132	-0.1526	0.9139	0.9453	1.0000							
F	0.2528	-0.0300	-0.1747	0.1742	0.0362	1.0000						
G	-0.0336	-0.3448	0.4289	0.7776	0.7107	0.5944	1.0000					
H	-0.1271	0.6712	-0.0082	-0.3482	-0.2157	-0.6498	-0.5978	1.0000				
I	-0.0884	0.0531	0.8525	0.7153	0.8281	-0.4516	0.2637	0.2259	1.0000			
J	0.8897	0.5088	-0.1506	-0.2157	-0.3775	0.1082	-0.4064	0.1070	-0.2873	1.0000		
K	-0.1544	-0.5423	0.5648	0.4594	0.5429	-0.5420	0.1834	-0.1525	0.5931	-0.3569	1.0000	
L	0.2666	-0.1172	0.3083	0.2652	0.2241	-0.0514	0.0082	-0.3131	0.2934	0.0834	0.1844	1.0000

Appendix B-7. Matrix of correlation among various characters in B3 plants of 9 wheat genotypes.

	A	B	C	D	E	F	G	H	I	J	K	L
A	1.0000											
B	0.0790	1.0000										
C	-0.4512	-0.1661	1.0000									
D	0.1573	0.0444	-0.0988	1.0000								
E	-0.3692	-0.2395	0.3411	0.7239	1.0000							
F	0.2584	0.0055	-0.2068	0.2212	0.2985	1.0000						
G	-0.3274	0.0011	0.1964	0.5544	0.7886	0.0907	1.0000					
H	-0.1176	0.3111	-0.1460	-0.4222	-0.6708	-0.4802	-0.5612	1.0000				
I	-0.1731	0.0733	-0.0924	0.0648	0.3083	-0.0222	0.7608	-0.4868	1.0000			
J	0.3885	-0.2093	-0.0284	-0.2510	-0.1725	0.1103	0.0052	-0.1106	0.2343	1.0000		
K	-0.1954	-0.2183	0.4214	0.5309	0.5971	0.1870	0.4626	-0.0876	-0.1372	-0.1543	1.0000	
L	0.0295	0.5237	0.2617	-0.6453	-0.5668	-0.0121	-0.2780	0.3923	-0.0310	0.1445	-0.1448	1.0000

A: days from emergence to double ridge stage;
B: anther length;
C: percentage of positive pollen reaction to iodine;
D: number of grains per two basal florets;
E: percentage of fertile florets;
F: spikelets per ear;
G: number of grains per ear;
H: 1000 grain weight;
I: grain yield;
J: [B] in whole tops at double ridge;
K: [B] in flag leaf at booting;
L: [B] in developing ear at booting.

Appendix C

Analysis of variance of pot experiments

Appendix C-1. Analysis over sowing dates

The procedure for combining data analysis over two sowing dates (24/10/91 and 15/1/92, respectively) is as follows:

--- to analyze two sets of data as a single factorial experiment involving sowing dates as a main plot factor and the others, such as plant B treatments and medium B treatments, as subplot factors;

--- to test for homogeneity of variance by comparing the mean square of error for main plot with the mean square of overall error (using F-test):

a) if it is non-significant, the homogeneity of variance is assumingly valid. The results of this analysis of variance are used for testing significance of treatments.

b) if it is significant, the homogeneity of variance is no longer valid. Thus, the data from two sowing dates is separately analyzed, *e.g.* the effects of treatments are examined independently for each sowing date. One Way ANOVA is used for testing effects of sowing dates.

Appendix C-2. Combined analysis for anther length, the number of pollen and the percentage of pollen positive reaction to iodine among locations in ear for two genotypes at two sowing dates

Source of variation		Anther length		The number of pollen		The percentage of pollen positive reaction to iodine	
	DF	MS	P	MS	P	MS	P
Date (A)	1	0.88674	0.109	3162500	0.0295	98.671	0.1243
Rep (B)							
A*B	4	0.20983		287610		26.202	
Boron (C)	3	0.14456	0.1565	175630	0.2076	82.344	0.0428
Genotype (D)	1	0.39063	0.0312	196	0.967	151.29	0.0252
Location (E)	2	2.861	0.0000	1275100	0.0001	341.49	0.0000
A*C	3	0.16021	0.1234	277350	0.0687	136.07	0.0046
A*D	1	0.013611	0.684	1200900	0.0016	13.322	0.5013
A*E	2	0.18991	0.1012	16391	0.8633	153.25	0.0071
C*D	3	0.12928	0.1971	240260	0.1029	100.2	0.0202
C*E	6	0.028281	0.9101	34147	0.9348	24.602	0.5424
D*E	2	0.059219	0.4911	29335	0.7762	43.17	0.2323
A*C*D	3	0.17903	0.0927	44776	0.7614	105.63	0.0161
A*C*E	6	0.0091493	0.9935	69523	0.7234	33.375	0.3446
A*D*E	2	0.029184	0.7052	71273	0.5423	19.414	0.522
C*D*E	6	0.01114	0.9897	96894	0.5357	18.109	0.7162
A*C*D*E	6	0.17898	0.0502	128740	0.3511	16.428	0.7613
A*B*C*D*E	92	0.08162		113910		29.232	

Appendix C-3. ANOVA of the pollen germination for two genotypes at sowing date 1.

Source of variation		Germinated pollen		Burst pollen		Ungerminated pollen	
	DF	MS	P	MS	P	MS	P
Plant B (A)	3	208.53	0.0053	1015.0	0.0000	454.40	0.0027
Genotype (B)	1	153.24	0.0662	2.0268	0.8889	120.07	0.2353
Medium B (C)	2	9289.9	0.0000	681.59	0.0029	8133.1	0.0000
Rep (D)	2	68.58	0.2160	148.66	0.2458	92.745	0.3360
A*B	3	23.661	0.6529	40.027	0.7610	48.155	0.6311
A*C	6	55.489	0.2843	112.59	0.3792	196.70	0.0446
B*C	2	234.56	0.0077	109.53	0.3527	366.68	0.0176
A*B*C	6	22.272	0.7942	145.87	0.2276	110.77	0.2616
A*B*C*D	46	43.284		102.74		83.029	

Appendix C-4. ANOVA of the pollen germination for two genotypes at sowing date 2.

Source of variation		Germinated pollen		Burst pollen		Ungerminated pollen	
	DF	MS	P	MS	P	MS	P
Plant B (A)	3	31.339	0.0444	523.45	0.0034	394.79	0.0373
Genotype (B)	1	34.113	0.0808	10.332	0.7462	82.033	0.4264
Medium B (C)	4	951.46	0.0000	562.2	0.0009	582.18	0.0039
Rep (D)	1	1.8301	0.6803	133.77	0.2479	104.22	0.3705
A*B	3	2.9982	0.8378	188.18	0.1398	176.56	0.26
A*C	12	17.425	0.1199	132.64	0.2242	194.01	0.1556
B*C	4	28.857	0.0434	220.59	0.0792	350.55	0.041
A*B*C	12	9.9061	0.525	80.667	0.6201	98.045	0.6743
A*B*C*D	39	10.616		97.221		126.96	

Appendix C-5. Combined analysis for the pollen germination at M20 and M100 for two genotypes at two sowing dates.

Source of variation		Germinated pollen		Burst pollen		Ungerminated pollen	
	DF	MS	P	MS	P	MS	P
Date (A)	1	2793	0.006	2568	0.0049	10717	0.0005
Rep (B)							
A*B	2	16.819		12.67		5.8129	
Plant B (C)	3	203.32	0.0065	374.99	0.0107	160.64	0.196
Genotype (D)	1	34.149	0.3687	1.5563	0.893	20.896	0.6452
Medium B (E)	1	1255.9	0.0000	1563.6	0.0002	16.841	0.6793
A*C	3	67.249	0.2009	80.18	0.4294	23.159	0.8679
A*D	1	60.353	0.2345	4.01	0.829	95.967	0.3269
A*E	1	482.41	0.0018	0.29431	0.9533	458.87	0.0373
C*D	3	4.5072	0.9536	18.34	0.8838	27.154	0.8386
C*E	3	53.674	0.2896	23.08	0.8443	62.018	0.594
D*E	1	101.88	0.1254	0.007225	0.9927	100.63	0.3156
A*C*D	3	31.386	0.5223	98.279	0.3401	48.919	0.6808
A*C*E	3	18.045	0.7259	104.17	0.315	171.5	0.1731
C*D*E	3	13.101	0.8111	103.52	0.3177	91.347	0.431
A*D*E	1	29.363	0.4041	45.765	0.4675	147.77	0.2257
A*C*D*E	3	21.323	0.6717	70.53	0.4854	23.787	0.8633
A*B*C*D*E	30	40.993		84.494		96.6	

Appendix C-6. ANOVA of pollen germination between field temperature and 30°C at M20 and M100 at sowing date 2.

Source of variation		Germinated pollen		Burst pollen		Ungerminated pollen	
	DF	MS	P	MS	P	MS	P
Plant B (A)	1	108.78	0.0029	334.76	0.0758	63	0.4499
Genotype (B)	1	17.701	0.173	9.7903	0.7488	53.303	0.4864
Temperature (C)	1	693.78	0.0000	2062.4	0.0003	363.83	0.082
Medium B (D)	1	5.2812	0.4467	883.05	0.0073	751.75	0.0171
Rep (E)	1	21.125	0.1389	61.328	0.427	10.465	0.7562
A*B	1	38.72	0.0515	51.258	0.4669	0.94531	0.9255
A*C	1	12.005	0.2571	55.915	0.4477	119.74	0.3017
A*D	1	15.125	0.2058	66.413	0.4089	144.93	0.2577
B*C	1	0.125	0.9059	89.445	0.3397	98.35	0.3478
B*D	1	42.32	0.0429	1.5753	0.8976	27.195	0.6177
C*D	1	5.78	0.4264	0.0078125	0.9928	5.8653	0.8161
A*B*C	1	3.0012	0.5646	1.0878	0.9148	0.52531	0.9445
A*B*D	1	7.8013	0.3573	199.5	0.1615	129.2	0.2841
B*C*D	1	24.151	0.1154	12.625	0.7162	71.103	0.4227
A*B*C*D	2	55.851	0.0095	9.7478	0.9001	58.268	0.5845
A*B*C*D*E	15	8.6477		91.988		104.67	

Appendix C-7. ANOVA of pollen tube length at M20 and M100 for two genotypes at sowing date 1.

Source of variation	DF	MS	P
Plant B (A)	3	2754.6	0.0000
Genotype (B)	1	1854.4	0.0000
Medium B (C)	1	387.81	0.0114
Rep (D)	1	68.445	0.2446
A*B	3	587.53	0.0002
A*C	3	157.51	0.0465
B*C	1	4.0613	0.772
A*B*C	3	581.56	0.0002
A*B*C*D	15	46.656	

Appendix C-8. Combined analysis of pollen tube length at M100 for two genotypes at two sowing dates.

Source of variation	DF	MS	P
Date (A)	1	897.82	0.0611
Rep (B)			
A*B	2	60.293	
Plant B (C	3	1349.6	0.0000
Genotype (D)	1	51.258	0.3127
A*C	3	629.54	0.0002
A*D	1	2728.8	0.0000
C*D	3	212.5	0.02
A*C*D	3	399.86	0.0018
A*C*D*E	14	46.731	

Appendix C-9. ANOVA of pollen tube under temperature treatments at M100 for two genotypes at sowing date 2

Source of variation	DF	MS	P
Plant B (A)	1	443.1	0.0001
Genotype (B)	1	1145.8	0.0000
Temperature (C)	1	2129.8	0.0000
Rep (D)	1	2.1025	0.6089
A*B	1	184.96	0.0015
A*C	1	5.29	0.4238
B*C	1	316.84	0.0003
A*B*C	1	7.0225	0.3604
A*B*C*D	7	7.3325	

Appendix C-10. Combined analysis of basal floret fertility and the percentage of fertile florets for two genotypes at two sowing dates.

Source of variation		Basal floret fertility		The percentage of fertile florets	
	DF	MS	P	MS	P
Date (A)	1	0.034133	0.2875	474.27	0.0465
Rep (B)					
A*B	4	0.022721		58.513	
Plant B (C)	3	0.18023	0.0014	541.78	0.0006
Genotype (D)	1	0.56333	0.0001	2363.8	0.0000
A*C	3	0.048672	0.1627	47.934	0.5593
A*D	1	0.010208	0.5394	79.156	0.2911
C*D	3	0.10476	0.0179	481.77	0.0011
A*C*D	3	0.016669	0.6015	18.294	0.8482
A*B*C*D	28	0.026445		68.364	

Appendix C-11. Combined analysis of yield and yield components for two genotypes at two sowing dates.

Source of variation		Spikelets/ear		Grains/ear		1000 grain weight	
	DF	MS	P	MS	P	MS	P
Date (A)	1	48.2	0.0002	0.12505	0.9237	2126.4	0.0000
Rep (B)							
A*B	4	0.25167		12.031		2.3132	
Plant B (C)	3	1.6442	0.0395	145.23	0.0204	52.964	0.0004
Genotype (D)	1	27.15	0.0000	10.129	0.6093	199.19	0.0000
A*C	3	2.9698	0.0034	7.6766	0.8938	3.2956	0.6746
A*D	1	2.7552	0.0287	311.87	0.0078	49.167	0.0097
C*D	3	1.2698	0.0841	116.46	0.0439	7.2397	0.3524
A*C*D	3	1.9603	0.0213	49.085	0.2956	7.175	0.3563
A*B*C*D	28	0.5178		37.905		6.386	

Appendix C-12. Combined analysis of [B] in tissue for two genotypes at two sowing dates.

Source of variation		Tillering stage		Double ridge		Flag leaf		Developing ear	
	DF	MS	P	MS	P	MS	P	MS	P
Date (A)	1	0.06627	0.1384	9.7921	0.0104	30.068	0.0005	1.3601	0.0637
Rep (B)									
A*B	4	0.19423		0.47141		0.27562		0.21014	
Plant B (C)	3	11.27	0.0000	4.2805	0	7.2299	0.0000	3.7641	0.0000
Genotype (D)	1	3.9331	0.0012	0.0096333	0.8404	0.9213	0.0949	1.92	0.0137
A*C	3	0.8633	0.0566	0.86769	0.0227	0.47835	0.2232	0.56452	0.1318
A*D	1	0.023408	0.7839	3.5752	0.0005	1.9643	0.0176	1.9441	0.0132
C*D	3	1.0349	0.0317	0.6691	0.0542	0.14839	0.6979	0.27537	0.4107
A*C*D	3	1.039	0.0313	0.37961	0.2052	0.05283	0.9149	0.52002	0.1569
A*B*C*D	28	0.30528		0.23315		0.30834		0.27748	

Appendix C-13. ANOVA of ears (mature and late) per pot of two genotypes for each sowing date

Source of variation		Mature ears				Late ears			
		Sowing date 1		Sowing date 2		Sowing date 1		Sowing date 2	
	DF	MS	P	MS	P	MS	P	MS	P
Plant B (A)	3	58.278	0.1165	3.0417	0.8422	355.6	0.0152	37.375	0.0016
Genotype (B)	1	73.5	0.1071	77.042	0.0194	26.042	0.5573	136.37	0.0000
Rep (C)	2	492.12	0.0001	8.6667	0.4754	263.38	0.0528	6.5417	0.2492
A*B	3	2.2778	0.9633	5.9306	0.6647	101.15	0.283	6.5972	0.2456
A*B*C	14	24.792		11.048		72.042		4.256	

Appendix C-14. One way ANOVA of sowing dates for ears (mature and late) per pot.

Source of variation		Mature ears		Late ears	
	DF	MS	P	MS	P
Between sowing dates	1	1074	0	1180	0.0002
Within sowing dates	46	40.49		71.77	

Appendix D

Analysis of [B] in tissue

Great care must be taken in sampling (see chapter 3), preparation of sample and analysis, in order to avoid contamination. Plant samples are oven dried at 80°C for 48 hours and ground.

1. Extraction

Dry-ashing technique is used to extract B from plant sample. Weigh approximately 300 mg oven-dried ground material into tall crucible and place in cold furnace. Sample of standard reference materialand blanks are included with each batch of samples.

Remove crucibles from furnace to electric frying pans. Rinse crucible wall down with 2 ml HCl (1:1 conc. HCl as to deionized (D.I.) water).

Adjust frying pan temperature to 50°C and heat gently for approximately half an hour.

Cool and transfer solution from crucible to 10 ml graduated polystyrene vial with small washes of D.I. water. Dilute to 10 ml volume. Cap vial and shake well.

Allow to settle by standing overnight or centrifuge at 2,500-3,000 rpm for 4 minutes.

2 Boron determination

The azomethine-H method is used to determine boron content in extracted solution.

2.1 Reagent

1) Buffer-masking reagent

Dissolve 140 g ammonium acetate
10 g potassium acetate
10 g tetra sodium salt of DETA
4 g nitrilotriacetic acid
in 200 ml of D.I. water.

After contents are completely dissolved, slowly add 63 ml conc. acetic acid.

Dilute solution to 1 litres.

Stand overnight at room temperature

Filter through a #1 filter paper.

2) Azomethine-H reagent (Prepare 24 hours before being used)

Dissolve 2 g of fresh azomethine-H (Merk) and 5 g ascorbic acid in 120 ml D.I. water.

Dilute solution to 250 ml.

Store in polypropylean bottle wrapped in aluminum foil and place in a refrigerator.

This reagent is useable for 14 days.

3) N Hydrochloric acid

Dilute 97 ml of conc. HCl (A.R. grade) to 1 litre with D.I. water.

4) Boron solution (100 ppm)

Dissolve 0.5716 g boric acid (A.R. grade) in 1 litre D.I. water.

This solution is used to prepare a series of boron standards in N HCl (0, 1, 2, 3, 4, and 5 ml of the 100 ppm boron standard, respectively, to 100 ml).

5) Mixed reagent

Mixed two parts (by volume) of the buffer-masking reagent with one part of the azomethine-H reagent.

This reagent must be used within 4 hours.

2.2 Procedure

Pipette 1 ml of the extracted solution (a) into a vial. Add 3 ml of the mixed reagent in a strong jet with a eppendorf to mixed well with sample.

Stand for 1 hour before boron content is determined colorimetrically at 420 nm.

Sample readings are compared with a series of boron standards (0, 1, 2, 3, 4 and 5 ppm) prepared at the same time and treated in the same way as sample solutions.

2.3 Cleaning

All of plastic containers are cleaned with D.I. water left in 20 % conc. HCl overnight, rinsed with D.I. water and air-dried.

Curriculum Vitae

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